Diagnosis of Tuberculous Pleural Effusion by the Detection of Tuberculostearic Acid in Pleural Aspirates*

Wing Wai Yew, M.B., F.C.C.P.; Chiu Yeung Chan, Ph.D.;
Susan Yuk-lin Kwan, M.B., F.C.C.P.; Stu Wai Cheung, B.Sc.;
Gary L. French, M.D., F.R.C. Path

Detection of TBSA was attempted in pleural aspirates of 74 patients with tuberculous and 44 patients with nontuberculous pleural effusion by gas chromatography/mass spectrometry with selected ion monitoring. The results were disappointing with a test sensitivity of 67.6 percent and a specificity of 52.3 percent. In contrast, histologic examination of pleural biopsies gave a diagnostic sensitivity of 71.0 percent. Pleural biopsy remains a better investigational procedure for the diagnosis of tuberculous pleural effusion. (Chest 1991; 100:1261-63)

Tuberculostearic Acid Detection in Pleural Fluid

Each of the 118 patients had 10 ml of pleural fluid aspirated and stored at −40°C for TBSA detection in batches. In our experience this does not influence the result. The reason for carrying out the detection in batches was purely for economy of time. Except for three patients who developed effusion during treatment, fluid samples were obtained before commencement of chemotherapy. Tuberculostearic acid was extracted from 0.5 to 1.0 ml of pleural aspirate by saponification,9 derivatized by boron trichloride methanalysis and examined by gas chromatography/mass spectrometry as described previously.10,11 Methylated TBSA was detected by selected ion monitoring at mass/charge ratios of 312 and 167 at a retention time of 27.2 min.

Pleural Biopsy and Pleural Fluid Cytologic Examination

All 118 patients had fluid cytologic examination for malignant cells. Sixty-nine of 74 in the group with tuberculous effusion had pleural biopsies, whereas 34 of 44 in the group with nontuberculous effusion had the procedure performed.

Diagnostic Criteria of Studied Patients

The prospective criteria for diagnosis of tuberculous pleural effusion included (1) positive sputum smear or pleural fluid for AFB and/or (2) granulomatous pleuritis on histologic examination of tissue obtained by closed pleural biopsy. Fifty-seven patients met these criteria. The remaining 17 patients had a compatible clinical picture, chest radiographic appearance (with parenchymal lesions present in 11) and positive Mantoux reaction: all had ≥13 mm induration and 5 had ≥18 mm induration. Subsequently, five were found to have a culture positive for AFB in sputum or pleural fluid, leaving 12 patients with tuberculous effusion diagnosed solely on clinical and radiographic grounds who subsequently all responded definitely well to antituberculosis chemotherapy. Pleural biopsies showing granulomatous changes were assumed to be tuberculous in origin even in the absence of stainable AFB because (1) most had evidence of caseous necrosis and (2) other causes of granulomatous pleuritis such as sarcoidosis and fungal infection are extremely uncommon in Hong Kong. However, fungal culture of the fluid was not performed for any of these cases. (3) Most importantly, all effusions resolved following antituberculosis chemotherapy.

In the nontuberculous group, 41 patients had malignant pleural effusion confirmed cytologically and/or histologically; 39 had primary lesions in the lung; one had a secondary tumor but the primary source was unknown; one had a mesothelioma and one had

*From the Tuberculosis and Chest Unit, Grantham Hospital, Aberdeen, Hong Kong (Drs. Yew and Kwan); the Department of Microbiology, Chinese University of Hong Kong and Clinical Pathology Unit, Prince of Wales Hospital, Shatin, Hong Kong (Dr. Chan, Mr. Cheung and Dr. French). Dr. French is currently with the Department of Microbiology, United Medical and Dental Schools, Guy's Hospital, London, England.

Reprint requests: Dr. Yeung, Department of Microbiology, Chinese University of Hong Kong Prince of Wales Hospital, Shatin, NT, Hong Kong.

