Clearance of *Pneumocystis carinii* Cysts in Acute *P. carinii* Pneumonia* 
Assessment by Serial Sputum Induction

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**Study objectives:** To determine the feasibility of repeat sputum induction in acute *Pneumocystis carinii* pneumonia (PCP) and to define the rate of clearance of *P. carinii* cysts from the respiratory tract of HIV-seropositive patients with acute PCP.

**Design:** Prospective cohort evaluation.

**Setting:** University medical center.

**Participants:** Twenty-four HIV-seropositive subjects with acute PCP.

**Measurements:** Sputum induction for *P. carinii* 2, 3, 4, and 6 weeks after initial diagnosis, and follow-up for 1 year.

**Results:** Eighty-eight percent of subjects had residual cysts at 2 weeks, 76% at 3 weeks, 29% at 4 weeks, and 24% at 6 weeks postdiagnosis. A prior AIDS-defining illness (p = 0.033) or prior PCP (p = 0.004) predicted relapse within 6 months, but persistent cysts at 3 weeks did not; 8 of 16 sputum-positive subjects and 1 of 5 sputum-negative subjects experienced a relapse within 6 months (p = 0.34). Secondary prophylaxis with trimethoprim-sulfamethoxazole was associated with a reduced risk of relapse.

**Conclusions:** Serial sputum induction coupled with direct fluorescent antibody staining is a feasible, noninvasive method of respiratory tract surveillance for the eradication of *P. carinii* during and after acute PCP. Three-quarters of HIV-seropositive patients with acute PCP have persistent cysts in their lungs at the end of antimicrobial treatment, despite clinical recuperation, but only one quarter have residual cysts 6 weeks postdiagnosis. A prior AIDS-defining illness and prior PCP are positively associated, and subsequent trimethoprim-sulfamethoxazole prophylaxis is negatively associated, with relapse within 6 months, while persistent organisms at 3 weeks do not appear to be a significant predictor of relapse risk.

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Key words: HIV infection; *Pneumocystis carinii* pneumonia; sputum induction

Abbreviations: DFA = direct fluorescent antibody; LDH = lactate dehydrogenase; NS = not significant; PCP = *Pneumocystis carinii* pneumonia

*Pneumocystis carinii* pneumonia (PCP) emerged in the early 1980s as the most common manifestation of AIDS and the leading cause of death in individuals infected with HIV. The prognosis for an individual with acute PCP has improved significantly over the past decade, due to the introduction of antiretroviral agents, earlier recognition of the symptoms and signs of PCP, more rapid and accurate diagnostic techniques, and adjunctive therapies such as corticosteroids. PCP remains the second leading cause of death in HIV-infected persons in the United States. Despite >15 years of experience with AIDS-related PCP, the time course of organism eradication after initiation of treatment for acute PCP remains incompletely defined.

Persistence of *P. carinii* organisms despite antimicrobial therapy was documented before the advent of AIDS. Early in the AIDS epidemic, investigators established that AIDS patients with acute PCP had
more frequent clinical and histologic evidence of persistent infection than other immunocompromised hosts.7 Prospective studies in the mid-1980s involving a single follow-up bronchoscopy confirmed the high rate of organism persistence posttreatment.8,9

In the decade since these bronchoscopic studies were published, the diagnostic approach to the HIV-infected individual with acute PCP has evolved significantly. Sputum induction with hypertonic saline solution can now provide a definitive noninvasive diagnosis of PCP in 70 to 80% of HIV-infected individuals with acute PCP.10 The diagnostic sensitivity of sputum induction coupled with direct fluorescent antibody (DFA) staining of the specimen can exceed 90%,11 approaching that of bronchoscopy with BAL.12

We sought to determine the feasibility and utility of serial sputum induction in acute PCP. Employing this procedure in a prospective cohort design, we undertook to determine the rate of clearance of P. carinii organisms in HIV-seropositive patients with acute PCP in the present era.

MATERIALS AND METHODS

Inpatients and outpatients with documented HIV infection who had PCP definitively diagnosed (by demonstration of P. carinii cysts in respiratory secretions) at Brigham and Women's Hospital were considered for enrollment. Potential subjects were excluded if any one of the following exclusion criteria were present: inability to give written informed consent, life expectancy <3 months, or inability to return for appointments. The study was approved by the Human Research Committee of Brigham and Women's Hospital.

