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ORIGINAL ARTICLE

Clinical significance of *Aspergillus* species isolated from respiratory specimens in patients with *Mycobacterium avium* complex lung disease

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Abstract Chronic pulmonary aspergillosis (CPA) is associated with mortality in patients with *Mycobacterium avium* complex lung disease (MAC-LD). An *Aspergillus*-positive respiratory specimen often reflects colonization, and thus the clinical significance of *Aspergillus* isolation in MAC-LD patients is not well understood. The objective of this study was to investigate the clinical characteristics and outcomes of MAC-LD patients in whom *Aspergillus* was isolated from respiratory specimens. We performed a retrospective review of the medical records of 329 MAC-LD patients. We compared the characteristics and mortality rates between patients with *Aspergillus* isolation and those without. All *Aspergillus* species detected from respiratory specimens within the follow-up period were reviewed. *Aspergillus* was detected in 40 (12.2%) of the 329 patients. There were no significant differences in the clinical characteristics and mortality rates between patients with and without *Aspergillus* isolation. Among the 40 patients with *Aspergillus* isolation, 9 (22.5%) developed CPA. CPA was most often caused by *A. fumigatus*. In the 40 *Aspergillus*-positive patients, patients with *A. fumigatus* isolation had a significantly higher mortality rate than those without ($P < 0.001$). The multivariate Cox proportional hazards model showed older age ($P = 0.050$), presence of respiratory comorbidities ($P = 0.008$), hypoalbuminemia ($P < 0.001$), and isolation of *A. fumigatus* ($P = 0.005$) to be prognostic factors for mortality in MAC-LD patients. There was no significant difference in the mortality rates between patients with *Aspergillus* isolation and those without. However, isolation of *A. fumigatus* may be associated with poor prognosis in MAC-LD patients.

Keywords *Mycobacterium avium* complex · *Aspergillus* species · Aspergillosis · Prognosis

Introduction

Mycobacterium avium complex lung disease (MAC-LD) is the most common form of nontuberculous mycobacterial (NTM) pulmonary infection, and the incidence of MAC-LD is increasing worldwide [1, 2]. Patients with MAC-LD often have co-infections with various pathogenic microorganisms, including *Pseudomonas aeruginosa*, *Haemophilus influenzae*, and *Aspergillus* [3]. Chronic colonization with bacteria such as *P. aeruginosa* and *H. influenzae* is associated with bronchiectasis, causing recurrent exacerbations and leading to lung function decline [4, 5].

Aspergillus is a ubiquitous fungus that causes various clinical manifestations in humans. The manifestations range from colonization of the respiratory tract with no clinical symptoms to pulmonary aspergillosis that exhibits a clinical spectrum according to the level of immune competence [6]. Chronic pulmonary aspergillosis (CPA) is a slowly progressing lung disease caused by *Aspergillus* species, which occurs in patients with underlying lung disease [7]. The number of patients with MAC-LD complicated by CPA has recently increased [8, 9]. In addition, coexisting CPA is reportedly associated with poor prognosis in MAC-LD patients [10, 11].

Although microbiological examination of respiratory specimens is used for the diagnosis of CPA, *Aspergillus*-positive

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culture often reflects colonization. Conversely, *Aspergillus* isolation from respiratory specimens in critically ill patients was reportedly associated with severe underlying disease and high mortality [12, 13]. Thus, the clinical significance of *Aspergillus* isolation in MAC-LD patients is not well understood. Moreover, no studies have examined whether isolation of *Aspergillus* is associated with poor prognosis in MAC-LD patients.

Therefore, the aim of the present study was to examine the clinical characteristics and outcomes of MAC-LD patients in whom *Aspergillus* was isolated from respiratory specimens.

Materials and methods

Patients

All patients who were newly diagnosed with MAC-LD from January 2006 to December 2011 at Kurashiki Central Hospital, Kurashiki, Japan, were included in the analysis. All patients met American Thoracic Society/Infectious Diseases Society of America diagnostic criteria for MAC-LD [14]. Patients who did not have fungal cultures of respiratory specimens during the observation period were excluded.

