PROJECT MANAGEMENT OF LYME DISEASE THROUGH MONITORING OF SEROPREVALENCE OF ANTI-BORRELIA ANTIBODIES IN MACEDONIAN PATIENTS

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Abstract

Lyme disease is a worldwide zoonotic disease and the most common tick-borne illness in Europe and North America. It is caused by at least three species of bacteria belonging to the genus *Borrelia (Borrelia afzelii* and *Borrelia garinii* cause most European cases, whereas *Borrelia burgdorferi* - is more frequent in North America). *Borrelia* is transmitted to humans by bite of infected ticks belonging to a few species of the genus *Ixodes*. Typical symptoms include fever, headache, fatigue, and a characteristic skin rash - erythema migrans. If left untreated, infection can spread to joints, heart, and nervous system. There are very little data about the seroprevalence in our population, furthermore the relation to clinical data has not been previously studied. IFA is a standard test for antibody detection of *B burgdorferi sensu lato*, since cultural growth of the organism from skin samples and blood is very difficult.

The aim of this paper is to find correlation between Lyme disease and tick bites in Macedonian patients. It was done through determining the seroprevalence of *Borrelia* IgG and IgM antibodies using IFA test and relating that data to reported tick bites.

The study was conducted on a sample of 240 patients, after suspected or observed tick bite. Serum samples were collected in a four years period (June 2009-June 2013). *Borrelia* specific IFA test - RIDA[®]FLUOR *Borrelia burgdorferi* (sensu lato), R-biopharm, Germany, was performed for IgG and IgM antibody detection. The positive titer was 1:16 for IgM and 1:32 for IgG. The sera were taken at least 10 days after confirmed or suspected thick bite. All patients also answered a short questionnaire regarding the history of a thick bite and previous treatment. Seropositive were 45/240 (18,8%) patients. Concomitant *Borrelia* IgG and IgM seropositivity was noted in 9 cases, and only IgG or only IgM seropositive were 21 and 15 patients, respectively. A tick bite 10 -60 days prior the testing had been noted in 62/240 (25,8%) patients. The ticks were removed in hospital settings in approximately two thirds of the patients. Seropositivity in either or both classes of antibodies in patients who reported a thick bite was 28,9% (13/45). Erythema migrans as a symptom was noted in 9 patients, all of them reporting a thick bite and being IgG positive. Approximately 50% of the patients bitten by a thick received antibiotic therapy.

Having in mind that *B burgdorferi* in this area is being tested for only few years, the results demonstrate IgG and/or IgM antibody seropositivity of 18,8%, whereas approximately one third of those cases were associated with a tick bite. Very few of those seropositive patients reported any previous symptoms. Those findings suggest that exposure to *B burgdorferi* does occur in our population, mostly without giving rise to clinical manifestations of Lyme disease. The need of applying precise testing and treatment protocols is obligatory. Further seroprevalence studies on human, as well as on animals are needed in order to create a correlation between seroprevalence of *anti- Borelia* antibodies with tick bites, which ultimately means better health protection of the population.

JEL Classification: H75, I10, I15, I18,

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1. INTRODUCTION

Lyme disease is a worldwide zoonotic disease and the most common tick-borne illness in Europe and North America. It is caused by at least three species of bacteria belonging to the genus *Borrelia (Borrelia afzelii* and *Borrelia garinii* cause most European cases, whereas *Borrelia burgdorferi* - is more frequent in North America) (Table 1). *Borrelia* is transmitted to humans by bite of infected ticks belonging to a few species of the genus *Ixodes* (Figure 1).

Borrelia species	Vector	Borrelia species	Vector
B. afzelii	I. ricinus I. persulcatus	B. californiensis	I. pacificus, I. jellisonii, I.
			spinipalpis
B. americana	I. pacificus, I. minor	B. carolinensis	I. minor
B. andersonii	I. dentatus	B. garinii	I. ricinus, I. persulcatus, I.
			hexagonus, I. nipponensis
B. bavariensis	I. ricinus	B. japonica	I. ovatus
B. bissettii	I. ricinus, I. scapularis, I.	B. kurtenbachii	I. scapularis
	pacificus		
B. burgdorferi sensu	I. ricinus, I. scapularis, I.	B. lusitaniae	I. ricinus
stricto	pacificus		

