Published in final edited form as:

Vaccine. 2013 January 21; 31(5): . doi:10.1016/j.vaccine.2012.11.010.

Low opsonic activity to the infecting serotype in pediatric patients with invasive pneumococcal disease

Tomohiro Oishi^a, Naruhiko Ishiwada^b, Kousaku Matsubara^c, Junichiro Nishi^d, Kenji Okada^e, Takashi Nakano^f, Akihito Wada^g, Bin Chang^g, Yuka Koizumi^h, Kazuyo Tamura^h, Yukihiko Akeda^h, Moon H Nahm^k, Toshiaki Iharaⁱ, Kazunori Oishi^{h,j,*}, and The Japanese IPD study group

^aDepartment of Pediatrics, Niigata University Medical and Dental University, Tokyo, Japan

^bGraduate School of Medicine, Chiba University, Tokyo, Japan

^cNishi-Kobe Medical Center, Tokyo, Japan

^dKagoshima University Graduate School of Medicine and Dental Sciences, Tokyo, Japan

^eNational Fukuoka Hospital, Tokyo, Japan

^fKawasaki Medical University, Tokyo, Japan

^gDepartment of Bacteriology I, Osaka University, Tokyo, Japan

^hNational Institute of Infectious Diseases, Research Institute for Microbial Diseases, Osaka University, Tokyo, Japan

ⁱMie National Hospital, Tokyo, Japan

^jInfectious Disease Surveillance Center, National Institute of Infectious Diseases, Tokyo, Japan

^kUniversity of Alabama at Birmingham, AL, United States

Abstract

Serotype-specific protective immunity of pediatric patients with invasive pneumococcal disease (IPD) has not been fully investigated. To determine the protective immunity to the infecting serotype, the serotype-specific immunoglobulin G (IgG) levels and opsonophagocytic assay (OPA) titers were examined in twenty-four pediatric patients whose serum was collected within one month of IPD episode in between May 2008 and June 2011 in Japan. The median age (range) of IPD patients was 17 (10–108) months and 63% were boys. The levels of serotype-specific IgG to the infecting serotype were higher than 0.2 µg/ml in all of 17 patients tested. By contrast, the OPA titers were < 8 to the infecting serotype in all of 17 patients tested. The avidities of 19F or 6B-specific IgG level higher than 5.0 µg/ml, but undetectable OPA titers, were confirmed to be lower than those with high OPA titers. Our data demonstrated that although the levels of serotype-specific IgG to the infecting serotype were higher than 0.2 µg/ml in sera of pediatric patients with IPD, the OPA titers were low during one month after the IPD episode. Impaired opsonic activities in these patients may be, in part, explained by the low avidities of serotype-specific IgG. Impaired serum opsonic activity in children immediately after the episode of IPD suggests their susceptibility to the infecting serotype of pneumococci.

*Corresponding author at: Kazunori Oishi, MD, Infectious Disease Surveillance Center, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjyuku, Tokyo, 162-8640, Japan. Tel: 81-3-5285-1111; fax: 81-3-5285-1129 oishik@nih.go.jp (K. Oishi)..

Conflicts of interest

All authors reported no conflicts of interest. UAB retained the rights to bacterial strains used for MOPA and MHN is an employee of the university.

Keywords

invasive pneumococcal diseases; serotype-specific IgG; opsonophagocytic activity; pneumococcal vaccine; children

1. Introduction

Streptococcus pneumoniae is a leading human pathogen that causes a wide variety of diseases, ranging from otitis media to pneumonia, bacteremia, and meningitis in both children and adults [1]. Antibodies to pneumococcal capsular polysaccharide (CPS) and complement provide protection against pneumococcal strains with homologous or cross-reactive capsular serotypes [2]. Seven-valent pneumococcal conjugate vaccine (PCV7; Prevenar®, Pfizer) has been used for children in the USA since 2000 [3], and the incidence of invasive pneumococcal diseases (IPD) caused by the 7 vaccine serotypes (VTs) has declined markedly, although the incidence of non-VTs has not declined [4–6]. PCV7 was licensed in Japan in October 2009, and the Japanese government decided to subsidize PCV7 in February 2011.

