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Sirolimus combined with oseltamivir and corticosteroid treatment for a puerpera with severe pneumonia caused by 2009 pandemic H1N1: A case report

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ABSTRACT

Severe pneumonia in patients infected with the 2009 pandemic H1N1 (pH1N1) virus was partially attributed to excessive immune response. Anti-virus treatment for these patients was insufficient. Here we reported the therapy effect of sirolimus, an immunosuppressor, combined with oseltamivir and corticosteroid for a puerpera with severe pneumonia caused by pH1N1 virus. This patient has infected with the pH1N1 virus in late pregnancy, and antiviral therapy was not implemented timely. She developed severe pneumonia and ARDS rapidly and need receive a cesarean section on the 39th week after pregnancy. After giving birth to a healthy baby, she received a combination of oseltamivir, sirolimus and corticosteroid, and improved in the following days. Moreover, the cytokines in serum and viral loads in BALF decreased significantly. She recovered without infectious symptoms and was discharged. Sirolimus combined with oseltamivir and corticosteroid is likely responsible for lowering the viral loads, reducing the patient's cytokine level, and further improving her clinical outcomes. It provides evidence that adjuvant treatment was beneficial to patients with severe pneumonia induced by the pH1N1 virus.

1. Introduction

Pneumonia is the most common complication of pH1N1 infection, which can further develop into aggressive respiratory failure, acute respiratory distress syndrome (ARDS), and fatal outcomes, especially during pregnancy and the early postpartum period [1,2]. “Cytokine storm” induced by hyperactive immune has been reported in patients with severe influenza pH1N1 [3,4]. Although early antiviral therapy with a neuraminidase inhibitor (NAI) is associated with improved outcomes in patients hospitalized with influenza, a significant number of deaths still occurred [5]. Therefore, other therapeutic regimens such as immunoregulation should be considered to decrease morbidity and mortality. It has been reported that sirolimus, an immunosuppressor, combined with oseltamivir and corticosteroid treatment, effectively improved the outcome of patients with severe pH1N1-induced pneumonia and respiratory failure [6]. To our knowledge, this is the only clinical trial about sirolimus administration for the patients with severe pH1N1 infection so far, without using puerpera. Here we reported a puerpera with severe pH1N1-induced pneumonia recovered

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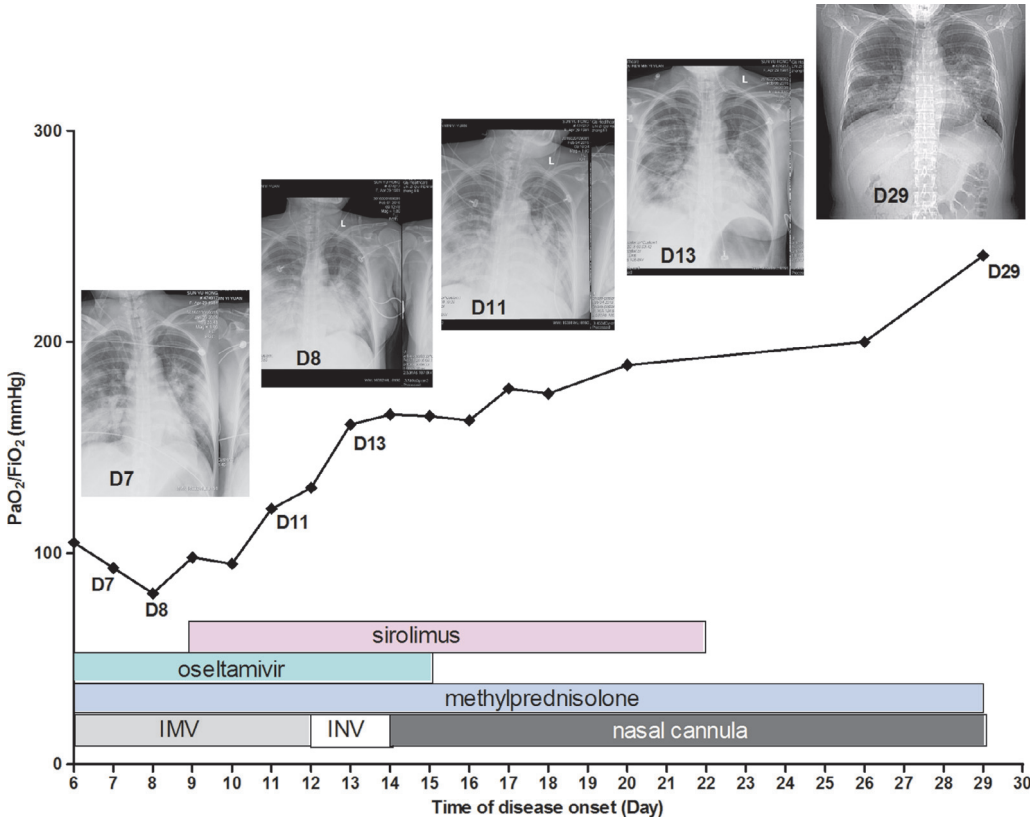


