

CLINICAL STUDY

Significance of specific antibody determination in Lyme borreliosis diagnosis

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*Institute of Epidemiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia. bazovska@fmed.uniba.sk***Abstract**

The diagnosis of Lyme borreliosis, except in cases characterised by pathognomonic clinical manifestation, usually requires confirmation by means of microbiological diagnostic assay, mainly by antibody detection methods. In our study antibodies to *B. burgdorferi* were tested in neurological patients with suspected Lyme borreliosis, depending on syndrome and clinical diagnosis.

Antibodies were tested with IFT, ELISA and immunoblot.

Blood samples of patients tested with IFT and ELISA tests were positive in 88 patients. Positive indirect immunofluorescence tests were found in 83 patients; in 5 patients the antibody level was borderline. Of these, 40 were positive also in ELISA but a correlation between IF titers and ELISA-positivity was not established. The immunoblot method confirmed specific antibody positivity in 36 of 88 patients (45.45 %) who were positive (or borderline positive) in the indirect IF test, and in 28 of 40 (70 %) ELISA-positive patients. Antibody specificity was found in 8 indirect IF-positive patients who were ELISA negative. This may be explained by the higher immunoblot sensitivity in comparison with ELISA. The Lyme borreliosis diagnosis was clinically established in 19 patients; antibodies to *B. burgdorferi* were only found in 13 patients in all three tests, and in 4 patients only in the indirect IF test.

The results of serological tests for antibodies to *B. burgdorferi* should be interpreted with caution, as the tests are not standardized and may show false positive or false negative results. A two-step serological examination with the immunoblot test is recommended, whereby some nonspecific reactions may be eliminated. The results of serological tests have only supportive value and cannot be deemed conclusive when establishing an etiological diagnosis.

Key words: Lyme borreliosis, neurological patients, screening tests, immunoblot.

Serological tests for presence of *Borrelia burgdorferi* antibodies are routinely used to support the Lyme borreliosis (LB) diagnosis. However, these tests do not establish the base of positive diagnosis and therapy (Brown et al., 1999). Their results indicate that antibodies are present, not necessarily due to an acute infection but possibly as the consequence of persistence of antibodies from an earlier, potentially inapparent infection. On the other hand, a patient with active borreliosis may show negative serological tests in certain cases, mainly in the early phases when antibodies are not yet formed, or their generation may be suppressed by early antibiotic treatment, or the intensity of their generation remains below positivity level. According to Honegr et al. (1997), ELISA fails to show antibodies in 20 % of individuals with positive borreliae presence in the serum or liquor. Indirect IF, ELISA, haemagglutination and immunodot are con-

sidered screening tests; they are not standardized, their sensitivity and specificity vary (Hauser et al., 1997) as well as the results obtained with test sets from different producers. The borreliae found in Europe are considerably antigen-variable, hence selection of the species and the antigen preparation method may influence the results (Magnarelli et al., 1994). Also, when determining anti-borrelia antibodies, potential cross-responses with

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Tab. 1. Comparison of results of serum examinations for *B. burgdorferi* antigens by the indirect IF test, ELISA and immunoblot.

titre of IF antibodies	1:2048 and above	1:1024	1:512	1:256	total
IF+/ ELISA+	41/19	34/13	7/5	6/3	88/40-45,45%
IF+/immunoblot+	41/20	34/12	7/3	6/1	88/36-40,90%
	IgM+IgG+	IgM-IgG+	IgM+IgG-	IgM-IgG-	total
ELISA+	10	22	8		40
ELISA+/immunoblot+	9	17	2		28-70,0%
ELISA-/immunoblot+				48/8	

Tab. 2. Antibodies to individual antigens found in serum examinations with immunoblot recomBlot Borrelia IgG Microgen.

antigens	p100 B.afz	p41 B.burg.	p39 B.afz.	p31 B.afz.	p22 B.afz. B.burg. B.gar.	p20 B.gar.	p18,5 B.afz.	p18 B.afz.
posit. serums	16	11	16	3	7	5	8	8
negat. serums	29	-	22	-	-	-	-	-
total	45							

other antigens in autoimmune diseases or in other bacterial, rickettsial and viral infections should be taken in account, as these may induce false-positive responses (Hulínska, 1997). Therefore positive or borderline positive screening tests should be supplemented with Western blot examination eliminating nonspecific positive serological reactions, although the results are not always conclusive even in such cases.

The objective of our work was to mutually compare serum examination results obtained by various serodiagnostic methods with the results of our commercially available tests and with those of the indirect IF test prepared by our laboratory, and to evaluate their significance in support of Lyme borreliosis diagnoses.

