Original Article

Pleural fluid lysozyme as a diagnostic biomarker of pleural tuberculosis: A systematic review and meta-analysis

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ABSTRACT

Objective: Pleural fluid lysozyme (LP) and its ratio to serum lysozyme (LP/LS) have been proposed as potential biomarkers for diagnosing tuberculous pleural effusion (TPE). We assessed the diagnostic accuracy of LP and LP/LS for TPE and evaluated their ability to differentiate TPE from other effusions. **Methods:** We queried the PubMed and Embase databases for studies indexed until October 2021. We included studies that (a) provided information regarding the sensitivity and specificity of LP or LP/LS for the diagnosis of TPE, or (b) compared LP or LP/LS between TPE and malignant or parapneumonic effusions. We used hierarchical summary receiver operating characteristic plots to model summary sensitivity and specificity. Random effects modeling was employed to pool standardized mean differences (SMD) across descriptive studies comparing TPE and other effusions. **Results:** We included 11 publications in our review, most of which were small and of poor quality. The summary estimates for sensitivity, specificity, and diagnostic odds ratio (DOR) were 0.94 (95% confidence interval [CI] 0.53–1.00), 0.89 (95% CI 0.63–0.97), and 129.88 (95% CI 6.26–2695), and 0.98 (95% CI 0.58–1.00), 0.91 (95% CI 0.84–0.96), and 708.47 (95% CI 11.42–43946), respectively, for LP and LP/LS. Mean LP and LP/LS in TPE were significantly higher than in malignant effusions (summary SMD 1.51 [95% CI 1.04–1.98] and 1.77 [95% CI 1.31–2.22], respectively), and parapneumonic effusions (summary SMD 0.86 [95% CI 0.51–1.22] and 1.15 [95% CI 0.64–1.66], respectively). **Conclusion:** There is low-quality evidence of good diagnostic accuracy for both LP and LP/LS in identifying TPE, the latter being marginally superior.

KEY WORDS: Diagnostic tests, lysozyme, meta-analysis, pleural tuberculosis, sensitivity and specificity

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INTRODUCTION

Tuberculosis (TB) is a common cause of exudative pleural effusion, particularly in regions with a high TB burden. A definitive diagnosis of tuberculous pleural effusion (TPE) requires demonstration of mycobacteria in pleural fluid (by nucleic acid amplification methods, microscopy, or culture), or documentation of granulomatous inflammation on pleural biopsy. The yield from microbiological testing is quite suboptimal, whereas the latter is invasive and not widely performed.[1] Therefore, physicians use surrogate laboratory biomarkers for initiating empiric anti-tuberculous therapy (ATT) among those with suspected TPE. Pleural fluid adenosine deaminase (ADA)

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is one such commonly used investigation having good sensitivity and specificity.[2] More recently, pleural fluid interferon-gamma levels too have shown good accuracy for identifying TPE.[3] However, none of these tests is a perfect discriminator, and there is an unmet need to identify other biomarkers for pleural TB.

Lysozyme is a low molecular weight bacteriolytic protein distributed in several body fluids and passively enters the pleural space through blood. Activated macrophages in tuberculous granulomas actively secrete lysozyme into the pleural fluid in patients with TPE, and both pleural fluid lysozyme levels (LP) and pleural fluid to serum lysozyme ratio (LP/LS) are thus greater in TPE than in other effusions.[4] However, lysozyme assays are poorly automated and time-consuming, and different studies report significant variability in diagnostic accuracy. Therefore, although the test is considered useful in differentiating tuberculous from non-tuberculous pleural effusions, it has not still been widely adopted.[4] Recent proteomics studies on pleural fluid and pleural biopsy samples, however, suggest significantly greater expression of lysozyme precursor in TPE compared to other pleural effusions.[5,6] Higher LP levels in patients with TPE also correlate with residual pleural thickening.[7] We, therefore, conducted a systematic review and meta-analysis to evaluate the utility of LP and LP/LS estimation in the diagnosis of TPE. We also specifically explored if LP or LP/LS could differentiate TPE from parapneumonic or malignant pleural effusions. Both these disorders are frequent diagnostic considerations during the assessment of pleural effusions suspected to be due to TB.

METHODS

We registered our systematic review and meta-analysis protocol with the PROSPERO database (registration number CRD42021287632) and followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for this review.[8,9] An approval was not required from our Ethics Committee as we used only summary data from studies already published.

Search strategy

We explored the PubMed and Embase electronic databases on October 31, 2021, using the following free-text search terms: (lysozyme, or muramidase); (tuberculosis, tubercular, tuberculous, TB, *Mycobacterium*, or mycobacterial); and (pleura, pleural, pleuritis, or pleurisy).

Study selection

After excluding duplicate records, two reviewers (ANA and RA) screened all titles and abstracts retrieved through the search process. We excluded publications in non-English languages and studies not focused on pleural TB. We also excluded review articles, conference abstracts, case reports, letters to editors not describing original data, and editorials. Full texts of all articles judged potentially eligible were then retrieved for independent evaluation by both reviewers.

We included a study for data synthesis if it (a) included patients with TPE and at least one other cause for exudative pleural effusion, (b) employed a microbiologic (presence of acid-fast bacilli, or positivity for *Mycobacterium tuberculosis* on nucleic acid amplification tests or culture, in pleural fluid, pleural biopsy, or another clinical specimen), histopathologic (pleural biopsy demonstrating granulomatous inflammation), and/or a clinical (compatible clinical profile with adequate resolution of effusion after empiric anti-tubercular treatment) reference standard for diagnosing TPE, and (c) provided numerical data for calculating both sensitivity and specificity of LP or LP/LS for diagnosis of TPE, or provided measures of central tendency (mean or median) and dispersion (standard deviation [SD], or interquartile range [IQR], or range) of LP levels or LP/LS in patients with TPE and other pleural effusions. If the same patient population was evaluated in two or more studies, only the one assessing the largest dataset was selected. In case of any disagreement, study inclusion was decided through consensus between the two reviewers.

