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Developing a method to detect lipoarabinomannan in pleural fluid and assessing its diagnostic efficacy for tuberculous pleural effusion

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ABSTRACT

Objectives: The diagnosis of tuberculosis pleural effusion (TPE) remains challenging, traditional diagnostic tests have limited diagnostic efficacy. This study aimed to assess the diagnostic performance of pleural fluid (PF) lipoarabinomannan (LAM) in TPE.

Methods: A diagnostic method for PF LAM (LAM-PF) was established using LEDBIO's AIMLAM kit. The diagnostic performance of LAM-PF was evaluated in 162 HIV-negative patients with suspected TPE.

Results: The LAM-PF method established in this study exhibited good linearity and recovery rate, with a limit of detection (LOD) of 2.90 pg/mL. Using a cut-off value of 5.33 pg/mL, the sensitivity and specificity of LAM-PF in diagnosing TPE (n = 128) were 47.7% and 100.0%, respectively. The sensitivity in patients with probable TPE (n = 29) and definite TPE (n = 99) were 41.4% and 49.5%, respectively. LAM-PF displayed a significantly higher sensitivity in probable TPE compared to other tuberculosis detection methods. Combined testing of adenosine deaminase (ADA)and LAM increased the detection sensitivity of TPE to 68.0%, and the area under the curve was 0.84 (0.77–0.89).

Conclusion: This study successfully established a method for detecting LAM in PF, which exhibited favorable diagnostic performance for TPE, particularly in challenging cases of probable TPE. Combined detection of LAM and ADA in PF significantly improves TPE diagnostic efficiency.

1. Introduction

Tuberculosis (TB) remains a significant contributor to global mortality as a singular infectious pathogen. In 2021, TB was responsible for an estimated 1.6 million deaths and 10.6 million cases, representing a 4.5% increase from the previous year [1]. Tuberculosis pleural effusion (TPE) is a frequently encountered extrapulmonary manifestation of TB in adults, resulting from the dissemination of the bacteria or its metabolites into the pleural cavity [2,3], TPE accounts for approximately 40% of PF cases in China [4]. Prompt recognition of TPE in undiagnosed PF patients is imperative as a delayed diagnosis can result in severe complications, including pleural thickening, empyema, calcification, and long-term pulmonary dysfunction [5].

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The diagnosis of TPE is typically challenging. Current gold standard methods for TPE diagnosis include culturing *Mycobacterium tuberculosis* (*Mtb*) from PF, performing Ziehl-Neelsen staining, and conducting pleural biopsy using an internal medicine thoracoscope, computed tomography, or ultrasound guidance. Although pleural biopsy is an effective diagnostic tool for TPE, its invasiveness may give rise to procedure-related complications, thus limiting its clinical utility [4]. While NAAT and culture exhibit a specificity of nearly 100%, their sensitivity is only about 30% [6]. Hence, the identification of novel diagnostic biomarkers in PF, the development of innovative diagnostic methods, or the refinement of existing diagnostic tests are essential for enhancing patient prognosis and TPE management.

Lipoarabinomannan (LAM) is a glycolipid present in the cell wall of Mtb, and it plays a role in mycobacterial growth, cell wall integrity, and host immune regulation [7]. LAM can be found in various body fluids of TB patients, including urine [8], serum [9], cerebrospinal fluid [10], and PF [11], and can serve as a biomarker for TB diagnosis, indicating active tuberculosis disease [7,12]. Currently, there are two primary LAM detection assays on the market, Alere Determine TB LAM Ag (AlereLAM, Abbott Laboratories, Chicago, USA) and Fujifilm SILVAMP TB LAM (FujiLAM, Fujifilm, Tokyo, Japan) [7,13], both of which can detect LAM in urine samples. There have been few reports of using other body fluid samples to detect LAM for TB diagnosis.

AIMLAM (LEDBIO, Guangzhou, China) is a chemiluminescent immunoassay reagent kit developed by LEDBIO for the detection of LAM in urine samples. This study aimed to expand diagnostic options for TPE by establishing a detection method for LAM in PF (LAM-PF) using the AIMLAM detection kit and assessing its diagnostic performance for TPE in clinical samples.

