Intern Med 51: 1773-1778, 2012 DOI: 10.2169/internalmedicine.51.7621

Table 1. **Laboratory Findings on Admission**

Hematology			Biochemistry			Serology		
WBC	23,000	/μL	TP	7.1	g/dL	CRP	0.91	mg/dL
Neut	88	%	Alb	4.2	g/dL	ANA	<40	
Lym	11	%	LDH	303	IU/L	RF	<10	IU/mL
Mon	1	%	AST	47	IU/L	PR-3-ANCA	<3.1	EU
Bas	0	%	ALT	33	IU/L	MPO-ANCA	<3.1	EU
Eos	0	%	BUN	23.5	mg/dL	IgG	871	mg/dL
RBC	474 $\times 10^4$	/μL	Cre	0.56	mg/dL	IgA	167	mg/dL
Hb	13.6	g/dL	Na	141	mEq/L	IgM	112	mg/dL
Ht	43.5	%	K	4.0	mEq/L	IgE	<35	mg/dL
Plt	36.3$\times 10^4$	/μL	Cl	103	mEq/L	Troponin T	0.038	ng/mL
			CPK	95	IU/L			

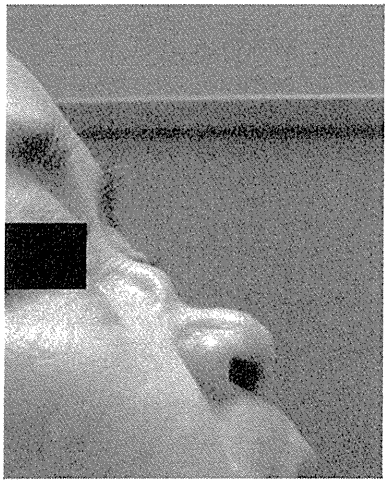

Figure 1. **Appearance of the affected part showing nasal chondritis (saddle nose).**

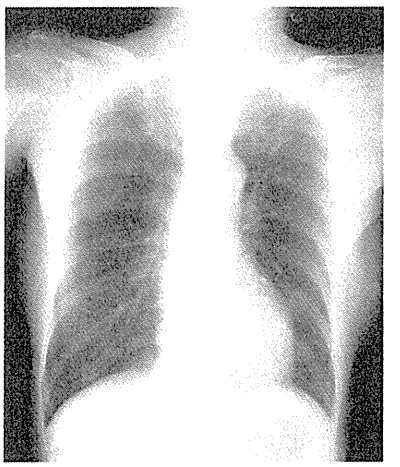

Figure 2. **Chest radiograph on admission showing narrowing of bronchial lucency. There is no abnormal finding in the lung fields.**

course several times she noticed flare/swelling of the ear auricles although she or her doctor did not realize that the symptom could be related with the dose of the corticosteroid. As for her saddle nose, she realized it when she was about 60 years old.

On admission, her height, weight and body temperature were 149.5 cm, 35.3 kg and 37.1℃, respectively. Her blood pressure was 154/92 mmHg with SpO_2 80% under 12 L/min of oxygen flow by reservoir mask. Slight flare/swelling of the bilateral ear auricles as well as saddle nose (Fig. 1) was observed without abnormal findings in the palpebral or bulbar conjunctivae. By auscultation, stridor was audible on the bilateral sides with no abnormal heart sounds. Edema was not detected in either lower limb. Laboratory data on admission is listed in Table 1. The white blood cell (WBC) count was markedly increased to 23,000/μL while the C-reactive protein (CRP) level was 0.91 mg/dL. The patient's serum was negative for antinuclear antibody and antineutrophil cytoplasmic antibody (ANCA). Although the chest X-ray (Fig. 2) demonstrated no abnormalities in the bilateral lung fields, stenosis of the left and right principal bronchi was noted.

Due to respiratory failure, she was intubated and connected to a ventilator on the day of admission. Treatment with methylprednisolone at a dose of 500 mg/day for 3 days was initiated. After confirming improvement in respiratory condition, the dose of corticosteroid was gradually decreased. Extubation was conducted 7 days after admission. The pattern of a flow-volume curve (Fig. 3) recorded at an outpatient clinic before this admission indicated reversible intrathoracic stenosis, which became flat in the descending limb after a sharp peak associated with the collapse of central airways (6, 7), suggesting tracheobronchomalacia had been present. In addition, the swelling of the auricles and saddle nose lead to a tentative diagnosis of RPC.

Thoracic computed tomography (CT) (Fig. 4) performed after extubation revealed thickening of airway walls from trachea to bilateral principal bronchi with the luminal diameter reduced to approximately 5 mm, consistent with RPC. CT on expiration exhibited applanation of the lumen, more marked stenosis, and tracheobronchomalacia in comparison with findings on inhalation.

Electrocardiography after admission (Fig. 5) revealed negative T waves and QT prolongation with I-, aVL-, II-, III-, aVF-, and V_2- to V_6-lead with a slight increase in the

Intern Med 51: 1773-1778, 2012 DOI: 10.2169/internalmedicine.51.7621

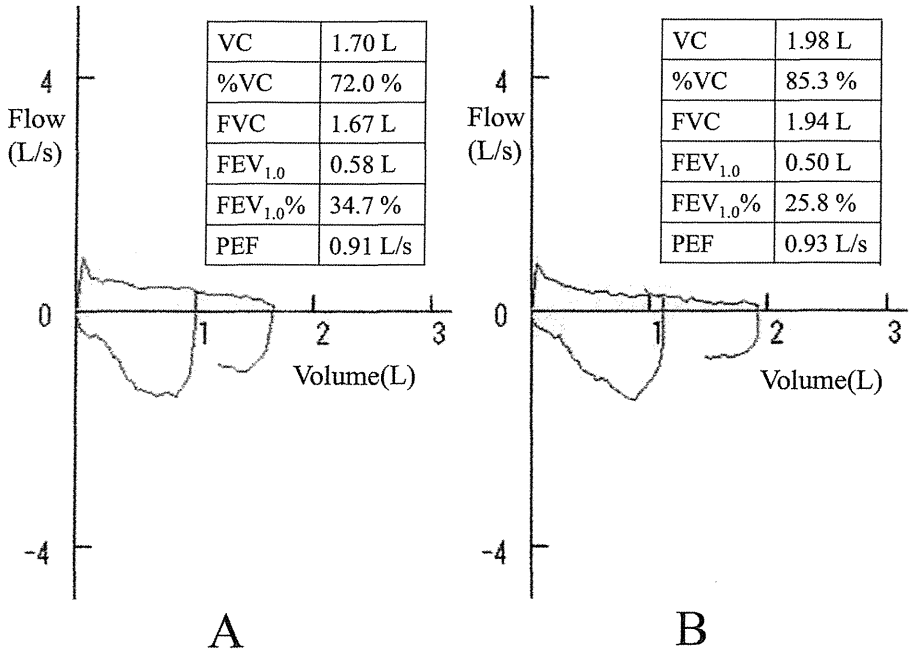

Figure 3. **Flow-volume curve before admission (A) and one month after admission (B) showing a constrictive pattern in the upper airway.**

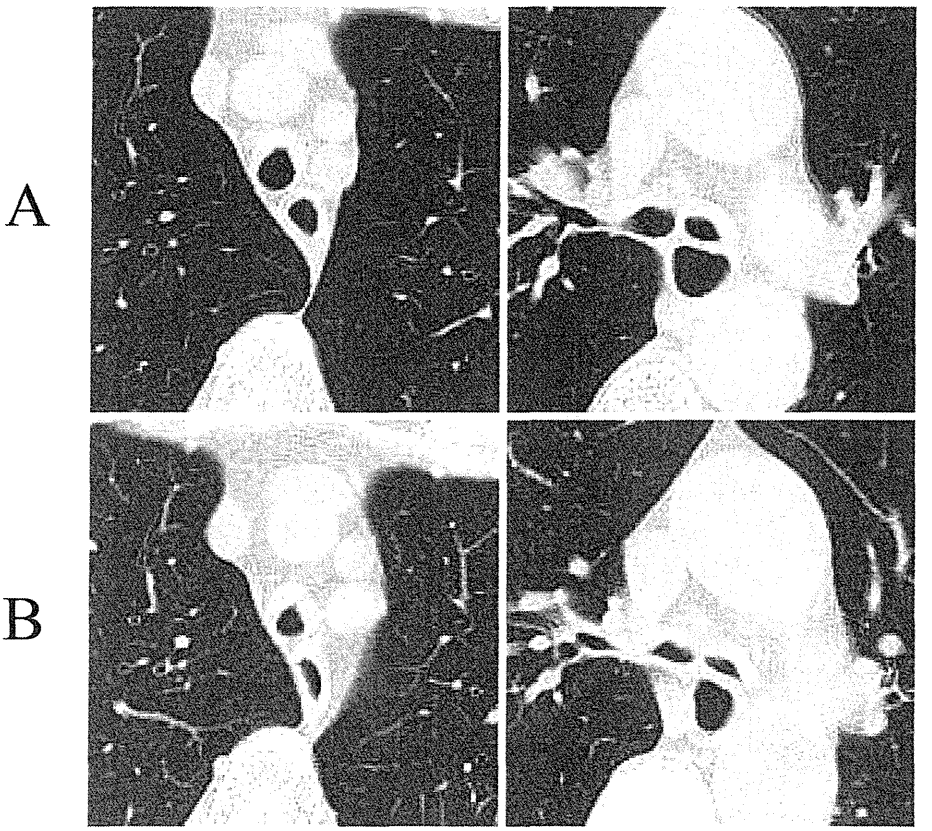

Figure 4. **Transverse (A) end-inspiratory and (B) expiratory CT scans. Chest CT scans showing thickening and edema of the tracheal and bronchial wall. Both main bronchi show severe stenosis in an expiratory CT scan.**

myocardial troponin-T level but without any increase in the other serum biomarkers of cardiac injury. In addition, echocardiography indicated akinesis of the left ventricular anterior wall and ventricular septum (intermediate to cardiac apex regions) and a decrease in the ejection fraction. Both the electrocardiographic and echocardiographic findings gradually and spontaneously subsided; echocardiography confirmed recovery of cardiac systolic function within a