Fig. 1. The oxygenation index variation and the parallel respiratory support treatment, chest X-ray, methylprednisolone, oseltamivir, and sirolimus administration in the puerpera during the therapy process. IMV = invasive mechanical ventilation, INV = intermittent non-invasive ventilation.

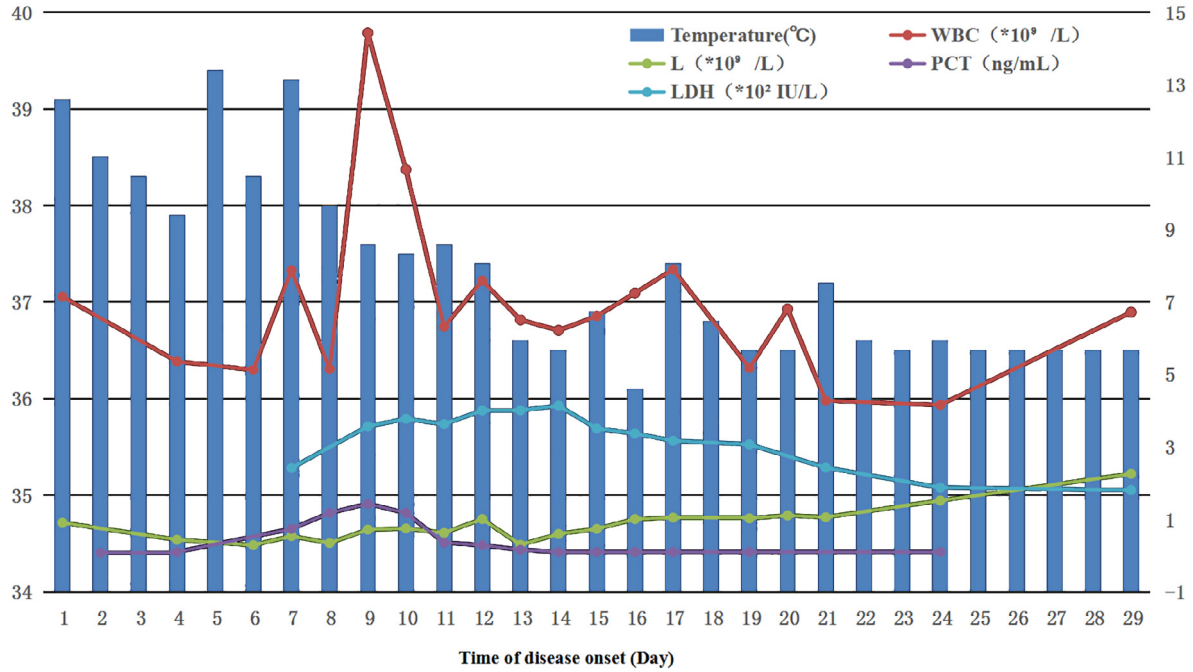


Fig. 2. The change of leading infection indicators of the patient from admission to discharge.WBC = white blood cell (normal range: 3.5×10^9–9.5×10^9/L), PCT = procalcitonin (normal range: 0–0.5 ng/mL), L = lymphocyte (normal range: 1.1×10^9–3.2×10^9/L), LDH = lactate dehydrogenase (normal range: 1.09×10^2–2.45×10^2U/L).

