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Case Report

Laboratory diagnosis of avian influenza virus H7N9 infection in a renal transplant recipient

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Abstract: A renal transplant recipient who had atypical clinical manifestations, unclear epidemiological exposure history and negative results from influenza virus antigen and nucleic acid amplification in throat swab specimens was admitted into our hospital on April 17, 2013. He was finally diagnosed as avian influenzavirus H7N9 infection. Here, we reviewed the epidemiological, clinical and laboratory findings of this patient. We speculated that the specimens should be collected repeatedly at different sites for suspected cases or special cases needing differential diagnosis; different methods or kits should be used for laboratory testing; atypical clinical manifestations caused by the special nature of patients such as long-term use of immunosuppressive agents and autoimmune diseases should also be taken into account.

Keywords: Avian influenza, subtype H7N9, testing, diagnosis

Introduction

On March 31, 2013, National Health and Family Planning Commission of People's Republic of China [1] informed that 3 patients were infected by avian influenza (H7N9) virus in Shanghai and Anhui province since February, and this was also the first report of human infection with avian influenza H7N9 in the world. On April 2 and 10, National Health and Family Planning Commission issued and revised *Diagnostic and Treatment Protocol for Human Infections with Avian Influenza A (H7N9)* in succession in order to provide guidance for diagnosis and treatment [1]. Before May 7th, 11 provinces and cities in China reported a total of 131 cases of H7N9 avian influenza infection, including 31 death cases.

Bird Flu or Avian Influenza with the full name of Avian Influenza in Poultry and Wild Birds is an animal-borne disease caused by a virus which usually only infects the birds. Most avian influenza viruses do not infect humans; however some such as H5N1 may cause serious infection in people. This disease has attracted global attention since human infection of bird flu was found in Hong Kong in 1997 [2, 3]. As human infection with avian influenza H7N9 virus first reported in China in March 2013, it has been confirmed that chicken in the live poultry markets are one of the sources of human infection of H7N9 virus [4]. There is no evidence that this infection can be spread among people through properly cooked food. Controlling this disease in animals is the first and a key step in decreasing risks to humans.

Case report

On April 17, a patient infected by avian influenza H7N9 virus was admitted to our hospital. He was a renal transplant recipient with atypical clinical manifestations and negative results from multiple tests of influenza virus antigen and nucleic acid amplification of throat swab specimens. Herein, relevant medical history and laboratory test results are reported as follows in order to provide a peer reference.

General history: the male patient (age, 62 years; from Pengbu Town of Hangzhou, Zhejiang

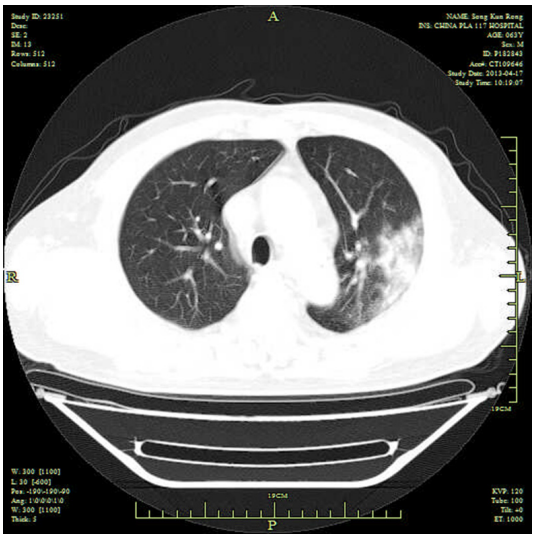

Figure 1. Findings from chest CT scan of the renal transplant recipient with avian influenza (H7N9) virus infection. Increased density shadows were found in the left upper lung with indistinct borderline and part consolidation.

