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NOTES

Semiquantitative Technique for Estimating *Pneumocystis carinii* Burden in the Lung

ROBERT P. BAUGHMAN,* SUSAN STROHOFER, GREGORY COLANGELO, AND PETER T. FRAME
Department of Medicine, University of Cincinnati Medical Center, Cincinnati, Ohio 45267-0564

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We developed a technique to estimate the amount of *Pneumocystis carinii* found in bronchoalveolar lavage fluid. *P. carinii* associated with 500 nucleated cells in the bronchoalveolar lavage fluid had little between-observer and within-observer variation. Varying the technique of the lavage did not change the amount of *P. carinii* recovered. This technique was used in patients treated for *P. carinii* pneumonia. Those patients who did not respond to treatment had more *P. carinii* in their bronchoalveolar lavage fluid than those who responded.

Although the diagnosis of *Pneumocystis carinii* pneumonia may be readily made with bronchoscopy and bronchoalveolar lavage (BAL) (7, 17), treatment is associated with a significant failure rate (9, 12, 13, 15). In patients with suboptimal response, establishing efficacy of treatment for *P. carinii* pneumonia is difficult because *P. carinii* is present in follow-up BAL specimens in over half of the patients who have been successfully treated (1, 16).

We established a method for comparing numbers of *P. carinii* with those of inflammatory cells. We then checked whether the lavage procedure would affect the determination of *P. carinii* concentration. Finally, the quantitation technique was used to compare the numbers of *P. carinii* found in the BAL before and after therapy in two groups of patients with *P. carinii* pneumonia: those who did well and those who did not.

Patients were recruited from those with human immunodeficiency virus infection with pulmonary symptoms and in whom a diagnosis of *P. carinii* pneumonia was established by BAL. Sixteen patients underwent repeat bronchoscopy. In some cases, patients were bronchoscoped after 3 weeks of treatment because they were thought to be failing to respond to therapy, while other patients were asked to undergo repeat bronchoscopy 21 to 42 days after completion of therapy for *P. carinii* pneumonia. These patients gave written consent to a protocol approved by our institutional review board. Bronchoscopy and lavage were performed in a standard fashion, and the lavage specimen was handled as described previously (18).

The initial and follow-up BAL specimens were handled similarly. Specimens, 200 to 400 μl, were spun onto glass slides, using a cytocentrifuge (Cytospin II; Shandon, Sewickley, Pa.). The slides were air dried and then stained with a modified Wright-Giemsa stain (Diff-Quik; American Scientific Products, McGaw Park, Ill.). The slides were studied and differential cell counts were performed on 200 nucleated cells per slide, using standard criteria (3). The number of clusters of *P. carinii* (4, 19) in relation to the nucleated cells was also noted for each slide.

To determine how many nucleated cells needed to be counted to provide a reproducible estimate of the percentage of *P. carinii* found on the slide, we chose 20 slides from patients with a variable amount of *P. carinii* present. An observer read the 20 slides (identified only by number) three separate times, noting the number of *P. carinii* clusters seen after counting 200, 500, and 1,000 nucleated cells. Two other observers counted the number of *P. carinii* associated with 500 nucleated cells on all 20 slides.

Other methods to estimate *P. carinii* burden were also studied in BAL specimens of 24 patients who were lavaged with 120 ml of saline, and a standard aliquot (200 μl) of unspun BAL fluid was cytocentrifuged onto the slide. After the slide was stained with modified Wright-Giemsa, a diagonal of the cytospin dot was examined at ×45. The number of *P. carinii* clusters encountered was noted (Pc_{DIAG}). The total volume of retrieved fluid was also noted, and an estimate of the total amount of *P. carinii* recovered (Pc_{TOT}) was derived by multiplying Pc_{DIAG} by the total volume retrieved.

To determine the effect of lavage volume on *P. carinii* recovery, patients were studied by using a previously described protocol (6). Four 60-ml boluses of normal saline were introduced and immediately aspirated with a hand-held syringe. Specimens from all four aliquots were used to prepare modified Wright-Giemsa-stained cytocentrifuge slides, which were randomly read.

In 16 patients, BAL was performed before and 21 to 42 days after therapy for *P. carinii* pneumonia. Patients were considered responders when they had significant improvement in their chest roentgenogram and arterial oxygen. Patients were considered to have failed therapy when they were clinically worse despite 21 days of therapy (nonresponders). The slides from all 32 lavages were counted randomly.

Cytocentrifuge slides from 20 patients undergoing lavage and subsequently found to have *P. carinii* infection were studied. *P. carinii* clusters ranging from zero to 18 clusters per 500 nucleated cells were seen. Intersubject variation was significantly different for all three counts (F ratio, 9.0, 13.3, and 16.8; $P < 0.001$ for 200, 500, and 1,000 cells, respectively). There was lower intrasubject variation when 500 cells were counted rather than 200 cells (F ratio, 1.03 and 0.29, respectively). The correlation coefficient among the three readers reading 500 nucleated cells on all 20 slides varied

* Corresponding author.

