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Epidemiology of *Pneumocystis* Colonization in Families

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Abstract

Whether *Pneumocystis* colonization is transmitted in families with HIV-infected members is unknown. Using nested polymerase chain reaction of oropharyngeal or nasopharyngeal samples, we detected colonization in 11.4% of HIV-infected adults and 3.3% of their children, but there was no evidence of clustering.

Keywords

Pneumocystis; colonization; HIV; children

Introduction

The development of sensitive molecular techniques has led to the discovery of a colonization or carrier state of *Pneumocystis jirovecii* in which low levels of the organism are detected in subjects without clinical *Pneumocystis* pneumonia (PCP)[1]. Colonization may be an important step in the organism's life cycle, and transmission of disease could occur via colonized individuals. In addition, *Pneumocystis* (Pc) colonization might increase risk of developing PCP in a susceptible host. *Pneumocystis* colonization has been detected in HIV-infected adults and in non-immunosuppressed children [2-4], but whether transmission of *Pneumocystis* colonization occurs in families with HIV-infected members is unknown.

We examined a cohort of HIV-infected adults and their HIV-infected and HIV-negative children to determine the prevalence and risk factors for *Pneumocystis* colonization in families.

Methods

Subjects

Subjects were recruited from the Maternal, Child, and Adolescent clinic at the University of Southern California from September 2004 through August 2005. This clinic consists of HIV-infected adults, primarily women, and their HIV-infected and HIV-negative offspring. Subjects were enrolled at routinely scheduled and urgent care medical appointments. Informed consent

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was obtained from all subjects, and the Institutional Review Board of the University of Southern California approved the study.

Data collection

Clinical data were collected by subject interview and medical record review. Demographic data consisted of age, gender, and race/ethnicity. Medical history obtained included previous episodes of PCP or other opportunistic infections, smoking history or smoke exposure (in children), current respiratory symptoms, and use of *Pneumocystis* prophylaxis and antiretroviral medications. Laboratory data for the HIV-infected subjects included most recent CD4 cell count and serum HIV viral RNA level.

Specimen collection

Nasopharyngeal aspirates were obtained from children less than three years of age with 3cc of sterile saline using a 6.0 or 8.0 French suction catheter. Oropharyngeal washes were obtained from older children and adults by a one-minute gargle with 10cc of sterile saline.

DNA extraction and PCR amplification

DNA was extracted from oropharyngeal washes or nasopharyngeal aspirates using the DNeasy kit (Qiagen, Valencia, CA). *Pneumocystis* colonization was determined by nested PCR of the mitochondrial large subunit rRNA (mtLSU) as previously described [2]. In order to prevent contamination, all steps of DNA extraction and PCR amplification were carried out in separate rooms. Negative and positive controls (DNA from lung tissue known to contain human *Pneumocystis*) were included in all reactions. PCR products were purified and sequenced as previously described and determined to be human *Pneumocystis* [2]. PCR for the human beta-globin gene was performed to test for the presence of DNA and lack of PCR inhibitors [5].

Statistical analysis

Data were double-entered and analyzed using SAS version 9.1 (SAS Institute Inc., Cary, NC). Continuous variables were described using mean and standard deviation or median and range depending on normality of data. Univariate analyses were performed to determine clinical variables related to Pc colonization using either t-tests/Wilcoxon ranksum or chi-square/ Fisher's exact test. Significance was determined for a p-value of less than 0.05.

Results

Forty-four HIV-infected adults were enrolled. Pc colonization was detected in 5 of 44 (11.3%) adults. There were no significant differences between the colonized and non-colonized adults in terms of demographic or clinical characteristics (Table 1). Most were females (93.2%) and of a minority ethnic group or race (86.4%). The mean years since HIV diagnosis was 5.7 and 34.1% had ever had an AIDS diagnosis. Only 11.4% had a CD4 cell count below 200 cells/ μl and many (63.6%) had a serum HIV viral RNA level below 400 copies/ml.

