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Abstract: Abstract
The investigation of pleural effusion has been greatly assisted by advancements in pleural fluid analysis. In the case of tuberculous pleural effusion, diagnosis traditionally requires the demonstration of acid fast bacilli in the pleural space using microbiological or histological techniques. In recent years there has been progress in pleural fluid analysis in suspected tuberculous effusions with particular interest in adenosine deaminase and interferon-γ. These individual tests are quite sensitive and specific, however data is sparse on the benefits multiple parameter testing may have when analysed in combination. We reviewed the literature to investigate the evidence for multiple parameters testing, both biochemical and clinical, in the evaluation of tuberculous effusion.

Authors' Response to Reviewers' Comments
Click here to download Authors' Response to Reviewers' Comments: response to reviewers March 2010.doc

Dear Editor,

Thank you for giving us the opportunity to submit a revised manuscript. I very much appreciated the reviewers' comments and have made every effort to address them. I have highlighted the changes to the manuscript in red font and will specifically address each comment below chronologically. As ever, we would be only too delighted to make any further changes the reviewers' recommend.

Yours faithfully

Dr. Emmet McGrath MB PhD MRCPI
NIHR Clinical Lecturer in Respiratory Medicine
Sheffield

Reviewer #1 comments addressed:

I have now critically appraised each individual study as requested.

Major comments

1. I have now cited Diacon reference as recommended.
2. I have now described that it is the combination of all 4 features of thoracoscopy which offers significant advantages over other biopsy techniques.
3. I have followed the reviewer's advice re. Introduction-- last sentence.
4. I have now included a critical appraisal of the strengths and weaknesses of each study.
5. I was talking about closed pleural biopsy (Abrams etc) and I have now made this point clearer.
6. I have removed the text concerning Porcel's recommendation. Both reviewers felt it was not required. I have appraised 2 of Porcel's research studies instead.
7. I have now specified which biopsy type I was referring to in the text.
8. I have now linked in the lack of specificity of ADA to the section on "disadvantages" of the tests.
9. I have again pointed out several advantages of thoracoscopy as recommended.
10. I have discussed the problems of variable prevalence, lack of gold standard controls in all but one study, variable study design (retro- versus prosp-) as well as the variable and generally low n numbers.

Minor comments:

1. I have shortened the title further "diagnostic tests for tuberculous pleural effusion"
2. I have adjusted the last sentence of the abstract which did not read so well: "...to investigate the evidence for multiple parameters..."

3. I have replaced "efficiency" with "accuracy"?
4. I have now included the cost of the ADA test (to give an an idea of "cheap")
5. I have improved the sentence in the discussion, para 8, line 5 (page 18) to read as " at present there is insufficient evidence to solely use combinations for diagnosis..." as recommended by the reviewer.

Reviewer #2 comments addressed:

1. I have now made it clear that IFN tests have not been accepted as a way to diagnose PLEURAL TB.

2. In the conclusion, the 3rd paragraph detailing the view of Porcel et al – I have now removed this as per the reviewer's advice.

*Manuscript
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Diagnostic tests for tuberculous pleural effusion

Word count- 4310

Running title : Pleural fluid analysis

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Diagnostic tests for tuberculous pleural effusion

Abstract

The investigation of pleural effusion has been greatly assisted by advancements in pleural fluid analysis. In the case of tuberculous pleural effusion, diagnosis traditionally requires the demonstration of acid fast bacilli in the pleural space using microbiological or histological techniques. In recent years there has been progress in pleural fluid analysis in suspected tuberculous effusions with particular interest in adenosine deaminase and interferon-γ. These individual tests are quite sensitive and specific, however data is sparse on the benefits multiple parameter testing may have when analysed in combination. We reviewed the literature to investigate the evidence for multiple parameters testing, both biochemical and clinical, in the evaluation of tuberculous effusion.

