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Case Report

Fatal overwhelming postsplenectomy infection due to *Streptococcus pneumoniae* serotype 10A with atypical polysaccharide capsule in a patient with chromosome 22q11.2 deletion syndrome: A case report

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ABSTRACT

We report the first case of a teenage patient with chromosome 22q11.2 deletion syndrome who died of overwhelming postsplenectomy infection (OPSI) by *Streptococcus pneumoniae* despite appropriate prevention by pneumococcal vaccine. He had congenital heart disease and underwent several surgeries. Immunodeficiency had not been noticed clinically. Two years prior to death, splenectomy was performed for a drug-resistant idiopathic thrombocytopenic purpura and he was immunized with 23-valent pneumococcal polysaccharide vaccine (PPV23) 4 months after splenectomy. He died suddenly after a mild flu-like symptom. Autopsy was performed and OPSI was diagnosed. Blood culture was positive for *S. pneumoniae*. This isolated *S. pneumoniae* strain was serotypically un-typable by polyvalent serum agglutination test. On the contrary, multilocus sequence typing followed by DNA sequencing indicated the molecular serotype as 10A. Additional testing using monovalent and factor-specific sera confirmed the strain as serotype 10A. Ultrastructural observation of this *S. pneumoniae* strain showed that the polysaccharide capsule was thin and sparse. We speculate that the abnormal morphology of the capsule may have accounted for the polyvalent serum agglutination failure and may possibly be associated with severity of OPSI observed in this case. Chromosome 22q11.2 deletion syndrome is associated with certain immunodeficiency, especially susceptible to *S. pneumoniae* infections; however, fatal OPSI has not been reported. In addition to vaccination, prophylactic antibiotics may be necessary for these patients who are at risk of immunodeficiency.

Abbreviation: PPV23, 23-valent pneumococcal polysaccharide vaccine; IPD, Invasive pneumococcal disease; OPSI, Overwhelming postsplenectomy infection; MLST, Multilocus sequence typing; ST, sequence type; PCV13, 13-valent pneumococcal conjugate vaccine; IDSA, Infectious Diseases Society of America.

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1. Introduction

Streptococcus pneumoniae is a gram-positive bacterium responsible for many types of infections other than pneumonia including otitis media, meningitis, and sepsis [1]. *S. pneumoniae* has a polysaccharide capsule, which acts as the major virulence factor. There are over 90 different capsular serotypes [1], and the capsule can influence interactions with host cells, including invasion, adhesion, and serum sensitivity [2].

Purified preparations of pneumococcal capsular polysaccharide or polysaccharide conjugated to a nontoxic variant of diphtheria toxin are used to prepare pneumococcal vaccines [3]. The 23-valent pneumococcal polysaccharide vaccine (PPV23) contains capsular polysaccharides from 23 pneumococcal capsular types of *S. pneumoniae* [3]. PPV23 can theoretically prevent 73% of invasive pneumococcal disease (IPD) cases based on the 2007 American serotype distributions [1].

Immunocompromised children with functional or anatomic asplenia are at a very high risk of developing IPD [4]. Overwhelming postsplenectomy infection (OPSI) is a fulminant infection commonly caused by *S. pneumoniae*. Therefore, after splenectomy, patients are recommended to have pneumococcal vaccination [4].

Although significant protection from pneumococcal infections has been achieved with pneumococcal vaccines, capsule-independent protection has been limited by either serotype replacement or switching along with diseases caused by nonencapsulated *S. pneumoniae* [1,3,5]. Here, we describe the first fatal case of OPSI caused by *S. pneumoniae* in a 13-year-old boy with chromosome 22q11.2 deletion syndrome despite PPV23 vaccination. Polyvalent serum agglutination analysis could not identify the serotype, but its molecular serotype was deduced to be as 10A. Based on this result, we performed an agglutination analysis using a monovalent serum for serotype 10. Further testing with factor-specific sera confirmed the *S. pneumoniae* strain as serotype 10A. Ultrastructural observation revealed that the bacterium has a thin and a sparse capsular structure.

