

# Paper4 Dr. Anna

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

## Abstract

**Background:** Chronic pulmonary aspergillosis (CPA) due to *Aspergillus* spp., causing slowly progressive destruction to lung parenchyma, is a major complication of pulmonary tuberculosis (TB). The 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 *Aspergillus* Western Blot (Asp-WB) IgG kit (LDBio Diagnostics, Lyon, France) for CPA diagnosis in 63 post-TB patients. The analysis was carried out by comparing Asp-WB with *Aspergillus* ELISA IgG (Asp-ELISA) and fungal culture as 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 the 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 showed positive Asp-WB test result in low sensitivity of Asp-WB test.

**Conclusion:** The Asp-WB has potential to use as confirmatory test to assist diagnosis of CPA in post-TB patients.

**Keywords:** Western blot, chronic pulmonary aspergillosis, tuberculosis

## INTRODUCTION

Pulmonary tuberculosis (PTB) is still a serious problem in Indonesia. About 8.5% of global TB cases are located in Indonesia, a country with the second-highest TB burden in the world.<sup>1</sup> Pulmonary TB can cause damage to lung tissue, making it easier for the adhesion and invasion of *Aspergillus*, which can lead to chronic pulmonary aspergillosis (CPA) in certain cases.<sup>2-6</sup> It affects approximately 1.2 million individuals with CPA as a sequel of PTB.<sup>7</sup>

The prevalence of CPA is predicted at 378,700 cases in Indonesia.<sup>8</sup> The incidence rate of CPA was 8% at the end of TB therapy in previous study.<sup>9</sup> The CPA diagnosis is still challenging because of the atypical clinical manifestations, radiology findings and the low sensitivity of the fungal culture. Detection of antibodies against *Aspergillus* might facilitate the accurate diagnosis of CPA. Prior study categorised the diagnosis of CPA into probable and proven CPA. Probable CPA may represent early-stage CPA or the limited radiology information due to lack of thorax CT scan, so the lung cavity is undetectable.

The enzyme-linked immunosorbent assays (ELISAs) and immunoprecipitin detection (IPD) are frequently used for detection of specific anti-*Aspergillus* antibodies.<sup>10-13</sup> However, those tests have drawbacks, including time-consuming, lack of standardisation, and extended turnaround times. The Asp-WB IgG kit has been commercialised (LDBio Diagnostics, Lyon, France) using *A. fumigatus* glycoprotein antigen with a molecular weight of 16 kD, 18-20 kD, 22 kD, and 30 kD.<sup>14-16</sup> Study on Asp-WB for diagnosing of CPA are still limited in Indonesia. This study aimed to evaluate the accuracy of Asp-WB compare 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. Sera were collected from 63 previous TB patient who had persistent symptoms after the completion of TB treatment. Probable CPA was diagnosed built upon 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, dyspnea, chest pain, haemoptysis, and/or fatigue within  $\geq 3$  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 endorsed by 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 controlled was added by 10  $\mu$ l and incubated for 90 minutes. The solution was washed by buffer solution (1:10) three times. Then, 1,2 ml 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 to 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 (Fig. 1A). The Asp-WB global intensity were scored from 1 to 4 by summing the results of each specific band (Fig. 1B). Asp-WB intensity was categorized 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.<sup>16</sup>

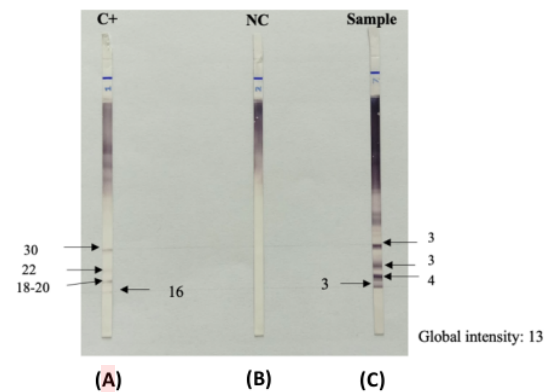


Figure 1. Asp-WB results.

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

***Aspergillus* ELISA IgG test:** The sera were tested using the IgG-specific *Aspergillus* antibody with ELISA indirect method. As 100  $\mu$ l diluted sample, standard and controlled solution 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 added a substrate solution. It was incubated for 15 minutes, then added a stopping solution. It was read using ELISA reader with wavelength 450 nm which gain the result such as cut-off positive result  $\geq 120$  AU/ml, negative  $<80$  AU/ml, and inconclusive 80-110 AU/ml.

