Diagnostic Accuracy Study

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Diagnostic value of polymerase chain reaction/acid-fast bacilli in conjunction with computed tomography–guided pleural biopsy in tuberculous pleurisy

A diagnostic accuracy study

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Abstract

Patients with tuberculous pleurisy often remain undiagnosed even after blind thoracentesis and closed pleural biopsy (PB). In this study, we assessed the value of computed tomography (CT)-guided core needle biopsy of pleural lesion and evaluated the diagnostic accuracy of polymerase chain reaction (PCR)/staining for acid-fast bacilli (AFB) in suspicious tuberculous pleurisy undiagnosed in blind thoracentesis.

Patients with exudative pleural effusion (PE) without specific etiology after blind thoracentesis and closed PB were enrolled in this study. PB specimens were obtained through CT-guided core needle biopsy of pleural lesion, then underwent PCR, AFB, histopathological examination, and some routine tests. Diagnostic values were evaluated through sensitivity, specificity, negative predictive value, positive predictive value, and accuracy.

A total of 261 participants (TB group: 241, non-TB group: 20) were recruited. In this cohort, the sensitivity, specificity, and accuracy were 56.0%, 95.0%, and 59.0%, respectively for PCR, whereas 57.3%, 95.0%, and 60.2%, respectively for AFB. Their parallel test achieved an improved sensitivity (76.8%) and accuracy (77.8%), with a slight decrease in specificity (90.0%). In histopathological examination, granuloma was the most common finding in TB group (88.4%, 213/241), but also observed in non-TB group (10.0%, 2/20). In addition, pleural lymphocyte percentage in TB group was significantly higher than that of non-TB group (92% vs 61%, respectively; $P = .003$). However, no significant differences were found for other biomarkers.

CT-guided core needle PB is essential for patients with exudative PE but undiagnosed after blind thoracentesis. Combining with PCR and AFB, it strongly improves the diagnosis of tuberculous pleurisy.

Abbreviations: ADA = adenosine deaminase, AFB = acid-fast bacilli, AUC = area under the curve, CRP = C-reactive protein, CT = computed tomography, ESR = erythrocyte sedimentation rate, LDH = lactate dehydrogenase, NPV = negative predictive value, PB = pleural biopsy, PCR = polymerase chain reaction, PE = pleural effusion, PF = pleural fluid, PPV = positive predictive value, TB = tuberculosis, ZN = Ziehl Neelsen.

Keywords: image guidance, pleural biopsy, polymerase chain reaction, staining for acid-fast bacilli, tuberculous pleurisy

1. Introduction

Tuberculosis (TB) is a major public health problem at a global scale, causing considerable morbidity and mortality especially in Africa and Asia.[1–3] As the most common type of extrapulmonary TB,[4] tuberculous pleurisy is difficult to distinguish from malignancy, bacterial infection, and connective tissue–related pleurisy,[5–7] delaying effective medications and aggravating prognosis.

The detection of *Mycobacterium tuberculosis* and/or caseating granuloma[8,9] is considered as a criterion standard for tuberculous pleurisy, but often results in an unstable diagnostic rate with different samples and technologies. The false negative rate of Ziehl Neelsen (ZN) stain for acid-fast bacilli (AFB) remains high either in pleural fluid (PF) samples (sensitivity <10%)[9–12] or in pleural biopsy (PB) samples (sensitivity <50%).[13–15] In addition, it cannot distinguish *M tuberculosis* from non-TB *Mycobacterium* species.[16–18] Histological examination has the same drawback, with a sensitivity varying from 50% to 90% (PB sample).[10,19–21] TB-polymerase chain reaction (PCR) works

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well in discriminating *Mycobacterium* species and achieves an outstanding specificity (>95%), whereas the sensitivity remains changeable (PF: 3%–50% vs PB: 90%).[9,22–25] Laboratory culture, both sensitive (PF: 15% vs PB: 90%) and specific,[9,26] takes 2–5 weeks[26–28] before reaching a definite result. Some routine biomarkers [including lymphocyte percentage, lactate dehydrogenase (LDH),[29] and adenosine deaminase (ADA)[30]] and innovative methods (e.g., interferon-γ release assay[31]) have been applied to facilitate diagnosis, but none performs robust enough in tuberculous pleurisy. Approximately 25% of cases with exudative pleural effusion (PE) remain undiagnosed despite the availability of all aforementioned tests.[32,33]

