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Evaluation and comparison of automated and manual ELISA for diagnosis of chronic pulmonary aspergillosis (CPA) in Indonesia



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ABSTRACT

Pulmonary tuberculosis (TB) is one of the common risk factors for chronic pulmonary aspergillosis (CPA). A positive *Aspergillus* IgG is a key element of the diagnosis of CPA but this has not been studied in Indonesia. We conducted studies with patients at the end of TB therapy in Indonesia. We performed receiver operating curve (ROC) analysis to determine the optimum cutoff of the *Aspergillus*-specific IgG level (Immulite and Dynamiker ELISA) in those patients who met criteria of CPA in relation to control groups. In 203 TB patients, 26 (13%) patients had clinical and radiological features of CPA. We derived optimum cutoffs for Immulite *Aspergillus*-specific IgG of 11.5 mg/L and Dynamiker anti-galactomannan IgG of 106.8 AU/mL (sensitivity 89% and 83%, specificity 78% and 51%, respectively). The currently accepted *Aspergillus*-specific IgG cutoff of Immulite and Dynamiker assays for CPA diagnosis may require slight adjustment for the Indonesian population.

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1. Introduction

Chronic pulmonary aspergillosis (CPA) is a progressive lung disease but responsive to antifungal therapy and surgery (Bongomin et al., 2018; Farid et al., 2013; Ohba et al., 2012). It is estimated that ~3 million CPA cases occurred worldwide (Brown et al., 2012; Denning et al., 2011). The 5-year mortality is 50–85% indicating CPA can be fatal especially for patients with underlying lung diseases such as pulmonary tuberculosis (TB) (Lowes et al., 2017; Page et al., 2019a). CPA as sequelae of TB is common with the estimated global 5-year period prevalence range from 0.8 to 1.37 million cases (Denning et al., 2011).

Diagnosis of CPA depends on several different aspects, persistent clinical symptoms, microbiology evidence of *Aspergillus*, and lung lesion seen on radiology (Denning et al., 2015, 2018). Since the culture rate is low, *Aspergillus* IgG detection is the most sensitive diagnostic test (Cucchetto et al., 2015; Richardson and Page, 2018; Vergidis et al., 2019). Many commercial platforms of *Aspergillus*-specific IgG are

available now but are not well distributed in areas where most needed (Denning et al., 2018; Godet et al., 2017; Page et al., 2015). Immulite is one of the recommended automated ELISA assay for measurement of *Aspergillus* IgG, and was available in Indonesia (Denning et al., 2018; Page et al., 2018). Recent studies have described performance of Dynamiker anti-galactomannan IgG, a manual ELISA assay, in China (Guo et al., 2019; Li et al., 2019; Ma et al., 2019). The cutoffs of this test also varied across different group of patients from different countries and backgrounds (Ma et al., 2019; Page et al., 2015, 2018, 2019b).

There is the possibility that CPA is misdiagnosed as smear negative TB (Oladele et al., 2017), because of the similar clinical and radiological features. Early diagnosis of CPA is important to optimize patients' outcome. There is no data about *Aspergillus*-specific IgG cutoffs in the Indonesian population where TB is an endemic disease. It is estimated that ~83,000 CPA cases occur in Indonesia based on extrapolation from UK data in the 1960's and 1-year survivors of TB (Wahyuningsih et al., 2017; WHO, 2018). Therefore, this study aimed to establish the optimal cutoffs for *Aspergillus* IgG using Immulite and anti-galactomannan IgG assays and evaluate the performance of these assays in pulmonary TB patients in Indonesia. Our hypotheses were: 1) there

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are differences in the cutoffs of *Aspergillus*-specific IgG assay in the Indonesian population compared to published cutoffs from other countries and 2) there are differences in the performances of the automated and manual ELISA of different *Aspergillus*-specific IgG assays.

2. Materials and methods

2.1. Study population

We conducted 2 (prospective and cross-sectional) studies in parallel in patients treated for pulmonary TB in Indonesia. We recruited patients at the end of (4–6 months after starting) TB therapy. The study was conducted in 6 centers in Indonesia, namely National Referral Centre Persahabatan Hospital, National Referral Centre Ciptomangunkusumo Hospital, Grha Permata Ibu Hospital, Universitas Kristen Indonesia Hospital, Cempaka Putih Jakarta Islamic Hospital, and MH Thamrin Hospital. In addition, healthy controls and diseased controls (patients with respiratory symptoms but without a history or evidence of TB) were included.

