


Diagnostic cut-off value for *Aspergillus fumigatus*- and *flavus*-specific IgG with clinical relevance in chronic pulmonary *Aspergillus* infection: A pilot study in Taiwan

Shiang-Fen Huang^{1,2}  | Shi-Yi Li^{3,4} | Yu-Jiun Chan^{3,4} | Yu-Chi Huang¹ |
Ying-Ying Yang^{5,6} | Fu-Der Wang^{1,3}

¹Division of Infectious Disease, Department of Internal Medicine, Taipei Veterans General Hospital, Taipei, Taiwan

²School of Medicine, National Yang-Ming University, Taipei, Taiwan

³Institute of Public Health, National Yang-Ming University, Taipei, Taiwan

⁴Division of Microbiology, Department of Pathology and Laboratory Medicine, Taipei Veterans General Hospital, Taipei, Taiwan

⁵Department of Clinical Education, Taipei Veterans General Hospital, Taipei, Taiwan

⁶Department of Medicine, Institute of Clinical Medicine, National Yang-Ming University, Taipei, Taiwan

Correspondence

Shiang-Fen Huang and Fu-Der Wang, Division of Infectious Disease, Department of Internal Medicine, Taipei Veterans General Hospital, Taipei, Taiwan.
Emails: sfhuang6@vghtpe.gov.tw (S-FH); fdwang@vghtpe.gov.tw (F-DW)

Funding information

Ministry of Science and Technology, Taiwan, Grant/Award Number: 108-2635-B-075-002; Taipei Veterans General Hospital, Grant/Award Number: 109DHA0100248

Abstract

Background: The diagnostic cut-off value for chronic pulmonary Aspergillosis (CPA) by *Aspergillus fumigatus*-specific IgG has never been evaluated in Taiwan. The cut-off value for *Aspergillus flavus*-specific IgG has not been evaluated worldwide.

Objectives: Evaluate diagnostic cut-off value of *Aspergillus* IgG and its application characteristics.

Patients/Methods: Blood from control groups and treatment-naïve patients with CPA infections was collected for *Aspergillus*-specific IgG measurements. Controls were patients who had chest radiographic abnormalities and signs of respiratory tract infection, but were negative for *Aspergillus* and resolved without anti-mould therapy. Confirmation and probability of CPA were defined according to radiological features and positivity for an *Aspergillus* or galactomannan index. Chest computer tomography patterns were recorded for the presence of aspergilloma or nodules, subacute invasive aspergillosis, chronic cavitary pulmonary aspergillosis and chronic fibrotic pulmonary aspergillosis.

Results: A total of 35 cases and 50 disease controls were included. The levels of *A. fumigatus*- and *A. flavus*-specific IgG correlated with CPA progression ($P < .05$) but not with the presence of *Aspergillus* species from clinical specimens ($P > .05$). The best cut-off value for *A. fumigatus* IgG was 21.7 mg/L with area under curve (AUC) for receiver operating characteristic curve (ROC) 0.934 and had 85.7% sensitivity and 92.0% specificity. For *A. flavus* IgG, the best cut-off value was 22.1 mgA/L and the AUC was 0.928 with 88.2% sensitivity and 94.1% specificity.

Conclusion: The level of *Aspergillus*-specific IgG correlated with radiographic characteristics in patients with CPA and the best cut-off values compared to controls were 21.7 mgA/L for *A. fumigatus*-specific IgG and 22.1 mgA/L for *A. flavus*-specific IgG.

KEYWORDS

Aspergillus flavus, *Aspergillus fumigatus*, cut-off value, pulmonary *Aspergillus* infection

1 | INTRODUCTION

Aspergillus is a globally distributed genus of fungi that causes various sequels, depending on host immunity. The pulmonary manifestation ranges from allergic reactions and tracheobronchitis in healthy people to chronic infection with lung parenchymal involvement and pleural thickening. In hosts who are extremely immunosuppressed, it can progress to systemic invasion from chronic pulmonary aspergillosis or from nasal-sinusoidal colonisation.^{1,2} Chronic pulmonary aspergillosis (CPA) is typically defined as fibro-bronchiectasis in the lung parenchyma with pleural thickening, with or without fungal balls in the cavity (chronic cavitary pulmonary aspergillosis, CCPA), and can be interchanged with subacute invasive aspergillosis (SIA) or necrotising pneumonia, or progress to fibrosis without recovery and with poor survival.

By mathematical estimation, the prevalence of CPA according to World Health Organization statistics is suggested to be 20% of the tuberculosis (TB)-treated population with cavitary lesions.³ In Taiwan, the incidence of MTB (43/100 000 in 2016)⁴ is lower than in countries with similar attitudes such as India and China,⁵ with estimates of 20% of post-*Mycobacterium tuberculosis* (MTB)-infected patients being CCPA in South-East Asian countries.³ Based on a previous investigation that evaluated serum or bronchoalveolar lavage fluid (BALF) by culture or galactomannan index, acute or subacute pulmonary *Aspergillus* infection in Taiwan with 10%-15%⁶ and European countries with 7%-20% of the incidence after severe influenza infection⁷⁻⁹ were observed. However, no investigations have described an underlying *Aspergillus* infection or colonization prior to a severe influenza episode. Thus, the 20% chance of developing CPA after completion of anti-TB treatment might be a misestimate.

The predominant species isolated in pulmonary *Aspergillus* infections are *Aspergillus fumigatus* and *Aspergillus flavus*.¹⁰⁻¹² Clinical diagnosis of *A. fumigatus*- or *A. flavus*-related infections is difficult because of low sensitivity in mycological and pathological surveys. Serological examination for *Aspergillus* infection provides a sensitive method for detecting fungal infection. For *A. fumigatus*, a wide range of cut-off values for *A. fumigatus*-specific IgG have been reported.¹³⁻¹⁷ Some reports have a high cut-off value (70 mgA/L) for a study that enrolled patients with CCPA and compared them to controls with anti-MTB treatment history in European countries.¹⁷ One study reported a lower cut-off value (27 mgA/L) when compared with controls with cavity lesions on chest film in Asia but extremely high TB incidence.¹⁶ Most control groups in the previous survey were healthy donors, and these 'control' cases were without pulmonary infection.

