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DIAGNOSTIC STRATEGIES IN PNEUMOCYSTIS CARINII PNEUMONIA

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1. ABSTRACT

Pneumocystis carinii (P. carinii) remains a major pulmonary pathogen for the immunocompromised patient. In HIV infected patients, *P. carinii* represents the most commonly diagnosed cause of pneumonia. In the AIDS patient, empiric therapy based on clinical presentation has its proponents. However, this approach has been associated with a worse overall prognosis for the at risk patient. Because *P. carinii* can not be cultured, specific identification relies on examining respiratory specimens ranging from expectorated sputum to bronchoscopy with bronchoalveolar lavage (BAL). The low sensitivity of conventional stains has led to the search for antibodies to *P. carinii* and the use of immunofluorescent techniques. In addition, the polymerase chain reaction (PCR) is successfully being used in the diagnosis of *P. carinii*. Overall, these techniques allow the clinician to tailor the diagnostic testing for the individual patient.

2. INTRODUCTION

The major mechanism of host defense against *Pneumocystis carinii* (*P. carinii)* is through cell- mediated immunity. Therefore, conditions such as HIV infection and solid organ transplant place the patient at increased risk for infection (1). For the HIV infected patient, *P, carinii* is no longer the ominous diagnosis it once was (2,3). However, it remains a commonly identified cause of lower respiratory tract infection (4) and is a significant contributor to the cost of care of the HIV patient with pulmonary symptoms (5). In the past few years, several methods have been used to better detect this infection. The purpose of this review is to summarize these findings.

The diagnosis of *P. carinii* pneumonia requires the identification of the micro-organism. Attempts, to date, have still failed to culture this micro-organism reliably in an acellular medium. Thus, the diagnosis relies on either empiric diagnostic standards or the use of diagnostic techniques that rely on visualization of the organism. The empiric approach has been mostly used in the HIV-infected population, especially those patients with low CD4+ lymphocytes. The best approach appears to be individualized for patients and their physician.

3. HISTORY/PHYSICAL EXAMINATION/ ROUTINE LABORATORY TESTS

3.1. History

The use of certain physical examination and laboratory tests have been shown useful in suggesting the diagnosis (table 1). The patient's current symptoms are useful in establishing the relative risk. It has been clear, for some time, that HIV-infected patients may have a prolonged prodrome of symptoms prior to being diagnosed (6). In the transplant and lymphoma population, the symptoms are more abrupt. In the transplant and lymphoma patients, acute symptoms leading to the diagnosis of *P. carinii* pneumonia are often encountered after reducing the dose of corticosteroids.

The relative risk of P. carinii pneumonia (PcP) for HIV-infected patients is related to their anti-pneumocystis prophylaxis. For a patient with pneumonia, the risk of recurrent PcP within the next year following infection is 50% if no prophylaxis is given (7,8). If

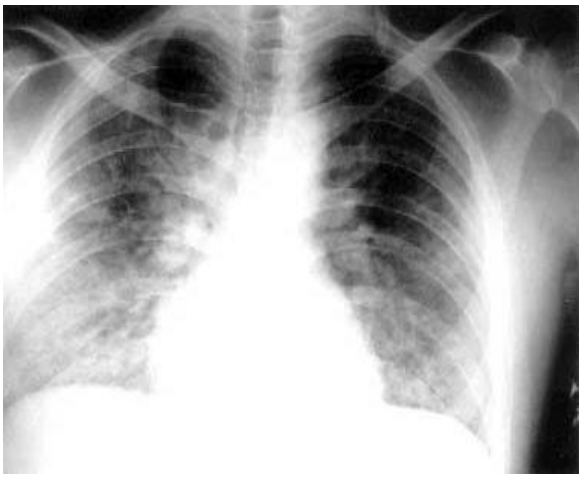

Figure 1: Chest roentgenogram of patient with *P. carinii* pneumonia, demonstrating diffuse infiltrates.

