Using targeted next-generation sequencing to diagnose severe pneumonia due to Tropheryma Whipplei and Human Metapneumovirus: a case report and literature review

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Case Report

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Abstract

**Background:** In addition to the well-known Whipple’s disease (WD) infection with Tropheryma whippelii (TW) can also lead to acute pneumonia, but due to the lack of specific clinical manifestations and diagnostic measures, diagnosing TW-related pneumonia is extremely difficult.

**Clinical Presentation and Intervention:** This is an elderly patient with multiple injuries caused by falling from a building, and was transferred to intensive care unit (ICU) for mechanical ventilation and empirical anti-infection treatment due to severe hospital-acquired pneumonia (HAP), and then the patient’s bronchoalveolar lavage fluid (BALF) targeted next-generation sequencing (tNGS) suggested TW and human metapneumovirus (HMPV) infection, and after switching to anti-infective therapy for TW, the patient was successfully extubated and transferred out of the ICU.

**Conclusion:** This is the first case that using tNGS to diagnose severe pneumonia caused by TW and HMPV. We hope that our study can serve as a reference for the diagnosis and treatment of related cases in the future.

**Background**

Whipple's disease was originally named by George Hoyt Whipple in 1907 [1]. It is a rare chronic systemic disease caused by Whipple's disease, mainly manifested by joint pain, chronic diarrhea, and weight loss [2], and its prevalence is about 1 in 1 million [3]. After TW infection, most of the body will produce asymptomatic seroconversion, and a few will cause acute TW infection, mainly manifested by acute gastroenteritis, pneumonia and bacteremia. Finally, in most cases, the body will complete bacterial clearance, and in rare cases, it will develop into chronic asymptomatic carrier or chronic TW infection [4]. However, since TW is a caustic bacterium and difficult to culture in vitro, we mainly rely on histopathology and PCR for the diagnosis of TW infection [4]. However, in recent years, with the clinical use of mNGS, cases of TW pneumonia have occasionally been reported [6][7][8], TW is often found in BALF of patients with other pathogens, and they work together to cause pneumonia.

In our case report, a patient was diagnosed with severe pneumonia caused by TW and HMPV through tNGS testing of BALF, and with targeted treatment, the patient was successfully extubated and transferred out of the ICU. At the beginning of 2023, there was a spike in HMPV detection in various regions of the United States, and through this case, we would like to share with you the experience of diagnosis and treatment of severe pneumonia caused by the co-infection of TW and HMPV.

**Case report**

**Patient presentation**
On August 19, 2023, a 60-year-old man was hospitalized in the Department of Orthopedics of Jiangxi Provincial People’s Hospital because of multiple fractures in his body caused by falling from the 3rd floor, but while waiting for surgery, the patient had symptoms such as fever, cough, shortness of breath, and vomiting, and the pulse oxygen dropped to 86–92%, and the re-examination of CT on August 29 showed that pneumonia had progressed significantly compared with before. Considering severe pneumonia, he was transferred to the ICU for invasive ventilation, prone position, imipenem combined with tigecycline for anti-infective therapy. However, the patient still has recurrent fever (moderate-high fever). The patient has no previous history of underlying medical conditions and surgery, and no history of immunosuppressant use.

**Diagnosis**

After the patient was admitted to the ICU, the body temperature was 39.2°C, the respiratory rate reached 27 times/min, the oxygenation index was about equal to 150 (in the case of high-flow nasal canula oxygen therapy, the fraction of inspired oxygen was 60%), a large number of wet rales could be heard on auscultation of both lungs, the blood routine showed that the white blood cell count was 10.46×10⁹/L, the percentage of neutrophils was 89.2%, IL-6 was 126.36pg/ml, the procalcitonin level was 2.47ng/ml. 1-3-β-D glucan detection, aspergillus galactomannan detection and Covid-19 nucleic acid test and sputum culture was negative. The CD4-positive cell count was 203/ul, the blood lactate level was normal, chest CT showed multiple pneumonia in both lungs (as shown in Fig. 1), BALF tNGS (as shown in Table 1) showed TW (sequence number 867) and human metapneumovirus (sequence number 127).

