The Optimal Number of Pleural Biopsy Specimens for a Diagnosis of Tuberculous Pleurisy

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**Study objectives:** To determine the optimal number of pleural biopsy (PLBX) specimens for a diagnosis of tuberculous pleurisy.

**Design:** Retrospective review.

**Setting:** County hospital.

**Methods:** We reviewed all percutaneous needle biopsy specimens of the parietal pleura in 30 patients who had tuberculous pleurisy. Data are reported as mean±SEM and statistical comparisons are done with the Mann-Whitney test. We accepted \( p < 0.05 \) as statistically significant.

**Results:** The number of biopsy specimens obtained from each patient ranged from 4 to 10 with 1 sample submitted for mycobacterial culture and the rest submitted for histologic study. Sixty percent of patients had pleural cultures positive for *Mycobacterium tuberculosis* and 80% had diagnostic histology. Overall pleural biopsy sensitivity (histology and culture) for tuberculous pleurisy was 87%. On average, 40.4%±4.7% of all PLBX specimens contained pleura. Diagnostic PLBX procedures compared to false-negative procedures produced more tissue specimens (7.1±0.3 vs 4.8±0.5, \( p = 0.005 \)) containing more pleural specimens (2.4±0.2 vs 0.8±0.5, \( p = 0.01 \)). If only PLBX procedures yielding more than six tissue specimens (n=18) or more than two pleural specimens (n=12) were analyzed, then the diagnostic sensitivity of PLBX for pleural tuberculosis was 100%. There seemed to be a direct relationship between the sensitivity of PLBX and the number of specimens submitted.

**Conclusions:** The sensitivity of percutaneous needle biopsy for diagnosis of tuberculous pleurisy is highest when more than six specimens are obtained which, on average, contain more than two specimens of parietal pleura. There are no conclusive data indicating how many tissue specimens to submit for mycobacterial culture, but one specimen seems sufficient. *(CHEST 1997; 112:702-06)*

**Key words:** pleural biopsy; pleural tuberculosis; tuberculous pleurisy

**Abbreviations:** AFB=acid-fast bacilli; MTB=*
Mycobacterium tuberculosis*; PLBX=pleural biopsy; PLTB=pleural tuberculosis; PPD=purified protein derivative; TB=tuberculosis; TBPL=tuberculous pleurisy

Percutaneous needle biopsy of the parietal pleura (PLBX) was first described in 1955 and has subsequently proved helpful in the diagnosis of tuberculous pleurisy (TBPL). The reported diagnostic sensitivity of PLBX for TBPL ranges from 60 to 95%, with best sensitivity (80 to 95%) when pleural histologic study is combined with PLBX culture. However, there is no agreement in the literature and no data regarding the optimal number of PLBX specimens required to yield the highest diagnostic sensitivity for TBPL. We, therefore, retrospectively reviewed our results of PLBX for the diagnosis of TBPL.

**Materials and Methods**

We retrospectively (1988 to 1994) and prospectively (1995 to 1996) reviewed all percutaneous needle biopsy specimens of the parietal pleura done by the Division of Respiratory and Critical Care Medicine at a 350-bed county hospital. PLBX was done exclusively in patients with exudative, radiographically demonstrable pleural effusions. Specimens of parietal pleura were obtained with the Cope needle* and Abrams needles as originally described or with the modified Abrams needle technique as described by our group. The modified Abrams technique simply allows suctioning of each specimen into a syringe placed at the hub of the needle without the necessity of complete
needle withdrawal after each sample. We reviewed all sputum and pleural fluid specimens and classified pleural fluid as exudate or transudate according to standard criteria. Routinely, one specimen was cultured for mycobacteria in both liquid (Middlebrook 7H12) and solid (Lowenstein-Jensen) media and all other tissue specimens obtained by PLBX were submitted for histologic study and appropriate special stains.

TBPL was diagnosed if the patient satisfied any one of the following criteria as described previously: (1) Mycobacteria tuberculosis (MTB) grew from sputum, pleural fluid, pleural tissue, or culture of any other body site (eg, cervical lymph node); (2) the PLBX specimen showed granulomata and acid-fast bacilli (AFB); and (3) the patient had a positive purified protein derivative (PPD) skin test (≥10 mm) and a PLBX specimen showed granulomata.

All patients with a diagnosis of TBPL, who had tissue available, had PLBX material retrieved and reexamined to determine the number of specimens containing pleura, the number of pleural specimens that contained granulomata, and the number of specimens that had demonstrable AFB. All cultures of PLBX specimens, sputum, pleural fluid, and other material were reviewed. All patients with a diagnosis of TBPL who had a false-negative PLBX had available tissue specimens completely sectioned and resubmitted for histologic study.

