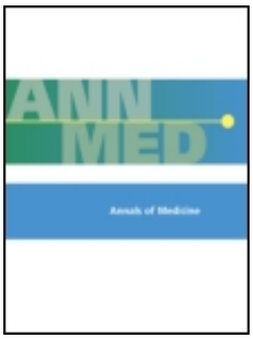

Annals of Medicine

ISSN: 0785-3890 (Print) 1365-2060 (Online) Journal homepage: informahealthcare.com/journals/iann20

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To cite this article: Bettina Wilske (2005) Epidemiology and diagnosis of Lyme borreliosis, Annals of Medicine, 37:8, 568-579, DOI: 10.1080/07853890500431934

To link to this article: https://doi.org/10.1080/07853890500431934

Published online: 08 Jul 2009.

Annals of Medicine. 2005; 37: 568–579

Taylor & Francis
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MINI REVIEW

Epidemiology and diagnosis of Lyme borreliosis

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Abstract
The multisystem disease Lyme borreliosis is the most frequent tick-transmitted disease in the northern hemisphere. In Europe Lyme borreliosis is most frequent in Central Europe and Scandinavia (up to 155 cases per 100,000 individuals) and is caused by the species, *B. burgdorferi* sensu stricto, *B. afzelii* and *B. garinii*. The recently detected genospecies A14S may also play a role in skin manifestations. Microbiological diagnosis in European patients must consider the heterogeneity of borreliae for development of diagnostic tools. According to guidelines of the USA and Germany, serological diagnosis should follow the principle of a two-step procedure (enzyme-linked immunosorbent assay (ELISA) as first step, if reactive; followed by immunoblot). The sensitivity and standardization of immunoblots has been considerably enhanced by use of recombinant antigens (p100, p58, p41i, VlsE, OspC, DbpA) including those expressed primarily *in vivo* (VlsE and DbpA) instead of whole cell lysates. VlsE is the most sensitive antigen for IgG antibody detection, OspC for IgM antibody detection. At present, detection rates for serum antibodies are 20%–50% in stage I, 70%–90% in stage II, and nearly 100% in stage III Lyme disease. Detection of the etiological agent by culture or polymerase chain reaction (PCR) should be confined to specific indications and specialized laboratories. Recommended specimens are skin biopsy specimens, cerebrospinal fluid (CSF) and synovial fluid. The best results are obtained from skin biopsies with culture or PCR (50%–70%) and synovial tissue or fluid (50%–70% with PCR). CSF yields positive results in only 10%–30% of patients except when the duration of symptoms is shorter than 2 weeks (50% sensitivity). Methods which are not recommended or adequately documented for diagnosis are antigen tests on body fluids, PCR of urine, and lymphocyte transformation tests.

Key words: *Borrelia, Borrelia burgdorferi, epidemiology, Lyme borreliosis, microbiological diagnosis, serology*

Introduction

Lyme borreliosis is a multisystem disease involving many organs such as the skin, the nervous system, the joints and the heart (1–3). The disease is caused by *Borrelia burgdorferi* s.l. detected in the early eighties by Burgdorfer et al. in the American tick vector *Ixodes scapularis* (4). Due to the diversity of clinical symptoms, Lyme disease is often considered in differential diagnosis. Laboratory tests for diagnosis of Lyme borreliosis are thus in high demand, and are among the most frequently requested tests in microbiological laboratories.

Frequency of Lyme borreliosis

Lyme borreliosis is the most frequent tick-borne disease in the northern hemisphere including North America and Eurasia. Lyme disease is not a reportable disease in most European countries in contrast to the United States. However, in studies performed in Scandinavia and in Slovenia, disease incidence was assessed as up to 155 per 100,000 inhabitants (1,5,6). Lyme borreliosis occurs with similar frequencies in women and men with the exception of acrodermatitis chronica atrophicans (ACA) which is more frequent in women (7–9). Early neuroborreliosis cases showed a bimodal age distribution with a lower frequency in the age range of 20 to 29 years (7) whereas ACA occurs primarily in older patients (7,8).

Causative agents

In Europe Lyme borreliosis is caused by at least three species: *B. burgdorferi* sensu stricto, *B. afzelii* and *B. garinii*. In contrast, *B. burgdorferi* sensu stricto

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ISSN 0785-3890 print/ISSN 1365-2060 online
DOI: 10.1080/07853890500431934

is the only human-pathogenic species in the United States (Table I) (10). The three human-pathogenic species comprise at least 7 OspA-serotypes in Europe (Figure 1) (11). Skin isolates primarily belong to *B. afzelii* (OspA-type 2), especially those from patients with ACA, a chronic skin disease not present in America (11–13). Isolates from CSF and ticks are heterogeneous with a predominance of *B. garinii* (11,14–16). Sequence analysis of polymerase chain reaction (PCR) *ospA* amplicons from synovial fluid of Lyme arthritis patients revealed considerable species- and OspA heterogeneity (17,18) (Figure 1), whereas some other studies found a prevalence of *B. burgdorferi* s.s. (19–21). The most frequent genomic groups in Europe, *B. afzelii* and *B. garinii* occur across the continent and the islands, whereas the third frequent group *B. burgdorferi* s.s. has only rarely been isolated in Eastern Europe (for a survey see (22)). Strains may be very heterogeneous even within small areas (14,23–26). On the other side a focal prevalence of certain species or subtypes was also observed (24,27). Mixed infections have been repeatedly observed in ixodid ticks (for a survey see (22)) and sometimes also in specimens from patients (16,18,28,29).

