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Brief Original Article

Pneumocystis jirovecii colonization in bronchoalveolar lavage among naïve non-small cell lung cancer from tertiary respiratory hospital in Jakarta, Indonesia

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Abstract

Introduction: *Pneumocystis jirovecii* pneumonia (PJP) is a lung mycosis commonly found in immunocompromised patients (e.g., HIV patients); however, its role in solid cancer remains unclear. This study aims to identify *Pneumocystis jirovecii* colonization among naïve non-small-cell lung cancer (NSCLC) through bronchoalveolar lavage (BAL) and explore its correlation with clinical parameters.

Methodology: This cross-sectional study recruited newly diagnosed naïve NSCLC patients who had not been given systemic treatments. We tested BAL from patients for *P. jirovecii* colonization with nested PCR targeting the mtLSU rRNA gene. Demographic and clinical characteristics were obtained from medical records, and the correlation between *P. jirovecii* colonization and clinicopathological data were analyzed. Kaplan-Meier analyses were done to evaluate survival.

Results: Among 56 newly diagnosed, naïve NSCLC patients enrolled, the prevalence of *P. jirovecii* colonization was 17.9% (10 subjects). There was no statistically significant difference in demographic and clinical characteristics between the *P. jirovecii* colonization group versus no colonization (p value > 0.05). The overall survival duration for both groups demonstrated no significant difference.

Conclusions: This study demonstrated a relatively high prevalence of *P. jirovecii* colonization among BAL samples of naïve Indonesian NSCLC patients. Further study is needed to delineate its implications for the potential transmission source, lung cancer pathogenesis, and prognosis.

Key words: *Pneumocystis jirovecii*; bronchoalveolar lavage; lung neoplasms.

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Introduction

Lung cancer is one of the leading causes of cancer death globally [1]. The incidence of lung cancer is decreasing in developed countries due to successful tobacco control, unlike in developing countries such as Indonesia, where lung cancer incidence is around 8.6 % of all recorded cancer cases, and it is predicted to increase in coming years, due to the high prevalence of cigarette smoking [2].

Patients with cancer have impaired innate and adaptive immune responses and are prone to infection, including bacterial and fungal infections. Mycosis could affect the quality of life and might also correlate with mortality in patients with cancer [3]. *Pneumocystis* is a ubiquitous organism initially classified as a protozoan, but it was reclassified as a fungus based on genetic DNA homology with fungal organisms. *Pneumocystis* is species-specific and infects only mammals. The human-specific form is known as *Pneumocystis jirovecii* [4].

Pneumocystis jirovecii pneumonia (PJP) is a lung mycosis caused by *P. jirovecii* based on a clinical, radiological, and serological examination, in addition to organism detection in respiratory samples [4]. PJP was initially recognized in AIDS patients, but recent data shows it also occurs in non-HIV immunocompromised states, such as patients with solid tumors, immunosuppressive therapy, and organ transplant [5]. Furthermore, PJP incidence in non-HIV patients was reported below 25 per 100,000 cases annually, 6% of which were present in cancer patients [6]. PJP was also reported in lung cancer patients [7,8].

The biology of *Pneumocystis* infection in humans has not been widely studied due to the lack of the propagation system of the organism [4,8,9]. It was thought that PJP occurred via the activation of latent infection early in childhood. Reactivation is possible when the immune system is weakened or compromised. Another possibility is *de novo* exposure from individuals with PJP, individuals with colonization, or acquired from the environment [4,9,10].

Pneumocystis colonization is defined as detecting the organism in subjects without signs and symptoms of acute pneumonia [4,9]. *Pneumocystis* colonization had been found in HIV, malignancy, organ transplantation, pregnancy, chronic lung disease, or childhood respiratory infection [9,10]. Colonization has important clinical implications. It might be involved in disease transmission or disease development [4,9,10]. It may also play a role in developing or progressing various lung diseases [10]. Therefore, Pneumocystis colonization has been an essential subject for research [9,10]. Data regarding *Pneumocystis* colonization in lung disease in tropical and developing countries is limited. This study aimed to evaluate the prevalence of *P. jirovecii* and its clinical relevance in newly diagnosed, treatment-naive NSCLC in Persahabatan National Respiratory Referal Hospital, Jakarta, Indonesia.

Methodology

Setting and Eligibility Criteria

This cross-sectional study aimed to identify *Pneumocystis jirovecii* colonization among newly diagnosed naïve-NSCLC patients admitted between February 2019 and April 2019 at the Persahabatan National Respiratory Referal Hospital. The inclusion criteria were as follows: adult patients (> 18 years), histologically confirmed NSCLC who did not yet receive any chemotherapy (therapy naïve) and were eligible for bronchoscopic evaluation.

Pneumocystis colonization was defined as the detection of *Pneumocystis* DNA from BALF samples.

