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Pneumocystis carinii Carriage in Immunocompetent Patients with Primary Pulmonary Disorders as Detected by Single or Nested PCR

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Ninety-five bronchoalveolar lavage specimens from 63 immunocompetent adult patients with primary pulmonary disease were analyzed for *Pneumocystis carinii* colonization by primary and nested PCR. Twelve of 63 patients (19%) were PCR positive. None of them developed *P. carinii* pneumonia. These results suggest that *P. carinii* carriage may exist in immunocompetent patients with underlying pulmonary disease.

Pneumocystis carinii is an opportunistic pathogen causing serious and even life-threatening pneumonia (*P. carinii* pneumonia [PCP]) in immunosuppressed patients. PCP is speculated to result either from a de novo infection or from reactivation of a latent childhood infection. Seroconversion usually happens during early childhood, leading to high rates of seroprevalence (9). Autopsy studies using microscopy or immunofluorescence, however, revealed no evidence or only a very low rate of prevalence of *P. carinii* (less than 1%) in adults without predisposing diseases (6, 7, 10). More-sensitive methods like PCR may be able to detect even low numbers of *P. carinii* organisms in clinically silent, but colonized, persons. It is conceivable that lung tissue already damaged by different conditions such as bacterial pneumonia, fibrosis, and chronic obstructive pulmonary disease is at increased risk for *P. carinii* colonization. In an autopsy study using PCR, however, no *P. carinii* DNA could be isolated (8).

To evaluate the prevalence of *P. carinii* colonization in immunocompetent patients with primary pulmonary diseases, 95 bronchoalveolar lavage (BAL) specimens of 63 immunocompetent patients (21 females, 42 males; median age, 53 years [Table 1]) suffering from primary acute (37 patients) or chronic (26 patients) lung disease were examined microscopically and by primary and nested PCRs. Inclusion criteria were the absence of underlying malignant disease (except bronchial carcinoma), systemic disease, and human immunodeficiency virus (HIV) infection; no use of immunosuppressive or cytotoxic medication; and normal immunological function. None of the patients had received antipneumocystic chemoprophylaxis.

Clinical specimens were centrifuged at 4,000 rpm for 10 min in an Omnifuge (2.0 RS; Heraeus, Munich, Germany). Portions of the pellets were smeared on slides and Giemsa and Grocott stained for microscopy. The other parts of the pellets were stored at −20°C until PCR analysis. Following proteinase K digestion, DNA was extracted with a Qiagen (Hilden, Germany) tissue kit. A two-step protocol with the external primers pAZ 102E and pAZ 102H (11) and the nested primers pLE1 and pLE2 (12) was applied as described elsewhere (12). Products of both primary and nested PCRs were investigated by agarose gel electrophoresis, stained with ethidium bromide, and analyzed under UV light. Precautions against contamination included the use of aerosol barrier pipette tips and the performance of the steps of the procedure (master mix preparation, DNA extraction, PCR, and specimen detection) in separate rooms. Several positive (from BAL specimens of PCP patients) and negative (autoclaved water and the PCR mixture minus the DNA template) controls were tested simultaneously. All experiments were performed at least twice. Nested-PCR-positive samples were identified as *P. carinii* specific following DNA sequencing of the amplicons and sequence comparison with the BLAST program (1). To further assess the specificities of the PCR primers, the nested PCR was performed on human and *Candida albicans* DNAs, yielding negative results.

With the BAL specimens of 3 of the 63 patients, both primary and nested PCR yielded a positive result, while with the specimens of 9 patients, only the more sensitive nested PCR could detect *P. carinii*-specific DNA. In none of the 12 PCR-positive specimens could *P. carinii* organisms be visualized microscopically. The characteristics of the 12 PCR-positive patients are summarized in Table 2. None of them presented with PCP according to criteria of the Centers for Disease Control and Prevention (4) or received antipneumocystic chemoprophylaxis and/or therapy. They either improved with adequate treatment aimed at the underlying pulmonary disease or were not curable. In the latter cases, pulmonary material obtained intra vitam or at autopsy did not justify assignment of the cause of death to PCP. Improved patients did not develop PCP within the follow-up period indicated in Table 2. Of the 12 patients with PCR-positive BAL specimens, 6 suffered from chronic pulmonary disease, with preexisting long-term lung tissue damage, and 6 were afflicted with more acute respiratory diseases of both infectious and noninfectious natures.

TABLE 1. Characteristics of 63 immunocompetent patients with primary pulmonary disease

Primary pulmonary disease[a]	No. of patients
Bacterial pneumonia	18
Chronic lung disease[b]	9
COPD	8
ARDS	5
Tuberculosis	4
Bronchial carcinoma	3
Aspergillosis	2
Other[c]	14

[a] ARDS, acute respiratory distress syndrome; COPD, chronic obstructive pulmonary disease.
[b] Including sarcoidosis, chronic lung fibrosis, and chronic alveolitis.
[c] Including pulmonary edema, viral pneumonia, lung embolism, and postsurgical respiratory failure.

