A Comparison of Induced and Expectorated Sputum for the Diagnosis of Pneumocystis carinii Pneumonia*

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Study objectives: To compare the sensitivities of expectorated and induced sputum for the diagnosis of Pneumocystis carinii pneumonia (PCP).

Design: Retrospective review.

Setting: Academic medical center.

Patients: Forty-five patients diagnosed as having PCP who had direct fluorescent antibody testing for P carinii on either expectorated or induced sputum.

Results: Patients were stratified according to the method of sputum production (induced vs expectorated). The two groups were similar with respect to demographic characteristics, use of prophylaxis with aerosolized pentamidine, serum lactate dehydrogenase level, and arterial oxygen level. When only the initial sputum for each patient was analyzed, there was a similar sensitivity of induced sputum, positive in 10 of 18 samples (56%), and expectorated sputum, positive in 14 of 27 samples (52%) (p>0.05).

Conclusion: There was no significant difference in the sensitivity of induced and expectorated sputum for the diagnosis of PCP when the direct fluorescent antibody method of staining was used.

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Key words: AIDS; Pneumocystis carinii; pneumonia; sputum

Abbreviations: DFA=direct fluorescent antibody; PCP=Pneumocystis carinii pneumonia

Pneumocystis carinii pneumonia (PCP) remains an important cause of morbidity and mortality in HIV-infected individuals.1 It was reported in the mid-1980s that the examination of sputum induced by the inhalation of hypertonic saline solution was frequently diagnostic for PCP.2,3 Thereafter, this method of diagnosis generally became the first employed when PCP was suspected. While sputum induction may be less invasive and less expensive to perform than bronchoscopy, its cost is not trivial, having been reported to be approximately 40% as costly as bronchoscopy.4 In many centers, the prevalence of PCP in patients referred for sputum induction is <50%,1,4-6 leading to further questions regarding the cost-effectiveness of the procedure.1,5

The yield of sputum induction has been reported to be improved with the use of some of the more recently developed, more sensitive methods of identifying P carinii, such as the direct fluorescent antibody7 (DFA) or the polymerase chain reaction.8 The increased sensitivity of these methods introduces the possibility that expectorated sputum obtained without the use of induction would be adequate for diagnosis of PCP, but there are little available data that address this issue. However, using oropharyngeal washings, Wakefield et al9 reported a 78% yield of DNA amplification for the diagnosis of PCP. These results suggest that some lower respiratory tract secretions containing the organism frequently reach the oropharynx and might be produced without the need for induction.

We recently reported a sensitivity of 56% using spontaneously expectorated sputum stained with the PCP-DFA,10 comparable to the sensitivity reported with induced sputum by many centers.2-4 However, the conclusions that could be drawn from this study were somewhat limited by the lack of a comparison group who underwent sputum induction. In the current study, we report the

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sensitivity of induced and expectorated sputum for the
diagnosis of PCP over a 5-year period at a
different institution.

MATERIALS AND METHODS

This study was performed at the University of Connecticut
Health Center, a 232-bed teaching hospital. A retrospective
review of cases of PCP diagnosed between January
1, 1991, and January 31, 1996, was performed. Cases were identified by two
methods. Review of the microbiology laboratory records yielded
all confirmed cases of PCP, including those treated on an
outpatient basis. In addition, a medical records search using the
diagnostic code for PCP yielded all patients discharged from the
hospital with a diagnosis of PCP, allowing capture of patients who
were treated empirically after nondiagnostic studies. Patients
were considered for inclusion in the study if they had sputum
induction performed or if they had expectorated sputum sent to
the laboratory for PCP-DFA staining. Samples obtained by
endotracheal suctioning were not considered. Patients who were
not HIV infected were excluded from the study. Data abstracted
from the chart included demographics, risk factors for HIV
infection, methods of PCP diagnosis, and clinical data, including
type of PCP prophylaxis, hospital admission arterial blood gas
analysis, lactate dehydrogenase level, chest radiograph results,
and outcome (survival or death).

A case of PCP was defined as occurring if a respiratory sample
was diagnostic for PCP. Additionally, patients with a hospital
discharge diagnosis of PCP without a confirmatory clinical sample
were considered as having PCP if review of medical records
revealed a clinical course consistent with PCP unless the following
occurred: (1) the patient’s condition improved without anti-
Pneumocystis therapy; (2) an autopsy showed no evidence of
PCP; or (3) a definitive alternate diagnosis was obtained. The
case definition was purposely made inclusive rather than exclusive
in order to prevent exclusion of patients who had false-
negative results of diagnostic studies. In this way, we hoped to
avoid reporting a falsely elevated yield of sputum studies.

