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【 CASE REPORT 】

The Diagnosis of *Legionella pneumophila* Serogroup 5 Bacteremic Pneumonia during Severe Neutropenia Using Loop-mediated Isothermal Amplification

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Abstract:
A 60-year-old man developed pneumonia after undergoing autologous peripheral blood stem cell transplantation for diffuse large-B cell lymphoma. A urinary antigen test and sputum culture were both negative for *Legionella pneumophila*; however, a sputum sample that was examined by loop-mediated isothermal amplification (LAMP) was positive for *Legionella* spp. On admission, the results of blood culturing using a BACTEC system were negative for 7 days. However, *L. pneumophila* serogroup 5 was detected in a blood subculture using WYOα medium. The patient was successfully treated with a fluoroquinolone-based regimen. LAMP is useful for the diagnosis of *Legionella* spp.

Key words: *Legionella* pneumonia, *Legionella pneumophila* serogroup 5, loop-mediated isothermal amplification, hematological malignancy, malignant lymphoma

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Introduction

Legionella pneumophila, which can cause severe and fatal disease, was first reported at the American Legion Convention in Philadelphia in 1976 (1, 2). Because of the difficulties in diagnosing *Legionella* pneumonia, delays in the initiation of appropriate therapy, which have been associated with increased mortality, can occur (3). Recently, polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP) methods have been gaining attention as diagnostic tools that allow for the early detection of the DNA of *Legionella* spp. (4, 5). LAMP is simple, easy to perform, cost-effective and amplifies DNA with high specificity and efficiency under isothermal conditions (60-65℃). Furthermore, the efficacy of LAMP is not affected by the co-presence of non-target DNA (6). In the present study, we reported a case of bacteremic pneumonia that was caused by *L. pneumophila* serogroup 5 and which was detected using LAMP before the blood culture results were obtained.

Case Report

A 60-year-old man underwent high-dose chemotherapy followed by autologous peripheral blood stem cell transplantation (auto-PBSCT) for relapsed diffuse large B-cell lymphoma following rituximab-combined chemotherapy. He had no history of diabetes mellitus, chronic kidney disease, or chronic liver disease. In addition, he had never smoked. He was discharged from the hospital 48 days after auto-PBSCT, after recovering from myelosuppression after auto-PBSCT. However, he was readmitted 60 days after discharge due to fever, impaired consciousness, and low blood pressure. On admission, his temperature was 40.1℃, his blood pressure was 87/46 mmHg, his pulse was 144 beats per minute, his respiratory rate was 38 breaths per minute, his consciousness was impaired [Glasgow Coma Scale 12 (E3V3M6)], and he required 2 L/min of oxygen maintain an oxygen saturation of ≥90%. Coarse crackles were heard in the left upper lung field. The laboratory data revealed neutropenia (131/μL), elevated of liver enzymes [aspartate aminotrans-

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Table. **Laboratory Findings on Admission (Day1).**

Hematology		Biochemistry		Coagulation	
WBC	410 /μL	TP	5.7 g/dL	APTT	36.1 second
Neu	37 %	Alb	3.2 g/dL	PT	59.7 %
Eos	0 %	BUN	39 mg/dL	FDP	61.5 μg/mL
Bas	0 %	Cre	2.31 mg/dL		
Mono	4 %	AST	256 IU/L		
Lym	58 %	ALT	81 IU/L	Arterial blood gas analysis	
Aty Lym	1 %	LDH	784 IU/L	O_2: 2L/minute nasal cannula	
RBC	1.86×10^6 /μL	ALP	216 IU/L	pH	7.51
Hb	6.2 g/dL	γGT	172 IU/L	pCO_2	19 Torr
Plt	6.000 /μL	Na	134 mmol/L	pO_2	95 Torr
		K	5.0 mmol/L	HCO_3^-	16 mmol/L
		Cl	97 mmol/L		
		Ca	8.4 mg/dL		
		CK	5,985 IU/L		
		CRP	34.2 mg/dL		

WBC: white blood cell, RBC: red blood cell, Plt: platelet, TP: total protein, Alb: albumin, BUN: blood urea nitrogen, Cre: creatinine, CK: creatine phosphokinase

