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Breakthrough of False Negative: A Case of Protracted H1N1 Influenza with Chest Pain Onset Implications for The Diagnosis and Treatment of Encapsulated Empyema

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Abstract

Background: Influenza A (H1N1) infection usually has a self-limiting course (5–7 days), but some patients may progress to severe pneumonia or complications, resulting in a significantly prolonged course of the disease. This case reports a special case of protracted H1N1 infection lasting more than one month. The patient presented with persistent chest pain (>1 month) as the initial symptom. The preliminary diagnosis was "encapsulated empyema." The results of multiple PCR tests on respiratory samples (swabs from the throat and sputum) in the early stage of fever were negative, masking the existence of the disease, and secondary bacterial mixed infections occurred. Through targeted next-generation sequencing (tNGS) of bronchoalveolar lavage fluid (BALF), it was finally confirmed as H1N1 virus infection. This highlights the crucial diagnostic value of lower respiratory tract sample testing in overcoming false negatives in upper respiratory tract testing. Further analysis revealed that the patient's weakened immunity might be a new risk factor for protracted influenza, providing clinical implications for the delayed initiation of antiviral treatment and the management of encapsulated empyema.

Case Presentation: This article reports a 49-year-old male patient who had a history of gastric perforation surgery and appendicitis surgery. He was admitted to the hospital with chest pain for more than 1 month and fever for 2 days. The final diagnosis was influenza A (H1N1) combined with purulent pleural effusion caused by *Fusobacterium nucleatum* and *Klebsiella pneumoniae* infection. The patient initially presented with a large amount of encapsulated pleural effusion on the right side. Empirical use of "ceftriaxone" for 5 days of anti-infection treatment was ineffective. The pleural fluid tNGS indicated "Fusobacterium nucleatum sequence number 9, relative abundance 49.9%." Subsequently, the treatment was adjusted to "piperacillin-tazobactam" for 3 days. The patient no longer had fever, but on the 18th day of treatment, the patient had a fever again. The re-examination of nasopharyngeal swabs for influenza A and B antigens was all "negative." The treatment continued with "piperacillin-tazobactam." On the 21st day, the patient still had a fever. Bronchoalveolar lavage fluid tNGS indicated "influenza A virus H1N1 sequence number 1344, relative abundance 76.2%, Klebsiella pneumoniae sequence number 50, relative abundance 0.3%." On the 22nd day, oseltamivir was added for antiviral treatment for 1 day. The fever was controlled, and the

patient's condition improved and was discharged. On the 29th day of follow-up after discharge, the patient's pulmonary infection and pleural effusion had significantly absorbed.

Conclusion: This case suggests that for patients with refractory pleural effusion and repeated fever, the possibility of mixed viral and bacterial infection should be considered. When traditional respiratory tract tests are negative, molecular techniques such as BALF tNGS should be actively adopted to clarify the etiological diagnosis and guide precise treatment.

Keywords

Influenza A H1N1 Virus, Coinfection, Empyema, Targeted next-generation sequencing(tNGS), Bronchoalveolar Lavage Fluid, BALF

Abbreviations

CT: Computer Tomography; Kp: Klebsiella Pneumonia; tNGS: Targeted Next-Generation Sequencing; H1N1: Influenza A H1N1 Virus; BALF: Bronchoalveolar Lavage Fluid

Introduction

The Influenza A (H1N1) virus (hereinafter referred to as H1N1) is a significant infectious respiratory pathogen that has caused global epidemics on multiple occasions in history [1]. Since 2009, H1N1 pneumonia has also sporadically occurred in China [2]. H1N1 is an acute disease that affects the upper respiratory tract. It can cause inflammation in the upper respiratory tract, the trachea, and even the lower respiratory tract [3]. The incubation period of H1N1 is known to range from 1 to 4 days. The average incubation period for most people is approximately 2 days, while a few cases can last up to 7 days. The contagious period for adults typically begins about 1 day before the onset of symptoms and lasts for approximately 5 to 7 days after the patient shows symptoms. In individuals with a weaker immune system and children, the contagious period may be longer (1–2 weeks) [4].

