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Comparison of Processing Techniques for Detection of *Pneumocystis carinii* Cysts in Open-Lung Biopsy Specimens

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Methenamine silver stain was used to compare the number of cysts of *Pneumocystis carinii* contained in lung concentrate smears of homogenized lung tissue with the number in impression smears. Results were also compared with histopathological examination of methenamine silver-stained paraffin-embedded sections. Of slides from 23 preparations, a greater number of cysts were contained in concentrate smears than in impressions ($P < 0.001$). In four preparations, cysts were noted in concentrate smears only. All concentrate smears were positive, whereas 11 of the 23 histopathological sections were negative ($P < 0.01$). The ability to detect the cyst phase of *P. carinii* in lung tissue is enhanced by the use of concentrate smears.

Pneumocystis carinii was first identified as a cause of interstitial pneumonia in premature infants (3). Now, it is most commonly observed in individuals who are severely immunosuppressed, such as patients receiving chemotherapy and transplant recipients. In homosexuals and others with the acquired immunodeficiency syndrome, *P. carinii* has become a major problem (6).

To improve patient survival, rapid diagnosis and early institution of therapy can be crucial. The principal means of diagnosis is the demonstration of the organisms in pulmonary tissue, and diagnosis may be most accurate in open lung biopsy specimens taken from areas of active disease (11). Investigators from this institution have compared the number of cysts in lung tissue impression smears with the number of cysts contained in concentrates of homogenized lung tissue by using a mouse model. It was found that the concentrate preparations contained more cysts per microscopic field than the impression smears ($P < 0.01$) (12). Because of these results from the mouse model, the concentration technique is now routinely used in our laboratory for the processing of open-lung biopsies. The purpose of this report is to compare the results of lung concentrate smears and lung impression smears, as well as lung concentrate smears and histological sections for the identification of *Pneumocystis* cysts in our patient population over 28 months.

MATERIALS AND METHODS

Smears of both lung tissue concentrates and impressions were made. The impressions were prepared by imprinting the freshly cut open-lung biopsy tissue surfaces on a glass slide. The concentrates were prepared by homogenization and subsequent centrifugation of the pulmonary tissue. Briefly, the same portion of lung tissue which was used to make the impressions was homogenized in 2 ml of nutrient broth by blending for 2 min in a stomacher (Tekmar Co., Cincinnati, Ohio) (8). The homogenate was then centrifuged (15 min, 2,525 × *g*), and the sediment was resuspended by vortexing (15 s) in 0.2 to 0.3 ml of nutrient broth. With a 1/100 wire loop, the sediment was spread in a 1.5-cm area alongside the touch preparation on the same slide. The smears were stained with methenamine silver (5). The slides were randomized, and the cysts in 14 microscope fields (×400) or in the entire smear were counted three times, and the results were averaged. The reviewer was readily able to determine whether a concentrate or an impression smear was being examined, because the concentrate smears were always placed at the same end of the glass slides adjacent to the impression smears. Also, the concentrate smears usually were of greater density than the impression smears. Because of these limitations, it was not possible to carry out the review in a totally blind fashion. The remaining portion of the lung was fixed in 10% Formalin, embedded in paraffin, sectioned, and stained with methenamine silver. The entire section was then completely searched (×400) for *P. carinii* cysts by routine examination in the surgical pathology section of the clinic. Statistical analysis of the impression versus concentrate smears was performed by using the Wilcoxon matched-pairs signed-rank test. The comparison of the positively rated concentrate smears to histopathological sections was made with the sign test (9).

RESULTS

During a 28-month period, 232 open-lung biopsies were processed. Of these specimens, 24 preparations (10.3%) contained cysts of *P. carinii*. Slides of 23 preparations from 20 patients were available for review. In all instances, a greater number of cysts were identified in the concentrate smear than in the impression smear ($P < 0.001$) (Table 1). All concentrate smears were positive, whereas 11 of the 23 histopathological sections were negative ($P < 0.01$). In one case, the histological features were suggestive of *Pneumocystis* infection. There were five instances in which *P. carinii* was not detected in impression smears; in four of these cases, the histopathological examination of fixed tissue was also negative. In four preparations, cysts were identified in concentrate smears only.

The above statistical analyses were repeated with only the first specimen on each of the 20 patients. As before, the concentrate smear was always more positive than the impression smear ($P < 0.001$), and in nine instances, the histopathological section was negative when the concentrate smear was positive ($P < 0.01$).

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TABLE 1. Comparison of methods used to detect *P. carinii* cysts[a]

Case no.	No. of cysts in[b]:		Histopathological examination of fixed tissue[c]
	Impression smear	Concentrate smear	
1	71	163	+
2	22	225	+
3	5	61	−
4	155	350	+
5	180	365	+
6	320	~1,900	+
7	170	239	+
8	16	48	+
9	5	30	+
10	1	2	−
11	1	7	−
12	0	2	−
13	5	12	+
14	0	3	−
15	48	285	+
16	1	11	−
17	0	8	−
18	0	4	−
19	1	12	±
20	2	15	−
21	2	20	−
22	4	16	−
23	0	3	+

[a] Methenamine silver-stained smears and sections.
[b] Numbers of cysts were counted per 14 microscope fields (cases 1 to 8) or per entire impression or concentrate (cases 9 to 23).
[c] +, Cysts identified in tissue; −, cysts not present in tissue; ±, suggestive of cysts.

DISCUSSION

Several different methods of laboratory examination have been utilized for the diagnosis of *Pneumocystis* infection. Generally, examination of sputum is not rewarding. Many observers report that antibody titers are not clinically useful (7). Likewise, owing to the fastidious growth requirements of the organisms, cell culture techniques do not provide an aid in clinical diagnosis (11). Bronchial washings are very infrequently positive, and the transthoracic needle biopsy has not been considered to be an optimal method (1). Transbronchoscopic biopsy ordinarily requires several specimens to ensure adequate sampling (4). Open-lung biopsy has been considered to be the most accurate specimen for the definitive diagnosis of *Pneumocystis* infection (10).

As a result of the previous animal model which was studied in this laboratory (12), our routine technique incorporated the use of a concentrate as well as an impression smear to search for *Pneumocystis* cysts. In the present review, we have found that the concentrate technique is very useful. In 5 of the 23 preparations which were studied, the impression smear was negative and the concentrate was positive. Further, in four of five of the latter group, the histopathological material was also negative. The concentrate smears were more productive than the histopathological slides, since the latter group contained 11 false-negative results. In addition, histological evidence was only suggestive for *Pneumocystis* in another case.

Processing of open-lung biopsy tissue by grinding in a mortar and pestle should be at least as adequate or perhaps even a superior method to the homogenization of specimens by the stomacher (12).

Recently, Domingo and Waksal have reported the use of Wright stain in the diagnosis of *Pneumocystis*. It was stated that the advantages of this method are that it is a rapid, readily available technique which does not require special stain procedures. They showed a 100% correlation when compared with conventional silver stains and permanent histological sections. However, there was one false-negative result (2). Based upon our findings, we believe that the methenamine silver-stained concentrate is a valuable component of our open-lung biopsy processing. There were no false-negative concentrate smears. The use of the concentrate has increased our ability to detect the cyst phase of *P. carinii* compared with standard impression smears and histopathological sections.

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