TABLE 1. Cell differential and *P. carinii* cluster counts per bolus

Bolus	% (mean ± SEM)				*P. carinii* per 500 cells (mean ± SEM)
	Macrophages	Lymphocytes	Neutrophils	Epithelial cells	
1	62 ± 2.3	24 ± 2.0[a]	6 ± 2.5	8 ± 1.9[b]	8 ± 2.6
2	59 ± 2.8	34 ± 3.0	5 ± 2.1	2 ± 0.6	7 ± 2.7
3	59 ± 3.3	34 ± 3.0	3 ± 0.7	1 ± 0.4	8 ± 2.5
4	58 ± 3.0	35 ± 2.8	3 ± 0.7	1 ± 0.3	6 ± 2.2

[a] Significantly differs from other boluses, $P < 0.05$ (analysis of variance).
[b] Significantly differs from other boluses, $P < 0.01$ (analysis of variance).

between 0.70 and 0.84 ($P < 0.01$ for all). The average time it took to count 500 nucleated cells for any observer was <5 min.

For the 24 patients studied by using a fixed lavage and cytocentrifuge volume, there was a significant correlation between the number of *P. carinii* clusters associated with 500 cells and the total counted on one diagonal and the total number of *P. carinii* retrieved (Pc_{DIAG}, $r = 0.60$ and $P < 0.01$; Pc_{TOT}, $r = 0.55$ and $P < 0.01$).

The cellular and *P. carinii* yields for the sequential lavages performed on 14 patients with 15 episodes of acute *P. carinii* pneumonia are summarized in Table 1. There was no difference between the amount of *P. carinii* recovered per bolus, regardless of the lavage volume.

Sixteen patients underwent repeat BAL either because of failure to respond to therapy (4 patients) or as a follow-up of successful therapy (12 patients). The number of *P. carinii* clusters seen before and after therapy is shown in Table 2. For the patients who responded to therapy, all had a 75% or greater reduction in *P. carinii* clusters in the second BAL ($P < 0.02$). Of 12 patients, 8 (67%) still had detectable *P. carinii* when the whole specimen was screened. Those patients who failed to respond to therapy had a 75% or lower reduction in the numbers of *P. carinii* associated with 500 cells. Three of the four died of *P. carinii* pneumonia within the next 2 weeks. The fourth patient responded to a change in anti-*P. carinii* therapy.

Direct examination of lung tissue has been useful in assessing the *P. carinii* burden (10, 14). Previously reported techniques are tedious and require a rigid, standardized lavage protocol. Alterations in the lavage procedure will change the total number of cells and *P. carinii* recovered. We found a reasonable correlation between our technique and that reported by others (P_{TOT} and P_{DIAG} [8, 11]).

Volume of lavage makes a difference in the cellular content measured in the lavage specimen (5, 6). In the current study, we found that larger-volume lavages gave a significantly higher percentage of lymphocytes and lower percentage of epithelial cells. In contrast, we found that the proportion of *P. carinii* was unaffected by the volume of lavage.

From 20 to 40% of patients with acquired immunodeficiency syndrome treated for *P. carinii* pneumonia fail to respond to initial drug therapy for their infection (9, 12, 15). In many of these patients, persistent symptoms and roentgenographic abnormalities are seen after 3 weeks of therapy. In studies to date, over half of patients with acquired immunodeficiency syndrome successfully treated for *P. carinii* pneumonia will have persistent organisms when studied at the end of 3 weeks of therapy (1, 16). One study to determine the value of repeat bronchoscopy in the persistently symptomatic acquired immunodeficiency syndrome patient reported no criteria for documenting failure of *P. carinii* therapy but performed repeat bronchoscopy only to identify a new pathogen (2). We studied four patients whose clinical courses suggested failure to respond to therapy; these four patients had little change in the amount of *P. carinii* recovered by lavage.

In conclusion, we established a semiquantitative technique for counting the clusters of *P. carinii* in association with 500 nucleated cells in the BAL fluid. The method was associated with little variation between or within reader. The number of clusters seen was not significantly affected by the volume of lavage fluid instilled. In follow-up BAL studies 21 to 42 days after patients had begun treatment for *P. carinii*, responders all had a >75% reduction in *P. carinii* clusters at repeat BAL and nonresponders had little reduction in *P. carinii* clusters.

TABLE 2. Number of *P. carinii* clusters associated with 500 nucleated cells before and after treatment

Patient group	No. of clusters	
	Pretreatment	Posttreatment
Responders	1	0
	10	0[a]
	2	0
	12	3
	7	0[a]
	4	0
	12	0[a]
	3	0[a]
	6	1
	1	0[a]
	2	0
	10	2
Nonresponders	8	5[b]
	3	2[b]
	14	13[b]
	30	12

[a] *P. carinii* seen when entire slide was reviewed.
[b] Died within 2 weeks of follow-up bronchoscopy.

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