Increased pleural fluid adenosine deaminase (ADA) activity is classically associated with tuberculous pleuritis. However, increased activity can also occur in a number of other diseases and this may negatively affect the diagnostic utility of ADA measurements and decrease its specificity for the diagnosis of tuberculosis (TB). The presence of ADA in pleural fluids reflects the cellular immune response in the pleural cavity and in particularly, the activation of T lymphocytes. Different disease entities are typically associated with the presence of particular types of leukocytes.

Objective: To determine whether the combined use of ADA activity and differential cell counts would provide a more efficient means for diagnosing tuberculous pleurisy than the use of ADA levels alone.

Methods: Biochemistry, cytology, and microbiology studies were performed on 472 consecutive pleural fluids. ADA and differential cell counts were determined on all exudative effusions.

Results: ADA activity in tuberculous effusions was significantly higher than in any other diagnostic group (p<0.005). At a level of 50 U/L, the sensitivity, specificity, positive predictive value (ppv), negative predictive value (npv), and efficiency for the identification of TB were calculated at 91%, 81%, 84%, 89%, and 86%, respectively. When the additional requirement of a lymphocyte neutrophil ratio of 0.75 or greater was included, the sensitivity, specificity, ppv, npv, and efficiency for the identification of TB were calculated at 88%, 95%, 95%, 88%, and 92%, respectively.

Conclusion: ADA, especially when combined with differential cell counts and lymphocyte/neutrophil ratios, remains a useful test in the diagnosis tuberculous pleuritis.

Key words: adenosine deaminase activity; differential cell count; lymphocyte/neutrophil ratio; tuberculous pleuritis

Although numerous studies have demonstrated the diagnostic significance of increased adenosine deaminase (ADA) levels in tuberculous pleurisy, other studies have shown that ADA is of limited value as raised levels are also associated with a number of other diseases, including malignancies (especially those of hematologic origin), bacterial infections, empyemas, and collagen vascular diseases (including systemic lupus erythematosus [SLE] and rheumatoid arthritis). ADA isoenzymes have been suggested to increase the overall diagnostic value of ADA determination. However, ADA isoenzyme patterns seen appear to be a reflection of the difference in the immune response and of the corresponding predominant cell populations in the pleural fluid.

Pleural effusions may arise secondary to pulmonary or systemic disease, and their development is classically associated with an influx of inflammatory cells into the pleural space. Different disease entities are typically associated with the presence of particular types of leukocytes. In the case of parapneumonic and empyematosus effusions, neutrophils constitute more phagocytic cell types in the pleural space. Lymphocytes predominate in malignant and tuberculous pleural effusions.

In view of the controversy surrounding the diagnostic utility of ADA in pleural fluids, we endeavored to determine whether the combined use of ADA activity and differential cell counts would provide a more efficient means for diagnosing tuberculous pleurisy than the use of ADA levels alone.

Materials and Methods

A study was carried out during 1993 at Tygerberg Hospital, South Africa. Four hundred seventy-two consecutive pleural fluid specimens from patients admitted to medical, surgical, gynecologic, and pediatric wards were analyzed. Posteroanterior and lateral chest
radiographs were done in all cases. Total protein and lactate dehydrogenase levels were obtained for both serum and pleural fluid specimens to distinguish exudates from transudates according to Light’s criteria. These were determined using a multichannel analyzer (Technicon DAX 48; Bayer-Miles Diagnostics, Tarrytown, NY). Total protein concentration (gram per liter) was estimated using the biuret method, and lactate dehydrogenase activity (unit per liter) was measured using an enzymatic ultraviolet optimized method.

Previous studies have shown that the measurement of ADA activity in transudative effusions is not clinically useful, and they were thus excluded from our study population. ADA activity (unit per liter) was determined on all exudative pleural fluid specimens according to the method described by Giusti. Adenosine is deaminated by ADA and the free ammonia is estimated by Berthelot’s reaction. One unit of ADA is defined as the amount of enzyme required to release 1 μmol of ammonia per minute from adenosine at standard assay conditions.

