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ORIGINAL RESEARCH

Clinical, Laboratory, and Imaging Characteristics of Tropheryma Whipplei Detection in Bronchoalveolar Lavage Fluid Using Next-Generation Sequencing: A Case-Control Study

Ya Shen[1,2,*], Shun-Shun Cui[3,*], Xiao-Bao Teng[2,*], Ming-Feng Han[2], Yan-Bei Zhang[1]

[1]Department of Geriatric Respiratory and Critical Care Medicine, The First Affiliated Hospital of Anhui Medical University, Hefei, 230000, People's Republic of China; [2]Department of Respiratory and Critical Care Medicine, Fuyang Infectious Disease Clinical College of Anhui Medical University, Fuyang, 236000, People's Republic of China; [3]Department of Respiratory and Critical Care Medicine, Fuyang People's Hospital, Fuyang, 236000, People's Republic of China

*These authors contributed equally to this work

Correspondence: Yan-Bei Zhang, Department of Geriatric Respiratory and Critical Care Medicine, The First Affiliated Hospital of Anhui Medical University, No. 218 of Jixi Road, Shushan District, Hefei, 230000, People's Republic of China, Tel +86-13093340370, Email zhangyanbeizyb@126.com; Ming-Feng Han, Department of Respiratory and Critical Care Medicine, Fuyang Infectious Disease Clinical College of Anhui Medical University, No.1088 of Yinghexi Road, Yingzhou District, Fuyang, 236000, People's Republic of China, Tel +86-16655800262, Email mingfeng_han@126.com

Objective: The aim of this study was to assess the prevalence of *Tropheryma whipplei* (TW) infection in the population and to investigate the clinical symptoms, as well as the laboratory and imaging characteristics of patients testing positive for TW using next-generation sequencing (NGS).

Methods: A retrospective review was conducted on 1346 bronchoalveolar lavage fluid (BALF) samples collected between January 2021 and September 2023. The case group comprised patients with TW detected using NGS while the control group included 65 randomly chosen Gram-positive bacterial infection patients without TW. Comparative analyses were carried out on the basic demographics, laboratory parameters, and imaging findings between the two groups. Additionally, the case group underwent an in-depth examination of underlying diseases, pathogens, final diagnoses, treatment strategies.

Results: The case group comprised of 51 patients with TW, constituting 3.8% of the total. There was no significant difference in gender and age between the case and control groups ($P = 0.84$, $P = 0.07$). Symptoms such as coughing, expectoration, wheezing, fever, and hemoptysis are less commonly detected in the case group with a higher incidence of chest pain when compared to the control group ($P > 0.05$). The case group exhibited decreased albumin levels and increased C-reactive protein and D-dimer levels compared to normal levels. Imaging findings in the case group commonly included nodules, patchy images, and interstitial changes, the most common underlying disease is cardiovascular disease, and the most frequently co-occurring pathogen is the human herpesvirus. Among the case group, 27 patients received a final diagnosis of pneumonia, and 3 patients clinically diagnosed with Whipple's disease demonstrated improvement in both symptoms and imaging after treatment.

Conclusion: NGS revealed a relatively low overall detection rate of TW-positive patients using BALF. TW was more prevalent in middle-aged and elderly male patients characterized by symptoms such as cough, expectoration, shortness of breath, and fever. Chest imaging in these cases typically showed nodules and interstitial changes.

Keywords: bronchoalveolar lavage fluid, feature, image, NGS, *Tropheryma whipplei*

Introduction

Tropheryma whipplei (TW), a Gram-positive bacterium, is commonly found in soil, waste water, and polluted environments.[1] Infections with TW can give rise to classic Whipple's disease, characterized by symptoms such as joint pain, abdominal pain, diarrhea, and weight loss.[2] Some patients may exhibit chronic infections, including endocarditis, encephalitis, arthritis, lymphno-ditis, and uveitis. The disease may also manifest as asymptomatic carriage or acute infections,[3,4] such as gastroenteritis,

pneumonia, and bacteremia. Lagier et al found in their study conducted between 2006 and 2014 that 65 (37.1%) out of 175 patients with acute TW infection presented with pneumonia.[5]

TW is challenging to culture, requiring specialized laboratory conditions. As a result, the current diagnostic methods primarily rely on the periodic acid-Schiff (PAS) stain with duodenum biopsy specimens and the real-time quantitative polymerase chain reaction (qPCR) detecting system, recommended in recent years.[6] In 2007, Harris et al, for the first time, detected TW in the bronchoalveolar lavage fluid (BALF) of a pediatric patient with pneumonia using PCR.[7] Subsequently, in 2010 Fenollar et al reported the first positive TW culture in BALF[8] from a 70-year-old female patient utilizing qPCR alongside traditional culture methods.