Table 1. Parameters aiding the diagnosis of *P. carinii* pneumonia

PARAMETER	ABNORMALITY
Acute History	❑ Gradual onset dyspnea (days to weeks)
	❑ Nonproductive cough
	❑ Weight loss
Past Medical History	❑ Immunosuppression
	❑ History of CD4 count < 250 cells/cu mm
	❑ Use of no anti-pneumocystis prophylaxis
Physical Examination	❑ Cough and crackles on deep inspiration
Laboratory testing	❑ Elevated LDH
	❑ Lymphopenia
	❑ Hypoxemia worsening with exercise
Chest Roentgenogram	❑ Diffuse infiltrates
	❑ Bilateral upper lobe infiltrate if patient on pentamidine Pneumothorax

trimethoprim/sulfamethoxazole (TMP/SMX) is given for prophylaxis, the risk of recurrence is less than 1% (7,9). Aerosol pentamidine leads to a reduction of incidence of reinfection (8), but up to 30% of patients will have recurrence within the two years subsequent to infection (7). Dapsone is associated with less adverse reactions than TMP/SMX and is often used as an alternative to TMP/SMX. Dapsone is more effective than aerosol pentamidine, but less effective than even a low dose TMP/SMX (10,11).

3.2. Physical examination

In the appropriate clinical setting, the physical examination may provide some information. Patients usually are tachypneic. Fever may be present in the majority of patients, often for several days to weeks prior to the diagnosis (6). Patients with advanced PcP may have dry crackles and often cough with deep inspiration (12). However, this is often not apparent in the patient with a mild infection.

3.3. Laboratory tests

Routine laboratory testing reveals limited information. The most useful test appears to be the serum level of lactate dehydrogenate (LDH), which is elevated during infection (13). However, this test is relatively nonspecific and elevated levels of LDH are often encountered in other conditions, including other pneumonias and lymphoma (14,15). However, serial changes in LDH may be useful in follow up of the patient. Also, the degree of elevation appears to be a useful marker for prognosis (16,17). Patients with a value two to three times greater than the upper normal levels of LDH have a significantly higher mortality (16). Using multi-regression analysis, LDH was not an independent risk factor for mortality from *P. carinii* pneumonia (18).

The presence of lymphopenia is an indication of immunosuppression. In the HIV-infected patient, the determination of the CD4 lymphocyte count is a useful way to determine the patient's risk for *P. carinii* pneumonia (19). Two prospective studies of HIV infected individuals have demonstrated that the risk of *P. carinii* rises sharply as the CD4 count falls below 250 cells/cu mm (20,21).

Hypoxemia is a characteristic feature of PcP as well as many other pneumonias. The use of exercise oximetry is a useful way to evaluate the respiratory complaints of a patient, since even in mild *P. carinii* infection desaturation with exercise is seen (22). The arterial blood gases also provide an indication of level of severity of illness (16,17). Since hyperventilation is a common in *P. carinii* infected patients, one should calculate the alveolar-arterial (A-a) gradient to detect an early lung disease which may still have a normal oxygen saturation. The A-a gradient may also be markedly abnormal in a patient with few other clinical signs of pneumonia. A calculated A-a gradient for oxygen that is greater than 35 mm Hg has been associated with increased mortality. In such a situation, the use of corticosteroids have been shown to improve survival (23).

The chest roentgenographic pattern of *P. carinii* pneumonia is variable. The classic x-ray pattern in PcP is a bilateral fluffy infiltrate, resembling pulmonary edema (figure 1) (24). With the widespread use of aerosol pentamidine, an upper lobe infiltrate can predominate (25,26). An unusual finding is pneumothorax. However, the finding of a spontaneous pneumothorax in an HIV-infected patient should suggest *P. carinii* until proven otherwise (27). *P. carinii* pneumonia may be associated with a normal chest roentgenogram (4,28); therefore, the chest roentgenogram should not be the only screening test in evaluating AIDS patients with pulmonary symptoms (29).

