Diagnosis of *Pneumocystis. carinii* Pneumonia by Bronchoalveolar Lavage in AIDS Patients

Comparison of Diff-Quik, Fungifluor Stain, Direct Immunofluorescence Test and Polymerase Chain Reaction

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OBJECTIVE; To assess the sensitivity, specificity and accuracy of Diff-Quik, fungifluor stain, the direct immunofluorescence test (RIFT) and the polymerise chain reaction (PCR) in the diagnosis of Pneumocystis carinii pneumonia (PCP) in human immunodeficiency virus (HIV)–infected patients. STUDY DESIGN: From December 1992 through November 1993, 112 bronchoalveolar lavage fluid (BALF) samples were obtained from 80 HlV-infected patients. BALF samples were processed for cytologic and microbiologic analysis and for PCR. Cytologic examination was carried out on Diff-Quik–stained cytocentrifuge preparations and with May-Griinwald-Giemsa staining and fungifluor staining. For diagnosis of PC infection, DIFT and PCR were used.

RESULTS: Thirty-two of 112 acute episodes were caused by P carinii. Diff-Quik had the highest sensitivity (84.8%) as compared to fungifluor stain (60.0%), DIFT (59.4%) and PCR (65.6%). The specificity was 98.7% with Diff-Quik, 100% with fungifluor stain, and 98.6% and 973% with DIFT and PCR, respectively. Accuracy was high with every method (94.4% with Diff-Quik, 88.3% with fungifluor stain, 86.7% with DIFT and 87.6% with PCR).

CONCLUSION: Diff-Quik is a good diagnostic tool in the diagnosis of PCP. The combination of Diff-Quik and fungifluor stain is recommended because of its cost-effectiveness and because of its rapid diagnosis of severe PCP. PCR and DIFT should be used only on patients judged clinically to have PCP with discrepant results in Diff-Quik and fungifluor stain in BALF samples. (Acta Cytol 1995; 39:1089-1093)

PCR and DIFT are time consuming and expensive and therefore not useful for rapid detection of P. carinii in cases of severe pulmonary disease.

Keywords: pneumonia, *Pneumocystis carinii*; bronchoalveolar lavage fluid; HIV; AIDS; stains and staining; polymerase chain reaction.

Pneumocystis carinii pneumonia (PCP) has a great impact on morbidity and mortality in acquired immunodeficiency syndrome (AIDS) patients.[19] The extensive use of prophylaxis has changed the inci-

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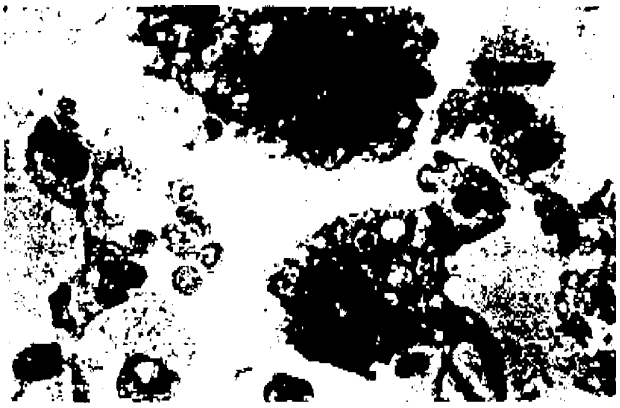

Figure 1 Bronchoalveolar lavage specimen. Alveolar macrophages, erythrocytes, *P carinii* organisms (cysts, with dot-like structures within) (Diff-Quik, X630).

dence and clinical presentation of PCP.[1,14,20,23] Based on effective treatment[4,10] and rapid diagnosis,[18] the mortality rate decreased from 24% in 1984 to 3% in 1989.[20] Nevertheless PCP is the most frequent index diagnosis for AIDS.20

Several new techniques have been described for detecting the microorganisms especially if their numbers are small.[25,27] The aim of our prospective study was to assess the diagnostic value of Diff-Quik, fungifluor stain, direct immunofluorescence test (DIFT) and polymerase chain reaction (PCR) in bronchoalveolar lavage fluid (BALF) specimens from patients with pulmonary diseases,

Materials and *Methods*

From December 1992 through November 1993, 112 RALF samples were obtained from 80 HIV-infected patients. Only patients with < 200 CD_4+ lymphocytes/mm^3 and who were not on aerosolized pentamidine as prophylaxis were included in the study in order to examine a homogeneous group of patients. None of the patients diagnosed as having PCP had prophylaxis with either aerosolized pentamidine or with trimethoprim/sulfamethoxazole regularly. The patients were followed for six months or until death.