In recent years, next-generation sequencing (NGS) has played a crucial role in clinical practice, particularly in the diagnosis of infectious diseases. Its significance is evident in the detection of rare pathogens.[9] One noteworthy study, led by Lin et al, examined 1725 BALF samples, identifying 70 patients as positive for TW using metagenomic NGS (mNGS).[10] This study delved into the clinical characteristics of these patients. However, there is limited research on the epidemiology and clinical aspects of TW in China. Our study aims to fill this gap by providing insights into TW infections within the population. We investigated the clinical symptoms as well as the laboratory and imaging features of affected patients. Additionally, we aim to highlight the diagnostic value of NGS in identifying TW-induced pneumonia, offering valuable references for clinicians.

Materials and Methods
Case Data
This study involved the selection of 1346 BALF samples obtained from Fuyang No. 2 People's Hospital and Fuyang People's Hospital, China spanning the period from January 2021 to September 2023. Collection of BALF samples adhered to clinical operation standards and aseptic principle. During electronic bronchoscopy 20–50 mL of normal saline was injected into the bronchus containing the lesion, followed by the prompt recovery of the lavage fluid. Subsequently, no less than 5 mL of BALF samples were aseptically stored at −20 °C for subsequent NGS detection.

Patients who tested positive for TW were identified and constituted the case group. Their baseline data encompassing demographic information, laboratory results, NGS findings, clinical symptoms, imaging examination outcomes, and history of diagnosis and treatment, were systematically collected. Additionally, 65 patients (except TW-positive patients) who tested positive for Gram-positive bacteria using NGS during the same period were randomly selected using a number table and formed the control group. Their basic data, clinical symptoms, NGS results, as well as laboratory and imaging data were collected. Immunodeficiency was defined based on specific criteria, including malignancies of solid organs, hematological malignancies, solid organ transplantation, hematopoietic stem cell transplantation, human immunodeficiency virus (HIV) infection/acquired immunodeficiency syndrome (AIDS), end-stage renal disease, congenital immunodeficiency, and autoimmune diseases (rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, multiple sclerosis, polymyalgia rheumatica, psoriasis, autoimmune thyroiditis, type 1 diabetes mellitus, vasculitis, and other autoimmune/collagen connective tissue diseases), among others.[11]

NGS Detection Process and Biological Information Analysis
NGS detection process: Bronchoalveolar lavage fluid was first lysed, followed by centrifugation to collect 600 μL of supernatant. DNA extraction was performed using DNA extraction kit (1901, Genskey, Tianjin). The extracted DNA was fragmented to sizes of 200–300 bp using sonication, followed by end repair, adapter ligation, and PCR amplification using NGS library preparation kit (1906, Genskey, Tianjin). Quality control of the amplified libraries and insert sizes was conducted using Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA), and DNA library concentration was quantified using fluorescent PCR. The constructed libraries were pooled and sequenced on an Illumina NextSeq 550 sequencer, with sequencing mode set to SE75, generating no less than 20 million reads.

Bioinformatics analysis: Sequences with low quality, low complexity, and lengths <70 bp were filtered out. Human reference genome sequences were removed, and the remaining high-quality sequencing data were aligned against microbial genome databases for identification. The microbial genome database includes 12,895 bacterial species, 11,120 viral species, 1582 fungal species, 312 parasitic species, 184 chlamydial species, and 177 mycobacterial species. Tropheryma whipplei was considered positive when at least three reads mapped to the species level.

Observational Indexes

Comparisons between the case group and the control group were conducted based on laboratory and imaging observations. White blood cell counts, neutrophil percentages, lymphocyte counts, platelet counts, hemoglobin, albumin, C-reactive protein (CRP), and D-dimer levels were measured in the blood and compared to established reference ranges.[12] Additionally, the number of cases in each group displaying specific imaging features such as nodules, patches, interstitial changes, cavities, masses, and pleural effusion was recorded.

Statistical Analysis

Categorical data were expressed as frequencies and percentages (n(%)), and compared using chi-square test or Fisher's exact test. Normally distributed continuous data were presented as mean ± standard deviation, and intergroup comparisons were conducted using independent samples t–test. Non-normally distributed continuous data were expressed as median and interquartile range (M(QL, QU)), and intergroup comparisons were performed using the Mann–Whitney U–test. Data were analyzed using SPSS 21.0 and GraphPad Prism, and $P < 0.05$ was considered as a statistically significant difference.

Results

General Data and Symptoms

In the case group, comprising 51 patients with an overall positive rate of 3.8%, demographic distribution revealed 36 males (70.6%) and 15 females (29.4%), aged 15 to 83 years. The mean age was 47.43 ± 17.59 years, with the highest proportion of patients falling within the 40 to 60 age range (41.2%). In the control group, which consisted of 47 males (72.3%) and 18 females (27.7%), the mean age was 53.29 ± 16.27 years, with the highest proportion of patients aged 40–60 years (49.2%). While there was no significant difference in gender and age between the two groups ($P = 0.84$, $P = 0.07$). Among the patients in the case group, 22 patients (43.1%), had a history of smoking, a slightly higher prevalence compared to the 41.5% observed in the control group, though this difference did not reach statistical significance ($P = 0.25$).