Diagnosis is also impacted by thoracentesis techniques. When using blind thoracentesis and closed needle biopsy, >40% malignant cases (sensitivity <60%)[34] get negative results. Local anesthetic thoracoscopy improves this sensitivity (approximately 95% in pleural malignancy),[19,35–38] but invasiveness and complications are inevitable in this process.[39–42] Considering as its surrogate, image guidance [such as computed tomography (CT) and ultrasound] has been applied widely in various diseases. However, further evidences are needed to clarify its advantage in diagnosing tuberculous pleurisy.

In this study, we enrolled patients with exudative PE without specific etiology after blind thoracentesis and closed PB. CT-guided core needle PB was performed to obtain PB samples, used for both PCR and AFB. Histopathology examination and biomarkers were also tested as comparisons. This study was aimed to provide a possible solution for patients without clear etiology after blind thoracentesis.

2. Methods

2.1. Study population

Patients with exudative PE were prospectively and continuously enrolled in West China Hospital, Sichuan University from April 2016 to October 2017. Standard diagnostic procedures were conducted, including history taking; physical examination; and biochemical, cytological, and bacteriological investigations. As shown in Figure 1, only those with unclear etiology after blind thoracentesis were eventually included in the present study. Others with specific diagnosis were excluded. This study was approved by the Institutional Review Board of West China Hospital, and written informed consents were obtained from every participant in this study. Authors of this research were blind to clinical courses. They had access to information that could identify individual participants only after data collection.

2.2. Diagnostic criteria

The diagnosis of exudative PE was conducted according to Light's criteria. TB pleurisy was diagnosed with *M tuberculosis*–positive culture, caseating granuloma, and/or positive response to anti-TB medications.[8,9] Notably, patients enrolled in this study were those without clear etiology after blind thoracentesis. Therefore, evidences in sputum, PF, and PB samples from blind thoracentesis were inapplicable here. Only PB samples from CT-guided core needle PB were taken into consideration. Anti-TB treatment was applied upon pathological/bacteriological evidences. For those lacking such proofs, anti-TB treatment was given only when tuberculous pleurisy was highly suspected and non-TB diseases were all excluded. At least 6 months were needed to evaluate therapeutic responses. In addition, diagnosis in non-TB group was confirmed according to the histological, bacteriological, and/or therapeutic evidences.

2.3. Sample collection

All PB samples were obtained through CT-guided core needle biopsy of pleural lesion, preserved in sterile normal saline and used for pathological examination, *M tuberculosis* culture, AFB smear, and PCR for TB-DNA. In addition, other specimens (including plasma, sputum, and PF) were also collected for biochemical [protein, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), LDH, and ADA], cytological (white blood cell count and lymphocyte percentage), and bacteriological (*M tuberculosis* culture and AFB smear) investigations. Results in these samples were used as comparisons when estimating diagnostic performances of AFB/PCR.

2.4. Polymerase chain reaction

A DNA extraction kit (Qiagen, Valencia, CA) was adopted to gather DNA fragments from PB samples, according to manufacturer's instruction. Samples preserved in normal saline were first centrifuged at 5000 g for 10 minutes, and then resuspended using 162 μL lysis buffer. Subsequently, 20 μL Proteinase K was added and incubated for 1 hour. After centrifuging, supernatant was transferred to mini spin columns, coming out as pure and concentrated DNA fragments.

These DNA fragments were amplified and processed using a Care TB Diagnostic Kit for *M tuberculosis* DNA (Qiagen, Shenzhen, China).[43] Afterwards, LightCycler 480 Real-time PCR System (Roche Diagnostics, Mannheim, Germany) was used to detect DNA (IS 6110 insertion) specific for *M tuberculosis*. In this process, a reaction volume of 20 μL was implemented, containing 2 μL template DNA fragments, 0.2 μL Taq DNA polymerase, 0.03 μL uracil N-glycosylase enzyme and 17.8 μL master mix. Other detailed information about DNA extraction and PCR has been reported in our previous studies.[16,44] Technicians conducting this test were blind to the results of other tests and patients' clinical information.