Intern Med 51: 1773-1778, 2012 DOI: 10.2169/internalmedicine.51.7621

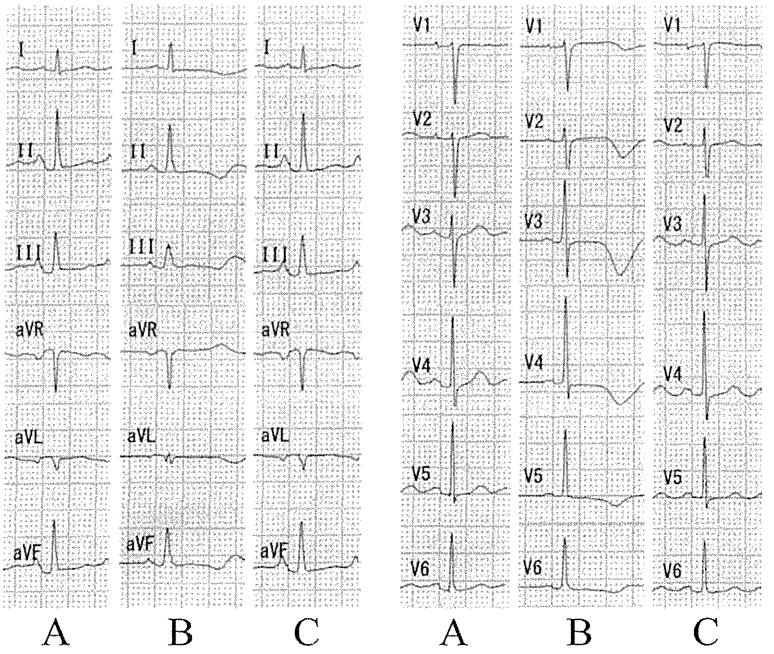

Figure 5. **Electrocardiography before admission (A), two days after admission (B), and two months after admission (C). (B): Negative T waves and QT interval prolongation in leads I, aVL, II, III, aVF, V_2-V_6. (C): Negative T waves improved.**

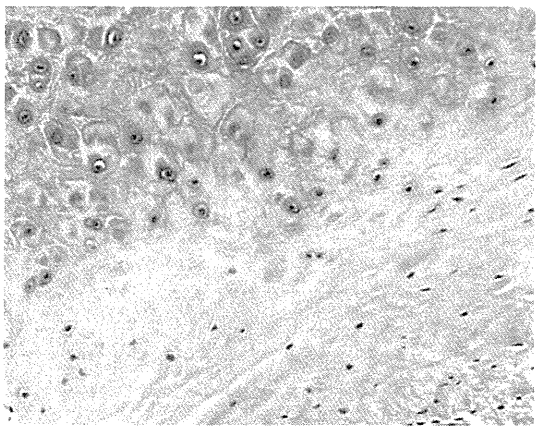

Figure 6. **Biopsy sample of the right auricle shows cartilage destruction and degeneration (Hematoxylin and eosin stain, ×40).**

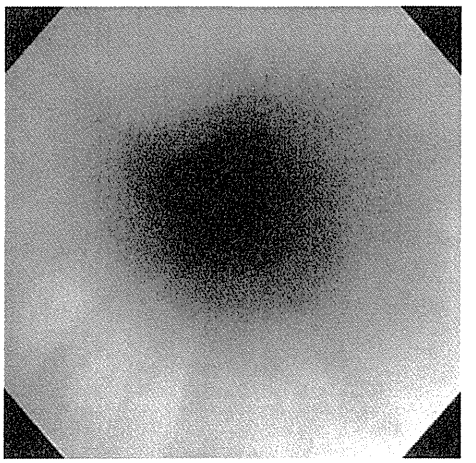

Figure 7. **Bronchoscopic finding of the trachea, showing the disappearance of cartilaginous rings.**

week, and negative T in electrocardiography persisted for about a month and was gradually normalized. Contrast-enhanced coronary CT revealed the absence of arteriosclerosis and stenosis in 3 vessels. Therefore, the cardiologists diagnosed she had takotsubo cardiomyopathy.

Since biopsy of the auricle (Fig. 6) demonstrated destruction of the cartilage and rupture of elastic fibers, a definitive diagnosis of RPC was made on 28th days after admission, based on the clinical and pathological findings. On the same day, the dose of oral prednisolone was increased to 30 mg combined with 100 mg of cyclosporine. The dose of prednisolone was decreased by 5 mg every 2 weeks until the maintenance dose was established as 15 mg.

The anti-type-II-collagen antibody was revealed to be negative at the concentration of 8.9 EU/mL (positive: >25 EU/mL) on the 62nd day after admission. Bronchoscopy (Fig. 7), performed on the 105th day after admission, did not indicate flare or swelling on the tracheal luminal surface although the disappearance of the tracheal cartilage rings was noted.

Discussion

RPC causes repetitive inflammation in the cartilage tissues of the whole body and in ocular/cardiovascular systems, which contain a high concentration of mucopolysaccharides, and it is likely to respond to steroids and immunosuppressive agents. Anti-type-II-collagen antibody was de-

Intern Med 51: 1773-1778, 2012 DOI: 10.2169/internalmedicine.51.7621

tected in approximately 30 to 50% of patients with RPC (8), suggesting an autoimmune disease.

McAdam et al. (9) established the diagnostic criteria in which patients with RPC were defined as having 3 or more of the following 6 items plus histological evidence of cartilage inflammation: 1) bilateral auricular chondritis, 2) non-erosive sero-negative inflammatory polyarthritis, 3) nasal chondritis, 4) ocular inflammation, 5) respiratory tract chondritis and 6) audiovestibular damage. In the present patient, auricular chondritis, nasal chondritis, and respiratory tract chondritis were noted in addition to cartilage destruction identified with the auricular cartilage biopsy, leading to a diagnosis of RPC. Although there was no increase in the anti-type-II-collagen antibody level, this could be because steroids had been frequently administered under a diagnosis of bronchial asthma.

Trentham and Le reported that the mean interval from the first visit to the diagnosis of RPC was 2.9 years (5). The present patient had been treated for bronchial asthma for about 3 years after her first visit at a local clinic with dyspnea at the age of 59 years. Since then, corticosteroid was administered for the treatment of suspected asthma attack and decreased and discontinued after symptoms subsided. The steroid dose-reduction or discontinuation had deteriorated not only her respiratory conditions but auricular swelling and saddle nose, which emerged during the course involving remission and exacerbation of her "asthma". As Segel et al. indicated (4), steroid administration to RPC patients might transiently improve a respiratory symptom that was related to RPC.

In the present case, the diagnosis may have been delayed for the following reasons: 1) auricular chondritis and saddle nose emerged after the onset of airway symptoms, 2) symptoms (auricular swelling/saddle nose) other than airway symptoms were underestimated, and 3) the patient had been diagnosed as bronchial asthma due to steroid therapy-related improvement. Previous case reports of relapsing polychondritis misdiagnosed as bronchial asthma (10-12) suggested similar reasons for the misdiagnosis. The present case exhibited saddle nose and flare/swelling of ear auricles, which was not connected with her airway symptoms by her doctor. Several studies reported that the incidence of airway symptoms in RPC patients ranged from 20 to 50%, and that airway symptoms were initially present in 10 to 15% (3, 9). Other common sites involved in RPC included the auricles, joints, and nasal cartilage although many patients might not show all symptoms at onset.

Clinical features of relapsing polychondritis, different from typical bronchial asthma, include the following: 1) inhaled bronchodilator and corticosteroid are ineffective and oral corticosteroid is required, 2) lung function test reveals upper airway obstruction, and 3) CT scan demonstrates stenosis and edema of large airways. Based on the present case report, we strongly suggest that relapsing polychondritis should be differentiated from intractable bronchial asthma by physical examination, lung function test, and imaging technique.

Concerning the prognosis of RPC patients, the 5- and 10-year survival rates were 74 and 55%, respectively (13). Airway involvement is considered to be a major prognostic factor (14). Inflammation and destruction of tracheobronchial cartilages caused airway edema, airway collapse (tracheobronchomalacia), and cicatricial stenosis of the airways. In patients without advanced cartilage destruction, treatment might normalize respiratory function (4). In the present case, repeated airway chondritis led to irreversible tracheobronchomalacia. The disappearance of the tracheal cartilage ring by bronchoscopy suggested advanced cartilage destruction, consistent with a flow-volume curve indicating the pattern of intrathoracic airway stenosis. Since common causes of death in RPC patients included respiratory failure and airway infection, insertion of a tracheobronchial stent must be considered (3, 14).

In the present case, the results of coronary CT, electrocardiography, echocardiography, and serum biomarkers of cardiac injury suggested the concomitant development of takotsubo cardiomyopathy. According to studies reported (15, 16), aortic regurgitation, mitral valve regurgitation, or pericarditis was detected in approximately 10% of patients with RPC while no study has reported the concomitant development of takotsubo cardiomyopathy. Physical/mental stress may be involved in the pathogenesis. In the present patient, severe dyspnea may have induced takotsubo cardiomyopathy. Furthermore, β2 stimulants administered before and after admission may also have been an etiological factor (17). This is the first report of takotsubo cardiomyopathy in the patient with RPC. Takotsubo cardiomyopathy should be considered when differentiating heart diseases in patients with RPC.