None of these tests are specific in the diagnosing *P. carinii*. By merely relying on clinical criteria alone, about 40-50% of patients may suffer from pneumonia other than that caused by *P. carinii*. In the majority of cases, no specific etiology is identified by bronchoscopy and lavage. Therefore, some have argued for the use of an empiric treatment (12,30). Although overall mortality may be higher, this strategy appears to be cost effective for some patients (31). This includes the patient with diffuse lung infiltrates, chronic symptoms, who is not allergic to TMP/SMX. In such a setting, the patient either has *P. carinii* or no pathogens identified.

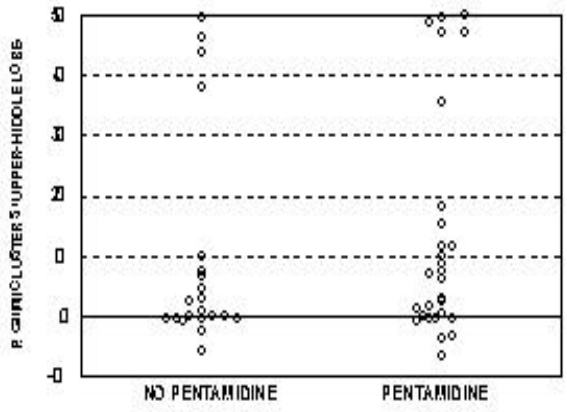

Figure 2. Comparison of the amount of *P. carinii* in the upper lobe versus the middle lobe of patients with *P. carinii* pneumonia. The amount of infection was measured using the semi-quantitative technique of clusters per 500 nucleated cells in the BAL fluid (72). There was significantly more *P. carinii* in the upper than middle lobes for both patients receiving pentamidine aerosol prophylaxis (Pentamidine) and those receiving no prophylaxis (NO Pentamidine) (26).

Table 2. Comparison of empiric versus diagnostic approach in HIV-infected patients with possible *P. carinii* pneumonia

	EMPIRIC THERAPY	BRONCHOSCOPIC DIAGNOSIS
Cost	Initially less expensive	Fixed cost
	Cost of failures to respond may be higher	Leads to directed therapy
Accuracy	Miss a significant number of non-P carinii cases	Does not miss a high number of P. carinii pneumonia
	Overestimates incidence of P. carinii pneumonia	Higher incidence of "non-diagnostic " cases
Outcome	May have higher mortality	Complications associated with bronchoscopy

Table 3. Diagnostic yield using silver stain for each specimen

SOURCE OF SPECIMEN	DIAGNOSTIC YIELD
Sputum	50 % (15-94%) *
Bronchial Wash	65% (60-70%)
Bronchoalveolar lavage A single area lavaged	90% (60-100%)
Bronchoalveolar lavage Two areas lavaged	95% (85-100%)
Transbronchial biopsy	97% (89-100%)

* Median (Range)

Table 4. Comparison of diagnostic yield using methenamine silver or equivalent stain for sputum samples

DIAGNOSTIC YIELD, PROPHYLAXIS NOT SPECIFIED	DIAGNOSTIC YIELD, NO PROPHYLAXIS	DIAGNOSTIC YIELD, AEROSOL PENTAMIDINE	Ref
11/20 (55%) *			(36)
14/25 (56%)			(37)
21/25 (84%)			(35)
2/13 (15%)			(39)
	36/39 (92%)	18/28 (64%)	(40)
	12/19 (63%)	35/55 (64%)	(44)
	17/23 (74%)	18/23 (78%)	(42)
	27/57 (48%)	52/110 (47%)	(43)

* Number positive/number studies (percentage)