Ethical approval was obtained from the ethic committee of the Faculty of Medicine, University of Indonesia (FMUI) with reference number 672/UN2.F1/ETIK/2016 and the University Research Ethics Committee University of Manchester with reference number 16416. Informed verbal and written consent was obtained from each patient after they received explanation about the study.

2.2. Study design

Symptoms such as cough, hemoptysis, chest pain, dyspnea, fatigue, and weight loss were recorded. Together with their duration, *Aspergillus*-specific IgG level in serum was measured using the Immulite 2000 *Aspergillus*-specific IgG testing system (Siemens, Berlin, Germany) with a recommended cutoff of 10 mg/L and also with the anti-galactomannan IgG (Dynamiker IgG ELISA, Tianjin, China) using the manufacturer cutoff of 120 AU/L. Sputum was collected, and then processed for fungal culture and TB PCR (GeneXpert MTB/RIF, Cepheid, CA, USA). We analyzed all chest x-rays (CXRs) and CT scans if available for cavities, pleural thickening adjacent to a cavity, any area of consolidation, aspergilloma, nodules, and/or bronchiectasis. Chest x-rays were assessed by an experienced radiologist and clinician, independent of and prior to serology and clinical analyses.

Two additional control groups were also recruited, healthy subjects without any history of chest disease or symptoms and a diseased controls group. Diseased control group were patients who came to the lung clinic without any chronic symptoms of CPA and no history of TB. The TB PCR results were all negative in patients with available sputum.

2.3. Case definitions

Proven CPA was diagnosed based on the 3 parameters: 1) at least one of these symptoms including cough, hemoptysis, chest pain, dyspnea, fatigue, and/or weight loss ≥ 3 months, AND 2) positive *Aspergillus* IgG and/or positive *Aspergillus* spp. culture AND 3) radiological features indicative of CPA (at least one of cavitation, fungal ball or pleural thickening). Probable CPA was diagnosed in patients with all of: 1) at least one of these symptoms including cough, hemoptysis, chest pain, dyspnea, fatigue, and/or weight loss ≥ 3 months, 2) progressive cavitation on serial chest radiographs, 3) negative TB PCR test for *M. tuberculosis*, but without microbiological or serological confirmation of CPA. We used this definition because proven CPA is a difficult concept as histopathology is rare and culture is insensitive (Vergidis et al., 2019). Proven and probable CPA were classified together as the CPA group.

Pulmonary TB was diagnosed if the patient had a persistent cough for more than 2 weeks and a positive sputum TB PCR MTB/RIF (Cepheid, CA, USA) polymerase chain reaction (PCR) test for MTB or a positive sputum smear test for acid and alcohol fast bacilli (AAFB), and/or any of infiltrates, cavitation, or nodules in chest radiology based on the

national guideline for TB diagnosis. It is acknowledged that the GeneXpert and/or AFB smear test may be positive for species in the nontuberculous mycobacteria (NTM) complex such as *M. abscessus*, *M. marinum*, *M. smegmatis*, *M. aurum* and *M. phlei* – the relative frequency of these pathogens in Indonesia is not known (Pang et al., 2017).

Intra-assay variation was measured using 2 samples selected for each assay, with expected low and high results (supplement Table 1). *Aspergillus*-specific IgG samples were repeated 10 times and anti-galactomannan IgG samples were repeated 6 times due to volume constraints. Co-efficient of variation was calculated as (standard deviation/mean) $\times 100$. Correlation between these 2 assays is measured by Spearman's rank analysis.

2.4. Data analysis

Statistical analysis was performed with the use of IBM SPSS 22 statistic software. Data were presented using frequencies and percentages for binary and categorical variables, medians and range for non-normally distributed, continuous variables. The difference between continuous variables was analyzed using Student *t*-tests. Fisher's exact tests or chi-squared tests were used for categorical variables.

To determine the optimum cutoff, we compared the *Aspergillus* IgG level in only those patients with cavities and/or pleural thickening and persistent symptoms at the end of TB therapy with a) healthy controls, b) other patients seen at the end of TB therapy without persistent symptoms and cavities and/or pleural thickening and c) diseased controls (patients with respiratory symptoms but having no history or evidence of TB), and all control groups combined.

We plotted a ROC curve using the best Youden's J statistic (sensitivity + specificity – 1). The differences between *Aspergillus*-specific IgG across different groups were analyzed using the Kruskal-Wallis test and post hoc by the Dunn-Bonferroni tests on each pair of groups. A *P* value < 0.05 was considered as statistically significant.