The clinical manifestations of Influenza A (H1N1) are diverse, ranging from mild flu symptoms to severe respiratory symptoms and even death. It has been reported that after seasonal influenza infection, there may be severe bacterial infections. Studies have shown that there is a significant synergistic pathogenic effect between respiratory virus infections and bacterial infections. The risk of severe bacterial infections after seasonal influenza virus infection may be related to the following mechanisms: 1. The influenza virus causes damage and shedding of respiratory epithelial cells, providing channels for bacterial colonization; 2. Disruption of the function of the pulmonary surfactant barrier; 3. The shed cells provide nutrients for bacterial growth. In addition, the polymorphism of the influenza virus may change tissue tropism, promoting combined infections in the upper and lower respiratory tracts, thereby further increasing the incidence of viral and bacterial combined infections [5].

This case report describes a patient with a course of more than one month due to H1N1 infection combined with empyema and pulmonary infection. The infection was caused by H1N1 virus, *Fusobacterium nucleatum*, and *Klebsiella pneumoniae*. The disease course was long, and the patient eventually recovered after antibiotic treatment and antiviral treatment. Additionally, this article reviews the clinical characteristics and treatment strategies of this disease based on existing literature.

Case Presentation

A 49-year-old male patient presented to the emergency department with “chest pain for 1 month and fever for 2 days”. One month ago, the patient began to experience right chest and back pain, which worsened with deep breathing, accompanied by coughing and expectoration, mostly white mucus. At that time, he did not pay much attention and did not undergo further examination. He took painkillers for symptomatic treatment, but the symptoms progressively worsened. Therefore, he came to our outpatient department for treatment. A chest enhanced CT scan suggested “Encapsulated pleural effusion in the right lung”. The patient was admitted to the emergency

department and was treated with “ceftriaxone” for infection control for 1 day. After that, the patient developed a fever. On the 3rd day of treatment, both chest pain and fever did not alleviate. The patient had undergone surgery for "gastric perforation" due to alcohol consumption 10 years ago and “appendicitis surgery” 20 years ago.

Vital signs: body temperature 37.2℃, heart rate 126 beats per minute, respiratory rate 20 breaths per minute, blood pressure 122/88 mmHg, blood oxygen saturation 97%. Physical examination showed rapid breathing, asymmetrical breath sounds in both lungs, low breath sounds in the right lung, no dry or wet rales heard in both lungs, a 10-cm-long surgical scar in the middle of the abdomen, and a 5-cm-long surgical scar in the right lower abdomen. Other system examinations showed no obvious abnormalities.

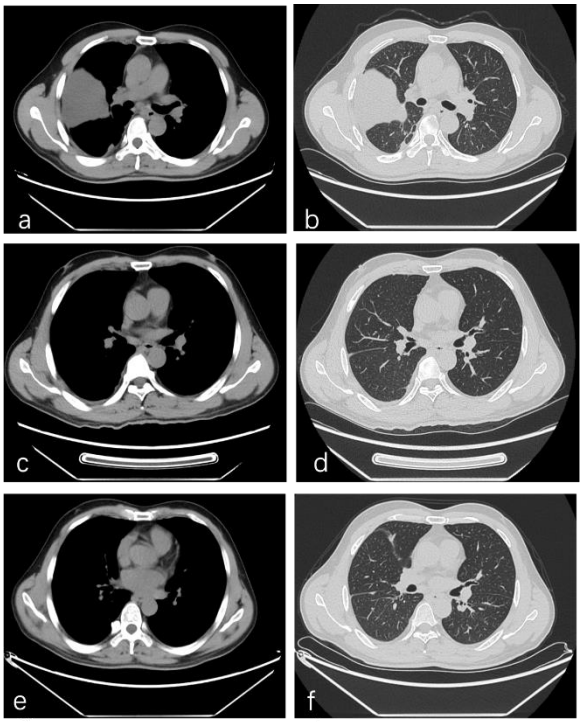

Fig-1:

Chest CT on admission showing pleural effusion combined with atelectasis. (a,b); Chest CT on day 17 showing less pleural effusion on the right side (c,d); Chest CT on day 30 days after discharge showing further less pleural effusion on the right side (e,f).

The enhanced CT scan of the chest (**Fig-1**) showed a large amount of effusion in the right thoracic cavity, partially encapsulated, with some of the fluid located in the interlobar fissure area, causing compression and poor gas retention of the right lung in multiple lobes. There were patchy nodules in the upper lobe of the right lung, suspected to be chronic inflammatory lesions or tumors. There were a few inflammatory lesions scattered in the right lung. Small nodules in both lungs were mostly inflammatory in nature, and there were increased and enlarged lymph nodes in the mediastinum. The right pleura was thickened.

