Letters to the Editor

PCR for Detection of Pneumocystis carinii in Blood Cells

We read with interest the article of Roux et al. concerning their experience with the PCR technique for detection of Pneumocystis carinii DNA in bronchoalveolar lavage, induced sputum, and blood samples collected from patients with P. carinii pneumonia (PCP).

The authors found that PCR did not detect P. carinii in blood, sera, or cells purified by Ficoll-Hypaque centrifugation from 13 (92.8%) of 14 patients with PCP. A positive result was found for only one patient who was found to have disseminated P. carinii infection. Thus it can be concluded that pneumocytosis appears to be an exclusively pulmonary disease in human patients (3).

We conducted a similar study by investigating P. carinii in an in vitro study with A549 cell lines inoculated with infected peripheral blood mononuclear cells (PBMC) isolated from patients with PCP.

Single or multiple blood samples were obtained from 44 AIDS outpatients (mean age, 34.7 years; range, 26 to 52 years). Of these, 38 had morphologically and clinically proven PCP or extrapulmonary pneumocystosis (one patient); 6 presented signs and respiratory features of pneumonia, but definitive diagnoses could not be made because of severe hypoxia; and 12 were AIDS patients with other opportunistic infections.

PBMC were purified from heparinized whole blood by Ficoll-Hypaque density centrifugation (Lymphoprep; Nycomed, Oslo, Norway) and cultured onto confluent A549 cell monolayers as previously described (1). Supernatants from ongoing cultures were sampled daily and evaluated for P. carinii by an immunofluorescence method (IIF) and by nested PCR using the primers described by Lipschick et al. (3).

Viabilities of organisms were determined by using a combination stain with fluorescein diacetate and ethidium bromide (4).

Both the IIF and PCR provided evidence of infection in 95.1% (98 of 103) of culture supernatant samples from patients with proven PCP including disseminated P. carinii infection and in 66.6% (16 of 24) of those with suspected pneumonia. Supernatants with positive results by IIF gave positive PCR signals with documentation of a single band of DNA with the expected size on autoradiographs (310 bp). Furthermore, PCR scored positive 24 to 32 h after PBMC inoculation, rather than the 48 h required by the IIF.

Pneumocystis was not seen in culture supernatants from the other 12 patients with AIDS, thus confirming recent reports in which organisms could not be detected in advanced AIDS patients without a history of PCP (5).

In a recent study, P. carinii was isolated by PCR DNA amplification from blood or sera of rats and humans with PCP, thus improving the possibility of diagnosis and providing insights into the pathogenesis of parasitic dissemination (4).

We conclude that contrary to the statements of Roux et al., PCR has been shown to be a highly sensitive and specific method which detects microorganisms in PBMC isolated from the blood of patients with PCP. The cultivation of these cells in the above system seems also to support growth and long-term propagation of P. carinii. Considering that most of the samples tested were collected from patients with PCP only, these results clearly demonstrate that even during pneumonia, P. carinii isolates spread through blood.

REFERENCES

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Author’s Reply

We read with interest the article by Contini et al. (1) concerning P. carinii detection in blood cultures from AIDS patients with PCP.

In our study, we observed only two clinically and biologically confirmed cases of extrapulmonary pneumocystosis. Detection in blood by PCR with primers described by Wakefield et al. (3) was positive for one patient. For the other patient, who had kidney problems, P. carinii DNA was detected in the urine. This patient had been receiving pentamidine intravenously for 15 days. Twenty-five blood samples from other patients with simultaneous PCP were negative.

The differences between our report and Contini’s report may be due to the different therapies administered to patients as prophylaxis. Aerosolization of pentamidine increases the occurrence of extrapulmonary pneumocystosis (2).

The in vitro culture used in this study seems unnecessary, as it doesn’t allow multiplication. DNA detection could be performed directly by using samples, as we did in our study. Nested PCR may improve the sensitivity of detection.

