Microarray immunoblot in the diagnosis of pediatric Lyme neuroborreliosis

Lenka Krbková[1], Michal Kýr [2], Iva Stoklásková[3], Lenka Pokorná[3]

[1]Masaryk University, Faculty of Medicine, Department of Children's Infectious Diseases, Faculty Hospital, Brno, CZ, [2] Department of Pediatric Oncology, Faculty of Medicine and University Hospital, Masaryk University, Brno, CZ, [3] TestLine, Clinical Diagnostics, Brno, CZ,

1. Introduction

The aim of the study was to analyse and compare humoral immune response in serum and cerebrospinal fluid samples (CSF) of 51 pediatric patients with clinically well defined Lyme neuroborreliosis (LNB) by multiplex assay and recombinant enzyme immunoassay (EIA). The multiplex assay (microarray) is used to profile the humoral immune response to *Borrelia* species. Several proteins and their homologs have been described [1, 2]. Immunodominant antigens from different *Borrelia* spp could be detected on the microarray plates [3].

2. Patients

A total of 102 paired samples of serum and CSF were obtained from children with LNB (n = 51). Lymphocytic pleocytosis was detected in all of them. Intrathecally synthesized borrelial antibodies were calculated for IgG using recombinant EIA (specific CSF/serum IgG ratios greater or equal than 1.4 were considered as positive). Sera and CSF of children (n = 36) with other neuroinflammatory conditions (mostly aseptic meningitis of viral etiology) were used as controls.

3. Methods

The microblot-array is a new generation of immunoblot methods. This multiplex assay is based on highly specific recombinant antigens spotted on the nitrocellulose membrane using a microdispensing method. Altogether, 19 recombinant antigens from three major borrelial species and *B. spielmanii* were used for the application. The recombinant antigens, which are considered major immunodominant markers for main *Borrelia* species, were used for the application: antigens are listed in FIGURE 1. The test was compared to the recombinant EIA test based on the selected antigen fragments from three major borrelial species: p17, OspC, p39, p41i, p83 and VlsE.

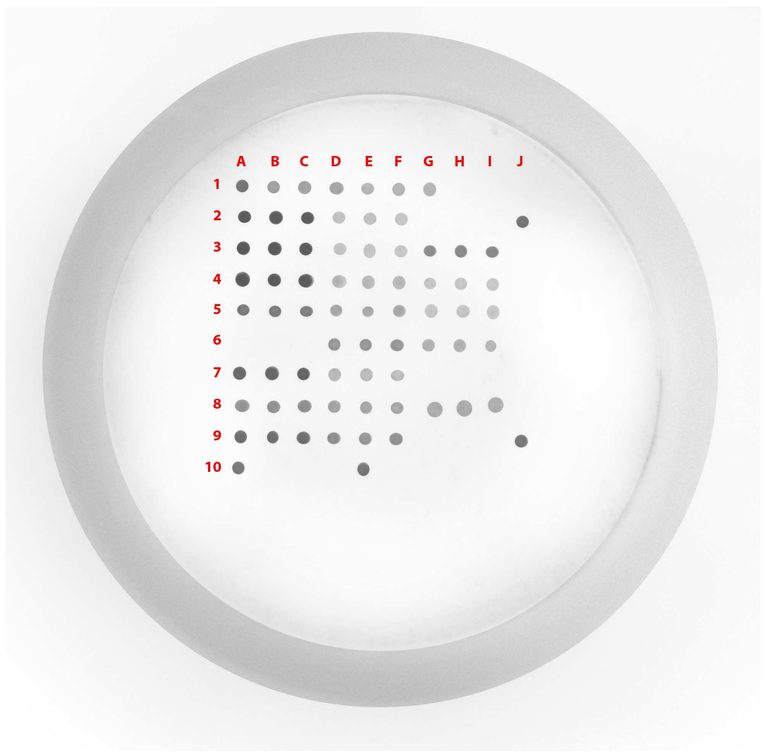

FIGURE 1. Microarray of positive cerebrospinal fluid sample. Antigen distribution (reactive antigens are typed in bold):

