

Pneumocystis carinii Carriage among Cystic Fibrosis Patients, as Detected by Nested PCR

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A total of 137 sputa from 95 consecutive cystic fibrosis (CF) patients undergoing routine bacteriological surveillance were analyzed for *Pneumocystis carinii* colonization using nested PCR. Seven of 95 patients (7.4%) were PCR positive, suggesting that *P. carinii* carriage may exist among CF patients due to their underlying pulmonary disease.

Cystic fibrosis (CF) is the most common life-shortening autosomal recessive disorder in Caucasians; it is caused by different mutations in the CF transmembrane conductance regulator gene, which is on chromosome 7 (4). Since the CF transmembrane conductance regulator gene encodes a protein functioning as a cyclic AMP-regulated chloride channel in the apical membrane of epithelial cells, several systems, including the sinopulmonary system, the gastrointestinal tract, and the male urogenital tract, can be affected in CF. The chronic bronchopulmonary manifestation of the disease poses the most serious clinical problem and causes most morbidity and mortality in CF patients. One major factor contributing to bronchopulmonary disease in CF patients is the persistent colonization and infection with typical CF bacterial pathogens, like mucoid and nonmucoid *Pseudomonas aeruginosa*, *Staphylococcus aureus*, nontypeable *Haemophilus influenzae*, and *Burkholderia cepacia*.

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 latent childhood infection. Seroconversion usually happens during early childhood, leading to high seroprevalence rates (3). Autopsy studies using microscopy or immunofluorescence, however, revealed no or only a very low prevalence of *P. carinii*, less than 1%, in adults without predisposing diseases (1, 2, 10). More sensitive methods like PCR may be able to detect even low numbers of *P. carinii* organisms in clinically asymptomatic but colonized persons. In a recent study it was shown that a considerable percentage (19%) of immunocompetent adult patients with primary pulmonary disease but without overt PCP were colonized by *P. carinii*, as established by primary and nested PCR on bronchoalveolar lavage (BAL) specimens (6). This suggests that *P. carinii* carriage may exist in immunocompetent patients with underlying pulmonary disease. Since it is conceivable that preexisting lung tissue damage may favor colonization by *P. carinii*, we hypothesized that *P. carinii* DNA might be detected in respiratory

samples from CF patients. In a previous study on *P. carinii* carriage among CF patients, however, Varela et al. were not able to detect *P. carinii* using four different staining methods on sputa from 45 consecutive CF patients (8).

To evaluate the prevalence of *P. carinii* colonization in CF patients, 137 sputa from 95 consecutive CF patients (43 females and 52 males; median age, 23.2 years) who were not receiving trimethoprim-sulfamethoxazole for treatment of underlying bacterial infections were examined by nested PCR. The sputa were collected for routine bacteriological surveillance of the CF patients. None of the patients suffered from over PCP.

Sputa were divided for conventional staining (Giemsa staining and Grocott silver staining) and nested PCR. Nested PCR was performed as described previously (7). Briefly, clinical specimens were centrifuged at $3,430 \times g$ for 10 min. The pellet was stored at -20°C until PCR analysis. Following proteinase K digestion, DNA was extracted using the Qiagen (Hilden, Germany) tissue kit. A two-step protocol using the external primers pAZ 102E and pAZ 102H and the nested primers pLE1 and pLE2 was applied as described previously (7). Products of nested PCR were investigated by agarose gel electrophoresis, stained with ethidium bromide, and analyzed under UV light. Contamination precautions included use of aerosol barrier pipette tips and the performance of master mix preparation, DNA extraction, PCR, and specimen detection in separate rooms. Additionally, all steps during DNA extraction and amplification were performed in laminar flow cabinets. Several positive (from BAL specimens from 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. Positive nested PCR findings were verified by DNA sequencing of the amplified gene products.

Seven of 95 (7.4%) CF patients were found to harbor *P. carinii* DNA in their sputa, while Giemsa staining and Grocott silver staining were negative for sputa from all patients. Bacteriological findings for *P. carinii* DNA-positive and -negative CF patients did not differ significantly. Of the six patients who were available for follow-up reevaluation by *P. carinii* PCR 4 to 6 weeks after their previous positive nested PCR result, two patients remained PCR positive. Taking into account that sputa are—compared to BAL fluid—not the diagnostic mate-

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rial of choice and that specimen inhibition of nested PCR was not assessed in this study, it may be speculated that the rate of *P. carinii* carriage among CF patients is actually higher than the observed percentage of nested PCR-positive sputa. The only previously performed study on *P. carinii* carriage among CF patients was not able to identify *P. carinii* carriers among 45 consecutive CF patients by using four different diagnostic methods, including toluidine blue O staining, modified Giemsa staining, methenamine silver staining, and immunofluorescence (8).

Interestingly, none of our *P. carinii* DNA-positive CF patients suffered from overt PCP or developed PCP within at least 2 months, although two of six patients with a previous positive PCR result who were reevaluated for *P. carinii* DNA in their sputa remained PCR positive after 4 weeks. Therefore, the immunostatus of CF patients seems to control *P. carinii* carriage without development of PCP. Accordingly, so far only one case, involving a 15-week-old CF patient in whom *P. carinii* cysts were visualized on BAL fluid, is reported in the literature (5); the authors, however, state that the contribution of *P. carinii* to the patient's symptoms could not be clearly defined.

In conclusion, a significant percentage of CF patients are colonized by *P. carinii*, as detected by nested PCR on sputum samples. A higher prevalence might be expected if material from deeper airways or—as recently suggested in a study on *P. carinii* DNA transmission to immunocompetent contact persons of a PCP patient (9)—deep nasal swabs are analyzed. *P. carinii* carriage, however, does not lead to PCP in CF patients.

It may be speculated that asymptomatic *P. carinii* carriers are a reservoir for *P. carinii*.

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