2. Methods

2.1. Study design and populations

This study retrospectively collected medical records of hospitalized patients who were 16 or older and presented with pleural effusion and suspected TPE. These patients were admitted to the Affiliated Hangzhou Chest Hospital, Zhejiang University School of Medicine, between April 2022 and October 2022. Standard thoracentesis was performed to collect PF samples from all patients. To assist in making the final diagnosis, imaging tests such as CT and ultrasound, as well as TB-related PF tests including acid-fast bacilli staining, mycobacterial culture, Xpert MTB/RIF (Xpert) test, and histopathological examination, as well as IFN-γ release assays (IGRAs) testing were performed on some patients. Biochemical, cytological, and LAM tests were performed on all PF samples. All commercial assays were performed according to the manufacturer's instructions.

2.2. Diagnostic criteria for TPE

Patients were divided into three groups according to diagnostic criteria [14,15] : (1) Definite TPE: *Mycobacterium tuberculosis* (Mtb) is isolated from PF, sputum, or pleural tissue through culture, microscopic examination, or Gene-Xpert, or pleural biopsy shows caseous granulomas. (2) Probable TPE: Pathogens are not confirmed in PF, but all patients are empirically treated for tuberculosis based on clinical suspicion (such as typical clinical symptoms, significant radiographic/pleural effusion, elevated PF adenosine deaminase (ADA), and positive immunological results (interferon-gamma release assays or tuberculin skin test)). (1) and (2) are considered the composite reference standard (CRS) for diagnosing TPE. (3) Non-TPE: The case is definitively diagnosed with other diseases, and all tests do not indicate tuberculosis.

2.3. The workflow for the collection, pre-processing, and detection of samples in LAM-PF testing

Process PF within 4 h and store it at −80 °C until LAM analysis. Thaw the samples on ice before use. Centrifuge the PF at 400g for 5 min and dilute the resulting supernatant 1:4 with normal saline. Heat the diluted sample at 95 °C for 5 min. After cooling to room

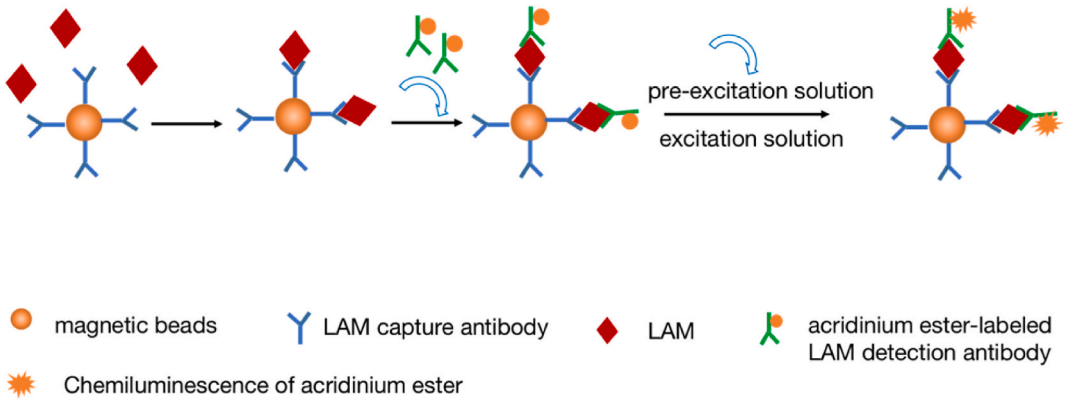

Fig. 1. Schematic diagram of LAM detection principle. LAM, lipoarabinomannan.

temperature, centrifuge the sample at 12,000 rpm for 5 min, and collect the supernatant for testing. Mix 1.5 mL of pre-treated sample supernatant with 50 μl of magnetic beads coated with LAM capture antibodies in a 2 mL centrifuge tube, and incubate at room temperature with agitation at 30 rpm for 2 h. Following incubation, place the tube on a magnetic stand for magnetic separation, and discard the supernatant. Add 200 μL of 50 mM Tris buffer (pH 7.4) to the tube, and vortex thoroughly. Sample testing should be conducted within 5 min according to the operating manual.