Key messages

- *Epidemiology and etiological agent.* Lyme borreliosis is the most frequent tick-borne disease in the northern hemisphere. The etiological agent of this multisystem disease is *B. burgdorferi* sensu lato which comprises at least three pathogenic species (*B. burgdorferi* sensu stricto, *B. afzelii* and *B. garinii*) and the recently detected genospecies A14S.
- *Antibody detection.* Serology is the most commonly used diagnostic tool. Sensitivity of antibody detection is 20%–50% in stage I, 70%–90% in stage II, and nearly 100% in stage III. Currently, a two-step approach is recommended (ELISA as first step, if reactive, followed by immunoblot). The detection of highly immunogenic, primarily *in vivo*-produced, proteins provided new diagnostic tools. Recombinant proteins as VlsE, OspC, DbpA and BBK32 were successfully used as ELISA antigens. A recombinant immunoblot (with p100, p58, p41i, VlsE, OspC, and DbpA as antigens including several homologues of these proteins) was more sensitive than the whole-cell sonicate immunoblot and is easier to standardize. Use of recombinant VlsE, DbpA and BBK32 as antigens for ELISA and immunoblot increased especially IgG antibody detection in early disease (erythema migrans (EM) and acute neuroborreliosis).
- *Culture and polymerase chain reaction (PCR).* Detection of borreliae using PCR or culture is confined to specific indications and specialized laboratories. The best results are obtained from skin biopsies with culture or PCR (50%–70%) and synovial tissue or fluid (50%–70% with PCR). CSF yields positive results in only 10%–30% of patients with the exception when duration of symptoms is shorter than 2 weeks (50% sensitivity).