Arterial blood gas analysis showed respiratory alkalosis, with pH 7.513, carbon dioxide pressure (PCO_2) 28.8 mmHg, oxygen pressure (PO_2) 159.9 mmHg, oxygen saturation (SPO_2) 99.9%. Laboratory tests showed a white blood cell count of 12.52 × 10^9/L, a neutrophil percentage of 73.4%, an absolute neutrophil value of 9.19 × 10^9/L, a lymphocyte percentage of 13.1%, and a lymphocyte absolute value of 1.67 × 10^9/L. Hemoglobin was 145 g/L, and platelets were 271 × 10^9/L. CRP was 191.78 mg/L. Total bilirubin was 26.9 μmol/L, direct bilirubin 7.8 μmol/L, indirect bilirubin 19.1 μmol/L, globulin 41.1 g/L, white-to-red cell ratio 0.98, high-density lipoprotein 0.94 mmol/L, alkaline phosphatase 172 U/L, gamma-glutamyl transpeptidase 129 U/L, creatine kinase 40 U/L, and serum potassium 3.29 mmol/L. The throat swab PCR tests for both H1N1 and influenza B virus were negative.

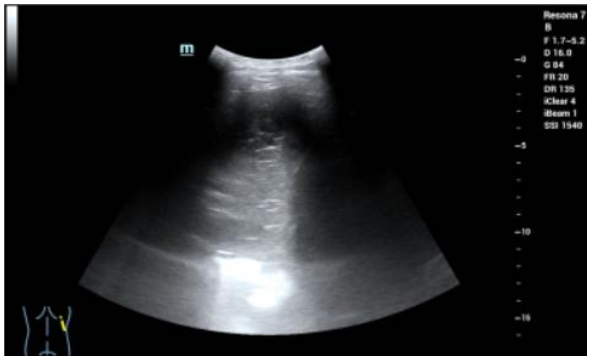

Fig-2:

Chest cavity color Doppler ultrasound on admission showing on the third day of treatment, there was encapsulated pleural effusion.

Coagulation function showed a prothrombin time of 12.9 seconds, fibrinogen of 9.57 g/L, and D-dimer of 3.21 mg/L FEU. Antibody IgG for *Mycoplasma pneumoniae* was >300 AU/ml, and IgG for *Chlamydia pneumoniae* was 163.9 AU/ml. Chest ultrasound of the thorax (**Fig-2**) suggested a maximum depth of approximately 3.0

cm of anechoic area below the 5th rib on the right side, with many septa visible and poor acoustic transmission; no obvious anechoic area was found on the left side. There was effusion in the septa of the right thorax. Procalcitonin was 0.14 ng/ml.

The emergency department administered ceftriaxone for anti-infection treatment. The patient's chest pain was relieved temporarily, but breathing difficulty gradually worsened, and there was still fever, with the highest temperature reaching 38.7℃. On the 4th day of "ceftriaxone" anti-infection treatment, the patient was admitted to the respiratory department for treatment.

During hospitalization, comprehensive nucleic acid testing for 13 respiratory viruses (influenza A, bocavirus, rhinovirus, parainfluenza virus, H1N1 [2009], H3N2, influenza B, *Mycoplasma pneumoniae*, coronavirus, respiratory syncytial virus, adenovirus, and *Chlamydia*) using sputum samples returned negative. Blood and sputum cultures were sterile. Urine protein qualitative test showed weak positive; urobilinogen was positive (+). Stool routine showed yeast-like positive result. Hepatitis B surface antibody was positive; hepatitis B e antibody and core antibody were semi-quantitatively positive. Hepatitis B surface antigen and e antigen were negative. HIV 24 antigen, antibody, and antigen-antibody combo tests were negative. Antibodies for *Treponema pallidum* and hepatitis C were negative. Tumor markers (CA-125, SCC, CEA, CA19-9, CYERA21-1, NSE) were all negative.

After 5 days of treatment, color ultrasound-guided thoracic puncture and catheter drainage along with pleural biopsy were performed. Pleural effusion smear and culture for bacteria and fungi, TB nucleic acid qualitative detection, and TB/RIF resistance gene detection (Xpert) were all negative. Thoracic fluid tNGS showed 9 sequences of *Fusobacterium nucleatum*, with a relative abundance of 49.9%. TB culture was negative. Exfoliated cell analysis revealed many neutrophils and lymphocytes. Pleural biopsy showed fibrous tissue hyperplasia with scattered chronic inflammatory cell infiltration and focal inflammatory exudation. Special stains: acid-fast (−), PAS (−), hexamine silver (−), TB qPCR (−).