Study design

This was a retrospective cohort study. We abstracted clinical data including age, sex, smoking history, body mass index (BMI), complications, baseline steroid use, laboratory data, bacteriological data, radiographic features, treatment history, and outcomes from medical records. Baseline clinical parameters were obtained within 2 months of the diagnosis of MAC-LD. Radiographic features of MAC-LD were classified according to four patterns based on chest computed tomography (CT) images: nodular/bronchiectatic (NB), fibrocavitary (FC), FC + NB, and unclassifiable [15]. All chest CT images were independently reviewed by four pulmonologists and discrepancies in their readings were resolved by a consensus. For the diagnosis of MAC-LD, respiratory samples were processed using Ziehl-Neelsen staining, solid medium culture (2% Ogawa medium), and the liquid-based BACTEC MGIT 960 system (Becton Dickinson Co., USA). MAC isolates were identified using DNA-DNA hybridization for the genetic identification of mycobacteria (DDH *Mycobacterium*; Kyokuto Pharmaceutical Industrial Co. Ltd., Tokyo, Japan). Results of culture using 2% Ogawa medium were recorded using the following criteria as previously described [16]: 0, solid medium growth with 0 colonies; 1+, 1–199 colonies; 2+, 200–499 colonies; 3+, 500–2000 colonies; and 4+, >2000 colonies.

The main objective of this study was to assess the prognostic impact of *Aspergillus* species isolated from respiratory specimens (sputum, endotracheal aspirate, or bronchial washing) in patients with MAC-LD. Patients were followed through August 2015. The survival time was measured from the date of MAC-LD diagnosis until the date of death or censoring.

In addition, all patients in whom *Aspergillus* species were detected from cultured respiratory specimens within the follow-up period were reviewed and classified into the following three patterns according to clinical conditions: CPA other than simple aspergilloma (SA), SA, and colonization.

The diagnosis of CPA was based on a combination of the following: compatible clinical symptoms, compatible radiological findings, and a positive serum *Aspergillus* precipitin test (*Aspergillus* Immunodiffusion System; Mercia Diagnostics Ltd., Surrey, UK) or isolation of *Aspergillus* species from respiratory samples. SA was defined as a single fungal ball in a single pulmonary cavity, with minor or no symptoms and no radiological progression over at least 3 months of observation. These criteria are consistent with the recently published European guideline on CPA [17]. Patients were diagnosed as being colonized when *Aspergillus* was isolated without clinical symptoms and compatible radiological findings.

This study was approved by the ethics committee of Kurashiki Central Hospital (no. 2091) and was performed in accordance with the Declaration of Helsinki.

Statistical analyses

Continuous variables are expressed as medians and interquartile ranges (IQR). Categorical variables are presented as numbers and percentages. Comparisons between groups were performed using the Mann-Whitney U-test for continuous variables and Fisher's exact tests for categorical variables. The overall survival rates were calculated using the Kaplan-Meier method, and differences in the survival rates between groups were compared by the log-rank test. Univariate and multivariate analyses were performed to identify prognostic factors on the overall survival by using the Cox proportional hazards model. Variables that reached statistical significance (P value <0.05) in the univariate analysis were entered into the multivariate analysis. A P value <0.05 was considered to be significant. All statistical analyses were performed using R version 2.13.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Patient characteristics

During the study period, 358 patients were diagnosed with MAC-LD. Twenty-nine patients were excluded from the

analysis because they did not have fungal cultures, leaving 329 patients who met the inclusion criteria for analysis. The median follow-up period was 3.7 years (IQR, 2.0–5.1 years) and the median number of samples examined per case was 4 (IQR, 2–8). During the follow-up period, *Aspergillus* species were detected from 40 (12.2%) of the 329 patients. The baseline clinical characteristics of all patients are presented in Table 1. Of these 329 patients, 196 (59.6%) were women (median age, 73 years) and 211 (64.1%) had never smoked. We compared the characteristics of patients with *Aspergillus* isolation and those without. There were no significant differences in the clinical characteristics including age, sex, comorbidities, radiographic features, and induction rates of antibiotic treatment for MAC-LD between the two groups. Among the 40 patients with *Aspergillus* isolation, 9 (22.5%) patients developed CPA. In addition, 11 (3.8%) of the 289 patients without *Aspergillus* isolation were diagnosed with CPA by the *Aspergillus* precipitin test.