2.5. Acid-fast bacilli

PB specimens for AFB were fixed in formalin and embedded in paraffin within 12 hours after CT-guided core needle biopsy of pleural lesion. Before staining, paraffin blocks were sliced into 8-μm sections, and deparaffinzed with xylene and ethanol. ZN method was used for staining. Afterwards, the stained slides were examined under oil immersion for the presence of AFB in a blind manner by 2 sophisticated pathologists independently. They were also blind to patients' information and results of other tests.

2.6. Statistical analysis

The statistical analysis was conducted using SPSS 16.0 (SPSS Inc, Chicago, IL). Baselines were compared between different groups through Pearson Chi-square test and nonparametric Mann-Whitney test. Diagnostic efficacies were evaluated with sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy. Receiving operating characteristic curve and area under the curve (AUC) were also conducted for biomarkers in blood and PE. In order to improve the diagnostic

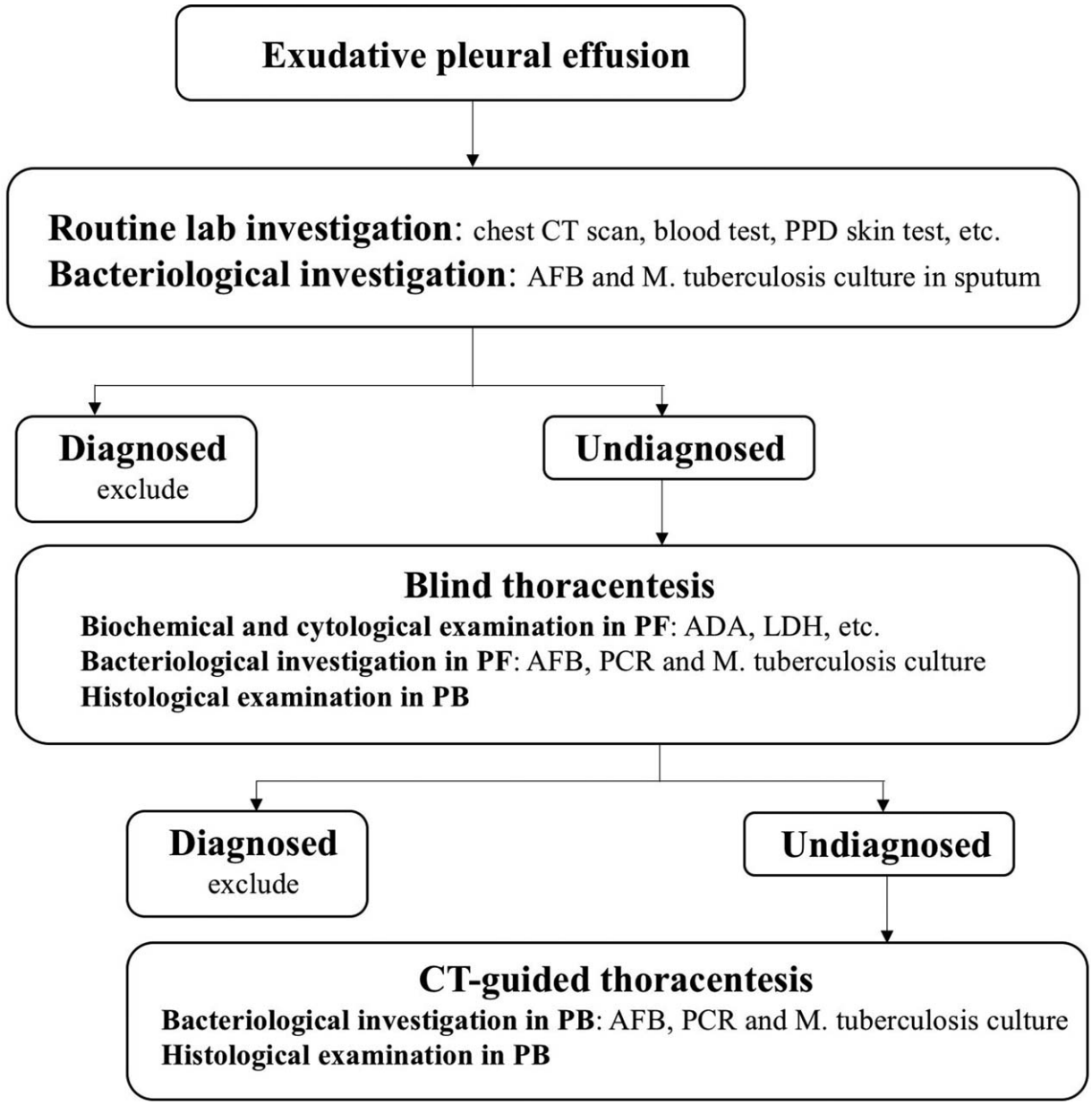

Figure 1. Inclusion and exclusion criteria of this study. ADA = adenosine deaminase, AFB = staining for acid-fast bacilli, CT = computed tomography, LDH = lactate dehydrogenase, PB = pleural biopsy, PCR = polymerase chain reaction, PF = pleural fluid, PPD = tuberculin pure protein derivative.