Routine pleural effusion examination revealed turbid fluid with clots and ++ nucleated cells. Pus cells and 1780 × 10^6/L red blood cells were observed. Multinucleated cells accounted for 95%, mononuclear for 5%. No mesothelial cells were found. Biochemical analysis of pleural fluid showed: total protein 60.6 g/L, glucose 0.06 mmol/L, LDH 3271 U/L, ADA 42.0 IU/L, albumin 32.7 g/L — indicating exudate. Pus cells were confirmed, and tNGS again suggested *Fusobacterium nucleatum* (9 sequences, 49.9% abundance).

The patient continued on "ceftriaxone" for 2 more days' post-admission. On day 6, the patient had repeated fever and breathing difficulty, peaking at 39.4℃. Antibiotics were switched to "piperacillin-tazobactam". Fever peaks gradually decreased. Lab tests showed WBC 5.02 × 10^9/L, neutrophils 53.1%, platelets 295 × 10^9/L, hemoglobin 123 g/L, RBC 4.02 × 10^{12}/L. Interleukin-6 was 15.60 pg/ml, procalcitonin 0.060 ng/ml, CRP 21.65 mg/L. ALT 119 U/L, total protein 61.7 g/L, albumin 30.5 g/L, white/red ratio 0.98, uric acid 139 μmol/L, AST 104 U/L, ALP 150 U/L, GGT 138 U/L, urea 2.0 mmol/L, calcium 2.07 mmol/L, inorganic phosphorus 0.64 mmol/L, HDL-C 0.50 mmol/L.

Coagulation showed APTT 35.0 s, fibrinogen 7.50 g/L, FDPs 8.4 mg/L, D-dimer 3.58 mg/L FEU. Cardiac markers and BNP were normal. On day 7, antibiotics were upgraded to "piperacillin-tazobactam sodium". After 9 days, the patient no longer had fever. "Polyene phosphatidylcholine" and "magnesium isoglycyrrhizinate" injections were administered for liver protection. On day 12, the thoracic drainage tube was removed.

Treatment continued with "piperacillin-tazobactam sodium." On day 18, the patient experienced recurrent fever (up to 38.7°C). WBC was 4.91 × 10^9/L, neutrophils 73.3%, CRP 35.44 mg/L, albumin 32.8 g/L, potassium 3.36 mmol/L, IL-6 18.80 pg/ml, procalcitonin 0.110 ng/ml. COVID-19 and influenza antigen tests were negative. ESR was 102 mm/h.

On day 21, a painless fiberoptic bronchoscopy was performed. No abnormalities were found in the epiglottis or main bronchus. White sticky phlegm was observed in bilateral bronchi, cleared by suction. BALF was collected. Smear, culture, GM test, TB nucleic acid, fungal culture, and TB identification were all negative.

However, BALF tNGS revealed 1344 sequences of H1N1 (76.2% relative abundance) and 50 sequences of *Klebsiella pneumoniae* (0.3%).

On day 22, oseltamivir was added. Fever resolved. The combination therapy of "oseltamivir + piperacillin-tazobactam sodium" was continued. The patient's condition gradually improved. After 26 days of treatment, he was discharged.

Post-discharge, the patient was prescribed oral moxifloxacin and followed up regularly. At 28 days after discharge, chest CT (**Fig-1**) showed significant reduction in the right pleural effusion, which was partially encapsulated and largely absorbed. Laboratory data are provided in **Table-1**.

Discussion

Empyema refers to the presence of bacterial infection in the pleural cavity and the surrounding tissues. It is usually caused by insufficient treatment of pneumonia, increased pleural permeability, and the leakage of fluid into the pleural space, or it is related to the persistent inflammation of the pleura. It often presents with bacterial invasion, activation of immune cells in the pleural cavity, and the formation of fibrin clots in the pleural cavity. The typical clinical manifestations include coughing, expectoration, fever, and symptoms such as chest pain and breathing difficulties [6]. All cases of empyema were treated by thoracentesis, thoracic tube insertion, or surgical drainage [7].

The most common symptoms of patients infected with H1N1 are fever, dry cough, sore throat, headache, muscle or joint pain, chills, fatigue, diarrhea, and vomiting. These symptoms are similar to those of seasonal influenza. The H1N1 virus has two distinct characteristics: Firstly, it mainly affects children and young adults; secondly, young to middle-aged people can develop lower respiratory tract infections, followed by a rapidly progressing pneumonia [8,9].

