Antigenicity of borrelial protein OppA2 and NapA fragments in pediatric Lyme arthritis

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1. Introduction

Laboratory diagnosis of Lyme arthritis (LA) is based on the detection of a high level of immunoglobulin G (IgG) antibodies against *Borrelia burgdorferi* sensu lato [1]. Whole-cell antigens contain epitopes which may cross-react with epitopes of other bacterial species. Recombinantly produced borrelial protein OppA2 (oligopeptide permease A2, p58) and NapA (neutrophil-activating protein A) fragments were found to be highly conserved among the three major pathogenic species [2,3]. OppA2 shared 70 to 80% sequence homology among genospecies [3]. NapA of *B. burgdorferi* is able to manage the expression of proinflammatory cytokines and might play a role in the pathogenesis of LA [4]. Reactivity with NapA protein was observed predominantly in serum of patients with LA [2]. The chromosomal gene of *B. burgdorferi* encoding NapA was found to be essential for the persistence of spirochete within ticks [5].

2. Patients

The reactivity to each of the recombinant proteins as antigens was examined in order to evaluate their diagnostic value in pediatric Lyme arthritis (LA). Arthritis (minimum one-time or repeated swelling of large joints) was the presenting feature in all of them (n = 41). Patients with solely arthralgia and positive Lyme serology were excluded. All children were treated by one course of antibiotics (ceftriaxone or doxycycline intravenously). None of the patients was classified as antibiotic-refractory LA. In total, 133 samples obtained from 41 children were divided into 3 groups. First group contained pretreatment samples (n = 9) from early acute stage of LA. In the second group, samples (n = 39) obtained in early convalescent stage were tested. The third group represented 85 samples, which were obtained repeatedly in follow-up visits during the period of more than 12 months up to several years after the therapy of LA.

3. Methods

BLOT-LINE technology is based on highly specific recombinant antigens applied on a nitrocellulose membrane and following analysis using special analyzer. Reactivity of the protein fragments OppA2 originated from *B. garinii* and NapA originated from *B. burgdorferi* sensu stricto was analyzed in 133 serum samples of 41 children with LA. Reactivity of these antigens was compared to the recombinant EIA test based on the selected antigen fragments p17, OspC, p39, p41i, p83 and VlsE for IgG.

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.

5. Results

The antibody responses to both specific antigens are presented in FIGURE 1. A total of 100 samples (86.2%) have showed a positive result for OppA2 and in recombinant EIA test compared to NapA, where only 33 (28.4%) of samples were positive in both tests. Diagnostic value of OppA2 was higher (OR = 1.5, P = 0.44) than of NapA (OR = 27.7, $P < 0.0001$) compared to recombinant EIA. The difference was statistically significant for OppA2 (TABLE 1 and 2).

	OppA2 Neg	OppA2 Pos
EIA Neg	6 (35.3%)	11 (64.7%)
EIA Pos	16 (13.8%)	100 (86.2%)

OR = 1.5, P = 0.4414

TABLE 1: Negative and positive antibody response to recombinant OppA2 in 133 serum samples of 41 children with Lyme arthritis.
EIA = enzyme immunoassay, OppA2 = oligopeptide permease A2, Neg = negative, Pos = positive.

	NapA Neg	NapA Pos
EIA Neg	14 (82.3%)	3 (17.7%)
EIA Pos	83 (71.6%)	33 (28.4%)

OR = 27.7, P < 0.0001

TABLE 2: Negative and positive antibody response to recombinant NapA in 133 serum samples of 41 children with Lyme arthritis.
EIA = enzyme immunoassay, NapA = neutrophil-activating protein A, Neg = negative, Pos = positive.