Key words

Tuberculous pleural effusion

Adenosine deaminase

Interferon-γ

Polymerase chain reaction

Diagnosis

Decision tree

Introduction

Tuberculosis continues to be an important cause of morbidity and mortality in developing countries. Areas of high epidemiologic burden (annual incidence >25/100,000) include South Africa 480/100,000 and Swaziland 1,155/100,000 which contrast with the low burden areas of the United States and Monaco where annual incidence is 4.6 and 2/100,000 respectively (1,2).

Mycobacterium tuberculosis (TB) related pleural effusion results from infiltration of the pleural space by *Mycobacterium tuberculosis* antigens or bacilli. Traditional investigation involves microscopic examination for acid fast bacilli (AFB), fluid culture, cytological examination of fluid for inflammatory cells and microbiological and histological examination of biopsied tissue.

With pleural fluid analysis, acid fast bacilli are detected in <5% of cases, whereas mycobacterial culture of fluid has a sensitivity of <60% with wait times up to 2 months for culture results (**3,4**).

Other methods traditionally used for TB lung infection also have disappointing diagnostic ability. The culture of spontaneous or induced sputum has a sensitivity of <30% and 52% respectively and approximately one third of patients with tuberculous pleural effusion have a negative tuberculin skin test (**5**).

Histological analysis and mycobacterial culture of closed pleural biopsied tissue have traditionally been the gold standard investigation with sensitivities of 71-80% and 39-79% respectively (6). Combining both together has a detection rate of 91.3% for TB (**7**). The introduction of thoracoscopy has had a very important impact on diagnosis which was particularly highlighted by Diacon *et al* who performed a direct comparative study of the diagnostic tools used in tuberculous pleurisy and found that

sensitivity of histology, culture and combined histology/culture was 66, 48 and 79%, respectively for closed needle biopsy and 100, 76 and 100%, respectively for thoracoscopy. Both were 100% specific. (8). Apart from achieving superior histological samples, the technique of thoracoscopically biopsying large areas of abnormal pleura also increases the likelihood of positive diagnosis especially when macroscopic abnormalities such as "tapioca pleura" are observed. This macroscopic appearance combined with the microscopic appearance of granulomas often allows the commencement of therapy while culture results are awaited. Thoracoscopy, unlike other forms of pleural biopsy, also facilitates the removal of pleural fluid thereby relieving troublesome dyspnoea.

In more recent times, additional pleural fluid biomarkers have been discovered including adenosine deaminase (ADA) which reportedly has a sensitivity of 88% and a specificity of 85.7% and interferon-γ (IFN-γ) which has a sensitivity of 85.7% and a specificity of 97.1%. These studies were reported from an area where prevalence was 59 per 100,000 population (6,9). A more recent meta-analysis of 63 studies found that ADA has a sensitivity and specificity of 92% and 90% respectively and a meta-analysis of 22 studies found that IFN-γ has a sensitivity and specificity of 89% and 97% respectively (10,11). Acceptance of these tests for the diagnosis of pleural TB has not been universal and remains contentious in some countries with diagnostic performance very variable between populations (12).

Used individually, the different cheap and easy to perform pleural fluid tests have a wide range of sensitivity and specificity. We hypothesised that these tests used in combination might improve the ability to diagnose this condition.

Methods

In this review, we searched for publications describing pleural fluid test combinations that may help with the diagnosis of tuberculous pleural effusion. Publications from a MEDLINE search and references from relevant articles were reviewed on the clinical investigation of tuberculous pleural effusion. All articles that adhered to the criteria of multiple tests or parameter combinations in the diagnosis of tuberculous effusions were included in this review. Papers were separately selected by author 1 (EMG) and author 2 (PA) with common papers included in the review. Papers found by only one author were assessed by both authors together for their suitability. Keywords searched were *tuberculous effusion, pleural fluid, diagnosis, adenosine deaminase, interferon-γ, pleuritis and empyema*

Results

As summarised in Table 1, twelve studies describing the use of combination fluid/clinical parameter analysis were found (6, **13-23**). Here we describe and appraise these studies in chronological order.