Data extraction

We extracted the following data from the studies finally eligible for inclusion: study design, year of publication, countries where the studies were performed, the etiology of non-tuberculous pleural effusions, human immunodeficiency virus (HIV) status, lysozyme assay method and its threshold, blinding, the proportion of TPE patients diagnosed using microbiologic or pathologic criteria (referred to hereafter as having "definite TB"), number of subjects in each group, the number of positive and negative assay results for each category of subjects, and the mean and SD of pleural fluid lysozyme for tuberculous, malignant, and parapneumonic effusions. If data dispersion was expressed as a range of values, or as a standard error of the mean, we approximated the SD assuming a normal distribution of data.[10]

Assessment of study quality

We graded the methodological quality of studies reporting on diagnostic accuracy through the QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies, version 2) tool.[11] We employed the Newcastle–Ottawa Scale to assess the methodological quality of studies describing differences in LP levels or LP/LS between TPE and other effusions. Any study with a score of at least 7 (out of a maximum possible score of 9) was judged as having good quality.[12]

Statistical analysis

We computed sensitivity, specificity, and diagnostic odds ratio (DOR) for all studies reporting on diagnostic accuracy data. We calculated the 95% confidence interval (95% CI) for each study using the Clopper–Pearson approach.[13] We applied a continuity correction of 0.5

before any logarithmic or logit transformation in studies describing zero cell frequencies. We used the Rutter and Gatsonis hierarchical model to pool diagnostic accuracy data across these studies.[14]

For studies comparing LP levels or LP/LS between different categories of pleural effusion, we estimated the standardized mean difference (SMD) and their 95% CI as bias-adjusted Hedges'*g*.[15] We made formal pairwise comparisons between TPE and malignant and parapneumonic pleural effusions. We calculated summary effect sizes for SMDs using DerSimonian and Laird random effects model.[16]

We expressed between-study heterogeneity using Higgins' inconsistency index (I^2) and judged it high if the I^2 value exceeded 0.75.[17] We explored heterogeneity through subgroup analysis if 10 or more studies were retrieved for any analysis. For this, we stratified data based on prespecified covariates that included study design, the national burden of TB (high or not), the prevalence of TB in the entire study population (below 50% or more), the robustness of reference standard (definite TB or composite clinical criteria), the inclusion of transudative effusions, a technique of lysozyme analysis, and blinding in study. The World Health Organization (WHO) guidelines were used to designate countries as high-burden.[18] We conducted sensitivity analysis by excluding one study at a time from the analysis to evaluate if the summary results were unduly influenced by any single publication. We assessed publication bias using Deek's plot for diagnostic accuracy studies, funnel plots, and the non-parametric rank correlation (Begg's) test for descriptive studies. We utilized the GRADE (Grading of Recommendations, Assessment, Development and Evaluation) approach to report the quality of evidence on diagnostic accuracy.[19,20]

We analyzed our data using the statistical package Stata (Intercooled edition 12.0, Stata Corp, Texas, USA). The MetaDAS macro was additionally applied to fit the hierarchical summary receiver operating characteristic (HSROC) model in the SAS environment (SAS OnDemand for Academics, SAS Institute Inc., North Carolina, USA).[21]

RESULTS

Our literature search yielded 89 citations [Figure 1], of which we ultimately included 11 studies reporting information on diagnostic accuracy ($N = 7$) or comparing LP data between tuberculous and other effusions ($N = 10$).[22-32] The major attributes of these 11 publications are summarized in Table 1. The maximum number of studies (5, 45.5%) were conducted in Spain.[22,24,30-32] Only two studies (18.2%), both from India, were conducted in a country with a high burden of TB.[27,29] One Indian study reported data exclusively from pediatric subjects.[27] Blinding was reported in only one (9.1%) study.[32] Only one (9.1%) Spanish study

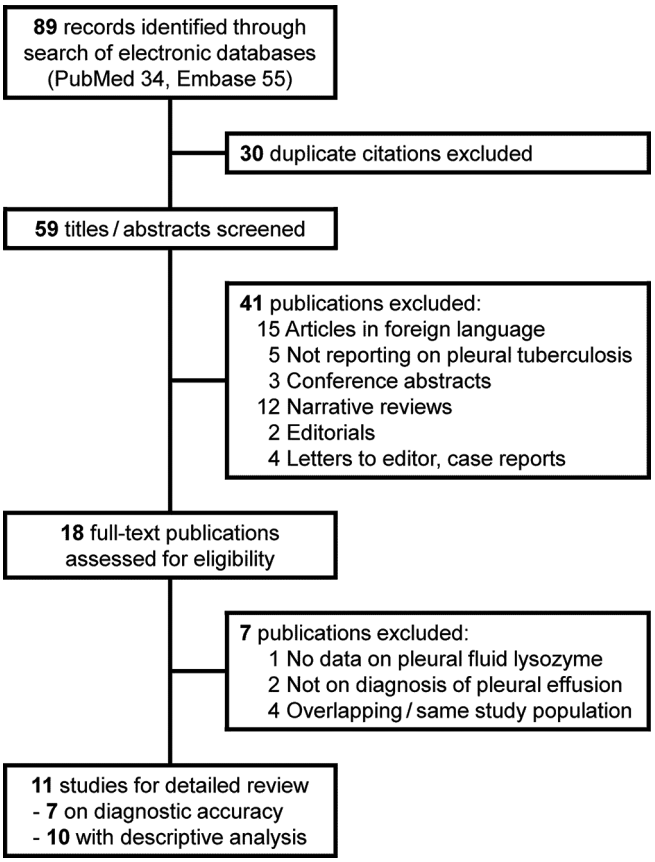

Figure 1: Study selection process

reported the inclusion of HIV seropositive patients.[32] Two (18.2%) studies employed definite (microbiologic and pathologic) reference criteria for diagnosing TPE.[24,30] Most studies (7, 63.6%) assayed lysozyme through a turbidimetric method [Table 1].[22,26-29,31,32]

