Diagnosis of Pneumocystis carinii Pneumonia*  
Roentgenographic-Pathologic Correlates Based on Fiberotic Bronchoscopy Specimens from Patients with the Acquired Immunodeficiency Syndrome  
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We analyzed the diagnosis of Pneumocystis carinii pneumonia by fiberoptic bronchoscopy in a large series of patients with the acquired immunodeficiency syndrome (AIDS). Transbronchial biopsy fragments, as opposed to endobronchial specimens, were found to have high diagnostic value. Their optimal number for diagnosis was determined by a simple statistical principle. It varied from a minimum of two in cases of severe pneumonia to a maximum of four when roentgenographic manifestations were altogether absent. The diagnostic yield of the transbronchial biopsy alone was 97 percent and that of "touch" preparations of the biopsies 88 percent; when both techniques were combined, the accuracy rose to 98 percent. By comparison, bronchial "washings" and "brushings" had a much lower yield, 59 percent and 57 percent, respectively. Because their diagnostic contribution was negligible, we conclude that the latter two procedures represent an unnecessary expense and waste of technical and professional effort.

Pneumocystis carinii pneumonia (PCP) is a major manifestation of the acquired immunodeficiency syndrome (AIDS) for which therapy is currently available. Early and prompt diagnosis of this pulmonary infection is therefore a matter of great practical importance, particularly in medical centers serving large metropolitan areas where AIDS is more prevalent.

Although PCP can be suspected with a high degree of confidence on clinical and roentgenographic grounds, the ultimate diagnosis is pathologic and rests on the demonstration of the protozoa in the pulmonary lesions or secretions thereof. Fiberoptic bronchoscopy (FOB) is a highly reliable and relatively simple technique to obtain diagnostic material. Recently, the advantages of bronchoalveolar lavage (BAL) for the same purpose in patients with a bleeding diathesis have been pointed out.

We presently report our experience in the pathologic diagnosis of PCP by FOB in a large series of patients with AIDS. Our pathologic findings have been correlated with the roentgenographic manifestations of the disease at the time of FOB. Major aims of the study were to clarify important methodologic aspects, namely: (a) the quality and optimal number of biopsy fragments required for the diagnosis; (b) to estimate the severity of infection in the tissue material by a semiquantitative method, and to correlate this severity with; (c) the diagnostic yield and the severity of the infection in "touch" preparations of the biopsies (TP), bronchial "washings" (BW), and bronchial "brushings" (BB).

Materials and Methods

The 95 patients on whom the present study is based were seen at Jackson Memorial Hospital of Miami in the 1981 to 1984 period. They all conformed to the definition of AIDS as set forth by the Center for Disease Control, Atlanta, Georgia. Eighty-two patients were men (86 percent) and 13 women (14 percent). The ages ranged from 21 to 60 years (median = 32). Homosexuals accounted for 47 percent of the patients and Haitians for 37 percent. Intravenous drug abusers comprised 11 percent and transfusion-related illness 2 percent. In 3 percent of the cases (two patients), there were no known risk factors, although there was a question of blood transfusions in the past.

Roentgenographic Observations

A roentgenographic estimate of the severity of the pneumonia was obtained in 81 patients in whom x-ray films at the time of FOB were available for review. The grading of the chest roentgenograms was exclusively the responsibility of one of us (SAO) working independently and unaware of the histopathologic observations. Four grades of severity were defined: 0 (normal roentgenogram), 1 (mild), 2 (moderate), and 3 (severe). Grade 1 was characterized by purely interstitial finely reticulor or reticulonodular infiltrates distributed in a more or less uniform manner throughout both lungs. In grade 2, in addition to the interstitial changes, there were one or more patchy areas of alveolar consolidation. In grade 3 infection, there were many confluent areas of alveolar consolidation and the appearance was that of the adult respiratory distress syndrome (ARDS). Unusual roentgenographic findings were noted in some patients and they included pleural effusions, cavitation of the parenchymal infiltrates, and hilar-
mediastinal lymph node enlargement. Because these findings were later found to be unrelated to PCP they will not be discussed further.

Pathologic Observations

Bronchoscopic biopsy specimens fixed in 10 percent buffered formalin were received together with air-dried TP of the biopsy fragments, BW, and BB. The biopsy specimens were processed by routine histologic techniques and cut at a thickness of 5 μm. Two to three sections were stained with hematoxylin and eosin (H&E).

