

Pneumocystis jiroveci: Epidemiology and diagnosis

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The *Pneumocystis* organism was initially described in 1909 by Chagas. *Pneumocystis jiroveci* is an uncommon opportunistic organism, which induces a severe and mostly fatal pneumonia in immunocompromised people. Pneumocystosis comes from reactivation of latent infection acquired while childhood or active acquisition while immunosuppression state. The epidemiology of *Pneumocystis* pneumonia (PCP) has changed considerably through the course of the HIV/AIDS epidemic. The establishments of PCP prophylaxis in 1989 and effective combination antiretroviral therapy in 1996, have resulted in substantial reduces

in the incidence of PCP. Due to the insufficient specificity of clinical symptoms as well as the possibility of toxicity with therapy, the affirmation of PCP is important details for correct patient management. The traditional method for diagnosis of PCP depends on the microscopic visualization of organisms in respiratory samples. PCR diagnosis of *P. jiroveci* was introduced experimentally in 1990. Molecular methods, could detect *P jirovecii* DNA in respiratory samples from patients without clinically apparent PCP. In this review both epidemiology and diagnosis of *Pneumocystis jiroveci* will be discussed.

INTRODUCTION

Pneumocystis jiroveci is an uncommon opportunistic organism, which induces a severe and mostly fatal pneumonia in immunocompromised people [1]. The *Pneumocystis* organism was initially described in 1909 by Chagas in Guinea pigs, and after that by Carini in rat lung [2]. Until 1980 *Pneumocystis* pneumonia (PCP) was unusual and primarily noticed in association with syndromes of immunodeficiency or intensive immunosuppression, particularly cancer chemotherapy. With the HIV-1 pandemic, however, PCP become the major AIDS defining disease in developed countries[3]. *Pneumocystis jiroveci*, consequently, continues to be an organism of clinical significance in today's practice [4]

ORGANISM

Pneumocystis organisms were primarily known and named *Pneumocystis carinii* at the beginning of last century [5]. In 2002, human *Pneumocystis* organism was recalled

Pneumocystis jiroveci by the identification of its genetic and functional distinctness, in honour of Otto Jirovec, who is recognized for identifying the microbe in humans [6,7].

Pneumocystis was for an extended time defined as a protozoae according to morphologic features and the resistance to classical antifungal agents. Then again, in the late eighties, *P. carinii* was positioned in the fungal kingdom depending on phylogenetic analysis of ribosomal RNA (rRNA) sequences and observations of genome size. [8]. The organism has currently been fixed in a group of fungi entitled the Archiascomycetes. [9]

In the lung, two main forms of the organism could be identified by microscopy: They are usually called cystic form (cysts) and trophic form (trophozoites), however they more appropriately, considering the fungal reclassification, might be defined as sporangium and yeast cells[10].

The cystic form (sporangium) is usually thick-walled oval, about 5 to 8 μ in diameter and include approximately eight daughter forms (spores or endospores, initially called intracystic bodies or sporozoites), which will eventually become trophic forms after excystation. The trophic form (yeast, previously trophozoite) is small (2 to 5 μ m), thin-walled, pleomorphic and commonly has an eccentric nucleus. The trophic forms are usually seen in clusters. A third form, the precyst, is infrequently encountered, but is believed to constitute an intermediate stage. There is no precise knowledge of the lifecycle as well as the mode of replication has not been absolutely demonstrated, but both asexual and sexual life cycles have been suggested according to electromicrographic observations of synaptonemal complexes [11,12]

TRANSMISSION

Reactivation of Latency

Traditional concepts have retained that pneumocystosis comes from reactivation of latent infection acquired while childhood. Detectable antibodies are found in most of children by four years of age. Moreover, presence of *P. jiroveci* in respiratory samples from clinically normal population sustains the reactivation theory [13,14]. Besides, host immune evasion by variation of major surface glycoprotein (MSG), species specificity of *P. jiroveci* and complicated in vitro cultivation are constant with extended carriage or latency in the host [15,16].

Active Acquisition

Almost all attempts to transfer *P. jiroveci* from one animal species to another have been failed. In natural environment, *P. jiroveci* deoxyribonucleic acid (DNA) has been found in water, air and in soil samples, however, whole organism has been practically never recognized from these sources. Navin et al [17] discovered that patients with *P. jiroveci* were usually have history of recent gardening, therefore suggesting soil exposure. Outbreaks in oncology and transplant units have increased the possibility of person to person transmission. Besides clinical cases, sub-clinical cases and a few number of immunocompetent persons with no evidence of clinical disease accommodate *P. jiroveci*, forming the reservoir. This source should not be disregarded without the presence of other evidences. In any case, the transmission occurs

via an airborne route as alveolus is the site of infection. The vertical transmission is rarely reported and it couldn't be demonstrated by animal experiments [18].