performance, AFB and PCR were further combined in 2 manners, serial test (AFB and PCR) and parallel test (AFB or PCR).[16] The former was positive only if both AFB and PCR were positive, whereas the later was positive when either AFB or PCR was positive. Results were considered significant when $P < .05$.

3. Results

3.1. Demographic and clinicopathological features

A total of 300 eligible participants with exudative PE were enrolled from April 2016 to October 2017, receiving CT-guided core needle biopsy of pleural lesion for PB samples (Fig. 2). Among them, 39 participants without specific diagnosis were excluded. Only 261 participants were finally included, divided into TB group (n = 241) and non-TB group (n = 20). Most TB cases (n = 225, 93.4%) were diagnosed according to positive response to anti-TB medications, whereas evidences of culture (n = 7, 2.9%) and histology (n = 9, 3.7%) were only attained in a small proportion. Non-TB cases were diagnosed with malignancy (n = 5), bacterial infection (n = 8), parasitic infection (n = 2), pulmonary embolism (n = 1), and connective tissue–related pleurisy (n = 4). No adverse events were observed in this procedure.

Detailed demographic and clinicopathological information of participants is shown in Table 1 and Supplemental file 1, http://links.lww.com/MD/D98. There was a trend that patients in non-TB group were older than that of TB group (medium, 50 vs 45), with a higher percentage of male (75.0% vs 74.7%). However, no significant differences were found (sex, $P = .98$; age, $P = .89$). Statistical differences were only noticed in clinical symptoms,

