ORIGINAL RESEARCH

Paraffin-embedded Sample Test Promotes the Diagnosis of Tuberculous Pleurisy

Jing Wei, MD; Tianyu Chen, MD; Xiaolin Chen, MD; Ganzhu Feng, MD

ABSTRACT

Context • Tuberculous pleurisy (TP) is the most common manifestation of extrapulmonary tuberculosis and the most frequent cause of pleural effusion (PE). Clinicians make a definitive diagnosis of TP based on the isolation of the mycobacterium tuberculosis (MTB) from PE or a pleural biopsy. Since the currently available tests for TP all have limitations in making a definitive diagnosis, clinicians urgently need new diagnostic tests.

Objective • The study intended to compare the value in clinically diagnosing TP of the paraffin-embedded sample test (PEST), using pleural-effusion samples; an adenosine deaminase assay (ADA) using pleural fluid; and the T cell enzyme-linked immunospot test (T-SPOT), using peripheral-blood.

Design • The research team performed a retrospective observational study.

Setting • The study took place at the Sir Run Run Hospital, Nanjing Medical University in Nanjing, Jiangsu, China.

Participants • Participants were 37 patients with suspected TP who had been admitted to the hospital between September 2018 and December 2022.

Outcome Measures • The research team assessed the diagnostic performance of PEST, ADA, and T-SPOT in the TP group, calculating the positive rate, sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) of the tests.

Results • Among the 37 participants, the testing confirmed that 24 had TP (64.86%), with 13 not having TP (35.14%). The PEST test produced a sensitivity of 83.3% for TP, with 20 out of 24 participants in the TP group testing positive (95% CI: 61.8 to 94.5), which was superior to the ADA, with only 9 out of the 24 participants (37.5%) in the TP group testing positive (95% CI: 19.6 to 59.2), with $P < .001$.

Conclusions • The PEST test possesses a high diagnostic value, and clinicians can use it as a time-saving, noninvasive, and highly sensitive method for TP diagnosis. It can be adjunct method to the currently used tests for diagnosing TP. A combination of several detection methods could promote effective treatment. (*Altern Ther Health Med.* 2023;29(5):153-157).

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The accumulation of fluid in the pleural cavity due to a mycobacterium tuberculosis (MTB) infection occurs as a result of a true pleural infection and an effusive hypersensitivity reaction. The clinical manifestations can be a large-scale pleural effusion (PE), cystic PE, or empyema.[1]

Tuberculous pleurisy (TP) is the most common manifestation of extrapulmonary tuberculosis and the most frequent cause of pleural effusions (PEs), with a tuberculous pleural effusion (TPE) being the second most common extrapulmonary TB.

The main clinical manifestations are fever, cough, unilateral pleurisy chest pain, night sweats, dyspnea, and

weight loss. The low bacterial nature of TPE makes it a diagnostic challenge,[2] and early diagnosis and timely initiation of treatment is of great importance.

Clinicians make a definitive diagnosis of TP based on the isolation of MTB from PE or a pleural biopsy. The isolation of MTB is difficult because it's a paucibacillary manifestation of tuberculosis. Although researchers have made great strides in the diagnosis of tuberculous pleurisy (TP), no single test has been sufficient so far to diagnose it in any setting where it's prevalent.[3]

Diagnostic Methods

Medical practitioners can harvest pleural tissues using closed pleural biopsy, thoracoscopy, or open surgical biopsy. Thoracoscopy and open surgical biopsy, however, are invasive manipulations and can cause complications, such as pneumothorax, pain, wound bleeding, and other complications.[4]

Baba et al evaluated and compared the performance of a real-time polymerase chain reaction (RT-PCR) to that of a nested polymerase chain reaction (N-PCR) for the diagnosis of pleural TB in formalin-fixed archival pleural biopsies from patients living in high TB and HIV endemic areas.[5] Their results suggested that RT-PCR could help to achieve a rapid diagnosis of TB pleuritis.

Another study revealed that PCR was useful for the rapid diagnosis of mycobacterial infection and differentiation of MTB from nontuberculous mycobacteria (NTM) in formalin-fixed, paraffin-embedded specimens, especially in acid-fast staining-positive specimens.[6]

Both of the above studies, however used lung tissues, lymphoid tissues, or tissues of other organs.

