Comparison of EIA Borrelia recombinant with VlsE lipoprotein with EIA *Borrelia garinii* using whole cell antigen in the diagnosis of neuroborreliosis

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1. Introduction

Aseptic meningitis, cranial neuritis and radiculitis are typical for neuroborreliosis, nevertheless, none of these involvements are pathognomonic. Diagnostic investigation includes assessment of antibodies in serum and CSF, cytology of CSF and intrathecal antibody synthesis (AI). During the early disseminated neuroborreliosis, expression of antigens by borreliae occurs at variable times. Assays using only one recombinant protein with the correct sequence may not detect antibodies at each stage of infection [1].
VlsE (variable major protein-like sequence, expressed), the variable surface lipoprotein of *B. burgdorferi* s.l. contains variable and invariable domains. Within the VlsE variable domain there are six invariable regions (IR 1-6). IR 6 is exposed on the surface of VlsE and is immunodominant in patients with Lyme borreliosis [2].
The description of variability of the antibody response among patients with Lyme borreliosis is the subject of many studies [3,4,5,6]. The purpose of our study was to evaluate a new kit of the 3rd generation, EIA Borrelia recombinant IgM and IgG (TEST-LINE, Brno, CZ) in serum and cerebrospinal fluid (CSF) of children with neuroborreliosis.

2. Patients

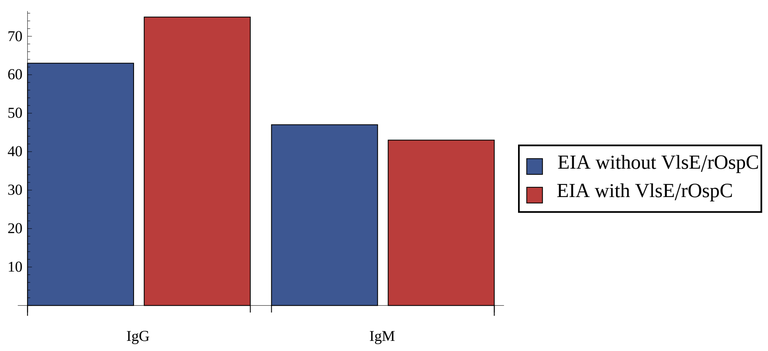
182 children were divided into 3 groups. In the first group, 86 children with proven neuroborreliosis were included, manifested by aseptic meningitis and facial palsy or both. Pleocytosis, elevated protein level, BBB (blood-brain barrier) disruption were the CSF findings. Antibody indices for IgM and IgG were determined in all CSF samples as gold standard for the definition of neuroborreliosis.
The second group represents 30 children, who were admitted with suspicion for neuroborreliosis. Following clinical symptoms and signs were present: solitary or multiple erythema migrans, headache, neck stiffness, positive meningeal signs, parestesia, tremor, fever, vomiting, fatigue. Symptoms were either acute or longlasting. All children had positive antiborrelial antibodies in ELISA test confirmed by immunoblot.
132 samples of 66 children with other neuroinfections than neuroborreliosis were used as controls. These children were diagnosed as tick-borne encephalitis, viral meningitis, isolated facial palsy of other than borrelial etiology, myelitis or children with negative CSF findings, where neuroinfection was excluded.

3. Methods

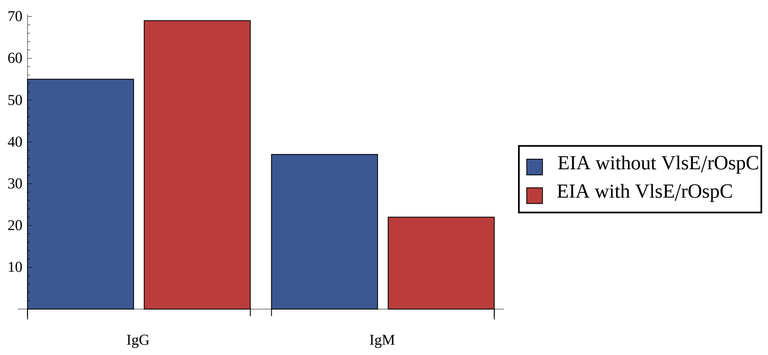
The newly tested EIA is based on the selected antigen fragments: rOspC, p39 and internal flagellin (p41i) for IgM and p17, rOspC, p39, p41i, p83 and VlsE for IgG. Recombinantly produced protein fragments were selected according to the most frequent borrelial subspecies in the Czech Republic: *B. garinii, B. afzelii* and *B. burgdorferi* sensu stricto. Selected antigen fragments are typical for early and late antibody response with production of IgM/IgG antibodies.
The antigen is bound in wells of a divided microtitre plate and 0.1 ml of 1:100 diluted serum or 1:1 CSF sample is added. The plate is incubated at 37°C for 30 minutes. The antigen-antibody reaction is probed by using a conjugate (SwAHu/IgM or IgG Px, 30 minutes by 37°C) and for the detection of a colour reaction by using a one component substrate (TMB-Complete, 15 minutes by 37°C). The positivity is indicated by the appearance of blue colour which changes to yellow by the addition of the stopping solution (H_2SO_4). The colour intensity is measured using a photometer at 450 nm wavelength.
The cut-off control is a component of the set which makes positivity index (IP) evaluation possible. Positive results are considered to be those which were indicated as borderline (IP 0.9-1.1) or positive (IP > 1.1). Samples having IP < 0.9 were evaluated as negative. The enriched EIA was compared with the EIA using whole cell antigen of *B. garinii* without VlsE.
McNemar's nonparametric test and the odds ratio (OR) were used for statistical analysis.