Vaccine-induced protective immunity is currently estimated by measuring the concentrations of serotype-specific immunoglobulin G (IgG) by enzyme-linked immunosorbent assay (ELISA) [7] and the opsonophagocytic assay (OPA) titers is measured using the multiplex-opsonophagocytic assay (MOPA) [8]. The World Health Organization working group suggested a serotype-specific IgG of concentration of 0.35 µg/ml for as a putative measure of protection against invasive disease in infants after immunization with pneumococcal conjugate vaccine at a population level [9]. This working group also reported that antibody concentrations of 0.2–0.35 µg/ml measured with the non-22F inhibition ELISA correlated best with an OPA titer of 8, which in turn correlates best with protective efficacy. Henckaerts, al. proposed a protective threshold concentration of 0.20 µg/ml with the 22F inhibition ELISA as a measure of for the serotype-specific IPD efficacy for the pneumococcal conjugate vaccine [10]. A recent study also reported that the serological response rate following a 3-dose PCV7 primary vaccination as determined using threshold of 0.2 µg/ml IgG and an OPA titer 8 corresponded well with overall effectiveness against IPD [11]. Although this threshold may not be necessarily applied to the individual case, it is curious to determine the protective immunity to the infecting serotype in sera of pediatric patients with IPD during the acute phase.

In this study, we therefore examined the IgG levels and the OPA titer to the infecting serotype in sera of pediatric patients with IPD within one month of the infectious episode. We here report that the OPA, but not serotype-specific IgG, is the better indicator of protective immunity against the infecting serotype than is the serotype-specific IgG level in these patients.

2. Methods

2.1. Patients

A total of thirty-two pediatric patients whose cultures from sterile sites, such as blood or cerebrospinal fluid, were positive for *S. pneumoniae* between May 2008 and January 2012 at 22 hospitals in Japan, were investigated in this study. All of the pneumococcal isolates were serotyped using coagglutination tests with rabbit antisera (Statens Serum Institute, Copenhagen, Denmark) at Department of Bacteriology I, National Institute of Infectious Diseases. Serotype 6C was confirmed by a home-made factor antisera [12]. The sera were obtained from these enrolled 32 patients after the episode of IPD. To examine the protective

immunity to the infecting serotype in these patients at the time of IPD episode, we excluded six patients that had more than one month of the period from the onset of IPD to the time of serum collection, one patient that received intravenous immunoglobulin for the treatment of IPD, and one patient with underlined condition of hypogammaglobulinemia. Consequently, we evaluated 24 patients with pediatric IPD for the measurement of anti-pneumococcal IgG and the OPA titers to the infecting serotype.

Vaccine failure of the 23-valent pneumococcal polysaccharide vaccine (PPV23; Pneumovax®, MSD) was defined where a patient who had prior PPV23 vaccination had an episode of IPD where the isolate was a PPV23 serotype. PCV7 breakthrough infection was defined where a patient who received at least one dose of PCV7 had an episode of IPD for which the pneumococcal isolate was a PCV7 serotype, and PCV7 vaccine failure was defined as the subset of breakthrough infections in which the patient had completed an Advisory Committee on Immunization Practice (ACIP)-recommended PCV7 vaccine schedule at least 2 weeks before the IPD [13,14]. An instance of an IPD patient who had at least one dose of PCV7 and for whom the pneumococcal isolate was not a PCV7 serotype was defined as PCV7 non-VT infection.

This study was reviewed and approved by the Ethics Committees of Research Institute for Microbial Diseases, Osaka University, and conducted according to the principles expressed in the Declaration of Helsinki.