2.4. The test principle for detecting LAM in body fluids using chemiluminescent immunoassay

The principle of LAM testing is depicted in Fig. 1. Magnetic beads coated with monoclonal LAM capture antibodies bind to LAM in the sample, resulting in the formation of a magnetic bead-antibody-antigen complex. Subsequently, an acridinium ester-labeled LAM detection antibody is added to generate a magnetic bead-antibody-antigen-acridinium ester labeled LAM detection antibody immune complex, which is then washed. Finally, the pre-excitation solution and excitation solution are added to the reaction mixture, and the chemiluminescence value is measured.

2.5. LAM-PF specificity verification, standard curve establishment, and analytical verification

We conducted a specificity validation of the LAM-PF assay using multiple strains of nontuberculous mycobacteria (NTM) and Mtb. The bacterial strains grown on solid culture media were adjusted to a McFarlane density of 1 with normal saline, diluted 1000-fold, boiled for 20 min, and cooled before centrifugation to measure the LAM in the supernatant. All bacterial strains were isolated from clinical samples and confirmed by sequencing.

The purified LAM from Mtb was obtained from LEDBIO located in Guangzhou, China. Briefly, LAM ranging from 0 to 600 pg/mL was added to the PF of TPE-negative patients, and the standard sample was pre-treated in the same way as the PF. The LAM concentration was obtained by inserting the relative light unit (RUL) value corresponding to the sample from the standard curve using linear fitting. Each sample was tested twice, and the results were averaged. Results below the limit of detection (LOD) were recorded as "0″ for analysis. The patient inclusion criteria are presented in Fig. 3.

The determination of LAM spike recovery and dilution linearity was performed using purified LAM added to PF. Specifically, LAM was spiked into PF of TPE-negative patients at a concentration of 1200 pg/mL, serially diluted 2-fold, and added to pure pleural effusion to achieve a final LAM concentration range of 0–1200 pg/mL for testing using the aforementioned protocol. Limits of detection (LOD) were calculated from the mean background value of the standard curve plus 2.5 times the standard deviation [9].

2.6. Data analysis

Participant characteristics were summarized using median, interquartile range (IQR), and proportion. Skewed continuous variables were analyzed using the Mann-Whitney *U* test, while categorical variables were analyzed using the Chi-square test. We estimated the test sensitivity, specificity, and area under the curve (AUC) for the diagnosis of TPE with exact 95% confidence intervals (CI). All tests were two-sided with an α level of 0.05. All analyses were performed using SPSS v24.0 and data visualization was done using GraphPad Prism (version 9.4.1).

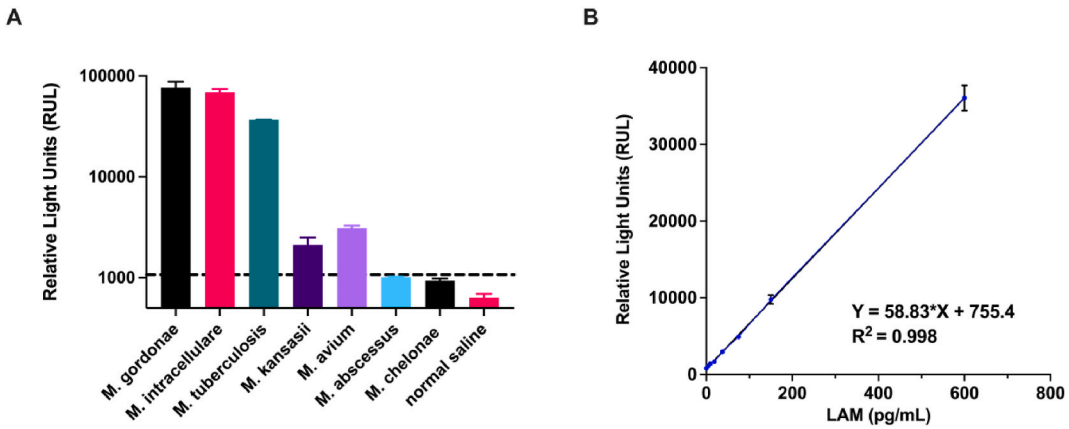

Fig. 2. Methodological Validation of LAM-PF. (A) Validation of specificity of the LAM-PF assay. (B) Establishment of a standard curve for LAM-PF in Pleural fluids. R^2 = 0.998. The points plotted were mean ± SD of 600, 150, 75,37.5,18.8,9.4, 4.6, 0 pg/mL. Each point represents 3 technical replicates.