Table 1 Patient characteristics

Characteristics[a]	Patients without *Aspergillus* isolation (N = 289)		Patients with *Aspergillus* isolation (N = 40)		*P* value
Age, years	73	(65–78)	74	(67–79)	0.508
Male	116	(40.1)	16	(40.0)	1.000
Smoking status (former or current)	103	(37.3)	15	(39.5)	0.859
BMI, kg/m^2	19.5	(17.6–21.7)	18.5	(16.7–20.4)	0.181
Comorbidity					
Respiratory disease					
Old pulmonary tuberculosis	24	(8.3)	5	(12.5)	0.373
Pulmonary emphysema	29	(10.0)	5	(12.5)	0.584
Interstitial pneumonia	22	(7.7)	5	(12.5)	0.351
Asthma	8	(2.8)	2	(5.1)	0.339
Systemic disease					
Diabetes mellitus	36	(12.5)	5	(13.2)	0.800
Rheumatoid arthritis	16	(5.5)	3	(7.5)	0.714
Steroid use	25	(8.7)	3	(7.5)	1.000
Laboratory data					
Albumin, g/dl	4.00	(3.5–4.3)	4.00	(3.5–4.4)	0.644
Hb, g/dl	12.5	(11.3–13.6)	12.5	(11.5–13.1)	0.686
Bacteriological examinations					
Culture score ≥ 2+	59	(20.4)	8	(20.0)	1.000
Positive smear result	39	(13.5)	5	(12.5)	1.000
Radiographic features					0.649
FC	29	(10.0)	5	(12.5)	
NB	241	(83.4)	32	(80.0)	
FC + NB	7	(2.4)	2	(5.0)	
Unclassifiable	12	(4.2)	1	(2.5)	
Treatment for MAC	150	(51.9)	27	(67.5)	0.090
Deaths	12	(30.0)	55	(19.0)	0.140
Cause of death					0.434
Pneumonia	15	(27.3)	4	(33.3)	
Progression of MAC	4	(7.3)	2	(16.7)	
Pulmonary aspergillosis	2	(3.6)	2	(16.7)	
Lung cancer	6	(10.9)	0	(0.0)	
Other pulmonary disease	6	(10.9)	1	(8.3)	
Non-pulmonary diseases	18	(32.7)	3	(25.0)	
Unknown causes	4	(7.3)	0	(0.0)	

BMI body mass index, *Hb* hemoglobin, *FC* fibrocavitary disease, *NB* nodular/bronchiectatic disease, *MAC* *Mycobacterium avium* complex

[a] Data are presented as *n* (%) or median (interquartile range)

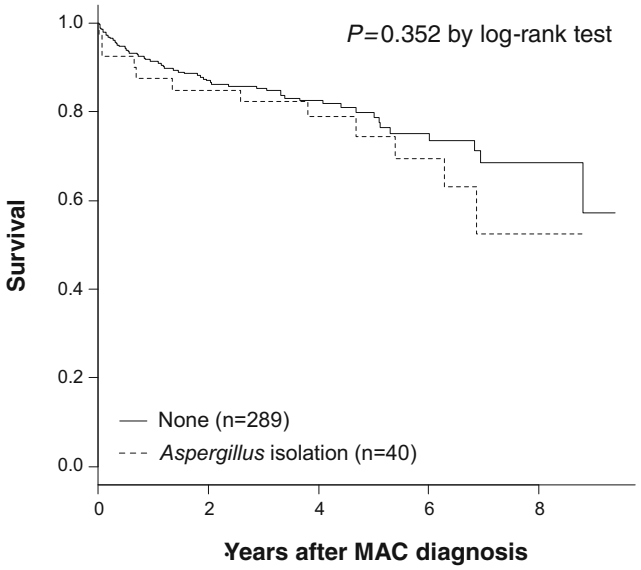

Fig. 1 Kaplan-Meier analyses of the overall survival based on the presence or absence of *Aspergillus* isolation in patients with *Mycobacterium avium* complex lung disease. The difference between the survival curves was not significant ($P = 0.352$, log-rank test)

Mortality

During the observation period, 12 (30.0%) of 40 patients with *Aspergillus* isolation and 55 (19.0%) of 289 patients without *Aspergillus* isolation died ($P = 0.140$). There was no significant difference in survival curves between patients with or without *Aspergillus* isolation ($P = 0.352$, log-rank test) (Fig. 1).

Death from any cause occurred in 67 patients. The causes of death included pneumonia ($n = 19$, 28.4%), progression of MAC-LD ($n = 6$, 9.0%), CPA ($n = 4$, 6.0%), lung cancer (n = 6, 9.0%), other pulmonary diseases ($n = 7$, 10.4%), non-pulmonary diseases ($n = 20$, 30.0%), and unknown causes ($n = 5$, 7.5%).

