A Cluster of *Pneumocystis* Infections Among Renal Transplant Recipients: Molecular Evidence of Colonized Patients as Potential Infectious Sources of *Pneumocystis jirovecii*

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**Background.** Eighteen renal transplant recipients (RTRs) developed *Pneumocystis jirovecii* infections at the renal transplantation unit of Brest University Hospital (Brest, Brittany, France) from May 2008 through April 2010, whereas no cases of *P. jirovecii* infection had been diagnosed in this unit since 2002. This outbreak was investigated by identifying *P. jirovecii* types and analyzing patient encounters.

**Methods.** The identification of *P. jirovecii* internal transcribed spacer (ITS) types was performed on *P. jirovecii* isolates from the 18 RTRs (12 patients with Pneumocystis pneumonia [PCP], 6 colonized patients), 22 unlinked control patients (18 patients with PCP, 4 colonized patients), and 69 patients (34 patients with PCP, 35 colonized patients) with contemporaneously diagnosed *P. jirovecii* infections in the Brest geographic area. A transmission map was drawn up. Its analysis was combined with the results of *P. jirovecii* typing.

**Results.** *P. jirovecii* ITS type identification was successful in 14 of 18 RTRs, 15 of 22 control patients, and 48 of the 69 patients. Type Eg was the most frequent type in the 3 patient groups. However, its frequency was significantly higher in the first patient group than in the 2 other groups (**P** < .05 and **P** < .01, respectively). Fourteen encounters between RTRs who harbored an identical type were observed. Ten patients were considered as possible index patients, of whom 3 were colonized by the fungus, and 7 presented PCP.

**Conclusions.** The results provide to our knowledge the first data on the role of colonized patients as potential sources of *P. jirovecii* in a context of nosocomial acquisition of the fungus.

*Pneumocystis jirovecii* is an opportunistic fungus that causes severe pneumonia in immunocompromised patients. *Pneumocystis* pneumonia (PCP) remains the most frequent AIDS-defining illness in developed countries in human immunodeficiency virus (HIV)–infected patients. In France, 32% of AIDS cases, which were registered with the Institut de Veille Sanitaire in 2009, were related to PCP [1]. PCP is also observed in non–HIV-infected patients who receive immunosuppressive therapy particularly for organ transplantation [2]. In the absence of prophylaxis, PCP occurs in 2%–24% of renal transplant recipients (RTRs), with a mortality rate of up to 49% [2]. These data show that PCP remains a public health issue and needs measures to prevent it in immunocompromised individuals.

Currently, PCP is considered to result from de novo acquisition of the fungus rather than from reactivation of latent forms of *P. jirovecii* present in the lungs [3–6]. Because *Pneumocystis* organisms infecting each mammalian species are host specific [7], an animal reservoir...
for *P. jirovecii* is excluded and PCP in humans can be considered as an anthroponosis.

No exosaprophytic form of *Pneumocystis* species has been identified so far, whereas *P. jirovecii* seems to be widespread within human communities. Indeed, *P. jirovecii* infections cover a large spectrum of clinical presentations of which PCP represents only a part, and mild infections such as pulmonary colonization may be the main part [8]. The potential human sources of *P. jirovecii* may be represented by all infected patients. Host-to-host transmission of *Pneumocystis* species via the airborne route has been demonstrated in models [9, 10] and was suspected in humans through the occurrence of clusters of PCP cases in hospitals [11–19].

Studies of PCP case clusters among RTRs—in the course of which analysis of patient encounters were combined with *P. jirovecii* genotype identification—support the hypothesis of nosocomial acquisition and patient-to-patient transmission of the fungus [12, 13, 15, 17–19]. However, the potential role of colonized patients as *P. jirovecii* sources was not investigated.

