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Case Report

Two pediatric cases of *Pneumocystis jirovecii* pneumonia diagnosed by polymerase chain reaction of gastric lavage☆

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ABSTRACT

Detecting *Pneumocystis jirovecii* by bronchoalveolar lavage or lung biopsy is the gold standard for diagnosis of *P. jirovecii* pneumonia (PJP); however, these techniques are not always applicable in children because of their high invasiveness. We report two pediatric cases of PJP diagnosed by polymerase chain reaction (PCR) of gastric lavage that were successfully treated. To date, there are no reported cases of using PCR of gastric lavage to diagnose PJP. On the day of PJP onset, both the infants required respiratory support and infiltrative shadows were observed in both lung fields on chest radiography. Furthermore, their (1 → 3)-β-D glucan levels were elevated. *P. jirovecii* was detected by PCR of gastric lavage and trimethoprim-sulfamethoxazole was administered for 3 weeks, following which their condition improved. They were long-term steroid users, but without any prophylaxis. PCR of gastric lavage in cases of suspected PJP may help in confirming the diagnosis in children who have mild to moderate airway symptoms, or have difficulty with invasive examination like bronchoscopy.

1. Introduction

Pneumocystis jirovecii causes respiratory infection in cellular immunodeficient patients. It is a severe infection with a mortality rate of 100% without treatment [1]. Pathogenic diagnosis is important before administering appropriate antibiotics. However, it is difficult to detect *P. jirovecii* in non-HIV immunocompromised children [2]. Therefore, clinical diagnosis and treatment are given based on their symptoms, image examination, and blood examination. Diagnosis of *P. jirovecii* pneumonia (PJP) based on visualization of *P. jirovecii* organisms using microscopic examination of stained gastric lavage specimen has been reported [3]. On the other hand, evidence from various studies suggests that Polymerase Chain Reaction (PCR) is more sensitive in detecting *P. jirovecii* than staining method [4,5]. However, to the best of our knowledge, no previous studies have reported the diagnosis of PJP by PCR of gastric lavage which is an easier and less invasive method to collect a sample without anesthesia. We report two cases with long-term NICU hospitalization exhibiting respiratory symptoms, diagnosed with PJP by PCR of gastric lavage, and administered appropriate treatment. They were long-term steroid users, but without any prophylaxis.

2. Case report

Case 1 was a one-year-old, preterm, low birth weight male infant with Down syndrome. He was born at 34 weeks of gestation and weighed 1988 g at birth. He underwent a surgery for congenital duodenal atresia when he was one day old. At one month of age, he had an intestinal tract resection due to necrotizing enteritis and had an artificial anus, and an intestinal fistula created. He underwent pulmonary artery banding at 3 months of age for Fallot tetralogy. Pericardial fluid appeared at 6 months of age and he was diagnosed with pericardiotomy syndrome for which he was administered prednisolone (PSL) intravenously from the age of 7 months. He needed positive pressure ventilation by continuous positive airway pressure (CPAP) due to tracheomalacia but 9 months onward it was changed to high flow nasal cannula (HFNC), and he had long-term hospitalization at NICU. According to his medical records, his chief

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complaint was desaturation. His SpO_2 fell gradually to 90–94%. On the thirteenth day of the infection, respiratory support was strengthened for CPAP. On the 14th day of the admission, a chest radiograph showed significant infiltrative shadows spreading in both the lungs (Fig. 1). Blood tests showed that the C-reactive protein (CRP) was 0.30 mg/dL indicating that the inflammatory response was low. Based on his clinical symptoms and X-ray, he was diagnosed with pneumonia and was put on treatment with Ampicillin/sulbactam (ABPC/SBT) (150 mg/kg/day). However, his respiratory condition did not improve even after treatment with ABPC/SBT. We suspected pulmonary fungal infection and PJP from the background that (1 → 3)-β-D glucan was as high as 53.65 pg/mL, and also due to long-term use of steroid. The patient was then administered liposomal amphotericin B (3 mg/kg/day) and Trimethoprim-sulphamethoxazole (15 mg/kg/day) (in terms of Trimethoprim). On the 17th day of the infection, investigations were done for serum Aspergillus antigens, Candida mannan antigen, Cryptococcus antigen, anti-acid bacterial stain, acid-fast bacterial culture, May Giemsa staining, and PCR of gastric lavage for *P. jirovecii*. Although no trophoblast of *P. jirovecii* was detected from May Giemsa staining, results of *P. jirovecii* PCR of gastric lavage were positive on the 25th day of infection. His respiratory condition showed improvement. On the 23rd day of the infection, respiratory support was returned to original HFNC. Liposomal amphotericin B course was completed because fungal antigen tests were negative and no fungus was detected in blood culture. Based on the patient's background of chronic steroid use, the result of gastric lavage PCR, and good clinical response to Trimethoprim-sulphamethoxazole administration, PJP was diagnosed as the cause of his respiratory distress. We administered Trimethoprim-sulphamethoxazole to him for 3 weeks and confirmed recovery of clinical symptoms, improvement of chest X-ray findings, and negative results of *P. jirovecii* PCR of gastric lavage. Since then, he was administered PJP preventive treatment of trimethoprim-sulphamethoxazole (5 mg/kg/day) three times a week. It was observed that PJP did not reoccur.

