Amount of *Pneumocystis carinii* and Degree of Acute Lung Inflammation in HIV-associated *P. carinii* Pneumonia*

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Correlations between semiquantitative amounts of *Pneumocystis carinii* (PC), the degree of inflammation, and the severity of pneumonia were analyzed in 58 patients with PC pneumonia (PCP). Material from both transbronchial biopsies (TBBs; n = 39) and bronchoalveolar lavage fluid (BALF; n = 57) was examined. In the TBB the amount of PC correlated strongly with overall inflammation in the interstitium (Kendall correlation coefficient [Kcc] = 0.59; p < 0.0001), type 2 pneumocyte proliferation, and edema formation. The amount of PC in the TBB also correlated with interstitial accumulation of neutrophils (Kcc = 0.54; p = 0.0001), lymphocytes, and macrophages. In BALF the amount of PC correlated with edema formation and type 2 pneumocyte proliferation in the TBB but not with the percentage of neutrophils, lymphocytes, or macrophages in BALF. The amount of PC in the BALF and the percentage of neutrophils in the BALF correlated significantly with PO₂ and the serum lactate dehydrogenase (LDH) level. Neither short-term nor long-term survival was affected by the amount of PC, inflammatory markers in the TBB, or the serum LDH levels. In conclusion, the amount of PC is associated with the extent of the acute inflammatory reaction in the lung in PCP associated with human immunodeficiency virus (HIV).

(Best 1993; 104:109-13)

Several characteristics of *Pneumocystis carinii* pneumonia (PCP) in patients with AIDS have been proposed as prognostic indicators, partly supported by studies correlating especially the percentage of neutrophils in bronchoalveolar lavage fluid (BALF) with the severity and prognosis of PCP. The amount of *Pneumocystis carinii* (PC) could also be a prognostic indicator, although our present knowledge does not support this. No study has attempted to correlate the following three aspects of PCP associated with the human immunodeficiency virus (HIV): (1) amounts of PC; (2) inflammatory changes in the lung; and (3) the severity and prognosis of PCP.

In this study, we examined the relationship between the amount of PC with markers of inflammation in transbronchial biopsies (TBBs) and BALF of HIV-associated PCP. In order to study the clinical relevance of these findings, the amount of PC and the degree of inflammation were correlated with indicators of the severity of the pneumonia, ie, PO₂ and the serum lactate dehydrogenase (LDH) level at the time of diagnosis, and with short- and long-term survival of the patients.

**Materials and Methods**

**Patients**

There were 189 fiberoptic bronchoscopic procedures performed on 135 HIV-positive patients with pulmonary symptoms in the period from September 1989 to June 1991. Thirty-six procedures were follow-up examinations due to either persistent symptoms or just to assess the effect of the treatment. Sixty-nine patients had PCP during the period, and of those, we report the findings in a group of 58 consecutive patients who had a primary episode of PCP. Clinical data, including PCP prophylaxis prior to bronchoscopy, were collected prospectively. Chest x-ray films and laboratory values, including CD4 count, serum LDH level, and arterial blood gas levels, were gathered less than 24 h before bronchoscopy. Outcome was recorded from the medical files.

**Bronchoscopy**

Bronchoscopy was done under local anesthesia as previously described. The BAL and TBB were performed in the right middle lobe in the case of diffuse infiltrates on the chest x-ray film (otherwise from the site of localized infiltration). The BAL was performed with instillation of up to 240 ml of warmed saline solution in 4 to 6 aliquots. The recovered BALF was pooled; 60 to 80 percent of the instilled volume was aspirated. Two or three TBBs were taken in another subsegment in the same lobe.