2A-C = VlsE *B. afzelii*,
3A-C = VlsE *B. garinii*,
4A-C = VlsE *B. burgdorferi* ss,
5A-C = OspA *B. garinii*,
6A-C = NapA,
7A-C = OppA2,
8A-C = p41 *B. afzelii*,
9A-C = p41 *B. burgdorferi* ss,
4D-F = OspB,
5D-F = OspA *B. afzelii*,
6D-F = OspA *B. burgdorferi* ss,
7D-F = OspC *B. burgdorferi* ss,
8D-F = OspC *B. afzelii*,
9D-F = OspC *B. spielmanii*,
3G-I = p83,
4G-I = p17,
5G-I = OspE,
6G-I = p39,
8G-I = OspC *B. garinii*,
1A, 2J, 9J, 10A, 10E = Ref. spots,
1B-D, 1E-F = Test controls,
2D-F,3D-F,2G-I = HGA antigens,
7G-I, 9G-I = No antigens.

4. Statistical analysis

The data were analysed using software R version 3.1.3. For categorical data, comparison was made using Fisher's exact test, McNemar's nonparametric test and odds ratio. P values of less than 0.05 were considered significant. Sensitivity was calculated from the confirmed LNB (n = 51). Specificity was calculated from control serum and CSF samples (n = 36).

5. Results

The presenting symptom in 51 of the LNB patients (age median 7 years, range from 2 to 15 years) was intermittent or continuous headache. Eleven patients had erythema migrans (untreated or partially treated) and 28 had unilateral or bilateral facial nerve paralysis. Both symptoms were present in eight patients. Tick bite was mentioned by 30/51 (59%) of parents. The median duration of symptoms before the first CSF sample was 10 days, with the range from one to 78 days. LNB patients have been treated with intravenous ceftriaxone for 14 to 21 days. Intrathecally synthesized antibodies were negative in 5 CSF samples (the range between 0.44 to 1.2). The duration of symptoms was from 1 to 4 days and the children were 3 to 6 years old. Two of them had clinically well defined erythema migrans. The diagnosis was confirmed either from the second CSF sample or by the seroconversion of antibodies. IgG microarray immunoblot was positive in four of them in serum, negative result in microarray was detected in one child with erythema migrans followed by meningoneuritis. The diagnostic value for serum (OR = 1.2, $P \sim 1.0$) and CSF (OR = 2.0, P = 0.505) was comparable, though positive result was twice more likely for CSF if tested by microarray immunoblot (TABLE 1 and 2). The sensitivity/specificity for IgG microarray immunoblot in pediatric LNB is 78.4%/77.8% for sera and 68.6%/88.9% for CSF. The sensitivity/specificity for IgG recombinant EIA in the same cohort is 82.4%/86.1% for sera and 68.6%/97.2% for CSF (TABLE 3).

	S-EIA Neg	S-EIA Pos
S-MA Neg	33 (84.6%)	6 (15.4%)
S-MA Pos	7 (14.6%)	41 (85.4%)

OR = 1.2, P ~ 1.0

TABLE 1: Positive and negative serum samples (n = 51) by microarray immunoblot and recombinant enzyme immunoassay; S = serum, MA = microarray, EIA = enzyme immunoassay, Neg = negative, Pos = positive.

	CSF-EIA Neg	CSF-EIA Pos
CSF-MA Neg	45 (93.8%)	3 (6.2%)
CSF-MA Pos	6 (15.4%)	33 (84.6%)

OR = 2, P = 0.505

TABLE 2: Positive and negative cerebrospinal fluid samples (n = 51) by microarray immunoblot and recombinant enzyme immunoassay; CSF = cerebrospinal fluid, MA = microarray, EIA = enzyme immunoassay, Neg = negative, Pos = positive.

