INTERNAL MEDICINE

□ CASE REPORT □

Primary A (H1N1) pdm09 Influenza Pneumonia Diagnosed on Reverse Transcription-polymerase Chain Reaction (RT-PCR) of Bronchoalveolar Lavage Fluid but not Rapid Tests with Nasopharyngeal Swabs

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Abstract

A 47-year-old man with a fever was highly suspected of having influenza A infection since his wife and son who lived with him had been diagnosed with influenza A. Although repeated rapid tests with a nasopharyngeal swab showed negative findings, the patient developed bilateral pneumonia and reverse transcription polymerase chain reaction (PCR) for A (H1N1) pdm09 virus in the bronchoalveolar lavage fluid was positive. We therefore diagnosed him with primary influenza pneumonia and initiated treatment with peramivir plus corticosteroids, which rapidly improved his condition. During the influenza season, sample collection from the lower airway and PCR should be considered for the definitive diagnosis of primary influenza viral pneumonia.

Key words: A (H1N1) pdm09, PCR, primary influenza pneumonia

Introduction

A pandemic caused by A (H1N1) pdm09 influenza virus occurred during the 2009/2010 seasons. Infection with A (H1N1) pdm09 influenza virus results in a broad spectrum of clinical syndromes, ranging from afebrile upper respiratory illnesses to fulminant viral pneumonia. Dyspnea, tachypnea, chest pain, hemoptysis or purulent sputum, prolonged or recurrent bouts of fever, an altered mental status, manifestations of dehydration and the reappearance of lower respiratory tract symptoms after improvement are signs of progression to more severe disease and/or complications. The principal clinical syndrome leading to hospitalization and intensive care is diffuse viral pneumonitis associated with severe hypoxemia, acute respiratory distress syndrome (ARDS) (1). Many reports have described the clinical diagnosis of A (H1N1) pdm09 influenza virus. Although nasopharyngeal aspirates or swabs collected early after the onset of symptoms provide suitable samples, endotracheal or bronchoscopic aspirates have higher yields in patients with lower respiratory tract illnesses (2, 3). The sensitivity of rapid tests (immunofluorescent antigen assays) with nasopharyngeal swabs is low in cases of A (H1N1) pdm09 infection (2, 4, 5), and repeated collection of multiple respiratory specimens is recommended in the patients with a high clinical suspicion of H1N1 infection (1). We herein report the case of a 47-year-old man with primary A (H1N1) pdm09 influenza viral pneumonia diagnosed on reverse transcription polymerase chain reaction (RT-PCR) of the bronchoalveolar lavage fluid (BALF) but not rapid tests with nasopharyngeal swabs.