Table 1 Currently known Borrelia burgdorferi sensu lato complex

Infection occurs primarily during the late spring and summer months when nymphs are most active and persons spend the most time outdoors, but cases have been reported throughout the year¹. The duration of tick attachment is a critical factor affecting the risk of transmission². After attachment, the tick feeds and becomes enlarged, discharging its saliva into the bite wound. It takes 36 to 48 hours after attachment for *B. burgdorferi* to migrate from the midgut of the tick to the salivary glands³. How long the tick is attached (usually at least 36 hours) and whether it is enlarged are two of the most important factors to consider when assessing the risk of transmission.

Figure 1. Ixodes ricinus tick feeding on human skin



Source: https://en.wikipedia.org/wiki/Ixodes (3.04.2014)

¹ Bacon RM at all; 2008,57(10):1–9

² Piesman J et all;1991,163(4):895–897),(Sood SK et al; 1997,175(4):996–999

³ Murray TS& Shapiro ED; 2010,30(1):311–328

1.1 Clinical Manifestations

Symptoms of early Lyme disease usually begin one to two weeks after a tick bite (range of three to 30 days)⁴. There are three well-recognized clinical stages of Lyme disease, and clinical manifestations are different at each stage (*Table 2*)⁵. Approximately 80 percent of patients develop the characteristic erythema migrans rash (Figure 2). Erythema migrans is classically reported as a single lesion, and most commonly appears as a uniform erythematous oval to circular rash with a median diameter of 16 cm (range of 5 to 70 cm). Nearly 19 percent of erythema migrans rashes are a "bull's-eye" rash. Multiple erythema migrans lesions may occur in up to 10 to 20 percent of patients. Associated symptoms are similar to a nonspecific viral illness and often include fatigue, malaise, fever, chills, myalgia, and headache. Following this initial stage, the bacteria disseminate systemically via the lymphatic system or blood. With untreated disease, the most common sites of extracutaneous involvement are the joints, nervous system, and cardiovascular system.

Figure 2. An erythema migrans rash in a patient with Lyme disease



Source: http://lymepedia.org/lyme-disease-bullseye-rash-erythema-migrans-rarely-seen-during-relapse-in-chronic-cases-of-the-disease/ (2.04.2014)

Common manifestations of early disease include transient oligoarticular symptoms of arthralgia or myalgia that may include joint swelling. Musculoskeletal symptoms are the most common extracutaneous manifestations of disseminated disease and can occur with early or late disease Arthritis is usually a manifestation of late disease. Patients approximately six months after infection are affected with joint pain and swelling, and synovial fluid findings that suggest an inflammatory process. Chronic arthritis primarily involves the knees and hips. Neurologic involvement, can include lymphocytic meningitis, cranial neuropathies, motor or

⁴ Murray TS& Shapiro ED; 2010,30(1):311–328),(DePietropaolo DL et all; 2005,72(2):297–304

⁵ Bratton RL et all; 2008,83(5):566–571),(Murray TS& Shapiro ED; 2010,30(1):311–328

sensory radiculoneuropathy, cerebellar ataxia, and myelitis. Patients may present with headaches neck pain and stiffness⁶. Lyme carditis is a less common complication of systemic disease, occurring in approximately 4 to 10 percent of patients. It may present as chest pain, dyspnea on exertion, fatigue, palpitations, or syncope, and often includes some form of atrioventricular block (Table 2)⁷

STAGE	SYMPTOM		
Early localized	Erythema migrans		
	Virus-like illness (e.g., fatigue, malaise, fever, chills, myalgia, headache)		
Early disseminated	Cardiac (e.g., atrioventricular block)		
	Dermatologic (e.g., multiple erythema migrans lesions) Musculoskeletal (e.g., arthralgia, myalgia)		
	Neurologic (e.g., lymphocytic meningitis, facial nerve palsy, encephalitis)		
Late	Arthritis (e.g., monoarticular, oligoarticular)		
	Neurologic symptoms (e.g., encephalomyelitis, peripheral neuropathy)		

