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INTERNAL MEDICINE

ORIGINAL ARTICLE

Importance of Functional Assessment in the Management of Community-acquired and Healthcare-associated Pneumonia

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Abstract

Objective In Japan, the number of elderly people who have difficulties performing the activities of daily living (ADLs) is increasing. The objective of this study was to assess the relationship between ADL and the clinical characteristics of pneumonia.

Methods We conducted a retrospective study of 219 adult patients hospitalized due to pneumonia [151 patients with community-acquired pneumonia (CAP) and 68 patients with healthcare-associated pneumonia (HCAP)]. CAP, HCAP, and all the patients were stratified into two groups using a modified version of the Katz index of five ADLs as follows: independent in all ADLs or dependent in one to three ADLs (CAP-A, HCAP-A, and All-A groups) and dependent in four or five ADLs (CAP-B, HCAP-B, and All-B groups). Disease severity, microbiological findings, and mortality were compared between the groups.

Results As the ability to perform ADLs declined, A-DROP scores (the CAP severity measurement index) increased significantly in CAP (CAP-A: 1.1±1.1, CAP-B: 2.6±1.1), HCAP (HCAP-A: 2.0±1.0, HCAP-B: 2.8±1.0), and all patients (All-A: 1.3±1.1, All-B: 2.8±1.0). Thirty-day mortality was higher in the CAP-B (23.1%) and All-B (19.2%) groups than in the CAP-A (0.7%) and All-A (1.8%) groups, respectively. A multivariate Cox proportional hazards analysis showed an ADL score ≥ four to be a significant predictor of 30-day mortality in CAP patients [hazard ratio (HR), 19.057; 95% confidence interval (CI), 1.930-188.130] and in all patients (HR, 8.180; 95% CI, 1.998-33.494).

Conclusion A functional assessment using a modified version of the Katz index is useful for the management of CAP and HCAP patients.

Key words: activities of daily living, respiratory tract infections, frail elderly, mortality, healthcare

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Introduction

In Japan and other developed countries, the number of elderly people who have difficulties and require supports in their activities of daily living (ADLs) is growing (1, 2). To manage patients with pneumonia who have frequent or chronic contact with the healthcare system and are found to be at risk of having drug-resistant pathogens with high mortality, healthcare-associated pneumonia (HCAP) that requires broad-spectrum antimicrobial drugs was included as a category of pneumonia according to the 2005 American Thoracic Society (ATS)/Infectious Diseases Society of America (IDSA) guidelines (3).

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However, the HCAP population is heterogeneous and overlaps with that of community-acquired pneumonia (CAP) in the elderly (4-6). Additionally, physical functional status is not included in the HCAP definition, even though it is a crucial predictor of drug-resistant pathogens and patient outcome (4, 7, 8).

El Solh et al. previously analyzed 88 patients with culture-positive, severe nursing home-acquired pneumonia (NHAP) and reported that the degree of ADL decline was one of the important predictors of drug-resistant pathogens (9). In addition, Lim et al. evaluated 437 patients with NHAP and CAP in the UK and found that NHAP patients had greater mortality related to poor functional status (10).

It is conceivable that ADLs are an important factor in the management of elderly patients with pneumonia. Nevertheless, few studies have evaluated the relationship between ADLs and the clinical characteristics of CAP and HCAP in Japan. The objective of this study was to evaluate differences in the clinical characteristics of pneumonia among patients classified by stratification of ADL before admission and to determine how ADL is related to clinical outcome in CAP and HCAP patients.

Materials and Methods

Study design, subjects, and definitions

We conducted a retrospective study of 219 patients with pneumonia who were hospitalized at Nijigaoka Hospital (a 150-bed community hospital in Nagasaki, Japan) between July 2009 and March 2012.

All patients were divided into CAP and HCAP groups based on the 2005 ATS/IDSA guidelines. Briefly, a patient with HCAP was defined as any patient with pneumonia who satisfied one of the following criteria: (1) hospitalization for two or more days in the preceding 90 days, (2) residence in a nursing home or extended care facility, (3) home infusion therapy (including antibiotics), (4) chronic dialysis within 30 days, or (5) home wound care (3). Long-term care facilities (LTCFs) included nursing homes, homes with more medical services, chronic-care hospitals, and psychiatric hospitals.

The ADL dependency of all hospitalized patients was routinely evaluated by nurses according to a uniform format used in Nijigaoka Hospital. ADL decline was defined as the need for personal assistance in performing one or more ADLs. The physical functional status was measured using a simplified and modified version of the Katz index of five ADLs: bathing, dressing, moving from a bed to a chair, using a toilet, and eating (11). CAP patients, HCAP patients, and all patients were divided into two groups according to their level of dependence on assistance to perform ADLs before admission as follows: patients with pneumonia who were independent in all ADLs or dependent in one to three ADLs (CAP-A, HCAP-A, and All-A groups) and those who were dependent in four or five ADLs (CAP-B, HCAP-B, and All-B groups).

We compared the baseline characteristics, identified pathogens, and clinical outcomes between the groups. The study was approved by the institutional review board of Nijigaoka Hospital. Informed consent was not required because the study was retrospective and the data were obtained within the context of normal daily practice. Pneumonia was defined as the appearance of a new infiltrate on chest images that was accompanied by clinical symptoms, such as cough, sputum and fever, or inflammatory reactions (e.g., leukocytosis, leukopenia, or increased C-reactive protein levels) on laboratory tests. Patients who were diagnosed with hospital-acquired pneumonia (HAP), lung cancer-associated obstructive pneumonia, interstitial pneumonia, organizing pneumonia, or eosinophilic pneumonia were excluded. Probable aspiration was defined as aspiration witnessed, confirmed by the water-drinking test on hospital admission, or strongly suspected based on the patient's clinical course (12). The outcome measures were 30-day mortality and initial treatment failure. Initial treatment failure was defined as death during initial treatment or change of therapeutic agents from initial agents to others due to clinical ineffectiveness (e.g., lack of response or worsening of fever pattern, respiratory condition, and/or radiographic findings). Therapy was deemed inappropriate if the identified pathogens were resistant to the initially prescribed antibiotics based on in vitro susceptibility testing or if the initially administered antibiotics were not recommended for treatment of the identified pathogens according to the Japanese CAP and HAP guidelines (13, 14).

Severity evaluation

Pneumonia severity was evaluated using the predictive rule of a five-point scoring system for CAP: the A-DROP [age, dehydration, respiratory failure, orientation disturbances, and low blood pressure (BP)], which was proposed by the Japanese Respiratory Society (13). These are basically modified versions of the CURB-65 (13, 15, 16).

Microbiological evaluation

Pathogens in samples obtained from sputum, blood, or other body fluids were investigated using standard microbiological procedures. The results of blood cultures were accepted as an etiological diagnosis if no other source could be identified for the positive culture. Sputum samples were cultured in a quantitative manner, and positive bacterial culture results for sputum were documented from medical records. Serologic methods using single sera were used to detect immunoglobulin (Ig)M antibodies against *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae*. Rapid immunochromatographic assays were used to detect the influenza virus antigen in nasopharyngeal swabs, *Streptococcus pneumoniae* in urine and *Legionella pneumophila* serogroup 1 antigen in urine. These examinations were ordered by attending physicians as needed for each patient. Methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas* species, *Acinetobacter* species, and extended-spectrum beta-

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Table 1. Baseline Characteristics of Patients with Pneumonia

	CAP (n = 151)		p value	HCAP (n = 68)		p value	All patients (n = 219)		p value
	CAP-A n = 138	CAP-B n = 13		HCAP-A n = 29	HCAP-B n = 39		All-A n = 167	All-B n = 52	
Age (years)	67.6 ± 18.9	88.0 ± 8.3	<0.001	79.8 ± 9.7	82.7 ± 12.5	0.076	69.7 ± 18.2	84.0 ± 11.7	<0.001
Sex (male / female)	77/61 (55.8)	5/8 (38.5)	0.257	17/12 (58.6)	15/24 (38.5)	0.141	94/73 (56.3)	20/32 (38.5)	0.027
Tube feeding	0 (0.0)	1 (7.7)	0.086	0 (0.0)	6 (15.4)	0.034	0 (0.0)	7 (13.5)	<0.001
Antibiotic use within 90 days	0 (0.0)	0 (0.0)	-	10 (34.5)	5 (12.8)	0.042	10 (6.0)	5 (9.6)	0.357
Use of gastric acid-suppressants[a]	40 (29.0)	5 (38.5)	0.530	19 (65.5)	20 (51.3)	0.323	59 (35.3)	25 (48.1)	0.105
Probable aspiration	10 (7.2)	6 (46.2)	0.005	9 (31.0)	25 (64.1)	0.014	19 (11.4)	31 (59.6)	<0.001
Comorbidities									
Cerebrovascular disease	11 (8.0)	5 (38.5)	0.005	5 (17.2)	16 (41.0)	0.040	16 (9.6)	21 (40.4)	<0.001
Chronic pulmonary disease	42 (30.4)	3 (23.1)	0.756	15 (51.7)	5 (12.8)	0.001	57 (34.1)	8 (15.4)	0.009
Congestive heart failure	14 (10.1)	4 (30.8)	0.051	2 (6.9)	7 (17.9)	0.282	16 (9.6)	11 (21.2)	0.050
Chronic renal dysfunction	4 (2.9)	1 (7.7)	0.367	2 (6.9)	8 (20.5)	0.171	6 (3.6)	9 (17.3)	0.002
Chronic liver disease	7 (5.1)	2 (15.4)	0.174	3 (10.3)	2 (5.1)	0.644	10 (6.0)	4 (7.7)	0.746
Diabetes mellitus	22 (15.9)	2 (15.4)	1.000	4 (13.8)	10 (25.6)	0.364	26 (15.6)	12 (23.1)	0.215
Gastrectomy	5 (3.6)	0 (0.0)	1.000	3 (10.3)	2 (5.1)	0.644	8 (4.8)	2 (3.8)	1.000
Malignancy	6 (4.3)	2 (15.4)	0.143	5 (17.2)	3 (7.7)	0.272	11 (6.6)	5 (9.6)	0.541
Immunosuppression[b]	9 (6.5)	0 (0.0)	1.000	0 (0.0)	6 (15.4)	0.034	9 (5.4)	6 (11.5)	0.204

Values are expressed as mean ± standard deviation or the number (%).
CAP: community-acquired pneumonia, HCAP: healthcare-associated pneumonia
[a] Gastric acid-suppressants included histamine H_2-receptor blockers or proton pump inhibitors.
[b] Immunosuppression was defined as administration of corticosteroids (5 mg/day or more) or other immunosuppressive agents.

lactamase (ESBL) producing *Enterobacteriaceae* were considered as multidrug-resistant (MDR) pathogens (3, 17).

