



A Typical Pneumonia Caused by *Chlamydia psittaci* during the COVID-19 Pandemic

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Abstract

Background: *Chlamydia psittaci* can bring about serious clinical manifestations, including a typical Pneumonia, Chills, sore throat, and headache and so on. It is possible that *Chlamydia psittaci* pneumonia was confused with COVID-19 during the COVID-19 pandemic.

Methods: Metagenomic Next-Generation Sequencing (MNGS) was performed and thirty-two patients who were diagnosed with *Chlamydia* parrot's pneumonia were collected. The patients, clinical characteristics and key points of diagnosis and treatment of the disease were summarized.

Results: All of patients showed atypical pneumonia. Frequent symptoms included headache (17/32), fever (2/32), myalgia (3/32), cough and expectoration (26/32). Laboratory data showed elevated WBC (4/32), percentage of neutrophils (30/32), CRP (32/32), PCT (15/26), BUN (10/32), CRE (4/32), ALT (21/32), AST (25/32), TB (4/32), DB (14/32). All patients experienced complete recoveries after doxycycline or doxycycline-based therapy. MNGS provided the results of the exact pathogen, while conventional etiological tests did not.

Conclusion: Facing the covid-19 pandemic, atypical pneumonia except for COVID-19 should also be considered. MNGS technology for the diagnosis of *Chlamydia parrots* pneumonia is of great significance, especially suitable for people with unknown pulmonary infection pathogen, timely to make the appropriate antimicrobial therapy effective.

Introduction

Parrot disease is a Zoonotic disease caused by *Chlamydia psittaci*. The disease is also known as parrot fever. Clinical manifestations of human parrot disease can range from mild to fulminant and are characterized by flu-like symptoms such as fever, headache, fatigue, joint pain, and loss of appetite. Human parrot disease is most commonly transmitted through inhalation of contaminated substances, such as dry faces or nasal secretions. The most commonly used treatments for human parrot disease are doxycycline, tetracycline, or Chloramphenicol [1].

The low sensitivity and complexity of *Chlamydia* parrot culture make it difficult to perform routinely in most diagnostic laboratories. Other laboratory tests, including serological tests and polymerase chain reaction-based methods, have limitations in sensitivity and specificity. MNGS has been increasingly used in the diagnosis of infectious diseases, and has become one of the most promising strategies for discovering new infectious agents in clinical specimens, especially when traditional diagnostic methods are limited [2-3]. Parrots fever may present as rapidly progressing severe pneumonia, acute respiratory distress syndrome, sepsis, and multiple organ failure. Heng Zhan et al. retrospectively analyzed the diagnosis and treatment of a fulgent pneumonia complicated with multiple organ failure complicated with severe pneumonia. Imaging findings showed unilateral lung consolidation, indistinguishable from Community-Acquired Pneumonia (CAP) caused by common pathogens [4]. Gu et al. reported 5 cases of *Chlamydia pneumonia*. Common symptoms and signs in the 5 patients included fever, cough, and muscle pain throughout the body, most notably chest CT and X-ray showing inflammatory infiltrates in the lungs. Metagenomic Next-Generation Sequencing (MNGS) revealed lung biopsy in 3 cases and Bronchoalveolar lavage fluid in 2 cases. Three patients responded to doxycycline and moxifloxacin. Two patients responded to moxifloxacin alone [5]. Cheng et al. identified 9 cases of severe *Mycoplasma pneumonia* infection using MNGS. Common symptoms included chills and relaxation fever (100%), cough and fatigue (100%), headache and myalgia (77.8%). All patients had severe parathyroid fever pneumonia

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complicated with respiratory failure, and 6/9 had sepsis. Laboratory data showed normal or slight increases in white blood cells, neutrophils, and procalcitonin, but high levels of C-reactive protein. Computed Tomography (CT) scan revealed consolidation and ground glass opacity in the upper lobes of the lung, with spread to both lungs, with miliary, nodular, or consolidation. One patient died of secondary *Klebsiella pneumonia* infection, while the other eight patients made a full recovery [6]. Shanshan Su et al. retrospectively studied 17 cases of *Chlamydia sitorii* pneumonia diagnosed by MNGS and 9 cases of atypical community-acquired pneumonia caused by *Legionella*. In terms of extrapulmonary manifestations, biological characteristics and prognosis, *Nautilus helicobacteriosis* and *Legionella Pneumonia* (LP) have many similarities [7]. Xiaojing Wu et al. reported a case of severe pneumonia caused by *Mycoplasma psittaci* infection in a pregnant woman who was diagnosed by NGS and cured without side effects of tetracycline in the infant [8]. Three cases of nautilus pneumonia admitted to Tongling People's Hospital were retrospectively analyzed. All three patients were infected by exposure from pets or animals. All symptoms include high fever (body temperature 39°C), cough, expectoration, chest tightness, and difficulty breathing. The disease progress rapidly, with severe Acute Respiratory Distress Syndrome (ARDS) and shock as the main manifestations, but the damage to the heart, liver and kidney is mild. Laboratory tests showed C-reactive protein (CRP, all >200 mg/L) and neutrophils (Neut %, >0.90), while WBC and PCT were not significantly increased. Chest computed Tomography (CT) shows inflammatory infiltrates with interstitial changes, either unilateral or bilateral. Chest radiograph shows an extensive inflammatory infiltrate with a fan-shaped or wedge-shaped pleural margin. After 7 days of treatment, bedside Computerized X-Ray (CR) showed infiltration and absorption. CT was reexamined 11 to 13 days later, and the pulmonary infection was basically absorbed. Sensitive to quinolones and tetracyclines. The patient's temperature returned to normal after 2 to 3 days of antibiotic administration, and all patients were extubated and transferred to normal wards after 10 days. The overall course of disease is 20 to 30 days [9].