Province, China) exhibited cough on April 15, 2013. Then he was diagnosed with the common cold and was given antitussive drug, hormone (Methylprednisolone, 8 mg, thrice daily) and antibiotics (Cefradine, 50 mg, thrice daily) in a local hospital on the same day. On April 17, he presented with fever (38.8°C) and then was diagnosed initially as lung infection after he came to our hospital. Then he received anti-infective (experience-based medication at an early stage: piperacillin sodium and sulbactam sodium, azithromycin, voriconazole, ganciclovir) and symptomatic treatment (tacrolimus, the dose of immunosuppressants was reduced to the minimum level for maintenance [0.05 mg/kg, twice daily], and the dose of intravenous methylprednisolone was increased to control lung inflammation). He received comprehensive imaging and laboratory examinations on the same day. On April 19, he exhibited dyspnea together with persistent low-grade fever (37.8°C-38.7°C) and was diagnosed as severe pneumonia. Then, he was transferred to the intensive care unit (immunosuppressant treatment was stopped. Meropenem and ambroxol hydrochloride were used). From 17 April to 20 April, throat swab specimens were collected repeatedly for testing relevant indicators (sputum culture, influenza A/B virus antigen typing and nucleic acid amplification for universal primer of influenza A virus). All these tests showed negative results. On April 20 and 21, he was given a consultation from a group of experts, and the sputum specimens from the lung (lower respiratory tract secretions) was collected on April 22 for influenza A/B virus antigen typing, nucleic acid amplification for universal primer of influenza A virus and the subtype of avian influenza (H7N9) virus tests, and these tests all showed a positive results, thus obtaining a definitive diagnosis.

Previous medical history: the patient received renal transplantation (recipient) due to uremia in our hospital 18 months ago (October 2011), who suffered from diabetes for 12 years and renal hypertension for 5 years and had been taking the immunosuppressive agent Tacrohmus (FK506), hypoglycemic agents and anti-hypertensive drugs.

Epidemiological history or exposure history: he had no history of exposure to the poultry and live-bird within half a month before cough, and exhibited cough caused by cold due to sweating during labor on April 10.

Imaging examination: Chest CT scan and chest X-ray, ultrasound and ECG were performed on the day of admission, and chest CT scan or chest X-ray was given every two days to observe the progress of lung inflammation.

No obvious abnormality was observed by ultrasound and ECG examination. The diagnostic findings from chest CT scan and chest x-ray were that the lesions of the left lung showed an apparent progressive development, but did not extend to the right lung (**Figure 1**).

Laboratory examination: the specimens were collected on the day of admission to perform routine testing including blood, sputum, throat swab culture (fungi and bacteria), common antigens of pathogen in respiratory tract (Influenza A virus, influenza B virus, parainfluenza 1,2,3 virus, respiratory syncytial virus, adenovirus), and common IgM antibodies against pathogens in the serum (mycoplasma pneumonia, chlamydia pneumonia, cytomegalovirus, herpes simplex virus type I/II, rubella virus, legionella pneumophila, mycobacterium tuberculosis), nucleic acids and routine blood, serum C-reactive protein (CRP), etc, and samples were collected continuously for some indicators to observe the progress of disease condition. The

Table 1. Primers and probes for detection of H7N9 avian influenza virus and influenza A virus

Virus type	ID	Sequence
FluA	F Primer	5'-GACCRATCCTGTCACCTCTGAC-3'
	R Primer	5'-AGGGCATTYTGGACAAAKCGTCTA-3'
	Probe	5'-FAM-TGCAGTCCTCGCTCACTGGGCACG-BHQ1-3'
H7	F Primer	5'-AGAAATGAAATGGCTCCTGTCAA-3'
	R Primer	5'-GGTTTTTTCTTGTATTTTTATATGACTTAG-3'
	Probe	5'-FAM-AGATAATGCTGCATTCCCGCAGATG-BHQ1-3'
N9	F Primer	5'-TAGCAATGACACACACTAGTCAAT-3'
	R Primer	5'-ATTACCTGGATAAGGGTCATTACACT-3'
	Probe	5'-FAM-AGACAATCCCCGACCGAATGACCC-BHQ1-3'

Conditions for reaction: 50°C 30 min, 95°C 5 min and a total of 45 cycles of 95°C, 10 s and 55°C, 40 s.

primers and probes for detection of H7N9 avian influenza virus and influenza A virus by real-time RT-PCR are shown in **Table 1**.