We recorded all data on a standard worksheet and all numeric data are reported as mean±SEM. The number of pleural specimens and the percentage of biopsy specimens containing pleura were determined histologically. Statistical comparisons of continuous data were done with the Mann-Whitney test and comparisons of categorical data were done with Fisher's Exact Test. We accepted a p<0.05 as statistically significant.

RESULTS

There were 56 PLBX procedures done in 55 patients over an 8-year period and results from 47 of these patients have been reported, in part, previously. A total of 31 patients were proven to have TBPL, of whom 30 had PLBX tissue available for reexamination. Two patients with a negative PPD were treated empirically for tuberculosis (TB) after a PLBX specimen showed granulomata without diagnostic AFB on smear or culture. There were 22 patients who were determined not to have pleural TB (PLTB), and 15 of 22 had alternate diagnoses based on a constellation of signs, symptoms, laboratory data, and empiric response to therapy. Seven of the 22 patients without a diagnosis of PLTB had no proven or presumptive alternative diagnosis to explain their pleural effusions. One of these seven patients with idiopathic exudative pleural effusions had 41% eosinophils in the pleural fluid, which is not typical for PLTB. These seven patients were followed up for 1 month to 6 years without any evidence of active TB.

The 30 patients with PLTB who had pleural tissue available for analysis constituted the study group. Twenty-five of the 30 patients have been described, in part, in a previous publication. There were 19 men and 11 women with an average age of 37.5±3.0 years (range, 19 to 76 years). The total number of biopsy samples obtained was distributed as follows: four (three patients); five (four patients); six (five patients); seven (nine patients); eight (five patients); nine (two patients); and ten (two patients). Figure 1 shows the breakdown of histologic and culture results in all 30 patients who had a diagnosis of PLTB. The sensitivity of PLBX for PLTB was 87% (26/30), using a combination of pleural histologic study and PLBX culture. There were two patients whose PLBX did not obtain pleural tissue; therefore, the sensitivity of PLBX for PLTB was 93% (26/28) in patients who had pleura for examination. Sixty percent (18/30) of all patients with PLTB and 69% (18/26) of the patients with a diagnostic PLBX had a positive PLBX culture for MTB; 80% (24/30) had diagnostic histology. All patients who had demonstrable AFB in tissue (n=9) had granulomata seen on pleural histologic section as well. Only 38% (9/24) of patients with demonstrated granulomata had AFB seen in histologic sections. Eighty-nine percent (16/18) of pa-

![Figure 1. Diagnostic findings for 30 patients with proven PLTB. CX = culture; PL FL = pleural fluid.](image-url)
patients with a positive PLBX culture for MTb had diagnostic pleural histologic features. There were four patients (including the two whose PLBX did not obtain pleura) who had a false-negative PLBX specimen for PLTB and the diagnosis of PLTB was established by growth of MTb from sputum culture in three patients and from pleural fluid culture in one patient. Blocks of tissue from the four patients with a false-negative PLBX specimen were retrieved and were completely sectioned, but no further diagnostic information was gained.

On average, 40.4%±4.7% of all tissue specimens obtained by PLBX and submitted for histologic study contained pleura. Although 43.3%±4.8% of tissue specimens obtained by diagnostic PLBX contained pleura compared with 21.7%±15.7% for false-negative PLBX, this difference was not significant (p=0.1). All four patients who had false-negative PLBX specimens were women (p=0.01) who were older (51.3±5.6 years) than those who had diagnostic PLBX specimens (35.4±3.2 years, p=0.04). Figure 2 shows that more tissue specimens (7.1±0.3 vs 4.8±0.5, p=0.005) containing more pleural specimens (2.4±0.2 vs 0.8±0.5, p=0.01) were obtained from diagnostic compared with false-negative PLBX procedures. If only PLBX procedures yielding more than six tissue specimens (n=18) or more than two pleural specimens (n=12) were analyzed, then the diagnostic sensitivity of PLBX for PLTB was 100%. There seemed to be a direct relationship between the sensitivity of PLBX and the number of specimens submitted, as shown in Figure 3.

**DISCUSSION**

Initial studies of percutaneous needle PLBX used tissue only for histologic study and sensitivity for TBPL ranged from 40 to 80%. Later workers discovered the utility of tissue culture for MTb as an adjunct to histologic study, which may boost diagnostic sensitivity to 95%.

