Clinical and Immunohistochemical Evaluation of Acute Interstitial Pneumonia, Bronchiolitis Obliterans Organizing Pneumonia, and Acute Exacerbation of Idiopathic Pulmonary Fibrosis

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Acute interstitial pneumonia (AIP), bronchiolitis obliterans organizing pneumonia (BOOP), and acute exacerbation of idiopathic pulmonary fibrosis (IPFex) all present with acute onset and a rapidly progressive clinical course. Since it is sometimes difficult to differentiate among these three entities, the present study was designed to analyze differences.

Four AIP, seven BOOP, and eight IPFex patients were studied clinically and pathologically with semi-quantitative scoring methods using immunohistochemistry.

Clinically, there were no significant differences, except that the patients with BOOP had lower chest roentgenogram scores and lung injury scores (p < 0.05).

Pathologically, the mean epithelial basement membrane damage score in the AIP patients was higher than that of the other patients (p < 0.05). Among the intraluminal organizing exudate scores, the polypoid type was highest in the BOOP patients (p < 0.01), and the incorporative/obliterative type was highest in the AIP patients (p < 0.005). The epithelial basement membrane damage scores correlated with the intraluminal organizing exudate scores (polypoid type, r = -0.57; incorporative/obliterative type, r = 0.81).

Although there are clinical similarities among the three disorders, pathological differences also exist and are demonstrated by the present findings.

Key words: acute lung injury, epithelial basement membrane, intraluminal organizing exudate

INTRODUCTION

Although most patients with interstitial lung disease experience a gradual evolution of symptoms over a period of months or years,¹¹ some patients may present a more acute onset and rapid course with progressive hypoxemia and bilateral infiltrates on chest x-rays over several days or weeks. Two idiopathic disorders, acute interstitial pneumonia (AIP) and idiopathic bronchiolitis obliterans organizing pneumonia (BOOP) have been recognized, and they occasionally present an acute onset and rapid course with progressive respiratory failure. AIP, which was reported by Katzenstein et al.,²) is recog-
nized as an idiopathic adult respiratory distress syndrome, and the clinical course is rapidly progressive toward severe respiratory failure with diffuse pulmonary infiltration. Idiopathic BOOP, as reported by Epler et al., typically shows flu-like symptoms, dyspnea and hypoxemia with patchy pulmonary infiltration on chest x-rays. Some patients with BOOP deteriorate rapidly, as patients with AIP.

Idiopathic pulmonary fibrosis (IPF) is generally a slowly progressive disorder. However, it sometimes abruptly worsens and becomes fatal within several days or weeks. We recently reported cases of acute exacerbation of IPF which showed a rapidly progressive dyspnea with newly developing pulmonary infiltrates during this chronic course.

Since these three entities occasionally share similar acute respiratory distress with diffuse pulmonary infiltrates, it is important to evaluate and to differentiate them because of their different prognoses and therapeutic responses.

Pathologically, AIP is characterized by interstitial fibrosis with extensive fibroblast proliferation; a feature of idiopathic BOOP is polypoid masses of granulation tissue in the lumens of the small airways, alveolar ducts and some alveoli. Katzenstein et al. suggested that AIP and BOOP be categorized as an acute lung injury pattern which might evolve from epithelial and endothelial necrosis to alveolar collapse and might eventually result in fibroblast proliferation. Because of their histologic similarities and temporal uniformities, distinguishing between these two disorders may be difficult in some patients. The histologic features of acute exacerbation of IPF (IPFex) are also acute lung injury patterns which show diffuse interstitial changes with various degrees of intraluminal organizing exudates superimposed on the usual interstitial pneumonia (UIP). It has recently been shown that intraluminal organizing exudates are commonly recognized in acute lung injury patterns, and that disruption of the epithelial basement membrane plays an important role in the process of intraluminal organization. In the present study, we evaluated the degree of epithelial basement membrane damage, and the type and degree of intraluminal organizing exudates by semi-quantitative methods, using immunohistochemistry in order to reveal differences and similarities among the three disorders and the relationships among the histologic findings along with their clinical characteristics.

