Bronchoalveolar Lavage Galactomannan for the Diagnosis of Chronic Pulmonary Aspergillosis

Running title: PA EIA from BALF in CPA

[1]Koichi Izumikawa, [1]Yoshihiro Yamamoto, [1]Tomo Mihara, [1]Takahiro Takazono,

[2]Yoshitomo Morinaga, [3]Shintaro Kurihara, [1]Shigeki Nakamura, [1]Yoshifumi Imamura,

[1]Taiga Miyazaki, [1]Tomoya Nishino, [3]Misuzu Tsukamoto, [1]Hiroshi Kakeya, [2]Katsunori

Yanagihara, [4]Mariko Mine, [3]Akira Yasuoka, [5]Takayoshi Tashiro, and [1]Shigeru Kohno

[1]Department of Molecular Microbiology and Immunology, Nagasaki University

Graduate School of Biomedical Sciences, Nagasaki, JAPAN

[2]Department of Laboratory Medicine, Nagasaki University Hospital, Nagasaki, JAPAN

[3]Nagasaki University Infection Control and Education Center, Nagasaki University

Hospital, Nagasaki, JAPAN

[4]Division of Scientific Data Registry, Atomic Bomb Disease Institute, Nagasaki

University Graduate School of Biomedical Sciences, Nagasaki, JAPAN

[5]Department of Health Sciences, Nagasaki University Graduate School of Biomedical

Sciences, Nagasaki, JAPAN

Corresponding author:

Koichi IZUMIKAWA, M.D., Ph.D.

Department of Molecular Microbiology and Immunology

Nagasaki University Graduate School of Biomedical Sciences

1-7-1 Sakamoto, Nagasaki 852-8501, JAPAN

Phone: +81-95-819-7273, Fax: +81-95-849-7285

E-mail: koizumik@nagasaki-u.ac.jp

Conflicts of interest:

All authors declare no conflicts of interest in this study.

SUMMARY

Diagnosing chronic pulmonary aspergillosis (CPA) is complicated, and limited data regarding the detection of galactomannan (GM) in clinical specimens are available. The purpose of this study was to evaluate the detection of GM in bronchoalveolar lavage fluid (BALF) and serum and to assess its utility for diagnosing CPA. We retrospectively reviewed the diagnostic and clinical characteristics of 144 patients with and without CPA whose BAL and serum were examined for the presence of GM in Nagasaki University Hospital, Japan. The Platelia *Aspergillus* enzyme immunoassay (PA EIA) was performed according to the manufacturer's instructions. Of the 144 patients, 18 had CPA and 126 patients did not have CPA. The mean values of BALF GM antigen were 4.535 (range, 0.062–14.120) and 0.430 (range, 0.062–9.285) in CPA and non-CPA patients, respectively. The mean values of serum GM antigen were 1.557 (range, 0.232–5.397) and 0.864 (range, 0.028–8.956) in CPA and non-CPA patients, respectively. PA EIA with BALF is superior to PA EIA with serum, according to receiver operating characteristics analysis; the optimal cut-off values for BALF and serum were 0.4 and 0.7, respectively. The sensitivity and specificity of PA EIA with BALF at a cut-off of 0.4 were 77.2% and 77.0%, respectively. The sensitivity and specificity of PA EIA with serum at a cut-off of 0.7 were 66.7% and 63.5%, respectively.

GM testing using BALF showed reasonable sensitivity and specificity as compared to

that using serum. Thus, assessing GM levels in BALF may enhance the accuracy of

diagnosing CPA.

INTRODUCTION

Chronic forms of pulmonary aspergillosis (CPA) are known as a slowly progressing inflammatory pulmonary syndrome caused by *Aspergillus* [1, 2]. These chronic forms include semi-invasive aspergillosis [3], chronic necrotizing pulmonary aspergillosis (CNPA) [4], chronic cavitary pulmonary aspergillosis (CCPA), chronic fibrosing pulmonary aspergillosis (CFPA), and simple and complex aspergilloma [5]. Hope *et al*. indicated [2] that apparently distinct entities do not exist for these subtypes, and they are usually overlapped. The common characteristics of these forms are as follows: (1) underlying pulmonary disorders (e.g., tuberculosis sequelae, bronchiectasis, chronic obstructive pulmonary disease, cystic lesions, and pulmonary fibrosis); (2) low-grade immunosuppression (e.g., low-dose steroid administration, diabetes, collagen diseases, renal disorders, or alcohol consumption) that is related to reduced host immunity; and (3) less severe findings than those of angioinvasion in histopathology [1, 5, 6].

