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ORIGINAL ARTICLE

Comparison of *Aspergillus* galactomannan antigen testing with a new cut-off index and *Aspergillus* precipitating antibody testing for the diagnosis of chronic pulmonary aspergillosis

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ABSTRACT

Background and objective: **The usefulness of two tests in the serodiagnosis of chronic pulmonary aspergillosis (CPA) was compared. The tests were the serum *Aspergillus* galactomannan antigen test (Platelia (R) *Aspergillus*) by enzyme-linked immunoassay (EIA) using old and new cut-off indexes, and the *Aspergillus* precipitating antibody test.**
Methods: **Both *Aspergillus*-precipitating antibody and Platelia *Aspergillus* EIA positivity were measured in the sera of 28 patients at the time of diagnosis of CPA.**
Results: **Serum *Aspergillus* precipitating antibody positivity was 89.3% (25/28) in CPA patients. Serum Platelia *Aspergillus* EIA positivity was 21.4% (6/28) using the old cut-off index (≥1.5) and 50% (14/28) using the new cut-off index (≥0.5)—still less than that for *Aspergillus* precipitating antibody. Three of the 28 CPA patients had positive reactions in the Platelia *Aspergillus* EIA using the old cut-off index but not in the *Aspergillus* precipitating antibody test. Positivity for (1,3) β-δ glucan was 15.4%, and that for culture on CHROMagar Candida was 17.9%. One patient with pulmonary actinomycosis had a false-positive reaction in the Platelia *Aspergillus* test with the new cut-off index.**
Conclusions: **For the diagnosis of CPA, *Aspergillus* precipitating antibody testing is more sensitive than the Platelia *Aspergillus* EIA, even with the new cut-off index. False-positive reactions are observed with the Platelia *Aspergillus* EIA in patients with conditions such as pulmonary actinomycosis. Results should be interpreted with care when patients are positive for the Platelia *Aspergillus* EIA but negative for *Aspergillus* precipitating antibody.**

SUMMARY AT A GLANCE

The *Aspergillus* galactomannan antigen test (Platelia *Aspergillus*), using old and new cut-off indices, and the *Aspergillus* precipitating antibody test were performed on sera of 28 patients with chronic pulmonary aspergillosis (CPA). For serodiagnosis of CPA, *Aspergillus* precipitating antibody testing was more sensitive than the Platelia *Aspergillus* test, even with the new cut-off index.

Key words: *Aspergillus* galactomannan antigen, *Aspergillus* precipitating antibody, chronic necrotizing pulmonary aspergillosis, chronic pulmonary aspergillosis, pulmonary aspergilloma.

INTRODUCTION

Chronic pulmonary aspergillosis (CPA) is characterized by: (i) the existence of underlying bronchopulmonary disease (e.g. a previous tuberculosis infection, COPD, bronchiectasis) or systemic disorders (e.g. diabetes mellitus, alcohol dependence, chronic hepatitis, use of corticosteroids), which cause deterioration in local or systemic defences against infection; and (ii) various radiographic findings (e.g. cavitation, fungus balls, consolidation, pleural thickening).[1–4] Although CPA has been given a variety of names (e.g. simple aspergilloma,[5] complex aspergilloma,[5] semi-invasive aspergillosis,[6] chronic necrotizing pulmonary aspergillosis,[7] or chronic cavitary and fibrosing pulmonary and pleural aspergillosis[2]), in Japan it has now been classified into pulmonary aspergilloma (PA) and chronic necrotizing pulmonary aspergillosis (CNPA)

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in the recently published guidelines for the management of deep-seated mycoses.[8]