There is uncertainty regarding the optimum number of total PLBX specimens and the number or fraction that should be cultured. Methods from original research, with attendant overall diagnostic sensitivities for PLTB, and suggestions from review articles are indicated in Table 1. To our knowledge, there is no study that affords any data on which to base an informed opinion. One group performed repeated PLBxs until “three reasonable biopsies” were obtained and another group suggests “multiple

![Figure 2](image_url) Patients with a positive PLBX specimen for PLTB (n=26) compared with patients who had a false-negative PLBX specimen for PLTB (n=4) had more PLBX specimens submitted (7.1±0.3 vs 4.8±0.5, p=0.005) which contained more pleural specimens (2.4±0.2 vs 0.8±0.5, p=0.01).

![Figure 3](image_url) “Dose-response” type of direct relationship between the number of PLBX specimens submitted and the diagnostic sensitivity for PLTB. The number of patients in each category is as follows: four specimens (three patients); five specimens (four patients); six specimens (five patients); and more than six specimens (18 patients).

<table>
<thead>
<tr>
<th>Source</th>
<th>PLBX Specimens</th>
<th>Specimens for Culture</th>
<th>Sensitivity for PLTB, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iles and Ogilvie** (R)</td>
<td>2</td>
<td>1</td>
<td>N/A</td>
</tr>
<tr>
<td>Wichelhausen et al** (R)</td>
<td>2</td>
<td>1</td>
<td>N/A</td>
</tr>
<tr>
<td>Levine et al**</td>
<td>2</td>
<td>1</td>
<td>95</td>
</tr>
<tr>
<td>Scurro et al**</td>
<td>2</td>
<td>1</td>
<td>71</td>
</tr>
<tr>
<td>Bueno et al**</td>
<td>≥2</td>
<td>1</td>
<td>84</td>
</tr>
<tr>
<td>Ball* (R)</td>
<td>3</td>
<td>1</td>
<td>N/A</td>
</tr>
<tr>
<td>Poe et al**</td>
<td>≥3</td>
<td>≥1</td>
<td>90</td>
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<td>Scharrer and McClement**</td>
<td>3 to 4</td>
<td>50%</td>
<td>80</td>
</tr>
<tr>
<td>Tomlinson and Sahn** (R)</td>
<td>3 to 5</td>
<td>50%</td>
<td>N/A</td>
</tr>
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<td>Light* (R)</td>
<td>≥4</td>
<td>1</td>
<td>N/A</td>
</tr>
<tr>
<td>Silver and Bone** (R)</td>
<td>4 to 5</td>
<td>1</td>
<td>N/A</td>
</tr>
<tr>
<td>Walsh et al**</td>
<td>6</td>
<td>1</td>
<td>N/A</td>
</tr>
</tbody>
</table>

*The number of PLBX histology and culture specimens suggested by the medical literature. R=review article without primary data; N/A=not available.
specimens. Sahn\textsuperscript{3} states that “a paucity of data is available concerning the optimum number of biopsies at a single site.”

We retrospectively reviewed our data to determine how many total PLBX specimens were needed to yield the highest diagnostic sensitivity for PLTB. Our routine clinical practice, which is consistent with that of other groups,\textsuperscript{2,4,8,22,23-27} was to submit only one PLBX specimen for culture. We found that our overall diagnostic sensitivity of PLBX for PLTB (87%) was well within the range reported in the literature\textsuperscript{3} and that our sensitivity increased to 93% if we included only PLBX procedures that actually yielded pleural tissue. We have shown that PLBX procedures diagnostic for PLTB obtain significantly more tissue specimens that contain more specimens of pleura when compared with false-negative procedures. Our sensitivity was 100% if more than six tissue specimens were obtained. We believe that the diagnostic sensitivity of PLBX for PLTB depends on whether the operator obtains pleural tissue as opposed to fat and intercostal muscle for analysis. In support of this theory, we found that about half of our PLBX specimens contained pleura and that the same 100% diagnostic sensitivity exists if the operator obtains more than two specimens that contain pleural tissue. Therefore, we found that more than six total PLBX specimens are necessary for optimal diagnostic sensitivity because only about half of these contain pleura and more than two pleural specimens are needed to maximize diagnostic sensitivity. We found an increase in diagnostic sensitivity with increasing number of PLBX specimens in a direct “dose-response” type relationship from n=4 to n=6 samples submitted for analysis (Fig 3). The relatively low diagnostic sensitivity for n=4 and n=5 samples may be due to the small sample size for these categories. However, the dose-response relationship does indicate that the diagnostic sensitivity can be increased by increasing the number of specimens obtained and that greater than six specimens may provide 100% diagnostic sensitivity. Retrieval of more than six pleural samples to maximize overall diagnostic sensitivity should not be precluded by presumed limitations of the percutaneous needle biopsy technique. Removal of 10 specimens at a single intercostal site has been reported.\textsuperscript{28} We\textsuperscript{17} have shown that the acquisition of multiple pleural samples at a single intercostal site can be facilitated by a modification of Abrams’ original technique. The modified Abrams needle method allows acquisition of biopsy samples without the necessity of needle removal after each specimen. We agree with Mun-gall et al\textsuperscript{29} that further sectioning of false-negative tissue specimens for histologic study is unrewarding.