Diagnosing CPA is problematic, which causes difficulty in establishing clear definitions of the previously stated subtypes of CPA. There are no reliable diagnostic methods for CPA, and the presence of underlying pulmonary diseases may make the problem worse, because these underlying diseases allow other microorganisms to cause co-infection with *Aspergillus*. Only *Aspergillus* antibody testing with the *Aspergillus*

immunodiffusion system is useful, as reported by several previous studies [2, 5, 7, 8].

Aspergillus galactomannan (GM) is a polysaccharide component of the fungal cell wall that is released during tissue invasion by *Aspergillus* hyphae. A commercially available Platelia *Aspergillus* enzyme-linked immunosorbent assay (PA EIA; Bio-Rad Laboratories, Redmond, WA) detects the GM of most *Aspergillus* species. The overall sensitivity of PA EIA with serum is approximately 61–71%, with a specificity of 89–93% for invasive aspergillosis [9]. However, a fairly low positive rate was obtained among CPA patients in limited studies [7, 8, 10]; therefore, this method is currently not considered useful for CPA diagnosis.

Bronchoscopy with bronchoalveolar lavage (BAL) is an important tool for diagnosing pulmonary infections including fungal infection. The use of testing BAL fluid (BALF) for diagnosing invasive pulmonary aspergillosis (IPA) of various backgrounds has been reported previously [11-18]. Moreover, BALF testing compensates for the moderate sensitivity and relatively low positive predictive value (PPV) of serum GM testing.

To date, very few studies have been performed on PA EIA with BALF in CPA patients. The objective of this study was to assess the use of the PA EIA with BALF for the diagnosis of CPA.

MATERIALS AND METHODS

Study design, BAL procedure, and GM measurement

This retrospective study was conducted between May 1, 2005 and March 31, 2009. All adult inpatients (age, ≥15 years) in the respiratory ward who had undergone BAL for the diagnosis of pulmonary infectious diseases and PA EIA with both BALF and serum at the Second Department of Internal Medicine, Nagasaki University Hospital were eligible for inclusion in this study.

Approximately 350–500 patients, including 150–200 new patients, are admitted to this respiratory department ward per year, and the most commonly observed lung disease in this department is lung cancer. BAL was performed according to the methods of individual pneumologists. The bronchus of the lobe in which consolidation or a newly appeared shadow was imaged by chest radiography or computed tomography (CT), and 20–50 ml of 0.9% sterile prewarmed saline solution was instilled with a syringe through the working channel of the bronchoscope. The total volume of saline solution instilled into the lungs was typically 150 ml, and 10–100 ml of BALF was recovered. BALF and serum samples were directly used for evaluating GM by using the PA EIA according to the manufacturer's instructions. Each BALF sample was centrifuged for 10 min at 3000 rpm, and a total of 300 µl of supernatant was used for the measurement. Patients with a

provisional diagnosis of lower respiratory infection were assessed by each study

investigator to confirm the diagnosis. Clinical information such as age, sex, existing

underlying diseases, complications, prior usage of drugs (such as antibiotics including

antifungal drugs, anti-cancer drugs, immunosuppressive agents, biological agents, and

steroids), and presence of cavitary lesions and/or fungus balls on chest X-ray films,

including CT, was recorded retrospectively.

The diagnostic criteria of Hope *et al.* for all subtypes of CPA, including CNPA, CCPA,

CFPA, and simple aspergilloma, were used in this study [2]. Proposed enrollment

criteria for prospective clinical studies of CPA by Denning were also used for this study

with minor modifications [5]. Patients with CPA had to fulfill the following conditions:

(1) presence of at least one of the symptoms from a complex of fever, weight loss,

sputum, cough, hemoptysis, fatigue, and shortness of breath; (2) new infiltrates, cavity

formation, or expansion of pre-existing cavities with or without pericavitary infiltrates

and adjacent pleural thickening; (3) either a positive result in the *Aspergillus*

immunodiffusion system (Microgen Bioproducts, Ltd., Camberley, United Kingdom)

for anti-*Aspergillus* antibody in serum or for the isolation of *Aspergillus* spp. from

BALF; (4) positive findings for at least one of the inflammation markers such as white

blood cell counts, C-reactive protein levels, and erythrocyte sedimentation rate; and (5)

lack of improvement of symptoms or signs after at least 3 days of broad-spectrum

antibiotics administration. (1,3)-β-D-glucan test (cut-off = 20 pg/ml for the Fungitec G

Test, Seikagaku Corporation, Tokyo, Japan) by using serum was also performed to

obtain supportive data.

