Molecular Epidemiology of Pneumocystis carinii Pneumonia

Pneumocystis carinii pneumonia (PCP) was first recognized as a distinct clinical entity in orphanages in Europe during World War II (1). Today it is the most frequent serious opportunistic infection in AIDS patients. Despite advances in research, numerous questions remain regarding the basic biology and epidemiology of P. carinii.

Transmission and Patient Care

Although reactivation of latent infections has long been considered the primary explanation for PCP in immunosuppressed patients, over the years a steady flow of reports has described clusters of PCP cases (2). In addition, recent studies have suggested that the duration of latency is very limited, i.e., usually less than 1 year (3,4). Still other studies have demonstrated genetic variation in PCR-amplified P. carinii DNA from the lungs of patients during subsequent PCP episodes (5). Together, these observations provide strong circumstantial evidence of person-to-person transmission of P. carinii. Consequently, establishing the role that person-to-person transmission plays in the epidemiology of PCP is urgent.

Another important area of PCP epidemiology is determining the predisposing factors for disease. The most frequently discussed predictor of disease is CD4+ cell count, specifically as it relates to care and management of AIDS patients (6); however, it has long been known that malnutrition can be an important contributor (7). The degree to which other factors such as viral infections or pneumonitis of other causes, may come into play, is yet to be shown.

Much can also be learned regarding the epidemiology of PCP in HIV-infected infants. Recent studies report that primary infections in these infants often develop when the child is 3 to 6 months old (8,9). The source of these patients’ P. carinii infections (i.e., the hospital setting, their mothers, other children, or an environmental source) is not known.

Clinicians working with AIDS patients need a sensitive, reliable, and noninvasive tool for early detection and diagnosis of PCP infections (10,11). Besides the standard procedures of bronchoalveolar lavage (BAL) and induced sputum (IS) sampling, recent studies indicate that it is possible to amplify P. carinii DNA sequences by polymerase chain reaction (PCR) directly from blood or serum samples and from nasopharyngeal aspirates of PCP patients (11,12). Further studies are needed to confirm that a PCR-based diagnostic tool superior to microscopy can be adapted for use in clinical settings. A serologic tool that will distinguish recent PCP infections from those past is also needed.

Prophylaxis failures have been reported for both trimethoprim-sulfamethoxazole (TMP-SMX) and pentamidine (13-16). Studies evaluating these cases, however, are frequently complicated by the difficulties in assessing and confirming patient compliance with the prophylaxis regimen. The only factor that has a significant correlation with failure in most cases, however, is the patient’s CD4+ T lymphocyte count (14). Although this correlation would be expected because of the general increased risk for PCP associated with CD4+ cell depletion (6) and the increase in prophylaxis complications in HIV-infected patients (17), these drugs may not eliminate all organisms, and some degree of patient immunity may be required to clear or control the infection. What role, if any, specific antimicrobial resistance mechanisms play in the reported treatment failures has not been shown; however, the emergence of resistance is always a threat. Likewise, long-term TMP-SMX prophylaxis increases the possibility for the selection of antimicrobial resistance in bacterial pathogens, some of which are important potential causes of pneumonia in HIV-infected patients (18). Identifying potential antimicrobial resistance mechanisms in P. carinii is difficult because of the lack of an established culture system for human P. carinii that would allow traditional antimicrobial sensitivity testing.

At least three separate lines of data suggest that P. carinii is a commonly encountered organism: the high seroprevalence rates reported in normal populations (19), the rapid rate at which infants acquire primary infections (8) and AIDS patients become reinfected after successful treatment (20), and the amplification of P. carinii-specific DNA from ambient air sampled from the environment (e.g., an apple orchard) (21) and from rooms of animals and patients with PCP (22). Airborne transmission has been demonstrated for PCP in rats (23-26) and is by far the most likely mechanism proposed for natural exposure to P. carinii in humans (2,22). Given the similarities
between P. carinii and various fungal agents and the enigma surrounding the issue of environmental sources for P. carinii, it has been suggested that P. carinii may in fact be a dimorphic fungus, ubiquitous in the environment and disseminated by airborne spores (27). Identifying the specific environmental source or sources of P. carinii is critical to understanding the epidemiology of PCP and establishing guidelines for preventing its transmission.