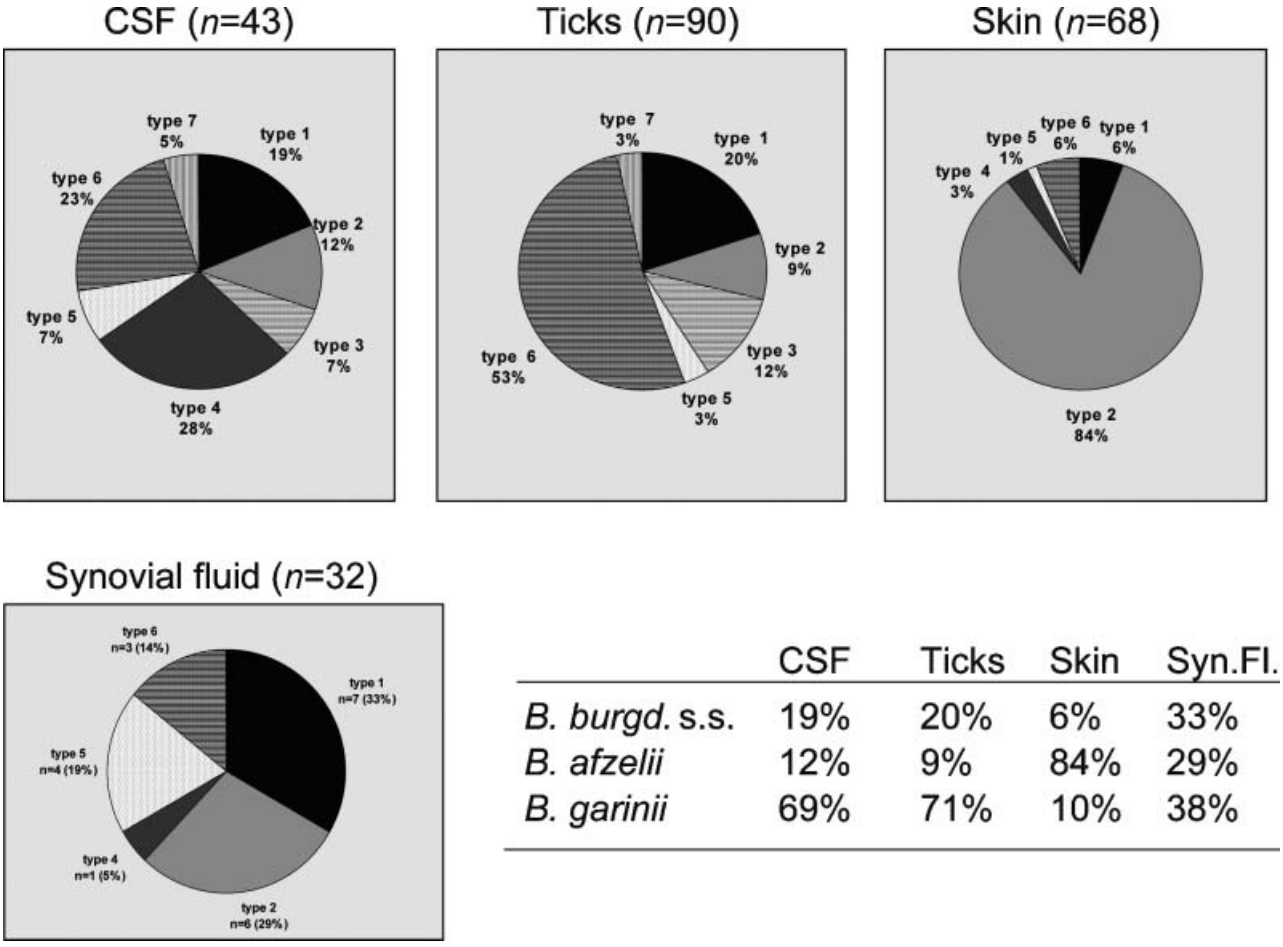
Vectors and reservoirs

Borrelia burgdorferi s.l. is transmitted by hard ticks (genus *Ixodes*). The larvae and nymphs feed primarily on small rodents whereas adult ticks feed on a variety of larger animals. The feeding period of *Ixodes* species ticks is rather long (several days to over a week) and contributes to their geographic dispersal along with the movement of the host. Birds, particularly migratory seabirds, can transport the ticks (*I. uriae*) over very long distances and thus distribute borreliae (especially *B. garinii*) worldwide (30). There appears to be an association between certain *Borrelia burgdorferi* s.l. species and certain vertebrate hosts: *B. afzelii* and small rodents and *B. garinii* and birds, possibly due to different serum-sensitivities of the borreliae (31,32). Complement-resistant Borreliae bind complement regulators and prevent the formation of toxic activation products which kill the borreliae (33). In unfed ticks *B. burgdorferi* s.l. lives in the midgut. During the blood meal on humans or mice molecular changes (e.g. switch from OspA to OspC expression) are induced in the borreliae that lead to their migration to the salivary glands (34,35). The migration process takes >36 h in *I. scapularis* (36). In *I. ricinus* nymphs, however, spirochete migration within the tick and transmission to the mammalian host has been observed with ticks feeding for a few as 17h (37). Infection rates of larvae are usually very low (ca 1%). Borrelial infection is mostly acquired by feeding on infected reservoir hosts leading to much higher infection rates in nymphs and adults. In a study from southern Germany prevalence of borreliae increased from 1% in larvae to 10% in nymphs and 20% in adult ticks (7).

Table I. Geographical distribution of *B. burgdorferi* sensu lato species and tick vectors.

Borrelia *species*	Tick species *Ixodes*	Geographic origin
B. burgdorferi sensu stricto [a]	*I. scapularis, I. pacificus, I. ricinus*	North America, Europe
B. garinii [a]	*I. ricinus, I. Persulcatus, I. uriae*	Eurasia
B. afzelii [a]	*I. ricinus, I. persulcatus*	Eurasia
genospecies A14S [a, b]	*I. ricinus*	Europe
B. lusitaniae	*I. ricinus*	Europe, North Africa
B. valaisiana	*I. ricinus*	Eurasia
B. bissettii	*I. scapularis, I. pacificus*	North America
B. andersonii	*I. dentatus*	North America
B. japonica	*I. ovatus*	Japan
B. tanukii	*I. tanukii, I. ovatus*	Japan
B. turdi	*I. turdi*	Japan
B. sinica	*I. ovatus*	China

[a] pathogenic for humans. [b] tentatively named *B. spielmanii (100)*.



	CSF	Ticks	Skin	Syn.Fl.
B. burgd. s.s.	19%	20%	6%	33%
B. afzelii	12%	9%	84%	29%
B. garinii	69%	71%	10%	38%

Figure 1. Distribution of species of *Borrelia burgdorferi* sensu lato as well as of OspA types in European isolates from ticks, CSF, skin and synovial fluid specimens [17;18;101]. Clinical data for the skin specimens are known in 46 patients: 30 cases with erythema migrans (of which there were 1, 26, 1 and 2 cases infected with OspA-types 1, 2, 4 and 6 respectively; 16 cases with acrodermatitis chronica atrophicans (ACA) (of which there were 1 and 15 cases infected with OspA-types 1 and 2 respectively). *B. burgdorferi s.l.* speciation from synovial fluid samples is based on *ospA* PCR results. Culture isolates from this tissue are too few to estimate species distribution. Figure 1 is modified from figures 5 and 6 in reference (102).

Implications of heterogeneity of borreliae for diagnosis

The heterogeneity of the causative strains (Figure 1) is a challenge for the microbiological diagnosis of Lyme borreliosis in Europe and must be kept in mind for development of diagnostic tools such as PCR primers and diagnostic antigens. For example, *ospA* PCR has been widely used. Here it is important to be sure that not only representatives of the three species are detected, but also the different *ospA*-types of the heterogeneous *B. garinii* group (14). In addition, PCR should detect *B. valaisiana* and the recently detected new genospecies A14S (24) since *B. valaisiana* and genotype A14S might also be pathogenic for humans, as suggested by positive PCR results or cultures obtained from skin biopsy specimens in a few studies (38,39). Recently A14S-like organisms have been found in four patients with erythema migrans from Germany confirming the pathogenic potential of this new genospecies (Fingerle and Wilske, unpublished results).