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

## RESULTS

Sixty-three sera from post-TB patients were included in this study (Table 1). Twenty-six (41%) out

of 63 patients fulfil the criteria for probable CPA. The primary symptoms in the probable CPA cases were dyspnea (38%) and fatigue (38%).

Table 1. Demography and clinical characteristic of post-TB patients

Symptoms	ALL (N=63)	CPA (n=26)	Non-CPA (n=37)	p-value
Gender				
Male	42 (67%)	19 (73%)	23 (62%)	0.366
Female	21 (33%)	7 (27%)	14 (38%)	
Mean age (range in years)				
≥ 60 years	16 (25%)	7 (27%)	9 (24%)	0.816
< 60 years	47 (75%)	19 (73%)	28 (76%)	
Sign & symptoms (>3 months)				
Cough	19 (30%)	9 (35%)	10 (27%)	0.518
Haemoptysis	11 (18%)	4 (15%)	7 (19%)	1
Dyspnea	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 rate of Asp-WB tests were 13% (n=8) equally, which together result in 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 the significant difference of 50% vs. 8% ( $p < 0.001$ ). Fifteen percent

(n=4) of probable CPA group showed three bands in Asp-WB test, which is significantly higher compare with no patients (0%) in non-CPA group ( $p=0.025$ ).

The 16 kDa WB band was the most prevalent (30%) bands among 63 patients (Table 2). All four Asp-WB bands showed the proportion of 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. It was remarkably higher in the CPA group compared to non-CPA group ( $p < 0.05$  in all the bands).

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-value
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 Asp-ELISA and Asp-WB tests require sensitivity and specificity values for becoming proper diagnostic tools. The tests should have good accuracy, precision, and reliability. The sensitivity and specificity of Asp-ELISA and Asp-WB compared with the combination methods (fungal culture results and positive Ab detection, either with one or both methods) as a standard diagnostic method.

## DISCUSSION

The evaluation of Asp-WB test performance to diagnose CPA in post-TB patients. The assay showed 50% sensitivity and 92% specificity for diagnosis of probable CPA. The relatively lower sensitivity compare with previously described study might be related to different population and the criteria used to diagnose CPA. Previous study using 88 post-TB patients with GeneXpert negative showed 80% sensitivity and 70% specificity of Asp-WB test to diagnose proven CPA.<sup>15</sup> Asp-WB test showed high specificity with only three false positive. These three patients had weak positive band of Asp-WB test, but the radiology and the symptoms were not fulfilled criteria of CPA.

However, the growths of *Aspergillus* could be 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 came to the clinic with pulmonary symptoms such as cough, dyspnea, and chest pain, but 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* were solely found in five patients, while *A. niger* grew together with *A. flavus* and *A. fumigatus* in three patients. Previous study revealed 38% of *Aspergillus* section *Nigri* as the etiology of CPA in Indonesia.<sup>17</sup> All the Asp-antibody detection kit that available now are based on the *A. fumigatus* antigen.<sup>14</sup> Therefore, these eight patients appeared as false negative results was likely because the presence of antibody that specific to non-fumigatus infections (for example *A. niger*). These types of antibodies were not detected using

the Asp-WB test that was specific for *A. fumigatus* infection.

The Asp-WB test is part of 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 because it is very simple, fast and inexpensive. The LFA-based test is more suitable for routine CPA screening. Meanwhile, the high specificity of Asp-WB test make it more feasible for confirmatory test of CPA. The serial Asp-WB test also has more potential for assessing the disease course of CPA, as well as evaluating treatment, in accordance with the clinical judgment.

## STUDY LIMITATION

The limitation of this study including the lack of serial Asp-WB tests and availability of thorax CT-scan. We used chest x-ray to detect suggestive CPA findings which might not reliably detect the cavities as good as CT-scan.

## CONCLUSION

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

## CONFLICT OF INTERESTS

There is no conflict of interests.

## FUNDING

This study received financial support from The Indonesia Pulmonary Mycoses Centre (IPMC) FMUI - Persahabatan National Respiratory Referral Hospital for the purpose of an epidemiological survey.

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