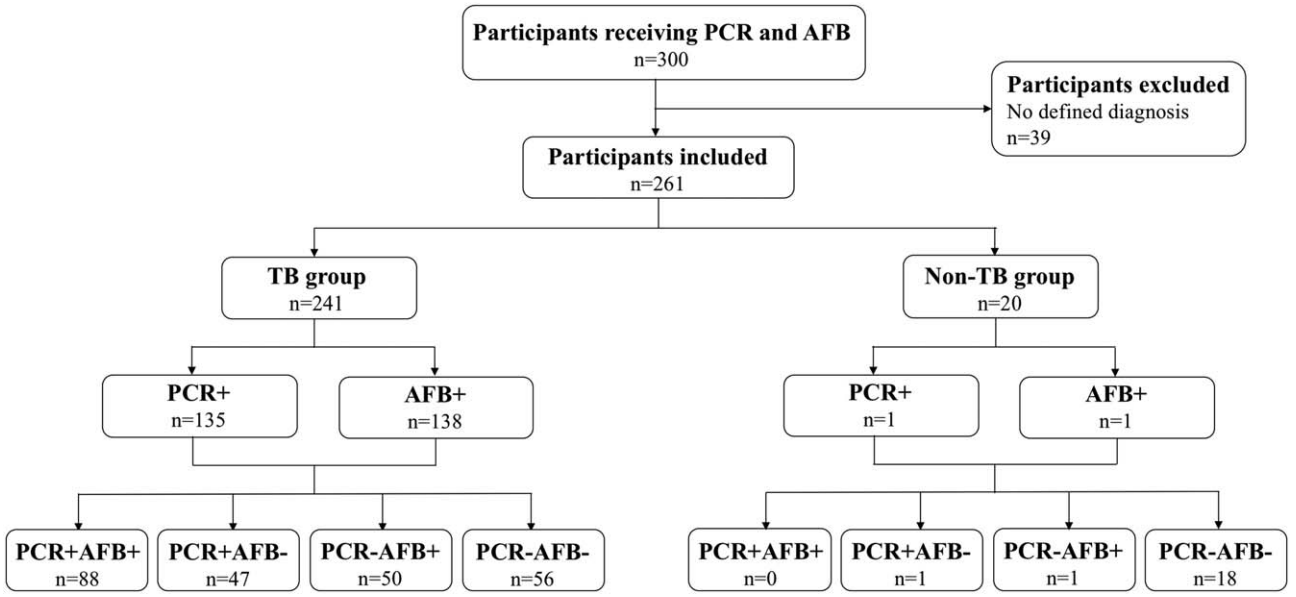

Figure 2. Study flow chart and diagnostic classification of eligible participants. −=negative, +=positive, AFB=staining for acid-fast bacilli, PCR=polymerase chain reaction, TB=tuberculosis.

including fever ($P=.02$), site of PE ($P=.006$), and comorbidity of lung TB ($P=.003$).

3.2. Diagnostic performance of PCR and AFB in pleural biopsy specimens

As shown in Table 2, AFB and PCR achieved an equal specificity (95.0%) and PPV (99.3%). However, neither was robust enough, yielding poor sensitivities (AFB, 57.3% vs PCR, 56.0%), NPVs (AFB, 15.6% vs PCR, 15.2%) and accuracies (AFB, 60.2% for vs PCR, 59.0%). One PCR false-positive case and 1 AFB false-positive case were observed in non-TB group. Both were proved to be bacterial infection, with positive responses to antibiotics. Neither received anti-TB medication.

We then compared diagnostic efficacies between AFB and PCR (Table 2). PCR successfully recognized 47 of 103 (45.6%) AFB-negative TB cases, whereas AFB identified 50 of 106 (47.2%) PCR-negative TB cases. A total of 56 TB cases were neither PCR nor AFB positive. Two tests achieved comparative diagnostic performances in this analysis.

At last, we tried to combine PCR and AFB in different manners (Table 2). The parallel test yielded an increased sensitivity (76.8%) and NPV (24.3%), with an approximate specificity (90.0%) and PPV (98.9%). As a comparison, the serial test slightly improved specificity (100.0%) and PPV (100.0%) at the expense of decreasing sensitivity (36.5%), NPV (11.6%), and accuracy (41.4%).

Table 1

Clinical characteristics of studied groups.

Category	TB group n=241	Non-TB group n=20	Total n=261	*P*
Sex, N (%)				
Male	180 (74.7)	15 (75.0)	195 (74.7)	.98
Female	61 (25.3)	5 (25.0)	66 (25.3)	
Age, avg (range)	45 (14, 88)	50 (23, 83)	46 (14, 88)	.89
Clinical symptom, N (%)				
Fever	127 (52.7)	5 (25.0)	132 (50.6)	.02*
Cough	189 (78.4)	14 (70.0)	203 (77.8)	.38
Site of pleural effusion, N (%)				
Unilateral	201 (83.4)	12 (60.0)	213 (81.6)	.006*
Bilateral	40 (16.6)	8 (40.0)	48 (18.4)	
Amount of pleural effusion, N (%)				
Mild	74 (30.7)	10 (50.0)	84 (32.2)	.13
Moderate	75 (31.1)	7 (35.0)	82 (31.4)	
Massive	92 (38.2)	3 (15.0)	95 (36.4)	
Comorbidity				
Lung TB	75 (31.3)	0 (0.0)	75 (28.7)	.003*
Diabetes mellitus	11 (4.6)	0 (0.0)	11 (4.2)	.33
HIV	2 (0.8)	0 (0.0)	2 (0.8)	.68

avg=average, HIV=human immunodeficiency virus, N (%) = number (percentage), TB=tuberculosis.
* $P<.05$.

