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. Therefore, physicians use surrogate laboratory biomarkers for initiating empiric anti-tuberculous therapy (ATT) among those with suspected TPE. Pleural fluid adenosine deaminase (ADA)
is one such commonly used investigation having good sensitivity and specificity.[5] 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 pleuriyis).

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 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].\[22,26-28,31,32\]

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.\[24\] 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

![Figure 1: Study selection process](image-url)
Table 1: Characteristics of studies selected for analysis

| Primary author, year of publication | Country of design | 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, Comparative |
| Klockars, 1979[25]                | Finland           | Prospective  | NS                | NS | 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, receiving ATT or steroids | CMP | Immunodiffusion assay | LP, LP/LS | Accuracy, Comparative |
| Verea Hernando, 1987[31]          | Spain             | Prospective  | No definite diagnosis, Final diagnosis not available | NS | 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 fluidido 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 [Figure S2 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 (F 92.24%), as well as LP/LS (F 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 6.26–2695), respectively. The summary positive and negative likelihood ratios were 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].
Mean LP/LS values were similarly higher among TPE patients for all pairwise comparisons, except in a single study involving Indian children. 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 (I² 79.18% and 75.21%, respectively). There was lesser heterogeneity for LP, as well as LP/LS, for comparisons involving parapneumonic pleural effusions (I² 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]. 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 (I² 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]. 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 scenarios.
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. 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 use clinical 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 |
|---------|---------------------------------|--------------|------------------------------------------------------|---------------------------------|-----------------------|
| 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 | 4 studies (267 patients) | Cross-sectional (cohort type accuracy study) | Serious\(^a\) Not serious Serious\(^b\) Not serious None | 3 (0 to 23) 28 (2 to 233) | LOW |
| 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 | | | | | |
| 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 | 7 studies (630 patients) | Cross-sectional (cohort type accuracy study) | Serious\(^a\) Not serious Serious\(^b\) Not serious None | 1 (0 to 21) 7 (0 to 210) | LOW |
| 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 | | | | | |

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
F 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
| Author, lysozyme parameter | Patient number | Etiology of non-tubercular effusions | Threshold | TP  | FN  | TN  | FP  |
|----------------------------|----------------|--------------------------------------|-----------|-----|-----|-----|-----|
|                            |                | TPE Others                            |           |     |     |     |     |
|                            |                | Malignant Empyema / Transudate         |           |     |     |     |     |
| 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  |
| 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±0.7 | 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               |
|                 | SMD (95% CI)            | SMD (95% CI)                          | SMD (95% CI)            | SMD (95% CI) |
|                 | $I^2$ (%)                | $I^2$ (%)                             | $I^2$ (%)                | $I^2$ (%)    |
| None            | 1.51 (1.04-1.98)        | 0.86 (0.51-1.22)                      | 1.77 (1.31-2.22)         | 1.15 (0.64-1.66) |
|                 | 79.18                   | 32.91                                | 75.21                    | 53.42        |
| Alegre, 2001    | 1.63 (1.11-2.14)        | 0.80 (0.34-1.26)                      | 1.74 (1.19-2.30)         | 1.08 (0.51-1.66) |
|                 | 78.26                   | 37.46                                | 79.22                    | 64.06        |
| Asseo, 1982     | 1.42 (0.93-1.91)        |                                      | 1.70 (1.19-2.21)         |               |
|                 | 76.74                   |                                      | 78.16                    |               |
| Klockars, 1979  | 1.53 (1.00-2.05)        | 0.86 (0.44-1.28)                      | 1.70 (1.19-2.21)         | 1.08 (0.51-1.66) |
|                 | 82.14                   | 49.30                                | 78.16                    | 64.06        |
| Lew, 1983       | 1.55 (1.03-2.06)        |                                      | 1.66 (1.19-2.13)         |               |
|                 | 82.11                   |                                      | 76.06                    |               |
| Mishra, 2000    | 1.51 (1.01-2.02)        |                                      | 1.93 (1.63-2.23)         |               |
|                 | 82.12                   |                                      | 41.82                    |               |
| Moriwaki, 1989  | 1.36 (0.93-1.79)        |                                      | 1.66 (1.19-2.13)         |               |
|                 | 73.34                   |                                      | 76.06                    |               |
| Rajpal, 1981    |                         | 0.96 (0.57-1.34)                      | 30.29                    |               |
| Valdes, 1983    | 1.63 (1.16-2.11)        | 0.98 (0.52-1.43)                      | 1.71 (1.15-2.27)         | 1.08 (0.47-1.69) |
|                 | 72.54                   | 32.32                                | 77.80                    | 63.52        |
| Verea Hernando, 1987 | 1.47 (0.94-2.00)   | 0.76 (0.47-1.05)                      | 1.71 (1.18-2.24)         | 1.39 (0.99-1.79) |
|                 | 80.06                   | 0.00                                 | 78.34                    | 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.