Diagnosis of tuberculous pleurisy (TP) using a smear and culture has low sensitivity. Conventional smear microscopy with Ziehl-Nielsen or Auramine stains of pleural fluid has a low yield of <10% and that of a culture with a solid culture media, such as the Lowenstein–Jensen medium, is about<30%.[1] Smears, pleural fluid and PEST in this study can get from thoracic close drainage in the ward with local anesthesia. The procedure is less invasive and it is different from obtaining pleural tissues. The latter needs to operate in the operating room under general anesthesia.

Two Common Clinical Tests

Adenosine deaminase assay (ADA). ADA, an enzyme involved in purine metabolism, occurs in high concentrations in tuberculous effusions and has a high overall diagnostic sensitivity and specificity, 92% and 90%, respectively.[7] An ADA level of ≥40 U/L in lymphocyte-predominant pleural fluid is the commonly accepted threshold for diagnosing TP.[8] ADA ≥40 U/L in pleural fluid was present in this article. ADA ≥40 U/L was a threshold reference.

Clinicians have used ADA as an auxiliary diagnostic indicator for TB pleural effusion for a long time.[9] However, elevated pleural fluid ADA can also occur in lung cancer, lymphoma, and rheumatoid arthritis.[10]

T cell enzyme-linked immunospot test (T-SPOT). TP's main immune mode is cellular immunity. After an in-vitro culture and antigen stimulation, the MTB antigens stimulate T cells, and the patient's body secretes cytokine interferon gamma (IFN-γ), participating in the immune response. The patient's peripheral blood shows the corresponding MTB-specific T cells.[11]

The T-SPOT detects the concentration of IFN-γ using the corresponding antibodies to determine the presence of the MTB infection. It can have a high value for diagnosing extrapulmonary tuberculosis and evaluating the therapeutic effects of treatments, because immunosuppression affects it less.[11]

The results of the T-SPOT can show differences in latent tuberculosis (TB), active TB, and old TB, but the T-SPOT can't clearly distinguish between active and latent tuberculosis.[12]

Paraffin-embedded Sample Test (PEST)

The above-mentioned methods all have limitations in detecting TP, so new assays or biomarkers may offer an appealing alternative for TP diagnosis. Given the paucibacillary manifestation of tuberculosis, centrifugation of pleural-effusion samples may help focus the evidence needed to diagnose TP.

The PEST refers to single or multiple centrifugal pleural effusions, stained acid-fast with sediments. Surgeons can perform this procedure after a patient has undergone a pleural puncture or closed drainage, and it doesn't increase the operating frequency or pain. PEST can be another way to diagnose TP. Meanwhile, the procedure has a low cost and can be repeated several times.

Current Study

The current study intended to compare the value in clinically diagnosing TP of PEST, using pleural-effusion samples; an ADA assay, using pleural fluid; and the T-SPOT, using peripheral-blood.

METHODS

Participants

The research team performed a retrospective observational study, which took place at Sir Run Run Hospital, Nanjing Medical University in Nanjing, Jiangsu, China. Potential participants were patients with suspected TP who had been admitted to the hospital between September 2018 and December 2022.

The study included potential participants if they: (1) were adults over the age of 18 years, and (2) had clinical records with complete information available for data collection; (3) had ultrasound-confirmed PE. 84 patients who had completed PEST were included at first, the reason that excluded is incomplete value of ADA, T-SPOT and follow-up disconnection.

All participants provided written informed consent for use of their medical information. The hospital's Institutional Review Board (2021-SR-013) approved the study's protocols.

Procedures

Data collection. The research team extracted all clinical data from participants' medical records and also tracked the treatment process of all patients.

TP diagnosis. The research team classified participants as definitely having TP if: (1) the testing detected MTB in their sputum; (2) their pleural tissue was positive for TP; or (3) they had had effective antituberculosis treatment confirmed in a subsequent follow-up examination.

The team classified participants as possibly having TP if: (1) the pathological examination had demonstrated granulomas in the pleura biopsies but MTB wasn't identified, (2) their ADA level was over 40 U/L in PE, or (3) they had had a good response to antituberculosis (TB) chemotherapy and clinicians couldn't exclude a diagnosis of active tuberculosis.