As described above, early diagnosis/drug therapy for RPC may prevent or delay progression to tracheobronchomalacia. On the other hand, RPC is easily misdiagnosed as bronchial asthma because of its response to corticosteroid. It is important to differentiate RPC from bronchial asthma based on physical examination, detailed imaging, and respiratory function test findings.

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

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RESEARCH PAPER

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Characterization and long-term persistence of immune response following two doses of an AS03$_A$-adjuvanted H1N1 influenza vaccine in healthy Japanese adults

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Keywords: adjuvant, AS03, H1N1, long-term, pandemic, persistence

Abbreviations: AESI, adverse event of special interest; ATP, according to protocol; CBER, Center for Biologics Evaluation & Research; CHMP, Committee for Medicinal Products for Human Use; CI, confidence interval; GMFR, geometric mean fold rise; GMT, geometric mean titre; HA, hemagglutinin; HI, hemagglutination inhibition; pIMD, potential immune-mediated disease; RBC, red blood cell; SAE, serious adverse event; SCR, seroconversion rate; SPR, seroprotection rate; TVC, total vaccinated cohort; VRR, vaccine response rate; WHO, World Health Organization

Background: Long-term persistence of immune response and safety of two doses of an A/California/07/2009 H1N1 pandemic influenza vaccine adjuvanted with AS03 (an α-tocopherol oil-in-water emulsion-based Adjuvant System) administered 21 d apart was evaluated in Japanese adults [NCT00989612].

Methods: One-hundred healthy subjects aged 20−64 y (stratified [1:1] into two age strata 20−40 y and 41−64 y) received 21 d apart, two doses of AS03-adjuvanted 3.75 μg haemagglutinin (HA) H1N1 2009 vaccine. Immunogenicity data by haemagglutination inhibition (HI) assay six months after the first vaccine dose (Day 182) and microneutralization assay following each of the two vaccine doses (Days 21 and 42) and at Day 182 are reported here.

Results: Persistence of strong HI immune response was observed at Day 182 that met the US and European regulatory thresholds for pandemic influenza vaccines (seroprotection rate: 95%; seroconversion rate: 93%; geometric mean fold-rise: 20). The neutralizing antibody response against the A/Netherlands/602/2009 strain (antigenically similar to vaccine-strain) persisted for at least up to Day 182 (vaccine response rate: 76%; geometric mean titer: 114.4) and paralleled the HI immune response at all time points. No marked difference was observed in HI antibody persistence and neutralising antibody response between the two age strata. The vaccine had a clinically-acceptable safety profile.

Conclusion: Two priming doses of H1N1 2009 pandemic influenza vaccine induced an immune response persisting for at least six months after the first vaccine dose. This could be beneficial in evaluating the importance and effect of vaccination with this AS03-adjuvanted pandemic influenza vaccine.

The H1N1 2009 epidemic in Japan started off as isolated outbreaks in small clusters between May and July 2009. The number of cases rose steadily from mid-August 2009 and peaked in November 2009.[1] An estimated 20 million cases (as of February 05, 2010)[2] and 202 deaths related to H1N1 2009 (as of the end of H1N1 2009 pandemic)[3] were recorded in Japan. The majority of infections were recorded in school children and young adults, with the hospitalization rates being highest in children aged 5−9 y.[4,5] Although, adults appeared to be less susceptible to the H1N1 clinical disease, H1N1 2009 related fatality peaked in adults aged 40−49 y in addition to children aged <10 y (as of August 10, 2010).[3,5]

Clinical effectiveness of neuraminidase inhibitors, zanamivir and oseltamivir has been reported.[6-8] These drugs are able to mitigate morbidity and mortality caused by an influenza pandemic. However, mass immunization is an effective intervention against pandemic influenza. Identifying the necessity to make available a large number of vaccine doses worldwide and the potential for cross-reactive immunity, the World Health Organization (WHO) supported the development of adjuvanted pandemic influenza vaccines in parallel with non-adjuvanted vaccines.[9,10] A H1N1 2009 pandemic vaccine utilizing 3.75 μg A/California/07/2009 (H1N1)v-like haemagglutinin (HA) antigen

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adjuvanted with AS03 Adjuvant System (an α-tocopherol oil-in-water emulsion-based Adjuvant System) was developed based on GlaxoSmithKline Biologicals' previous experience with the AS03-adjuvanted prepandemic H5N1 vaccine.[11-13] This H1N1 2009 vaccine has demonstrated strong immunogenicity (fulfilling the US and European regulatory guidance criteria for pandemic influenza vaccines) and a clinically acceptable safety profile in different populations.[14,15]

In an open-label, single group study (NCT00989612) in Japanese adults aged 20−64 y, two doses of this H1N1 2009 pandemic influenza vaccine administered 21 d apart was found to be well-tolerated and highly immunogenic (all subjects seroconverted/ were seroprotected 21 d after the second vaccine dose), achieving the US and European regulatory guidance criteria for pandemic influenza vaccines in adults.[16] This manuscript presents follow-up data from the same population (stratified into 20−40 y and 41−64 y) on the persistence of humoral immune response in terms of HI antibody titers against the vaccine-homologous strain six months after primary vaccination with two doses of this AS03-adjuvanted H1N1 2009 vaccine (at Day 182), as well as neutralising antibody titers against the vaccine-homologous strain following each of the two doses and six months later (Days 21 and 42; Day 182). Data on the safety profile of the vaccine up to Day 182 is also presented here.

Results

Study population. The six month follow-up phase of this study was completed on April 19, 2010 (up to Day 182).

All 100 subjects who were part of the primary assessment and had received two doses of the H1N1 2009 vaccine completed the study up to Day 182 and were included in the according to protocol (ATP) cohort for persistence. The median age of subjects at the time of enrolment was 40.5 y (range: 21 to 59 y); 64% of subjects were female and all were of Japanese heritage.

Immunogenicity. *HI antibody immune response.* The haemagglutination inhibition (HI) immune response against the H1N1 2009 strain after six months after the first vaccine dose (Day 182) is presented in **Table 1**. The seroprotection rate (SPR) was 95%, seroconversion rate (SCR) was 93%, with a corresponding geometric mean titer (GMT) of 175.1 and geometric mean fold rise (GMFR) of 20. These values still met and exceeded the Center for Biologics Evaluation and Research (CBER) and Committee for Medicinal Products for Human Use (CHMP) guidance criteria for pandemic influenza vaccines. There was no appreciable difference in HI antibody persistence between the two age strata (overlapping 95% confidence intervals [CIs]). It is to be noted that the samples from Day 0, Day 21 and Day 42 were tested at the same time, while the Day 182 samples were tested later without an assessment of variability from earlier time points. Due to potential assay variability, a comparative interpretation of the HI response at Day 182 with earlier time points should be done with caution.

Neutralizing antibody response. Prior to receiving vaccination, 51% of subjects were seropositive for neutralising antibodies against the A/Netherlands/602/09 strain and the corresponding geometric mean titers (GMT) was low (8.5). Twenty-one days after the first vaccine dose (Day 21), the GMT rose to 136.9, with a vaccine response rate (VRR) of 74%. Following the second vaccine dose, these values increased to 305.8 and 96%, respectively. Six months after the first vaccine dose, persistence of neutralizing antibody response against the A/Netherlands/602/09 strain was evident (overall, GMT of 114.4 and VRR of 76%). No difference in neutralizing immune response was observed between the two age strata at any of the time points (overlapping 95% CIs) (**Table 2**). The proportion of subjects with antibody titers equal or above different threshold of positivity have been presented. The reverse cumulative curves for neutralizing antibodies 21 d after each of the two vaccine doses and at Day 182 (**Fig. 1**) and the neutralizing antibody titers for all time points (**Table 3**) showed a large proportion of subjects with titers equal or above the thresholds of 1:8, 1:16, 1:32 and 1:64, for six months after the first vaccine dose.

Safety and Reactogenicity. Overall, at least one unsolicited symptom was reported in 46 subjects (20−40 y: 21 subjects; 41−64 y: 25 subjects) during the 84 d post-vaccination follow-up period, of which 18 were considered to be vaccine-related.

Diarrhea, nasopharyngitis and headache (five subjects each) were the most frequently reported unsolicited symptoms. Of these, four cases of diarrhea and one case of headache were considered to be causally related to vaccination. One subject reported an unsolicited symptom of Grade 3 intensity (urticaria) which required medical attention and was unrelated to vaccination.