We report herein a cluster of *Pneumocystis* infections in 18 RTRs, which occurred in Brest University Hospital, Brest, France from May 2008 through April 2010. No cases of PCP had been diagnosed among RTRs followed up in our institution since 2002. PCP was diagnosed in 12 of the 18 patients, and 6 patients were colonized by *P. jirovecii*. To investigate the potential nosocomial acquisition and transmission of *P. jirovecii* in the course of this cluster, we identified *P. jirovecii* types based on the analysis of 2 loci. Encounters between RTRs were analyzed, and a transmission map combined with the results of *P. jirovecii* type identification was drawn up.

**METHODS**

**Hospital Settings**

Brest University Hospital is a 2100-bed healthcare facility, located at 2 main sites 5 km apart, Cavale Blanche Hospital and Morvan Hospital. The renal transplantation unit is located at the first site. Specialized care is provided for 550 RTRs. Because outpatient clinic and hospitalization department are located on the same floor in the same building, outpatients and inpatients may encounter each other.

**Patients and *P. jirovecii* Isolates**

Eighteen RTRs were involved in the cluster. The patients’ median age was 62 years (range, 44–77 years); the male-female ratio was 11:7; the median posttransplantation period was 39.5 months (range, 13–183 months). None of the patients were submitted to PCP prophylaxis at the time of *P. jirovecii* infection diagnoses. Biological diagnoses of *P. jirovecii* infection were based on *P. jirovecii* detection in bronchoalveolar lavage (BAL) specimens by microscopy using Wright-Giemsa and toluidine blue O stains, and a real-time polymerase chain reaction (PCR) assay. The PCR assay was performed, after DNA extraction procedure using NucliSens easyMag system (Bio-Mérieux), with specific probe and primers of the gene encoding the mitochondrial large subunit ribosomal RNA (rRNA), as reported elsewhere [20]. The fungus was detected by both microscopy and the PCR assay in 7 patients who developed PCP. It was detected only by PCR in 5 other patients with PCP and 6 colonized patients. In the colonized patients, improvement was obtained despite the absence of specific treatment for *P. jirovecii*, alternative diagnoses of PCP were available, and the fungus was only detected by the PCR assay because of its low apparent burden (Table 1).

Twenty-two other patients hospitalized at Morvan Hospital and with contemporaneously diagnosed *P. jirovecii* infection were also enrolled. They had no a priori encounters with RTRs and represented a control group. Their median age was 58 years (range, 3–79 years), and their male-female ratio was 13:9. Biological diagnoses of *P. jirovecii* infection were performed as described above. The fungus was detected by both microscopy and PCR in 10 patients who developed PCP. It was detected only by PCR in 8 other patients with PCP and 4 colonized patients (Table 2).

The frequency of occurrence of the different *P. jirovecii* genotypes in the Brest geographic area was determined by analysis of *P. jirovecii* isolates from 69 patients with *P. jirovecii* infections diagnosed contemporaneously in our laboratory (from 3 October 2007 to 10 February 2010). The median age of these patients was 63 years (range, 3–87 years); the male-female ratio, 43:26. Risk factors for *P. jirovecii* infection included hematological malignancies (24 patients), cancers (14 patients), immunosuppressive therapy (13 patients), chronic bronchopulmonary diseases (7 patients), HIV infection (4 patients), malnutrition (2 patients), and liver transplantation, heart transplantation, congenital immunodeficiency, alcoholism, and pregnancy (1 patient each). Thirty-four patients developed PCP, whereas 35 patients were colonized by the fungus. Alternative diagnoses of PCP in these 35 patients were bacterial pneumonia (20 patients), bronchial carcinoma (5 patients), pulmonary aspergillosis (2 patients), drug-induced pneumonia (2 patients), and sarcoidosis, tuberculosis, exacerbation of chronic bronchitis, Eisenmenger syndrome, pulmonary edema, and pulmonary fibrosis (1 patient each). Biological diagnoses of *P. jirovecii* infection were performed as described above. The fungus was detected by both microscopy and PCR in 17 patients who developed PCP. It was detected only by PCR in 17 other patients with PCP and 35 colonized patients. This patient population is designated as the third patient group in the subsequent sections.

