

## Original Article

# Application of metagenomic next-generation sequencing (mNGS) combined with rapid on-site cytological evaluation (ROSCE) for the diagnosis of *Chlamydia psittaci* pneumonia

Qian Zhang\*, Shuo Li\*, Wei Zhou, Liwen Zheng, Yi Ren, Lixia Dong, Jing Feng, Jie Cao

Department of Respiratory and Critical Care Medicine, Tianjin Medical University General Hospital, Tianjin 300052, China. \*Equal contributors.

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**Abstract:** We evaluated the application of metagenomic next-generation sequencing (mNGS) combined with rapid on-site cytological evaluation (ROSCE) in the diagnosis of *Chlamydia psittaci* pneumonia, to provide a basis for an accurate diagnosis. Clinical data from three patients with *C. psittaci* pneumonia diagnosed by the combination of mNGS and ROSCE from June 2019 to June 2020 in the Department of Respiratory and Critical Care Medicine of Tianjin Medical University General Hospital were reviewed. Three patients with community-acquired pneumonia failed to respond to the initial treatment, and were finally diagnosed by bronchoscopic lung biopsy and alveolar lavage fluid (BALF) mNGS. According to the ROSCE cytologic characteristics, the scope of the lesions was narrowed to inflammatory lesions excluding tumor and other non-infectious lesions. Subsequently, mNGS results confirmed that the three patients were infected by *C. psittaci*, sequence numbers 2066, 126, and 1077, respectively. Two patients developed severe pneumonia and required organ function support. The other patient had recurrent high fever and severe headache, which significantly complicated clinical diagnosis and treatment. Eventually, the treatment plan was adjusted according to the mNGS results, resulting in gradual improvement of symptoms and satisfactory prognosis. mNGS combined with ROSCE is effective for the accurate diagnosis and treatment of *C. psittaci* pneumonia, having significant advantages in comparison with other detection methods, particularly in the cases of rare pathogens, mixed pathogen infections, and immunodeficient patients.

**Keywords:** Metagenomic next-generation sequencing (mNGS), rapid on-site cytological evaluation (ROSCE), *Chlamydia psittaci* pneumonia

## Introduction

*Chlamydia psittaci* is an intracellular parasitic pathogen. After inhalation into the lung, the pathogen enters the blood, followed by proliferation in the mononuclear phagocytic system in the liver and spleen, finally spreading through the circulation to the organs of the entire body, including the lung, liver, spleen, kidney, and central nervous system [1]. Pneumonia caused by *C. psittaci* is a zoonotic disease, disseminated mostly by the dust of excreta of parrots, pigeons, and canaries. Additionally, chickens and peacocks can carry this pathogen and cause disease transmission, as well as the possibility of interpersonal transmission [2, 3].

With the recent increase in the number of pet birds, *C. psittaci* infections are on the rise. The detection methods for *C. psittaci* are limited and time-consuming. The detection of antibodies against *Chlamydia* provides a retrospective diagnosis, and the absence of an early test delays the treatment of the disease. In addition, considerable drug resistance of *C. psittaci* complicates clinical treatment, negatively affecting the prognosis and the cost of therapy. Therefore, rapid and timely diagnosis is the key to the successful management of *C. psittaci* pneumonia.

At present, the commonly used methods for the detection of pathogens utilize body fluid smears

and cultures. However, these traditional techniques are time-consuming and many are characterized by a low positive rate and frequent false-positive results. Therefore, if no positive results are obtained using conventional detection methods, clinicians desire to quickly and accurately identify the pathogenic bacteria and provide an appropriate and timely anti-infection treatment to avoid adverse outcomes. This is especially true for rare diseases, critically ill patients, and immunodeficient patients, and early diagnosis is essential. In this report, we review three cases of *C. psittaci* pneumonia treated in our department during the last year. We have analyzed the role of metagenomic next-generation sequencing (mNGS) and rapid on-site cytological evaluation (ROSCE) in the diagnosis and treatment of this disease and summarized the steps of the procedure and clinical significance of these technologies.

### Medical records

Case 1, an 83 year-old female, was admitted to the hospital on November 21, 2019, due to fever and wheezing lasting for six days. She had a history of cerebral infarction in 2015, and an occasional cough when she was eating and drinking water. The patient had had fever for six days before admission, with the highest temperature of 39°C, accompanied by headache, runny nose, sore throat, whole-body ache, and weakness. Additionally, she was experiencing nausea and vomiting, occasional cough, expectoration, wheezing, and a small amount of white sticky sputum. She could lie on her back at night without edema of her lower limbs. After admission, the patient received cefixime, moxifloxacin, and tazocin for the infection and dexamethasone as an antipyretic treatment. Intermittent fever was still present, especially in the afternoon, with the body temperature reaching 41°C. Physical examination on admission: temperature 39°C, heart rate 120 bpm, respiratory rate 25 bpm, and blood pressure 171/80 mmHg. The respiratory sounds of both lungs were thick, and moist rales could be heard in the right lung. Blood gas analysis documented type I respiratory failure (inhaled oxygen concentration: 83%, pH 7.42,  $\text{paO}_2$  63.90 mmHg,  $\text{pCO}_2$  30.80 mmHg,  $\text{HCO}_3^-$  19.50 mmol/L). Routine blood testing showed the following: white blood cells  $13.86 \times 10^9/\text{L}$ , neutrophils 94.9% (high), C-reactive protein (CRP)