San Jose *et al* analysed the use of serum and pleural ADA and lysozyme in tuberculous pleurisy in 271 patients in Spain, of which 53 had tuberculosis. They found that pleural ADA had a sensitivity of 100% and a specificity of 93% for the diagnosis of TB and that combining the use of pleural ADA and total protein in the form of a ratio increased the specificity to 95% at the expense of a reduction in sensitivity to 93%. Using a ratio involving pleural fluid ADA and lysosyme also increased the specificity to 95% with a sensitivity of 80%. A ratio of pleural fluid lysosyme to total protein had a sensitivity of 60% and a specificity of 42% (13).

While these combinations improved specificity, it was at the expense of a reduction in sensitivity. This was a retrospective study involving a small number of tuberculosis cases. The authors used robust statistics but failed to describe parameters such as age and sex. There is also little information given regarding the diagnostic methods used in the tuberculous effusion group. The cases appear to be a mix of microbological/histological and clinical diagnosis, the latter having not been proven. The ADA cut-off was different to the other studies described at 43U/l.

Muranishi *et al* investigated the simultaneous measurement of ADA activity and tuberculostearic acid (TSA) in pleural effusions for the diagnosis of tuberculous pleuritis. Pleural effusions from 18 patients with tuberculous pleuritis, 16 patients with suspected tuberculous pleuritis, 14 patients with malignant effusions, 6 patients with parapneumonic effusions, 5 with cardiac failure related effusions, 3 with collagen disease related effusions and 5 miscellaneous effusions were studied. They found that when they analysed pleural fluid ADA and TSA in combination, sensitivity increased from 56% (ADA alone) to 83%. Specificity remained at 76% (14). The authors felt that the simultaneous measurement of both these parameters was useful in the diagnosis of pleural effusions. There were a number of weaknesses with this study. It was a very small study and the sensitivity and specificity were relatively low when compared to the more recent data. It was retrospective and included cases where definitive proof of TB was absent relying solely on high clinical suspicion. Therefore, some of the suspected TB cases will have been due to other aetiologies. They used an ADA cut-off level of 50 U/l which differs from many of the other studies and did not describe the study population adequately (age/sex).

In 1993 in Brazil, De Olivereia *et al* studied the usefulness of pleural fluid ADA in combination with pleural fluid lymphocyte proportion in 276 patients of which 54 had TB. They found that using these tests in combination resulted in a sensitivity of 90.7% and a specificity of 97.7%. They concluded that the use of these tests in combination was a highly efficient diagnostic strategy of low cost that merits wider use (15).While this study benefitted from its prospective nature, there were a number of important weaknesses. Only 48% of the TB cases included in the study were actually proven either by microbiology or histology. Cardiac failure and very small effusions were excluded from this study which has implications on the interpretation of the results. Only 61% of the neoplastic effusions had positive cytological or histological evidence of malignancy. The balance was made up of presumed malignancy. The ADA cut-off level was different to many other previous studies at 40U/l. Finally, the statistical analysis was not well explained.

Villena *et al* measured the concentration of pleural ADA and the ratio of pleural lysozyme (PL) to serum lysozyme (SL) in consecutive patients (49 tuberculous and 179 nontuberculous) (**16**). Using sensitivity and specificity curves, they established cut-off values at 33 U/L (units per litre) for ADA and 1.7 for the PL/SL ratio. The sensitivity of ADA activity for tuberculous effusion was 90% with a specificity of 85%. Combining ADA with the PL/SL ratio enhanced specificity to 99% (**16**). However, they concluded that these markers should only suggest a diagnosis and point the clinician in the direction of pleural biopsy and pleural fluid cultures, and that values for ADA and lysozyme ratios are not, alone or in combination, sensitive or specific enough to replace pleural biopsy or culture of pleural fluid for the diagnosis of tuberculous empyema. This work had a number of good qualities. It was

