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mNGS: A novel method for the diagnosis of Chlamydia psittaci infection

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Abstract: Psittacosis is a zoonotic disease caused by Chlamydia psittaci infection; human infections with Chlamydia psittaci usually originate from contact with infected animals, and studies have confirmed the existence of the human-to-human route of transmission, which also suggests that Chlamydia psittaci infections have a high risk of causing public health and safety incidents. Previous methods for detecting Chlamydia psittaci include polymerase chain reaction (different targets), serological assays (complement binding reaction, enzyme-linked immunosorbent assay, immunofluorescence assay, and immunoperoxidase assay), and isolation cultures, with the emergence of macrogene second-generation sequencing mNGS as a new diagnostic tool. Since the emergence of mNGS, there has been a significant increase in the number of studies on Chlamydia psittaci infections. In this paper, we will review the above case reports to help public health and medical practitioners to identify the disease earlier and provide timely and appropriate treatment, so as to reduce the risk to the public health and the pressure on the patients' medical care.

1. Introduction

Psittacosis is a zoonotic disease caused by Chlamydia psittaci, and human Chlamydia psittaci infections usually originate from close contact with infected birds (e.g., parrots, poultry) or from inhalation of aerosols of nasal secretions and dust from faeces or feathers of infected birds, etc. Moerover the human-to-human transmission of psittacosis has been confirmed recently [1]. Since Chlamydia psittaci is a strictly intracellular parasitic bacterium that cannot grow on ordinary media and cannot be detected by techniques such as conventional smear staining, there is a lack of clinical means to confirm the diagnosis at an early stage. One systematic review and Meta-analysis [2] showed that the current international diagnostic methods for related Chlamydia psittaci infections cover serological diagnostic methods such as complement assays and fluorescent staining, as well as polymerase chain reaction (PCR) and culture of the pathogen, but are unsatisfactory in terms of timeliness, sensitivity and specificity [3]. In 2014, the New England Journal of Medicine published the first clinical application case of macrogenomic second-generation sequencing (mNGS) to confirm

the diagnosis of leptospirosis, firing the first shot of mNGS for pathogens [4]. In 2018, Professor Zhang Wenhong's team from the Department of Infection at Shanghai Huashan Hospital used mNGS to assist in the clinical diagnosis of hepatic tuberculosis [5], which promoted the application of mNGS in China for the accurate diagnosis of clinical infectious disease-causing bacteria [6]. Since then, reports of Chlamydia psittaci pneumonia have been increasing, and this review focuses on summarising the research results of Chinese scholars on Chlamydia psittaci infections at this stage.

2. Epidemiological characteristics

Psittacosis, as a zoonosis, has been reported globally in scattered or outbreaks, and it has been widely believed that human infections with psittacosis generally originate from contact with infected birds. However, with further scientific research and significant advances in genomic testing technology, recent studies have confirmed that Chlamydia psittaci can be transmitted not only by the route of person-to-person transmission, but may even extend to secondary contacts [7].The identification of this transmission route significantly increases the risk of outbreaks of Chlamydia psittaci infection, making the development of effective preventive measures urgent.

According to the current study about Chlamydia psittaciosis infection, most of them are caused by a clear history of parrot or avian contact [8], poultry farming, animal husbandry, domestic pets, and farmers are high-risk factors, and other less common routes of exposure include slaughter of poultry animals, cooking, and contact with the entrails of killed poultry [9]. Most reports in China show that psittacosis infection occurs mostly in middle-aged and elderly people, with no significant difference in the incidence between men and women. Elderly people with multiple underlying diseases often develop severe community-acquired pneumonia (CAP), which can cause multiple organ damage and even death [10]. Psittacosis has been reported in a small number of pregnant women, causing serious adverse outcomes for both mothers and fetu [11]. In China, it has been noted that the incidence of Chlamydia psittaci pneumonia is seasonal, generally occurring in the winter during the colder seasons, with the incidence of the disease being significantly higher in the south of the Qinling-Huaihe River than in the north of the country [12].