Table 1. Immune response in terms of haemagglutination inhibition antibodies against the vaccine homologous A/California/07/2009 strain at Day 182 (ATP cohort for persistence)

Age strata	Time point	Seroprotection rates		Seroconversion rates		Geometric Mean titers		Geometric Mean Fold Rise	
		N	% (95% CI)	N	% (95% CI)	N	Value (95% CI)	N	Value (95% CI)
Overall	Pre-vaccination	100	6 (1.9–13.6)	–	–	100	8.8 (7.3–10.5)	–	–
	Day 182	100	95 (88.7−98.4)	100	93.0 (86.1−97.1)	100	175.1 (144.2−212.7)	100	20.0 (16.8−23.8)
20−40	Pre-vaccination	50	6 (1.3–16.5)	–	–	50	8.9 (7.1–11.1)	–	–
years	Day 182	50	98.0 (89.4−99.9)	50	96.0 (86.3−99.5)	50	182.6 (141.1−236.4)	50	20.6 (16.0−26.5)
41−64	Pre-vaccination	50	6 (1.3–16.5)	–	–	50	8.6 (6.8–10.9)	–	–
years	Day 182	50	92.0 (80.8−97.8)	50	90.0 (78.2−96.7)	50	167.9 (124.5−226.5)	50	19.4 (15.1−25.1)

N, number of subjects with available results; CI, confidence interval; ATP: according to protocol

Table 2. Immune response in terms of neutralising antibodies against the A/Netherlands/602/09 strain [antigenically homologous to the vaccine strain] (ATP cohort for immunogenicity)

Age strata	Time point	Vaccine Response Rates		Geometric Mean titers	
		N	% (95% CI)	N	Value (95% CI)
Overall	Pre-vaccination	100	–	100	8.5 (7.1–10.2)
	Day 21	100	74.0 (64.3–82.3)	100	136.9 (97.0–193.3)
	Day 42	100	96.0 (90.1–98.9)	100	305.8 (242.5–385.6)
	Day 182	100	76.0 (66.4–84.0)	100	114.4 (89.3–146.5)
20–40 y	Pre-vaccination	50	–	50	8.7 (6.6–11.4)
	Day 21	50	78.0 (64.0–88.5)	50	146.6 (90.1–238.6)
	Day 42	50	96.0 (86.3–99.5)	50	336.6 (247.8–457.1)
	Day 182	50	84.0 (70.9–92.8)	50	133.5 (97.3–183.1)
41–64 y	Pre-vaccination	50	–	50	8.4 (6.6–10.7)
	Day 21	50	70.0 (55.4–82.1)	50	127.8 (77.1–211.9)
	Day 42	50	96.0 (86.3–99.5)	50	277.7 (194.3–397.0)
	Day 182	50	68.0 (53.3–80.5)	50	98.0 (66.6–144.3)

N, number of subjects with available results; CI, confidence interval; ATP: according to protocol

No potential immune mediated diseases (pIMD) or adverse events of special interest (AESIs) were recorded during the study period. Three serious adverse events (SAEs) were reported in two subjects during the entire study period. One male subject aged 44 y presented with ureteric calculi, approximately four months after the second vaccine dose which was resolved within three days and a female subject aged 36 y had a viral infection and pharyngeal ulceration approximately four and half months after the second vaccine dose which resolved in seven and 11 d, respectively; none of the SAEs were considered by the investigators to be vaccine-related. No fatalities were reported.

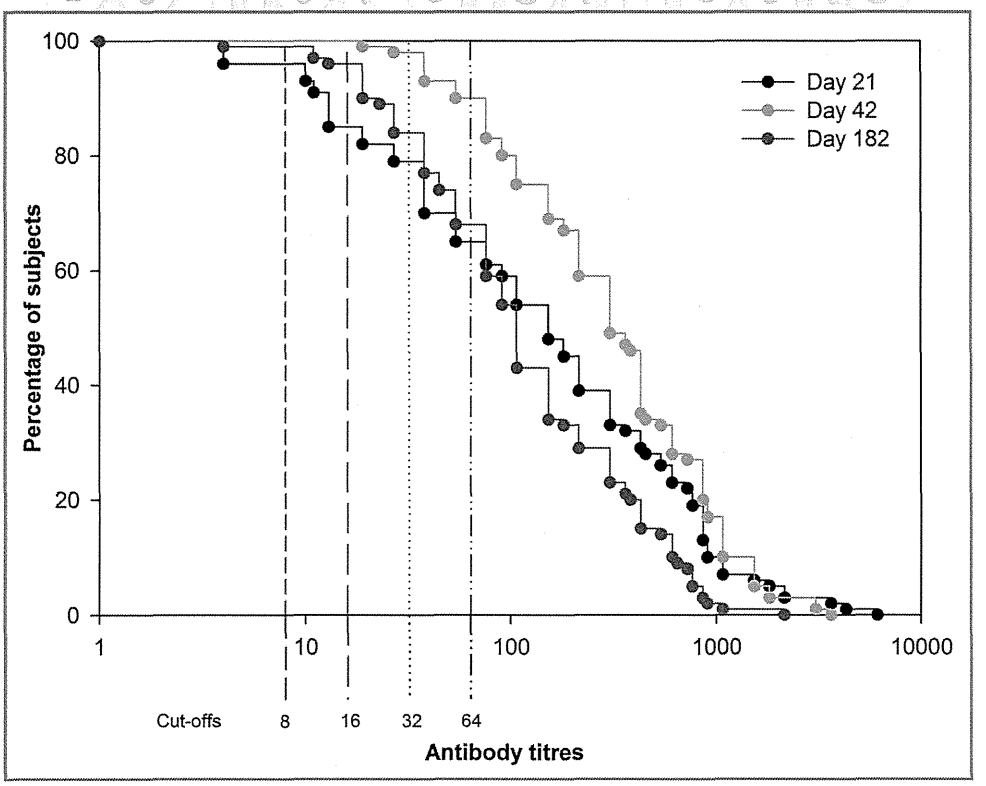

Figure 1. Reverse cumulative curves for neutralising antibody responses 21 d after each of the two vaccine doses (Days 21 and 42) and six months after the first vaccine dose (Day 182) (ATP cohort for immunogenicity). ATP, according to protocol.

Table 3. Percentage of subjects aged 20−64 y with neutralising antibodies titers ≥ 1:8, ≥ 1:16, ≥ 1:32 and ≥ 1:64 against the A/Netherlands/602/09 strain [antigenically homologous to the vaccine strain] at all time points (ATP cohort for immunogenicity)

Time point		≥ 1:8	≥ 1:16	≥ 1:32	≥ 1:64
	N	% (95% CI)			
Pre-vaccination	100	51.0 (40.8−61.1)	20.0 (12.7−29.2)	12.0 (6.4−20.0)	6.0 (2.2−12.6)
Day 21	100	96.0 (90.1−98.9)	85.0 (76.5−91.4)	79.0 (69.7−86.5)	65.0 (54.8−74.3)
Day 42	100	100 (96.4−100)	100 (96.4−100)	98.0 (93.0−99.8)	90.0 (82.4−95.1)
Day 182	100	99.0 (94.6−100)	96.0 (90.1−98.9)	84.0 (75.3−90.6)	68.0 (57.9−77.0)

N, number of subjects with available results; CI, confidence interval; ATP: according to protocol

Discussion

This is the first study assessing the persistence of immunological response against the A/California/07/2009 strain in Asian adults, six months after vaccination with the AS03-adjuvanted H1N1 2009 pandemic influenza vaccine.

Persistence of HI immune response against the A/California/07/2009 strain was observed for six months after the first vaccine dose (SPR: 95%; SCR: 93%); the CHMP and CBER guidance criteria for pandemic influenza vaccines were met and exceeded at Day 182. The observations from this study is in agreement with available data from studies in other adult populations that reported that the immune response induced by two doses of the 3.75 μg HA AS03-adjuvanted H1N1 2009 vaccine persists for six months after vaccination.[17,18] These observations are important for assessment of disease management strategies in the context of the WHO recommendations for the post-pandemic period which stresses on continuous vigilance, surveillance and disease management of circulating influenza strains.[19]

A previous head-to-head comparison study in UK between a similar AS03-adjuvanted H1N1 2009 vaccine and a non-adjuvanted whole-virion H1N1 2009 vaccine in adults (including those aged ≥65 y) reported that a single dose of the adjuvanted vaccine was sufficient to induce immune responses meeting the US and European regulatory criteria while two doses of the whole-virion vaccine were required. In addition, a large proportion of the participants were found to have protective levels of antibodies against the vaccine strain even six months after vaccination with two doses (although age-related decline was evident), indicating that pandemic influenza vaccines can potentially confer immunity against successive waves of the same virus.[20] This is in agreement with previous studies using the AS03-adjuvanted H1N1 2009 vaccine that have demonstrated substantial benefits in terms of induction of rapid, strong and long-lasting immune responses.

Theoretically, neutralization assays can capture a broad range of anti-influenza antibody activities and are able to interrupt several steps of the infectious life cycle of the virus. In contrast, HI assays are largely restricted to measuring the receptor-binding blocking activity of antibodies.[21] However, many different neutralising assays with different variations in protocols and expression of endpoints have been described[22] and it is likely that the biological activity of antibodies measured by these different assays is variable. The assay used in this study is characterized by a short incubation time between the virus and the tested serum. Although the extent of method-specific variation in neutralisation titer and its clinical significance is unknown, assay validation demonstrated good assay specificity (97% with 95% CI: 91.48–99.38%) and a good correlation with the HI assay (r = 0.64) (unpublished GSK data). The study demonstrated strong neutralising antibody response as evident from the high VRRs following each of the two vaccine doses and persistence of high VRRs for six months after the first vaccine dose. Neutralizing antibody responses observed in the study population confirmed the robust immunogenicity of the vaccine and persistence of anti-A/California/07/2009-like antibodies. Overall, the vaccine had a clinically acceptable safety profile in the study population.

The epidemiology and characteristics of the H1N1 2009 virus in Japan has been similar to that observed in other countries in the northern hemisphere and the trends in incidence mirrored those observed worldwide. Although there were fewer laboratory-confirmed H1N1 2009 cases after February−March 2010, and the last reported death due to H1N1 2009 in the pandemic period was in July 2010,[3] the virus continued to circulate in the post-pandemic phase, making it essential to investigate whether pandemic vaccination programs led to long-term persistence of immune response against the H1N1 2009 virus.

The present study advances information on the safety, immunogenicity and long-term immunological persistence of this AS03-adjuvanted H1N1 2009 pandemic influenza vaccine in an Asian population. Contrary to the observations made by Nicholson et al. using a similar vaccine in adults including the elderly,[20] no age-related declined immunological response was observed at Day 42 in the present study, and the data indicated that the immunological response was persistent up to Month 6 in both age strata (20–40 and 41–64 y). The safety profile of the vaccine in Asian adults was comparable to previous reports and no-Asia-specific safety concerns were reported. Thus, the data obtained from this study provides a holistic worldwide dimension to the safety and immunogenicity profile of the study vaccine observed across different populations, now including this Japanese population.