In patients with CPA, it is often difficult to obtain microbiological proof of the presence of the fungus,[8,9] and in many cases there is no choice but to depend on serodiagnosis using *Aspergillus* antibody or antigen tests. Generally, serum *Aspergillus* precipitating antibody tests tend to show positive reactions in patients with PA.[8,10–13] In contrast, many cases of CNPA are diagnosed by positive reactivity to serum *Aspergillus* galactomannan antigen testing (Platelia (R) *Aspergillus*).[8] Few studies have investigated the rates of positivity to both serum *Aspergillus* precipitating antibody and Platelia *Aspergillus* tests among patients with CPA.[14–16] Moreover, the cut-off index in the Platelia *Aspergillus* enzyme-linked immunoassay (EIA), the most popular of the *Aspergillus* galactomannan antigen tests, was recently decreased in France and Japan, in light of the report by Marr *et al.*[17] However, there has not yet been sufficient examination of the influence of this change on the early diagnosis of CPA. We therefore considered it necessary to evaluate and compare the rates of positivity between the *Aspergillus* precipitating antibody test and the Platelia *Aspergillus* EIA test (using both the old and new cut-off indexes) in the serodiagnosis of CPA.

METHODS

Collection of patient records and inclusion criteria

A retrospective review of medical records was performed, and data were extracted from 80 patients who had been diagnosed with pulmonary aspergillosis (excluding allergic bronchopulmonary aspergillosis[18]) by chief physicians at the National Hospital Organization Fukuoka-Higashi Medical Center between January 1995 and December 2007. The patient records were checked by two of the authors (Y.K. and Y.T.) to confirm that they satisfied the diagnostic criteria for CPA.

In accordance with the Japanese guidelines[8] and the recent report by Camuset *et al.*,[16] the following criteria were defined as diagnostic for CPA: (i) existence of underlying bronchopulmonary disease (e.g. history of previous tuberculosis infection, COPD, bronchiectasis) or systemic disorders (e.g. diabetes mellitus, alcohol dependence, chronic hepatitis, use of corticosteroids) causing a deterioration in local or systemic defences against infection; (ii) non-acute progression (more than 2 weeks); (iii) compatible CXR findings (e.g. cavitation, fungus balls, consolidation, pleural thickening); and (iv) positive results in serological tests for *Aspergillus* (*Aspergillus* precipitating antibody or *Aspergillus* galactomannan antigen).

Aspergillus precipitating antibody tests were performed in the hospital clinical laboratory, using an *Aspergillus* immunodiffusion system (Microgen Bioproducts Ltd., Camberley, Surrey, UK); tests were judged to be positive if immunoelectrophoresis showed at least one distinct precipitation arc (Fig. 1). Platelia *Aspergillus* tests were performed by BML Inc. (Tokyo, Japan) using the Platelia *Aspergillus* EIA (Bio-Rad, Hercules, CA, USA). The old cut-off index was ≥1.5 and the new cut-off index was set as ≥0.5.

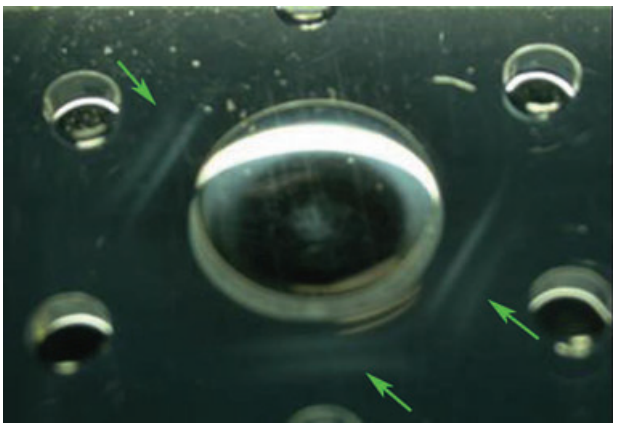

Figure 1 Typical example of a positive result for *Aspergillus* precipitating antibody. Three precipitation arcs (arrows) are visible.

Patients who satisfied all of the above criteria (i–iv) were assumed to have had a probable clinical diagnosis of CPA (defined as 'probable disease' in this study). If there was documented evidence of *Aspergillus* species in samples (sputum, bronchial washings, or other pulmonary or pleural specimens) by culture or pathological examination, then patients were considered to have definite, mycologically proven CPA (defined as 'definite disease').