Seven studies reported information regarding diagnostic test accuracy of LP/LS on 224 TPE patients and 630 patients having pleural effusions due to other disorders [Table S1 of online supplement].[23,24,27,28,30-32] Four of these studies also provided data for diagnostic test accuracy of LP on 88 TPE patients and 267 patients having pleural effusions due to other disorders [Table S1 of online supplement].[23,27,28,30] Only one (14.3%) study was published from a high TB burden country.[27] Five (71.4%) studies included patients only with an exudative pleural effusion.[23,24,30-32] Only one (14.3%) study performed assays in a blinded fashion.[32] Four (57.1%) studies employed a composite reference standard to diagnose TPE.[24,27,31,32] Most studies (4, 57.1%) assayed lysozyme through a turbidimetric method.[27,28,31,32]

The diagnostic thresholds varied widely between 10.0 mg/L and 15.0 g/mL for LP, and between 1.0 and 2.0 for LP/LS [Table S1 of online supplement]. A high risk of bias was observed in all studies, except one, when assessed through the QUADAS-2 tool [Figure S1 of online supplement].[32] The bias was primarily related to the absence of blinding and failure to specify diagnostic thresholds before the

Table 1: Characteristics of studies selected for analysis

Primary author, year of publication	Country of study	Study design	Inclusion criteria	Exclusion criteria	Reference standard for TPE diagnosis	Analytical technique for lysozyme	Parameters studied	Diagnostic information
Alegre, 2001[22]	Spain	Prospective	Inpatients over 18 years of age with effusion	NS	CMP	Turbidimetric assay	LP/LS	Comparative
Asseo, 1982[23]	Greece	Prospective	NS	NS	MP	Lysoplate assay	LP, LP/LS	Accuracy, Comparative
Caballero, 1999[24]	Spain	Prospective	NS	NS	CMP	Bead array	LP	Accuracy
Klockars, 1979[25]	Finland	Prospective	Inpatients with effusion	No definite diagnosis, receiving ATT or steroids	MP	Lysoplate assay	LP/LS	Comparative
Lew, 1983[26]	Switzerland	Prospective	Patients undergoing diagnostic thoracentesis	NS	CMP	Turbidimetric assay	LP	Comparative
Mishra, 2000[27]	India	Prospective	Children below 14 years of age with effusion	NS	CMP	Turbidimetric assay	LP, LP/LS	Accuracy, Comparative
Moriwaki, 1989[28]	Japan	Prospective	NS	NS	MP	Turbidimetric assay	LP, LP/LS	Accuracy, Comparative
Rajpal, 1981[29]	India	Prospective	Inpatients with effusion	NS	CMP	Turbidimetric assay	LP/LS	Comparative
Valdes, 1993[30]	Spain	Prospective	Inpatients with effusion	No definite diagnosis	MP	Immunodiffusion assay	LP, LP/LS	Accuracy, Comparative
Verea Hernando, 1987[31]	Spain	Prospective	Inpatients with effusion	Final diagnosis not available	CMP	Turbidimetric assay	LP, LP/LS	Accuracy, Comparative
Villena, 1996[32]	Spain	Prospective	NS	Empyema	CMP	Turbidimetric assay	LP, LP/LS	Accuracy, Comparative

ATT Anti-tuberculous treatment, LP Pleural fluid level, LP/LS Pleural fluidto serum ratio, NS Not specified, TPE Tuberculous pleural effusion. Reference standard for diagnosis: C Clinical, M Microbiologic, P Pathologic

start of the study. All these studies additionally showed applicability concerns in the patient selection domain as well. There was no publication bias [FigureS 2 of online supplement].

Table S2 of the online supplement provides the diagnostic accuracy estimates calculated from individual studies. There was substantial heterogeneity between the studies providing information on LP (I^2 92.24%), as well as LP/LS (I^2 86.42%). The sensitivity of LP for diagnosis of TPE varied from 0.63 to 1.00, and specificity from 0.62 to 1.00 [Figure 2]. The summary sensitivity, specificity, and DOR were 0.94 (95% CI 0.53–1.00), 0.89 (95% CI 0.63–0.97), and 129.88 (95% CI 6.26–2695), respectively. The summary positive and negative likelihood ratios were 8.30 (95% CI 2.14–32.15) and 0.06 (95% CI 0.01–0.80), respectively. A low positive likelihood ratio (below 10) and a low negative likelihood ratio (below 0.1) for the summary estimate indicate that LP might prove useful for excluding, but not confirming, TPE. The sensitivity of LP/LS for diagnosis of TPE varied from 0.72 to 1.00, and specificity from 0.70 to 1.00 [Figure 2]. The summary sensitivity, specificity, and DOR were 0.98 (95% CI 0.58–1.00), 0.91 (95% CI 0.84–0.96), and 708.47 (95% CI 11.42–43946), respectively. The summary positive and negative likelihood ratios were 11.31 (95% CI 5.76–22.22) and 0.02 (95% CI 0.00–0.75), respectively. A high positive likelihood ratio (above 10) and a low negative likelihood ratio (below 0.1) for the summary estimate suggest that LP/LS could be useful both for confirming and excluding TPE. The HSROC plots [Figure 3] appeared symmetrical implying that test accuracy was not dependent on the test threshold for either LP or LP/LS. However, the HSROC plot for LP/LS exhibited a much narrower 95% confidence zone and was positioned closer to the desired upper left corner of the graph [Figure 3], implying that LP/LS had better accuracy for diagnosing TPE than LP. Our summary estimates for LP/LS were robust in the sensitivity analysis and did not change much after excluding any single study from the meta-analysis [Table S3 of online supplement]. Sensitivity analysis could not be performed on data for LP levels due to the insufficient number of studies. Because of the small number of studies, a subgroup analysis was also not conducted for any of the prespecified covariates.