Case 2 was an 8-month-old, extremely low birth weight (ELBW) male infant with a birth weight of 798 g at 28 weeks of gestation. He had a gastrointestinal perforation at day 2 of birth for which he had an artificial anus created, and continued hospitalization at NICU with central venous and enteral nutrition. From the age of 5 months, he was taking hydrocortisone (1.4 mg/kg) for persistent hypoglycemia. According to his medical records, his chief complaint was desaturation. He had a confirmed drop in SpO_2 from the first day of infection. On the third day of the infection, we started respiratory support with HFNC because of respiratory distress and tachycardia. He showed a decline in SpO_2, tachypnea, and his chest radiographs showed infiltrative shadow spreading in both lungs (Fig. 2). Based on his history of long-term steroid use and X-ray findings, assuming lung fungal infection and PJP, investigations were done for serum fungal antigen, anti-acid bacterial stain, acid-fast bacterial culture, May Giemsa stain, and PCR of gastric lavage for *P. jirovecii*. Liposomal amphotericin B (3.5 mg/kg/day) and Trimethoprim-sulphamethoxazole (15 mg/kg/day) were then administered to him. On the 10th day of infection, PCR of gastric lavage was positive for *P. jirovecii.* He was diagnosed with PJP because he showed improvement in the respiratory condition with treatment, and we were able to gradually decrease the respiratory support to only oxygen administration on the same day. Oxygen administration was also terminated on day 12 of the infection. Trimethoprim-sulphamethoxazole was administered for 3 weeks, after which improvement of chest X-ray findings and negative results of PCR of gastric lavage for *P. jirovecii* were confirmed. He was administered oral Trimethoprim-sulphamethoxazole (5 mg/kg/day) three times a week as PJP prophylaxis, after which PJP did not reoccur. Summary of the two cases is shown in Table 1.

3. Discussion

From the two cases presented it was observed that both had some commonalities: NICU long-term hospitalization, cellular immunodeficiency from long-term use of steroids, respiratory distress (that was not very severe and could be managed without intubation), and prominent weight gain failure which occurred as a result of the underlying infection. Based on our experience with case 1, we could intervene at an early stage for case 2 examination and treatment assuming PJP. In the end, both the cases were appropriately treated which prevented further severity.

Regarding the risk of PJP in long-term steroid users, there are some indications from previous reports. In children, PSL dosage of 2 mg/kg/day for more than 14 days is a risk factor for the development of PJP because it disrupts the cellular immunity [6]. PSL

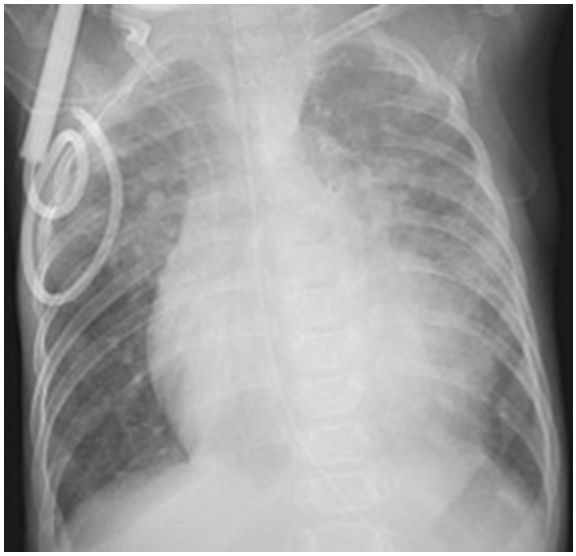

Fig. 1. Chest X ray of case 1. The chest X-ray shows significant infiltrative shadows spreading in both the lungs.