Statistical analysis

Continuous variables are expressed as mean ± standard deviation, and differences between groups were statistically analyzed by Student's *t*-tests when variables were normally distributed and Mann-Whitney U tests when variables were not normally distributed. Fisher's exact tests were used to compare categorical data between the groups. Survival rates within 30 days were estimated using the Kaplan-Meier method and compared between the groups with log-rank tests. We conducted univariate and multivariate analyses using a Cox proportional hazards model. Variables with p values <0.2 in univariate analysis were selected and adjusted by forward stepwise selection in multivariate analysis to identify the predictors of 30-day mortality. The data were analyzed using SPSS 16.0J for Windows (SPSS Inc., Chicago, USA), and p values of 0.05 were considered statistically significant.

Results

Patient characteristics

The characteristics of the 219 patients enrolled in the study are presented in Table 1. The mean age was significantly higher in the CAP-B and All-B groups than in CAP-A and All-A groups. All patients who received tube feeding were included in the reduced ADL group. As inactivity advanced, the proportion of aspiration pneumonia increased. About 40% of CAP-B and HCAP-B patients had cerebrovascular disease as a comorbidity. Immunosuppression was significantly higher in the HCAP-B group compared to the HCAP-A group. Conversely, the incidence of chronic lung disease was lower in the HCAP-B and All-B groups than in the HCAP-A and All-A groups. Chronic renal dysfunction was higher in the All-B group compared to the All-A group.

Regarding ADL dependency, a total of 75.5% of CAP patients and 27.9% of HCAP patients were independent in all ADLs. Conversely, 7.3% of CAP and 44.1% of HCAP patients needed support in all five ADLs.

HCAP-A patients were previously administered antibiotics within 90 days more frequently than HCAP-B patients. Gastric acid-suppressants were similarly prescribed between the groups.

Symptoms, clinical, laboratory, and radiographic findings

Cough was the most common symptom in CAP patients (CAP-A group: 80.4%, CAP-B group: 61.5%, p=0.150), with a lower frequency in HCAP patients with declined ADL (HCAP-A group: 69.0%, HCAP-B group: 43.6%, p= 0.050). In contrast, the percentage of disoriented patients significantly increased along with ADL decline in CAP (6.5% in CAP-A group, 53.8% in CAP-B group, p<0.001) and HCAP patients (10.3% in HCAP-A group, 56.4% in HCAP-B group, p<0.001).

Table 2 shows the physical findings and laboratory data of the study subjects. Hypoxia was observed in 53.8% of the CAP-B group but only 23.9% of the CAP-A group. More than 60% of HCAP patients had hypoxia, which was similar for the HCAP-A and B groups. The frequency of low BP was higher in the HCAP-B group compared to the HCAP-A group.

The laboratory data indicated that serum albumin decreased with worsening physical function in CAP and

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Table 2. Physical Examinations and Laboratory Findings of the Patients

	CAP (n = 151)		p value	HCAP (n = 68)		p value	All patients (n = 219)		p value
	CAP-A n = 138	CAP-B n = 13		HCAP-A n = 29	HCAP-B n = 39		All-A n = 167	All-B n = 52	
Clinical parameters									
Temperature (°C)	38.4 ± 1.0	37.9 ± 1.1	0.201	38.2 ± 0.8	38.3 ± 0.7	0.610	38.3 ± 1.0	38.2 ± 0.9	0.458
Pulse rate (/min)	88.5 ± 16.6	78.3 ± 15.3	0.035	85.4 ± 18.7	89.4 ± 18.4	0.378	87.9 ± 16.9	86.6 ± 18.2	0.636
Systolic BP ≤ 90 mmHg	1 (0.7)	0 (0.0)	1.000	1 (3.4)	9 (23.1)	0.036	2 (1.2)	9 (17.3)	<0.001
$SpO_2 \leq 90\%$	33 (23.9)	7 (53.8)	0.042	18 (62.1)	25 (64.1)	1.000	51 (30.5)	32 (61.5)	<0.001
Laboratory data									
White blood cell (/μL)	10,891.3 ± 5,301.4	10,092.3 ± 3,929.7	0.779	12,073.4 ± 5,111.8	11,723.1 ± 5,422.0	0.788	11,096.6 ± 5,273.0	11,315.4 ± 5,103.3	0.609
Red blood cell ($\times 10^4$/μL)	402.2 ± 53.1	387.5 ± 45.8	0.336	378.6 ± 43.2	369.8 ± 61.7	0.204	398.1 ± 52.2	374.2 ± 58.2	0.006
Platelets ($\times 10^4$/μL)	21.6 ± 8.0	24.1 ± 6.3	0.162	21.2 ± 9.9	20.6 ± 9.4	0.968	21.5 ± 8.3	21.5 ± 8.8	0.987
Total protein (g/dL)[a]	6.9 ± 0.6	6.6 ± 0.7	0.135	6.5 ± 0.8	6.3 ± 0.7	0.055	6.8 ± 0.7	6.3 ± 0.7	<0.001
Albumin (g/dL)[a]	3.6 ± 0.6	3.0 ± 0.4	0.002	3.1 ± 0.6	2.8 ± 0.5	0.037	3.5 ± 0.6	2.8 ± 0.5	<0.001
BUN (mg/dL)	16.2 ± 8.5	23.3 ± 6.9	<0.001	20.3 ± 10.4	27.3 ± 25.5	0.611	16.9 ± 9.0	26.3 ± 22.3	<0.001
Creatinine (mg/dL)	0.9 ± 0.4	1.0 ± 0.4	0.399	0.9 ± 0.4	1.1 ± 0.6	0.802	0.9 ± 0.4	1.0 ± 0.6	0.396
Na (mEq/L)	136.6 ± 3.9	132.1 ± 7.4	0.013	137.7 ± 4.6	136.4 ± 6.1	0.329	136.8 ± 4.0	135.3 ± 6.6	0.188
K (mEq/L)	3.9 ± 0.5	4.4 ± 0.9	0.053	4.0 ± 0.6	4.2 ± 1.0	0.424	3.9 ± 0.5	4.3 ± 1.0	0.024
Cl (mEq/L)	99.3 ± 4.4	94.5 ± 8.4	0.035	100.6 ± 4.7	100.5 ± 6.1	0.937	99.5 ± 4.5	99.0 ± 7.2	0.843
CRP (mg/dL)	12.0 ± 9.3	9.1 ± 5.8	0.427	9.7 ± 8.6	10.5 ± 8.7	0.522	11.6 ± 9.2	10.2 ± 8.1	0.457

Values are expressed as mean ± standard deviation or the number (%).

CAP: community-acquired pneumonia, HCAP: healthcare-associated pneumonia, BP: blood pressure, SpO_2: pulse oximetric oxygen saturation, BUN: blood urea nitrogen, CRP: C-reactive protein

[a] Serum total protein and albumin were measured in 79.5% and 82.2% of all patients, respectively.

HCAP patients. High levels of blood urea nitrogen (BUN) and hyponatremia were observed in the CAP-B group compared to the CAP-A group. A significant difference in potassium levels was seen between All-A and All-B patients.

Radiography demonstrated that the incidence of bilateral involvement was similar between the groups in CAP (CAP-A group: 31.9%, CAP-B group: 30.8%, p=1.000), HCAP (HCAP-A group: 51.7%, HCAP-B group: 56.4%, p=0.807), and all patients (All-A group: 35.3%, All-B group: 50.0%, p=0.073). Pleural effusion tended to occur more frequently in ADL-declined groups in CAP (CAP-A group: 26.1%, CAP-B group: 53.8%, p=0.051), HCAP (HCAP-A group: 27.6%, HCAP-B group: 46.2%, p=0.138), and all patients (All-A group: 26.3%, All-B group: 48.1%, p=0.006).

Distribution of identified pathogens

Streptococcus pneumoniae was the most frequently isolated pathogen in all patients, and the isolation rate was similar between the CAP (20.5%) and HCAP groups (23.5%). *Mycoplasma pneumoniae* was the second most commonly detected pathogen in the CAP (12.6%) and HCAP (14.7%) groups.

The microbes identified in each group are shown in Table 3. MRSA was significantly more frequently identified in the All-B group (13.5%) compared to the All-A group (3.0%). An inter-group comparison indicated no significant differences between the groups in terms of the numbers of identified pathogens.

Among patients with identified pathogens, MDR pathogens were identified more frequently in the All-B group (36.0%, nine of 25 patients) than the All-A group (13.4%, nine of 67 patients) (p=0.035).

Disease severity, antibiotic treatment, and clinical outcome

Pneumonia severity and the clinical outcomes are presented in Table 4. As physical function diminished, the A-DROP score increased, with statistically significant differences between groups. The 30-day mortality was higher in the CAP-B and All-B groups compared to the CAP-A and All-A groups. A similar was observed in the comparison of the HCAP-A and B groups, although the difference was not significant.

Initial treatment failure occurred more frequently in the CAP-B group than in the CAP-A group, and the duration of antibiotic therapy and length of hospital stay were much longer in the CAP-B and All-B groups than in CAP-A and All-A groups.

No significant differences were seen between the groups in terms of overall antimicrobial therapy by monotherapy in CAP patients (CAP-A group: 55.8% and CAP-B group: 69.2%, p=0.396) or in HCAP patients (HCAP-A group: 58.6% and HCAP-B group: 71.8%, p=0.305). A total of 67.3% of patients in the All-B group received monotherapy with beta-lactams. In particular, carbapenem was administered to 13.5% of patients in All-B and only 3.6% of the All-A group (p=0.015). Conversely, combination therapy with beta-lactams plus tetracyclines was administered to 17.4% of patients in the All-A group and 3.8% of patients in the All-B group (p=0.012).