MATERIALS AND METHODS

Subjects
A total of 19 patients (11 men and 8 women, mean age ± S.D., 59.4 ± 10.6 years, ranging from 29 to 74) participated in this study. The patients had one of three disorders: AIP (n = 4), BOOP (n = 7), or IPFex (n = 8). Their pathological diagnoses, confirmed by open lung biopsy, were made by at least two pathologists according to the criteria for AIP, BOOP, and IPFex. AIP was characterized by diffuse alveolar wall thickening with proliferation of fibroblasts (organizing diffuse alveolar damage, DAD). BOOP showed intraluminal organizing buds with structural preservation. IPFex was indicated by an organizing DAD superimposed on UIP lesions. As diagnoses of each cases, there were no differences among the pathologists. Infectious causes were excluded by bacteriological cultures of sputa and bronchoalveolar lavage fluids before administration of antibiotics, and by serological examinations of bacterial and viral infection.

Methods

1. Clinical Courses and Data — The clinical findings in each case, including the initial symptoms, the duration of the symptoms before admission, white blood cell (WBC)
counts, C-reactive protein (CRP), blood gas levels (PaO₂/fractional concentration of oxygen in inspired gas [FIO₂]), and a pulmonary function test on admission were analyzed. Chest roentgenogram scores and lung injury scores on admission, as described by Murray et al., were also analyzed. The chest roentgenogram scores were estimated by degree of the extent of roentgenographic densities. It was evaluated by two clinicians, who did not know about the aim of this study. There were no significant differences between their scores. The lung injury score consisted of four components: The chest roentgenogram score, hypoxemia score (converted by PaO₂/FIO₂ levels), the positive end-expiratory pressure (PEEP) score (when ventilated), and the respiratory system compliance score (when available). The final lung injury score was obtained by dividing the aggregate sum by the number of components that were used.

2. Immunohistochemistry — Open lung biopsy specimens were taken from normal to mildly-affected areas and moderately-affected areas in each case. All specimens were gently inflated with formalin using a syringe and needle through the pleura. One or two representative histologic sections were stained with hematoxylin-eosin (HE) and immunostained with type IV collagen, which is the major collagenous component of the epithelial basement membrane. Immunostaining was performed according to the avidin-biotin-peroxidase complex method of Hsu et al. Sections were pretreated with 0.4% pepsin (Sigma Chemical Co., St. Louis, MO) at 37°C for 60 min, and 0.05% pronase (Sigma, Protease type VII) at room temperature for 15 min. Ten percent normal rabbit serum was used as a blocking antibody. Anti-type IV collagen mouse monoclonal antibody (DAKO, diluted 1:500), biotinylated rabbit anti-mouse monoclonal antibody (Nichirei, Tokyo, Histfine kit), and avidin-biotin-peroxidase complex (Nichirei, Histfine kit) were also used.

3. Semi-quantitative Evaluation and Scoring of Pulmonary Lesions — Using HE staining and immunostaining slides, we checked the severity of epithelial basement membrane disruption, and the types and degree of intraluminal organizing exudate in all fields. Intraluminal organizing exudate was divided into two types; the polypoid type and the incorporative/obliterative type. The polypoid type exudate was demonstrated by intraalveolar buds, and the incorporative/obliterative type showed obliterative changes and mural incorporation, as reported the study of intraluminal fibrosis by Basset et al.8

Epithelial basement membrane damage...
Statistical Analysis

The results are expressed as mean ± S.D. and statistical comparisons among the groups were analyzed using the Kruskal-Wallis test and the Student’s unpaired t-test. A correlation coefficient analysis was performed, and differences were considered significant at the p < 0.05 level.

