Transbronchial Lung Biopsy in Pulmonary Sarcoidosis*

Is It An Evaluable Method in Detection of Disease Activity?

Venerino Poletti, M.D.; Marco Patelli, M.D.; Luigi Spiga, M.D.; Romano Ferracini, M.D.; and Valeria Manetto, M.D.

To assess the value of transbronchial lung biopsy in the evaluation of disease activity in pulmonary sarcoidosis, lung biopsy specimens obtained from 37 patients with this disease and their cellular patterns of bronchoalveolar lavage were studied. Morphologic analysis has showed peculiar lesions: predominant interstitial alveolitis consisting of mononuclear cells and scattered neutrophils, and eosinophils, diffuse in 13 cases and focal in 11 cases, interstitial nodular clusterings of mononuclear cells (five cases), diffuse intraalveolar infiltration of macrophages (one case), granulomas (27 cases), hyaline membranes (one case), intralveolar (two cases) and interstitial (six cases) fibrosis, and finally cuboidal metaplasia of alveolar lining cells (eight cases). Hyaline membranes were always combined to a diffuse alveolitis consisting of some neutrophils. Only diffuse alveolitis was significantly associated with a high lymphocytosis in BAL (p<0.05).

Alveolitis represents one of the first pathologic manifestations of pulmonary sarcoidosis. This lesion is characterized by chronic accumulation of mononuclear cells (T-lymphocytes and activated macrophages) within the alveolar structures. It plays a central role in the pathogenesis of the disease, mediates the formation of granulomata, and in the more active forms, the appearance of fibrosis and structural derangements. Its evaluation is therefore needed for making rational therapy decisions and expressing an accurate prognosis. Three methods have been used to serve this purpose: open lung biopsy, gallium-67 scanning, and bronchoalveolar lavage.

Open lung biopsy represents the more direct approach to the study of alveolitis. However, because of discomfort, risk, and cost, it is rarely carried out more than once during a patient's course, and thus, it is not useful as a means to sequentially assess the alveolitis. Gallium-67 scanning emphasizes the activated macrophage component of the inflammation, and data provided by this technique are not always reliable since it can lead to a large number of "false positives." Bronchoalveolar lavage assesses the status of the T-cell population in sarcoidotic alveolitis. Because it can be used easily, safely, and repetitively, it has become the primary method in the evaluation of inflammation of the lower respiratory tract. However, it can only give a rough estimate of the density of effector cells in lung parenchyma and it is not reliable in the presence of inflammation of the upper respiratory tract.

Histopathologic alterations of pulmonary sarcoidosis are heterogenous at the 2 to 4 mm level. Transbronchial lung biopsy specimens are, therefore, generally considered not representative of the extent or intensity of the alveolitis present. A recent study has, however, demonstrated that clinical methods are firmly related to morphologic changes of pulmonary sarcoidosis determined by transbronchial lung biopsy, and in this study, we examine the value of this method in the evaluation of the disease activity.

MATERIALS AND METHODS

The study comprises 37 patients (46 ± 26 yr of age; 15 males, 22 females) with biopsy-proved pulmonary sarcoidosis (transbronchial lung biopsy, lymph node, liver and skin biopsies). Roentgenologic features of cases are summarized in Table 1. No patient has been treated with steroids in the previous six months, and all underwent at least a transbronchial lung biopsy under local anesthesia by transnasal approach with a fiberoptic instrument using the wedging technique. Informed consent was obtained. Biopsies were obtained with elliptical forceps without fluoroscopic control from the basilar segments of the right lower lobe. At least six and usually eight specimens were obtained from at least two segments and they were stored in 10 percent buffered formalin.

Bronchoalveolar lavage was accomplished during the same examination using a total of 80 ml saline solution in 40 ml aliquots. The lavage that was carried out in our patients is comparable to that performed by Abe et al, and this makes it possible to directly compare our results with theirs.