After giving informed consent, each subject was scheduled to undergo repeated sputum induction at 2 weeks, 3 weeks, 4 weeks, and 6 weeks after the date of initial diagnosis of PCP (Fig 1). When the induced sputum specimen showed no cysts, the subject was considered to have cleared the organisms, and no further inductions were performed. The subject was then followed up for 1 year after the initial diagnosis for evidence of PCP relapse and death.

Each of the repeated visits for sputum induction and specimen evaluation was performed according to a standard protocol. The subject inhaled a 3% saline solution mist from an ultrasonic nebulizer (DeVilbiss Ultra-Neb 99; Somerset, PA) for up to 45 min until a minimum of 5 mL of sputum was produced. The collected specimen was processed and stained with a fluorescein isothiocyanate-conjugated murine anti-P. carinii monoclonal antibody specific for an antigen on the cyst wall (Genetic Systems; Seattle, WA). The slides were read by experienced personnel (W.P. and J.S.) using fluorescence microscopy. A sputum specimen was considered to be positive for P. carinii if two or more typical cysts were clearly visualized on the slide.11

Demographic and clinical data were collected for each patient and have been summarized as mean ± SD unless otherwise specified. The chest radiographic pattern was considered atypical for PCP if the staff radiologist interpreted it as normal or noted predominantly upper-lobe, asymmetric, or cystic disease.13

The major outcomes of interest were PCP relapse within 6 months of diagnosis and death within 1 year of the initial episode. Relapse of PCP was defined as the recurrence of symptoms and radiographic findings consistent with acute PCP and the demonstration of P. carinii in sputum or bronchoscopic specimens. The presence or absence of P. carinii cysts at 3 weeks was analyzed both as a predictor of relapse or death and as an outcome itself, to explore possible predictors of persistent organisms. Highly skewed variables were transformed prior to inference testing. Continuous variables were compared using Student’s t test and categorical variables using Fisher’s Exact Test. All analyses were two tailed and were performed using software (SAS; SAS Institute; Cary, NC).

RESULTS

Twenty-two men and one woman were enrolled during 24 episodes; one subject participated during 2 episodes of acute PCP. Mean age was 37.8±6.4 years (Table 1). Seventeen were white, 4 were African-American, 1 was Hispanic, and 1 was Asian. HIV risk groups represented included the following: 16 homosexual men, 3 IV drug users, 2 hemophiliacs, 1 man with high-risk heterosexual contact, and 1 man with unknown risk factors. The mean duration of HIV serodiagnostics was 2.6±2.5 years, and the mean CD4 count was 69.8±83×10⁶/L. Nearly half (46%) had previously suffered an AIDS-defining illness, 29% had a history of prior PCP, 50% were receiving anti-PCP prophylaxis (either dapsone or aerosolized pentamidine or both), and 38% were receiving antiretroviral therapy. One-third of subjects were diagnosed as HIV-seropositive during this acute illness.

Clinical characteristics at presentation were notable for the following: symptoms for a mean of 19.0±20.1 days, mean lactate dehydrogenase (LDH) level of 466±336 IU/L (reference range, 107 to 231 IU/L), a chest radiograph pattern atypical for PCP in 5 (21%), and an initial room-air oxygen saturation of ≤ 90% in 25%. All subjects received 3 weeks of treatment with one or more generally accepted antimicrobial regimens (trimethoprim-sulfamethoxazole, dapsone-trimethoprim, pentamidine, clindamycin-primaquine), at the discretion of the subject’s primary care physician. All subjects were begun on a regimen of anti-Pneumocystis treatment within 12 h of diagnosis. Fifty-eighth percent received corticosteroids for significant hypoxemia, as judged by the primary physician. One-third of subjects were diagnosed and treated entirely as outpatients.
Table 1—Clinical Data and Outcomes for the Entire Cohort and for Sputum-Positive and Sputum-Negative Subgroups