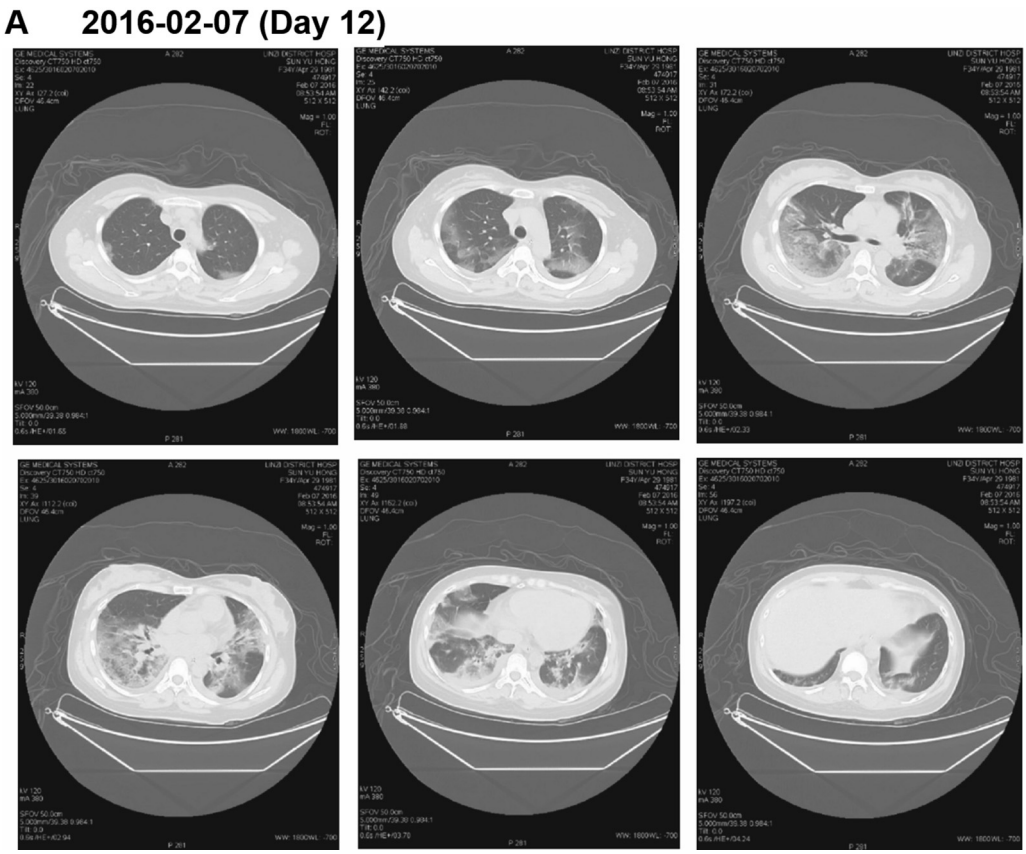


Fig. 3. Lung computed tomography test of the patient. **(A)** On Day 12, lung computed tomography revealed a large patch of ground-glass opacity. **(B)** On Day 19, it also showed a large patch of ground-glass ambiguity but absorbed partially than before. **(C)** On Day 25, a large patch of ground-glass opacity also existed, but it was allivated compared with the CT scan of the lung on day 19. **(D)** On Day 44, the date of the patient re-examination, lung computed tomography showed patchy and streaky opacity, which improved significantly than that on Day 25.

by the sirolimus treatment combined with oseltamivir and corticosteroid.

2. Case report

A 34-year-old pregnant woman with cough and fever was admitted to the Obstetric ward at Linzi District People's Hospital, Shandong Province, on Jan 25th, 2016 (Day 1). The patient was pregnant for 35 weeks and four days. She had a dry and itchy nose three days ago, with a cough but no sputum production. Fever emerged 5 h ago with the highest temperature of 39.1 °C. In the following four days, she developed excess sputum, shortness of breath, and tonsil swelling. Blood tests showed a normal white blood cell (WBC) count (5.46×10^9/L) but decreased lymphocyte count (0.36×10^9/L) and increased CRP (60.4 mg/L) level. Arterial blood gas test exhibited the following results: pH 7.18 (the normal ranges, 7.35–7.45), partial pressure of carbon dioxide 53 mmHg (the normal ranges, 35–45 mmHg), partial pressure of oxygen 105 mmHg (the normal ranges, 80–100 mmHg) and PO_2/FiO_2 105 mmHg (the normal ranges, 400–500 mmHg). Though ceftriaxone (2 g/day, iv drip), dexamethasone (2 mg/d, iv drip), budesonide (4 mg/d, inh), oxygen therapy, and other supportive treatments were given, her high fever stilled progressed. Worst still, Color Doppler Ultrasound showed reduced amniotic fluid and poor intrauterine environment in the patient. She succeeded in the cesarean section surgery and gave an infant in good condition on Jan 30th (Day 6). The patient was admitted to the Intensive Care Unit (ICU) for her persistent high fever (with a body temperature of 39.1 °C), the requirement of mechanical ventilation for severe acute respiratory distress syndrome (ARDS). Chest radiograph showed bilateral pulmonary patchy clouding opacity, and a large amount of bloody sputum was suctioned by trachea cannula. The patient was given oseltamivir (150 mg/12 h), broad-spectrum antibiotic (Sulperazone, 3 g/8h, iv drip and linezolid, 0.6 g/12 h iv drip), antifungal drugs (voriconazole, 0.2 g/d), methylprednisolone (80 mg/d, iv drip) and human immunoglobulin (12.5 g/d iv drip) for anti-infection therapy immediately. Subsequently, pH1N1 virus infection was laboratory-confirmed on a pharyngeal swab using real-time polymerase chain reaction assay (DAAN Gene, Guangzhou, China) on Day 7. Considering that pH1N1 virus infection was known to induce cytokine storm and excessive inflammation, combinations of antiviral and immunomodulatory therapies would be beneficial in treating severe influenza pneumonia, sirolimus (2 mg/d, nasogastric tube feeding) was added on Day 9. By implementing the combination therapy, the patient's clinical manifestation improved gradually, finally recovered, and was discharged on Feb 21st, 2016 (Day 28). Eighteen days later (Day 47), re-examination showed normal blood indices, arterial blood gas, and improving chest radiograph.