Lavage cell differentials were determined by Papanicolaou stain of cytocentrifuge preparations. Lung biopsy specimens were embedded in paraffin: five slides were prepared from serial sections of each

Table 1—Roentgenologic Pattern of the 37 Patients with Pulmonary Sarcoidosis

<table>
<thead>
<tr>
<th>Rx pattern</th>
<th>No Cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>11</td>
<td>30</td>
</tr>
<tr>
<td>Stage II</td>
<td>15</td>
<td>41</td>
</tr>
<tr>
<td>Stage III</td>
<td>10</td>
<td>27</td>
</tr>
<tr>
<td>Pseudoneoplastic</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Mass</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*From the Service of Pathology, Service of Bronchology and Respiratory Physiopathology, Ospedale Bellaria, Bologna, Italy. Manuscript received January 24; revision accepted July 9.
Reprint requests: Dr. Poletti, Servizio di Isto-patologia, Osp. Bellaria, Via Allure 3, Bologna, Italy 40139
block and they were stained with hematoxylin-eosin, Ziehl-Neelsen, periodic acid-Schiff, Mallory and Weigert methods. Each parenchymal specimen allowed the study of at least ten respiratory spaces. The slides were examined without knowledge of clinical and laboratory data. Morphologic lesions were independently evaluated. The alveolitis was classified as "diffuse" if it was present in most of the parenchymal tissue, as "focal" if it was present in less than one third of the alveolated lung parenchyma, and as "absent" if it was present in a very minimal amount or not at all. Intraalveolar and interstitial fibrosis were considered separately from granulomata. In the case of a pseudoneoplastic lesion (sarcoïdosis), transbronchial biopsy specimens were taken in the peripheral pulmonary mass and in the right lower lobe.

RESULTS

Pathologic changes present in lung biopsy specimens (Fig 1) and their detection rate are listed in Table 2. Alveolitis was characterized by a predominantly interstitial accumulation of mononuclear cells (lymphocytes and macrophages) and scattered neutrophilic and eosinophilic granulocytes. Diffuse alveolitis was observed in 13 (35 percent) of the 37 biopsy specimens, focal alveolitis was present in 11 specimens (30 percent), and alveolitis was classified as absent in 13 patients (35 percent). In comparing the mean percentage of lymphocytes in bronchoalveolar lavage to range of alveolitis in biopsy specimens, in patients with diffuse alveolitis, this value was higher than that in patients with focal or absent alveolitis (p<0.05 and p<0.01 respectively) (Fig 2). Some authors consider the level of 28 percent of lymphocytes in bronchoalveolar lavage as representative of the difference between high intensity and low intensity alveolitis. If we accept this value, we see that only three patients with diffuse alveolitis present a low intensity alveolitis, while six and 11 patients with focal or absent alveolitis, respectively, show a low intensity alveolitis. In order to predict the minimum number of alveolated biopsy specimens needed for an adequate evaluation of diffuse alveolitis, we have considered the ratio: number of positive alveolar samples for diffuse alveolitis/total number of alveolar samples in 13 cases. Then we have applied the probability theorem used by Murray and Wanga “p = 1 − (1 − ρ)n”.

Thirty-six of 66 (54.5 percent) alveolar samples showed an interstitial diffuse alveolitis. If we chose the diagnostic yield of p to be 95 percent, it appears that 3.79 alveolar biopsy specimens (n) is the minimum number adequate for diffuse interstitial alveolitis evaluation. Interstitial clusterings of mononuclear cells have been considered representative of developing granulomas. In one of our cases, mononuclear cells

