Figure 2. Comparison of inflammatory marker levels in the azithromycin-oseltamivir combination therapy and oseltamivir monotherapy groups. Serial test results (on days 0, 2, and 5) for the 8 inflammatory markers were compared between the groups with and without AZM. The horizontal and vertical lines depicted in the scattergram represent the central 50% range (25–75 percentiles) and the median, respectively. In the graphs for interleukin 8 (IL-8), IL-12, and tumor necrosis factor α (TNF-α), missing central boxes or concordance of the left end of the box with the median indicate that the majority of the test results were lower than the limit of detection.
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General Hospital; 6, NHO Ureshino Medical Center; 5, Koseikai Hospital; 0, Isahaya Health Insurance General Hospital (Not approved until the end of the study); 6, Nagasaki Municipal Hospital; 20, Onitsuka Naika Clinic; 0, Hayashida Naika Clinic; 20, Tomonaga Naika Clinic; 13, Irihune Clinic; and 5, Kawamura Clinic. All patients were recruited after the relevant project approval date.

The patients were randomized into the mono-group (56 patients) or combo-group (51 patients), and all enrolled patients were included in the ITT population (Figure 1). All patients were diagnosed with influenza A virus infection, and none of the patients had comorbid pneumonia. The participants included 50 male patients and 57 female patients, and their mean age was 43.5 years. The 2 treatment groups did not differ significantly in terms of their clinical characteristics, sex, age, underlying diseases, or disease severity (Table 1).

Inflammatory markers

The baseline values of IL-6, IL-8, IL-12, TNF-α, IL-1β, TGF-β1, PCT, and HMGB1 on day 0 before treatment allocation did not differ between the groups (Figure 2). However, the baseline values of TNF-α were statistically significantly higher in the combo-group than in the mono-group ($p = 0.03$). No statistically significant differences were observed between the 2 groups in the expression of any of the inflammatory cytokines or chemokines on days 2 and 5.

Although TNF-α levels were statistically different between the 2 groups on day 5, its value decreased below measurable limits in almost all patients, and thus no clear difference was observed.

Resolution time of influenza-related symptoms

The baseline maximal temperature on day 0 did not differ between the 2 groups ($p = 0.984$). Comparison of the maximum temperatures on days 1 to 5 showed no significant differences on

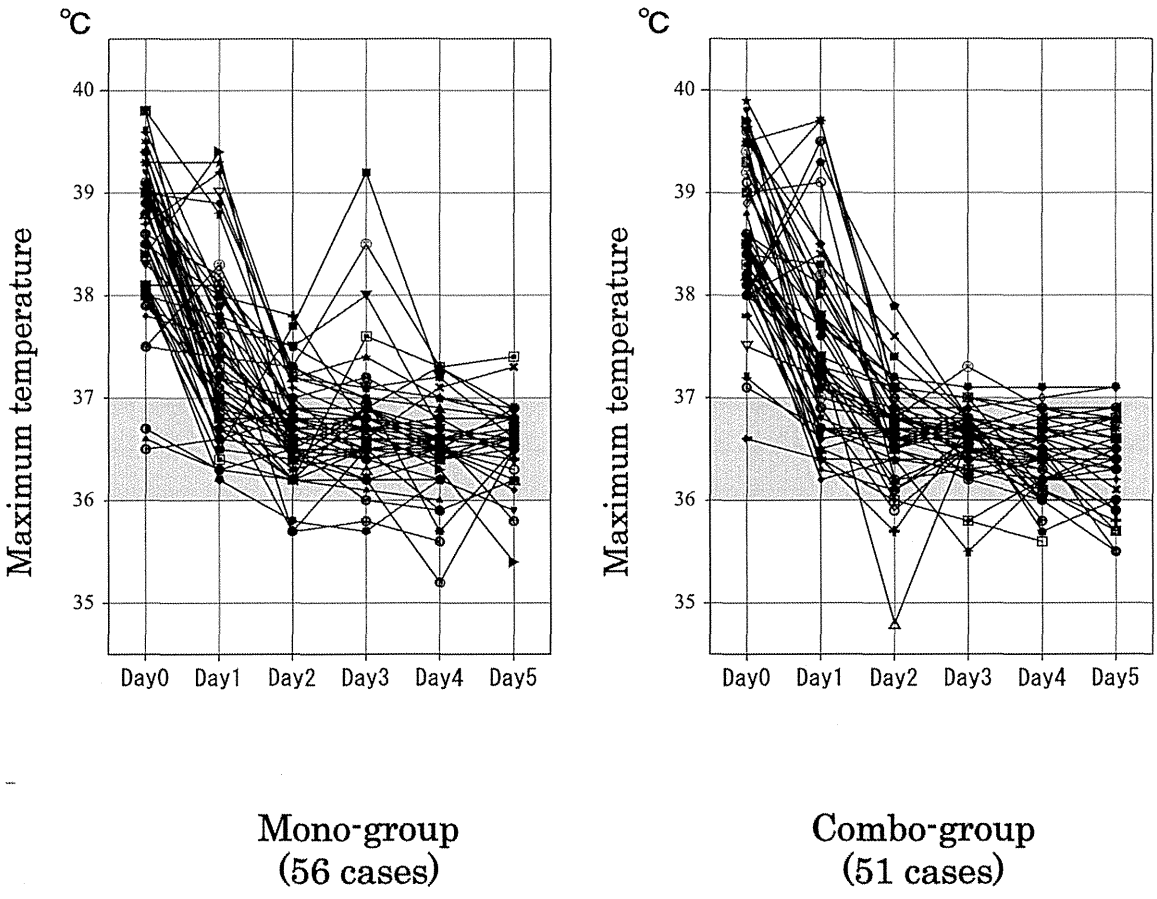

Figure 3. Comparisons of maximum temperature in the azithromycin-oseltamivir combination therapy and oseltamivir monotherapy groups. The maximum temperatures on day 1 through day 5 were compared between groups. No significant differences were detected between the groups on days 1, 2, 3, and 5. However, our analysis revealed a significant decrease in the maximum temperature on day 4 in the combo-group compared to that in the mono-group ($p = 0.037$). In addition, a mixed-design ANOVA indicated that the maximum temperature on days 3 through 5 was significantly lower in the combo-group than in the mono-group ($p = 0.048$).
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Table 2. Improvement of influenza-related symptoms in the azithromycin-oseltamivir combination therapy and oseltamivir monotherapy groups.

		Azithromycin		
		−mean ± S.D	+ mean ± S.D	p value
Headache	Day0	1.48±0.86	1.48±0.89	0.9648
	ΔDay2	0.68±0.68	0.74±0.95	0.7890
	ΔDay5	1.23±0.99	1.30±0.96	0.8649
Muscle/Joint pain	Day0	1.76±0.95	1.70±0.92	0.6611
	ΔDay2	1.08±0.92	1.15±0.99	0.7968
	ΔDay5	1.60±0.93	1.6±0.95	0.9970
Heat sensation	Day0	1.98±0.86	2.24±0.85	0.1039
	ΔDay2	1.43±0.91	1.78±1.11	0.0506
	ΔDay5	1.8±0.94	2.13±1.00	0.0609
Feeling of fatigue	Day0	2.06±0.81	2.11±0.77	0.7373
	ΔDay2	0.98±0.84	1.20±1.07	0.2211
	ΔDay5	1.58±0.93	1.76±1.04	0.1871
Sore throat	Day0	1.19±0.85	1.20±0.86	0.8020
	ΔDay2	0.34±0.73	0.70±0.87	0.0323
	ΔDay5	0.81±0.83	1.02±0.88	0.2138
Nasal congestion	Day0	1.26±0.97	1.2±0.86	0.2138
	ΔDay2	0.43±1.05	0.44±0.89	0.8630
	ΔDay5	0.79±1.12	0.93±1.04	0.3890
Cough	Day0	2.00±0.71	1.62±0.78	0.0143
	ΔDay2	0.52±0.83	0.60±0.86	0.6645
	ΔDay5	1.06±0.78	1.07±0.93	0.8783

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days 1 ($p = 0.864$), 2 ($p = 0.864$), 3 ($p = 0.741$), or 5 ($p = 0.068$). However, a significant decrease in the maximum temperature was observed on day 4 between the combo-group and the mono-group ($p = 0.037$; Figure 3). In addition, the maximum temperature on days 3 through 5 was significantly lower in the combo-group than in the mono-group ($p = 0.048$).

Improvements in sore throat were observed more frequently on day 2 among patients in the combo-group than in the mono-group ($p<0.05$). No significant differences were observed between the 2 groups in the resolution time of other influenza-related symptoms (headache, muscle or joint pain, heat sensation, feeling of fatigue, sore throat, nasal congestion, and cough). However, compared to the mono-group, the combo-group showed a trend toward earlier resolution of fever ($p = 0.05$ on day 2 and $p = 0.06$ on day 5, Table 2).

Laboratory tests

The baseline hematological test values (hemoglobin, Ht, WBC, neutrophil count, lymphocyte count, total protein and albumin) on day 0 before treatment allocation were not significantly different between the 2 groups. Only the baseline RBC was statistically significantly higher in the combo-group than in the mono-group (($p<0.05$). In addition, the combo-group showed statistically significant increases in the RBC and hemoglobin and Ht values on days 2 and 5 and a statistically significant decrease in the levels of Alb and T-P on day 2 ($p<0.05$ and $p<0.01$, respectively; Table 3).

Safety

Adverse events (AEs) occurred in 11 of the 56 patients (19.6%) in the combo-group and in 9 of the 51 patients (17.6%) in the mono-group (Table 4). There was no significant difference in the incidence of AEs between the 2 groups. AEs for which a causal relationship with the study drugs could not be ruled out (known plus unknown causal relationships) occurred in 9 patients (16.1%) in the combo-group and 4 patients (7.8%) in the mono-group. No severe AE occurred in either group and no patients discontinued treatment because of an AE. The most common AEs were diarrhea ($n = 3$ in the combo-group) and decreased WBC ($n = 5$ in the combo-group and $n = 3$ in the mono-group). Only 1 patient in the mono-group developed secondary pneumonia.

Discussion

In this study, we present the findings of a randomized clinical trial of combination therapy with oseltamivir and AZM in patients with influenza virus infection. The primary endpoint of this study was variation in the expression of inflammatory markers (i.e., inflammatory cytokines and chemokines). Although the combination of oseltamivir plus AZM did not show any early reduction in the levels of inflammatory markers compared to that with oseltamivir alone, the combination treatment showed a potential early resolution of influenza-related symptoms such as fever and sore throat.

Macrolides have antibiotic and immunomodulatory activities *in vitro* and *in vivo*, and thus may have a favorable effect on the clinical outcome of patients with severe infection [6]. CAM decreases the

Table 3. Laboratory data for the azithromycin-oseltamivir combined therapy and oseltamivir monotherapy groups.

