Artikel Penelitian

Point-of-Care Diagnosis of Chronic Pulmonary Aspergillosis in Respiratory Disease Patients: Hope in a Resource-Limited Setting

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Abstract

Introduction: *Chronic pulmonary aspergillosis (CPA) is a global health burden, however the diagnosis is still challenging due to non-specific clinical and radiological features. To overcome this problem, the lateral flow assay to detect Aspergillus-specific antibodies has the potential for rapid CPA diagnosis (point-of-care test/ POCT). The aim of this study is to describe the profile of POCT Aspergillus-specific antibody detection in various populations as part of CPA diagnosis in Indonesia.*
Method: *This study is a part of previous CPA studies conducted in Jakarta between 2018-2021. The tests were carried out at the Laboratory of Parasitology Department, Faculty of Medicine Universitas Indonesia. All sera were tested using Aspergillus-specific immunochromatography lateral flow assay (LDBio, Diagnostics, Lyon, France) according to the manufacturer's instructions.*
Result: *There were 314 patients' sera derived from four groups: post-TB (n=82), active TB (n=105), uncontrolled-asthma (n=50), and lung cancer (n=77). The positive results of LDBio Aspergillus ICT were 43% in post TB, 11% in active TB, 16% in uncontrolled asthma, and 14% in lung cancer patients. Further analysis revealed that the prevalence of CPA was 6-22% in those populations.*
Conclusion: *The LDBio Aspergillus ICT has the potential as a POCT for early diagnosis of CPA. The risk of CPA was found highest in the post-TB group compared to others.*

Keywords: *Chronic pulmonary aspergillosis, Antibody detection, Point-of-care test*

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Diagnosis Cepat Aspergilosis Paru Kronik pada Pasien Penyakit Respirasi: Harapan bagi Daerah Bersumber Daya Terbatas

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Abstrak

Pendahuluan: *Aspergillosis paru kronik (APK) merupakan beban kesehatan global, namun diagnosisnya tidak mudah karena gambaran klinis dan radiologis yang tidak khas. Untuk mengatasi masalah tersebut, pemeriksaan antibodi spesifik Aspergillus berbasis lateral flow assay (LFA) dapat menjadi alternatif dalam mendiagnosis APK secara cepat. Penelitian ini bertujuan untuk mengetahui profil deteksi antibodi spesifik Aspergillus sebagai bagian dari diagnosis APK pada berbagai populasi pasien di Indonesia.*
Metode: *Penelitian ini merupakan kompilasi dari riset sebelumnya tentang APK di Jakarta selama periode 2018-2021. Pemeriksaan serologi dilakukan di Laboratorium Departemen Parasitologi Fakultas Kedokteran Universitas Indonesia, Jakarta. Semua serum pasien yang memenuhi kriteria inklusi diuji menggunakan kit deteksi antibodi spesifik Aspergillus (LDBio, Diagnostics, Lyon, Prancis) metode immunochromatography (LDBio Aspergillus ICT) sesuai petunjuk produsen.*
Hasil: *Diperoleh 314 serum pasien sesuai kriteria inklusi yang terbagi dalam empat kelompok pasien: bekas TB (n=82), TB aktif (n=105), asma tidak terkendali (n=50), dan kanker paru (n=77). Hasil positif LDBio Aspergillus ICT adalah 43%, 11%, 16%, dan 14% berturut-turut pada kelompok bekas TB, TB aktif, asma tidak terkontrol, dan kanker paru. Analisis klinis menunjukkan bahwa prevalensi APK berkisar 6-22% pada seluruh populasi tersebut.*
Kesimpulan: *Pemeriksaan LDBio Aspergillus ICT berpotensi menjadi point-of-care-test untuk diagnosis dini APK di tempat dengan sumber daya terbatas, termasuk Indonesia. Pasien bekas TB paling berisiko mengalami APK dibandingkan populasi pasien lain.*