Case Report

A 47-year-old man complained of fever and headache at a nearby clinic in January 2014. His wife and son who lived with him also exhibited a fever during the same time period and were diagnosed with influenza A using rapid tests with nasopharyngeal swabs. Three rapid tests with nasopharyngeal swabs were performed at the clinic on three different days; however, all results were negative. While antibiotics
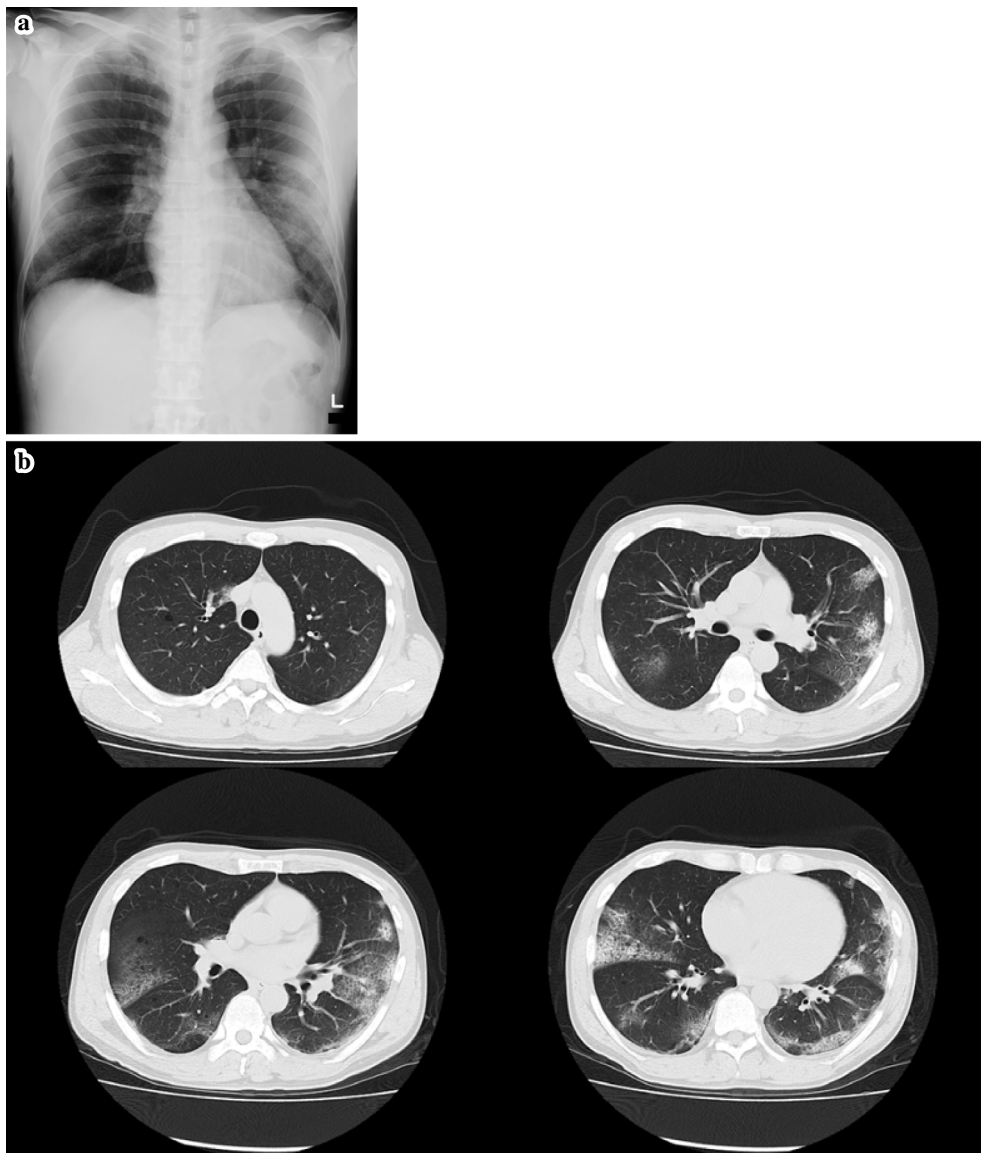
Figure 1.   **Chest imaging findings on admission. A chest X-ray showed bilateral patchy opacity (a). Chest computed tomography demonstrated ground glass opacity, a crazy-paving pattern and consolidation in the bilateral lung fields (b).**

(cefcapene pivoxil and azithromycin) were prescribed, the patient's symptoms persisted for seven days. Since a chest X-ray showed consolidation in both lungs (Fig. 1a), he was referred to our hospital. He had no significant underlying diseases, although he had smoked from 20 to 47 years of age. He did not receive the influenza vaccine during the relevant season. On admission, his respiratory rate was 20 breaths per minute, with a heart rate of 75 beats per minute, blood pressure of 97/56 mmHg and percutaneous oxygen saturation of 96%. Chest auscultation revealed inspiratory fine crackles on the left side, and chest computed tomography (CT) showed bilateral ground-glass opacity, a crazy-paving pattern and consolidation (Fig. 1b). The laboratory data disclosed a white blood cell count of 3,480/μL (neutrophils: 73.8%, lymphocytes: 16.7%, eosinophils: 0%, monocytes: 8.9%, basophils: 0.6%), hemoglobin level of 13.0 g/dL, platelet count of 17.7×10^4/μL, total protein level of 7.0