**BALF Cell Differential Counts, and TBBs**

Immediately after bronchoscopy, a standardized volume of the obtained BALF (3.5 ml) was centrifuged for 5 min at 1,500 g, and smears were prepared from the deposit by aliquoting 1 drop per slide. The following staining methods were used: May-Grunwald-Giemsa stain (MGG); Papanicolaou stain; Grocott methenamine-silver stain; and an immunoperoxidase stain using monoclonal antibodies against PC (Dako-Pneumocystis, M 778; DAKO-DK). The biopsies were fixed in 4 percent buffered Formalin, embedded in paraffin, and stained with hematoxylin-eosin, van Gieson-Hansen stain, periodic acid-Schiff (PAS), Grocott methenamine-silver stain, and the immunoperoxidase stain. Biopsies were considered satisfactory for evaluation if they contained more than 10 well-preserved alveolar lumina. Evaluation of the BALF and TBBs was done prospectively and without knowledge of the patient's clinical status. Also, the observer was blinded to the information on the BALF when studying the TBB and vice versa.
The presence of PC was diagnosed if a honeycombed foamy material (ie, trophozoites) could be demonstrated by either MGG or Papanicolaou stain and confirmed on the immunoperoxidase stained slide or if cysts were found in the silver-stained smear (or both). In both BALF and TBB, trophozoites contributed mostly in the overall score of PC. They were graded in either MGG staining or using immunoperoxidase staining. In only two cases were only cysts detected. The amount of PC was graded semiquantitatively on a scale of 0 to 3 as described in Table 1. In a previous series of patients with PCP, the intraobserver variation for grading the amount of PC and indices of inflammation was examined. For the amount of PC in both the TBB and BALF, a κ value of 0.81 was found.

In the BALF Cytospin precipitates, a differential count of the inflammatory cells was performed in a representative area of the MGG-stained slide containing a monolayer of cells. In the TBB the inflammatory reaction in the alveolar walls was estimated semiquantitatively and graded on a scale of 0 to 3 by recording the following parameters: edema in the alveolar walls (0, not present; 1, slight widening of the alveolar septa with a loose texture of the tissue; increasing to 3, severe increase of the thickness of the alveolar wall due to interstitial fluid accumulation); type 2 pneumocyte proliferation (0, not present; 1, scattered cuboidal cells protruding into the alveolar space; increasing to 3, numerous cuboidal cells protruding into the alveolar space in a hobnail fashion); and the number and type of inflammatory cells (0, no inflammatory cells present; 1, scattered inflammatory cells in few alveolar walls; increasing to 3, numerous inflammatory cells in all alveolar walls present). The presence of fibrin and the number and type of inflammatory cells in the alveolar lumina were evaluated in the same way. For inflammation, pneumocyte proliferation, and edema in the TBB, we have previously found κ varying from 0.52 to 0.67. For cell types involved in inflammation, κ varied from 0.59 to 1.00.

**Statistical Methods**

A nonparametric measure of correlation, ie, the Kendall rank-order correlation coefficient\(^{37}\) (Kcc) has been used; this test makes no assumptions about linear associations and distributions. The logrank test\(^6\) was used for analysis of survival.

**Figure 1.** Association between semiquantitative evaluation of PC in TBBs and semiquantitative score of inflammation in TBB (A), pneumocyte proliferation in TBB (B), edema in TBB (C), neutrophils in interstitium of TBB (D), macrophages in interstitium of TBB (E), and lymphocytes in interstitium of TBB (F). Values for Kcc and p are shown.
Results
The total group consisted of 58 consecutive patients with PCP for whom median values and ranges for background data are shown in the following tabulation:

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>38 (22-76)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO2, mm Hg</td>
<td>8.4 (5.1-14.4)</td>
</tr>
<tr>
<td>PCO2, mm Hg</td>
<td>4.3 (3.3-5.4)</td>
</tr>
<tr>
<td>Serum LDH</td>
<td>567 (259-1,293)</td>
</tr>
<tr>
<td>CD4 count (mm3)</td>
<td>14 (0-200)</td>
</tr>
<tr>
<td>No. of deaths</td>
<td>5 (9%)</td>
</tr>
</tbody>
</table>

No differences in these characteristics were found when comparing all patients with the 57 patients with complete information on the BALF and the 39 patients with complete information on the BALF and TBB. All patients had low CD4 counts, i.e., 200/mm3 or less. Within the range 0 to 200/mm3, no association between the CD4 count and the amount of PC was seen, either in the BALF or in the TBB. Only two patients had received PCP prophylaxis, i.e., pentamidine inhalation; in these patients the amounts of PC in the BALF and TBB were similar to those of the others.