3. Results

3.1. Analytic validation of the LAM-PF

Based on the cut-off value (derived from the RUL value of 1069 corresponding to 100% specificity in clinical samples), Fig. 2A demonstrates that the LAM-PF assay is capable of detecting Mtb and some slow-growing non-tuberculous mycobacteria, namely M. gordonae, M. intracellulare, *M. avium*, and M. kansasii, while rapidly growing mycobacteria, such as *M. chelonae* and M. abscessus, yield negative results.

We utilized purified LAM processed under the same protocol as clinical samples to generate a standard curve (Fig. 2B). The limit of detection (LOD) was determined by calculating the mean background value of the calibration curve plus 2.5 times the standard deviation, yielding a LOD of 2.90 pg/mL. In order to evaluate spike/recovery and dilution linearity, spiked LAM (1200 pg/mL) was serially diluted and added to mixed PF from non-TPE patients (PF dilution factors 2, 4, 8, 16, 32, 64, 128, 256) to measure a concentration range of 1.02–1045.27 pg/mL. All sample recoveries were found to be within the range of 80–120% [recovery = (measured value at each dilution - blank pleural effusion sample without spiked LAM)/theoretical value]. The linear range of dilution factors, except for 128 and 256, was determined to be within 80–120% [linear range = (measured value at each dilution factor × dilution factor)/original concentration value] (Table 1).

3.2. Characteristics of participants

A total of 162 patients were enrolled in the study, including 128 cases of TPE comprising 29 suspected cases and 99 confirmed, and 34 cases of Non-TPE (Fig. 3). Among the TPE patients, 60.9% (78/128) presented with concurrent pulmonary tuberculosis. Overall, the research comprised 134 male patients (79.8%), with a median age of 64 years (range, 16–98 years). TPE patients were relatively younger and had a higher proportion of males compared to non-TPE patients. Before PF collection, 45.3% of TPE patients had received anti-tuberculosis treatment, and probable and definite TPE accounted for 62.1% and 40.4%, respectively. The incidences of pneumonia, pericardial disease, and hypoalbuminemia did not differ between non-TPE and TPE patients (all $p > 0.05$). Non-TPE patients had a higher incidence of heart failure and cancer than TPE patients. The baseline characteristics of all patients are presented in Table 2.

3.3. Diagnostic performance of LAM and other tests for TPE in pleural fluid

Fig. 4 illustrates the comparison of LAM concentrations measured in the different sample groups. To achieve a WHO-recommended target product profile specificity of ≥98% for non-sputum-based biomarker-based tuberculosis detection, a cutoff value of 5.33 pg/mL was used in this study to achieve a specificity of 100.0% [16]. The LAM concentration in the Non-TPE group ranged from 0 to 5.30 pg/mL (median 0, IQR 0–3.12), while in the probable TPE group, it ranged from 0 to 167.40 pg/mL (median 4.23, IQR 0–21.71), and in the definite TPE group it ranged from 0 to 461.40 pg/mL (median 4.92, IQR 0–16.49). The PF LAM concentrations in probable TPE and definite TPE patients did not differ significantly ($P > 0.05$) but were significantly higher than those in the Non-TPE patients (all $P < 0.001$). The concentration of LAM in the TPE group receiving anti-tuberculosis treatment ranged from 0 to 378.40 pg/mL (median 4.44, IQR 3.04–10.81), whereas in the TPE group not receiving anti-tuberculosis treatment, it ranged from 0 to 461.40 pg/mL (median 6.49, IQR 0–24.74). There was no statistically significant difference in LAM concentrations between the group receiving anti-TB treatment and the group not receiving anti-TB treatment ($P > 0.05$).

