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Pneumocystis Jirovecii: A Retrospective Evaluation Of The Last 15 Years In Istanbul, Turkey

KEYWORDS: Pneumocystis jirovecii, Retrospective, Turkey

Hayriye Kırkoyun Uysal
Istanbul University, Istanbul Faculty of Medicine, Department of Medical Microbiology, Istanbul, Turkey

Özer Akgül
Uskudar University, Vocational School of Health Services, Istanbul, Turkey *Corresponding Author

Sevim Purisa
Istanbul University, Istanbul Faculty of Medicine, Department of Biostatistics and Medical Informatics, Istanbul, Turkey

Yaşar Ali ÖNER
Istanbul University, Istanbul Faculty of Medicine, Department of Medical Microbiology, Istanbul, Turkey

ABSTRACT *To evaluate the epidemiological and significance characteristics of Pneumocystis jirovecii in the Istanbul area. We retrospectively collected data from the cases with Pneumocystis jirovecii pneumonia (PJP) diagnosed at Istanbul University Hospital during a 15 year period (between 1998 and 2012) in Istanbul, Turkey. Total of 387 samples were examined by Giemsa staining and direct immunofluorescence method. Eight-one of all (20.9%) were found positive for Pneumocystis jirovecii. Statistically highly significant increasing was found at the positivity ratio of Pneumocystis jirovecii ($p<0.001$). Although risk factors, person to person transmission and effective prophylaxis being known, Pneumocystis jirovecii remains a important infectious disease associated with HIV and in other immunosppressive agents as well. Continuous efforts in infection control are still needed to keep the incidence down. Further and epidemiological surveillance studies with larger patient groups are needed to elucidate the actual role of Pneumocystis jirovecii in infectious diseases*

Introduction

Pneumocystis jirovecii (formerly known as Pneumocystis carinii), was first identified by Carlos Chagas in 1909 from the lungs of guinea-pigs infected by Trypanosoma cruzi [1]. In 1942, Pneumocystis was redescribed as a human pathogen [2]. Pneumocystis was initially misclassified as a protozoan on the basis of the morphologic features, the larger cyst form, the resistance profile to anti-fungal drugs and the phenotypic fungal characteristics [3]. Currently, based on DNA sequence analysis, Pneumocystis jirovecii is genetically more closely aligned with the fungi, thus has been reclassified as an ascosporogenous yeast [4].

The life cycle of Pneumocystis jirovecii (PJ) is complicated. After inhalation of PJ, the organism reaches the alveoli where the trophozoite form attaches to type 1 pneumocytes via the adhesion molecules fibronectin and vitranectin. In a host with an intact immune system the organism is eliminated; in the immunodeficient host PJ pneumonia will develop [5]. PJ has 2 different forms called as trophozoite and cyst, the trophozoite forms measure 1-5 µm and are ameboid in shape, the cyst forms also measure 5-10 µm diameter and contain eight trophozoites when mature. After the disintegration of the mature cysts, trophozoites remain free and generate the trophozoite forms, so that the life cycle of PJ continues [6].

The transmission of pneumocystis is not fully understood. Person to person transmission is the most likely mode of acquiring new infections, although acquisition from environmental sources may also occur [7]. Studies in animals demonstrate that pneumocystis is transmitted from person to person via an airborne route [8].

Infection in humans is caused by PJ, and is called as Pneumocystis jirovecii pneumonia (PJP). Common signs and symptoms of pneumocystis pneumonia include progressive dyspnea, nonproductive cough, low-grade fever, and cheast pain. Physical examination characteristically indicates tachypnea, tachycardia, and normal findings on lung auscultation. Blood count test is generally not helpful in the evaluation of patients with PJ, although usually mild leukocytosis with eosinophilia may reveal [9].