Materials and methods

Determination of anti-borrelia antibodies

ELISA: Commercial Borrelia test (Biomedika) with recombinant antigens: for testing IgG p21, p41i *B. garinii*, p41i *B. afzelii*, p18, p100, for IgM p21, p41i *B. garinii*, p41i *B. afzelii*.

Indirect immunofluorescence test: Standard strain of *B. burgdorferi* s,s B 31 and endemic *B. garinii* and/or other endemic strains (Kmety, 2000). Serums with titres 1 : 512 and higher were evaluated as positive samples.

Western blot: The commercial recomBlot Borrelia IgG (Microgen) test was used with 10 recombinant *B. garinii*, *B. burgdorferi* and *B. afzelii* antigens, along with the anti-Borrelia burgdorferi IgG test (Euroimmun), with 19 separated borrelia antigens after solubilization with sodium dodecylsulphate.

Patients

Serums were obtained mostly from patients with neurological diseases, where borreliosis was a possibility within the differential diagnosis. Positive screening tests were supplemented with the Western blot. The clinical diagnosis was based on the epidemiological anamnesis and clinical process of the disease. All patients were subjected to complete neurological and neurophysiological examination (evoked potentials, electromyography, EEG) and to MRI, and categorised as follows, depending on the results: I - Lyme borreliosis diagnosis (with a typical clinical picture - neuroborreliosis in the form of meningitis, encephalitis, radiculitis, brain nerve affections), II - suspected diagnosis of borreliosis or of an inflammatory disease of unclear etiology, III - potentially different disease requiring long-term observation (SM-like), IV - improbable diagnosis, V - other confirmed diagnosis.

Results

Screening tests (indirect IF and ELISA) showed *B. burgdorferi* antibodies in the serum of 88 patients (Tab. 1). The indirect IF test showed positive antibodies in 83 and borderline positives in 5 cases. Of these, 40 patients were also ELISA-positive, but a direct dependence between the titre of indirect IF and ELISA positivity was not found. The immunoblot test confirmed presence of specific antibodies in 36 of the 88 indirect IF-positives and borderline positives (45.45 %), and in 28 of the 40 ELISA-positives (70.0 %). Antigen specificity was confirmed in 8 patients who had positive indirect IF but negative ELISA results.

All serums were Western blot-tested with Euroimmun IgG anti-*B. burgdorferi* or with Microgen recomBlot Borrelia IgG (Tab. 2, 3). Of the specific antigens, Microgen most frequently showed antigen p100 positivity, and Euroimmun antigen p83 and p31 positivity. Of antibodies found in ELISA, immunoblot confirmed specificity in 70.0 % of all cases, whereas indirect IF-positive antibodies were confirmed in substantially less cases.

Tab. 3. Antibodies to the individual antigens in serum examinations with immunoblot Euroimmun IgG anti *B. burgdorferi*.

antigens	p83	p75	p62	p57/59	p50	p47	p43	p41	p39	p36	p34	p32	p31	p29	p28	p25	p21/22	p18	p17	
posit. serums	21	16	7	12	16	-	3	2	19	9	5	14	2	14	6	1	-	10	6	2
negat. serums	31	-	7	9	11	1	-	-	13	1	1	1	-	-	1	-	-	-	-	2

Tab. 4. Comparison of the results of serological tests for antibodies to *B. burgdorferi* in patients with clinical diagnoses.

Clinical diagnosis	No. of patients				Total
	+++	++	+-	-	
I	13	1	1	4	19
II	7	4	3	7	21
III	5	-	3	6	14
IV	2	1	1	6	10
V	2	2	3	8	15
Total	29	8	11	31	79

+++ posit. IF, ELISA, immunoblot

++ posit. IF, immunoblot, negat. ELISA

+- posit. IF, ELISA, immunoblot negat.

- posit. IF, negat. ELISA, immunoblot

The serums of 9 patients examined by both immunoblot tests showed identical results, although involving different antigens. Patients who were antibodies-positive in the screening tests but whose immunoblot test did not confirm specific antibodies to *B. burgdorferi* most frequently showed antibodies to the 41 kDa flagellate antigen (Tab. 2, 3) and to antigens in the p57-59, p62 fields. Part of the patients (7 in Microgen and 8 in Euroimmun tests) showed no antibodies to any of the antigens investigated. This indicated that other nonspecific or unidentified antigens participated in the positivity of the screening tests.

Comprehensive documentation on the clinical process of the disease could be compiled for 79 of 88 completely serologically examined cases (Tab. 4). Lyme borreliosis was clinically diagnosed in 19 patients, 13 of whom showed antibodies to *B. burgdorferi* in all three tests, but four were only positive in the IF test. Of the 25 patients with improbable or clearly other diagnoses only 4 had antibodies confirmed in all three tests.