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TABLE 2. Characteristics of 12 immunocompetent patients with primary pulmonary disease and *P. carinii* colonization as detected by single and nested PCRs

Patient	Age (yr)	Sex[a]	Primary pulmonary disease(s)[b]	Length of intubation (days)	Length of follow-up time without PCP	Single PCR result	Nested PCR result
1	47	M	Chronic lung fibrosis	2	19 mo	Positive	Positive
2	71	M	Carnified bacterial pneumonia	8	LF[d]	Negative	Positive
3	76	F	Bacterial pneumonia	8	1 mo	Negative	Positive
4	25	F	ARDS, lung embolism	NI[c]	LF	Negative	Positive
5	21	F	Bacterial pneumonia	4	LF	Negative	Positive
6	37	M	Bacterial pneumonia	7	1 mo	Negative	Positive
7	32	M	Chronic lung fibrosis, COPD	12	3 mo (died)	Negative	Positive
8	64	F	Lung edema, postsurgical pneumonia	10	LF	Negative	Positive
9	73	M	ARDS, COPD	12	NF[e]	Positive	Positive
10	45	M	SIRS, COPD	7	LF	Negative	Positive
11	77	M	Hamman-Rich syndrome	NI	14 days (died)	Negative	Positive
12	26	M	Lung fibrosis, pleuraempyem	NI	5 mo	Positive	Positive

[a] M, male; F, female.
[b] ARDS, acute respiratory distress syndrome; COPD, chronic obstructive pulmonary disease; SIRS, systemic inflammatory response syndrome.
[c] NI, no intubation.
[d] LF, lost to follow-up.
[e] NF, no follow-up due to death of patient.

The percentage of PCR-positive patients among the 63 immunocompetent patients without clinical PCP, 19%, is relatively high. In a study of 50 patients with chronic bronchial disease, 10% were found to be *P. carinii* positive by sputum sampling (3). However, it was not stated whether *P. carinii* contributed to or even was responsible for pulmonary disease in these patients. Moreover, *P. carinii* carriage in these patients was defined by the simultaneous determination of positivity by four techniques less sensitive than PCR with sputum samples, which usually yield fewer positive results than more invasively obtained material. These results might suggest at least some pathological role for *P. carinii* in these patients. Furthermore, three of the five *P. carinii* patients had received inhalative corticoid therapy, which may have led to some degree of local immunosuppression among elderly patients. Therefore, a higher rate of prevalence might have been found in this study by a more sensitive PCR on material from deeper airways.

An even lower rate of prevalence of *P. carinii* carriage was found by Armbruster et al. (2). In 5 of 77 (6.5%) HIV-negative immunocompetent patients with acute respiratory illness *P. carinii* colonization was detected by immunofluorescence and PCR of microscopically negative BAL specimens. However, underlying pulmonary disease in four of these five patients was a rather short-term condition. In contrast, the *P. carinii* DNA-positive patients described here were more equally distributed among those with acute and those with chronic underlying pulmonary diseases, possibly explaining the higher level of prevalence of *P. carinii* carriage in our study population.

A level of prevalence of *P. carinii* colonization similar to that found in our study was found among immunocompetent HIV-negative children with chronic respiratory disorders (5). Seven of 28 (25%) patients had PCR-positive nasopharyngeal aspirates. In at least five of them, *P. carinii* may have contributed to pulmonary disease since antipneumocystic therapy led to clinical and microbiological improvement. Those authors concluded that the pathologic changes in chronic lung diseases might permit colonization of *P. carinii* and that the pathogenic role of *P. carinii* in exacerbations of chronic lung diseases needs further clarification.

In contrast, our study suggests a less severe pathogenic effect of *P. carinii* in immunocompetent patients with underlying pulmonary diseases, as the 12 *P. carinii*-colonized patients either recovered without antipneumocystic therapy or died from non-PCP-related diseases. None of the seven *P. carinii*-colonized patients not lost to follow-up developed PCP in a mean period of 5 months.

In conclusion, we found a relatively high percentage of *P. carinii* DNA-positive BAL specimens among immunocompetent patients with primary respiratory diseases, suggesting that lung tissue damage may favor *P. carinii* colonization. The pathogenic impact of colonization on immunocompetent patients, however, may not be significant as long as the immunostatus of the patient has not deteriorated dramatically. In this scenario, reactivation might occur. In addition, it can be speculated that patients with underlying pulmonary disease might serve as carrier reservoirs for *P. carinii*.

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