In addition, 10 studies provided comparative data on biomarker estimation in pleural effusions due to TB or other disorders.[22,23,25-32] Eight and five studies each compared LP levels between TPE and malignant or parapneumonic pleural effusions, respectively [Table S4 of online supplement]. Seven and four studies each compared LP/LS between TPE and malignant or parapneumonic pleural effusions, respectively [Table S4 of online supplement]. Only five (50.0%) of these studies had a Newcastle–Ottawa Scale score of 7/9 or higher and were thus considered of high quality [Table S5 of online supplement].[22,26,28,29,32] There was no significant publication bias [Figure S3 of online supplement].

Mean LP levels were higher among TPE patients for all pairwise comparisons [Table S6 of online supplement].

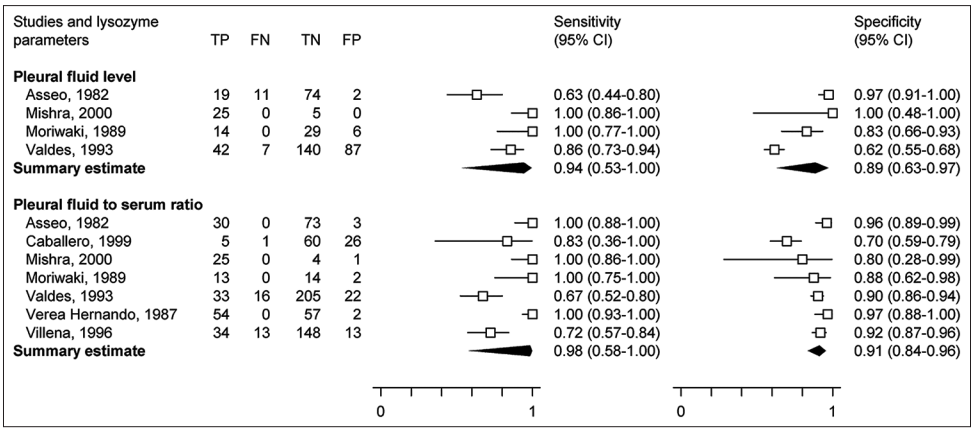

Figure 2: Coupled forest plot from studies reporting on the diagnostic accuracy of pleural fluid lysozyme levels (top panel) and pleural fluid to serum lysozyme ratio (bottom panel). Individual study estimates are depicted by hollow squares, and the horizontal lines correspond to their 95% confidence intervals (95% CI). Solid diamonds represent the summary sensitivity and specificity estimates

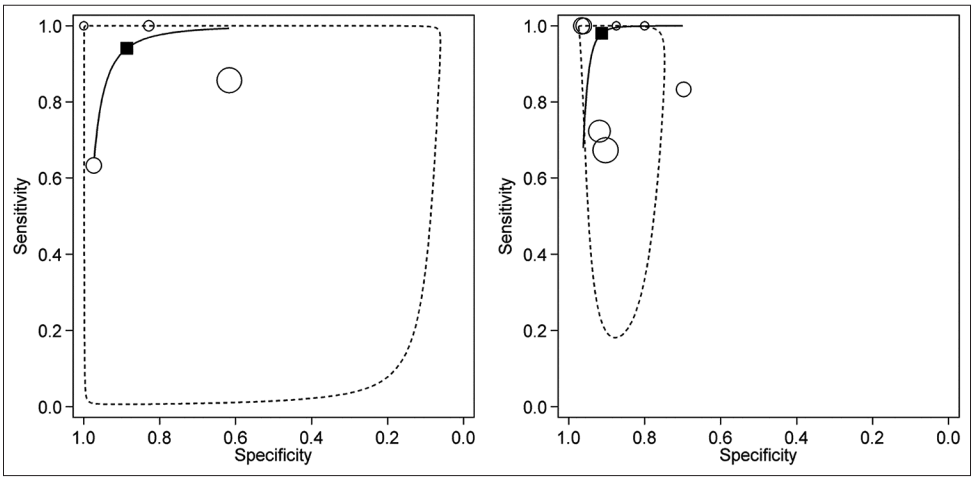

Figure 3: Hierarchical summary receiver operating characteristic (HSROC) plots to summarize diagnostic accuracy for pleural fluid lysozyme (left panel) and pleural fluid to serum lysozyme ratio (right panel) in diagnosing tuberculous pleural effusion. Each open circle represents an individual study, with a circle size proportionate to the inverse standard error of sensitivity and specificity. Summary estimates of diagnostic accuracy are indicated by black squares, and the surrounding dashed regions outline the zone of 95% confidence around this estimate

Mean LP/LS values were similarly higher among TPE patients for all pairwise comparisons, except in a single study involving Indian children.[27] The SMDs exhibited a substantial variability for LP levels, as well as LP/LS values, between TPE and other pleural effusions [Figure 4]. There was considerable heterogeneity for LP, as well as LP/LS, for comparisons involving malignant pleural effusions (I^2 79.18% and 75.21%, respectively). There was lesser heterogeneity for LP, as well as LP/LS, for comparisons involving parapneumonic pleural effusions (I^2 32.91% and 53.42%, respectively). After pooling observations from different studies, LP levels were significantly greater in TPE than in malignant pleural effusions (summary SMD 1.51, 95% CI 1.04–1.98) or parapneumonic pleural effusions (summary SMD 0.86, 95% CI 0.51–1.22) [Figure 4]. Similarly, LP/LS was significantly higher in TPE than in malignant pleural effusions (summary SMD 1.77, 95% CI 1.31–2.22) or parapneumonic pleural effusions (summary SMD 1.15, 95% CI 0.64–1.66). A single outlier result (SMD − 0.11) was noted among the studies comparing this ratio between TPE and malignant pleural effusions [Figure 4].[27] Removal of this study improved the summary SMD from 1.77 to 1.93 (95% CI 1.63–2.23) with a considerable reduction in heterogeneity (I^2 41.82%). Apart from this, our sensitivity analysis did not suggest any appreciable alteration in summary SMD if any one study was eliminated from that meta-analysis [Table S6 of online supplement]. However, the removal of a single Spanish study markedly improved the homogeneity in comparisons between TPE and parapneumonic pleural effusions [Table S6 of online supplement].[31] A formal subgroup analysis was not undertaken for any comparison due to the small number of studies.

