Eighty-one bronchoalveolar lavage (BAL) specimens obtained from 26 HIV-infected, 45 non-HIV immunosuppressed and 10 immunocompetent patients with primary pulmonary diseases were analysed for the presence of *Pneumocystis carinii* by staining and by *P. carinii* 5S rDNA determined by PCR. *P. carinii* was observed by staining of BAL specimens from HIV-infected patients significantly more frequently than those from immunocompromised hosts without HIV infection (57.7% versus 20.0%, respectively). *P. carinii* 5S rDNA was detected by PCR assay in seven (26.9%) HIV-infected individuals, which was significantly more frequent than for four (8.9%) immunosuppressed patients without HIV infection, for whom staining was negative. None of these patients developed *P. carinii* pneumonia (PCP) within the follow-up period. BAL specimens from 10 immunocompetent patients with pulmonary disorders were negative for PCP by both staining and PCR assay.

**Introduction**

*Pneumocystis carinii* f. sp. *hominis* (*P. carinii*) is an opportunistic pathogen that causes critical and life-threatening infection in immunocompromised patients with human immunodeficiency virus (HIV) type-1 infection [1], haematological malignancies, organ transplantation or connective tissue diseases. The frequency of *P. carinii* pneumonia (PCP) has decreased since the introduction of highly active antiretroviral therapy [2]. PCP results from either reactivation of latent infection or from *de novo* infection [3]. However, several autopsy investigations that used microscopic and immunofluorescent examinations showed no evidence or only a very low rate (<1%) of *P. carinii* detection in patients without immunodeficiency [4–6]. PCR, a more sensitive assay than microscopic and immunofluorescent tests, may be able to detect even lower levels of *P. carinii* trophozoites. Nevertheless, it has been demonstrated that *P. carinii* DNA detection by PCR on post-mortem pulmonary tissues from immunocompetent individuals failed to provide positive results [7]. On the other hand, low levels of the trophozoites, not determined by microscopic examinations, are occasionally detected by PCR assay of clinical respiratory samples including bronchoalveolar lavage (BAL) specimens from immunocompromised patients with no evidence of *P. carinii* infection [8]. Low numbers of the trophozoites may represent pulmonary colonisation [8]. As PCR assay is infrequently applied as a diagnostic method for PCP on respiratory samples, colonised patients with *P. carinii* may be missed [8]. Huang et al. [9] have recently reported that a high proportion of mutant genotypes of *P. carinii* among HIV-infected individuals in a localised region provides the evidence of person-to-person transmission of the organism either directly or through a common environmental source. Transmission of *P. carinii* DNA from a patient with PCP to immunocompetent contact health-care workers has also been described [10]. Therefore, this study evaluated the prevalence of *P. carinii* colonisation, as well as PCP infection, among hospitalised immunosuppressed patients and a control group. It reports the results of microscopic examinations and PCR assay for immunocompromised patients.
with and without HIV infection and immunocompetent patients with primary pulmonary diseases.

Patients and methods
All patients (n = 81) with respiratory symptoms and abnormal findings on chest radiography who underwent BAL between April 1997 and August 2001 were enrolled in this study. BAL specimens were centrifuged at 1300 rpm for 5 min and portions of the pellets were smeared on slides. Diff-Quik staining (American Scientific Products, Chicago, IL, USA), a rapid Giemsa-like staining method that detects P. carinii trophozoites, or toluidine blue O staining that reveals the cyst forms, were used for the microscopic examinations. The results of microscopic examinations were considered to be positive when the organism was found with either Diff-Quik or toluidine blue O staining and were considered negative when it was not detected by either stains. The remaining pellets were stored at −80°C until use. P. carinii SS rDNA was amplified by PCR as described previously [11]. Diluted DNA samples with only a few organisms per PCR solution efficiently amplified P. carinii SS rDNA, demonstrating a high sensitivity of the PCR method for P. carinii. PCR was able to detect P. carinii in sputum samples that were not detectable by Diff-Quik staining [11]. After DNA extraction with proteinase K and phenol-chloroform on the remaining BAL fluid sediments, a PCR assay was performed with a pair of specific oligonucleotide primers (5S sense, 5'-AGTTACGGCCATACTCTCAGA-3'; 5S antisense, 5'-AACGCTACAGCAGCCTCGTAF-3') generating a 120-bp PCR product. Denaturation was at 94°C for 1 min with annealing at 55°C for 2 min and extension at 72°C for 2 min; amplification was done for 40 cycles. To avoid contamination of the PCR mixtures, mixture preparation and template DNA addition were performed in separate rooms. Positive (P. carinii-infected mouse lung) and negative (autoclaved water) controls were tested simultaneously. The PCR products were analysed by polyacylamide 12% gel electrophoresis, stained with ethidium bromide and visualised under UV light.