The following patients were carefully excluded from the study: (i) patients with negative results in both *Aspergillus* precipitating antibody and Platelia *Aspergillus* testing; (ii) patients with obvious invasive pulmonary aspergillosis (IPA),[19–21] 'airway colonization'[22] or allergic bronchopulmonary aspergillosis;[18] and (iii) patients with false-positive reactions in the Platelia *Aspergillus* EIA test.

Review of patient records in accordance with these diagnostic criteria resulted in the exclusion of 17 patients. Thirteen did not satisfy the diagnostic criteria, two had IPA, one had airway colonization, and one patient with pulmonary actinomycosis had a false-positive reaction in the Platelia *Aspergillus* test. Among the remaining 63 patients with CPA, 28 were positive for both serum *Aspergillus* precipitating antibody and Platelia *Aspergillus* EIA at the time of diagnosis. Among these patients, we classified those who satisfied the following diagnostic criteria as having CNPA, and the remaining patients as having PA.

Diagnostic criteria for CNPA

On the basis of the Japanese guidelines[8] and the recent report by Kohno *et al.*,[23] the following criteria were defined as diagnostic for CNPA: (i) chronic symptoms, including at least one of fever, cough, excessive sputum production, haemoptysis and dyspnoea; (ii) radiological findings such as bronchopulmonary cavitation and/or consolidation, developing over a time frame of several weeks to several months; (iii) positive

results in serological tests (*Aspergillus* precipitating antibody and/or *Aspergillus* galactomannan antigen) or documented evidence of *Aspergillus* species in a sample (sputum, bronchial washings, or other pulmonary or pleural specimens) by culture or pathological examination; (iv) elevated inflammatory markers, including at least one of elevated WCC, CRP or ESR; and (v) exclusion of other pulmonary diseases such as other mycoses, *Mycobacterium* infections or other bacterial infections. The classification of CPA patients into PA and CNPA groups was performed by two of the authors (Y.K. and Y.T.).

Data collected

The following data, recorded at the time of diagnosis, were collected from the 28 patients included in the study: age, gender, presence of underlying pulmonary diseases, presence of systemic disorders, history of treatment with systemic or inhaled corticosteroids, presence of symptoms (temperature ≥37.5°C, cough, sputum, haemoptysis, dyspnoea), laboratory data (WCC, CRP), results of serum *Aspergillus* precipitating antibody testing, Platelia *Aspergillus* EIA testing and (1,3) β-D glucan testing (Fungitec G test; Seikagaku Corporation, Tokyo, Japan, measured by BML Inc.; ≥20 pg/mL judged as positive), the results of examination of mycological samples by positive direct microscopy and/or positive mycological culture on CHROMagar Candida (CHROMagar, Paris, France), CXR and CT findings (presence of bronchopulmonary cavitation, fungus balls, consolidation, pleural thickening, or interstitial or fibrotic changes) and the number of affected lung lobes. These data were collected anonymously, with consideration for the protection of personal privacy. This retrospective clinical study was approved by the internal review board of the National Hospital Organization Fukuoka-Higashi Medical Center.

Statistical analysis

All data were expressed as means (±SD) or medians (range). Categorical variables were expressed as percentages and compared using Fisher's exact test or Student's *t*-test. A *P*-value <0.05 was considered to indicate statistical significance.

RESULTS

Characteristics of the patients

Twenty-two were men and six were women. All patients except two had at least one underlying pulmonary disease or disorder, including previous tuberculosis infection, COPD, bronchiectasis or previous surgery for lung cancer. All patients presented with at least one of the following CXR and/or chest CT findings: bronchopulmonary cavitation ($n = 26$), consolidation of lung fields ($n = 25$), pleural thickening ($n = 23$) or fungus balls ($n = 18$) (Table 1). On the basis of the criteria described previously, 16 patients (57.1%) were diagnosed with CNPA and 12 (42.9%) were diagnosed with PA.

