



Clinical Performance of the *Aspergillus* Western Blot IgG Kit for Serodiagnosis of Chronic Pulmonary Aspergillosis in Post-Tuberculosis Patients

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Abstract

Background: Chronic pulmonary aspergillosis (CPA) by *Aspergillus* spp., which causes slowly progressive destruction of lung parenchyma, is a major complication of pulmonary tuberculosis (TB). Clinical and radiological features of CPA are not typical and might resemble TB. Therefore, detecting *Aspergillus*-specific IgG is critical for diagnosing CPA.

Methods: This cross-sectional study was conducted to evaluate the performance of the *Aspergillus* Western Blot (Asp-WB) IgG kit (LDBio Diagnostics, Lyon, France) for CPA diagnosis in 63 post-TB patients. The analysis was performed by comparing Asp-WB with *Aspergillus* ELISA IgG (Asp-ELISA) and fungal culture as the standard method.

Results: Of the 63 patients studied, twenty-six (41%) met the probable CPA criteria. The Asp-WB results were positive in 13 probable CPA patients and 3 non-CPA patients, with a significant difference of 50% vs. 8% ($P < 0.001$). The sensitivity and specificity of Asp-WB were 50% and 93%. False-negative results of Asp-WB were detected from non-*fumigatus* CPA that grew *Aspergillus niger*. CPA patients with mild symptoms (less than 3 months) indicated early progression of CPA might show positive Asp-WB test results in low sensitivity of Asp-WB test.

Conclusion: The Asp-WB has the potential to be used as a confirmatory test to assist diagnosis of CPA in post-TB patients.

Keywords: chronic pulmonary aspergillosis, tuberculosis, Western blot

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INTRODUCTION

Pulmonary tuberculosis (PTB) remains a serious problem in Indonesia. About 8.5% of global TB cases occurred in Indonesia, a country with the second-highest TB burden in the world.¹ Pulmonary TB may damage lung tissue, making it easier for the adhesion and invasion of *Aspergillus*, which can lead to chronic pulmonary aspergillosis (CPA) in certain cases.^{2–6} It affects approximately 1.2 million individuals with CPA as a sequel of PTB.⁷

The prevalence of CPA is estimated at 378,700 cases in Indonesia.⁸ The incidence rate of CPA at the end of TB therapy in the previous study was 8%.⁹ The CPA diagnosis is still challenging because of the atypical clinical manifestations, radiology findings and low sensitivity of the fungal culture. Detection of antibodies against *Aspergillus* might facilitate an

accurate diagnosis of CPA. The prior study categorized the diagnosis of CPA into probable and proven CPA. Probable CPA may represent early-stage CPA or limited radiology information due to lack of thorax CT scan, so the lung cavity is undetectable.

The enzyme-linked immunosorbent assays (ELISAs) and immunoprecipitation detection (IPD) are widely used to detect specific anti-*Aspergillus* antibodies.^{10–13} However, those tests have drawbacks, including time-consuming, lack of standardization, and extended turnaround times. The *Aspergillus* Western Blot IgG kit (Asp-WB) has been commercialized (LDBio Diagnostics, Lyon, France) using *A. fumigatus* glycoprotein antigen with a molecular weight of 16 kD, 18–20 kD, 22 kD, and 30 kD.^{14–16} Study on Asp-WB for diagnosing of CPA are still limited in Indonesia. This study aimed to evaluate

the accuracy of Asp-WB compared to consensus results of fungal cultures and Asp-ELISA to support CPA diagnosis in post-TB patients.

METHODS

This cross-sectional study is part of the prior study on CPA diagnostics in Indonesia. Clinical material was obtained from the sera of post-TB patients from several hospitals in Jakarta, which were delivered to the Parasitology Laboratory FMUI. The previous study recruited patients by consecutive sampling in April-December 2019.

Inclusion criteria were previous TB patients who had persistent symptoms after completing TB therapy and negative HIV tests. Probable CPA was diagnosed based on three parameters: 1) *Aspergillus* culture positive and/or positive *Aspergillus* antibody test including immunochromatography (ICT) or Western Blot (WB) methods, and 2) at least one of these symptoms including cough, dyspnoea, chest pain, hemoptysis, and/or fatigue within ≥ 3 months, OR 3) radiological features indicative of CPA (at least one of cavitation and/or fungal ball). The study was performed at the Parasitology Laboratory, FMUI. It was approved by the Health Research Ethics Committee of FMUI through the Ethics Review No. 95/UN2.F1/ETIK/2019.