Kata kunci: *Aspergilosis paru kronik, Deteksi antibodi, Point-of-care test*

Introduction

In 2022, the World Health Organization (WHO) launched a global warning about fungal infections that can pose a threat to public health as they are becoming increasingly common and resistant to treatment. The WHO fungal priority pathogens list (WHO FPPL) is the first global effort to systematically prioritize fungal pathogens, which are categorized into three groups: critical, high, and medium priority. Most fungal pathogens lack rapid and sensitive diagnostics, and the existing ones are not widely available or affordable globally.[1-3]

Aspergillus fumigatus is one of the critical fungal pathogens groups and has become the most common cause of pulmonary aspergillosis. In individuals with intact immune status, Aspergillus can be eliminated and rarely cause diseases. However, the risk of pulmonary aspergillosis increases significantly in immune-deficient patients. Chronic pulmonary aspergillosis (CPA) is an important clinical spectrum of pulmonary aspergillosis characterized by slowly progression and destruction of lung tissue. It affects over three million people worldwide, approximately 1.2 million of whom have had tuberculosis. The five-year mortality reaches 85%, depending on co-morbidities and disease severity.[1,2]

CPA typically occurs in non-immunocompromised or minimally immunocom-

promised patients who have pre-existing or co-existing lung disease, such as post-primary tuberculosis (TB) infection, active TB, asthma, lung cancer, and other chronic respiratory diseases. Accurate diagnosis of CPA remains a challenge, as the clinical and radiological features can resemble those of other lung diseases, including pulmonary TB. Indonesia is one of the countries with the highest burden of TB in the world and may potentially have a significant of CPA cases as well. Unfortunately, data on CPA in Indonesia is still lacking, and the diagnostic facilities are also still limited.[4,5]

The diagnosis of CPA relies on a combination of clinical symptoms, radiological features, and mycological evidence. The current criteria for CPA diagnosis are based on the three parameters that are present for more than three months: 1) at least one of the following clinical symptoms: weight loss, persistent cough, hemoptysis, chest pain, fatigue; 2) positive Aspergillus IgG assay result or other evidence of Aspergillus infection; 3) chest imaging showing progressive cavitary infiltrates and/or a fungal ball and/or pericavitary fibrosis or infiltrates or pleural thickening.[2,6]

Serological testing, particularly the detection of Aspergillus-specific antibodies, plays a crucial role in CPA diagnostics, but it should not be solely relied upon. The commonly used methods are the precipitin test enzyme immunoassay (EIA) or enzyme-linked immunoassay (ELISA). However, the performance levels of these tests can vary, and the cut-off values may differ among different populations. Unfortunately, these tests require specific facilities and trained personnel, making them relatively expensive and unsuitable for resource-constrained settings, including Indonesia. In this article, we present the potential of using immunochromatography (ICT) testing to profile of Aspergillus-specific antibodies (IgG and IgM). This method has the advantage of being a point-of-care (POCT) and could be a valuable part of CPA diagnosis in Indonesia.[6,7]

Methods

This study is part of a series of previous studies on CPA involving various populations, including patients with post-TB, active TB, uncontrolled asthma, and lung cancer in Jakarta and surrounding cities from 2018 to 2021. The study involved consecutive sampling of 314 patients' sera collected at the Mycology Laboratory, Department of Parasitology, Faculty of Medicine Universitas Indonesia (FMUI), Jakarta. All collected sera were tested using the Aspergillus-specific immunochromatography lateral flow assay (LDBio, Diagnostics, Lyon, France) following the manufacturer's instructions (Figure 1). The inclusion criteria for clinical material were as follows: sera from adult patients with chronic respiratory diseases (including active TB, post-TB, uncontrolled asthma, and lung cancer); patient who did not have HIV/AIDS, and patients who had not received systemic antifungal agents within the last one month.