Pneumocystis jirovecii is an opportunistic organism causing severe pneumonia, especially in immunocompromised patients infected with human immunodeficiency virus (HIV) or HIV-uninfected. Before the acquired immunodeficiency syndrome (AIDS) epidemic, which began in 1982, this organism was known to cause interstitial plasma cell pneumonia in premature and malnourished children as well as in cancer patients on chemotherapy [10,11]. At the present time, although the incidence of PJP has decreased, it remains an important cause of pneumonia in both HIV-associated and non-associated immunosuppressed patients. Furthermore, PJP is now more frequently seen for the increasing numbers of organ transplants [12]. The aim of the present study was to describe the incidence and the significance of Pneumocystis jirovecii from 1998 to 2012 in Istanbul, Turkey.

Materials and methods

In Istanbul University Istanbul Medical Faculty Medical Microbiology Department Istanbul, Turkey, BAL samples are routinely came from different departments, such as pulmonary disease, internal medicine, transplantation unit and pediatrics. We collected retrospectively the medical data of all patients who were preliminary diagnosed as PJP. Patients tested positive and treated for PJP, or treated only on clinical suspicion, were identified. In our study, we could not clearly recorded all demographic data of study patients, so we were focused on mainly parasitological datas. For this reason, only demographic data (age, sex),

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and the clinical department treating the patient were registered.

During the 15 years (January 1998 to December 2012), 387 bronchial alveolar lavage (BAL) samples were examined for identification of the existence of Pneumocystis jirovecii. The identification of Pneumocystis jirovecii in patients suspected of pneumocystosis was achieved by parasitological investigation in BAL. BAL samples were examined by Giemsa staining and immunofluorescence test kit MONOFLUO™ (Bio-Rad Laboratories, Marnes-la-Coquette, FR) following the manufacturer's instructions. Statistical calculation of confidence limits was performed using SPSS, version 19.0.

Results

Between 1998 and 2012, total of 387 samples (mean 25.8 cases/year) were included in our study. Two hundred and twenty-one of these patients were men and 166 were women. The average age at the time of identification of the 387 episodes was 45 years (range 0 to 83 years) (median = 47 years). In the present study, 207 samples (53%) were sent from the internal medicine, 158 samples (41%) the pulmonary disease, 14 samples (4%) the transplantation unit, and 8 samples (2%) were sent from the pediatrics.

Of the 387 BAL samples analyzed microscopically following Giemsa staining and immunofluorescence test kit, 81 (20.9%) were overall positive with both methods.

The annual number of Pneumocystis jirovecii detected samples and the positive ratio is shown in Table 1. Maximum positive ratio (47.6%) was detected in 2012, although there were no samples detected as positive in 2003 and 2004. Furthermore, a variable distribution was found in Pneumocystis jirovecii positive patients, although the number of positive patients and the positive ratio were continuously increased between 2005 and 2012 (Figure 1).

Because a different number of samples was came from the clinical departments in each year, the grouping was done by total count of samples for statistical analysis. All collected datas were divided into 3 groups. Group 1 was included 118 samples between 1998 and 2004, and positivity rate of this group was found as 6.8%. Group 2 was included 100 samples between 2005-2008, and this group had 15% positivity rate. Group 3 was included 169 samples between 2009-2012, and 34.3% positivity rate was found in this group (Table 2). The results of the present study clearly demonstrate that there is a statistically highly significant increasing at the positivity ratio of Pneumocystis jirovecii with time ($p<0.001$).

Table 1. Yearly incidence of PJ investigation in Istanbul.

Years	Number of BAL Samples (n)	Number of PJ Positive BAL Samples (n)	Positive Ratio (%)
1998	35	2	5.7
1999	17	1	5.9
2000	20	2	10.0
2001	20	2	10.0
2002	8	1	12.5
2003	12	0	0
2004	6	0	0
2005	21	2	9.5
2006	26	4	15.4
2007	24	4	16.7
2008	29	5	17.2
2009	35	8	22.8
2010	40	12	30.0
2011	52	18	34.6
2012	42	20	47.6
Total	387	81	20.9

Figure 1. Pneumocystis jirovecii positive status

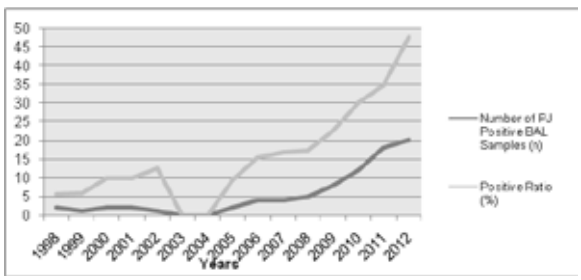

Table 2. The positive/negative distribution between the groups.