Symptomatically, in the case group, 39 patients exhibited cough, 31 patients had expectoration, 24 patients experienced shortness of breath, and 18 patients presented with fever. Additional symptoms such as chest pain, weight loss, hemoptysis, abdominal pain, diarrhea, and joint pain were also reported. In the control group, the most common symptoms were cough, expectoration, shortness of breath, and fever, mirroring those in the case group. The number of patients in the control group who coughed up sputum was significantly higher ($P = 0.04$), as shown in Table 1.

Table 1 Comparison of General Features and Clinical Manifestations Between the Case Group (n=51) and the Control Group (n=65)

Group	Case Group	Control Group	P Value
Gender (case)			
Male	36 (70.6%)	47(72.3%)	0.84
Female	15 (29.4%)	18(27.7%)	
Age (year)			
Mean age	47.43±17.59	53.29±16.27	0.07
<40	18 (35.3%)	11 (16.9%)	
40–60	21 (41.2%)	32 (49.2%)	
>60	12 (23.5%)	22 (33.8%)	

(Continued)

Table 1 (Continued).

Group	Case Group	Control Group	P Value
Smoking (case)			
Yes	22 (43.1%)	27 (41.5%)	0.25
No	29 (56.9%)	38 (58.5%)	
Symptom (case)			
Cough	39 (76.5%)	54 (83.1%)	0.38
Expectoration	31 (60.8%)	51 (78.5%)	0.04
Shortness of breath	24 (47.1%)	30 (46.2%)	0.92
Fever	18 (35.3%)	24 (36.9%)	0.85
Chest pain	13 (25.5%)	12 (18.5%)	0.36
Hemoptysis	6 (11.8%)	9 (13.8%)	0.79
Weight loss	8 (15.7%)	–	–
Abdominal pain and diarrhea	2 (3.9%)	–	–
Joint pain	1 (2.0%)	–	–
None	2 (3.9%)	–	–

Laboratory Data and Imaging Manifestations

In the case group, the laboratory examination Results indicated a decrease in serum albumin levels (38.10 ± 5.12 g/L), alongside increased levels of CRP (28.76 ± 52.58 mg/L) and D-dimer (0.68 ± 0.84 mg/L), compared to normal values. Conversely, the control group exhibited higher levels of white blood cells, neutrophilic granulocyte percentage, and CRP, however, the difference was not statistically significant (all with $P > 0.05$), along with lower levels of hemoglobin and serum albumin ($P < 0.05$), compared to the case group.

Regarding imaging manifestations, the case group predominantly displayed nodules, patchy images, and interstitial changes. Some patients exhibited cavities, mass images, enlargement of mediastinal lymph nodes, and pleural effusion, as shown in Figure 1. In the control group, patchy images were the most common manifestation, accounting for 83.1%, which was significantly higher than the 52.9% observed in the case group ($P = 0.00$). However, the proportion of interstitial changes and pulmonary nodules in the control group was lower than that in the case group, with no statistically significant difference ($P > 0.05$), as shown in Table 2.