3. Clinical manifestation

Patients with Chlamydia psittaci pneumonia can be classified as common or severe depending on the severity of the disease, and clinical symptoms can range from general lower respiratory tract infection symptoms to secondary severe acute respiratory syndrome and systemic organ function damage, or even death [10]. The clinical symptoms can range from general lower respiratory symptoms to severe acute respiratory syndrome and systemic organ damage and even death. (Table 1)

Table 1: Summary of possible signs and symptoms of Chlamydia psittaci pneumonia

common symptom	Other atypical manifestations	signs
have a high temperature cough phlegm fatigue shivering and chills gastrointestinal distress muscle pain headaches and dizziness	myocarditis hearing loss prostatitis Abdominal pain as the predominant manifestation of pancreatitis Chest pain, dyspnoea and other signs of pulmonary thrombosis Secondary Guillain-Barre syndrome, etc. Irregular vaginal bleeding [22]	relatively slow pulse Dampness of the lungs cyanosis (blue skin due lack of oxygen in blood) cervical rigidity breath sounds thick infective shock

3.1. Typical clinical presentation of the common type

Most patients start with respiratory symptoms such as cough, sputum or fever, and the cough and sputum are non-specific compared to CAP caused by other pathogenic infections. The majority of patients with Chlamydia psittaci pneumonia have a high fever, which may be as high as 40 ℃ or higher, and are often accompanied by systemic symptoms of toxicity, such as chills or chills, malaise, and muscle aches and pains, but the white blood cell count is mostly in the normal range. Severe symptoms of infectious toxicity and a normal white blood cell count may be considered a feature of Chlamydia psittaci infection.

3.2. Other rare clinical conditions include

Prostatitis as the first symptom [13] pancreatitis with abdominal pain as the first symptom [14]. headache and meningitis irritation [15]; pulmonary thrombosis with chest pain and dyspnoea [16]; Myocarditis with markedly elevated markers of myocardial injury [17]; Greene-Barre Syndrome with limb weakness and respiratory muscle weakness [18] etc.

3.3. Clinical manifestations of the severe form

Perhaps because Chlamydia psittaci belongs to atypical pathogens, conventional tests cannot confirm the diagnosis at an early stage, and when empirical antibiotic therapy fails to effectively cover the disease, the disease may rapidly progress to severe CAP, with varying degrees of respiratory failure or infectious shock, requiring respiratory and circulatory support, or even extracorporeal membrane pulmonary oxygenation (ECOM) supportive therapy [19]. Other common complications, such as rhabdomyolysis syndrome, hepatic and renal failure, and abnormal coagulation mechanisms, require renal replacement or component blood transfusion. It has been shown that age >65 years and being male are risk factors for progression of Chlamydia psittaci pneumonia to severe pneumonia [20]. Serum d-dimer, IL-2, IL-6, and IL-10 levels, as well as lymphocyte, CD3+ T-cell, and CD4+ T-cell counts, are strongly associated with disease severity [21].

3.4. Clinical signs

Chlamydia psittaci pneumonia has no specific signs, some studies [8] suggest that the performance of a relatively slow pulse (71%) may be a more prominent sign, more than half of the patients lung examination can be found shortness of breath, local dry and wet rales, or respiratory sounds rough; the combination of severe complications may appear cyanosis, hypotension, oliguria and other signs.

4. Laboratory tests

In Chlamydia psittaciosis, leukocytes are usually absent or only slightly elevated, or there is a mild leukocyte decline, neutrophil ratio is mildly elevated, CRP is usually markedly elevated, and mild elevations of calcitonin and interleukin-6 may be seen in mild CAP; in severe CAP patients, there are marked elevations of calcitonin and interleukin-6, accompanied by a decrease in lymphocyte counts [23]. In severe CAP, calcitonin and interleukin-6 are significantly elevated, accompanied by decreased lymphocyte count.

More than half of the mild cases of psittacosis CAP may show mild elevation of aminotransferases, CK, MYO, etc., and the normal range of albumin in general; severe patients may show significant abnormalities of aminotransferases or bilirubin, and patients with secondary rhabdomyolysis syndrome may have significant elevation of CK and MYO; patients with secondary renal failure may

have significant elevation of creatinine and urea nitrogen, with electrolyte disorders; some patients may have abnormalities of coagulation mechanism and elevation of D-dimer; blood gas analysis may show varying degrees of hypoxaemia, alkaline imbalance, and electrolyte disorders. Abnormalities in coagulation mechanism and elevated D-dimer can be seen in some patients; different degrees of hypoxaemia, acid-base imbalance and electrolyte disorders can be seen in blood gas analysis [24]. The blood gas analysis may show different degrees of hypoxaemia, acid-base imbalance and electrolyte disorders.