In this study, the majority of PF samples were subjected to Xpert and culture testing, with sensitivities of 24.0% and 12.2%, respectively, and both AUCs were below 0.7. ADA and LAM testing was performed on all PF samples, with specificities of 100.0% each. The sensitivities for diagnosing TPE were 46.1% and 47.7% for ADA and LAM, respectively, with no significant difference between

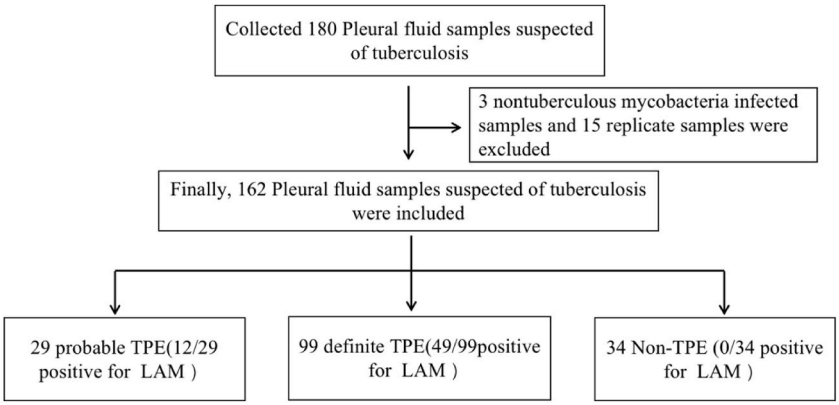

Fig. 3. Flow chart of patient enrollment scheme. TPE, tuberculous pleural effusion; LAM, lipoarabinomannan.

Table 1
Spike recovery and dilutional linearity of LAM-PF assay.

LAM standard	Dilution factors	LAM measured value	Recovery rate	Dilution linearity
1200	1	1045.27	87.00%	
600	2	599.84	99.80%	114.80%
300	4	281.95	93.60%	107.90%
150	8	153.08	101.40%	117.20%
75	16	70.53	92.70%	108.00%
37.5	32	37.49	97.30%	114.70%
18.75	64	16.52	82.70%	101.10%
9.38	128	11.27	109.30%	138.00%
4.69	256	6.37	114.10%	156.00%
0		1.02		

LAM, lipoarabinomannan.

Table 2
Baseline characteristics and demographics of participants.

Characteristics	probable TPE (n = 29)	definite TPE (n = 99)	Non-TPE (n = 34)	*p*-Value*	*p*-Value#
Male	24 (82.8%)	85 (85.9%)	25 (73.5%)		
Female	5 (17.2%)	14 (14.1%)	9 (26.5%)		
Age, years; Median (IQR)	60.0 (45.5–77.5)	61.0 (40.0–69.0)	80.5 (67.5–89.5)	<0.001	<0.001
TB treatment initiated	18 (62.1%)	40 (40.4%)			0.203
Pneumonia	8 (27.6%)	22 (22.2%)	9 (26.5%)	0.921	0.613
Cancer	4 (13.8%)	10 (10.1%)	9 (26.5%)	0.215	0.019
Pericardial disease	7 (24.1%)	20 (20.2%)	3 (8.8%)	0.189	0.130
Heart failure	7 (24.1%)	2 (2.0%)	9 (26.5%)	0.832	<0.001
Pulmonary tuberculosis	21 (72.4%)	57 (57.6%)			0.150
Hypoalbuminemia	24 (82.8%)	69 (69.7%)	26 (76.5%)	0.539	0.451
Pleural fluid, Median (IQR)					
Lymphocyte ratio	65.0 (34.5–92.5)	89.0 (63.5–98.0)	68.0 (34.0–88.8)	0.927	0.003
White blood cell*106	630.0 (155.0–1030.0)	1290.0 (445.0–3083.0)	520.0 (197.5–1153.0)	0.715	0.001
Red blood cell*106	1665.0 (812.5–14250.0)	2500.0 (975.0–5175.0)	1265.0 (287.5–4525.0)	0.277	0.136
Total protein (g/L)	28.0 (21.9–48.8)	48.3 (38.7–53.3)	31.4 (27.1–49.0)	0.220	<0.001
Albumin (g/L)	15.8 (10.3–29.2)	25.1 (18.9–29.0)	17.7 (14.9–24.6)	0.312	0.002
LDH(U/L)	153.0 (98.0–224.5)	364.0 (219.0–555.0)	139.0 (113.8–277.0)	0.745	<0.001
ADA (U/L)	7.7 (4.0–11.0)	37.7 (20.4–52.3)	7.4 (5.3–13.7)	0.393	<0.001
Glucose (mmol/L)	7.5 (5.5–9.0)	6.2 (4.7–7.9)	6.7 (5.8–8.6)	0.581	0.119
hs-CRP (mg/L)	9.2 (3.5–20.6)	17.1. (7.5–32.7)	10.4 (2.7–18.7)	0.975	0.005

TPE, tuberculous pleural effusion; IQR, interquartile range; TB, tuberculosis; LDH , Lactate dehydrogenase; ADA, adenosine deaminase; hs-CRP, high-sensitivity C-reactive protein; *Indicates the comparison; # Indicates the comparison between Non-TPE and definite TPE group between Non-TPE and probable TPE group.