DNA samples of BAL specimens from all patients were stored at −80°C until typing.
Table 1. Characteristics of 18 Renal Transplant Recipients Involved in a Cluster of *Pneumocystis jirovecii* Infections and Results of *P. jirovecii* Type Identification

<table>
<thead>
<tr>
<th>Patient Code</th>
<th>Age, y</th>
<th>Sex</th>
<th>Posttransplant Period, mo</th>
<th>Immunosuppressive Regimens</th>
<th>Date of BAL Retrievala</th>
<th>Presentation of <em>P. jirovecii</em> Infection (Alternative Diagnosis of PCP)</th>
<th><em>P. jirovecii</em> ITS Type (No. of Clones)b</th>
<th><em>P. jirovecii</em> DHPS Typeb</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>69</td>
<td>M</td>
<td>84</td>
<td>Sirolimus, prednisone</td>
<td>20/05/08</td>
<td>Pulmonary colonization (bacterial pneumonia)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>P2</td>
<td>72</td>
<td>M</td>
<td>140</td>
<td>Sirolimus</td>
<td>15/11/08</td>
<td>PCP</td>
<td>Gg (5)</td>
<td>Wild</td>
</tr>
<tr>
<td>P3</td>
<td>67</td>
<td>M</td>
<td>50</td>
<td>Everolimus, ciclosporin, prednisone</td>
<td>27/01/09</td>
<td>PCP</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>P4</td>
<td>45</td>
<td>M</td>
<td>23</td>
<td>Ciclosporin, MMF, prednisone</td>
<td>25/02/09</td>
<td>PCP</td>
<td>Eg (5)</td>
<td>Wild</td>
</tr>
<tr>
<td>P5</td>
<td>61</td>
<td>F</td>
<td>80</td>
<td>Tacrolimus, MMF, prednisone</td>
<td>25/02/09</td>
<td>PCP</td>
<td>Eg (5)</td>
<td>Wild</td>
</tr>
<tr>
<td>P6</td>
<td>77</td>
<td>M</td>
<td>26</td>
<td>Belatacept, MMF, prednisone</td>
<td>27/02/09</td>
<td>PCP</td>
<td>Eg (5)</td>
<td>Wild</td>
</tr>
<tr>
<td>P7</td>
<td>60</td>
<td>F</td>
<td>27</td>
<td>Sirolimus, MMF, prednisone</td>
<td>10/04/09</td>
<td>Pulmonary colonization (drug induced pneumonia)</td>
<td>Eg (5)</td>
<td>Wild</td>
</tr>
<tr>
<td>P8</td>
<td>63</td>
<td>F</td>
<td>29</td>
<td>Belatacept, MMF, prednisone</td>
<td>16/05/09</td>
<td>Pulmonary colonization (viral pneumonia)</td>
<td>...</td>
<td>Wild</td>
</tr>
<tr>
<td>P9</td>
<td>54</td>
<td>M</td>
<td>15</td>
<td>Ciclosporin, MMF, prednisone</td>
<td>28/05/09</td>
<td>PCP</td>
<td>Eg (5)</td>
<td>Wild</td>
</tr>
<tr>
<td>P10</td>
<td>54</td>
<td>M</td>
<td>16</td>
<td>Ciclosporin, MMF, prednisone</td>
<td>28/05/09; 17/06/09c</td>
<td>PCP</td>
<td>Eg (2), d ''N''eg (1), Eh (1), IV'g (1); Eg (5)c</td>
<td>Wild; Wildc</td>
</tr>
<tr>
<td>P11</td>
<td>53</td>
<td>F</td>
<td>62</td>
<td>Ciclosporin, MMF, prednisone</td>
<td>03/06/09</td>
<td>PCP</td>
<td>Eg (5)</td>
<td>Wild</td>
</tr>
<tr>
<td>P12</td>
<td>62</td>
<td>M</td>
<td>69</td>
<td>Tacrolimus, MMF, prednisone</td>
<td>16/06/09</td>
<td>PCP</td>
<td>Eg (3), d E''g1''h (1)</td>
<td>Wild</td>
</tr>
<tr>
<td>P13</td>
<td>62</td>
<td>F</td>
<td>183</td>
<td>Ciclosporin, MMF, prednisone</td>
<td>06/07/09</td>
<td>Pulmonary colonization (bacterial pneumonia)</td>
<td>...</td>
<td>Wild</td>
</tr>
<tr>
<td>P14</td>
<td>48</td>
<td>M</td>
<td>69</td>
<td>Sirolimus, MMF, prednisone</td>
<td>08/07/09</td>
<td>Pulmonary colonization (drug induced pneumonia)</td>
<td>Eg (4), d E''g2''h (1)</td>
<td>Wild</td>
</tr>
<tr>
<td>P15</td>
<td>65</td>
<td>M</td>
<td>25</td>
<td>Ciclosporin, MMF, prednisone</td>
<td>14/07/09</td>
<td>Pulmonary colonization (bacterial pneumonia)</td>
<td>Eg (4), d Lg (1)</td>
<td>Wild</td>
</tr>
<tr>
<td>P16</td>
<td>66</td>
<td>F</td>
<td>13</td>
<td>Tacrolimus, MMF, prednisone</td>
<td>23/10/09</td>
<td>PCP</td>
<td>Eg (5)</td>
<td>Wild</td>
</tr>
<tr>
<td>P17</td>
<td>44</td>
<td>F</td>
<td>21</td>
<td>Tacrolimus, MMF, prednisone</td>
<td>01/03/10</td>
<td>PCP</td>
<td>Eg (5)</td>
<td>Wild</td>
</tr>
<tr>
<td>P18</td>
<td>77</td>
<td>M</td>
<td>71</td>
<td>Sirolimus, MMF, prednisone</td>
<td>11/03/10; 21/04/10c</td>
<td>PCP</td>
<td>Eg (4); Eg (5)c</td>
<td>Wild; Wildc</td>
</tr>
</tbody>
</table>