Variables such as age, sex, smoking history, histopathology, and staging were taken from patients' medical records. The Eastern Cooperative Oncology Group Performance Status (ECOG-PS) scoring system was used to evaluate patients' performance [11]. Lung cancer TNM staging version 8 was used as a reference for staging. Smoking status was divided into three groups: non-smokers, active smokers, and passive smokers.

Ethical approval was granted by The Ethics Committee of the Faculty of Medicine, Universitas Indonesia, with reference number KET-753/UN2.F1/ETIK/PPM.00.02/2019. Written informed consent was obtained from all study participants.

Bronchoalveolar lavage (BAL) procedure, DNA extraction, and PCR test

The BAL procedure was carried out according to a previously published protocol [12]. The patient was anesthetized before undergoing the BAL. The procedure was initiated with the instillation of 100-150 mL of NaCl 0.9% at 37 ºC to prevent cough, bronchospasm, and decreasing lung function. The fluid was inserted as a bolus using a syringe with a velocity of 5 mL/s or naturally driven in hydrostatic force from the reservoir. The collected fluid was aspirated with negative pressure of 25-100 mmHg or gravity force. A minimum of 30-50 cc of BALF was collected during the procedure.

DNA in BALF was extracted using kit DNA purification (GeneJET, Pittsburgh, US) following the manufacturer's protocol. After the extraction, a nested-PCR test was done using a primer for rRNA large mitochondrial subunit/mtLSU [9]. The length of the fragment produced on the first cycle was 346 bp (pAZ102-E), whereas the second cycle was 260 bp (pAZ102-X).

Follow up data

Overall survival (OS) was measured as duration (in days) from the pathological diagnosis until death. If the event occurred in the subject's home, the family was contacted by phone.

Statistical Analysis

Descriptive statistics for demographic and clinicopathological data were analyzed using SPSS 27.0 IBM (Corp, 2020). The Chi-Square test was used to compare positive colonization vs. negative ones. Kaplan-Meier analysis and the Log-rank test were applied to evaluate survival analysis. Statistically significant differences were defined as p value < 0.05.

Results

Seventy-five subjects were diagnosed with lung cancer during the study period, but 15 subjects were ineligible for the bronchoscopic procedure due to clinical conditions. Bronchoscopic procedures were done among 60 naïve lung cancer patients. However, four patients with small-cell lung carcinoma were excluded, and 56 patients were included in the final analysis. The subjects' characteristics are shown in Table 1. The subjects were predominantly less than 65

years old (75%, aged 18-65 years), male (62.5%), and active smokers (62.5%). Adenocarcinoma was the most common histological type (78.2%). Furthermore, performance status 1 (39.2%) and late-stage lung cancer (76.8%) were frequently found. All subjects exhibited lung cancer-related respiratory symptoms, but none had any signs of acute pneumonia infection such as fever. Imaging studies also found no sign of pneumonia in this study population.

The prevalence of pneumocystis colonization in this study was 17.9% (10 subjects). There was no statistically significant correlation between Pneumocystis colonization vs. non-colonization in demographic and clinical characteristics (p value > 0.05).

The mean and median overall survival rates for the pneumocystis-positive group were 119 and 215 days (Figure 1). The mean and median survival rates for the pneumocystis-negative group were 122 days and 211 days, respectively. There is no statistically significant difference in survival between both groups (p value = 0.99).

Discussion

P. jirovecii colonization is defined as the detection of the organism by molecular or conventional methods in respiratory samples without signs and symptoms of pneumonia [4,8,9]. Immunosuppression, chronic disease, pregnancy, and immature immune system such as in newborns are the main risk factors for colonization. People with *P. jirovecii* colonization are potential sources for transmission to susceptible and vulnerable individuals [10]. Colonization also served as a possible initial step in disease progression for affected individuals [8,9]. Studies have reported that colonization rates of *P. jirovecii* in non-HIV groups

Figure 1. The Overall Survival Duration (OS) in patients with non-small cell lung cancer (NSCLC), according to the PCR results (Blue line: Negative group; Green: Positive group).

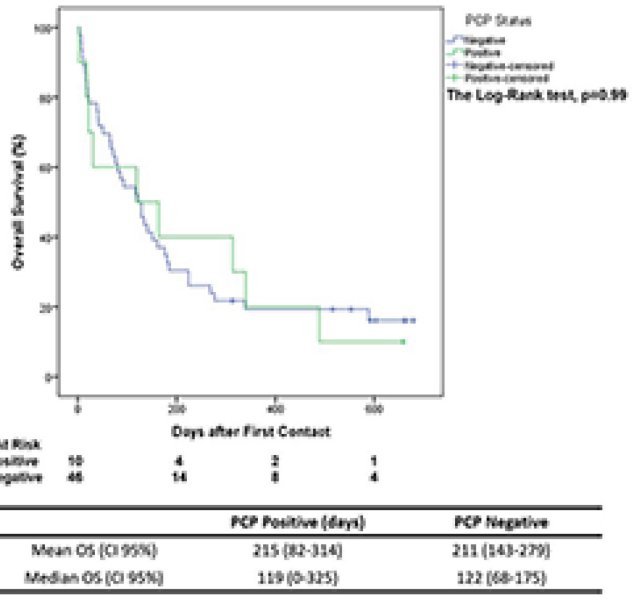

Table 1. Subject's Demographical and Clinical Characteristics.