In conclusion, this study presents novel data on persistence of immunological response against the H1N1 2009 virus in adults and on neutralising antibody response induced by this H1N1 2009 pandemic influenza vaccine. It was established that following two doses of a 3.75μg HA AS03-adjuvanted H1N1 2009 pandemic influenza vaccine in adults aged 20−64 y, immune response against the vaccine homologous A/California/07/2009 strain persisted for at least six months after the first vaccine dose. The immunological response met the US and

European guidance criteria for pandemic influenza vaccines up to six months after the first vaccine dose. These results will be beneficial in evaluating the importance and effect of vaccination with this AS03-adjuvanted pandemic influenza vaccine.

Materials and Methods

Study design and subjects. In the primary phase, 100 healthy Japanese adults aged 20−64 y without history of clinically-confirmed influenza infection or previous vaccination with a novel H1N1 2009 vaccine or any seasonal influenza vaccination within 14 d prior to study start were enrolled to receive 21 d apart, two doses of a monovalent AS03-adjuvanted 3.75μg HA A/California/07/2009 pandemic influenza vaccine. The subjects were further stratified by age (stratification ratio: 1:1) into 20−40 y and 41−64 y age strata.

Written informed consent was obtained from all subjects prior to conducting any study-related procedures. The study was conducted in accordance with the Good Clinical Practice guidelines, the Declaration of Helsinki and local regulations. All study-related documents were approved by Institutional Review Boards.

Study vaccine. The H1N1 2009 pandemic influenza vaccine was a monovalent, inactivated, split-virion antigen adjuvanted with $AS03_A$ (*Arepanrix*™, a trademark of the GlaxoSmithKline group of companies). The H1N1 viral seed for the vaccine was prepared from the reassortant virus NYMC X-179A (New York Medical College, New York) generated from the A/California/07/2009 strain, as recommended by the WHO.[23] $AS03_A$ is an oil-in-water emulsion-based Adjuvant System containing α-tocopherol (11.86mg tocopherol).

Both vaccine doses were administered intramuscularly at alternate deltoid muscles sides.

Immunogenicity assessments. Blood samples were collected before vaccination, 21 d after each of the two vaccine doses and six months after the first vaccine dose.

Serum samples collected six months after the first vaccine dose (Day 182) were tested at GSK Biologicals Central Laboratory using a validated in-house HI assay [cut-off: ≥ 1:10] that used chicken erythrocytes as previously described.[24]

The viral microneutralisation assay was performed on serum samples collected at all time points at Viroclinics Biosciences (Rotterdam, The Netherlands).[25] The sera were subjected to heat treatment at 56°C for 30 min and then tested in triplicate. The assay used a constant amount of A/Netherlands/602/2009 pandemic H1N1 Influenza virus (A A/California/07/2009-like virus) mixed with serial 2-fold dilutions of serum samples. The mixture of virus and serum was added to Madin-Darby Canine Kidney (MDCK) cell cultures (10^4 cells per well) and incubated for one hour at 37°C, following which the virus-antibody mixture was removed from the wells by aspiration, cells were fed with fresh culture medium and further incubated for 6 d at 37°C. After the incubation period, the well supernatants were transferred into 96 well plates and a suspension of turkey red blood cells (RBCs) was added to it; following an incubation for 60 min at 4°C, the culture supernatants (virus replication) were visualized by haemagglutination of RBCs. The 50% neutralisation titer of a serum was calculated by the Reed and Muench method.[26] The assay cut-off was 1:8.

The evaluation of outcome measures of immune response was based on the immunogenicity criteria for pandemic influenza vaccines in adults as required by the CHMP: point estimates for HI antibody SCR: > 40%, SPR: > 70% and GMFR: > 2.5 and CBER: lower bound of 95% CI for HI antibody for SCR: ≥ 40% and SPR: ≥ 70%.[27,28] SPR was defined as percentage of subjects with a post-vaccination titer ≥ 1:40, SCR as percentage of subjects with pre-vaccination titer < 1:10 and post-vaccination titer ≥ 1:40 or pre-vaccination titer > 1:10 and at least 4-fold increase in post-vaccination titer and GMFR as post-vaccination fold increase in GMTs for HI antibodies. For neutralising antibodies, immunological assessments were based on the VRRs defined as percentage of subjects with either a pre-vaccination titer < 1:8 and a post-vaccination titer ≥ 1:32, or a pre-vaccination titer ≥ 1:8 and at least a 4-fold increase in post-vaccination titer.

Safety and reactogenicity assessments. Unsolicited adverse events were recorded up to 84 d following the first vaccine dose; pIMD (which are a subset of adverse events that include both autoimmune diseases and other inflammatory and/or neurologic disorders which may or may not have an autoimmune etiology), AESI and SAEs occurring during the entire study period were recorded.

Statistical analyses. The analyses of immunogenicity in terms of HI antibodies at Day 182 were performed on the per-protocol cohort for persistence, analyses of immunogenicity in terms of neutralising antibodies at all time points were performed on the per-protocol cohort for immunogenicity and the analyses of safety were performed on the total vaccinated cohort (TVC). The according to protocol cohort for immunogenicity included all subjects who received both vaccine doses and met all protocol-defined eligibility criteria and procedures and for whom data was available at Days 21 and 42. The according to protocol cohort for persistence included all subjects who received both vaccine doses and met all protocol-defined eligibility criteria and procedures and for whom data was available at Days 21, 42 and 182. The TVC included all vaccinated subjects for whom data was available. For the purpose of GMT calculations, antibody titers below the cut-off value of each assay were substituted by half of the cut-off value.

Disclosure of Potential Conflicts of Interest

Drs. Hideyuki Ikematsu and Hideaki Nagai were the principal investigators of this study and disclose having received honoraria/paid expert testimony and travel grants from the commercial entity that sponsored the study. Drs. Masahiro Kawashima and Yasunobu Kawakami disclose having no conflict of interest. All participating institutions received compensation for study involvement. Drs. Paul Gillard, François Roman, Karl Walravens, Kazuyoshi Tenjinbaru and Ping Li are employees of GlaxoSmithKline Biologicals. P. Gillard and F. Roman report ownership of stock options.

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All authors participated in the implementation of the study including substantial contributions to conception and design, the gathering of the data, or analysis and interpretation of the data. All

authors were involved in the drafting of the article or revising it critically for important intellectual content, and final approval of the manuscript.

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Trade mark statement

Arepanrix is a trade mark of GlaxoSmithKline group of companies. Zanamivir *(Relenza)* is a trade mark of GlaxoSmithKline group of companies. Oseltamivir *(TamiFlu)* is a trade mark of Roche. ClinicalTrials.gov Identifier: NCT00989612

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ORIGINAL ARTICLE

Reevaluation of the Japanese guideline for healthcare-associated pneumonia in a medium-sized community hospital in Japan

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Abstract The Japanese guidelines for nursing- and healthcare-associated pneumonia (NHCAP) categorize patients by risk of resistant bacteria and defined antimicrobials to be used, similar to the healthcare-associated pneumonia (HCAP) guidelines of the United States. The data were collected in large-scale hospitals, possibly a cause of inconsistency with everyday practice in medium-sized community hospitals. To test the feasibility of this guideline based on a retrospective study performed in a medium-sized community hospital in Japan, the medical records of pneumonia patients were retrospectively studied [718 patients: NHCAP, 477, 66.4 %; community-acquired pneumonia (CAP), 241, 33.4 %). Factors related to patients' background, clinical and laboratory findings, treatment, and outcome were compared between NHCAP and CAP. The A-DROP system, scored by age, dehydration, respiratory failure, disorientation, and low blood pressure, evaluated the severity of pneumonia. In contrast to CAP patients, NHCAP patients included more elderly patients requiring nursing care and revealed higher rates of poor nutrition, dementia, aspiration, severe cases, detection of drug-resistant bacteria, and mortality. For NHCAP, the success rate did not differ between those receiving and not receiving proper initial treatment (76.9 vs. 78.5 %) nor did mortality rate within 30 days differ (13.1 vs. 13.8 %). Risk factors for mortality within 30 days for NHCAP were diabetes [adjusted odds ratio (AOR) 2.394, $p = 0.009$], albumin <2.5 g/dl (AOR 2.766, $p = 0.002$), A-DROP very severe (AOR 1.930, $p = 0.021$), and imaging showing extensive pneumonia (AOR 2.541, $p = 0.002$). The severity of pneumonia rather than risk of resistant bacteria should be considered, in addition to ethical concerns, in initial treatment strategy in NHCAP to avoid excessive use of broad-spectrum antimicrobials.

Keywords: Severity of pneumonia · Drug-resistant bacteria · Performance status · Healthcare-associated pneumonia · Nursing and healthcare-associated pneumonia

Introduction

Healthcare-associated pneumonia (HCAP) is considered to fall between community-acquired pneumonia (CAP) and hospital-acquired pneumonia (HAP) [1, 2]. It has a poor prognosis and a high rate of detection of drug-resistant bacteria, as does HAP, according to reports from the United States [3, 4]. In contrast, the British Thoracic Society guidelines have documented that patients with nursing home-acquired pneumonia (NHAP), which is considered as a counterpart of HCAP, should be treated in the same way as CAP [5, 6]. In addition, a report from Spain indicated that HCAP more closely resembled CAP [7]. Differences in results might be derived from the differences in medical and healthcare systems among countries. In Japan, one report stated that more drug-resistant bacteria were detected as causative organisms for HCAP compared to CAP [8], whereas another report found that HCAP was pneumonia with a poor prognosis in the elderly rather than pneumonia caused by drug-resistant bacteria, and therefore closer to CAP [9].