g/dL, urea nitrogen level of 13.1 mg/dL, creatinine level of 0.7 mg/dL, lactic dehydrogenase level of 410 IU/L, total bilirubin level of 0.2 mg/dL, aspartate aminotransferase (AST) level of 121 U/L, alanine aminotransferase (ALT) level of 65 U/L and C-reactive protein (CRP) level of 6.2 mg/dL. Urinary antigen tests for both *Legionella pneumophila* and *Streptococcus pneumoniae* were negative, and a rapid influenza virus test (ImunoAce, TAUNS, Numazu, Japan) with a nasopharyngeal swab was negative. In addition, sputum smears and culture were negative for bacteria, including acid-fast bacteria, and the results of isothermal loop-mediated amplification of *Legionella pneumophila* and *Mycoplasma pneumonia* in the sputum were negative. In contrast, fiberoptic bronchoscopy performed on the second hospital day showed abnormal findings, including inflammation of the bronchial mucosa in the bronchial lumen. A total of 53 mL/150 mL of BALF recovered from the left superior
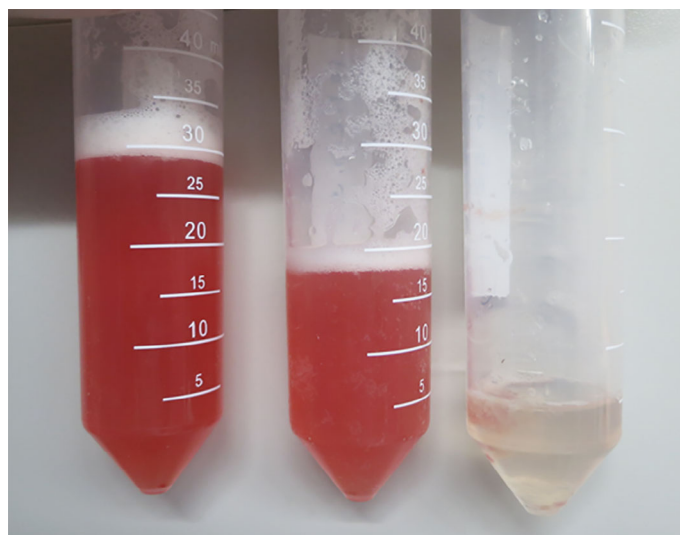
Figure 2. Appearance of the bronchoalveolar lavage fluid (BALF). Bronchoscopy performed on the second hospital day. BALF from the left superior lingular region displayed hemorrhagic features on sequential samples.
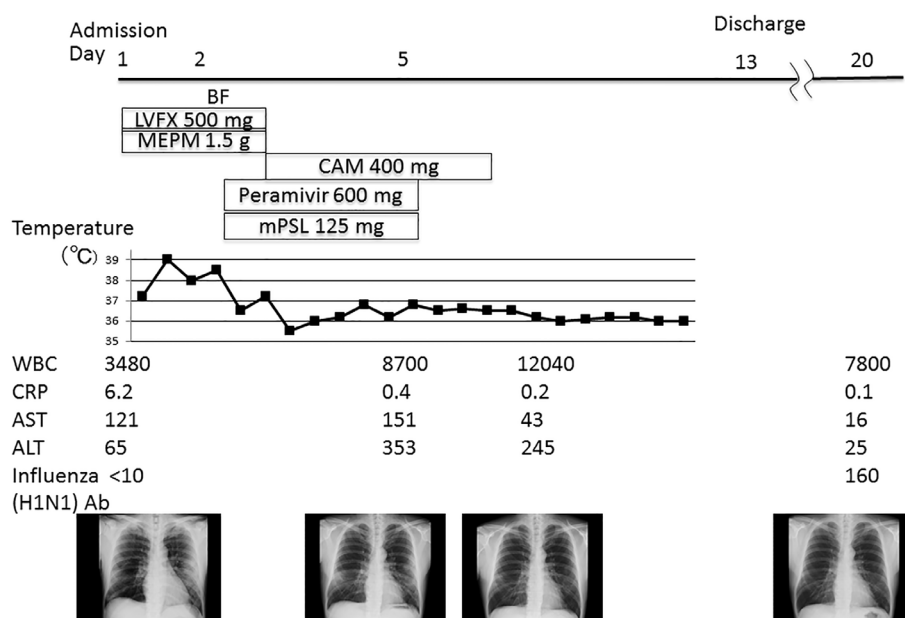

Figure 3. Clinical course. The patient's clinical course showed improvements in the fever, radiological findings, inflammatory response and hepatopathy following the administration of a neuraminidase inhibitor and corticosteroids. The patient was discharged on hospital day 13. Three weeks after admission, the serum antibody titer for influenza H1N1 had increased significantly. Abbreviations: ALT: alanine aminotransferase, AST: aspartate aminotransferase, CAM: clarithromycin, CRP: C-reactive protein, LVFX: levofloxacin, MEPM: meropenem, mPSL: methylprednisolone, WBC: white blood cell

lingular region disclosed hemorrhagic features on sequential samples (Fig. 2). The white blood cell count in the BALF was 5.3×10^5 cells/mL (macrophages: 44.3%, lymphocytes: 55.7%, neutrophils: 0%, eosinophils: 0%), although a BALF smear and culture for bacteria, including acid-fast bacteria, were negative. While the findings of RT-PCR for the influenza virus with a nasopharyngeal swab and rapid test using BALF were negative, RT-PCR with BALF was positive for the A (H1N1) pdm09 influenza virus. We therefore diagnosed the patient with primary influenza viral pneumonia and initiated therapy with an intravenous neuraminidase inhibitor, peramivir (600 mg/day), plus corticosteroids (mPSL: 125 mg/day) for three days. Consequently, the patient's fever, CRP, AST, and ALT levels and consolidation on chest X-rays improved, and he was discharged on hospital day 13. Three weeks after admission, the serum hemagglutinin (HA)

antibody titer for influenza H1N1 had increased from <10 to 160 (Fig. 3).