Pneumocystis jirovecii Positive (n, %)	Pneumocystis jirovecii Negative (n, %)	P	
value			
Group 1 (n=118)	8 (%6.8)	110 (%93.2)	<0.001
Group 2 (n=100)	15 (%15)	85 (%85)	
Group 3 (n=169)	58 (%34.3)	111 (%67.7)	

Discussion

Pneumocystis jirovecii is an opportunistic pathogen especially in immunocompromised patients who are AIDS or are not infected with HIV but are receiving chemotherapeutic medications or who have an underlying acquired/inherited immunodeficiency [13]. PJ cannot be effectively cultured so conventional typing methodology cannot be applied. An empiric diagnosis based on clinical presentation, chest radiographic and arterial blood gas abnormalities is widely used for HIV-associated PJP, but it is rarely, if ever, used for non HIV-associated disease. Typically, the chest radiograph shows diffuse bilateral infiltrates; occasionally appearances are atypical, showing multiple nodules [14]. In the present study, the annual incidence rate of the PJ distribution has been discussed.

PJP remains an important cause of HIV-associated pneumonia, but in some reports suggested that the rates of PJP have decreased. However, the mortality associated with PJP remains high [15]. On the other hand, earlier reports have been shown that the incidence of PJP in non-HIV patients is increasing [16]. Besides, a decrease in the overall incidence of PJP infections has been observed in HIV patients in previous studies [17]. In addition, immunosppressive treatment (corticosteroids, anti-TNFα antibodies, methotrexate, etc.) is a reputed risk factor for PJP in non-HIV infected patients and the mechanism could be a decrease of blood CD4+ lymphocyte count [18]. We herein report a statistically highly significant increasing at the positivity ratio of Pneumocystis jirovecii but we could not

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evaluate the relationship between underlying disease and PJ frequency, owing to the fact that the limitations in earlier patients' data.

Panizo et al. have shown that the 23.3% general frequency rate in 129 Venezuelan patients utilizing a retrospective study during a six year period. Furthermore, the researchers have processed by direct immunofluorescence technique to detection of Pneumocystis jirovecii. The direct immunofluorescence is a useful technique for pneumocystosis diagnosis, however, researchers have also highlighted that this method requires an optimal sample and qualified staff in the laboratory [19]. We found that the PJ positivity rate as a %20.9 by direct immunofluorescence technique. Direct immunofluorescence tests are being used in our laboratory for the microscopic detection of PJ with respect to achieved some benefits, such as lower price, shorter process time, higher sensitivity and specificity rates.

The diagnosis of Pneumocystis jirovecii is generally based on a direct microscopic examination of BAL and the sensitivities of such examinations range from 48% to 92% [20, 21]. Indeed, Toper et al. performed the diagnosis of Pneumocystis jirovecii in 41 patients with standard coloration and/or immunofluorescence analysis of BAL. The researchers have highlighted the difficulty to define risk of PJP in non HIV-infected patients [22]. Molecular techniques, such as PCR, have been described in order to improving the detection of Pneumocystis jirovecii [23]. Recently, many publications have reported that PCR has a greater sensitivity and a better specificity than conventional parasitological analyses [24, 25].

In conclusion, Pneumocystis jirovecii remains a critical reason of illness and death in immunosuppressed patients. Regardless of the reason for suppressed immune function, PJP keeps a high mortality rate in immunocompromised patients. The number of opportunistic infections such as PJP, in immunosuppressed non-HIV infected patients has been increasing. Careful monitoring and definitive diagnosis of immunosuppressed patients for pulmonary infections is recommended.

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