3.3. Diagnostic efficacy of histopathological examination

Granuloma was the most sensitive finding in TB group (88.4%, n=213, Table 3), but it was also determined in bacterial infection (n=1) and malignancy (n=1). Caseating granuloma yielded the highest specificity (100%), coming with an extremely low sensitivity (3.7%). Intriguingly, the most common finding in non-TB group was nonspecific pleurisy (n=11, 55.0%).

3.4. Diagnostic values of biomarkers in plasma and pleural effusion

As shown in Table 4, pleural lymphocyte percentage in TB group was significantly higher than that of non-TB group ($P=.003$), achieving a limited AUC (0.768). No significant differences were found in other biomarkers, including ESR, CRP, protein in plasma and ADA, nucleolus cell, protein, and LDH in PE.

Table 2

Diagnostic performance of polymerase chain reaction and staining for acid-fast bacilli in pleural biopsy specimens.

	TB group n = 241	Non-TB group n = 20	Total n = 261	Sensitivity, %	Specificity, %	PPV, %	NPV, %	Accuracy, %
AFB								
Positive	138	1	139	57.3	95.0	99.3	15.6	60.2
Negative	103	19	122					
Total	241	20	261					
PCR								
Positive	135	1	136	56.0	95.0	99.3	15.2	59.0
Negative	106	19	125					
Total	241	20	261					
Serial test (AFB and PCR)								
Positive	88	0	88	36.5	100.0	100.0	11.6	41.4
Negative	153	20	173					
Total	241	20	261					
Parallel test (AFB or PCR)								
Positive	185	2	187	76.8	90.0	98.9	24.3	77.8
Negative	56	18	74					
Total	241	20	261					
PCR in AFB negative								
Positive	47	1	48	45.6	94.7	97.9	24.3	53.3
Negative	56	18	74					
Total	103	19	122					
AFB in PCR negative								
Positive	50	1	51	47.2	94.7	98.0	24.3	54.4
Negative	56	18	74					
Total	106	19	125					

AFB = staining for acid-fast bacilli, NPV = negative predictive value, PCR = polymerase chain reaction, PPV = positive predictive value, TB = tuberculosis.

4. Discussion

In this study, we enrolled patients with pleural exudates without clear etiology after blind thoracentesis and closed PB. PB samples were collected to evaluate the necessity of CT-guided core needle biopsy of pleural lesion, and different tests were compared to achieve an optimal solution for these patients. The parallel test of PCR/AFB using PB samples performed best in this cohort, with a sensitivity of 76.8% and a specificity of 90.0%. Other tests, including histological findings and biomarkers in plasma and PE, were either insensitive or nonspecific when used alone for diagnosing tuberculous pleurisy. It is noteworthy that caseating granuloma, which yielded the highest specificity (100%), was proved to be extremely insensitive (sensitivity 3.7%). Combing it with other robust markers may be a good choice for future studies.

Our research shows that image-guided PB is essential for patients without definite diagnosis after blind thoracentesis. Not only CT[45] but also thoracoscopy[38,42] and ultrasound[46] are effective image guidance. They provide clear visions for physicians and facilitate locating candidate tuberculous lesions. Our results also demonstrate the value of PB samples in diagnosing tuberculous pleurisy. Compared to PF samples, they contain much more *M tuberculosis* and yield a higher sensitivity. The underlying reason is that PF is primarily caused by delayed hypersensitivity to tuberculous protein.[26,47] Few *M tuberculosis* enters the pleural cavity directly.[12,48] Therefore, CT-guided core needle PB combined with PB samples can be an optimal choice for patients undiagnosed in blind thoracentesis.

Table 3

Histological findings of the studied groups.

Finding	TB group, N (%)	Non-TB group, N (%)
Nonspecific pleurisy	28 (11.6)	11 (55.0)
Granuloma	213 (88.4)	2 (10.0)
Necrotizing granuloma	113 (46.9)	0 (0.0)
Caseating granuloma	9 (3.7)	0 (0.0)

TB = tuberculosis.