The overall benefits of empiric therapy versus bronchoscopic diagnosis followed by therapy are summarized in table 2. The initial cost is less with empiric therapy (12). This has led to reduced utilization of bronchoscopy in patients with certain insurance coverages (32). However, the final cost is not so clear. For example, the cost of missed diagnoses is considerable. In one series of 894 lavages in HIV infected patients with pulmonary symptoms, patients often had a treatable pathogen other than *P. carinii*. Overall, patients either had *P. carinii* alone (39%), *P. carinii* plus another pathogen (8%), or another pathogen alone (12%) (4). A similar high incidence of *P. carinii* with other pathogens has been reported by others (33). Empiric therapy will probably treat all cases of *P. carinii*, but will overestimate the incidence of this infection. For the individual patient, this may represent a problem. The diagnosis of *P. carinii* pneumonia could mean that an HIV-infected individual is considered to have AIDS. For the institution looking at its prophylaxis regimen, physicians may underestimate its effectiveness if they use response to empiric therapy as an indicator of failure of prophylaxis. Bronchoscopy misdiagnoses a smaller number of *P. carinii* cases. However, bronchoscopy will be left with a larger number of cases in whom the diagnosis is unclear. The outcome of empiric therapy versus bronchoscopy is controversial. A prospective study comparing empiric versus bronchoscopic diagnosis found that patients not undergoing bronchoscopy had a higher mortality (31). In another study, a significant number of patients with suspected *P. carinii* and negative induced sputum had a different diagnosis, usually tuberculosis (33). Limited use of bronchoscopy associated with a certain insurance coverage was associated with a higher mortality rate (32).

4. DIAGNOSTIC SAMPLING

The analysis of cytologic samples requires that a sample of infected tissue be examined. In PcP, this usually means respiratory samples. The specimens may be obtained by the non-invasive examination of induced sputum, the bronchoscopic acquired bronchial washing, transbronchial biopsy, and bronchoalveolar lavage (BAL), to open lung biopsy. There is a range reported for the sensitivity of these techniques using the silver stain that is summarized in table 3. Later, we will discuss the use of various staining techniques. The silver stain is considered to be specific, but perhaps not as sensitive compared to other techniques such as immunofluorescence (34,35).

4.1. Induced sputum

The induction of sputum in the diagnosing of *P. carinii* has become an extremely popular method, especially in areas with a high incidence of disease (36-38). Others have reported a much lower yield (39). Using sputum for detecting *P. carinii* appears to require a dedicated team. Lower diagnostic yield may also be affected when the patient is receiving aerosol pentamidine (40). This has been associated with a different roentgenographic and clinical presentation in HIV patients with *P. carinii* (26,41,42). Several studies using experienced respiratory therapist and pathologist have shown that aerosolized pentamidine does not significantly affect the yield of *P. carinii* (42-44). These results are summarized in table 4.

Table 5. Yield of bronchoalveolar lavage for diagnosis of *P. carinii* pneumonia: effect of prophylaxis and number of lavages

PENTAMIDINE	NUMBER OF LAVAGES	YIELD	REF
None	1	82%	(68)
None	1	86%	(55)
No comment	1	97%	(59)
No comment	1	89%	(69)
No comment	1	97%	(34)
No comment	1	86%	(70)
No	1	100%	(53)
Yes	1	62%	(53)
No	2	100%	(40)
Yes	2	98%	(40)
No	2	100%	(26)
Yes	2	100%	(26)
Yes	2	100%	(65)
Yes	1	65%	(62)
Yes	2	95%	(62)
No comment	2	94%	(63)

In a study of 1700 cases of suspected cases of *P. carinii* at San Francisco General Hospital, 80% of the cases with *P. carinii* were diagnosed by induced sputum. In patients with negative sputum (600), two thirds underwent diagnostic testing, presumably because of persistent symptoms. A third of these patients had *P. carinii* identified in their BAL or transbronchial biopsy and 20% had another pathogen. In 64% of cases of *M. tuberculosis*, bronchoscopy provided either an earlier or sole means of diagnosis (33). As found by others (45), this study points out that pathogens other than *P. carinii* and *M. tuberculosis* are poorly identified by induced sputum. Because of the variable yield and the relatively limited detection rate, it appears that induced sputum may have little to offer over empiric treatment. Sputum remains useful at institutions with a large enough number of potential cases of *P. carinii* pneumonia to maintain dedicated personnel for acquisition and interpretation of samples.