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The healthcare system differs greatly between Japan and the United States, and it is difficult to apply the same definitions and analyses for HCAP. In Japan, many elderly and physically handicapped people receive care at home [10]. In addition, nursing homes are not yet popular, so a great many elderly patients are admitted to general hospitals and tend to stay longer [11]. Because more than half of cases referred to as HCAP in the United States would be defined as HAP in Japan, a divergence between actual medical practice and HAP guidelines has been observed. Taking those backgrounds into account, HCAP guidelines [called nursing- and healthcare-associated pneumonia (NHCAP) guidelines] were formulated in Japan based on data extracted on HAP patients in long-term convalescent or psychiatric wards and CAP patients who were elderly and physically handicapped, requiring nursing care or receiving intravascular treatment continuously on an outpatient basis.

There were only a few reports on HCAP in Japan [8, 9]; reports on HCAP in the United States were mainly analyses of severely ill patients in intensive care units (ICU) or in large hospitals. Therefore, sufficient data from medium-sized or smaller community hospitals were lacking. The present study was conducted to address the following two points in a medium-sized community hospital: first, to study clinical differences between NHCAP and CAP; and second, to determine which is more important, severity or resistant bacteria, to the prognosis of NHCAP.

Patients and methods

Patient data

Data were collected and analyzed retrospectively on pneumonia patients admitted between January 2008 and June 2010 to Sekishinkai Sayama Hospital, which is a community hospital designated as a secondary emergency hospital with 350 beds in Sayama City, Saitama Prefecture, Japan.

Pneumonia cases were divided into two groups according to guidelines from the Japanese Respiratory Society: NHCAP [12] and CAP. HAP and cases diagnosed as diseases other than infectious pneumonia during the course of the study were excluded.

For eligible patients, information on patients' background, clinical findings and laboratory data on admission, severity of pneumonia, detected bacteria, initial antibiotics administered and outcome were collected. The antibiotics were selected by the attending physicians, generally, but not strictly, based on the CAP guidelines. Failure of the initial treatment was defined as escalation of antibiotics within 48–72 h of hospitalization, poor clinical improvement (no defervescence, start of mechanical ventilation, use of vasopressor), or death. Adequacy of the initial treatment was determined such that, when the bacteria detected before treatment were sensitive to the initial antibiotics, the treatment was defined as appropriate and when not sensitive, the treatment was defined as inappropriate. For outcome, mortality within 30 days and total hospital mortality were examined.

Use of data for this study was permitted by the Information Systems Division of Sekishinkai Sayama Hospital. Also, this study was approved by the institutional review board of the National Hospital Organization Tokyo National Hospital.

Criteria for NHCAP and CAP

Patients in the NHCAP group met one or more of the following criteria: (1) admitted to long-term convalescent ward or nursing home (including psychiatric wards); (2) discharged from hospital within preceding 90 days; (3) elderly and physically handicapped, requiring nursing care; or (4) receiving continuous intravascular treatment on an outpatient basis (dialysis, antibiotics, chemotherapy, immunosuppressive agents). The CAP was defined as pneumonia other than NHCAP (defined above) or HAP, which was defined as pneumonia occurring more than 48 h after admission to a hospital. Within these categories, patients requiring nursing care were higher than grade 3 performance status (PS) of the Eastern Cooperative Oncology Group (ECOG).

Microbiological evaluation

Microbiological diagnosis was performed by cultures (sputum, blood, bronchial wash, pleural effusion) and Gram stain. Indigenous bacteria were excluded from culture-positive cases. We diagnosed pneumococcal pneumonia when *Streptococcus pneumoniae* was isolated from the sputum or urine antigen test was positive. *Mycoplasma* pneumonia (caused by *Mycoplasma pneumoniae*) and *Chlamydophila* pneumonia (*Chlamydophila pneumoniae*) were diagnosed only when there were significant findings in a single serum or paired serum test. *Legionella* pneumonia was diagnosed by a urinary antigen test that detects only serotype 1. Drug-resistant bacteria in NHCAP were defined as *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Acinetobacter* spp., and extended-spectrum β-lactamase-producing *Enterobacteriaceae*, which have a risk of drug resistance according to the guidelines [12].

Evaluation of severity

Severity of pneumonia was evaluated by the A-DROP system (old age, dehydration, respiratory failure, orientation

disturbance, low blood pressure), which is used in the CAP guidelines of the Japanese Respiratory Society [13]. Condition is mild if none of the items is present, moderate with one or two items present, severe with three items present, and very severe if there are four or five items present. If there is only one item present but it is shock (5), the condition is deemed very severe. In a multicenter prospective study ($n = 1{,}875$), ADROP score was correlated well with the mortality rate; mortality rates were 0 % for mild cases, 3.1 % for moderate, 9.9 % for severe, and 19.6 % for very severe cases [14]. For this study, patients were divided into three groups: mild-to-moderate, severe, and very severe groups.

NHCAP guidelines for empirical antimicrobial selection

The NHCAP guidelines divided patients into four groups, designated as Groups A to D, and recommended the choice of antimicrobials based on the category. Group A can be properly treated with outpatient care with oral medication. For Group D, at the greatest risk, multidisciplinary treatment, such as mechanical ventilation or ICU care, is needed with one or more anti-pseudomonal antimicrobials. For patients other than those in Groups A and D, (1) antibiotics administration within the past 90 days and (2) tube feeding are considered as risk factors for drug-resistant bacteria. Group B has no risk factors of drug-resistant bacteria; therefore, narrow-spectrum antibiotics are recommended. Group C has at least one risk factor. Because there is risk of drug-resistant bacteria, broad-spectrum antibiotics are recommended.

Statistical analysis

Data analysis software (IBM SPSS for Windows, version 19.0J; IBM SPSS, Chicago, IL, USA) was used for all statistical analyses. To compare categories between two groups, the chi-square test or Fisher's exact test was used. Continuous variables were shown as an interquartile range (IQR) or mean ± SD. The two-sample t test for a normal distribution and the Mann–Whitney test for a nonnormal distribution were used. Multiple logistic analysis was performed for the risk factors involved in mortality within 30 days and detection of drug-resistant bacteria in NHCAP. An α error of less than 5 % was considered significant.

Results

Criteria of NHCAP (Table 1)

Of the 718 patients who were evaluated, 477 (66.4 %) had NHCAP and 241 (33.4 %) had CAP. Elderly and physically handicapped patients who required nursing care accounted for the majority of NHCAP (418/477 patients, 87.6 %) and met two or more inclusion criteria for NHCAP.

Table 1 Criteria of nursing home- and healthcare-associated pneumonia (NHCAP)

NHCAP ($n = 477$)	n (%)
Number of criteria met for NHCAP[a] (IQR)	2 (1–2)
Admitted to long-term convalescent wards or nursing home	186 (39.0 %)
Discharged from the hospital within 90 days	142 (29.8 %)
Elderly and physically handicapped who require nursing care	418 (87.6 %)
Receiving intravascular treatment continuously on outpatient basis	24 (5.0 %)

IQR interquartile range

[a] Indicates inclusion of duplicated cases

Patient background and severity classification on admission (Table 2)

Significantly more patients with old age, poor PS, dementia, involvement of aspiration, gastrostoma, low serum albumin level, greater number of complications, chronic kidney disease, and central nervous system disease were found in the NHCAP group than in the CAP group. Significantly more patients had a history of previous administration of antibiotics or broad-spectrum antibiotics within 90 days and detection of MRSA in the NHCAP compared to the CAP group. Severity on admission was significantly higher in the NHCAP than in the CAP group.

Detected bacteria (Table 3)

Detected bacteria in NHCAP were more frequent, of the order of *S. pneumoniae*, MRSA, *Klebsiella* spp., and *Pseudomonas aeruginosa*; in CAP the frequency was of the order of *S. pneumoniae*, *Haemophilus influenzae*, and *Klebsiella* spp. Drug-resistant bacteria were detected in 60 cases (12.6 %) in the NHCAP group, which was significantly higher than the 5 cases (2.1 %) in the CAP group ($p < 0.001$). Although *S. pneumoniae* was detected most frequently in both NHCAP and CAP, the frequency was significantly higher in the CAP than in the NHCAP group ($p < 0.001$). *H. influenzae* was also detected with higher frequency in CAP than in NHCAP ($p = 0.025$).

Risk factors involved in the detection of drug-resistant bacteria in patients with NHCAP (Table 4)

Multiple logistic analysis was performed for risk factors involved in drug-resistant bacteria in the 195 NHCAP

Table 2 Background and severity classification on admission

	NHCAP ($n = 477$)	CAP ($n = 241$)	p value
Age (IQR)	84 (77–90)	74 (66–82)	<0.001
Male	274 (57.4 %)	153 (63.5 %)	0.119
PS (IQR)	4 (3–4)	0 (0–1)	<0.001
PS 0	29 (6.1 %)	163 (67.6 %)	
PS 1	13 (2.7 %)	45 (18.7 %)	
PS 2	19 (4.0 %)	33 (13.7 %)	
PS 3	93 (19.5 %)	0 (0 %)	
PS 4	323 (67.7 %)	0 (0 %)	
Complications (IQR)	2 (1–3)	1 (1–2)	<0.001
Malignant tumor	54 (11.3 %)	20 (8.3 %)	0.209
Chronic lung disease	141 (29.6 %)	101 (41.9 %)	0.001
Chronic heart disease	282 (59.1 %)	127 (52.7 %)	0.101
Chronic kidney disease	45 (9.4 %)	5 (2.1 %)	<0.001
Chronic liver disease	27 (5.7 %)	22 (9.1 %)	0.082
Central nervous system disease	253 (53.0 %)	38 (15.8 %)	<0.001
Diabetes	70 (14.7 %)	50 (20.7 %)	0.039
Immunodeficiency	32 (6.7 %)	9 (3.7 %)	0.105
Two or more underlying diseases	299 (62.7 %)	115 (47.7 %)	<0.001
Dementia[a]	342 (71.7 %)	23 (9.5 %)	<0.001
Involvement of aspiration[b]	358 (75.1 %)	24 (10.0 %)	<0.001
Gastrostoma	55 (11.5 %)	1 (0.4 %)	<0.001
History of previous administration of antibiotics within 90 days	231 (48.4 %)	87 (36.1 %)	0.002
History of previous administration of broad-spectrum antibiotics within 90 days[c]	125 (26.2 %)	39 (16.2 %)	0.003
History of MRSA detection	50 (10.5 %)	5 (2.1 %)	<0.001
Serum albumin level (g/dl) (IQR*)	3.1 (2.7–3.5)	3.4 (3.0–3.8)[d]	<0.001
A-DROP Score (IQR*)	3 (0–2–4–5)	0–2 (0–2–3)	<0.001
Mild to moderate (Score 0–2)[e]	160 (33.5 %)	167 (69.3 %)	
Severe (Score 3)	158 (33.1 %)	59 (24.5 %)	
Very severe (Score 4–5)	159 (33.3 %)	15 (6.2 %)	