A sample of pleural fluid was sent to microbiology for Gram’s staining and bacterial and tuberculosis (TB) culture. An additional sample was sent to cytology where a differential cell count was also done. The fluid was spun down and four slides were made from the sediment. Of these slides, two were fixed and stained with Papancolou stain; the other two were air dried and stained with May-Grunwald stain. The slides were then mounted and examined microscopically. The different cells were counted per hundred cells on various fields throughout the slide. A total of 300 cells were counted and then an average taken. The differential cell count was reported as percentages. Further investigations, including histology, were done at the discretion of the primary physician.

The hospital records of all patients having exudative effusions were reviewed and a diagnosis made according to the following predetermined criteria.

**Tuberculous pleuritis** was classified into three diagnostic subclasses: (1) identification of the bacillus in pleural fluid or biopsy specimen by stain or by culture, or by the presence of granulomas in pleural biopsy tissue; (2) positive sputum culture in the presence of clinical and radiologic evidence for TB and in the absence of any other obvious cause associated with pleural effusions; and (3) clinical and radiologic evidence for TB in the absence of any other obvious cause associated with pleural effusions and associated with a positive response to antituberculous therapy.

** Infective effusions** included the following: pneumonia effusions associated with acute febrile illness, pulmonary pneumonic infiltrates, purulent sputum and responsiveness to antibiotic treatment or identification of the organism in the pleural fluid; sepsis, characterized by radiologic evidence of pulmonary infiltrates and multisystem involvement in the presence of positive blood cultures; and other obvious infective conditions in the absence of any other cause associated with pleural effusions. Empyematosus effusions, characterized by the finding of frank pus in the pleural cavity, were included provided that the specimen’s turbidity did not interfere with the relevant investigations.

**Neoplastic effusions** were diagnosed when one of the following criteria was met: (1) the presence of cytologic or histologic evidence of a malignant pleural effusion or (2) histologic proof of a malignant tumor with exclusion of any other cause known to be associated with pleural effusions.

**Other exudates** were defined by effusions that were clearly caused by pancreatitis, Dresler’s syndrome, collagen vascular disease, pulmonary embolus or infarction, and various other rare but well-documented causes of exudative pleural effusions. In all cases, there was an absence of malignancy, pulmonary infiltrates, and diseases causing transudates.

Patients having multiple superimposed diseases or effusions of unknown origin were classified as “undiagnosed.” Patients having ‘hepatomoraces or empyemas too turbid for analysis were excluded.

ADA activity in tuberculous effusions was compared with that in the other diagnostic groups by the Wilcoxon two-sample test. Data are given as median (25th, 75th percentile) values. The utility of ADA as a diagnostic tool for TB was evaluated at various cutoff levels by calculating sensitivity, specificity, positive predictive value (ppv), negative predictive value (npv), and efficiency. These were compared by means of relative operating characteristic curves and the cutoff value that maximized the true-positive rate was selected.

Differential cell counts were obtained for all effusions. From these values, a lymphocyte/ neutrophil (L/N) ratio was calculated. ADA values were then compared with various L/N ratios and evaluated at various cutoff levels for ADA and L/N ratios by calculating sensitivity, specificity, ppv, npv, and efficiency. These were again compared by means of relative operating characteristic curves and the cutoff value that maximized the true-positive rate was selected.

The efficiencies of the two tests were compared.

**RESULTS**

Although 472 specimens were received for analysis, 127 patients were excluded due to diagnoses of hemathorax (5), grossly turbid empyemas (14), or transudative effusions (106). An additional 42 patients were excluded as no differential cell counts were available. The remaining 303 patients consisted of 194 patients of mixed race (64%), 61 blacks (20%) and 48 whites (16%), of which 176 were men (58%) and 127 were women (42%). The average age of the patient population was 49 (SD 20,72) years (range, 6 months to 98 years).

