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Antibodies to decorin-binding protein B (DbpB) in the diagnosis of Lyme neuroborreliosis in children

Heidi Sillanpää [a,b,*], Barbro H. Skogman [c], Heikki Sarvas [b], Ilkka J.T. Seppälä [b], Pekka Lahdenne [d]

[a] Immunobiology, Research Program Unit, University of Helsinki, Helsinki, Finland
[b] Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki, Helsinki, Finland
[c] Center for Clinical Research Dalarna, Falun, Sweden
[d] Children's Hospital, University of Helsinki, Helsinki, Finland

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SUMMARY

Background: Laboratory support is needed to confirm the clinical diagnosis of Lyme neuroborreliosis (LNB). Antibodies to Borrelia-specific proteins have been used to improve serological diagnostics. The aims of this study were to assess the occurrence of antibodies to decorin-binding protein B (DbpB) in serum and cerebrospinal fluid (CSF) in children with LNB and to evaluate the performance of DbpB variants in the diagnosis of LNB in children.

Methods: Serum and CSF sample pairs were available from 57 children evaluated for LNB. Based on the presence of anti-flagella antibodies and pleocytosis in the CSF, patients were divided into three different groups: confirmed LNB ($n = 24$), possible LNB ($n = 16$), and non LNB ($n = 17$). Recombinant DbpBs from three *Borrelia burgdorferi* sensu lato species – *Borrelia burgdorferi* sensu stricto, *Borrelia garinii*, and *Borrelia afzelii* – were used in an ELISA to detect IgG antibodies.

Results: The sensitivity of variant recombinant DbpBs in serum and CSF samples varied between 0% and 46% and between 0% and 42%, respectively. In CSF, the most sensitive antigen was the DbpB variant from *B. garinii*.

Conclusions: Serum or CSF antibodies to DbpB do not appear to be beneficial in the laboratory diagnosis of LNB in children.

1. Introduction

Lyme disease (LD) is an infectious disease transmitted via a tick bite. It is caused by a group of *Borrelia burgdorferi* sensu lato spirochaetes. At least five *Borrelia* species (*B. burgdorferi* sensu stricto, *B. afzelii*, *B. garinii*, *B. spielmanii*, and *B. bavariensis*) may cause LD in Europe.[1] Due to the genotypic and phenotypic heterogeneity of the *B. burgdorferi* sensu lato species, clinical symptoms vary.[2] In the disseminated stage of the disease, one of the most common manifestations, especially in Europe, is neuroborreliosis (LNB).[3] Of the several *Borrelia* species, *B. garinii* is known for its neurotropism and is associated most frequently with LNB.[4,5]

The diagnosis of LNB may be challenging because the patient may not have noticed a tick bite or erythema migrans rash. In children in Europe, acute facial palsy and meningitis are common manifestations of LNB, even without preceding erythema migrans.[6–8] However, these manifestations are not specific to LNB.[6,9–12] Thus, laboratory testing (serum and cerebrospinal fluid (CSF)) is needed to confirm the diagnosis of LNB and exclude other aetiologies of the neurological manifestations.

In Europe, three criteria have been suggested for the diagnosis of definite LNB by the European Federation of Neurological Societies: (1) neurological symptoms indicative of LNB, (2) lymphocytic pleocytosis in CSF, and (3) demonstration of

* Corresponding author.
E-mail address: Heidi.Sillanpaa@helsinki.fi (H. Sillanpää).

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intrathecally produced antibodies against *B. burgdorferi* sensu lato.[13] Two of these criteria should be fulfilled for possible LNB.

Different types of antigen have been used for ELISA and immunoblotting methods on serum and CSF samples.[5,13,14] RevA, DbpA, DbpB, OspC, OspC peptide, VlsE, and IR6 peptide have been studied for diagnostic purposes in LD.[9,15–20] Such antigens, produced as recombinant proteins or synthetic peptides, have increased the specificity of laboratory testing for LD, but the sensitivity of new antigens has been unsatisfactory. Our earlier study on adult patients suggested that antibodies to decorin-binding protein B (DbpB) in CSF might be beneficial in the diagnosis of LNB, especially as a marker of active infection.[17] The aims of this study were to assess the occurrence of antibodies to DbpBs in children with LNB and to evaluate the performance of DbpBs in the diagnosis of LNB in children.