Overall, we found low-grade evidence regarding the diagnostic performance of LP and LP/LS for the diagnosis of TPE [Table 2]. Based on our pooled data for LP, the false-positive rate was quite high for scenarios with low pre-test probabilities of TPE. The false-positive rate was somewhat lower, but still substantial, for LP/LS in such

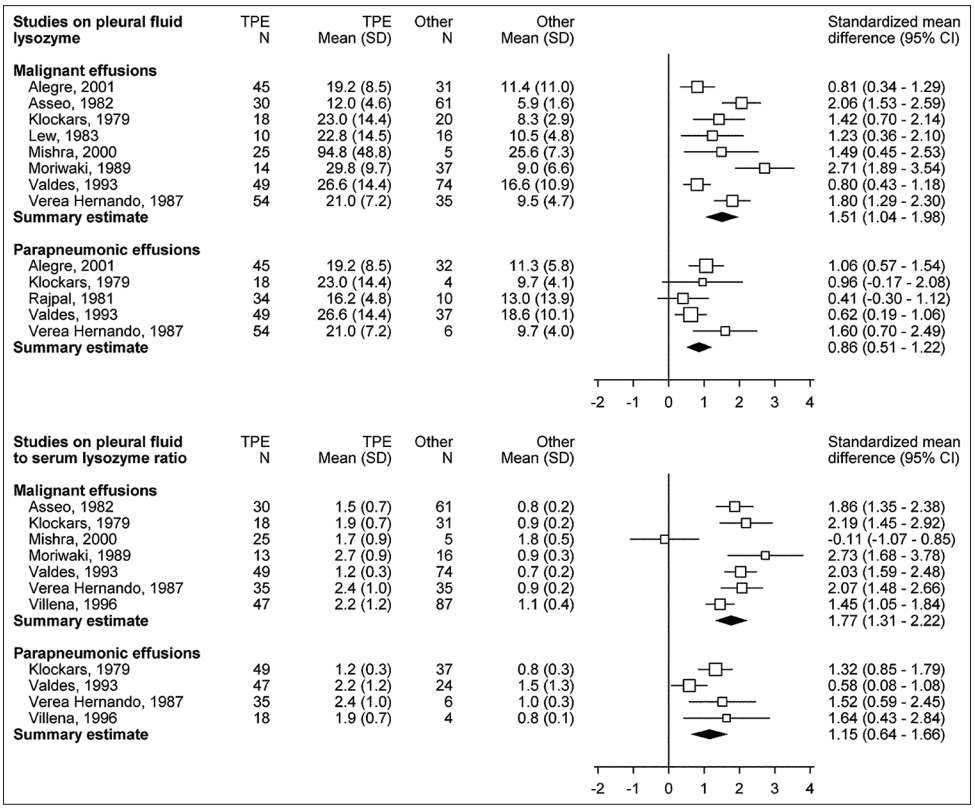

Figure 4: Forest plots from studies comparing pleural fluid lysozyme levels (top panel) and pleural fluid to serum lysozyme ratio (bottom panel) in tuberculous pleural effusions (TPE) and malignant effusions or parapneumonic effusions. Individual standardized mean difference estimates from each study are depicted by hollow squares, and the horizontal lines correspond to their 95% confidence intervals (95% CI). Solid diamonds represent the summary estimates

situations [Table 2]. The diagnostic performance of both tests appeared much better in settings with a higher pre-test probability of TPE.

DISCUSSION

To our knowledge, the diagnostic utility of lysozyme for identifying TPE has never been systematically reviewed. Our meta-analysis suggests that LP exhibits good sensitivity (0.94) and moderate specificity (0.89) for diagnosing TPE [Table 2]. LP/LS shows better diagnostic discrimination (sensitivity 0.98, specificity 0.91). These results suggest a marginally better sensitivity and similar specificity, as compared to pleural fluid ADA estimation.[2] Further, both LP concentration and LP/LS were significantly higher in TPE than in malignant or parapneumonic pleural effusions.

Pleural fluid analysis is always the initial investigation while evaluating any patient suspected to have pleural TB. Because microbiological testing on pleural fluid has a low yield, clinicians useclinical details and findings from other pleural fluid investigations while judging the need for ATT. We, therefore, combined microbiologic, pathologic, and clinical criteria as study inclusion parameters to represent information relevant to real-life scenarios. Unfortunately, this also led to a rather imperfect reference standard for TPE diagnosis in several studies, and therefore we cannot entirely rule out misclassification bias. Some studies also included patients with transudative effusions, whereas others enrolled only malignant pleural effusions as a comparator. Because this is not the usual spectrum of clinical scenarios where pleural TB is suspected, specificity figures from such studies could be erroneous. Almost all studies enrolled a small number of subjects, and several were of poor quality. Some of these factors may compromise the validity and applicability of our findings. There was also considerable heterogeneity across the included publications. Because of the small number of eligible studies, we could not further explore potential reasons for heterogeneity. Also, various investigators employed a very wide range of diagnostic thresholds, mostly in a post-hoc fashion, and it was not feasible to identify a clinically acceptable range of values that could optimize diagnostic test performance.

How do our observations impact routine clinical practice? In a setting of low TPE prevalence (e.g., 5% pre-test probability), nearly 70% of positive LP test results and more than 60% of LP/LS results are likely to be falsely positive, implying that a large proportion of these patients may not undergo more definitive evaluation for an alternate etiology and could be unnecessarily prescribed empiric ATT [Table 2]. However, both tests are unlikely to be

Table 2: Summary of findings from studies evaluating the diagnostic accuracy of lysozyme in tuberculous pleural effusion