<table>
<thead>
<tr>
<th></th>
<th>All Subjects (n = 24)</th>
<th>Sputum Positive at 3 wk (n = 16)</th>
<th>Sputum Negative at 3 wk (n = 5)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>37.8 ± 6.4</td>
<td>37.0 ± 6.6</td>
<td>37.6 ± 3.1</td>
<td>NS*</td>
</tr>
<tr>
<td>Male, No. (%)</td>
<td>23 (96)</td>
<td>15 (94)</td>
<td>5 (100)</td>
<td>NS*</td>
</tr>
<tr>
<td>Nonwhite, No. (%)</td>
<td>6 (25)</td>
<td>5 (31)</td>
<td>1 (20)</td>
<td>NS*</td>
</tr>
<tr>
<td>IV drug use, No. (%)</td>
<td>3 (13)</td>
<td>3 (19)</td>
<td>0 (0)</td>
<td>NS*</td>
</tr>
<tr>
<td>Hospitalized, No. (%)</td>
<td>16 (67)</td>
<td>11 (69)</td>
<td>4 (80)</td>
<td>NS*</td>
</tr>
<tr>
<td>Duration of HIV diagnosis, yr</td>
<td>2.6 ± 2.5</td>
<td>2.5 ± 2.6</td>
<td>3.4 ± 2.5</td>
<td>NS*</td>
</tr>
<tr>
<td>Prior AIDS-defining illness, No. (%)</td>
<td>11 (46)</td>
<td>8 (50)</td>
<td>2 (40)</td>
<td>NS*</td>
</tr>
<tr>
<td>Prior PCP, No. (%)</td>
<td>7 (29)</td>
<td>6 (38)</td>
<td>1 (20)</td>
<td>NS*</td>
</tr>
<tr>
<td>Antiretroviral therapy, No. (%)</td>
<td>9 (38)</td>
<td>7 (44)</td>
<td>1 (20)</td>
<td>NS*</td>
</tr>
<tr>
<td>Prior PCP prophylaxis, No. (%)</td>
<td>12 (50)</td>
<td>8 (50)</td>
<td>2 (40)</td>
<td>NS*</td>
</tr>
<tr>
<td>Duration of symptoms, d</td>
<td>19.0 ± 20.1</td>
<td>19.8 ± 23.2</td>
<td>13.6 ± 9.6</td>
<td>NS*</td>
</tr>
<tr>
<td>Initial oxygen saturation (room air)</td>
<td>93.9 ± 4.3</td>
<td>93.5 ± 5.0</td>
<td>94.4 ± 3.6</td>
<td>NS*</td>
</tr>
<tr>
<td>LDH, IU/mL</td>
<td>465 ± 336</td>
<td>495 ± 399</td>
<td>394 ± 107</td>
<td>NS*</td>
</tr>
<tr>
<td>Serum sodium, mmol/dL</td>
<td>136 ± 2.8</td>
<td>137 ± 3.1</td>
<td>135 ± 1.5</td>
<td>NS*</td>
</tr>
<tr>
<td>Serum albumin, g/L</td>
<td>34 ± 5</td>
<td>35 ± 5</td>
<td>36 ± 2</td>
<td>NS*</td>
</tr>
<tr>
<td>WBC count, ×10^9/L</td>
<td>5.6 ± 2.5</td>
<td>5.7 ± 1.8</td>
<td>6.4 ± 4.0</td>
<td>NS*</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.35 ± 0.6</td>
<td>0.35 ± 0.06</td>
<td>0.38 ± 0.07</td>
<td>NS*</td>
</tr>
<tr>
<td>Corticosteroid therapy, No. (%)</td>
<td>14 (58)</td>
<td>10 (63)</td>
<td>3 (60)</td>
<td>NS*</td>
</tr>
<tr>
<td>Helper T-cell count, ×10^9/L</td>
<td>69.8 ± 53.3</td>
<td>61.7 ± 60.4</td>
<td>89.6 ± 150.9</td>
<td>NS*</td>
</tr>
<tr>
<td>Secondary prophylaxis with T-S,† No. (%)</td>
<td>7 (29)</td>
<td>4 (25)</td>
<td>1 (20)</td>
<td>NS*</td>
</tr>
<tr>
<td>Atypical chest radiograph, No. (%)</td>
<td>5 (21)</td>
<td>1 (6)</td>
<td>2 (40)</td>
<td>p = 0.13</td>
</tr>
<tr>
<td>Relapse within 6 mo, No. (%)</td>
<td>9 (38)</td>
<td>8 (50)</td>
<td>1 (20)</td>
<td>NS*</td>
</tr>
<tr>
<td>Death within 1 y, No. (%)</td>
<td>7 (29)</td>
<td>4 (25)</td>
<td>2 (40)</td>
<td>NS*</td>
</tr>
</tbody>
</table>

*NS = p > 0.20.
†T-S = trimethoprim-sulfamethoxazole.