For *A. flavus* (ImmunoCAP), no cut-off value is available for estimates in South Asian countries and no cut-off value is provided by the manufacturer. Diagnosis of CPA might be underestimated since both *A. fumigatus* and *A. flavus* are not easily found in clinical specimens. Furthermore, the occurrence of CPA may be underestimated. One reason is that pulmonary lesions associated with CNPA are rarely treated with the CPA by clinicians. Another reason is the

lack of a cut-off value for *A. fumigatus* IgG levels, which is suitable in Taiwan. Only CCPA with fungal balls rather than CFPA is considered an *Aspergillus* infection by most physicians. In most cases with CCPA, physicians generally did not take a comprehensive diagnostic approach for mycological survey due to lack of consideration for anti-mould therapy for CPA in the past year.

We aimed to improve the diagnostic sensitivity by enrolling more patients with possible CPA. We evaluated cut-off values for *A. fumigatus*- and *flavus*-specific IgG by enrolling patients with typical CFPA, CCPA, CNPA or simple aspergilloma or nodules, and some selected patients with consolidation on chest CT with clinical radiological improvement after anti-mould triazole monotherapy. The control groups were individuals with bacterial respiratory tract infections or known fungi infections such as cryptococcus or *Candida* pneumonia with resolution after non-anti-mould triazole, regardless of a history of anti-TB treatment. In this study, we also evaluated the clinical relevance of the levels of *Aspergillus*-specific IgG.

2 | METHOD

2.1 | Ethics statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received by the Institute Ethics Committee approved the study protocol (TVGH IRB: 2018-02-004BC), and written informed consent was obtained from all the study participants.

2.2 | Study population

This was a prospective study conducted from May 2018 to December 2019 in a Tertiary Medical Center in Taiwan. Individuals with pulmonary abnormalities who visited a hospital or had the presence of symptoms and signs such as fever, respiratory tract infection, cough, recurrent hemoptysis, shortness of breath, dyspnoea on exercise, respiratory distress, weight loss or malaise were enrolled. After a complete clinical history and physical evaluation, peripheral blood was collected and serum was obtained prior to initiating anti-fungal treatment. Individuals with newly diagnosed active tuberculosis, history of asthma or immunoglobulin deficiency (IgG < 500 mg/L) were excluded.

2.3 | Clinical evaluation and measurement

A microbiological survey was conducted upon entering the study. For every enrolled patient, fungal culturing from the nasal swabs for one specimen and from sputum for one times per day for 3 days were collected. Endotracheal aspirate or BALF were collected if

patients had respiratory failure with mechanical ventilator support. *Aspergillus* galactomannan antigen (GM) was measured for blood samples, and for BALF if available. *Aspergillus* GM was detected using Platelia *Aspergillus* Ag assays (Bio-Rad Laboratories, Marnes-La-Coquette, France) for serum with a positive cut-off value of an optical density index ≥ 0.5 in blood and ≥ 1 in BALF. The presence of *Mycobacterium tuberculosis* by sputum smear or culture and MTB-specific nucleic acid assay Xpert MTB/RIF (Cepheid Inc.) was also evaluated. Patients with active TB who underwent anti-TB therapy were excluded for cut-off value estimations. Serological tests for *A. fumigatus*- and *A. flavus* IgG assays (ImmunoCAP system, Thermo Fisher Scientific) were performed. Pulmonary function was evaluated by questionnaire recording (chronic obstructive pulmonary disease [COPD] assessment test) and demand for oxygenation was recorded. Chest radiograph and computer tomography (CT) of the thorax and sinus radiograph by Waters' view were arranged. Patients without pulmonary abnormality but with sinusitis were excluded.

2.4 | Definitions

CPA was defined by typical chest radiographic findings, presence of clinical symptoms or progression in respiratory symptoms, and by mycological, pathological or GM detection as the European Respiratory Society (ERS)¹ and Infectious Disease Society in America (IDSA)² recommended. We defined CPA in three categories: (a) laboratory-confirmed CPA (cCPA) with presence or culture of *Aspergillus* of the same species as the IgG (ie, fungal culture-positive for *A. fumigatus* or *A. flavus*); (b) possible CPA (pCPA) with positive galactomannan (≥ 0.5 in serum and ≥ 1 in BALF) or presence of *Aspergillus* other than *A. fumigatus* or *A. flavus*; and (c) probable cases with pulmonary radiological patterns compatible with CPA categories and respiratory symptoms or signs of unresponsiveness to appropriate anti-bacterial antibiotics for more than 2 weeks but resolved with anti-mould monotherapy. For probable cases, the clinical specimens may have grown filamentous fungi other than known *Aspergillus* species or might have a borderline serum galactomannan index (> 0.1 and < 0.5). Both possible and probable CPA were labelled as pCPA. Confirmed CPA and possible CPA were grouped as a 'Training' group and probable CPA cases were characterised as a 'Validation' group when determining cut-off values.

Patients with CPA were recorded by the presence of four types of CPA on chest CT, defined according to European Respiratory Society guidance: (a) *Aspergillus* granuloma or nodules, (b) SIA or chronic necrotic pulmonary aspergillosis (CNPA), (c) chronic cavitary pulmonary aspergillosis (CCPA) or (d) chronic fibrotic pulmonary aspergillosis (CFPA).¹ The presence of radiological characteristics on chest CT was also recorded for all patients, including fungal balls, cavities, nodules or granuloma with or without halo sign, bronchiectasis, pleural thickening, lung-volume reduction and consolidation. Severe lung fibrosis with traction bronchiectasis was categorised as fibro-bronchiectasis.

Patients with allergic bronchopulmonary aspergillosis (ABPA; with stimulated IgE for *Aspergillus*, central bronchiectasis and reversibility in bronchodilator tests by spirometry, and culture-positive for *Aspergillus* spp.) or allergic asthma were excluded.

Controls were individuals who sought medical help for respiratory tract symptoms or infection with presence of pulmonary infiltration and without mycological evidence (fungus culture with *Aspergillus* species, biopsy or galactomannan index < 0.1) and recovered without anti-mould therapy. Controls were randomised into two subgroups with two-thirds (training) and one-third (validation) for cut-off value evaluation.

2.5 | Statistical analysis

For clinical data, significance values and statistical tests are described in the text, associated figures and figure legends. Continuous variables were compared using ANOVA or non-parametric tests and results are presented as means \pm standard deviation. Categorical variables were compared using chi-squared or Fisher's exact tests. Receiver operating characteristic (ROC) curves were drawn to determine cut-off values for *A. fumigatus* and *A. flavus* IgG, and the best cut-off value was determined by Youden's J statistic for highest area under the ROC curve.