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**Figure 1. (right) Transbronchial lung biopsy specimens consisting of peripheral lung tissue showing:**
(A) diffuse interstitial infiltration of mononuclear cells: lymphocytes and macrophages (hematoxylin-eosin, original magnification ×250). (B) A clustering of mononuclear cells with abundant cytoplasm around a pulmonary vessel (hematoxylin-eosin, original magnification ×250). (C) Diffuse infiltration of macrophages with obliteration of most of the respiratory spaces (hematoxylin-eosin, original magnification ×100). (D) Interstitial pneumonitis and presence of hyaline membranes bordering alveolar septa (hematoxylin-eosin, original magnification ×250). (E) Buds of intraalveolar fibrosis and focal interstitial fibrosis (hematoxylin-eosin, original magnification ×250).
concentrated around a vessel. Increased intraalveolar macrophages are frequently observed in lung biopsy specimens. This lesion is entirely nonspecific when seen in association with pulmonary or bronchial infiltrative lesions. Mural and luminal macrophage infiltration was predominant only in one lung biopsy specimen of our report. Chest film showed large, rather poorly defined lesions. This unusual pattern is sometimes referred to as “alveolar sarcoidosis.” There is no large series of cases of this type of sarcoid correlated with pathology reported in the literature: closely packed mononuclear cells within the alveolar air space have been suggested as the cause of the acinar pattern, but there is also evidence that it is brought out by interstitial granulomas compressing the air spaces. In our case, a transbronchial lung biopsy made six months after demonstrated noncaseating granulomas in lung tissue. The characteristic morphologic feature of pulmonary sarcoidosis is the presence of multiple noncaseating granulomas in the lung. We have observed an inverse ratio between alveolitis and granuloma (Table 3). In the five cases that were submitted to transbronchial lung biopsies in successive times, we

Table 2—Pathologic Lesions and Their Detection Rate in Transbronchial Lung Biopsy Specimens

<table>
<thead>
<tr>
<th>Pathologic Lesions</th>
<th>No Cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alveolitis diffuse</td>
<td>13</td>
<td>35</td>
</tr>
<tr>
<td>focal</td>
<td>11</td>
<td>30</td>
</tr>
<tr>
<td>Interstitial clusters of mononuclear cells</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>Intraalveolar macrophage infiltration</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Granulomas</td>
<td>27</td>
<td>73</td>
</tr>
<tr>
<td>Hyaline membranes</td>
<td>7</td>
<td>19</td>
</tr>
<tr>
<td>Intraalveolar fibrosis</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Interstitial fibrosis</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>Cuboidal metaplasia of alveolar lining cells</td>
<td>8</td>
<td>22</td>
</tr>
</tbody>
</table>

Table 3—Granuloma Positivity in Transbronchial Lung Biopsy Specimens Compared to the Alveolitis Present

<table>
<thead>
<tr>
<th>Pathologic Lesions</th>
<th>No Cases</th>
<th>Pres</th>
<th>Granulomas</th>
<th>Abs</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. diffuse</td>
<td>13</td>
<td>7</td>
<td>54</td>
<td>6</td>
</tr>
<tr>
<td>A. focal</td>
<td>11</td>
<td>8</td>
<td>73</td>
<td>3</td>
</tr>
<tr>
<td>A. absent</td>
<td>13</td>
<td>12</td>
<td>92</td>
<td>1</td>
</tr>
</tbody>
</table>

have noted the preceding of alveolitis compared to granuloma. The relationship between the percentage of lymphocytes in BAL of patients with granuloma in lung biopsy specimens and that of patients without granuloma emphasized that these percentages were not statistically different (p>0.05, Fig 3). We have observed the presence of hyaline membranes in seven patients (19 percent). In these cases, the lesion was always associated with a diffuse alveolitis in lung specimens, and in detail, neutrophilic component of the inflammation was evident although not yet prominent.

Intraalveolar and interstitial fibrosis were focally present and not associated with structural derangement. In one patient submitted to transbronchial lung biopsies at 1½-month intervals, we first observed a diffuse alveolitis and the presence of hyaline membranes and then the appearance of endoalveolar and interstitial fibrosis and of a granuloma.

Finally, cuboidal metaplasia of alveolar epithelium was uncommon and only focal. The cuboidal cells did not have a corresponding granuloma. We have observed these changes in two patients who were referred for evaluation of chronic respiratory symptoms. In both cases, the lesions were thought to be consistent with sarcoidosis. However, the absence of granulomas in these cases has not excluded the possibility of a more diffuse disease process. Further study is needed to determine the significance of these findings.

Figure 2. Lymphocytosis in bronchoalveolar lavage compared to alveolitis as seen in transbronchial lung biopsy specimens.

Figure 3. Lymphocytosis in bronchoalveolar lavage of patients without granulomas in transbronchial lung biopsy specimens compared to that of patients with granulomas in the lung biopsy fragments.
not present dysplastic alternations of the nuclei.