The team classified participants as not having TP if: (1) they had received an alternative diagnosis or (2) clinical improvement had occurred in the absence of anti-TB chemotherapy.[13]

The team considered all participants with definite and possible TP as having TP and used those results as the gold standard for calculation of sensitivity and specificity.

Groups. The research team divided participants into 2 groups: (1) those with definite and possible TP became the TP group, and (2) those who didn't have TP became the non-TP group.

Outcome measures. The research team assessed the diagnostic performance of PEST, ADA, and T-SPOT in the TP group, calculating the positive rate, sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) of the tests.

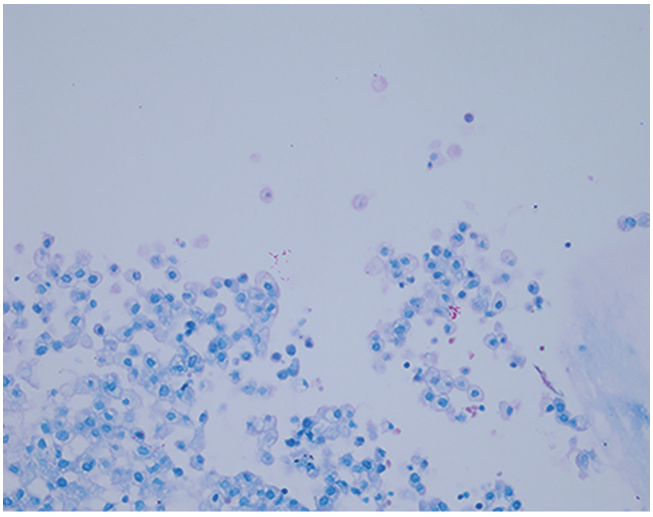
All participants received testing: (1) using pleural fluids, an ADA test (Ningbo Ruiyuan Biological Technology, Ningbo, China), for which the cut-off value for diagnosis was 40 U/L; (2) using serum samples, the T-SPOT (Wantai Kairui Biotechnology, Wantai, China), and (3) PEST, using pleural-effusion samples.

PEST. The research team: (1) collected participants' PE samples using pleural punctures and closed drainage; (2) centrifuged the samples at 3000 rpm for 5 minutes; (3) after removing the upper layer of liquid, fixed the sediments in alcohol for 5 minutes; (4) removed the fixation solution; (5) embedded the sediment and sliced it; and (6) performed hematoxylin-eosin (HE) staining and acid-fast staining (Figure 1). The team used an exudate of all of the participants' effusions.

Statistical Analysis

The research team analyzed all data using the R software, version 4.0.1 (R Foundation for Statistical Computing, Vienna, Austria). The team used the Chi-square test for comparisons between groups. $P < .05$ indicated that a difference was statistically significant.

Figure 1. Acid-fast Staining for PEST (×400). Rose-red and rod-shaped acid-fast bacilli can be seen in the pleural effusion treated with PEST.



Abbreviations: PEST, paraffin-embedded sample test.

RESULTS

Participants

The research team included and analyzed the data of 37 participants suspected of having TP (Table 1). No participants received a diagnosis of definite TP, and 24 received a diagnosis of possible TP (64.86%); 13 participants didn't have TP (35.14%). Among the 13 participants who didn't have TP, five received a diagnosis of cancer, according to the outcomes of cytological examinations; two had cardiac insufficiency, and six had parapneumonic effusion.

The research team made a judgment that all of the possible TP patients did have TP and used them as the gold standard for calculation of specificity and sensitivity.

The non-TP group included 4 females (30.77%) and 9 males (69.23%), with a median age of 67 and a range from 57 to 72 years. The TP group included 6 females (25.00%) and 18 males (75.00%), with a median age of 53 and a range from 24.50 to 72 years. The difference in age wasn't statistically significant ($P = .232$).

For the ADA, 13 participants in the non-TP group had a value of <40 (100.00%), and none had a value of ≥40 (0.00%); 15 participants in the TP group had a value of <40 (62.50%), and nine had a value of ≥40 (37.50%). The TP group had significantly more participants with a value of ≥40 than the non-TP group did ($P = .033$).

For the PEST, two participants in the non-TP group were positive (15.38%), and 11 were negative (84.62%); 20 participants in the TP group were positive (83.33%), and 4 were negative (16.67%). The TP group had significantly more participants with a positive result than the non-TP group did ($P < .001$).