3 | RESULTS

3.1 | Characteristics of study population

From May 2018 to December 2019, 85 patients and controls were enrolled (Table 1). Most patients with CPA had respiratory symptoms or signs longer than 3 months (85.7%), and five patients with CPA had no previous medical history but typical respiratory presentations including fibro-bronchiectasis or cavities in chest image when entering the study. Overall, 31.4% of patients with CPA had positive cultures for *Aspergillus* species and 37.1% of patients had filamentous fungi other than *Aspergillus* species. *A. flavus* (14.3%) and *A. fumigatus* (14.3%) were the first two frequently isolated *Aspergillus* species (Table 1). Several patients were found concurrently culture-positive for *A. fumigatus* and non-*fumigatus* *Aspergillus*, or with more than one non-*fumigatus* *Aspergillus* in respiratory tract specimens.

Among patients with CPA, only three patients were tested positive for galactomannan in peripheral blood samples. In the control group, the proportions of those with filamentous fungi (36%) and yeast-form fungi (54.5%) in the respiratory tract were similar to those of CPA patients (36% for filamentous fungi and 58% for yeast form fungi; $P > .05$). Distributions for age, gender and demand for oxygen were not different between the cCPA, pCPA and control groups. More patients with CPA had COPD than controls ($P < .05$). Otherwise, underlying comorbidities were similar between CPA patients and controls ($P > .05$).

TABLE 1 Clinical characteristics and laboratory data in study patients

Characteristics	Chronic pulmonary Aspergillus infection (CPA) (N = 35)		Diseased control (N = 50)		P-value
	n	%	n	%	
Gender					.8
Male	21	61.8	32	64.0	
Female	13	38.2	18	36.0	
Age					.23
≤30	0	0.0	0	0.0	
31-50	1	2.9	7	14.0	
51-70	10	29.4	10	20.0	
70-90	14	41.2	24	48.0	
>90	9	26.5	9	18.0	
History of anti-TB therapy					.4
Yes	8	22.9	8	16.0	
No	27	77.1	42	84.0	
Respiratory symptoms					
Abnormal chest film > 3 mo	30	85.7	26	52	<.01
Bronchodilator > 3 mo	11	31.4	19	38	.59
Chronic cough > 3 mo	32	91.4	33	66	<.01
History of pneumonia in 3 mo	22	62.9	23	46	.12
Demand of oxygen					.33
Respiratory failure	8	23.5	14	28.0	
Moderate (FiO ₂ > 32%)	11	32.4	8	16.0	
Mild (FiO ₂ < 32%)	11	32.4	16	32.0	
Room air	5	14.7	12	24.0	
Comorbidities					
Malignancy	9	26.5	10	20.0	.5
Diabetes	7	20.6	8	16.0	.6
Autoimmune disorders	7	20.6	8	16.0	.6
COPD	24	70.6	18	36.0	.003
Dialysis	3	8.8	9	18.0	.2
Fungal isolates					
Aspergillus fungi					.01
<i>A. fumigatus</i>	5	14.3	1	2.0	
Non-fumigatus <i>Aspergillus</i>					
<i>A. flavus</i>	5	14.3	0		
<i>A. niger</i>	2	5.7	0		
<i>A. terreus</i>	1	2.9	0		
Others	6	17.1	1	2.0	
All <i>Aspergillus</i> spp.	11	31.4	2	4.0	
Non-Aspergillus moulds	13	37.1	18	36.0	.91
<i>Cladosporium</i> spp.	3	8.6	4	8.0	
<i>Exophiala</i> spp.	1	2.9	1	2.0	
<i>Geotrichum</i> spp.	3	8.6	1	2.0	
<i>Penicillium</i> spp.	1	2.9	2	4.0	
<i>Trichoderma</i> spp.	1	2.9	1	2.0	

(Continues)

TABLE 1 (Continued)

Characteristics	Chronic pulmonary Aspergillus infection (CPA) (N = 35)		Diseased control (N = 50)		P-value
	n	%	n	%	
Others	5	14.3	8	16.0	
Yeast form fungi	23	65.7	29	58.0	
Pathological proven					NA
Yes	3	8.6			
No	32	91.4			
CPA in current study					NA
Confirmed	9	26.5			
Possible	14	41.2			
Probable	11	32.4			
ERS category					NA
Aspergilloma or nodules	11	32.4			
Subacute IA or CNPA	20	58.8			
CCPA	11	32.4			
CFPA	9	26.5			
Unable to be grouped	5	14.7			