Fusobacterium nucleatum is a Gram-negative anaerobic bacterium. It has specific species distribution in the human oral cavity, gastrointestinal tract, and other parts. For a long time, due to its frequent isolation and identification in anaerobic samples from different infected patients, *Fusobacterium nucleatum* has been regarded as an opportunistic pathogen [10]. Recent studies have shown that the detection rate of *Fusobacterium nucleatum* in thoracic infectious diseases has been gradually increasing, and at the same time, other pathogen infections may occur concurrently during thoracic infection [11]. This further complicates the severity of the condition.

In this case, we detected *Fusobacterium nucleatum* in the patient's pleural effusion through tNGS. The patient's immune status was related to this. When the patient's immune system was weakened, opportunistic pathogenic bacteria such as *Fusobacterium nucleatum* could cause pleural effusion and pulmonary inflammation, damaging the lung tissue, disrupting the

Table-1: Blood Investigations of the Patient

Laboratory tests	DAY-1	DAY-4	DAY-6	DAY-10	DAY-15	DAY-19
White blood (10^9/L)	12.52	8.82	N/A	5.02	7.13	4.91
Neutrophils (%)	73.40%	77.6	N/A	53.1	62.5	73.3
Lymphocytes (%)	13.3	10.5	N/A	32.7	26.1	14.5
Platelet count(10^9/L)	271	N/A	N/A	295	393	263
C-reactive protien(mg/l)	191.78	N/A	196.73	21.65	10.29	35.44
ALT(U/L)	46	N/A	121	119	78	31
AST9(U/L)	44	N/A	93	104	41	24
Albumin(g/l)	40.2	N/A	32.1	30.5	31.5	32.8
Globulin(g/l)	41.1	N/A	29.9	31.2	31.1	24.6
Albumin/Globulin	0.98	N/A	1.07	0.98	1.01	1.33
Procalcitonin(ng/ml)	N/A	0.14	N/A	0.06	N/A	0.11
Interleukin-6(pg/ml)	N/A	N/A	N/A	15.6	N/A	18.8

local immune microenvironment, and interfering with the normal functions of lung immune cells and immune factors, such as neutrophils and macrophages, which consume a large amount of energy and substances during the process of eliminating *Fusobacterium nucleatum*, resulting in the body being unable to make an effective immune response when facing H1N1 infection. The infection of *Fusobacterium nucleatum* triggers a febrile inflammatory response that continuously releases inflammatory mediators, affecting the functions of respiratory epithelial cells and endothelial cells, making it easier for H1N1 to adhere and invade cells, creating conditions for viral replication and spread, thereby prolonging the course of H1N1.

The microbiological diagnosis of pandemic H1N1 pneumonia is based on rapid and sensitive nucleic acid amplification tests of respiratory tract samples, mainly nasopharyngeal aspirates or nasopharyngeal swabs [12]. False negatives may be caused by incorrect collection, improper handling or transportation, or it could be due to a low viral load in the specimen [13]. Previous studies have reported cases of H1N1 infection where the virus was mainly detected in lower respiratory tract samples, but no virus was found or the viral level was very low in nasopharyngeal swab samples [14].

Common H1N1 mainly affects the upper respiratory tract or the trachea and bronchi. For some variant strains, the lower respiratory tract may be the preferred site of viral infection. The H1N1 virus not only has a preference for infecting tracheal and bronchial cells, but also for type I and type II lung cells, thereby causing extensive peeling of the epithelial layer [15]. A study reported that 18 patients diagnosed with the H1N1 influenza virus were admitted to the internal medicine and surgery ICU of a community hospital. Among them, 15 patients developed severe pneumonia. At the time of admission to the ICU, respiratory specimens were collected from the upper respiratory tract. The results of reverse transcription polymerase chain reaction (RT-PCR) were positive in 12 cases and negative in 3 cases. Bronchoalveolar lavage (BAL) tests were conducted on the positive patients, and the results were positive. Fiberoptic bronchoscopy is a safe procedure for critically ill patients and has no absolute contraindications. However, it is not recommended to use in cases of severe hypoxemia [13]. In a subgroup of 21 critically ill patients from Australia and New Zealand, the incidence of false negative results in upper respiratory tract samples of critically ill patients was recorded at 19%. All BAL samples of the patients were positive [16]. Another study recorded that the false negative rate of nasopharyngeal swabs was 10% [17].