Abbreviations: BAL, bronchoalveolar lavage; DHPS, dihydropteroate synthase; F, female; ITS, internal transcribed spacer; M, male; MMF, mycophenolate mofetil; PCP, *Pneumocystis jirovecii* pneumonia.

a Dates are given as date/month/year.

b Ellipses indicate that *P. jirovecii* typing failed to give positive results.

c Patients P10 and P18 underwent 2 consecutive BAL procedures, for which dates of retrieval and *P. jirovecii* type identification are separated by a semicolon.

d Major type within mixed infections due to >1 type in patients P10, P12, P14, and P15.

e ''N'' differs from ITS1 allele N reported elsewhere by Lee et al [21] by the presence of a G residue at position 15.

f IV is an ITS1 allele described elsewhere by Robberts et al [22] (Genbank access no. AY328046).

g ''g1'' differs from ITS2 allele g reported elsewhere by Lee et al [21] by the presence of a G residue at position 62.

h ''g2'' differs from ITS2 allele g reported elsewhere by Lee et al [21] by the presence of G residues at positions 62 and 72.

**P. jirovecii** Typing

The *P. jirovecii* dihydropteroate synthase (DHPS) locus was examined using PCR-restriction fragment length polymorphism (RFLP) analysis. First, the DHPS locus was amplified using a nested-PCR assay using primer pairs F1 [23] and B45 [24] (first round) and Ahum [24] and BN [23, 24] (second round).
Reagent and amplification conditions of the 2 rounds of PCR were described elsewhere [23]. Second, the RFLP assay was performed using restriction enzymes, *Accl* and *Hae*III (New England Biolabs), which made possible the detection of mutations at nucleotide positions 165 and 171 respectively, as described elsewhere [25].
To amplify the internal transcribed spacer (ITS) 1 and 2 sequences, a nested-PCR assay was performed with 2 pairs of primers specific for *P. jirovecii*, N18SF and N26SRX (first round) and ITSF3 and ITS2R3 (second round) [4, 26]. The 2 rounds were performed under reagent and amplification conditions described elsewhere [26, 27] but with the following modifications. The second PCR round was done with denaturation at 94°C for 1.5 minutes, annealing at 55°C for 1.5 minutes, and extension at 72°C for 5 minutes, for 35 cycles.