Test	S-MA	S-EIA	P-value	CSF-MA	CSF-EIA	P-value
Sensitivity	78.4%	82.4%	<0.0001	68.6%	68.6%	<0.0001
Specificity	77.8%	86.1%	<0.0001	88.9%	97.2%	<0.0001

TABLE 3: Sensitivity and specificity of microarray (MA) immunoblot and recombinant enzyme immunoassay (EIA) in serum (S) and cerebrospinal fluid (CSF)

FIGURE 2 and 3 represent the evaluation of anti-*Borrelia* IgG antibodies in serum and CSF samples of LNB patients and controls.

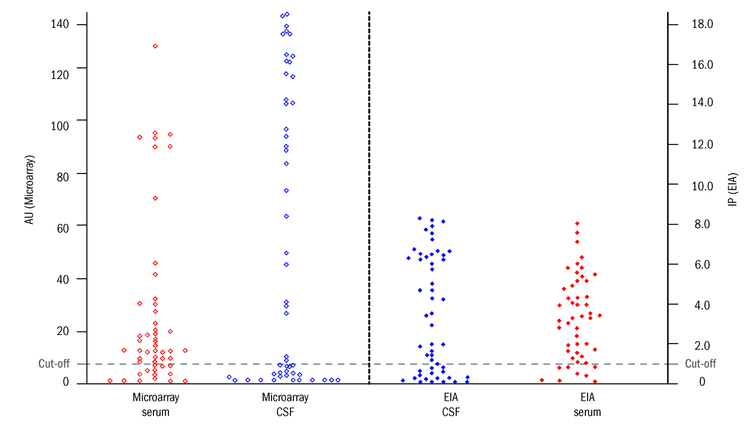

FIGURE 2. Serum and cerebrospinal fluid (CSF) samples of 51 children with Lyme neuroborreliosis (LNB): Arbitrary units (AU) of microarray test compared with index of positivity (IP) of recombinant EIA. Detection of IgG antibodies by microarray immunoblot method in serum samples [**Microarray serum** (◊)] and in cerebrospinal fluid samples [**Microarray CSF** (◊)]. Detection of IgG antibodies by EIA using recombinant *Borrelia burgdorferi* sensu lato antigens in cerebrospinal fluid samples [**EIA CSF** (♦)] and in serum samples [**EIA serum** (♦)].

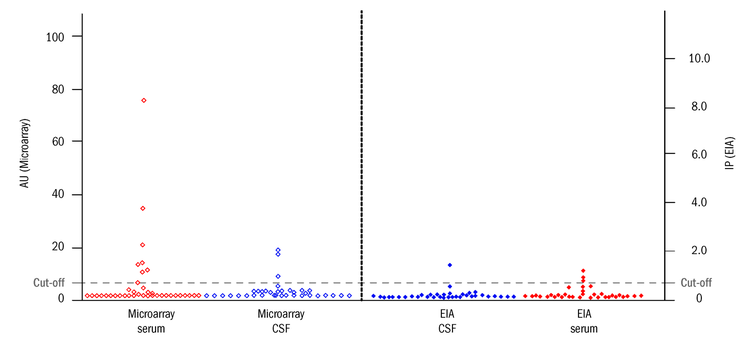

FIGURE 3. Control serum and cerebrospinal fluid (CSF) samples of 36 children: Comparison of positivity index of recombinant EIA and arbitrary units of microarray test calculated for IgG antibodies. See footnotes to FIGURE 2.

6. Discussion and conclusion

The microarray with 19 recombinant antigens from four *Borrelia* species includes not only widely used outer surface proteins but even some of new recombinant peptides containing specific epitopes (NapA, OppA2, OspE) which may play a role in the immune response to the pathogen and may increase the specificity of the test [4,5]. Production of antibodies against surface antigens VlsE and OspC expressed by borreliae during the early phase of mammalian infection is predominant immune response. Microarrays for their serologic detection represent a promising new technology. Generally, immunoblot assay shows only low detection rate in CSF, whereas the microarray technology with new antigens increases sensitivity of antibody detection in CSF. Despite of high sensitivity of microarray test, one child with erythema migrans and meningoneuritis could not be diagnosed from CSF sample. Both tests have comparable statistical significance. Microarray immunoblot assay enriched by 19 specific antigens is an appropriate confirmation test after screening by recombinant EIA.

References

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