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Distribution of *Pneumocystis jirovecii* in lungs from colonized COPD patients☆,☆☆,★

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Abstract

Pneumocystis jirovecii has been detected in lung tissue from patients with chronic obstructive pulmonary disease (COPD) and is associated with disease severity. The regional distribution of the organism in lungs is unknown, but differences in distribution of *Pneumocystis* could affect estimates of colonization prevalence. We examined the distribution of *Pneumocystis* in the lungs of 19 non–HIV-infected patients with COPD who were undergoing lung transplantation. DNA was extracted from explanted lungs. We found *Pneumocystis* colonization in lung tissue of 42.1% of patients with advanced COPD; however, there was significant regional variation in colonization between lung segments of individual patients. Colonization was detected more commonly in the lower and middle lobes than in the upper lobes. These findings suggest that single samples from an individual may underestimate the prevalence of *Pneumocystis* colonization and future studies may obtain a higher yield of *Pneumocystis* colonization detection when sampling the lower lobes.

Keywords: Chronic obstructive pulmonary disease; *Pneumocystis jirovecii*; Lung

1. Introduction

The presence of *Pneumocystis jirovecii* (Pc) in respiratory specimens in the absence of clinical infection has been defined as colonization (Morris et al., 2004). Polymerase chain reaction (PCR) assays are very sensitive, enabling detection of limited numbers of organisms, even in cases where routine histochemical staining methods are negative (Wakefield et al., 1990). PCR can detect colonization in diverse respiratory samples (e.g., sputum, bronchalveolar lavage, and lung tissue), but the operating characteristics of these assays may be influenced by the type and location of the respiratory sample, as well as the number and volume of samples. A recent study found that the sensitivity of PCR for detection of Pc colonization in the lungs of normal subjects was increased by analyzing a large volume of lung tissue obtained from the right upper lobe (Ponce et al., 2010). Combined analysis of both an oropharyngeal wash and a nasal swab has also been reported to improve detection of Pc in a population of older, healthy adults (Vargas et al., 2010). The distribution of Pc colonization within the lung, however, has not been well studied, and it is unknown whether sampling of upper lobe versus lower lobe or of apical versus basal lung regions within or among lobes affects Pc detection.

Sample site may be particularly important in lung diseases such as chronic obstructive pulmonary disease (COPD) that may have differential expression throughout the lung. Pc colonization has been associated with development of COPD-like changes in nonhuman primates and mouse models and with increased severity of COPD in humans (Calderón et al., 2007; Christensen et al., 2008; Morris et al., 2004; Shipley et al., 2010), although not all studies have corroborated this association (Maskell et al., 2003). This discrepancy may be due in part to differences in study

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populations, but might also result from regional lung variation in colonization. In addition, there is growing interest in the lung microbiome in an effort to understand the relationship of microbes to the lung in health and disease (Friaza et al., 2010). Microbial flora has been shown to vary within specific locations of the skin, the gastrointestinal tract, and the mouth (Cowan and Talaro, 2008). It is not known whether the same regional variation occurs in the lung, and it remains unclear whether detection of colonization in a single lung sample accurately reflects the entire lung. Regional differences in microbial detection throughout the lung would have important implications for the design and interpretation of studies of the lung microbiome or of specific organisms. We performed a cross-sectional study to determine the differential anatomic distribution of Pc as a representative organism in the lungs of patients with COPD.

2. Materials and methods

Subjects were undergoing lung transplantation for end-stage COPD at the University of Pittsburgh Medical Center (Pittsburgh, PA) between March 2008 and August 2009. Clinical data were collected prospectively, prior to transplantation, and included demographic information, history of previous pneumonia, medications and chemical exposures, as well as completion of St. George's Respiratory Questionnaires, pulmonary function tests, and chest radiographs. Chest computed tomography (CT) scans were visually scored for emphysema presence and severity based on the National Emphysema Treatment Trial (Fishman et al., 2003). CT scans were rated as normal, trace (1–10%), mild (11–25%), moderate (26–49%), marked (50–74%), and severe (>75%) emphysema (National Emphysema Treatment Trial website). The University of Pittsburgh Institutional Review Board approved this study, and all participants provided informed consent.