		Azithromycin		
		- mean ± S.D (median)	+ mean ± S.D (median)	p value
WBC	Day0	4154±3442 (5045)	4137±3430 (5175)	0.7904
	ΔDay2	−1013±2091 (−1400)	−2138±1472 (−2350)	0.0103
	ΔDay5	−614±1648 (−910)	−754±2203 (−1100)	0.5667
Neutrophil	Day0	4720±1707 (4320)	4745±1524 (4540)	0.7565
	ΔDay2	−1692±2154 (−1987)	−2771±1462 (−2635)	0.0081
	ΔDay5	−1503±704 (−1286)	−1729±2143 (−1825)	0.5802
Lymphocyte	Day0	948±429 (847)	851±343 (767)	0.2722
	ΔDay2	633±540 (694)	751±346 (797)	0.1856
	ΔDay5	940±79 (918)	1170±557 (1189)	0.0614
RBC	Day0	474±46 (476)	458±40 (453)	0.0455
	ΔDay2	−3.53±20.0 (−1.36)	14.4±23.3 (13.5)	0.0002
	ΔDay5	−9.53±19.8 (−1.50)	5.9±24.1 (9.0)	0.0008
Hgb	Day0	14.3±1.47 (14.2)	13.8±1.68 (13.8)	0.1744
	ΔDay2	−0.33±0.65 (0.00)	0.43±.71 (0.50)	0.0012
	ΔDay5	−0.30±0.63 (−0.30)	0.16±0.72 (0.20)	0.0010
Hct	Day0	42.8±3.81 (42.6)	41.2±4.36 (41.4)	0.1195
	ΔDay2	−0.36±1.81 (−0.30)	1.36±2.20 (1.45)	0.0001
	ΔDay5	−1.10±1.81 (−1.30)	0.22±2.11 (0.20)	0.0010
Total Protein	Day0	7.3±0.45 (7.3)	7.2±0.44 (7.2)	0.2910
	ΔDay2	−0.22±0.33 (−0.20)	0.01±0.41 (0.05)	0.0026
	ΔDay5	−0.10±0.36 (−0.20)	0.06±0.48 (0.00)	0.0831
Alb	Day0	4.5±0.34 (4.5)	4.4±0.30 (4.5)	0.5207
	ΔDay2	−0.24±0.26 (−0.20)	−0.12±0.24 (−0.10)	0.0155
	ΔDay5	−0.20±0.22 (−0.30)	−0.02±0.29 (0.00)	0.0026
AST	Day0	24.8±9.36 (22.0)	22.6±12.38 (19.5)	0.0204
	ΔDay2	1.36±6.31 (1.0)	0.84±6.10 (2.0)	0.7559
	ΔDay5	−1.79±8.61 (−1.0)	−0.75±9.49 (0.0)	0.0609
ALT	Day0	23.3±15.02 (19.0)	24.0±21.41 (16.0)	0.4339
	ΔDay2	2.36±6.89 (1.0)	0.34±4.78 (1.0)	0.4868
	ΔDay5	1.02±7.50 (0.5)	−1.16±12.58 (0.5)	0.6777
BUN	Day0	11.3±3.60 (10.8)	11.5±4.65 (11.2)	0.9093
	ΔDay2	0.76±3.00 (1.20)	1.20±3.18 (0.80)	0.6140
	ΔDay5	0.51±3.15 (0.95)	1.57±3.11 (1.65)	0.1293
Cr	Day0	0.8±0.20 (0.8)	0.7±0.18 (0.8)	0.7492
	ΔDay2	−0.05±0.10 (−0.05)	−0.04±0.09 (−0.05)	0.5743
	ΔDay5	−0.08±0.08 (−0.06)	−0.07±0.08 (−0.06)	0.4516

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ratio of serum IL-10 to serum TNF-α in patients with ventilator-associated pneumonia (VAP) and sepsis caused by gram-negative bacteria [16]. In addition, AZM significantly reduces the expression of the proinflammatory cytokine IL-1β and the chemokine C-C motif ligand (CCL)-2 and TNF-α in M1-induced cystic fibrosis alveolar macrophages in patients with cystic fibrosis [17]. In addition, AZM decreases acute and chronic airway inflammation in a mouse model of paramyxoviral bronchiolitis without any association with antiviral activity [18]. Azithromycin has a large volume of distribution, although serum concentrations remain low, and its half-life is much longer than that of clarithromycin. Therefore, a single dose of azithromycin is an effective and convenient dosing schedule that improves patient compliance [19]. Although we expected AZM to reduce inflammatory cytokine expression in patients with influenza virus infection, compared to oseltamivir alone, the combination of oseltamivir plus AZM did not result in an early reduction in the levels of inflammatory markers. The baseline values of each inflammatory cytokine differed for each patient, and the median value was relatively low, and therefore we suspect that variability in the patient backgrounds might have affected the study outcomes.

The present study has several potential limitations. Our study was performed during 1 winter season. Further, the number of

Table 4. List of adverse events in the present study.

AZM	Adverse Event	Severity	Causality	Treatment
+	Secondary bronchitis	Mild	No	Continue
+	Bronchitis	Mild	No	Continue
+	Abdominal pain	Mild	Unknown	Continue
+	Abdominal pain/Diarrhea	Mild	Unknown	Continue
+	Diarrhea	Mild	Yes	Continue
+	Diarrhea	Mild	Unknown	Continue
+	Leucopenia	Mild	Unknown	Continue
+	Leucopenia	Moderate	Unknown	Continue
+	Leucopenia	Moderate	Unknown	Continue
+	Leucopenia	Mild	Unknown	Continue
+	Leucopenia	Mild	Unknown	Continue
−	Sinusitis	Moderate	No	Continue
−	Pneumonia	Mild	No	Continue
−	Bronchitis	Mild	No	Continue
−	Leucopenia	Mild	No	Continue
−	Leucopenia	Mild	Unknown	Continue
−	Leucopenia	Mild	Unknown	Continue
−	Eosinophilia	Mild	Unknown	Continue
−	Hepatic dysfunction	Mild	Unknown	Continue
−	Hepatic dysfunction	Mild	No	Continue

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patients was limited. We planned a sub-group analysis of older individuals, particularly patients with underlying respiratory disease. However, the number of patients who met this definition was limited, and thus the analysis was not possible. A randomized controlled trial of such patients should be performed in the future. Moreover, the enrollment criteria included patients with a wide variety of backgrounds to ensure the feasibility of patient enrollment. The timing of enrollment from the onset of an influenza-related symptom was different for each patient. Although the inclusion criteria stipulated that a patient had to be enrolled within 48 h after the onset of an influenza-related symptom, there were still 48 hours between the symptom onset and enrollment. Additionally, it was difficult to prepare a specially blinded drug for the AZM extended-release formulation because of its unique size and shape. Therefore, the study was conducted in an open-label manner. Although it cannot be denied that AZM may have had some placebo effect, we do not believe that this affected our results.

High body temperature is a common influenza-related symptom. Although no statistically significant differences in fever reduction were observed between the 2 groups until day 3, compared to oseltamivir alone, the oseltamivir plus AZM combination group showed a statistically significant early antipyretic effect on day 4. The mechanism of action of the early antipyretic effect associated with AZM is difficult to determine in our patients, but we present 2 hypotheses. The first is an anti-inflammatory effect exerted by AZM [6], and the second is a conventional antibiotic effect giving due consideration to a bacterial superinfection. In this study, we were unable to show that AZM decrease the levels of inflammatory markers in influenza patients compared to those in controls. However, macrolide therapy has been reported to improve the outcomes of patients with VAP [20] and acute lung injury (ALI) [21]. Several studies have also shown that compared to administration of beta-lactams alone, fluoroquinolones alone, or beta-lactams in combination with fluoroquinolones, administration of beta-lactams in combination with macrolides improves the survival of patients with severe community-acquired pneumonia (CAP) [22–24]. These reports suggest that macrolides produce an effect (i.e., anti-inflammatory or immunomodulatory) other than a conventional antibiotic effect. The potential therapeutic value of the anti-inflammatory effects of macrolides is supported by murine models of ALI induced by endotoxin [25,26] as well as murine models of influenza [13,27] and VAP caused by *Pseudomonas aeruginosa* [28]. These studies have shown increased survival after macrolide therapy. The possible involvement of a bacterial superinfection cannot be ruled out because bacteriological examinations such as Gram stains and cultures of respiratory samples were not required to be performed at baseline in this study. Rates of influenza-related pneumonia are generally less than 10% [29,30], and it is very rare in Japan (1-2%) because patients tend to consult physicians at an early stage because of the broad coverage by the health insurance system. In this study, we analyzed about 100 patients, but none developed pneumonia.

The incidence of bacterial pneumonia as a secondary infection after influenza is well known as a major cause of increased morbidity and mortality. Concomitant administration of macrolides, including AZM, to treat influenza may contribute to the prevention of secondary bacterial pneumonia by preventing airway epithelial cell damage because of an overactive immune response. Macrolides exert immunomodulatory effects via inhibition of neutrophil oxidative bursts, decrease of elastase activity, and suppression of granulocyte macrophage-colony stimulating factor [6]. In this study, only 1 patient who received oseltamivir monotherapy developed secondary pneumonia, but the sample size was not sufficiently large to adequately detect secondary infection after influenza.

Decreases in serum albumin and total protein levels were significantly modulated by the addition of AZM to oseltamivir therapy. The relationship between AZM and such modulation is unclear, but AZM could have contributed to early improvement in the general condition of the patients.

In conclusion, to our knowledge, this is the first prospective, randomized, clinical trial of oseltamivir and AZM combination therapy for influenza. Although no statistically significant difference was observed in the expression levels of inflammatory cytokines and chemokines, the combination therapy showed a trend toward the earlier resolution of some symptoms.

Supporting Information

Checklist S1 CONSORT checklist.
(DOC)

Protocol S1 Trial Protocol.
(DOC)

Acknowledgments

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Author Contributions

Conceived and designed the experiments: HK, MS, KI, KY, TT, S. Kohno. Performed the experiments: KK, YM, S. Kurihara, SN, YI, TM, MT. Analyzed the data: KI, KK, YM, S. Kurihara, SN, YI, TM, MT, KI, KY, TT. Wrote the paper: HK, MS, S. Kohno.

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Original article

Pathogenesis and clinical features of chronic pulmonary aspergillosis – Is it possible to distinguish CNPA and CCPA clinically?

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ABSTRACT

Background: The pathogenesis of chronic pulmonary aspergillosis (CPA) including chronic necrotizing pulmonary aspergillosis (CNPA), chronic cavitary pulmonary aspergillosis (CCPA), and simple aspergilloma (SA) has been poorly investigated. We examined all types of CPA cases with histopathological evidence to clarify the differences in pathogenesis and clinical features.
Method: We searched for cases diagnosed as pulmonary aspergillosis by histopathological examination in Nagasaki University Hospital between 1964 and September 2010. All available clinical information including radiological findings were collected and analyzed.
Result: We found 7, 5, 8, and 7 cases of proven CNPA, probable CNPA, CCPA, and SA, respectively. The radiograph of proven and probable CNPA was initially infiltrates or nodules that progress to form cavities with or without aspergilloma, whereas the radiograph of CCPA showed pre-existed cavities and peri-cavitary infiltrates with or without aspergilloma. The patients with proven and probable CNPA exhibited not only respiratory symptoms but also systemic symptoms and malnutrition. *Aspergillus fumigatus* was the most frequently isolated *Aspergillus* species ($n = 14$), however, *Aspergillus niger* was the predominant isolated species in proven CNPA cases ($n = 4$).
Conclusion: Our data indicate that the cases with chronic infiltration, progressive cavitation, and subsequent aspergilloma formation should be diagnosed as CNPA, and the cases with pre-existed cavities showing peri-cavitary infiltrates with or without aspergilloma would mean CCPA. However, it may be difficult to distinguish the two subtypes if a series of adequate radiography films are not available. We propose the term "chronic progressive pulmonary aspergillosis (CPPA)" for the clinical syndrome including both CNPA and CCPA.