3. Results

3.1. Clinical characteristics of CPA

Two hundred three patients (132 from the prospective study and 71 from the cross-sectional) were assessed at the end of TB therapy from February 2017 to February 2019 (Table 1). Ninety samples from healthy participants and 100 samples from diseased control participants were included in the study (supplement Table 2). Twenty-six (13%) patients met the criteria for CPA, comprising 24 (12%) with proven CPA and 2 (1%) patients with progressive cavitation and *Aspergillus* specific-IgG < 10 mg/L as probable CPA. The median level of *Aspergillus*-specific IgG in 203 TB patients was 9.1 mg/L (3.2–630 mg/L). There was a moderate correlation ($r_s = 0.504$, $P < 0.001$) between Immulite and Dynamiker.

The most common pulmonary diseases found in all patients enrolled were COPD, asthma and bronchitis, with bronchitis ($n = 42$, 42%) the most frequent in the diseased control group. In the non-CPA group, TB was clinically diagnosed (negative TB PCR and/or AFB smear at the start of TB therapy) in 53 (29%) patients and in 5 (22%) patients in the CPA group. However, the TB PCR and/or AFB smear tests result was only available for the 93 patients (from the total 203 patients) because of limited volume of sputum samples from patients. TB cultures were not done. The main symptoms in the CPA cases were cough ($n = 18$, 69%), fatigue ($n = 15$, 58%) and dyspnea ($n = 8$, 31%) (Table 1). The dominant features of the CXRs were infiltrates ($n = 24$, 92%) and cavitation ($n = 22$, 85%).

3.2. Determination of the *Aspergillus*-specific IgG cutoff point

Based on prior experience in other countries, we used the Immulite *Aspergillus*-specific IgG assay as the reference assay. Optimal cutoffs, sensitivity and specificity for the diagnosis of CPA ($n = 26$) were then

Table 1
Demography of end of treatment TB patients.

Symptoms	ALL (n = 203)	CPA (n = 26)	non CPA (n = 177)	P value
Gender				
Male	91 (45%)	16 (62%)	75 (42%)	
Female	112 (55%)	10 (39%)	102 (58%)	0.067
Mean age (range in years)	39.5 (17–83)	48.1 (18–83)	38.2 (17–71)	0.014
≥ 60 years	22 (11%)	6 (23%)	16 (9%)	
< 60 years	181 (89%)	20 (77%)	161 (91%)	0.043
Sign and symptoms (>3 months)				
Cough	39 (19%)	18 (69%)	21 (12%)	< 0.001
Hemoptysis	4 (2%)	1 (4%)	3 (2%)	0.425
Dyspnea	23 (11%)	8 (31%)	15 (9%)	0.003
Chest pain	10 (5%)	2 (8%)	8 (5%)	0.62
Fatigue	32 (16%)	15 (58%)	17 (10%)	< 0.001
Weight loss	4 (2%)	0	4 (2%)	1
Hemoptysis (<3 months)	15 (7%)	3 (12%)	12 (7%)	0.416
Radiology				
Cavitation	82 (40%)	22 (85%)	60 (34%)	< 0.001
Air fluid level in cavities	6 (3%)	2 (8%)	4 (2%)	0.171
Paracavitary fibrosis	43 (21%)	13 (50%)	30 (17%)	< 0.001
Pleural thickening	56 (28%)	12 (46%)	44 (25%)	0.023
Aspergilloma	2 (1%)	2 (8%)	0	0.016
Bronchiectasis	27 (13%)	8 (31%)	19 (11%)	0.01
Nodules	29 (14%)	4 (15%)	25 (14%)	0.771
Infiltrates	171 (84%)	24 (92%)	147 (83%)	0.385
Other diseases				
Asthma	9 (4%)	1 (4%)	8 (5%)	1
COPD	3 (2%)	1 (4%)	2 (1%)	0.339
Diabetes mellitus	33 (16%)	3 (12%)	30 (17%)	0.775
Hypertension	17 (8%)	4 (15%)	13 (7%)	0.244
CAD	1 (1%)	0	1 (1%)	1
CKD	3 (2%)	0	3 (2%)	1

COPD: chronic obstructive pulmonary disorder; CAD: coronary artery disease; CKD: chronic kidney disease.

calculated in relation to healthy controls, other patients at the end of TB therapy and diseased controls, as individual control groups and all combined as a single control group using ROC analysis. We tested 393 sera with Immulite and the range of *Aspergillus* IgG levels ranged from 2.64 mg/L to 630 mg/L (median 8.3 mg/L; SD: 36.4 mg/L). A significant difference in *Aspergillus*-specific IgG levels was found across the 4 different groups with $P < 0.001$ (Kruskal-Wallis test) (supplement Table 3). The highest median *Aspergillus*-specific IgG level (20.3 mg/L) was detected in CPA cases, while 3 other groups showed median levels of 8.5 mg/L (end of treatment TB), 7.1 mg/L (healthy controls), and 7.7 mg/L (diseased controls).