IQR interquartile range, *CAP* community-acquired pneumonia, *MRSA* methicillin-resistant *Staphylococcus aureus*, *PS* performance status

PS 0, can be active without any problems or limitations, daily life the same as before the onset; PS 1, intense activity limited, but can walk and perform light work or work while sitting; PS 2, can walk and perform all personal care, but cannot work; more than 50 % of daytime hours out of bed; PS 3, can only do limited personal care; more than 50 % of daytime hours spent in bed or chair; PS 4, cannot move at all or perform personal care, all day spent in bed or chair

[a] Dementia: diagnosed by revised Hasegawa's Dementia Scale (HDS-R)

[b] Involvement of aspiration: dysphagia or aspiration confirmed or strongly suspected

[c] History of previous administration of broad-spectrum antibiotics: history of administration of anti-pseudomonal penicillin, third- and fourth-generation cephalosporin injection, new quinolone or carbapenem was present

[d] Score 0, 2 NHCAP cases, 34 CAP cases; Score 1–2, 158 NHCAP cases, 133 CAP cases

[e] Serum albumin level was measured in 238 cases

Mann–Whitney test

patients from whom 231 bacteria (more than 195 because of multiple isolation) were isolated, and their drug sensitivity was determined.

PS, central nervous system disease, gastrostoma, history of previous administration of antibiotics within 90 days, and being discharged from the hospital within 90 days were independent variables for the increased detection rate of drug-resistant bacteria.

Initial antibiotics (Table 5)

Monotherapy accounted for the majority of antibiotic therapies in both groups. The rate of monotherapy was significantly higher in NHCAP than in CAP ($p = 0.001$). The most frequent choice of drug was sulbactam/ampicillin (SBC/ABPC) in both groups, and the frequency of SBC/ABPC usage was significantly higher in NHCAP than in

Table 3 Detected bacteria

Detected bacteria	NHCAP ($n = 477$)	CAP ($n = 241$)
Gram-positive cocci	133 (27.9 %)	78 (32.4 %)
Streptococcus pneumoniae	76 (15.9 %)	71 (29.5 %)
MSSA	23 (4.8 %)	8 (3.3 %)
MRSA	38 (8.0 %)	2 (0.8 %)
Streptococci other than *Streptococcus pneumoniae*	10 (2.1 %)	0 (0 %)
Gram-negative bacilli	101 (21.2 %)	38 (15.8 %)
Pseudomonas aeruginosa	27 (5.7 %)	4 (1.7 %)
Klebsiella sp.	34 (7.1 %)	9 (3.7 %)
Klebsiella sp. ESBLs	1 (0.2 %)	0 (0 %)
Haemophilus influenzae	16 (3.4 %)	17 (7.1 %)
BLNAR	1 (0.2 %)	3 (1.2 %)
Enterobacter sp.	9 (1.9 %)	8 (3.3 %)
Escherichia coli	16 (3.4 %)	1 (0.4 %)
Escherichia coli ESBLs	0 (0 %)	0 (0 %)
Serratia sp.	3 (0.6 %)	1 (0.4 %)
Stenotrophomas maltophilia	0 (0 %)	0 (0 %)
Acinetobacter sp.	1 (0.2 %)	0 (0 %)
Citrobacter sp.	2 (0.4 %)	0 (0 %)
Moraxella catarrhalis	6 (1.3 %)	2 (0.8 %)
Proteus sp.	3 (0.6 %)	0 (0 %)
Anaerobic organisms	0 (0 %)	0 (0 %)
Other organisms	5 (1.0 %)	5 (2.1 %)
Atypical pathogens	0 (0 %)	4 (1.7 %)
Mycoplasma pneumoniae	0 (0 %)	2 (0.8 %)
Chlamydophila pneumoniae	0 (0 %)	0 (0 %)
Legionella pneumoniae	0 (0 %)	2 (0.8 %)
Unknown	280 (58.7 %)	132 (54.8 %)
Drug-resistant bacteria[a]	60 (12.6 %)	5 (2.1 %)

MSSA methicillin-sensitive *Staphylococcus aureus*, *MRSA* methicillin-resistant *Staphylococcus aureus*, *ESBLs* extended-spectrum β-lactamases, *BLNAR* β-lactamase-negative ampicillin-resistant *Haemophilus influenzae*

[a] Drug-resistant bacteria: *Pseudomonas aeruginosa*, MRSA, *Acinetobacter*, ESBL-producing *Enterobacteriaceae* were defined. When more than one organism was detected in the same patient, it was counted as one

Table 4 Risk factors involved in the detection of drug-resistant bacteria in NHCAP patients by multiple logistic analysis

	Adjusted odds ratio (95 % CI)	p value
Performance status	1.592 (1.111–2.282)	0.011
Central nervous system disease	2.756 (1.249–6.084)	0.012
Gastrostoma	5.459 (1.921–15.510)	0.001
History of previous administration of antibiotics within 90 days	4.108 (1.852–9.112)	0.001
Discharged from hospital within 90 days	3.448 (1.537–7.736)	0.003

Drug-resistant bacteria: *Pseudomonas aeruginosa*, MRSA, *Acinetobacter*, ESBLs. Multiple logistic analysis was performed for factors involved in drug-resistant bacteria in 195 cases of NHCAP in which microorganisms were detected (*CI* confidence interval). Model chi-square test, $p < 0.001$; Hosmer–Lemeshow test, $p = 0.983$; discriminant accuracy rate, 76.8 %

Table 5 Initial antibiotics

	NHCAP ($n = 467$)[a]	CAP ($n = 241$)
Monotherapy	433 (90.8 %)	198 (82.2 %)
Sulbactam/ampicillin	308 (64.6 %)	95 (39.4 %)
Cephalosporin	68 (14.3 %)	47 (19.5 %)
Carbapenem	34 (7.1 %)	27 (11.2 %)
Quinolone	10 (2.1 %)	13 (5.4 %)
Macrolide	1 (0.2 %)	10 (4.1 %)
Others	12 (2.5 %)	6 (2.5 %)
Combination therapy	34 (7.1 %)	43 (17.8 %)
β-Lactam + quinolone	14 (2.9 %)	18 (7.5 %)
β-Lactam + macrolide	8 (1.7 %)	17 (7.1 %)
β-Lactam + clindamycin	5 (1.0 %)	0 (0 %)
β-Lactam + vancomycin	1 (0.2 %)	0 (0 %)
Others	6 (1.3 %)	8 (3.3 %)
Anti-pseudomonal agents[b]	82 (17.2 %)	68 (28.2 %)
Anti-MRSA agents[c]	1 (0.2 %)	0 (0 %)

[a] Ten cases in NHCAP were excluded because antibiotics used were unknown

[b] Anti-pseudomonal agents: antibiotics with a spectrum against *Pseudomonas aeruginosa*

[c] Anti-MRSA agents: antibiotics with a spectrum against MRSA

CAP ($p < 0.001$). Frequency of choice for combination therapy and an anti-pseudomonal agent were significantly lower in NHCAP than in CAP ($p < 0.001$ and $p = 0.001$, respectively). An anti-MRSA agent was used in only one case of NHCAP.

Success or failure of initial treatment (Table 6)

Rates of improper treatment and of failure despite proper initial treatment were significantly higher in NHCAP compared to CAP. However, the failure rate of improper initial treatment did not differ significantly between groups. Mortality within 30 days and total hospital mortality among patients who received proper initial treatment were significantly higher in NHCAP than in CAP, but there was no significant difference between the two groups regarding patients who received improper initial treatment. Within the NHCAP group, success rates for patients who did and did not receive proper initial treatment were 76.9 and 78.5 %, respectively; mortality rates within 30 days were 13.1 and 13.8 %, respectively; and total hospital mortality was 24.6 and 21.5 %, respectively; there were no

Table 6 Success or failure of initial treatment in patients with NHCAP and CAP in which microorganisms were identified

	NHCAP	CAP	*p* value
Proper initial treatment	130/195 (66.7 %)	100/108 (92.6 %)	
Success	100/130 (76.9 %)	91/100 (91.0 %)	
Failure	30/130 (23.1 %)	9/100 (9.0 %)	0.005
Mortality within 30 days	17/130 (13.1 %)	3/100 (3.0 %)	0.007
Total hospital mortality	32/130 (24.6 %)	3/100 (3.0 %)	<0.001
Improper initial treatment	65/195 (33.3 %)	8/108 (7.4 %)	<0.001
Success	51/65 (78.5 %)	6/8 (75.0 %)	
Failure	14/65 (21.5 %)	2/8 (25.0 %)	0.562[a]
Mortality within 30 days	9/65 (13.8 %)	0/8 (0 %)	0.329[a]
Total hospital mortality	14/65 (21.5 %)	1/8 (12.5 %)	0.478[a]

[a] Fisher's exact test

significant differences between the two groups. Among NHCAP patients who received proper initial treatment, serum albumin levels were 3.1 ± 0.5 and 2.7 ± 0.7 g/dl in successful and failed cases, respectively, with levels significantly lower in failed cases ($p = 0.001$; data not shown). No significant difference in serum albumin levels in NHCAP patients who received improper initial treatment was found between successful cases and failed cases.