prospective. All patients followed the exact same diagnostic algorithm with difficult cases discussed in a blinded manner by a multidisciplinary panel. However, on the negative side, this study included cases where definitive histology or microbiology was not forthcoming and clinical suspicion alone was used. The non-tuberculous group was approximately 3.5 times the size of the tuberculous group. The findings were only relevant when diagnosing TB cases from a mix that includes parapneumonic, neoplastic and transudative effusions only. The ADA cut-off level of 33 U/l was again different to other studies. The analysis of pleural fluid lysozyme is also a contentious issue with discrepancies in results noted in previous work, possibly attributed to the method of analysis (turbimetric v lysoplate). In this work the sensitivity of lysozyme was reported as much higher than previous reports. Again, in this study, there was inadequate description of the study population. Statistical analysis was robust and explained.

Burgess *et al* analysed the differential cell counts and ADA on 303 of 472 consecutive pleural fluid samples after hemothorax, transudate and unsuitable samples were excluded. They found that when ADA levels were combined with the lymphocyte/neutrophil ratio results, the accuracy of identifying TB increased compared with isolated ADA analysis (**17**). When a positive ADA result was combined with a lymphocyte neutrophil ratio of 0.75 or greater, the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy for the identification of TB were calculated at 88%, 95%, 95%, 88%, and 92%, respectively (**17**). The authors concluded that pleural fluid which met these criteria was very

suggestive of TB. The good qualities of this work included the fact that it was a prospective study with a higher number of TB cases that were evenly distributed between men and women. The ADA detection method followed the well established Giusti method (used by all but one of the listed studies) although the cut-off used was 50 U/l (**18**). The main problems with the work include the inclusion of suspected rather than definitive (microbiology or histology) cases. Transudates were excluded from the study affecting its sensitivity and specificity when compared with other studies. Conclusions can only be drawn on exudative effusions. The study population was 68% mixed race with a low number of Caucasian patients (16%), therefore the findings may not be as relevant in the Caucasian population. The statistics used were not well described.

Villegas *et al* evaluated the individual use of ADA, IFN-γ levels and polymerase chain reaction (PCR) in the diagnosis of tuberculous effusion in 140 cases of pleural effusion. They found that these tests had a sensitivity of between 73%-88% when used alone, but that sensitivity and specificity increased when all three were used in combination (**6**). Incidentally, they also found that a lower age and fever was associated with TB related effusion. Pleural biopsy was also performed and had a sensitivity of 64.3% and a specificity of 98%. The authors concluded that their findings supported the use of new and more efficient diagnostic strategies in the management of pleural TB. This was a prospective study with a good male/female balance and a good mix of TB and non TB cases. The ADA was analysed in a standardised fashion (Giusti), however like the previous studies described, the cut-off level was not standardised with a cut-off level of 45.3 U/l. Only 42 patients had confirmed TB, the rest were included due to high clinical suspicion. There was a

significant frequency of false-negative results of PCR in the study (26%). This is most likely attributable to the inefficient recovery of genomic DNA and the extraction of DNA from frozen samples. Nonetheless, this will have had a significant effect on the results of this study. Statistical analysis was robust and well explained.

Porcel *et al* retrospectively reviewed 106 patients with tuberculous effusions and 286 patients with malignant effusions over a 9 year period. They used two scoring systems, using clinical and laboratory variables where the main difference was the inclusion (model 2) or exclusion (model 1) of ADA (**19**). They found that using the scoring system from model 1 which was calculated by attributing scores to the results of measured variables including an adenosine deaminase ≥ 40 U/L (5 points), age <35 years (2), temperature ≥ 37.8 degrees C (2), and pleural fluid red blood cell count < 5 x 10^9/L (1).