Underlying Diseases in the Case Group

The prevalent underlying disease among the patients was cardiovascular diseases (27.5% of the total cases). This category encompassed conditions such as hypertension, coronary heart disease, and arrhythmia. The other common underlying diseases included diabetes mellitus (13.7%), chronic respiratory disease (9.8%), and liver and kidney diseases (9.8%). A smaller proportion of patients presented conditions related to the nervous system, digestive system, thyroid, malignancy, rheumatic immune diseases, and other miscellaneous diseases, as shown in Figure 2. According to the definition of immunodeficiency,[11] there were 3 patients identified, including 2 males and 1 female, aged between 38 and 52 years, with a mean age of 43.67 ± 7.37 years. Two patients had underlying conditions of malignancy, specifically gastric cancer and non-Hodgkin lymphoma, while one patient had end-stage chronic kidney disease.
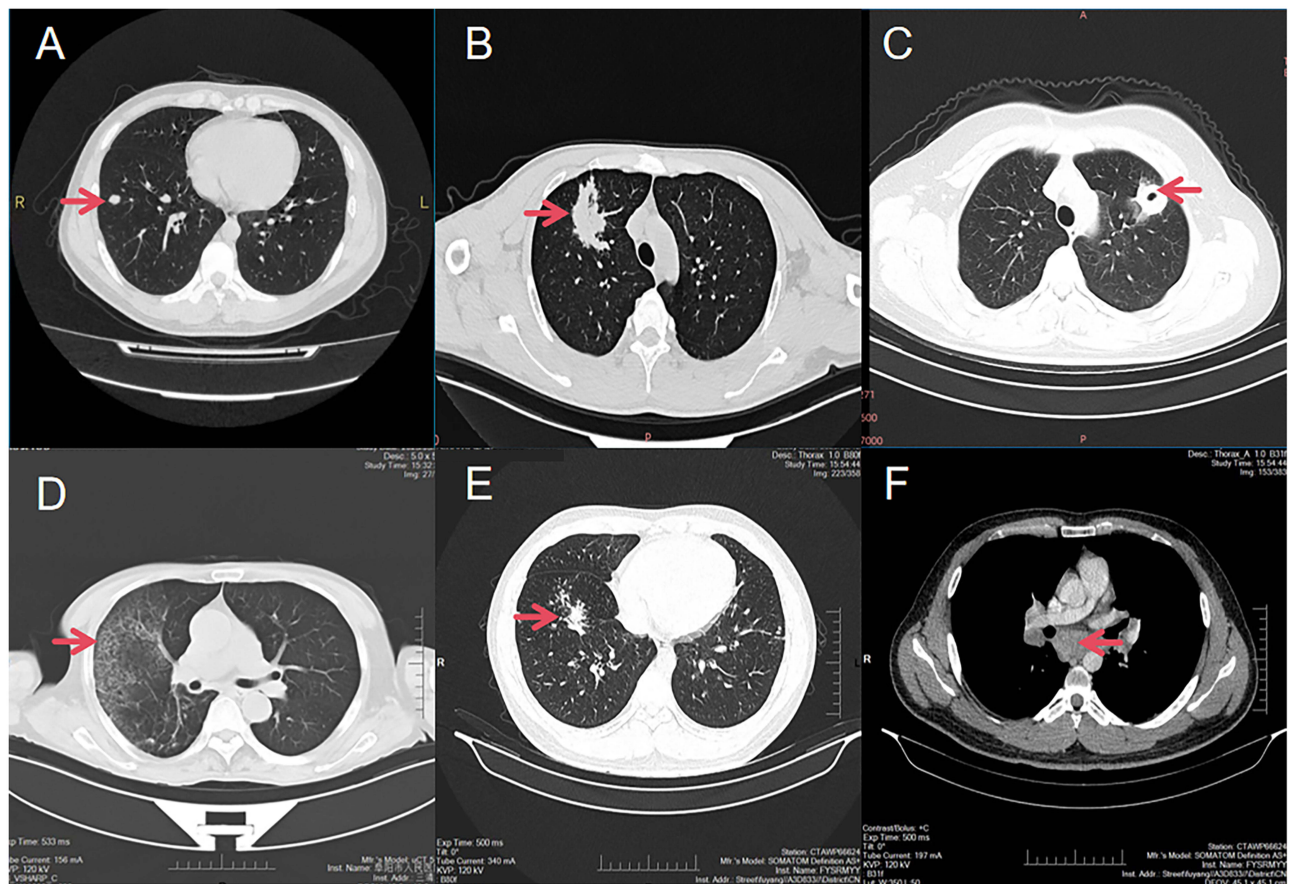
Figure 1 Imaging manifestations in the case group, indicated with arrows, including the nodule image (**A**), mass image (**B**), cavity image (**C**), interstitial change (**D**), patchy image (**E**), and enlargement of mediastinal lymph nodes (**F**).

Pathogen Distribution in the Case Group

Except for 8 patients with TW simple infection, the most frequently identified pathogens included human herpesvirus, *Haemophilus influenzae*, *Mycobacterium tuberculosis* (MTB)/non-tuberculosis mycobacteria (NTM), *Aspergillus*, and other infectious agents (Table 3).

Final Diagnosis and Treatment

Ultimately, 27 patients received a diagnosis of pneumonia, while the remaining cases were attributed to various conditions such as pulmonary tuberculosis, pneumomycosis, pulmonary sarcoidosis, bronchiectasis, pneumoconiosis, radiation pneumonia, and NTM disease, as shown in Figure 3. Whipple's disease was initially considered in the clinical diagnosis of 3 patients; however, the final diagnosis ruled out Whipple's disease as these patients also exhibited other infections. In 3 other patients, clinical diagnosis supported Whipple's disease, although they did not undergo further pathological examination. Among the 3 patients, 1 patient had immunodeficiency, diagnosed with non-Hodgkin lymphoma. The imaging findings in all 3 patients mainly showed interstitial changes and pulmonary nodules. Following diagnosis, these patients underwent treatment with ceftriaxone sequential compound sulfamethoxazole for a total course of 1 year. Subsequently, their clinical symptoms and imaging findings gradually improved.