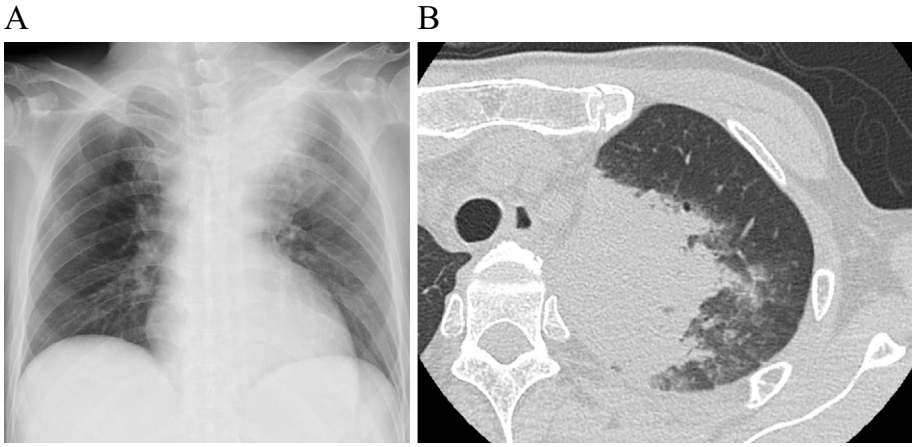

Figure. **Chest radiography revealed pneumonia in the left upper lung field (A), and chest CT revealed consolidation and centrilobular nodules in the left upper lobe of the lung (B).**

ferase (AST) 256 IU/L, alanine aminotransferase (ALT) 81 IU/L], lactate dehydrogenase (LDH) (784 IU/L), creatine kinase (5,985 IU/L), serum creatinine (2.31 mg/dL), serum urea nitrogen (39 mg/dL), C-reactive protein (34.2 mg/dL), and mild hyponatremia (134 mmol/L) (Table). Chest radiography revealed pneumonia in the left upper lung field (Figure A), and chest CT revealed consolidation and centrilobular nodules in the left upper lobe of the lung (Figure B). The clinical diagnosis on admission was pneumonia with septic shock and neutropenia. Empirical antimicrobial agents were administered immediately after obtaining two sets of blood cultures, as well as sputum and urine samples. Intravenous cefepime, ciprofloxacin, and amikacin were administered as an empirical treatment. The results from initial blood cultures, sputum Gram staining, and sputum cultures were all negative. Urine antigen tests for *Streptococcus pneumoniae* and *L. pneumophila* were also negative. Furthermore, the serum level of *Aspergillus* galactomannan antigen and β-D glucan were below the cut-off value.

On day 3, his impaired consciousness was found to have worsened. No white blood cells were found in a cerebrospinal fluid (CSF) examination. The glucose concentration, CSF protein levels, brain CT and MRI findings were normal. *Legionella* pneumonia was considered to be the most important differential diagnosis because the patient had headache, confusion, hyponatremia, and creatine kinase elevation, which are reported to be useful in the diagnosis of *Legionella* pneumonia. Thus, LAMP for the *Legionella* spp. (Eiken Chemical, Tokyo, Japan) was added to the stored sputum sample on day 3. The sputum sample was obtained on admission (day 1) and had been stored in the laboratory until day 1. However, it was difficult to distinguish whether impaired consciousness was a symptom of pneumonia or ciprofloxacin-associated encephalopathy. Ciprofloxacin was switched to azithromycin on the same day. The sputum sample was found to be positive for *Legionella* spp. by LAMP

on day 8 (8 days after admission). As a result, combination therapy consisting of levofloxacin and azithromycin was initiated immediately after withdrawing cefepime on day 8. The BACTEK 9240 (Becton, Dickinson and Company, Sparks, USA) culture bottles were negative after 7 days of incubation. Subsequently, a subculture of the blood samples was initiated using Wadowsky-Yee-Okuda-α-ketoglutarate (WYOα) agar (Eiken Chemical). As a result, a *Legionella* strain with Gram-negative rod detected by Gram staining was cultured on the media but not on blood agar. The strain was identified as *L. pneumophila* serogroup 5 using monovalent immune sera (Denka Seiken, Tokyo, Japan). Levofloxacin and azithromycin were administered for 3 weeks and 7 days, respectively. After starting the treatment, the patient's consciousness impairment gradually improved, and the use of oxygen and vasopressors was stopped. Chest CT on day 23 (15 days after the diagnosis of *L. pneumophila*) revealed that the consolidation in the left upper lobe had decreased in size in comparison to the chest CT image that was obtained on admission. Although the pneumonia was treated successfully, the recurrence of lymphoma was discovered during admission.