Nineteen subjects participated in all visits of the trial until completion or death, including two subjects who died during the fourth week postdiagnosis. The five subjects who dropped out (three after the 2-week visit and two after the 3-week visit) were available for ascertainment of relapse and death. These subjects were similar in clinical and laboratory characteristics to the majority completing the trial (age 37.9 vs 37.6 years, duration of symptoms 19.2 vs 18.2 days, oxygen saturation 93.3% vs 96.0%, LDH 486.2 vs 350.5 IU/mL, CD4 count 73.6 vs 60.2 × 10^6/L, corticosteroid therapy in 63% vs 40%, post-episode prophylaxis with trimethoprim-sulfamethoxazole in 26% vs 40%, relapse in 42% vs 20%; p = not significant [NS] for all comparisons).

During the 3 weeks of antimicrobial treatment, most subjects had persistent organisms, but thereafter the proportion of sputum-positive patients declined abruptly (Fig 2). Twenty-one of 24 subjects (88%) were sputum positive by the end of the second week of treatment, and 16 of 21 (76%) remained sputum positive at the 3-week point. By the end of the fourth week, 5 of the remaining 17 subjects (29%) had evidence of P. carinii in their sputum. Six weeks post-diagnosis (3 weeks after completion of a standard 3-week course), 4 of the 17 subjects (24%) were still sputum positive. Despite these findings of persistent infection, all subjects had experienced resolution of symptoms and radiographic improvement, and had been discharged from the hospital by the 3-week point. Two of the 24 subjects (9%) developed an early relapse of acute PCP during week 4 and died of hypoxemic respiratory failure. Seven

![Figure 2: Cyst clearance from sputum. Percentage of subjects with an induced sputum specimen positive for P. carinii cysts at 2, 3, 4, and 6 weeks following the diagnosis of PCP.](http://JournalPublications.Chestnet.org/pdfsaccess.aspx?url=data/journals/chest/21874/ on 04/03/2017)
more subjects (25%) suffered a relapse within 6 months after initial PCP diagnosis. The 1-year mortality rate for all enrollees was 29% (7/24).

No demographic or clinical variable features were significantly associated with persistence of cysts in the sputum at 3 weeks (Table 1). While 8 of 16 subjects with persistent organisms at 3 weeks suffered a relapse within 6 months, only 1 of 5 with a negative 3-week sputum examination suffered a relapse, but this difference did not reach statistical significance (p = 0.34). Relapse within 6 months was significantly associated with a prior AIDS-defining illness—7 of 11 (64%) experienced a relapse vs 2 of 13 (15%) without prior AIDS (p = 0.033)—and with a prior episode of PCP (6 of 7 [86%] vs 3 of 17 [18%], p = 0.004). Post-episode prophylaxis with trimethoprim-sulfamethoxazole was associated with a reduced risk of recurrent PCP (0 of 7 [0%] vs 7 of 15 [47%], p = 0.05).

**DISCUSSION**

In this prospective study of HIV-seropositive patients with acute PCP, serial sputum induction and DFA staining demonstrated that three quarters of the subjects had evidence of persistent organisms in their lungs at the end of a standard 3-week course of antimicrobial therapy. Six weeks post-diagnosis, 24% still had positive findings on sputum examinations. Subjects with persistent organisms at 3 weeks had a higher frequency of early relapse, but this did not reach statistical significance. A history of a prior AIDS-defining illness or PCP was associated with an increased risk of early relapse, while patients receiving secondary prophylaxis with trimethoprim-sulfamethoxazole experienced a reduced risk of relapse.