Pathologic Correlations
Figure 1 shows that semiquantitative evaluation of PC in the TBB correlated with the semiquantitative score of inflammation in the TBB (Fig 1A), the type 2 pneumocyte proliferation in the TBB (Fig 1B), edema in the TBB (Fig 1C), neutrophils in the interstitium of the TBB (Fig 1D), lymphocytes in the interstitium of the TBB (Fig 1E), and macrophages in the interstitium of the TBB (Fig 1F). Values for Kcc and p are shown. The amounts of PC in the TBB were significantly associated with a semiquantitative score of macrophages in the alveolar space in the TBB (Kcc = 0.27; p = 0.03). No significant numbers of neutrophils or lymphocytes were detected in this area of the TBB. The association between semiquantitative evaluation of PC in the BALF and the percentage of neutrophils in the BALF was insignificant (Kcc = -0.01; p = 0.46), as was the association between PC in the BALF and lymphocytes (Kcc = -0.08; p = 0.25) and macrophages (Kcc = 0.05; p = 0.46) in the BALF. Associations between PC in the BALF and inflammatory markers in the TBB were also examined. The PC in the BALF correlated significantly with type 2 pneumocyte proliferation in the TBB (Kcc = 0.35; p = 0.006) and edema in the TBB (Kcc = 0.26; p = 0.03).

Clinical Findings at Time of Diagnosis
Primarily the PO2 but also the serum LDH level at the time of bronchoscopy were studied as markers of the severity of PCP. Information on the PO2 and the serum LDH level at time of bronchoscopy was available for 53 and 43 patients, respectively; missing data were unrelated to any of the features under study. The amounts of PC in the BALF, neutrophils in the BALF, and edema in the TBB correlated significantly with PO2, whereas only PC in the BALF correlated significantly with the serum LDH (Table 2). Inflammatory markers other than edema in the TBB were consistently associated with PO2 and LDH, but correlations were only marginally significant.

Bacteria were found in the BALF of five patients. Neither inflammatory markers in the TBB (including neutrophils) nor the mean PO2 or serum LDH in these patients differed from patients without bacteria in the BALF. Cytomegalovirus (CMV) was cultured from the BALF of 12 patients; a positive culture was not related to cell differential counts in the BALF, TBB findings, PO2, or serum LDH.

Survival
The amount of PC and the inflammatory changes detected in the BALF and TBB at the time of diagnosis were without prognostic information for survival from the PCP episode. Fifty-three patients survived the initial PCP. They have been followed for 77 to 730 days (median, 346 days); 16 patients have died during this follow-up (12-month survival rate, 65 percent). Neither significant associations nor any trend could be found when looking at associations between long-term mortality and the following variables: the amount of PC in the BALF or TBB; inflammatory markers in the TBB; cell types in the TBB; cell differential counts in the BALF; PO2 at the time of bronchoscopy; or serum LDH at the time of bronchoscopy.

Discussion
To our knowledge, this study is the first to correlate the amount of PC with the appearance of inflammation in the lung of patients with AIDS who had PCP. Descriptions of the histologic findings in PCP usually emphasize the infiltration of lymphocytes and macro-

| Table 2—Associations Between PC in Both BALF and TBBs, Inflammatory Markers in TBB, Neutrophils in Both BALF and TBB, and Markers of Severity of PCP |
|---|---|---|---|---|
| Data | PO2 | Serum LDH |
| BALF | | |
| PC | -0.23 | 0.02 | 0.51 | 0.005 |
| Neutrophils | -0.21 | 0.01 | 0.17 | 0.06 |
| TBB | | |
| PC | -0.17 | 0.09 | 0.18 | 0.11 |
| Alveolar space | | |
| Macrophages | 0.04 | 0.37 | 0.14 | 0.19 |
| Interstitium | | |
| Overall inflammation | -0.10 | 0.21 | 0.11 | 0.22 |
| Edema | -0.26 | 0.02 | 0.23 | 0.06 |
| Pneumocyte proliferation | -0.16 | 0.10 | 0.11 | 0.21 |
| Neutrophils | -0.19 | 0.07 | 0.04 | 0.39 |
| Macrophages | -0.11 | 0.21 | 0.01 | 0.46 |
phages into the pulmonary interstitium.\textsuperscript{1,9,10} In this study the amount of PC in the TBB correlated with the degree of inflammation in the interstitium, including the accumulation of neutrophils and lymphocytes. The amount of PC in the TBB correlated with semiquantitative measures of alveolar macrophages seen in the TBB but not with the percentage of inflammatory cells in the BALF. Unfortunately, the total number of inflammatory cells per milliliter of BALF was not available; thus, we were not able to analyze for quantitative measures of the inflammatory reaction in the BALF. Also, quantitating the amount of PC in the BALF appears to be more unreliable than quantitating the amount of PC in the TBB, since PC adheres to the alveolar wall.\textsuperscript{11} Other investigators have described ways of quantitating the amount of PC in the BALF, either relative to the number of inflammatory cells\textsuperscript{12} or per milliliter of BALF,\textsuperscript{4} but neither of these methods seems superior to the relatively simple but standardized technique used in this report. A variety of possible causes for the increase in the number of neutrophils in PC-infected pulmonary interstitium exists, eg, tobacco smoke and contemporary infection with bacteria, CMV, or herpes simplex virus. In our patients, no data were available on exposure to tobacco smoke. The presence of bacteria was associated with higher percentages of neutrophils in the BALF but not in the TBB. Cytomegalovirus was not associated with the accumulation of neutrophils in our study.