Model 2 involved a scoring system based on model 1 (excluding ADA) plus the following: no history of malignancy (3), pleural protein ≥ 50 g/L (1), and pleural fluid to serum lactate dehydrogenase ratio ≥ 2.2 (1). Total scores ≥ 5 in model 1 and ≥ 6 in model 2 resulted in a sensitivity and specificity of 95% and 94% and 97% and 91% respectively in the discrimination of tuberculous from malignant effusions (**19**). This work while involving larger numbers, was retrospective and again included cases of high clinical suspicion rather than exclusively involving confirmed cases. The other important weakness with this work was that their diagnostic tree method was not validated by the authors.

Ghanei *et al* investigated the combination of pleural fluid ADA, LDH and lymphocyte to neutrophil ratio. In the combination of three methods, with a positive result by either three of the methods considered to be indicative of a positive diagnosis of pleural TB, ADA activity plus LDH levels plus lymphocyte to neutrophil ratio in the pleural fluid yielded a sensitivity of 100%. This combination requiring all three methods to be positive for diagnosis to be made was 100% specific for pleural TB.

They also found that using a combination of pleural fluid ADA and LDH yielded a sensitivity of 91.4% and a specificity of 100% (**20**). This was a very small study where only 17 patients had a tuberculous effusion. They concluded that using these parameter combinations could lead to a diagnosis of pleural TB with an acceptable degree of confidence. While this was a prospective study with a good male/female balance and well described robust statistical method, the main criticism with this work is the small number of confirmed TB cases which were compared to a non TB group that was approximately 3.5 times its size (17). The ADA detection method was as per Giusti but again the cut-off level was different to other studies at 47 U/l.

Neves *et al* prospectively analysed 215 patients with pleural effusions from 1997-2001, of which 104 were tuberculous, and found that the analysis of ADA, total serum leukocytes, percentile of lymphocytes, protein and duration of disease combined, yielded a sensitivity and specificity of > 95%. (**21**)**.** This was a retrospective study which excluded a large proportion of cases due to inadequate specimen collection or inappropriate handling of ADA specimens. ADA detection was as per Giusti with a cut-off of 39 U/l. Of the TB cases included in the study, less than 10% of pleural fluid cultures and 58% of biopsy cultures were positive for TB. Acid fast staining was negative in all cases. Like Porcel's work from 2003, the main weakness of this study

was that the authors did not validate their predictive model. Statistics were well described and thee was a good balance between TB and non TB cases in the study.

Daniil *et al* investigated the measurement of ADA, IFN-γ, C-reactive protein (CRP), carcinoembryonic antigen, interleukin-6, tumour necrosis factor-α and vascular endothelial growth factor concentration in pleural fluid and found that the combination of ADA and CRP increased the percentage of correctly classified individuals as having tuberculous rather than malignant or parapneumonic effusions in 92% of cases (**22**). The study involved 72 patients of which only 12 were tuberculous. The authors recommended further and larger studies in this area. While this was a prospective study using robust statistical method, it was extremely small. ADA detection was again as per Giusti with a cut-off of 42.2 U/l. The study involved twice as many males as females. The non TB group was approximately 4.5 times the size of the TB group and the non TB malignant group was highlighted by the unusual absence of lymphomatous effusions which will have had an impact on the sensitivity and specificity.

In 2008 Porcel *et al*, having already published in this area in 2003, described a decision tree for differentiating tuberculous from malignant effusions. They retrospectively compared clinical and pleural features of 238 adults with pleural effusion who satisfied diagnostic criteria for tuberculosis or malignancy (**23**). They found that the combination of age > 35 years, pleural fluid ADA > 38 U/L, temperature ≥ 37.8 ^{o}C, and a pleural fluid LDH > 320 U/L yielded a sensitivity of 92.2% and a specificity of 98.3% allowing them to conclude that the use of this decision tree could help in the differential diagnosis of malignant and tuberculous

effusions but not of other forms of effusions (**23**). This was a good sized retrospective study which only involved confirmed TB cases and not suspected ones. Importantly, it included a derivation cohort followed by a validation cohort. The limitations of this work are few but involve the important fact that this model is only useful in differentiating TB from malignancy and not from other forms of effusions. The ADA detection system was not like those of the previous studies described and the cut-off used with the detection system was 38 U/l. While it is limited by its retrospective nature, the study involved only objective clinical characteristics and laboratory parameters strengthening the data. The author's model is only relevant in areas of high prevalence and could lead to very different findings if used in the USA for example, where prevalence is 3.4 /100,000.