2. Case report

The patient was a 13-year-old boy with chromosome 22q11.2 deletion syndrome. Tetralogy of Fallot was diagnosed soon after birth and surgically corrected at age 2 years. During the surgery, the thymus was not evident. Recurrent tonsillitis occurred until tonsillectomy at 3 years of age. The frequency of infections subsided afterward. At the age of 10 years, he developed idiopathic thrombocytopenic purpura, which was resistant to immunosuppressive therapy, and he eventually underwent splenectomy 5 months after diagnosis. After splenectomy, he took prophylactic antibiotics (benzylpenicillin benzathine hydrate) for 4 months. The immunological status was evaluated by measuring serum IgG, IgA, IgM levels and T cell counts, which were all confirmed to be within normal range. He then received PPV23 at the age of 11 years, and the prophylactic antibiotics were discontinued. At the age of 12 years, he required tissue valve replacement owing to stenosis of the corrected pulmonary valve. His last outpatient visit was 10 days prior to death, where improved body function was reported. On the day of death, he complained of malaise and appetite loss in the morning and vomited several times in the evening; he was without fever. The same night, he was found unconscious and died soon after arriving at the emergency department.

Autopsy was performed 9 hours after death. A non-occlusive partially organizing thrombus with dense white blood cells was detected in the large pulmonary vessel near the hilum of the lung (Fig. 1). Multiple small foci of gram-positive cocci were detected within the thrombus (Fig. 2), as well as in the alveoli and small vessels of other organs. No inflammatory cells were observed along with these bacteria. A blood sample was collected by cardiocentesis during the autopsy for culture, and was positive for *S. pneumoniae*. Severe hemorrhage was not observed in the skin or adrenal glands. There was neither vegetation nor enclosed bacteria in the cardiac valves, including the replaced tissue valve at the main pulmonary artery. Enlargement of multiple abdominal lymph nodes was found. Microscopic examination revealed that these lymph nodes showed follicular hyperplasia without bacteria. The postmortem diagnosis was sepsis due to *S. pneumoniae*.

Initial antiserum serotyping of the *S. pneumoniae* strain was performed by agglutination test with 8 sets of polyvalent sera (Denka Seiken, Japan). The test was repeated 6 times using multiple different lots. However, no agglutination was observed for the *S. pneumoniae* strain with all of the polyvalent sera. In addition, we could barely visualize the capsule by the India ink staining method. Therefore the result of initial serotyping indicated that the strain was un-typable.

Next, the genomic DNA of the *S. pneumoniae* strain was extracted and used as a template for PCR. Multilocus sequence typing (MLST) was performed as previously described [6]. The internal fragments of 7 housekeeping genes (*aroE*, *gdh*, *gki*, *recP*, *spi*, *xpt*, *ddl*) were amplified from the chromosomal DNA by PCR using previously reported primer sets [6]. The allelic profiles were as follows: *aroE* 15, *gdh* 13, *gki* 4, *recP* 16, *spi* 6, *xpt* 1, and *ddl* 17. From the MLST database [7], the sequence type (ST) was determined as ST 1263 with corresponding allelic profiles. Thirty-one isolates have been deposited in the MLST database as ST1263, including three serotypes of 10A (29 isolates), 10C (1 isolate), or 1 (1 isolate) [7]. PCR was performed using the primers sets for *wzg* (primers cpsA-f and cpsA-r), *wzx* (primers 10F/(10C/33C)-f, 10F/(10C/33C)-r), *wzy* specific for serotype 10 (primers 10-YS-seq and 10-YA-seq), and *wcrG* specific for serotypes 10A and 10B (primers 10A-f and 10A-r, and prepared primer sets specific for the 5' region of *wcrG*: 10A10B-wcrG-N-F1; GCTGACTCTGGTTTAACAG 10A10B-wcrG-N-R-1; TTCCCCAATAAATAGGAAC), to determine the serotype at the molecular level [8,9]. The *wzg*, *wzy*, and *wcrG* were amplified whereas *wzx* was not amplified. The amplified *wzg* indicated the common capsular gene of *S. pneumoniae*. The amplified PCR fragments of *wzy* and *wcrG* were purified and the sequences were determined using an ABI 3130 Genetic Analyzer (Life Technologies). The DNA sequence of the *wzy* confirmed the molecular serotype as 10. Non-amplification of the *wzx* indicated exclusion of serotypes 10F and 10C among serotype 10. The DNA sequence of *wzy* was compatible with serotype 10A or 10B, and the sequenced region of *wcrG* was