Rates of positivity in serum *Aspergillus* precipitating antibody testing and Platelia *Aspergillus* EIA

Among the 28 patients with CPA, positivity for serum *Aspergillus* precipitating antibody was 89.3% (25/28) (Table 2). It is noteworthy that 4 of these 25 patients were initially negative in the *Aspergillus* precipitating antibody tests but became positive later. Of these four patients, two had stable PA, and two had CNPA. No patient had community-acquired *Aspergillus* pneumonia, which might evolve into CPA. There was no evidence that patients with seroconversion showed faster disease progression. Figure 2 shows the results of serum *Aspergillus* galactomannan antigen testing (Platelia *Aspergillus* EIA) in patients with PA ($n = 12$) and CNPA ($n = 16$). Positivity for serum *Aspergillus* galactomannan antigen was 21.4% (6/28) with the old cut-off index (≥1.5) and improved to 50.0% (14/28) with the new cut-off index (≥0.5), but the rate of positivity was still inferior to that of the *Aspergillus* precipitating antibody test ($P = 0.0015$). Among the 12 patients with PA, positivity in Platelia *Aspergillus* testing was 0% using the old cut-off index and 41.7% (5/12) using the new cut-off index. Among these patients, positivity for *Aspergillus* precipitating antibody was 100%.

Among the 16 patients with CNPA, positivity for *Aspergillus* precipitating antibody was 81.3% (13/16), whereas positivity in Platelia *Aspergillus* testing with the new cut-off index was 56.3% (9/16). Although positivity in the Platelia *Aspergillus* test was lower than that in the *Aspergillus* precipitating antibody test, this difference was not significant ($P = 0.13$). Moreover, at the time of diagnosis, three patients showed negative reactions for *Aspergillus* precipitating antibody but were positive in the Platelia *Aspergillus* EIA with the old cut-off index (Table 3).

Clinical data (age, gender, underlying diseases, symptoms, laboratory data, radiological findings, number of affected lobes and prevalence of CNPA) were compared between the 14 CPA patients who showed positive reactions in the Platelia *Aspergillus* test using the new cut-off index, and the 14 who showed negative reactions. There were no significant differences between the two groups (data not shown). Positivity for (1,3) β-D glucan at the time of diagnosis was 15.4% (4/26). Figure 3 shows the levels of (1,3) β-D glucan in serum of patients with PA ($n = 12$) or CNPA ($n = 14$). The (1,3) β-D glucan test was not performed at the time of diagnosis in 2 of 16 CNPA patients. Positive culture findings (all *Aspergillus* spp.) were obtained for only five patients (17.9%).

No data were available on *Aspergillus* antigen in BAL fluid, total serum IgE or *Aspergillus*-specific IgE in the 28 CPA patients. *Aspergillus* antibody can also be measured using the Phadia ImmunoCap system (Phadia, Uppsala, Sweden), which can provide data on *Aspergillus*-specific IgE. However, that system was not used in this study.

Table 1 Characteristics of the 28 patients with chronic pulmonary aspergillosis (CPA)