Tuberculous pleurisy accounted for 143 (55%) of the exudative effusions, neoplastic conditions 59 (19%), infective conditions 54 (18%), and other exudates 16 (5%). The group of “other exudates” included the following etiologic factors: pulmonary emboli (7), systemic lupus erythematosus (SLE) (3), pancreatitis (2), asbestosis (2), Dresler’s syndrome (1), and chylothorax (1). In 31 cases (10%), patients were found to have multiple superimposed diseases or effusions of unknown origin.

In the case of patients having tuberculous pleuritis, three diagnostic subclasses were identified: (1) identification of the bacillus in pleural fluid or biopsy specimen by stain or by culture, or by the presence of granulomas in pleural biopsy tissue (83); (2) positive sputum culture in the presence of clinical and radiologic evidence for TB and in the absence of any other obvious cause associated with pleural effusions (26); and (3) clinical and radiologic evidence for TB in the absence of any other obvious cause associated with pleural effusions and associated with a positive response to antituberculous therapy (34). The distribution of ADA activity for the various subclasses of TB is shown in Figure 1. No significant difference was found in ADA activities among these diagnostic groups (p>0.05).

The median (25th, 75th percentile) values for ADA activity were determined for each of the five diagnostic groups. ADA activity was significantly higher for tuberculous effusions than for the other diagnostic...
groups (p<0.005 for each group). The group of patients having tuberculous pleurisy had median (25th, 75th percentile) values of 103 (75, 141) U/L. Corresponding values for patients with infective, malignant, and other exudative effusions were 30 (20, 66) U/L, 26 (19, 34) U/L, and 21 (15, 43) U/L, respectively. The distribution of ADA activity for each of the diagnostic classes is shown in Figure 2. ADA activity was significantly higher for TB than for the other diagnostic groups (p<0.005 for each group).

Various levels of ADA were tested as a cutoff level for the diagnosis of TB. As in previous studies done at Tygerberg Hospital, using a cutoff level of 50 U/L for ADA activity yielded the best results. At this level, 130 (of 143) patients having TB were correctly diagnosed. Nine of these patients were found to be positive for HIV. The sensitivity, specificity, ppv, npv, and efficiency were calculated as 91%, 81%, 84%, 89%, and 86%, respectively (Table 1).

Thirteen patients having TB were found to have ADA activities less than 50 U/L (range, 12 to 49 U/L). In five of these cases, definitive microbiologic evidence for TB was found; four had positive pleural fluid cultures and one was diagnosed by pleural biopsy speci-

Figure 1. Distribution of ADA activity for the TB diagnostic subclasses. Diagnosis made by (1) identification of the bacillus in pleural fluid or biopsy specimen or by the presence of granulomas in biopsy tissue (103 [69,149] U/L); (2) positive sputum culture in the presence of clinical and radiologic evidence for TB (90 [55,117] U/L); and (3) clinical and radiologic evidence for TB associated with a response to antituberculous therapy (113 [96,136] U/L). Corresponding median (25th, 75th percentile) values are in brackets.

Figure 2. Distribution of ADA activity for the various diagnostic subclasses. TB (103 [75,141] U/L); Inf=infective (30 [20,66] U/L); Ca=malignancy (26 [19,34] U/L); Ex=miscellaneous exudates (21 [15,43] U/L); Undx=undiagnosed (18 [12,34] U/L). Corresponding median (25th, 75th percentile) values are given in brackets.

men. Six patients had positive sputum cultures for *Mycobacterium tuberculosis* in the presence of clinical and radiologic evidence thereof; two had only clinical and radiologic evidence accompanied by a positive response to treatment. Six (of these 13) patients were also found to be HIV positive. Of the nontuberculous patients, 25 were found to have ADA activities of 50 U/L or greater (range, 51 to 402 U/L). These included 18 patients with infective effusions (5 of that had empyemas); 5 with malignant effusions (3 of that had lymphomas); 1 with chylothorax; and 1 with SLE.