Prior to the era of AIDS, open-lung biopsy studies had documented the persistence of *P. carinii* cysts despite the institution of therapy.6 Subsequent lung biopsy and autopsy reports suggested that AIDS patients cleared the organisms more slowly than other immunosuppressed patients.7 Several later drug treatment trials employed a single follow-up bronchoscopic examination in HIV-seropositive patients with acute PCP. Residual organisms were found in the lungs of 63 to 73% of AIDS patients with acute PCP at the conclusion of 3 weeks of treatment with various conventional and experimental regimens.8,14,15

To our knowledge, this study is the first in which repeated sampling of the lower respiratory tract was performed during and after a standard course of therapy to determine the rate of *P. carinii* clearance in acute PCP. The diagnostic efficacy of the sampling technique, sputum induction with DFA specimen staining, is well established, with sensitivity rates of ≥ 90% for *P. carinii* in HIV-seropositive patients11,16 and 94% at our institution (unpublished data). In prior studies, the invasive nature of bronchoscopic surveillance may have reduced subject participation; in a recent report, only 24% of subjects submitted to the 3-week bronchoscopy.15 Sputum induction, in contrast, is simple, noninvasive, and well tolerated; 88% of our subjects completed the protocol through the end of therapy (3 weeks), a rate exceeding the best figure for the proportion of participants submitting to a single post-treatment bronchoscopy (82%).8

Our results obtained via serial sputum induction confirm and extend the data from single time point bronchoscopic studies of a decade ago. The finding in this study that nearly 90% of subjects with acute PCP harbor persistent cysts at 2 weeks post-diagnosis represents an original observation, as no other study entailed reassessment at that interval. Detection of residual cysts in 76% of our subjects at 3 weeks and in 29% at 4 weeks post-diagnosis agrees with the single time point bronchoscopic results of Wharton et al9 and Delorenzo et al9 who found persistent cysts in 64% and 38% at those intervals, respectively. The progressive decline in the proportion of subjects with residual cysts in this study is consistent with both animal and human data documenting eventual eradication of cysts during the year following an acute bout of PCP.17,18

Persistent cysts post-treatment in this study did not correlate with clinical severity at presentation or with the risk of subsequent relapse or death. Residual cysts after 3 to 4 weeks of treatment was associated with an increased risk of death within 8 months in an early report by Brenner et al.19 However, that retrospective case-series summarized findings from patients who had usually undergone follow-up examination for persistent or recurrent symptoms. In the present study, as in prior prospective bronchoscopic studies, there was little correlation between clinical severity at presentation or clinical status at 3 weeks and the presence of persistent organisms.

Furthermore, the failure to clear cysts from the lung in this study did not predict relapse, a finding consistent with those of previous studies.8,9 Follow-up for 1 year post-diagnosis in the present investigation makes it unlikely that a significant adverse association was missed. Other clinical features such as a prior AIDS-defining illness appear to be more powerful determinants of relapse risk than persistent organisms. The use of trimethoprim-sulfamethoxazole as secondary prophylaxis was associated with a reduced risk of relapse and might have attenuated any potential risk associated with persistent cysts. Thus, we cannot exclude the possibility
that an HIV-seropositive patient with persistent *P. carinii* at the end of therapy who fails to receive subsequent trimethoprim-sulfamethoxazole prophylaxis might be at an increased risk of relapse in the next 6 months, especially in the context of AIDS-defining illness.

This study has limitations in the patient population studied and the procedures employed. Five subjects failed to complete all aspects of the study; however, their presenting characteristics and subsequent course were not significantly different from those who finished the trial, and the completion rate equaled or exceeded that for any analogous study. The sample size precluded multivariate analysis or the assessment of predictors of clearance at other time points, and may have limited our ability to define the prognostic importance of persistent organisms. However, to demonstrate a significant difference in relapse rates between those with persistent or eradicated organisms at 3 weeks would have required a cohort of > 100 subjects.20 False-positive findings due to cross-reactivity with Candida species21 or false-negative results in the absence of a confirmatory bronchoscopy are possible, but performance of smear readings by experienced personnel and the concordance of our results with those from prior bronchoscopic series makes such misinterpretations unlikely. Finally, quantification of sputum cyst burden and determination of cyst viability were not performed, because such methods are not well established.

This study demonstrates the feasibility and utility of repeated sputum induction in the setting of PCP, and suggests that this inexpensive and noninvasive procedure may be useful in future investigations to clarify the natural history of PCP during and after acute treatment. The high frequency of persistent cysts despite treatment suggests that detection of *P. carinii* immediately after therapy for acute PCP should not be considered *de facto* residual or relapsed disease. Residual organisms would be expected to clear without further treatment in three quarters of cases by 6 weeks post-diagnosis. Future trials incorporating one or more repeated sputum inductions for *P. carinii* post-diagnosis might explore strategies such as short-course therapy in those who cleared their infection within 2 weeks.

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REFERENCES