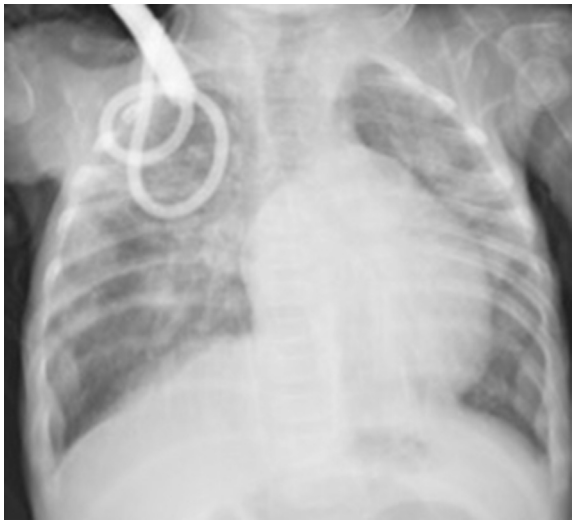

Fig. 2. Chest X ray of case 2. The chest X-ray shows significant infiltrative shadows spreading in both the lungs.

Table 1
Summary of the two cases.

	Case 1	Case 2
Background	12-month-old NICU inpatient	8-month-old NICU inpatient
Body weight at onset of PJP	2048 g	1380 g
Quantity and duration of prescribed steroid for PSL conversion	PSL 1.2 mg/kg/day, 5 months	PSL 0.35 mg/kg/day, 3 months
Chief complaint at onset of PJP	O_2 demand	O_2 demand
Respiratory support during PJP	CPAP	HFNC
Reason for suspecting PJP	X-ray, increase in BDG (53.65 pg/mL)	X-ray, increase in BDG (305.6 pg/mL)
Detection method of *P. jirovecii*	PCR of gastric lavage	PCR of gastric lavage

PJP, *Pneumocystis jirovecii* pneumonia; NICU, neonatal intensive care unit; PSL, Prednisolone; O_2, Oxygen; CPAP, continuous positive airway pressure; HFNC, high flow nasal cannula; BDG, (1 → 3)-β-D glucan; PCR, polymerase chain reaction.

dosage of 30 mg/day for 12 weeks or more, 0.4 mg/kg/day for several months or more, and 20 mg/day for 3 weeks or more is reported as a risk factor for PJP [6,7]. Since both the cases were administered PSL dosage of 1.2 mg/kg/day (Case 1) and 0.35 mg/kg/day (Case 2) for several months, prophylaxis for PJP might be necessary. It was also suspected that a marked failure to gain weight may have been a result of cellular immunity dysfunction due to malnutrition, which in turn amplified the risk of PJP.

Detection of *Mycobacterium tuberculosis* by gastric lavage is commonly used in childhood tuberculosis but for other respiratory pathogens, examination of gastric lavage is uncommon. Detecting *P. jirovecii* from bronchoalveolar lavage (BAL) or lung biopsy is the gold standard to diagnose PJP. Its sensitivity is 75–100% [1], but it is not always possible in children due to the high invasiveness involved to obtain a specimen. *P. jirovecii* has been diagnosed by Grocott staining of gastric lavage [3] because it has antacidity [8], but has low sensitivity (23%) [3], then, staining may be required several times. On the other hand, PCR test of *P. jirovecii* is currently commercially available and testing of airway specimen is common. It is a useful method for detecting *P. jirovecii* since the sensitivity of PCR is higher than staining methods [5].

Further, the number of children who carry *P. jirovecii* in the respiratory tract is high with the carrier rate of 10–30% in immunocompetent children. In immunosuppressed patients, depending on the underlying diseases it is in the range of 60–70% [9]. Therefore, when *P. jirovecii* is detected, it is important to distinguish infection from colonization.

We did not test for cellular immunity in the two cases. In HIV/AIDS positive children with PJP, the number of CD4 positive T cells are 1500/μL or more which usually decrease before progression of PJP. Therefore, it is difficult to evaluate cellular immunity by the number of CD4 positive T cells [10]. In non-HIV/AIDS patients, it is important to evaluate the risk of cellular immunodeficiency as a result of steroids and immunosuppressants dosage, and administration period [10].

In conclusion, when PJP is suspected in pediatric patients but symptoms are minor, intubation is not needed, and collection of lower respiratory tract specimens is difficult, PCR of gastric lavage to detect *P. jirovecii* can be a useful method. The two cases diagnosed by this method were steroid users admitted to NICU. PJP was diagnosed based on the patient's background, chest X-ray findings, reaction to Trimethoprim-sulphamethoxazole therapy, and PCR of gastric lavage. Therefore, when *P. jirovecii* is detected, it is necessary to comprehensively judge the patient's background and progress, examination results, and treatment course together to distinguish infection from colonization. These cases also might have a background of cellular immunodeficiency accompanying long-term use of steroids, suggesting the need for PJP prophylaxis.

Ethical approval

This work was approved by the Ethical Committee of Chiba University (approval number: 3262).

Conflicts of interest

None.

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