2. Materials and methods

2.1. Patient samples

Fifty-seven children (aged 2–17 years) with clinically suspected LNB from a highly endemic area in Sweden were included in this study. CSF and serum samples were obtained before the start of antibiotic treatment. Laboratory testing included CSF cell counts and the measurement of anti-flagella antibodies in serum and CSF, together with exclusion of other neurological aetiologies.[9] Based on clinical features at admission and laboratory results, patients were divided into three groups: confirmed LNB ($n = 24$), possible LNB ($n = 16$), and non LNB (originally not determined) ($n = 17$).[9] In brief, the classification of patients with clinical symptoms indicative of LNB was as follows: The confirmed LNB group of patients had pleocytosis (median 195, range 7–442 $\times$ 10^6 mononuclear cells/l in CSF) and either IgG or IgM or both anti-flagella antibodies in CSF. The possible LNB group of patients had pleocytosis in CSF (median 80, range 6–301 $\times$ 10^6 cells/l in CSF) and five of them had either IgG or IgM or both anti-flagella antibodies in serum, but none in CSF. The non LNB group of patients had no anti-flagella antibodies in CSF and no pleocytosis in CSF. One of them had IgG and another had IgM anti-flagella antibodies in serum. None of them had erythema migrans. This group of 17 patients was considered to have an aetiology other than LNB for the symptoms.

The clinical characteristics of the patients have been described in detail previously.[9,21] For the anti-flagella antibody ELISA, an index value of >0.3 between serum and CSF was used (as recommended by the manufacturer Dako (at present Oxoid), Glostrup, Denmark) to ensure intrathecal synthesis of anti-flagella antibodies.[22] The very same panel of serum and CSF samples has been studied previously for antibodies to IR6 peptide and recombinant proteins DbpA, OspC, and BBK32; CXL13 levels have also been measured.[9,21]

2.2. Serum disease control samples

Serum samples without anti-flagella antibodies were used as disease controls ($n = 36$). These samples were obtained from Finnish adult patients serologically positive for *Yersinia enterocolitica* infection ($n = 3$), Salmonella infection ($n = 3$), or anti-nuclear antibodies ($n = 10$), and from healthy adults with no proven infection ($n = 20$).

2.3. CSF disease control samples

CSF samples without anti-flagella antibodies were used as disease controls ($n = 57$). Disease control samples were available from children with other neurological diseases such as confirmed viral meningitis or convulsions/epilepsy ($n = 10$) and from healthy adults with no proven infection ($n = 16$). In addition, samples from adult patients with neurosyphilis ($n = 6$), syphilis ($n = 13$), and viral meningitis ($n = 12$) were used as disease controls.

2.4. DbpB antigens and ELISA

Recombinant antigen DbpB originated from three different pathogenic borrelial species: *B. burgdorferi* sensu stricto Ia (ss), *B. afzelii* A91 (afz), and *B. garinii* 40 (gar). The cloning, production, and purification of these recombinant proteins have been described previously.[23,24] ELISA tests were performed as described previously.[23] Samples from 20 healthy individuals were used to define the cut-off value for each antigen in serum ELISA (mean plus 2 SD). Samples from 10 disease control children and from 16 healthy adults were used to define the cut-off value for each antigen in the CSF ELISA (mean plus 3 SD). OD/cut-off values were used to present data in the figures.

2.5. Statistics

GraphPad Prism 4 (GraphPad Software Inc., USA) and Excel XP (Microsoft, USA) were used for calculations and illustrations. Sensitivity was calculated from the confirmed LNB and possible LNB groups ($n = 40$). Specificity was calculated from healthy adults with no proven infection and disease control samples in CSF ($n = 57$) and serum ($n = 36$).