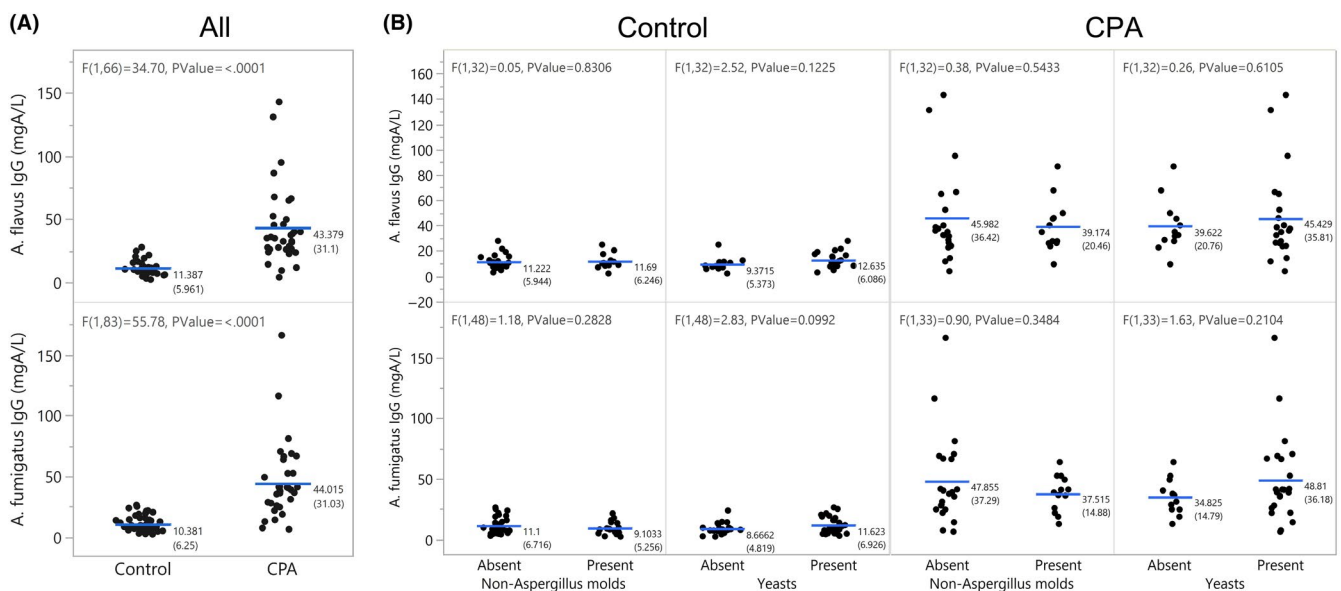


FIGURE 1 Distribution of *Aspergillus*-specific IgG in the study population. A, *Aspergillus* IgG distribution was significantly different between the control group and CPA patients. B, The *Aspergillus*-specific IgG levels were not different between yeast-form fungi and non-*Aspergillus* moulds isolated from the respiratory tract either in control groups or in CPA patients. The numbers in the figures represent the mean (standard error) for each group

The levels of *A. fumigatus* IgG (44 ± 31 and 10.4 ± 6.3 mgA/L) and *A. flavus* IgG (43.4 ± 31.1 and 11.4 ± 5.9 mgA/L) were higher in CPA than in control groups, respectively (Figure 1A, $P < .05$). The IgG levels were not associated with the presence of different types of fungi such as non-*Aspergillus* moulds or yeast-form fungi (Figure 1B, $P > .05$). In CPA patients, the levels of *A. fumigatus* and *A. flavus* IgG were not correlated to the presence of corresponding *Aspergillus* species (Figure 2A,B). However, a slight correlation

between *Aspergillus* IgG levels and number of *Aspergillus* species (Figure 2C) was observed.

Most patients with CPA presented with CNPA or SIA (58.9%), followed by CCPA (32.4%) and aspergilloma or nodules (32.4%) on chest CT. Only 26.5% (9/35) of patients presented with CFPA on chest CT. Overall, the median levels of *A. fumigatus* IgG were 30.9, 38.5 and 8.67 mgA/L for the cCPA, pCPA and controls, with levels for *A. flavus* IgG of 27.5, 37.4 and 10.5 mgA/L.

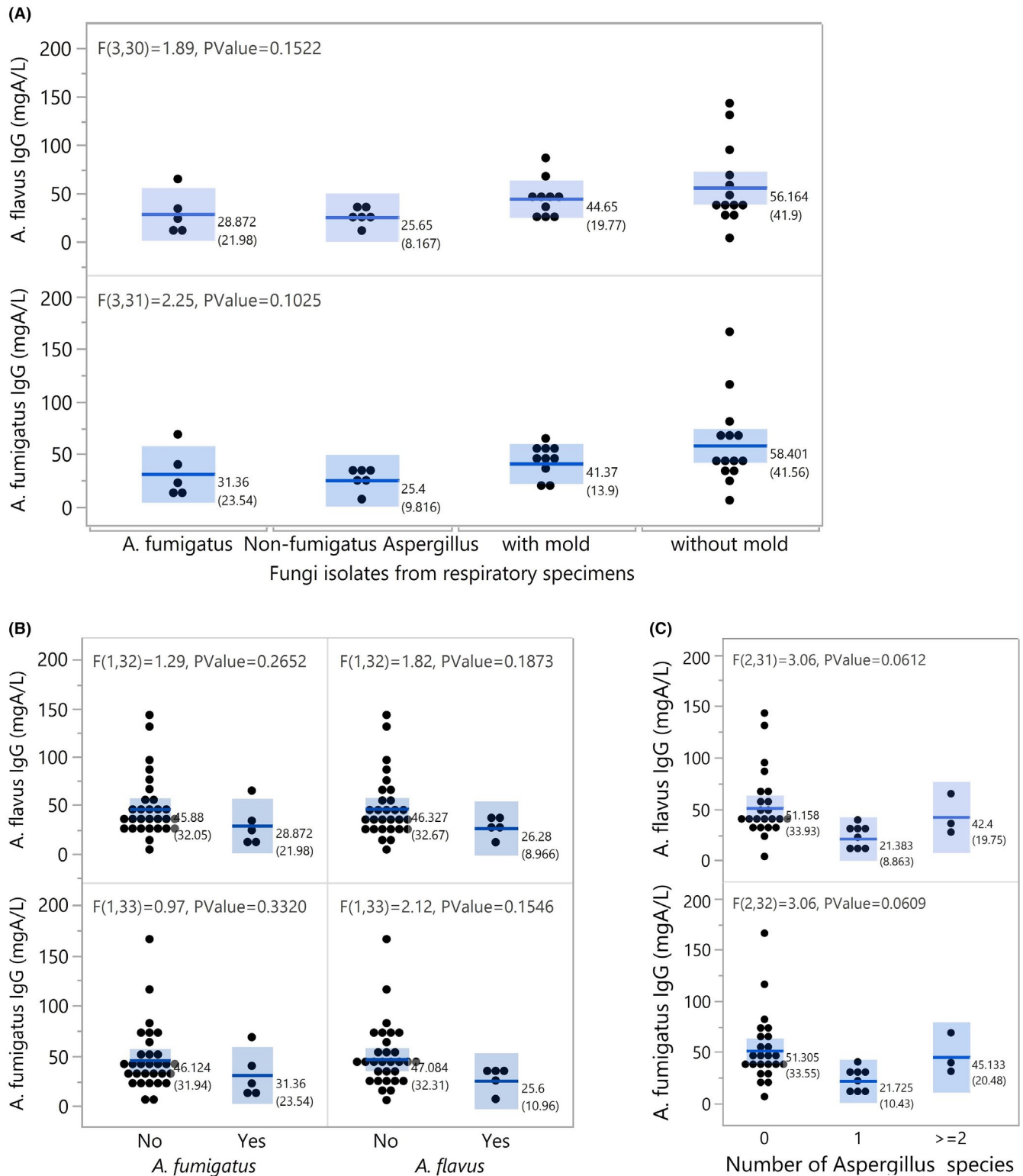


FIGURE 2 Distribution of Aspergillus-specific IgG by presences of *A. fumigatus* and non-fumigatus Aspergillus fungi in respiratory specimens in CPA patients. A, Aspergillus-specific IgG levels were not different by presence of *A. fumigatus* or non-fumigatus fungi ($P > .05$) in CPA patients. B, The *A. flavus* or *A. fumigatus* IgG levels were not influenced by the presence of *A. flavus* or *A. fumigatus*, respectively ($P > .05$). C, The Aspergillus-specific IgG was slightly influenced by the number of isolates with Aspergillus species ($P = .06$) from respiratory specimens in CPA patients