Aspergillus species detected from respiratory specimens

A total of 49 *Aspergillus* strains (sputum, $n = 47$; endotracheal aspirate, $n = 1$; and bronchial washing, $n = 1$) were detected from 40 MAC-LD patients during the study period (Table 2). The most frequently detected species were *A. niger* (28/49, 57.1%) and *A. flavus* (10/49, 20.4%). Thirty-seven (90.2%) of the 41 non-*fumigatus Aspergillus* strains caused colonization, whereas 5 (62.5%) of the eight *A. fumigatus* strains caused CPA. CPA was most often caused by *A. fumigatus*.

Characteristics of the 40 patients with Aspergillus isolation

A. fumigatus was detected from 8 (20.0%) of the 40 patients. Compared to patients without *A. fumigatus* isolation, patients with *A. fumigatus* isolation more frequently had interstitial pneumonia and FC disease (Table 3). In addition, patients with *A. fumigatus* isolation significantly more frequently developed CPA than those without (5/8, 62.5% vs 4/32, 12.5%; $P = 0.008$). Of the eight patients with *A. fumigatus* isolation, six died. Five of the six patients died from respiratory infectious diseases (pneumonia, $n = 2$; MAC-LD, $n = 1$; and CPA, $n = 2$).

Prognostic impact of *A. fumigatus* isolation from respiratory specimens

The overall survival of the 40 *Aspergillus*-positive patients based on the presence or absence of *A. fumigatus* isolation was estimated (Fig. 2). Patients with *A. fumigatus* isolation had a significantly higher mortality rate than those without *A. fumigatus* isolation ($P < 0.001$, log-rank test).

We also conducted univariate and multivariate analyses of factors associated with mortality in the 329 MAC-LD patients. Prognostic variables were chosen with reference to previous reports [15, 18]. In the multivariate Cox proportional hazards model, age ≥ 70 years ($P = 0.050$), presence of respiratory comorbidities ($P = 0.008$), serum albumin level < 3.5 g/dL ($P < 0.001$), and isolation of *A. fumigatus* ($P = 0.005$) were found to be independent negative prognostic factors (Table 4).

Discussion

In the present study, we aimed to investigate the clinical significance of *Aspergillus* species isolated from respiratory

Table 2 *Aspergillus* species isolated from respiratory specimens

Aspergillus species	Number of positive cultures according to clinical condition			
	CPA other than SA ($n = 8$)	SA ($n = 1$)	Colonization ($n = 40$)	Total ($n = 49$)
A. niger	2	1	25	28
A. flavus	1	0	9	10
A. fumigatus	5	0	3	8
A. terreus	0	0	3	3

CPA chronic pulmonary aspergillosis, *SA* simple aspergilloma

Table 3 Characteristics of the 40 patients with *Aspergillus* isolation

Characteristics[a]	Isolation of *A. fumigatus* (N = 8)		Isolation of non-*fumigatus* species (N = 32)		*P* value
Age, years	75.5	(71–78)	73	(67–77)	0.520
Male	4	(50.0)	12	(37.5)	0.690
Smoking status (former or current)	5	(62.5)	10	(33.3)	0.223
BMI, kg/m^2	17.5	(15.7–24.8)	18.7	(16.9–20.1)	0.783
Comorbidity					
Respiratory disease					
Old pulmonary tuberculosis	2	(25.0)	3	(9.4)	0.257
Pulmonary emphysema	2	(25.0)	3	(9.4)	0.257
Interstitial pneumonia	3	(37.5)	2	(6.2)	0.046
Asthma	0	(0.0)	2	(6.5)	1.000
Systemic disease					
Diabetes mellitus	1	(12.5)	4	(12.5)	1.000
Rheumatoid arthritis	2	(25.0)	1	(3.1)	0.096
Steroid use	2	(25.0)	1	(3.1)	0.096
Laboratory data					
Albumin, g/dl	3.80	(3.4–4.0)	4.15	(3.7–4.4)	0.312
Hb, g/dl	12.4	(12.0–12.8)	12.5	(11.4–13.3)	0.985
Bacteriological examinations					
Culture score ≥ 2+	3	(37.5)	5	(15.6)	0.320
Positive smear result	2	(25.0)	3	(9.4)	0.257
Radiographic features					0.004
FC	3	(37.5)	2	(6.2)	
NB	3	(37.5)	29	(90.6)	
FC + NB	1	(12.5)	1	(3.1)	
Unclassifiable	1	(12.5)	0	(0.0)	
Treatment for MAC	6	(75.0)	18	(56.2)	0.439
Deaths	6	(75.0)	6	(18.8)	0.005
Cause of death					0.740
Pneumonia	2	(33.3)	2	(33.3)	
Progression of MAC	1	(16.7)	1	(16.7)	
Pulmonary aspergillosis	2	(33.3)	0	(0.0)	
Other pulmonary disease	0	(0.0)	1	(16.7)	
Non-pulmonary diseases	1	(16.7)	2	(33.3)	