This study was approved by the ethical committee of Nagasaki University Hospital,

and informed consent was obtained from each patient before bronchoscopic

examination.

Statistical analysis

The sensitivity, specificity, PPV, and negative predictive value (NPV) were calculated

for PA EIA with serum and BALF. The Mann-Whitney U test was used to compare

paired data (means of GM testing with serum and BALF between CPA and non-CPA

groups). False-positive fractions were compared using univariate analysis, and the

chi-square test was used for qualitative variables. Correlation coefficients between

variables were calculated. Multivariate logistic regression analysis was performed using

the variables that were selected from univariate analysis ($P < 0.1$) and correlation

analysis ($|r| > 0.224$). The optimal threshold level for PA EIA with serum and BALF was

determined by receiver operating characteristics (ROC) analysis. This analysis was

performed by SPSS ver. 16, software. A *P*-value < 0.05 was considered statistically

significant.

RESULTS

Profile of recruited patients

We studied 18 CPA patients and 126 non-CPA patients (total, 144 patients). Table 1 shows the mean age, sex, and underlying diseases and conditions of the evaluated patients. The mean age of all patients was relatively high at 64.8 years, and 61.8% of these patients were men. Patients with bronchiectasis were the most dominant population, followed by patients with interstitial pneumonia, chronic obstructive pulmonary disease (COPD), and lung cancer. The mean age of the 18 CPA patients was 68.6 years, and 88.9% of these patients were men. COPD, bronchiectasis, and tuberculosis sequelae were predominant as underlying diseases in CPA patients. Other underlying diseases and conditions in non-CPA patients are also listed in Table 1.

Characteristics of CPA patients

The characteristics of CPA patients are indicated in Table 2. All the 18 patients met the disease criteria for CPA for this study. Eleven (61.1%) patients were positive for *Aspergillus* antibody titer, and 7 (38.9%) were positive for serum β-D-glucan. *Aspergillus* was isolated from the BALF of all CPA patients excluding 1 patient (case no. 8), and the most frequent isolate from BALF was *A. fumigatus* (11 patients), followed

by *A. niger* (2 patients), *A. terreus* (2 patients), *A. flavus* (1 patient), *A. nidulans* (1 patient), and unidentified *Aspergillus* spp (1 patient). Radiological analysis showed cavitary lesions with fungus balls in 12 patients (66.7%). Prior usage of antibiotics was recognized in 1 patient (case no. 8), and beta-lactams without beta-lactamase inhibitors were used by this patient. Antifungal drugs were not used by any CPA patient before PA EIA was performed.

Comparison of GM antigen titers between patients with and without CPA

The mean values of GM antigen in BALF were 4.535 (range, 0.062–14.120) in CPA patients and 0.430 (range, 0.062–9.285) in non-CPA patients. The mean values of GM antigen in serum were 1.557 (range, 0.232–5.397) in CPA patients and 0.864 (range, 0.028–8.956) in non-CPA patients. The mean GM values of PA EIA with both BALF and serum were statistically higher in CPA patients than in non-CPA patients ($P < 0.01$ for both serum and BALF).

The ROC curve in the generalized estimation equation model for PA EIA is presented in Figure 1. The area under the curve (AUC) was larger for PA EIA with BALF (0.775) than for PA EIA with serum (0.711). The optimal cut-offs of PA EIA calculated from the ROC curve were 0.4 and 0.7 for BALF and serum, respectively. Table 3 shows the

sensitivity, specificity, PPV, and NPV of PA EIA with BALF and serum for diagnosing CPA with a cut-off established by ROC analysis (0.4 for BALF and 0.7 for serum). Both the sensitivity and specificity of PA EIA were superior for BALF than for serum, which was also confirmed by the larger AUC in the ROC analysis.

