Pneumocystis carinii Pneumonia Diagnosed by Non-Induced Sputum Stained with a Direct Fluorescent Antibody*

ALBERT L. RAFANAN, M.D.†
PAULA KLEVJER-ANDERSON, Ph.D.‡
and MARK L. METERSKY, M.D.§

†Pulmonary and Critical Care Division
Cleveland Clinic Foundation
Cleveland, OH 44106
and
‡Department of Pathology and Laboratory Medicine
St. Francis Hospital
Hartford, CT 06105
and
§Pulmonary Division
University of Connecticut School of Medicine
Farmington, CT 06030

ABSTRACT

Non-induced expectorated sputum (NIS) stained with tinctorial stains is not considered useful in the diagnosis of Pneumocystis carinii pneumonia (PCP). The diagnostic yield of NIS was evaluated in human immunodeficiency virus (HIV)-infected patients, when stained with a more sensitive direct fluorescent antibody for PCP-direct fluorescent antibody (PC-DFA). A retrospective analysis was carried out on fifty-five HIV-infected patients with PCP, who had NIS submitted for staining with PC-DFA. Thirty had positive NIS with PC-DFA and all had clinical courses consistent with PCP. Twenty-five had negative NIS with PC-DFA and were diagnosed as having PCP, by autopsy (n = 2), by a positive bronchoalveolar lavage (n = 10), or by having a clinical course consistent with PCP (n = 13). Thus, the sensitivity of NIS stained with PC-DFA was 55 percent (30/55). This is within the range reported in the literature for induced sputum for the diagnosis of PCP. Non-induced sputum stained with PC-DFA can be useful for the diagnosis of PCP in HIV-infected patients.

Introduction

During the initial years of the acquired immunodeficiency syndrome (AIDS) epidemic, Pneumocystis carinii pneumonia (PCP) was most commonly diagnosed by specimens obtained with bronchoscopy. This approach was based on prior studies in oncology patients that reported that the sensitivity of non-induced spontaneously expectorated sputum (NIS) stained with Grocott’s modified Gomori methenamine silver stain (GMS) to be poor.¹² The NIS from HIV-infected patients was

* Send reprint requests to: Mark Metersky, M.D., Pulmonary Division MC-1225, University of Connecticut Health Center, 263 Farmington Ave., Farmington, CT 06030.
believed to have a similar poor sensitivity, and it had even been suggested that NIS should not be accepted for processing. The sensitivity of sputum stained with GMS was increased to 55 percent, by induction with inhaled hypertonic saline. Thereafter, induced sputum stained with GMS was recommended as the initial diagnostic test for PCP. However, this has not been uniformly accepted as many centers have reported sensitivities as low as 10 to 34 percent.

A further improvement in the sensitivity of diagnostic tests was achieved with the development of a direct fluorescent antibody to human \textit{P. carinii} (PC-DFA). It was shown that PC-DFA testing improved the sensitivity of induced sputum, compared to GMS and Giemsa. To our knowledge, the clinical utility of NIS stained with the more sensitive PC-DFA has not yet been studied. The examination of NIS with PC-DFA has been routinely used to diagnose PCP in HIV-infected patients at Saint Francis Hospital and Medical Center (SFHMC) since 1989. This reports our experience with this technique.

**Methods**

**Patients**

St. Francis Hospital and Medical Center (SFHMC) is an inner city, 420 bed teaching hospital located in Hartford, CT, with a high AIDS diagnosis rate. The medical records of hospitalized patients with a Diagnosis Related Group (DRG)-coded diagnoses of PCP, between 1992 to 1994, were reviewed. Charts were then selected for HIV-infected patients; only patients with NIS sent to the laboratory for PC-DFA were studied, as sputum induction was rarely performed. Cross reference with records in the microbiology department was done to ensure that no case of PCP was missed. Data abstracted included demographics, risk factors for HIV acquisition, methods of diagnosis, admission pO2 (mmHg), lactate dehydrogenase (U/L), chest roentgenograms, clinical courses, treatment responses and autopsy reports.