It is generally accepted that P. carinii strains from rats do not infect humans and that human strains do not infect rats; however, we do not know the host boundaries for a given P. carinii strain or if all isolates from a given host display the same degree of host restrictions (28,29). In fact, a careful evaluation of the available data concerning P. carinii of numerous hosts suggests that P. carinii may represent a collection of diverse fungal species (30). Like drug resistance research, studies aimed at strain/species characterization are generally hindered by the difficulties in culturing human P. carinii and the lack of refined molecular biological methods that allow strain identification and characterization.

**Molecular Biologic Techniques and Specific Epidemiologic Issues**

One of the essential reasons for cultivating any particular pathogen is for strain identification and characterization that would elucidate such specific phenotypic characteristics as virulence factors, antimicrobial sensitivity levels, and factors associated with transmissibility. The isolation and cultivation of individual strains, and ultimately of clones, would provide a homogeneous population of organisms from which the desired information can be obtained and a pure source of genetic material for constructing DNA libraries and identifying relevant genes.

In the absence of cultivation, investigators have been able to begin addressing some of the basic epidemiologically important issues by applying PCR-based technology. In these studies, the DNA sequence of specific genetic loci from P. carinii is usually amplified from BAL, IS, or serum samples from PCP patients, using highly specific oligonucleotide primers. Inherent problems exist in this approach (which are discussed below); however, the approach has allowed the identification of genotypic differences in P. carinii populations sampled from the lungs of different patients and even from the lungs of the same patient during different PCP episodes. Great potential exists in applying this technology to develop molecular profiles of P. carinii isolates that could ultimately allow the particular genotypes to be linked to specific epidemiologically relevant phenotypes.

**Molecular Typing**

Five to ten different genetic loci have been identified as potentially informative for molecular characterization and typing (30-33). Concerning the typing that has actually been performed on human samples, the primary loci evaluated include: 1) a 346-bp region of the mitochondrial large subunit rRNA gene (mt lsrRNA) (10) and 2) a 550-bp fragment containing the nuclear ribosomal internal transcribed spacer regions 1 and 2 (ITS1 and ITS2) (34). When these loci are considered collectively, nucleotide variation can be detected at approximately 37 different positions. Work is in progress in several laboratories, both to type patient isolates according to the available loci and to identify additional genetic loci to more thoroughly define a given genotype.

The primary obstacles to the development of a molecular typing scheme based on PCR-amplified DNA sequence data obtained from PCP patients include the following: 1) multiple strains may infect a single patient at a given time; 2) a diploid organism of a single strain may be heterozygous with respect to a particular polymorphic locus; 3) presumed single genes could have multiple copies in a single genome, which could give the appearance of genetic polymorphism; 4) amplified DNA sequence data might be confounded because of other fungal agents such as Cryptococcus or Candida; and 5) inferences that can be drawn from restricted sequence data (i.e., gene typing versus strain typing) are limited. Although these problems are not insurmountable, they must be considered when evaluating data obtained by this approach. We propose the following recommendations.

**Recommendations**

1. Recent advances in molecular-based typing should be combined with epidemiologic studies to investigate the transmission of P. carinii and new strategies for control.
2. Additional genomic regions must be identified for use in typing, along with the genetic loci that are available. These new loci must be shown to represent single-copy genes. Also, new molecular approaches should be developed that will increase the current capacity to resolve genotypic variation among P. carinii strains.

3. Genetic variation should be investigated among P. carinii strains that could be linked to variations in factors such as strain virulence, drug resistance, or transmissibility.

4. The critical issue regarding person-to-person transmission is not so much whether it occurs, as whether it contributes to infection significantly more than airborne sources in the environment. Thus, it must be determined whether there is any benefit to establishing complex protocols that ensure that patients are carefully protected from each other if they can become infected from other sources in the environment. Consequently, the importance of person-to-person transmission in the epidemiology of PCP should be defined.