Sixty children, ages 2 weeks to 17.6 years, were enrolled. Colonization was detected in two (3.3%) pediatric subjects (Table 2). These subjects were HIV-negative females less than six months of age with HIV-infected mothers. Neither had a mother with a history of PCP. One mother was receiving PCP prophylaxis. Both subjects had upper respiratory tract symptoms at the time of colonization. Colonized children were significantly more likely to be less than one year of age (p=0.04). None of the colonized adults or children were members of the same family. Interestingly, all colonized adults and children were Hispanic (p=0.04 for comparison to all other race/ethnicities).

Discussion

This study is one of the first to examine *Pneumocystis* colonization in HIV-infected children and in families with an HIV-infected member. Somewhat surprisingly, we found no evidence of transmission in families and a low prevalence of colonization in HIV-infected adults and their offspring. There were also no clinical characteristics that distinguished colonized from non-colonized subjects in the adult population, but when examining the adult and pediatric cohorts together, colonized subjects were more likely to be Hispanic. Colonization in the pediatric population was associated with age less than one year, and there was a tendency for colonized children to have upper respiratory symptoms.

We found no evidence of clustering of colonization within families despite previous work demonstrating that person-to-person transmission of *Pneumocystis* likely occurs. Other studies have found that health care workers caring for patients with PCP can develop *Pneumocystis* colonization [14,15], and there has been a reported case of probable maternal-infant transmission of PCP from an HIV-infected mother [16]. Animal studies support the theory that transmission of colonization to a normal adult host can result from exposure to an infected one [17-19], and newborn mice rapidly acquire *Pneumocystis* colonization after birth, suggesting transmission from a maternal source [20]. Our results indicate that if person-to-person transmission is occurring, it may be transient or the burden of organisms in asymptomatic individuals may be quite low.

The prevalence of colonization we found in HIV-infected adults is much lower than previously reported. In an autopsy study, 46.2% of subjects were colonized and another study of HIV-infected subjects with pneumonia found that 68.8% were colonized [2,3]. Other studies have reported prevalence of colonization in HIV-infected adults ranging from 10.0% to 43.8% [1].

In the pediatric HIV-infected population, only one previous study has reported prevalence of colonization and found that 9 of 45 (20.0%) HIV-infected children were colonized when examined at autopsy after dying of a respiratory illness [6]. In the non-HIV-infected pediatric population, *Pneumocystis* colonization prevalence ranges from 15.9% to 32.0% using nasopharyngeal and/or oropharyngeal samples in children with bronchiolitis or acute respiratory syndromes [1]. Colonization in autopsy series of children has found a range of colonization from 25% to 100% [21,22]. The colonization prevalence we found was much lower than in any of these populations.

There are several possible explanations for our low prevalence of colonization. First, geographic variation might contribute to a lower level of colonization. We have previously shown that colonization risk varies by city, with Los Angeles, our study site, having a lower prevalence than other cities [2]. Therefore, our results might be different if repeated in a different area. Season might also affect colonization as has previously been shown with PCP [7], although we did not find variation in colonization throughout the study period. In addition, our population was fairly healthy and consisted entirely of outpatients. Many previous studies have examined subjects with advanced HIV or with lower respiratory tract symptoms. Colonization in a relatively healthy population might be rapidly cleared by the immune system. Finally, nasopharyngeal and oral washes might lack sufficient sensitivity. More invasive samples such as bronchoalveolar lavage might have detected evidence of colonization, but were not feasible in this outpatient study. However, other studies using similar PCR techniques have shown that oropharyngeal washes and nasopharyngeal aspirates adequately detect colonization [4,8-10], and there was no evidence of PCR inhibitors. Collection of serial samples might also have increased our ability to detect colonization or clustering in families.