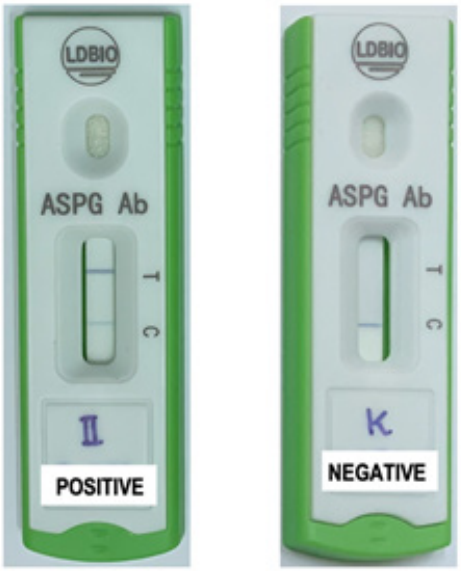


Figure 1. Aspergillus-specific Antibody IgG IgM Detection (LDBio, Diagnostics, Lyon, France). The test result is considered positive when two lines appear: a positive control line ("C") and a positive text line ("T"). A negative result is indicated when only the positive control line ("C") appears. However, if the "C" line does not appear at all, the test result is considered invalid.

In the study, a total of 15 µl sera was dispensed onto the ICT sample application pad, and then four drops of eluting solution (provided by each kit) were applied. The mixture was left to stand for 20-30 minutes. After that, the final point reading was performed. The results were visually interpreted by three individuals at almost the same time. Furthermore, CPA diagnosis was confirmed by experienced specialist clinicians followed the guideline provided. The study was conducted in accordance with the principles outlined in the Declaration of Helsinki and was approved by The Ethics Committee of the Faculty of Medicine, Universitas Indonesia (ND 071/UN2.F1/ETIK/PPM.00.02/2021).

Results

This study included a total of 314 patients' sera, which derived from different groups: post-TB patients (n=82), active TB patients (n=105), uncontrolled-asthma patients (n=50), and lung cancer patients (n=77). The positive results obtained using LDBio Aspergillus ICT showed a proportion of 43% in post-TB, 11% in active TB, 16% in uncontrolled asthma, and 14% in lung cancer patients (Figure 2). Further analysis, taking into account clinical manifestations, radiological results, and mycological evidence (including LDBio Aspergillus ICT results), revealed that the prevalence of CPA varies between 6-22% in these populations.