The second round PCR products were purified (Microcon PCR columns, Millipore) and cloned into the plasmid pGEMT (pGEMT easy Vector System II kit; Promega) [27]. Recombinant plasmids were sequenced from the 2 strands using T7P and SP6 universal primers on an automatic sequencer (BigDye Terminator Method, Applied Biosystems Sequencer 3700, Applied Biosystems). Sequence alignment was performed with BioEdit software, version 7.0.0, using the Clustal W program. ITS1 and ITS2 alleles were identified using the score described by Lee et al [21]. *P. jirovecii* ITS types are defined by a combination of the alleles of the 2 loci.

### Transmission Map

A transmission map was drawn up to reveal encounters and *P. jirovecii* transmission between patients. We assumed that transmission took place via the airborne route. Dates of outpatient visits and patient hospitalization were extracted from the database of the Laboratory and Medical Information Systems (Hexaflux Galaxie II [5.3.0] and Susie V4). All infected patients were regarded as possible index patients. A possible index patient was defined as a patient for whom a diagnosis of *Pneumocystis* infection was confirmed by a positive result of *P. jirovecii* detection in a BAL specimen, and who had, contemporaneously with the course of *P. jirovecii* infection, ≥1 encounter with a potential susceptible patient. A susceptible patient was defined as a patient who had ≥1 encounter with an index patient and who later developed *P. jirovecii* infection, as revealed by a positive result of *P. jirovecii* detection in BAL specimen. We considered that encounters and transmission conditions were combined if index patients and susceptible patients visited the outpatient clinic or the hospitalization department at the same day and harbored identical *P. jirovecii* types.

### Statistical Analysis

*P. jirovecii* ITS type frequencies in RTRs, the control group, and the third group were compared using Fisher exact test and \( \chi^2 \) test with Yates’ correction. Differences were considered significant at \( P \leq .05 \).

### RESULTS

#### P. jirovecii Typing

Positive results of *P. jirovecii* DHPS type identification were obtained in 16 of the 18 RTRs (Tables 1 and 3), in 18 of the 22 patients of the control group (Tables 2 and 3), and in 52 of the 69 patients of the third group (Table 3). A wild type was
identified in all 16 RTRs, in the 18 patients of the control group, and in 50 of the 52 patients (96.2%) in the third group. Mutant types were identified in the 2 remaining patients. Thus, there were identical characteristics of *P. jirovecii* genotypes in RTRs and in the patient control group.

Positive results of *P. jirovecii* ITS type identification were obtained in 14 of the 18 RTRs of whom 11 developed PCP and 3 were colonized by the fungus. Eight ITS types—Eg, Eh, Gg, IVg, Lg, “N”g, E”g1”, E”g2”—were identified. Type Eg, Eh, Gg, and Lg result from the combination of ITS1 and ITS2 alleles described elsewhere by Lee et al [21]. Type IVg results from the combination of ITS1 allele IV described by Robberts et al [22] with ITS2 allele g. Type “N”g results from the combination of ITS2 allele g with a new ITS1 allele that we designated as “N”. Type E”g1” results from the combination of ITS1 allele E with a new ITS2 allele that we designated as “g1”. Type E”g2” results from the combination of ITS1 allele E with a new ITS2 allele that we designated as “g2” (sequence data of new ITS1 and 2 alleles are described in Table 1). More than 1 type was detected in 4 of the 14 patients (28.6%), suggesting mixed infections. Type Eg, which was detected in 13 of the 14 patients (92.8%), was the most frequent type in RTRs.