The L/N ratio in the combined tuberculous group was analyzed. Only five patients, of whom four had both positive pleural fluid cultures and an ADA level...
of 50 U/L or greater, had a L/N ratio less than 0.75 (range, 0.12 to 0.74). The remaining patient was diagnosed on the grounds of positive sputum cultures in combination with clinical and radiologic evidence for TB (ADA, 36 U/L).

These two criteria were combined. No correlation could be found between ADA activity and differential lymphocyte counts or L/N ratio. Using the finding of a pleural fluid ADA activity of 50 U/L or greater and a pleural fluid L/N ratio of 0.75 or greater as being diagnostic for tuberculous pleuritis yielded a test efficiency of 92%. Corresponding sensitivity, specificity, ppv, and npv were 88%, 95%, 95%, and 88%, respectively (Table 1). Various other combinations of ADA activity and L/N ratio were tested, but the resultant test efficiencies were all less than those yielded by the former combination (data not shown).

At this level, 126 (of 143) patients having TB were correctly diagnosed. Six nontuberculous patients were misclassified. These included four patients with malignant effusions (of which 3 were due to lymphomas), 1 with SLE, and 1 with a traumatic chylothorax. Application of this combined use of ADA activity and L/N ratio resulted in 17 patients with tuberculous effusions being omitted. Thirteen of these patients were also misdiagnosed by the use of ADA activity alone (ADA levels <50 U/L). The additional four patients who were excluded had all definitive microbiologic evidence for TB. The ADA levels were significantly elevated (ranging from 116 to 220 U/L) but the L/N ratios were low.

**DISCUSSION**

ADA (Enzyme Commission 3.5.4.4) is a polymorphic enzyme involved in purine catabolism, catalyzing the deamination of adenosine and deoxyadenosine to produce inosine and deoxynosine, respectively. Although found in most human tissues, its activity is greatest in the lymphoid tissues,22 where it plays a role in the differentiation of lymphoid cells23,24 and the maturation of monocytes to macrophages.25 Increased ADA activity in pleural effusions is classically associated with TB.1,5 However, it may occur due to a number of causes and this may negatively affect the diagnostic utility of ADA measurements and decrease its specificity in the diagnosis of TB.2,8-10

Our results show that, at a cutoff level of 50 U/L, ADA has a sensitivity, specificity, ppv, npv, and efficiency of 91%, 81%, 84%, 89%, and 86%, respectively, for the diagnosis of TB. These statistical results are lower than those obtained by similar studies. However, it must be noted that only exudates have been included in this study. All transudates, which are characterized by low ADA levels (<20 U/L),19 have been excluded.

The presence of ADA in pleural fluids reflects the cellular immune response in the pleural cavity and in particular, the activation of T lymphocytes.3 The identification of specific pleural fluid ADA isoenzyme patterns in different pleural diseases emphasizes this relationship between ADA and the different cellular and immunologic responses occurring in the pleural cavity.13 When the L/N ratio (≥0.75) was considered together with the ADA activity (≥50 U/L), the results improved considerably for the diagnosis of tuberculous pleuritis. The sensitivity, specificity, ppv, npv, and efficiency were 88%, 95%, 95%, 88%, and 92%, respectively.

Numerous studies have shown that the pathogenesis of a pleural effusion, due to pulmonary and systemic causes, is associated with the recruitment of phagocytic cells and the sequential influx of cells into the pleural space.14 As a result, different disease entities are associated with the presence of particular types of leukocytes.15-18 Tuberculous pleurisy is an HIV helper cell lymphocyte-dominant pleurisy, based on delayed-type hypersensitivity caused by M tuberculosis and characterized histopathologically by granuloma formation.26 Pleural fluid lymphocytosis is also found in malignant conditions (including lymphomas and leukemias), collagen vascular diseases, sarcoidosis, and up to one third of all transudates.16-18 Parapneumonic and empyematosus effusions are characterized by neutrophil-predominant, exudative effusions.15-17