Most of the proteins relevant for serodiagnosis are heterogeneous. Interspecies amino acid sequence identities are for example only 40%–44% for DbpA and 54%–68% for OspC for representative strains of *B. burgdorferi* sensu stricto, *B. afzelii*, and *B. garinii* (40). However, highly heterogeneous proteins sometimes have conserved immunogenic epitopes (e.g. the C6 peptide of VlsE and the pepC10 peptide derived from OspC) (41–43).

Microbiological diagnosis of Lyme borreliosis

Except in cases with the pathognomic clinical manifestation erythema migrans the diagnosis of Lyme borreliosis usually requires confirmation by means of a microbiological diagnostic assay. Antibody detection methods mainly are used for this purpose, whereas detection of the causative agent by culture isolation and nucleic acid techniques is confined to special situations. Since the present review is limited in space the reader who wants to go deeper into this field is referred to more comprehensive reviews (44,45).

Specimens for the microbiological diagnosis. For culture and PCR, skin biopsy samples are the most promising specimens (Table II). In general poor results are obtained from body fluids with the exception of PCR from synovial fluid. Examination of urine is not recommended (see last section). Examination of ticks should be performed only for epidemiological or other scientific studies. Ticks removed from patients should not be examined in order to decide antibiotic prophylaxis (46,47). For antibody determination, serum or CSF can be investigated. CSF examination should always be done together with serum antibody analysis (determination of the CSF/serum antibody index).

Direct detection methods

Culture. B. burgdorferi can be cultivated in modified Kellys medium (44,48,49). This, however, is a very

Table II. Specimen types used for the diagnosis of Lyme borreliosis.

	Specimens for	
Clinical manifestation	Direct pathogen detection (culture, PCR)	Antibody detection
Stage I (early / localized), *(days through weeks after tick bite)*.		
Erythema migrans	Skin biopsy	Serum
Stage II (early / disseminated) *(weeks through months after tick bite)*		
Multiple erythemata	Skin biopsy	Serum
Borrelial lymphocytoma	Skin biopsy	Serum
Lyme carditis	Endomyocardial biopsy	Serum
Neuroborreliosis	CSF	Paired serum/CSF [a]
Stage III (late / persistent) *(Months through years after tick bite)*		
Arthritis	Synovial fluid, synovial biopsy	Serum
Acrodermatitis chronica atrophicans	Skin biopsy	Serum
Chronic neuroborreliosis	CSF	Paired serum/CSF [a]

[a] from the same day for CSF/serum index determination.

time-consuming method (generation time of *B. burgdorferi* is about 7–20 h) characterized by low sensitivity, especially in body fluids (50–53) (Table III). Only under special conditions (3 specimens of 3 ml plasma cultured in a 70 mL medium for 12 weeks) have positive cultures been derived from about 50% of patients with erythema migrans (54).

Culturing may be of help in individual cases if the clinical picture suggests Lyme borreliosis despite a negative antibody assay (seronegative Lyme borreliosis), e.g. in atypical erythema migrans, suspected acute neuroborreliosis without detection of intrathecal antibodies or in the case of suspected Lyme borreliosis in patients with immune deficiencies.

Polymerase chain reaction (PCR). For DNA amplification under experimental conditions various target sequences have been used, e.g. from plasmid-borne genes such as *ospA* and *ospB*, or chromosomal genes such as the genes for the flagellar protein or p66, or from gene segments of the 16S rRNA or the 5S/23S rRNA intergenic spacer region (for surveys see (55,56)). Borrelia PCR should allow diagnosis of the *Borrelia* species, i.e. the medical report should contain information as to which of the species pathogenic for humans has been found.

Sensitivity of culture and PCR. Table III gives a survey about sensitivity of direct detection methods in clinical specimens from patients with Lyme borreliosis. Borreliae are detected with much more difficulty from body fluids than from tissue specimens (19,50,51). Culture and PCR have the highest detection rates (50%–70%) in skin biopsies from patients with erythema migrans or ACA (15,53,57,58). In contrast, borreliae are detected by PCR or culture in the CSF of only 10%–30% of patients with acute neuroborreliosis (14,51,59). CSF isolates are more frequently obtained from patients with short duration of disease than from patients with disease of long duration (51). Accordingly CSF-PCR is positive in up to 50% of patients with disease duration of less than 2 weeks compared with only 13% patients in whom the illness duration was greater than 2 weeks (60). Borreliae are detected by PCR in 50%–70% of the synovial fluids of Lyme arthritis patients but culture is rarely successful (17,18,61). The best PCR results are obtained from synovial tissue, not fluid (19).