Discussion

Whipple's disease is considered clinically rare with a higher prevalence among individuals of white descent and lower incidence among Asians and Africans.[13] A study conducted in the United States from 2016 to 2018 reported an incidence of 4.6 cases of Whipple's disease per one million inpatients.[14] In northwest Italy, the incidence from 2002 to 2008 was

Table 2 Comparison of Laboratory and Imaging Manifestations Between the Case Group (n=51) and the Control Group (n=65)

Group	Case Group	Control Group	P Value
Laboratory examination			
White blood cell count (*10^9/L)	6.78±2.57	7.38±3.15	0.42
Neutrophilic granulocyte percentage (%)	64.61±12.46	66.66±14.96	0.42
Lymphocyte count (*10^9/L)	1.64±0.74	1.54±0.69	0.47
Platelet count (10^9/L)	241.67±96.27	236.98±111.98	0.50
Hemoglobin (g/L)	131.80±18.25	122.19±18.83	0.01
Albumin (g/L)	38.10±5.12	36.00±5.88	0.04
C-reactive protein (mg/L)	28.76±52.58	52.91±77.87	0.24
D-dimer (mg/L)	0.68±0.84	1.00±1.50	0.22
Imaging manifestation (%)			
Nodule	24 (47.1%)	20 (31.3%)	0.08
Patch	27 (52.9%)	54 (83.1%)	0.00
Interstitial changes	11 (21.6%)	6 (9.2%)	0.11
Cavity	8 (15.7%)	11 (16.9%)	0.86
Mass	6 (11.8%)	11(16.9%)	0.44
Pleural effusion	5 (9.8%)	9(13.8%)	0.51
Double lungs	36 (70.6%)	47 (72.3%)	0.84
Single lung	15 (29.4%)	18(27.7%)	0.84
Single lung lobe	13 (25.5%)	11 (16.9%)	0.53
≥ 2 lung lobes	38 (74.5%)	54 (83.1%)	0.53
Enlargement of mediastinal/hilar lymph nodes	8 (15.7%)	11 (16.9%)	0.86

documented as 3 cases per million.[15] At present, there is a scarcity of epidemiological studies on Whipple's disease in China, with only a few case reports available.[16–18]

The widespread use of NGS in infectious diseases has led to an increased detection of cases with TW in recent years.[19] In this study, 1346 BALF samples collected from January 2021 to September 2023 revealed 51 samples positive for TW-through NGS, yielding an overall positive rate of 3.8%. This result aligns with the findings of Lin et al[10] (70/1705, 4.1%) and Bousbia et al[20] (6/210, 2.9%). Xing et al[19] analyzed respiratory tract samples from 119 patients using mNGS and tNGS, reporting positive TW in 11 patients (11/119, 9.3%). Lagier et al[21] employing PCR to assess 1438 BALF samples, detected TW in 88 patients, with an overall detection rate of 6.1%. Both studies reported higher detection rates compared to our findings. It is hypothesized that variations in baseline case characteristics and the relatively small sample size in our study may contribute to these differences, emphasizing the need for more extensive data for validation.

Prior research indicates that classic Whipple's disease predominantly affects male patients (approximately 86%), with an initial diagnosis typically occurring between the ages of 48 to 54 years.[6,13] In our study, the case group exhibited a higher proportion of male patients (70.6%) as compared to females, with a mean age of 47.43 ± 17.59 years, notably lower than the mean age in the control group (53.29 ± 16.27 years). The majority of patients in the case group fell within
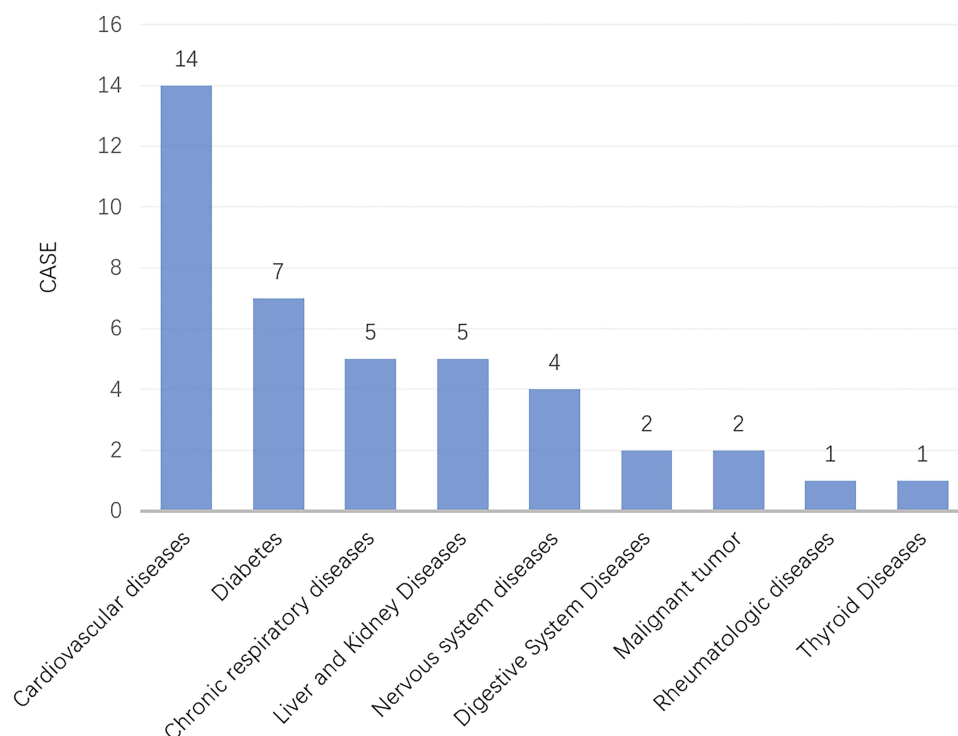
Figure 2 Distribution of underlying diseases in the case group.