<table>
<thead>
<tr>
<th>Type</th>
<th>specific name</th>
<th>Latin name</th>
<th>Sequence number</th>
<th>signal strength</th>
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<tbody>
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<td>Gram-positive bacteria</td>
<td>Tropheryma</td>
<td>Tropheryma whipplei</td>
<td>867</td>
<td>Medium</td>
</tr>
<tr>
<td>RNA virus</td>
<td>human metapneumoviru</td>
<td>human metapneumovirus</td>
<td>127</td>
<td>Medium</td>
</tr>
</tbody>
</table>

**Table 1**
The patient’s tNGS report.

**Therapy**

While hospitalized in the orthopedic ward, the patient began to have repeated fever, accompanied by cough and shortness of breath on August 26. The patient was given cefoperazone (2.0g,Q8h) as anti-infection treatment, but the symptoms did not improve. After being transferred to the ICU on August 29, the patient was given high-flow nasal canula (HFNC), imipenem (0.5gQ6h) as anti-infection treatment. Invasive mechanical ventilation treatment was given on August 30, and tigacycline (100mg Q12h) was added on August 31. The patient’s tNGS indicated TW and HMPV infection, but due to insufficient understanding of this bacteria, the anti-infection treatment regimen was not changed in time, but the
patient still presented with medium-high fever. On September 03, imipenem and tigacycline were stopped, and meropenem combined with co-trimoxazole SMZ was used to fight infection. co-trimoxazole, and then the tracheal intubation was removed, and he was transferred back to the general ward for further treatment on September 06.

**Discussion**

We first learned about TW mainly because of WD, a rare infectious disease\[^9\]. There is now growing evidence that TW is associated with acute pneumonia. J. Kirk Harris et al.\[^10\] first detected the sequence of TW in BALF in pediatric patients with acute interstitial lung disease. Subsequently, Sabri Bousbia et al.\[^11\] tested BALF from ICU pneumonia patients in France by 16S rDNA and quantitative polymerase chain reaction (qPCR), and found that among the 210 BALF specimens, TW DNA was detected in 6 samples, and only TW DNA was detected in 1 sample, which highly suggests that TW can cause pneumonia. In 2010, Florence Fenollar et al.\[^12\] detected TW DNA in saliva, BALF, and lung biopsy specimens from an elderly female patient with fever, night sweats, dyspnea, and arthralgia, and TW was cultured in BALF as the only bacterium. With the use of mNGS, there have been several case reports of TW pneumonia in various hospitals in China\[^6\][^7][^8].

In this case report, considering that the patient had HAP, the etiology might be relatively limited, and the patient's family was not wealthy, we chose to conduct tNGS detection on the patient's BALF instead of mNGS. Because of recurrent fever, we empirically added tigecycline to cover multi-resistant gram-negative bacteria. Since we had not seen TW pneumonia or WD cases before, so we didn't adjust the antibiotic regimen based on tNGS results. There was no significant improvement in the patient's symptoms. Fortunately, the patient was eventually diagnosed with pneumonia caused by the co-infection of TW and HMPV by the chief physician, meropenem combined with SMZ was used to target TW infection. Subsequently, the patient's condition improved and the ventilator was successfully removed and transferred to the general ward for further treatment. The patient's treatment results further supported the diagnosis of TW pneumonia.

Compared with mNGS, tNGS uses a screening process to enrich microbial sequences of interest before library preparation and sequencing\[^13\], it has higher sensitivity\[^14\]. Currently, tNGS can cover more than 300 kinds of bacteria and 200 kinds of viruses\[^15\][^16]. To this end, I have also learned from the genetic medical testing company we are currently cooperating with, and TW is also covered by tNGS.