Age (years), mean	65.9 ± 11.6 (range 46–87)
Gender	
Male	22 (78.6%)
Female	6 (21.4%)
BMI (kg/m^2), mean	17.9 ± 2.7
Subtypes of CPA	
Pulmonary aspergilloma	12 (42.9%)
Chronic necrotizing pulmonary aspergillosis	16 (57.1%)
Underlying pulmonary diseases or disorders (including recurrences)	Total 26 patients (92.9%)
Post-tuberculous infection	12 (42.9%)
COPD	6 (21.4%)
Post-surgery for lung cancer	4 (14.3%)
Chronic bronchitis/bronchiectasis;	3 (10.7%)
Interstitial pneumonias	3 (10.7%)
Bulla/cystic lung disease	2 (7.1%)
Non-tuberculous mycobacterium	2 (7.1%)
Pneumoconiosis	1 (3.6%)
Underlying systemic diseases or disorders (including recurrences)	Total 13 patients (46.4%)
Diabetes mellitus	7 (25.0%)
Alcohol dependence	4 (14.3%)
Chronic hepatitis	2 (7.1%)
Post-gastrectomy	2 (7.1%)
Other	4 (14.3%)
Use of systemic corticosteroids (10 and 5 mg/day of oral prednisolone)	2 (7.1%)
Respiratory symptoms	
Cough	17 (60.7%)
Fever ≥ 37.5°C	14 (50.0%)
Excessive sputum production	8 (28.6%)
Dyspnoea	8 (28.6%)
Haemoptysis	3 (10.7%)
Radiological findings	
Cavitation	26 (92.9%)
Consolidation	25 (89.3%)
Pleural thickening	23 (82.1%)
Fungus ball	18 (64.3%)

Table 2 Results of serological tests

	Positive for *Aspergillus* precipitating antibody	Positive for Platelia *Aspergillus* EIA	*P*-value
Total number 28	25 (89.3%)	Old COI: 6 (21.4%)	<0.001
		New COI: 14 (50.0%)	0.0015
PA (n = 12)	12 (100.0%)	Old COI: 0 (0.0%)	<0.001
		New COI: 5 (41.7%)	0.0022
CNPA (n = 16)	13 (81.3%)	Old COI: 6 (37.5%)	0.0015
		New COI: 9 (56.3%)	n.s. (0.1300)

CNPA, chronic necrotizing pulmonary aspergillosis; COI; cut-off index; n.s., not significant; PA, pulmonary aspergilloma.

False-positive reaction in Platelia *Aspergillus* testing with the new cut-off index

A 60-year-old man had a negative reaction for serum *Aspergillus* precipitating antibody and a false-positive reaction for Platelia *Aspergillus* with the new cut-off index (the result was 0.8). Cultures of sputum and bronchial washings were obtained several times, but *Aspergillus* was not detected. A pneumonectomy was performed. Histopathological examination revealed a cluster of Gram-positive filamentous *Actinomyces* inside the chest cavity.

DISCUSSION

Chronic pulmonary aspergillosis develops in the presence of, or after, underlying bronchopulmonary

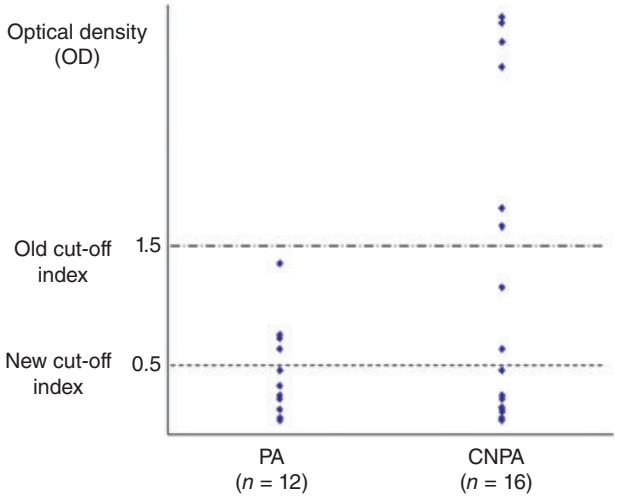

Figure 2 The results of Platelia *Aspergillus* EIA in patients with pulmonary aspergilloma (PA) and chronic necrotizing pulmonary aspergillosis (CNPA).

or systemic diseases, and it has various radiographic manifestations, including bronchopulmonary cavitation, fungus balls, infiltration and pleural thickening.[1–4] The results from this study highlighted the existence of various underlying diseases and radiographic findings in CPA.