Recuts were stained with the Grocott's modification of Gomori's methenamine silver (GMS) stain for the demonstration of Pneumocystis carinii organisms. Other special stains routinely performed included Ziehl-Neelsen and Kinyoun for acid-fast bacteria, Brown-Brenn for pyogenic organisms, and Wright-Giemsa. The TP, BW (after ultracentrifugation), and BB were stained in a similar manner and the results were available 12 to 24 hours in advance of the biopsy slides. Usually the BW included oropharyngeal secretions since the specimen trap was attached to the suction from the beginning of the procedure. As a rule, from 10 to 20 ml of sterile saline solution were administered to wash before terminating the procedure.

Number of Fragments: The total number of fragments in the biopsy specimen was determined by examining the glass slides with the naked eye followed by confirmation under the microscope. The "typical" specimen obtained in our institution utilizing a bronchoscope (Olympus BF2) and forceps (Olympus BF-19C) is an approximately round, square, or rectangular tissue fragment measuring on the average 1 mm in largest transversal dimension (the size of a pinhead). Occasionally, however, fragments measuring 2 or even 3 mm can be obtained with this instrument. The amount of tissue available for diagnosis, i.e., the number as well as the size of the biopsy fragments, was considered a matter of crucial importance in this study; therefore, it was decided to carefully compare these larger fragments with "typical" ones under the scanning power of the microscope and to count them, accordingly, as two or three fragments depending on their size. Only cases in whom all the biopsy fragments came from the same area were included, the latter usually being the lower lobes. Fragments composed solely of bronchial mucosa with or without cartilage and containing no alveolar tissue were found of little diagnostic value and were disregarded. Also not counted were fragments composed of blood cells, fibrin, mucus, and bronchial epithelial cells.

The optimal number of transbronchial biopsy (TBB) fragments needed to establish a diagnosis of PCP was calculated by a probability theorem, according to which:

\[ P = 1 - (1 - p)^n \]

where

\[ P \]

represents the probability of obtaining a positive specimen in \( n \) fragments, \( n \) representing the minimal number of fragments required to achieve \( P \). The estimated probability of obtaining positive biopsy fragment in our sample material is represented by \( p \) (Table 1). Values for \( n \) can be solved from this formula for different probability rates.

Severity of Infection in TBB: The severity of infection in the biopsy specimens was rated semiquantitatively in four grades: grade 0 (negative), grade 1 (mild), grade 2 (moderate), and grade 3 (severe). In grade 1 infection, the alveolar tissue was fundamentally normal except for a nonspecific alveolitis, i.e., increased numbers of mononuclear cells (lymphocytes, plasma cells) in the alveolar septae. Occasionally, and on high power magnification, bits of proteinaceous foamy exudate could be detected attached to alveolar walls. The GMS stains revealed few organisms in small groups of less than ten cysts, or in rows attached to the alveoli (Fig 1).

In grade 2 infection, in addition to the interstitial changes, a few small and isolated masses of foamy exudate containing up to 50 cysts were seen in alveoli. They could only be recognized at high magnification and on close scrutiny of foci of atelectasis. Organisms were readily identifiable by GMS stains (Fig 2).

Grade 3 infection was characterized by large, confluent masses of foamy exudate that filled and distended the great majority of air spaces. The GMS stains revealed numerous cysts, in the hundreds or thousands, within the exudates (Fig 3). Because the severity of the infection could vary from fragment to fragment or within an

<table>
<thead>
<tr>
<th>Roentgenographic Involvement (Grade)</th>
<th>No. Patients</th>
<th>NP/TF*</th>
<th>Probability</th>
<th>95%</th>
<th>99%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
<td>13/17</td>
<td>77%</td>
<td>n = 3</td>
<td>n = 4</td>
</tr>
<tr>
<td>1</td>
<td>27</td>
<td>83/97</td>
<td>86%</td>
<td>n = 2</td>
<td>n = 3</td>
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<tr>
<td>2</td>
<td>35</td>
<td>120/130</td>
<td>92%</td>
<td>n = 2</td>
<td>n = 2</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>37/38</td>
<td>97%</td>
<td>n = 1</td>
<td>n = 2</td>
</tr>
<tr>
<td>TOTAL</td>
<td>81</td>
<td>233/292</td>
<td>90%</td>
<td></td>
<td></td>
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</tbody>
</table>

*Number of positive fragments (NP) over total number of fragments (TF) in transbronchial biopsies.