We also made a corresponding analysis on how the patient contracted TW pneumonia. We suspect that the patient is likely to be an asymptomatic carrier of TW, TW can exist in the saliva of asymptomatic carriers\[^17\][^18], and TW may co-cause aspiration pneumonia with other oral flora\[^5\]. J.-C. Lagie et al.\[^19\] determined by TW PCR on 1438 BALF samples in hospitals, and the positive TW DNA of BALF was often associated with aspiration pneumonia. Although the patient's lung CT images did not show typical signs of aspiration pneumonia, combined with the patient's clinical manifestations such as vomiting, poor mental state, bed rest, and the presence of two common oral bacteria in the background microorganisms...
provided by tNGS, such as Abiotrophia defectiva and Veillonella parvus, all suggested the possibility of aspiration pneumonia in the patient. Unfortunately, at that time, we did not test the stool and saliva of patients for TW DNA, and it is not clear whether the patient is an asymptomatic carrier of TW. In addition, TW may colonize in the airways of healthy people, and when patients are hit by trauma and blood loss, it becomes an opportunistic pathogen, which I have considered.

As for the treatment of TW pneumonia, We often refer to the antibiotic treatment regimen for WD, while these treatment options have certain limitations, because WD is a rare disease, the sample size of randomized controlled trials was limited. The current recommended treatment regimen for WD is either ceftriaxone (1 dose of 2 g/day) or meropenem (3 doses of 1 g/day) for 14 days, followed by oral SMZ for 12 months. In vitro tests have shown that TW may be resistant to trimethoprim, in this case, we can replace SMZ with doxycycline. After referring to the antibiotic regimen for TW pneumonia reported by Wei Li et al., and considering the patient’s own situation, we also chose meropenem combined with SMZ. Unlike us, Sheng Wang et al. reported that TW pneumonia was successfully treated with imipenem. Areen Boulos et al. found that TW had great differences in sensitivity to imipenem in vitro, among the three strains, only Twist strains was sensitive to imipenem (MIC was 0.5 g/ml), while Endo2 and Slow strains were resistant to imipenem (MIC was 10 g/ml). I think this may be the reason why we all use imipenem, but the effect is not the same. Secondly, Kalliopi Foteinogiannopoulou et al. had successfully treated a WD patient who was resistant to trimethoprim by long-term intravenous use of tigecycline followed by oral doxycycline combined with hydroxychloroquine, so we also considered whether tigecycline could continue to be used as a part of treatment of TW pneumonia. Considering that the patient’s tNGS did not indicate MDR-GNB infection, we did not chose tigecycline in consideration of economic and adverse drug reactions and other factors. For the special medical setting of the ICU, tigecycline may also become a new option for the treatment of TW.

HMPV is a common cause of respiratory infections, and its infection is self-limited, the mainstay of treatment is supportive care measures with supplemental oxygen, antipyretic agents, and hydration with intravenous fluids if needed.

In conclusion, this case report is the first reported case of severe pneumonia caused by TW and HMPV infection confirmed by tNGS. For the diagnosis of special pathogen infection, tNGS has higher sensitivity and economic cost saving advantages compared with mNGS, and we should also pay attention to the existence of bias by tNGS. At present, there is no uniform standard for anti-infective treatment plan and course of treatment for TW pneumonia. Piperacillin tazobactam, imipenem, meropenem combined with doxycycline, meropenem combined with SMZ, and ceftriaxone combined with SMZ have all been reported to have successfully treated TW pneumonia, however, subsequent antibiotic treatment plan, TW re-infection and TW complete elimination is not clear, and this also needs further study.

Declarations
Since the data were anonymous, the need for ethics approval was waived by Ethics Committee of the Jiangxi Provincial People's Hospital.

**Consent for publication:**

Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor in-Chief of this journal.

**Availability of data and material**

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

**Competing interests**

The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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**Authors’ contributions**

FL and XY conceived and drafted the study. XY and CY revised the manuscript. All authors read, edited, and approved the final manuscript. All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. XY and CY are responsible for the overall content of the manuscript, and serve as the guarantors. All authors reviewed the manuscript.

**References**


Figures

Figure 1

Chest CT Scan: a. the first column, August 20th; b. the second column, August 29th; c. the third column, September 22nd