21.80 mg/dL (high), procalcitonin 10.31 ng/mL (high), plasma D-dimer > 10000 ng/mL (high), and B-type natriuretic peptide (BNP) 2160.0 pg/mL (high). Additional laboratory tests indicated: IgM antibody against *Legionella pneumophila* negative, IgG antibody against *Legionella pneumophila* negative, *Chlamydia pneumoniae* antibody negative, *Mycoplasma pneumoniae* antibody negative, fungi (1-3)- $\beta$ -D glucan < 37.5 pg/mL, *Aspergillus galactomannan* < 0.25 ug/L; antinuclear antibody positive (nuclear granule type 1:80), anti-SSA antibody positive, anti-Ro-52 antibody positive, rheumatoid factor 24.50 IU/ml (high), and anti-neutrophil cytoplasmic antibody (ANCA) negative. The levels of tumor markers were as follows: ferritin 529.25 ng/mL (high), cytokeratin 19 fragment 7.17 ng/mL (high), squamous cell carcinoma antigen 3.30  $\mu\text{g}/\text{L}$  (high), and human epididymal epithelial secretory protein 4 (HE4) 249.20 pmol/L (high). Chest CT (**Figure 1**) showed a large area of increased density in the upper lobe of the right lung and multiple patchy consolidations in the middle and lower lobes of the right lung. Diagnosis at admission indicated severe community-acquired pneumonia (SCAP), type I respiratory failure, and heart failure (heart function grade IV). The patient was given piperacillin-tazobactam 4.5 g every eight hours and moxifloxacin 0.4 g daily as an empirical broad coverage anti-infection therapy. Bronchoscopy, sputum smear, and culture of airway secretions, rapid on-site cytological evaluation of lung tissue by transbronchial lung biopsy (TBLB), and second-generation sequencing of the bronchoalveolar lavage fluid (BLAF) were performed. The results of ROSCE (**Figure 2**) revealed the presence of fibroblasts, neutrophils, macrophages, monocytes, and lymphocytes. Inflammatory lesions were identified, and an organizing pneumonia trend was observed; however, tumor lesions and other non-infectious diseases were definitively excluded. mNGS detected the presence of *C. psittaci* DNA (sequence number 2066). In view of the results of the tests, the treatment plan was adjusted to moxifloxacin (0.4 g per day) and doxycycline enteric-coated capsules (0.1 g every 12 hours). Additionally, nasal high-flow ventilation assisted respiration was initiated, medications for heart failure and hypoproteinemia were given, and nutritional support was provided. The patient's temperature gradually decreased, and the respiratory function improved. After eight

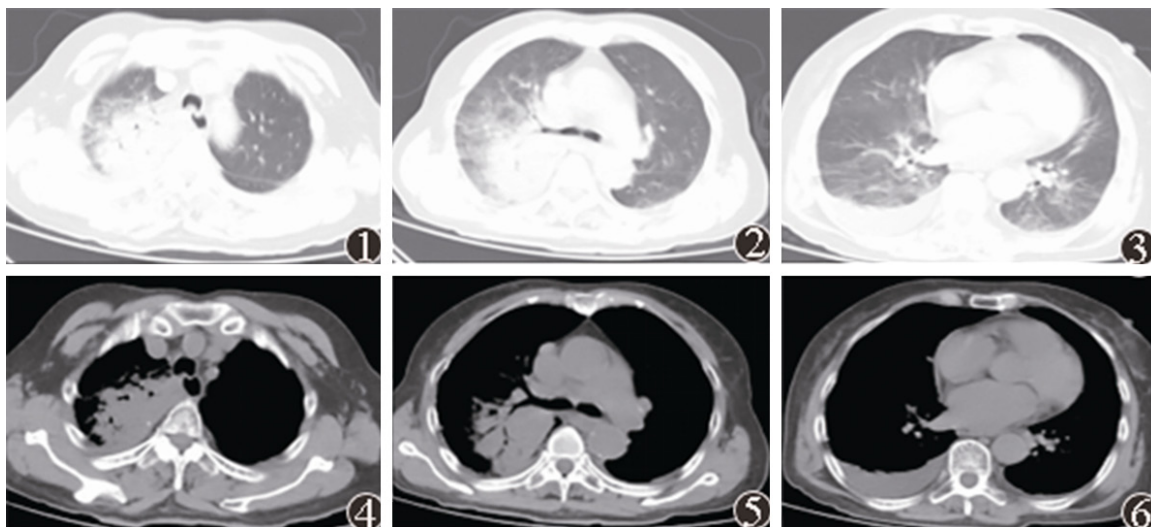


Figure 1. Case 1: chest CT at admission (November 21, 2019).

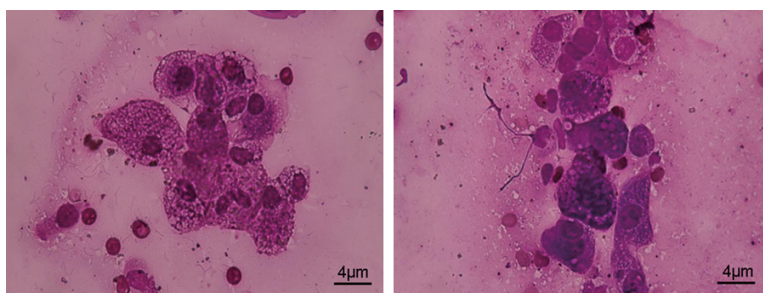


Figure 2. Case 1: ROSCE results.

days of treatment, a chest CT (Figure 3) scan demonstrated that the lesions were clearly absorbed. One month after discharge, the patient was free from fever, cough, and expectoration.