Outcome	No. of studies (No. of patients)	Study design	Factors that may decrease the certainty of evidence					Effect per 1,000 patients tested		Certainty of evidence
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	5% pre-test probability	50% pre-test probability	
A. Pleural fluid lysozyme										
True positives	4 studies (88 patients)	Cross-sectional (cohort type accuracy study)	Serious[a]	Not serious	Serious[b]	Not serious	None	47 (27 to 50)	472 (267 to 498)	LOW
False negatives								3 (0 to 23)	28 (2 to 233)	
True negatives	4 studies (267 patients)	Cross-sectional (cohort type accuracy study)	Serious[a]	Not serious	Serious[b]	Not serious	None	842 (598 to 924)	443 (315 to 486)	LOW
False positives								108 (26 to 352)	57 (14 to 185)	
B. Pleural fluid to serum lysozyme ratio										
True positives	7 studies (224 patients)	Cross-sectional (cohort type accuracy study)	Serious[a]	Not serious	Serious[b]	Not serious	None	49 (29 to 50)	493 (290 to 500)	LOW
False negatives								1 (0 to 21)	7 (0 to 210)	
True negatives	7 studies (630 patients)	Cross-sectional (cohort type accuracy study)	Serious[a]	Not serious	Serious[b]	Not serious	None	867 (794 to 908)	456 (418 to 478)	LOW
False positives								83 (42 to 156)	44 (22 to 82)	

Figures in parentheses in the effect estimate columns are 95% confidence intervals. [a]Most studies failed to employ pre-specified thresholds for diagnosis, and/or did not report if the index test or reference standard was interpreted without knowledge of the results of the other test. [b]Estimates of sensitivity and specificity showed substantial variability that could not be fully explained by the study population, assay techniques, or quality of included studies

falsely positive for patients with nontuberculous pleural effusions. Conversely, in a high prevalence situation (e.g., 50% pre-test probability), about 6% of patients who test negative with LP will actually have a disease (but would be denied appropriate therapy); this rate is much lower at around 1.5% for LP/LS. More than 10% of positive LP test results and nearly 8% of LP/LS results are likely to be falsely positive. Overall, both LP and LP/LS appear to be reasonably good biomarkers for pleural TB, more so in high TB prevalence settings. LP/LS also seems to be a better discriminator than LP. However, our analysis was limited to evaluating the performance of lysozyme as a single isolated assay and we cannot comment on its additive utility when considered along with other test results. There are some data to suggest that the diagnostic accuracy improves further if it is combined with pleural fluid ADA estimation.[32-34] Finally, there is a need for standardizing simpler automated assays for lysozyme determination, given its good diagnostic performance in TPE.

CONCLUSION

In conclusion, findings from our meta-analysis provide low-quality evidence that both LP and LP/LS exhibit good diagnostic accuracy for diagnosis of TPE, the latter being marginally superior. Good-quality studies are needed to better define clinically useful thresholds for LP and LP/LS.

Author contributions

ANA Conceptualization; methodology; investigation; formal analysis; data curation; supervision; writing-original draft; writing-review/editing.
RA Methodology; investigation; formal analysis; data curation; writing- original draft; writing- review/editing.
SD Methodology; investigation; formal analysis; writing- original draft; writing- review/editing.
KTP Methodology; investigation; formal analysis; writing- original draft; writing- review/editing.
IPS Methodology; investigation; formal analysis; writing-original draft; writing-review/editing.
VM Methodology; investigation; formal analysis; writing-original draft; writing-review/editing.

List of abbreviations

95% CI 95% confidence interval
ADA Adenosine deaminase
ATT Anti-tuberculous therapy
DOR Diagnostic odds ratio
HIV Human immunodeficiency virus
HSROC Hierarchical summary receiver operating characteristic
I^2 Higgins' inconsistency index
LP Pleural fluid lysozyme
LP/LS Pleural fluid to serum lysozyme ratio
PRISMA Preferred Reporting Items for Systematic Reviews and Meta-Analyses
QUADAS-2 Quality Assessment of Diagnostic Accuracy Studies, version 2
SD Standard deviation
SMD Standardized mean difference
TB Tuberculosis
TPE Tuberculous pleural effusion.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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ONLINE SUPPLEMENT

Pleural fluid lysozyme as a diagnostic biomarker of pleural tuberculosis: a systematic review and meta-analysis

Table S1. Characteristics of studies providing data on diagnostic test accuracy of pleural fluid lysozyme.

Author, lysozyme parameter	Patient number		Etiology of non-tubercular effusions			Threshold	TP	FN	TN	FP
	TPE	Others	Malignant	Empyema / parapneumonic	Transudate					
Pleural fluid level										
- Asseo, 1982	30	76	Yes	Yes	Yes	76 mg/L	19	11	74	2
- Mishra, 2000	25	5	Yes	No	No	50 U/L	25	0	5	0
- Moriwaki, 1989	14	35	Yes	No	No	35 mg/L	14	0	29	6
- Valdes, 1993	49	227	Yes	Yes	Yes	15 g/mL	42	7	140	87
Pleural fluid to serum ratio										
- Asseo, 1982	30	76	Yes	Yes	Yes	1.0	30	0	73	3
- Caballero, 1999	6	86	Yes	Yes	Yes	2.0	5	1	60	26
- Mishra, 2000	25	5	Yes	No	No	1.1	25	0	4	1
- Moriwaki, 1989	13	16	Yes	No	No	1.2	13	0	14	2
- Valdes, 1993	49	227	Yes	Yes	Yes	1.1	33	16	205	22
- Verea Hernando, 1987	54	59	Yes	Yes	Yes	1.2	54	0	57	2
- Villena, 1996	47	161	Yes	Yes	Yes	1.7	34	13	148	13

FN False negative, FP False positive, TN True negative, TP True positive, TPE Tuberculous pleural effusion

Table S2. Diagnostic accuracy estimates from the included studies.