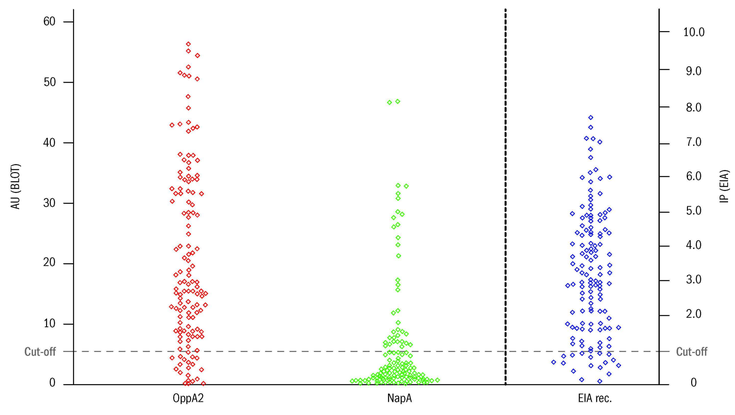

FIGURE 1. Comparison of arbitrary units of BlueBLOT-LINE measurement of OppA2 and NapA antigen and positivity index of recombinant EIA calculated for IgG antibodies. Serum samples (n = 133) of children (n = 41) with Lyme arthritis. IP = index of positivity AU = arbitrary units
OppA (◊) = Detection of IgG antibodies against OppA2 (Oligopeptide permease A2) antigen of *Borrelia garinii*
NapA (◊) = Detection of IgG antibodies against NapA (Neutrophil activating protein A) antigen of *Borrelia burgdorgferi* sensu stricto
EIA (◊) = Detection of IgG antibodies using *Borrelia burgdorferi* sensu lato recombinant antigens

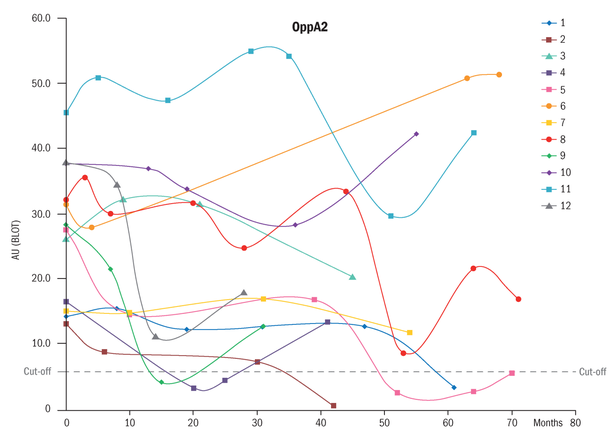

FIGURE 2. Antibody responses to OppA2 during acute onset of Lyme arthritis and in follow-up of 12 children.

Twelve children were followed-up from 1 to 5.8 years after the treatment of LA. Dynamic longitudinal antibody responses to OppA2 and NapA during acute infection and after antibiotic treatment are shown in FIGURE 2 and FIGURE 3. Recurrent attacks were diagnosed in five cases before or during the treatment.

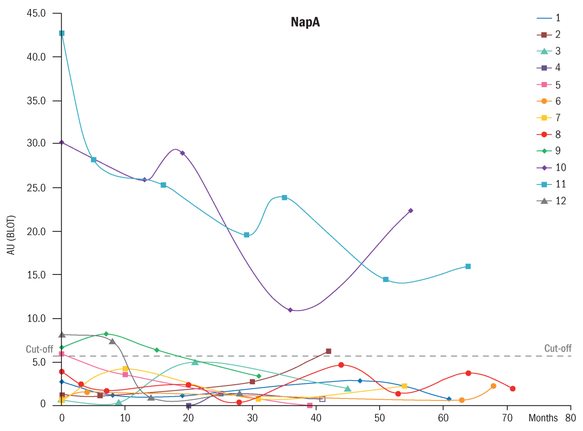

FIGURE 3. Antibody responses to NapA during acute onset of Lyme arthritis and in follow-up of 12 children.

6. Discussion and conclusion

Recombinant EIA enriched by OppA2 and NapA might exceed the performance of routinely used screening test. We assume that OppA2, a surface-localized lipoprotein, is one of the significant recombinant proteins indicative for the diagnosis of LA. Specific IgG antibodies to OppA2 could be determined not only early in the course of LA, but even several years after treated infection in tested serum samples of children with LA. Similar results with slow and incomplete antibody decline to OppA2 were demonstrated in nonhuman primates [6]. The production of IgG antibodies to OppA2 did not correlate with the severity of LA-infection in children. Anti-NapA IgG levels were not significantly increased in children with LA and appeared to decline with antibiotic treatment. The statistical significance was at $P < 0.0001$. We can not confirm diagnostic value of NapA for humoral immune response in children with LA.

References

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