The origin of the increased ADA activity found in tuberculous effusions is uncertain. Many investigators have attributed the origin of these high levels to the fact that tuberculous pleurisy is a T-cell-mediated response.2-4,8 However, insignificant or inconclusive results have been obtained for studies attempting to show a correlation between number of lymphocytes or lymphocyte populations and ADA levels.3,27,28 Other investigators have suggested a monocyte-macrophage

Table 1—Comparison of ADA Activity Alone vs ADA Activity in Combination With L/N Ratio as a Means for Diagnosing TB Pleuritis

<table>
<thead>
<tr>
<th>Criteria Used to Diagnose TB</th>
<th>ADA Level, U/L</th>
<th>L/N Ratio</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>ppv, %</th>
<th>npv, %</th>
<th>Efficiency, %</th>
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<tbody>
<tr>
<td>≥50</td>
<td>—</td>
<td></td>
<td>91</td>
<td>81</td>
<td>84</td>
<td>89</td>
<td>86</td>
</tr>
<tr>
<td>≥50</td>
<td>≥0.75</td>
<td>88</td>
<td>95</td>
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<td>88</td>
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origin of ADA$^{13,20,30}$ reflecting an increased cellular activity or turnover.$^{13}$

Blood ADA levels are also increased in certain T-cell lymphoproliferative disorders and this can be expected to spill over to the pleura in these conditions.$^{9}$ In the para-infective effusions, ADA probably originates from lymphocytes or neutrophils.$^{2,13}$

In the present study, ADA activity was highest among the tuberculous group. Para-infective conditions were also seen to be associated with high ADA activities. The relative cell count or L/N ratio could be used to distinguish between these two entities.$^{15-18}$ In the case of tuberculous pleurisy, a predominant lymphocyte count was usually found, resulting in a L/N ratio of 0.75 or greater whereas in the case of para-infective effusions, a predominant neutrophil count was usually found (L/N ratio <0.75).

As already mentioned, malignant effusions may also be associated with high lymphocyte counts.$^{16,18}$ The distinction between malignant and tuberculous effusions can usually be made on the grounds of ADA activity. In general, malignant effusions have lower ADA levels than those found in TB. However, effusions secondary to lymphomas and leukemias were generally associated with higher ADA activities than nonhematologic malignancies, and could be confused with tuberculous effusions on the grounds of ADA activity and L/N ratios.

Another source of false-positives could be the presence of rheumatoid pleuritis. Although absent in our group of patients, rheumatoid pleurisy appears to be a unique entity in that it could not be differentiated from pleural TB on the basis of ADA activity alone. In addition to studying ADA activity in these patients, Ocaña et al$^{31}$ also determined differential cell counts on these effusions. The L/N ratio can thus be determined and in three (nine cases), the L/N ratio is greater than 0.75. However, other parameters (such as glucose and rheumatoid factor) can be used to make this distinction.

TB pleurisy is traditionally diagnosed by either identification of $M$ tuberculosis in pleural fluid and/or biopsy specimen cultures or from the presence of granulomas in pleural biopsy tissue. Pleural fluid cultures have a sensitivity 20 to 30%;$^{32}$ pleural biopsy specimens, 50 to 80%,$^{33}$ depending on the clinician's proficiency. Because of the long culture periods required, clinical and therapeutic decisions are often made before these laboratory results become available. Polymerase chain reaction, having a sensitivity of 78% for active disease,$^{34}$ has not been found to be an efficient alternative. Use of ADA level, especially in conjunction with the L/N ratio, is therefore a valuable diagnostic tool in this regard, as it provides a rapid and accurate means of detecting TB pleurisy.

In conclusion, we suggest that the pleural fluid ADA values be used, in conjunction with differential cell counts, in the following way: (1) a lymphocytic exudate (L/N ratio ≥0.75) with a high ADA value (≥50 U/L) is highly suggestive of TB pleurisy; (2) a lymphocytic exudate with low ADA values (<50 U/L) is suggestive of nonhematologic malignancies; and (3) a neutrophilic exudate (L/N <0.75) with a high ADA concentration (≥50 U/L) is suggestive of parainfective effusions.

**References**