The results obtained seem interesting and have to be confirmed, with further studies taking into account therapy, clinical aspects, and evolution.
REFERENCES


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**Lactobacillus GG Vaginal Suppositories and Vaginitis**

*Lactobacillus GG*, a variant of *Lactobacillus casei*, has been used as therapy and prophylaxis for disorders, including traveler’s diarrhea (2) and antibiotic-associated colitis (1, 3, 5). Other lactobacilli have been cited as possible therapeutic agents in the prophylaxis of vulvovaginal candidiasis (2). This pilot study examined the effects of topically applied *Lactobacillus GG* when used as treatment for recurrent vaginitis.

Twenty-eight women who suffered from recurrent vaginitis (greater than five infections per year) were enrolled in a trial. Inclusion criteria included symptoms and signs of vaginal candidiasis or inflammation, including pruritus, a thick vaginal discharge, erythema on exam, and a low vaginal pH. Specimens were collected from the posterior fornix and plated onto chocolate and Sabouraud’s agars (Becton-Dickenson Microbiology Systems, Cockeysville, Md.). After informed consent was obtained, the women were then given 14 glycerol suppositories impregnated with $10^9$ *Lactobacillus GG* organisms. They were instructed to insert the suppositories twice a day for 7 days. The patients returned 7 days after the completion of therapy for a repeat examination with microbiological sampling.

All of the women enrolled reported subjective improvement after the use of the suppositories. Additionally, all of the women showed decreased erythema and discharge on repeat examination. Microbiologically, only five women had significant colony counts of *Candida albicans* isolated from their pretreatment samples. No other pathogens were isolated. Four of the five with positive cultures had negative cultures on reexamination following therapy.

The paucity of organisms isolated from pretreatment specimens (i.e., vaginal cultures did not yield any *Candida* species) may have been due to secondary to prior treatment with antifungal agents. Fifteen of the women had just completed courses of antifungal medications, but symptoms persisted even in the absence of yeasts on repeat-cultured specimens. Another possibility is that some of these patients may have had another cause of their persistent symptoms, although this is less likely, as they were selected from a population who had documented recurrent candidal vulvaginitis. It is possible that lactobacilli, in restoring the normal flora, may prove helpful in the treatment of female genital infections caused by candidal species. Further study is needed to determine the efficacy of *Lactobacillus GG* for the treatment of vaginitis.

**REFERENCES**


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Usefulness of PCR for Detection of *Pneumocystis carinii* DNA

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Received 22 November 1993/Returned for modification 4 January 1994/Accepted 3 June 1994

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, inefficacy, 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 immunosuppressed 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.

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). PCR 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.

In PCP occurred in the 46 HIV-seronegative patients studied. Although HIV-seronegative patients usually have lower parasite burdens than HIV-seropositive patients, SS are

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

<table>
<thead>
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<th>Result for BAL samples (n = 65) by standard staining</th>
<th>No. of samples with the following result by PCR:</th>
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<tbody>
<tr>
<td>+ (n = 27)</td>
<td>+ (n = 27)</td>
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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

<table>
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<th>Result for BAL samples (n = 67) by standard staining</th>
<th>No. of samples with the following result by PCR:</th>
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<tr>
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<td>16</td>
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<td>1</td>
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TABLE 3. Comparison of PCR and IFI results for 14 IS samples from patients with PCP

<table>
<thead>
<tr>
<th>Result for IS samples (n = 14) by IFI</th>
<th>No. of samples with the following result by PCR:</th>
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<td>+ (n = 6)</td>
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<tr>
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<td>- (n = 2)</td>
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<tr>
<td></td>
<td>5</td>
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<tr>
<td></td>
<td>1*</td>
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<tr>
<td></td>
<td>7</td>
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* Patient treated for 3 weeks.

* Poor-quality (salivary) sample for the detection of *P. carinii*.

This work was supported by the ANRS (Agence Nationale de Recherche sur le SIDA).

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