†Estimated probability of obtaining a positive biopsy fragment.

Figure 1. Microscopic appearance of grade 1 PCP. Note the basic normalcy of lung parenchyma except for an increase in the number of mononuclear cells in alveolar septae and luminae ("mild nonspecific alveolitis"). No intra-alveolar exudate is recognizable. The inset shows few organisms attached to alveolar septae (H&E, original magnification ×190; inset: GMS, original magnification ×800).
individual fragment, we chose to rate the case by the most severely involved area.

"Touch" Preparations: The TPs of the biopsy fragments were largely composed of bronchial epithelial cells admixed with mucus, alveolar macrophages, red blood cells, and inflammatory cells. The slides were read systematically in a driving stage under a convenient scanning power of 200 x (eyepiece 10 x, objective 20 x) followed by closer scrutiny of suspicious structures at higher magnifications.

The TPs were rated as grade 0 (negative), grade 1 (mild), grade 2 (moderate), or grade 3 (severe) depending on the number of organisms found and their distribution. Grade 1 TP was characterized by rare or occasional organisms in an isolated manner, no more than ten per slide. Grade 2 TP was characterized by frequent (10 to 50) organisms per slide usually isolated and rarely in small clusters of less than five to ten organisms. In grade 3 TP, there were many readily identifiable organisms characteristically in clusters corresponding to the intra-alveolar foamy masses in the biopsy specimens. The results of TPs were compared to those of the corresponding TBB according to Kendall's tau b test for statistical significance.

Bronchial "Brushings" and "Washings." The severity of infection in BB and BW was rated in a manner similar to that for TP and the results compared to the corresponding TBB according to Kendall's tau b test.

RESULTS

Roentgenologic Findings

At the time of FOB, five patients (6 percent) had normal roentgenograms (grade 0 infection), 27 patients had grade 1 infection (33 percent), 35 patients had grade 2 infection (43 percent), and 14 patients had grade 3 infection (18 percent).

Pathologic Observations

The diagnosis of PCP was established in 95 patients by FOB (100 percent). In 92 patients, the TBB was positive (97 percent). Two other patients had negative TBB, but TPs were positive. The remaining patient had both negative TBB and TP but organisms were present in the BW (Table 2).

Number of Fragments in the Biopsy: The total number of TBB fragments in 81 patients with available chest roentgenograms was 282, of which 253 were positive for PCP (90 percent). A breakdown of the material according to radiologic grade of infection is

Figure 2. Microscopic appearance of grade 2 PCP in a patient with emphysematous changes. Few isolated masses of intraalveolar proteinaceous exudate (arrow) are noted in addition to the nonspecific alveolitis. Clusters of organisms are demonstrated in the inset (H&E, original magnification x 190; inset: GMS, original magnification x 800).

Figure 3. Microscopic appearance of grade 3 PCP. Large and confluent masses of foamy exudate fill and distort alveolar spaces. Collections of macrophages and occasional giant cells are present within the exudate. A multitude of micro-organisms is shown in the inset. (H&E, original magnification x 190; inset: GMS, original magnification x 800).
presented in Table 1. Also presented in Table 1 are the different values of n for probability rates of 95 and 99 percent.

Severity of Infection in the Transbronchial Biopsy (TBB): In 95 patients with TBB, there were 69 cases with grade 3 infection (73 percent), 20 patients with grade 2 infection (21 percent), and three patients with grade 1 infection (36 percent). The remaining three patients had grade 0 infection (3 percent).

"Touch" Preparations (TP): The TP were available in 67 patients of whom 59 were positive (88 percent) and eight were negative (12 percent). When the severity of the infection was compared with that of the corresponding TBB, TP grade results were significantly associated with TBB results only in cases of severe, grade 3 infection (Kendall’s tau b = .720; p<0.0001). There was a tendency to underestimate the severity of the infection in the TP as compared with the TBB. Nevertheless, two negative TBB were positive by TP.

Bronchial “Washings” (BW) and “Brushings (BB):” The BW were available in 29 patients in whom 17 were positive (59 percent) and 12 negative (41 percent). No significant association in regard to severity was noted when BW results were compared with the corresponding TBB results (Kendall’s tau b = .255; .10>p>0.05).

The BBs were available in 21 patients of whom 12 were positive (57 percent) and nine were negative (43 percent). No association was noted when the BBs were compared with the corresponding TBB in regard to severity (Kendall’s tau b = .143; .3>p>0.2).