**DISCUSSION**

Rosen et al\(^1\) observed a mononuclear cell interstitial pneumonitis as a predominating or prominent histopathologic finding in 62 percent of 128 granuloma-containing specimens of open lung biopsies obtained from patients with sarcoidosis. The authors noted other pathologic changes in lung tissue too: interstitial fibrosis and small lesions consisting of interstitial poorly circumscribed group of mononuclear cells considered as developing granulomas. Hyaline membranes were not seen. In this study, an inverse ratio between alveolitis and granulomas and between alveolitis and fibrosis was noted. Intertitial pneumonitis was thus considered a relatively early lesion in pulmonary sarcoidosis. Now, it is assumed to be an early lesion and probably the initial lesion anetading the appearance of characteristic, noncaseating granulomas.\(^1,2\)

Histopathologic features of pulmonary sarcoidosis can be heterogenous at the 2 to 4 mm level.\(^2\) This recognition is on the basis of the assertion that transbronchial lung biopsy specimens often are not representative of the extent or intensity of the alveolitis present.

Transbronchial biopsy is now the method of choice for obtaining intrathoracic tissue demonstrating noncaseating granulomas—a necessary criterion for the diagnosis of pulmonary sarcoidosis.\(^1,3\) In our experience (181 cases of pulmonary sarcoidosis), we have detected epithelioid granuloma in 69 percent of the reentgenologic stage I patients, in 73 percent of the stage II patients, and in 84 percent of the stage III patients. Transbronchial lung biopsy through the fiberoptic bronchoscope has been shown to be an extremely safe and effective method for obtaining lung tissue. Biopsy specimens are small but it is possible to make quite a lot of bites. Lung tissue comes from centrilobular regions, and the chances to study alveolated parenchyma are higher when specimens are serially sectioned.

In our study, diffuse alveolitis results statistically correlated to a higher lymphocytosis in bronchoalveolar lavage fluid (p<0.05). This is not true for focal or absent alveolitis. The presence of interstitial clusterings of mononuclear cells is not a frequent finding in transbronchial lung biopsy specimens. While interstitial alveolitis does not have apparent perivascular or peribronchiolar localization, in one case, this lesion centered around a vessel. We have not found correlations between the percentage of lymphocytes in bronchoalveolar fluid and the presence or absence of granulomas in biopsy specimens. This is in agreement with some authors\(^1,3\) who do not consider the lesion an expression of the disease developing capacity.

Buchalter et al\(^4\) have indirectly shown the presence of alveolar edema and capillary leak in the lower respiratory tract of patients with sarcoidosis. We have seen hyaline membranes in lung parenchyma in 19 percent. The morphologic basis for the formation of hyaline membranes is represented by the destruction of type I epithelial cells.\(^5\) This lesion was always associated with a diffuse alveolitis consisting of mononuclear cells and scattered neutrophils.

The role of neutrophilic cells in pulmonary sarcoidosis is not clear, but they can cause substantial damage of the alveolar wall and precede the appearance of fibrosis and the destruction of lung parenchyma.\(^5,6\)

Focal intraalveolar and interstitial fibrosis has been found less commonly (two and six cases, respectively). The observation proves that the alveolar damage in pulmonary sarcoidosis is not heavy. Also, cuboidal metaplasia of alveolar epithelium has not been so evident as in other infiltrative lung diseases (idiopathic interstitial fibrosis, eosinophilic pneumonia, and hypersensitivity pneumonia).\(^7,8\) In one case, it was possible to highlight the transformation of a fibrinous alveolar exudate into intraalveolar and interstitial fibrosis.

In conclusion, our findings suggest that alveolar exudative damage may be present in pulmonary sarcoidosis and that transbronchial lung biopsy can contribute to the study of early lesions and to an evaluation of the disease activity. For this last purpose, about four alveolar samples appear sufficient.

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**REFERENCES**

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The Seventh Annual Meeting of the Society will be held May 11-14 in Montreal, Quebec. For information, contact Ms. Mary A. Finch, Meeting Coordinator, Society for Clinical Trials, Inc., 600 Wyndhurst Avenue, Baltimore 21210.