Factors associated with the false positivity of GM tests in non-CPA patients

Of the 126 non-CPA patients studied, 29 (23.0%) had a positive GM titer in BALF with a cut-off of 0.4. Age, sex, and various conditions such as underlying diseases, drug usage, radiological findings, and fungal isolates from BALF were evaluated to detect the factors associated with false positivity of PA EIA with BALF. Table 4 shows the results of univariate analysis. The presence of bulla in the lungs according to radiological findings and the presence of *Penicillium* spp. in respiratory specimens statistically influenced the false-positive results of PA EIA with BALF. Multivariate analysis also identified these 2 factors as being significantly associated with false positivity of PA EIA with BALF. The *P* values for the presence of bulla and *Penicillium* spp. were 0.03 and 0.04, respectively.

DISCUSSION

CPA usually occurs in middle-aged to elderly patients with underlying chronic pulmonary diseases. It slowly progresses over months and even years, causing lung destruction as evidenced by progressive cavitation, fibrosis, and pleural thickening [10]. The 5-year survival rate of CPA patients in Korea is reported to be approximately 50%; however, epidemiological studies are limited [19]. Pre-existing or residual cavities after mycobacterial infection are common dwelling sites of *Aspergillus*. Other pulmonary diseases such as emphysematous bullae, COPD, and bronchiectasis that result in cavitation can cause CPA [2, 20]. Although early diagnosis of CPA is important, the complexity of the background of underlying pulmonary diseases may preclude a rapid and precise diagnosis of CPA. Although pathological examination is required for a definitive diagnosis of CPA, acquiring tissues by using invasive procedures may be difficult because of the underlying pulmonary diseases mentioned above. In our study, diagnosis of definite CPA cases by pathological examination was not made in all CPA cases. However, in practice most CPA patients are diagnosed using clinical, radiological, and mycological factors except pathological findings in practice.

PA EIA has been approved for the diagnosis of aspergillosis, especially IPA, with a high sensitivity and specificity at a cut-off of 0.5 by using serum. Our previous clinical study

showed a low positive rate (48.9% at a cut-off of 0.5) of detecting CPA by using serum.

Similarly, a study in Japan reported that PA EIA positivity was only 50.0% at a cut-off

of 0.5 [8]. Thus, PA EIA is not suitable for diagnosing CPA by using serum as a sample

[7]. Although the use of BALF instead of serum for the diagnosis of IPA of various

backgrounds has been reported previously [11-18], the potential use of this test in CPA

patients is very limited. The primary objective in this study was to compare the utility of

PA EIA with BALF and PA EIA with serum.

The features of CPA patients in this study were as follows: (1) various underlying

conditions such as previous tuberculosis infection, COPD, and bronchiectasis; (2)

middle-aged to elderly patients; (3) primarily male patients; and (4) a relatively high

positive rate for the *Aspergillus* antibody test (61.1%). These features matched the

findings reported in previous studies, including our recent clinical study [7, 19, 20].

Although beta-lactams with beta-lactamase inhibitors influence the result of PA EIA

with serum [21, 22], no such drugs were used in this study. Furthermore, antifungal

drugs were not administered to any patient until at least 4 weeks prior to timing of PA

EIA with BALF and serum. Thus, influence of the prior treatment of antibiotics and

antifungals on the results of PA EIA can be ignored in this study.

A comparison of actual GM antigen titers between patients with and without CPA

indicated that GM titer in the BALF and serum of CPA patients was statistically higher than that in the BALF and serum of non-CPA patients. Our attempt to estimate optimal cut-off values for PA EIA with BALF and serum from ROC curves indicated that the optimal cut-off values for BALF and serum were 0.4 and 0.7, respectively. The sensitivity and specificity of PA EIA by using the new cut-offs showed that BALF is superior to serum as the target sample for diagnosing CPA. However, no dramatic increase was observed in its utility for diagnosing CPA as compared to that reported in a similar study by Park *et al.* [23]. Park *et al.* reported that the sensitivity of PA EIA with BALF for a cut-off of 0.5 in 36 patients with single pulmonary cavity containing a fungus ball was 92% [23]. Hence, our result in 18 CPA patients showed a lower sensitivity of 72.2% at a cut-off of 0.4 (61.1% at a cut-off of 0.5). The major difference in the present study and the study by Park *et al*. was the patient population (subtypes of CPA). In our study, almost all patients had multiple cavities with or without fungus balls; moreover, the complexity of lesions in the lungs due to underlying diseases may have influenced the results of our study. Moreover, because our study was retrospective, the ability to estimate the power of the diagnostic tool was limited.