Oseltamivir was stopped on Day 15; Sirolimus was stopped on Day 22; methylprednisolone was reduced to 4 mg/d before discharge and stopped on Day 44. Also, invasive ventilation weaned off on Day 15, with resolved chest X-ray manifestation indicated by the upward trend of PO_2/FiO_2 (Fig. 1). Inflammation infection-related indicators, such as

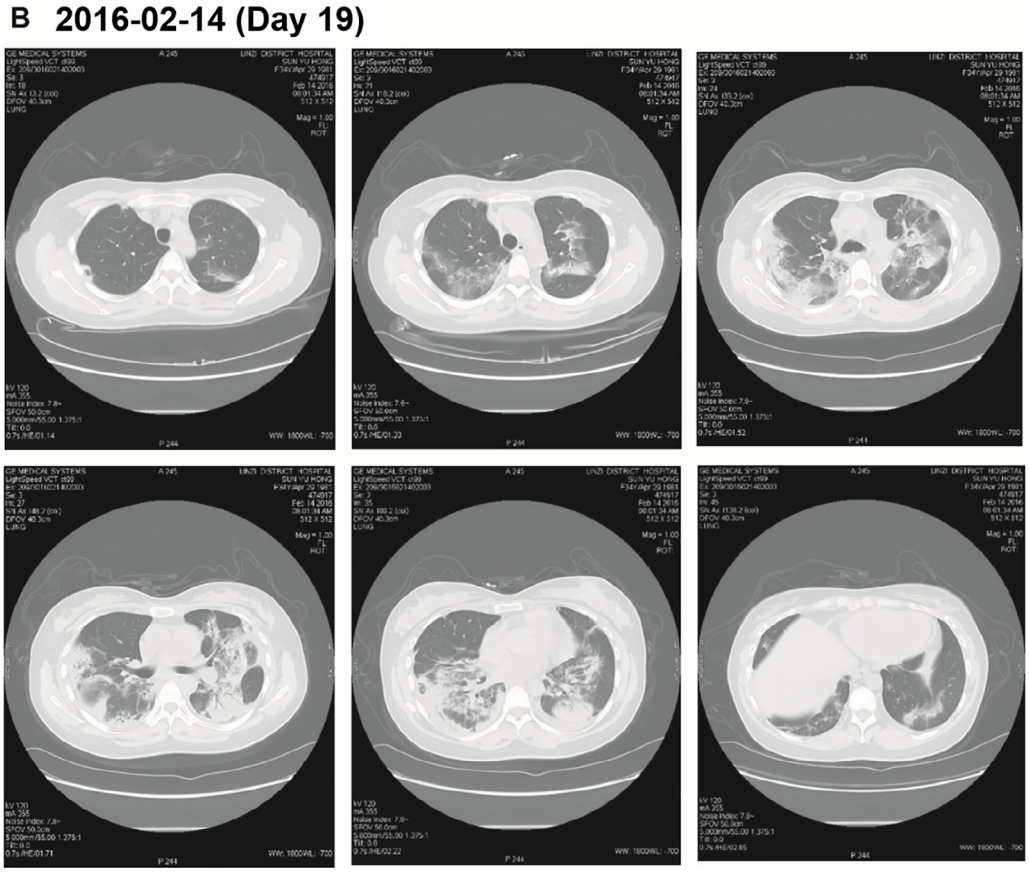


Fig. 3 (*continued*)

body temperature and procalcitonin, were prone to return to an average level (Fig. 2). Besides, Computed tomography (CT) showed persistent absorption of pulmonary inflammation as well (Fig. 3). Furthermore, serum cytokines including IL-6, IL-10, IL-15, IL-21, INF-γ, and MIP-3a decreased after antiviral therapy, though some increased again on Day 8. Therefore, sirolimus was added on Day 9, IL-6, IL-10, IL-12, IL-15, IL-17, IL-13, IL-21, IL-27, INF-γ, TNF-a, and MIP-3a in serum were reduced gradually to become regular with the combined oseltamivir and sirolimus therapy (Fig. 4).