Discussion

As a characteristic in the present case, PCR of a sample obtained from the lower airway (BALF) was positive for the A (H1N1) pdm09 influenza virus. However, rapid tests (immunofluorescent antigen assays) and PCR of samples from the upper airway were negative, as was a rapid test of a sample from the lower airway. The type of assay and the origin of the sample may affect the accuracy of diagnosis of A (H1N1) pdm09 influenza virus infection (2). Indeed, it has been reported that nasopharyngeal aspirates or swabs collected early after the onset of symptoms provide suitable samples, whereas endotracheal or bronchoscopic aspirates have higher yields in patients with lower respiratory tract illnesses (2, 3). According to a previous report, 19% of patients with detectable A (H1N1) pdm09 viral RNA in bronchoscopic samples had negative upper respiratory tract samples. Furthermore, direct or indirect immunofluorescence tests are less sensitive than RT-PCR (2). The efficacy of several commercial influenza rapid tests for assessment of the upper airway has been evaluated in case of A (H1N1) pdm09, demonstrating low sensitivity for the detection of A (H1N1) pdm09 with a sensitivity and specificity of 48.7% and 96.5% (4) versus, 47% and 95% for PCR analyses (5), respectively. Negative results for single respiratory specimens do not rule out the possibility of A (H1N1) pdm09 influenza virus infection, and repeated collection of multiple types of respiratory specimens is recommended if clinical suspicion is high (1).

The findings of ground glass opacity, a crazy-paving pattern and consolidation in the bilateral lung fields on CT observed in the present case are consistent with those of primary viral pneumonia reported previously (6). However, these findings on CT are not specific to A (H1N1) pdm09 influenza viral pneumonia. Nevertheless, the potential for influenza viral pneumonia should be taken into consideration in the differential diagnosis, especially during the pandemic season.

In a report of lung autopsy findings of A (H1N1) pdm09 influenza virus pneumonia (7), pathological changes associated with H1N1 infection were localized to the lungs, in which three distinct histological patterns could be identified: diffuse alveolar damage (DAD), necrotizing bronchiolitis and DAD with intense alveolar hemorrhage. In addition to the presence of infectious cells in the upper respiratory and tracheobronchial epithelium and mucosal glands, the A (H1N1) pdm09 influenza virus targets cells of the alveolar lining (type I and II pneumocytes) (8). Clinically, one of the most frequent causes of diffuse alveolar hemorrhage in cases of infectious disease is A (H1N1) influenza virus (9). The features of hemorrhagic BALF noted in the present case were consistent with the pathological findings of A (H1N1) pdm09 influenza virus infection.

The present patient improved with adjunctive corticosteroid therapy. Corticosteroids possess anti-inflammatory properties and are known to modulate inflammation, suppress the immune system and regulate the stress response. Their use in patients with influenza infections has only been evaluated in retrospective studies, with controversial results. In ARDS patients, with and without confirmed H1N1 influenza infection, prolonged low- to moderate-dose corticosteroid treatment is well tolerated and associated with significant improvements in scores for lung injury and multiple organ dysfunction as well as low hospital mortality (10). In contrast, in a retrospective cohort study of hospitalized patients with A (H1N1) pdm09 influenza virus infection in China, the early use (less than 72 hours after onset) of glucocorticoids for fever reduction and pneumonia prevention was found to be associated with the subsequent development of critical illnesses, even after adjusting for the presence of underlying diseases or risk factors (hazard ratio: 2.9, 95% CI: 1.3-6.2) (11). Further research is necessary to improve patient selection and facilitate the identification of subgroups that may benefit from adjunctive steroid therapy.

During the influenza pandemic season, sample collection from the lower airway and PCR should be performed to obtain a definitive diagnosis of primary influenza viral pneumonia, even if rapid tests (immunofluorescent antigen assays) with nasopharyngeal swabs are negative.

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

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