**Sputum Preparation and Staining**

At SFHMC, it is general practice to attempt to obtain a NIS for PC-DFA from patients suspected of having PCP. Sputum samples were collected in a sterile collection cup. Patient preparation was not observed, nor were samples screened prior to staining with PC-DFA. Mucolysis, concentration, and preparation of smears were performed similarly to methods described by Zaman et al. Specimens were mucolysed (0.5 percent N-acetyl-L-cysteine, 15 min, room temperature) and concentrated by centrifugation (1500 x g, 10 min). Duplicate smears were made from the pellet. The smears were air dried, heat fixed, then placed in cold acetone for 10 min. Each slide was stained for \textit{P. carinii} with a fluorescent monoclonal antibody reagent according to the manufacturer's instructions.* Smears were examined using a fluorescent microscope and were considered positive if at least 3 characteristic fluorescing cysts were observed.

**Definition of \textit{P. Carinii} Infection**

A patient with a DRG-coded diagnosis of PCP was presumed to have PCP unless; (1) the patient improved without any specific anti-\textit{P. carinii} treatment, (2) a bronchoalveolar lavage (BAL), within seven days was PC-DFA negative, (3) an autopsy report was negative for PCP, or (4) the clinical course was not consistent with PCP and a definitive alternate diagnosis was made.

**Statistical Analysis**

The unpaired t-test was used to compare the group with positive NIS versus the group with negative NIS with regard to age, serum lactate dehydrogenase, and pO2. The chi square test

---

* Genetic Systems Inc., Redmond, WA 98052-5039.
was used to compare the two groups with regard to sex, race, HIV risk factors, and mode of PCP prophylaxis.

**Results**

A total of 142 patients had a DRG coded diagnosis of PCP, of which 133 were HIV-infected. Sixty-seven HIV-infected patients (50 percent) had produced NIS for PC-DFA. The charts of the 67 patients were reviewed; for 12 patients with negative NIS by PC-DFA, the DRG code of PCP was found to be erroneous. Two of these 12 patients had no evidence of PCP at autopsy; five had BAL with negative PC-DFA and had an atypical clinical course; the remaining five initially suspected of PCP had an alternate diagnosis made—bacterial pneumonia (3), congestive heart failure (1), and fungal meningitis and pneumonia (1). Thus, there were 55 episodes of PCP included in this study (table I).

Of the 55 patients, 30 had NIS positive for PC-DFA, had a clinical course and presentation consistent with PCP, and were considered true positives. Twenty-five patients had negative NIS by PC-DFA. Of these, two were positive for PCP at autopsy; 10 had a BAL positive for P. carinii; and 13 had clinical courses consistent with PCP. All 25 were considered false negatives. Thus, the sensitivity of NIS stained with PC-DFA was 55 percent (30/55).

Comparisons of age, HIV risk factors, race, pO2, lactate dehydrogenase (LDH) and mode of PCP prophylaxis were made between the two groups (NIS PC-DFA positive and NIS PC-DFA false negative). No statistically significant differences were found except that women were more likely to produce false negative NIS with PC-DFA (table I).

**Discussion**

Before the AIDS epidemic, the diagnosis of PCP was usually made by open lung biopsy as all other methods had a very poor yield. However, in HIV-infected patients, less invasive procedures were satisfactory in establishing the diagnosis. This was attributed to the larger burden of organisms that proliferated in the lungs. Bronchoalveolar lavage became the standard against which other nonsurgical methods were compared, having a sensitivity of 97 percent. In immunosuppressed non-HIV infected patients, examination of NIS stained with GMS for P. carinii has a reported sensitivity of 1 to 14 percent. Similarly, NIS from AIDS patients is considered to be not useful. Indeed, the limited data available suggest a poor yield, although the exact sensitivity cannot be determined as there is little data regarding the yield of NIS in the diagnosis of PCP in HIV-infected patients.
In 1986, Pitchenik et al.\textsuperscript{3} and Bigby et al.\textsuperscript{4} showed that in patients with PCP, induced sputum stained with GMS had a sensitivity of 55 percent. Since then, several centers have investigated the use of induced sputum in the diagnosis of PCP. Some have reported a sensitivity greater than 75 percent;\textsuperscript{17} however, many have noted difficulty reproducing these results, reporting yields as low as 10 to 34 percent.\textsuperscript{6,7,8,9,10}