In the All-A group, 11 of 67 patients with at least one identified pathogen received inappropriate therapy. Initial treatment failure occurred in five of 56 patients (8.9%) who received appropriate therapy, while four of 11 patients (36.4%) in the All-A group received inappropriate therapy (p=0.034). In the All-B group, nine of 25 patients with iden-

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Table 3. Pathogens Identified in Patients with Pneumonia

	CAP (n = 151)		p value	HCAP (n = 68)		p value	All patients (n = 219)		p value
	CAP-A n = 138	CAP-B n = 13		HCAP-A n = 29	HCAP-B n = 39		All-A n = 167	All-B n = 52	
Gram-positive pathogens									
Streptococcus pneumoniae	30 (21.7)	1 (7.7)	0.306	6 (20.7)	10 (25.6)	0.775	36 (21.6)	11 (21.2)	1.000
Staphylococcus aureus	8 (5.8)	1 (7.7)	0.566	3 (10.3)	7 (17.9)	0.498	11 (6.6)	8 (15.4)	0.085
MSSA	5 (3.6)	0 (0.0)	1.000	1 (3.4)	1 (2.6)	1.000	6 (3.6)	1 (1.9)	1.000
MRSA	3 (2.2)	1 (7.7)	0.305	2 (6.9)	6 (15.4)	0.451	5 (3.0)	7 (13.5)	0.009
Gram-negative pathogens									
Haemophilus Influenzae	11 (8.0)	0 (0.0)	0.600	2 (6.9)	1 (2.6)	0.571	13 (7.8)	1 (1.9)	0.196
Klebsiella pneumoniae	2 (1.4)	0 (0.0)	1.000	2 (6.9)	4 (10.3)	1.000	4 (2.4)	4 (7.7)	0.093
Pseudomonas aeruginosa	1 (0.7)	1 (7.7)	0.165	1 (3.4)	2 (5.1)	1.000	2 (1.2)	3 (5.8)	0.088
Escherichia coli	1 (0.7)	0 (0.0)	1.000	0 (0.0)	2 (5.1)	0.504	1 (0.6)	2 (3.8)	0.141
Acinetobacter baumannii	0 (0.0)	0 (0.0)	-	2 (6.9)	0 (0.0)	0.178	2 (1.2)	0 (0.0)	1.000
Moraxella catarrhalis	1 (0.7)	0 (0.0)	1.000	0 (0)	0 (0)	-	1 (0.6)	0 (0.0)	1.000
Other gram negative pathogens	1 (0.7)	0 (0.0)	1.000	0 (0.0)	1 (2.6)	1.000	1 (0.6)	1 (1.9)	0.419
MDR pathogens	5 (3.6)	2 (15.4)	0.112	4 (13.8)	7 (17.9)	0.747	9 (5.4)	9 (17.3)	0.016
Atypical pathogens									
Mycoplasma pneumoniae	17 (12.3)	2 (15.4)	0.669	6 (20.7)	4 (10.3)	0.305	23 (13.8)	6 (11.5)	0.817
Chlamydophila pneumoniae	1 (0.7)	1 (7.7)	0.165	1 (3.4)	0 (0.0)	0.426	2 (1.2)	1 (1.9)	0.558
Legionella pneumophila	0 (0.0)	0 (0.0)	-	0 (0.0)	0 (0)	-	0 (0.0)	0 (0.0)	-
Influenza virus	2 (1.4)	0 (0.0)	1.000	0 (0.0)	0 (0.0)	-	2 (1.2)	0 (0.0)	1.000
The number of identified pathogens									
At least one	52 (37.7)	5 (38.5)	1.000	15 (51.7)	20 (51.3)	1.000	67 (40.1)	25 (48.1)	0.337
Single	32 (23.2)	4 (30.8)	0.510	9 (31.0)	13 (33.3)	1.000	41 (24.6)	17 (32.7)	0.281
Double	16 (11.6)	1 (7.7)	1.000	4 (13.8)	3 (7.7)	0.449	20 (12.0)	4 (7.7)	0.458
Triple	4 (2.9)	0 (0.0)	1.000	2 (6.9)	4 (10.3)	1.000	6 (3.6)	4 (7.7)	0.253

Values are expressed as the number (%).

CAP: community-acquired pneumonia, HCAP: healthcare-associated pneumonia, MSSA: methicillin-susceptible *Staphylococcus aureus*, MRSA: methicillin-resistant *Staphylococcus aureus*, MDR: multidrug-resistant

Table 4. Disease Severity according to the A-DROP Scoring System, and Clinical Outcomes

	CAP (n = 151)		p value	HCAP (n = 68)		p value	All patients (n = 219)		p value
	CAP-A n = 138	CAP-B n = 13		HCAP-A n = 29	HCAP-B n = 39		All-A n = 167	All-B n = 52	
Severity evaluation									
A-DROP score	1.1 ± 1.1	2.6 ± 1.1	<0.001	2.0 ± 1.0	2.8 ± 1.0	0.002	1.3 ± 1.1	2.8 ± 1.0	<0.001
Outcome									
Initial treatment failure	8 (5.8)	5 (38.5)	0.002	5 (17.2)	4 (10.3)	0.481	13 (7.8)	9 (17.3)	0.063
30-day mortality	1 (0.7)	3 (23.1)	0.002	2 (6.9)	7 (17.9)	0.282	3 (1.8)	10 (19.2)	<0.001
Duration of intravenous antibiotics (days)	6.6 ± 3.5	10.0 ± 5.7	0.013	6.5 ± 2.7	8.6 ± 4.3	0.064	6.6 ± 3.3	8.9 ± 4.7	0.001
Hospitalization (days)	14.7 ± 19.5	21.8 ± 16.9	0.008	20.5 ± 18.0	29.8 ± 24.0	0.061	15.7 ± 19.3	27.8 ± 22.5	<0.001

Values are expressed as mean ± standard deviation or the number (%).

CAP: community-acquired pneumonia, HCAP: healthcare-associated pneumonia, A-DROP: age, dehydration, respiratory failure, orientation disturbances and low blood pressure

tified pathogens received inappropriate therapy. The frequencies of initial treatment failure were similar in patients with appropriate therapy (two of 16 patients, 12.5%) and those with inappropriate therapy (one of nine patients, 11.1%) (p= 1.000) in the All-B group.

Survival analysis and independent prognostic factors in CAP and HCAP patients

Kaplan-Meier curves showed that survival rates in the CAP-B and All-B groups were significantly lower than those in the CAP-A and All-A groups (Figure a, c). These trends were similar for the HCAP group (Figure b), although the difference was not significant.

Table 5 shows the results of the univariate and multivariate analyses of prognostic factors for 30-day mortality in patients with CAP and HCAP. On multivariate analysis, the independent predictors of 30-day mortality were ADL score ≥ four in CAP patients, malignancy, systolic BP ≤90 mmHg, and creatinine in HCAP patients and malignancy, ADL score ≥ four, and oxygen saturation (SpO_2) ≤90% in all patients.

Discussion

The present study demonstrates the differences in baseline characteristics, identified pathogens, disease severity, and clinical outcome among patients with pneumonia divided according to their ability to perform ADLs.

In our study, A-DROP scores and the 30-day mortality

Intern Med 53: 1613-1620, 2014 DOI: 10.2169/internalmedicine.53.2499

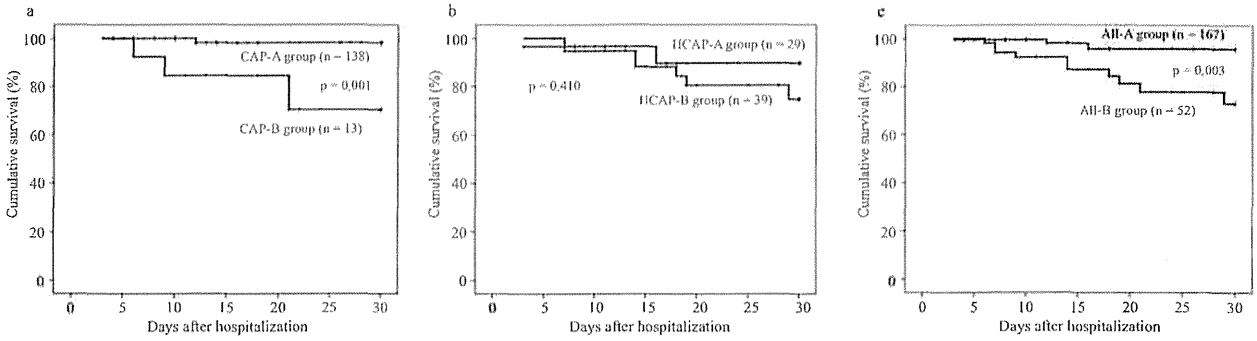

Figure. Kaplan-Meier survival analysis of patients with pneumonia according to activities of daily living (ADLs) in community-acquired pneumonia (CAP) patients (a), healthcare-associated pneumonia (HCAP) patients (b), and all CAP and HCAP patients (c). The survival rates of the CAP-B and All-B groups were significantly lower than those in CAP-A and All-A groups, respectively. The prognosis in HCAP-B patients tended to be worse compared to HCAP-A patients, although the difference was not significant.