2.2. ELISA

Anti-pneumococcal IgG antibodies were measured with the 22F inhibition ELISA using a standard reference serum (89-SF) as previously described [7]. The levels of serotype-specific IgG for the infecting serotype such as 6B, 9V, 14, 19F, and 23F were determined.

2.3.MOPA

A multiplexed opsonophagocytic killing assay (MOPA) based on antibiotic-resistant target bacteria was performed for the infecting serotype as previously described at Research Institute for Microbial Diseases, Osaka University [8]. The OPA titers were defined as the serum dilution that killed 50% of bacteria. Only the OPA titer results for the infecting serotype such as 6B, 6C, 14, 19A, and 19F were used in this study.

2.4. Measurement of protective immunity

Both the serotype-specific IgG and the OPA were not available in one patient with serotype 15B and another with serotype 24F infection. Only the serotype-specific IgG was not available in 3 patients with serotype 19A and 2 patients with serotype 6C infection. The OPA titers were not determined in 5 patients because their sera contained antibiotics. Consequently, both the levels of serotype-specific IgG and OPA titers to the infection serotype were measured for each 17 patients.

2.5 Avidity of serotype-specific IgG

The avidity of serotype-specific IgG in sera was evaluated by using ELISA by the previously published method with a minor modification [15]. The serum samples preadsorbed to C-polysaccharide and 22F CPS were added to the coated microtiter plates, and the plates were incubated for 1 h at 37 °C. After washing of the plates, sodium thiocyanate at concentrations between 0 to 1.0 M was added to each well and the plates were incubated for 15 min at room temperature. After washing of the plates, diluted goat anti-human IgG HRP-conjugate was added to each well. After incubation for 1 h at room temperature, the substrate solution was added to the plates, followed by incubation for 20 min at room temperature. The optical density at 405 nm was measured. The avidity of

serotype-specific IgG was expressed as the percentages of remained absorbance after treatment with different concentrations of sodium thiocyanate.

3. Results

The clinical characteristics of the 24 pediatric patients with IPD are shown in Table 1. Diagnosis of these patients included meningitis (n = 11), bacteremia (n = 10), and bacteremic pneumonia (n = 2) and septic arthritis (n=1). The median age (range) was 17 (10–108) months, and 63% were boys. Four patients (17%) were associated with comorbid conditions and these included immune thrombocytopenia and splenectomy, meningoencephalocele, asplenia and single ventricle, asplenia, and hydrocephalus (V-P shunt). Among 24 cases examined, the most common serotype was 6B for 9 isolates (38%), followed by 19F for 4 isolates (17%), 3 isolates for 19A (13%), 2 isolates for serotype 6C (8%) and 14 (8%) and 9V, 15B, 23F, 24F for each 1 isolate (4%). The median (range) period from the onset of IPD to the time of serum collection was 2 (0–23) days.

Before the episode of IPD, two patients with serotype 19F and one patient with serotype 9V received PPV23. Because PPV23 contains serotypes 19F and 9V, all 3 cases were considered PPV23 vaccine failure (VF). Of 10 patients who received PCV7 previously, four cases (Cases 11, 15, 19 and 21) were classified as PCV7 VF, and two cases (Cases 8 and 20) were classified as PCV7 breakthrough infection (BTI) [13]. The other four cases (Cases 11, 15, 23 and 24) were classified as PCV7 non-VT infection.

The levels of serotype-specific IgG and the OPA titers for the infecting serotype were determined for both 17 of 24 cases, respectively. The levels of specific IgG for the infecting serotype ranged widely from 0.34 to 6.53 µg/ml. In all of 17 cases, the levels of specific IgG for the infecting serotype were higher than 0.20 µg/ml, the putative threshold for preventing IPD [10,11]. The geometric mean concentration was 1.35 µg/ml for 17 cases. By contrast, the OPA titer for the infecting serotype was <8 in all of 17 cases. In particular, obvious discrepancies between the level of serotype-specific IgG higher than 5 µg/ml and undetectable OPA titer were found in two patients with serotype 19F (Cases 1 and 2) and one patient with serotype 6B (Case 22).