Others have implemented an increased percentage of neutrophils in the BALF as a predictor of lower Po\textsubscript{2} at the time of diagnosis.\textsuperscript{4} We found a similar association when correlating the percentage of neutrophils in the BALF and initial Po\textsubscript{2}. There was a statistically weaker inverse correlation between the amount of neutrophils in the interstitium and Po\textsubscript{2} (p = 0.07), which may be explained by the smaller number of TBBs than BALs performed (39 vs 57). Also, the amount of PC correlated with Po\textsubscript{2} (BALF, p = 0.02; TBB, p = 0.09). These findings are somewhat in contrast to those of a previous study by Limper et al,\textsuperscript{4} who found no association between the number of cysts per milliliter of BALF and the initial Po\textsubscript{2} in patients with AIDS who have PCP. In the TBB, only the degree of interstitial edema in our patients correlated significantly with Po\textsubscript{2}. The associations between the amount of PC, the accumulation of neutrophils, and edema may suggest that PC and a resultant acute inflammation lead to edema formation in the pulmonary interstitium and consequently to impaired oxygen transportation; however, it is noteworthy that this often severe inflammatory response in HIV-associated PCP is not sufficient or effective in clearing the causal microorganism.

Serum LDH was included in this study as a marker of the severity of the pneumonia based on previous studies\textsuperscript{13-15} and appeared to have prognostic significance. Serum LDH values were markedly higher in 3 of our patients with a fatal course of the pneumonia than in 40 surviving patients (means, 1,065 and 603 U/L, respectively); the small number of deaths precludes statistical analysis. In our study the amount of PC in the BALF correlated with serum LDH levels. Also, the percentage of neutrophils in the BALF and the degree of edema formation in the pulmonary interstitium were associated with serum LDH. These associations have not previously been described but could be due to backflow of LDH from pulmonary cells over the alveoloarterial membrane, as suggested by Smith et al.\textsuperscript{8}

We did not find the percentage of neutrophils in the BALF nor the initial Po\textsubscript{2} to predict survival from the PCP episode, whereas several previous studies have found a high percentage of neutrophils and a low Po\textsubscript{2} to be associated with poor survival.\textsuperscript{2,3,13,16-18} We did not find any association with short-term survival when studying changes in the TBB, as others have failed to do;\textsuperscript{17} however, only 5 (9 percent) of our patients died in the course of the PCP. We also studied whether changes in the TBB and BALF could predict changes in long-term survival, but our data failed to show such an association. These negative results contrast with previous findings,\textsuperscript{17} where patients with HIV-related PCP who had large amounts of PC and a severe degree of edema in the initial TBB had significantly lower long-term survival (ie, observation time, 12 months). The time span between the present study and the studies by Brenner et al\textsuperscript{17} and others\textsuperscript{2,3,13} may explain the discrepancies, since antiviral therapy, treatment of opportunistic infections, and presumably earlier diagnosis of PCP have improved in the last 5 years.\textsuperscript{19-41}

The data on morphology presented in this report could have been influenced by the pathologist studying both the amount of PC and the degree of inflammation in the same specimen; however, several points argue against the data being confounded. First, the amount of PC and the changes in host cells were evaluated using different stains of the material. Secondly, the study was done prospectively, and the hypotheses tested in this material were addressed by the clinicians. Thirdly, the finding that the degree of edema and type 2 pneumocyte proliferation in the TBB correlated with the amount of PC in the BALF strongly argues for a real association between these parameters in the lung of patients with AIDS who have PCP. Future investigations should focus on the identification of mediators and cells of importance in the inflammatory component of the development of PCP.

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