4. Results

I. Group (neuroborreliosis): The probability of a positive result by the EIA Borrelia recombinant test using VlsE for IgG corresponds to the OR=7.0 in serum and OR=4.5 in CSF. The difference was significant for both serum (p=0.006) and CSF (p=0.006) . There was no statistically significant difference in the IgM positivity of of serum (p=0.54), only in CSF (p=0.001).
Negative results of EIA with VlsE correlate with negative antibody indices in samples of 8 children, the serum and CSF specimen were obtained either early in the infection or after the therapy. Neuroborreliosis was proven from the first, resp. the second specimen. Clinically, meningitis with (7 children) or without (1 child) facial palsy was diagnosed in 8 children alltogether. Only one child had a negative AI in CSF (IgG = 0.82) but with inflammatory changes in CSF without BBB disruption (Q_{alb} = 2.4). Erythema migrans and facial palsy were still present at the time of admission.
AI IgM positivity without IgG production was detected in 6 CSF samples, 5 of which had a positive result in enriched EIA test with VlsE.
Identical results were obtained by immunoblots. The difference in the detection of positive sera for IgG was p=0.037 and for CSF p<0.001.



Graph A: Comparison of two EIA methods in sera



Graph B: Comparison of two EIA methods in CSF

II. Group (neuroborreliosis excluded): No CSF sample was IgM positive with enriched EIA B. recombinant. No child had inflammatory changes or positive AI in CSF. These results correspond to the diagnosis of excluded neuroborreliosis despite some suspicious clinical findings in favour of neurological involvement of Lyme borreliosis or skin manifestations typical for dermatoborreliosis. The difference in the detection of positive results in serum resp. CSF for IgG amounted to p=0.228 resp. p=0.077.
III. Group (controls): There was no statistical difference between both tests either in serum or in CSF samples (p~1.0) among controls.
Results of EIA with/without VlsE in children with neuroborreliosis are shown in Graphs A (serum) and B (CSF) for IgM and IgG antibodies.
The sensitivity of enriched EIA B. recombinant IgG increased from 73% to 87% in serum samples and from 64% to 80% in CSF samples. The specificity in IgG antibodies was 82% for sera and 97% for CSF.

5. Discussion

The diagnosis of neuroborreliosis is based on clinical signs which are typical not only for neuroborreliosis. CSF examination is therefore determinative for the definite diagnosis. Tests using a peptide based on a immunodominant conserved region of *B. burgdorferi* VlsE were developed to improve the diagnosis of Lyme borreliosis [3,4].The diagnostic sensitivity ranges from 70% to 88% [5,6,7,8].
The heterogeneity of borreliæ in Europe has to be taken in consideration in development of new diagnostic tests. Recombinantly produced protein fragments were selected according to the most frequent borrelial subspecies in the Czech Republic, therefore the sensitivity of the EIA Borrelia recombinant (87% for sera and 80% for CSF) in the detection of antibody response in children with early disseminated neuroborreliosis is comparable with immunoblot.
In the study [9] using sera of 36 neuroborreliosis patients, the diagnostic sensitivity (86.1%) of the recombinant immunoblot was increased without loss of specificity and the improvement was mainly due to the presence of VlsE followed by DbpA.
Improved sensitivity results from the use of recombinant proteins that are expressed primarily in vivo (VlsE). The recombinant VlsE seems to be the most sensitive antigen for the detection of IgG antibodies. The EIA Borrelia recombinant test with highly specific antigen VlsE shows an increased sensitivity in samples obtained from children with proven neuroborreliosis with comparable specificity.

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