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CASE REPORT

Recurrent bacterial meningitis by three different pathogens in an isolated asplenic child

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Abstract Isolated congenital asplenia (ICA) is a rare condition at risk for overwhelming infection. When complicated by invasive infection, the mortality remains high, at greater than 60%. We describe a girl with ICA who developed recurrent meningitis by three different pathogens. The first, meningitis by *Escherichia coli*, occurred 4 days after premature birth. The other two pathogens were serotype 6B *Streptococcus pneumoniae* and *Haemophilus influenzae* type b (Hib), at 18 and 25 months of age, respectively. The patient was successfully treated with prompt antimicrobial therapy in all episodes. Serum anti-polyribosylribitol phosphate (PRP) and anti-6B-type pneumococcal antibodies were below the levels for protective activity after natural infections. Although anti-PRP antibody was significantly increased after Hib vaccination, two (6B and 19F) of seven serotype-specific pneumococcal antibodies were not elevated to protective levels after the second 7-valent pneumococcal conjugate vaccine (PCV7). We, therefore, added a third PCV7. To our knowledge, this is the first neonatal ICA patient with invasive infection and the first case of bacterial meningitis occurring three times. Our findings indicate that monitoring of immune responses after natural infections and vaccinations, and reevaluations of vaccine schedule, are important for ICA patients to prevent subsequent invasive infections.

Keywords Isolated congenital asplenia · Bacterial meningitis · Immunological response · Recurrence · Neonate · Vaccine

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Introduction

Congenital asplenia often occurs as part of a recognized malformation syndrome with anomalies of the heart, great vessels, and viscera [1]. The best known among these syndromes is the asplenia/polysplenia syndrome associated with visceroarterial heterotaxy, and its incidence is estimated at approximately 1/10,000 to 1/40,000 live births [2]. In contrast, isolated congenital asplenia (ICA) occurs fairly more infrequently. A recent French nationwide study indicated that the prevalence is 0.51 per million births [2]. Both conditions have an increased susceptibility to overwhelming invasive infections, carrying considerable mortality. However, the diagnosis of ICA is sometimes difficult because of the lack of other anomalies; therefore, such individuals may be unrecognized until postmortem autopsy.

Practice guidelines for the prevention of life-threatening infections in children with hyposplenia and asplenia advocate antibiotic prophylaxis and immunizations against *Streptococcus pneumoniae* and *Haemophilus influenzae* type b (Hib), the most common causative organisms for

these patients [3]. However, given that several asplenic cases of overwhelming infections that could be considered as vaccine failures have been documented [4, 5], the immunogenicity of vaccination for asplenic patients is still an important concern.

We present here a girl with ICA who developed multiple episodes of meningitis caused by three different pathogens, namely, *Escherichia coli*, *S. pneumoniae* (serotype 6B), and Hib. She was successfully treated with prompt initiation of antibiotics in all episodes. We also present the details of immune responses to natural infections by Hib and serotype 6B *S. pneumoniae* and those to immunizations of Hib conjugate vaccine and 7-valent pneumococcal conjugate vaccine (PCV7).

Case report

A 4-day-old girl, who was born of nonconsanguineous parents as their first child, weighing 1,742 g at the 34th week of gestation, presented with repetitive apnea during admission because of prematurity. Physical examination showed that heart rate was 135/min and body temperature was 37.2°C. Laboratory data showed WBC of 5.8×10^9/l with 28.5% neutrophils, C-reactive protein (CRP) of 4.3 mg/dl, and blood glucose of 95 mg/dl. Cerebrospinal fluid (CSF) examination showed 3,947 cells/μl with 96% polymorphonuclear cells, 197 mg/dl protein, and 44 mg/dl glucose. Two days later, isolates from the CSF and blood were identified as *E. coli* OX:K1:H–, and the same bacterium was also subsequently isolated from the stool of her asymptomatic mother. The patient was diagnosed as having early-onset *E. coli* meningitis that was vertically transmitted. We treated the patient with cefotaxime (CTX) for 21 days. Auditory brainstem response examination at 28 days of age revealed profound hearing impairment at the right ear. The patient was discharged at 38 days of age. Genetic analysis [6, 7] showed that the strain harbored virulence factor genes such as *iroN*, *papG3*, *afa*, and *kps*, but not *cnf1*, *sfa*, or *ibeA*.