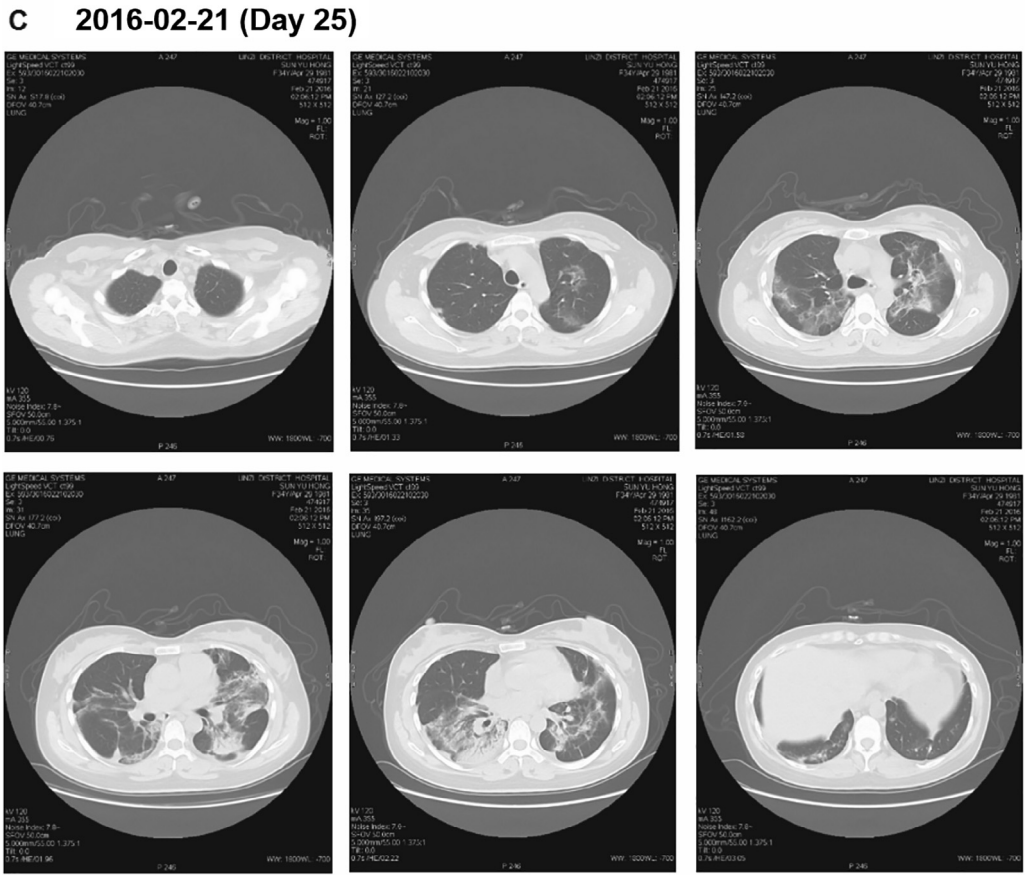
Hypokalemia, diarrhea, transient elevation of serum triglycerides, hypercholesterolemia, and hyperlipemia are the common side-effects of sirolimus. While during the two weeks treatment, only transient elevation of lactate dehydrogenase was observed in this patient but rapidly returned to normal after sirolimus withdrawal.

Secondary infection of bacteria was investigated by repeated bacterial culture of sputum, blood, and bronchoalveolar lavage fluid (BALF) sample from this patient, but no pathogenic strain was isolated. At the same time, metagenomic sequencing of BALF and blood samples showed *Pseudomonadaceae* (PAE) sequences. The reads amount variation of PAE and influenza virus was the same during the treatment. Both could not be detected in BALF when the patient was discharged (on Days 15 and 21) but re-emerged on Day 44, which might be associated with sirolimus's immunosuppressive effect. Besides, Paramyxoviridae, adenoviridae or nidovirale, retroviridae, etc. were also detected in BALF. But both of them had no significance after clinical evaluation, which included symptoms, signs, imaging tests and laboratory detection, etc. Sequence amount of PAE in blood was not affected by a combined therapy (Fig. 5). Therefore, more pathogens could be detected by metagenomic sequencing than traditional tests, but clinical evaluation should determine their pathogenicity.