A separate analysis of the four patients with a false-negative PLBX specimen showed that they were all older (p=0.04) women (p=0.01). These PLBX procedures yielded a smaller number of specimens (p=0.005) with no difference in the percentage of specimens containing pleural tissue (p=0.1) when compared with patients who had a diagnostic PLBX. It is possible that the lack of a demonstrated significant difference in percentage of specimens containing pleura is due to a small number of false-negative PLBX patients (type II or beta error). We calculate that we would need a total of 54 patients in our study, assuming an overall diagnostic sensitivity of 87% (47 diagnostic and 7 false-negative PLBX) with unchanged means/SEM, to obtain a significant difference (p=0.04) in percentage of pleural specimens. This would require nearly doubling the size of our study population. It is possible that fewer biopsy specimens were obtained in the false-negative group of older women because of some technical problem or because they did not tolerate the procedure very well. Despite the possible problems of PLBX in the false-negative group, our main observations are unchanged regarding the optimal number of PLBX specimens.

The optimal number or fraction of total PLBX specimens that should be cultured is still unclear and our data do not allow us to directly answer this question. The literature indicates that one specimen to 50% of all specimens should be cultured for MTb (Table 1). Many recommendations are not based on any referenced data. One group indicates that PLBX tissue culture is 68% sensitive for confirmed cases of PLTB if 50% of all tissue specimens are submitted for culture.\textsuperscript{9} This compares with our culture sensitivity of 60% when we submit only one specimen for each patient. We, and Poe et al\textsuperscript{11} found that PLBX culture is 67 to 70% sensitive in patients with a diagnostic PLBX specimen for PLTB. We agree with other workers\textsuperscript{8,9,22,26,29} that a negative histologic analysis does not preclude a positive culture for MTb by other specimens obtained at the same biopsy procedure. Presumably, the positive tissue cultures for MTb in this situation are due to submission of all specimens containing pleura for culture, leaving none for histologic analysis. Two of our four false-negative procedures did not yield any pleura for histologic analysis and therefore would not likely have provided positive cultures even if more of the obtained specimens were cultured. Two of our four false-negative procedures yielded one and two specimens of pleura for histologic analysis, which showed no granulomata. This histologically negative pleural tissue might have grown MTb in culture. Therefore, we estimate that PLBX missed, at most, two cases of PLTB in our series because of our use of only one tissue specimen for culture. It is possible that use of
50% of all PLBX specimens for culture might have precluded detection of granulomata in those samples without an increase in culture sensitivity. There may be an increased diagnostic sensitivity provided by liquid vs solid media for culture of MTb in PLBX specimens. We used both types of culture media in our patients to maximize our culture sensitivity. If one must choose between a diagnosis based on histology alone and one based on culture alone, various advantages and disadvantages emerge. A diagnosis based on histology alone provides an immediate support for therapy, but provides no data on drug sensitivity of the MTb organism. A diagnosis based on positive tissue culture alone may delay empiric therapy but provides drug susceptibility data later. The use of pleural fluid adenosine deaminase levels and polymerase chain reaction technology may provide immediate support for empiric therapy while cultures are growing.

We examined the four patients in our group who had a diagnosis of PLTB based only on a positive PPD skin test and demonstrable pleural granulomata without AFB seen on PLBX specimen and without MTb on PLBX tissue culture. Three of these four patients had confirmatory cultures of sputum (n=2) and pleural fluid (n=1) that yielded MTb. Therefore, only one patient, in this study, was determined to have PLTB who had neither AFB seen on histologic specimen nor detected in tissue culture.

In conclusion, the sensitivity of closed needle biopsy for diagnosis of PLTB is directly related to number of samples submitted. The diagnostic sensitivity of PLBX for PLTB is highest when more than six specimens are obtained which can be expected to contain, on average, more than two samples of parietal pleura. There are no conclusive data indicating how many tissue samples to submit for MTb culture, but one specimen seems sufficient. There seems to be no advantage to resecting pleural tissue obtained by percutaneous needle biopsy if initial histologic results are nondiagnostic for PLTB.

References

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