

# Tuberculous pleural effusions: increased culture yield with bedside inoculation of pleural fluid and poor diagnostic value of adenosine deaminase

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## Abstract

**A prospective study of 111 adult patients with a pleural effusion was carried out in an area with a high prevalence of tuberculosis to compare the yield of bedside with laboratory inoculation of pleural fluid, and the yield and speed of a radiometric mycobacterial culture system (BACTEC) with that of conventional culture. The use of adenosine deaminase activity in pleural fluid as a diagnostic test for tuberculosis was also evaluated. In the 62 cases of tuberculosis confirmed histologically or by culture, or both, the BACTEC system had the same culture yield as conventional mycobacterial culture (positive in 14 cases—23%), but was significantly faster (18 versus 33 days). Bedside inoculation had a culture yield significantly higher than laboratory inoculation in the 24 patients tested (11 versus four). The remaining three diagnostic categories were malignant (28), miscellaneous (10), and undiagnosed (11). Median adenosine deaminase activity was significantly higher in tuberculous effusions than in any of the other categories, but there was considerable overlap between the groups. It is concluded that the BACTEC system is significantly faster than conventional mycobacterial culture and that bedside inoculation of pleural fluid substantially increases culture yield. Adenosine deaminase does not provide as valuable a diagnostic test of pleural tuberculosis as has been suggested.**

The diagnosis of tuberculous pleural effusions is usually established by closed pleural biopsy, granulomas being found in 60–80% of initial biopsy specimens.<sup>1-4</sup> Mycobacterial culture of pleural biopsy specimens has a similar yield and may be positive when histological examination yields negative results.<sup>2-4</sup> Acid and alcohol fast bacilli are rarely seen in Ziehl-Neelsen stained smears of pleural fluid<sup>5</sup> and culture is positive in only about a quarter of cases.<sup>2,3</sup> An improved yield from culture of pleural fluid would reduce the number of cases in which the diagnosis has to be made on clinical grounds (14–20%).<sup>2,3</sup>

Several studies of tuberculous serositis have reported higher than expected yields from fluid cultures when standard methods were modified: 48% when two or more pleural

aspirations were used,<sup>4</sup> 71% when 200 ml of ascitic fluid was cultured,<sup>6</sup> and 58% for pericardial effusions with bedside inoculation into mycobacterial culture medium.<sup>7</sup> None of these studies, however, made direct comparisons of the conventional and the modified methods, using them with the same specimens.

With conventional media culture may take up to eight weeks before results are obtained. Radiometric culture techniques take about half as long,<sup>8</sup> but this is still too long for many clinical purposes. There is a need therefore for a reliable, rapid diagnostic test.

Measurement of adenosine deaminase activity has been widely reported as a useful diagnostic test for tuberculous pleural effusions, with a sensitivity approaching 100%.<sup>9-13</sup> Adenosine deaminase is a product of activated lymphocytes, so that high activity is also found in effusions due to rheumatoid arthritis, lymphoma, empyema, parapneumonic effusions and mesothelioma.<sup>10-14</sup> Not surprisingly, therefore, one study in a low prevalence area found adenosine deaminase to be of little value as a diagnostic test.<sup>14</sup>

The current study was performed to compare prospectively (1) the yield from bedside and from laboratory inoculation of pleural fluid; (2) the sensitivity and speed of a radiometric culture method (BACTEC, Johnson Laboratories) for recovering *Mycobacterium tuberculosis* from pleural fluid versus those of conventional culture media; and (3) the sensitivity and specificity of measuring adenosine deaminase activity as a diagnostic test of tuberculous effusions in an area with a high prevalence of tuberculosis.