3.2 | Clinical and mycological relevance of *A. fumigatus* and *A. flavus* IgG serum levels

The level of *A. fumigatus* or *A. flavus* IgG was correlated to the types of CPA, such as aspergilloma or CFPA (Figure 3A,B, $P < .05$). The IgG levels also correlated with radiographic characteristics. Both *A. fumigatus*- and *A. flavus*-specific IgG were higher in patients with fibro-bronchiectasis or volume reduction than with other radiological characteristics on chest CT (Figure 3C, $P < .05$). Age, gender and comorbidities including COPD, autoimmune disorders, end-stage renal disease and diabetes ($P > .05$, data not shown) were not related to serum *Aspergillus* IgG. The presence of non-*fumigatus* moulds was not correlated to serum levels of *A. fumigatus* or *A. flavus* IgG (Figure S1) in patients with CPA. History of anti-TB treatment did not influence differences in *Aspergillus*-specific IgG levels (Figure S2) between the CPA and control group.

3.3 | Cut-off value and cross-reactivity

Patients with cCPA and two-thirds of the controls were used to determine the cut-off value for a positive result for *A. fumigatus* and *A. flavus* IgG testing (training). The AUC for *A. fumigatus* IgG was 0.941 for the training group and 0.941 for the validation group (Figure S3A). With the cut-off value at 21.7 mgA/L, sensitivities were 72.2% and 88.2% for the training and validation groups and specificities were 96.7% and 89.4%, respectively. AUCs for *A. flavus*-specific IgG were 0.952 and 0.923 for

the training and validation group (Figure S3B). Under the cut-off value of 22.1 mgA/L, the sensitivities were 82.3% and 94.1%, and specificities were 100% and 88.8% for the training and validation groups, respectively.

For all 35 CPA cases compared with 50 controls, the AUCs were 0.934 (Figure 3A,B) and 0.928 (Figure 4C,D) for *A. fumigatus* IgG and *A. flavus* IgG testing, respectively. According to the cut-off values of 21.7 and 22.1 mgA/L, the sensitivities were 85.7% and 88.2% and specificities were 92.0%, 94.1% for *A. fumigatus* IgG and for *A. flavus* IgG testing, respectively (Table 2).

By regression analysis, the levels of *A. fumigatus* and *A. flavus* IgG were cross-related both in control (Figure 4D, $R^2 = 0.67$, $P < .01$) and in CPA groups (Figure 4F, $R^2 = 0.82$, $P < .01$).

4 | DISCUSSION

This was the first study in Taiwan to evaluate the diagnostic cut-off value of *Aspergillus* IgG by defining confirmed cases and treatment responsiveness for pulmonary *Aspergillus* infection, rather than using non-diagnostic epidemiological data without a history of treatment for filamentous fungi. This study also provided the best cut-off value for *A. flavus* IgG. The best cut-off value was 21.7 mgA/L for *A. fumigatus* IgG and 22.1 mgA/L for *A. flavus* IgG with higher sensitivity to enroll more patients with pulmonary infection that might be due to *Aspergillus* spp. infection who need anti-mould therapy as early as possible.

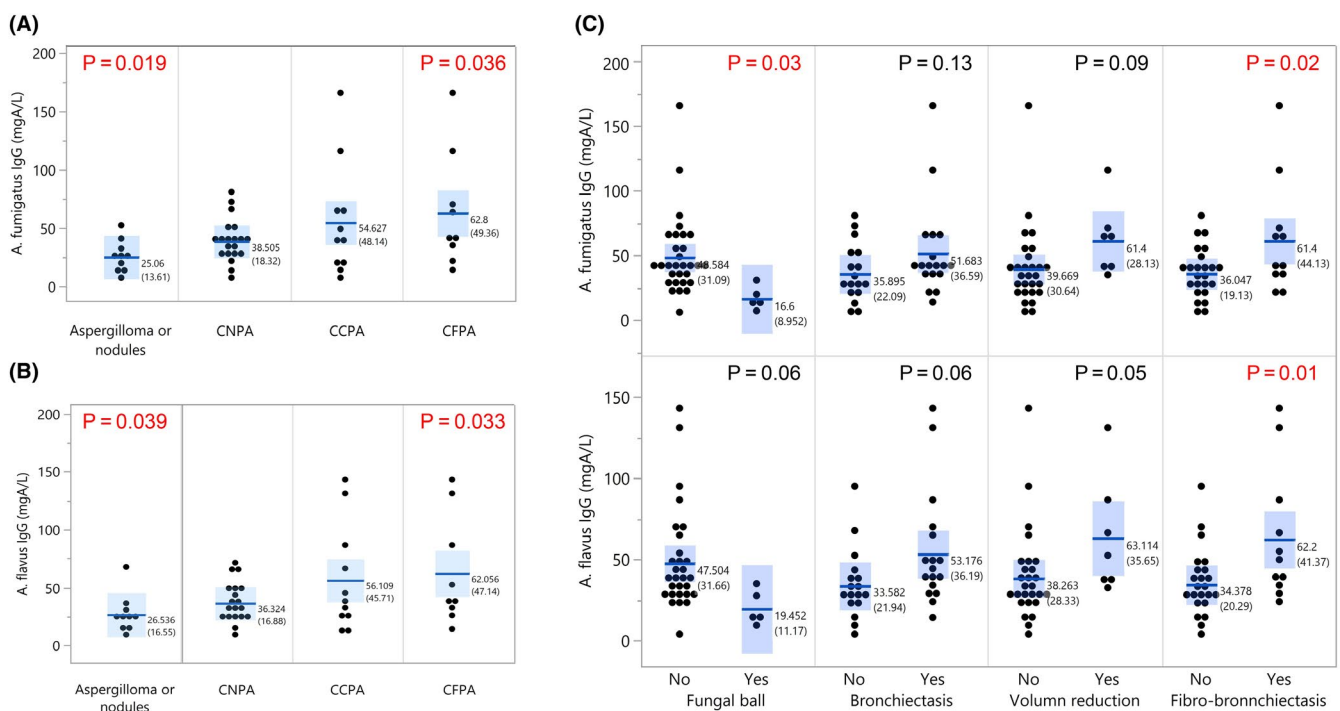


FIGURE 3 Relationship between *Aspergillus*-specific IgG and radiographic characteristics on chest CT. A, Level of *A. fumigatus* IgG by the presence of CPA categories. B, Level of *A. flavus* IgG by presences of CPA categories. C, Level of *A. fumigatus*- and *flavus*- IgG by the presence of radiographic characteristics on chest CT