We found no clinical risk factors that were associated with colonization in adults. We have previously reported that cigarette smoking increases the risk of colonization, but this result was

not duplicated in the current study [2]. Interestingly, both current and previous work demonstrate that CD4 cell count, history of PCP, and use of PCP prophylaxis do not impact colonization [2,3]. In the non-HIV-infected population, underlying lung disease and pregnancy have been shown to increase colonization risk [11-13], but these subject groups were not included in the current cohort. Previously reported risk factors in non-HIV-infected children include presence of respiratory symptoms and young age [4], similar to our pediatric population. Interestingly, when we examined the cohort as a whole, colonized subjects were more likely to be Hispanic and in fact, there were no colonized adults or children who were not Hispanic. Race or ethnicity has not been previously demonstrated to influence *Pneumocystis* colonization risk, and it is unclear if this finding is due to environmental or biologic causes. Because the number of colonized subjects was low, we may have lacked sufficient power to detect all clinical characteristics associated with colonization.

In conclusion, this study demonstrates that *Pneumocystis* colonization is relatively uncommon in healthy HIV-infected outpatient adults and their children. Transmission of colonization does not seem to be occurring within families. Colonization was more common in Hispanic adult and pediatric subjects, but no other clinical characteristics were associated with colonization in the adult population. The pediatric subjects who were colonized were young HIV-negative infants who had respiratory symptoms at the time of colonization.

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Table 1

Characteristics of adult subjects by *Pneumocystis* colonization status.

Characteristic	Colonized (n= 5)	Non-colonized (n=39)
Female gender, n (%)	5 (100.0)	36 (92.3)
Mean age in years, (SD)	26.1 (8.1)	32.9 (6.9)
Ethnicity, n (%)		
White	0 (0.0)	4 (10.3)
Black	0 (0.0)	12 (30.8)
Hispanic	5 (100.0)	21 (53.8)
Other	0 (0)	2 (5.1)
Years HIV-infected, mean (SD)	5.8 (6.1)	5.7 (4.4)
AIDS, n (%)	1 (20.0)	14 (35.9)
History of PCP, n (%)	0 (0.0)	1 (2.6)
PCP prophylaxis, n (%)	0 (0.0)	3 (7.7)
HAART use, n (%)	4 (80.0)	27 (69.2)
Median serum HIV viral level, log copies/ml (range)	1.7 (1.7-2.8)	2.6 (0.9-5.2)
Median CD4 count, cells/μl (range)	783 (483-953)	497 (0-1090)
CD4 count <200 cells/μl, n (%)	0 (0)	5 (12.8)
Respiratory symptoms, n (%)	1 (20.0)	22 (56.4)
Current smoker, n (%)	0 (0.0)	11 (28.2)

Abbreviations: HAART, highly active antiretroviral use; PCP, *Pneumocystis* pneumonia; SD, standard deviation.

Table 2

Characteristics of pediatric subjects by *Pneumocystis* colonization status.

Characteristic	Colonized (n= 2)	Non-colonized (n=58)
Female gender, n (%)	2 (100)	28 (48.3)
Mean age, years (range)*	0.22 (0.2, 0.3)	6.8 (0.0-17.6)
Ethnicity, n (%)		
White	0 (0)	4 (6.9)
Black	0 (0)	20 (34.5)
Hispanic	2 (100)	33 (56.9)
Other	0 (0)	1 (1.7)
HIV-infected, n (%)	0 (0.0)	9 (15.5)
AIDS, n (%)^	NA	1 (11.1)
History of PCP, n (%)^	NA	0 (0.0)
PCP prophylaxis, n (%)^	NA	2 (22.2)
HAART use, n (%)^	NA	8 (88.8)
Median serum HIV viral level, log copies/ml (range)	NA	3.1 (1.7-5.0)
Median CD4 count, cells/μl (range)*	NA	593 (104-2171)
CD4 count <200 cells/μl, n (%)^	NA	1 (11.1)
Respiratory symptoms, n (%)	2 (100.0)	24 (41.3)
Tobacco exposure, n (%)	1 (50.0)	16 (27.6)

* p= 0.04 for comparison of children less than or greater than one year of age.

^ Percentage of those who are HIV-infected.

Abbreviations: HAART, highly active antiretroviral use; NA, not applicable; PCP, *Pneumocystis* pneumonia.