3. Discussion

Pregnant women were considered at high risk for influenza virus infection and influenza-associated death, especially in the third trimester of pregnancy. Therefore, antiviral therapy with oseltamivir within 48 h from symptom onset could benefit the clinical efficacy [2,7]. However, oseltamivir often is administrated beyond the recommended window in clinical practice. This case involved a pregnant woman with severe pH1N1 infection and was rescued successfully by sirolimus along with oseltamivir and corticosteroid. As far as we know, this is the first case report on adjuvant sirolimus treatment for a pregnant woman with pH1N1 virus-induced severe pneumonia.

Sirolimus, also known as rapamycin, is an inhibitor of kinase mTOR (mammalian target of rapamycin) which plays a critical role in virus replication and regulating host innate and adaptive immune system [8]. A prospective clinical study indicated that adjuvant therapy with sirolimus, oseltamivir, and corticosteroids effectively treated pH1N1 patients with ARDS and respiratory failure [6]. Our case also demonstrated the process of a pregnant woman with severe pneumonia recovered by sirolimus, oseltamivir, and corticosteroid administration,



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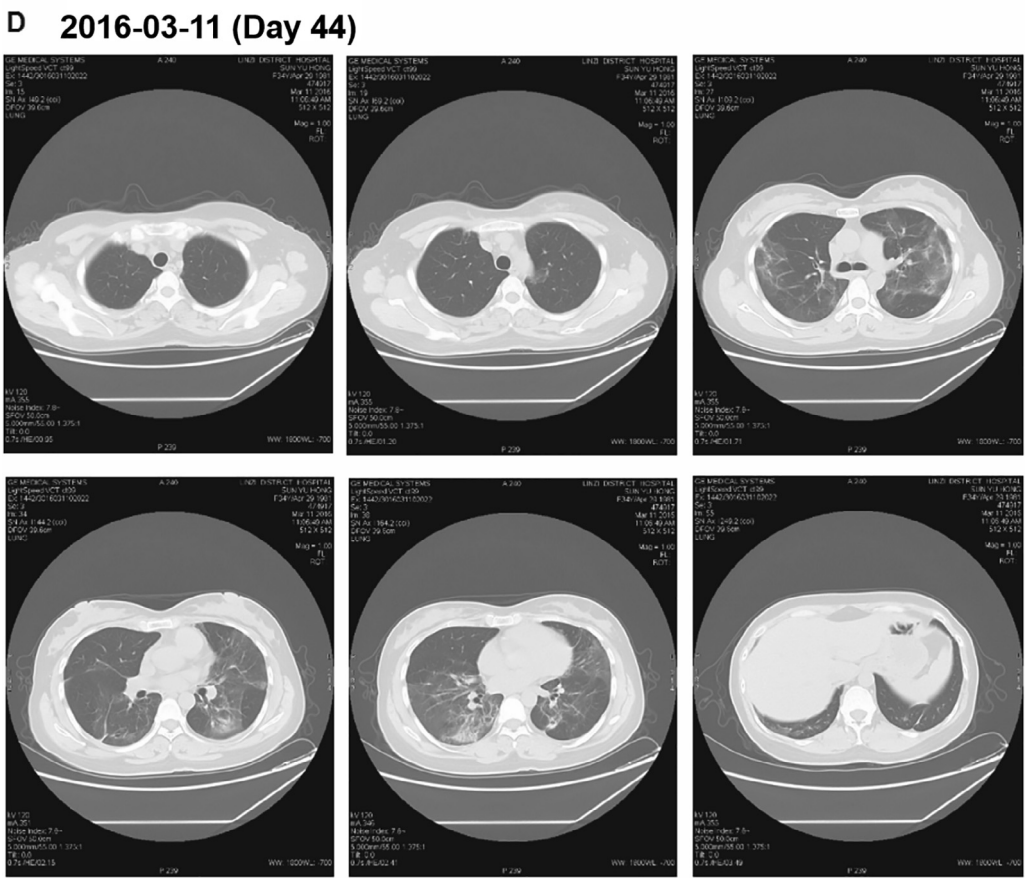
indicating that sirolimus was beneficial to the patients with severe viral pneumonia. Besides, we revealed that delayed oseltamivir plus sirolimus treatment protects mice against lethal pH1N1 infection by attenuating severe lung injury. Furthermore, the mechanism inhibited NLRP3 inflammasome activation and inflammatory response cell infiltration [9]. However, Alsuwaidi et al [10] claimed that sirolimus treatment exacerbates respiratory function by increasing viral titer and worsening lung inflammation. But in their study, oseltamivir was not used. Therefore, the adverse outcome was regarded as viral replication due to immunosuppression.