Because it is also important to assess the false-positive rate for diagnosing CPA by using the PA EIA with BALF, we examined the factors associated with false positivity

in non-CPA patients. Our data indicated that presence of bulla on a chest radiograph and detection of *Penicillium* spp. in BALF were significantly associated with false-positive results of PA EIA with BALF. Both univariate and multivariate analyses confirmed the significance of these factors. Bulla lesions can be colonized by *Aspergillus*, and PA EIA can detect GM from colonized *Aspergillus*. Moreover, because PA EIA showed cross-reactivity with some *Penicillium* spp. in an in vitro study [24], it is possible to detect *Penicillium* spp. antigens in BALF by using this method. To the best of our knowledge, this is the first study to identify a relationship between the presence of *Penicillium* spp. in BALF and false positivity of PA EIA with BALF. There were only four cases of which *Aspergillus* spp. (*A. niger*, *A. versicolor*, and *A. flavus*) were isolated from BALF by culturing BALF from 126 non-CPA patients; of these, 2 patients were indicated positive by using PA EIA (data not shown). Because *Aspergillus* is a ubiquitous fungus and may exist in any environment, it is possible that it colonizes the respiratory tract or contaminates the samples during handling. Therefore, careful assessment of the results of PA EIA with BALF is still required. Because some studies, including our previous clinical study (88.6% positivity), have reported the use of the serum *Aspergillus* precipitins test for detecting antibodies to *Aspergillus* for CPA patients [1, 7, 8, 19], a combined use of an antibody test and PA EIA with BALF but not

serum may prove potentially useful.

In conclusion, this is the first report to address the use of PA EIA with BALF for the diagnosis of CPA. This test has reasonable sensitivity and specificity at a cut-off of 0.4 as compared to the use of serum. Diagnosis of CPA requires synthetic assessment of clinical, radiological, mycological, and serological factors, and it is apparent that no single serological test is universally applicable. However, PA EIA with BALF may have a potential use for the diagnosis of CPA. Prospective and large-scaled studies are still warranted, and newer biomarkers that reflect a more precise state of CPA are also expected.

Figure legend

Figure 1. Receiver operating characteristic curve for the galactomannan test with serum and bronchoalveolar lavage fluid (BALF).The area under the curve was larger for the Platelia *Aspergillus* enzyme-linked immunosorbent assay with BALF (0.775) than that for the assay with serum (0.711).

Table 1. Characteristice of patients			
	Total (144 cases)	CPA cases (18 cases)	non-CPA cases (126 cases)
Age	64.8 (range: 23-84)	68.6 (range: 53-83)	64.2 (range: 23-84)
Sex, # of male	89 (61.8)	16 (88.9)	73 (57.9)
underlying diseases and conditions			
tuberculosis sequelae	15 (10.4)	8 (44.4)	7 (5.6)
COPD	29 (20.1)	11 (61.1)	18 (14.3)
non-mycobacterium infection	18 (12.5)	4 (22.2)	14 (11.1)
bronchiectasis	39 (27.1)	11 (61.1)	28 (22.2)
interstitial pneumonia	32 (22.2)	1 (5.6)	31 (24.6)
lung cancer	26 (18.1)	1 (5.6)	25 (19.8)
abnormal findings in chest X-ray films without symptoms			18 (14.3)
organizing pneumonia			11 (8.7)
pulmonary cryptococcosis			8 (6.3)
pneumonia	8 (5.6)	1 (5.6)	7 (5.6)
pneumocystis jirovecii pneumonia			6 (4.8)
tuberculosis			3 (2.4)
ABPM			3 (2.4)
sarcoidosis			2 (1.6)
hypersensitivity pneumonitis			2 (1.6)
drug induced pneumonitis			2 (1.6)
others			8 (6.3)