Case 2, a 60 year-old male, was admitted to the hospital on September 30, 2019 for “fever for one week”. He had a history of acute glomerulonephritis 40 years previously that was cured and a history of hypertension for 10 years that was well controlled. Fifteen years previously, he had had drainage of a liver abscess, and six months previously, surgery for a right femoral shaft fracture that was healing well. One week before admission, the patient developed fever with a temperature of 38.6°C and headache, but no other associated symptoms. After treatment with cephalosporins, the fever returned. Lung CT examination in the hospital showed a consolidation shadow in the middle lobe of the right lung. After treatment with moxifloxacin

and methylprednisolone for three days, he continued to have a recurrent fever. Physical examination: temperature 38.6°C, heart rate 100 bpm, respiratory rate 22 bpm, and blood pressure 140/80 mmHg. Breath sounds were thick in both lungs, and moist rales could be heard in the right lung. Supplementary examinations included routine blood testing which showed

a white blood cell count of  $5.92 \times 10^9/L$ , neutrophils 75%, and CRP 13.3 mg/dL. Additional laboratory tests indicated: *Legionella pneumophila* IgG antibody suspected positive, *Mycoplasma pneumoniae* antibody negative, *Chlamydia pneumoniae* antibody negative, *Cryptococcus capsular antigen* negative, tumor makers negative, and antinuclear antibody positive (nuclear granule type 1:80). Chest CT (Figure 4) showed patchy consolidation near the right cardiac margin. Diagnosis at admission indicated community-acquired pneumonia (CAP), *Legionella pneumonia* not excluded, and hypertension grade III (high risk). After intravenous treatment with moxifloxacin (0.4 g daily), the body temperature did not decrease, and chest pain, headache, and other symptoms occurred. Chest CT re-examinations after three and twelve days of treatment (Figures 5, 6) showed that the consolidation was larger than before. After a detailed review of the patient’s

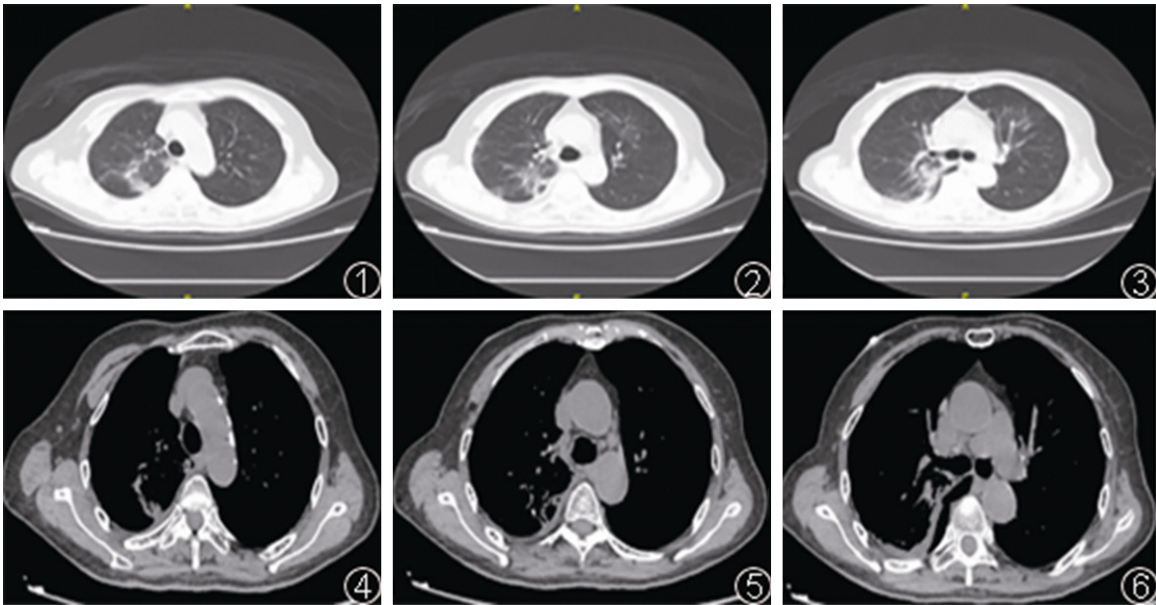


Figure 3. Case 1: chest CT Re-examination after 8 days of treatment (December 2, 2019).

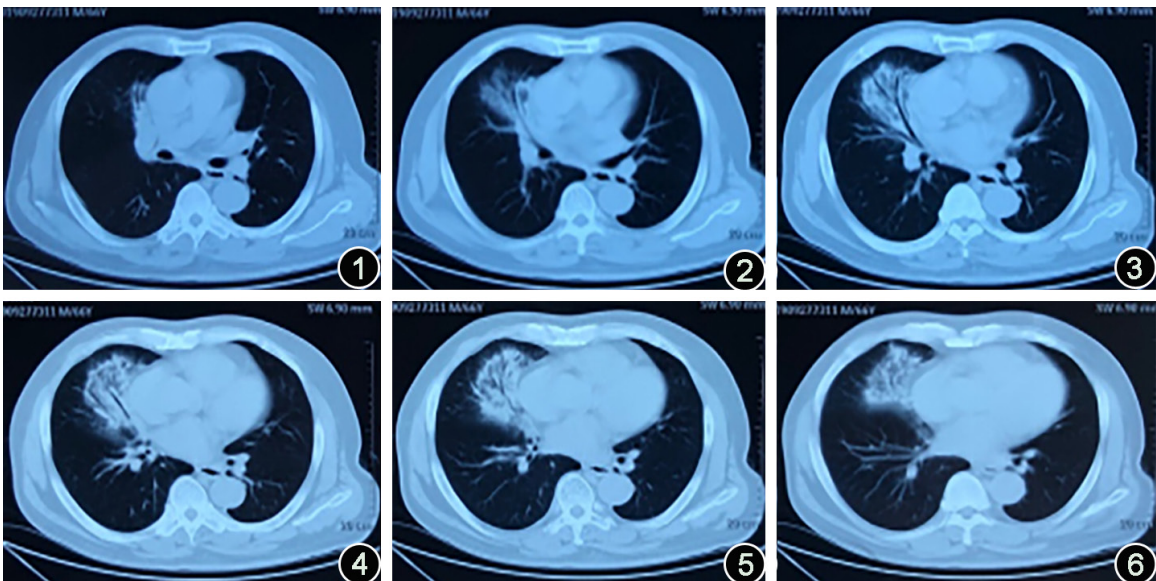


Figure 4. Case 2: chest CT at admission (September 30, 2019).