5. The role of latent P. carinii infection as a source of PCP in immunocompromised persons should be clarified.

**Diagnosis, Treatment, and Prevention**

1. New tools for noninvasive early diagnosis of PCP, including culture systems, molecular approaches, and serologic tests that can distinguish recent and past PCP infections are needed.

2. In the United States, clinician compliance with recently published U.S. Public Health Service/Infectious Diseases Society of America guidelines on the treatment and prophylaxis of PCP should be evaluated.

3. Studies should be initiated to develop additional drugs for PCP treatment and prophylaxis.

4. New approaches for improving patient compliance with prescribed PCP prophylaxis must be devised and evaluated.

5. Methods for detecting the possible emergence of drug resistance to P. carinii should be standardized.

6. Standard decontamination procedures for respiratory therapy equipment and pulmonary diagnostic instruments should be evaluated to confirm that they effectively eliminate all viable P. carinii.

**Environmental Reservoirs and General Biology**

1. Environmental sources and the coinciding infective stage(s) of P. carinii should be detected and evaluated.

2. The host range of P. carinii from various sources (i.e., to what degree are humans susceptible to P. carinii from nonhuman sources?) should be determined.

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**References**

1. Walzer PD, editor. Pneumocystis carinii pneumonia. New York: Marcel Dekker, 1994.
2. Cushion MT. Transmission and epidemiology. In: Walzer PD, editor. Pneumocystis carinii pneumonia. New York: Marcel Dekker, 1994:123-40.
3. Chen W, Gigliotti F, Harmens AG. Latency is not an inevitable outcome of infection with Pneumocystis carinii. Infect Immun 1993;61:5406-9.
4. Vargas SL, Hughes WT, Wakefield AE, Oz HS. Limited persistence in and subsequent elimination of Pneumocystis carinii from the lungs after P. carinii pneumonia. J Infect Dis 1995;172:506-10.
5. Keely SP, Stringer JR, Baughman RP, Linka MJ, Walzer PD, Smulian AG. Genetic variation among Pneumocystis carinii hominisia isolates in recurrent pneumocystosis. J Infect Dis 1995;172:595-8.
6. Phair J, Muñoz A, Detels R, Kaslow R, Rinaldo C, Saah A. The risk of Pneumocystis carinii pneumonia among men infected with human immunodeficiency virus type 1. N Engl J Med 1990;322:161-5.
7. Hughes WT, Price RA, Havron SF, Sisko F, Havron SF, Kafatos AG, Schonland M, et al. Protein-calorie malnutrition: a host determinant for Pneumocystis carinii infection. Am J Dis Child 1974;128:44-52.
8. Hughes WT. 1994. Clinical manifestations in children. In: Walzer PD, editor. Pneumocystis carinii pneumonia. New York: Marcel Dekker, 1994:319-29.
9. Simonds RJ, Lindegren ML, Thomas P, Hanson D, Caldwell B, Scott G, et al. Prophylaxis against Pneumocystis carinii pneumonia among children with perinatally acquired human immunodeficiency virus infection in the United States. N Engl J Med 1995;332:786-90.
10. Wakefield AE, Pixley FJ, Banerji S, Sinclair K, Miller RF, Moxon ER, et al. Detection of Pneumocystis carinii with DNA amplification. Lancet 1990; 336:451-3.

11. Atzori C, Lu J-J, Jiang B, Bartlett MS, Orlando G, Queener SF, Smith J W, et al. Diagnosis of Pneumocystis carinii pneumonia in AIDS patients by using polymerase chain reactions on serum specimens. J Infect Dis 1995;172:1623-6.

12. Richards CGM, Wakefield AE, Mitchell CD. Detection of pneumocystis DNA in nasopharyngeal aspirates of leukaemic infants with pneumonia. Arch Dis Child 1994;71:254-5.

13. Montgomery AB, Feigal DW, Sattler F. Pentamidine aerosol versus trimethoprim-sulfamethoxazole for Pneumocystis carinii in acquired immune deficiency syndrome. Am J Respir Crit Care Med 1995;151:1068-74.

14. Saah AJ, Hoover DR, Peng Y, Phair JP, Visscher B, Kingsley LA, et al. Predictors for failure of Pneumocystis carinii pneumonia prophylaxis. JAMA 1995;273:1197-1202.