COPD, chronic obstructive pulmonary diseases; NTM. Non-tuberculosis mycobacterium; ABPM, allergic bronchopulmonary mycoses

CPA, chronic pulmonary aspergillosis

Case	Age	Sex	*Aspergillus* galactomannan antigen		*Aspergillus* antibody	serum β-D-glucan	*Aspergillus* isolated from BALF	Chest radiological findings		Prior treatment	
			BALF	serum		(pg/ml)		cavitary lesion	fungus ball	beta-lactams	antifungals
1	73	M	0.062	0.718	-	4.2	*A. fumigatus*	+	+	-	-
2	81	M	0.111	2.113	-	4.3	*A. fumigatus*	+	-	-	-
3	61	M	0.154	0.786	+	26.7	*A. nidulans*	+	+	-	-
4	76	M	0.209	3.032	-	7.4	*A. niger*	-	-	-	-
5	77	M	0.254	0.607	-	7.2	*A. fumigatus*	+	-	-	-
6	72	M	0.426	3.310	-	8.6	*A. fumigatus*	+	+	-	-
7	55	M	0.452	0.232	+	312.9	*A. fumigatus*	+	+	-	-
8	70	M	0.607	0.285	+	5.1	N.D.	+	+	+	-
9	75	M	0.736	1.302	+	17.3	*A. fumigatus*	+	+	-	-
10	60	F	1.112	2.083	+	8.4	*A. niger*	-	+	-	-
11	55	M	1.628	0.267	+	372.2	*A. fumigatus*	+	+	-	-
12	76	M	7.314	2.360	-	9.2	*A. flavus*	+	+	-	-
13	65	M	8.409	2.391	+	7.9	*A. fumigatus A. terreus*	+	+	-	-
14	53	M	8.882	0.496	+	153.7	*A. fumigatus*	+	+	-	-
15	65	M	11.370	0.650	+	39.5	*A. fumigatus*	+	+	-	-
16	80	M	11.950	1.150	-	5.4	*Aspergillus* spp.	+	-	-	-
17	57	F	13.837	5.397	+	92.3	*A. fumigatus*	+	+	-	-
18	83	M	14.120	0.852	+	28.3	*A. terreus*	+	-	-	-

Table 2. Characteristice of patients with chronic pulmonary aspegillosis

N.D., not detected; +, positive; -, negative

Table 3. Sensitivity, specificity, positive predictive value, and negative predictive value of galactomannan antigen test

	serum (%)	BALF (%)
Sensitivity	66.7	72.2
Specificity	63.5	77.0
PPV	20.7	31.0
NPV	93.0	95.1

PPV, positive predictive value; NPV, negative predictive value

Table 4. False-positive factors of galactomannan test using BALF in non-CPA cases□

	number of positive in non-CPA cases	number of GM positive cases (%)	number of GM negative cases (%)	P
Age, >65	74	19 (25.7)	55 (74.3)	0.398
Sex, Male	73	15 (20.5)	58 (79.5)	0.440
tuberculosis sequelae	7	2 (28.6)	5 (71.4)	0.719
COPD	18	3 (16.7)	15 (83.3)	0.489
NTM infection	14	6 (42.9)	8 (57.1)	0.061
bronchiectasis	28	8 (28.6)	20 (71.4)	0.428
interstitial pneumonia	31	10 (32.2)	21 (67.8)	0.159
diabetes	26	5 (19.2)	21 (80.8)	0.607
steroid	34	8 (23.5)	26 (76.5)	0.934
immunosuppressive agents	13	5 (38.5)	8 (61.5)	0.162
neutropenia	5	2 (40.0)	3 (60.0)	0.357
anti-cancer drug	5	1 (20.0)	4 (80.0)	0.870
renal failure	7	1 (14.3)	6 (85.7)	0.572
biological agents	1	0 (0.0)	1 (100.0)	0.583
cavitary lesions	13	2 (15.4)	11 (84.6)	0.490
consolidation	47	12 (25.5)	35 (74.5)	0.605
bulla	22	9 (40.9)	13 (59.1)	0.032
Penicillium spp. detection	12	6 (50.0)	6 (50.0)	0.020

