Molecular Evidence of Pneumocystis Transmission in Pediatric Transplant Unit

Britta Höcker,* Constanze Wendt,† Aimable Nahimana,‡ Burkhard Tönshoff,* and Philippe M. Hauser‡

We describe an outbreak of *Pneumocystis jirovecii* pneumonia in a pediatric renal transplant unit, likely attributable to patient-to-patient transmission. Single-strand conformation polymorphism molecular typing showed that 3 affected patients had acquired the same 2 strains of *Pneumocystis*, which suggests interhuman infection. An infant with mitochondriopathy was the probable index patient.

Despite intensive medical treatment, *Pneumocystis jirovecii* pneumonia (PCP) is still a severe disease in immunocompromised patients, with a high death rate of up to 50% (1). The first report of human *Pneumocystis* infection appeared in 1909; nevertheless, its epidemiology is poorly understood to date. In the 1950s, reports on PCP epidemics in malnourished infants in hospitals and orphanages aroused suspicion of interhuman transmission. In addition, animal studies have demonstrated airborne transmission of *Pneumocystis* (2). A case-control study conducted for a cluster of 5 PCP cases in transplant recipients suggested transmission of *P. jirovecii* from AIDS patients to other immunosuppressed persons (3). However, molecular typing methods for *P. jirovecii* were lacking so that patient-to-patient transmission could not be assessed at the molecular level. When such techniques were developed in the 1990s, 3 analyses showed different *P. jirovecii* genotypes within clusters (4–6). A recent analysis at the molecular level of a cluster of 10 PCP cases strongly suggested that HIV-infected persons with active PCP transmitted *P. jirovecii* to renal transplant recipients (7). The role of interhuman transmission of *P. jirovecii* in the epidemiology of PCP is still unclear.

The Outbreak

Having observed no occurrence of PCP in our pediatric renal transplant unit for the last 20 years and only 1 case in all German pediatric renal transplant units during the last 10 years, we encountered 3 consecutive incidents of PCP during a 5-month period. The first patient was a 13-year-old girl, who had received her second renal graft because of cystic kidney disease; PCP developed 4 months after transplantation. The second patient, a 14-year-old boy, fell ill in the ninth posttransplant month; he had bilateral vesicoureteral reflux as underlying renal disease. The third patient was a 13-year-old girl, who had a transplant 2 years before contracting PCP because of cystic renal dysplasia occurring in the context of Bardet-Biedl syndrome.

All 3 children had been given cyclosporine A (average dose 6.7 mg/kg/day), mycophenolate mofetil (1,060 mg/m²/day), and methylprednisolone (3.2 mg/m²/day), as maintenance immunosuppression. One patient had also received induction therapy with the interleukin-2-receptor-antibody basiliximab. All 3 children had been treated with methylprednisolone pulses for acute rejection episodes 2, 3, and 15 months before PCP was diagnosed.

Clinically, all patients showed nonspecific symptoms, such as mild fever, dyspnea, and dry cough in the absence of auscultatory anomalies. Laboratory tests showed an elevation of lactic dehydrogenase activity, C-reactive protein concentration in blood, and pronounced hypercalcemia (2.7–3.5 mmol/L), which was interpreted as an extrarenal production of 1,25-dihydroxyvitamin D₃ by activated alveolar macrophages. We found a significant reduction of S-adenosylmethionine concentration in plasma (6 mmol/L; normal range 86–128 mmol/L), which appears to be specific to PCP, unlike bacterial or other atypical pneumonias (8). We measured the blood count of CD4+ and CD4/DR-T lymphocytes in the third patient to indicate the degree of immunosuppression, since antirejection therapy had been administered 15 months before the occurrence of PCP. The number of CD4+T cells was normal at the time of PCP diagnosis (1,100 cells/µL; normal range 505–1,151 cells/µL), while the number of activated T-helper cells was slightly decreased (24 CD4/DR+ cells/µL; normal range 29–87/µL). Only in the course of PCP did the numbers of CD4+ and CD4/DR+ T lymphocytes drop significantly (308 CD4+ T cells/µL and 8 CD4/DR+ cells/µL). Chest radiographs and thorax computed tomographic scans of the 3 children showed typical signs of interstitial pneumonia, e.g., ground-glass opacity.

Diagnosis of PCP was confirmed by the presence of cysts and vegetative forms in bronchoalveolar lavage fluid, proved by immunofluorescence staining, and through detection of *Pneumocystis* DNA by means of polymerase chain reaction (PCR). In spite of intensive antimicrobial therapy, 2 of our 3 renal transplant patients died, at 10 and 28 days, respectively, after the onset of PCP.