4.2. BAL and bronchial washing

With bronchoscopy, BAL is superior to bronchial washing alone in the diagnosis of *P. carinii* (46). The bronchial washings are the pooled samples from the airways, collected during the entire bronchoscopy, including those obtained after the lavage. BAL is a specific task of wedging a bronchoscope in a distal airway and instilling aliquots of saline and immediately retrieving the fluid either by a low suction or a hand held syringe. Since the bronchial washing also collects samples after the lavage, the yield is perhaps even lower in those patients who never underwent lavage. When compared to BAL, bronchial washing had a significantly lower yield (34,47). The bronchial brush technique also has a low yield and has been basically abandoned (48).

4.3. Transbronchial biopsy

The use of transbronchial biopsy allows sampling of lung tissue. This is particularly useful in detecting microorganisms other than *P. carinii*, such as *M. tuberculosis* and fungi (49-51). In *P. carinii* infection, the transbronchial biopsy is complimentary to lavage. Many series document that both techniques are over 90% sensitive (52-54). In a large series, Broaddus *et al* found only 3% of patients were documented to have *P. carinii* only on the basis of the transbronchial biopsy (55). In 9% of patients, pneumothorax occurred, half of whom required chest tubes. Griffiths *et al* encountered pneumothorax in 22% of patients undergoing lung biopsy (56). Others have reported that in patients undergoing pentamidine prophylaxis, BAL has a much lower yield than transbronchial biopsy (53). Transbronchial biopsy was complementary to lavage even in site directed BAL (54). Others have not found biopsy to add to the yield of BAL, except when malignancy is suspected (56,57). In the transplant patients, transbronchial biopsies are more frequently done and appears more useful since these patients have a lower burden of *P. carinii* than the HIV patient (58).

4.4. BAL alone

In the diagnosis of *P. carinii*, the use of BAL alone has its proponents (59). Although this may be true for *P. carinii*, there still is the issue of other infections. As pointed out above, up to 20% of time, an additional or sole pathogen other than *P. carinii* may be found (4,33). For *M. tuberculosis*, bronchial washing offers a significantly higher yield (60). In one center, BAL was not found to be cost effective for examination or culture for *M. tuberculosis* (61).

The proper technique of BAL has recently undergone scrutiny. The rationale for this was a study demonstrating a low yield for lavage alone in patients with *P. carinii* pneumonia who received pentamidine prophylaxis (53). In that study, lavage was done in the middle lobe, a technically easy area to lavage, and the yield was significantly lower for those patients on aerosol prophylaxis (table 5).This lower yield for middle lobe was confirmed by others (26,62). In patients with *P. carinii*, the upper lobes may be more prominently involved (26); this seems to be particularly true in patients who receive aerosol pentamidine prophylaxis (25,41,53). Several subsequent studies have demonstrated that an increased yield may be obtained by lavaging two or more areas of the lung. This can be done in two ways. The first method is to perform one subsegmental lavage in each lung (63). Others have preferred to perform a site-directed lavage, that is lavage in the most involved area (64). The common practice is to perform two lavages in the same lung, usually one in the upper lobe, the other in the middle lobe. This has resulted in a higher number of cases diagnosed since the upper lobe is often the more affected area (26,62,64,65). In a systematic study of this issue, we routinely performed lavages in the upper and middle lobes (or lingula for the left side) of patients with possible *P. carinii* pneumonia (26). We characterized the number of *P. carinii* identified using a semi-quantitative technique (66). As can be seen in figure 2, most patients had more *P. carinii* in the upper lobes. In six of the fifty patients, *P. carinii* was not detected in the mid-lung. Thus, the two lobe lavage technique was more sensitive in the diagnosis of *P. carinii* infection (26,65). Bronchoscopy and BAL can induce transient hypoxemia, which can be impressive in a patient with respiratory failure (63,67). In patients with moderate to severe hypoxemia from pneumonia, it is best to only lavage the most involved area, usually the upper lobe.

