Pneumocystis carinii Pneumonia

Cytologic Manifestations and Rapid Diagnosis in Routinely Prepared Papanicolaou-Stained Preparations

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In this study of 28 immunocompromised patients, it was found that Pneumocystis carinii pneumonia could be easily and reliably diagnosed by examination of routinely prepared, Papanicolaou-stained cellular samples obtained by bronchoalveolar lavage, bronchial brushing, and bronchial washing. The distinctive intra-alveolar exudate of pneumocystosis observed in lung biopsy specimens was readily discernible in all of the cellular samples that demonstrated P. carinii by special stains. The exudate was not present in any of the P. carinii-negative samples. Routinely prepared, Papanicolaou-stained cellular samples can be relied upon for the rapid diagnosis of P. carinii pneumonia.

Infection with Pneumocystis carinii accounts for a large proportion of the rapidly progressive and often fatal pneumonias occurring in a variety of immunocompromised patients [1,2]. Recently, additional attention has been focused on pneumocystosis as a major complication of the acquired immune deficiency syndrome (AIDS) [3]. The diagnosis of pneumocystosis rests on the microscopic demonstration of the protozoa in closed or open lung biopsy specimens, or in cellular samples obtained primarily by bronchoalveolar lavage, endobronchial brushing and washing, and transthoracic fine needle aspiration. In such specimens, the P. carinii cysts are commonly detected by employing the methenamine silver nitrate stain [4] or one of its modifications [5,6], the Gram-Wiegart stain [7], or the toluidine blue O stain [8]. The Giemsa, Wright, and Wright-Giemsa stains [9,10] demonstrate not only the cysts, but also the sporozoite and trophozoite forms. Most recently, Ghali and associates [11] used direct immunofluorescence to demonstrate the cyst forms. With the exception of the reports of Pintozzi et al [12] and Greaves and Strigle [13], the detection of P. carinii in Papanicolaou-stained cellular samples has received little attention. In this report, we give an account of the diagnostic usefulness of this preparation.

PATIENTS AND METHODS

All specimens submitted to the Cytopathology Laboratory over the last 16 months from immunosuppressed patients in whom pneumocystosis was suspected were reviewed. Such suspicion was based on abnormal chest roentgenograms, a history of dyspnea or fever or both, or positive findings on gallium lung scanning. Forty-eight specimens (21 bronchoalveloar lavages, 12 bronchial washings, eight bronchial brushings, five sputum samples, and two transtracheal aspirates) were received from 28 patients (**Table I**). The clinical course and follow-up of each patient were recorded by chart review and, in three patients, by review of necropsy protocols.

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TABLE I Underlying Disorders of 28 Immunosuppressed Patients

Diagnosis/Clinical Condition	Number of Patients
Acquired immune deficiency syndrome	14
Lymphoma/leukemia	6
Renal/cardiac transplantation	2
Primary immunodeficiency disorders	2
Wegener's granulomatosis	2
Progressive systemic sclerosis	1
Adenocarcinoma (colon)	1

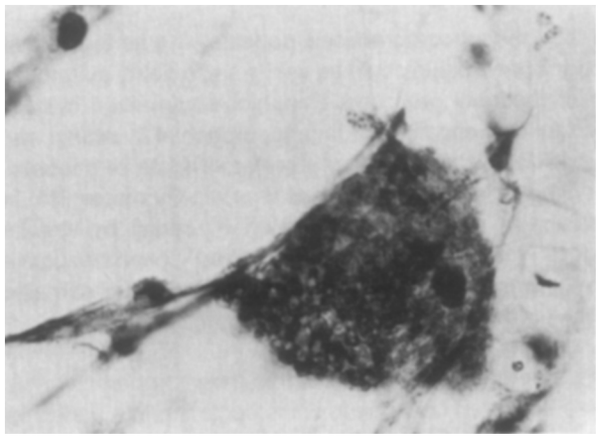

Figure 1. *Detailed view of the granular exudate. The outlines of the cyst walls of P. carinii are clearly discernible (Papanicolaou stain; original magnification × 500).*