Antibody detection

It is generally accepted that serological examination should follow the principles of a two-step approach (44,46,62,63): 1) A serological screening assay, and 2) in the event of a positive or equivocal result a confirmatory assay. A sensitive ELISA is recommended, which – in case it is reactive – should be confirmed by the immunoblot. The new development of highly sensitive and specific ELISAs based on recombinant antigens or synthetic peptides raises the question whether two ELISAs could be the two-step procedure. However the two ELISA results might have a common error source that would be difficult to discover. Therefore immunoblotting has merits because the error sources are at least partly different and there is possibility to see background binding artifacts. In addition immunoblotting allows analysis of the antibody pattern against different borrelial proteins possibly associated with different stages of the disease. New technologies (as the Luminex bead system) may provide the possibility of quantitative multiple parameter analysis combining features of ELISA and immunoblot.

ELISA. The ELISA tests used for screening should be at least second generation tests (46), which have been improved with respect to cross reactivity with other bacteria (e.g. extract antigen with previous Reiter treponeme adsorption) (64) or purified intact flagella as antigen (65) or third generation tests using specific and sensitive recombinant antigens or synthetic peptides. Strains used as antigen source should express OspC the immunodominant antigen of the IgM response and DbpA an immunodominant antigen of the IgG response (46). Specific recombinant antigens (i.e. VlsE, DbpA, BBK23, and OspC) or synthetic peptides (i.e. the pepC10 peptide derived from OspC and the C6 peptide derived from VlsE) have been successfully used (42,43,66–70). The most sensitive

Table III. Sensitivity of direct pathogen detection methods in Lyme borreliosis.

Specimens	Sensitivity
Skin (erythema migrans, acrodermatitis)	50%–70% when using culture or PCR
CSF (neuroborreliosis, stage II)	10%–30% when using culture or PCR [a]
Synovial fluid [b] (Lyme arthritis)	50%–70% when using PCR (culture is only extremely seldom positive)

[a] up to 50% in patients with disease duration of less than 2 weeks compared with only 13% patients in whom the illness duration was greater than 2 weeks (60). [b] higher sensitivity of direct pathogen detection from synovial biopsy specimen.

antigens were for IgM antibody detection OspC (or the pepC10 peptide) and for IgG antibody detection VlsE (or the C6 peptide) respectively. Since VlsE is not present in relevant amounts in cultivated borreliae, sensitivity of IgG ELISAs based on whole cell lysates or detergent extract antigens might be increased with recombinant VlsE or C6 peptide. ELISAs using the C6 peptide, VlsE enriched antigens or VlsE alone are commercially available.

Immunoblot. As a confirmatory assay the immunoblot should have high specificity (at least 95%). If a whole cell lysate is used as antigen, diagnostic bands must be defined by monoclonal antibodies or other reliable identification. In case of recombinant antigens, identification of diagnostic bands is much easier. For the whole cell lysate blot, strains expressing immunodominant variable antigens (OspC, DbpA) in culture should be used (46).

The immunoblot criteria recommended by the Centers of Disease Control (CDC) for use in the United States cannot be used for Europe (71–73). The immune response of European patients is restricted to a narrower spectrum of Borrelia proteins, compared with that shown by American patients (74). Hauser et al. demonstrated in two studies (first serum panel from Germany, second serum panel from various European countries) that strain-specific interpretation rules must be defined (71,72). Interpretation criteria for the immunoblot recommended by the German Society for Hygiene and Microbiology (DGHM) are published in the 'MiQ 12 Lyme-Borreliose' (46) which is available in English via internet (http://www.dghm.org/red/index.html?cname=MIQ).

Patients with early manifestations of acute neuroborreliosis have an immune response restricted to only a few proteins. Patients with late disease such as ACA or arthritis have IgG antibodies to a broad spectrum of antigens (Figure 2). Recombinant antigens for the immunoblot have several advantages compared to whole cell lysate antigens (specific antigens can be selected, homologous antigens derived from different strains can be combined, and antigens primarily expressed *in vivo* can be used (75,76). Commercial recombinant antigen immunoblots are better standardized than the conventional ones. If a broad panel of recombinant antigens (including the recently described VlsE) is used the recombinant blot is at least as sensitive as the conventional one. An in-house recombinant IgG immunoblot could be significantly improved by addition of recombinant VlsE and an additional DbpA homologue (75). Using the line blot technique which allows detection of antibodies against antigens with identical molecular weight (i.e. homologues of the same borrelial protein) (Figure 3) the recombinant IgG immunoblot became even significantly more sensitive than the conventional IgG sonicate immunoblot (i.e. 91.7% *versus* 68.8% in patients with early neuroborreliosis for the detection of IgG antibodies) (77). In both immunoblots the criterion for a positive test was reactivity of at least two different proteins. Using this criterion none of the control sera shown in Table V was reactive (reactivity of controls was restricted to maximal one protein). In this study the immunodominant protein of the IgM response was OspC followed by VlsE (Figure 3b). For the IgG response VlsE was the immunodominant antigen in all stages (80%–100%) whereas OspC is reactive only in 20 to 50% (Figure 3 and Table V). Other proteins have low reactivities in early manifestations compared to those in late manifestations. This is especially apparent for p58 where reactivities increase from 7% (stage I) to 54% (stage II) and to 95% in stage III. The combination of different homologues from one protein was especially efficient in case of DbpA. Out of 50 patients with acute neuroborreliosis 39 (78.0%) were positive with at least one of the 4 DbpAs, but only 6 (12%) with *B. burgdorferi* s.s. DbpA, 17 (34%) with *B. afzelii* DbpA and 32 (64.0%) with at least one of the 2 *B. garinii* DbpAs. Thus a panel of DbpAs representing the four major DbpA groups of *B. burgdorferi* s.s., *B. afzelii* and *B. garinii* appears to provide optimal detection rates (78). The prevalence of reactivity with *B. garinii* DbpA is in agreement with the fact that *B. garinii* is prevalent in patients with neuroborreliosis. Future investigations will show whether quantitative multiantigen assays i.e. such as those based on the Luminex bead technology can give reliable information about the stage of the disease and the causative *Borrelia* species or type. Such assays might also help to discriminate between present and past infections or to control success of antibiotic therapy which is not possible with the presently available tests.