Tissue was obtained from surgical explants of the native lungs. Samples were randomly acquired from subpleural apical and basal regions in each lobe of the explanted lung (s). Two 200-mg pieces of tissue were obtained from the upper, middle, and lower lobes in explanted right lungs and/or upper and lower lobes of the explanted left lung. Individual sterile equipment was used to obtain samples within the same lung, and lungs were processed individually on separate days. DNA was extracted from approximately 200 mg of tissue from each site. Tissue was homogenized with a Bullet Blender™ (Next Advance, Averill Park, NY, USA) using extraction buffer (10× PCR buffer, 50 μm $MgCl_2$, and distilled water) and stainless steel beads for 2 min. New beads and tubes were used for each sample. DNA extraction was performed as previously described (Morris et al., 2004).

Nested PCR was performed at the *Pneumocystis* mitochondrial large subunit (mtLSU) rRNA gene using first-round primers PAZ 102-E and PAZ102-H and second-round primers PAZ 292-R and PAZ 102-X as previously reported (Morris et al., 2004). Negative and positive samples (DNA from lung tissue known to contain human *P. jirovecii*) were included in all reactions. Positive results were determined by visual inspection and confirmed by sequencing. All PCR was performed by personnel blinded to subject identities and was performed in duplicate. DNA extraction and PCR were carried out in separate rooms, and all PCR procedures were performed in an ultraviolet box. The presence of adequate DNA and lack of PCR inhibitors in each sample were confirmed by performing PCR for human beta-globin (Spencer et al., 2008).

Subjects were considered Pc-colonized if at least 1 lung sample had *P. jirovecii* identified by sequencing. Gene Codes Sequencher® version 4.9 sequence analysis software (Gene Codes Corporation, Ann Arbor, MI USA) was used to identify the mtLSU rRNA genotypes based on polymorphisms at nucleotide positions 85 and 248 (Board et al., 2003).

Stata version 8.2 (StataCorp, College Station, TX, USA) was used for analyses, and statistical significance was defined as $P < 0.05$. Clinical, physiologic, and radiographic data were compared between Pc-colonized and Pc-negative subjects using Wilcoxon rank sum tests for continuous or ordered variables or Fisher's exact and chi-square tests for dichotomous variables.

3. Results

Demographic and clinical parameters of the 19 lung transplant recipients from whom lung specimens were obtained are detailed in Table 1. Subjects received either single or double lung transplants. All subjects quit smoking

Table 1
Characteristics of subjects according to *Pneumocystis* colonization status

Subject characteristics	Pc+ (n = 8)	Pc− (n = 11)
Male, n (%)*	8 (61.5)	5 (38.5)
Caucasian, n (%)	8 (100)	11 (100)
Age, median years (range)	65 (52–74)	61 (55–69)
Pack years, median (range)	42 (30–80)	60 (25–106)
Inhaled corticosteroids, n (%)	6 (46.1)	7 (53.5)
Systemic steroid use, n (%)	3 (37.5)	5 (62.5)
Current macrolide use, n (%)	0	2 (18.2)
Prior pneumonia, n (%)	6 (75.0)	11 (100)
Environmental exposures, n (%)[a]	7 (87.5)	10 (90.9)
Post bronchodilator FEV_1, median (range)	0.68 (0.58–2.07)	0.67 (0.41–1.41)
FEV_1 percent predicted, median ml (range)	19.9 (13.8–63.4)	22.7 (14.4–35.7)
DLco percent predicted, median (range)	27.1 (16.5–34.3)	31.5 (17.3–45)

DLco = Diffusing capacity for carbon monoxide; FEV_1 = forced expiratory volume in 1 s; Pc = *Pneumocystis*.

[a] Asbestos, berrylium, coal dust, cadmium, cobalt, diesel engine exhaust fumes, silica.

* $P = 0.02$.