**Table 2—Diagnostic Yield of Fiberoptic Bronchoscopy in Pneumocystis carinii Pneumonia**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>No. Patients</th>
<th>Positive (%)</th>
<th>Negative (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBB</td>
<td>95</td>
<td>92 (97)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>TP</td>
<td>67</td>
<td>59 (88)</td>
<td>8 (12)</td>
</tr>
<tr>
<td>TBB + TP</td>
<td>67</td>
<td>66 (98)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>BW</td>
<td>29</td>
<td>17 (59)</td>
<td>12 (41)</td>
</tr>
<tr>
<td>TBB + TP + BW</td>
<td>21</td>
<td>21 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>BB</td>
<td>21</td>
<td>12 (57)</td>
<td>9 (43)</td>
</tr>
</tbody>
</table>


Discussion

It would be ideal to know prior to FOB the optimal number of TBB fragments required to establish a particular diagnosis with both certainty and expediency. For example, by applying statistical criteria Gillman and Wang, were able to determine that four fragments are needed to diagnose stage 2 sarcoidosis with a high degree of accuracy. Presently, a comparable approach has been applied for the diagnosis of PCP taking into account the roentgenographic manifestations of the disease (Table 1).

In those rare instances of PCP with a normal chest roentgenogram, we found that the optimal number of randomly-taken tissue fragments required for an almost certain diagnosis was four. In cases with roentgenographic grade 1 involvement, the required number was three tissue fragments taken from the representative interstitial infiltrates. In grade 2 PCP, two tissue fragments were required from areas of alveolar consolidation, and this was also the case in grade 3 infection with features of ARDS. By adhering to these criteria and provided that the biopsy fragments consisted of TBB, ie, purely or fundamentally alveolar tissue, the diagnosis of PCP could be accomplished with almost absolute certainty while sparing the patients unnecessary discomfort and the potential threats of pneumothorax or bleeding.

Our observations also confirm the high degree of efficacy off FOB in the diagnosis of PCP in patients with AIDS. This diagnosis was established in 97 percent of our cases by TBB alone. While TP was positive in 88 percent of the cases, the diagnostic yield could be improved to 98 percent when the results of TBB and TP were pooled (Table 2). For these reasons, we presently rely solely on the combined results of TBB and TP for the diagnosis of PCP. Moreover, TP allows a diagnosis the same day of FOB in a great majority of patients (88 percent). A rapid diagnosis might still be important in deciding therapy in some cases, although treatment is frequently started prior to the pathologic results because of the high degree of awareness of PCP among clinicians in institutions like ours.

It has not been the purpose of the present investigation to establish comparisons with BAL because of our limited experience with this technique. BAL is seldom performed in our institution because of the reliance of our clinicians on the TBB and the absence of major bleeding complications in their experience except for patients with Kaposi’s sarcoma of the tracheobronchial tree.

There were two patients in whom the TBB was negative and yet the TP was positive, a somewhat perplexing situation. It is possible that the particular aspect of the TBB "touched" for imprints contained organisms but the histologic sections were obtained from a disease-free level within the same biopsy. Such contention was tested after the completion of our study by obtaining "deeper" sections of the biopsies in these two cases and finding foci of positivity in both of them.

Conversely, the biopsy results can be positive and yet the TP negative, a situation which was observed in seven of our cases. An explanation is that either a purely endobronchial specimen or biopsy fragments free of disease were used for the touch imprint. To avoid this pitfall, we recommend that all biopsy fragments be used for TP in one single glass slide and the
latter be stained by the GMS technique.

A most unusual situation is exemplified by one of our cases having both TBB and TP negative while organisms were noted in BW. We speculate that organisms contained within airways draining both involved and noninvolved lung tissue were harvested during washing but the TBB was obtained, by chance, from the noninvolved lung parenchyma.

The diagnostic yield of BW alone was a mere 59 percent, and adding the results of BW to the combined yield of TBB plus TP (98 percent) raised the diagnostic accuracy only 2 percent more (Table 2). No advantage was gained by adding the results of BB. For these reasons, and taking into account the considerable amount of time and expense involved in the study of BW and BB, we have decided against searching for Pneumocystis carinii and use these specimens exclusively for cytologic and microbiologic investigations. An exception to this approach is probably the patient with a bleeding diathesis in whom TBB might be contraindicated. Under these circumstances, BW and certainly BAL are alternatives worthy of consideration.

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