Each cellular sample had been smeared and stained according to the standard Papanicolaou method; additional smears from each specimen were stained with either methenamine silver [4] or toluidine blue O [8]. Each Papanicolaou-stained sample was also examined by fluorescent microscopy [11]. Two observers independently recorded the cytologic features of each smear and the presence or absence of P. carinii cysts. Lung biopsy specimens concomitantly obtained from 22 of the 28 patients were also examined by the methenamine silver stain for the presence of P. carinii cysts.

RESULTS

The cyst forms of P. carinii were detected in 14 Papanicolaou-stained cellular samples (five bronchoalveolar lavages, five bronchial washings, three bronchial brushings, and one sputum sample) obtained from nine of the 28 patients. In each case, silver methenamine and toluidine blue O stains as well as direct fluorescence examination confirmed the presence of the cysts. P. carinii was also present in the lung biopsy specimens from seven of the nine patients (biopsy was not performed in the other two patients).

Each smear contained irregularly shaped clumps of granular exudate that contained a definite honeycomb architecture due to the subtle outlines of the enmeshed cysts (**Figure 1**). The presence of the cysts were corroborated by direct fluorescence microscopy and the special stains. This material, which varied in amount from case to case, was most readily found in the samples obtained from the patients with AIDS (six of the nine patients), and was identical to that characteristically observed filling the alveoli of lung biopsy specimens of patients with pneumocystosis.

The cellular samples from 19 of the 28 patients did not show P. carinii. Lung biopsy specimens obtained from 15 of these 19 patients similarly showed no abnormalities. Ten of the patients with negative cytologic results did not receive treatment at any time for P. carinii pneumonia; all subsequently improved clinically.

COMMENTS

Almost without exception, P. carinii infects the immunocompromised host [14]. As Burke [15] stated, the single most valuable aid in the diagnosis of P. carinii infection is a high index of suspicion; however, demonstration of the organism remains at present the only unequivocal diagnostic evidence of infection. In our experience, the amorphous granular exudate we have described in cytologic specimens stained by the Papanicolaou method is specific for P. carinii infection. The formation of fairly well-defined clumps of exudate with its internal "honeycomb" structure is quite distinctive and unlike exudate we have seen in cellular samples from the respiratory tract from patients with other conditions. Our experience is shared by Greaves and Strigle [13] who were similarly impressed by the specificity of the cytologic features. Clumps of inflammatory exudate are often found in cellular samples obtained from patients with pneumonia due to other causes; however, in such cases, the exudate does not have the granularity of architecture as just described in patients with pneumocystosis. Examination of this exudate by fluorescence microscopy or special stains confirmed the presence of the cysts in all cases.

A careful search with special stains did not reveal cysts in those cellular samples in which the distinctive exudate was absent, and the corresponding lung biopsy specimens also did not demonstrate the cyst forms. Although false-negative results were not obtained in this study, it is well to remember that such results may occur as the characteristic intra-alveolar exudate is often not observed in patients with pneumocystosis [16].

A variety of histologic stains may be used to demonstrate P. carinii. Many of these stains often require special

expertise in either their preparation or their interpretation. Although their diagnostic efficacy, when properly performed and interpreted, is unquestioned, these special stains are not as easily employed for the rapid screening of cytologic specimens. In contrast, Papanicolaou-stained smears are easily and quickly prepared in a routine manner and an unequivocal diagnosis may be rapidly established. In view of the increasing numbers of immunocompromised patients encountered in clinical practice and the usefulness of bronchoalveolar lavage [17,18] in the diagnosis of pulmonary infiltrates in such patients, the use of routinely stained Papanicolaou preparations may prove to be of great value in identifying those patients with P. carinii pneumonia.

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