medical history, it was discovered that the patient had been clearing feces from a pigeon shed 20 days before admission. Although moxifloxacin was given intravenously, it failed to work. Because infection by special pathogens could not be excluded, fiberoptic bronchoscopy was performed again, and the secretion in the airway was sampled and cultured. TBLB was used to collect lung tissue for ROSCE, and BALF was sent out for mNGS. ROSCE (Figure 7) showed the presence of large numbers of fibroblasts, histiocytes, macrophages, monocytes,

and lymphocytes under the microscope. The BALF was negative for *Mycobacterium tuberculosis* DNA, and the BALF smear, bacterial culture, and acid-fast staining were also negative. mNSG detected *C. psittaci* DNA (sequence number 126). The revised diagnosis was *C. psittaci* pneumonia and secondary organizing pneumonia. The treatment regimen was adjusted to intravenous moxifloxacin (0.4 g per day), intravenous minocycline (100 mg twice a day), methylprednisolone (40 mg twice a day for five days, then 40 mg daily for three days and 20

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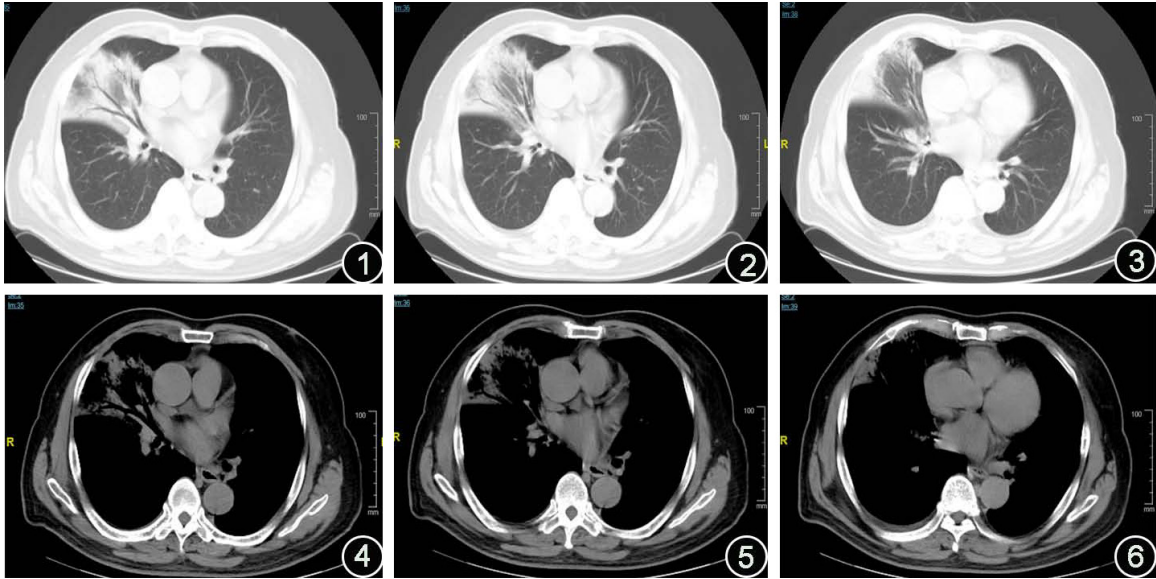


Figure 5. Case 2: chest CT re-examination after 3 days of treatment (October 2, 2019).

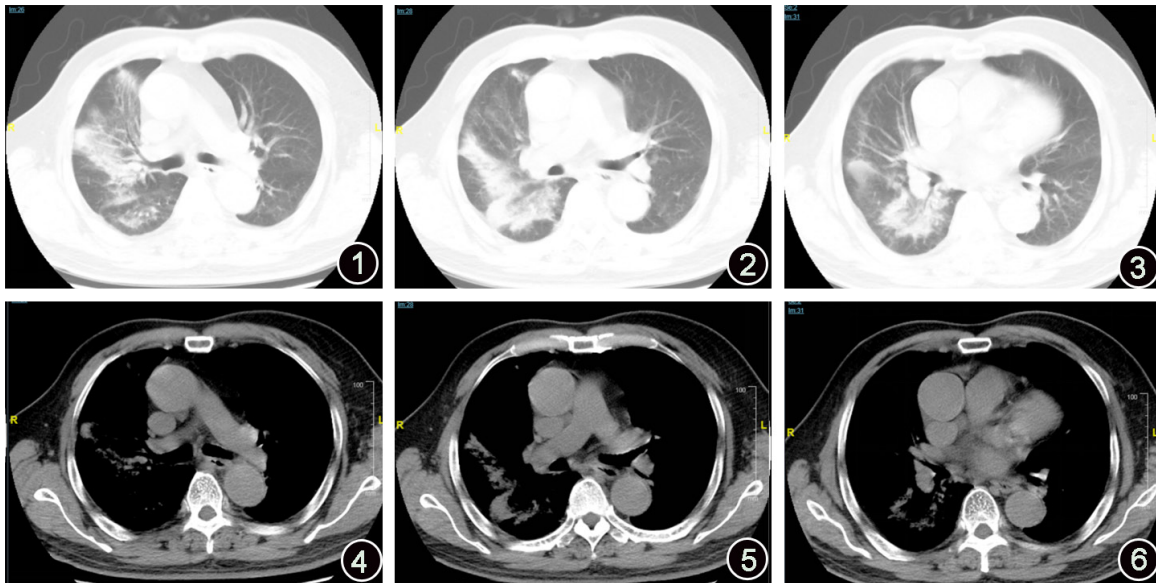


Figure 6. Case 2: chest CT re-examination after 12 days of treatment (October 12, 2019).