Dunn's pairwise tests were carried out for the 4 pairs of groups. There is strong evidence ($P < 0.001$, adjusted using Bonferroni correction) of differences between the *Aspergillus*-specific IgG in the CPA group compared to the other groups. The *Aspergillus*-specific IgG was higher in the end of TB treatment group compared to healthy controls ($P = 0.001$), with no other significant differences between groups (Supplement Fig. 1).

Healthy controls and end of treatment TB therapy secondary analyses produced a cutoff of 11.5 mg/L, while the diseased control showed a cutoff of 12.2 mg/L (Table 2), compared with the manufacturer's cutoff of 10 mg/L. All control groups combined also identify 11.5 mg/L as the optimal cutoff with a sensitivity of 89% and specificity of 78%. If a cutoff of 12.2 is used, only one CPA serum is misclassified as negative in the proven CPA group.

The comparisons of AUC of CPA diagnosis in the different populations with a cutoff of 11.5 mg/L are explained in supplement Table 4. The highest sensitivity and specificity were observed in the CPA cases

Table 2

Immulite *Aspergillus*-specific IgG performances at various points on the ROC AUC curve, using the 26 patients with CPA as cases in the comparison with three different control groups (manufacturer cutoff 10 mg/L).

Cutoff (mg/L)	Sensitivity (%)	Specificity (%)	Youden's J statistic
Healthy controls			
9.6	92	77	0.69
10.2	92	78	0.70
11.5	89	88	0.77
11.9	85	88	0.72
12.9	69	90	0.59
14	58	93	0.51
End of treatment TB			
9.6	92	59	0.52
10	92	63	0.55
10.2	92	65	0.57
11.5	89	72	0.61
12.1	85	74	0.59
13	69	76	0.46
14	58	78	0.36
Diseased controls (non TB)			
9.9	92	70	0.62
10.5	92	74	0.66
11.5	88	79	0.68
11.6	88	80	0.69
12.2	85	84	0.69
13.1	69	85	0.54
All controls			
10	92	69	0.62
10.5	92	71	0.63
11.5	89	78	0.67
12	85	80	0.65
13	69	82	0.50

vs healthy controls group with AUC 0.93, sensitivity 86% and specificity 88% using the 11.5 mg/L cutoff (Fig. 1). The diagnostic odds ratio of this cutoff in the end of treatment TB group was 31.3 (95% CI 7.14–137.65).

3.3. Determination of the anti-galactomannan IgG cutoff point

We had sufficient sera remaining for testing in 381 samples: 191 from end of treatment TB, 100 from diseased controls, and 90 from healthy controls, including 23 (12%) CPA patients from the end of treatment TB group. The range of *Aspergillus* IgG levels varied from 31.3 AU/mL to 500 AU/mL (median 108.6 AU/mL; SD: 131.9 AU/mL). A significant difference in *Aspergillus* IgG levels was found across the 4 different groups ($P < 0.001$) (Kruskal-Wallis test) (supplement Table 5). The highest median *Aspergillus* IgG level (140.9 AU/ml) was detected in CPA cases, while the 3 other groups showed median levels of 127.7 AU/mL (end of treatment TB), 80.6 AU/mL (healthy control), and 88.7 AU/mL (disease control).

Dunn's pairwise tests were carried out for the 4 groups. The *Aspergillus*-specific IgG in the CPA group was higher compared to healthy controls and diseased controls ($P < 0.05$, adjusted using Bonferroni correction). The *Aspergillus*-specific IgG at the end of TB treatment group was higher than the healthy and diseased controls ($P < 0.05$), but no other significant differences between groups (supplement Fig. 2).

A median level of 131.7 (31.3–500) AU/mL was found in the 191 samples from end of treatment TB using the anti-galactomannan IgG assay. Healthy controls, end of treatment TB therapy, and all control groups combined produced a cutoff of 106.8 AU/mL, while the diseased control showed a cutoff of 91.6 AU/mL (the optimal cutoffs are highlighted in Table 3). The diagnostic odds ratio of this cutoff in the end of treatment TB group was 3.15 (95% CI 1.03–9.67).