Abnormal "cytokine storm" plays a critical role in influenza-induced severe pneumonia. High levels of pro-inflammatory cytokines have been reported in patients with severe pH1N1 virus infection. These cytokines may be induced by the viral infection (primary cytokines), such as type I and III interferons, or immune response (secondary cytokines), including INF-γ, IL-10, etc. [11]. Corticosteroids have been suggested as an anti-inflammatory treatment for early ALI/ARDS induced by influenza infection. However, other studies showed that high doses or sustained corticosteroids might be harmful to these patients by increasing viral load and secondary bacterial infection [12,13]. In our case, methylprednisolone was given after cesarean section (Day 6), the dose was reduced gradually from 80 to 0 mg/d. Oseltamivir was also administrated immediately after operation. Cytokines in serum were progressively reduced to an average level with the combined therapy. Sirolimus inhibits cytokines production is associated with the mTOR signaling pathway, which has a vital role in regulating pro- and anti-inflammatory responses in innate immune cells. Blocking mTOR or its downstream signaling molecules impairs the production of type I IFN from plasmacytoid DCs [14]. Inhibiting mTOR also suppressed TNF-α and IL-6 in serum, alleviated lung injury, and improved mouse survival with LPS induced ALI [15–17]. Further research was needed to elucidate the mechanism of sirolimus inhibiting cytokine expression in patients with severe pneumonia caused by pH1N1 infection.

Influenza virus infection increases the risk of second bacterial infection by causing impaired immune responses in the lung [18–20]. In our case, no bacteria were detected by traditional bacteria or fungi culture, but metagenomic sequencing identified PAE sequence in blood and BALF. Because the patient in our case had been discharged without infection-related symptoms when the sequencing results were made, no antibiotic was specially used to target the PAE. Still, it could be covered by the broad-spectrum antibiotics empirically given to this patient. Consequently, the PAE sequence number in BALF was gradually decreased to zero, while its percentage showed relatively small variation in blood, which we evaluated as no clinical significance.

Ethics statement

The ethics committee approved this case study of Linzi District People's Hospital, Zibo City, Shandong Province, China, and written informed consent was obtained from the patient to publish this case report.



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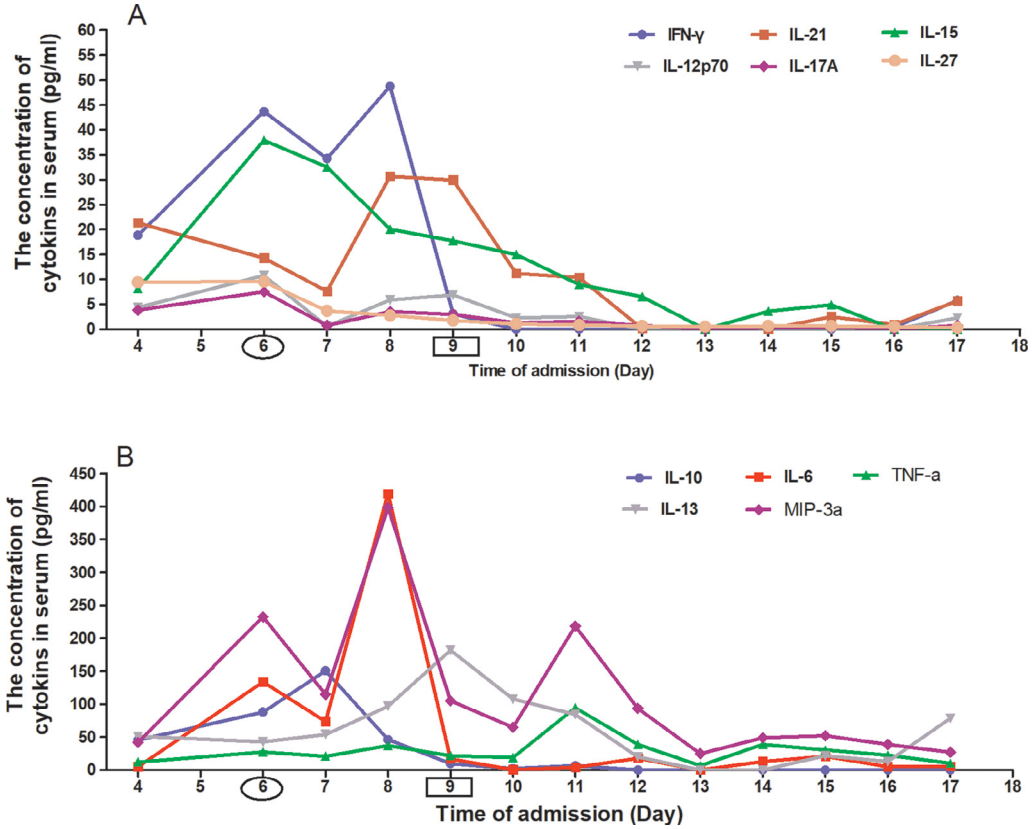