Determination of the CSF/serum index. Methods taking into account potential dysfunction of the blood-CSF barrier are suitable for the detection of intrathecal antibody production (79–81). Determination of the CSF/serum index should be performed if neuroborreliosis is considered, since a positive CSF/serum index confirms involvement of the nervous system. It may be positive in some cases when serum antibody tests are negative or equivocal, especially if the patient's illness has been of short

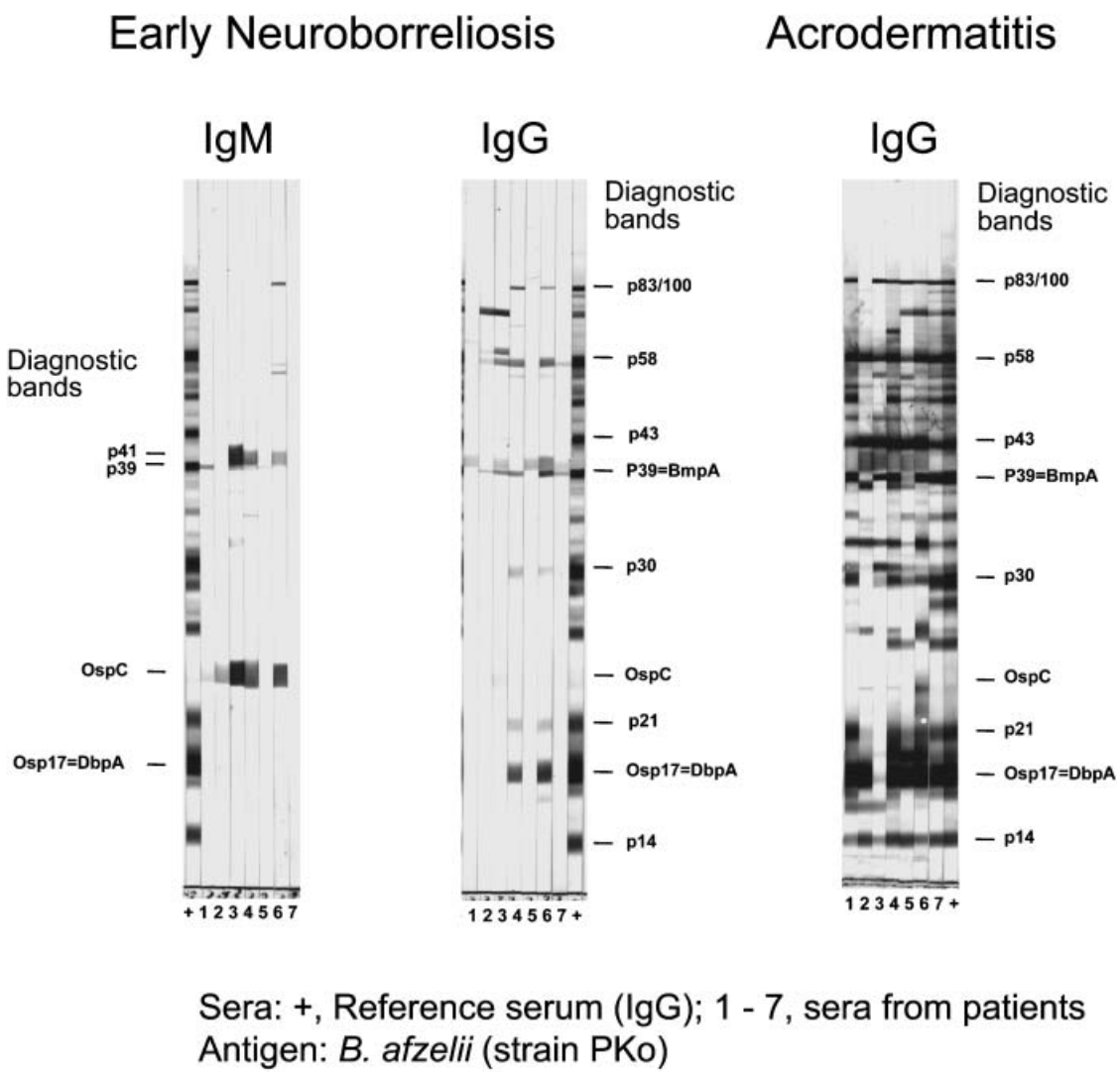


Figure 2. Whole cell immunoblot from patients with early neuroborreliosis (stage II) and acrodermatitis (stage III). Note, the antigen used is *B. afzelii* strain PKo and the sera are from European patients. Figure 2 is modified from Figure 5 of reference (71).