2.6. Ethics

The retrospective examination of serum and CSF samples was approved by the ethics committees of Helsinki University Central Hospital, Finland (licence number 472/E0/01) and the Faculty of Health Sciences at Linköping University, Sweden (Dnr 03-546).

3. Results

3.1. DbpB antibodies in serum

Antibodies to DbpB variants in serum ELISA were found in 13 of the 24 patients with confirmed LNB and in four of the 16 patients with possible LNB (Figure 1, Table 1). One disease control sample

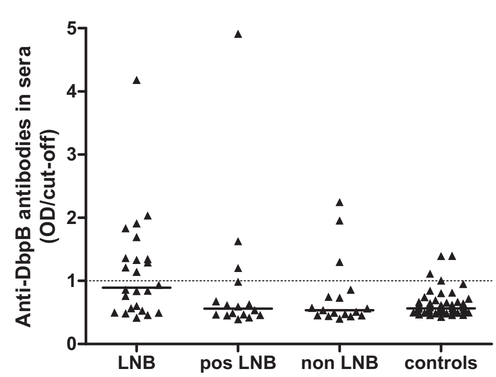

Figure 1. Serum samples tested with DbpB variants shown as OD_{405nm}/cut-off values. For each patient or control sample, only the highest value of the three DbpB variants is presented. All values above 1 are considered positive. The horizontal bars represent median values. Control samples were obtained from 16 adult patients serologically positive for *Yersinia enterocolitica*, Salmonella, or anti-nuclear antibodies, and from 20 healthy adults with no proven infection. Four of the controls were positive (range of OD/cut-off values 1.004–1.396). The cut-off is indicated with a dashed line. LNB, neuroborreliosis; pos LNB, possible neuroborreliosis; non LNB, other aetiology.

Table 1
Number of positive samples (%) to different DbpB variants in serum and in CSF ELISA

	n	No. (%) of positive samples			
		DbpB-afz	DbpB-gar	DbpB-ss	DbpBs[a]
Serum					
Confirmed LNB	24	4 (17)	11 (46)	2 (8)	13 (54)
Possible LNB	16	0	4 (17)	0	4 (17)
Non LNB	17	2 (12)	1 (6)	2 (12)	3 (18)
Disease controls	16	0	1 (6)	0	1 (6)
Healthy adults	20	1 (5)	1 (5)	2 (10)	3 (15)
CSF					
Confirmed LNB	24	1 (4)	10 (42)	0	10 (42)
Possible LNB	16	1 (6)	1 (6)	0	1 (16)
Non LNB	17	0	0	0	0
Disease controls	41	2 (10)	2 (10)	2 (10)	2 (10)
Healthy adults	16	1 (6)	1 (6)	1 (6)	1 (6)

DbpB, decorin-binding protein B; afz, *B. afzelii*; gar, *B. garinii*; ss, *B. burgdorferi* sensu stricto Ia; CSF, cerebrospinal fluid; LNB, Lyme neuroborreliosis.
[a] Samples positive with at least one DbpB variant.

(from a patient with *Yersinia enterocolitica* infection) and three healthy patient control samples were positive. The sensitivity of the serum DbpB ELISA (all three antigens together) was 43% (confirmed NB + possible LNB) or 54% (confirmed LNB only), and the specificity was 89%. All four positive samples from the possible LNB group were positive with the DbpB-gar variant (Table 1).

3.2. DbpB antibodies in CSF

Antibodies to DbpB variants in CSF ELISA were found in 10 of the 24 patients with confirmed LNB and in one of the 16 patients with possible LNB (Figure 2, Table 1). Three control samples were low positive, one from a healthy individual with no proven infection and two from patients with viral meningitis. The sensitivity of the CSF DbpB ELISA (all three antigens together) was 28% (confirmed NB + possible LNB) or 42% (confirmed LNB only), and the specificity was 95%.

In the confirmed LNB group, five of the 13 samples positive in the serum ELISA were also positive in the CSF ELISA. In the possible LNB group, one of the four samples positive in the serum ELISA was positive in the CSF ELISA. The sensitivity of DbpB serum and CSF ELISAs combined (all three antigens together) was 53% (confirmed LNB + possible LNB) or 71% (confirmed LNB only).