1. Introduction

Chronic pulmonary aspergillosis (CPA) was originally established in the early 1980s by Binder as chronic necrotizing pulmonary aspergillosis (CNPA) [1] and semi-invasive aspergillosis (SIA) by Gefter [2]. CNPA/SIA is characterized by a slow progressive cavitating process in the lungs due to *Aspergillus* spp. infection. In the last decade, new clinical nomenclature and definition of chronic forms of aspergillosis have been proposed [3–5], and recent guidelines from the Infectious Diseases Society of America (IDSA) have indicated 3 major subtypes of chronic forms of pulmonary aspergillosis, namely CNPA (categorized in subacute invasive form of aspergillosis; subacute IPA), chronic cavitary pulmonary

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aspergillosis (CCPA), and aspergilloma [6]. Aspergilloma was traditionally classified as simple or complex in the surgical literature, and complex aspergilloma is considered CCPA by current IDSA guidelines [6,7]. Updated IDSA guidelines and textbook have indicated that the differences between CNPA and CCPA include prolonged time frame (CNPA, 1–3 months vs. CCPA, >3 months) [8]. The original CNPA cases defined by Binder are not equal to those of CNPA cases defined by Denning. Establishing the precise subclassifications of CPA, while challenging, is important for clinical trials as well as the development of tools for its diagnosis and treatment. The pathogenesis of each type of CPA examined by pathological samples has been poorly investigated. In this study, we investigated all types of CPA cases including CNPA, CCPA, and simple aspergilloma (SA) diagnosed by histopathological examination to clarify the differences in the pathogenesis and clinical features of each type of CPA. In particular, revealing the difference between CNPA and CCPA is the most important of goal of our study. This study was performed in a single medical teaching hospital in Japan.

2. Material and methods

2.1. Case collection and analysis

We searched for cases diagnosed as "pulmonary aspergillosis" by histopathological examination of respiratory specimens acquired by biopsy, surgical resection, or autopsy in Nagasaki University Hospital. Nagasaki University Hospital is a Japanese teaching hospital that contains 860 beds and is located in the southwestern part of Japan. All cases registered in the database between 1964 and September 2010 were screened, and patient data such as sex, age, underlying diseases, clinical symptoms, clinical course, laboratory and radiological findings, and treatment was collected from medical records and analyzed. This retrospective study was approved by the ethics committee of Nagasaki University Hospital.

2.2. Definition of pulmonary aspergillosis subtypes by histopathological and clinical aspects

The histopathological and clinical definition of invasive pulmonary aspergillosis (IPA), CNPA, CCPA, and SA in this study is indicated in Fig. 1. A diagnosis of IPA and CNPA requires extensive hyphal invasion in the lung parenchyma and localized hyphal invasion in cavity walls and/or destroyed lung tissue, respectively. Non-invasive CPA was subdivided into three groups as Group A, CCPA and SA. CCPA requires pre-existing cavities and disease progression within 3 months. SA requires a solitary cavitary legion with an aspergilloma and no disease progression within 3 months. Group A does not match to either of CNPA and CCPA. This group requires a newly developed necrotic lung cavity and no hyphal invasion in any lung tissue. The existence of pre-existing cavity was confirmed by X-ray films taken prior to the disease onset.

2.3. Statistical analysis

The chi-square test was used for qualitative variables. Multivariate logistic regression analysis was performed using the variables that were selected from univariate analysis ($P < 0.1$). This analysis was performed by SPSS ver. 16 software. P values <0.05 were considered statistically significant.

3. Results

3.1. Characters of CPA cases and patients

A total of 60 cases of which histopathological findings indicated pulmonary aspergillosis were found in the database. Then the cases of IPA and allergic bronchopulmonary aspergillosis (ABPA) were excluded. We searched the patients' medical records including X-ray films and accepted 27 cases of CPA for further analysis due to their availability of medical information and X-ray films. We found 7, 5, 8, and 7 cases of CNPA, Group A, CCPA, and SA, respectively. They were histopathologically diagnosed by surgical resection in 19, biopsy in 4, and autopsy in 4 cases, respectively.

Table 1 shows sex, age, body mass index, symptoms, and disease duration prior to diagnosis. The cases of CNPA, Group A and CCPA demonstrated a male predominance, and the body mass index values of patients with CNPA and Group A were statistically lower than those of patients with SA. Several patients with SA complained of cough, hemoptysis, and sputum; however, the symptoms or radiographic findings did not indicate disease progression within 3 months. All cases with CNPA, Group A and CCPA complained respiratory symptoms. Progressive fever and weight loss were found

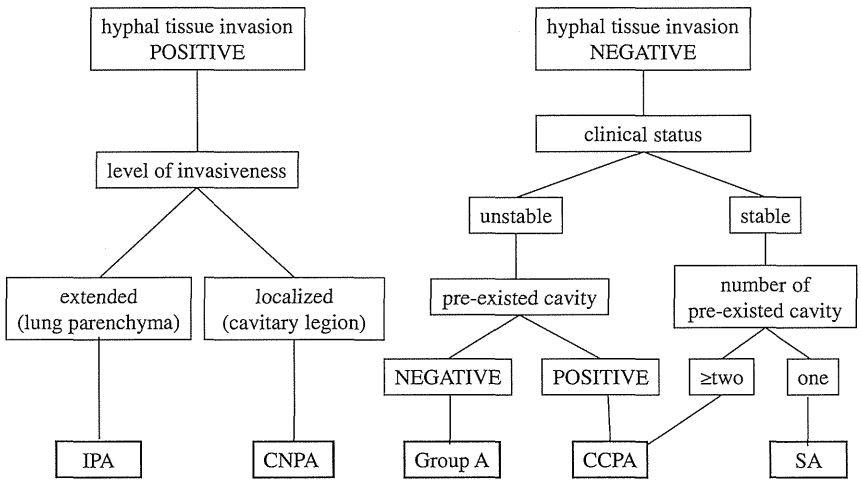

Fig. 1. Histopathological and clinical diagnosis of pulmonary aspergillosis in this study. A diagnosis of invasive pulmonary aspergillosis and chronic necrotizing pulmonary aspergillosis (CNPA) requires extensive hyphal invasion in the lung parenchyma and localized hyphal invasion in cavity walls and/or destroyed lung tissue, respectively. Group A, chronic cavitary pulmonary aspergillosis (CCPA), and simple aspergilloma (SA) require no hyphal invasion in any lung tissue; however, Group A requires new cavity formation and clinical deterioration without a pre-existing cavitary lesion in the lung. CCPA requires pre-existing cavities and disease progression and SA requires a solitary cavitary legion with an aspergilloma and no clinical deterioration within 3 months.

Table 1
Characteristics of CPA patients.

	CNPA ($n = 7$)	Group A ($n = 5$)	CCPA ($n = 8$)	SA ($n = 7$)
Sex; Male/Female	6/1	4/1	7/1	3/4
Age; mean (range)	59.1 (45–75)	67.0 (50–77)	69.7 (58–80)	56.6 (40–68)
Body mass index; mean (range)	18.0* (13.0–21.4)	16.9* (13.9–18.8)	20.2 (15.7–25.7)	21.3 (18.0–23.9)
Symptoms				
Cough	5	5	7	4
Sputum	5	2	3	3
Hemoptysis	1	5	6	4
Dyspnea	3	1	2	0
Fever	4	2	0	0
Malaise	3	0	0	0
Weight loss	1	2	0	0
Duration; median (range)	3 m (1 m–6 y)	24 m (17 m–6 y)	29 m (6 m–5 y4 m)	24 m (6 m–4 y)

CNPA, chronic necrotizing pulmonary aspergillosis; CCPA, chronic cavitary pulmonary aspergillosis; SA, simple pulmonary aspergilloma; m, months; and y, years.
*: Statistical difference between CNPA, Group A and SA was indicated ($p < 0.05$).

in only CNPA and Group A cases, which may reflect progressive tissue destruction and more direct inflammatory responses than CCPA and SA does.

The underlying conditions of patients with CPA are indicated in Table 2. Patients with CNPA most frequently had chronic obstructive pulmonary disease (COPD) or emphysema, whereas patients with CCPA and SA most frequently had prior tuberculosis. A history of pneumonia was detected in 3/7 and 4/5 cases of CNPA and Group A, respectively, and may have been caused by a direct or indirect response to *Aspergillus* spp. A history of thoracic surgery or pneumothorax was also detected in CNPA and Group A cases. Diabetes mellitus was the predominant underlying systemic disease in all subtypes of CPA cases. Cigarette smoking was also seen in all subtypes of CPA cases. Malnutrition was seen in 4/7, 4/5 and 3/8 cases of Group A, CNPA and CCPA, respectively.

3.2. Radiological findings of patients with CPA

Typical radiographic images of each subtypes of CPA are presented in Fig. 2. The radiograph of CNPA and Group A cases was initially infiltrates that progress to form cavities with or without aspergilloma. The radiograph of CCPA cases showed pre-existed cavities and peri-cavitary infiltrates with or without aspergilloma, and SA cases showed a single cavity with aspergilloma. Radiological findings at the time of diagnosis are summarized in Table 3. The upper lobes were affected in all cases of CPA, while both the right and left upper lobes were affected in 4/7 and 1/5 cases of CNPA and Group A, respectively. Although the upper lobes were most commonly affected, the middle and the lower lobes were also affected in CCPA and SA cases. Cavitary infiltrates with aspergilloma was the predominant finding in CNPA and Group A cases. Cavitary nodules with aspergilloma were also recognized in CNPA cases. Cavities with aspergilloma were most commonly found in CCPA cases. All SA cases showed a single cavity with aspergilloma. Aspergillomas were recognized in 4/7, 5/5, 6/8, and 7/7 cases of CNPA, Group A, CCPA, and SA, respectively.

Table 2
Underlying conditions of CPA patients.

	CNPA ($n = 7$)	Group A ($n = 5$)	CCPA ($n = 8$)	SA ($n = 7$)
Respiratory conditions				
Prior tuberculosis	2	1	3	4
Bronchiectasis	1	3	2	3
Prior pneumonia	3	4	0	2
Pneumothorax or bullae	1	3	2	2
COPD or emphysema	5	0	1	0
Prior thoracic surgery	2	2	1	1
Other respiratory conditions[a]	2	4	9	4
Systemic conditions				
Diabetes Mellitus	3	2	2	2
Steroid usage	2	0	2	0
Other systemic conditions[b]	4	0	3	8
Smoking (>20 y)	5	2	7	2
Malnutrition (BMI <18.5)	4	4	3	1

CNPA, chronic necrotizing pulmonary aspergillosis; CCPA, chronic cavitary pulmonary aspergillosis; SA, simple pulmonary aspergilloma; COPD, chronic obstructive pulmonary disease.
[a] Bronchial asthma (4 cases), lung cancer (4 cases), chronic parasinusitis (4 cases), prior pleuritis (2 cases), interstitial pneumonia (2 cases), eosinophilic pneumonia (1 case), non-tuberculous mycobacteriosis (1 case) and pneumoconiosis (1 case).
[b] Gastric cancer (2 cases), colon cancer (1 case), prostate cancer (1 case), rheumatoid arthritis (1 case), Harada disease (1 case), ovarial cyst (2 cases), nasal disease (3 cases), liver disease (1 case), alcoholism (2 cases), and acoustic neuroma (1 case).

3.3. Laboratory findings of patients with CPA

Laboratory findings are presented in Table 4. *Aspergillus* spp. were isolated in 6/7 (86%), 4/5 (80%), 7/8 (88%), and 4/7 (57%) from CNPA, Group A, CCPA, and SA cases, respectively. *A. fumigatus* was the most frequently isolated *Aspergillus* species ($n = 14$), followed by *A. niger* ($n = 5$), *A. flavus* ($n = 2$), and *A. terreus* ($n = 1$) in all CPA cases; while *A. niger* was the predominant isolated species in CNPA cases ($n = 4$). Anti-*Aspergillus* antibody tests were positive in 3/5 (60%), 3/4 (75%), 4/8 (50%), and 1/6 (17%) cases of CNPA, Group A, CCPA, and SA, respectively. *Aspergillus* antigen tests were positive in 5/6 (83%), 4/5 (80%) and 6/6 (100%) cases of CNPA, Group A and CCPA, respectively. However, it was negative in all SA cases. A few CPA cases had a positive β-D-glucan test. The inflammatory marker results indicated a lower positive rate of leukocytosis compared to C-reactive protein and erythrocyte sedimentation rate (ESR), although the number of cases in which ESR was measured was low. The number of cases in which any of the 3 inflammatory markers tested positive was 5/7 (71%), 5/5 (100%), 4/8 (50%), and 2/7 (29%) in CNPA, Group A, CCPA, and SA, respectively.