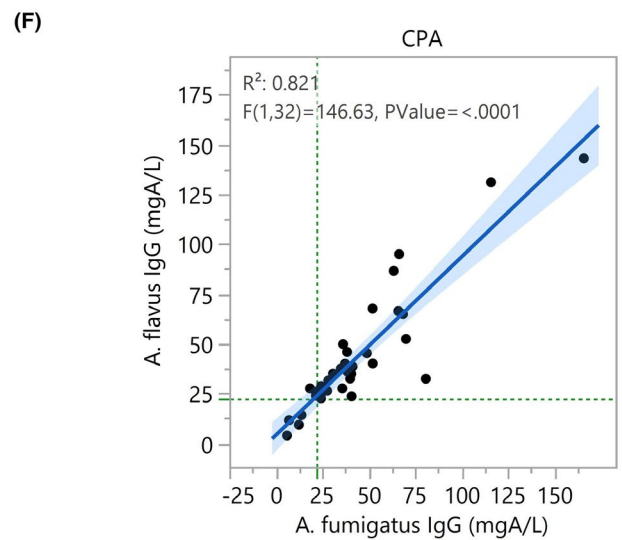
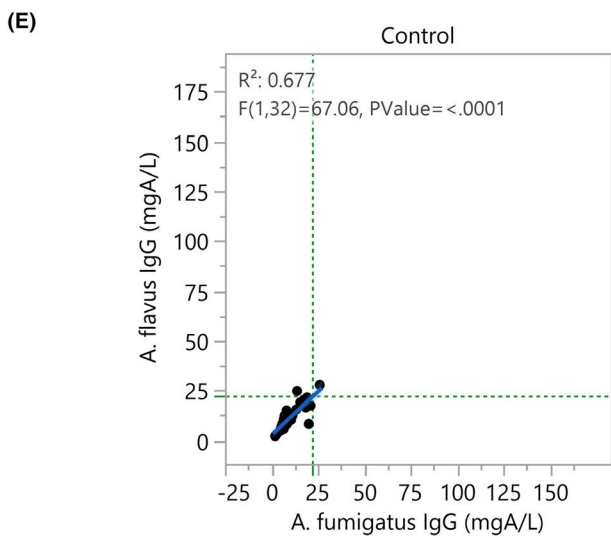
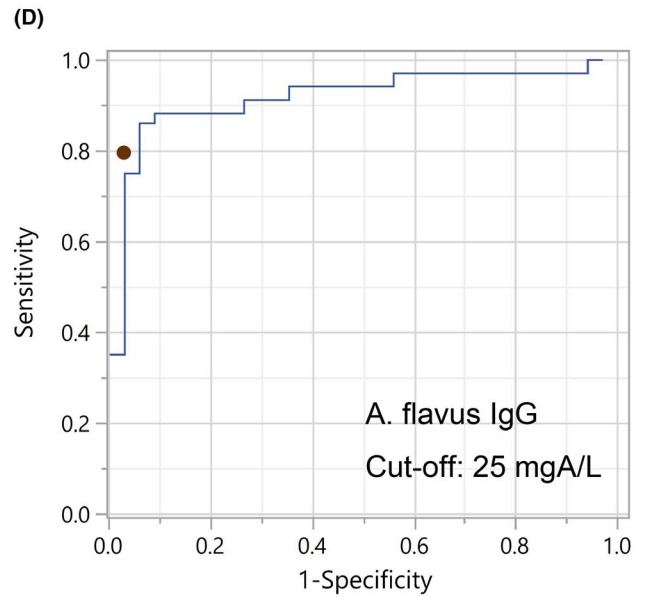
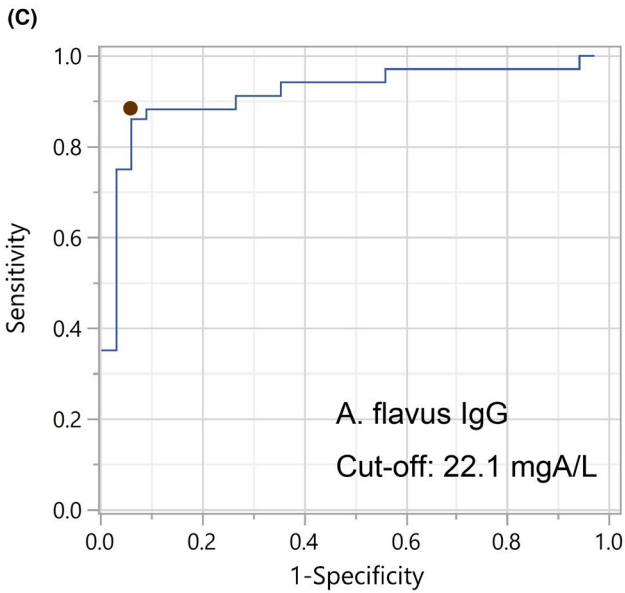
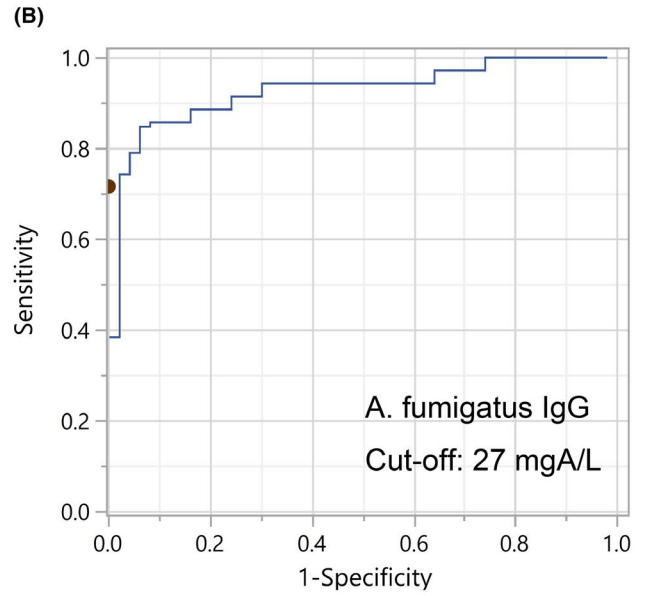
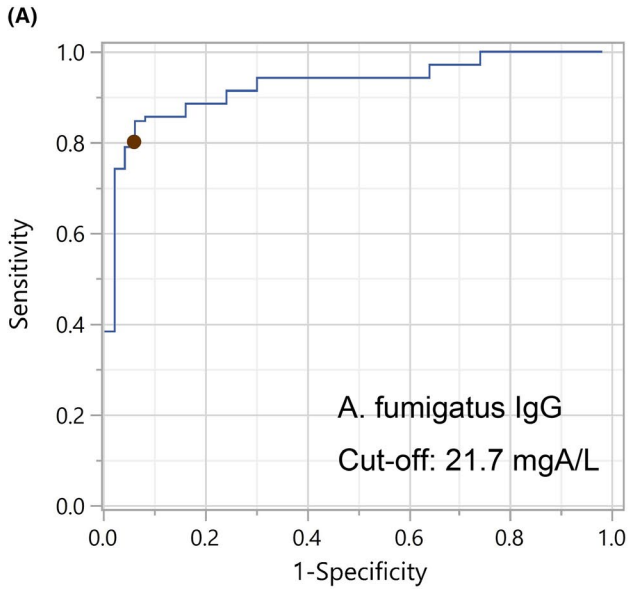