Bronchoscopy was done under local anesthesia in cases of acute respiratory illness presenting one or more of the following symptoms: abnormal chest radiograph, abnormal chest signs, cough, shortness of breath, fever or abnormal lung function tests. Treatment of PCP was started after bronchoscopy, Bronchoalveolar lavage was performed by instilling 20-mL aliquots of lukewarm saline (NaCI 0.9%) into the middle lobe or lingula with an Olympus fiberoptic bronchoscope (Austria Gesellschaft M.B.H., Vienna, Austria). A maximum of 200 mL saline was used; the recovery rate was 5D-60%,

BALF samples were processed for cytologic and microbiologic analysis and for PCR. Cytologic examination of BALF specimens was carried out by Diff-Quik-stained cytocentrifuge preparations, May-Grünwald-Giemsa staining and fungifluor staining (Polyscience Inc., Warington, Pennsylvania, U.S.A.) (Figures 1 and 2).

We employed both Diff-Quik and May-Grünwald Giemsa stain according to the suggestions of Hopewell et al.[13]

For diagnosis of PC infection we used DIFT (Cellabs Pty. Ltd., Sydney, Australia) (Figure 3) and PCR according to Wakefield et al[27] (Figure 4).

To detect viral infections we used a monoclonal antibody-based immunofluorescence method for cytomegalovirus-specific early antigen.[11] *Toxoplasma gondii* was detected by DIFT (Cellabs) and by PCR according to Burg et al.[5]

Smears and cultures were carried out for isolation of fungi and bacteria, including mycobacteria.

Sensitivity, specificity and accuracy of Diff-Quik, fungifluor stain, DIFT and PCR results for the detection of PC were calculated from a 2 x 2 table.[2] A P value of < .05 was considered statistically significant.

Results

Our study included 80 HIV-infected patients, who underwent 112 bronchoscopies for acute respiratory illness. On the basis of clinical data, response to

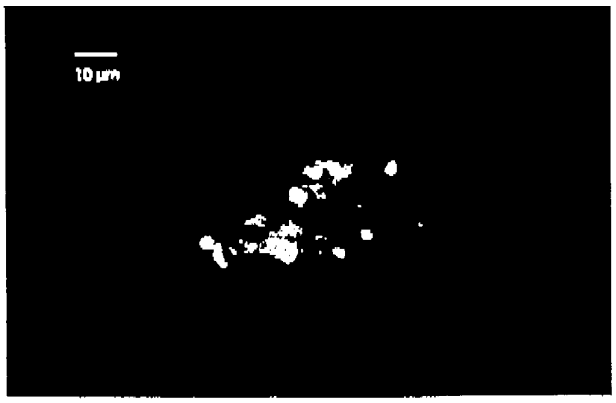

Figure 2 *P carinii* cysts, fungifluor stain. Note the two typical, kidney-shaped, intracellular bodies.

Figure 3 *P carinii* cysts and trophozoites, direct immunofluorescence stain. Note the typical honeycomb structure.

treatment and results of follow-up, 32 acute episodes were caused by PCP.

Sixty-five of the 80 patients were male, and 15 were female. Their mean age was 38.0±4.5 years (mean ±SD). Forty-six were homosexual and 21, intravenous drug users; 8 acquired HIV infection by heterosexual contact with an HIV-infected partner and two by blood products. Three patients were without high-risk behavior. All patients except one had AIDS according to the revised classification system of the Centers for Disease Control.[7] The CD, cell count was <200/mm^3 in every patient, and none of them diagnosed as having PCP receives prophylaxis regularly. The pulmonary diseases are

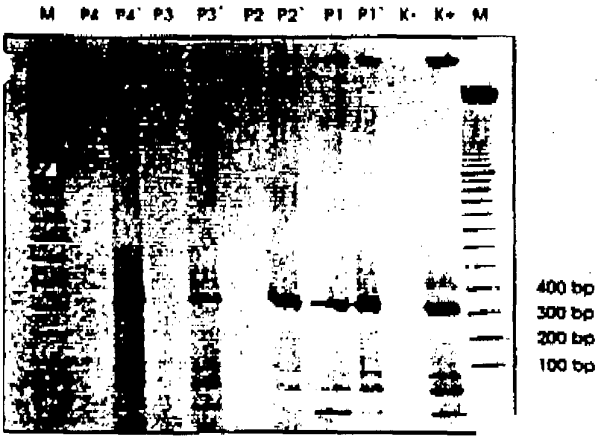

Figure 4 High-resolution polyacrylamide gel electrophoretic separation and consecutive silver staining of PCR products. *P carinii* DNA-specific band: -346 base pairs (bp}, m=DNA marker, K= controls, P= samples, P+ = internal control for detection of inhibition.

summarized in Table I. Eighty-three of 112 bronchoscopies led to diagnosis of an acute pulmonary illness; the main pathogens were P *carinii* and bacteria.