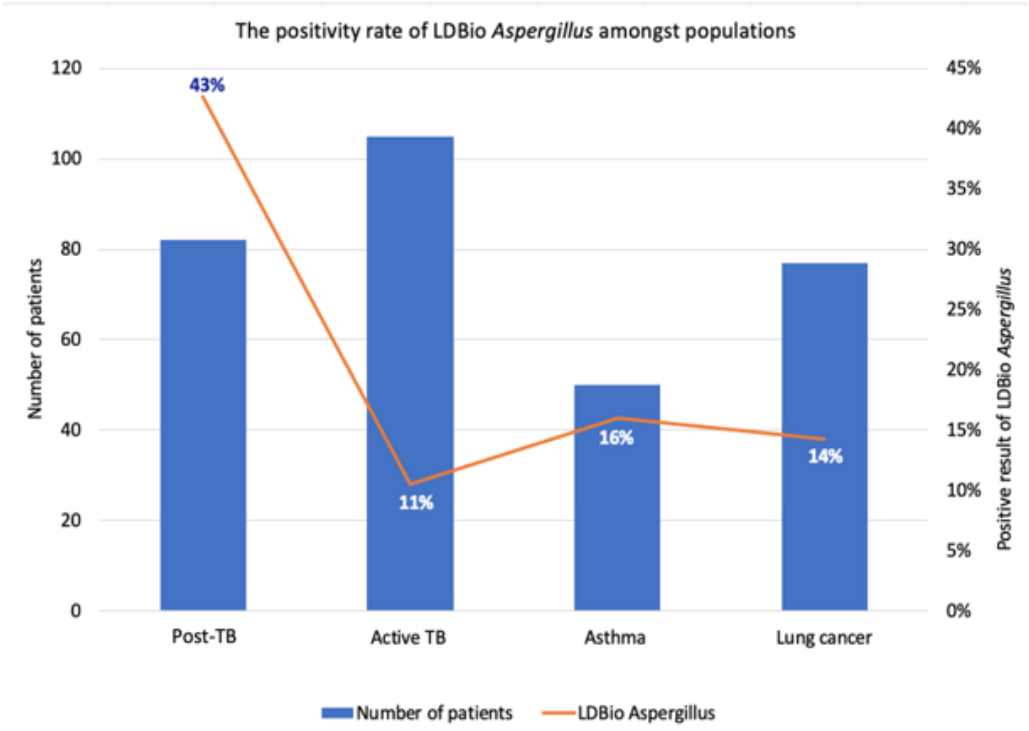
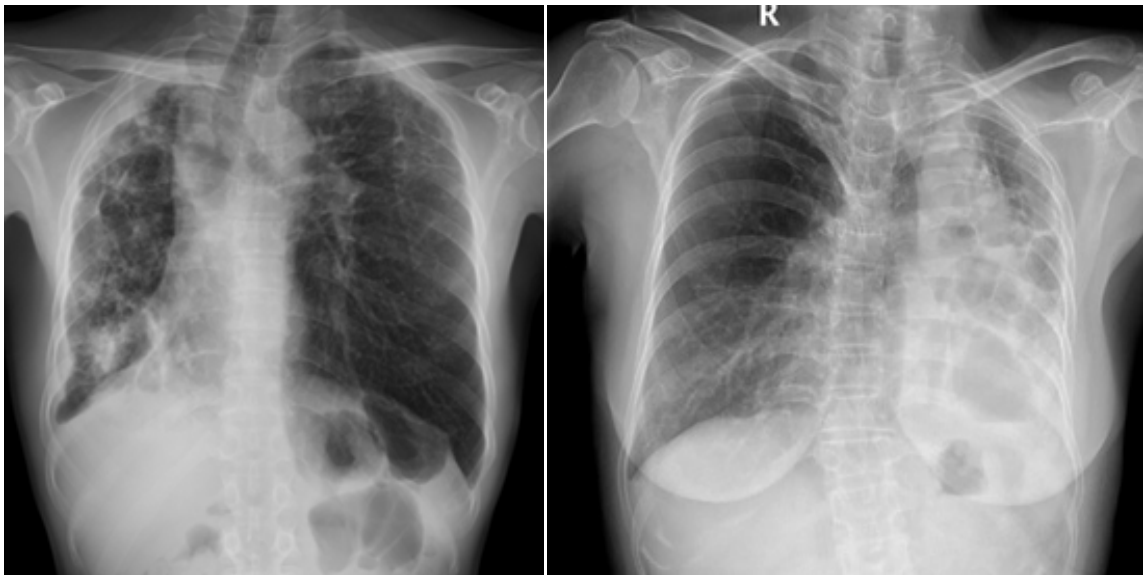


Figure 2. The Positive Results of LDBio Aspergillus ICT from Various Populations (n=314). ICT, immunochromatography; TB, tuberculosis.