Author, lysozyme parameter	Sensitivity	Specificity	Positive likelihood ratio	Negative likelihood ratio	Diagnostic odds ratio
Pleural fluid level					
- Asseo, 1982	0.63 (0.44-0.80)	0.97 (0.91-1.00)	24.07 (5.97-97.05)	0.38 (0.23-0.60)	63.91 (13.05-312.99)
- Mishra, 2000	1.00 (0.86-1.00)	1.00 (0.48-1.00)	6.74 (1.10-41.44)	0.04 (0.01-0.30)	156.00 (8.49-2865.04)
- Moriwaki, 1989	1.00 (0.77-1.00)	0.83 (0.66-0.93)	4.96 (2.51-9.77)	0.08 (0.01-0.52)	64.29 (7.23-571.56)
- Valdes, 1993	0.86 (0.73-0.94)	0.62 (0.55-0.68)	2.24 (1.83-2.73)	0.23 (0.12-0.46)	9.66 (4.15-22.45)
Pleural fluid to serum ratio					
- Asseo, 1982	1.00 (0.88-1.00)	0.96 (0.89-0.99)	18.89 (7.26-49.17)	0.03 (0.00-0.23)	573.50 (61.60-5339.2)
- Caballero, 1999	0.83 (0.36-1.00)	0.70 (0.59-0.79)	2.76 (1.70-4.46)	0.24 (0.04-1.44)	11.54 (1.28-103.70)
- Mishra, 2000	1.00 (0.86-1.00)	0.80 (0.28-0.99)	3.37 (1.04-10.90)	0.05 (0.01-0.38)	65.00 (4.90-861.45)
- Moriwaki, 1989	1.00 (0.75-1.00)	0.88 (0.62-0.98)	5.60 (1.98-15.87)	0.08 (0.01-0.54)	70.00 (6.49-754.44)
- Valdes, 1993	0.67 (0.52-0.80)	0.90 (0.86-0.94)	6.95 (4.46-10.82)	0.36 (0.24-0.54)	19.22 (9.16-40.34)
- Verea Hernando, 1987	1.00 (0.93-1.00)	0.97 (0.88-1.00)	19.97 (6.62-60.23)	0.02 (0.00-0.13)	1063.33 (107.3-10532)
- Villena, 1996	0.72 (0.57-0.84)	0.92 (0.87-0.96)	8.96 (5.17-15.53)	0.30 (0.19-0.48)	29.78 (12.67-69.97)

Figures in parentheses are 95% confidence intervals

Table S3. Sensitivity analysis for studies included in meta-analysis of diagnostic test accuracy data for pleural fluid to serum lysozyme ratio.

Study removed	Sensitivity (95% CI)	Specificity (95% CI)	I^2 (%)
None	0.98 (0.58-1.00)	0.91 (0.84-0.96)	86.42
Asseo, 1982	0.97 (0.62-1.00)	0.90 (0.80-0.95)	82.57
Caballero, 1999	0.99 (0.62-1.00)	0.93 (0.91-0.95)	89.20
Mishra, 2000	0.97 (0.59-1.00)	0.92 (0.83-0.96)	80.05
Moriwaki, 1989	0.97 (0.59-1.00)	0.92 (0.83-0.96)	81.77
Valdes, 1993	1.00 (0.38-1.00)	0.91 (0.82-0.96)	85.24
Verea Hernando, 1987	0.96 (0.63-1.00)	0.89 (0.80-0.95)	80.43
Villena, 1996	1.00 (0.42-1.00)	0.91 (0.81-0.96)	87.53

Figures in columns 2 and 3 are summary estimates from hierarchical modeling, after exclusion of a single study mentioned in the first column
95% CI 95% confidence interval, I^2 Higgins' inconsistency index

Table S4. Observations from studies providing comparative data on pleural fluid lysozyme in tuberculous and other pleural effusions.

Study	Pleural fluid lysozyme						Pleural fluid to serum lysozyme ratio						Units
	Tuberculous effusions		Malignant effusions		Parapneumonic effusions		Tuberculous effusions		Malignant effusions		Parapneumonic effusions		
	No.	Mean±SD	No.	Mean±SD	No.	Mean±SD	No.	Mean±SD	No.	Mean±SD	No.	Mean±SD	
Alegre, 2001	45	19.24±8.45	31	11.37±11.0	32	11.25±5.79							mg/L
Asseo, 1982*	30	12.04±4.6	61	5.91±1.64			30	1.52±0.66	61	0.77±0.16			mg/L
Klockars, 1979	18	23.0±14.4	20	8.3±2.9	4	9.7±4.1	18	1.9±07	31	0.9±0.2	4	0.8±0.1	mg/L
Lew, 1983*	10	22.8±14.5	16	10.5±4.8									mg/L
Mishra, 2000	25	94.8±48.8	5	25.6±7.3			25	1.7±0.9	5	1.8±0.5			U/L
Moriwaki, 1989	14	29.8±9.7	37	9.0±6.6			13	2.7±0.9	16	0.9±0.3			mg/L
Rajpal, 1981	34	16.17±4.76			10	12.98±13.9							mg/L
Valdes, 1983	49	26.6±14.4	74	16.6±10.9	37	18.6±10.1	49	1.2±0.3	74	0.7±0.2	37	0.8±0.3	g/mL
Verea Hernando, 1987	54	21.0±7.2	35	9.5±4.7	6	9.7±4.0	35	2.39±0.97	35	0.93±0.18	6	0.98±0.27	mg/dL
Villena, 1996*							47	2.2±1.15	87	1.1±0.41	24	1.5±1.26	-

*Mean and standard deviation (SD) values indirectly computed from standard error of mean, or range, data

Table S5. Assessment of quality of ten publications providing descriptive data, using an adapted version of the Newcastle-Ottawa scale for case-control studies.

Study	Selection (maximum 4 points)	Comparability (maximum 2 points)	Exposure (maximum 3 points)	Total (maximum 9 points)
Alegre, 2001	4	1	2	7
Asseo, 1982	4	0	2	6
Klockars, 1979	3	0	2	5
Lew, 1983	4	1	2	7
Mishra, 2000	4	0	2	6
Moriwaki, 1989	4	1	2	7
Rajpal, 1981	4	1	2	7
Valdes, 1983	3	1	2	6
Verea Hernando, 1987	3	0	2	5
Villena, 1996	4	1	3	8

Table S6. Sensitivity analysis for meta-analyses of ten studies providing descriptive data.