4. Discussion

The pathogenesis of CPA, especially CNPA and CCPA, has not been widely investigated, and the non-committal definition criteria and overlapping clinical features make it difficult to distinguish the two subtypes. The clinical time course, radiological findings, and defects in innate immunity may be indexes of differences as suggested by Denning and others [3,5,6,8,9], however, subclassification of CPA should be made according to the pathogenesis of the disease on the basis of pathological and radiological features. Although this study is retrospective, we collected a total of 27 CPA cases with complete pathological, radiological, microbiological, and serological findings.

The clinical distinction between SA and other CPA subtypes may not be difficult because of it presents with obvious radiological findings and clinical features. All 7 SA cases in our series showed a solitary cavity with an aspergilloma. On the other hand, discerning the difference between CNPA and CCPA is challenging. Binder et al. described that CNPA is defined as an indolent, cavitating process in the lungs due to the invasion of lung tissue by a fungus of the *Aspergillus* spp. Gefter et al. reported that the radiographic features of SIA include chronic infiltration, progressive cavitation, and subsequent mycetoma formation, and that the absence of a previous cavity distinguishes such cases from secondary non-invasive mycetomas. While, Denning et al. defined CNPA as subacute invasive form of aspergillosis, occurring over 1–3 month, and CCPA as non-invasive chronic cavitary aspergillosis, occurring >3 months.

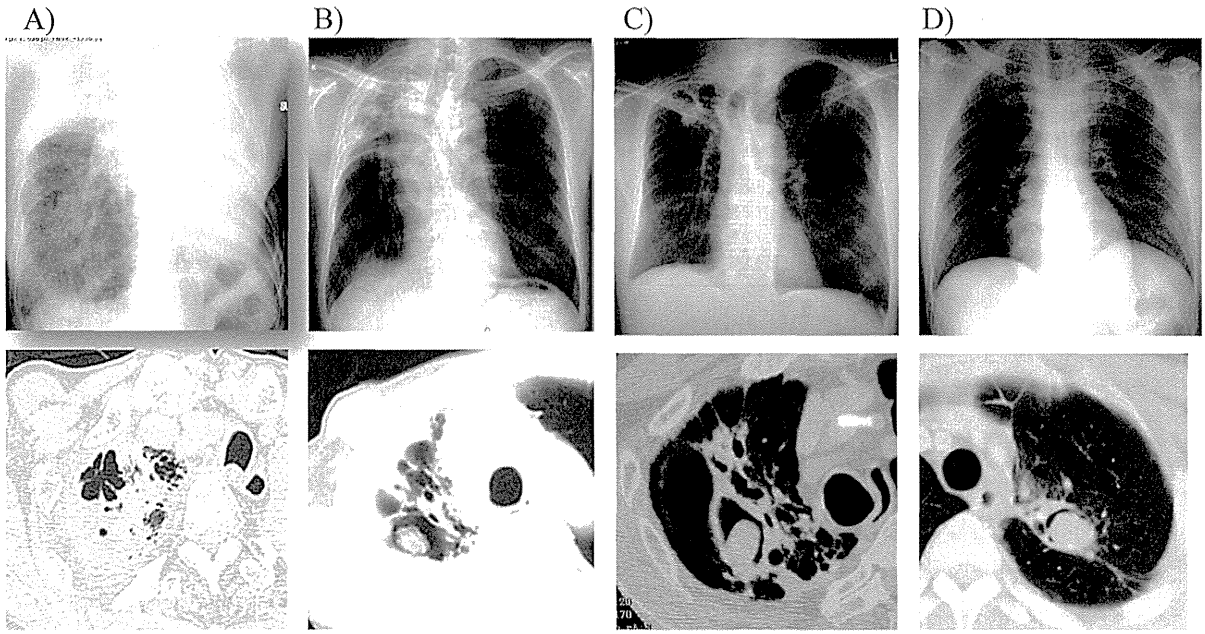

Fig. 2. Chest radiographic images of CPA cases. A) CNPA: the patient (75-year-old man) had chronic obstructive lung disease and a history of left pneumonectomy for intractable pulmonary tuberculosis and aspergilloma 7 years before admission. The radiographs show consolidation around bullae and pleural thickening in the right upper lung. B) Group A: the patient (75-year-old man) had diabetes mellitus and a history of pneumonia 2 years before admission. The radiographs show consolidation around cavities with aspergilloma and pleural thickening in the right upper lung. C) CCPA: the patient (69-year-old man) had a history of pulmonary tuberculosis. The radiographs show multiple cavities with aspergilloma in the right upper lung. D) Simple aspergilloma: the patient (40-year-old man) had a history of bullectomy for left pneumothorax 6 years before admission. The radiographs show a single cavity with an aspergilloma in the left upper lung.

According to Denning et al., the development of CCPA lesions involves two possible ways of cavity formation: 1) the infiltrates were initially ill-defined areas of consolidation that progressed to form well-defined cavities; and 2) the cavities were pre-existing (i.e., in cases of previous tuberculosis or bronchiectasis) [4]. However, the former radiographic features are concordant with those of Binder's CNPA or Gefter's SIA. Thus, the consensus of definition of CNPA is not established, and some cases of Denning's CCPA may be Binder's CNPA.

In this study, we defined the cases with progressive cavitating processes in the lung without a pre-existing cavity and presence of hyphal invasion into lung parenchyma as CNPA, and the cases with pre-existing cavities and peri-cavitary infiltrates and presence of hyphae in a cavity but not in lung parenchyma as CCPA. However, whether group A in our series is classified into CNPA or CCPA is controversial. The radiographic features of CNPA and Group A cases are similar; they are chronic infiltration, progressive cavitation and subsequent aspergilloma formation. The pathological features of the cavity wall of CNPA and Group A cases are similar except hyphal invasion; they are acute and chronic inflammation, granulation, parenchymal necrosis and subsequent cavity formation with or without fragments of destroyed lung tissue in the cavity. The hyphal invasion into cavity wall was observed only in CNPA cases but not Group A cases. Antifungals treatment before the pathological examinations were conducted in 3/7 and 4/5 cases of CNPA and

Table 3
Radiological findings of CPA patients.

	CNPA ($n = 7$)	Group A ($n = 5$)	CCPA ($n = 8$)	SA ($n = 7$)
Affected sites				
RUL + LUL	4	1	0	0
RUL	3	3	3	2
RML	0	0	2	2
RLL	0	0	1	1
LUL	0	1	0	2
LLL	0	0	2	0
Cavities with aspergilloma	2	1	5	0
Cavity with aspergilloma	1	0	1	7
Cavities without aspergilloma	1	1	0	0
Cavitary infiltrates with aspergilloma	3	4	0	0
Cavitary infiltrates without aspergilloma	1	0	0	0
Cavitary nodules with aspergilloma	2	0	0	0
Multiple nodules	1	0	0	0
Bronchiectasis and infiltrate[a]	0	0	1	0
Bronchiectasis and atelectasis[a]	0	0	1	0

CNPA, chronic necrotizing pulmonary aspergillosis; CCPA, chronic cavitary pulmonary aspergillosis; SA, simple pulmonary aspergilloma. RUL, right upper lobe; LUL, left upper lobe; RML, right middle lobe; RLL, right lower lobe; LLL, left lower lobe.
[a] Intrabronchial aspergilloma.

Table 4
Laboratory findings of CPA patients.

	CNPA ($n = 7$)	Group A ($n = 5$)	CCPA ($n = 8$)	SA ($n = 7$)
Isolation of *Aspergillus* spp.				
A. fumigatus	2	4	5	3
A. niger	4	0	1	0
A. flavus	0	0	1	1
A. terreus	1[a]	0	0	0
Not identified	1	1	1	3
Serological findings				
Anti-*Aspergillus* antibody (+)	3/5	3/4	4/8	1/6
Aspergillus antigen (≥0.5 C.O.I.)	5/6	4/5	6/6	0/5
β-D-glucan (≥20 pg/ml)	2/6	1/4	1/5	1/4
Inflammatory makers				
Leukocytosis (WBC>9000/mm^3)	2	0	1	0
CRP (>0.3 mg/dl)	5	4	4	0
ESR (>20 mm/h)	1/1	2/2	3/3	2/4

CNPA, chronic necrotizing pulmonary aspergillosis; CCPA, chronic cavitary pulmonary aspergillosis; SA, simple pulmonary aspergilloma; CRP, C-reactive protein; and ESR, Erythrocyte sedimentation rate.
[a] *A. terreus* was isolated with *A. fumigatus* from the same patient.

Group A, respectively. Additionally, duration of treatment was relatively longer in Group A cases (6 weeks–8 months) compared to that of CNPA(10 days for two case) except one case (approximately 14 months). It is possible that antifungal treatment prior to the pathological examination may block the hyphal invasion of *Aspergillus* in Group A cases. Moreover, systemic symptoms such as fever and weight loss, and underlying conditions such as history of pneumonia or thoracic surgery are common to both CNPA and Group A, but not to CCPA. Thus, the pathological and clinical features and backgrounds of Group A cases were resemble to those of CNPA cases; which means the pathogenesis of CNPA and Group A cases is considered the same.

It is supposed that *Aspergillus* infects air spaces, such as emphysematous bullae, then destroys lung tissue by invasion and/or mycotic toxins, proteolytic enzymes, and metabolites produced by *Aspergillus*. Proteolytic enzymes and oxidant derived from neutrophils and macrophages may also cause tissue necrosis. Thus, the cavity formation can be developed even though the hyphal invasion is minimal or absent. Therefore, the cases with chronic infiltration, progressive cavitation, and subsequent aspergilloma formation should be diagnosed as CNPA; the cases in which hyphal invasion of lung tissue is demonstrated are proven CNPA and the cases in which the invasion is not demonstrated are probable CNPA. Hence, we diagnose the Group A in our series as probable CNPA. The median duration of the disease was 3 and 24 months in proven CNPA and probable CNPA (Group A) cases, respectively. As Binder et al. mentioned that the disease is usually of 1–6 months duration but can be present for years prior to diagnosis, CNPA is not always subacute (1–3 months).

There were pre-existing cavities such as sequelae of pulmonary tuberculosis, bronchiectasis, COPD, bullae, and interstitial pneumonia in all 8 of CCPA cases, and the wall of these cases was composed of chronic inflammatory, granulation, and fibrous tissue layers, and the inner surface of the cavity is covered by bronchial epithelium or metaplastic squamous cell epithelium in part. Thus, CCPA is synonymous with complex aspergilloma. Our study results indicate that CNPA can be distinguishable from CCPA by careful observation of progression of the cavitary lesion if a series of adequate radiography films are available. In some cases, however, it will be difficult to distinguish the two subtypes because adequate films are not always available.

The limitations of our study include its retrospective nature, the number of cases being insufficient for establishing definite criteria, and the need for additional information. The importance of differentiating between CNPA and CCPA is scientific; however, it may be difficult because of the non-committal definition criteria and existence of overlapping findings. From the management perspective, its elucidation does not provide deeper insight into the differentiation, except for executing clinical trials. Although we respect and follow the original definition of CNPA and the concept of CCPA, there is a limitation to distinguishing these two entities in actual clinical settings as discussed above. Hope et al. indicated that apparent distinct entities do not exist for the CPA subtypes and that these forms usually overlap; therefore, the requirement and importance of the rigorous sub-classifications of CPA are unclear [5]. Hence, we propose the term "chronic progressive pulmonary aspergillosis (CPPA)" for the clinical syndrome including both CNPA and CCPA [10]. Therefore, CPA is consisted of CPPA and SA.

