Detection of *Legionella pneumophila* serogroup 1 in blood cultures from a patient treated with tumor necrosis factor-alpha inhibitor

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ABSTRACT

A 65-year-old man was admitted to our hospital with a fever of 39.3°C, cough, sputum and pharyngeal discomfort that had persisted for three days. Rheumatoid arthritis had been treated with methotrexate and adalimumab for two years and pancreatic metastasis of gastric cancer had been treated with S-1 (tegafur, gimeracil, and oteracil potassium) for two months. Regardless of the underlying pathologies, his general condition was good and he had worked as an electrician until two days before admission. However, his appetite had suddenly decreased from the day before admission, and high fever and hypoxia were also evident upon admission. A chest X-ray and computed tomography scan revealed left pleural effusion and consolidation in both lungs. The pneumonia severity index score was 165 and risk class was V. Accordingly, we started to treat the pneumonia with a combination of levofloxacin and meropenem. Thereafter, we received positive urinary antigen test (UAT) findings for *Legionella pneumophila*. Despite the application of appropriate antibiotics, vasopressors and oxygenation, the hypotension and hypoxia progressed and the patient died eight hours after admission. Even after his death, blood cultures had been continued to consider the possibility of bacterial co-infection. Although no bacteria were detected from blood cultures, Gimenez staining

revealed pink bacteria in blood culture fluids. Subsequent blood fluid culture in selective medium revealed *L. pneumophila* serogroup 1.

Key words: Legionnaires' disease, community-acquired pneumonia, blood culture, tumor necrosis factor-alpha inhibitor

INTRODUCTION

Since the first outbreak of Legionnaires' disease (LD) at the American Legion convention in 1976 [1], *Legionella pneumophila* has been a relatively common pathogen of community-acquired pneumonia (CAP). The reported incidence of LD in CAP ranges from 1.6% to 7.5% [2-6] and that in severe CAP is between 14% and 22.8%) [6-8]. Legionnaires' disease has appeared in hospitals, long-term care facilities and other types of accommodation [9-15], causing serious problems. Rapid diagnosis is important to prevent LD outbreaks. Among diagnostic tests for LD, the urinary antigen test (UAT) is the most useful [15] and it supposedly improves the outcomes and mortality rates of patients involved in LD outbreaks [16]. However, detection of the organism by culture is still important, because UAT can detect only *L. pneumophila* serogroup 1 and approximately 14% to 20% of LD is caused by non-serogroup 1 *Legionella* species [15, 17].

In order to use examinations such as UAT effectively, it is important to suspect LD early. Although one review describes clinical clues that increase the possibility of identifying LD [18], to suspect clinical LD remains difficult because clinical

manifestations are non-specific. Therefore, risk factors for LD are important to understand. Smoking has been reported as the most common risk factor for LD in some studies [19-21]. However, other recent reports have described LD arising in patients administered with tumor necrosis factor-alpha (TNF-α) inhibitors [22-25], and these drugs are now recognized as a risk factor for LD [22]. The US Food and Drug Administration (FDA) has updated the indications for the use of TNF-α inhibitors to include risk of *Legionella* infection [26].

We detected *L. pneumophila* serogroup 1 in blood cultures from a Japanese patient with severe CAP who had received a TNF-α inhibitor. Although there have been no report of such a case in Japan, it might have to be recognized that *Legionella pneumophila* might be a pathogen of severe CAP, considering the increased frequency of TNF-α inhibitor use.

Case report

A 65-year-old man developed pharyngeal discomfort that persisted for three days. He was admitted to hospital with a fever of 39.3°C, cough and sputum. He had been treated for rheumatoid arthritis with methotrexate and adalimumab (TNF-α inhibitor) for two years. He had undergone distal gastrectomy to treat gastric cancer four months previously, but pancreatic metastasis discovered two months later resulted in treatment with S-1 (tegafur, gimeracil, and oteracil potassium). He smoked one pack of cigarettes per day, and consumed alcohol socially. There was no history of Hot Springs or travel. Regardless of the underlying pathologies, his general condition was good and he had worked as an electrician until two days before admission. However, his appetite suddenly decreased from the day before admission, when he presented with a temperature of 39.3°C and hypoxia (SpO_2, 85%, room air).