Positive results of *P. jirovecii* ITS type identification were obtained in 15 of the 22 control patients, of whom 13 developed PCP and 2 were colonized by the fungus. Thirteen ITS types—Eg, Gg, IVg, Ec, Ne, Ai, Bi, Jf, Di, Ng, “E”g, E”b”, “E”g”—were identified. Type Eg, Gg, IVg, Ec, Ne, Ai, Bi, Jf, Di, Ng result from the combination of ITS1 and ITS2 alleles described elsewhere by Lee et al [21] and Robberts et al [22]. Type “E”g results from the combination of ITS2 allele g with a new ITS1 allele that we designated as “E”. Type E”b” results from the combination of ITS1 allele E with a new ITS2 allele that we designated as “b”. Type E”g” results from the combination of ITS1 allele E and a new ITS2 allele designated “g3” (sequence data of new ITS1 and 2 alleles are described in Table 2). More than 1 type was detected in 6 of the 15 patients (40%), suggesting mixed infections. Type Eg, which was detected in 9 of the 15 patients (60%), was the most frequent type.

Positive results of *P. jirovecii* ITS type identification were obtained in 48 of the 69 patients of the third group. Twenty-six patients developed PCP, and 22 were colonized by the fungus. Thirty-one ITS types were identified. Types Ai, Bi, Di, Eb, Ec, Ed, Ei, Eg, Eh, Ei, Em, Gg, Hf, Hg, Ig, Ir, It, Ki, Ne, Ng, Nh, and IVg resulted from the combination of ITS1 and ITS2 alleles described elsewhere by Lee et al [21], Robberts et al [22], and Siripattanapipong et al [28]. Nine ITS types result from the combination of a new ITS1 allele with a previously described ITS2 allele, or a previously described ITS1 allele with a new ITS2 allele (complete list of types not shown; sequence data available on request). More than 1 type was detected in 17 of the 48 patients (35.4%), suggesting mixed infections. Type Eg, which was detected in 23 of the 48 patients (47.9%), was the most frequent type. Types Ir, Ec, and Ai were identified in 5 patients each. Other types were detected in 1, 2, or 3 patients each.

Frequencies of mixed infection in the 3 patient groups did not differ (4 of 14 RTRs vs 6 of 15 control patients [P = .4]; 4 of 14 RTRs vs 17 of 48 patients of the third group [P = .44]). Conversely, the frequency of type Eg is higher in RTRs than in the control group (13 of 14 vs 9 of 15; P = .049) or in the third group (13 of 14 vs 23 of 48; P = .007) (Table 3).

**Transmission Map**

Taking into account the above definitions of index and susceptible patients, a total of 25 encounters involving 16 patients were initially observed (Figure 1). The analysis of encounters combined with the results of typing enable us to rule out 11 of the 25 encounters as the cause of *P. jirovecii* transmission. Four encounters involved patients from whom *P. jirovecii* ITS types did not match. Indeed, P2 was infected with type Gg, whereas the potential susceptible patients P4, P5, P6, and P12 were infected with type Eg. Moreover, 7 encounters involved patients from whom ITS typing failed to give positive results for at least 1 patient. Finally, 14 encounters occurred between patients who developed *Pneumocystis* infections due to identical *P. jirovecii* ITS and DHPS types (Figure 1, arrows). Ten patients were considered as index patients (patients P4, P5, P6, P7, P9, P10, P12, P14, P15, and P16), 7 were considered as susceptible patients (patients P7, P9, P11, P14, P15, P16, and P18). Patients P7, P9, P14, P15, and P16 were considered as either index patients or susceptible patients. Three of the index patients were colonized by *P. jirovecii* (patients P7, P14, and P15), whereas 7 presented PCP (patients P4, P5, P6, P9, P10, P12, and P16).