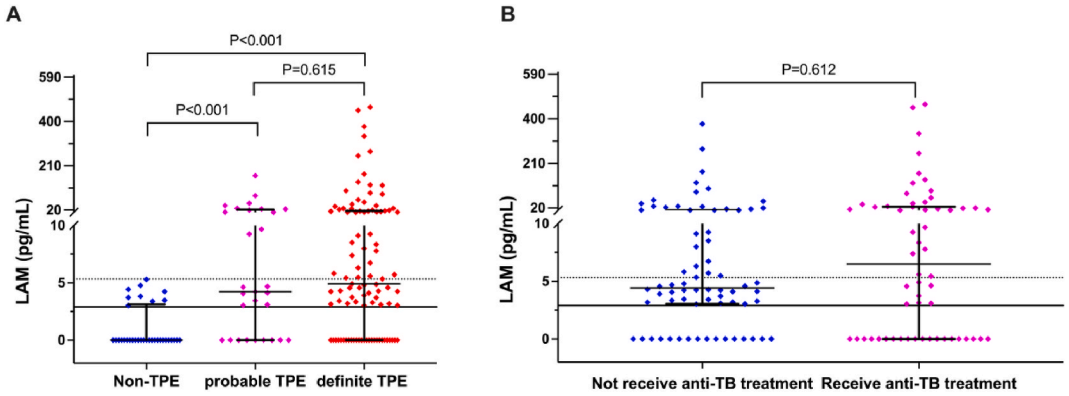

Fig. 4. The scatter plot depicts the distribution of LAM values from pleural fluid samples of different participants. Median and interquartile range (IQR) lines are displayed, the horizontal dashed line indicates the selected cutoff (5.33 pg/mL) and the solid horizontal line represents the LOD (2.90 pg/mL). (A) Grouped by diagnostic criteria (B) TPE patients were grouped according to whether they received anti-tuberculosis treatment. LAM, lipoarabinomannan; TPE, tuberculosis pleural effusion.

them ($P > 0.05$). The AUC for diagnosing TPE with LAM (0.74, 95% CI 0.66–0.80) was similar to that with ADA (0.73, 95% CI 0.66–0.80), and significantly higher than that with Xpert and culture (Table 3, Table 4). The sensitivity of ADA for diagnosing definite TPE was 57.6%, but only 6.9% for probable TPE, with a significant difference between the two groups ($P < 0.001$). The sensitivities of LAM for diagnosing probable TPE and definite TPE were 41.4% and 49.5%, respectively, with no significant difference between the two groups ($P > 0.05$). The combined use of ADA and LAM for diagnosing TPE increased the sensitivity to 68.0%, and the AUC was 0.84 (95% CI 0.77–0.89). The sensitivities of the combined use of ADA and LAM for diagnosing probable TPE and definite TPE were 44.8% and 74.8%, respectively (Table 5).

4. Discussion

In this study, the presence of LAM in PF was determined using a chemiluminescent immunoassay. To further augment the assay's sensitivity, a pair of high-affinity monoclonal antibodies was employed in the assay kit. These antibodies were generated by immunizing rabbits with cell wall components of the Mtb H37Rv strain. The antibody immobilized on magnetic beads exhibited an affinity constant of 4.34×10^{-10}, and the antibody conjugated with acridinium ester displayed an affinity constant of 3.35×10^{-10}. The test offers the advantages of simple operation, with results reported within 2.5 h. Furthermore, the magnetic bead capture provides the benefit of accommodating larger sample volumes.