5. Imaging manifestations

Most patients with Chlamydia psittaci pneumonia have lobular solid lesions on chest CT, usually with air bronchial signs and ground-glass shadows around the solid lesions, which may be unilateral or bilateral, and depending on the severity of the disease, the area of the affected lungs varies, with severe patients showing a wide distribution of multiple lobes and combined with prepleural effusions [10]. The disease is often combined with pleural effusion. Psittacosis has also been found to be associated with a halo sign along the bronchovascular bundles, as well as thickening of the interlobular septa, paving stone sign, and reverse halo sign [25]. Other manifestations include thickening of the interlobular septum, paving stone sign and reverse halo sign.

6. Etiological diagnosis

Diagnostic methods for Chlamydia psittaci infection have changed markedly over time. A review, by finding and analysing global literature on outbreaks of Chlamydia psittaci infection between 1986-2017, concluded that laboratory tests for Chlamydia psittaci infection include polymerase chain reaction (different targets), serological tests (complement binding reaction, enzyme-linked immunosorbent assay, immunofluorescence test, and immuno peroxidase test) and culture, in various combinations, but there is no gold standard for confirming Chlamydia psittaci infection, and in most psittacosis outbreaks, Chlamydia psittaci infection cannot be confirmed or ruled out as the causative pathogen in a significant number of cases [26].

The earliest method of diagnosing Chlamydia psittaci pneumonia was the complement fixation test, which was later developed into methods such as the immunofluorescence test or the enzyme-linked immunosorbent assay (ELISA), but its sensitivity and specificity are unsatisfactory [27]. However, its sensitivity and specificity are not satisfactory. Pathogen culture has always been the gold standard for the diagnosis of bacterial infections, but Chlamydia psittaci is an intracellular parasitic bacterium, which is difficult to cultivate and technically demanding, and it is difficult to isolate and culture it in general laboratories [28]. However, Chlamydia psittaci is an intracellular parasite that is difficult to isolate and culture in the general laboratory. Polymerase chain reaction (PCR) of sputum samples from all CAP patients with different targets is a rapid method of confirming Chlamydia psittaci infection and allows genotyping to find the source of infection [29]. However, the disadvantages are that the sensitivity is only high in the acute phase and Chlamydia psittaci, as a rare causative agent, is not usually routinely used in China for the identification of the causative agent of CAP in adults.

mNGS, which performs non-targeted sequencing of DNA/RNA of all microorganisms including bacteria, fungi, viruses and parasites, has been used in clinical applications since the beginning of its use, and has increasingly demonstrated its unique advantages in the rapid identification of acute and difficult pathogenic bacteria [3]. Studies have shown that early adoption of appropriate specimen delivery mNGS for confirmatory pathogen detection in adults with CAP is more likely to improve pathogen identification in severe community-acquired pneumonia (SCAP) compared to serology and traditional microbiological cultures [30]. The detection rate of pathogens by mNGS is higher than

that of microbiological culture and PCR alone, and alveolar lavage or lower respiratory secretions are more desirable specimens than blood or body fluids for clinical specimens [31]. The use of alveolar lavage or lower respiratory tract secretions is preferable to blood and body fluids for clinical specimen selection. Especially in immunocompromised patients, NGS is superior to conventional microbiological tests in terms of diagnostic positivity, diagnosis of mixed infections, detection of pathogens, and guidance for the adjustment of anti-infective treatment strategies, etc. It is worthwhile to promote the use of clinical application [32]. It is worth promoting the clinical application.

7. Treatment

The latest edition of the Chinese Guidelines for the Diagnosis and Treatment of Community-Acquired Pneumonia in Adults states that tetracycline antibiotics are preferred for the treatment of Chlamydia psittaci, with secondary options including macrolide antibiotics, quinolone antibiotics, and chloramphenicol [33]. Because Chlamydia psittaci is a rare causative agent of CAP, some cases of Chlamydia psittaci pneumonia have been treated empirically with β-lactam antibiotics prior to diagnosis [10], the disease is not controlled in a timely manner, and some patients are admitted to the hospital with quinolone antibiotics to cover Chlamydia, but the patient's condition still does not improve effectively. Chlamydia psittaciosis may not be effective on conventional antimicrobials because the pathogenic Chlamydia psittaciosis carries drug resistance genes [34]. The patient's condition did not improve despite the use of quinolone antibiotics.