Table 2. Stages and Symptoms of Lyme Disease

1.2. Diagnostic Testing

Direct and indirect approaches have been used in the laboratory to assist in the diagnosis of Lyme disease. Direct methods involve culture or techniques that detect *B. burgdorferi*-specific proteins or nucleic acids, whereas indirect methods involve serology to detect antibodies⁸. Although culture remains the diagnostic standard, it is not routinely available and its usefulness has been limited to skin biopsy samples in patients with a single early erythema migrans lesion or plasma in patients with multiple erythema migrans lesions. European and American protocols recommend serology as the preferred initial diagnostic test. Currently, the CDC recommends a two-tier protocol using an enzyme-linked immunosorbent assay initially, followed by the more specific Western blot to confirm the diagnosis when the assay samples are positive or equivocal(Centers for Disease Control and Prevention (CDC); 1995,44(31):590–591). Polymerase chain reaction testing has the highest sensitivity for Lyme disease in synovial fluid samples from patients with untreated late Lyme arthritis, for patients with neurologic Lyme disease⁹.

⁶ Steere AC; 2001,345(2):115–125

⁷ Bratton RL et all; 2008,83(5):566–571), Murray TS& Shapiro ED; 2010,30(1):311–328).

⁸ Murray TS& Shapiro ED; 2010,30(1):311-328

⁹ Dumler JS. 2001;6(1):1–11

1.3. Immune response

It is necessary to understand the immune response to *Borrelia*, which will explain the reasons for false-positive and false-negative results with serology and the limitations inherent in this testing that often lead to an erroneous diagnosis and unnecessary antimicrobial treatment¹⁰. During infection, *B. burgdorferi* is capable of disseminating and persisting in a variety of host tissues, suggesting that B. burgdorferi has developed efficient mechanisms for evading the host innate immune response. In fact, with regard to the major borrelial genospecies that cause Lyme isease, B.burgdorferi and B. afzelii are resistant to the complement-mediated bactericidal activity of serum, while most strains of *Borrelia garinii* are killed by human serum¹¹ Immunoglobulin M (IgM) and IgG produced in response to B. burgdorferi may persist for years following standard antimicrobial therapy. These persistently elevated levels are not an indication of ineffective treatment or chronic infection. Repeated serologic testing for documentation of treatment effectiveness or cure is not recommended in current guidelines. The immune response of Lyme Disease can be divided into three stages. These stages can turn into each other, but they can also be separated by periods of a symptom-free latency. The course of the disease is not obligate. Symptoms generally found in specific stages can be missed and spontaneous recovery may occur at any point. With serological diagnostics it has to be considered, that in stage I 50 - 60 % as well as in stage II 10 - 30 % of the patients with clinical manifestations have no sufficient antibody titers against Borrelia burgdorferi. In stage III of the disease high antibody titers can be detected generally. Furthermore, it has to be considered, that an early antibiotic therapy can prevent the occurrence of serious and chronic disease manifestations. But, it reduces also the production of antibodies. Therefore, a prophylactic antibiotic therapy should be avoided if only a tick bite has been reported.

2. AIM

There are very little data about the seroprevalence in our population, furthermore the relation to clinical data has not been previously studied. IFA is a standard test for antibody detection of *B burgdorferi sensu lato*, since cultural growth of the organism from skin samples and blood is very difficult. The aim of this paper is to find correlation between Lyme disease and tick bites in Macedonian patients. It was done through determining the seroprevalence of *Borrelia* IgG and IgM antibodies using IFA test and relating that data to reported tick bites.

¹⁰ Murray TS& Shapiro ED; 2010,30(1):311-328

¹¹ Alitalo, A. et all; 2001, 693685-91

3. MATERIALS AND METHODS

The study was conducted on a sample of 240 patients, after suspected or observed tick bite. Serum samples were collected in a four years period (June 2009-June 2013). *Borrelia* specific IFA test - RIDA[®]FLUOR *Borrelia burgdorferi* (sensu lato), R-biopharm, Germany, was performed for IgG and IgM antibody detection. The positive titer was 1:16 for IgM and 1:32 for IgG. The sera were taken at least 10 days after confirmed or suspected thick bite. All patients also answered a short questionnaire regarding the history of a thick bite and previous treatment.