Comparisons of AUC of CPA diagnosis using anti-galactomannan IgG in different populations with the anti-galactomannan IgG cutoff of 120 mg/L are explained in supplement Table 6. The highest sensitivity and specificity were observed in the CPA cases vs healthy controls group with AUC 0.758, sensitivity 83% and specificity 69% with proposed

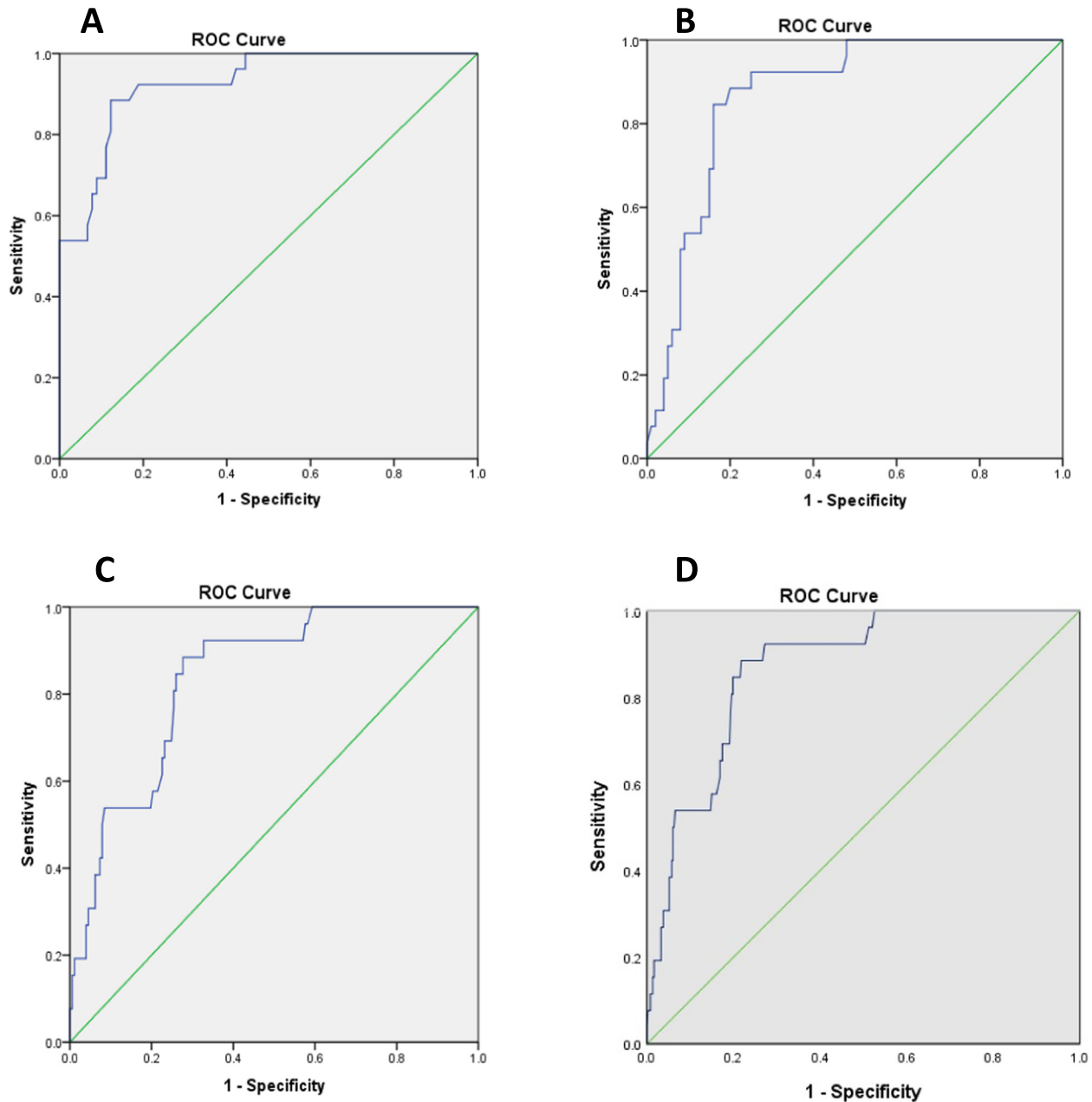


Fig. 1. Receiver operating curve (ROC) of *Aspergillus*-specific IgG using Immulite in (A) healthy control with AUC 0.926, (B) diseased control with AUC 0.837, (C) end of treatment tuberculosis with AUC 0.87, and (D) all control groups combined with AUC 0.87. The highest AUC was observed in the healthy control and all control groups combined with the cutoff of 11.5 mg/L.

cutoff of 106.8 AU/mL. The ROC curve using the 106.8 AU/mL as a cutoff for each control group and together is shown in Fig. 2. The difference in specificity between the Immulite and Dynamiker with all control groups combined was statistically significant ($P < 0.001$).