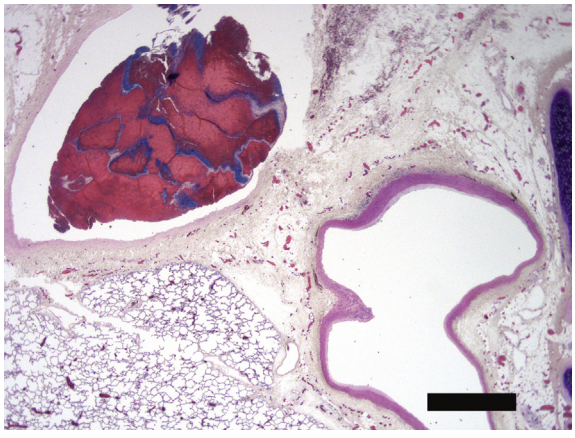

Fig. 1. Non-occlusive partially organizing thrombus with dense white blood cells seen in the large pulmonary vessel near the hilum (Giemsa stain). Bars: 2 mm.

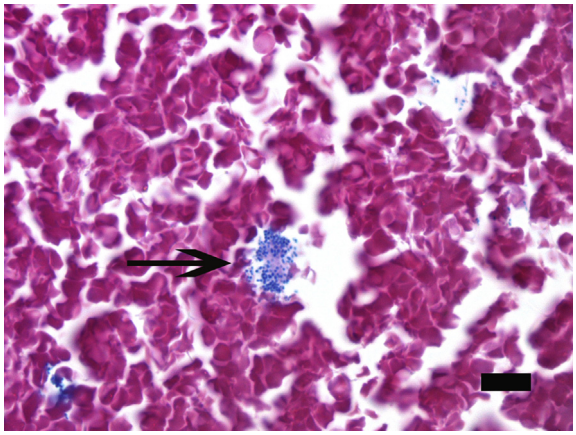

Fig. 2. Small focus of gram-positive cocci (arrow) within the thrombus (Gram stain). Bars: 10 μm.

compatible with 10A (identity 541/541 bp: 100%) and 10B (identity 505/541 bp: 93%). Together with the MLST data and DNA sequences of the capsular genes, we concluded that the molecular serotype of the *S. pneumoniae* strain was 10A.

We then performed an antiserum serotyping using monovalent serum for serotype10, and agglutination was observed for the *S. pneumoniae* strain. Additional testing with factor-specific sera for group 10 revealed that factor 10b was negative whereas factor 10c was positive, confirming the *S. pneumoniae* strain as serotype 10A (Denka Seiken, Japan) [10].

To further clarify the characteristic of the capsule of this particular *S. pneumoniae* strain, we performed electron microscopic examination. Bacteria were collected by centrifugation at 5000 rpm and fixed by the lysine-acetate-based formaldehyde-glutaraldehyde ruthenium red-osmium fixation procedure as previously described [11]. Samples were dehydrated with a graded series of ethanol and then infiltrated with epoxy resin and embedded for 48 h at 65 °C. Ultrathin sections were cut and stained with saturated uranyl acetate and lead citrate solution. Electron micrographs were taken with a JEOL JEM-1011 transmission electron microscope (80 kV). The bacterial capsule of the *S. pneumoniae* strain was thin and sparse (Fig. 3A) compared with the wild-type capsule of serotype 10A, identified in the sputum of a patient with pneumonia (Fig. 3B).