At 18 months old, the patient was rehospitalized because of a 6-h history of fever and generalized tonic-clonic convulsion lasting 3 min. On admission, 30 min after the convulsion, heart rate was 170/min and body temperature was 39.4°C. Her consciousness had become clear. Laboratory findings showed WBC of 21.7×10^9/l and CRP of 6.0 mg/dl. CSF examination showed no pleocytosis, with normal concentrations of protein (10 mg/dl) and glucose (85 mg/dl). Treatment with intravenous CTX was empirically initiated under the tentative diagnosis of occult bacteremia. The day after admission, serotype 6B *S. pneumoniae* was isolated from the blood but not from the CSF. Resistance to penicillin was established by microbiological [minimum inhibitory concentration (MIC), 2 μg/ml] and genotypic (mutations in *pbp1a*, *pbp2X*, and *pbp2b* [8]) analyses, and CTX was substituted with panipenem–betamipron. On day 3, prolonged fever and frequent vomiting led us to perform a second CSF examination, showing 14,500 cells/μl, protein of 58 mg/dl, and glucose of 63 mg/dl. The CSF was positive for *S. pneumoniae* antigen test (Binax NOW *S. pneumoniae*; Binax), but yielded no organisms in culture. The blood WBC and CRP were elevated to 21.7×10^9/l and 22.1 mg/dl, respectively. We diagnosed her disease as pneumococcal meningitis following bacteremia and increased the doses of panipenem–betamipron with good clinical response. She received antimicrobial therapy for 14 days and was discharged without any additional sequelae.

At 25 months of ages, the patient was referred to the emergency department in another hospital with a 2-h history of fever, vomiting, and tonic–clonic convulsion of 2-min duration. At arrival, heart rate was 180/min and body temperature was 39.4°C. Her consciousness soon became clear. Laboratory examination showed WBC of 3.5×10^9/l and CRP of 0.6 mg/dl. After blood culture was obtained, the patient received intravenous sulbactam/ABPC. On day 3, the blood culture yielded β-lactamase-non-producing ABPC-resistant (BLNAR) Hib, and the laboratory examinations showed marked deterioration: WBC of 26.6×10^9/l and CRP of 21.5 mg/dl. CSF examination showed 4,992 cells/μl, 164 mg/dl protein, and 34 mg/dl glucose with positive culture for Hib. Thus, the diagnosis of a third bacterial meningitis was made. The patient thereafter received intravenous meropenem for 14 days and was discharged on day 16 after onset without any additional sequelae. Molecular analysis of the strain identified three amino acid substitutions: His-517, Thr-385, and Ile-377, in *ftsI* [9]. This substitution pattern was classified as subgroup III BLNAR by a recent nationwide study of childhood meningitis in Japan [9].

The multiple episodes of meningitis prompted us to evaluate immunological functions. The results after the second episode of meningitis showed that serum levels of IgG (639 mg/dl), IgA (65 mg/dl), IgM (97 mg/dl), IgG_2 (80 mg/dl), C3 (140 mg/dl), C4 (24 mg/dl), and CH50 (36.1 U/ml) were within normal limits. T/B-cell subsets (65/28%), CD3/CD4/CD8 lymphocyte subsets (61%/44%/14%), natural killer cell activity (25%), neutrophil phagocytic activity using fluorescence bead test by flow cytometry (70.0%), and neutrophil bacteriocidal activity (93.4%) were also normal. Computed tomography (CT) of the skull and inner ears did not show any deformity or defects. To screen interleukin-1 receptor-associated kinase 4 deficiency and myeloid differentiation primary response protein 88 deficiency, we performed flow cytometric analysis [10], resulting in normal intracellular tumor necrosis factor-α

production of monocytes after lipopolysaccharide stimulation. After the third meningitis, ultrasonography and CT of the abdomen finally revealed asplenia without visceroarterial anomalies. Howell–Jolly body-containing RBCs were exceedingly rarely found (<0.1% of RBCs) in peripheral blood. Ultrasonographic examinations of her parents detected normal size and normal position of the spleen.