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Original article

Clinical features of pulmonary cryptococcosis in non-HIV patients in Japan

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ABSTRACT

Objective: To clarify the clinical features of pulmonary cryptococcosis in Japanese non-HIV population.
Methods: Retrospective investigation of 151 pulmonary cryptococcosis cases between 1977 and 2012 was executed. The underlying disease (UDs), aggravating factors, radiological characteristics, and treatment were examined.
Results: Sixty-seven patients (44.4%) had no UDs. The common UDs were diabetes (32.1%) followed by hematologic disease (22.6%), and collagen disease (22.6%). Peripherally distributed pulmonary nodules/masses were most commonly seen. Lesions in the right middle lobe ($p = 0.01$) and air bronchogram ($P = 0.05$) were significantly more frequent, respectively, in patients with UDs than patients without them. Azoles were mainly selected for the patients without meningoencephalitis. Mean treatment duration for patients with and without UDs was 6.64 and 2.87 months, respectively. Patients whose pulmonary nodules improved after treatment continued to experience gradual reduction of cryptococcosis antigen titers, even if antigen titers were positive at the time of treatment cessation. The average time for antigen titers to become negative after treatment cessation was 13.1 and 10.7 months for patients with and without UDs, respectively. When groups were compared according to the presence of meningoencephalitis complications, deaths, and survivals, factors contributing to cryptococcosis prognosis included higher age, hypoproteinemia, hypoalbuminemia, steroid use, high C-reactive protein levels, and meningoencephalitis complications.
Conclusions: It is crucial to consider the presence of UDs and meningoencephalitis for the choice of antifungals and treatment duration for cryptococcosis in non-HIV patients. Three- and six months-administration of azoles for pulmonary cryptococcosis with or without UDs, respectively is reasonable.

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1. Introduction

Cryptococcus neoformans is a nonmycelial, budding encapsulated yeast-like fungus found in soil contaminated with pigeon and chicken excreta [1–4]. Inhalation of cryptococcal particles from contaminated soil into the lung is considered the usual route of human infection [2,3]. The organism may cause isolated pulmonary infection or hematogenous dissemination involving the central nervous system (CNS), bones, and skin, mostly depending on the host immunity [2,3]. Although cryptococcal infection can occur in individuals with normal immunity, it most commonly occurs in immunocompromised hosts. Predisposing factors are acquired immune deficiency syndrome (AIDS) and other causes of impaired T cell–mediated immunity, e.g., transplant-related immunosuppression, hematological malignancies, corticosteroid administration, and diabetes mellitus [4–6].

Although the clinical characteristics and natural history of cryptococcosis in HIV patients have been described elsewhere due to its large number, those in non-HIV patients have rarely been reported [7]. To date, few studies have reported comparative data regarding the clinical manifestations, laboratory findings, radiographic findings and survival of patients with pulmonary cryptococcosis in Japan [8]. Additionally, very few research comparing clinical manifestation of cryptococcosis between HIV and non-HIV patients [9–11].

In Japan, the number of HIV/AIDS patients is relatively lower compared to those of other countries. However, it is increasing recently and over 20,000 of the cumulative patients are registered in Japanese government database to date (http://www.nih.go.jp/niid/ja/aids-m/aids-iasrd/2274-kj3888.html). Hence, the study of clinical manifestation of cryptococcal diseases in non-HIV background possess high impact. We reviewed 151 cryptococcal cases among non-HIV background and investigated the clinical features, including clinical manifestations, underlying conditions, laboratory findings, radiological features, treatment, survival, and outcomes.

2. Materials and methods

2.1. Patients

A retrospective cohort study was conducted by reviewing the medical records of patients who had been diagnosed with pulmonary cryptococcosis at Nagasaki University Hospital and its affiliated hospitals during the 35-year period between 1977 and 2012. The patients were grouped into 2 populations based on positivity of underlying diseases. Definite case of pulmonary cryptococcosis requires isolation or detection of *Cryptococcus* by lung specimen culture and/or by histopathological examination, and only definite cases are recruited in this study. This retrospective study including analysis and release of clinical data was approved by the ethical committee of Nagasaki University Hospital.

2.2. Clinical data

All available patient records were reviewed from the time of cryptococcal diagnosis until the patients died or were lost to follow up.

The data included clinical manifestations, underlying conditions, laboratory findings (age, lymphocyte count, neutrophil count, immunoglobulin, serum protein, serum albumin, CD4/8 ratio, CD4 count, C-reactive protein [CRP], cryptococcal serum antigen titers) at the timing of diagnosis, radiological findings, treatment, survival, and outcome were recorded.

Eiken Latex® (Eiken Kagaku Co., Tokyo, Japan) was used for the qualitative and semi-quantitative detection of the *C. neoformans* capsular polysaccharide antigen in serum and CSF according to the manufacturer's instructions.

2.3. Interpretation of chest CT scans

The findings of chest CT scans were assessed for 1) the presence and distribution of parenchymal lesions, including nodules, masses, and consolidation; 2) the characteristics of nodules and masses; and 3) related thoracic abnormalities such as pleural effusion and lymphadenopathy according to previous reports [12]. Based on the predominant parenchymal findings from the CT scans, the morphological characteristics were classified as solitary nodule/mass (type I), multiple nodules/masses (type II), and consolidation (type III). In addition, type II was subdivided into distribution in a single lobe (type IIa) and distribution in multiple lobes (type IIb).

2.4. Statistical analysis

We used FREQ, NPAR1WAY, and ANOVA in SAS. The chi-square test was used to compare the frequency of categorical variables (e.g., underlying disease, steroid usage). Wilcoxon's test was used to compare age, lymphocyte count, neutrophil count, serum protein, serum albumin, CD4/8 ratio, CD4 count, CRP, and cryptococcal serum antigen titers. The Eiken Latex® latex agglutination test was used to detect cryptococcal polysaccharide. Antigen titers were transformed to the logarithm to the base 2 ($Log_2[Ag + 1]$). Ag (cryptococcal antigen titer) is expressed as 0, 1, 2, 4, ... as powers of 2 and Ag + 1 was expressed as $Log_2(0 + 1) = 0$.

For radiographic analysis, a chi-square test was employed to compare the presence and distribution of parenchymal lesions, nodule and mass characteristics except their number, and related thoracic abnormalities between the 2 groups. A Cochran–Armitage test was used to analyze the differences among 4 groups based on the number of nodules and masses, and among 4 morphological types based on the CT classification between the 2 patient populations. For all statistical tests, $p < 0.05$ indicated a significant difference.

3. Results

3.1. Patients

One hundred fifty-one patients were diagnosed with pulmonary cryptococcosis during the 35-year period between 1977 and 2012. Sixty-seven (44.4%) occurred in the patients without underlying diseases. Forty-two were men and 25 were women. Eighty-four cases (56.6%) were the patients with underlying disease. Thirty-eight were men and 46 were women.

3.2. Underlying diseases

Among 84 patients with underlying diseases, diabetes mellitus was most dominant (32.1%) followed by hematological diseases including human T-cell leukemia virus type-I carrier (22.6%), collagen disease including systemic lupus erythematosus, rheumatoid arthritis and others (22.6%), renal failure (16.7%), solid tumor (13.1%), chronic lung diseases including bronchiectasis, sequel pulmonary tuberculosis, and interstitial pneumonia (13.1%), liver disease including cirrhosis or hepatitis (9.5%), renal transplantation (2.4%), and other diseases (9.5%). Treatment with glucocorticoids (5–40 mg/day or pulse therapy) were recorded in 31 (37.0%) patients. Total of 5 patients were administrated glucocorticoids concomitantly with immunosuppressant such as cyclosporine and azathioprine.

3.3. Clinical symptoms

In 67 patients without underlying diseases, 43 (64.2%) patients were asymptomatic and detected accidentally by mass screening examination. Others had pulmonary symptoms such as cough ($n = 15$; 22.3%), sputum ($n = 4$; 6.0%), chest pain ($n = 7$; 10.4%), fever ($n = 2$; 3.0%), and others. In 84 patients with underlying disease, 39 patients (46.4%) were asymptomatic and found by abnormal chest radiograph findings taken as during routine examination of underlying diseases. Others had pulmonary symptoms such as cough ($n = 15$; 17.6%), sputum ($n = 15$; 17.6%), chest pain ($n = 3$; 3.6%), fever ($n = 20$; 23.8%), and other symptoms ($n = 19$; 22.6%).

3.4. Laboratory findings

The laboratory findings of the patients with and without underlying disease at the timing of diagnosis are shown in Table 1. The patients without underlying disease are statistically younger, better nutrition status reflected by higher total protein and albumin value, compared to those with underlying diseases. Serum antigen titers (Log_2[Antigen titer + 1]) were not different statistically in both arms.

Compared to steroid non-usage patients ($n = 114$), steroid usage patients ($n = 36$) were statistically significantly older ($p < 0.0001$), had lower lymphocyte count ($p = 0.03$), higher neutrophil count ($p = 0.02$), lower blood serum protein ($p = 0.0002$), lower blood serum albumin ($p < 0.0001$), and higher CRP ($p = 0.001$). There was no significant difference in IgG, IgA, or IgM between the two groups.

3.5. CT findings

Table 2 shows the detail of CT findings between patients with or without underlying diseases. The CT findings of 81 of 151 pulmonary cryptococcosis patients were analyzed. Forty-two and 39 patients were without and with underlying diseases, respectively. The frequency of the four CT classification types based on predominant parenchymal findings and lobar distribution of the lesions is listed in Table 2. Type IIb and type III lesions occurred more frequently in patients with underlying diseases than in those without underlying diseases. The main finding of this study is the presence of peripherally distributed multiple pulmonary nodules or masses with predominant lower lobe involvement in both patients without and with underlying diseases ($P < 0.0001$, Data not shown). Parenchymal lesions in the right middle lobe ($P = 0.01$), masses and more extensive lung involvement such as multiple lobes in distribution ($P = 0.06$) were more common in patients with underlying disease than those without underlying diseases. The number of masses (>30-mm diameter) ($P = 0.02$) and air bronchogram ($P = 0.05$) was significantly more common in patients with underlying diseases than those without underlying diseases.

Table 1
Characteristics of patients with cryptococcosis with or without underlying disease.

		State of underlying conditions						
		Patients without underlying diseases			Patients with underlying diseases			Wilcoxon test
	Criteria	n	Median	IQR	n	Median	IQR	p Value
1	Age	67	41	31	84	63	18.5	<0.0001
2	lymphocyte counts	54	1985.5	573.0	74	1429.0	1218.0	0.03
3	Neutrophil counts	55	3245.0	2403.0	75	4680.0	4273.0	0.02
4	IgG	32	1262.0	435.0	37	1343.0	891.0	0.31
5	IgM	32	142.0	71.3	37	145.0	96.0	0.49
6	IgA	31	249.0	142.0	37	275.0	169.0	0.39
7	Total protein	48	6.90	0.70	65	6.40	1.40	0.0002
8	Serum albumin	42	4.39	0.54	60	3.60	1.29	<0.0001
9	CD4/CD8	31	1.50	0.79	37	1.42	0.90	0.53
10	CRP	40	0.21	0.33	49	0.84	3.60	0.001
11	Cryptococcal antigen	56	16.00	124.00	63	32.00	252.00	0.35
12	CD4 counts	17	874.80	282.20	19	637.00	915.20	0.99

IQR: Inter Quartile Range, CRP, C-reactive protein.