Physical findings were normal, except for a slight clouding of consciousness and moist rales in the lower lung field. A chest X-ray and computed tomography (CT) scan revealed left pleural effusion and consolidation accompanied by ground-glass opacity (GGO) in both lungs (Fig. 1). The physical and radiological findings indicated a

diagnosis of pneumonia. Laboratory data showed a decrease in white blood cells (WBC) and platelets (PLT) (WBC, 2700 /μL; PLT, 139,000 /μL), renal dysfunction (blood urea nitrogen, 35.1 mg/dL; serum creatinine, 1.5 mg/dL), and serum lactic dehydrogenase (LDH) and C-reactive protein (CRP) levels were respectively elevated at 544 U/L and 31.77 mg/dL. The pneumonia severity index (PSI) for adults [27] was 165 and risk class was V. Accordingly, we started treating the pneumonia with levofloxacin and meropenem. Thereafter, the UAT findings for *L. pneumophila* were positive. However, the hypotension and hypoxia progressed, and the patient refused intubation due to the underlying diseases. Despite the use of appropriate antibiotics, a vasopressor and oxygenation, the patient died eight hours after admission.

Even after his death, blood cultures had been continued to consider the possibility of bacterial co-infection. The blood cultures appeared negative for co-infective bacteria and Gram staining, but Gimenez staining revealed positive (pink) bacteria (Fig. 2A). Subsequently, *L. pneumophila* serogroup 1 was detected in the blood culture fluid. The process was as follows. Seven days after starting cultures, the content fluid was withdrawn, separated by centrifugation and applied to Wadowsky-Yee-Okuda (WYO)

medium, blood agar, bromothymol blue lactose (BTB) agar and chocolate agar media. Colonies were evident after four days only in WYO medium (Fig. 2B). Gimenez staining again revealed pink bacteria (Fig. 2C), and Gram staining revealed gram-negative rods (Fig. 2D). An antigen-antibody reaction then confirmed the bacteria as *L. pneumophila* serogroup 1.

Discussion

The two key findings of this case are that *L. pneumophila* serogroup 1 was detected from blood cultures and that *L. pneumophila* caused severe CAP in a patient under treatment with a TNF-α inhibitor.

We diagnosed LD using UAT and cultures. The primary method of LD diagnosis is UAT since this modality identifies 81% to 97% of LD infections compared to only 5% to 9% by culture [15, 28]. The UAT is simple and it can rapidly and accurately (sensitivity, 60% to 80%; specificity, >99%) identify *L. pneumophila* serogroup 1 [17, 28]. However, culture remains important because 14% to 20% of LD infections are caused by non-serogroup 1 *Legionella* species [15, 17] that UAT cannot detect. Notably, *L. pneumophila* from the present patient survived in a non-selective medium for one week and another study has shown that *L. pneumophila* could survive in standard blood culture broth medium [29]. Although the number of report of detection of *L. pneumophila* was actually very few [30-32], this case suggests that blood culture is worth trying when UAT results are negative and sputum is not discharged when LD is suspected.

Diabetes mellitus, current smoker, traveling abroad was reported as risk factors for LD [19-21]. Our patient was current smoker, and it was reported as the most common risk factor for LD [19-21]. Multivariable analysis has identified current cigarette smoking as a dose-dependent risk factor (multivariable odds ratio [OR] for currently smoking >70 cigarettes/week, 13.5; 95% confidence interval [CI], 5 – 36) [20]. Our patients received S-1. However, the incidence of LD arising in patients under therapy with anti-cancer drugs (such as S-1) does not seem to be increased. A previous study did not find an unusually high incidence of LD among patients with neutropenia, acute leukemia or HIV [33].