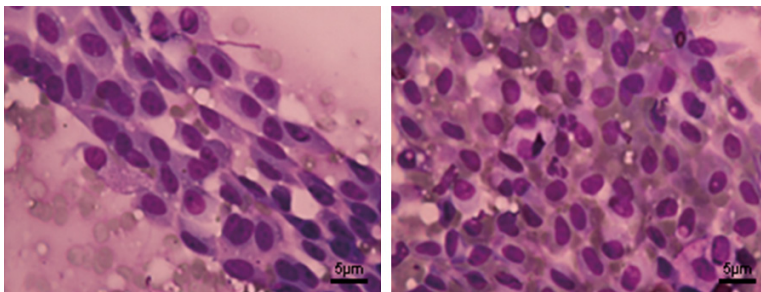
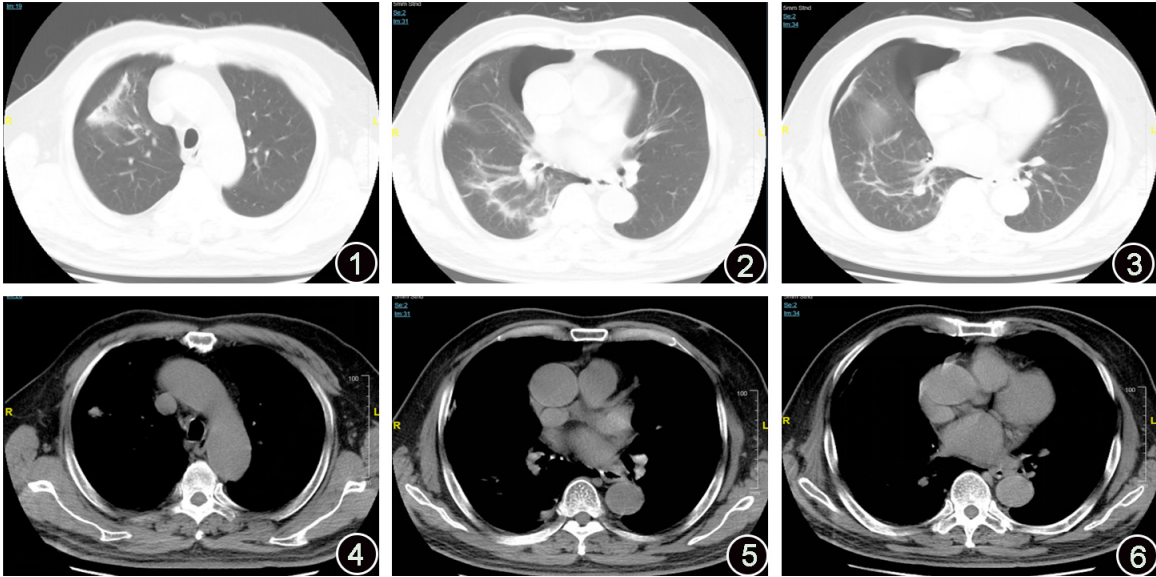


Figure 7. Case 2: ROSCE results.

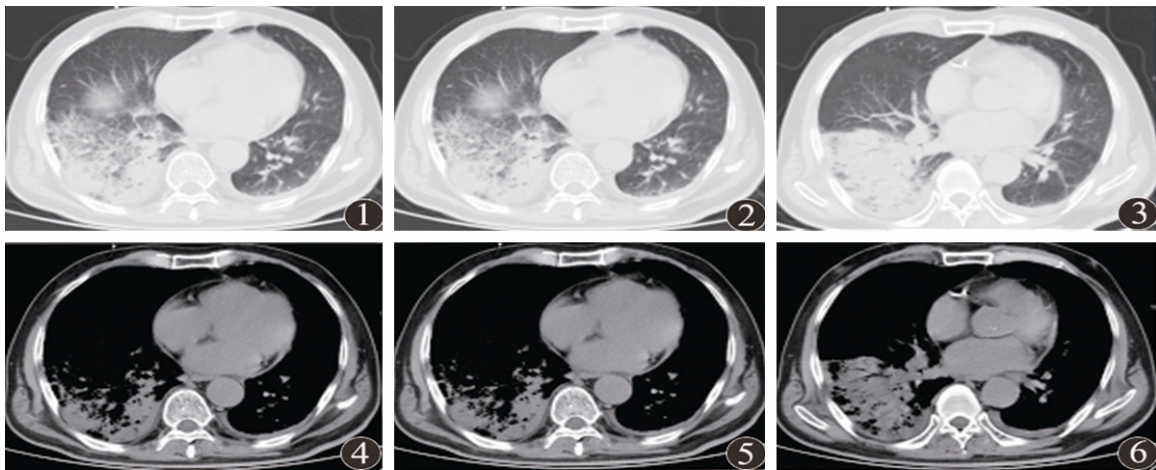
mg daily for three days, followed by a gradual reduction. Chest CT scan after four days of minocycline treatment (Figure 8) showed evident absorption of the lesions.

Case 3, a 72 year-old male patient, was admitted to hospital on October 28, 2020, due to fever, vomiting, and

## Diagnosis of *C. psittaci* pneumonia by mNGS combined with ROSCE



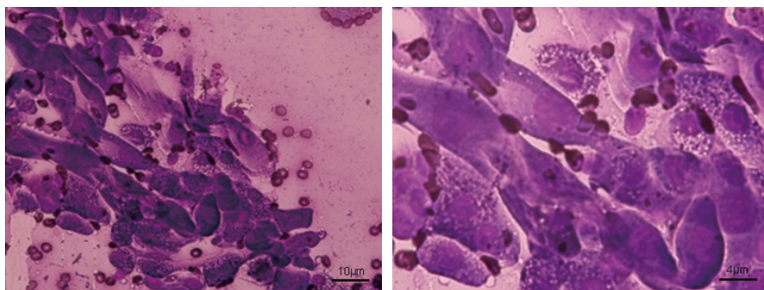
**Figure 8.** Case 2: chest CT after 4 days of minocycline treatment (October 22, 2019).