## Methods

### PATIENTS

Groote Schuur Hospital is a large community based teaching hospital serving an area where annual tuberculosis notifications are 300–400/100 000 population. Ninety four adults (older than 12 years) from the department of medicine whose attending physicians had requested diagnostic pleural aspiration and biopsy during the six months December 1988 to June 1989 were entered into the study. A further 17 patients known to have malignant disease and with a new pleural effusion were enrolled from the department of radiotherapy. Only six of these patients from the radiotherapy department were subjected to pleural biopsy.

### SPECIMENS

Clinical details were recorded, and closed

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pleural biopsy was performed with an Abrams needle. Ninety biopsies were performed by one of us (GM) and the remaining 10 by registrars and interns. In each case three or four biopsy samples were taken: one sample was crushed and inoculated into mycobacterial culture media (Kirchner, Lowenstein-Jensen, and pyruvate enriched Lowenstein-Jensen); the remaining two or three samples were fixed in formaldehyde and sectioned, and haematoxylin and eosin and Ziehl-Neelsen staining was carried out. All the sections were reviewed by a histopathologist experienced in interpreting pleural biopsy specimens.

Two samples of pleural fluid from each patient were centrifuged; the spun deposits were stained by the Ziehl-Neelsen method and one was inoculated into conventional mycobacterial culture media as above and the other into 12B BACTEC mycobacterial culture bottles. Clots frequently formed when the fluid was centrifuged, and these had to be crushed before injection through the rubber stopper of the 12B bottles. In 40 patients 5 ml of pleural aspirate was inoculated at the bedside into 13A BACTEC bottles (12B bottles accept only 1 ml). Antimicrobials were added to the Kirchner and BACTEC media. Cytological analysis was done on heparinised samples. Adenosine deaminase activity was measured by a standard method.<sup>15</sup>

Conventional mycobacterial culture media were aired and read weekly for eight weeks. BACTEC bottles were analysed on a BACTEC 460 analyser three times a week for two weeks and then weekly for eight weeks. Identification of *M tuberculosis* was confirmed by the niacin test.

#### STATISTICAL ANALYSIS

The time taken for positive cultures to be obtained after laboratory inoculation by conventional and by radiometric methods was compared with the signed rank test. McNemar's  $\chi^2$  test was used to compare the yields from bedside and of laboratory inoculation.<sup>16</sup> Adenosine deaminase activity in tuberculous effusions was compared with the levels of activity in the other diagnostic categories by the Wilcoxon two sample test. Median values are given for data that were not normally distributed, with 25th and 75th percentiles.

## Results

### TUBERCULOUS EFFUSIONS

Tuberculosis was confirmed histologically or by culture, or both, in 62 patients. The median age of the patients was 37.5 (range 14–83) years and the age distribution was bimodal. Thirty three patients were black and 28 of mixed race and one was white—a racial distribution similar to that found in tuberculosis notifications in our area. Nine patients had tuberculosis at sites outside the lungs, four of them having disseminated disease. Effusions were bilateral in six patients. Mantoux tests produced positive reactions (over 9 mm) in 18 of 23 recorded cases (78%).

Table 1 Yield of diagnostic procedures in patients with tuberculous effusions

| Procedure                | No (%) positive |
|--------------------------|-----------------|
| Pleural biopsy histology | 52 (84)         |
| Pleural biopsy culture   | 44 (71)         |
| Pleural fluid culture*   | 29 (47)         |

\*Combined total of radiometric and conventional culture and bedside inoculation.

### DIAGNOSTIC YIELD

The yields of diagnostic procedures are summarised in table 1. Caseation was present in 41 biopsies, and acid and alcohol-fast bacilli in 27. Pleural histology was diagnostic in all 3 patients with ascites. In 2 patients all tests on pleural biopsy and fluid were negative, and the diagnosis was established by supraclavicular lymph node biopsy and aspiration of a "cold" sternal abscess respectively.

Pleural fluid culture was positive in four patients with negative pleural biopsy culture and histological findings, thus increasing the diagnostic yield from 58 to 62 of the 111 patients investigated. Scanty acid and alcohol fast bacilli were found in one pleural fluid sample (only when all the slides were reviewed).