LAM-PF detection demonstrated good performance with recovery rates within the range of 80–120% in the 0–1200 pg/mL range. Linear ranges of 80–120% were observed for all dilution factors except for 128 and 256 when conducting consecutive 2-fold gradient dilutions within the 0–1200 pg/mL range, indicating good dilution linearity. The LOD for LAM-PF detection in PF was 2.90 pg/mL. The low LOD of the detection can be attributed to the following factors: the utilization of a highly sensitive chemiluminescence immunoassay by AIMLAM and the use of high-affinity rabbit monoclonal antibodies labeled on magnetic nanoparticles to capture and enrich LAM antigens in the sample, despite a 5-fold sample dilution resulting in a final detection sample volume of 300 μL.

In this study, the cutoff value of 5.33 pg/mL was used to determine the sensitivity and specificity of LAM, which were 47.7% and 100%, respectively. The sensitivity of LAM was slightly higher than ADA and significantly higher than other Mtb pathogen detection methods, such as Xpert. Due to the paucibacillary nature of TPE, methods such as centrifugation for separating Mtb for genomic DNA detection and culture yielded poor results [3,17,18]. As a result, the sensitivity of PF methods such as Xpert and culture was low. The sensitivity of LAM in the definite TPE group (49.5%) was lower than that of ADA (57.6%), and the diagnostic advantage was not significant. However, in the probable TPE group, the sensitivity of LAM (41.4%) was significantly higher than that of ADA (6.9%) and other detection methods, such as Xpert. A significant statistical difference was observed between ADA in the definite TPE and probable TPE groups ($P < 0.001$), while no statistical difference was found in LAM between the definite TPE and probable TPE groups ($P > 0.05$). Probable TPE is a challenging clinical case to diagnose, and the existing methods have low diagnostic sensitivity. LAM has the potential to become a better pathogen diagnostic indicator.

In this study, the sensitivity of LAM in the probable TPE group was comparable to that in the definite TPE group, which could be attributed to the release of LAM bound to PF protein during the pre-processing steps [9,19], or the mechanisms of Mtb's existence and clearance of LAM in PF, which require further investigation to optimize the pre-processing methods and improve sensitivity. Although ADA demonstrated good diagnostic ability in definite TPE, its diagnostic efficacy was limited in probable TPE. Considering the diagnostic efficacy of LAM in probable TPE and the diagnostic advantage of ADA in definite TPE, combined detection of the two could enhance the sensitivity of TPE detection up to 68.0%, with an AUC of 0.84.

Thus far, only one study has been conducted on the detection of LAM in PF. Despite the use of a heating step to ensure LAM dissociation, the sensitivity of AlereLAM for detecting LAM antigen in PF was only 7% (1/14) [11]. While our study has made significant advancements in terms of sensitivity, achieving a sensitivity of 47.7%, it is still relatively modest and may be influenced by the presence of paucibacillary feature in PF and the sample pre-processing steps. Previous research has elucidated the formation of LAM-protein complexes in body fluids, which spatially hinder the capture or labeling of LAM in immunoassays [19]. To mitigate interference from host antibodies in the sample and to fully release LAM bound to proteins [9,11], a 95 °C heat treatment was performed on the samples before LAM assay analysis. Pleural effusion, unlike urine, contains higher protein content, and direct heating would result in protein denaturation and coagulation, rendering detection impossible. Considering that the protein content in pleural effusion is generally slightly lower than that in serum, we referred to a study by Brock et al. [9] , which involved a 6-fold dilution and heat treatment at 85 °C for 10 min for LAM detection in plasma samples. Based on this, we conducted tests using different dilution factors to establish the pre-processing workflow for pleural effusion. Our workflow included a 5-fold dilution of the sample with saline solution, followed by heat inactivation at 95 °C for 5 min. Prior studies have suggested that pre-treating serum samples with strong inorganic acids (HCl, HNO3, H2SO4, CF3CO2H and HClO4), followed by dilution and heat treatment, can improve LAM detection in serum [20–22]. However, considering the practical limitations of strong acid pre-treatment and the subsequent need for neutralization prior to LAM detection, we initially attempted a simplified pre-processing method involving dilution and heat treatment to achieve LAM detection in PF. There may exist more optimal processing methods, such as the use of proteinases like proteinase K or pronase to degrade proteins, and we will further optimize the sample pre-processing workflow for different sample types to enhance sensitivity. Additionally, while theoretically this method can detect LAM in other serous cavity fluid samples (such as ascites, cerebrospinal fluid, and pericardial fluid), it should be noted that the quantitative method used in this study was developed using PF as the substrate sample. Therefore, the detection procedures for other sample types would need to be developed accordingly.