The sputum induction technique was performed using a
modification of the technique reported by Zaman et al.11 Patients
were instructed to gargle with tap water to clear the oropharynx.
A minimum of 100 mL of 3% saline solution was added to a jet
nebulizer (Bard-Parker; Professional Medical Products; Green-
wood, SC) that was run using compressed oxygen at a flow rate of
10 L/min. This resulted in aerosolization of approximately 1 mL/min.
After approximately 15 min, if there had been no spontaneous cough with sputum production, the patient
was asked to take several deep breaths to promote coughing. If no
sputum was produced, then the procedure was continued for a
maximum of 30 min. Induction was done at the request of the
treating physician; there was no screening performed.

In general, any sample was accepted for processing, even if
microscopically, the specimen appeared to be mostly saliva. For
unclear reasons, during the period studied, one sputum sample
was not processed due to its microscopic appearance. Specimens
were processed by mixing equal volumes of sputum and normal
saline solution and then vortexing for 1 min. From each sample,
a 200-μL aliquot was cytopsued onto a glass slide at 750 rpm for
6 min. Slides were air dried, heat fixed, and then placed in
acetone for 10 min. The acetone-fixed slides were stained with
the fluorescein monoclonal antibody reagent according to the
manufacturer’s instructions (Genetic Systems; Redmond, Wash.).
The entire smear was scanned at ≥400X magnification with a
fluorescent microscope using a wavelength range of 420 to 490
nm with a 515-nm barrier filter. To be defined as positive,
characteristic round to elliptical-shaped cysts or trophozoites,
stained bright apple-green, needed to be present. No other
staining techniques were used routinely.

Statistical analysis was performed using the unpaired t test or
Fisher’s Exact Test, except for the comparison of chest radiograph patterns, for which the χ² test was used. Statistical
significance was accepted at p<0.05. Results are presented as
mean ± SD.

RESULTS

During the study period, 54 cases of PCP were identified that met the inclusion criteria. Of these,
nine had an unsuccessful sputum induction and no
sputum sample was obtained subsequently. Seven of
these cases were confirmed at bronchoscopy and two
patients were treated empirically.

There were 45 cases of PCP in which one or more
sputum samples were obtained. Of these, 35 (78%)
were confirmed by a diagnostic sputum or BAL,
while 10 (22%) were diagnosed on clinical grounds
and the patients were treated empirically after non-
diagnostic studies. For the purposes of the data
analysis, patients were stratified according to the
method that was used to obtain the first sputum
sample if more than one sputum sample was ob-
tained. This was done because of the possibility that
patients would be more likely to have sputum induc-
tion performed if an initial expectorated sputum
sample was negative. This could lead to bias as
patients who produced a nondiagnostic sputum sam-
ple might be likely to continue to do so with subsequent attempts.

Table 1 shows the demographic and clinical charac-
teristics of the patients when stratified according

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<th>Table 1—Baseline Patient Characteristics*†</th>
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<tr>
<td>Age, yr</td>
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<tr>
<td>Risk factor for HIV infection</td>
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<tr>
<td>IV drug use</td>
</tr>
<tr>
<td>Male homosexual</td>
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<tr>
<td>Other</td>
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<td>No. (%) receiving prophylaxis with aerosolized pentamidine</td>
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<td>Lactate dehydrogenase, U/L</td>
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<td>Arterial PO₂ on room air†</td>
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<td>Chest radiograph pattern (%)</td>
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<tr>
<td>Diffuse infiltrates</td>
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<tr>
<td>Upper lobe or focal infiltrates</td>
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<tr>
<td>No infiltrates</td>
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<tr>
<td>Mortality</td>
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*Patients were stratified according to method of obtaining first sputum sample if more than one sample was obtained.
†p>0.05 for all comparisons.
‡Values not available for total patient group.
to the method of obtaining the first sputum sample (induced vs expectorated). There were no statistically significant differences between the groups. Figure 1 shows the ultimate outcome for each patient. Table 2 reveals that there was no difference between the sensitivity of the initial induced and expectorated sputa in these patients. When patients with proved PCP are analyzed separately, the apparent sensitivities increase for both expectorated sputum, of which 14 of 22 (64%) were diagnostic, and induced sputum, of which 10 of 13 (77%) were diagnostic, but there remains no significant difference between the two groups. When sputa obtained subsequent to the initial sample are considered, one of seven induced sputa (14%) were positive and four of nine expectorated sputa (44%) were positive (p>0.05). There were no significant complications of sputum induction, although one patient complained of nausea such that he refused a subsequent attempted induction, but instead underwent bronchoscopy.