Patients and Methods

Patients

We retrospectively analyzed 32 patients with *Chlamydia psittaci* pneumonia diagnosed by MNGS who were admitted to Zhejiang Provincial People's Hospital from April 2020 to June 2021. This study was approved by the ethics committee of the hospital.

Clinical data collection

Information collection, including clinical data, demographic characteristics, basic medical conditions, clinical signs and symptoms chest radiograph results, clinical laboratory test results, travel history, recent contact with animals, and the results.

Genome sequencing

Nucleic acids were extracted from each sample with the Direct-zol RNA Miniprep kit (Zymo Research, Irvine, CA, USA) and Trizol LS (Thermo Fisher Scientific, Carlsbad, CA, USA) according to the manufacturer instructions in a biosafety III laboratory. A 50- mL elution was obtained from each sample. The DNA/RNA concentrations were measured by a Qubit Fluorometer (Thermo Fisher Scientific). The sequencing library was constructed by a transposase-based methodology and sequenced on an Illumina sequencing platform (Illumina, San Diego, CA, USA). At least 25

million single-end 76-bp reads were generated for each sample on the Illumina NextSeq platform. Quality control processes included removal of low-complexity reads by BBDuk (entropy = 0.7, entropy-window = 50, entropy k = 5; version: January 25th, 2018), adapter trimming, low quality reads removal, short reads removal by Trimmomatic (adapter: TruSeq3-SE.fa:2:30:6, LEADING: 3, TRAILING: 3, SLIDING WINDOW: 4:10, MINLEN: 70, version: 0.36), host removal by BMTagger (using human genome GRCh38 and the specific sequences as reference), and ribosomal reads removal by SortMeRNA (version: 2.1b). Taxonomic assignment of the clean reads was performed with Kraken 2 against the reference databases, including Archaea, bacteria, fungi, human, plasmid, protozoa, univec, and virus sequences (software 2.0.7-beta, database version: August 2, 2019). A negative control sample was processed and sequenced in parallel for each sequencing run as a contamination control. The data were classified by simultaneous alignment to the microbial genome databases comprising viruses, bacteria, fungi, and parasites after filtering of the adapters and human-origin reads.

Result

Patient characteristics

The 32 patients included 20 males and 12 females, with a median age of 63 years (range 45 to 84 years). Twenty patients had underlying diseases. Regard to the history of exposure, 7 of the patients had a history of direct exposure and raise chickens or ducks in their homes. Notably, 25 patients had no history of direct exposure. Seventeen patients had fever with a body temperature of more than 38.5°C, accompanied by cough and expectoration of yellow-white sputum. Three patients had myalgia, two patients had headache, and two patients had hypotension on admission (90/60 mmHg). All of patients showed atypical pneumonia, including inflammatory infiltration, Pleural effusion, and multiple inflammatory exudative lesions with interstitial edema, lung abscess, and White lung (Table 1).

Technical investigations

On admission, neutrophils and C-reactive protein were significantly increased, while leukocytes and procalcitonin were not significantly increased (Table 2). The patients had a mean white blood cell count was $6.87 \times 10^9/L$, and the neutrophil percentage was 86.3%, C-Reactive Protein (CRP) level of 170.85 mg/l and Procalcitonin (PCT) level of 0.715 ng/ml. No pathogen was found in blood culture of all patients. Ten patients had elevated urea nitrogen, 4 patients had elevated creatinine, and 25 patients had elevated liver enzymes. Pulmonary imaging this is an inflammatory infiltration of the lungs with interstitial changes, unilateral or bilateral, with pleural effusion.

Table 1: Patient characteristics.

Characteristics	Patients, n (%)	Median value, (range)
Male/female	20/32	
Age		63 (45-84)
History of exposure to poultry	7/32	
Underlying diseases	20/32	
Fever (>38.5°C)	17/32	38.5°C (36-40.1°C)
Headache	2/32	
Myalgia	3/32	
Cough and expectoration	26/32	
Hypotension (<90/60 mmHg)	2/32	
Atypical pneumonia	32/32	

Table 2: Technical investigations.