Table 5. Univariate and Multivariate Analysis of Prognostic Factors for 30-day Mortality in Patients with Community-acquired and Healthcare-associated Pneumonia

	Univariate analysis			Multivariate analysis		
	HR	95% CI	p value	HR	95% CI	p value
CAP						
ADL score ≥ 4	19.057	1.930-188.130	0.012	19.057	1.930-188.130	0.012
Temperature (°C)	0.088	0.011-0.692	0.021			
Altered mental status	14.871	1.475-149.908	0.022			
K (mEq/L)	4.414	1.339-14.548	0.015			
Initial treatment failure	18.487	1.869-182.871	0.013			
HCAP						
Systolic BP ≤ 90 mmHg	3.159	0.789–12.648	0.104	7.762	1.567-38.450	0.012
Chronic renal dysfunction	4.315	1.152–16.159	0.030			
Malignancy	5.264	1.299-21.340	0.020	8.739	1.558-49.004	0.014
Immunosuppression	4.541	1.133–18.195	0.033			
BUN (mg/dL)	1.019	1.000–1.039	0.048			
Creatinine (mg/dL)	4.400	1.627–11.898	0.004	5.107	1.800-14.491	0.002
All patients						
ADL score ≥ 4	6.001	1.620–22.231	0.007	8.180	1.988-33.494	0.003
Congestive heart failure	3.216	1.049–9.866	0.041			
Chronic renal dysfunction	4.283	1.310–14.001	0.016			
Malignancy	6.411	1.964–20.932	0.002	13.370	3.291-54.313	<0.001
Systolic BP ≤ 90 mmHg	4.161	1.139–15.197	0.031			
SpO_2 ≤ 90%	4.774	1.034–22.040	0.045	7.464	1.422-39.185	0.018
BUN (mg/dL)	1.026	1.008–1.043	0.004			
Creatinine (mg/dL)	2.522	1.250–5.086	0.010			
K (mEq/L)	2.170	1.304–3.610	0.003			
Initial treatment failure	3.537	1.144–10.943	0.028			

HR: hazard ratio, CI: confidence interval, CAP: community-acquired pneumonia, HCAP: healthcare-associated pneumonia, ADL: activity of daily living, BP: blood pressure, BUN: blood urea nitrogen, SpO_2: pulse oximetric oxygen saturation

rate tended to be higher in patients with diminished ADL. A multivariate analysis indicated that ADL decline was an independent predictor of 30-day mortality in CAP patients and all patients. A previous report identified ADL dependency as an independent risk factor for both in-hospital and post-discharge mortality (18). Our results support those of a previous study in which functional status was the main determinant of outcome in elderly patients with pneumonia (7). Additionally, previous reports have indicated that performance status (PS) evaluation is useful for predicting the outcome of patients with pneumonia (19, 20). Therefore, PS is one of the criteria of nursing and healthcare-associated pneumonia (NHCAP), which was newly categorized in the 2011 Japanese Respiratory Society guidelines (6, 21). Although PS could not be documented from the medical records in the present study, our results, which focus on ADL dependency, correspond with the concept of NHCAP which includes patients with diminished PS in addition to HCAP patients.

We did not detect a significant difference between the 30-day mortalities of HCAP-A (6.9%) and HCAP-B (17.9%) patients, and ADL decline was not identified as an inde-

Intern Med 53: 1613-1620, 2014 DOI: 10.2169/internalmedicine.53.2499

pendent risk factor for 30-day mortality in HCAP patients. Therefore, we should consider other factors when predicting prognosis in HCAP patients, a group that mainly includes patients with diminished ADL.

We found that malignancy was an important predictor of 30-day mortality in HCAP and all patients. A recent study reported that it was an independent risk factor for in-hospital mortality in HCAP patients (22). The number of outpatients undergoing cancer therapy is increasing as a result of dramatic advances in cancer treatment and care (23). Therefore, we should recognize malignancy as a risk factor for mortality in patients with pneumonia, excluding those with HAP.

Previous studies reported that serum albumin was an independent prognostic factor in CAP, NHAP, and HAP patients (19, 20). In the present study, the low serum albumin levels observed in the diminished ADL group could be indicative of malnutrition, which is probably related to the high frequency of tube feeding in these patients. However, we could not adequately assess it as a prognostic factor because serum albumin was not measured in 17.8% of the patients. Aspiration pneumonia, defined by the presence of risk factors for aspiration and chest computed tomography (CT) findings, is also an independent risk factor for 30-day mortality among CAP and HCAP patients (24). However, we could not apply a strict definition for aspiration pneumonia in this study because CT was not performed in all patients.

In the All-B group, the mortality rate within 30 days (19.2%) tended to be higher than the proportion of patients in whom primary treatment failed (17.3%). Primary antibiotic therapy was successful in seven of 10 patients who subsequently died, with the ultimate poor outcome in these patients attributed to the development of secondary complications or exacerbation of comorbidities. Therefore, the systemic management of these complications and preventing the onset of pneumonia, such as by prophylaxis against aspiration or vaccination, are as essential as treatment with appropriate antibiotics in patients with diminished ADL.

The identities of the causative pathogens are also important when considering pathophysiology in patients with pneumonia. Lopez et al. previously described that MDR pathogens are implicated in a variable percentage of HCAP patients and do not seem to be the unique or direct cause of their increased mortality (25). In the present study, the rate of MRSA isolation was significantly higher in All-B patients. However, MRSA was not identified from the sputum of any of the All-B patients who died within 30 days of admission (except from fecal culture in one of the patients who died). This indicates that the high 30-day mortality of the All-B patients was not directly associated with the increased frequency of MRSA isolation from sputum.

Because our study was retrospective, there are some limitations associated with it that should be considered. First, microbiological evaluations were not uniformly and sufficiently performed, particularly with respect to anaerobic pathogens that are an important cause of aspiration pneumonia. However, because about 60% of patients in the All-B group were suspected to have developed aspiration pneumonia, treatment to cover anaerobic pathogens should be prescribed for patients with decreased ADL. Second, previous studies indicated that the sensitivity of IgM antibodies against *Mycoplasma pneumoniae* was 33.3% (26). Therefore, our diagnosis using IgM against *Mycoplasma pneumoniae* might be insufficient. Third, two of 12 patients with MRSA isolation in this study ultimately required treatment with anti-MRSA drugs, but we were not able to assess whether empirical broad-spectrum antibiotic therapy with anti-MRSA drugs could improve the outcome of patients with ADL decline. Additionally, the frequency of HCAP (31.1%) was lower than that of CAP (68.9%) in the present study. Because the proportion of HCAP or NHCAP compared to CAP is very diverse and varies according to the geographic region and medical environment, some of our results might not apply to other institutions (12, 21, 22).

In conclusion, our findings revealed that ADL dependency correlates with high mortality and that subgrouping based on ADL score is useful for predicting the outcome in CAP and all CAP and HCAP patients. Furthermore, patients with decreased ADL have several disadvantages, including multiple comorbidities and malnutrition (hypoalbuminemia). Because they are predisposed to a poor outcome, appropriate antibiotic usage and systemic management, including prophylaxis, are required for adult patients with diminished ADL.

Functional assessment using the modified version of the Katz index is simple, useful, and important for predicting outcome and managing CAP and HCAP patients.

The authors state that they have no Conflict of Interest (COI).

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Intern Med 53: 1613-1620, 2014 DOI: 10.2169/internalmedicine.53.2499

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INTERNAL MEDICINE

□ CASE REPORT □

Concurrent Subcutaneous Candidal Abscesses and Pulmonary Cryptococcosis in a Patient with Diabetes Mellitus and a History of Corticosteroid Therapy

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Abstract

A 50-year-old man with a history of long-term corticosteroid treatment following adrenalectomy for Cushing's syndrome and uncontrolled diabetes mellitus was admitted for an examination of an abnormal thoracic shadow. Cryptococcal serum antigens were positive, and the histopathology of a lung biopsy showed encapsulated yeast resembling *Cryptococcus neoformans*. On admission, the serum β-D-glucan level was approximately twice the cutoff value, several nodules were observed on both legs and magnetic resonance imaging revealed subcutaneous abscesses. *Candida albicans* was identified from needle aspirates, and the patient was successfully treated with fluconazole and flucytosine. We herein report the first case of concurrent *C. albicans* skin abscesses and pulmonary cryptococcosis.

Key words: subcutaneous candidal abscess, pulmonary cryptococcosis, serum 1,3-β-D-glucan

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Introduction

Cryptococcus neoformans is a ubiquitous, encapsulated, yeast-like fungus found worldwide, particularly in soils that are contaminated with pigeon droppings and decaying wood. Pulmonary cryptococcosis is caused by the inhalation of *Cr. neoformans* into the lungs, with subsequent hematogenous dissemination that may induce central nervous system infection. Although pulmonary cryptococcosis can occur in both healthy individuals and immunocompromised patients, it is frequently recognized as an opportunistic pathogen, particularly in patients with lymphohematological disorders, those receiving steroids or immunosuppressants and those with acquired immunodeficiency syndrome (AIDS) (1, 2). Another *Cryptococcus* subspecies, *Cr. gattii* has been cultured from river red gum trees (*Eucalyptus camaldulensis*) and forest red gum trees (*Eucalyptus tereticornis*) in Australia (3, 4). In addition, an outbreak of *Cr. gattii* infection was reported in Vancouver Island, British Columbia in 1999 (5).

Candida albicans is the most common cause of candidiasis; however, there has been an increase in the isolation of non-albicans *Candida* species (i.e., *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei*) in recent years (6, 7).

The clinical manifestations of candidiasis range from local mucosal membrane infection to widespread dissemina-

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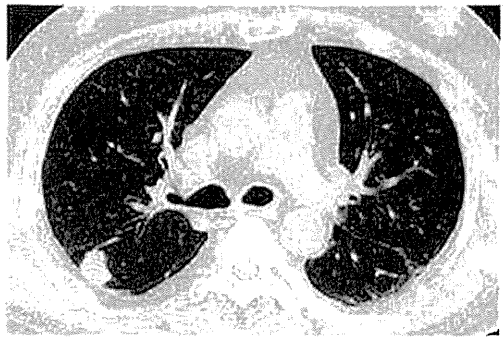

Figure 1. Thoracic computed tomography (CT) image obtained on admission showing a solitary well-defined, pleural-based nodule in the right S2 region. No pleural effusion or cavitation were observed.