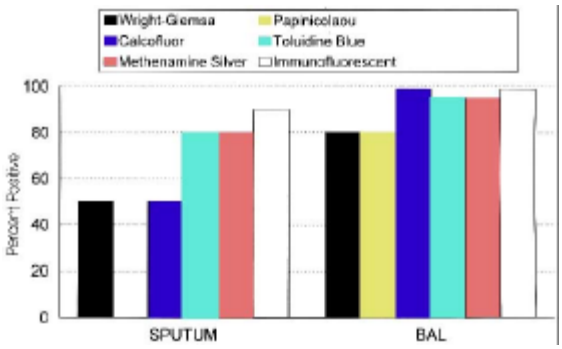

Figure 3. Relative sensitivity for the various stains used to detect *P. carinii* infection. The reported percentages are the median value of those reported in the literature.

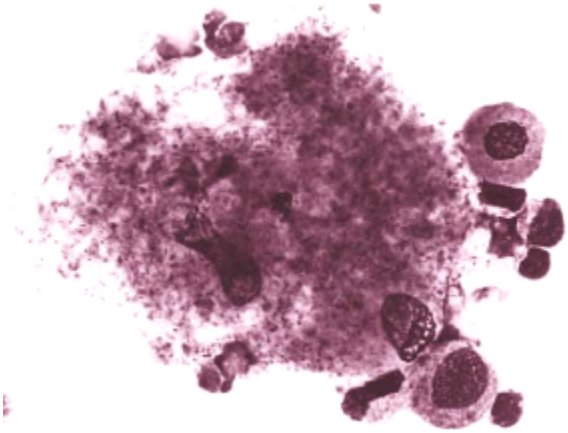

Figure 4. Modified Wright-Giemsa stain (Diff-Quik) of cluster of *P. carinii* from HIV-infected patient. The individual trophozoites can be seen, but there is no staining of the cyst wall. Original magnification was 80X.

Prominence of *P. carinii* in the upper lobe of patients on pentamidine suggests poor delivery of the drug by aerosol to the upper lobes (71). This has become less of a problem with the use of more efficient delivery via nebulization (72,73). Although drug delivery may be partly responsible for the upper lobe prominence of *P. carinii*. It may also be due to preferential localization of the microorganism. As can be seen in figure 2, in both patients who received pentamidine prophylaxis or did not, *P. carinii* was preferably found in the upper lobes of the lungs.

Another technical aspect of the lavage procedure is the volume of instilled fluid. In studies of noninfectious inflammatory diseases, it is clear that the first 20 ml of instilled fluid samples the bronchial area and less the alveolar component (22). The first 60 ml samples a higher proportion of large airways and the results are different from the next 60-180 ml in normals and inflammatory conditions such as sarcoidosis (74). Interestingly, the first 60 ml seems to have an adequate diagnostic yield of *P. carinii* compared to a larger volume lavage, with no difference in the number of clusters of *P. carinii* found in the first 60 ml versus the rest of the lavage (66). Therefore, a small volume lavage may be quite adequate in the diagnosis of *P. carinii* (75).

4.5. Open lung biopsy

Although the open lung biopsy remains as a standard for comparing with other techniques, it is rarely performed for the diagnosis of *P. carinii* pneumonia in AIDS patients. It may still have a role in other immunocompromised groups, although bronchoscopy should still be part of the initial approach (76). The few cases missed by bronchoscopy can be detected by open lung biopsy (77), however, the risks of the procedure are significant (78). This is even true in the setting of the "minimally invasive" procedure, video-assisted thoracoscopy surgery (VATS).

5. STAINS FOR IDENTIFICATION

For the identification of *P. carinii* several stains exist. These vary in cost, sensitivity, speed in which they can be done, and specificity. The staining procedure can be divided into three groups based on their characteristics: stains for the cyst wall, stains for the individual trophozytes, and immunofluorescent stains. Figure 3 summarizes the various stains for each group and their relative sensitivity. For the purpose of this table, both sputum and BAL are shown. This allows for a comparison for the less sensitive stains when there are more organisms.