The results, of Diff-Quik, fungifluor staining, DIFT and PCR are reported in Table II. Diff-Quik stain showed the highest sensitivity (84.4%) and was therefore considered the best screening method. The specificity did not differ between the four methods. Accuracy was highest with Diff-Quik stain (94.4%) and lowest with DIFT (86.7%). No statistically significant difference was calculated between the results of first episodes and of the total number of episodes, These results demonstrate that preceding treatment does not influence the diagnostic value of the examinations.

We recommend the combination of Diff-Quik and fungifluor stain for a rapid, effective laboratory diagnosis of severe PCP using BALF samples.

According to Hopewell et al,[13] May-Grünwald Giemsa stain *is* a very sensitive means of detecting *Pneumocystis* organisms. It has proved to be a very reliable way of detecting intracystic and extracystic organisms.[6] Diff-Quik has the advantage of taking only a few minutes, but it is reported to be not as reliable as May-Grünwald-Giemsa stain.[6] At our institution both stains are used, and no discrepant results were observed.

No patient died of PCP, either during an acute episode of respiratory illness or during follow-up. No patient who was not fudged initially to have PCP developed this pulmonary complication during follow-up.

Discussion

In PCP the choice of diagnostic test depends on multiple factors, including - the type of specimen being examined, the kind of prophylaxis against PCP, the level of technical expertise required and the experience of the laboratory staff.[12,29] Since 1986, induced sputum has been used to diagnose PCP.[3,15] Since 1989, HIV-infected patients have been inhaling aerosolized pentamidine as primary and secondary prophylaxis for PCP.[16]

The sensitivity of May-Grünwald-Giemsa staining in induced sputum is low,[3,22] but the diagnostic

yield ran be increased by using monoclonal antibodies[15] and PCR.[18] In contrast, conventional staining methods, such as toluidine blue O and May-Grünwald-Giemsa stain, are efficient diagnostic means in BALF samples from patients without aerosolized pentamidine prophylaxis.[17]

In the case of inhaled pentamidine as prophylaxis and at certain stages of the infection, the structure of Pneumocystis organisms may be changed, and the disease may be missed if the diagnosis is based on the presence of cysts alone.[6,17,21] Therefore, new diagnostic methods, including immunofluorescence tests,[25] PCR[27] and fungifluor staining,[24] were developed, Several authors have reported that immunofluorescence tests are useful with induced sputum,[9,28,29] but they seem to offer no advantage over conventional staining methods in BALF samples, as described above.[6,26]

PCR is likely to be more efficient than the immunofluorescence test, but the results of PCR on induced sputum and blood[18] are quite different from those of PCR used on BALF samples.[27]

In this study only BALF samples were tested by different methods, and none of the patients were on inhaled pentamidine as prophylaxis for PCP regularly. Diff-Quik had the highest sensitivity (84.4%) and is therefore useful as a screening *test.*

DIFT and PCR are characterized by high specificity and should be used on patients judged clinically to have PCP in whom it is necessary to verify the diagnosis in the case of discrepant results of Diff-Quik and fungifluor staining in BALF samples, A comparison of the sensitivity, specificity and accuracy of the four methods in a prophylactic aerosolized pentamidine group was riot done because only patients who did not inhale aerosolized pentamidine prophylactically were included in the study.

To our knowledge, this was the only study comparing these four methods in the diagnosis of PCP. The sensitivity of Diff-Quik was comparable to that reported by Murray et al.[19] The sensitivity of DIFT and PCR were poorer as compared with that reported by other authors.17,27

PCR and DIFT have several disadvantages in comparison to Diff-Quik and fungifluor stain: they are time consuming and expensive and therefore not useful for rapid detection of *P carinii in* cases of severe pulmonary disease 3[,24] We recommend the combination of Diff-Quik and fungifluor stain for a rapid, effective laboratory diagnosis of severe PCP using BALF samples. This recommendation is based on the high sensitivity of Diff-Quik and high specificity of fungifluor stain.

Table I *Acute Pulmonary Diseases*

Disease	No. of acute episodes (%)
Bacterial pneumonia	32 (28,6)
P carinii infection	32 (28,6)
Mycobacterial infection (including MAC)	14 (123)
Kaposi's sarcoma	4 (3.6)
CMV pneumonitis	1 (0,9)
None	25 (25,8)

MAC=Mycobacterium avium complex.
CMV =Cytomegalovirus.
CMV pneumonitis was diagnosed by open lung biopsy.

Table II *Results of Diff-Quik, Fungifluor Staining, DIFT and PCR in BALF Specimens*

Method	Sensitivity	Specificity	Accuracy
Diff-Quik	84.4% (91.7%)	98.7% (98.1%)	94.4% (96.2%)
Fungifluor	60.0% (56.5%)	100.0% (100.0%)	88.3% (86,8%)
DIFT	59.4% (58.3%)	98.6% (100.0%)	86.7% (87.0%)
PCR	65.6% (62,5%)	97.3% (100.0%)	87.6% (88.3%)

Numbers in parentheses are the results of the examinations during the first episode.
N=112,

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