COPD, chronic obstructive pulmonary disease; NTM, not-tuberculosis mycobacterium

immunosuppressive agent includes alkylating agents and methotrexate

Figure 1. Receiver operating characteristic curve for the galactomannan test with serum and bronchoalveolar lavage fluid
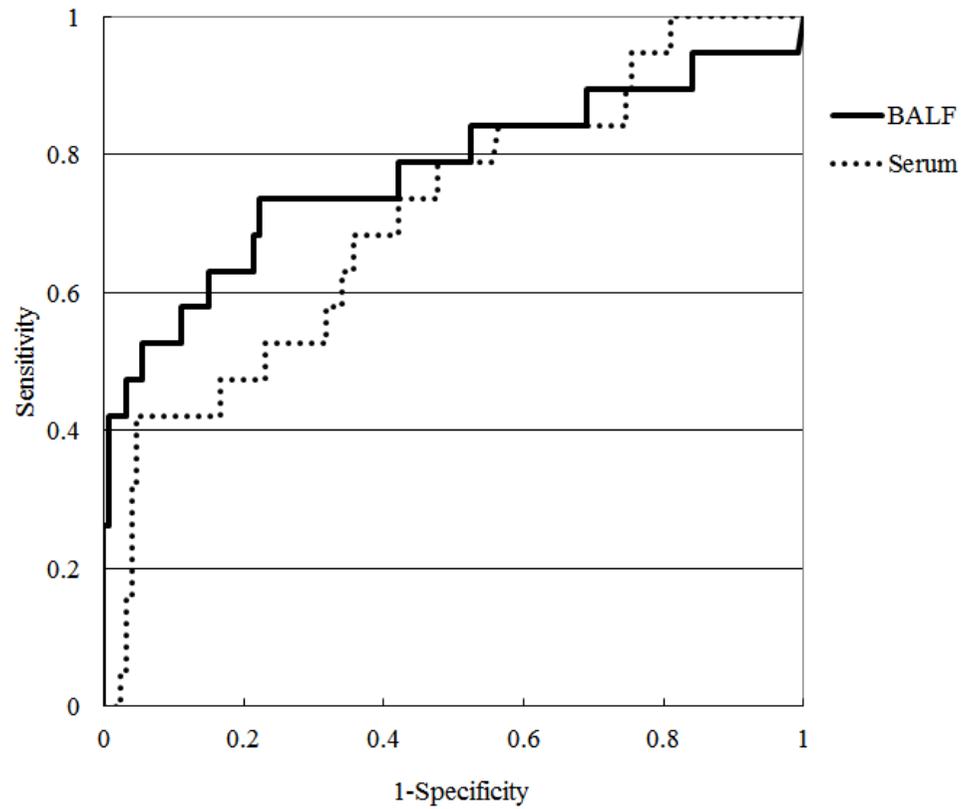
REFERENCES

1.   Denning DW. Chronic forms of pulmonary aspergillosis. *Clin Microbiol Infect* 2001; **7 Suppl 2**: 25-31.

2.   Hope WW, Walsh TJ, Denning DW. The invasive and saprophytic syndromes due to *Aspergillus* spp. *Med Mycol* 2005; **43 Suppl 1**: S207-38.

3.   Gefter WB, Weingrad TR, Epstein DM, Ochs RH, Miller WT. "Semi-invasive" pulmonary aspergillosis: a new look at the spectrum of aspergillus infections of the lung. *Radiology* 1981; **140**: 313-21.

4.   Binder RE, Faling LJ, Pugatch RD, Mahasaen C, Snider GL. Chronic necrotizing pulmonary aspergillosis: a discrete clinical entity. *Medicine (Baltimore)* 1982; **61**: 109-24.

5.   Denning DW, Riniotis K, Dobrashian R, Sambatakou H. Chronic cavitary and fibrosing pulmonary and pleural aspergillosis: case series, proposed nomenclature change, and review. *Clin Infect Dis* 2003; **37 Suppl 3**: S265-80.

6.   Saraceno JL, Phelps DT, Ferro TJ, Futerfas R, Schwartz DB. Chronic necrotizing pulmonary aspergillosis: approach to management. *Chest* 1997; **112**: 541-8.

7.   Kohno S, Izumikawa K, Ogawa K*, et al.* Intravenous micafungin versus voriconazole for chronic pulmonary aspergillosis: a multicenter trial in Japan. *J Infect* 2010; **61**: 410-8.