To explain these discrepancies, we next examined the avidities of serotype 19F-specific IgG in sera from Cases 1 and 2, and the avidities of serotype-6B specific IgG in sera from Case 22. The remained absorbance to 19F CPS of the positive control serum (IgG 7.25 µg/ml, OPA titer 2336) collected from a patient (Case 6) after two doses of PCV7 vaccination gradually decreased linearly according to the increasing concentrations of sodium thiocyanate (Figure 1A). By contrast, the remained absorbance of sera from Cases 1 (IgG 6.53 µg/ml, OPA titer 2) and 2 (IgG 5.10 µg/ml, OPA titer 2) to 19F CPS declined sharply according to the increasing concentrations of sodium thiocyanate.

The remained absorbance to 6B CPS of the positive control serum (IgG 4.16 µg/ml, OPA titer of 4626) collected from Case 6 after two doses of PCV7 modestly decreased after treatment with the increasing concentrations of sodium thiocyanate (Figure 1B). By contrast, the remained absorbance of serum from Case 22 before PCV7 vaccination (IgG 5.62 µg/ml OPA titer 2) were much lower than those of the positive control serum. The remained absorbance of serum from Case 22 after two doses of PCV7 vaccination (IgG 2.37 µg/ml, OPA titer 562) were lower than those of the positive control serum, but slightly higher than those of serum from Case 22 before vaccination (IgG 5.62 µg/ml, OPA titer 2). Collectively, our data demonstrated that sera containing 19F or 6B-specific IgG levels higher than 5.0 µg/ml, but lacking opsonic activity, contained lower avidities of serotype-specific IgG, compared with the positive control sera with high OPA titers.

4. Discussion

In pediatric patients with IPD during one month after the episode, the serum OPA titers for the infecting serotype were <8 in all of 17 patients tested for OPA, although the levels of IgG for the infecting serotype were higher than 0.2 µg/ml in all of 17 patients tested for serotype-specific IgG. Undetectable OPA titers suggest that these patients are susceptible to the infecting serotype, and that the serotype-specific IgG in sera are largely nonfunctional in these pediatric patients with IPD episode.

Three patients received PPV23 before PCV7 was licensed in Japan in 2009 because of their increased risk for pneumococcal disease. Although the current guideline of the Advisory Committee on Immunization Practices (ACIP) recommends that children aged 2–18 years with underlying medical conditions should receive PPV23 after completing all recommended doses of PCV13 [16], pediatricians should be aware of the possible induction of nonfunctional IgG by PPV23 in high-risk children aged >2 years. Two patients with PCV7 BTI received 1 or 2 doses of PCV7 9–11 months after birth, and two patients with PCV7 VF received only 1 dose of PCV7 26–28 months after birth. All of these four patients comprised the catch-up cases for PCV7. Interestingly, all cases with BTI or VF were caused by serotype 6B. A recent study from the US reported that 155 of 753 (21%) pediatric IPD cases were PCV7 BTIs caused predominantly by serotypes 6B (32%) and 19F (29%) [13]. The PCV7 BTIs caused by serotype 6B were more likely to have occurred in children who received only 1 or 2 PCV7 doses (84%) compared with infections caused by other VT (61%).

In this study, we demonstrated a low avidity of serotype 6B or 19F-specific IgG in sera from three IPD patients showing the serotype-specific IgG levels higher than 5.0 µg/ml. These findings are in agreement with previously published reports [17, 18], and may explain, in part, the discrepancies between high serotype-specific IgG levels and undetectable OPA titers in sera collected from Cases 1, 2 and 22 within one month of the IPD episode.