Lastly, Kupeli *et al* have recently examined concurrent measurement of ADA and dipeptidylpeptidase (DPP) IV (T cell associated enzyme) activity in the diagnosis of tuberculous pleural effusion (**24**). They found that using both of these markers together improved the sensitivity, specificity and diagnostic accuracy (77%, 94% and 91% respectively), when compared to analysing these markers separately (**24**). This study involved 87 patients of which 18 had TB. Apart from the small number of patients involved, Kupeli et al included known transudates in their study thus increasing the diagnostic yield of the test. They concluded that in an area of high prevalence, ADA levels in combination with that of DPP can be useful in the diagnosis of tuberculous pleural effusion with high specificity and accuracy. The strengths of this study include its prospective nature, robust statistics and its focus on definite TB cases only. Like many of the previous studies it is limited by its extremely small number of TB cases (19). The ADA analysis method was also different to other

studies as was the ADA cut-off level of 40 U/l. While this work was performed in an area of high prevalence, the age of the TB group was young with an age range of 17 – 30 yrs and the male to female ratio was 3:1. Another limitation of the work is that parameter testing of each case occurred depending on the clinical scenario rather that blindly testing all cases similarly.

Marker combinations	Result	Author	Year	Compared with biopsy or culture	Study design	Patient no.	Prev. per 100/000
ADA and fluid lysozyme	↑ specificity to 95% P < 0.05	San Jose	1992	No	Pro	271	39
ADA and fluid tuberculostearic acid	↑ sensitivity to 83% P < 0.05	Muranishi	1992	No	Pro	67	58
ADA and fluid lymphocyte proportion	↑ sensitivity to 90.7% ↑ specificity to 97.7% P < 0.05	De Oliveira	1993	No	Pro	276	108
ADA, ratio of pleural to serum lysozyme	↑ specificity to 99% P < 0.05	Villena	1996	No	Pro	228	32
ADA, ratio of fluid lymphocytes to neutrophils	↑ sensitivity to 88% ↑ specificity to 95% P < 0.05	Burgess	1996	No	Pro	303	480
ADA and IFN-γ, PCR (any 2 markers combined)	↑ sensitivity to 90.5% P < 0.05	Villegas	2000	Yes	Pro	140	50
ADA, fever, age, fluid RBC	↑ sensitivity to 95% ↑ specificity to 94% P < 0.05	Porcel	2003	No	Retro	392	32
ADA, fluid LDH, ratio of fluid lymphocytes to neutrophils	↑ sensitivity to 100% (1 of 3 +ve) ↑ specificity to 100% (all 3+ve) P < 0.05	Ghanei	2004	No	Pro	88	32
ADA, fluid % lymphocytes, serum leukocyte count, fluid protein, symptom duration	↑ sensitivity > 95% ↑ specificity > 95% P < 0.05	Neves	2007	No	Pro	215	59
ADA and fluid CRP	↑ specificity > 91.7% P < 0.05	Daniil	2007	No	Pro	72	16
ADA, fluid LDH, age, temperature	↑ sensitivity to 92.2% ↑ specificity to 98.3% P < 0.05	Porcel	2008	No	Retro	238	32
ADA, fluid dipeptidylpeptidase IV	↑ sensitivity to 77% ↑ specificity to 94% P < 0.05	Kupeli	2009	No	Pro	87	33

Table 1. Studies examining the role of combination tests in the diagnosis of tuberculous pleural effusion.