4. Discussion

Our study provides the first analysis of *Aspergillus*-specific IgG cutoffs in the Indonesian population of approximately 275 million. We compared optimal diagnostic cutoffs through ROC analysis across healthy controls, diseased controls and an end of treatment TB group of patients. There is near consensus in the published papers about the optimal cutoff for *Aspergillus*-specific IgG assay. A recent study recommends a cutoff of 20 mg/L based on a study from European and African population (Page et al., 2019b). Another study conducted using CPA patients from the UK National Aspergillosis Centre and

blood donors from Uganda showed 10 mg/L as the optimum cutoff for Immulite *Aspergillus*-specific IgG assay (Page et al., 2015). The cutoff produced based on ethnicity is crucial as there is a big gap in cutoff between Ugandan healthy controls (10 mg/L and 15 mg/L) and European healthy controls (25 mg/L) using Immulite *Aspergillus*-specific IgG assay (Page et al., 2019b). We derived an optimal cutoff in our study (11.5 mg/L), as this showed highest sensitivity and specificity compared to other cutoffs (Denning et al., 2018). It will need confirmation in other patients.

There was no *Aspergillus* detected in the culture from 26 patients in the CPA group. The reason is probably the limited volume of sputum in most of the patients. This sputum was used for both fungal culture and TB PCR tests. We recommend high volume culture for diagnosis of CPA especially in the population at risk of CPA (Vergidis et al., 2019). There were 3 patients with a positive *Aspergillus* culture from a single sample at the end of TB treatment but all of them had an Immulite *Aspergillus*

Table 3

Dynamiker anti-galactomannan IgG performances at various points on the ROC AUC curve, using the 23 patients with CPA as cases in the comparison with three different control groups (manufacturer cutoff 120 AU/mL).

Cutoff (AU/mL)	Sensitivity (%)	Specificity (%)	Youden's J statistic
Healthy controls			
90.4	87	60	0.47
100.2	83	63	0.46
106.8	83	69	0.52
110.4	70	69	0.39
120.4	57	71	0.28
End of treatment TB			
90.3	87	33	0.20
99.5	83	38	0.21
106.8	83	40	0.23
110.4	70	42	0.11
120	57	46	0.02
Diseased controls (non TB)			
85.2	87	48	0.35
91.6	87	51	0.38
106.2	83	55	0.38
107.3	78	55	0.33
110.5	70	57	0.27
119.6	61	63	0.20
All controls			
90	87	45	0.32
100	83	48	0.31
106.8	83	51	0.34
110	70	53	0.22
120	57	57	0.14

IgG level below 11.5 mg/L and one of them had no radiological characteristics of CPA. We categorized these three patients as non-CPA. The 2 patients with probable CPA were included in the longitudinal study, and follow up after 6 months showed an increasing level of *Aspergillus*-specific IgG (from early in their TB course) and progression of cavitation, most consistent with CPA and showing that *Aspergillus*-specific IgG develops over time. This finding is consistent with another study which showed anti-galactomannan IgG seronegative CPA cases could be caused by non-*A. fumigatus* species (*A. flavus* and/or *A. niger*) proven by cultures (Guo et al., 2019). Negative results of *Aspergillus*-specific IgG were observed in all three patients of probable CPA in our study using 11.5 mg/L (*Aspergillus*-specific IgG) and 106.8 AU/mL (anti-galactomannan IgG) except one patient with anti-galactomannan IgG value 107.1 AU/mL (low positive). Future research is required to explore diagnosis of non-*A. fumigatus* CPA with the *Aspergillus*-specific non-*A. fumigatus* IgG.