BMI body mass index, *Hb* hemoglobin, *FC* fibrocavitary disease, *NB* nodular/bronchiectatic disease, *MAC* *Mycobacterium avium* complex

[a] Data are presented as *n* (%) or median (interquartile range)

specimens in MAC-LD patients. *Aspergillus* was detected in 40 (12.2%) of the 329 patients. *A. fumigatus* was most commonly isolated in patients with CPA, whereas non-*fumigatus* *Aspergillus* species were more frequently found in colonized patients. We found no significant difference in the clinical characteristics and mortality rate according to the presence or absence of *Aspergillus* isolation. Conversely, MAC-LD patients with *A. fumigatus* isolation were likely to have CPA and worse mortality. To our knowledge, this is the first study to describe the prognostic impact of *Aspergillus* isolation in MAC-LD patients.

Recently, reports on NTM and *Aspergillus* co-infection have raised concern. NTM infection was reportedly associated with *Aspergillus*-related disease in patients with bronchiectasis [19]. Several studies showed that the presence of CPA is an independent prognostic factor in MAC-LD patients [10, 11]. Furthermore, Lowes et al. recently reported that NTM infection was also associated with high mortality in patients with CPA [20]. Therefore, early diagnosis of CPA in MAC-LD patients is critical. Although respiratory specimen examination is used for the diagnosis of CPA, a positive result often reflects colonization [21].

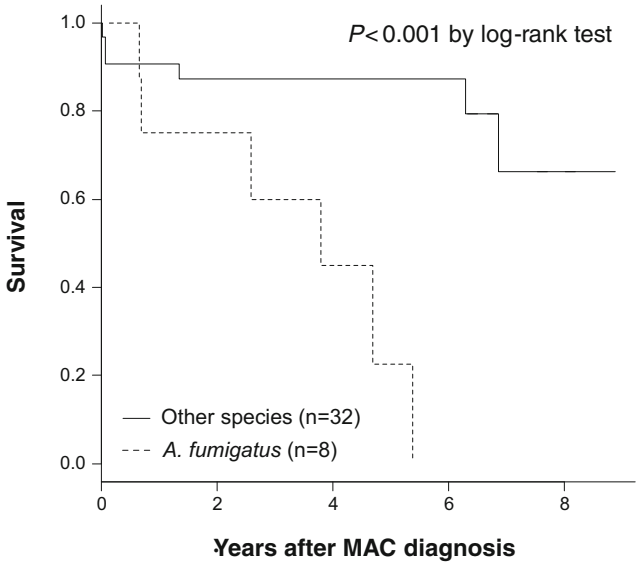

Fig. 2 Kaplan-Meier analyses of the overall survival based on the presence or absence of *A. fumigatus* isolation in patients with *Aspergillus* isolation. The difference between the survival curves was significant ($P < 0.001$, log-rank test)

In this study, *A. fumigatus* was the most frequent causative pathogen of CPA. Among hundreds of *Aspergillus* species, *A. fumigatus* is responsible for approximately 90% of pulmonary aspergillosis cases [22]. There are several reports on the pathogenesis of *A. fumigatus* in pulmonary aspergillosis. Whereas *A. fumigatus* has small conidia (2–3 μm) that allow it to reach pulmonary alveoli, other *Aspergillus* species produce larger conidia that can be removed more easily by mucociliary clearance in the upper respiratory tract [23]. Furthermore, *A. fumigatus* conidia were found to bind significantly better to both lung cell basal lamina and fibronectin than those of other *Aspergillus* species [24].