Detection of *Aspergillus* antibody and/or antigen is used frequently for the serodiagnosis of CPA.[8,10–13] However, there have been few comparisons of the rates of serum positivity for *Aspergillus* antibody and antigen in patients with CPA.[14–16] In addition, EIA methods such as the Platelia *Aspergillus* test measuring serum *Aspergillus* galactomannan antigen have become mainstream and have recently replaced conventional latex agglutination assays such as the Pastrex *Aspergillus* test because of their superior sensitivity.[24,25] Marr *et al.* recently examined the performance of galactomannan EIA using 986 serum samples from 67 patients with invasive aspergillosis; the results showed that decreasing the cut-off index for positivity to 0.5 increased the sensitivity of the test with minimal loss of specificity.[17] On the basis of this report, the cut-off index has been changed from ≥1.5 to ≥0.5 in France (since March 2006) and Japan (since November 2006). However, this change of index was based on clinical data from patients with invasive aspergillosis due to immune disorders, and the effect of the change on the diagnosis of early CPA has not been examined.

Fujiuchi *et al.* reported that in Japan, the rate of serum positivity for *Aspergillus* antigen in patients with PA and underlying pulmonary disease was 21.8% (14/64).[14] Ogawa *et al.* reported that the rate of serum positivity for *Aspergillus* antigen in CPA patients was 11% (5/45).[15] In a recent study from France, Camuset *et al.* tested for *Aspergillus* antigen in 15 of 24 CPA patients (all of whom were positive for *Aspergillus* precipitating antibody); the results were negative in 13 patients (87%) and positive in only two.[16] However, in these reports, details of the methods used to measure *Aspergillus* antigen and the cut-off indices used were unclear. Therefore, the present study is the first to have evaluated the rates of positivity for the Platelia *Aspergillus* EIA using the old and new cut-off indices, and also for *Aspergillus* precipitating antibody testing, in order to assess the usefulness of each method in the serodiagnosis of CPA. The results suggest that the rate of positivity in the Platelia *Aspergillus* EIA for patients with CPA improved from 21.4% to 50.0% when the new cut-off index was used, but this was still inferior to that of *Aspergillus* precipitating antibody testing and was especially low in patients with PA.

In patients with CNPA, although the rate of positivity for Platelia *Aspergillus* testing using the new cut-off index was lower than that of *Aspergillus* precipitating antibody testing, the difference between the methods was not significant. Moreover, three patients showed negative reactions for *Aspergillus* precipitating antibody and positive reactions for Platelia *Aspergillus*. Therefore, it may be meaningful to measure both Platelia *Aspergillus* and *Aspergillus* precipitating antibody, especially when CNPA is suspected. In addition, the rate of positivity for (1,3) β-D glucan at the time of diagnosis was 15.4%. This result supports previous reports of low rates of positivity for (1,3) β-D glucan among patients with CPA.[8,14,15]

Some patients with very slowly progressive CPA may progress to IPA with the addition of corticosteroids to their treatment, particularly if they are not receiving antifungal agents. It is also possible that these patients with slowly progressive CPA may show positive reactions in Platelia *Aspergillus* tests. Therefore, two patients with CPA who had received systemic corticosteroid therapy for other diseases were evaluated; a patient with CNPA and another patient with PA were treated with oral prednisolone at doses of 5 and 10 mg per day, respectively. These two patients showed positive reactions in *Aspergillus* precipitating antibody tests but negative results for the Platelia *Aspergillus* EIA, even with the new cut-off index. Moreover, these two CPA patients did not show progression to IPA, as they had been treated with antifungal agents. Further studies will be needed to verify whether patients with CPA progress to IPA with the addition of corticosteroids to their treatment, as only two patients were examined in this study.

Because this was a retrospective clinical study, we cannot rule out the possibility of subsequent bias, and our results were based on a relatively small number of patients. In addition, some other potential limitations of this study need to be addressed. First, the disease entity and diagnostic criteria for CNPA were based on recent Japanese guidelines.[8] The initial disease entity criteria for CNPA, as proposed by Binder *et al.*,[7] were based on histopathological diagnosis. Moreover, CNPA was sometimes described as a kind of IPA.[22] More recently the disease entity of CNPA has been expanded to include the concept of 'semi-invasive pulmonary aspergillosis',[6] and CNPA and PA have been recognized as a series of processes within CPA.[1,16,26] In the recent Japanese guidelines based on this interpretation,[8] CNPA was defined and described as a clinical rather than a histopathological diagnosis.