Discussion

Determination of the presence of antibodies to *B. burgdorferi* is used to support the diagnosis of Lyme borreliosis. Serological tests are generally unsuitable in early stages of the disease due to their low sensitivity; they are more significant in advanced stages, when the sensitivity and specificity of tests is higher. The importance of serological tests in Lyme borreliosis diagnoses is frequently overestimated and may result in incorrect diagnosis and therapy in cases when their strong and weak points are incorrectly balanced. Reid et al. (1998) evaluated a group of 209 patients in retrospect with LB diagnosed in 1994 and 1995, and failed to confirm the diagnosis in up to 60 % of all cases. After ATB therapy (usually 42 days), 55 % of these patients showed low-intensity and 6 % more intense accessory symptoms.

ELISA is the most frequently used screening test. Testing, mainly in the past, had been based on processing whole cells of *B. burgdorferi* of various strains; the present trend is to use tests with several subunit or recombinant antigen, as they show hi-

gher specificity (Rauer et al., 1998; Magnarelli et al., 1996). Application of the antigen mix enables to compensate for a possible reduction in the production of antibodies to a specific antigen, as it takes the variability of immune response of patients and antigens of several strains in account. It also enables to significantly reduce the false-positivity ratio, e.g. from 23 % to 10 % in EBV infections. Increased OD values improve discrimination between positive and negative serums. As the result, test specificity may reach as much as 94 % according to certain authors (Kaiser and Rauer, 1999). Positivity found in our ELISA tests with recombinant antigens was confirmed by detecting specific antibodies with immunoblot in 70.0 % of all cases. Positive immunoblot was also found in 8 patients with negative ELISA results, confirming the higher sensitivity of this test in accordance with other authors (Treib et al., 1998).

The indirect IF test showed high occurrence of positivity in our patients in comparison with ELISA; however, only 45.45 % of the patients had identical results in both tests. This may have been influenced by selecting patients with high proportion of demyelinating diseases and of diseases with autoimmune components, as these could contribute to the false positivity of the results. On the other hand, immunoblot confirmed presence of specific antibodies in 8 patients with positive IF antibodies and negative ELISA, indicating that this test, while being obviously less specific, is more sensitive than ELISA.

We used two commercial immunoblot tests, and certain patients were examined by both tests. The weak lines obtained in these tests are sometimes difficult to interpret (Engstrom et al., 1995), and we tried to compensate this by applying both tests and/or by repeating them after two months. Given both the variability the borreliae appearing in our territory and the variable antibody response of patients, negativity in this test cannot exclude a *B. burgdorferi* infection.

Contemporary serodiagnostics often feel the absence of effective tests able to prove an active infection. Prolonged retention of IgM antibodies and frequent appearance of cross-reactions, mainly when looking for such antibodies reduce their diagnostic utility in comparison with other infectious diseases. The comparison of results obtained by different diagnostic labs shows substantial variances (Brown et al., 1999). Our sample of neurological patients contained none with clinically diagnosed borreliosis and negative serological examination, although such cases have been sufficiently documented (Oksi et al., 1995).

Lyme borreliosis was clinically diagnosed in 19 patients; however in determining the diagnosis the fact must be taken in account that clinical symptoms indicating the disease may have different etiologies which could not be confirmed in the framework of broad diagnostic procedures. Also, the evaluation of clinical symptoms of certain persistently infected patients in a causal connection with Lyme borreliosis is problematic. Further laboratory tests are therefore urgently needed to support the diagnosis. Determination of the presence of intrathecal antibodies in the liquor is an additional important diagnostic criterion; however, none of the direct-proof methods that show various sensitivity (cultivation, IEM, PCR) can intercept all patients, besides

being difficult to practise (Honegr et al., 2001, Mateicka and Kmety, 2000).

To diagnose LB in neurological patients is a difficult process, considering the wide scale of clinical symptoms. Proof of anti-borrelia antibodies in itself does not establish the base of a causal relationship between the infection and its clinical manifestation. Results of serological tests should be evaluated very carefully, as these tests are not standardised and correlation of results among the various commercial sets and laboratories is weak, requiring consideration of possible false-positive or false-negative data. Some of the problems can be resolved by immunoblot examination that may confirm the presence of specific antibodies and exclude certain nonspecific ones, however when taking the variability of borreliae circulating through Europe and variability of the immune response of patients in account even a negative immunoblot test cannot exclude the disease. Presently it is recommended to use immunoblot, as this test has higher specificity in the framework of a two-step serological examination. Results of serological tests have only supportive value in the diagnosis, their correct evaluation requires knowledge of their strong and weak points, thereby emphasizing the need for close co-operation of clinical and laboratory workers.

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