The samples were collected repeatedly from multiple parts for testing. The test results showed that the white blood cell count and blood lymphocyte count had a continuous decrease trend, and CRP in the blood rose continuously, while all the multiple tests of influenza antigens and nucleic acids by using throat swab showed a negative result. Finally, the sputum of the lung (lower respiratory tract secretions) was collected and then a definitive diagnosis was obtained. See **Table 2** for the details.

Common antigens against influenza B virus, parainfluenza 1,2,3 virus, respiratory syncytial virus, adenovirus were negative in multiple detections, and common IgM antibodies against mycoplasma pneumonia, chlamydia pneumonia, cytomegalovirus, herpes simplex virus type I/II, rubella virus, legionella pneumophila, and mycobacterium tuberculosis were also negative in serum. However, common antigen against influenza A virus, universal primer for influenza A virus, nucleic acid amplification of the subtype of avian influenza (H7N9) virus in the lower respiratory tract secretions showed positive results after repeated confirmation.

Post-diagnosis outcome

This patient was finally diagnosed as H7N9 avian influenza virus infection after repeated laboratory examinations and expert consultation. Treatment with piperacillin sodium, sulbactam sodium, azithromycin and voriconazole was discontinued. Oral oseltamivir phosphate was administered for consecutive 5 days (150 mg, twice daily), and other symptomatic support treatment. Unfortunately, this patient died due to respiratory failure and renal failure on May 12, 2013.

Discussion

Clinical diagnosis of H7N9 avian influenza virus infection in human is usually made according to the medical history (history of exposure), symptoms of influenza (fever, cough, little phlegm; body temperature of higher than 39°C in critically ill patients), findings in imaging examinations (disease progresses rapidly in critically ill patients and severe pneumonia will be present within 5-7 days; symmetrical signs in bilateral lungs, and dyspnea is also present), results of laboratory examinations (unchanged or reduced white blood cell count, increased CRP, or reduction in total white blood cell count and lymphocyte count in critically ill patients), findings in pathogen examinations (positivity in antigen against influenza A virus, positivity in common primers for influenza A virus or H7N9 in RT-PCR, positive result in virus culture, type H7N9 avian influenza virus specific antibody is increased by 4 times in both serum samples during the dynamic examinations) [1]. However, in the present patient, he had several atypical characteristics: 1) Although he was in the epidemic area, he had no evident exposure history; 2) Although he had fever during the whole course of disease, the body temperature was no higher than 38°C; 3) Manifestations and signs of severe pneumonia were unilateral; 4) Negative results were shown in the pathogen examination of throat swab specimens. It is these atypical characteristics (especially the negative result in repeated examination of throat swab specimens) that result in delayed diagnosis and misdiagnosis. We speculate that this might be attributed to the long-term use of immunosuppressants and recent use of steroid.

The patient is a renal transplant recipient with long-term use of immunosuppressive agents, and lung infection is the most common complication of organ transplantation [5] and has an obviously increased risk in elderly patients with

Table 2. Main findings in laboratory examinations of this patient with subtype of avian influenza (H7N9) virus infection