The patients enrolled in the study were classified into three groups: group A comprised HIV-infected individuals, group B comprised immunosuppressed patients without HIV infection, and group C comprised immunocompetent patients with primary pulmonary diseases. Details of age, sex, underlying diseases, pulmonary complications, presence of PCP development, PCP therapy or prophylaxis and blood CD4+ cell counts at the time of BAL procedure were obtained by retrospective medical chart survey. PCP diagnosis was based on findings of dyspnoea and a diffuse ground-glass opacity on chest radiograph in addition to positive staining results. Patients whose diagnosis was not consistent with PCP were defined as colonised with P. carinii when the BAL specimens showed a positive PCR test with a negative stain for the organism. The difference in prevalence of infection and colonisation among the different groups was analysed by Fisher's exact test. CD4+ cell counts were analysed by an unpaired Student's t-test. A p value <0.05 was considered statistically significant.

Results
The demographic characteristics of the three groups of patients are shown in Table 1. Group A consisted of 26 HIV-infected individuals, whereas the underlying diseases included leukaemia, lymphoma, connective tissue diseases and renal transplantation state in group B. The underlying diseases in group C included bacterial pneumonia, pulmonary tuberculosis, pulmonary Mycobacterium avium complex (MAC) infection and sarcoidosis. In group B, immunosuppressive agents were administered to 40 subjects to treat their underlying diseases before the BAL procedure except for 5 patients with leukaemia. No patients in group C received immunosuppressive therapy for the pulmonary diseases.

Positive results of staining and PCR testing for P. carinii were observed in BAL specimens from HIV-infected individuals significantly more frequently than in those from immunosuppressed patients without HIV infection (57.7% versus 20.0%, p = 0.002) (Table 2). These cases were diagnosed as having PCP and

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean age (range)</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>42.6 (27–61)</td>
<td>HIV infection 26</td>
</tr>
<tr>
<td></td>
<td>25 (1)</td>
<td>Leukaemia 31</td>
</tr>
<tr>
<td>B</td>
<td>41.5 (12–77)</td>
<td>Lymphoma 4</td>
</tr>
<tr>
<td></td>
<td>34 (11)</td>
<td>Connective tissue diseases 6</td>
</tr>
<tr>
<td>C</td>
<td>41.1 (23–72)</td>
<td>Pulmonary tuberculosis 3</td>
</tr>
<tr>
<td></td>
<td>7 (3)</td>
<td>Pulmonary MAC infection 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sarcoidosis 2</td>
</tr>
</tbody>
</table>

HIV: human immunodeficiency virus; MAC, Mycobacterium avium complex.
Table 2. Results of microscopic studies and PCR test for *P. carinii* among different groups

<table>
<thead>
<tr>
<th>Staining</th>
<th>PCR</th>
<th>Group A (%)</th>
<th>Group B (%)</th>
<th>Group C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>15 (57.7)</td>
<td>9 (20.9)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>−</td>
<td>+</td>
<td>7 (26.9)</td>
<td>4 (8.9)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>−</td>
<td>−</td>
<td>4 (15.4)</td>
<td>32 (71.1)</td>
<td>10 (100)</td>
</tr>
</tbody>
</table>

*Group A versus group B showing positive results of staining and PCR test, p = 0.002.