**Figure 9.** Case 3: chest CT on admission.

diarrhea for two days. The patient had a 20-year history of diabetes. Fever had occurred two days before admission, with the highest temperature of 38.7°C, accompanied by vomiting of stomach contents and, diarrhea, but no abdominal pain, no obvious cough and expectoration, no chest tightness and chest pain, no hemoptysis, or other symptoms, so he was hospitalized. Physical examination: temperature 38.0°C, blood pressure 135/78 mmHg, respiratory rate 23 bpm, heart rate 115 bpm, and poor mental health. The physical examination coordination was poor, the breathing sounds of both lungs were thick, a small amount of moist rales could be heard in the right lung, the heart sound was normal, the abdominal physical

examination was negative, neither of the lower limbs was swollen, and pathologic signs were not induced. On the supplementary examination, the results of the routine blood tests were as follows: white blood cell count  $9.76 \times 10^9/L$ , neutrophil percentage 85.7%, blood gas analysis showed pH 7.543, ( $paO_2$  55 mmHg,  $paCO_2$  23.6 mmHg), BNP 11.7 pg/ml, CRP 75.26 mg/l, procalcitonin 0.55 ng/ml, lactate dehydrogenase 293 u/L, albumin 21 g/L,  $Na^+$  123 mmol/L. Legionella IgM antibody was suspected positive, antinuclear antibody was positive, the carcinoembryonic antigen level was 6.81 ng/ml, ferritin 1611.67 ng/ml, and the cytokeratin fragment 6.38 ng/ml. Chest CT (**Figure 9**) showed a large consolidation in the lower lobe



**Figure 10.** Case 3: ROSCE results.

of the right lung. Diagnosis at admission indicated SCAP, type I respiratory failure, *Legionella* pneumonia, electrolyte disorder, and hypoproteinemia. The patient was given moxifloxacin, oxygen therapy, blood glucose regulation, basic support, and other treatment. Although the patient's symptoms improved, there was still intermittent fever, dry cough symptoms, and pulmonary burst sound. Bronchoscopic lung biopsy, ROSCE, and mNGS were performed. ROSCE (**Figure 10**) showed that a large number of fibroblasts, fibroblasts, histiocytes, and macrophages under the microscope. The BALF was negative for *Mycobacterium tuberculosis* DNA, and the BALF smear, bacterial culture, and acid-fast staining were also negative. mNSG detected *C. psittaci* DNA (sequence number 1077). The revised diagnosis was *Chlamydia psittaci* pneumonia, secondary organizing pneumonia, and type I respiratory failure. Doxycycline (100 mg, bid) was added to the treatment. The patient's temperature gradually dropped to normal, and the respiratory symptoms improved.

### Discussion

*C. psittaci* pneumonia is a zoonotic disease typically caused by the excretory dust of parrots and pigeons. The epidemiological history is very important, although some patients may not have a clear or direct bird contact history. In the early stage, *C. psittaci* pneumonia causes inflammatory reactions mediated mostly by neutrophils. The pathogenesis involves mainly perivascular inflammation, which spreads throughout the body, causing lobular and interstitial pneumonia that is obvious in the lower part. Most cases have a sudden onset, typically manifested by fever, dry cough, myalgia, and headache, with the latter being the most characteristic feature. In some cases, however, only

fever of unknown origin is present, respiratory symptoms are absent, and the clinical manifestations are quite different. In critical cases, patients can develop severe pneumonia with dyspnea, cyanosis, and coma, and prognosis is often poor [4]. Moreover, the disease can be easily misdiagnosed as *Legionella* pneumonia due to the involvement of multiple organ systems

and imaging features of the lungs [5]. Also, lung imaging reveals mainly single nodules, consolidation, or ground-glass opacity distributed along the subpleural space [6], features that can be readily confused with invasive pulmonary fungal diseases such as cryptococcal infection. However, in the three patients discussed here, the tests for fungi were negative, and there was no fungal sequence found in the mNGS results. The identification of *C. psittaci* in patients mainly depends on antibody detection. The international standard for the serologic diagnosis of *C. psittaci* is the micro-immunofluorescence method, which not only needs to be carried out in a specialized laboratory but can be used solely for retrospective diagnosis. Tetracyclines (doxycycline and minocycline) are the first-choice drugs for the treatment of *C. psittaci* pneumonia. The treatment lasts 10 to 14 days, and 3-4 weeks is required in special cases. However, a significant increase in the resistance of *Chlamydia trachomatis* to tetracyclines has been documented recently. In vitro studies have demonstrated that moxifloxacin has strong antibacterial activity against *Chlamydia* species, particularly *C. pneumoniae*, *C. trachomatis*, and *C. psittaci* [7]. At present, the effectiveness of quinolones in the treatment of *C. psittaci* has been reported in only a few cases [8]. Although moxifloxacin was given to the three patients on admission in the early stage of the disease, this therapy was not effective, and the symptoms, such as fever, did not improve. The treatment of *C. psittaci* pneumonia caused by a pathogen with high drug resistance is challenging. In addition, the mortality rate in severe cases is high. Therefore, early diagnosis is particularly important.