Since the diagnosis of ICA at 26 months of age, chemoprophylaxis with amoxicillin of 20 mg/kg/day was introduced as well as vaccinations of Hib vaccine and PCV7. Subsequent to the introduction of these strategies, the patient has not suffered from any invasive infections for more than 2 years. At 36 months of age, we assessed her neurodevelopmental status using the New Edition of the Kyoto Scale of Psychological Development, indicating a normal developmental quotient of 88 (normal range, >80).

We evaluated immune responses to natural infections with Hib and serotype 6B pneumococcus and those to immunizations of Hib vaccine and PCV7 (Table 1). Despite natural infections, serum anti-polyribosylribitol phosphate (PRP) (0.60 μg/ml) and anti-serotype 6B (0.191 μg/ml) antibodies were below the levels of long-term protective activity (1.0 μg/ml [11] and 0.34 μg/ml [12, 13], respectively) 4 and 6 months after each infection, respectively. At 1 month after administration of the second Hib and PCV7 vaccination, anti-PRP antibody was significantly elevated to 3.15 μg/ml, but two (6B and 19F) of seven serotype-specific pneumococcal antibodies were still below the protection levels. We therefore added a third PCV7. Because antibodies to pneumococcal capsular polysaccharide protect the host by opsonizing pneumococci for phagocytosis, we concomitantly performed the opsonophagocytic killing assay (OPA) [14] after the third PCV7. Table 1 shows significantly high OPA titers against types 6B and 19F were observed, findings inconsistent with the low anti-6B and anti-19F IgG antibody levels. OPA titers against five other types were also elevated to the levels for protection (>8) [12, 13].

Discussion

We report a girl with non-familial ICA with recurrent bacterial meningitis. ICA is a rare anomaly. Mahlaoui et al. [2] recently documented 20 ICA cases in France and reviewed the literature. In addition to the 65 cases in their report and references therein [2], we found reports of 5 other ICA patients [5, 15] in the literature between January 1960 and April 2011 using the Medline database. Thus, we can here review 70 ICA cases in total. Compared with these patients [2, 5, 15], our case is informative and interesting in several respects.

First are the multiple episodes of meningitis caused by three different pathogens. Of the previous 70 cases, 48 (69%) experienced invasive bacterial infection at least once. Of these 48 patients, only 8 had multiple episodes of invasive bacterial infections, two times in 5 cases and three times in 3 cases (Table 2) [2, 16–20]. Our patient is the first described for whom all three episodes were bacterial meningitis. To better understand the underlying pathogenesis, we characterized the causative pathogens by molecular analysis. Penicillin-resistant serotype 6B pneumococcus and BLNAR Hib subgroup III were among the most prevalent strains causing childhood meningitis in Japan [8, 9]. In contrast, *E. coli* is extremely rare among ICA patients, and we are aware of only one such case, which resulted in death at 4 months of age [21]. *E. coli* in our case possessed capsular antigen K1 and the siderophore receptor gene, *iroN*, which contribute to the bacteremic step in *E. coli* neonatal meningitis [7, 22]. Because the same strain was isolated from the stool of her asymptomatic mother, we confirmed the route of contagion. Besides asplenia, prematurity of the host and high pathogenic factors of the *E. coli* strain might have contributed to this infection.

Second is the good prognosis, despite our patient developing meningitis three times, one of which occurred 4 days after premature birth. Our neonatal case is the youngest at the first invasive infection among the previously reported ICA patients. There have been only 3 ICA patients

Table 1 Serum serotype-specific IgG antibody concentrations and opsonophagocytic killing assay titer before and after 7-valent pneumococcal conjugate vaccine

Serotype	4		6B		9V		14		18C		19F		23F	
	IgG conc.	OPA	IgG conc.	OPA	IgG conc.	OPA	IgG conc.	OPA	IgG conc.	OPA	IgG conc.	OPA	IgG conc.	OPA
Before PCV7 (6 months after natural infection)	0.132	NA	0.191	NA	0.062	NA	0.366	NA	4.229	NA	0.295	NA	0.14	NA
1 month after 2-dose PCV7	2.809	NA	0.263	NA	4.040	NA	6.767	NA	3.949	NA	0.356	NA	0.233	NA
1 month after 3-dose PCV7	1.37	536	0.137	557	1.199	326	5.075	2367	1.89	210	0.295	192	0.471	769

PCV7 7-valent pneumococcal conjugate vaccine, *IgG conc.* anti-serotype-specific IgG antibody concentration (μg/ml), *OPA* opsonophagocytic killing assay (titer), *NA* not assessed (under treatment with antimicrobial agents)