5.1. Traditional pathologic stains

The Wright-Giemsa stain and its modifications have been used for some time to examine white blood cell morphology (79). The stain has been modified and can be performed in a rapid manner (Diff-Quik) for examining cytocentrifuge preparation of BAL samples (figure 4) (35,70,80). Since no special fixation is required, the slide can quickly be read by the laboratory allowing a rapid diagnosis of *P. carinii* (58). Unfortunately, the Wright-Giemsa stain is an indirect stain and has to be interpreted with some caution. Neutrophils, especially entrapped in mucus, can be confused for clusters of *P. carinii*. The overall sensitivity of Wright-Giemsa staining is significantly lower than other staining techniques (35,47,70,80). This is particularly true when there are only a small number of microorganisms, such as in transplant patients (58).

Papanicolaou stain also can be used to identify the foamy material associated with *P. carinii* infection (69,81,82). This stain is most useful in identifying changes in cellular morphology, such as is seen in cytomegalovirus infection (83). However, if there are sufficiently large clumps of organisms a skilled cytologist can often recognize *P. carinii*. Its overall diagnostic yield is similar to the Wright-Giemsa stain, but significantly less useful that cyst stains such as silver stains (47,48,84).

Overall, the stains for cyst wall are more accurate in the diagnosis of *P. carinii* microorganisms. Although other microorganisms, especially fungi, often have positive stain, distinct morphologic characteristics allow diagnosis of *P. carinii* versus fungi (85). The size of the cyst is uniform and is similar to the size of red blood cells. The microorganisms tend to form clumps, often, but not always held together by a proteinaceous material (figure 5) (86). The microorganism are not seen within cells. On the other hand *Histoplasmosis capsulatum*, which looks morphologically similar to *P. carinii*, clumps within alveolar macrophages.

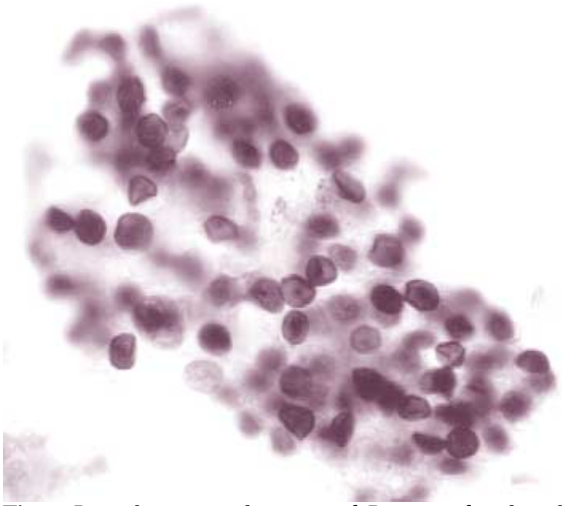

Figure 5. Methenamine silver stain of *P. carinii* found in the BAL fluid of HIV-infected patient. The cyst walls readily take up stain, but individual trophozoites do not stain. Original magnification was 80X.

The most commonly employed cell wall stain is the silver stain. This stain, and its modifications, is considered the "gold standard" stain for *P. carinii* since it is more sensitive and specific than other stains (79,80). It is unfortunately a somewhat tedious stain to perform (84). Even with modifications, it still takes several hours to do the staining (87).

Toluidine blue staining which also allows diagnosis of *P. carinii* is simpler to perform than the silver stain (87). It has been used instead of (35) or in addition to the silver stain (88). Although simpler than the silver stain technique, the use of cresyl violet stain has been limited to laboratories which use the stain frequently (89,90). The sensitivity of this stain is somewhat better than Wright-Giemsa (47).

Nonspecific fluorescent staining of the cyst wall can be used to identify *P. carinii* (91-93). However, certain cell walls such as those of fungi may also be stained. Its major advantage is in the rapid screening of slides for a few positive staining cells, such as when a sputum sample is screened. This stain is as sensitive as the silver stain (93), but not as sensitive as the antibody-directed fluorescent techniques (94).