Bronchoscopy was performed after negative sputum samples eight times, four after an initial negative expectorated sputum and four times after an initial negative induced sputum. Three of four (75%) yielded a diagnosis of PCP in each group. The two patients with normal bronchoscopies were nevertheless treated for PCP, due to their suggestive clinical presentation.

**DISCUSSION**

Although the use of sputum induction has simplified the diagnosis of PCP in many patients, the
associated costs approach 40% of the cost of using bronchoscopy. The noninvasive nature of the procedure when compared with bronchoscopy has led to its overuse in many centers, creating significant problems relating to resource utilization and cost. This issue is further exacerbated by the fact that in many centers, most patients for whom sputum induction is performed do not actually have PCP. These issues have prompted extensive research into the cost-effectiveness of various strategies for diagnosing PCP as well as the development of methods of restricting the use of sputum induction.

Surprisingly, given the introduction of more sensitive methods for identifying P. carinii in biological samples, these concerns have not prompted reexamination of the assumption that expectorated sputum is inadequate for the diagnosis of PCP. Although PCP rarely causes a cough productive of large amounts of sputum, it has been our experience that when asked, many PCP patients can produce scant but diagnostic samples, without the use of sputum induction. Indeed, we have submitted samples of expectorated sputum that upon visual inspection appeared to consist of saliva alone, but were unambiguously diagnostic for PCP.

In this retrospective study, we found that the sensitivity of induced sputum for the diagnosis of PCP was similar to that of expectorated sputum. Although others have reported sensitivities of induced sputum higher than the 55% obtained in the current study, for unclear reasons, many have reported sensitivities in that range or significantly lower, using both tinctorial staining procedures or the DFA. Furthermore, the reported sensitivities are often for the sputum analysis and therefore do not take into account the number of failed inductions. We and others have noted a significant failure rate of sputum induction, further impacting the diagnostic yield and cost-effectiveness of the procedure. It must be noted that since this was not a randomized study, the sensitivity of sputum induction may have been negatively impacted by preferential referral of patients who had already demonstrated an inability to produce an expectorated sputum. However, there was no way of reliably determining to what degree this effect was present from the chart review.

The results of this study may not be directly applicable to all other institutions that rely on sputum induction. Although the reported sensitivity of induced sputum for the diagnosis of PCP varies widely, it is unknown whether the sensitivity of expectorated sputum is also variable. However, our reported sensitivities of 52% in this study and 56% in our prior study, performed at a different institution, are quite consistent. Institutions that are not able to achieve a higher sensitivity using sputum induction might use fewer resources by relying on expectorated sputum, and if indicated, proceeding to bronchoscopy after a negative expectorated sputum sample. At our institution, the prevalence of PCP among HIV-infected individuals is approximately 30%, yielding a negative predictive value of only 84%, so bronchoscopy is frequently performed after a negative sputum study in patients with a high clinical likelihood of having PCP. Since the factors leading to the variable success with sputum induction are not well understood, each institution may need to examine its own results with expectorated and induced sputum as well as the prevalence of PCP in their patient population before determining the appropriate sampling method.

In summary, we did not find any difference between the sensitivity of induced and expectorated sputum for PCP at our institution. Because of the tremendous variation in the reported sensitivity of induced sputum, these results may not be applicable to all centers. However, given the large amount of resources expended performing sputum induction at many institutions, and the possibility introduced by the results of this study that such efforts may not always lead to an improved yield, further study of this issue is warranted. Such investigation may be especially productive if the extremely sensitive methods employing DNA amplification become widely used, as such methods might further increase the sensitivity of the examination of expectorated sputum.

**Table 2—Diagnostic Yield of Sputum Samples During 45 Episodes of PCP**

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<tr>
<th>Sample Type</th>
<th>Induced Sputum (%)</th>
<th>Expectorated Sputum (%)</th>
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<tr>
<td>Diagnostic yield of first sample</td>
<td>10/18 (56)</td>
<td>14/27 (52)</td>
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<tr>
<td>Diagnostic yield of subsequent samples</td>
<td>1/1 (14)</td>
<td>4/9 (44)</td>
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*p > 0.05 for comparisons between induced and expectorated sputum as well as for comparisons between first and subsequent samples.

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