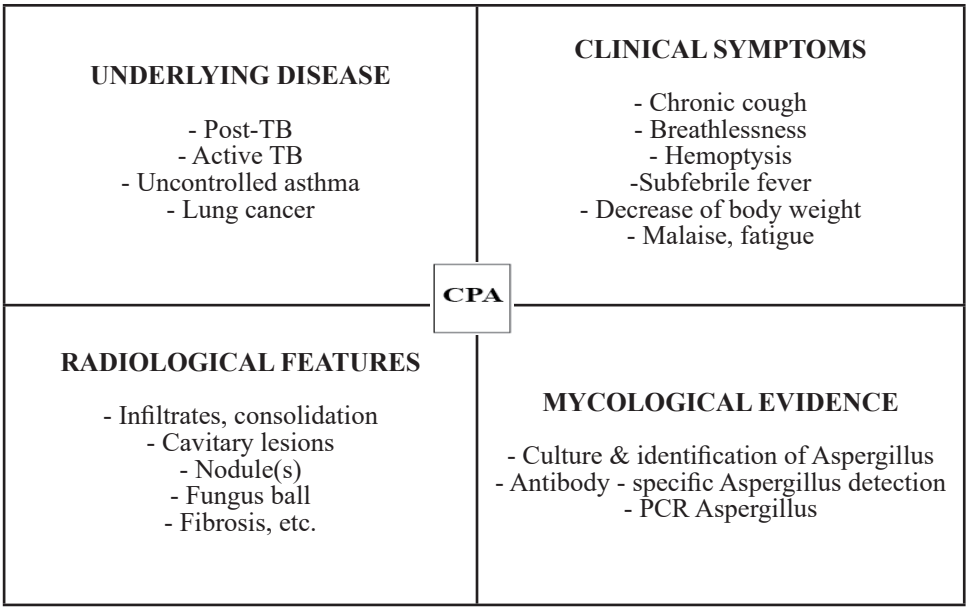
In this study, the clinical manifestations, radiological results, and mycological evidence of these patients showed significant variation among the different populations and were observed for a minimum of 3 months. The clinical symptoms were particularly challenging to distinguish from other chronic pulmonary diseases or non-pulmonary chronic diseases. Hemoptysis, which is often chronic, recurrent, or severe, was identified as a hallmark symptom, especially prevalent in post-TB patients with extensive cavitary lesion(s). On the other hand, radiological features also demonstrated variability, with typical characteristics being cavitary lesions or the presence of fungal balls as the hallmark indicators (Figure 3).

The complexity and overlap of clinical and radiological features emphasize the difficulty in diagnosing CPA accurately, underlining the need for comprehensive evaluation and mycological evidence to confirm the presence of this condition (Figure 4). The conventional mycological examination in this study involved the use of high-volume culture method. However, this approach proved to be less sensitive and time-consuming. Moreover, when Aspergillus grew in the culture media, the results had to be interpreted carefully to differentiate between an infection, colonization, saprophytes, or contamination. To overcome these limitations, antibody detection tests were conducted using LDBio Aspergillus, which offered several advantages. This method was easy to perform, simple, rapid, and cost-effective. However, the molecular examination was not routinely conducted in these studies. It was only partially carried out on Aspergillus fumigati complex (polymerase chain reaction/PCR test) to detect the cryptic species.



(A) (B)

(A) A-47-yo male with history of post-TB presented with symptoms of cough, dyspnea, and fatigue persisting for more than 3 months. The radiological findings revealed multiple cavitary lesions in both lungs, with a particular emphasis on the right lung, along with inhomogeneous consolidation in both lungs. Additionally, there was evidence of destruction in the right lung lobes.
(B) A-61-yo female with a history of post-TB complained of cough, dyspnea, and recurrent and massive hemoptysis. The radiological examination showed multiple cavitary lesions and inhomogeneous consolidation in the left lungs, with an apparent destruction of the left lung appearance. CPA, chronic pulmonary aspergillosis; TB, tuberculosis.



(Figure 4. The Profile of Clinical, Radiological, and Mycological Examinations Observed in This Study. CPA, chronic pulmonary aspergillosis; TB, tuberculosis; PCR, polymerace chain reaction.