3. Discussion

The highest incidence of IPD occurs in adults ≥65 years of age, in children <2 years of age, and in individuals with certain underlying conditions, such as HIV infection or asplenia [12]. Interestingly, the fulminant-type pneumococcal infection often lacks severe infiltration of inflammatory cells on pathological inspection [13] as seen in our autopsy findings. Performing a blood culture is the very first step in reaching the cause of death. The mortality rate of OPSI can be as high as 50% [13]. The spleen plays a primary role in the clearance of bacteria from the bloodstream and produces serum IgM antibodies against polysaccharide antigens [14]. From epidemiological observations, patients need protection from IPD after splenectomy [14]. In our case, the patient was given PPV23 following prophylactic antibiotics, which we thought was an appropriate preventive measures.

When we first learned that he died of sepsis by *S. pneumoniae* with un-typable strain, our first speculation was that he was infected with the serotype uncovered by PPV23. By thorough examination of the bacteria using molecular techniques, we deduced the *S. pneumoniae* strain as molecular serotype 10A. By adding agglutination test with monovalent and factor-specific sera, we confirmed *the S. pneumoniae* strain as serotype 10A. It is of note that in our case, although the polyvalent serum agglutination testing was not revealing, the monovalent and factor-specific sera agglutination testing could prove the serotype.

IPD caused by serotype 10A is still prevalent in Japan. According to the IPD Surveillance Study Group in Japan, serotype 10A was isolated from samples of 4.8% of adult patients with IPD from 2012 to 2013 [15]. This serotyping result led to two concerns. First, usually, serotype 10A can be detected by regular agglutination serotyping with polyvalent sera; however, here, repeated testing showed that it was un-typable. Second, although PPV23 covers serotype 10A, the vaccine could not protect the patient from fatal OPSI.

We found that the *S. pneumoniae* strain had an ultrastructurally atypical capsular structure. This morphological change of the capsule may have resulted in the failure of proper antigen presentation in initial polyvalent serum agglutination testing and in host immunity.

It is well known that the type of polysaccharide capsule is related to the invasiveness of the strain. However, Hammerschmidt et al. reported that invasive *S. pneumoniae* that entered cultured epithelial cells lost their capsular polysaccharide material, as determined by electron microscopic observation [11]. To achieve an intimate attachment and internalization into epithelial cells at a certain time, the polysaccharide capsule might be unnecessary for invasion by *S. pneumoniae*. IPD caused by nonencapsulated *S. pneumoniae* has also been reported [5]. This is consistent with the electron micrographs of the particular *S. pneumoniae* strain of our case, which showed a thin and sparse capsule but strong virulence clinically.

To prevent fatal IPD, a novel pneumococcal vaccine that covers un-typable or nonencapsulated *S. pneumoniae* is needed for pneumococcal infection in high-risk groups [3].

There is a possibility that the PPV23 vaccination could not provide enough protection for the development of IPD in this case. According to the guideline by the Infectious Disease Society of America (IDSA) 2013, the current recommendation for the prevention of OPSI is that 13-valent pneumococcal conjugate vaccine (PCV13) should be administered to asplenic patients followed by PPV23 8 weeks later. These vaccines should be administered ≥2 weeks prior to splenectomy. The second dose of PPV23 should be administered 5 years later [16]. Prophylactic antimicrobial therapy, typically oral penicillin given twice daily is recommended for children younger than 5 years of age and may be considered for older children and adults during the initial 1–2 years after splenectomy. Immediate administration of an antimicrobial agent in the event of fever is also indicated [14]. In our case, although serotype 10A is not covered by PCV13, following the IDSA guideline, administering two vaccines prior to splenectomy, might have benefited the patient. For the administration of antibiotics, there was an interval of more than 2 years between splenectomy and his death. Moreover, the fever was not noted in his course of IPD. Hence, strictly speaking, he was not a subject for administration of antibiotics. However as discussed below, as a high-risk patient, he might have needed antibiotics even without fever.