Aspergillus Western Blot IgG test: 1,2 ml of buffer solution was added to each incubation tray with a strip in it. Samples and control were added by 10 μ l and incubated for 90 minutes. The solution was washed with buffer solution (1:10) three times. Then, 1,2 ml of anti-IgG conjugate was added and incubated for 60 minutes. The solution was rewashed, and 1,2 ml of substrate was dispensed depending on the strip coloration and incubated for 60 minutes. The strips were left to dry for at least 15 minutes at room temperature.

The results were contrasted with the positive control and determined as positive or negative according to the manufacturer. *Aspergillus*-specific sensitization was proved by four protein bands at 16, 18 to 20, 22, and 30 kDa. The result was positive when at least two of these bands were documented

(Figure 1A). The Asp-WB global intensity was scored from 1 to 4 by summing the results of each specific band (Figure 1B). Asp-WB intensity was classified as very high (>10), high (5 to 10), moderate (2 to 4), and weak (<2). Each test was carried out in duplicate and read by two experts.¹⁶

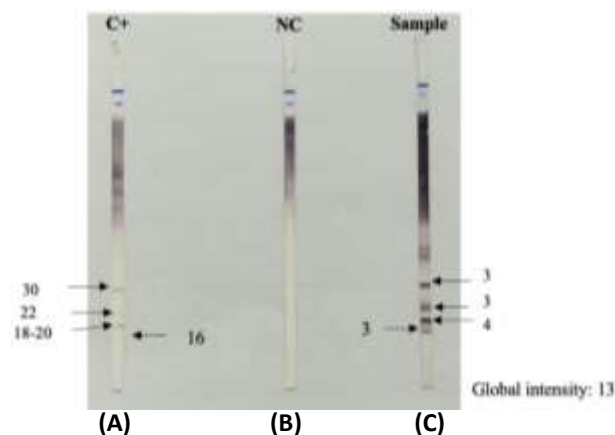


Figure 1. *Asp*-WB results. (A) C+ positive control; the mass (in kDa) of the specific bands shown by values next to the arrows. (B) NC, negative control. (C) Quantification of *Asp*-WB band intensity in a positive assay; the intensities of four specific bands were 3, 3, 4, and 3, respectively, yielding a global intensity of 13.

Aspergillus ELISA IgG test: The sera were tested using the IgG-specific *Aspergillus* antibody with an indirect ELISA method. As 100 μ l diluted sample, standard and controlled solutions were taken by pipets, then put in the well and incubated for 60 minutes. The solution was washed three times with washing solution (1:20). Anti-IgG conjugate was added to the solution and incubated for 30 minutes. The solution was rewashed and a substrate solution was added. It was incubated for 15 minutes, and then a stopping solution was added. It was interpreted by using an ELISA reader with 450 nm wavelength which resulted in a cut-off positive result ≥ 120 AU/ml, negative <80 AU/ml, and inconclusive 80-110 AU/ml.

Diagnostic potential in this study was assessed based on the sensitivity test and specificity test. Data were presented using frequencies and percentages for binary and categorical variables. Fisher's exact tests or chi-squared tests were used for categorical variables. Statistical analysis was performed by using IBM SPSS 22 statistic software.

RESULTS

Sixty-three sera from post-TB patients were included in the study (Table 1). Twenty-six (41%) out of 63 patients met the criteria for probable CPA. The main symptoms in the probable CPA cases were dyspnoea (n=10, 38%) and fatigue (n=10, 38%).

Table 1. Demography of post-TB patients

Symptoms	ALL (n=63)	CPA (n=26)	Non-CPA (n=37)	P
Gender				
Male	42 (67%)	19 (73%)	23 (62%)	
Female	21 (33%)	7 (27%)	14 (38%)	0.366
Mean age (range in years)				
≥ 60 years	16 (25%)	7 (27%)	9 (24%)	
< 60 years	47 (75%)	19 (73%)	28 (76%)	0.816
Sign & symptoms (>3 months)				
Cough	19 (30%)	9 (35%)	10 (27%)	0.518
Haemoptysis	11 (18%)	4 (15%)	7 (19%)	1
Dyspnoea	22 (35%)	10 (38%)	12 (55%)	0.621
Chest pain	10 (16%)	4 (15%)	6 (16%)	1
Fatigue	26 (41%)	10 (38%)	16 (43%)	0.704

Among 63 patients, the strong positive and weak positive rates of Asp-WB tests were 13% (n=8) equally, with a total result of 25% (n=16) positives. In the probable CPA group, 13 of 26 sera tested had positive results by Asp-WB with 50% sensitivity. In the non-CPA group, 34 of the 37 sera showed negative results by WB with 92% specificity. The Asp-WB results were positive in 13 probable CPA patients and three non-CPA patients, with a significant difference of 50% vs. 8% ($P<0.001$). Fifteen percent (n=4) of the probable CPA group showed three bands in the Asp-WB test, which is significantly higher compared to none patients (0%) in a non-CPA group ($P=0.025$).