the 40 to 60 age range, aligning with previous observations. The reasons for the higher incidence in male patients remain unclear, with some speculation linking it to X-linked susceptibility[22] or the genetic superiority of the HLA-B27 antigen in men,[23] although these hypotheses lack confirmation. The delayed diagnosis of Whipple's disease, typically occurring 2 to 6 years after the onset of symptoms, contributes to the higher mean age at diagnosis. The clinical symptoms of Whipple's disease often mimic those of other conditions making definitive diagnosis a challenge.[24]

Cough emerged as the most prevalent symptom in the case group accounting for 76.5%, along with expectoration, shortness of breath, fever, and chest pain. Individuals in the control group exhibited similar symptoms, with cough, expectoration, shortness of breath, and fever, being the most common. However, the incidence of chest pain was

Table 3 Pathogen Distribution in the Case Group

Pathogen	Case (%)
Simple TW	8 (15.7%)
Human herpes viruses (types 1, 4, 5, 6B and 7)	17 (33.3%)
Haemophilus influenzae	14 (27.5%)
Mycobacterium tuberculosis/Non-tuberculosis mycobacteria	9 (17.6%)
Aspergillus	8 (15.7%)
Streptococcus pneumoniae	8 (15.7%)
Klebsiella pneumonia	5 (9.8%)
Pneumocystis jiroveci	4 (7.8%)
Pseudomonas aeruginosa	3 (5.9%)
Torque teno virus	3 (5.9%)

(*Continued*)

Table 3 (Continued).

Pathogen	Case (%)
Staphylococcus aureus	3 (5.9%)
Acinetobacter baumannii	2 (3.9%)
Mucor	2 (3.9%)
Mycoplasma	2 (3.9%)
Legionella	1 (2.0%)
Nocardia	1 (2.0%)

significantly lower in the control group when compared to the case group. This suggests that chest pain may be more likely to occur following pulmonary infection with TW, consistent with some existing findings. The reported cases by Damaraju et al featured a 63-year-old male patient with a history of chronic cough for over two years who was ultimately diagnosed with Whipple's disease.[25] In the study conducted by Lin et al,[10] it was found that 32.9% of patients presented with cough and 18.6% exhibited expectoration, alongside other symptoms such as shortness of breath, fever, and chest pain. In the study conducted by Zhang et al,[17] which focused on 20 patients with Whipple's disease affecting the lungs, the distribution of clinical symptoms was as follows: 11 patients (55.0%) experienced cough, 10 patients (50.0%) had dyspnea, 5 patients (25.0%) reported chest pain, 3 patients (15.0%) exhibited expectoration, 1 patient (5.0%) had hemoptysis, and 2 patients (10.0%) were asymptomatic. The study conducted by Fang et al showed that all 5 patients with Whipple's disease involving the lungs experienced cough, 60% had expectoration, 20% had fever, 40% experienced shortness of breath, and 20% had hemoptysis.[15] These findings are generally consistent with the results obtained in our
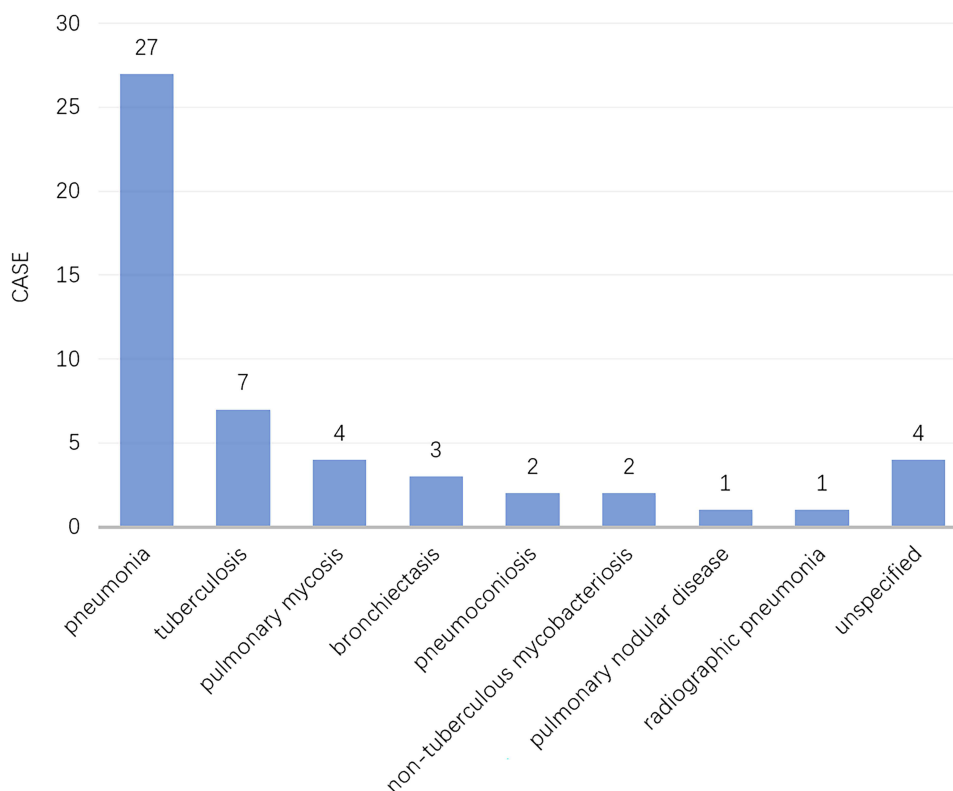
Figure 3 Final diagnosis in the case group.