Discussion

CPA was relatively neglected for a long time by physicians and frequently misdiagnosed as pulmonary TB or relapse TB. Recently, CPA has been recognized as a serious global health problem, particularly in low-middle-income countries where the TB burden is huge. Patients with TB have an increased risk of developing CPA due to lung damage. Moreover, concurrent CPA in TB patients leads to poorer clinical outcomes, reduced pulmonary function, and worsened health-related quality of life (HR-QoL).[8,9]

The diagnosis of CPA cannot be sole-

ly relied on clinical or radiological manifestations or laboratory results; instead, it must combine these three aspects as the presentation is non-specific and difficult to distinguish from TB or other chronic lung diseases. Mycological evidence for CPA diagnosis is considered through direct confirmation of Aspergillus infection by microscopy or culture of bronchoalveolar lavage (BAL) fluid/biopsy, or immune response to Aspergillus spp. Thus, serological tests are essential for CPA diagnosis because fungal culture results are frequently negative. The CPA diagnosis is often delayed due to some constraints. There is an urgent need for low-cost diagnostic methods for CPA as alternatives to expensive tests like CT scanning or molecular testing. Developing effective screening techniques is critical to identifying people who are most at risk of CPA, such as those living with TB or other chronic lung diseases with pre-existing lung damage. The health cost for CPA diagnosis in Indonesia is not sufficient. Mycological examination costs have not been included in the universal coverage (BPJS) and have become another obstacle.[6,9,10]

LDBio Aspergillus (Lyon, France) has been introduced to detect Aspergillus-specific antibodies (IgG and IgM) using immunochromatography (ICT) technology. This assay is a new point-of-care lateral flow assay (LFA) that complies with ASSURED (affordable, sensitive, specific, user-friendly, rapid/robust, equipment-free, and deliverable to end users). Improved clinical outcomes and lower costs make the POCT compatible with resource-limited settings. LDBio Aspergillus ICT has excellent performance due to its ability to detect both IgG and IgM of A. fumigatus. However, the sensitivity and specificity of LDBio Aspergillus ICT varies among studies: 91.6% and 98% in a French multicenter study, 85% and 72.1% in APICAL study (Indonesia), whereas 67.6% and 81%, respectively, in an Indian study.[3-5,11-13]

The positivity rate of LDBio Aspergillus showed the highest percentage (43%) in the post-TB patients compared to active TB (11%), uncontrolled asthma (16%), and lung cancer populations (14%). The result was consistent with the severe lung damage due to a post-TB infection, as well as in line with the radiological findings showing extensive lung damage in post-TB patients. The presence of multiple cavitary lesions provides a favorable environment for Aspergillus colonization, acting as a biofilm that might become a source of continuous infection.[5,7,14]

Early diagnosis of CPA is pivotal in reducing complications, mortality, and health costs. For this reason, several strategies efforts are mandatory. Increased awareness of clinicians and healthcare providers regarding CPA is crucial. It will encourage clinicians for consider early diagnosis of CPA, especially in patients with chronic respiratory diseases. The TB population in Indonesia is enormous, thus the double infections of TB and CPA would pose a very serious health problem. In addition, post-TB lung diseases (PTLD) have emerged and become important issues nowadays. Aspergillus colonization in difficult-to-treat asthmatic patients can complicate asthma control and interfere with the patient's quality of life. Similarly, Aspergillus colonization in lung cancer patients has the potential to become a source of infection, which can worsen the prognosis and cause disease complications.[15-17]

This paper represents the profile of CPA in various patient populations by compiling data from previous studies. This profile will complement Indonesia's data to reflect the global situation of CPA more adequately. Some limitations to be considered include variations in the number and characteristic of patients among populations. In addition, not all patients can excrete sputum adequately; making it difficult to perform Aspergillus culture. Fungal culture has the potential for false negative results, being less sensitive, and time-consuming. However, these limitations can be addressed by serological examination, which provides mycological evidence to fulfill the diagnostic criteria of CPA.

Conclusion

The LDBio Aspergillus ICT has the potential to be a POCT for early diagnosis of CPA in Indonesia and other resource-limited settings. The overall prevalence of CPA was found to range from 6 to 22% in the post-TB, active TB, uncontrolled asthma, and lung cancer populations. Among these groups, the post-TB group showed the highest risk of developing CPA. Implementing the aspergillosis POCT will benefit in reducing complications, mortality, and healthcare costs, as well as improving prognosis.

Conflicts of Interest

We declare no conflict of interest in this study.

Acknowledgment

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