Discussion

We successfully treated a patient who was diagnosed with bacteremic healthcare-associated pneumonia (HCAP) caused by *L. pneumophila* serogroup 5, which was detected using LAMP and a blood subculture. *Legionella* pneumonia is often severe in immunocompromised patients (1, 2), such as the present case. Monotherapy with azithromycin or fluoroquinolone is typically administered as the standard therapy for *Legionella* pneumonia (7). However, some experts have suggested that combination therapy with macrolide and fluoroquinolone is an important therapeutic option especially for patients with severe *Legionella* pneumonia (8). This was why we administered the combination of levofloxacin and azithromycin after obtaining the definite diagnosis.

Legionella pneumonia accounts for 2 - 9% of community-acquired pneumonia (CAP) cases (9). *L. pneumophila* was identified as the causative agent in more than 80% of *Legionella* pneumonia cases, and approximately 50% of the cases of *Legionella* pneumonia are caused by *L. pneumophila* serogroup 1. Among the serogroups of *L. pneumophila*, *L. pneumophila* serogroup 5 is reported to cause 0.6% and 0.7% of the cases of nosocomial and community-acquired Legionnaires' disease, respectively (10). *L. pneumophila* is less common in patients with HCAP than in patients with CAP. The prevalence of *L. pneumophila* is reportedly 2.4% and 8.8% in patients with HCAP and CAP, respectively (11). In addition, to the best of our knowledge, only a few cases of bacteremic pneumonia caused by *L. pneumophila* have been reported (12).

In the present case, the diagnosis of HCAP caused by *Legionella pneumophila* serogroup 5 was difficult, because the results of the blood sample cultured for 7 days using the BACTEC system, sputum culturing, and a urinary antigen test were negative. However, we strongly suspected *Legionella* pneumonia based on the patient's clinical characteristics, which included headache, confusion, hyponatremia, and elevated creatine kinase; these findings are reported to be useful in the diagnosis of *Legionella* pneumonia (13). Thus, LAMP for *Legionella* spp. (Eiken Chemical) was initially performed using the stored sputum sample (14). After obtaining a positive LAMP result, blood subculturing was performed on WYOα agar. This approach allowed for the isolation *L. pneumophila* serogroup 5 from the blood.

The methods used to detect *Legionella* infection include antibody titers, indirect immunofluorescence assays, enzyme-linked immunosorbent assays, sputum culture and blood culturing, urinary antigen tests, PCR, and LAMP (5). Blood and sputum cultures show 100% specificity and are considered to be the gold standard methods for the diagnosis of *Legionella* pneumonia (5). However, a culture diagnosis requires special media (which is buffered by activated charcoal and WYOα media), adequate samples and a suitable technique. Furthermore, positive results are obtained after several days (5, 15). The detection of a 4-fold increase in the antibody titers by serological assays, including indirect immunofluorescence assays and enzyme-linked immunosorbent assays, takes 3 to 4 weeks (5). The urinary antigen test is a rapid diagnostic test that can be performed within an hour; the sensitivity and specificity are approximately 80% and 99%, respectively (16). However, this test is only available for detection of the *L. pneumophila* serogroup 1 (17).

To the best of our knowledge, no well-designed studies have determined the sensitivity and specificity of a PCR of samples from the lower respiratory tract in the detection of Legionella among pneumonia patients. However, following the introduction of the routine PCR testing of respiratory specimens (instead of the routine culturing of *Legionella* spp. from the specimens) in Christchurch, New Zealand, the burden of Legionnaires' diseases was recognized to be much greater than previously thought (18). This implies that the PCR may have higher sensitivity than the culture methods.

LAMP was developed in Japan (6), which it has been approved for the diagnosis of *Legionella* pneumonia since October 2010. The detection limit of LAMP is comparable to that of a PCR (6). The sensitivity and specificity of LAMP in the detection of reference *Legionella* spp. strains were found to be high in a previous study (19). In this context, LAMP may enable clinicians to identify cases of *Legionella* pneumonia, that are difficult to diagnose using other conventional methods, as was seen in the present case and in a previous report, wherein routine PCR was indicated (18).

LAMP is simple and easy to perform and only requires a laboratory water bath or a heat block for the reactions (6). Thus, LAMP may be useful, particularly in geographical areas with limited medical resources, such as developing countries. In this context, the introduction of LAMP may add new insights into the epidemiology of pneumonia

through the detection of hidden Legionnaires'disease, particularly in developing countries.

In conclusion, to our knowledge, this is the first English-language report to provide a detailed description of the diagnosis of a case of *Legionella* pneumonia using LAMP. Few cases of bacteremic pneumonia caused by *L. pneumophila* serogroup 5 have been reported to date. LAMP is a useful diagnostic tool for detecting *Legionella* pneumonia, which is difficult to diagnose using other conventional methods.

The authors state that they have no Conflict of Interest (COI).

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