O'Brien, et al. recently reported the pneumococcal antibody status in a child with of PCV7 vaccine failure caused by serotype 14 [19]. In this patient, the serotype-specific IgG and the OPA titer in serum were 4.98 µg/ml and 1024, respectively, 35 days after three doses of PCV7. However, this patient developed occult bacteremia at 9.6 months of age, 53 days after the third dose of PCV7. The authors suggested that the existing functional antibodies during infection were consumed by binding to the serotype 14 antigens because of a slightly decreased serotype-specific IgG (4.25 µg/ml) and a significantly decreased OPA titer of 4 in serum of this patient after this episode of IPD. This finding also suggests that the ELISA assay detected some non-specific or non-functional IgG in the serum of this patient, and is in agreement with the findings in the sera of our pediatric patients with IPD.

The limitations of our study are the small number of IPD cases examined and the variable timing of serum collection although the sera were collected within 23 days after the IPD episode.

In conclusion, in all of 17 patients tested within one month of IPD episode, the serum OPA titers to the infecting serotype were <8, whereas the levels of serotype-specific IgG were higher than 0.2 µg/ml. Low avidities of serotype-specific IgG were confirmed in three patients associated with the serotype-specific IgG levels higher than 5 µg/ml, but undetectable OPA titers. Impaired serum opsonic activity in children within one month of IPD episode suggests that they are susceptible to the infecting serotype of pneumococci.

Acknowledgments

The authors are grateful to Tao Yu and Yumi Hattori and for technical assistance in the measurement of the serotype-specific IgG and OPA titers, and to Yasuyo Uemura for analyzing the clinical and laboratory data. This work was supported by research grants from the Ministry of Health, Labor and Welfare of Japan and by US NIH contract N01-AI-30021 to MHN.

References

- [1]. O'Brien KL, Wolfsan LJ, Watt JP, Henkle E, Deloria-Knoll M, McCall N, et al. Burden of disease caused by *Streptococcus pneumoniae* in children younger than 5 years: global estimates. *Lancet*. 2009; 374:893–902. [PubMed: 19748398]
- [2]. Musher DM, Chapman AJ, Goree A, Jonsson S, Briles D, Baughn RE. Natural and vaccine-related immunity to *Streptococcus pneumoniae*. *J Infect Dis*. 1986; 154:245–56. [PubMed: 3722865]
- [3]. American Academy of Pediatrics; Committee on Infectious Diseases. Policy statement: recommendations for the prevention of pneumococcal infections, including the use of pneumococcal conjugate vaccine (Prevnar), pneumococcal polysaccharide vaccine, and antibiotic prophylaxis. *Pediatrics*. 2000; 106:362–6. [PubMed: 10920169]
- [4]. Whitney CG, Farley MM, Halder J, Harrison KH, Bennet NM, Lynfield R, et al. Decline in invasive pneumococcal disease following the introduction of protein polysaccharide conjugate vaccine. *N Eng J Med*. 2003; 348:1737–46.
- [5]. O'Brien KL, Moulton LH, Reid R, Weatherholts R, Oski J, Brown L, et al. Efficacy and safety of seven-valent conjugate vaccine in American Indian children: group randomized trial. *Lancet*. 2003; 362:355–61. [PubMed: 12907008]
- [6]. Pilishvili T, Lexau C, Farley MM, Hadler LH, Bennett NM, Reingold A, et al. Sustained reductions in invasive pneumococcal disease in era of conjugate vaccine. *J Infect Dis*. 2010; 201:32–41. [PubMed: 19947881]
- [7]. Concepcion NF, Frasch CE. Pneumococcal type 22F polysaccharide absorption improves the specificity of a pneumococcal-polysaccharide enzyme-linked immunosorbent assay. *Clin Diagn Lab Immunol*. 2001; 8:266–72. [PubMed: 11238206]
- [8]. Burton RL, Nahm MH. Development and validation of a fourfold multiplexed opsonization assay (MOPA4) for pneumococcal antibodies. *Clin Vaccine Immunol*. 2006; 13:1004–9. [PubMed: 16960111]
- [9]. World Health Organization. Pneumococcal conjugate vaccines. Recommendations for the production and control of pneumococcal conjugate vaccines. WHO Tech. Rep. Ser. 2005; 927(annex 2):64–98.
- [10]. Henckaerts I, Goldblatt D, Ashton L, Poolman J. Critical differences between pneumococcal polysaccharide enzyme-linked immunosorbent assays with and without 22F inhibition at low antibody concentrations in pediatric sera. *Clin Vaccine Immunol*. 2006; 13:356–60. [PubMed: 16522777]
- [11]. Schuerman L, Wysocki J, Tejedor JC, Knuf M, Kim KH, Poolman J. Prediction of pneumococcal conjugate vaccine effectiveness against invasive pneumococcal disease using opsonophagocytic activity and antibody concentrations determined by enzyme-linked immunosorbent assay with 22F adsorption. *Clin Vaccine Immunol*. 2011; 18:2161–7. [PubMed: 21994351]
- [12]. Chang B, Otsuka T, Iwaya A, Okazaki M, Matsunaga S, Wada A. Isolation of *Streptococcus pneumoniae* serotypes 6C and 6D from the nasopharyngeal mucosa of healthy Japanese children. *Jap J Infect Dis*. 2010; 63:381–3. [PubMed: 20859012]
- [13]. Park SY, Beneden CAV, Pilishvili T, Martin M, Facklam RR, Whitney CG, et al. Invasive pneumococcal infections among vaccinated children in the United States. *J Pediatr*. 2010; 156:478–83. [PubMed: 19962156]
- [14]. Centers for Disease Control and Prevention; Advisory Committee on Immunization Practices (ACIP). Updated recommendation from the Advisory Committee on Immunization Practices (ACIP) for use of 7-valent pneumococcal conjugate vaccine (PCV7) in children aged 24–59 months who are not completely vaccinated. *MMWR Morb Mortal Wkly Rep*. 2008; 57:343–4. [PubMed: 18385642]