FIGURE 4 Diagnostic cut-off values for *A. fumigatus*- and *A. flavus*-specific IgG and cross-reactivity for chronic pulmonary *Aspergillus* infection. A, Sensitivity and (1-specificity) on ROC for *A. fumigatus* IgG at 21.7 mgA/L cut-off value. B, Sensitivity and (1-specificity) on ROC of *A. fumigatus* IgG at 27 mgA/L cut-off value. C, Sensitivity and (1-specificity) on ROC of *A. flavus* IgG at 22.1 mgA/L cut-off value. D, Sensitivity and (1-specificity) on ROC of *A. flavus* IgG at 25 mgA/L. E, Liner regression curve with 95% confidence interval (CI) for *A. flavus* IgG levels in response to *A. fumigatus* IgG levels in control groups. F, Liner regression curve with 95% confidence interval (CI) for *A. flavus* IgG levels in response to *A. fumigatus* IgG levels in CPA group

TABLE 2 Serological positives in study patients

Characteristics	Chronic pulmonary Aspergillosis (CPA) N = 35		Disease control N = 50		Youden's J
	n	%	n	%	
A. fumigatus IgG (AUC: 0.934)					
Cut-off: 21.7 mgA/L ^a					0.79
Positive	30	85.7	4	8.0	
Negative	5	14.3	46	92.0	
Cut-off: 27.1 mgA/L					0.70
Positive	25	71.4	0	0.0	
Negative	10	28.6	50	100.0	
A. flavus IgG (AUC: 0.928)					
Cut-off: 22.1 mgA/L ^a					0.823
Positive	30	88.2	2	5.9	
Negative	4	11.8	32	94.1	
Cut-off: 25 mgA/L					0.74
Positive	27	79.4	1	2.9	
Negative	7	20.6	33	97.1	

Abbreviation: AUC: Area under curve

^aBest cut-off value.

Our results agree with a previous report in India¹⁶ that 27.1 mgA/L had a good specificity (Table 2) for *A. fumigatus* IgG and were similar to a study in the United Kingdom¹⁵ in which the best cut-off value was near 20 mgA/L. However, some investigations in Europe focused on patients with late-stage CPA who presented with CFPA and CCPA and cut-off values were as high as 70 mgA/L,¹⁷ which were compared to the patients with MTB infection as control groups. We suggest that the differences in cut-off values among these studies might be attributed to some factors such as local prevalence of mould infection and racial differences in genetic diversity of Toll-like receptor (TLR)¹⁸ as well as immunoglobulin production for fungal antigens.^{19,20}

The second explanation might be differences in the stage of the CPA. In this pilot study, we recorded four categories of CPA based on image characteristics and ERS recommendations from aspergilloma to CFPA. Aspergilloma was assumed to have less antigenicity from *Aspergillus*, and CFPA was suggested to be involved in late-stage chronic inflammation with higher antigenicity from *Aspergillus*.

Our data demonstrated that when patients presented with CFPA and lung-volume reduction, the levels of *A. fumigatus* and *A. flavus* IgG were much higher than those who did not have CFPA or volume reduction. Among patients with simple aspergilloma or fungal balls with fewer peripheral lung parenchyma invasions, the level of *Aspergillus*-specific IgG was much lower than in other patients (Figure 3). In CCPA and CFPA, a greater area of pulmonary parenchyma was involved and the toxin produced by *Aspergillus* might lead to chronic inflammation and a higher level of *Aspergillus*-specific IgG. A recent investigation focused on COPD with (35.4 mg/L) or without bronchiectasis (17.4 mg/L) with different *Aspergillus* IgG levels²¹ also suggested that pulmonary structural deformity might be associated with the level of *Aspergillus*-specific IgG. Further investigation regarding the genetic diversity and immune response caused by racial differences could be considered.

The other explanation was that patients with aspergilloma were culture-negative either for *A. flavus* or *A. fumigatus* and only *Aspergillus* fungal hyphae were noted in biopsy tissue. Patients with low levels of *Aspergillus* IgG but presented with tissue confirmed aspergilloma might be due to *Aspergillus* other than *A. fumigatus* or *A. flavus*, which led to a low cross-antigenicity and a low IgG level by the current ImmunoCAP examination method.