study. Additionally, 1 patient in the case group encountered digestive tract issues, while another experienced joint pain. However, further histopathological examination would be needed to confirm the involvement of other issues. Urbanski et al observed that patients with Whipple's disease affecting the lungs often presented with clinical symptoms such as dry cough, chest pain, and shortness of breath, without gastrointestinal symptoms.[26]

Based on the laboratory findings, the case group commonly exhibited reduced serum albumin level and elevated levels of CRP and D-dimer. Notably the control group showed higher levels of white blood cells, neutrophilic granulocyte percentage, and CRP compared to the case group, however, the difference is not statistically significant. (P >0.05). Additionally, hemoglobin and serum albumin levels were lower in the control group than in the case group. In a study of American patients with Whipple's disease, the predominant abnormality was an increased CRP level (82%), along with other anomalies such as anemia (60%), elevated erythrocyte sedimentation rate (48%), and hypoalbuminemia (54%).[27]

Although, the current study did not reveal specific laboratory markers for Whipple's disease, it highlighted certain trends. Chest imaging indicated that nodule images, patchy images, and interstitial changes were common in the case group. Certain patients also displayed cavity images, mass images, enlargement of mediastinal lymph nodes, and pleural effusion. In contrast, the control group primarily exhibited patchy images, compared with the case group, the difference is statistically significant.(P<0.05). Furthermore, the proportion of nodules and interstitial changes was lower than that in the case group, indicating that nodules and interstitial changes may be distinctive clinical manifestations in patients testing positive for TW. However, since the sample size of this study was small and some patients in the case group were not definitively diagnosed, no statistically significant difference was found between the two group.

Research by Zhang et al found that the most frequent chest imaging manifestation in patients with pulmonary infection of TW was nodule images (10/20, 50%), followed by interstitial changes (5/20, 25%), and patchy images (5/20, 25%). Some patients also exhibited cavities, enlargement of mediastinal lymph nodes, pleural effusion, and other abnormalities.[18] These findings align with the current study, emphasizing the importance of considering and promptly identifying TW infection in clinical chest imaging for patients presenting with nodule images and interstitial changes.

Cardiovascular diseases were the most prevalent (14/51, 27.5%) among the comorbidities, encompassing conditions such as hypertension, coronary heart disease, and arrhythmia, followed by diabetes mellitus, chronic respiratory disease, and liver and kidney diseases. Bronchiectasis emerged as the predominant condition within the category of chronic respiratory diseases, suggesting a potential association between structural lung disease and TW infection. As there is a lack of sufficient studies exploring the influence of underlying diseases on patients with Whipple's disease, further research is warranted to elucidate the role of underlying diseases in the development and progression of Whipple's disease.