The three patients discussed in this paper had a tortuous course of the disease, including recurrent fever, severe headache, with no obvious cough and expectoration, and the respon-

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se to the hormonal antipyretic was poor. The presence of the pathogen in blood and sputum samples and BALF culture did not produce positive results. Eventually, *C. psittaci* was detected in the BALF by mNGS. Case 1 and Case 3 were complicated by respiratory failure, and Case 1 was complicated by heart failure at the same time. Given the clinical course and imaging findings, the two patients were diagnosed with severe pneumonia. A high number of sequences of *C. psittaci* were detected by mNGS, which was consistent with the diagnosis of severe *C. psittaci* pneumonia. The two patients were admitted to the respiratory ICU of Tianjin Medical University to provide high-flow respiratory support. In addition, although several immune indices of these two patients were abnormal, dominating the patients' medical histories, the lung imaging did not reveal obvious interstitial changes, and the characteristics of the lesions did not conform to the manifestations of immune-related lung injury. In case 2, obvious fever and severe headaches were present. The serum was weakly positive for the *Legionella* antibody. The imaging showed "atoll-like" changes without apparent exudation. Early examination of TBLB identified fibroblasts, lymphocytes, monocytes, and macrophages. The patient was initially diagnosed with *Legionella* pneumonia and secondary organizing pneumonia and was treated with moxifloxacin (0.4 g, per day) combined with methylprednisolone (60 mg, per day). The patient continued to have a recurrent high fever accompanied by a severe headache. The dosage of methylprednisolone (40 mg, twice a day) was increased but did not affect the fever. Therefore, TBLB was repeated, and the BALF was analyzed by mNGS. Large numbers of fibroblasts, macrophages, and lymphocytes were observed in ROSCE. mNGS detected *C. psittaci*, but not *Legionella*. Gram-negative bacteria were not found in the smear. *Legionella* pneumonia was ruled out, and a revised diagnosis of *C. psittaci* pneumonia with secondary organizing pneumonia was made. After adjusting the treatment plan by adding minocycline (100 mg, twice daily), the body temperature gradually decreased, the dose of methylprednisolone could be reduced, and the body temperature returned to normal. One month after discharge, the lung lesions were clearly absorbed.

ROSCE utilizes samples obtained by puncture, biopsy, and brush examination to prepare cyto-

logic specimens by direct tissue imprint or cell suspension smear. Rapid staining is used to evaluate the quality of the sample and provide a preliminary diagnosis. ROSCE is used mostly for the initial identification of benign and malignant lesions, to determine the effect of sampling and whether the number of samples is sufficient. Several methods for the preparation of ROSCE specimens are used. 1. Small tissue block printing is used for most tissue samples obtained by bronchoscopy or thoracoscopic mucosal biopsy and lung biopsy, percutaneous lung biopsy, or other percutaneous examination. Small tissue block printing is also used for the strip tissue block obtained by transbronchial needle aspiration (TBNA). 2. Brush film is utilized for most bronchoscope brush pieces or other thicker samples. 3. Spray film is used for most fine needle aspiration (FNA) samples and cytologic specimens obtained in percutaneous FNA lung biopsies or other percutaneous examinations. Spray film is also employed for cytologic specimens other than the strip-shaped tissue blocks from TBNA. Staining protocols in ROSCE involve very fast, lasting 30 seconds, modified Wright's staining. In most cases, the samples are ready for observation within 30 seconds to 1 minute [9]. The results are interpreted using a light microscope and a supporting image acquisition system. Full-time cytopathology experts are needed to evaluate the quality of the samples and to interpret the findings. Different subsequent tests are performed according to different results. For example, in the case of cell necrosis, large numbers of infiltrating neutrophils and lymphocytes can be seen under the microscope, suggesting that a bacterial infection may be involved, and specimen culture is required. The presence of fungal spores and hyphae indicates a fungal infection, and fungal antigen detection and silver staining should be carried out. The presence of purulent secretions and cheese-like granulomatous changes suggests the necessity to perform gene Xpert detection. If heterologous cells are seen under the microscope, it is necessary to determine the tumor type by immunohistochemistry and to identify its genotype. In addition, ROSCE can help in making a preliminary assessment of the disease, guide further examination, and provide information for a definitive diagnosis. However, the interpretation of ROSCE results is affected by the experience of the operator and cytologist, the quality of the microscope, the pathologic type of the



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specimen, the location and size of the lesion, and the staining reagent used.

mNGS is a non-biased method for pathogen detection based on second-generation sequencing technology. It directly extracts the DNA and RNA of all pathogenic microorganisms in clinical samples by standard genomics techniques and performs a high-throughput sequencing. By comparing the results with the sequences database and bioinformatic analysis, mNGS can achieve simultaneous detection of bacteria, fungi, viruses, parasites, and other pathogens [10]. Importantly, this technique is not affected by the use of antibiotics in the early stage of the disease, has a high detection rate, fast speed, wide coverage, high sensitivity, and is non-biased. mNGS has clear advantages in comparison with the current commonly methods for pathogen detection technology. For example, analysis of body fluid smears or cultures by enzyme-linked immunosorbent assay (ELISA) or polymerase chain reaction (PCR) is affected by multiple factors, is sensitive to contamination, frequently provides false-positive results, and often cannot distinguish between pathogenic bacteria and colonized bacteria. In addition, the properties of pathogens also can affect the culture results. Cultures of *Mycobacterium tuberculosis*, non-tuberculosis mycobacteria, mycoplasma, chlamydia, *Legionella*, *Cryptococcus*, fungi, viruses, and *Pneumocystis* not only require a long time but also yield a low positive rate, causing significant confusion and misdirecting the treatment. Thus, ELISA and PCR require the determination of the possible pathogens in advance, and then conducting respective detection assays; since the number of feasible tests is limited, it is easy to miss the correct diagnosis. In contrast, mNGS is not affected by other pathogenic factors and is better suited for the detection of pathogens responsible for lung diseases than the other methods. The clear advantages of mNGS for the diagnosis of unknown pathogens, special pathogens, and drug-resistant pathogens are currently well-documented [11, 12].