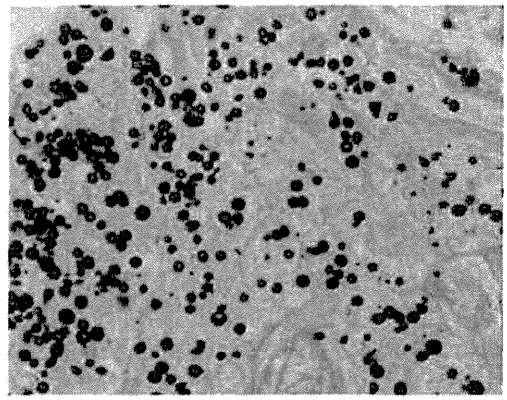

Figure 2. A histopathological examination of a lung biopsy specimen. Gomori's methenamine-silver stain showing encapsulated yeast forms that resemble *Cr. neoformans* (Magnification, ×400).

tion. Local overgrowth on mucous membranes (oropharyngeal involvement or vaginitis) is often observed in patients with changes in normal flora or deficiencies in cell-mediated immunity, as in AIDS. Invasive focal infections, such as pyelonephritis, endocarditis and meningitis, most often occur following hematogenous spread or in patients with anatomic abnormalities or implanted medical devices (e.g., prosthetic heart valves or central nervous system shunts). Widespread visceral dissemination occurs in patients with neutropenia when *Candida* species gain access to the bloodstream, and candidal infections can occur in various locations throughout the body; however, subcutaneous candidal abscess formation is very rare, even in immunocompromised patients. Some cases of subcutaneous candidal abscesses have been reported in patients with skin breakdown, such as that due to bacterial cellulitis or abscess formation, trauma, parenteral substance abuse, iatrogenic procedures or central venous catheter insertion (8).

This report describes the first reported instance of co-infection with *C. albicans* and *Cr. neoformans* in a patient without human immunodeficiency virus (HIV)/AIDS infection and speculates an association with diabetes mellitus and steroid therapy.

Case Report

A 50-year-old man with type II diabetes (undergoing insulin treatment), hypertension and a herniated lumbar disc was diagnosed with Cushing's syndrome. Right adrenalectomy was performed two months before the described hospital admission, and daily corticosteroid replacement therapy (40 mg/day of hydrocortisone, 0.5 mg/day of dexamethasone) was initiated. The patient had not received any antifungal or antimicrobial prophylaxis since the adrenalectomy. He was admitted to a local hospital due to lower leg palsy, suggesting exacerbation of the herniated lumbar disc. Chest radiography revealed a pulmonary nodule in the right upper field of the lung, and he was transferred to our hospital for a further examination.

On admission, a physical examination revealed full moon face, centripetal obesity and mild pretibial pitting edema. No abnormal respiratory sounds were heard in either lung field, and no heart murmurs were audible. An abdominal examination showed abdominal striae, and a neurological examination revealed no nuchal rigidity, cranial nerve deficits or papilledema. The patient's tendon reflexes were normal without pathological reflexes; however, proximal muscle weakness was observed. His body temperature was 37.0℃, his blood pressure was 140/91 mmHg and his heart rate was 90 beats/min. The results of laboratory tests were as follows: leukocyte count, 13,200/mm^3 (82% polymorphonuclear leukocytes); hemoglobin level, 8.7 g/dL; hematocrit concentration, 30.8%; serum Fe level, 42 μg/dL (suggesting iron deficiency anemia); platelet count, 645,000/mm^3; urea level, 11 mg/dL; creatinine level, 0.63 mg/dL; total protein level, 6.4 g/dL; albumin level, 3.8 g/dL; and C-reactive protein level, 0.10 mg/dL. The CD4 count was 533/μL. The fasting blood sugar level and HgbA1c concentration (Japan Diabetes Society) were 146 mg/dL and 7.7%, respectively, suggesting poorly controlled diabetes mellitus. Regarding blood gases, the PaO_2 and $PaCO_2$ values were 59.2 and 50.7 mmHg on room air, respectively. The cortisol level was 1.4 μg/dL, and the adrenocorticotropic hormone level was <5 pg/mL. A serum cryptococcal antigen test was positive, with a titer of 1:8 (Serodirect® "Eiken" Cryptococcus, Eiken Co., Tokyo, Japan), and the serum β-D-glucan level was 43.7 pg/mL (cutoff, <20 pg/mL; Fungitec G test, Seikagaku Kogyo, Tokyo, Japan). An HIV test was negative.

Thoracic computed tomography (CT) showed an 18-mm solitary, well-defined nodule in the right S2 region (Fig. 1). In order to examine the pulmonary nodule, a CT-guided biopsy was performed, followed by a histopathological examination of the lung biopsy specimen using Gomori's methenamine-silver stain, which showed encapsulated yeast forms that resembled *Cr. neoformans* (Fig. 2). Although the patient had no headaches or nuchal rigidity, lumbar puncture

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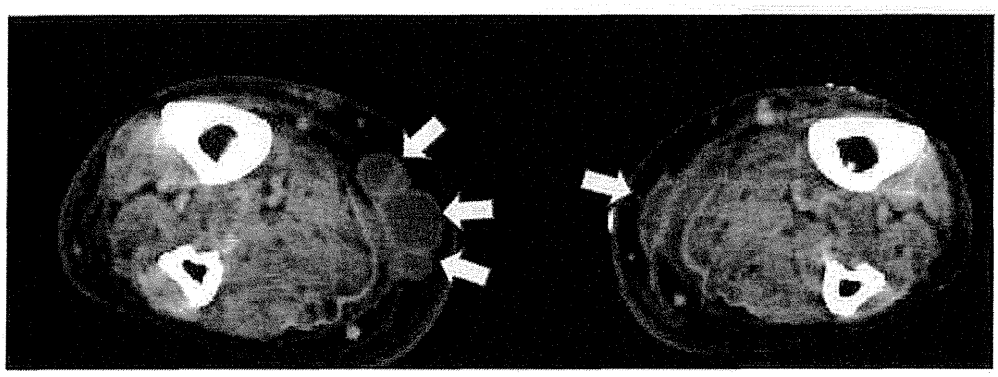

Figure 3. T1-weighted magnetic resonance image showing a well-demarcated collection of fluid in the soft tissue of both lower legs (arrows), suggesting a subcutaneous abscess.

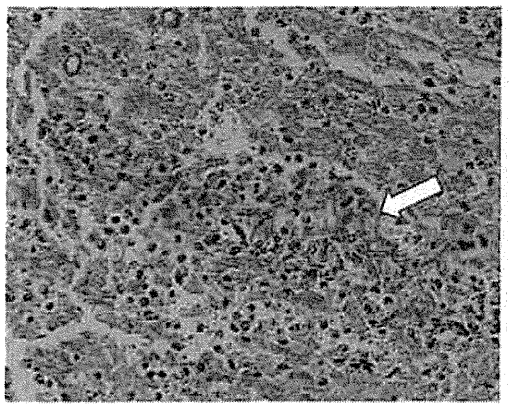

Figure 4. A histopathological examination of the needle aspirate of a lower extremity abscess. Periodic acid-Schiff stain showing yeast and hyphal forms (arrow) of the fungus (Magnification, ×400).

was performed to confirm the diagnosis of cryptococcal meningoencephalitis. However, a cerebrospinal fluid cryptococcal antigen test was negative, the total nucleated cell count was not elevated and no *Cr. neoformans* was cultured. The patient was consequently diagnosed with pulmonary cryptococcosis based on the positive cryptococcal antigen test and pulmonary histopathological findings.

The patient subsequently developed several firm subcutaneous nodules on both lower legs. The nodules each measured approximately 1.0 cm in diameter and were reddish, movable and warm. A T1-weighted magnetic resonance image revealed a rounded fluid-collection signal in the soft tissue of the legs, suggesting a subcutaneous abscess formation (Fig. 3). The image showed no evidence of osteomyelitis. Needle aspiration was performed, and a histopathological examination using periodic acid-Schiff stain showed both the yeast and hyphal forms of the fungus (Fig. 4). *C. albicans* was successfully cultured from the aspirate. No bacteria were isolated from aerobic or anaerobic cultures, and a blood culture was negative. Diabetic retinopathy, without candidal endophthalmitis, was observed. No other signs of candidal infection (i.e., of the oral cavity, esophagus, nails or gastrointestinal tract) were observed.

The minimum inhibitory concentration (MIC) of the isolated *C. albicans* revealed that the pathogen was susceptible to all antifungals tested: voriconazole, 0.015; amphotericin B (AMPH-B), 0.125; flucytosine (5-FC), 0.25; fluconazole (FLCZ), 0.125; itraconazole, 0.03; and micafungin, 0.03 mg/mL. Therefore, the patient was treated with FLCZ (400 mg/day) and 5-FC (8 g/day) for both fungal infections, and drainage of the subcutaneous abscesses was performed.

One month after the start of antifungal treatment, the size of the pulmonary cryptococcal nodule had generally decreased on thoracic CT, and the subcutaneous candidal abscesses had improved on lower extremity CT (Fig. 5A). In addition, the serum β-D-glucan level decreased to 19.6 and 27.1 ng/mL after one and three months, respectively.

Following a three-month course of FLCZ and 5-FC treatment, renal dysfunction was observed; therefore, both antifungals were stopped for a three-week period. Thereafter, only FLCZ was continued for a total of six months for treatment of pulmonary cryptococcosis according to the recommendations of the Japanese Mycology Study Group guidelines for patients with underlying disease (9). The titer of cryptococcal antigens gradually decreased over five months. After six months, the cryptococcal antigen test became negative and the pulmonary nodule reduced in size on thoracic CT (Fig. 5B).

The patient is currently receiving daily corticosteroid replacement therapy (5 mg/day of hydrocortisone and 0.5 mg/day of dexamethasone) without antifungal treatment. Periodic follow-up observations have revealed no trends towards relapse.

Discussion

Subcutaneous candidal abscesses are very rare, even in immunocompromised patients (10). However, cases of such lesions have been reported in patients with skin breakdown, such as that due to bacterial cellulitis or abscess formation, iatrogenic procedures, trauma or parenteral substance

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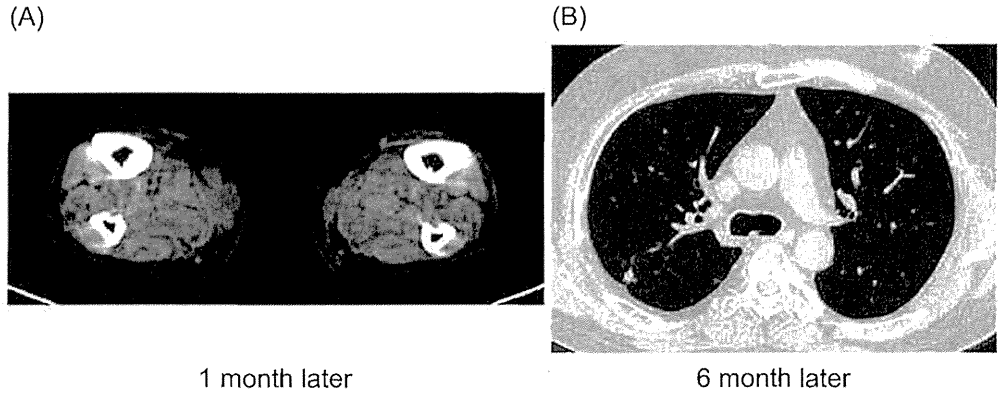

Figure 5. Antifungal therapy reduced the size of the patient's leg skin abscesses and lung nodule. (A) A leg CT image obtained one month after the initiation of antifungal therapy showing a reduction in the size of the skin abscesses. (B) A thoracic CT image obtained six months after the start of antifungal therapy showing a 5-mm (reduced from 18 mm) nodule in the right S2 region.

abuse (11-16).