Table 2 Isolated congenital asplenia patients with multiple episodes of invasive bacterial infections

Patient number	Gender	Infectious episodes	Age at onset	Type of infection	Organisms	Outcome	Reference
1	F	1	6 months	Meningitis	*Streptococcus pneumoniae*	Survived	[2]
		2	11 months	Meningitis, purpura fulminans	*S. pneumoniae*	Died	
2	M	1	10 months	Meningitis	*S. pneumoniae*	Survived	[2]
		2	11 months	Purpura fulminans	*S. pneumoniae*	Survived	
		3	1 year 7 months	Purpura fulminans	*S. pneumoniae*	Survived	
3	M	1	1 year 9 months	Meningitis	*S. pneumoniae*	Survived	[16]
		2	2 years 3 months	Meningitis	*S. pneumoniae*	Survived	
4	M	1	1 year 2 months	Meningitis	*S. pneumoniae*	Survived	[17]
		2	15 years	Meningitis	Not available	Died	
5	M	1	1 year	Meningitis	*S. pneumoniae*	Survived	[18]
		2	1 year	Meningitis	*S. pneumoniae*	Survived	
		3	1 year	Osteomyelitis	Culture negative	Survived	
6	F	1	6 months	Meningitis	*S. pneumoniae*	Survived	[19]
		2	2 years 6 months	Sepsis	Not available	Died	
7	F	1	1 year 6 months	Arthritis	*S. pneumoniae*	Survived	[19]
		2	1 year 9 months	Arthritis	*Haemophilus influenzae* type b	Survived	
		3	10 years	Sepsis	*S. pneumoniae*	Died	
8	M	1	5 years	Sepsis	*S. pneumoniae*	Survived	[20]
		2	9 years	Meningitis	*S. pneumoniae*	Died	
9	F	1	0 month (4 days)	Meningitis	*Escherichia coli*	Survived	Present case
		2	1 year 6 months	Meningitis	*S. pneumoniae*	Survived	
		3	2 years 1 month	Meningitis	*H. influenzae* type b	Survived	

who had overt infections under 3 months of age, which include 1 fatal case [21] and 2 with major sequelae (central nervous system deficit [23] or loss of foot and fingers [24]). Of the 45 childhood and adult patients with invasive infections whose outcomes were known, 29 (64%) died and 3 (7%) had serious sequelae [2, 5, 23, 24]. In contrast, our patient showed normal neurological development under non-serious sequelae of unilateral hearing loss. Such favorable outcome may be attributable to the early recognition and hospitalization. Fortunately, the first episode developed during the period of hospitalization under close monitoring because of prematurity. In addition, at both second and third infectious episodes, she could receive immediate antimicrobial treatment.

Finally, we meticulously investigated the immunological responses to natural infections with *S. pneumoniae* and Hib and those to vaccinations. Of the 70 cases we can review [2, 5, 15], there has been no report addressing this issue. The spleen is a pivotal organ for the phagocytosis of encapsulated bacteria and for the production of immunoglobulins against these pathogens [3]. Even after natural invasive infections of Hib and serotype 6B pneumococcus, serum antibody levels were not elevated to the levels of long-term protection against the pathogens, which may reflect the immunocompromised status of asplenia. This concept is supported by findings from Mikoluc et al. [25] that the congenital asplenic patients had significantly lower concentrations of serum anti-pneumococcal antibodies and reduced responses to PCV7, especially to serotypes 6B and 23F. Similar findings were also observed in adult asplenic patients with overwhelming infection caused by *S. pneumoniae*, representing vaccine failures [4, 5]. Serum antibody concentrations against 6B and 19F in our patient were significantly lower than those against five other serotypes. In contrast, when we evaluated OPA titers after the third PCV7 vaccination, they were at sufficient levels for protection against all serotypes including types 6B and 19F. OPA might be a more important indicator for protection against *S. pneumoniae* [13].

In conclusion, we described a girl with a rare case of ICA, who presented with recurrent meningitis caused by three different pathogens, and was successfully treated without severe sequelae. Exact determination of serum antibody concentrations of encapsulated bacteria and reevaluation of vaccine schedules should be important to protect against relevant infections in ICA patients.

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