Clinicians should consider conducting lower respiratory tract sample testing for patients with prolonged pulmonary infection treatment courses who have negative results from upper respiratory tract specimens. The detection rate of influenza by lower respiratory tract samples (BALF) is significantly higher than that by upper respiratory tract samples (throat swabs) [18]. The tNGS has been widely applied in the clinical diagnosis of many infectious diseases [12]. In a study, tNGS was performed on 18 samples, and it was possible to determine the consistent sequences of all viral genes with 100% coverage. The tNGS analysis results seemed not to be affected by the deviation of viral concentrations in each sample [19]. tNGS is a new tool that can rapidly and accurately identify potential pathogens. It can detect almost all pathogens from clinical samples with high sensitivity and accuracy and is particularly suitable for the diagnosis of rare, novel, and unknown pathogens, as well as those infectious diseases with atypical causes and complex conditions [20].

In this case, we repeatedly conducted throat swab antigen tests and sputum nucleic acid tests for H1N1, and all results were negative. We also collected sputum, BALF, and blood samples for smear and culture, but no pathogenic bacteria or fungi were found. The acid-fast staining of sputum and BALF was negative. Finally, through BALF submission for tNGS testing, we detected H1N1 and Kp. After adding oseltamivir antiviral treatment, the patient's condition was controlled. For patients with suspected H1N1 infection, when routine tests are negative, tNGS can be used as an effective supplementary detection method to help identify the cause early, guide clinical treatment, shorten the course of the disease, and improve the prognosis of the patients.

In this case, the patient presented with chest pain and

fever. Initially, there was a large amount of encapsulated pleural effusion on the right side. After empirical anti-infective treatment, the condition relapsed. Finally, through tNGS testing of pleural effusion, *Fusobacterium nucleatum* was positive, and through tNGS testing of bronchoalveolar lavage fluid (BALF), it was confirmed to be infected with H1N1 and Kp. The total course of the disease exceeded one month, which was significantly longer than the typical course of 1–2 weeks for common influenza A [4].

In the literature, a prolonged disease course is relatively rare. This might be related to the replication of the virus and immune evasion. Although the patient had no clear history of immunodeficiency, there were multiple indicators suggesting immune disorder. During the disease course, the increase in globulin (41.1 g/L) and the inversion of the white-to-globulin ratio (0.98) indicate that the patient had a chronic inflammation or immune activation state. Previous history of gastric perforation and appendicitis surgery may have led to potential impairment of the patient's immune function, increasing the risk of persistent viral infection. Literature reports on the clinical outcome analysis of H1N1 and non-H1N1 ARDS show that the positive rate of bacterial detection in H1N1-infected ARDS patients reached 52%, the proportion of receiving extrapulmonary support was higher, and the length of hospital stay was significantly longer [21].

This patient had concurrent pulmonary infection caused by *Fusobacterium nucleatum* (detected by thoracic fluid tNGS) and Kp (detected by BALF tNGS). The coexistence of bacterial and viral infections may interfere with the immune clearance process, prolonging the course of the disease. In this case, multiple upper respiratory tract PCR tests were all negative. The H1N1 was confirmed through BALF testing, indicating that the virus had invaded the lung parenchyma (positive in the lung lavage fluid), which might trigger a persistent inflammatory response within the lungs. At the same time, the viral infection might damage the respiratory mucosal barrier, leading to secondary bacterial infection (Pneumococcus, tNGS in the pleural effusion suggested), resulting in a synergistic damage between the virus and bacteria, causing the disease to persist.

The patient initially presented mainly with bacterial pleural effusion (pus cells and neutrophils accounted for 95%). Antimicrobial drugs such as ceftriaxone and piperacillin-tazobactam were used. Although the patient's condition was controlled for a short period, the viral infection was not intervened in time, and it was not until the 21st day of treatment that H1N1 infection was confirmed through bronchoscopy and oseltamivir was added, achieving virus clearance. This suggests that the absence of early viral diagnosis may delay targeted treatment and lead to a prolonged disease course.

In the diagnosis and treatment process of this case, although we could not clearly determine whether the H1N1, *Fusobacterium nucleatum*, and Kp were infected successively or simultaneously, for patients with persistent pleural effusion and repeated fever of unknown cause—especially those with unsatisfactory response to empirical anti-infection treatment—it is necessary to be vigilant of the possibility of mixed viral and bacterial infections. tNGS technology has advantages in the diagnosis of complex infection etiology and can provide important basis for clinical treatment adjustments. Early completion of respiratory virus testing (such as BALF tNGS) is necessary to avoid missed diagnosis of viral infection, shorten the patient's disease course, and improve prognosis.