EPIDEMIOLOGY

The epidemiology of PCP has changed considerably through the course of the HIV/AIDS epidemic. In the 1980s, PCP was the AIDS defining disease for about two-thirds of adults and adolescents with AIDS in the United States, and it was supposed that 75% of HIV-infected persons would acquire PCP through their lifetime [19]. At the beginning of the epidemic, the incidence of *Pneumocystis* pneumonia was approximately 20 cases per 100 person-years in HIV-infected persons with CD4+ cell counts under 200 cells/l [20]. The establishments of PCP prophylaxis in 1989 and effective combination antiretroviral therapy in 1996 have resulted in substantial reduces in the incidence of *Pneumocystis* pneumonia. In the Centers for Disease Control and Prevention (CDC) Adult and Adolescent Spectrum of Disease (ASD) Project, the incidence of *Pneumocystis* pneumonia declined 3.4% per year for the period from 1992 to 1995 and then decreased 21.5% annually during 1996 to 1998, a time when effective combinations of antiretroviral therapy were starting to be applied [21]. In the Euro SIDA study, the incidence of *Pneumocystis* pneumonia reduced from 4.9 cases per 100 person-years before March 1995 to 0.3 cases per 100 person-years after March 1998 [22]. Even with its decreased incidence, PCP continues to be the common serious opportunistic infection among HIV-infected persons, and a considerable proportion of persons who develop *Pneumocystis* pneumonia are unconscious of their HIV infection or are outside of medical care, consequently minimizing opportunities for additional reductions in the incidence of the disease. A consideration, *Pneumocystis* pneumonia has been significantly reported in low- or middle-income countries (LMIC). One clinical study from Uganda observed that 38.6% of 83 HIV-infected patients who were admitted to the hospital with pneumonia and who had three expectorated sputum smears which were negative for acidfast bacilli had *Pneumocystis* pneumonia detected on bronchoscopy with bronchoalveolar lavage (BAL) [23,24].

DIAGNOSIS

CLINICAL DIAGNOSIS

The fungal pathogen *Pneumocystis jirovecii* is the source of PCP in humans [25]. Patients with PCP generally present non-specific respiratory symptoms, such as dyspnea, cough, and fever. Immunocompromised hosts commonly present a triad of progressive dyspnoea of many weeks, non-productive cough or with clear sputum and low-grade fever. They are more apt to be in respiratory distress coupled with severe hypoxemia [4]. On medical exam, tachypnoea, tachycardia, cyanosis and fine dry rales can be present [2]. While extrapulmonary involvement is exceptional, eye, ear, spleen, bone marrow, lymph node, liver, and meningeal involvement, have been mentioned [4].

Chest X-ray generally reveals diffuse interstitial or peri-hilar reticulonodular infiltrates but could be normal in one-third of cases. Other findings, including spontaneous pneumothorax, effusions or cavitory lesions may be seen in few numbers of cases [26].

BIOLOGICAL DIAGNOSIS

Due to the insufficient specificity of clinical symptoms as well as the possibility of toxicity with therapy, affirmation of PCP is important an essential details for correct evidence-based patient management [1]. Diagnosis of *P. jirovecii* infection is hampered by the lack of a sustainable in vitro culture method. Elevated serum lactate dehydrogenase (LDH) > 460 IU/L has been noticed to be a sensitive test for this disease, but a variety of pathological conditions involving lungs indicate increased serum LDH level. Measurement of partial pressure of arterial oxygen (PaO₂) gives a perfect negative predictive value for exemption of *P. jirovecii* infection as PaO₂ < 75 mmHg correlates with the illness [27, 28].

Conventional methods

The traditional method for diagnosis of PCP depends on the microscopic visualization of *P. jirovecii* organisms in respiratory samples. Given that only a few organisms can be found within the upper respiratory tract, lower respiratory secretions are often essential for certain diagnosis. Bronchoalveolar lavage (BAL) coupled with colorimetric as well as an immunofluorescent stain of BAL fluid is recognized as the technique of choice with

sensitivity and specificity more than 95%. A different option is evaluation of material gotten by induced sputum. In spite of this, the sensitivity of that method is much more based on the experience of the personnel executing the procedure and checking the samples, with elevated variation in the diagnostic sensitivity noted (between 50 to 90%) [29,30,31,32].

The usage of cytochemical stains for diagnosis is very slow process, and it could be complicated to preserve laboratory diagnostic expertise due to the reduced incidence of PCP after the introduction of significantly active antiretroviral therapy [33,34].

PCR methods

Since BAL is an invasive and irritating procedure that is not without risk, other non-invasive test were approved. PCR diagnosis of *P. jirovecii* was introduced experimentally in 1990 once Wakefield et al first identified DNA amplification of the multicopy mitochondrial ribosomal RNA gene. Since that time, numerous studies have evaluated PCR detection of *Pneumocystis* DNA in respiratory samples from HIV-1 positive and negative patients. To improve the diagnostic sensitivity of non-BAL specimens, distinct PCR methods have been considered, such as different gene targets and the use of nested PCR, during which a second round of PCR reamplifies the primary PCR product [1]. Molecular methods, on the other hand, could detect *P. jirovecii* DNA in respiratory samples from patients without clinically apparent PCP [35,36] advocating asymptomatic carriage or "colonisation". Real-time PCR permits appropriate quantification of DNA as well as possibility to discriminate between asymptomatic carriage of *P. jirovecii* and clinical disease depending on pathogen load.

CONCLUSION

P. jirovecii is pathogen fungal organism witch continues to be an organism of clinical significance in today's practice and should not be disregarded because of the close link between this disease and HIV-infected persons. An earlier diagnosis with appropriate method could improve the prognosis of PCP and the correct patient therapy management.

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