RESULTS

1. Clinical Courses and Data

The clinical findings for the AIP, BOOP, and IPFex patients are summarized in Table 1. The age of the patients was 51.5 ± 16.0 years in the AIP group, 59.6 ± 10.0 years in the BOOP, and 63.3 ± 6.3 years in the IPFex. Dyspnea, cough and fever were observed as initial symptoms in most cases in all three groups. The duration of these symptoms from beginning to admission was 10.8 ± 10.3 months in the IPFex group, which was significantly longer than in the AIP (p < 0.05) and the BOOP (p < 0.01). But there was no significant difference among the groups in the duration from the onset of acute respiratory distress to admission. All of the patients’ conditions worsened progressively within three months. Although there were also no significant differences in WBC counts and CRP, the PaO₂/FIO₂ levels in the BOOP (361.4 ± 93.1) were significantly higher than in the AIP (191.4 ± 118.8) and the IPFex (253.3 ± 97.9) (p < 0.05). AIP, BOOP and IPFex were 1.5 ± 1.7, 0.3 ± 0.8, and 1.3 ± 1.5 in converting PaO₂/FIO₂ levels to hypoxemia score. There were significant differences among them (p < 0.05). On a pulmonary function test, the prediction of vital capacity (%)VC in the BOOP group (89.6 ± 21.2%) was higher than in the AIP (44.5 ± 11.6%) and the IPFex (61.8 ± 12.0%) (p < 0.01). The mean chest roentgenogram

scores were estimated from the percentage of disrupted lesions in all immunostained epithelial basement membranes on the slides. Intraluminal organizing exudates were scored according to the degree of area occupied by the polypoid or incorporative/obliterative type on the slides. The degree of each parameter was scored from 0 to 4 (0: 0% affected, 1: <10%, 2: 10% ≤ and <30%, 3: 30% ≤ and <50%, 4: 50% ≤). Two observers evaluated the slides independently on two separate times, at intervals of about three weeks, without any clinical information about the patients. The means of the four observations for each subject were used in the statistical analysis. Examples of some of the pathological features and their assigned scores are shown in Figs. 1 and 2. Old scarring areas were excluded from the study.

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Table 1 Clinical findings in AIP, BOOP, and IPFex patients.
AIP: acute interstitial pneumonia; BOOP: bronchiolitis obliterans organizing pneumonia; IPFex: acute exacerbation of idiopathic pulmonary fibrosis; WBC: white blood cell; CRP: C-reactive protein; %VC: prediction of vital capacity.

<table>
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<tr>
<th>Data</th>
<th>AIP</th>
<th>BOOP</th>
<th>IPFex</th>
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<td>3M, 4F</td>
<td>6M, 2F</td>
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<td>59.6±10.0</td>
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<td>8/8</td>
</tr>
<tr>
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<td>6/7</td>
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</tr>
<tr>
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<td>5/7</td>
<td>7/8</td>
</tr>
<tr>
<td>others</td>
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<td>0/8</td>
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<td>Duration of symptoms</td>
<td></td>
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<tr>
<td>before admission, mo</td>
<td>1.6±1.3</td>
<td>1.6±0.7</td>
<td>10.8±10.3</td>
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<tr>
<td>WBC, /mm³</td>
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<td>8214.3±2858.6</td>
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<td>PaO₂/FIO₂*</td>
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<td>361.4±93.1</td>
<td>253.3±97.9</td>
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<td>Pulmonary function</td>
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<td>%VC**</td>
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<td>89.6±21.2</td>
<td>61.8±12.0</td>
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<td>Chest roentgenogram</td>
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<td>score*</td>
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<td>1.9±0.7</td>
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<tr>
<td>Lung injury score*</td>
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<td>Outcome</td>
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<td>7 alive</td>
<td>3 dead, 5 alive</td>
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</table>

*: duration from acute exacerbation.  ** p<0.05,  *** p<0.01.

Score in the BOOP (1.9±0.7) was lower than in the AIP (3.0±0.8) and the IPFex (3.0±0.9). The mean lung injury score in the BOOP (1.1±0.5) was also lower than in the AIP (2.3±1.0) and the IPFex (2.2±1.1) (p<0.05). Corticosteroid therapy was administered to all patients. Nevertheless, one of the four AIP patients and three of the eight IPFex patients died, 3.5, 1, 5, and 24 months after beginning therapy, respectively.

2. Semi-quantitative Evaluation of Immunohistochemistry

In the semi-quantitative evaluation, there were significant correlations between the first and the second scores by both observers 1 and 2 (p<0.01). And the mean scores of the first and the second evaluation correlated significantly between observer 1 and 2 (p<0.0001). The epithelial basement membrane damage scores are shown in Fig. 3. The score of the AIP group (3.1±0.3) was significantly higher than that of the BOOP (1.6±0.3) and IPFex patients (1.9±0.2) (p<0.05). Both polypoid type and incorporative/obliterative
Fig. 4 Scores for intraluminal organizing exudate. A: The score for the polypoid type was highest in the BOOP group, followed by the IPFex and the AIP ($p < 0.01$). B: The incorporative/obliterative type score was higher in the AIP group than in the IPFex and the BOOP ($p < 0.005$).