**DISCUSSION**

The cluster of *Pneumocystis* infections occurred from May 2008 through April 2010, whereas no cases of PCP had been diagnosed in RTRs followed up in our institution since 2002. The apparent increase in *P. jirovecii* infection incidence may be partly due to recent use of PCR assay, which is more sensitive than microscopic techniques for *P. jirovecii* detection. Indeed, we started to use PCR assay for routine detection of *P. jirovecii* in our laboratory in October 2007; the fungus was detected with PCR in 11 of the 18 RTRs, whereas it was not detected with microscopy. Nonetheless, we diagnosed no *Pneumocystis* infection cases in RTRs using both PCR and microscopic techniques from October 2007, when PCR assay use was initiated, to May 2008, the date when the cluster began. Moreover, we detected *P. jirovecii* in 7 patients using conventional microscopic techniques that have not changed since 2002. Likewise, immunosuppressive and PCP chemoprophylaxis...
regimens have not changed recently. No patients were submitted to chemoprophylaxis when *P. jirovecii* infections occurred, because the posttransplantation period for all patients was 13 months, whereas the duration of recommended prophylaxis is limited to 4 or 6 months after transplantation [29, 30]. For all the above reasons, we believed that we were confronted with a genuine outbreak, revealed by the cluster in the course of which patient-to-patient transmission of the fungus may have occurred.

Amplifications of DHPS or ITS loci failed to give positive results in 23 patients, although they had tested positive with the PCR assay at the mtLSUrRNA gene. These sensitivity differences in PCR assays are due to the presence of the mtLSUrRNA gene in several copies within the *P. jirovecii* genome [31], contrary to DHPS and ITS loci [32, 33]. DHPS locus analysis was poorly informative because no RTRs were infected with a mutant. ITS loci were chosen because they are considered more informative than the mtLSUrRNA gene [34, 35]. Type Eg, which was the most frequent type in patients living in our geographic region, is also the most frequent type identified worldwide [21, 22, 26, 35–37]. However, its higher frequency in RTRs involved in the cluster represents a specific characteristic of *P. jirovecii* organisms infecting this group. The higher frequency may be explained by the fact that fungus acquisition in this group resulted from common sources. Choukri and colleagues recently provided arguments against environmental sources of *P. jirovecii*. They did not detect *P. jirovecii* in air samples collected in the hospital far from wards admitting patients with PCP or in the hospital yard. Conversely, they detected *P. jirovecii* in the air surrounding patients with PCP. The fungal burden in the air decreased with distance from patients, suggesting the airborne spread of the fungus within patients’ environment [31]. Several encounters were not taken into account in drawing up the transmission map, because it is unknown whether patients can transmit the fungus during the *P. jirovecii* infection incubation. An incubation duration of about 2 months has been suggested, but it varies greatly [17, 36]. Following our restrictive criteria of index patient definition and transmission conditions, we finally considered 14 encounters involving 12 patients. Seven patients who encountered a proven index patient and who later developed *P. jirovecii* infection,
whatever the postencounter period, were all considered to be susceptible patients. The postencounter period in the 7 susceptible patients theoretically represented the incubation duration, which varied from 1 to 4.5 months.

Three colonized patients were considered to be index patients. The possible transmission of *P. jirovecii* from colonized patients to susceptible patients who later develop PCP or pulmonary colonization is prompted by experimental *Pneumocystis murina* infections. *P. murina* can be transmitted from mice with severe combined immunodeficiency (SCID) with corticosteroid-induced PCP to Balb/c mice, which do not develop PCP but which are transiently colonized. Colonized Balb/c mice can afterward transmit *P. murina* to susceptible SCID mice [10].

We assumed that direct transmission of *P. jirovecii* from index patients to susceptible patients has occurred. The hypothetical *P. jirovecii* circulation via healthcare workers in contact with infected patients, as discussed by Miller and colleagues [38], was not investigated in this retrospective study.

Main clusters that have previously been reported were most frequently observed in RTRs (Table 4). This apparent higher risk for *P. jirovecii* infection outbreaks may be related to the high number of RTRs worldwide, their immunosuppression status, and their compliance with regular follow-up within hospital settings, combined with the consequent high rate of encounters and potential transmission of the fungus. We hypothesize that the causes of our PCP case cluster were the absence of PCP prophylaxis and measures to prevent *P. jirovecii* acquisition in the hospital combined with the occurrence of PCP during the late posttransplantation period. Causes of this occurrence—as observed by many teams and our own—were not the focus of the present study and are still subject to debate [16, 39].