Many risk factors for the development of candidal infection have been recognized, including immunosuppression, corticosteroid use, chemotherapy, prolonged neutropenia, broad-spectrum antibiotic use, indwelling central catheter placement, hyperalimentation, dialysis, abdominal surgery disrupting the integrity of the bowel mucosa, intravenous drug use, prosthetic intravascular implantation, severe burns and *Candida* spp. colonization (17, 18). Florescu et al. reviewed the risk factors associated with the development of subcutaneous candidal abscesses and found that these factors included indwelling central venous (CV) and intravenous catheter placement, antibiotic use, diabetes mellitus, gastrointestinal surgery, hyperalimentation, immunosuppression due to corticosteroid therapy or AIDS and *Candida* spp. colonization (19). In the present case, the patient had several of the above mentioned factors, i.e., uncontrolled diabetes mellitus and corticosteroid use, due to which, he was at risk of candidal abscess infection. Several firm subcutaneous nodules were observed on both lower legs. However, no apparent injuries or scars were noted. Lower extremity CT showed subcutaneous abscesses on both legs, suggesting that *Candida* had disseminated via the bloodstream. Sites of intravenous catheter placement and the gastrointestinal tract are major portals of entry for systemic *Candida* infection. However, the patient did not have a CV catheter for a long period. Therefore, the gastrointestinal tract may have been the portal of entry in this case.

Although cryptococcal infection can develop in individuals with normal immunity, it most commonly occurs in immunocompromised hosts. Cell-mediated immunodeficiency is an important underlying condition of cryptococcal infection. Predisposing factors include AIDS and other causes of impaired T cell-mediated immunity, e.g., transplant-related immunosuppression, hematological malignancies, corticosteroid administration and diabetes mellitus (20-23). Prolonged, high-dose corticosteroid use (i.e., 20 mg/day) is reported to be an independent factor for disseminated disease (24). In the present case, uncontrolled diabetes and corticosteroid use were co-factors of pulmonary cryptococcosis and candidal skin abscess formation.

Neutrophil chemotaxis and adherence to the vascular endothelium, phagocytosis, intracellular bactericidal activity, opsonization and cell-mediated immunity are all depressed in diabetes patients with hyperglycemia (25, 26). In addition, glucose-inducible proteins promote the adhesion of *C. albicans* to buccal or vaginal epithelium, which impairs phagocytosis, giving the organism an advantage over the host (27). Glucocorticoid administration also results in neutrophilic leukocytosis accompanied by dramatic reductions in circulating eosinophils, monocytes and lymphocytes (28). In the present case, CD4 lymphopenia was observed without HIV infection. CD4 lymphopenia is thought to be related to impaired T-cell-mediated immunity, resulting in disseminated candidiasis and cryptococcosis.

Little is known about other risk factors for the development of co-infection. TNF-α and interleukin (IL)-17 may be key factors for co-infection. TNF-α is a factor for the reduction of the pathogenic burden of *C. albicans* in animals (29, 30). In contrast, cryptococcal infection is reported to inhibit TNF-α production (31). Furthermore, cryptococcal infection may exacerbate candidiasis.

IL-17 is a proinflammatory cytokine produced by a subset of CD4 T-cells, termed Th17 cells. Increased IL-17 production is associated with a reduced cryptococcal burden, suggesting that IL-17 plays a significant role in the generation of a protective anti-cryptococcal immune response. In contrast, *C. albicans* is reported to dampen the host defense by downregulating IL-17 production (32). Candidal infection may alter cellular immunity and is recognized to be a predisposing factor for developing cryptococcosis. Altered host immunity may explain why cryptococcosis and candidiasis developed in this patient without HIV infection.

During a 35-year period at Nagasaki University hospital

Intern Med 53: 1385-1390, 2014 DOI: 10.2169/internalmedicine.53.1409

and its affiliates, the diagnosis of pulmonary cryptococcosis was confirmed in 151 patients. Of these patients, only the present subject exhibited pulmonary cryptococcosis and candidal skin abscess co-infection (0.66%, unpublished data).

Approximately two decades have passed since the introduction of the serum β-D-glucan assay for the clinical diagnosis of deep-seated mycosis in Japan (33). The assay is now widely accepted in Japan and other countries as an indispensable tool for managing febrile episodes in immunocompromised hosts. In addition, its use is included in the guidelines for the diagnosis and treatment of deep-seated mycosis (9, 34, 35). The presence of β-D-glucan in the serum signifies the presence of fungal invasion; however, the results are not specific for *Candida* species (36). False-positive findings can occur for a variety of reasons, including the use of glucan-contaminated blood collection tubes, gauze and depth-type membrane filters for blood processing, as well as *in vitro* tests using various antibiotics (e.g., some cephalosporins, carbapenems and ampicillin-sulbactam) (34, 37). Therefore, a serum β-D-glucan level exceeding the cutoff value even slightly may indicate the absence of deep-seated mycoses. A high serum β-D-glucan level is associated with cryptococcal meningitis (38) and cryptococcemia (39). The present patient was diagnosed with pulmonary cryptococcosis in addition to a moderate immunosuppressive state. A lumbar puncture did not reveal cryptococcal meningoencephalitis, and no microorganisms were cultured from his blood. On admission, the serum β-D-glucan level was approximately twice the cutoff value. Therefore, we interpreted this to be a false-positive result. However, a cautious medical examination revealed that he had candidal skin abscess co-infection.

Cryptococcus was not cultured in this case; therefore, we were unable to perform antifungal susceptibility tests for *Cryptococcus* isolates. It is difficult to distinguish between *Cr. neoformans* and *Cr. gattii* infections based on the results of histopathological examinations. Nevertheless, the first case report of a patient in Japan infected with *Cr. gattii* genotype VGIIa noted that the patient had no recent history of travel to any disease endemic areas (40), suggesting that the virulent strain may have spread to regions outside North America. *Cr. gattii* is generally geographically restricted. Furthermore, among cryptococcal infections in Japan, *Cr. neoformans* (serotype A) is the most common, with a frequency exceeding 95% (41). In addition, no azole-resistant *Cr. neoformans* isolates have been detected, even in the latest reports (42).

The *C. albicans* isolated from this patient was found to be susceptible to all antifungals tested. In the updated practical guidelines for the management of candidiasis issued by the Infection Disease Society of America, treatment with FLCZ or echinocandin is recommended for candidemia in patients with neutropenia as the initial therapy (43). The administration of a combination of the lipid formulation AMPH-B (LFAmB) or AMPH-B deoxycholate with 5-FC is recommended in cases of central nervous system (CNS) candidiasis, candidal endophthalmitis, candidal infection of the cardiovascular system, endocarditis and others (43). We chose the combination of FLCZ and 5-FC as the initial antifungal therapy in the present case based on the antifungal activity against both *Cr. neoformans* and *C. albicans* infection (44, 45). On the other hand, if a non-albicans *Candida* species e.g., *C. glabrata* or *C. krusei*, had been identified, these antifungal agents would have been changed to LFAmB. However, the patient's treatment was successful, as originally prescribed.

In conclusion, we herein reported, to the best of our knowledge, the first published case of pulmonary cryptococcosis and candidal skin abscess co-infection in an immunocompromised patient.

Author's disclosure of potential Conflicts of Interest (COI).
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Efficacy of Combination Therapy with Oseltamivir Phosphate and Azithromycin for Influenza: A Multicenter, Open-Label, Randomized Study

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Abstract

Background: Macrolides have antibiotic and immunomodulatory activities, which may have a favorable effect on the clinical outcome of patients with infections, including influenza. This study aimed to evaluate the effects of combination therapy with an anti-influenza agent, oseltamivir, and a single-dose formulation of azithromycin (AZM), which has been used for influenza-related secondary pneumonia, on influenza patients. The primary endpoint was a change in the expression levels of inflammatory cytokines. Secondary endpoints were the time required for resolution of influenza-related symptoms, incidence of complications, and adverse reactions.

Methods: Patients with seasonal influenza were enrolled in this multicenter, open-label, randomized study. Patients were stratified according to the presence of a high risk factor and were randomized to receive combination therapy with oseltamivir plus an extended-release formulation of AZM (combo-group) or oseltamivir monotherapy (mono-group).

Results: We enrolled 107 patients and randomized them into the mono-group (56 patients) or the combo-group (51 patients). All patients were diagnosed with influenza A infection, and none of the patients had comorbid pneumonia. Statistically significant differences were not observed in the expression levels of inflammatory cytokines and chemokines between the 2 groups. The maximum temperature in the combo-group was lower than that in the mono-group on day 3 through day 5 ($p=0.048$), particularly on day 4 ($p=0.037$).

Conclusion: To our knowledge, this is the first prospective, randomized, clinical trial of oseltamivir and AZM combination therapy for influenza. Although the difference in inflammatory cytokine expression level was not statistically significant, combination therapy showed an early resolution of some symptoms.

Name of registry: University hospital Medical Information Network (UMIN).

Trial Registration no.: UMIN000005371

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Introduction

Influenza virus infection is a major respiratory infectious disease that generally induces bronchitis, and occasionally leads to fatal pneumonia in the elderly when bacterial infections are involved [1]. Comorbid or secondary bacterial pneumonia is a severe complication related to the influenza virus infection, which suggests the importance of the latter infection in the morbidity and mortality in elderly patients with this disease [1,2]. High mobility group B1 (HMGB1), a known proinflammatory cytokine

and cytotoxic factor, is suggested to be involved in the development of influenza-related pneumonia [3]. In addition, increase in the levels of proinflammatory cytokines and monokines, including interleukin 1 (IL-1), IL-6, and IL-8, have been observed in the sera of patients and in the lungs of mice infected with the influenza virus [4]. These factors are suggested to be associated with the pathogenesis and severity of influenza virus infection [5].