Laboratory Examination	Patients, n (%)	Median value, (range)
Blood oxygen <60 mmHg	1/21	88.4 (59.9-159 mmHg)
Elevated WBC (4-10 × 10 ⁹ /l)	4/32	6.87 (2.99-29.5 × 10 ⁹)
Elevated percentage of neutrophils (45% to 75%)	30/32	86.3 (73.2% to 97.3%)
Elevated CRP (0-8 mg/l)	32/32	170.85 (44.3-245.4 mg/l)
Elevated PCT (0-0.5 ng/l)	15/26	0.715 (0.09-22.459 ng/l)
Elevated BUN (3.2-7.1 mmol/l)	10/32	5.175 (2.71-17.58 mmol/l)
Elevated CRE (40-133 mmol/l)	4/32	67.5 (15-115 mmol/l)
ALT (7-40 u/l)	21/32	58.15 (13-309.4 u/l)
AST (13-35 u/l)	25/32	70.4 (17-739.9 u/l)
TB (3.4-24 umol/l)	4/32	12.5 (7-48.3 umol/l)
DB (0-6.8 umol/l)	14/32	4.9 (2-27.6 umol/l)

Table 3: MNGS results.

Sample Type	Sample number	Median	Average number
BALF	18	308	1015
Blood	9	35	33
Sputum	5	12	233

Etiological examinations

Nasal swabs and alveolar lavage fluid samples were collected from each patient and SARS-CoV-2 nucleic acid was detected by real-time RT-PCR. Plasma IgM or IgG antibodies against SARS were detected by repeated ELISA for COV-2 at admission and 2 weeks after admission. In addition, specific IgM antibodies against common respiratory pathogens, isothermal amplification tests and indirect serum immunofluorescence tests were performed in conventional culture of alveolar lavage fluid, peripheral blood and sputum samples. All of the above tests reported negative results. MNGS was performed because the etiology did not identify the exact cause of pneumonia. Clinical specimens included blood, sputum bronchoalveolar lavage fluid. Alveolar lavage fluid was detected in 18 patients, peripheral blood was detected in 9 patients, and sputum was detected in 5 patients. All patients were positive for *Chlamydia psittaci* DNA fragments. The number of sequences detected by MNGS in alveolar lavage fluid was more than that detected by sputum and blood test.

Treatment

On admission, all patients were given empirical anti-infective therapy with Ceftriaxone, Piperacillin, Amoxicillin, Amoxicillin Sodium, Cefoperazone, Meropenem, Suprofen, Cefotiam etc. However, their clinical symptoms did not improve, and most of them were treated with upgraded antibiotics. The MNGS took 24 h to 48 h from the receipt of the sample to the reporting of the results. When patients were diagnosed with *Chlamydia psittaci* pneumonia, they were adjusted to doxycycline or doxycycline-based treatment regimens. The median course of antibiotic treatment was 11 days (4 to 30 days). Thirty-two patients' body temperature gradually dropped to normal, lung lesions gradually absorbed, improved and discharged from hospital.

Discussion

Chlamydia psittaci is responsible for 1% to 8% of community-acquired pneumonia [10,11]. Due to the lack of routine detection and common diagnostic methods for *Chlamydia psittaci* and the

differences in sensitivity and specificity, it is difficult to determine the exact incidence and prevalence of *Chlamydia psittaci* [12]. In addition to pigeons, poultry is an important source of infection of parrot fever, poultry including chickens and ducks. However, in the present study, 25 cases did not have a history of exposure to poultry or pigeons. We speculate that the reasons for this error, which may come from the patient's unclear concept of poultry exposure history. For example, a patient once saw geese and ducks in the vegetable market but did not report it until we got the MNGS results and ask again. In this study, there were 32 patients, 27 of whom had fever with cough and sputum, and 13 of whom had respiratory failure. Patients showed elevated white blood cells, significantly increased neutrophils and CRP, and elevated liver enzymes in some patients. All the patients progressed rapidly. Clinical symptoms and examination results were similar to those of COVID-19 patients [13]. *Chlamydia psittaci* belongs to the *Chlamydia* family [14]. Tetracyclines, macrolides or quinolones that interfere with DNA and protein synthesis can be selected as antimicrobial agents and doxycycline as first-line treatment [15]. In this study, 32 patients were adjusted to doxycycline or doxycycline-based therapy after diagnosis was confirmed. The clinical symptoms of these patients have been effectively controlled and improved the prognosis of all 32 patients were good. The methods for diagnosing *C. psittaci* infections have drawn attentions for a long time [16]. Multiple real-time polymerase chain reaction methods have been developed for the detection of *Chlamydia psittaci*, but they are sensitive only in the acute phase [17]. There has been no ideal tool recently. Isolation and culture of *Chlamydia psittaci* are inefficient. Serological examination requires titer comparison between acute and convalescence serological specimens, routine culture of respiratory specimens takes 5 to 7 days, which is time-consuming, the advantage of MNGS is that it can detect a wide range of pathogens and is suitable for the lack of suspected pathogenic microorganisms, and the results can be obtained within 48 h to 72 h. In this study, MNGS technology was used to diagnose *Chlamydia psittaci* pneumonia, which could quickly and accurately detect *Chlamydia psittaci*, shorten the diagnosis time and initiate antimicrobial treatment in time.

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