- [15]. Anttila M, Eskola J, Ahman H, Kayhty H. Avidity of IgG for *Streptococcus pneumoniae* type 6B and 23F polysaccharides in infants primed with pneumococcal conjugates and boosted with polysaccharide or conjugate vaccines. *J. Infect Dis.* 1998; 177:1614–21. [PubMed: 9607841]
- [16]. Nuorti JP, Whitney CG, Centers for Disease Control and Prevention (CDC). Prevention of pneumococcal disease among infants and children - use of 13-valent pneumococcal conjugate vaccine and 23-valent pneumococcal polysaccharide vaccine. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep.* Dec 10; 2010 59(RR-11): 1–18. [PubMed: 21150868]
- [17]. Usinger WR, Locas AH. Avidity as a determinant of the protective efficacy of human antibodies to pneumococcal capsular polysaccharides. *Infect Immun.* 1999; 67:2366–70. [PubMed: 10225896]
- [18]. Musher DM, Phan HM, Watson DA, Baughn RE. Antibody to capsular polysaccharide of *Streptococcus pneumoniae* at the time of hospital admission for pneumococcal pneumonia. *J Infect Dis.* 2000; 182:158–67. [PubMed: 10882593]
- [19]. O'Brien KL, Moisi J, Romero-Steiner S, Holder P, Carlone GM, Reid R, et al. Pneumococcal antibodies in a child with type 14 pneumococcal conjugate vaccine failure. *Vaccine.* 2009; 27:1863–8. [PubMed: 19171175]

Fig 1.

Avidity of serotype 19F-specific IgG (A) and serotype 6B-specific IgG (B) in sera from pediatric patients with invasive pneumococcal diseases. Two serum samples from Cases 1 (closed diamond) and 2 (closed square), and the positive control serum (closed triangle) from Case 22 (4 months after the episode of IPD and one month after two doses of PCV7 vaccination) were examined for the avidity of serotype 19F-specific IgG. Two serum samples from Case 22 before (open diamond) and after two doses of PCV7 vaccination (open square), and a positive control serum (open triangle) collected from Case 6 after two doses of PCV7 were used for the avidity of serotype 6B-specific IgG.