For the T-SPOT, two participants in the non-TP group were positive (15.38%), and 11 were negative (84.62%); 15 participants in the TP group were positive (62.50%), and

Table 1. Demographic and Clinical Characteristics of the study Participants

Characteristic	Non-TP n = 13 n (%) Median [IQR] Mean ± SD	TP n = 24 n (%) Median [IQR] Mean ± SD	*P* value
Gender			1.000
Female	4 (30.77)	6 (25.00)	
Male	9 (69.23)	18 (75.00)	
Age	67 [57.00, 72.00]	53 [24.50, 72.00]	.232
HIV			1.000
Negative	13 (100.00)	24 (100.00)	
Positive	0 (0.00)	0 (0.00)	
Exudation			1.000
Positive	13 (100.00)	24 (100.00)	
Negative	0 (0.00)	0 (0.00)	
Bacillus			1.000
Negative	13 (100.00)	24 (100.00)	
Positive	0 (0.00)	0 (0.00)	
ADA			
≥40	0 (0.00)	9 (37.50)	.033[a]
<40	13 (100.00)	15 (62.50)	
PEST			<.001[a]
Positive	2 (15.38)	20 (83.33)	
Negative	11 (84.62)	4 (16.67)	
T-SPOT			.016[a]
Positive	2 (15.38)	15 (62.50)	
Negative	11 (84.62)	9 (37.50)	

[a]$P < .05$, indicating that the TP group had significantly more participants with an ADA value of ≥40 and with positive results for the PEST and the T-SPOT than the non-TP group did

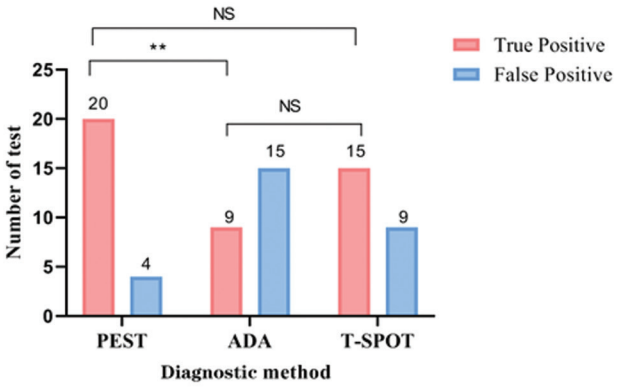
Abbreviations: ADA, adenosine deaminase assay; PEST, paraffin-embedded sample test; TP, tuberculous pleurisy; T-SPOT, T cell enzyme-linked immunospot test assay.

Table 2. Consistency Between PEST and Other Confirmatory Diagnosis Methods.

Diagnostic Methods		TP n = 24	Non-TP n = 13	Kappa
PEST	Positive	20 (83.33)	2 (15.38)	0.656
	Negative	4 (16.67)	11 (84.62)	
ADA	≥40	9 (37.50)	0 (0.00)	0.297
	<40	15 (62.50)	13 (100.00)	
T-SPOT	Positive	15 (62.50)	2 (15.38)	0.419
	Negative	9 (37.50)	11 (84.62)	

Abbreviations: ADA, adenosine deaminase assay; PEST, paraffin-embedded sample test; TP, tuberculous pleurisy; T-SPOT, T cell enzyme-linked immunospot test assay.

Figure 2. Sensitivity of Diagnosis Methods in Tuberculous Pleurisy (TP)



Abbreviations: ADA, adenosine deaminase assay; NS, not significant; PEST, paraffin-embedded sample test; T-SPOT, T cell enzyme-linked immunospot test assay.

nine were negative (37.50%). The TP group had significantly more participants with a positive result than the non-TP group did ($P = .016$).

Consistency of Results

Table 2 shows that 20 of the 24 participants in the TP group were positive for TP using PEST (83.33%); nine were positive using the ADA (37.50%), and 15 were positive using the T-SPOT (62.50%). Of the 13 participants in the non-TP group, two were positive for TP using PEST (15.38%); none were positive using the ADA (0.00%), and 2 were positive using the T-SPOT (15.38%). The Kappa values for 3 methods are positive numbers , confirms the consistency in diagnosis. However, the value of PEST is 0.656, higher than ADA and T-SPOT, indicating a better forcast result.