duration (46). Depending on the time elapsed since the first manifestation of neurological symptoms, the IgG CSF/serum index is positive for 80%–90% of patients (8–41 days after onset of the disease) up to 100% of patients (>41 days after onset) (81). Detection of intrathecally produced IgM antibodies shows a high degree of sensitivity in neuroborreliosis with short duration of symptoms, especially in children (81,82). However false positive IgM reativity has been observed in some cases with viral meningitis that are difficult to distinguish from specific borrelial antibodies without the presence of IgG class antibodies (82). CSF/serum index determination is especially important for diagnosis of chronic neuroborreliosis. A positive IgG CSF/serum index is essential for the diagnosis of chronic borreliosis of the central nervous system (see EUCALB case definitions (83)) whereas chronic peripheral polyneuropathy is usually negative for intrathecal antibody production (84).

Serological findings in various stages of the disease

Interpretation of serological test results must always be done in context with clinical data (Table IV). Here case definitions are helpful (46,83). In stage I (erythema migrans) only 20%–50% of the patients are seropositive for IgM and/or IgG antibodies (85,86). IgM antibodies usually prevail. An exception might be the immune response against some primarily *in vivo* expressed antigens. In American patients with erythema migrans IgG responses against VlsE are observed earlier than IgM responses (66). In European patients with erythema migrans an early IgG response to VlsE was observed in 20 of 23 (87%) culture-confirmed erythema migrans cases