0-9 years	5
10-19 years	79
20-29 years	65
30-39 years	40
40-49 years	51

Table 3. Age structure of the examined group of 240 patients

3.1. Test principle

Inactivated *Borrelia* are fixed in defined wells on the surface of test slides. Diluted serum samples were applied to the wells. Present anti-*Borrelia*-antibodies bind to cell surfaces and form antigen-antibody-complexes. After a washing step for removing unbound antibodies, the complexes become visible by adding FITCconjugated anti-human-immunoglobulines (anti-IgG or anti-IgM-conjugate). After a further washing step for removing excess conjugate, the test was analyzed by a fluorescence microscope with 400x magnification.

Figure 3. Borrelia seen by fluorescence microscope with 400x magnification



http://www.orbio.fr/catalogue_details.php?item_id=109&g=canin&t=ANALYSE&c=S%E9rol ogie%20chien (2.04.2014)

3.2. Evaluation and interpretation

Specific fluorescence is decisive for positive evaluation of a sample. The specific fluorescence is a bright green coloration visible on the cell surfaces of the *Borrelia* in a homogenous distribution within the wells. Intensity of the fluorescence can vary from weak (1+ reaction) to very strong (4+ reaction). If no specific fluorescence is visible, the test was valuated negative. The RIDA®FLUOR Borrelia sensu lato IgG, IgM tests detect antibodies against *Borrelia*. The fluorescence intensity did not always correlate with the clinical data. Titers of 1:>64 (IgG) and 1:>32 (IgM) were considered positive. Serum samples showing just a weak fluorescence (1+) with the recommended initial dilution were considered equivocal, whereas serum samples with titers of 1:<64 (IgG) and 1:<32 (IgM) were considered negative.

4. RESULTS AND DISCUSSION

Seropositive were 45/240 (18,8%) patients. Concomitant *Borrelia* IgG and IgM seropositivity was noted in 9 cases, and only IgG or only IgM seropositive were 21 and 15 patients, respectively (Chart 1).



Chart 1. Distribution of seropositivity in examined sera

A tick bite 10 - 60 days prior the testing had been noted in 62/240 (25,8%) patients. The ticks were removed in hospital settings in approximately two thirds of the patients. Seropositivity in either or both classes of antibodies in patients who reported a thick bite was 28,9% (13/45) (Chart 2). Erythema migrans as a symptom was noted in 9 patients, all of them reporting a thick bite and being IgG positive. Approximately 50% of the patients bitten by a thick received antibiotic therapy.



Chart 2. Seropositivity in patients bitten by a tick

A positive IgM result as well as a four fold IgG titer increase indicated an acute infection. However, a positive result did not exclude the presence of other pathogens as a cause of an illness. Due to the low antibody titers at the beginning of an infection, the test can show negative results, so negative antibody findings cannot exclude a *Borrelia* infection. Even in stage II of the disease, no detectable antibody levels might be found in up to 30% of cases. In the cases when there is a clinical suspicion for a *Borrelia* infection, a further patient sample after three weeks should be taken and tested. IgM-antibodies are usually detectable five days after a tick bite with Borrelia infection at the earliest. There were cases when antibiotics were given directly after the tick bite. There was a possibility that the immune response was reduced so that despite of an infection, antibody IgG as well as IgM titer could often decrease, evan in months after a successful therapy. Additionally, IgM-antibodies in these cases persist, too.

5. CONCLUSION

Prevalence of *Borrelia burgdorferi* sensu lato species in *Ixodes* ticks in Europe has been studied frequently (Rauter C & Hartung T;2005,71:7203–7216). However, investigations for seroprevalence of *B burgdorferi* in human population in this area are performed for only few years. Our results demonstrate IgG and/or IgM antibody seropositivity of 18,8%, whereas approximately one third of those cases were associated with a tick bite. Very few of those seropositive patients reported any previous symptoms. Those findings suggest that exposure to *B burgdorferi* does occur in our population, mostly without giving rise to clinical manifestations of Lyme disease. The need of applying precise testing and treatment protocols is obligatory. Further seroprevalence studies on human, as well as on animals are needed in order to create a correlation between seroprevalence of *anti- Borelia* antibodies with tick bites, which ultimately means better health protection of the population.

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