We also have to take into consideration the patient's genetic abnormality. Chromosome 22q11.2 deletion syndrome is characterized by heterogeneous clinical manifestations such as congenital heart disease, immunodeficiency, hypoparathyroidism, and facial abnormalities. Immunodeficiency is mostly due to thymic hypoplasia and impaired T-lymphocytic production. The degree of immunodeficiency is highly variable but complete T-cell depletion

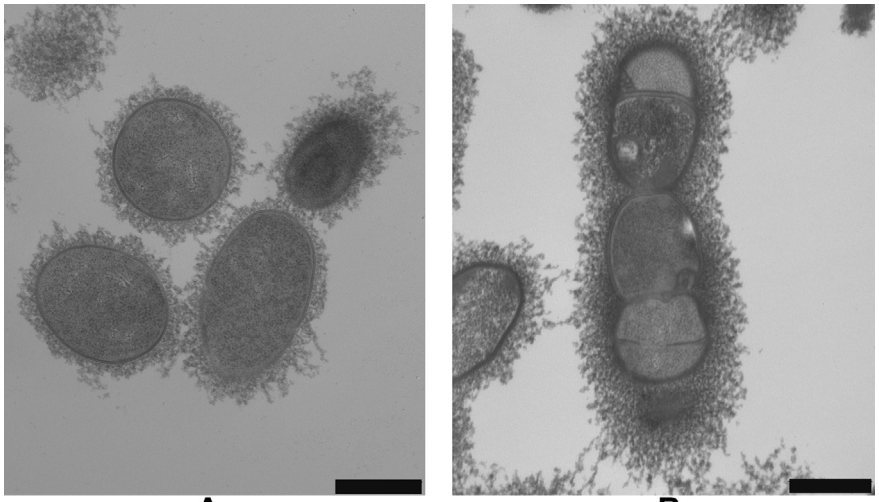

Fig. 3. Electron microscopic analysis of bacteria from the patient (3A). The capsule of the *S. pneumoniae* strain is thin and sparse compared to the wild-type capsule of serotype 10A from another patient (3B). Staining was performed with saturated uranyl acetate and lead citrate solution. Bars: 500 nm.

is rare and, in most cases, immune status improves with age [17]. In our case, although the thymus was not evident during his cardiac surgery, he showed no recurrent or severe infections after the age of 3 years, suggesting that persistent immunodeficiency was unlikely. Gennery et al. reported an absent or poor antibody response to pneumococcal polysaccharide vaccine in patients with chromosome 22q11.2 deletion syndrome, which predisposed these patients to recurrent sinopulmonary infection [18]. Because a specific antibody against pneumococcal polysaccharide antigen after vaccination was not evaluated in our case, we cannot exclude the possibility of impaired antibody production against *S. pneumoniae*. Idiopathic thrombocytopenic purpura is seen at a higher frequency (4%) among patients with chromosome 22q11.2 deletion syndrome than in the general population (0.02%) [19]. Individuals with chromosome 22q11.2 deletion syndrome who have undergone splenectomy may need additional protective remedies.

In conclusion, this is the first report of a patient with chromosome 22q11.2 deletion syndrome who died of OPSI caused by *S. pneumoniae* serotype 10A after PPV23 vaccination. Performing autopsy with a blood culture sampling was crucial for reaching the cause of death. Molecular serotyping was valuable when initial serotyping did not yield an informative result. Using the monovalent and factor-specific sera for agglutination test made it possible to confirm the serotype even after polyvalent sera could not detect the serotype. Ultrastructural observation helped us understand the inconsistent antigen presentation and invasiveness. Currently, patients who undergo splenectomy should better follow the IDSA guideline. In addition, asplenic patients with a possible immunodeficiency background may benefit from not only vaccination but also continuous or early administration of antibacterial agents after the slightest sign of infection.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Informed consent

Written informed consent was obtained from the patient's parents for the publication of this case with accompanying images.

ICMJE statement

All authors meet the ICMJE authorship criteria.

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