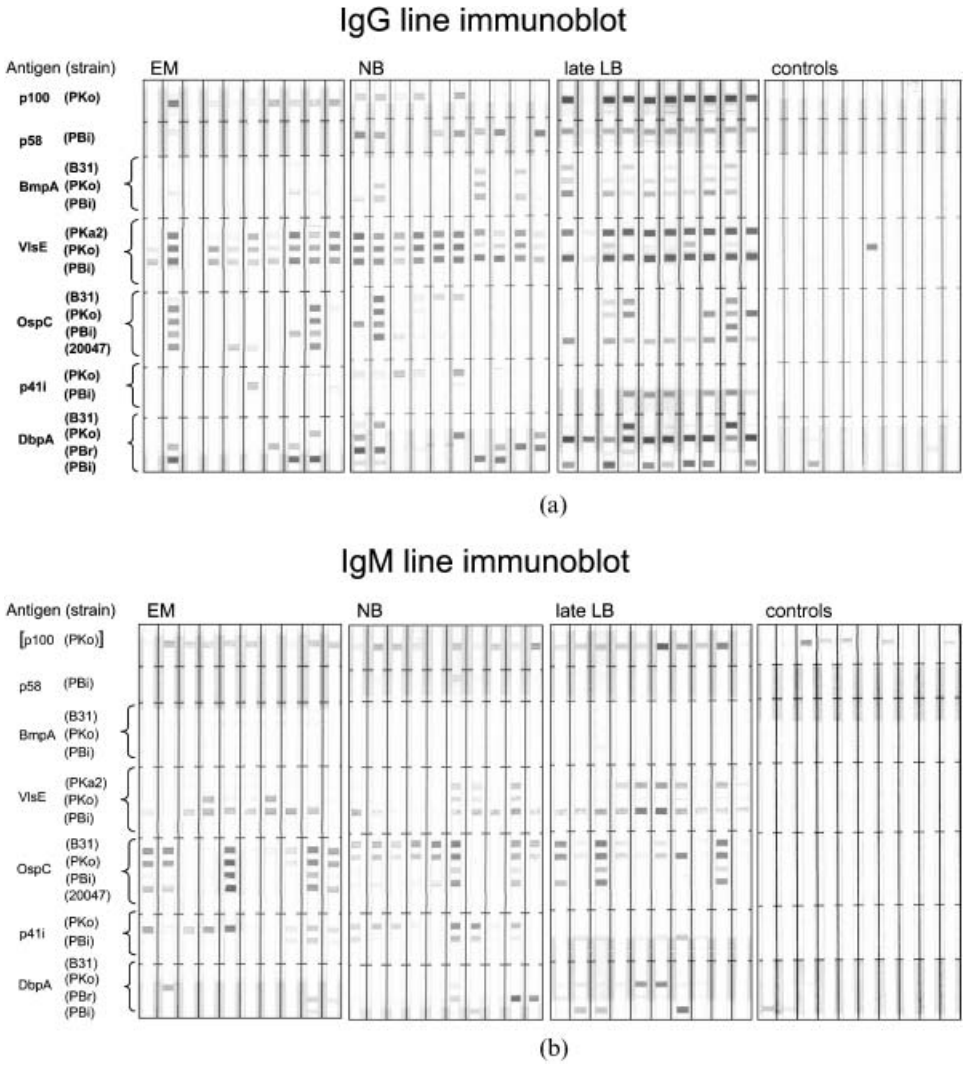


Figure 3. Recombinant line immunoblot. (**a**) Representative IgG blots and (**b**) IgM blots of patient and control sera. Strains belong to the following species: B31 and PKa2 to *B. burgdorferi* s.s., PKo to *B. afzelii,* PBr to *B. garinii* OspA-type 3, PBi to *B. garinii* OspA-type 4, and 20047 to *B. garinii* unknown OspA-type. Sera were obtained from patients with erythema migrans (EM), early neuroborreliosis (NB), Acrodermatitis chronica atrophicans or Lyme arthritis (late LB), and controls. Figures 3 a and b are modified from Figures 1 and 2 of reference (77). The Borrelia protein encoding genes used correspond to the B31 sequence database as follows: *p100*, BB0744; *p58*, BB0329, *bmpA*, BB0383; *ospC*, BBB19; *flaB* (p41) BB668; *dbpA*, BBA24, the *vlsE* gene is not in the database.

Table IV. Sensitivity of antibody detection methods in the diagnosis of Lyme disease.

Stage	Sensitivity	Remarks
I	20%–50%	Predominance of IgM
II	70%–90%	In cases of short disease duration predominance of IgM, in cases of long disease duration predominance of IgG
III	Nearly 100%	Usually solely IgG [a]

[a] the presence of IgM antibodies without IgG antibodies is not diagnostic for late disease: for possible exceptions see text.

(42). BBK32 is another antigen with considerable IgG reactivity (73%) in EM patients (87). In stage II (acute neuroborreliosis) seropositivity (IgM and/or IgG antibodies) increases to 70%–90% (59,65). Also here IgG antibody detection could be improved using recombinant VlsE or DbpA as antigens in the ELISA or the immunoblot (69,75,77). In principle, patients with early manifestations may be seronegative especially in case of short duration of symptoms. Then serological follow up is recommended and in

Table V. IgG reactivity of recombinant *Borrelia* proteins in the line immunoblot in early erythema migrans (EM) and acute neuroborreliosis (NB) and late manifestations acrodermatitis chronica atrophicans (ACA) or arthritis (AT). The table is based on data from reference (77).

Group	No. of sera	No. (%) of reactive sera with at least one homologue [a]					
		DbpA	p41i	OspC	VlsE	p58	p100
EM	15	5 (33.3)	1 (6.7)	3 (20.0)	12 (80.0)	1 (6.7)	4 (26.7)
NB II	50	39 (78.0)	17 (34.0)	9 (18.0)	46 (92.0)	27 (54.0)	26 (52.0)
ACA or AT	20	18 (90.0)	17 (85.0)	10 (50.0)	20 (100.0)	19 (95.0)	17 (85,0)
Negative controls [b]	110	4 (3.6)	0 (0.0)	0 (0.0)	4 (3.6)	1 (0.9)	2 (1.8)

[a] Multiple homologous proteins from different strains were used as antigens in case of DbpA, p41i, OspC, and VlsE (same as in Figure 3); [b] none of the controls were reactive with more than one *Borrelia* protein.

case of neurological symptoms the CSF/serum index should be determined. Six weeks or more after onset of symptoms, 100% of the patients with stage II neuroborreliosis were seropositive (65). In cases with late disease (stage III, ACA and arthritis) IgG antibodies were detectable in all patients tested (64,86). A negative IgG test argues against late Lyme borreliosis. Thus a positive IgM test without a positive IgG test is not diagnostic for late disease manifestations (46). Since serological findings vary considerably and antibodies may persist for a long time in successfully treated individuals, serological follow up is not suitable for therapy control. Recently the C6 peptide ELISA has been recommended from American authors for therapy control (88), however data were not convincing in a study from Europe (89). The presence of specific antibodies does not prove the presence of disease, a positive antibody test may also be due to clinical or subclinical infections in the past. The more nonspecific the symptoms, the lower is the predictive value of a positive serological test. Seropositivity in the normal healthy population varies with age and increased outdoor activities.

Methods which are not recommended or adequately documented for diagnosis

Recently, various methods have been used in commercially oriented laboratories which are not sufficiently evaluated for diagnostic purposes. Among them are the antigen tests in body fluids, PCR of urine, and lymphocyte transformation tests (90). The T-lymphocyte proliferation assays have been used in various scientific studies performed with blood from Lyme borreliosis patients to investigate the T-cell response to *Borrelia* antigens (91–93). However T-lymphocyte proliferation assays cannot be recommended as diagnostic tests due to their cumbersome nature and concerns about their specificity and standardization (40,90,94). Antigen detection tests have been used for the detection of borrelial antigen in body fluids from patients with Lyme borreliosis including CSF and urine (95,96). However, the validity of this technique is controversial and its use for diagnosis is no longer recommended for microbiological diagnosis (97). PCR from urine is unreliable too (98), borrelia DNA has been detected also from healthy seropositive individuals (99).

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