According to our findings, the parallel test of PCR/AFB outperformed other tests in diagnosing tuberculous pleurisy, which has only been proved in pulmonary TB[16] previously. We also found that our PCR sensitivity (56.0%) turned out lower than determined in previous studies.[9,49,50] It may be related to our strict inclusion criteria. Since only patients without distinct etiology after blind thoracentesis were enrolled, *M tuberculosis* in pleural cavity might be fewer in this cohort, therefore yielding a lower PCR positive rate. Potential inhibitory substances in PB samples (such as pus and heparin[51]) are another important reason. They interfere with nucleic acid and inactivate DNA polymerase, then cause false-negative cases. Notably, we observed several AFB-positive/PCR-negative cases, which may be caused by non-TB *Mycobacterium* (such as *M kansasii*, *M chelonae*, and *M avium*).[52–55] DNA Sequencing is needed to confirm this hypothesis.

Consistent with previous studies,[56,57] we found significant difference in lymphocyte percentage in PF samples, but its value proved limited with an unfavorable AUC. We also evaluated the diagnostic performance of ADA, which has been considered as an ideal marker for tuberculous pleurisy in many studies.[10,58] Unfortunately, no significant difference was found between TB group and non-TB group ($P = .93$). Notably, this ADA level (medium 15.5 IU/L) was much lower than previously reported (40–50 IU/L).[58,59] It may be caused by the minimum amount of *M. tuberculosis* in this cohort as well. Since ADA is activated by the infiltration of T lymphocyte in pleural space, less *M*

Table 4

Comparison of biomarkers in blood and pleural effusion.

	TB group medium (range)	No	Non-TB group medium (range)	No	*P*	AUC
Plasma						
ESR, mm/h	58 (2, 120)	217	55 (2, 120)	19	.70	.527
CRP, mg/L	41.3 (<1.0, 205.0)	186	17.8 (1.9, 211.0)	18	.27	.579
Protein, g/L	66.7 (33.1, 88.8)	235	69.6 (50.9, 80.8)	20	.96	.497
Pleural effusion						
WBC, 10^6/L	845 (2, >20000)	205	1070 (20, >20000)	16	.18	.396
Lymphocyte (%)	92 (1, 100)	175	61 (2, 94)	11	.003*	.768
Protein, g/L	48.1 (6.8, 63.9)	208	47.7 (29.0, 86.0)	16	.69	.470
LDH, IU/L	346 (31, 10374)	207	1421 (94, 14621)	16	.11	.379
ADA, IU/L	15.5 (0.2, 130.4)	205	12.5 (3.5, 118.3)	16	.93	.506

ADA = adenosine deaminase, AUC = area under the curve, CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, LDH = lactate dehydrogenase, TB = tuberculosis, WBC = white blood cell.
* $P < .05$.

tuberculosis may cause weaker immune responses and lower ADA level. Other factors, including age and smoking history,[60,61] may also impact ADA level.

Except for the aforementioned issues, lacking a criterion standard is another major problem for tuberculous pleurisy diagnosis. Based on distinctive diagnostic criteria, tests in different studies are difficult to be compared or integrated. Most studies diagnosed tuberculous pleurisy with AFB culture and/or caseating granuloma.[8,9] Because this standard is not sensitive enough, patients with tuberculous pleurisy may be diagnosed with non-TB diseases, and tests evaluated may yield higher sensitivities. In order to solve this problem, multiple parameters were added, including biomarkers (such as ADA and lymphocyte count), CT scan, and clinical symptoms.[58,62] Conversely, non-TB diseases may be mistakenly diagnosed as tuberculous pleurisy in these studies, resulting in lower sensitivities for evaluated tests.

There's a major limitation in our study. Results of *M tuberculosis* culture (using PB samples obtained in CT-guided core needle biopsy of pleural lesion) were unavailable in some participants. Therefore, a large proportion of TB cases were diagnosed according to their active response to anti-TB medications. Another limitation should be the small sample size in non-TB group. An optimized research with larger-scale samples should be conducted to further evaluate the values of different tests.

In summary, image guidance is essential for patients with pleural exudates undiagnosed in blind thoracentesis. In addition, both PCR and AFB should be conducted to improve the diagnosis of tuberculous pleurisy.

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