Table 2
Comparison of CT findings of cases with pulmonary cryptococcosis with or without underlying disease.

	Patients without underlying diseases		Patients with underlying diseases		p Value
	n = 42	%	n = 39	%	
Mean of age (range)	47.4 (15–80)		61.4 (19–79)		
Sex (men: women)	26:16:00		18:21		
Presence of parenchymal lesions					
Nodule and masses	42	100.0	39	100	
Consolidation	3	7.1	7	18.0	0.14
Solitary nodules/ mass(type I)	14	33.3	9	23.1	0.30
Multiple nodules	25	59.5	29	74.4	0.15
Single lobe limited (type IIa)	10	23.8	5	12.8	0.06
Multiple lobe limited (type IIb)	15	35.7	24	61.5	
Consolidation (type III)	3	7.1	7	17.9	0.14
Distribution of parenchymal lesions					
Lobar distribution					
Right upper lobe	13	31.0	16	41.0	0.34
Right middle lobe	6	14.3	15	38.5	0.01
Right lower lobe	26	61.9	28	71.8	0.34
Left upper lobe	10	23.8	6	15.4	0.34
Lingura	3	7.1	5	12.8	0.39
Left lower lobe	20	47.6	17	43.6	0.71
Contact with pleura	33	78.6	33	85.0	0.48
Size (mm)					
1–30	31	73.8	16	41.0	0.02
31-	5	11.9	11	28.0	
Number					
1	15	35.7	11	28.0	0.85
2–4	10	23.8	10	26.0	
5–9	7	16.7	7	18.0	
10-	9	21.4	11	28.0	
Border					
Well-defined/ ill-defined	34/8	(81/19)	34/5	(87/13)	0.44
Margin					
Smooth/irregular/ speculated	20/21/19	(48/50/45)	12/27/20	(31/69/51)	0.25
Convergence of bronchi and vessel	35	83.3	31	79.0	0.65
Pleural identification	19	45.2	22	56.0	0.32
Internal characteristics					
Air-bronchogram	21	50.0	28	72.0	0.05
Cavitation	12	28.6	16	41.0	0.24
Calcification	1	2.4	0	0.0	0.33
CT halo sign	25	59.5	18	46.0	0.22
Satellite lesion	27	64.3	26	67.0	0.82

3.6. Serum cryptococcal antigen titer and radiological findings

The relationship of serum cryptococcal antigen titer and radiological findings were analyzed. Data from patients with

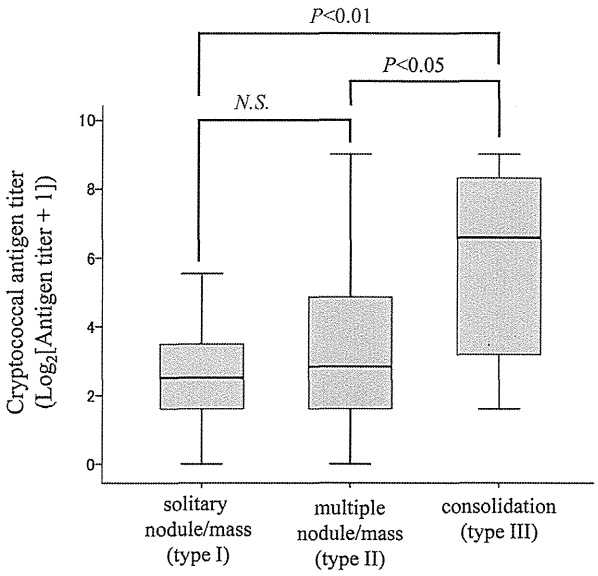

Fig. 1. The relationship of serum cryptococcal antigen titer and radiological findings. The morphological characteristics were classified as solitary nodule/mass (type I), multiple nodules/masses (type II), and consolidation (type III). Cryptococcal antigen titers were transformed to the logarithm to the base 2 (Log_2[Ag + 1]). Data from patients with meningoencephalitis was excluded.

meningoencephalitis was excluded. For patients with solitary nodules (N = 14), multiple nodules (N = 34) or consolidation (N = 8), comparison of the cryptococcal antigen titer (Log_2[Antigen titer + 1]) revealed no significant correlation between solitary and multiple nodules (N.S.); however, a significant higher cryptococcal antigen titer was observed in consolidation when compared with solitary nodules ($p < 0.01$) and multiple nodules ($p < 0.05$) (Fig. 1).

3.7. Antifungal treatment

The mainstay of initial treatment in both groups was azoles. Fifty-six (83.6%) patients without underlying diseases were initially treated with azoles, fluconazole (including Fos-fluconazole) (n = 21, 31.3%), itraconazole (n = 4, 5.9%), voriconazole (VRCZ) or miconazole (MCZ) (n = 10, 15.0%), azoles + 5-fluorocytosine (5-FC) (n = 15, 22.4%), or amphotericin B (n = 3, 4.5%). To patients with underlying diseases, fluconazole (including Fos-fluconazole) (n = 30, 35.7%), azoles plus 5-FC (n = 17, 20.2%), itraconazole (n = 6, 7.1%), VRCZ or MCZ (n = 10, 11.9%), and amphotericin B ± 5-FC (n = 4, 4.8%) were administered.

The median duration of fluconazole (Fos-fluconazole) treatment was 90 days (range 60–110 days) for patients without underlying disease. Five patients did not receive any antifungal drugs because they were initially suspected of having lung cancer and underwent pneumonectomy. Three patients without underlying disease were initially observed without any antifungals under informed consent, as the size and number of radiological abnormalities were reduced spontaneously and the serum cryptococcal antigen titers decreased within a few months.

Antifungal agents were administered for 6 months in all patients except three with refractory cryptococcosis among population with underlying diseases.

3.8. Transitional serum cryptococcal antigen titer before and after treatment

Forty definite cases (14 cases with underlying diseases and 26 cases were without underlying disease) which were followed until the serum cryptococcal antigen became negative or up to 45 months after treatment.

Titer changes in the latex agglutination test before and after therapy in patients without underlying disease and with underlying disease are depicted in Figs. 2 and 3, respectively. The mean duration of treatment for 14 patients without underlying diseases was 2.87 months. The cryptococcal antigen titer decreased for all cases after antifungal treatment. The cryptococcal antigen became negative in 13 of 14 cases following administration of antifungal agents. The mean period from treatment cessation to negative antigen observation was 10.7 months.

The mean duration of treatment for 26 patients with underlying diseases was 6.64 months. In the 22 cases where antigen titers

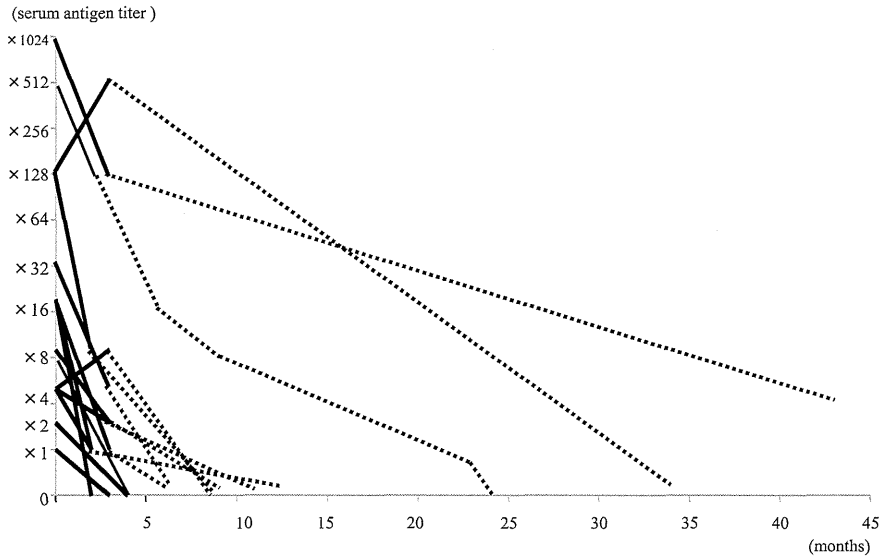

Fig. 2. Transitional change of the latex agglutination test after treatment in patients without underlying diseases. Solid line indicates the duration of treatment and dotted line indicates the following time after treatment. The mean duration of treatment for 14 patients without underlying diseases was 2.87 months. The cryptococcal antigen titer decreased for all cases after antifungal treatment. The cryptococcal antigen became negative in 13 of 14 cases following administration of antifungal agents. The mean period from treatment cessation to negative antigen observation was 10.7 months. The patients were followed until the serum antigen become negative or up to 45 months.

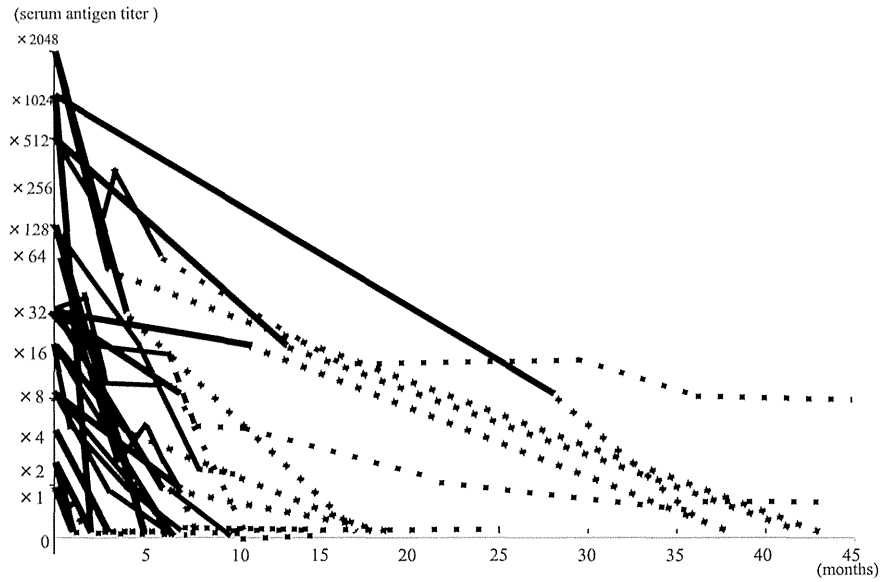

Fig. 3. Transitional change of the latex agglutination test after treatment in patients with underlying diseases. Solid lines and dotted lines denote treatment duration and duration of follow-up after treatment, respectively. The mean duration of treatment for 26 patients with underlying diseases was 6.64 months. In the 22 cases where antigen titers became negative after treatment, the mean period from treatment cessation to negative antigen observation was 13.1 months. The patients were followed until the serum antigen become negative or up to 45 months.

became negative after treatment, the mean period from treatment cessation to negative antigen observation was 13.1 months (Fig. 3). No significant difference was observed between two groups. The serum antigen titers were decreased after antifungals were discontinued.

3.9. Comorbid cryptococcal meningoencephalitis

In 151 of pulmonary cryptococcosis cases, 122 patients were performed lumbar puncture test. Fourteen patients (9.3%) presented CNS involvement. Four and 10 patients were without and with underlying disease, respectively (Table 3).

Fever (57.1%; 8/14), headache (35.7%; 5/14), and appetite loss or vomiting (35.7%; 5/14) suggesting CNS infection were observed. However, 2 patients had no CNS symptoms. The radiographic findings in patients with CNS involvement are shown in Table 3. Solitary or multiple nodules, consolidation, reticular shadow, granular shadow or mixed findings were observed in meningoencephalitis patients. Pulmonary shadows were seen in both lungs in 7 patients. In meningoencephalitis patients, all patients without

Table 3
Summary of cases with cryptococcal meningoencephalitis.