Our patients also received TNF-alpha inhibitor. The use of TNF-alpha inhibitor has been reported as a risk factor for serious infections [34]. Recently, some reports have described LD in patients receiving TNF-α inhibitors [22-24] and the relative risk for LD in patients treated with TNF-α inhibitor was between 16.5 and 21 compared to that for the overall population [22]. Although our patients received methotrexate and some reports described that the incidence of serious infections in patients with TNF-alpha inhibitor was not significantly higher than those with disease modifying anti-rheumatic

drugs (DMARDs) [35, 36], two in vivo studies have reinforced the notion of an increasing risk for LD among patients receiving TNF-α inhibitors. First, the administration of a TNF-α-neutralizing antibody to mice in vivo inhibited TNF-α activity and resulted in enhanced *L. pneumophila* growth in the mouse lung [37]. Secondly, the mortality rates of TNF receptor (TNFR) 1- and TNFR2-knockout mice infected with *L. pneumophila* [38] are higher than those of wild-type mice, and *L. pneumophila* clearance is slower among TNFR1- than TNFR2-knock-out and wild type mice. That study also indicated that TNF-α is involved in *L. pneumophila* infection, since *L. pneumophila* proliferated in peritoneal macrophages, neutrophil accumulation was decreased in bronchoalveolar lavage (BAL) fluids and cytokines (IFN-γ, interleukin-12 and TNF-α) were dysregulated in TNFR1-knock-out mice. In contrast, large amounts of neutrophils accumulated in BAL fluids from TNFR2-knock-out mice. These data indicated that a TNFR1 deficiency leads to compromised innate immunity against *L. pneumophila*, whereas a TNFR2 deficiency induces an excessive inflammatory response.

In conclusion, this case showed that blood culture remains valid for diagnosing LD

and the risk of LD in patients under treatment with TNF-α inhibitors. Blood culture is worth trying in the absence of other findings if LD is suspected. In addition, *L. pneumophila* should be considered as a causative pathogen if severe pneumonia develops in a patient undergoing treatment with TNF-α inhibitors.

Figure legends

Figure 1. Chest X-ray (A) and Computed tomography (CT) (B, C).

Left pleural effusion and consolidation accompanied by ground-glass opacity (GGO) in both lungs evident in all images.

Figure 2. Blood culture fluid staining.

Bacteria in blood culture fluid identified by pink Gimenez staining (A). Four days after starting culture in selective medium, colonies appeared in Wadowsky-Yee-Okuda (WYO) medium, but not in blood agar, bromothymol blue lactose (BTB) agar or chocolate agar media (B). Gimenez stain also stained bacteria from colonies in WYO medium pink (C) and Gram staining revealed gram-negative rods (D). (A), (C) and (D), ×1000 magnification.

