JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2000, p. 1307–1308 Vol. 38, No. 3
0095-1137/00/$04.00+0

Letters to the Editor

Very Low Frequence of *Pneumocystis carinii* DNA Detection by PCR in Specimens from Patients with Lung Damage

Data reported by Dr. Sing et al. (3) about *Pneumocystis carinii* carriage in immunocompetent adults with primary pulmonary disorders differ partially from our results. We have analyzed 81 bronchoalveolar lavage fluid samples (BALs) from 78 patients (37 male and 41 female; mean age, 57.8 years; range, 35 to 84 years) who underwent bronchoscopy from November 1997 to June 1998. Forty-four patients had experienced a worsening of their chronic obstructive lung disease (COLD), 15 patients had suspected pulmonary carcinoma (definitive diagnosis of lung neoplasm in 10 cases), and 18 patients had a respiratory syndrome with abnormal chest X ray (bacterial pneumonia in 15 cases and mycobacterial infection in 3 cases). All samples were directly treated for microbiological and cytological analysis; we tested for *P. carinii* by indirect immunofluorescence (IF) assay and by PCR. Twenty-microliter samples of BAL were digested with proteinase K and then amplified; a nested PCR was performed using mitochondrial LSU region primers (5). All experiments were repeated at least three times, and multiple negative controls were included in each amplification run.

All specimens were negative for *P. carinii* by IF assay and upon the first amplification; only two samples (2.5%) were positive for *P. carinii* by nested PCR. The definitive diagnoses for the two patients who supplied these samples were, respectively, COLD and microcitoma. The patients were not receiving corticoid therapy or anti-*P. carinii* prophylaxis or therapy, and they did not develop *P. carinii* pneumonia (PCP) within an 18-month surveillance period.

None of the specimens in our study were positive for *P. carinii* after the first amplification. In our experience PCP is defined by an acute respiratory syndrome that is confirmed by positive morphological staining or a positive result upon the first cycle of amplification, and under any of these conditions anti-*P. carinii* treatment is necessary for the recovery of the patients; moreover, previous data (2) have demonstrated that positivity upon the first amplification is consistent with the diagnosis of PCP.

Only 2.5% of our samples were *P. carinii* positive by nested PCR compared to the 19% described by Dr. Sing. We hypothesize that some technical differences between our methods exist. We did not perform DNA extraction, but samples were digested with proteinase K without any loss in sensitivity, as previously reported (4). Our nested PCR was performed with different internal primers, but both primer pairs belong to the mtLSU-rRNA region, and it seems unlikely that there would be a different sensitivity. Moreover, our nested PCR showed a sensitivity of 100% and a specificity of 62% in the diagnosis of PCP from BALs of human immunodeficiency virus (HIV)-positive patients; in addition, acute PCP is rarely defined solely on the basis of positive nested PCR results (E. Visconti et al., Conf. Rec. 12th World AIDS Conf., abstr. 22184, p. 299, 1998).

In conclusion, we question whether subclinical colonization by *P. carinii* occurs in HIV-positive and elderly patients. It may occur in a small number of immunocompetent adults with lung damage due to COLD or lung carcinoma; further studies need to define the role of *P. carinii* carriage in the diffusion of *P. carinii* and the possibility, as previously described (1), that a positive nested PCR could precede the debut of acute PCP even though none of our patients developed PCP within 18 months of follow-up.

REFERENCES

1. **Elvin, K., M. Olsson, C. Lidman, and A. Bjorkman.** 1996. Detection of asymptomatic *Pneumocystis carinii* infection by polymerase chain reaction: predictive for subsequent pneumonia. AIDS **10:**1296–1297.
2. **Peters, S. E., A. E. Wakefield, S. Banerji, and J. M. Hopkin.** 1992. Quantification of the detection of *Pneumocystis carinii* by DNA amplification. Mol. Cell. Probes **6:**115–117.
3. **Sing, A., A. Roggenkamp, I. B. Autenrieth, and J. Heesemann.** 1999. *Pneumocystis carinii* carriage in immunocompetent patients with primary pulmonary disorders as detected by single or nested PCR. J. Clin. Microbiol. **37:** 3409–3410.
4. **Tamburrini, E., P. Mencarini, A. De Luca, G. Maiuro, G. Ventura, A. Antinori, A. Ammassari, E. Visconti, L. Ortona, A. Siracusano, E. Ortona, and G. Vicari.** 1993. Diagnosis of *Pneumocystis carinii* pneumonia: specificity and sensitivity of polymerase chain reaction in comparison with immunofluorescence in bronchoalveolar lavage specimens. J. Med. Microbiol. **38:**449–453.
5. **Wakefield, A. E., F. J. Pixley, S. Banerji, K. Sinclair, R. F. Miller, E. R. Moxon, and J. M. Hopkin.** 1990. Amplification of mitochondrial ribosomal RNA sequences for *Pneumocystis carinii* DNA of rat and human origin. Mol. Biochem. Parasitol. **43:**69–76.