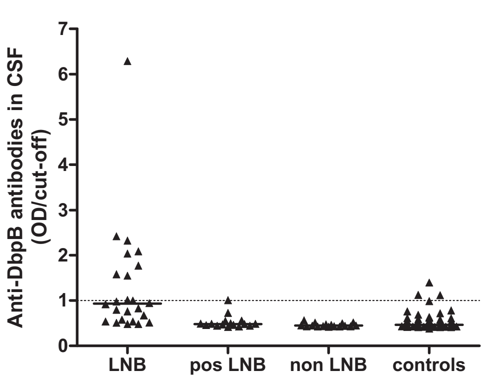

Figure 2. CSF samples tested with DbpB variants shown as OD_{405nm}/cut-off values. For each patient or control sample, only the highest value of the three DbpB variants is presented. All values above 1 are considered positive. The horizontal bars represent median values. Control samples were obtained from 10 children with other neurological diseases and 31 adults with syphilis, neurosyphilis, or viral meningitis, and from 16 healthy adults with no proven infection. Three control samples were positive (range of OD/cut-off values 1.118–1.400). The cut-off is indicated with a dashed line. LNB, neuroborreliosis; pos LNB, possible neuroborreliosis; non LNB, other aetiology.

4. Discussion

This study provides support to previous observations on the role of *B. garinii* as the predominant aetiological agent of European LNB. However, although the DbpB originating from *B. garinii* was the most sensitive antigen both in serum and in CSF, the overall sensitivity of this antigen in the laboratory diagnosis of early LNB remained at an unsatisfactory level.

This study aimed to improve the laboratory diagnostics of early LNB especially. Because symptoms in the early stages of infection are usually unspecific, the diagnosis is difficult to confirm on clinical grounds only.[6] The interval between the initiation of neurological symptoms and diagnostic evaluation is reported to be shorter in children than in adults.[11] Obviously, children more often present disseminated stages of the disease earlier than adults. Disappointingly, the present study showed that in the laboratory diagnosis of LNB in children, serum or CSF antibodies to DbpB appeared to be of limited value. The performance of the DbpB ELISA assays remained inferior to routine testing with anti-flagella antibodies. The intrathecal production of DbpB antibodies was not evaluated in this study. However, in provisional analyses, only two of all samples from patients with confirmed neuroborreliosis had a value $\geq$0.3 suggesting the intrathecal production of antibodies[22] (data not shown).

The sensitivity of the DbpB ELISA was highest in the children with confirmed LNB but remarkably low in those with possible LNB. These children had a short duration of symptoms, and it appears that DbpB antibodies are not helpful in the diagnosis of early LNB. As a whole, both CSF and serum DbpB antibody testing were much less sensitive compared to our previous results in adults with LNB and adolescents with Lyme arthritis.[17,25] Such manifestations can be considered as representing more advanced stages of the disease, and the sensitivity of the DbpB assay is improved with the longer disease duration. In adults the median disease course was 1 month or more,[17,25] whereas in our cohort it was 1–2 weeks. Furthermore, it has been reported that the intrathecal synthesis of anti-Borrelia antibodies increases with the duration of the disease.[26,27] The delay between the first appearance of symptoms and diagnostic evaluation, as well as the clinical outcome of the disease, have all been reported to be associated with the level of CSF pleocytosis.[28,29]

The low sensitivity of DbpB antibody testing can also be explained in part by the heterogeneity of DbpB variants between the different *Borrelia* species.[23] Among the three DbpB antigens, the recombinant protein originating from *B. garinii* outperformed the other variants in the diagnosis of LNB. This is in line with our earlier study on the diagnosis of adult patients with LNB.[17] Of the *B. burgdorferi* sensu lato species, *B. garinii* is not detected in the USA where LNB is uncommon.[30,31] In Europe, LNB in most cases is caused by *B. garinii*.[4,5]

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Conflict of interest: The authors declare that they have no conflict of interest.

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