Success rate of initial treatment in NHCAP with regard to initial treatment and detected bacteria (Table 7)

The success rate against typical nondrug-resistant bacteria in NHCAP was approximately 70 % in patients who received proper initial treatment. On the other hand, the success rate against drug-resistant bacteria in NHCAP was approximately 80 % in patients who received inappropriate initial treatment.

Clinical outcome (Table 8)

Mortality within 30 days and total hospital mortality were significantly higher in NHCAP than in CAP. Length of stay was significantly longer in NHCAP than in CAP. Mortality within 30 days in NHCAP with regard to severity was nearly identical in the mild-to-moderate and severe groups but was increased approximately twofold in the very severe group. Total hospital mortality in NHCAP with regard to severity tended to rise with increasing severity. The difference between mortality within 30 days and total hospital mortality was greater in NHCAP compared to CAP.

Table 7 Success rate of initial treatment in patients with NHCAP with regard to initial treatment and detected bacteria

Nondrug-resistant bacteria in NHCAP	Success rate of proper initial treatment group
Streptococcus pneumoniae	50/66 (75.8 %)
MSSA	14/19 (73.7 %)
Klebsiella sp.	22/26 (84.6 %)
Haemophilus influenzae	8/12 (66.7 %)
Escherichia coli	10/13 (76.9 %)
"Drug-resistant bacteria" in NHCAP	Success rate of improper initial treatment group
MRSA	29/37 (78.4 %)
Pseudomonas aeruginosa	16/20 (80.0 %)

There were 130 cases in the proper initial treatment group and 65 cases in the improper initial treatment group. Success rates of initial treatment for the detected bacteria in each treatment group are shown

Table 8 Outcome

	NHCAP	CAP	*p* value
Mortality within 30 days	67/477 (14.0 %)	10/241 (4.1 %)	<0.001
Total hospital mortality	118/477 (24.7 %)	13/241 (5.4 %)	<0.001
Length of stay (IQR)	17 (10–34)	9 (7–13)	<0.001*
A-DROP mild to moderate, mortality within 30 days	16/160 (10.0 %)	2/167 (1.2 %)	
A-DROP severe, mortality within 30 days	16/158 (10.1 %)	8/59 (13.6 %)	
A-DROP very severe, mortality within 30 days	35/159 (22.0 %)	0/15 (0 %)	
A-DROP mild to moderate, total hospital mortality	26/160 (16.3 %)	3/167 (1.8 %)	
A-DROP severe, total hospital mortality	37/158 (23.4 %)	8/59 (13.6 %)	
A-DROP very severe, total hospital mortality	55/159 (34.6 %)	2/15 (13.3 %)	

IQR interquartile range

* Mann–Whitney test

Risk factors involved in mortality within 30 days in patients with NHCAP (Table 9)

Multiple logistic analysis to identify factors involved in mortality within 30 days in 477 cases of NHCAP showed

Table 9 Risk factors involved in mortality within 30 days in patients with NHCAP by multiple logistic analysis

	Adjusted odds ratio (95 % CI)	*p* value
Diabetes	2.394 (1.241–4.622)	0.009
Albumin <2.5 g/dl	2.766 (1.431–5.348)	0.002
A-DROP very severe	1.930 (1.102–3.382)	0.021
Image of extensive pneumonia[a]	2.541 (1.419–4.551)	0.002

Multiple logistic analysis was performed for factors involved in mortality within 30 days in 477 cases of NHCAP

[a] Image of extensive pneumonia: shadow of more than two-thirds of the unilateral lung in a plain chest X-ray: model chi-square test $p < 0.001$; Hosmer–Lemeshow test $p = 0.686$; discriminant accuracy rate 86.8 %

that diabetes, albumin <2.5 g/dl, A-DROP very severe, and images indicating extensive pneumonia were independent variables involved in increased mortality within 30 days.

Discussion

A new concept of NHCAP was announced by the Japanese Respiratory Society in 2011 [12]. NHCAP guidelines were formulated to properly treat pneumonia patients who are in need of home-based or long-term medical treatment or care for their everyday conditions. In comparison to CAP, NHCAP patients were older and more likely to require nursing care. The rate of poor nutrition, dementia, aspiration, and severe cases were higher in NHCAP than in CAP patients (Tables 1, 2), suggesting that patients in the NHCAP group should have received extensive medical care for their pneumonia.

The detection rate of drug-resistant bacteria was significantly higher in NHCAP compared to CAP (Table 3). It was revealed that PS, central nervous system disease, gastrostoma, a history of prior treatment with antibiotics within 90 days, and being discharged within 90 days were independent variables involved in the increased detection rate of drug-resistant bacteria in NHCAP (Table 4). Patients with NHCAP often had difficulty in expectorating sputum and in undergoing invasive tests, making it difficult to obtain good specimens. Because isolates from these patients contained indigenous bacteria from the oral cavity and colonizers of the airways, interpretation of pathogenic bacteria and their drug susceptibility was difficult. Because there was the potential for excessive antimicrobial therapy, sufficient consideration was necessary in choosing antibiotics to avoid excessive use of broad-spectrum drugs.

Bacteriological examination in this study might have three limitations: half of cases did not reveal any pathogenic bacteria, atypical pathogens were not examined elaborately, and more than one pathogenic bacteria was detected in 57 of 195 patients. In HCAP, including many aspiration pneumonia cases, anaerobe infection may be more frequent, leading to less detection of the causative bacteria [15, 16]. Even if aspiration pneumonia would have been caused by anaerobic bacteria, the drug-resistant bacteria might be that which was isolated. This possibility may have affected the result that improper antimicrobial selection did not change the survival. As for atypical pathogens, it had been reported that *Legionella* infections and atypical pathogens were uncommon [17, 18]. Therefore, meticulous effort to detect atypical pathogens might not change the results. The last limitation was the multiple isolation of pathogenic bacteria. Because we could not determine which of these caused the pneumonia, the case with at least one bacteria resistant to the antibiotics was categorized as improper initial treatment.

Previous reports of HCAP showed a significantly poorer prognosis in patients who received improper treatment compared to patients who received proper treatment [3, 4, 7, 8, 19–21]. However, we found no differences in the success rate, mortality within 30 days, and total hospital mortality between NHCAP patients who did or did not receive proper initial treatment (Table 6). The success rate was approximately 70 % with initial proper antibiotic use and approximately 80 % with initial improper treatment (Table 7). Therefore, we suspect that the drug-resistant bacteria detected in NHCAP, such as *Pseudomonas aeruginosa* and MRSA, might be colonizers, not the pathogens. Thus, in many cases SBT/ABPC was effective even when MRSA was detected in sputum. Brito et al. [22] and Ewig et al. [23] also raised similar issues. In our hospital, a medium-sized community hospital, the variety and frequency of detected bacteria might be different from those in large-scale or university hospitals, which could be the reason for high efficiency of SBT/ABPC and low importance of resistant bacteria in this study.

Mortality within 30 days and total hospital mortality were significantly higher and length of stay was significantly longer in NHCAP than in CAP (Table 8). Risk factors involved in mortality within 30 days were diabetes, albumin <2.5 g/dl, an image of extensive pneumonia, and A-DROP score indicating a "very severe" state (Table 9). This result demonstrated that the patients' nutritional conditions at baseline, diabetes, and albumin, as well as severity of the pneumonia, were important for their survival, rather than the drug susceptibility of the bacteria detected in their sputum. Yende et al. [24] also reported a high rate of mortality from pneumonia in diabetic patients.

There are several issues regarding NHCAP in this study to be resolved in the future. First, the treatment success rate was approximately 70 % even when appropriate antibiotics were used against nondrug-resistant bacteria in NHCAP,

such as *S. pneumoniae*. The prospective clinical trial of a pneumococcal vaccine demonstrated that both incidence of all pneumonia and mortality from pneumococcal pneumonia were reduced [25]. The rate of pneumococcal vaccination in Japan was about 10 %. It is hoped that vaccination will be actively promoted.

Second, a new classification of NHCAP patients based on severity of pneumonia was needed. The A-DROP system may be potentially useful in considering total hospital mortality with regard to the severity of NHCAP. However, mortality within 30 days was almost the same in the mild-to-moderate and the severe groups. Therefore, a new index of severity must include the absence or presence of diabetes, serum albumin concentration, and the range of consolidation on chest X-ray. The use of serum albumin level was also recommended by Hedlund et al. [26].

Third, caution is urged with regard to gastrostomy. The risk of aspiration pneumonia in patients with NHCAP was high, and when oral intake becomes difficult, gastrostomy may be performed. However, we suggest that gastrostomy is not recommended as a precaution against pneumonia [27]. In this study, the presence of a gastrostoma was found to increase the risk of resistant bacteria.

NHCAP, primarily occurring in the elderly requiring nursing and home-based medical care, needed special consideration for treatment. We conclude that the severity of pneumonia, rather than the risk of resistant bacteria, should be considered as well as physicians' ethical judgment and end-of-life decisions of the patients and their families in the initial treatment strategy to avoid excessive use of broad-spectrum antimicrobials.

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Conflict of interest None.

Ethical approval National Hospital Organization Tokyo Hospital Department of Clinical Research.

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