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Author's Reply

I read with great interest the letter by Dr. Visconti et al. on our study of *P. carinii* carriage in immunocompetent patients with primary pulmonary disorders as detected by single or nested PCR (3). We agree with the authors that PCP is defined as an acute respiratory syndrome that is confirmed by positive morphological staining of *P. carinii* organisms on respiratory samples. However, the inclusion of a positive first cycle of amplification (single PCR) in their PCP definition has to be restricted, in our opinion, to defined patient groups, since in our experience of a yet-unpublished study with 334 patients of different immunostatus specificity rates of single PCR on BALs vary between HIV positive and immunosuppressed HIV negative (e.g., transplant patients or patients suffering from malignancies) patient groups. According to these results, the proposed inclusion of a positive first cycle of amplification (single PCR) in the definition of PCP is justified only for HIV-positive

patients. This conclusion may also be drawn from the study by Weig et al. who found nested PCR useful only in HIV-positive patients, i.e., not in otherwise-immunocompromised HIV-negative patients (4). Therefore, we feel comfortable recommending anti-*P. carinii* treatment only when conventional staining is positive; however, we report a positive PCR result to the clinician immediately and discuss the laboratory finding and the management of the patient. We agree with the authors that acute PCP is rarely defined solely on the basis of positive nested PCR results.

Dr. Visconti and colleagues report a very low frequency of *P. carinii* DNA in the BALs of 78 patients, as detected by a nested PCR using mtLSU-rRNA region primers after proteinase K digestion without a DNA extraction step. The technical differences might contribute to their differing results. However, we did not test for the influence of a DNA extraction step on the sensitivity of the nested PCR we performed (4). In our opinion, the sensitivity of the different primers used can only be compared by performing a study with both PCR methods on the same samples.

The nested PCR we used showed a sensitivity of 100% and a specificity of 97.9% on BAL samples of HIV-positive PCP patients. Weig et al. (4) found a 45.4% rate of positivity by nested PCR for BALs from HIV-negative immunosuppressed patients without clinical proof of PCP, suggesting a high sensitivity of their nested PCR. As discussed in our paper (3), several other studies on different HIV-negative immunocompetent patient groups with underlying pulmonary diseases found *P. carinii* carriage rates of 6.5% (1) to 25% (2) as shown by PCR.

Additionally, differences in the incidence of PCR positive samples might be explained by different patient collectives or geographical factors.

We agree with Dr. Visconti and colleagues that further studies are needed to define the role of *P. carinii* carriage in the diffusion of *P. carinii*.

REFERENCES

1. **Armbruster, C., A. Hassl, and S. Kriwanek.** 1997. *Pneumocystis carinii* colonization in the absence of immunosuppression. Scand. J. Infect. Dis. **29:**591–593.
2. **Contini, C., M. P. Villa, R. Romani, R. Merolla, S. Delia, and R. Ronchetti.** 1998. Detection of *Pneumocystis carinii* among children with chronic respiratory disorders in the absence of HIV infection and immunodeficiency. J. Med. Microbiol. **47:**329–333.
3. **Sing, A., A. Roggenkamp, I. B. Autenrieth, and J. Heesemann.** 1999. *Pneumocystis carinii* carriage in immunocompetent patients with primary pulmonary disorders as detected by single or nested PCR. J. Clin. Microbiol. **37:**3409–3410.
4. **Weig, M., H. Klinker, B. H. Bogner, A. Meier, and U. Gross.** 1997. Usefulness of PCR for diagnosis of *Pneumocystis carinii* pneumonia in different patient groups. J. Clin. Microbiol. **35:**1445–1449.

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