To determine if PCP could have been caused by patient-
Transmission in Pediatric Transplant Unit

To our knowledge, this report is the first published on an outbreak of PCP in a pediatric renal transplant unit, probably attributable to patient-to-patient transmission. However, we cannot exclude that the cases described were infected by the same environmental source. The presence of \textit{P. jirovecii} in the air of hospital corridors has been described (13), making an environmental reservoir in the hospital possible. Other potential sources of \textit{P. jirovecii} could be asymptomatic \textit{P. jirovecii} carriers, such as immunosuppressed patients (14,15). Our findings at the molecular level suggest that \textit{P. jirovecii} may be transmitted nosocomially and be acquired by immunosuppressed pediatric transplant recipients. The incubation periods of \textit{P. jirovecii} infection (17, 15, and 19 weeks for patients 1, 2, and 3, respectively) would be longer than those (2–12 weeks) suggested by the previously described clusters of PCP (3,7,16). This finding may reflect a difference between adults and children.

Until the outbreak of PCP outlined in this article, pediatric renal transplant recipients in our hospital and other pediatric renal transplant units in Germany were not given PCP prophylaxis routinely because of possible side effects, such as a rise of serum creatinine values, myelosuppression,

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|c|c|c|c|}
\hline
\textbf{Week 2002} & 10 & 12 & 14 & 16 & 18 & 20 & 22 & 24 & 26 & 28 & 30 & 32 & 34 & 36 & 38 & 40 & 42 & 44 & 46 & 48 & 50 & 52 \\
\hline
\textbf{Index Pat.} & \rightarrow \quad X \quad D \\
\hline
\textbf{Pat. 1} & RTx & \checkmark & R & R & \rightarrow & X & D \\
\hline
\textbf{Pat. 2} & RTx January 2002 & R & \checkmark & \rightarrow & X & D \\
\hline
\textbf{Pat. 3} & RTx December 2000 & \checkmark & \# & \rightarrow & X \\
\hline
\end{tabular}
\end{table}

Conclusions

To our knowledge, this report is the first published on an outbreak of PCP in a pediatric renal transplant unit, probably attributable to patient-to-patient transmission. However, we cannot exclude that the cases described were infected by the same environmental source. The presence of \textit{P. jirovecii} in the air of hospital corridors has been described (13), making an environmental reservoir in the hospital possible. Other potential sources of \textit{P. jirovecii} could be asymptomatic \textit{P. jirovecii} carriers, such as immunosuppressed patients (14,15). Our findings at the molecular level suggest that \textit{P. jirovecii} may be transmitted nosocomially and be acquired by immunosuppressed pediatric transplant recipients. The incubation periods of \textit{P. jirovecii} infection (17, 15, and 19 weeks for patients 1, 2, and 3, respectively) would be longer than those (2–12 weeks) suggested by the previously described clusters of PCP (3,7,16). This finding may reflect a difference between adults and children.

Until the outbreak of PCP outlined in this article, pediatric renal transplant recipients in our hospital and other pediatric renal transplant units in Germany were not given PCP prophylaxis routinely because of possible side effects, such as a rise of serum creatinine values, myelosuppression,
and Lyell syndrome. We had not observed any case of PCP in our transplant recipients for the last 20 years without prophylaxis. In the light of the high death rate for PCP, prophylactic treatment with trimethoprim-sulfamethoxazole is highly recommended for the first 6 posttransplant months and during the 4 months after antirejection therapy, in accordance with the guidelines for adults (1). According to these guidelines, patient 3, in whom PCP developed 15 months after steroid pulse therapy, would not have been protected by prophylaxis. Whether prophylaxis should be given for a longer period of time remains unknown, particularly since immunosuppression did not appear to be intensive in this patient at the onset of PCP, as indicated by the normal CD4+ T-lymphocyte count in peripheral blood and the only slightly decreased number of activated T-helper cells.

Dr. Höcker is a physician and research fellow at the University Children's Hospital, Heidelberg, Germany. Her research activities are focused on immunosuppressive therapy and diagnosis and treatment of opportunistic infections in pediatric renal transplant recipients.

References

1. EBPG Expert Group on Renal Transplantation. European best practice guidelines for renal transplantation. Section IV: long-term management of the transplant recipient. IV.7.1 Late infections. Pneumocystis carinii pneumonia. Nephrol Dial Transplant. 2002;17(Suppl 4):36–8.

2. Hughes WT. Current issues in the epidemiology, transmission, and reactivation of Pneumocystis carinii. Semin Respir Infect. 1998;13:283–8.

3. Chave JP, David S, Wauters JP, Van Melle G, Francioli P. Transmission of Pneumocystis carinii from AIDS patients to other immunosuppressed patients: a cluster of Pneumocystis carinii pneumonia in renal transplant recipients. AIDS. 1991;5:927–32.