Following the cut-off value identified in this study, we had a lower positivity for *A. fumigatus* IgG than in previous investigations.²² This difference might be due to: (a) not detecting the IgM level for *Aspergillus* via current ELISA methodology, which might lead to false negatives for patients with CPA; (b) infections with non-*fumigatus Aspergillus* having less cross-immunogenicity with *A. fumigatus* that might lead to lower *A. fumigatus* IgG levels; (c) *Aspergillus*-specific antibodies detected by current ELISA methods (ImmunoCAP) had lower sensitivity in the general population in Taiwan compared to other countries; (d) not enrolling patients with ABPA because of a limited sample size in our preliminary results; or (e) a study population that was not solely patients with a history of anti-TB chemotherapy who might have higher *Aspergillus* IgG levels²³ with more patients infected with *A. fumigatus* among them.²⁴ Nevertheless, this is the first investigation to evaluate the cut-off value for *A. fumigatus* IgG and *A. flavus* IgG to identify the CPA cases in Taiwan. We suggest more patients be included in the future as well as patients with a TB history concurrent with pulmonary aspergillosis.

This study excluded patients with a history of asthma because asthma or ABPA were mediated by type IV immune reactions, and IgE was increased in response to colonisation by *Aspergillus* and other fungi,²⁵ rather than chronic or subacute infection by *Aspergillus spp.* In an investigation of ABPA,²⁶ fungi other than

Aspergillus spp. also had a significant influence on acute exacerbation of asthma. In our study, we assumed that colonisation of non-*Aspergillus* fungi rather than infection could interfere with the estimation of the cut-off value for *Aspergillus*-specific IgG if including patients with asthma or ABPA. Further evaluation of fungal colonisation, IgG levels, comorbidities, central bronchiectasis and frequency of asthma acute exacerbation were considered in epidemiological research in Taiwan.

In this study, we demonstrated a high collinearity between serum *A. fumigatus*- and *A. flavus* IgG levels both in the CPA and control groups. Our data also demonstrated that culture positivity for *Aspergillus* was not correlated to serum levels of *Aspergillus* IgG. Disease status was the only major factor associated with levels of *Aspergillus* IgG. Accordingly, a high level of *Aspergillus* IgG can help identify individuals with pulmonary *Aspergillus* infection and these levels were CPA stage-related. We found two individuals with borderline levels of *A. fumigatus* IgG (less than 21.7 mgA/L) but with positive *A. flavus* IgG (>22.1 mgA/L). Both patients were presented with CPA on chest CT and responded to anti-mould monotherapy. We suggest that serum *A. flavus* IgG helped to improve diagnostic sensitivity and confirm CPA if identification of filamentous fungi or GM tests were not available or had negative findings.

In this study, the history of MTB infection was similar between control and CPA groups (16% controls, 22.9% CPA, $P > .05$). The cut-off value was not markedly different for patients with a history of anti-TB treatment. However, the status of the previous anti-TB treatment slightly influenced the sensitivity and specificity (Figure S2). According to the best cut-off values for *A. fumigatus* IgG (21.7 mgA/L) and *A. flavus* IgG (22.1 mgA/L), for individuals with anti-TB treatment, the test had higher sensitivity (83.3% for *A. fumigatus*, 100% for *A. flavus*) than for individuals without a history of anti-TB treatment (82.8% for *A. fumigatus*, 85.7% for *A. flavus*). In contrast, the specificity was lower for individuals with a history of anti-TB treatment (87.5%, 66.7% vs 97.2% for *A. fumigatus*, 100% for *A. flavus*). Further investigation into the study of patients with a history of anti-TB treatment to determine the cut-off value for CPA for MTB-infected patients should be considered.

In this study, we found more patients with CNPA or Aspergilloma than with CFPA, with a proportion of four categories of CPA similar to a previous study.²⁴ We suggest that this result might be due to mycological prevalence, immunological status of patients, patient selection in a hospital-based study and racial differences. Nevertheless, we enrolled patients and provided evidence that they could be considered to have *Aspergillus* infection.

Rather than the healthy donors, we enrolled control groups that presented with pneumonia and respiratory tract infections that resolved without anti-mould treatment. The reasons for choosing this group were the availability of a comprehensive mycological survey, to evaluate the existence of *Aspergillus* in the respiratory tract and to identify true control groups with pulmonary illness not related to *Aspergillus*. This scenario would be similar to a clinic or hospital.

Aspergillus is a well-known, air-transmitted fungus that may colonise the nasal cavity and cause a variety of outcome. In this study, we excluded patients who had sinusitis by checking sinus radiography or referring to otolaryngologists to exclude the possibility of chronic fungal sinusitis. However, among patients with high serum *Aspergillus*-specific IgG and normal chest radiography, one might expect to see other sources of *Aspergillus* colonisation such as the rhinal or sinus cavities. Further investigation of the relationship between *Aspergillus*-specific IgG levels, nasal colonisation and fungal sinusitis needs to be conducted.

The limitations of the study include a small sample size, lack of genetic sequencing and identification of unknown filamentous fungi, and lack of tests for blood GM for all individuals due to financial constraints and lack of coverage by National Health Insurance in Taiwan. Despite these limitations, we enrolled patients with comprehensive surveys for mycology and treatment response without anti-mould reagents to avoid misclassification, and cut-off values were similar to previous studies. Patients in disease control groups were frequently observed in clinical practice, and the results could help doctors to identify *Aspergillus*-infected patients and initiate appropriate anti-mould therapy.

ACKNOWLEDGMENTS

The study was funded by the Ministry of Science and Technology in Taiwan (MOST 108-2635-B-075-002) and Taipei Veterans General Hospital (109DHA0100248).

AUTHOR CONTRIBUTIONS

Dr SFH, the main author: Design, study, manuscript writing and proof-reading. Professor FDW, equal corresponding author: Enrolment of participants and funding. Professors YJC and YYY, co-authors: Laboratory resources and identification. SYL, a co-author: Fungi identification. YCH L, a co-author: Specimen collection and database establishment.

ORCID

Shiang-Fen Huang  <https://orcid.org/0000-0003-0390-9108>

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Huang S-F, Li S-Y, Chan Y-J, Huang Y-C, Yang Y-Y, Wang F-D. Diagnostic cut-off value for *Aspergillus fumigatus*- and *flavus*-specific IgG with clinical relevance in chronic pulmonary *Aspergillus* infection: A pilot study in Taiwan. *Mycoses*. 2020;63:1083-1093. <https://doi.org/10.1111/myc.13143>