Consent for Publication

Written informed consent was obtained from the patient's son for the publication of this case report and any accompanying images.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of West China Hospital of Sichuan University. Written informed consent was obtained from the individual and family members for the publication of any potentially identifiable images or data included in this article.

Data Availability Statement

The original contributions generated for the study are included in the article; further inquiries can be directed to the corresponding authors.

Conflict of Interest

The authors have read and approved the final version

of the manuscript. The authors have no conflicts of interest to declare.

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References

[1] Kong J, Hao Y, Wan S, Li Z, Zou D, Zhang L, Lu Y, Wang J, Chen X, Fu J. Comparative study of hematological and radiological feature of severe/critically ill patients with COVID-19, influenza A H7N9, and H1N1 pneumonia. J Clin Lab Anal. 2021 Dec;35(12):e24100. [**PMID**: 34762754]

[2] Kuang PD, Wang C, Zheng HP, Ji WB, Gao YT, Cheng JM, Ma JB, Liu YQ, Su MG, Ruan GX, Zhang MM. Comparison of the clinical and CT features between COVID-19 and H1N1 influenza pneumonia patients in Zhejiang, China. Eur Rev Med Pharmacol Sci. 2021 Jan;25(2):1135-45. [**PMID**: 33577070]

[3] Uyeki TM, Hui DS, Zambon M, Wentworth DE, Monto AS. Influenza. Lancet. 2022 Aug 27;400(10353):693-706. [**PMID**: 36030813]

[4] Jilani TN, Jamil RT, Nguyen AD, Siddiqui AH. H1N1 Influenza. 2024 Mar 4. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan–. [**PMID**: 30020613]

[5] McCullers JA. The co-pathogenesis of influenza viruses with bacteria in the lung. Nat Rev Microbiol. 2014 Apr;12(4):252-62. [**PMID**: 24590244]

[6] Addala DN, Bedawi EO, Rahman NM. Parapneumonic Effusion and Empyema. Clin Chest Med. 2021 Dec;42(4):637-47. [**PMID**: 34774171]

[7] Ampofo K, Herbener A, Blaschke AJ, Heyrend C, Poritz M, Korgenski K, Rolfs R, Jain S, Carvalho Mda G, Pimenta FC, Daly J, Mason EO, Byington CL, Pavia AT. Association of 2009 pandemic influenza A (H1N1) infection and increased hospitalization with parapneumonic empyema in children in Utah. Pediatr Infect Dis J. 2010 Oct;29(10):905-909. [**PMID**: 20407400]

[8] Karadeli E, Koç Z, Ulusan S, Erbay G, Demiroğlu YZ, Sen N. Chest radiography and CT findings in patients with the 2009 pandemic (H1N1) influenza. Diagn Interv Radiol. 2011 Sep;17(3):216-22. [**PMID**: 20703994]

[9] Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team; Dawood FS, Jain S, Finelli L, Shaw MW, Lindstrom S, Garten RJ, Gubareva LV, Xu X, Bridges CB, Uyeki TM. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. N Engl J Med. 2009 Jun 18;360(25):2605-15. Erratum in: N Engl J Med. 2009 Jul 2;361(1):102. [**PMID**: 19423869]

[10] Brennan CA, Garrett WS. Fusobacterium nucleatum - symbiont, opportunist and oncobacterium. Nat Rev Microbiol. 2019 Mar;17(3):156-66. [**PMID**: 30546113]

[11] Alvarez Otero J, Mandrekar J, Wolf MJ, Starkey JC, Carmona EM, Dyrhovden R, Kommedal Ø, Patel R. Pleural space infection microbiology as assessed using a clinical sequencing-based assay: *Fusobacterium nucleatum* group, *Streptococcus intermedius*, and other oral normal microbiota are the most common bacteria identified in community-acquired pleural space infections. J Clin Microbiol. 2024 Dec 11;62(12):e0069424. [**PMID**: 39584837]

[12] Gu W, Deng X, Lee M, Sucu YD, Arevalo S, Stryke D, Federman S, Gopez A, Reyes K, Zorn K, Sample H, Yu G, Ishpuniani G, Briggs B, Chow ED, Berger A, Wilson MR, Wang C, Hsu E, Miller S, DeRisi JL, Chiu CY. Rapid pathogen detection by metagenomic next-generation sequencing of infected body fluids. Nat Med. 2021 Jan;27(1):115-24. [**PMID**: 33169017]