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H histologic examination of pleural biopsy specimens has been accepted as the most satisfactory method for diagnosing tuberculous pleural effusion.1 The diagnostic yield may be further increased by culture of the biopsy and bacteriologic examination of sputum and pleural fluid.1,2 Pleural fluid concentrations of adenosine deaminase3 and lysozyme4 and the detection of mycobacterial antigens and antibodies5,6 and TBSA also may be helpful. Tuberculostearic acid is a structural component of mycobacteria7 and other members of the Actinomycetales8,9 and its presence in clinical specimens may be diagnostic of pulmonary tuberculosis10-12 and tuberculous meningitis.13,14 In this study we investigated the usefulness of TBSA for the rapid diagnosis of tuberculous pleural effusion.

Patients and Methods

We studied 74 patients with tuberculous and 44 patients with nontuberculous pleural effusion admitted into the Tuberculosis and Chest Unit of Grantham Hospital during the latter six months of 1988 and the first eight months of 1989.

Sputum and Pleural Fluid Bacteriologic Examination

All 118 patients had sputum smear and pleural fluid smear examined for AFB and auramine-rhodamine stain. Each patient also had sputum and pleural fluid collected for culture of AFB. For sputum culture, the modified Petroff method using NaOH decontamination and Lowenstein-Jensen medium was used. The pleural fluid sample was centrifuged at 3,000 g for 20 min and 2 loopful of deposit was directly inoculated onto two Lowenstein-Jensen media. The rest of the deposit was handled as for sputum culture.
Table 1 — Results of Investigations in 74 Cases of Tuberculous Pleural Effusion

<table>
<thead>
<tr>
<th>TBSA in Pleural Fluid</th>
<th>Pleural Biopsy</th>
<th>Sputum Smear for AFB</th>
<th>Sputum Culture for AFB</th>
<th>Pleural Fluid Smear for AFB</th>
<th>Pleural Fluid Culture for AFB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Granuloma</td>
<td>Nonspecific</td>
<td>Not Done</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>34 (16 ‡)</td>
<td>13*</td>
<td>3</td>
<td>7</td>
<td>43</td>
</tr>
<tr>
<td>n = 50*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>–</td>
<td>15 (7 ‡)</td>
<td>7 +</td>
<td>2</td>
<td>2</td>
<td>22</td>
</tr>
<tr>
<td>n = 24†</td>
<td></td>
<td></td>
<td></td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td>49 (23 ‡)</td>
<td>20</td>
<td>5</td>
<td>9</td>
<td>65</td>
</tr>
<tr>
<td>n = 74</td>
<td></td>
<td></td>
<td></td>
<td>32</td>
<td>42</td>
</tr>
</tbody>
</table>

*Eight cases diagnosed on clinical grounds.
†Four cases diagnosed on clinical grounds.
‡Numbers in parentheses represent stainable AFB present on histologic section.

A malignant thymoma. The other three patients had lupus pneumonitis (one) and paraneoplastic effusions (two). All these patients had a culture negative for AFB in sputum and fluid except one patient with malignant effusion and concomitant positive sputum culture. For those who had pleural biopsies, none showed granulomatous pleuritis.

RESULTS

Of the 74 patients with tuberculous effusion, only 50 patients (67.6 percent) had detectable TBSA in their pleural aspirates (Table 1). Twenty-one of 44 specimens (47.7 percent) from patients with nontuberculous effusion also contained TBSA (these included an aspirate from one patient who yielded *Mycobacterium tuberculosis* from sputum culture). The sensitivity of TBSA detection was thus 67.6 percent (50 of 74) (Table 1) and the specificity, 52.3 percent (23 of 44). The sensitivity of pleural biopsy alone was 71.0 percent (49 out of 69 tested [Table 1]) with a specificity of near 100 percent. The latter was based on virtual lack of false-positive cases exemplified earlier in the "Patients and Methods" section herein.

Acid-fast bacilli were seen in direct microscopy of only one third (23 of 69) of the pleural biopsies, 12 percent (9 of 74) of the sputum specimens and less than 2 percent (1 of 74) of the pleural fluid aspirates (Table 1). The biopsies were not cultured, but sputum and pleural fluid specimens yielded *M tuberculosis* in 43 percent (32 of 74) and 16 percent (12 of 74) of cases respectively (Table 1). The combined diagnostic sensitivity of tests for bacteriology of sputum and pleural fluid and histology of pleural biopsy was 83.8 percent (62 of 74). Table 2 depicts the correlation of bacteriology status of sputum, pleural fluid and granulomatous pleuritis on histology in patients who had positive TBSA results in fluid assessment. Data on 37 patients were presented; three did not, however, have pleural biopsies. Five other patients with detectable TBSA in pleural fluid had nonspecific pleuritis: one had positive fluid and sputum culture, two had positive sputum culture, one had positive fluid culture and one had positive fluid smear.