Clinical Characteristics of 24 Pediatric Patients with Invasive Pneumococcal Disease (IPD)

Table 1

No.	Age (months)	Sex	Diagnosis	Comorbid condition	Infecting serotype	Serum obtained days after IPD	Antibody to the infecting serotype		Vaccination before IPD (doses)	Age at each dose (month)	Category of IPD after PPV23	Category of IPD after PCV7D	Outcome
							IgG (µg/ml)	OPA titer					
1	108	M	Meningitis	ITP, splenectomy	19F	10	6.53	2	PPV23(1)	62	Vaccine failure	NA	Alive
2	50	M	Meningitis	Meningoencephaloele	19F	17	5.1	2	PPV23(1)	42	Vaccine failure	NA	Alive
3	75	M	Bacteremia	Asplenia, single ventricle	9V	1	0.57	NT	PPV23(1)	24	Vaccine failure	NA	Dead
4	14	M	Bacteremia	None	6B	11	0.34	2	None	-	NA	NA	Alive
5	38	M	Meningitis	None	19F	4	1.08	2	None	-	NA	NA	Alive
6	14	M	Bacteremia	None	14	5	2.1	5	None	-	NA	NA	Alive
7	13	M	Bacteremia	None	6B	4	2.25	NT	None	-	NA	NA	Alive
8	12	M	Meningitis	None	6B	20	1.81	7	PCV7(1)	10	NA	Breakthrough infection	Alive
9	10	M	Meningitis	None	19F	0	0.85	NT	None	-	NA	NA	Alive
10	17	M	Bacteremic pneumonia	None	19A	2	NA	NT	None	-	NA	NA	Alive
11	30	M	Bacteremic pneumonia	None	6B	0	0.53	2	PCV7(1)	28	NA	Vaccine failure	Alive
12	17	F	Meningitis	None	24F	1	NA	NA	PCV7(1)	16	NA	Non-VT infection	Alive
13	12	F	Meningitis	None	6B	12	0.78	2	None	-	NA	NA	Alive
14	10	M	Meningitis	None	15B	2	NA	NA	None	-	NA	NA	Alive
15	30	F	Bacteremia	None	6B	0	1.18	2	PCV7(1)	26	NA	Vaccine failure	Alive
16	26	F	Bacteremia	None	19A	1	NA	2	None	-	NA	NA	Alive
17	15	F	Bacteremia	None	14	0	1.75	2	None	-	NA	NA	Alive
18	10	M	Bacteremia	None	19A	0	NA	2	PCV7(3)	4,5,6	NA	Non-VT infection	Alive
19	30	F	Meningitis	Hydrocephalus (V-P shunt)	6B	23	0.92	2	PCV7(1)	28	NA	Vaccine failure	Alive
20	17	F	Meningitis	None	6B	0	1.38	2	PCV7(2)	9,11	NA	Breakthrough infection	Alive
21	11	F	Septic arthritis	None	23F	0	0.55	2	PCV7(3)	7,8,9	NA	Vaccine failure	Alive
22	16	F	Bacteremia	None	6B	0	5.62	2	None	-	NA	NA	Alive
23	49	M	Meningitis	None	6C	1	NA	2	PCV7(1)	36	NA	Non-VT infection	Alive
24	14	M	Bacteremia	None	6C	7	NA	NT	PCV7(2)	9,10	NA	Non-VT infection	Alive

OPA, opsonophagocytic assay; ITP, immune thrombocytopenia; PPV23, 23-valent pneumococcal polysaccharide vaccine; PCV7, 7-valent pneumococcal conjugate vaccine; NA, not applicable; NT, not tested because of antibiotic use; VT, vaccine type.