[13] Estella A. Bronchoalveolar lavage for pandemic influenza A (H1N1)v pneumonia in critically ill patients. Intensive Care Med. 2010 Nov;36(11):1976-77. [**PMID**: 20689914]

[14] Yeh E, Luo RF, Dyner L, Hong DK, Banaei N, Baron EJ, Pinsky BA. Preferential lower respiratory tract infection in swine-origin 2009 A(H1N1) influenza. Clin Infect Dis. 2010 Feb 1;50(3):391-94. [**PMID**: 20047483]

[15] Gill JR, Sheng ZM, Ely SF, Guinee DG, Beasley MB, Suh J, Deshpande C, Mollura DJ, Morens DM, Bray M, Travis WD, Taubenberger JK. Pulmonary pathologic findings of fatal 2009 pandemic influenza A/H1N1 viral infections. Arch Pathol Lab Med. 2010 Feb;134(2):235-43. [**PMID**: 20121613]

[16] ANZIC Influenza Investigators; Webb SA, Pettilä V, Seppelt I, Bellomo R, Bailey M, Cooper DJ, Cretikos M, Davies AR, Finfer S, Harrigan PW, Hart GK, Howe B, Iredell JR, McArthur C, Mitchell I, Morrison S, Nichol AD, Paterson DL, Peake S, Richards B, Stephens D, Turner A, Yung M. Critical care services and 2009 H1N1 influenza in Australia and New Zealand. N Engl J Med. 2009 Nov 12;361(20):1925-34. [**PMID**: 19815860]

[17] Rello J, Rodríguez A, Ibañez P, Socias L, Cebrian J, Marques A, Guerrero J, Ruiz-Santana S, Marquez E, Del

Nogal-Saez F, Alvarez-Lerma F, Martínez S, Ferrer M, Avellanas M, Granada R, Maraví-Poma E, Albert P, Sierra R, Vidaur L, Ortiz P, Prieto del Portillo I, Galván B, León-Gil C; H1N1 SEMICYUC Working Group. Intensive care adult patients with severe respiratory failure caused by Influenza A (H1N1)v in Spain. Crit Care. 2009;13(5):R148. [**PMID**: 19747383]

[18] Torres A, Niederman MS, Chastre J, Ewig S, Fernandez-Vandellos P, Hanberger H, Kollef M, Li Bassi G, Luna CM, Martin-Loeches I, Paiva JA, Read RC, Rigau D, Timsit JF, Welte T, Wunderink R. International ERS/ESICM/ESCMID/ALAT guidelines for the management of hospital-acquired pneumonia and ventilator-associated pneumonia: Guidelines for the management of hospital-acquired pneumonia (HAP)/ventilator-associated pneumonia (VAP) of the European Respiratory Society (ERS), European Society of Intensive Care Medicine (ESICM), European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and Asociación Latinoamericana del Tórax (ALAT). Eur Respir J. 2017 Sep 10;50(3):1700582. [**PMID**: 28890434]

[19] Takayama I, Nguyen BG, Dao CX, Pham TT, Dang TQ, Truong PT, Do TV, Pham TTP, Fujisaki S, Odagiri T, Hasegawa H, Nakajima N. Next-Generation Sequencing Analysis of the Within-Host Genetic Diversity of Influenza A(H1N1)pdm09 Viruses in the Upper and Lower Respiratory Tracts of Patients with Severe Influenza. mSphere. 2021 Jan 6;6(1):e01043-20. [**PMID**: 33408229]

[20] Chen Y, Feng W, Ye K, Guo L, Xia H, Guan Y, Chai L, Shi W, Zhai C, Wang J, Yan X, Wang Q, Zhang Q, Li C, Liu P, Li M. Application of Metagenomic Next-Generation Sequencing in the Diagnosis of Pulmonary Infectious Pathogens From Bronchoalveolar Lavage Samples. Front Cell Infect Microbiol. 2021 Mar 11;11:541092. [**PMID**: 33777827]

[21] Töpfer L, Menk M, Weber-Carstens S, Spies C, Wernecke KD, Uhrig A, Lojewski C, Jörres A, Deja M. Influenza A (H1N1) vs non-H1N1 ARDS: analysis of clinical course. J Crit Care. 2014 Jun;29(3):340-46. [**PMID**: 24508203]

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