According to the criteria for immunodeficiency,[11] three patients in the case group were identified as immunocompromised, consisting of two patients with malignancies and one patient with end-stage renal disease. Bousbia et al conducted studies that involved six patients with TW-induced pneumonia, three of whom were immunocompromised.[20] Additionally, Stein et al reported a case of pneumonia in a patient with concurrent HIV infection, where TW was identified as the pathogenic bacterium through BALFPCR testing and other relevant examinations.[28]

In a study, it was found that 43.4% of patients with AIDS tested positive for TW when pathogens were detected in phlegm or BALF using PCR. However, further analysis of inflammatory cytokines and chemokines in the samples indicated no elevation in these cells, suggesting that TW may act as colonizing bacteria rather than pathogenic bacteria in these patients.[29] Consequently, it can be inferred that immunocompromised patients are susceptible to TW colonization and infection. When TW is detected in HIV-infected patients a comprehensive analysis considering the symptoms of the patient and relevant examinations is crucial to ascertain whether it represents pathogenic bacteria. It is important to note that in our study the statistical results might be influenced by the limited number of immunocompromised patients in the case group.

NGS can identify a broad spectrum of pathogens. In addition to the detection of TW in eight patients from the case group mixed pathogens were also identified in other patients. The prevalent pathogens in these cases were human herpes viruses, encompassing types 1, 4, 5, 6B, and 7. It is currently established that human herpes viruses types 6 and 7 primarily infect individuals with a high prevalence but generally do not cause significant effects in immunocompetent

adults.[30,31] Within the mixed bacterial infections, the proportion of MTB/NTM was 18.4%. Following further testing seven patients were conclusively diagnosed with pulmonary tuberculosis and two patients with pulmonary NTM disease. Other bacteria agents included *Haemophilus influenzae, Streptococcus pneumoniae, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. Fungal pathogen encompassed *Aspergillus, Mucor, Pneumocystis jiroveci*, and atypical pathogens (such as *Mycoplasma* and *Legionella*). While cases of TW coexisting with tuberculosis and fungal infections have been documented, the specific mechanisms underlying these combinations remain unclear.[32,33]

We conducted a more in-depth investigation into 27 patients definitively diagnosed with pneumonia in the case group. We determined that 6 patients were likely infected with TW through thorough testing, treatment, and comprehensive analysis. Among these, 3 patients were not diagnosed with Whipple's disease due to co-infection with other pathogens. As for the remaining 3 patients, who declined further procedures such as enteroscopy, lung puncture, or a second tracheoscopy, no pathological results were obtained, and they were clinically diagnosed solely with pneumonia caused by TW. Following treatment with ceftriaxone sequential compound sulfamethoxazole, all 3 patients exhibited improved clinical symptoms and imaging results. The patient with the longest survival has been under observation for 13 months and the medication has been discontinued without any recurrence during this period, with resolution of lung lesions.

Upon analyzing the data from patients in the case group, we recommend the following principles to determine the clinical diagnostic value of BALFNGS in detecting TW in patients with pneumonia: (1) For immunocompromised patients with TW as the only pathogen, consideration should be given to TW as a potential pathogenic bacterium. PCR detection is recommended for further diagnosis if circumstances permit. (2) In cases where TW is not the only pathogen among immunocompromised patients, a comprehensive assessment based on sequence numbers and the presence of other pathogens is advised. If treatment remains ineffective when considering other pathogens, TW should be included in the diagnostic considerations. (3) For immunocompetent patients with TW as the only pathogen and with a high sequence may warrant consideration of TW as a potential causative agent. PCR detection is suggested if conditions allow. (4) For immunocompetent patients with TW not being the only pathogen, PCR detection is recommended for further diagnosis if sequence numbers are high. TW is not considered if sequence numbers are low.

Conclusion

There is a growing number of reports on TW detection in BALF. Patients commonly exhibit symptoms such as cough, expectoration, shortness of breath, chest pain, and fever. Laboratory examinations do not reveal distinctive features and chest imaging typically shows nodules or interstitial changes. Patients with underlying diseases and immunocompromised individuals are more likely to be infected with TW. NGS enables clinicians to identify pathogens effectively and promptly, however, a comprehensive assessment based on sequence abundance, along with patient examinations, and treatment considerations is essential. Patients with TW-induced pulmonary infections, when treated in a timely and effective manner often experience favorable outcomes. It is important to note that results may be somewhat inaccurate in our study as patients in the case group were not further diagnosed based on histopathology and other examinations. As the comprehension of the disease grows, future studies will aim to provide more relevant insights.

Abbreviations

TW, Tropheryma whipplei; NGS, next-generation sequencing; BALF, bronchoalveolar lavage fluid; ALB, albumin; CRP, C-reactive protein; DDIC D-dimer PAS, periodic acid Schiff; qPCR, quantitative polymerase chain reaction; PCR, polymerase chain reaction; WBC, white blood cell; N, neutrophil; HLB, hemoglobin; NTM, nontuberculous mycobacteria; ESR, erythrocyte sedimentation rate; HIV, human immunodeficiency virus.

Data Sharing Statement

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

This study was conducted with approval from the Ethics Committee of Fuyang No. 2 People's Hospital (No.20231127054). This study was conducted in accordance with the declaration of Helsinki. Written informed consent was obtained from all participants.

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Disclosure

The authors declare that they have no competing interests.

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