Conclusion

The combination of tests and biomarkers to diagnose tuberculous pleural effusion in endemic areas where resources are limited or in cases where traditional investigation is negative but suspicion is high certainly has merit. Even in cases where ADA is unavailable, the use of clinical and demographic information in combination with a cell differential count, in high incidence settings, can yield a diagnosis of TB with a high predictive value (**25**). This concept has been further advanced with a number of groups recommending the use of pleural fluid and clinical parameter combinations in the diagnosis of tuberculous pleural effusions which we described in this review.

We believe there are a number of advantages to using such scoring systems. They are cheap to perform (ADA costs $1.28 per test Bio-Quant INC San Diego USA), do not require clinical expertise (eg. thoracoscopist), are associated with minimal morbidity, give a speedy result and are beneficial in areas of poor resources or in investigating elderly frail patients not suitable for more invasive procedures. If positive, anti-tuberculous therapy may be started straight away in areas of high prevalence as culture ± histology results are awaited.

They are also useful in those highly suspicious few patients that do not yield a definitive diagnosis after closed pleural biopsy or fluid analysis and culture.

While the advantages described above might appear obvious it is also important to highlight a number of disadvantages with these tests. Firstly, the specificity of ADA is relatively low when compared to, for example, thoracoscopy and therefore it is important to remember that ADA in lymphocyte rich effusions has been described in rheumatoid arthritis, mesothelioma, psittacosis, brucellosis, histoplasmosis, coccidiomycosis, bronchoalveolar carcinoma, chlamydia and mycoplasma

pneumonia, Mediterranean fever and in the majority of patients with empyema (**26,27,28,29**).

The benefits described in the studies in figure 1 have in the majority of cases been performed in areas where TB prevalence is high and data is therefore lacking from countries with lower prevalence. As ADA makes up part of the combination in all the studies reviewed, it is important to highlight that that ADA is less sensitive in countries of low TB prevalence and may lead to an under-diagnosis of pleural TB (however TB may be excluded particularly if ADA is negative and suspicion is moderate or low) which can lead to further organ spread in 65% of patients (**30**). It is also possible that false positive cases will be commenced on anti-tuberculous therapy resulting in significant drug related adverse effects. Importantly the Infectious Diseases Society of America (IDSA) recommends the demonstration of *M. tuberculosis* in the sputum, pleural fluid or pleural biopsy specimen as their gold standard in diagnosing the condition and clearly do not recommend the replacement or substitution of this standard by pleural fluid and clinical combination tests (**31**). Additionally, one of the most important disadvantages of using these combinations alone in the diagnosis of pleural TB is the fact that these combinations bypass the culture of fluid or tissue and therefore drug susceptibility testing is not performed in an era when drug resistant TB is increasing. In the USA, Baumann *et al* described resistance to a first line treatment drug in 9.9% of pleural TB patients from 1993-2003 (**32**). This can lead to treatment failure and clinical deterioration. Certainly the use of combination tests may allow the commencement of four drug therapy while pleural fluid culture is awaited but if this is non-diagnostic, pleural biopsy will still be required especially in those groups who have a higher rate of drug resistance (**32**).

Also, the process of testing for these markers does not offer any immediate therapeutic benefit unlike thoracoscopy which is not only diagnostic but allows the removal of large pleural fluid collections giving immediate relief to the patient. Other advantages highlighted earlier included microscopic and macroscopic examination of tissue leading to early drug testing prior to culture results and drug susceptibility testing on biopsied tissue.

While the studies listed here appear to demonstrate a benefit of combination (particularly those involving ADA) over single parameter testing, the authors feel that there is currently insufficient evidence available to conclude whether the use of clinical and biochemical parameter combinations should routinely be used to diagnose tuberculous effusions. Studies have involved low numbers of patients and all have recommended the use of different parameters thus lacking consensus. Other problems with the studies include the variable prevalence rates associated with the different countries where the research took place, the different ADA detection methods and cut-off levels used, the variable study design (retrospective versus prospective), the regular inclusion of unproven TB cases in the studies as well as the lack of gold standard controls in all but one study.