Age	Sex	Symptoms	Radiographic findings	Site of shadow	Underlying diseases	Steroid usage	Cr Ag	Treatment	Prognosis
28	M	Headache	Solitary nodule	rt. Middle robe	(−)	(−)	NA	AMPH-B + 5-FC	Improved
59	M	Cough, sputum	Consolidation	Both lungs	(−)	(−)	NA	AMPH-B + 5-FC, MCZ	Improved
62	M	Headache, cough	Multiple nodules	Both lungs	(−)	(−)	512	FLCZ + 5-FC	Improved
80	M	Fever, headache, vomiting, lumbago	Consolidation	rt. Middle robe	(−)	(−)	2046	FLCZ + 5-FC	Improved
48	F	Fever, appetite loss	Reticular shadow	Both lungs	SLE, NS	(+)	NA	(−)	Death
75	F	Fever, headache, consciousness disorder	Granular shadow	Both lungs	ATL	(−)	2048	FLCZ + 5-FC	Death
60	F	No symptom	Solitary nodule	rt. Lower lobe	DM	(−)	1	MCZ + 5-FC, FLCZ	[a]
73	F	Fever, cervical lymph node enlargement	[a]	[a]	ATL	[a]	128	FLCZ + 5-FC	Death
61	M	Headache, change in personality	Consolidation	rt. Lower lobe	SLE、APS	(+)	1024	AMPH-B + 5-FC	Improved
76	F	Fever, vomiting	Multiple nodules + consolidation	Both lungs	DM, RA	(−)	NA	FLCZ + 5-FC	Improved
86	M	fever, respiratory discomfort	diffuse GGA	Both lungs	CRF	(−)	1024	F-FLCZ	Death
74	F	Fever	Multiple nodules	rt. Lower lobe	RA, CRF, amyloidosis	(+)	1024	FLCZ + 5-FC + AMPH-B	Death
74	M	Fever, appetite loss, general fatigue	Solitary nodule	lt. Lower lobe	Wegener's granulomatosis	(+)	256	FLCZ + 5-FC	Improved
60	M	Appetite loss, general fatigue	Multiple nodules	Both lungs	ATL, DM	(+)	NA	FLCZ + 5-FC	Death

ATL, Adult T cell leukemia; SLE, systemic lupus erythematosus; NS, nephrosis syndrome; DM, diabetes mellitus; RA, rheumatoid arthritis; CRF, chronic renal failure; APS, anti-phospholipid antibody syndrome, rt., right, and lt., left; Cr Ag, Cryptococcal antigen titer; NA, not available. AMPH-B, amphotericin B; 5-FC, flucytosine; FLCZ, fluconazole; F-FLCZ, fos-fluconazole; MCZ, miconazole.

[a] Data was missed.

Table 4
Summary of patients who died of cryptococcosis.

Age	Sex	Radiographic findings	Site of shadow	Underlying condition	Steroid usage	Treatment	Meningoencephalitis	Diagnostic methods
40	M	Consolidation	Both lungs	ML	(−)	MCZ + 5FC, AMPH-B+5FC	(−)	TBLB
57	M	Consolidation	Both lungs	DM.LC.ATL, HCC	(+)	AMPH-B + 5FC, ITZ, FLCZ	(−)	TBLB
64	F	Consolidation	Both lungs	ATL	(−)	FLCZ	(+)	Sputum culture
69	F	Consolidation	rt. Upper lobe	LK	(+)	FLCZ+5FC	not done	BALF culture
86	M	Diffuse GGA	Both lungs	CRF	(−)	F-FLCZ	(+)	Sputum, CSF, Urine, blood culture
48	F	Reticular shadow	Both lungs	SLE, NS	(+)	(−)	(+)	Autopsy
75	F	Consolidation	Both lungs	Bladder tumor	(−)	(−)	(−)	Autopsy
62	F	Consolidation	Both lungs	PN, ARF	(+)	FLCZ, MCZ	(−)	Autopsy
75	F	Granular shadow	Both lungs	ATL	(−)	(−)	(+)	Autopsy
82	F	Consolidation	both lungs	RA, miliary TB	(+)	(−)	not done	Autopsy
66	M	Interstitial shadow (by underlying disease)	both lungs	IP	(+)	(−)	not done	Autopsy
73	F	Consolidation	both lungs	ATL	[a]	FLCZ+5-FC	not done	Sputum culture
74	F	Multiple nodules	rt. lower lobe	RA, CRF, secondary amyloidosis	(+)	FLCZ+5-FC + AMPH-B	(+)	BALF, CSF, blood culture
60	M	Multiple nodules	both lungs	ATL, DM	(+)	FLCZ+5-FC	(+)	BALF, CSF, blood, prostatic fluid culture

GGA, ground-glass attenuation; rt., right; ML, malignant lymphoma; DM, diabetes mellitus; LC, liver cirrhosis; ATL, adult T cell leukemia; HCC, hepatocellular carcinoma; LK, lung cancer; CRF, chronic renal failure; SLE, systemic lupus erythematosus; NS, nephrosis syndrome; PN, polyarteritis nodosa; ARF, acute renal failure; RA, rheumatoid arthritis; TB, tuberculosis; IP, interstitial pneumonia; TBLB, transbronchial biopsy; BALF, bronchial alveolar lavage fluid, and CSF, cerebrospinal fluid. AMPH-B, amphotericin B; 5-FC, flucytosine; FLCZ, fluconazole; ITZ, itraconazole; F-FLCZ, fos fluconazole; MCZ, miconazole.
[a] Data was missed.

underlying disease were improved; however, 6 of 10 patients with underlying disease died. One patient did not have underlying disease; the other had a previous history of diabetes.

3.10. Pulmonary cryptococcosis patients who died

Overall cryptococcal-related mortality was 9.4% (14/151). Mortality in patients with underlying diseases was 16.7% (14/84). No patients without underlying diseases died of cryptococcal-related disease. Some patients harbored underlying conditions such as hematologic disease and malignant tumors; the cause of death in many cases could be traced to worsening underlying disease.

The radiographic finding of most of the patients who died revealed consolidation (57.1%; 8/14) and in both lungs (85.7%; 12/14), suggesting disseminated cryptococcal infection. Six of the deceased patients were diagnosed by autopsy without any anti-cryptococcal treatment. The progression of serum antigen titers could not be observed continuously in the patients that died. Only one patient relapsed (Table 4).

3.11. Laboratory data correlated to meningoencephalitis and outcome

The clinical features including laboratory data in the patients with and without cryptococcal meningoencephalitis, and outcome are compared in Table 5.

Older age, lower lymphocyte counts, higher neutrophil counts, lower serum total protein, lower serum albumin, low CD4/8 ratio, high CRP and higher cryptococcal antigen titer are related to comorbidity of meningoencephalitis. For prognosis, older age, higher neutrophil counts, lower serum total protein, lower serum albumin, and higher CRP are correlated to death. Comorbidity of meningoencephalitis and poor outcome shares same factors.

4. Discussion

In Japan, the majority of cryptococcosis is seen in non-HIV patients. In the present study, we reviewed the clinical features of 151 pulmonary cryptococcosis in non-HIV patients in Nagasaki, Japan.

Table 5
Comparison of clinical characters of patients with or without cryptococcal meningoencephalitis and prognosis.

		Comorbid meningoencephalitis							Prognosis						
		Without meningoencephalitis			With meningoencephalitis			Wilcoxon test	Improved			Died			Wilcoxon test
	Criteria	*n*	Median	IQR	*n*	Median	IQR	*p* Value	*n*	Median	IQR	*n*	Median	IQR	*p* Value
1	Age	110	54.5	30.0	14	67.5	15.0	0.004	123	55.0	30.0	13	69.0	13.0	0.004
2	lymphocyte counts	98	1914.0	1006.0	11	1023.0	926.0	0.005	108	1874.0	992.5	11	1370.0	2792.0	0.26
3	Neutrophil counts	99	3355.0	2658.0	11	7626.0	4280.0	0.001	109	3780.0	2780.0	11	7832.0	4908.0	0.003
4	IgG	61	1290.0	661.0	5	1140.0	468.0	0.78	63	1307.0	600.0	5	1343.0	694.0	0.87
5	IgM	61	142.0	79.0	5	103.0	115.3	0.33	63	142.0	81.0	5	157.0	115.3	0.80
6	IgA	60	259.0	179.0	5	328.0	133.0	0.74	62	259.0	167.0	5	454.0	256.0	0.34
7	Total protein	88	6.90	0.90	11	5.90	1.50	0.02	99	6.90	0.90	10	5.30	0.50	0.0003
8	Serum albumin	82	4.10	0.80	9	3.10	1.60	0.01	90	4.10	0.80	8	2.65	1.13	0.0006
9	CD4/CD8	60	1.47	0.74	3	0.96	0.43	0.03	61	1.45	0.72	5	1.34	0.85	0.45
10	CRP	69	0.26	1.02	4	10.59	13.44	0.004	76	0.29	1.08	5	4.12	12.29	0.003
11	Cryptococcal antigen	90	16.00	120.00	7	1024.00	1920.00	0.02	103	16.00	252.00	11	128.00	1016.00	0.15
12	CD4 counts	30	719.50	474.60	1	421.50		0.30	34	734.60	480.80	1	95.00		0.09

IQR: Inter Quartile Range, CRP, C-reactive protein.

Roughly half of the cryptococcosis patients did not have underlying diseases and almost half of patients (82/151, 54.3%) without respiratory symptoms were found accidentally by mass screening examination or routine chest X-ray check. In Japan, there is a unique medical insurance system which allowed people easy and cheap access to medical examination or annual medical check. This background may cause the potential bias in this study.

It is generally recommended that HIV-negative patients with cryptococcal pneumonia undergo routine lumbar puncture to attempt to identify asymptomatic or subclinical CNS involvement that may require more potent or aggressive therapy [13]. However, 2010 updated IDSA guidelines commented as follows, "In non-immunocompromised patients with pulmonary cryptococcosis, consider a lumbar puncture to rule out asymptomatic CNS involvement. However, for normal hosts with asymptomatic pulmonary nodule or infiltrate, no CNS symptoms, and negative or very low serum cryptococcal antigen, a lumbar puncture can be avoided (B-II)" [14]. Of the 14 patients with cryptococcal meningoencephalitis in this study, 4 did not have underlying disease. Six patients with meningoencephalitis were dead (eight were survived) and the correlation between comorbidity of meningoencephalitis and poor outcome shares same clinical factors. However, the possibility of existence of meningoencephalitis links significant poor prognosis was not evaluated due to the low number of cases. Of those, 1 patient had solitary nodules, 1× negativity for cryptococcosis antigens, and no CNS disorders. We believe that the necessity of CSF examinations should be debated thoroughly. There has been ongoing discussion regarding the need for lumbar punctures in patients without CNS symptoms. The 2007 Guidelines [15] also recommend lumbar puncture to identify asymptomatic CNS involvement.

To our knowledge, this review of pulmonary cryptococcosis constitutes the largest report to date describing and comparing chest CT findings in non-HIV patients both with and without underlying diseases. Additionally, since many cases were diagnosed from mass screening check-up, it is important to investigate the unique features of radiological findings. Similar to recent studies of immunocompetent hosts [16–19] and non-AIDS individuals [11,20,21], the most common CT feature was the presence of peripherally distributed multiple pulmonary nodules or masses with predominant lower lobe involvement in both patients without and with underlying diseases Although the number of nodules or masses in previous reports has varied, there was no significant difference in the frequency between multiple nodules or masses (type II) and single lesions (type I) in our series. Multiple nodules or masses distributed in multiple lobes (type IIb) also tended to occur more frequently in patients with underlying diseases than in patients without underlying diseases.