Inspected items	Inspection Date	Results	Unit	Specimen types
Blood culture	2013-4-17	*No growth		venous blood
	2013-4-19	*No growth		venous blood
	2013-4-21	*No growth		venous blood
Sputum culture	2013-4-17	△No growth		sputum
	2013-4-19	△No growth		sputum
	2013-4-21	△No growth		sputum
white blood cell	2013-4-17	7.22	10^9/L	venous blood
	2013-4-19	5.32	10^9/L	venous blood
	2013-4-21	4.75	10^9/L	venous blood
blood lymphocyte	2013-4-17	0.93	10^9/L	venous blood
	2013-4-19	0.48	10^9/L	venous blood
	2013-4-21	0.16	10^9/L	venous blood
CRP in the blood	2013-4-17	82.4	mg/L	venous blood
	2013-4-19	151.50	mg/L	venous blood
	2013-4-21	155.10	mg/L	venous blood
influenza A/B virus antigen typing	2013-4-17	Negative		throat swab
	2013-4-19	Negative		throat swab
	2013-4-20	Negative		throat swab
	2013-4-22	Positive		#sputum in the lung
universal primer for influenza A virus	2013-4-19	Negative		throat swab
	2013-4-20	Negative		throat swab
	2013-4-22	Positive		#sputum in the lung
nucleic acid amplification for the subtype of avian influenza (H7N9) virus	2013-4-19	Negative		throat swab
	2013-4-20	Negative		throat swab
	2013-4-22	Positive		#sputum in the lung

*No bacteria, △No pathogenic bacteria and fungus; #lower respiratory tract secretions.

diabetes, therefore, this condition is generally considered to be the lung infection complicated by organ transplantation during the initial diagnosis. But taking into account the area of bird flu, the possibility of infection by bird flu cannot be excluded. Thus multiple tests of influenza A/B virus antigen typing by sputum culture and throat swab and nucleic acid amplification for universal primer of influenza A virus was performed and showed negative results, and the patient exhibited ongoing low-grade fever and had unilateral pulmonary infection, without exposure history or atypical clinical manifestations of avian influenza virus infection (it may be caused by special immunization condition) [1, 6] therefore, the patient was diagnosed as lung infection and severe pneumonia after renal transplantation.

Currently, the diagnosis of human infection with avian influenza virus mainly depends on the laboratory examination results: influenza A/B virus antigen typing test, nucleic acid amplification test for universal primer of influenza A virus, the subtype of avian influenza (H7N9) virus isolated from the respiratory secretions or nucleic acid amplification test for the subtype of avian influenza (H7N9) virus. However, these tests would be interfered by many factors to cause the false-negative results (such as, swab itself quality, non-standardized sampling, time and temperature of sample collection, influence of medication, RNA enzyme contamination) [7].

Through the statistical analysis, some medical institutions in this region adopted the different methods (colloidal gold method, enzyme linked immunosorbent assay, virus culture, nucleic acid amplification, etc.) to detect the throat swab or sputum of the lung (lower respiratory tract secretions) in the patient infected by avian

influenza H7N9, and the results showed that the positive rate of colloidal gold method, enzyme linked immunosorbent assay, virus culture in testing influenza A/B virus antigen typing was about 60% and 80% of that of nucleic acid amplification or virus culture. Long-term use of immunosuppressive agents and antibiotics before sampling may cause loss of viral antigen expression and disappearance or variability of antigenicity in the upper respiratory tract to result in the negative result from the test of influenza virus antigen in throat swab specimens, while the negative result of nucleic acid amplification for universal primer of influenza A virus may be caused by abnormal sampling or RNA enzyme pollution; in addition, the epidemiological history still needs further verification and investigation, which may be opportunistic infection caused by low immunity in the body (taking immunosuppressant due to renal transplantation) [8, 9].

Conclusion

In summary, we believe that comprehensive consideration should be given during the diagnosis and treatment for special patients with atypical clinical manifestations (such as the long-term use of immunosuppressive agents, autoimmune diseases, etc.) to avoid the misdiagnosis, and specimens should be collected repeatedly from the different parts for inspection before medication, and different methods and kits should be used for laboratory testing for suspected cases or special cases that needed differential diagnosis [10]. Only in this way early discovery, early reporting, early diagnosis and early treatment can be made for patients infected by bird flu.

Disclosure of conflict of interest

None.

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