Further large studies are required to validate their use and studies in areas of low TB prevalence are also warranted to investigate the suitable use of these combination tests in these areas. In particular the study by Porcel in 2008 would benefit from validation in an area of low prevalence as it was one of the stronger studies performed by a world recognised research group with a good demographic distribution, robust statistics and large study size. Studies which intend to include ADA might benefit

from using the more specific ADA2 isoenzyme which may increase the sensitivity and specificity further. Furthermore, while at best these combinations may strongly suggest a diagnosis in endemic areas warranting commencement of drug therapy, their use as a replacement to the demonstration of *M. tuberculosis* in culture or histology will require randomised control trials comparing these parameters with the gold standard of thoracoscopic biopsy for microbiology and histology. If they performed well in these trials, their regular use may be validated however the problem still arises regarding susceptibility testing in areas of high drug resistance.

While we await these studies, we believe that combination tests may be used in current practice in areas of high prevalence to steer the physician towards a diagnosis allowing therapy to start and continue or be adjusted once definitive proof has been achieved with culture or histology. They may also be useful in endemic areas with poor resources, such as the Sub Saharan region where diagnosis is often made clinically or empirically as no other resources are available (**33**).

Clearly, the use of a combination of parameters to diagnose tuberculous effusions has great potential for both physicians and patients involved. The benefits in the acute setting, in patients with negative initial results despite high clinical suspicion, in frail patients unable to undergo pleural biopsy combined with their cheap cost are considerable in regions where TB is endemic. Their use in areas where the incidence of TB is low is less valid. As a result, further clinical assessment of these combinations through larger studies and randomised trials may validate their general and routine use in medical practice and in particular identify a robust combination that yields the highest sensitivity and specificity.

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Table

Marker combinations	Result	Author	Year	Compared with biopsy or culture	Study design	Patient no.	Prev. per 100/000
ADA and fluid lysozyme	↑ specificity to 95% P < 0.05	San Jose	1992	No	Pro	271	39
ADA and fluid tuberculostearic acid	↑ sensitivity to 83% P < 0.05	Muranishi	1992	No	Pro	67	58
ADA and fluid lymphocyte proportion	↑ sensitivity to 90.7% ↑ specificity to 97.7% P < 0.05	De Oliveira	1993	No	Pro	276	108
ADA, ratio of pleural to serum lysozyme	↑ specificity to 99% P < 0.05	Villena	1996	No	Pro	228	32
ADA, ratio of fluid lymphocytes to neutrophils	↑ sensitivity to 88% ↑ specificity to 95% P < 0.05	Burgess	1996	No	Pro	303	480
ADA and IFN-γ, PCR (any 2 markers combined)	↑ sensitivity to 90.5% P < 0.05	Villegas	2000	Yes	Pro	140	50
ADA, fever, age, fluid RBC	↑ sensitivity to 95% ↑ specificity to 94% P < 0.05	Porcel	2003	No	Retro	392	32
ADA, fluid LDH, ratio of fluid lymphocytes to neutrophils	↑ sensitivity to 100% (1 of 3 +ve) ↑ specificity to 100% (all 3+ve) P < 0.05	Ghanei	2004	No	Pro	88	32
ADA, fluid % lymphocytes, serum leukocyte count, fluid protein, symptom duration	↑ sensitivity > 95% ↑ specificity > 95% P < 0.05	Neves	2007	No	Pro	215	59
ADA and fluid CRP	↑ specificity > 91.7% P < 0.05	Daniil	2007	No	Pro	72	16
ADA, fluid LDH, age, temperature	↑ sensitivity to 92.2% ↑ specificity to 98.3% P < 0.05	Porcel	2008	No	Retro	238	32
ADA, fluid dipeptidylpeptidase IV	↑ sensitivity to 77% ↑ specificity to 94% P < 0.05	Kupeli	2009	No	Pro	87	33

Table 1. Studies examining the role of combination tests in the diagnosis of tuberculous pleural effusion.