8.   Kitasato Y, Tao Y, Hoshino T*, et al.* Comparison of *Aspergillus* galactomannan antigen testing with a new cut-off index and *Aspergillus* precipitating antibody testing for the diagnosis of chronic pulmonary aspergillosis. *Respirology* 2009; **14**:

701-8.

9. Pfeiffer CD, Fine JP, Safdar N. Diagnosis of invasive aspergillosis using a galactomannan assay: a meta-analysis. *Clin Infect Dis* 2006; **42**: 1417-27.

10. Izumikawa K, Takazono T, Kohno S. Chronic aspergillus infections of the respiratory tract: diagnosis, management and antifungal resistance. *Curr Opin Infect Dis* 2010; **23**: 584-9.

11. Musher B, Fredricks D, Leisenring W*, et al. Aspergillus* galactomannan enzyme immunoassay and quantitative PCR for diagnosis of invasive aspergillosis with bronchoalveolar lavage fluid. *J Clin Microbiol* 2004; **42**: 5517-22.

12. Salonen J, Lehtonen OP, Terasjarvi MR, Nikoskelainen J. *Aspergillus* antigen in serum, urine and bronchoalveolar lavage specimens of neutropenic patients in relation to clinical outcome. *Scand J Infect Dis* 2000; **32**: 485-90.

13. Sanguinetti M, Posteraro B, Pagano L*, et al.* Comparison of real-time PCR, conventional PCR, and galactomannan antigen detection by enzyme-linked immunosorbent assay using bronchoalveolar lavage fluid samples from hematology patients for diagnosis of invasive pulmonary aspergillosis. *J Clin Microbiol* 2003; **41**: 3922-5.

14. Siemann M, Koch-Dorfler M. The Platelia Aspergillus ELISA in diagnosis of invasive pulmonary aspergilosis (IPA). *Mycoses* 2001; **44**: 266-72.

15. Becker MJ, Lugtenburg EJ, Cornelissen JJ*, et al.* Galactomannan detection in computerized tomography-based broncho-alveolar lavage fluid and serum in haematological patients at risk for invasive pulmonary aspergillosis. *Br J Haematol*

2003; **121**: 448-57.

16.   Meersseman W, Lagrou K, Maertens J*, et al.* Galactomannan in bronchoalveolar lavage fluid: a tool for diagnosing aspergillosis in intensive care unit patients. *Am J Respir Crit Care Med* 2008; **177**: 27-34.

17.   Knox KS, Meinke L. Role of bronchoalveolar lavage diagnostics in fungal infections. *Clin Chest Med* 2009; **30**: 355-65, viii.

18.   Husain S, Kwak EJ, Obman A*, et al.* Prospective assessment of Platelia *Aspergillus* galactomannan antigen for the diagnosis of invasive aspergillosis in lung transplant recipients. *Am J Transplant* 2004; **4**: 796-802.

19.   Nam HS, Jeon K, Um SW*, et al.* Clinical characteristics and treatment outcomes of chronic necrotizing pulmonary aspergillosis: a review of 43 cases. *Int J Infect Dis* 2010; **14**: e479-82.

20.   Smith NL, Denning DW. Underlying conditions in chronic pulmonary aspergillosis, including simple aspergilloma. *Eur Respir J* 2010.

21.   Mattei D, Rapezzi D, Mordini N*, et al.* False-positive *Aspergillus* galactomannan enzyme-linked immunosorbent assay results in vivo during amoxicillin-clavulanic acid treatment. *J Clin Microbiol* 2004; **42**: 5362-3.

22.   Viscoli C, Machetti M, Cappellano P*, et al.* False-positive galactomannan platelia *Aspergillus* test results for patients receiving piperacillin-tazobactam. *Clin Infect Dis* 2004; **38**: 913-6.

23.   Park SY, Lee SO, Choi SH*, et al.* Serum and bronchoalveolar lavage fluid galactomannan assays in patients with pulmonary aspergilloma. *Clin Infect Dis*

2011; **52**: e149-52.

24.   Swanink CM, Meis JF, Rijs AJ, Donnelly JP, Verweij PE. Specificity of a sandwich enzyme-linked immunosorbent assay for detecting *Aspergillus* galactomannan. *J Clin Microbiol* 1997; **35**: 257-60.