After the diagnosis of Chlamydia psittaci infection was confirmed by mNGS, most cases were given targeted administration of tetracycline or fluoroquinolone or macrolide antimicrobial drugs, monotherapy or combination therapy [35]. The majority of patients were cured, while a few patients with severe disease failed to respond to treatment and eventually died.[20] The vast majority of patients recovered from the disease. This may be because SCAP patients infected with psittacosis are often associated with respiratory failure, shock, rhabdomyolysis syndrome, and often require invasive therapy such as mechanical ventilation, ECMO or renal replacement, and the body's immune function is impaired to varying degrees, and is often combined with complex infections with other pathogenic organisms, which can lead to therapeutic failure [36].

Combined with the symptoms and history of Chlamydia psittaci, the traditional Chinese medicine (TCM) diagnosis of the disease belongs to the category of "exogenous fever", accompanied by a series of pulmonary symptoms, so the disease is considered to be in the lungs, and the diagnosis is lung fever. In China, there existed some cases reporting that treatment with the help of TCM might achieve good curative effect.

8. Prognosis

CAP due to Chlamydia psittaciosis can be classified as common and severe according to the clinical manifestations, with a high rate of severe disease, and the targeted administration of tetracyclines or fluoroquinolones in the majority of cases after identification of the causative organisms disease by mNGS [37]. In most cases, tetracyclines or fluoroquinolones are targeted, and in a few cases, macrolides are used [38]. In the majority of cases, the prognosis is favourable with monotherapy or combination antimicrobial therapy, but in some cases, the disease continues to progress [9]. In some cases, the disease continues to progress and death occurs [39]. In some cases, the disease continued to progress and death occurred, and these deaths were due to mixed infections with other pathogens [40]. These deaths may be due to the presence of other pathogenic organisms in combination, or to a combination of multiple underlying diseases at an advanced age, or to interruption of treatment [41]. The majority of cases have a favourable prognosis, and in some cases deaths occur. Chlamydia psittaci infection in pregnant women may also progress to severe disease,

posing a serious threat to the safety of mother and child, with foetal and maternal mortality rates of 82.6% (19/23) and 8.7% (2/23), respectively [38].

9. Outlook

Most of the current studies on Chlamydia psittaci pneumonia are on adults, and there are a few reports on pregnant women after infection with psittacosis, but there are almost no reports on paediatrics and adolescents, and further studies are needed. Due to the adverse effects of tetracycline and quinolone antibiotics on foetuses and infants, macrolide antibiotics are preferred for pregnant women infected with Chlamydia psittaci, but the foetal mortality rate is still high at more than 80%, and it can pose a serious threat to the mother as well, and further studies are needed to see whether this is related to the drug resistance of Chlamydia psittaci.

In China, as an atypical pathogen causing CAP, Chlamydia psittaci is currently not included in the routine pathogen testing of CAP inpatients, which may lead to underdiagnosis and misdiagnosis in clinical diagnosis. In addition, the route of transmission of Chlamydia psittaci is not limited to human-animal contact, but also includes interpersonal transmission and the possibility of the presence of asymptomatic carriers of the pathogen. Together, these factors increase the potential risk of an outbreak of Chlamydia psittaci infection. Therefore, the potential threat posed by Chlamydia psittaciens should be of great concern to the public health and medical professions. To improve diagnostic accuracy and timeliness, it is recommended that Chlamydia psittaciella be incorporated into routine screening processes for respiratory pathogens. In addition, the establishment and improvement of public prevention mechanisms and the adoption of effective measures to prevent and control the spread of Chlamydia psittaci have become an urgent public health issue.

Author contributions

Jianhua Gong proposed the idea for the article, Lianlian Yu and Yuefeng Li reviewed the literature. Lianlian Yu was responsible for writing the first draft, while Jianhua Gong and Yuefeng Li were responsible for proofreading and revising. All authors read and approved the final manuscript.

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