Be that as it may, the extension of the period of chemoprophylaxis beyond the 4 or 6 months recommended by guidelines must be discussed [29, 30]. However, measures to prevent *P. jirovecii* infections based only on chemotherapy may not be enough to achieve prevention. Data on outbreaks

### Table 4. Main Clusters of *Pneumocystis* Pneumonia in Hospitals Reported During the Past 5 Decades

<table>
<thead>
<tr>
<th>Source (Year of Publication)</th>
<th>Patients, No.</th>
<th>Underlying Conditions of Patients</th>
<th>Genotyping Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ruskin et al (1968)a</td>
<td>2</td>
<td>Connective tissue diseases</td>
<td>NP</td>
</tr>
<tr>
<td>Brazinski et al (1969)a</td>
<td>2</td>
<td>Hematological malignancies</td>
<td>NP</td>
</tr>
<tr>
<td>Perera et al (1970)a</td>
<td>19</td>
<td>Cancers</td>
<td>NP</td>
</tr>
<tr>
<td>Yates et al (1975)a</td>
<td>2</td>
<td>Hematological malignancies</td>
<td>NP</td>
</tr>
<tr>
<td>Singer et al (1975)a</td>
<td>11</td>
<td>Hematological malignancies</td>
<td>NP</td>
</tr>
<tr>
<td>Ruebush et al (1978)a</td>
<td>10</td>
<td>Cancers</td>
<td>NP</td>
</tr>
<tr>
<td>Goesch et al (1990)a</td>
<td>2</td>
<td>Hematological malignancies</td>
<td>NP</td>
</tr>
<tr>
<td>Bensousan et al (1990)a</td>
<td>10</td>
<td>Renal transplantation</td>
<td>NP</td>
</tr>
<tr>
<td>Chave et al (1991)a</td>
<td>5</td>
<td>Renal transplantation</td>
<td>NP</td>
</tr>
<tr>
<td>Jacobs et al (1991)a</td>
<td>5</td>
<td>Hospitalization in ICU</td>
<td>NP</td>
</tr>
<tr>
<td>Glotz et al (1992)a</td>
<td>8</td>
<td>Renal transplantation</td>
<td>NP</td>
</tr>
<tr>
<td>Cheung et al (1994)a</td>
<td>3</td>
<td>Cancers</td>
<td>NP</td>
</tr>
<tr>
<td>Helweg-Larsen et al (1998)a,b</td>
<td>8; 6</td>
<td>Hematological malignancies; AIDS</td>
<td>Analysis of ITS1 and ITS2 loci (12 patients)</td>
</tr>
<tr>
<td>Olsson et al (2001)a,b</td>
<td>10 (3 + 7); 7</td>
<td>Renal transplantation; hematological malignancies</td>
<td>Analysis of mtLSUrRNA locus (17 patients)</td>
</tr>
<tr>
<td>Rabodonirina et al (2004)h,d</td>
<td>10; 39</td>
<td>Renal transplantation; AIDS</td>
<td>SSCP at 4 loci (9 patients; 30 patients)</td>
</tr>
</tbody>
</table>

Abbreviations: ICU, intensive care unit; ITS, internal transcribed spacers; MLST, multilocus sequence typing; mtLSUrRNA, mitochondrial large-subunit rRNA; NP, not performed; SSCP, single-strand conformation polymorphism; 


b Investigation of 2 or 3 clusters.

c No. of patients with positive results of *P. jirovecii* typing.

d Results of these studies are compatible with nosocomial acquisition of *P. jirovecii* through interhuman transmission.
in hospitals and Pneumocystis transmission in animal models currently seem sufficient to justify a policy of isolation [40]. This policy should be extended to patients in whom pulmonary colonization with P. jirovecii is diagnosed.

Notes

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