15. Lecuit M, Livartowski J, Vons C, Goujard C, Lamaigre G, Delfraissy J-F, et al. Resistance to trimethoprim-sulfamethoxazole and sensitivity to pentamidine therapy in an AIDS patient with hepatosplenic pneumocystosis. AIDS 1994;8:1506-7.

16. Torres RA, Barr M, Thorn M, Gregory G, Keily S, Chanin E, et al. Randomized trial of dapsone and aerosolized pentamidine for the prophylaxis of Pneumocystis carinii pneumonia and toxoplasmic encephalitis. Am J Med 1993;95:573-83.

17. Walker RE, Masur H. Current regimens of therapy and prophylaxis. In: Walzer PD, editor. Pneumocystis carinii pneumonia. New York: Marcel Dekker, 1994:331-59.

18. Schwartz RH, Khan WN, Akram S. Penicillin and trimethoprim-sulfamethoxazole-resistant pneumococci isolated from blood cultures of three infants in metropolitan Washington, DC: a harbinger of serious future problems? Pediatr Infect Dis J 1991;10:782-3.

19. Smulian AG, Walzer PD. Serological studies of Pneumocystis carinii infection. In: Walzer PD, editor. Pneumocystis carinii pneumonia. New York: Marcel Dekker, 1994:141-51.

20. Dohn MN, Frame PT. Clinical manifestations in adults. In: Walzer PD, editor. Pneumocystis carinii pneumonia. New York: Marcel Dekker, 1994:331-59.

21. Wakefield AE. Detection of DNA sequences identical to Pneumocystis carinii in samples of ambient air. J Euk Microbiol 1994;41:1165.

22. Bartlett MS, Lee C-H, Lu J-J, Bauer NL, Betts J F, McLaughlin GL, et al. Pneumocystis carinii detected in air. J Euk Microbiol 1994;41:75-24.

23. Hendley JO, Weller TH. Activation and transmission in rats of infection with Pneumocystis. Proc Soc Exp Biol Med 1971;137:1401-4.

24. Walzer PD, Schnelle V, Armstrong D, Rosen PP. Nude mouse: a new experimental model for Pneumocystis carinii infection. Science 1977;197:177-9.

25. Hughes WT, Bartlett DL, Smith BM. A natural source of infection due to Pneumocystis carinii. J Infect Dis 1983; 147:595.

26. Hughes WT. Natural habitat and mode of transmission. In: Pneumocystis carinii pneumonia, vol I. Boca Raton, FL: CRC Press, 1987:97-105.

27. Dei-cas E, Caliiiecz C, Palluault F, Aliouat EM, Mazars E, Soulle B, et al. Is Pneumocystis carinii a deep mycosis-like agent? Eur J Epidemiol 1992; 8:460-70.

28. Smith J W, Bartlett MS. Laboratory diagnosis of pneumocystosis. Clin Lab Med 1991;11:957-75.

29. Armstrong MYK, Cushion MT. In vitro cultivation. In: Walzer PD, editor. Pneumocystis carinii pneumonia. New York: Marcel Dekker, 1994:3-24.

30. Stringer JR. The identity of Pneumocystis carinii: not a single protozoan, but a diverse group of exotic fungi. Infect Agents Dis 1993;2:109-17.

31. Edman JC, Sogin ML. Molecular phylogeny of Pneumocystis carinii. Infect Agents Dis 1993;2:109-17.

32. The Pneumocystis Workshop. Revised nomenclature for Pneumocystis carinii. J Euk Microbiol 1994;41:1215-225.

33. Lu J J, Chen C-H, Bartlett MS, Smith J W, Lee C-H. Comparison of six different PCR methods for detection of Pneumocystis carinii. J Clin Microbiol 1995;33:2785-8.

34. Lu J, Bartlett MS, Shaw MM, Queener SF, Smith J W, Ortiz-Rivera M, et al. Typing of Pneumocystis carinii strains that infect humans based on nucleotide sequence variations of internal transcribed spacers of rRNA genes. J Clin Microbiol 1994;32:2904-12.