The Immulite *Aspergillus*-specific IgG performance is superior to the anti-galactomannan IgG with higher AUC, sensitivity and specificity. The AUC of *Aspergillus*-specific IgG (0.84–0.93) is comparable to that of other studies using healthy controls from Uganda and the Netherlands (Page et al., 2015, 2018). The anti-galactomannan IgG AUCs (0.58–0.76) in this study are lower than one prior study using the anti-galactomannan IgG assay (Ma et al., 2019; Page et al., 2015), but showed the same ROC (0.76) as a recent study from Guo with 16 proven CPA, 54 probable CPA, 28 fungal colonization, and 46 other respiratory disease cases included in the analysis (Guo et al., 2019). This study included a larger CPA sample than our study (70 vs 23 patients), but with fewer total cases and controls (144 vs 381 patients). The percentage of proven TB in our study is higher (50%) compared to the Guo study (21%). This study reported a sensitivity of 70% and 82.8% specificity using the manufacturer cutoff >60 AU/mL. While our study used the new version of anti-galactomannan IgG with manufacturer cutoff >120 AU/mL, we propose an optimum cutoff of 106.8 AU/mL with a sensitivity of 82.6% and specificity of 68.9% compared to healthy controls. This is different from a recent study from China which suggested a cutoff 89.3 AU/mL for anti-galactomannan IgG (Ma et al., 2019).

The limitation of the diseased control group is that CXRs reports were unavailable for many patients, and also sputum was not available

from some for TB PCR or fungal culture. It was presumed that all the diseased control patients did not have cavities since the diagnosis of TB was not considered. Patients with no pleural thickening and cavities on the CXRs have 100% negative predictive value for CPA (Page et al., 2019a). High levels of *Aspergillus*-specific IgG in this group might be caused by clinical forms of aspergillosis such as ABPA, SAFS, or *Aspergillus* bronchitis as they have underlying lung diseases (asthma, COPD, bronchitis) as the risk factors (Baxter et al., 2013; Chrdle et al., 2012).

We note that the *Aspergillus*-specific IgG level at the end of TB treatment and the CPA cases is significantly different in *Aspergillus*-specific IgG level but not with the anti-galactomannan IgG level. The high level of anti-galactomannan IgG in TB patients might be caused by cross reaction with non-*Aspergillus* species as galactomannan is the component of many pathogenic fungal cell walls including *Histoplasma*, *Penicillium*, and *Fusarium* (Cummings et al., 2007; Min et al., 2012). Additionally, cross-reactivity of *Aspergillus*-specific IgG and other commercial assays against non-*Aspergillus* species is not known (Richardson and Page, 2018). The moderate level of correlation between *Aspergillus*-specific IgG and anti-galactomannan IgG results was probably a function of different antigens used by these 2 assays. Culture extract antigens are used for *Aspergillus*-specific IgG (Immunitite) while only galactomannan is used in anti-galactomannan IgG (Dynamiker) (Richardson and Page, 2018).

The median value of both *Aspergillus*-specific IgG and anti-galactomannan IgG *Aspergillus*-specific IgG of healthy and disease controls showed nearly similar values and the difference is not significant, implying that our cutoffs are appropriate for use in both healthy people and people with chronic lung disease with TB excluded. This also reflects our strategy to reduce the probability of CPA cases in the diseased control by screening them based on the inclusion criteria and TB PCR test to exclude TB patients. There are only 2 positive Immunitite results (2%) of the *Aspergillus*-specific IgG level in the healthy controls, as found in Uganda (Page et al., 2019b).

Another limitation of this study is the small number of CPA cases. The median level of *Aspergillus*-specific IgG in the CPA group in this study is 20.3 mg/L, lower than the median level of previous published papers using 241 CPA cases with median of 392 mg/L. The high level from the previous study probably represents a referral bias of more complex cases and duration of disease at the National Aspergillosis Centre in the UK. Most of the CPA cases in this study are probably early stage CPA as the cavity was formed during TB infection and is stable under TB therapy, unless the TB diagnosis was incorrect and the correct diagnosis was CPA.

We used *Aspergillus* specific-IgG level as one of the CPA diagnosis criteria and in the same time measured the proposed cutoff of *Aspergillus* specific-IgG using these CPA cases. This approach might introduce bias. This problem was recognized as one of the challenges need to be solved in the validation of CPA serological tests (Godet et al., 2017). However this is the best possible approach in this study as we found no positive *Aspergillus* cultures in our 23 CPA patients (Chikere et al., 2019; Vergidis et al., 2019).

In summary, we have investigated the diagnostic performance of Immunitite and anti-galactomannan IgG for CPA for the first time in Indonesia. We propose cutoffs for both assays. Immunitite seems to have better diagnostic value than anti-galactomannan IgG, but the application in daily clinical practice is limited due to unavailability of the facilities in many resources-constrained settings. The proposed cutoffs require validation in different groups of patients with CPA.