### COMPARISON OF CULTURE METHODS

Conventional mycobacterial and 12B BACTEC cultures had the same yield (positive in 14 cases). The result was positive in 16 cases with one method and in six with both. The median time taken for culture to become positive was 18 (range 3–40) days with the 12B BACTEC and 33.5 (range 21–48) days with conventional media. The 12B BACTEC method was significantly faster in the six patients with a positive result with both methods ( $p = 0.036$ ).

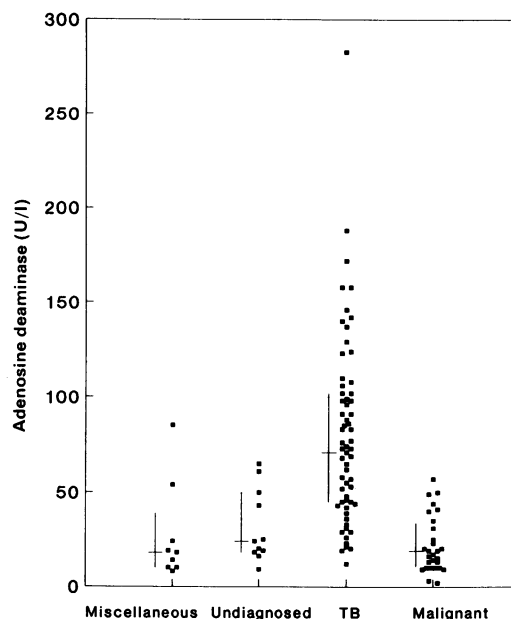
Bedside inoculation into 13A BACTEC bottles was performed in 24 cases where the final diagnosis was tuberculosis. Culture was positive in 11 of the 24 patients (46%), compared with four each (17%) for conventional media and 12B BACTEC. This difference of 29% was significant ( $p = 0.046$ ; 95% confidence interval of difference 3–55%). The median time taken for cultures to become positive in 13A bottles was 25 (range 17–56) days.

### OTHER DIAGNOSTIC CATEGORIES

Twenty eight patients had malignant effusions confirmed histologically or cytologically or by both methods (from pleural aspirate or biopsy specimen in 6). Twenty six had carcinoma at various sites, and there was one mesothelioma and one lymphoma. Two of the 17 patients from the radiotherapy department had non-malignant causes for their pleural effusions (tuberculosis and cardiac failure) and were categorised accordingly.

Miscellaneous causes were responsible for 10 effusions (one case each of systemic lupus erythematosus, empyema, pulmonary infiltrates with eosinophilia, cardiac failure, amoebic liver abscess, and sequelae of abdominal surgery, and two cases each of

Adenosine deaminase activity in the four diagnostic categories (TB—tuberculosis). The vertical lines against the individual values in each category extend from the 25th to the 75th percentiles, and the horizontal bars crossing these lines represent the median.



spontaneous pneumothorax and parapneumonic effusion). No diagnosis was made in 11 patients with exudative effusions, four of whom had repeat pleural biopsies (open in one case). Three patients in this undiagnosed group had probable tuberculous effusions with a good response to antituberculous treatment.

#### ADENOSINE DEAMINASE

Adenosine deaminase activity was measured in 109 patients. The sample from the patient with empyema was judged unsuitable for analysis, and one sample from a tuberculous effusion was mislaid. The results are represented graphically in the figure. Median (25th, 75th percentile) values for patients with tuberculosis were 71 (45, 102) U/l, compared with 19 (11, 34), 18 (10, 39), and 24 (18, 50) U/l in patients with malignancy, miscellaneous causes, and undiagnosed cause respectively. Adenosine deaminase activity was significantly higher for tuberculous effusion than for the other categories ( $p < 0.01$  for each category). Values of 45 U/l or more were considered to be raised.<sup>11</sup> The sensitivity of raised adenosine deaminase activity for identifying tuberculous effusions was 0.77 and the specificity 0.83. The eight false positive cases included three carcinomas, three undiagnosed (including two cases of probable tuberculosis), one parapneumonic effusion, and one spontaneous pneumothorax. The positive predictive value was 0.85 and the negative 0.74.

