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# Usefulness of PCR for Detection of *Pneumocystis carinii* DNA

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**Diagnosis of *Pneumocystis carinii* pneumonia is based on the identification of the various stages of the parasite in lung samples by standard staining techniques. We therefore assessed the value of the PCR for detection of *P. carinii* in bronchoalveolar lavage, induced sputum, and blood samples relative to that of standard staining techniques.**

*Pneumocystis carinii* pneumonia (PCP) in premature infants (4), immunodeficient subjects with hematologic cancers, graft recipients, and others (13) was described in the first half of this century. The incidence of PCP has sharply increased since the emergence of AIDS at the start of the 1980s (12).

After 1989, therapies improved and led to the recommendation of primary prophylaxis with trimethoprim-sulfamethoxazole or aerosolized pentamidine for human immunodeficiency virus (HIV)-seropositive patients with CD4 counts below 200 cells per μl (2, 3, 5–7, 18). Nevertheless, prophylaxis can fail because of allergy, inefficiency, or poor compliance. Thus, cases of atypical PCP (8), which does not involve the whole lung but is localized in the apices, have emerged; disseminated PCP can also occur, especially in subjects treated with pentamidine aerosols (14).

Classical forms of pneumocystosis are easily diagnosed by identification of the various stages of the parasite in lung samples such as bronchoalveolar lavage (BAL) and induced sputum (IS) samples prealably treated with mucolytic agent (Digest Eur; Eurobio) by using standard stains (SS) such as Giemsa and toluidine blue O (TBO) or indirect immunofluorescence (IFI) (15, 16). Atypical and disseminated forms are more difficult to diagnose; thus, standard sampling and staining techniques can lead to false-negative results (8, 9).

We therefore assessed the value of the PCR for detection of *P. carinii* in BAL, IS, and blood samples relative to that of standard techniques.

We tested samples from patients suspected of having PCP on the basis of clinical and radiological features (11). These samples comprised the following. A total of 178 BAL specimens were taken from 165 patients, of which 132 samples were from 120 HIV-seropositive patients. Ten patients underwent sequential BAL procedures; five had a positive BAL followed by a control some weeks later, and five underwent two (three patients) or three (two patients) BALs.

Of the BAL specimens, 46 were from 45 immunosupressed HIV-seronegative patients (one patient had two sequential BALs). A total of 51 IS samples were taken simultaneously with BAL samples from 51 HIV-seropositive patients, and 224 blood samples were taken from 205 immunodeficient patients. Of these 205 patients, 195 were HIV seropositive (14 of whom had progressive PCP), 10 were HIV seronegative, and 19 were immunocompetent controls.

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DNA was extracted from the pellets obtained by centrifugation of 10 ml of the BAL or IS samples and from the sera and buffy coats obtained by Ficoll density centrifugation of blood samples. After proteinase K digestion and phenol-chloroform extraction, amplification of a 346-bp fragment of the *P. carinii* mitochondrial ribosomal gene was performed by using primers described by Wakefield et al. (20, 21). The PCR product was identified with a radiolabeled oligonucleotide probe.

BAL samples were stained with SS; IS samples were only stained by IFI (Monofluokit *Pneumocystis*; Pasteur Diagnostics), because this is a better technique than the use of SS (10, 15). PCP was diagnosed if SS patterns were positive.

As previously reported (21), no cross-reaction with *Toxoplasma gondii*, *Cryptococcus neoformans*, or *Candida albicans* was observed when BAL samples containing such strains were tested.

For untreated HIV-seropositive patients (Table 1), *P. carinii* was detected in BAL fluid by means of PCR with a sensitivity and a specificity of 100%, but this technique displayed no particular advantage over the use of SS such as Giemsa; in addition, the latter method is more rapid and less expensive, and it has to be carried out in any case to test for other opportunistic organisms (e.g., *T. gondii* and *C. neoformans*).

A total of 67 BALs were performed on patients treated for more than one week (Table 2), and 16 of these still produced positive results by both techniques; this demonstrates that BAL could be performed and *P. carinii* could be detected even after some days of therapy. Among the 43 patients with negative results, 5 had had a first positive BAL about 3 weeks earlier. This illustrates that *P. carinii* can be cleared out.