Table 3 Characteristics of three patients with chronic necrotizing pulmonary aspergillosis who showed negative reactions for *Aspergillus* precipitating antibody and positive reactions for Platelia *Aspergillus* EIA

	Patient 1	Patient 2	Patient 3
Age (years)	78	87	85
Gender	Female	Male	Female
Underlying diseases or disorders	Post-tuberculous infection, bronchiectasis, chronic hepatitis	Post-tuberculous and non-tuberculous mycobacterial infections	Post-surgery for lung cancer, diabetes mellitus
Radiological findings	Cavitation, consolidation, fungus ball	Cavitation, consolidation, pleural thickening, fungus ball	Cavitation, consolidation, pleural thickening, fungus ball
Number of affected lobes	3	4	2
Platelia *Aspergillus* EIA result	>5.0	3.1	3.2

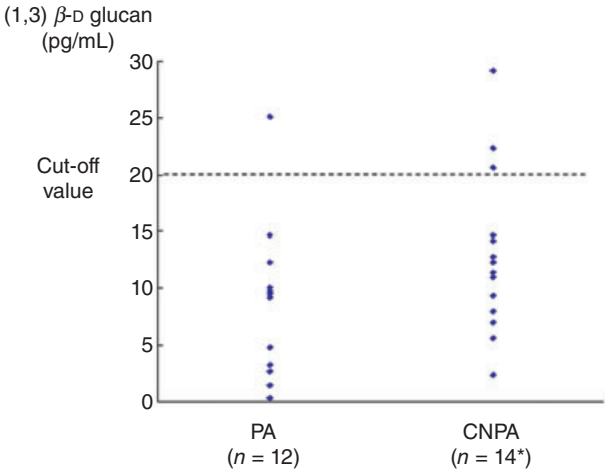

Figure 3 The concentrations of (1,3) β-D glucan in patients with pulmonary aspergilloma (PA) and chronic necrotizing pulmonary aspergillosis (CNPA). *The (1,3) β-D glucan test had not been performed for 2 of 16 patients with CNPA at the time of diagnosis.

An international consensus statement for the classification of pulmonary aspergillosis is needed.

Second, positive culture findings were obtained from only five patients, and most of the patients were clinically diagnosed as 'probable disease'. Culture on CHROMagar Candida was used for the detection of fungi and all samples were cultured for 1–3 weeks. Moreover, transbronchial samples were taken from nine patients. However, the rate of culture positivity for these transbronchial samples was also low at 22.2% (2/9). Mycological proof of pulmonary aspergillosis seemed to differ between the present study (17.9%) and previous reports.[14–16] In addition, Willinger and Manafi compared the effectiveness of CHROMagar Candida and Sabouraud glucose agar media for the detection of fungi; their results suggested that CHROMagar Candida was a useful isolation medium, but that for detection of *Aspergillus* species CHROMagar Candida was inferior to Sabouraud glucose agar.[27] Further studies to assess the most suitable agar medium for the detection of *Aspergillus* and to improve culture techniques are needed.