Table 2 Studies reporting adenosine deaminase activity in pleural fluid

| Reference                  | No of patients | No with tuberculosis | Sensitivity | Specificity |
|----------------------------|----------------|----------------------|-------------|-------------|
| Piras <sup>9*</sup>        | 96             | 21                   | 1           | 1           |
| Maritz <sup>12</sup>       | 368            | 107                  | 0.93        | 0.82        |
| Ocana <sup>11*</sup>       | 221            | 48                   | 1           | 0.97        |
| Pettersen <sup>10</sup>    | 90             | 19                   | 1           | 0.79        |
| Niwa <sup>18</sup>         | 58             | 28                   | 0.79        | 0.87        |
| van Keimpema <sup>14</sup> | 95             | 5                    | 0.8         | 0.91        |
| Stranginka <sup>13</sup>   | 86             | 10                   | 1           | 0.87        |

\*These studies included patients with ascites.

## Discussion

We confirmed that radiometric mycobacterial culture systems provide faster culture results than conventional methods without lowering sensitivity. Disadvantages include cost (which is likely to limit their use in developing countries, where such methods are most needed), the need to crush clotted exudate (and tissue samples) before injection through the rubber stopper, and the fact that the BACTEC system appears to be less sensitive for culturing some species of mycobacteria other than *M tuberculosis*.<sup>8</sup>

A more important finding of this study is that bedside inoculation of pleural fluid substantially increases the yield of mycobacterial culture. We elected to use BACTEC bottles, but bedside inoculation into conventional culture media should be as effective. Improved results have been reported with bedside inoculation of pericardial fluid into Kirchner medium.<sup>7</sup>

The reason why bedside inoculation improves the yield is not clear. Perhaps during the period between aspiration and laboratory inoculation the intracellular organisms are damaged by continued lymphocyte mediated cytotoxicity. The other methods that may increase the yield of serosal fluid culture are either uncomfortable for the patient (multiple aspiration<sup>4</sup>) or awkward for the laboratory (culturing large volumes of aspirate<sup>6</sup>).

Our finding that pleural biopsy was diagnostic in all three patients with tuberculous peritonitis is contrary to the experience of others.<sup>2,17</sup> Pleural effusions are not uncommon in tuberculous peritonitis<sup>6</sup> and pleural biopsy is less invasive than laparoscopy, the current procedure of choice for diagnosing tuberculous ascites.<sup>6</sup>

Results of studies reporting the diagnostic value of raised adenosine deaminase activity in tuberculous pleural effusions are shown in table 2. Our specificity of 0.83 was similar to the specificity found in most studies.<sup>10,12-14,18</sup> Only two studies,<sup>14,18</sup> however, reported sensitivities as low as the 0.77 we found. As can be seen from the figure, lowering the cut off value of adenosine deaminase to achieve a sensitivity approaching 1 would reduce specificity to a very low level. On the basis of the results of this study we believe that adenosine deaminase has limited usefulness as a diagnostic test.

Until more specific rapid diagnostic tests become available, clinicians must rely on a combination of conventional methods. These should include histology and culture of closed pleural biopsy specimens, and bedside inoculation of pleural fluid into mycobacterial culture media. If radiometric culture methods are available the time taken for cultures to become positive is substantially reduced.

John Kruger and Di Barlow carried out the mycobacterial cultures with bacteriological advice from Dr D Roditi; Gina Joubert of the South African Medical Research Council did the statistical analysis; Dr MG Hanly reviewed the pleural biopsy histological specimens; and Dr JIG Strang suggested bedside inoculation. We thank Professor D Werner for permission to study patients having radiotherapy.

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