Azithromycin (AZM), a 15-membered ring macrolide, is an azalide and is structurally related to the macrolide family of antibiotics. It binds to the 50S ribosomal subunit of susceptible organisms, thereby interfering with protein synthesis. AZM is approved worldwide as a broad-spectrum antibiotic for the treatment of a variety of community-acquired infections. A recently developed novel microsphere formulation of AZM (Zithromax SR 2 g) enables oral administration of high doses of AZM as a part of a single-dose regimen while maintaining tolerability.

Macrolides, including AZM and clarithromycin (CAM), a 14-membered ring macrolide, exert immunomodulatory effects on the host and antibacterial effects against the targeted microorganisms [6].

Viasus et al. reported that immunomodulatory therapies using corticosteroids and macrolides did not prevent the development of severe disease in patients with pandemic influenza A (H1N1) 2009 infection complicated by pneumonia [7]. Similarly, macrolide-based treatment has not been associated with improved survival in critically ill H1N1 patients with primary pneumonia in an intensive care unit (ICU) setting [8].

However, for patients with mild influenza, the duration of cough in patients without cough at the onset of pyrexia is significantly shorter with combined therapy with CAM and oseltamivir (Tamiflu) than that with oseltamivir monotherapy [9]. In addition, Kido et al. reported that while administration of CAM to influenza A virus (IAV)-infected mice decreases the production of tumor necrosis factor alpha (TNF-α) and increases the production of IL-12 in the blood, which results in the alleviation of flu symptoms [10], oral treatment with oseltamivir attenuates the induction of respiratory anti-IAV-specific secretory immunoglobulin A (S-IgA) immune responses [11]. Furthermore, a recent study showed that oral CAM increases the nasopharyngeal mucosal immune responses in IAV-infected children, while oseltamivir suppresses the production of mucosal anti-IAV S-IgA [12].

AZM may thus modulate airway inflammation induced by influenza virus infection. Basic studies have shown that AZM is effective against secondary bacterial pneumonia after influenza virus infection because of its inhibitory effect on the expression of various cytokines and its antibacterial activity [13].

In this study, we evaluated the efficacy of combination therapy with an anti-influenza agent, oseltamivir, and a single administration of an extended-release formulation of AZM and compared it with the efficacy of oseltamivir monotherapy in patients with influenza.

Methods

The protocol for this trial and supporting CONSORT checklist are available as supporting information (see Checklist S1 and Protocol S1).

Participants

We enrolled patients with influenza from the Nagasaki University Hospital and 13 of its affiliated hospitals and clinics. Patients aged 20 years and older with influenza A or B virus infection diagnosed by a positive rapid antigen test (RAT) for influenza were considered for enrollment. Patients had to have signs or symptoms of a seasonal flu or influenza A (H1N1) pdm 2009 virus infection with an axillary temperature ≥38.0°C and at least 2 of the following signs or symptoms at a moderate-to-severe degree: headache, muscle or joint pain, fever or chills, fatigue, cough, sore throat, and nasal stuffiness caused by influenza.

In addition, patients had to have accepted the treatment within 48 h from the onset of influenza symptoms, which were defined as follows: initial temperature elevation ≥1°C from the patient's normal body temperature or experience of at least 1 symptom included in the Influenza Symptom Severity scale (ISS) [14].

Patients with a history of hypersensitivity to AZM or oseltamivir and patients with bacterial infections were excluded. At screening, a complete history was recorded from all patients, including notes on flu vaccination, physical examination, chest radiographs, and blood chemistry. Assessment of clinical symptoms of influenza, including vital signs (body temperature, blood pressure, and pulse rate), was performed at baseline (day 0) and on days 2 and 5. Blood samples were collected on days 0, 2, and 5 for measurement of the levels of inflammatory cytokines and chemokines, HMGB1, and procalcitonin (PCT). Patients recorded their own influenza symptoms, maximal temperature, and activities of daily living using a 7-symptom ISS and a visual analogue scale (Influenza Impact Well-Being Score [IIWS]) ranging from 0 to 10.

Study design

This prospective, randomized, open-label, controlled, multicenter study was performed between December 2010 and March 2011.

Ethics

The trial was conducted in accordance with the Declaration of Helsinki and in compliance with the ethical guidelines for clinical studies issued by the Health, Labour and Welfare Ministry. Written informed consent was obtained from all patients before enrollment. The protocol, amendments, and informed consent documentation were approved by the institutional review board and/or independent ethics committee at each facility.

The project approval date for each Research Ethics Board is listed in brackets: Nagasaki University (October 13, 2010), The Japanese Red Cross Nagasaki Genbaku Isahaya Hospital (December 24, 2010), The Japanese Red Cross Nagasaki Genbaku Hospital (October 25, 2010), Hokusho Central Hospital (November 16, 2010), Sasebo General Hospital (January 17, 2011), NHO Ureshino Medical Center (November 22, 2010), Koseikai Hospital (November 22, 2010), Isahaya Health Insurance General Hospital (Not approved until the end of this study), Nagasaki Municipal Hospital (November 4, 2010), Onitsuka Naika Clinic (October 13, 2010), Hayashida Naika Clinic (March 12, 2011), Tomonaga Naika Clinic (October 13, 2010), Irihune Clinic (October 13, 2010), Kawamura Clinic (October 13, 2010).

The trial was first approved in October, 2010 by the ethics committee of Nagasaki University (accession number, 100100130), but was only finally approved by the other branch hospitals in March, 2011. Additionally, the trial was registered in the University Hospital Medical Information Network (UMIN) Center system. The UMIN accession number is UMIN000005371. The trial began in December, 2010.

Study intervention and randomization

Patient enrollment was performed using a central registration system through a computer-generated random listing of the two

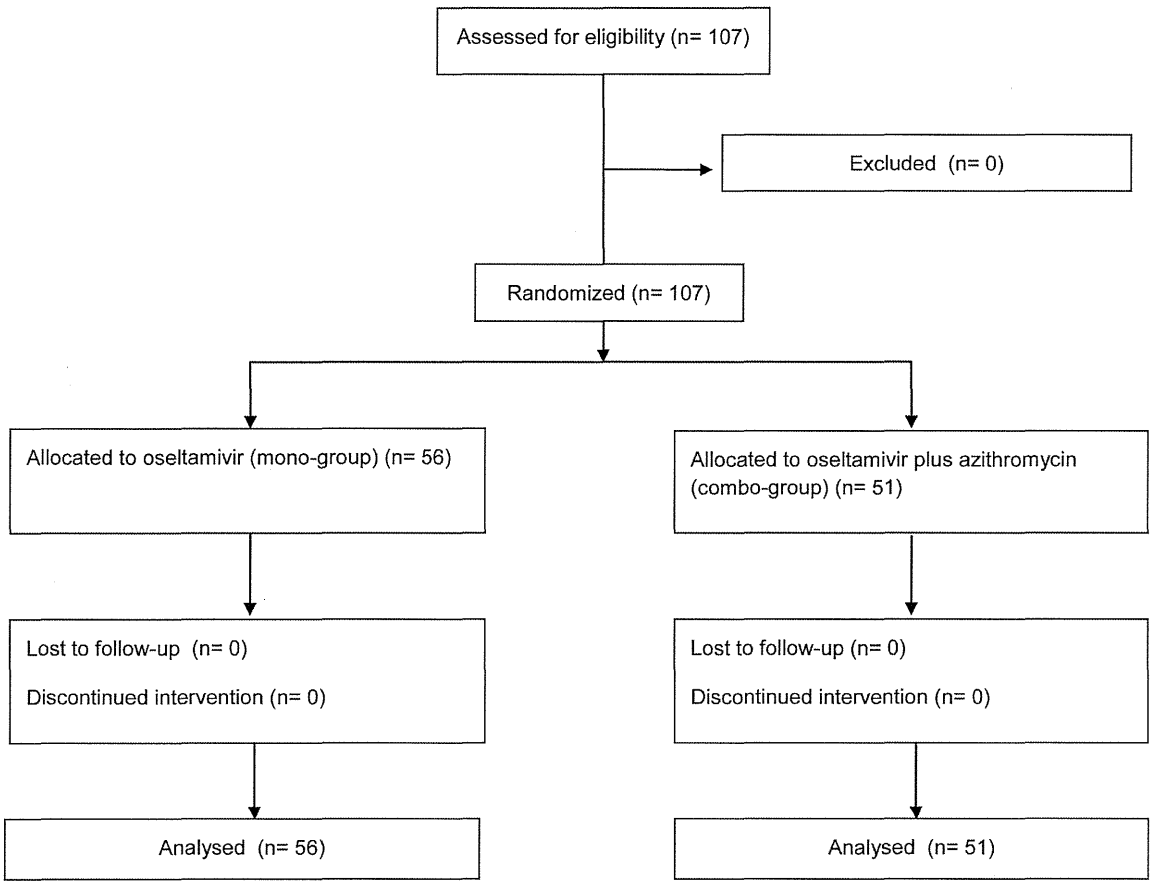

Figure 1. Trial Profile.
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treatment allocations. A minimization method [15] was used to randomize patients in a 1:1 ratio to receive oral oseltamivir 75 mg alone (mono-group) every 12 h or oral oseltamivir 75 mg every 12 h in combination with an extended-release formulation of single-dose oral AZM 2,000 mg (combo-group). For randomization, patients were stratified according to the presence of high risk factors such as age (≥65 years), underlying respiratory diseases (e.g., chronic obstructive pulmonary disease, bronchial asthma), use of steroids (equivalent to prednisolone >10 mg/day), and uncontrolled diabetes mellitus (hemoglobin A1c [HbA1c] level > 7.4; national glycohemoglobin standardization program [NGSP]). Oral oseltamivir was to be administered for 5 days in both groups.