Third, in the evaluation of Platelia *Aspergillus* EIA tests, the problems of false-positive reactions due to various factors, such as the use of tazobactam/piperacillin,[28] the use of clavulanic acid/amoxicillin,[29] the presence of bifidobacterial lipoglycan,[30] the presence of *Cryptococcus neoformans* galactoxylomannan[31] and tube feeding with nutrients containing soybean protein,[32] have been pointed out. It can be assumed that the frequency of these false-positive reactions will increase if the cut-off index is decreased. The results for the Platelia *Aspergillus* EIA test were >1.5 in all three patients who showed negative reactions for *Aspergillus* precipitating antibody at the time of diagnosis. Among 80 patients screened for this study, none of the above-mentioned factors that could cause false-positive reactions in the Platelia *Aspergillus* EIA was present. However, there was one patient with pulmonary actinomycosis who showed a false-positive reaction in the Platelia *Aspergillus* EIA at the new cut-off index (the result was 0.8), but was negative for *Aspergillus* precipitating antibody; this patient was initially misdiagnosed as having CNPA. These facts suggest that popularization of *Aspergillus* precipitating antibody testing is more important than improving the rate of positivity in Platelia *Aspergillus* testing by decreasing the cut-off index. At the very least, the results of Platelia *Aspergillus* tests should be compared with other clinical data before a judgment is made.

Fourth, manual techniques for *Aspergillus* precipitating antibody testing, such as the adjustment of antigen, have not been standardized. In addition, other methods such as complement fixation testing and immunoprecipitation methods are available for the detection of serum *Aspergillus* antibody. However, recent research aimed at finding the most useful method for the serodiagnosis of CPA is scarce.[33] A prospective study with a larger number of patients is needed to clarify these problems. Moreover, there were four patients who were initially negative in *Aspergillus* precipitating antibody testing but later became positive. For patients in whom CPA is suspected, careful follow up, without complete disregard of any one-off negative result, appears important for diagnosis.

Fifth, *Aspergillus fumigatus* is most commonly observed in CPA. Although other species of *Aspergillus*

are thought to be rare, species such as *Aspergillus flavus* and *Aspergillus terreus* may give false-negative results in the *Aspergillus* precipitating antibody test. This study included some patients who showed only one precipitin line in the *Aspergillus* antibody test. Patients with diseases other than CPA were carefully excluded. However, the possibility of a false-positive *Aspergillus* antibody test result, as reported previously,[33] could not be completely ruled out.

Sixth, there are different cut-off values for the different assay kits for (1,3) β-D glucan testing. In addition, the US Food and Drug Administration (FDA) approved a cut-off value higher than that originally proposed by the manufacturer. The cut-off values for the US FDA-approved Fungitell kit (Beacon Diagnostics Laboratory, East Falmouth, MA, USA) and the Japanese Fungitec G test kit used in this study are ≥80 and 20 pg/mL, respectively. The Fungitell kit and Fungitec G test kit use the Xephosura strains *Limulus polyphemus* and *Tachypleus tridentatus*, respectively. The different Xephosura strains used in these assay kits may result in different cut-off values.[34] As far as we know, no comparison has been made between these two (1,3) β-D glucan assay kits. Further studies will be required to evaluate the differences in cut-off values between different assay kits for (1,3) β-D glucan testing.

Recently, new antifungal drugs such as voriconazole[16,35,36] and micafungin[37] have been suggested to be effective in the treatment of CPA. Early diagnosis is likely to mean early treatment, and both are desirable for disease control. It is interesting that three CNPA patients showed negative reactions for *Aspergillus* precipitating antibody and positive reactions in the Platelia *Aspergillus* EIA. Therefore, for early detection of CNPA at least, we recommend measurement of both *Aspergillus* galactomannan antigen and *Aspergillus* precipitating antibody.

In conclusion, the rate of positivity for *Aspergillus* galactomannan antigen using the Platelia *Aspergillus* EIA in patients with CPA was increased with the new cut-off index, but it was still inferior to that obtained with *Aspergillus* precipitating antibody testing, especially in patients with PA. However, among patients with CNPA, some showed positive Platelia *Aspergillus* reactions but negative *Aspergillus* precipitating antibody reactions. Therefore, it may be useful to measure both Platelia *Aspergillus* and *Aspergillus* precipitating antibody, especially when CNPA is suspected. However, because false-positive reactions for Platelia *Aspergillus* can occur for various reasons, care is needed in assessing patients who show positive reactions for Platelia *Aspergillus* and negative reactions for *Aspergillus* precipitating antibody.

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