Cryptococcal antigen is widely recognized to have both diagnostic and prognostic value for cryptococcosis. Lu et al. reported on the CSF titer change in the latex agglutination test before and after therapy in non-HIV cryptococcal meningitis. The cryptococcal antigen titer in CSF decreased after therapy for every case and correlated with fungal clearance; however, cryptococcal antigen can remain at low titers for long periods after therapy, even when fungal smear and/or culture become negative. Previous study suggested that the cryptococcal antigen test may not be used as an index of cure [22].

In this study, serum cryptococcal antigen in pulmonary cryptococcosis patients can remain at low titers for long periods after therapy. However, the titers continuously decrease after effective therapy. Our data demonstrate that the cryptococcal antigens in pulmonary cryptococcosis remain detectable even months following successful therapy, suggesting that the cryptococcal antigen test may not be used as an index of cure or decision of discontinuation of treatment.

The first line antifungal drugs were selected because the facilities that reported these cases were conducting clinical trials for antifungal drug development. Moreover, because the guidelines for cryptococcosis management had not yet been presented before 2007, azole-type drugs + 5-FC were used as pulmonary cryptococcosis treatment for a certain period even in patients without meningoencephalitis.

After the 2007 Guidelines [15] was published, treatment was conducted according to these guidelines' recommendations.

The IDSA guideline for pulmonary cryptococcosis in non-HIV, non-immunosuppressed patients states that for mild-to-moderate symptoms, fluconazole (400 mg/day orally) should be administered for 6–12 months; persistently positive serum cryptococcal antigen titers are not criteria for continuance of therapy (B-II) [14]. Conversely, the 2007 Guidelines recommend administering 400 mg/day oral fluconazole for 3 months for immunocompetent pulmonary cryptococcosis patients, and for 6 months for patients with underlying disease [15].

In our experience, with the exception of some severe cases, the duration of treatments as recommended in the 2007 Guidelines [15] appears appropriate.

In the management of pulmonary cryptococcosis in non-HIV patients, it is important to confirm the presence of underlying disease and encephalomeningitis complications, and a careful treatment plan for patients with severe underlying diseases.

Conflict of interest

SK received honorarium, consultation fees and research grants from Pfizer Inc., and Dainippon Sumitomo Pharma Co.

HK received honorarium from Pfizer Inc. and Dainippon Sumitomo Pharma Co.

KI received honorarium from Pfizer Inc., and Dainippon Sumitomo Pharma Co.

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Brain Magnetic Resonance Imaging Screening Is Not Useful for HIV-1-Infected Patients Without Neurological Symptoms

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Abstract

We investigated the diagnostic usefulness of brain magnetic resonance imaging (MRI) screening in HIV-1-infected patients without neurological symptoms in detecting intracranial diseases at early stages. In this retrospective analysis, the study patients were HIV-1-infected patients who underwent brain MRI scan in clinical practice between 2001 and 2013. We excluded patients with MRI for (1) follow-up examination for prediagnosed intracranial diseases, (2) cancer staging, (3) screening mycobacterium/bacteria/fungi disease proliferation in the brain, and (4) evaluation for meningitis/encephalitis. The study patients ($n=485$) were classified into two groups: those who underwent brain MRI scan without any neurological symptoms/signs (asymptomatic patients, $n=158$) and those who underwent MRI due to such symptoms (symptomatic patients, $n=327$). Asymptomatic patients had lower CD4 counts than symptomatic patients (median 78 versus 241/μl). Intracranial diseases were detected in three (2%) of the asymptomatic patients [two toxoplasmosis and one progressive multifocal leukoencephalopathy (PML)] compared to 58 (19%) of the symptomatic patients (the χ^2 test, $p<0.01$). The latter included toxoplasmosis ($n=10$), PML ($n=7$), cytomegalovirus encephalitis ($n=3$), primary central nervous system lymphoma ($n=3$), cryptococcoma/meningitis ($n=3$), and HIV-associated dementia ($n=17$). Among symptomatic patients, intracranial diseases were common in those with slurred speech (3/6, 50%), seizure (4/10, 40%), eyesight/vision abnormality (5/16, 31%), altered mental status (8/31, 26%), and hemiplegia/numbness (13/50, 26%). For patients with CD4 count $<200/\mu$l, intracranial diseases were detected in only 3 (3%) of 144 asymptomatic patients, compared with 46 (32%) of 113 symptomatic patients ($p<0.01$). Brain MRI screening for HIV-1-infected patients without neurological symptoms is of little value.

Introduction

PATIENTS WITH ADVANCED HIV-1 INFECTION are prone to develop intracranial opportunistic diseases, such as toxoplasma encephalitis, primary central nervous system lymphoma (PCNSL), progressive multifocal leukoencephalopathy (PML), and cytomegalovirus (CMV) encephalitis.[1] Although the introduction of antiretroviral therapy (ART) substantially decreased the incidence of neurological opportunistic infections,[2,3] such diseases have high associated mortality even with appropriate treatment, and recurrences and residual neurological deficits can occur.[4,5] Because delayed diagnosis of these intracranial diseases has a detrimental effect on patients with HIV-1 infection,[5,6] early diagnosis, not to mention prevention, of such diseases is of importance.

Brain magnetic resonance imaging (MRI) is often preferred to computed tomography (CT) in establishing the diagnosis of many of these diseases due to its superior sensitivity to subtle white matter and meningeal disease.[7–10] However, there is no information on the utility of brain MRI screening for HIV-1-infected patients without neurological symptoms/signs in detecting intracranial opportunistic diseases at early stages. This observational study was designed to assess the usefulness of brain MRI screening of such patients with HIV-1 infection.

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Materials and Methods

Study design, setting, and participants

We conducted an observational single-center study to investigate the usefulness of brain MRI screening in HIV-1-infected patients without neurological symptoms who warrant investigation for intracranial diseases. The study was conducted at the AIDS Clinical Center, National Center for Global Health and Medicine (NCGM), Tokyo, the largest referral center for HIV care in Japan.[11] The study patients were those who fulfilled the following inclusion criteria: HIV-1-infected patients who underwent brain MRI scan in clinical practice between June 2001 and August 2013. In addition, the following exclusion criteria were applied: patients who underwent brain MRI for (1) follow-up examination during the study period because of intracranial diseases such as opportunistic infections, stroke, or malignancy, which were diagnosed prior to the referral to our clinic, (2) staging of malignant tumors, (3) screening mycobacterium/bacteria/fungi disease proliferation in the brain in patients who were already diagnosed with mycobacterial diseases or bacteremia or fungemia, and (4) evaluation of meningitis/encephalitis.

The study patients ($n=485$) were classified into those who underwent brain MRI scan without any neurological symptoms, such as seizure, altered mental status, hemiplegia/numbness, headache, or fever (asymptomatic patients, $n=158$), and those who underwent MRI due to the abovementioned symptoms, which can suggest a focal brain lesion[5] (symptomatic patients, $n=327$). Asymptomatic patients included those who underwent MRI due to positive antitoxoplasma IgG antibody ($n=38$) and positive serum cryptococcal antigen ($n=1$). At our clinic, patients with a low CD4 cell count (typically less than 200/μl) often underwent brain MRI even though they had no neurological symptoms/signs that would warrant a brain imaging examination to rule out intracranial opportunistic infections or malignancy at early stages.

The study was approved by the Human Research Ethics Committee of NCGM. All patients included in this study provided written informed consent for their clinical and laboratory data to be used and published for research purposes. The study was conducted according to the principles expressed in the Declaration of Helsinki.

Measurements

At our hospital, brain MRI was routinely read by one experienced radiologist and the findings were confirmed by another radiologist. Furthermore, the MRI diagnosis was confirmed by reviewing the medical records and follow-up brain imaging when available. The diagnostic criteria for cryptococcal meningitis, cytomegalovirus encephalitis, and toxoplasmic encephalitis were those adopted by the AIDS Clinical Trials Group (ACTG)-A5164.[12] HIV-associated dementia in this study was diagnosed based on the MRI findings, which included generalized atrophy and prominent white matter changes plus cognitive impairment based on the chart review, and not necessarily required neurocognitive function tests.[8] The reasons for conducting an MRI were also extracted from the medical records. Baseline characteristics and HIV-1-related variables at the time of brain MRI were also extracted from the medical records. They included age, sex, ethnicity, history of AIDS, route of HIV-1 transmission, treatment status for HIV-1 infection (either treatment naive or experienced), CD4 cell count, and HIV viral load. For CD4 count and HIV load, we used data collected closest to and preceding by up to 3 months the day of the brain MRI. In Japan, because the prescription period under the health care system is limited to 3 months, patients need to visit the HIV Clinic at least once every 3 months for prescriptions as well as monitoring CD4 cell count and HIV-1 load.[11]

Statistical analysis

Baseline characteristics were compared between asymptomatic and symptomatic patients using the Student's t-test and χ^2 test (Fisher's exact test) for continuous and categorical variables, respectively. Prevalence of intracranial diseases was calculated among asymptomatic patients and compared to that of symptomatic patients with the χ^2 test. The logistic regression model was used to estimate the associations of lack of neurological symptoms/signs over the presence of such symptoms/signs with the MRI findings of intracranial diseases. The model was adjusted for age, sex, CD4 count, HIV treatment status, and history of AIDS. Subgroup analysis included the prevalence of intracranial diseases in patients with a CD4 count $<200/\mu$l. Statistical significance was defined as two-sided p values <0.05. We used odds ratios (ORs) with 95% confidence intervals (95% CIs). All statistical analyses were performed with The Statistical Package for Social Sciences ver. 21.0 (SPSS, Chicago, IL).

Results

The study included 485 patients who underwent a brain MRI scan in clinical practice, of whom 158 had no neurological symptoms (asymptomatic) and 327 did have such symptoms (symptomatic). Of the total patients, 475 (98%) were Asians, 446 (92%) were males, and 365 (75%) were infected with HIV-1 through homosexual contact (Table 1). The median age of the study patients was 41 [interquartile range (IQR) 34–51]. Asymptomatic patients had a lower CD4 count [median 78/μl, interquartile range (IQR) 21–237, symptomatic: 241/μl, 60–470 ($p<0.01$)] and higher HIV-1 viral load [4.84 $\log_{10}$/ml, IQR 2.97–5.62, symptomatic: 2.95 $\log_{10}$/ml, 1.70–5.11 ($p<0.01$)] than symptomatic patients. Asymptomatic patients were more likely to be treatment naive (68% versus 41%, $p<0.01$) and have a history of AIDS (62% versus 47%, $p<0.01$). There was no significant difference in other baseline characteristics between the two groups (Table 1).

Among the 158 asymptomatic patients, brain MRI screening detected toxoplasmosis ($n=2$) and PML ($n=1$, with CD4 43/μl), i.e., a prevalence of intracranial diseases of 2%. The two patients with toxoplasmosis underwent brain MRI due to positive antitoxoplasma IgG antibody with a titer of 20,480 (CD4 168/μl) and 1,280 (CD4 16/μl) IU/ml. In asymptomatic patients who underwent brain MRI due to positive antitoxoplasma IgG antibody, intracranial diseases were detected in 3 (8%) out of 38 patients (Table 2). On the other hand, brain MRI for symptomatic patients detected 58 intracranial diseases with a prevalence of 19%. The cases included toxoplasmic encephalitis ($n=10$), PML ($n=7$), CMV encephalitis ($n=3$), PCNSL ($n=3$), cryptococcosis/meningitis ($n=3$), herpes simplex virus encephalitis ($n=1$), HIV-associated dementia ($n=17$), acute cerebral infarction ($n=8$), gummatous