For 8 patients, conflicts between the results with the different techniques appeared. For 7 treated patients, low parasite burdens (rare cysts with IFI) were found to be associated with negative PCR results. This could be interpreted as the persistence of empty cyst walls lacking DNA. These discrepancies underline the fact that staining interpretation is difficult in the absence of vital staining or in vitro culture. Therefore, PCR could be useful for patients with persistent low parasite burdens during treatment and should allow discrimination between specimens with and specimens without genetic material.

One patient with a positive PCR result and a negative stain result had had PCP some months earlier and probably relapsed after a clinical and parasitological cure; this latter point could not be confirmed, as the patient died in another center.

No PCP occurred in the 46 HIV-seronegative patients studied. Although HIV-seronegative patients usually have lower parasite burdens than HIV-seropositive patients, SS are

TABLE 1. HIV-positive patients without treatment: comparison of standard staining and PCR results for 65 BAL samples

| Result for BAL samples ($n$ = 65) by standard staining | No. of samples with the following result by PCR: | |
|---|---|---|
| | + ($n$ = 27) | − ($n$ = 38) |
| + ($n$ = 27) | 27 | 0 |
| − ($n$ = 38) | 0 | 38 |

generally sufficiently sensitive to detect the parasite; however, PCR may improve this sensitivity.

PCR didn't detect the parasite in the 132 BAL samples from immunodepressed patients without PCP. These findings are in agreement with those of earlier studies (9, 19) and do not support the reactivation of latent *P. carinii* from alveolar macrophage in the course of PCP. On the contrary, they suggest de novo contamination, which occurs in immunodeficient patients.

A total of 51 IS samples were tested in parallel with BAL samples. Among these 51 BAL samples, 37 were negative; the corresponding IS samples were found to be negative too by IFI and PCR. A total of 14 BAL samples were positive; these were from patients with confirmed PCP (Table 3).

Considering BAL the "gold standard," the specificity of PCR and IFI with regard to IS samples was 100%; the sensitivity of PCR was 86% versus 43% with IFI. With high-quality samples from untreated patients, the sensitivity of PCR was 100%: PCR could detect *P. carinii* DNA in all 12 available samples from untreated patients (2 samples were unavailable: one of these was salivary, and the other came from a patient treated for 3 weeks). Such results were reported by others (10, 19), suggesting that PCR may be the procedure of choice for detection of *P. carinii* in IS. But the extraction step needs to be optimized and the processing time needs to be reduced in order to permit rapid diagnosis. A recent study of BAL (1) indicates that PCR can be carried out after filtration without DNA extraction; the application of this technique to IS could also produce interesting results. Various groups in the United States have proposed such an approach, but in France IS is not routinely used for PCP diagnosis.

Among the 225 blood samples tested, 222 were negative; one of the positive samples was from a patient who had disseminated pneumocystosis (*P. carinii* in spleen, liver, and mesenteric vessels); the other two were probably positive because of contamination (both samples were taken in the same set of samples).

Detection of DNA in blood by PCR is of value (10), and it is the only available blood test for patients with extrapulmonary involvement (17). PCR did not detect *P. carinii* in blood, sera, or cells purified by Ficoll centrifugation in the case of any of the 13 other patients with PCP, and so pneumocystosis usually appears to be an exclusively pulmonary disease in human patients.

TABLE 2. HIV-positive patients treated for PCP: comparison of standard staining and PCR results for 67 BAL samples

| Result for BAL samples ($n$ = 67) by standard staining | No. of samples with the following result by PCR: | |
|---|---|---|
| | + ($n$ = 17) | − ($n$ = 50) |
| + ($n$ = 23) | 16 | 7 |
| − ($n$ = 44) | 1 | 43 |

TABLE 3. Comparison of PCR and IFI results for 14 IS samples from patients with PCP

| Result for IS samples ($n$ = 14) by IFI | No. of samples with the following result by PCR: | |
|---|---|---|
| | + ($n$ = 12) | − ($n$ = 2) |
| + ($n$ = 6) | 5 | 1[a] |
| − ($n$ = 8) | 7 | 1[b] |

[a] Patient treated for 3 weeks.
[b] Poor-quality (salivary) sample for the detection of *P. carinii*.

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