A. Polypoid intraluminal exudate

B. Incorporative/obliterative exudate

Fig. 5 Correlation between basement membrane damage scores and intraluminal organizing exudate scores. The basement membrane damage scores correlated negatively with the polypoid type scores, and positively with the incorporative/obliterative type scores. （○: AIP, ×: BOOP, △: IPFex）
type intraluminal organizing exudates were observed in all groups. The polypoid type score in the BOOP patients (3.0 ± 0.2) was significantly higher than that in the AIP (0.9 ± 0.9) and IPFex groups (1.6 ± 1.1) (p < 0.01) (Fig. 4A). In contrast, the incorporative/obliterative type score in the AIP group (3.0 ± 0.3) was significantly higher than in the IPFex (1.8 ± 0.2) and BOOP patients (0.9 ± 0.7) (p < 0.005) (Fig. 4B). The epithelial basement membrane damage score correlated negatively with the polypoid type intraluminal organizing exudate score (y = 3.88 —0.93x, r = —0.57, p < 0.05), but positively with the incorporative/obliterative type (y = —0.53 + 1.60x, r = 0.81, p < 0.0001) (Fig. 5).

DISCUSSION

Among the present clinical findings, there are some similarities among the AIP, BOOP and IPFex groups, but their mean PaO₂/FIO₂ levels, %VC, chest roentgenogram scores and lung injury scores showed significant differences. In the BOOP patients, the pulmonary impairment was milder than in the AIP and IPFex groups. These parameters might help us to differentiate these disorders in difficult cases with clinical similarities.

In the pathological semi-quantitative evaluation of the present study, the epithelial basement membrane damage score was highest in the AIP group. The epithelial basement membrane may play an important role in the process of lung remodeling after injury. It is possible that if the epithelial basement membrane damage is localized and mild, as it is in BOOP patients, effective repair can be expected. Conversely, if the epithelial basement membrane damage is diffuse and severe, as it is in AIP patients, the lung remodeling progresses and effective repair may be difficult.

The intraluminal organizing process may also play an important role in the pathogenesis of interstitial lung disorders. In the present evaluation of the intraluminal organizing exudate using a semi-quantitative analysis, both polypoid type and incorporative/obliterative type intraluminal organizing exudates were observed in all three groups. Thus, in some cases, it might still be difficult to differentiate AIP, BOOP patients, and from acute exacerbated area of IPF patients, all of which are categorized as acute lung injuries.

In the present study, the BOOP patients had a higher mean score for polypoid type intraluminal organizing exudate than did the patients with the other two diseases. Considering the pathological characteristics of BOOP, this is not unexpected. In contrast, the score of incorporative/obliterative type intraluminal organizing exudate was highest in the AIP patients and lowest in the BOOP patients. It has recently been hypothesized that the magnitude of epithelial damage or the degree of the disruption of the epithelial basement membrane may define the patterns of the intraluminal organizing process. Basset et al. has stated that intraluminal buds result from small defects in the epithelial structures of the alveolar walls, and that obliterative change occurs in more severe injuries. In our study, the epithelial basement membrane damage score correlated negatively with the polypoid type intraluminal organizing exudate score, and correlated positively with the incorporative/obliterative intraluminal organizing exudate score. These results might confirm the above hypothesis. That is, if the degree of disruption of the epithelial basement membrane is mild, a mainly polypoid type intraluminal organizing exudate could be observed. If the degree of disruption is severe, an incorporative/obliterative type intraluminal organizing exudate would be likely.

We investigated differences and similarities among AIP, BOOP and IPFex patients clinically and pathologically. The results suggest that there are pathological differences in
the severity of epithelial basement membrane damage, as well as in the types of intraluminal organizing exudates. With the present semi-quantitative evaluation of epithelial basement membrane disruption and patterns of intraluminal organizing exudates, differences in pathological severity became clearer for these three diseases.

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