Fig. 4. Serum cytokines variation of the patient during her hospitalization. **(A)** The variation of INF-γ, IL-21, IL-15, IL-12p70, IL-17A, and IL-27 in serum. **(B)** The variation of IL-10, IL-6, TNF-α, IL-13 and MIP-3α in serum. The number in the ellipse and rectangle meant the day that oseltamivir and sirolimus were given, respectively.

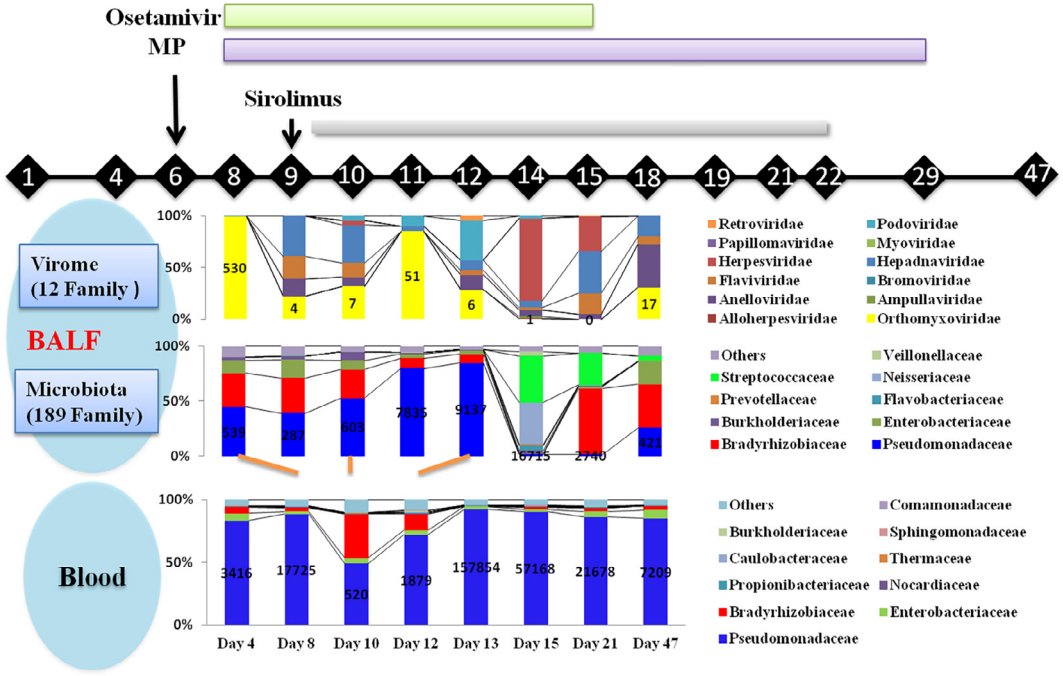

Fig. 5. The sequence variation assigned to bacteria and viruses in BALF and blood during the patient's therapy process detected by metagenomic sequencing. MP = methylprednisolone. The three rectangular boxes above present the period of oseltamivir, sirolimus, and methylprednisolone administration, respectively. The yellow bar represents the influenza H1N1 virus; the blue bar presents P. aeruginosa.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

Author contributions

Lili Ren, Bo Liu and Bin Cao: Conceptualization, Methodology, Writing - Review & Editing. **Lijun Suo, Xiaofeng Yu, Yongfeng Hu and Hongyun Cao:** Data Curation, Writing - Original Draft. **XiaoHui Zou:** Formal Analysis. **Peiquan Wang, Tao Xu, Xiangzhi Zhou and Yexin Wu:** Investigation, Writing - Review & Editing.

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