4. Olsson M, Eriksson BM, Elvin K, Strandberg M, Wahlgren M. Genotypes of clustered cases of Pneumocystis carinii pneumonia. Scand J Infect Dis. 2001;33:285–9.

5. Latouche S, Poirot JL, Maury E, Bertrand V, Roux P. Pneumocystis carinii hominis sequencing to study hypothetical person-to-person transmission. AIDS. 1997;11:549.

6. Helweg-Larsen J, Tsolaki AG, Miller RF, Lundgren B, Wakefield AE. Clusters of Pneumocystis carinii pneumonia: analysis of person-to-person transmission by genotyping. Q J Med. 1998;91:813–20.

7. Rabodonirina A, Vanhems P, Courray-Targe S, Gillibert RP, Ganne C, Nizard N, et al. Molecular evidence of interhuman transmission of Pneumocystis pneumonia among renal transplant recipients hospitalized with HIV-infected patients. Emerg Infect Dis. 2004;10:1766–73.

8. Skelly M, Hoffman J, Fabbi M, Holzman RS, Clarkson AB Jr, Merali S. S-adenosylmethionine concentrations in diagnosis of Pneumocystis carinii pneumonia. Lancet. 2003;361:1267–8.

9. Hauser PM, Francioli P, Bille J, Telenti A, Blanc DS. Typing of Pneumocystis carinii f. sp. hominis by single-strand conformation polymorphism of four genomic regions. J Clin Microbiol. 1997;35:3086–9.

10. Hauser PM, Blanc DS, Sudre P, Senggen Manoloff E, Nahimana A, Bille J, et al. Genetic diversity of Pneumocystis carinii in HIV-positive and -negative patients as revealed by PCR-SSCP typing. AIDS. 2001;15:461–6.

11. Nahimana A, Blanc DS, Francioli P, Bille J, Hauser PM. Typing of Pneumocystis carinii f. sp. hominis by PCR-SSCP to indicate high frequency of co-infections. J Med Microbiol. 2000;49:753–8.

12. Vargas SL, Ponce CA, Gigiotti F, Ulloa AV, Prieto S, Munoz MP, et al. Transmission of Pneumocystis carinii DNA from a patient with P. carinii pneumonia to immunocompetent contact health care workers. J Clin Microbiol. 2000;38:1536–8.

13. Bartlett MS, Vermund SH, Jacobs R, Durant PJ, Shaw MM, Smith JW, et al. Detection of Pneumocystis carinii DNA in air samples: likely environmental risk to susceptible persons. J Clin Microbiol. 1997;35:2511–3.

14. Hauser PM, Blanc DS, Bille J, Nahimana A, Francioli P. Carriage of Pneumocystis carinii by immunosuppressed patients and molecular typing of the organisms. AIDS. 2000;14:461–3.

15. Vargas SL, Ponce CA, Sanchez CA, Ulloa AV, Bustamante R, Juarez G. Pregnancy and asymptomatic carriage of Pneumocystis jirovecii. Emerg Infect Dis. 2003;9:605–6.

16. Goesch TR, Gotz G, Stellbrinck KH, Albrecht H, Weh HJ, Hossfeld DK. Possible transfer of Pneumocystis carinii between immunodeficient patients. Lancet. 1990;336:627.

*Bold letters signify the most abundant simple pattern with the complex one, as shown by silver staining. Bold numbers signify the most abundant P. jirovecii type. PCR, polymerase chain reaction; SSCP, multtarget single-strand conformation polymorphism.

Table. Pneumocystis jirovecii genotyping by PCR-SSCP of 4 genomic regions*

| Patient | Date    | ITS1 | 2S  | mt26  | β-tub | P. jirovecii type |
|---------|---------|------|-----|-------|-------|------------------|
| Index case-patient | 3/27/02 | A    | A, B| A, B, C| A, B | >2 types (nonidentifiable) |
| 1       | 7/26/02 | A    | A, B| A     | A     | 1, 2             |
| 2       | 10/30/02| A    | A, B| A     | A     | 1, 2             |
| 3       | 12/3/02 | A    | A, B| A     | A     | 1, 2             |
| 4       | 2/7/03  | A, B | A, B| B, C  | B, C  | >2 types (nonidentifiable) |
| 5       | 2/15/03 | A    | A, B| C     | A     | 6, 44            |
| 6       | 2/18/03 | A, B | A, B| A     | C     | 45, 46           |

Address for correspondence: Britta Höcker, University Children's Hospital, Im Neuenheimer Feld 150, 69120 Heidelberg, Germany; fax: 49-6221-564203; email: Britta_Hoecker@med.uni-heidelberg.de