Study removed	Pleural fluid lysozyme				Pleural fluid to serum lysozyme ratio			
	Malignant effusions		Parapneumonic effusions		Malignant effusions		Parapneumonic effusions	
	SMD (95% CI)	I^2 (%)	SMD (95% CI)	I^2 (%)	SMD (95% CI)	I^2 (%)	SMD (95% CI)	I^2 (%)
None	1.51 (1.04-1.98)	79.18	0.86 (0.51-1.22)	32.91	1.77 (1.31-2.22)	75.21	1.15 (0.64-1.66)	53.42
Alegre, 2001	1.63 (1.11-2.14)	78.26	0.80 (0.34-1.26)	37.46				
Asseo, 1982	1.42 (0.93-1.91)	76.74			1.74 (1.19-2.30)	79.22		
Klockars, 1979	1.53 (1.00-2.05)	82.14	0.86 (0.44-1.28)	49.30	1.70 (1.19-2.21)	78.16	1.08 (0.51-1.66)	64.06
Lew, 1983	1.55 (1.03-2.06)	82.11						
Mishra, 2000	1.51 (1.01-2.02)	82.12			1.93 (1.63-2.23)	41.82		
Moriwaki, 1989	1.36 (0.93-1.79)	73.34			1.66 (1.19-2.13)	76.06		
Rajpal, 1981			0.96 (0.57-1.34)	30.29				
Valdes, 1983	1.63 (1.16-2.11)	72.54	0.98 (0.52-1.43)	32.32	1.71 (1.15-2.27)	77.80	1.08 (0.47-1.69)	63.52
Verea Hernando, 1987	1.47 (0.94-2.00)	80.06	0.76 (0.47-1.05)	0.00	1.71 (1.18-2.24)	78.34	1.39 (0.99-1.79)	0.00
Villena, 1996					1.83 (1.28-2.37)	75.65	1.11 (0.37-1.86)	57.00

Figures are summary estimates after exclusion of a single study mentioned in the first column

95% CI 95% confidence interval, I^2 Higgins' inconsistency index, SMD Summary standardized mean difference

Fig S1. Risk of bias and applicability concerns summary for studies providing diagnostic accuracy data.

	Risk of Bias				Applicability Concerns		
	Patient Selection	Index Test	Reference Standard	Flow and Timing	Patient Selection	Index Test	Reference Standard
Asseo, 1982	Low	High	Low	Low	High	Low	Low
Caballero, 1999	Low	High	Low	High	High	Low	Low
Mishra, 2000	Low	High	Unclear	Low	High	Low	High
Moriwaki, 1989	Low	High	Low	Low	High	Low	Low
Valdes, 1993	Unclear	High	Low	Low	High	Low	Low
Verea Hernando, 1987	Unclear	High	Low	Low	High	Low	Low
Villena, 1996	Low	Unclear	Low	Low	Low	Low	Low

Fig S2. Deek's funnel plot assessment for evaluating potential publication bias for studies providing diagnostic accuracy data. These plots show a symmetric distribution of log of diagnostic odds ratios against inverse root of effective sample sizes (ESS) for studies evaluating pleural fluid lysozyme (right panel, slope coefficient 29.98, p=0.07) and pleural fluid to serum lysozyme ratio (right panel, slope coefficient 4.53, p=0.85), indicating absence of any significant publication bias.

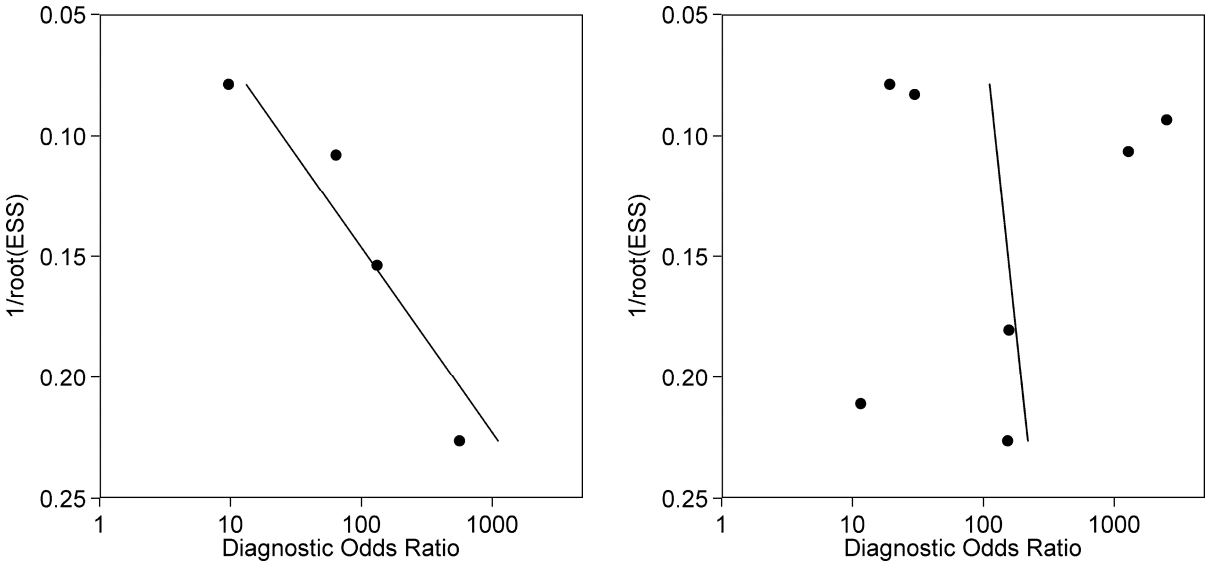

Fig S3. Funnel plot assessment for evaluating potential publication bias among studies reporting on pleural fluid lysozyme levels in patients having tuberculous pleural effusion and malignant effusion (upper left panel) or parapneumonic effusion (upper right panel), and pleural fluid to serum lysozyme ratio in patients with tuberculous pleural effusion and malignant effusion (bottom left panel) or parapneumonic effusion (bottom right panel). All graphs are symmetrical (p >0.05 on Begg's test) and hence not suggestive of any publication bias.