The limitations of this study are as follows: Firstly, this study is a retrospective case-control study, and due to limitations in the experimental design, 45.3% of TPE patients received anti-tuberculosis treatment. Although this study did not demonstrate any statistically significant difference in LAM concentrations and diagnostic efficacy of TPE between the treatment and non-treatment groups,

Table 3
Diagnostic performance of several tests in pleural fluid.

Test	CRS diagnosed TPE		Sensitivity	specificity	AUC
	Positive	Negative	% (95%CI)	% (95%CI)	% (95%CI)
Culture			12.2 (6.3–20.8)	100.0 (75.3–100.0)	0.56 (0.46–0.66)
Positive	11	0			
Negative	79	13			
Xpert			24.0 (16.0–33.2)	100.0 (71.5–100.0)	0.62 (0.52–0.71)
Positive	24	0			
Negative	76	11			
ADA≥35 (U/L)			46.1 (37.2–55.1)	100.0 (89.7–100.0)	0.73 (0.66–0.80)
Positive	59	0			
Negative	69	34			
LAM			47.7 (39.2–56.3)	100.0 (89.7–100.0)	0.74 (0.66–0.80)
Positive	61	0			
Negative	67	34			
LAM + ADA			68.0 (59.1–75.9)	100.0 (89.7–100.0)	0.84 (0.77–0.89)
Positive	87	0			
Negative	41	34			

TPE, tuberculous pleural effusion; LAM, lipoarabinomannan; ADA, adenosine deaminase; CI, confidence interval.

Table 4
Diagnostic performance comparison of LAM and culture, Xpert, ADA.

Test	sensitivity (p-value)	specificity (p-value)	AUC (p-value)
LAM vs culture	<0.001	1.000	<0.001
LAM vs Xpert	<0.001	1.000	<0.001
LAM vs ADA	0.802	1.000	0.786

LAM, lipoarabinomannan; AUC, area under the curve; ADA, adenosine deaminase.

Table 5
Comparison of diagnostic sensitivity of LAM and ADA in pleural fluid.

Test	probable TPE		definite TPE		P -Value
	correct/total	% (95%CI)	correct/total	% (95%CI)	
ADA≥35 (U/L)	2/29	6.9 (0.9–22.8)	57/99	57.6 (47.2–67.5)	<0.001
LAM	12/29	41.4 (23.5–61.1)	49/99	49.5 (39.3–59.7)	0.442
LAM + ADA	13/29	44.8 (26.5–64.3)	74/99	74.8 (65.0–82.9)	0.002

TPE, tuberculous pleural effusion; LAM, lipoarabinomannan; ADA, adenosine deaminase; CI, confidence interval.

a cautious attitude toward this finding is warranted. Future studies involving larger prospective cohorts are needed to further validate the impact of treatment on LAM detection. Secondly, this study used frozen samples, and potential differences between fresh and frozen samples must be taken into account.

To summarize, the LAM-PF method developed in this study demonstrates effective detection of LAM in PF and holds promising diagnostic potential for TPE, particularly for probable cases. As the pre-processing steps continue to be optimized, the detection limit is expected to decrease further, resulting in improved sensitivity. Compared to mycobacterial culture and Xpert, the LAM-PF assay exhibits superior sensitivity, cost-effectiveness, and rapid result reporting. LAM has the potential to become a pathogenetic indicator for the diagnosis of TPE in the future.

Ethical statement

This study is a retrospective study, and the waiver of informed consent has been approved by the Ethics Committee of the Affiliated Hangzhou Chest Hospital, Zhejiang University School of Medicine (Research ID: 2023–013). The study protocol strictly adheres to the requirements of the Helsinki Declaration of the World Medical Association. All members of our research team are committed to maintaining the confidentiality of all patient information collected in the electronic medical record database.

Author contribution statement

Long Cai: Conceived and designed the experiments.
Lijun Peng: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.
Lingshan Dai: Performed the experiments; Analyzed and interpreted the data.
Mingzhi Zhu; Tingting Fang; Haiqiong Sun; Yanqin Shao: Performed the experiments.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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