Outcome measures

The purpose of this study was to evaluate the efficacy and safety of combination therapy with an anti-influenza agent, oseltamivir, and AZM in patients with influenza.

The intent-to-treat (ITT) population was used prospectively for analysis. The ITT population included all patients who received 1 or more doses of the study medication. The primary endpoint was defined as variations in the levels of inflammatory markers (i.e., inflammatory cytokines and chemokines, HMGB1, PCT). Secondary endpoints were defined as follows: (1) the duration of influenza; (2) the incidence of influenza-related complications (sinusitis, otitis media, bronchitis, and pneumonia); (3) the time to alleviation of influenza symptoms; and (4) adverse events and adverse drug reactions.

Inflammatory marker assays

The levels of the cytokines IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, TGF-β, interferon γ (IFN-γ), and TNF-α were measured using the cytokine bead array.

Clinical laboratory tests

We performed hematological (measurements of red blood cell [RBC] count, Hb level, hematocrit [Ht] level, platelet count, white blood cell [WBC] count, and WBC fraction); biochemical (measurement of the levels of aspartate aminotransferase [AST], alanine aminotransferase [ALT], total bilirubin [T-Bil], blood urea nitrogen [BUN], creatinine [Cre], total protein [T-P], albumin [Alb], sodium [Na], chloride [Cl], and potassium [K]); and immunological (measurement of C-reactive protein [CRP] level) tests on days 1, 2, and 5.

The differences in values on day 2 (ΔDay2) and day 5 (ΔDay5) from those observed on day 0 were evaluated.

Statistical methods

The statistical analyses were performed by an expert biostatistician experienced in the subject studied. The mono-group included 56 patients, while the combo-group included 51 patients, which was the number estimated as the appropriate sample size (see the protocol).

This was an exploratory trial to assess the efficacy of oseltamivir plus AZM in the treatment of patients with influenza. Statistical analyses were performed using PASW Statistics 18 (SPSS Inc., Chicago, IL, USA). All tests were two-tailed, and a p value <0.05

Table 1. Study population in the azithromycin-oseltamivir combination therapy and oseltamivir monotherapy groups.

		Azithromycin		
		-	+	p value
No. of patients		56	51	
Age (years)	Range	20–87	20–91	0.734 (*t* test)
	Median	42	39	
	Mean ± SD	44.1±17.3	42.9±17.3	
Gender	M (%)	25 (39.3)	25 (49.0)	0.398 (Fisher)
	F (%)	31 (60.7)	26 (51.0)	
Chronic Lung Disease (%)		6 (10.7)	5 (9.8)	0.566 (Fisher)
Diabetes		3 (5.4)	0	0.140 (Fisher)
Steroid use		3(5.4)	2(3.9)	0.545(Fisher)
Maximal body temperature(mean± SD)		38.6±0.7	38.8±0.7	0.202 (*t* test)
Influenza Symptom Severity scale (ISS)				
Headache	None	8 (14.3)	7 (13.7)	0.985 (t test)
	Mild	17 (30.4)	15 (29.4)	
	Moderate	24 (42.9)	19 (37.3)	
	Severe	5 (8.9)	5 (9.8)	
Muscle/Joint pain	None	7 (7.1)	5 (9.8)	0.735 (*t* test)
	Mild	11 (19.6)	13 (25.5)	
	Moderate	24 (42.9)	19 (37.3)	
	Severe	12 (21.4)	9 (17.6)	
Heat sensation	None	3 (5.4)	2 (5.9)	0.135 (*t* test)
	Mild	11 (19.6)	6 (11.8)	
	Moderate	24 (42.9)	17 (33.3)	
	Severe	16 (28.6)	21 (41.2)	
Feeling of fatigue	None	2 (3.6)	2 (3.9)	0.738 (*t* test)
	Mild	10 (17.9)	5 (9.8)	
	Moderate	25 (44.6)	25 (49.0)	
	Severe	17 (30.4)	14 (27.5)	
Cough	None	1 (1.8)	3 (5.9)	0.014 (*t* test)
	Mild	10 (17.9)	16 (31.4)	
	Moderate	30 (53.6)	21 (41.2)	
	Severe	12 (21.4)	5 (9.8)	
Sore throat	None	11 (19.6)	10 (19.6)	0.852 (*t* test)
	Mild	26 (46.4)	19 (37.3)	
	Moderate	13 (23.2)	14 (27.5)	
	Severe	4 (7.1)	3 (5.9)	
Nasal congestion	None	16 (28.6)	11 (21.6)	0.732 (*t* test)
	Mild	12 (21.4)	17 (33.3)	
	Moderate	22 (39.3)	16 (31.4)	
	Severe	4 (7.1)	2 (3.9)	

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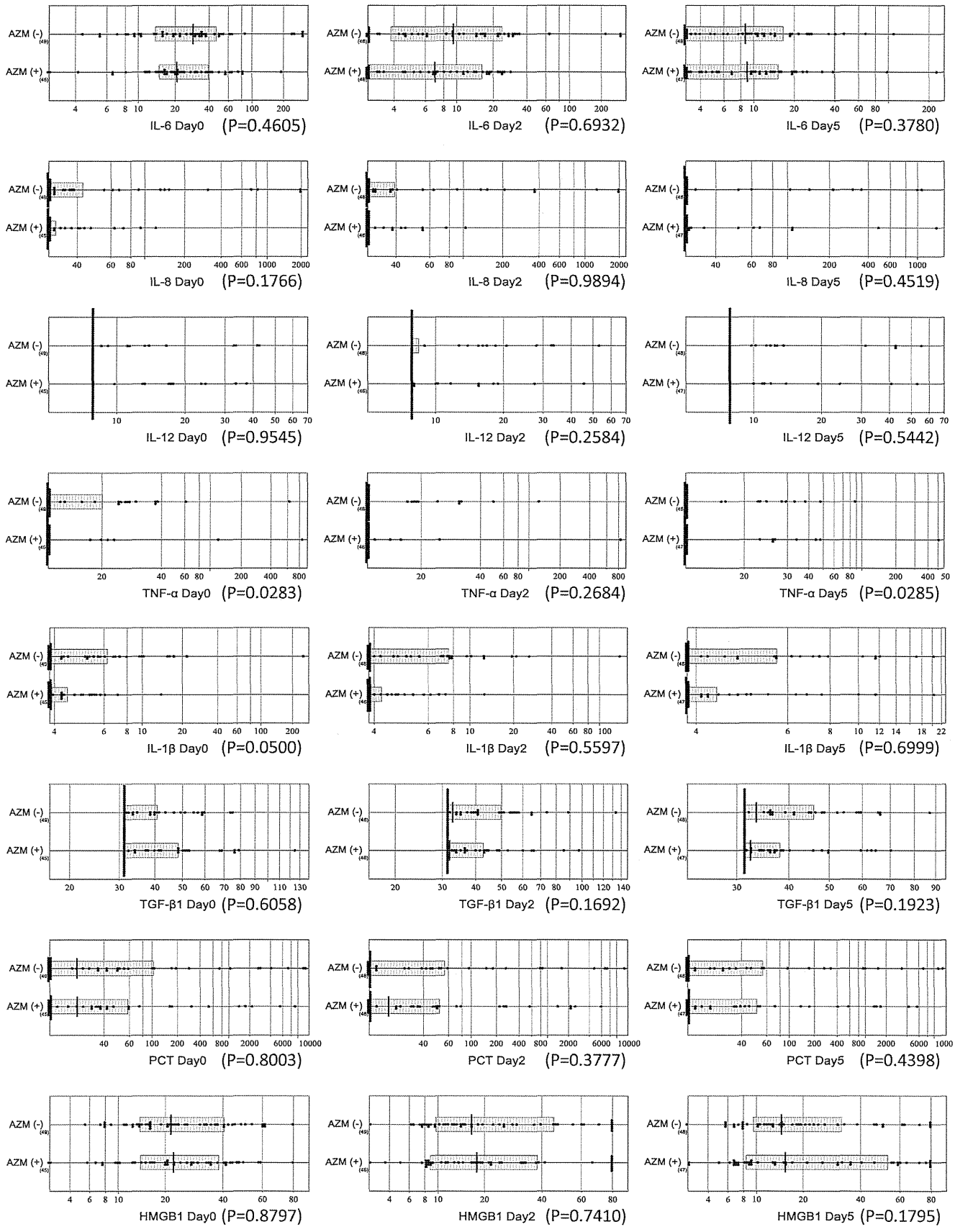
was considered statistically significant. The significance of differences in the expression levels of cytokines and chemokines and in influenza-related symptoms between the mono-group and combo-group were examined using the Mann–Whitney *U* test. In addition, significance of differences in the maximum temperature between the mono- and combo-groups on days 3 through 5 were using a mixed-design analysis of variance (mixed-design ANOVA).

Results

Study population

A total of 107 patients were enrolled in the study between December 2010 and March 2011.

The number of patients enrolled at each hospital was as follows: 4, Nagasaki University; 12, The Japanese Red Cross Nagasaki Genbaku Isahaya Hospital; 8, The Japanese Red Cross Nagasaki Genbaku Hospital; 2, Hokusho Central Hospital; 6, Sasebo


AZM (-)
AZM (+)
IL-6 Day0 (P=0.4605)
IL-6 Day2 (P=0.6932)
IL-6 Day5 (P=0.3780)
IL-8 Day0 (P=0.1766)
IL-8 Day2 (P=0.9894)
IL-8 Day5 (P=0.4519)
IL-12 Day0 (P=0.9545)
IL-12 Day2 (P=0.2584)
IL-12 Day5 (P=0.5442)
TNF-α Day0 (P=0.0283)
TNF-α Day2 (P=0.2684)
TNF-α Day5 (P=0.0285)
IL-1β Day0 (P=0.0500)
IL-1β Day2 (P=0.5597)
IL-1β Day5 (P=0.6999)
TGF-β1 Day0 (P=0.6058)
TGF-β1 Day2 (P=0.1692)
TGF-β1 Day5 (P=0.1923)
PCT Day0 (P=0.8003)
PCT Day2 (P=0.3777)
PCT Day5 (P=0.4398)
HMGB1 Day0 (P=0.8797)
HMGB1 Day2 (P=0.7410)
HMGB1 Day5 (P=0.1795)