5.2. Immunofluorescent stains

Although antibodies to *P. carinii* have been available for many years (95), the use of antibody-directed fluorescent stains started about ten years ago (35). The development of antibodies has been divided into two general categories: the direct fluorescent antibody and indirect fluorescent antibody stains. The direct fluorescent antibody technique often relies on a monoclonal antibody (35,96,97). On the other hand, the indirect antibody technique usually relies on the use of polyclonal antibodies which have a wider range of stains (34,98). Although occasional macrophages autofluoresce, using standard cytologic criteria, one can usually identify the *P. carinii*.

5.3. Identification using polymerase chain reaction (PCR)

Since the gene library of *P. carinii* has been developed (99), the potential of using this information in the diagnosis of pulmonary infection by *P. carinii* has been appreciated (100). The study of genetic markers of *P. carinii* have demonstrated distinct species specificity of *P. carinii* (101-103). By using genetic-based technologies, it has been possible to show that repeated infections of *P. carinii* may be due to new infection rather than reactivation of preexisting microorganisms (104,105).

Several different techniques for detecting *P. carinii* by PCR have been studied (106). The use of PCR in the diagnosis of *P. carinii* has been applied to sputum and bronchoscopy. As noted above, induced sputum has a low yield by conventional stains. Because of its convenience and low cost, it may become cost effective to screen sputum for *P. carinii* with PCR (107). Table 6 is a comparison of some of the studies reported to date. Overall, PCR appears more sensitive that fluorescent techniques in the diagnosis of *P. carinii* infection. This is more readily apparent when one looks at the sputum specimen (108,109).

Using PCR techniques, HIV patients who have no evidence of infection were found to have positive samples for *P. carinii* (112,118). In a post mortem study of lungs from patients with no evidence of *P. carinii* pneumonia, PCR was unable to detect any evidence of *P. carinii* (119). Thus, the *P. carinii* identified by PCR in the samples from HIV infected individuals may represent a subclinical infection (112,118). Another consideration is that the positive results may arise from nonviable microorganisms. *P. carinii* often persists from three to six weeks after a successful therapy (120,121). However, the level of detectable *P. carinii*-associated DNA seems to drop rapidly with therapy (122). Due to these considerations, the overall role of PCR in the diagnosis of *P. carinii* remains unclear (123).

6. PERSPECTIVE

The approach to the diagnosis of *P. carinii* pneumonia and its treatment remain controversial. Some advocate the use of clinical criteria alone in the diagnosis of *P. carinii*, however it has become increasingly clear that such an empiric regimen may be associated with an overall worse outcome for the patient. Diagnostic testing requires obtaining an adequate respiratory sample. The necessary steps for using sputum as a screening tool probably include a specific protocol for obtaining the sample and the use of more sensitive stains such as fluorescent markers or PCR to detect the microorganism. On the other hand, BAL is used to diagnose pneumonia due to *P. carinii* or other microorganisms. Since up to 20% of AIDS patients with *P. carinii* pneumonia may have another pulmonary pathogen, bronchoscopy should be considered, especially in patients who fail the initial empiric therapy.

Table 6. Comparison of polymerase chain reaction to immunofluorescence for diagnosing *P. carinii* pneumonia

SENSITIVITY				SPECIFICITY				
SPUTUM FA	SPUTUM PCR	BAL FA	BAL PCR	SPUTUM FA	SPUTUM PCR	BAL PCR	BAL PCR	REF
		82%	100%			85%	85%	(110)
53%	100%	100%	95%	100%	93%	93%	93%	(108)
43%	86%	100%	100%	N.A.	N.A.	N.A.	N.A.	(109)
		100%	89%			100%	100%	(111)
		97%	97%			100%	100%	(112)
	69%				95%			(113)
50%	74%			N.A.	N.A.			(114)
	100%				100%			(107)
		60%	66%			97%	100%	(80)
		100%	100%			100%	100%	(115)
		100%	100%			98%	100%	(116)
67%	100%			100%	100%			(117)

Abbreviations: FA: Fluorescent antibody, PCR: Polymerase chain reaction, N.A.:Not Available.

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