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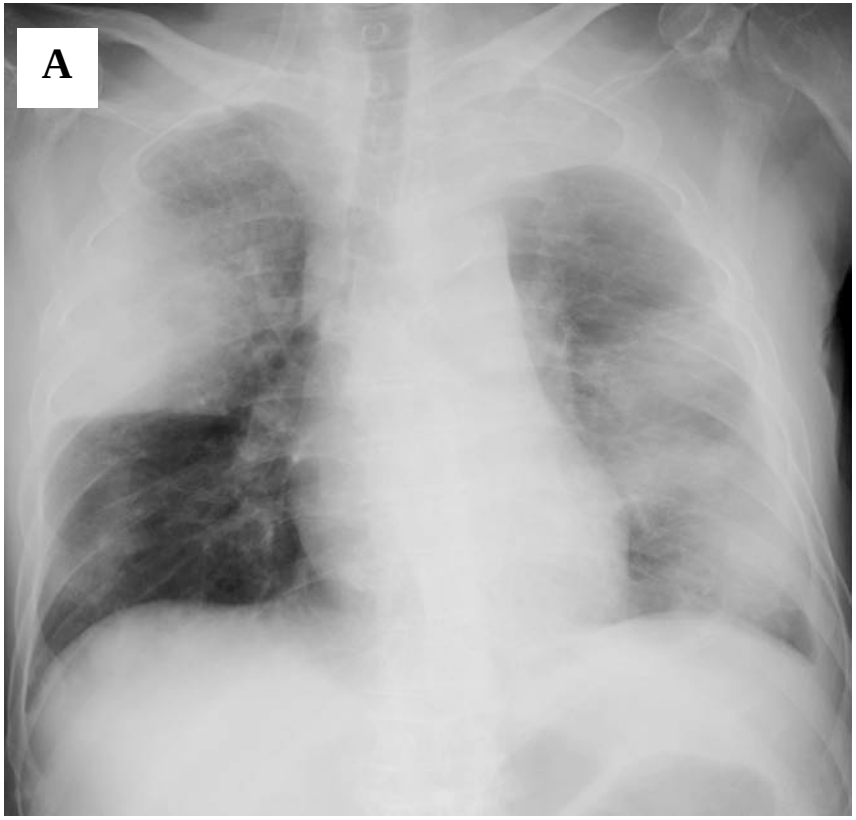
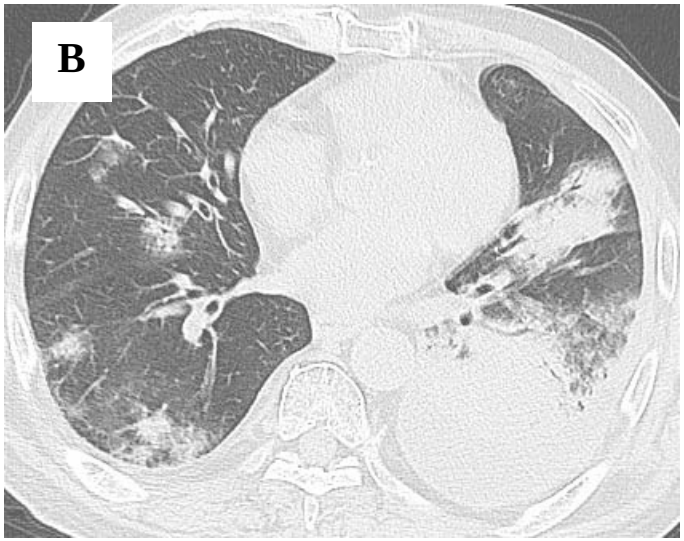
Figure 1.





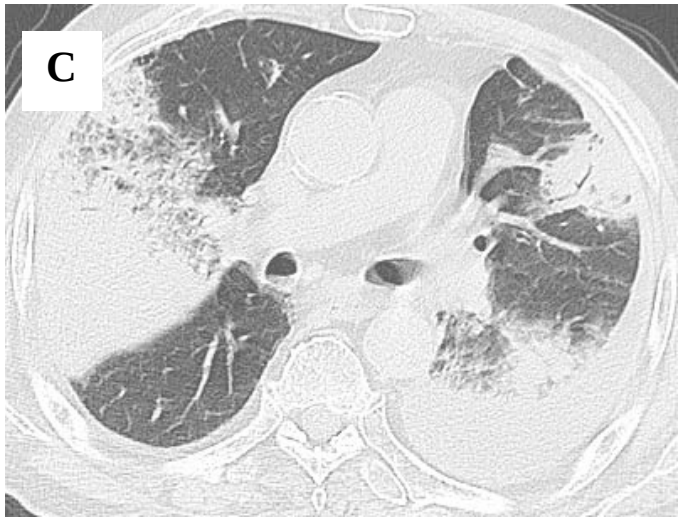


Figure 2.