The diagnosis and treatment of the three patients provide valuable experience. We demonstrated that mNGS has obvious advantages over other clinical methods for the detection of pathogens, particularly in the case of rare

pathogens, mixed pathogen infections, severely ill patients, and immunodeficient patients. In all these instances, mNGS can provide an accurate diagnosis. ROSCE can ensure precise cytological characterization of the lesion site. For example, in the case of patients showing fever with lung shadow at admission, in which the anti-infection treatment is not effective, ROSCE can exclude tumor lesions or other non-infectious diseases. In the three patients discussed in this study, ROSCE documented large numbers of fibroblasts, lymphocytes, and macrophages. The detection of *C. psittaci* by mNGS, in combination with the patient's history, was more in line with the secondary organized pneumonia after infection, thus changing the direction of treatment and providing the basis for more accurate diagnosis and more effective therapy. Therefore, the combination of ROSCE and mNGS can improve the accuracy and speed of diagnosis, and accelerate the initiation of treatment, thus minimizing the exposure to broad-spectrum antibiotics, reducing the therapy cost, shortening hospital stay, and improving patient outcome. Our center specializes in interventional treatment of respiratory diseases, and several studies have verified that the combination of ROSCE and mNGS is superior to other detection methods in the diagnosis of pulmonary infectious diseases [13-15]. However, due to the lack of recognized interpretation standards, complex procedures, and higher detection cost than that of traditional detection methods, the clinical application of mNGS remains limited and cannot be promoted on a large scale [16].

### Disclosure of conflict of interest

None.

### Abbreviations

mNGS, metagenomic Next-generation Sequencing; ROSCE, Rapid On-site Cytological Evaluation; *C. psittaci*, Chlamydia psittaci; BALF, bronchoalveolar lavage fluid; TBLB, transbronchial lung biopsy; CAP, community-acquired pneumonia; CRP, C-reactive protein; BNP, brain natriuretic peptide; SCAP, severe community-acquired pneumonia; TBNA, transbronchial needle aspiration; FNA, fine needle aspiration; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction.

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**Address correspondence to:** Jie Cao, Department of Respiratory and Critical Care Medicine, Tianjin Medical University General Hospital, Tianjin 300052, China. E-mail: tjcaojie@sina.com

### References

- [1] Fraeyman A, Boel A, Van Vaerenbergh K and De Beenhouwer H. Atypical pneumonia due to *Chlamydophila psittaci*: 3 case reports and review of literature. *Acta Clin Belg* 2010; 65: 192-196.
- [2] Ciftçi B, Güler ZM, Aydoğdu M, Konur O and Erdoğan Y. Familial outbreak of psittacosis as the first *Chlamydia psittaci* infection reported from Turkey. *Tuberk Toraks* 2008; 56: 215-220.
- [3] Yang J, Ling Y, Yuan J, Pang W and He C. Isolation and characterization of peacock *Chlamydophila psittaci* infection in China. *Avian Dis* 2011; 55: 76-81.
- [4] Petrovay F and Balla E. Two fatal cases of psittacosis caused by *Chlamydophila psittaci*. *J Med Microbiol* 2008; 57: 1296-1298.
- [5] Gacouin A, Revest M, Letheulle J, Fillatre P, Jouneau S, Piau C, Uhel F, Tattevin P and Le Tulzo Y. Distinctive features between community-acquired pneumonia (CAP) due to *Chlamydophila psittaci* and CAP due to legionella pneumophila admitted to the intensive care unit (ICU). *Eur J Clin Microbiol Infect Dis* 2012; 31: 2713-2718.
- [6] Strambu I, Ciolan G, Anghel L, Mocanu A and Stoicescu IP. Bilateral lung consolidations related to accidental exposure to parrots. *Pneumologia* 2006; 55: 123-127.
- [7] Donati M, Rodríguez Fermepin M, Olmo A, D'Apote L and Cevenini R. Comparative in-vitro activity of moxifloxacin, minocycline and azithromycin against *Chlamydia* spp. *J Antimicrob Chemother* 1999; 43: 825-827.
- [8] DE Boeck C, Dehollogne C, Dumont A, Spierenburg M, Heijne M, Gyssens I, VAN DER Hilst J and Vanrompay D. Managing a cluster outbreak of psittacosis in Belgium linked to a pet shop visit in The Netherlands. *Epidemiol Infect* 2016; 144: 1710-1716.
- [9] Feng J, Chen BY and Wu Q. Attention should be paid to the rapid field evaluation of interventional respiratory disease. *Tianjin Pharmaceutical* 2014; 42: 193-196.
- [10] Tao Y, Fu QH and Mo X. Application of the metagenomic sequencing in the detection of new coronavirus and its challenge. *Chin J Traumatol* 2020: 217-218.
- [11] Lavezzo E, Barzon L, Toppo S and Palu G. Third generation sequencing technologies applied to diagnostic microbiology: benefits and challenges in applications and data analysis. *Expert Rev Mol Diagn* 2016; 16: 1011-1023.
- [12] Deurenberg RH, Bathoorn E, Chlebowicz MA, Couto N, Ferdous M, García-Cobos S, Kooistra-Smid AMD, Raangs EC, Rosema S, Veloo ACM, Zhou K, Friedrich AW and Rossen JWA. Reprint of "Application of next generation sequencing in clinical microbiology and infection prevention". *J Biotechnol* 2017; 250: 2-10.
- [13] Huang J, Jiang E, Yang D, Wei J, Zhao M, Feng J and Cao J. Metagenomic next-generation sequencing versus traditional pathogen detection in the diagnosis of peripheral pulmonary infectious lesions. *Infect Drug Resist* 2020; 13: 567-576.
- [14] Wang J, Han Y and Feng J. Metagenomic next-generation sequencing for mixed pulmonary infection diagnosis. *BMC Pulm Med* 2019; 19: 252.
- [15] Liu N, Kan J, Cao W, Cao J, Jiang E, Zhou Y, Zhao M and Feng J. Metagenomic next-generation sequencing diagnosis of peripheral pulmonary infectious lesions through virtual navigation, radial EBUS, ultrathin bronchoscopy, and ROSE. *J Int Med Res* 2019; 47: 4878-4885.
- [16] Zhu YM and Zhang WH. Application of second generation sequencing in etiological diagnosis of sepsis. *Microorganism and Infection* 2018; 13: 97-101.