Characteristics	*P. jirovecii* PCR (n = 56)		*p* value
	Positive (n = 10)	Negative (n = 46)	
Age			
18-65 years	9 (21.4)	33 (78.6)	0.42
66-99 years	1 (7.1)	13 (92.9)	
Sex			
Male	5 (14.3)	30 (85.7)	0.48
Female	5 (23.8)	16 (76.2)	
Performance Status			
PS 0-1	5 (14.3)	30 (85.7)	0.48
PS 2-3	5 (23.8)	16 (76.2)	
Smoking History			
No smokers	3 (16.7)	15 (83.3)	0.11
Passive smokers	2 (66.7)	1 (33.3)	
Active smokers	5 (14.3)	30 (85.7)	
Brinkman index			
Mild	6 (24.0)	19 (76.0)	0.67
Moderate	2 (14.3)	12 (85.7)	
Severe	2 (11.8)	15 (88.2)	
Histopathological Type (NSCLC)			
Adenocarcinoma	9 (20.9)	34 (79.1)	0.78
Squamous cell carcinoma	1 (9.1)	10 (90.9)	
Neuroendocrine	0 (0.0)	2 (100.0)	
Staging			
Early stage (I-IIIA)	2 (15.4)	11 (84.6)	1.00
Late stage (IIIB-IV)	8 (18.6)	35 (81.4)	

vary between 0-65% worldwide depending on the population studied and laboratory techniques used for detection [4,8].

P. jirovecii colonization in lung diseases has been found in patients with cystic fibrosis, interstitial lung disease, and COPD [13]. The role of *P. jirovecii* colonization in pulmonary diseases is still unclear, but it is thought to have an essential role in chronic respiratory diseases [10]. Studies showed that colonization of *P. jirovecii* in COPD might modify the immune response and correlate with the frequency of exacerbation, lung function impairment, and disease severity [14]. Lung cancer patients were at risk of PJP due to immune dysfunction. Chemotherapeutic agents or other lung cancer therapeutics modalities can further reduce immune impairment [15].

Data regarding pneumocystis colonization in tropical countries or developing countries is scarce. This study evaluated the prevalence of *P. jirovecii* colonization among naïve NSCLC patients using nested-PCR through samples collected from BAL in Indonesia. The prevalence of *P. jiroveci* colonization in this study was high. (17.9%). De La Horra *et al.* detected *P. jirovecii* in 2 out of 10 samples from non-small cell lung cancer [16]. A study in Poland by Sokulska *et al.* [13], and a study from Japan found a similar prevalence with 20.9% *P. jirovecii* colonization among lung cancer [17]. However, a study from Korea found around 12/206 (5.8%) based on a bronchial wash of lung cancer patients [18]. This difference might be related to different populations, environments, and techniques to detect colonization.

In this study, we did not find a difference in survival between *P. jirovecii* colonization and noncolonization. As a small sample size may cause this, more extensive studies are required to verify the result. A meta-analysis reported that solid tumor was associated with an increase in in-hospital mortality from *P. jirovecii* pneumonia (OR 2.66, CI 95% 1.72 to 4.13) [19]. Interestingly, another study by De La Horra *et al.* also gave rise to the possibility of *P.jirovecii* colonization in lung carcinogenesis since *P. jirovecii* was detected in all small cell lung cancer samples tested [16].

There are some limitations of this study. First, the number of subjects with lung cancer was limited and might limit the generalization. Second, the detection of *P. jirovecii* was based on simple qualitative PCR; therefore, it could not differentiate between true colonization and infection, unlike quantitative PCR [20].

Despite negative results and limitations, this study is worth noting since limited data on *P. jirovecii* colonization in lung cancer is available in developing countries. Colonization in the airways in this population may develop into pneumocystis pneumonia if the host's immune status deteriorates. The presence of the pneumocystis in the lung might induce an inflammatory response, lung tissue damage, and modify lung disease progression, thus warranting further studies. This data also raises concern regarding the high prevalence of *Pneumocystis* colonization in lung cancer. Although asymptomatic, it poses a risk to public health. The colonized individuals may transmit and spread the pathogens to other individuals at risk. These issues need to be further studied.

Conclusions

This study demonstrated a relatively high prevalence of *P. jirovecii* colonization among naïve Indonesian NSCLC patients through BAL specimens. Its role in lung carcinogenesis, disease progression, and disease transmission warrants further investigation.

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Authors' Contributions

JZ, AR, and ES conceived the study. PP performed bronchoscopic procedures. AAA, TF, BH, and MRC collect samples, experimented, and analyzed the data. JZ, AR, MRF, RA, and FS analysed the laboratory/clinical data, and wrote the manuscript with input from all authors.

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