A commercially available PC-DFA was developed in 1990. This test offers the advantage of requiring less time to process and fewer reagents than tinctorial stains.\textsuperscript{17} Induced sputum stained with PC-DFA has been reported to have a specificity of >99 percent and to be more sensitive than GMS\textsuperscript{11,18} and as or more sensitive than Giemsa stain.\textsuperscript{12,17} The PC-DFA method is quicker and easier to interpret and may be more useful in institutions with little experience in examining smears for \textit{P. carinii}.\textsuperscript{17}

Nonetheless, centers using the PC-DFA also have had variable success with the evaluation of induced sputum, reporting sensitivities as low as 40 percent\textsuperscript{19} and as high as 72 to 95 percent.\textsuperscript{17,20,21} In our study, the sensitivity of PC-DFA on NIS in the diagnosis of PCP is 55 percent. This is within the range reported in the literature for PC-DFA and tinctorial staining of induced sputum.\textsuperscript{6,7,8,9,10,19} Unfortunately, this study does not enable us to compare the sensitivity of induced sputum with that of NIS as sputum induction was rarely performed in our institution.

The implementation of sputum induction in the diagnosis of PCP has not been uniformly applied owing to the variability of the results and technical difficulties.\textsuperscript{13,22,23} Even with induction, 27 to 29 percent of patients will not be able to produce a respiratory sample.\textsuperscript{6,7} The induction protocol is cumbersome, requires meticulous attention to detail, and a dedicated physiotherapist for optimum results.\textsuperscript{5,22} Few centers will have the case load of HIV-positive patients to justify hiring specially trained personnel to implement the sputum induction protocol.\textsuperscript{24} The costs associated with sputum induction are not trivial.

In our center, NIS stained with PC-DFA costs $31, while sputum induction adds approximately $80 to the patient's charges. This cost is further magnified when one considers that in some centers, more than 66 percent of patients who have sputum induction for suspected PCP have a final diagnosis other than PCP.\textsuperscript{7,10} This has prompted recommendations for appropriate screening of every request for sputum induction in order to minimize the expense associated with the procedure.\textsuperscript{25}

Moreover, sputum induction can be an unpleasant experience for the patient, often provoking nausea, prolonged coughing and dyspnea.\textsuperscript{3,4,6} It requires the patient's cooperation and is a difficult procedure for patients with severe hypoxemia.\textsuperscript{36}

Despite the difficulties described with the use of the sputum induction technique at many centers, its use has clearly obviated the need for countless bronchoscopies and associated morbidity and financial costs over the last decade. Nonetheless, our results suggest that the examination of NIS is useful when the DFA stain is used. Sputum induction may not be necessary if the patient is able to produce any sputum sample spontaneously. It is conceivable that patients who have a negative NIS are unlikely to have a positive result after induction; and that the most cost-effective procedure following a negative NIS (or following the inability to produce a NIS) would be BAL. This is speculation, but as new technologies become available, prior assumptions and practices need to be reexamined. As more sensitive methods for detecting \textit{P. carinii}, such as polymerase chain reaction,\textsuperscript{19,20} become widely used, the utility and cost-effectiveness of sputum induction for the diagnosis of PCP must be continually reassessed. The results of this study suggest that in patients who can provide NIS, DFA-PC can be frequently diagnostic.

\textbf{Acknowledgments}

Thanks are extended to Dr. Eytan Rubinstein for his helpful review of the manuscript.
References


11. Halfor JA, Shield PW, Wright RG. The value of direct fluorescent antibody (DFA) testing for the detection of *Pneumocystis carinii* in cytological specimens. Cytopathology 1994;5:234-42.