**DISCUSSION**

Pleural biopsy is widely reported to be the single best test for the diagnosis of tuberculous pleuritis. Histologic examination alone is successful in 50 to 80 percent of cases\(^{15-20}\) and biopsy specimens yield positive cultures of *M tuberculosis* in 55 to 80 percent.\(^{16-20}\) Thus, with combined histology and culture of pleural biopsies, a diagnosis can be established in 90 to 95 percent of patients.\(^{17,19,20}\) In the present study, 71 percent of cases were diagnosed by histology alone. Pleural biopsies were not routinely cultured for *M tuberculosis*, but 23 of 49 (46.9 percent) of granulomatous specimens contained stainable AFB.

Microscopy and culture of sputum seldom reveal AFB in patients with tuberculous effusion.\(^{17,20-22}\) Acid-fast bacilli are reported to be seen in less than 10 percent of pleural aspirates, but 25 to 75 percent of specimens yield *M tuberculosis* on culture.\(^{17,19,20,22,23}\) In our study, sputum microscopy was diagnostic in 9 of 74 (12.2 percent) and sputum culture in 32 of 74 (43.2 percent) cases (Table 1). Microscopy revealed AFB in only 1 of 74 (1.4 percent) aspirates, and only 12 of 74 (16.2 percent) were culture-positive for *M tuberculosis* (Table 1). Our relatively low recovery rate of *M tuberculosis* from pleural aspirates may have been because we used flat-bottomed containers for...

Table 2 — Correlation of Sputum, Pleural Fluid Culture Status with Granulomatous Pleuritis on Histology and Positive TBSA in Fluid

<table>
<thead>
<tr>
<th>Sputum Culture Status for AFB</th>
<th>Pleural Fluid Culture Status for AFB</th>
<th>Pleural Biopsy: Granulomatous Pleuritis</th>
<th>Total No. Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>+</td>
<td>+ Not done</td>
<td>+</td>
<td>1</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
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<td>2</td>
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<tr>
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<td>+</td>
<td>+</td>
<td>13</td>
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<td>37</td>
</tr>
</tbody>
</table>

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centrifugation, made cultures only on Lowenstein-Jensen medium, and processed only 20 ml or less of pleural fluid in each case.

Tuberculous pleural effusions may result from the development of a "hypersensitivity" reaction when a subpleural focus of *M tuberculosis* ruptures into the pleural space.24-28 This would explain the disappointing microbiological results in this condition, the frequent presence of stainable AFB in pleural biopsies, the development of pleural effusion in patients already on chemotherapy, and the usefulness of corticosteroids in treatment.27,28 In communities such as Hong Kong where the incidence of tuberculosis is still high (about 130 notifications annually per 100,000 population currently), hypersensitivity reactions to tuberculo-protein are probably common.

The detection of TBSA in pleural aspirate was not a very useful diagnostic test, with many false-positive and false-negative results in both tuberculous patients and controls. The low sensitivity (67.6 percent) may have been related to the small sample volume tested and the possible infrequency of AFB in acute pleural effusions as explained earlier. The cause for the low specificity (52.3 percent) seems more perplexing and invites contention. One possibility might be related to release of TBSA from old subvisceral pleural foci. This was suggested by higher incidence of Chon's foci in chest radiographs of patients in the false-positive group when compared with the true-negative group.

Although the detection of TBSA in CSF, sputum and bronchial washings has been used successfully for the diagnosis of tuberculous meningitis and pulmonary tuberculosis,10-14 in this study, the detection of TBSA in pleural aspirates was not found to be helpful in the diagnosis of tuberculous pleural effusion. The detection of TBSA in pleural biopsies might be more useful as a rapid diagnostic technique and is currently being evaluated in a separate sequential study.

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