Sensitivity

Table 3 and Figure 2 shows that the PEST produced a sensitivity of 83.3% for the TP group, producing a positive diagnosis for 20 of the 24 participants (95% CI: 61.8-94.5), which was significantly superior to the sensitivity of the ADA test, producing a positive diagnosis for 9 (37.5%) of the 24 participants (95% CI: 19.6-59.2), with $P < .001$ as shown in Table 1.

The PEST wasn't superior to the T-SPOT, which produced a positive diagnosis for 15 (62.5%) of the 24 participants in the TP group (95% CI: 40.8-80.4), with $P = .016$ as shown in Table 1.

Specificity, PPV, and NPV

For the TP group, the specificity, PPV, and NPV were: (1) 84.6%, 90.9%, and 73.3%, respectively, for PEST; (2) 100%, 100%, and 46.4% respectively, for the ADA test; and 84.6%, 88.2%, and 55% respectively, for the T-SPOT (Figure 2 and Table 3). No significant differences existed in the specificity, PPV, or NPV between these methods.

Table 3. Comparison of Sensitivity, Specificity, PPV, and NPV Among the Tests for the TP Group (n = 24)

Diagnostic Method	Sensitivity 95%CI	Specificity 95%CI	PPV 95%CI	NPV 95%CI
PEST	0.833 (0.618, 0.945)	0.846 (0.537, 0.973)	0.909 (0.694, 0.984)	0.733 (0.448, 0.911)
ADA ≥40	0.375 (0.196, 0.592)	1.000 (0.716, 1.000)	1.000 (0.629, 1.000)	0.464 (0.280, 0.658)
T-SPOT	0.625 (0.408, 0.804)	0.846 (0.537, 0.973)	0.882 (0.622, 0.979)	0.550 (0.320, 0.762)

Abbreviations: ADA, adenosine deaminase assay; PEST, paraffin-embedded sample test; PPV, positive predictive value; NPV, negative predictive value; T-SPOT, T cell enzyme-linked immunospot test assay.

DISCUSSION

The current study evaluated the diagnostic performance of PEST and compared it with the ADA test and T-SPOT for TP patients. All of the current study's results suggest that PEST was the most accurate of the tests evaluated.

The PEST showed a significantly higher positive rate than the other two test methods. It had significantly better sensitivity (83.33%) than that of the ADA test (37.5%), indicating excellent performance, but the PEST wasn't superior to the T-SPOT in sensitivity, which confirms the clinical value of T-SPOT.

The specificity, PPV, and NPV showed no statistically significant differences between the tests, probably because the small size of the study's sample, but the low positive rate for TP using the ADA suggests PEST may be more meaningful for ADA-negative patients who actually have TP.

To the best of the research team's knowledge, the current study was the first to report the use of the PEST to detect TP. Unlike two studies evaluating PCR for diagnosis but using lung tissues, lymphoid tissues, of tissues of other organs,[5,6] the present study used centrifuged pleural effusion for the PEST, creating no secondary trauma to the body, and therefore, an increased possibility of patient involvement.

Clinicians use the positive acid-fast staining of the PEST sample not only found for MTB but also for non-tuberculous mycobacteria (NTM). Therefore, the current research team combined the results with the patients' clinical features and posttreatment follow-up to confirm the diagnosis of MTB.

The current study had some limitations. First, clinicians may use positive acid-fast staining in both MTB and NTM. Therefore, they must consider next generation sequencing (NGS) technology when necessary. Second, the number of patients with TP in the current study was relatively small, so a selection bias could have existed. The research team needs a larger cohort to further validate the results.

CONCLUSIONS

The PEST test possesses a high diagnostic value, and clinicians can use it as a time-saving, noninvasive, and highly sensitive method for TP diagnosis. It can be adjunct method to the currently used tests for diagnosing TP. A combination of several detection methods could promote effective treatment.

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AVAILABILITY OF DATA AND MATERIALS

This article includes the majority of the data generated or analyzed during the study. Unpublished data are available from the corresponding author upon reasonable request.

AUTHORS' DISCLOSURE STATEMENT

The authors declare that they have no conflicts of interest related to the study.

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