No significant differences were observed in the clinical characteristics and mortality rates between patients with *Aspergillus* isolation and those without. One possible reason contributing to these results is the high colonization rates with non-*fumigatus Aspergillus* strains. In the present study, 90.2% (37 of 41) of the isolated non-*fumigatus Aspergillus* strains were found in cases representing colonization, and the most common colonizing species was *A. niger*. Tashiro et al. reported that *A. niger* was the dominant species in colonized patients, whereas *A. fumigatus* was most commonly isolated in patients with pulmonary aspergillosis [25]. Moreover, it is reported that *A. niger* is becoming more prevalent in respiratory tract samples [25]. Our results were consistent with these observations. Non-*fumigatus Aspergillus* species may be prone to colonize due to structural changes induced by MAC-LD, regardless of the radiographic features, and may not affect the natural course of MAC-LD.

Isolation of *A. fumigatus* was a significant prognostic factor, in addition to older age, hypoalbuminemia, and presence of respiratory comorbidities, which were previously reported as predictors of mortality in MAC-LD patients [15]. Furthermore, in the 40 *Aspergillus*-positive patients, patients with *A. fumigatus* isolation had a significantly higher mortality rate than those without. There are several possible reasons why *A. fumigatus* isolation was associated with high mortality. First, as mentioned above, *A. fumigatus* is more pathogenic than other species and more frequently caused CPA. Second, *A. fumigatus* might be more frequently found in patients with more severe conditions; in this study, patients with *A. fumigatus* isolation more frequently had interstitial pneumonia and FC disease. Third, *A. fumigatus* colonization may increase the risk of bacterial infection and lead to polymicrobial interactions that promote airway pathogenesis. In cystic fibrosis patients, colonization with *A. fumigatus* was associated with an increased risk for infection with *P. aeruginosa* [26]. Moreover, the presence of *A. fumigatus* enhanced *P. aeruginosa* cytotoxic elastase production, which damages host tissues [27]. In the

Table 4 Univariate and multivariate analyses of risk factors for mortality in 329 *Mycobacterium avium* complex lung disease (MAC-LD) patients

Variable	Univariate analysis			Multivariate analysis		
	HR	95%CI	*P* value	HR	95%CI	*P* value
Age ≥ 70 years	2.965	1.616–5.439	<0.001	1.919	1.001–3.680	0.050
BMI <18.5 kg/m^2	2.138	1.245–3.669	0.006			
Respiratory comorbidities[a]	3.485	2.151–5.646	<0.001	2.106	1.216–3.648	0.008
Albumin <3.5 g/dl	6.725	4.102–11.02	<0.001	4.780	2.741–8.335	<0.001
Hb <10.0 g/dl	2.731	1.425–5.235	0.002			
FC/FC + NB	2.279	1.297–4.004	0.004			
Isolation of *A. fumigatus*	4.728	2.033–11.00	<0.001	3.546	1.435–8.092	0.005

BMI body mass index, *Hb* hemoglobin, *FC* fibrocavitary disease, *NB* nodular/bronchiectatic disease, *HR* hazard ratio, *CI* confidence interval

[a] Respiratory comorbidities include old pulmonary tuberculosis, pulmonary emphysema, interstitial pneumonia, and asthma

present study, most patients with *A. fumigatus* isolation died from respiratory infectious diseases (pneumonia, MAC-LD, and CPA). MAC-LD patients occasionally have co-infections with various microorganisms including *P. aeruginosa* and *A. fumigatus*, thus such polymicrobial interactions may occur, leading to high mortality from infectious diseases.

There are several limitations associated with this study. First, the study was retrospective and conducted at a single center, thus the frequency of microbiological examination was not uniform. Second, we excluded patients who had not been examined by fungal culture testing of respiratory specimens. However, as most of the excluded patients had less expectoration of sputum, the results would reflect a symptomatic population. Finally, the present study included a relatively small number of patients with *Aspergillus* isolation. Low statistical power may have limited our ability to detect significant differences. Prospective studies with larger patient cohorts are required to overcome these limitations.

In conclusion, isolation of non-*fumigatus Aspergillus* strains from respiratory samples often reflected colonization and was not associated with mortality in MAC-LD patients. However, isolation of *A. fumigatus* more frequently suggests infection and may be associated with poor prognosis in MAC-LD patients.

Funding No financial support was received for this study.

Compliance with ethical standards

Conflict of interest Tadashi Ishida has received honoraria from Pfizer Japan Inc. The other authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Informed consent For this type of study formal consent is not required.

Data availability The datasets used and analyzed during this study are available from the corresponding author upon request.

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