†Group A versus group B showing positive PCR test with negative staining, p = 0.048.

Positive PCR tests with negative stains for *P. carinii* were observed in the BAL samples from individuals with HIV infection significantly more frequently than in those from immunocompromised patients without HIV infection (26.9% versus 8.9%, p = 0.048) (Table 2). These patients were considered to be colonised with *P. carinii*. Pulmonary complications included bacterial pneumonia (n = 3), pulmonary MAC infection (2), pulmonary Kaposi’s sarcoma (1) and interstitial pneumonitis (1) in group A, and pulmonary graft versus host disease (2), bacterial pneumonia (1) and cytomegalovirus pneumonia (1) in group B. Patients improved with appropriate treatment for the pulmonary complications despite the absence of PCR therapy, except for a patient with pulmonary Kaposi’s sarcoma in group A and a case of cytomegalovirus pneumonia in group B. No patients in either group A or group B developed PCP within a mean follow-up period of 25.4 months (range, 2–47 months) and 22.8 months (range, 1–38 months) after the BAL procedure, respectively. The seven colonised patients in group A received PCP prophylaxis before undergoing BAL, compared with two cases in group B who received prophylaxis. CD4+ cell counts in group A were significantly lower than in group B (mean: 58.6 SD 71.0 versus 281.5 SD 45.4, p < 0.001).

Negative results of staining and PCR tests for *P. carinii* were observed in BAL specimens from 10 immunocompetent patients with pulmonary diseases (group C), four patients in group A and 32 in group B (Table 2).

Discussion

Staining methods detected *P. carinii* in BAL specimens from HIV-infected individuals significantly more frequently than in those from immunosuppressed hosts without HIV infection (57.7% versus 20.0%, p = 0.002). These immunosuppressed patients were all diagnosed as having PCP. It has been reported that individuals without HIV infection have significantly fewer organisms in BAL specimens obtained during an episode of PCP than HIV-infected patients with PCP, and that it may be difficult to detect organisms in induced sputum and BAL samples from HIV-negative patients [12]. To improve the accuracy of PCP diagnosis among immunocompromised patients without HIV infection, it is necessary to detect *P. carinii* with transbronchial lung biopsy samples as well as BAL specimens.

The prevalence of pulmonary *P. carinii* colonisation among HIV-positive and HIV-negative patients was investigated previously and was reported to be not significant (11.4% versus 15.9%, respectively; Fisher’s test, p = 0.59) [13]. In the present study, *P. carinii* colonisation was observed among HIV-infected individuals significantly more frequently than among immunocompromised patients without HIV infection (26.9% versus 8.9%, p = 0.048). These results bolster the hypothesis that colonised immunosuppressed patients with and without HIV infection may serve as carrier reservoirs for *P. carinii* in the hospital environment. Pulmonary colonisation with *P. carinii* in an immunosuppressed HIV-negative patient and genotyping of the organism by PCR of BAL fluid has been reported [8]. Further investigation of this hypothesis by genotyping *P. carinii* isolates from colonised immunosuppressed patients might be useful.

It has been reported that blood CD4+ cell counts <400/μl seem to pose an increasing risk for pulmonary colonisation with *P. carinii* among patients without HIV infection [14]. *P. carinii* colonisation in HIV-positive homosexual men was shown to be associated with a low peripheral CD4+ cell count <400/μl [15]. The mean CD4+ cell counts among the colonised immunosuppressed individuals with and without HIV infection (58.6 and 281.5/μl, respectively) in the present study were consistent with the data from the latter reports [14, 15]. No significant difference in the presence of PCP prophylaxis was found between the colonised patients with and without HIV infection in the present study.

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References