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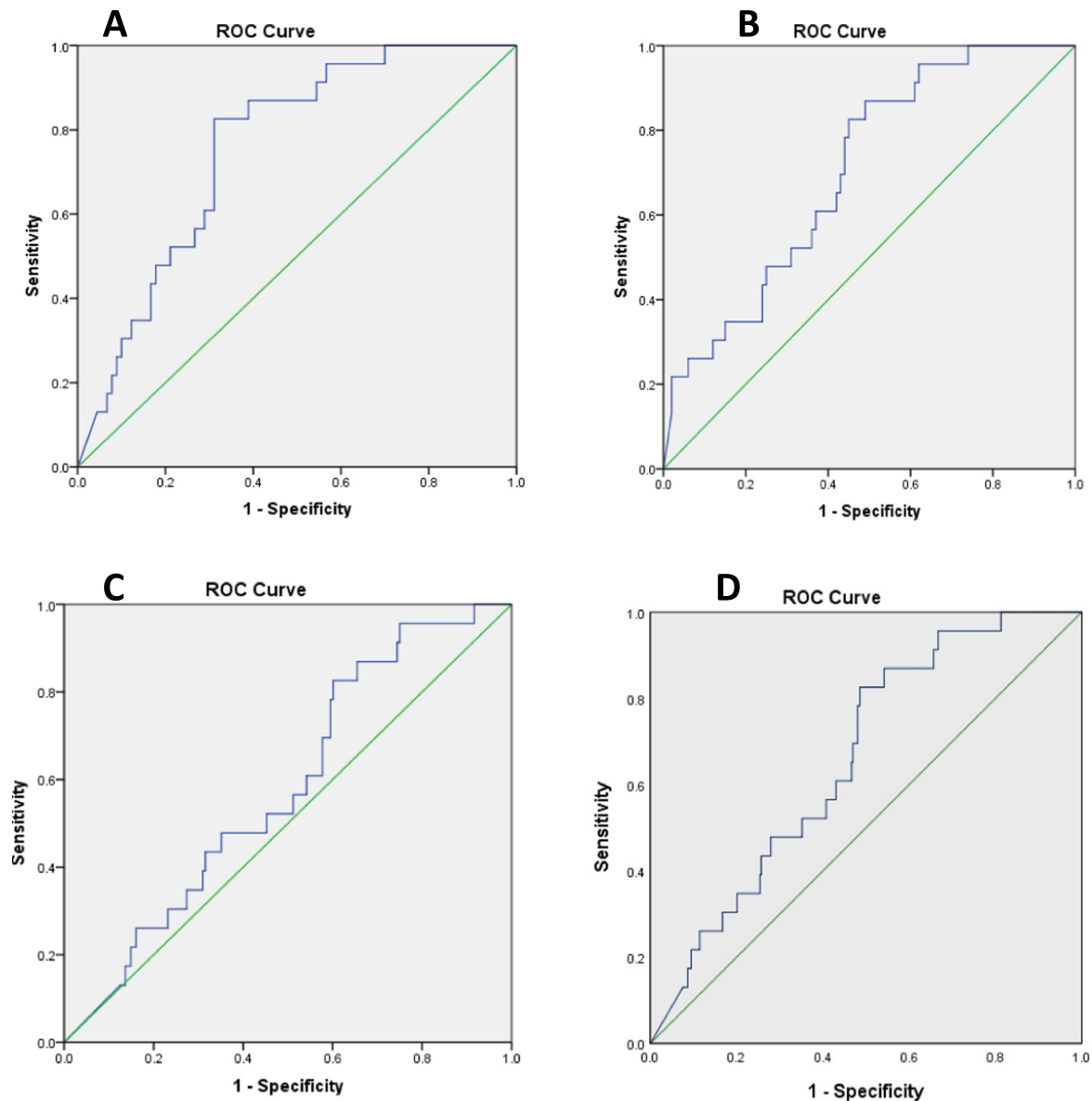


Fig. 2. Receiver operating curve (ROC) of anti-galactomannan IgG using Dynamiker in (A) healthy control with AUC 0.758, (B) diseased control with AUC 0.704, (C) end of treatment tuberculosis with AUC 0.581, and (D) all control groups combined with AUC 0.660. The highest AUC was observed in healthy control with the cutoff of 106.8 AU/mL.

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