Table 2. The result of Asp-WB based on antigen characterization

Antigen characterization (molecular weight)	Total (n=63)	CPA (n=26)	Non-CPA (n=37)	P
16 kD	19 (30%)	13 (50%)	6 (16%)	0.004
18-20 kD	17 (27%)	12 (46%)	5 (14%)	0.004
22 kD	10 (16%)	9 (35%)	1 (3%)	0.001
30 kD	9 (14%)	7 (27%)	2 (5%)	0.026

The 16 kDa WB band was the most prevalent (30%) band among 63 patients (Table 2). The proportion of all four WB bands (50% vs. 16% for 16 kDa, 46% vs. 14% for 18–20 kDa, 35% vs. 3% for 22 kDa and 27% vs. 5% for 30 kDa) was significantly

higher in the CPA group than in non-CPA group ($P<0.05$ in all the bands).

The Asp-ELISA and Asp-WB tests require sensitivity and specificity values to become proper diagnostic tools. The tests should possess good accuracy, precision, and reliability. The sensitivity and specificity of Asp-ELISA and Asp-WB are comparable to the combination methods (fungal culture results and positive Ab detection, either with one or both methods) as a standard diagnostic method. The diagnostic accuracy of the Asp-WB kit generally showed a sensitivity (88%) and specificity (94%), whereas other studies reported a sensitivity of 80%. Variations in those results may occur due to differences in population and study design.

DISCUSSION

The evaluation of Asp-WB test performance to diagnose CPA in post-TB patients has been carried out in this study. The assay showed 50% sensitivity and 92% specificity for diagnosis of probable CPA. The relatively lower sensitivity compared to prior studies might be related to different populations and the criteria applied to diagnose CPA.¹⁵

A previous study that included 88 post-TB patients with negative GeneXpert showed 80% sensitivity and 70% specificity of the Asp-WB test to diagnose proven CPA.¹⁵ The Asp-WB test showed high specificity with only three false positives. These three patients had weak positive bands of the Asp-WB test, but the radiology findings and the symptoms did not meet the criteria of CPA.

However, the growth of *Aspergillus* was documented in the sputum of those three patients; one patient had *A. flavus*, one patient had *A. niger*, and one patient had *A. flavus* and *A. niger*. All these patients presented to the clinic with pulmonary symptoms such as cough, dyspnoea, and chest pain, but in less than three months. The presence of minimal symptoms might indicate the early stage of CPA.

Eight out of thirteen patients with false negative Asp-WB test had *A. niger* in their culture. *Aspergillus*

niger was solely found in five patients, while *A. niger* grew together with *A. flavus* and *A. fumigatus* in three patients. A previous study revealed 38% of *Aspergillus* section *Nigri* as the etiology of CPA in Indonesia.¹⁷ All available Asp-antibody detection kits now are based on the *A. fumigatus* antigen.¹⁴ Therefore, these eight patients appeared as false negative results were likely because of the presence of antibodies specific to non-*fumigatus* infections (for example *A. niger*). These types of antibodies were undetected by the Asp-WB test that was specific for *A. fumigatus* infection.

The Asp-WB test is part of the diagnostic scheme, as the point-of-care test (POCT) of CPA, particularly in limited-resource settings. The Asp-WB test was then less popular for routine use as POCT due to several issues, including the limitation of ELISA facilities and less economical costs.

Further development of CPA diagnostic tests is by using the lateral flow assay (LFA) method. The test becomes more feasible for POCT of CPA since it is relatively simple, fast and inexpensive. The LFA-based test is more suitable for routine CPA screening. Meanwhile, the high specificity of the Asp-WB test makes it more feasible for confirmatory tests of CPA. The serial Asp-WB test also has more potential for assessing the disease course of CPA, as well as evaluating treatment, following the clinical judgment.

LIMITATION

The limitation of this study includes the lack of serial Asp-WB tests and the availability of thorax CT-scan. Chest x-ray was performed to detect suggestive CPA findings which might not be as reliable as a CT scan in identifying the cavities.

CONCLUSION

In conclusion, the clinical and radiological appearances of CPA might resemble TB. Therefore, an antibody test such as the Asp-WB test is critical to assist in the diagnosis of CPA. The specificity of the Asp-WB test is high, so this test can be used as a confirmatory test to diagnose CPA.

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CONFLICT OF INTERESTS

There is no conflict of interest.

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