However, pathological confirmation demonstrating noncaseating epitheliod cell granulomas is essential for its definitive diagnosis [1]. So far, a transbronchial approach using a flexible bronchoscope including transbronchial lung biopsy (TBLB) and endobronchial biopsy has been the most common method for sampling specimens in patients with suspected sarcoidosis [1]. However, the yield of these conventional methods for detecting noncaseating epithelioid cell granulomas is not satisfactory, especially for stage I sarcoidosis [2–5]. In addition, it is well known that TBLB sometimes causes pneumothorax and/or bleeding. Since the advent of ultrasound endoscopes which make it possible to perform transesophageal endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) and ultrasound bronchoscopes enabling endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA), many investigators have reported the usefulness of these procedures for diagnosing benign as well as malignant hilar/mediastinal lesions [6, 7]. The reported diagnostic yields of EUS-FNA and EBUS-TBNA for sarcoidosis demonstrating epithelioid cell granulomas are excellent, ranging from 82 to 100 [8–12] and from 71 to 94% [2, 4, 5, 12–16], respectively. Furthermore, these procedures rarely cause complications.

Recently, several investigators [17–22] suggested that needle aspiration procedure through the esophagus can be performed using an US bronchoscope instead of an US endoscope. The procedure may be preferable to EUS-FNA for some pulmonologists because it obviates the need for an ultrasound endoscope and an experienced endoscopist. The aim of this study was to evaluate the diagnostic accuracy, feasibility and safety of transesophageal endoscopic ultrasound with bronchoscope-guided fine needle aspiration (EUS-B-FNA) in patients with stage I/II sarcoidosis.

Patients and Methods

Patients

We carried out a prospective study which was approved by the institutional review board of Nagoya Medical Center (identifier 2009-254) and registered with the University Hospital Medical Information Network-Clinical Trials Registry (identifier UMIN000002883). From January 2010 until February 2011, a total of 33 patients with suspected stage I (bilateral hilar and/or mediastinal lymphadenopathy) or stage II (bilateral hilar and/or mediastinal lymphadenopathy and parenchymal abnormalities) sarcoidosis in the presence of a compatible clinical and radiological picture were enrolled in the study. Patients with already pathologically diagnosed sarcoidosis were excluded. All patients gave written informed consent for their participation.

The primary outcome of this study was to evaluate the pathological diagnostic yield of EUS-B-FNA for stage I and II sarcoidosis. The secondary outcome was to assess the safety and feasibility of this procedure.

Procedures

EUS-B-FNA was performed using a 7.5-MHz curvilinear scanning ultrasound bronchoscope (BF-UC260F-OL8; Olympus, Tokyo, Japan) connected to an ultrasound processor (EU-C2000; Olympus). The procedure was performed at the left lateral position, which is similar to that during esophagogastroduodenoscopy including EUS-FNA to prevent aspiration, under conscious sedation using midazolam by staff pulmonologists. After anesthetizing the upper airway with lidocaine, an ultrasound bronchoscope was inserted and advanced through the esophagus while examining the structure around the esophagus by ultrasound. Once the target lesion was identified, it was punctured through the esophagus with a 21-gauge needle under real-time ultrasound guidance. After that, the needle was manipulated back and forward within the lesion, applying suction by confirming the ultrasound image, and then retracted to collect the aspirated specimens.

Handling of sampled specimens was done in the same manner as in the EBUS-TBNA procedures we previously described [23]. The aspirated specimen in the needle was pushed out by a stylet and then expelled by blowing air through a syringe onto a glass slide. The visible tissue fragments on the glass slide were then collected and transferred into containers filled with formalin for histologic examination, and the remaining specimen on the glass slide was immediately smeared and fixed in 95% alcohol for cytologic examination. The remaining specimen stored at the lumen of the needle and catheter was then washed and flushed into saline for culture for microbiological analysis. The pathological specimens obtained by EUS-B-FNA in the containers labeled with the lymph node station were then submitted to the pathologist for interpretation. On-site cytologic evaluation was not used. Decision on the number of punctures or target lesions depended on the examiner. If oxygen saturation decreased to less than 90% for more than 20 s during the procedure, oxygen supplementation was provided to maintain oxygen saturation at more than 90%. The lymph node location examined [24], the number of punctures, supplemental oxygen administration and the time of the procedure measured were recorded.

Diagnosis

Each histologic and cytologic specimen was interpreted separately as 'positive', 'suspicious' or 'negative' for sarcoidosis with noncaseating epithelioid cell granulomas by an experienced pathologist. 'Suspicious' findings were analyzed as negative in this study. The final diagnosis of sarcoidosis was based on the clinical and radiological compatibility at the time of the procedures and during the clinical follow-up period as well as on the pathological findings of noncaseating granulomas and on exclusion of other causes of granulomas.

Data Analysis

EUS-B-FNA may be promising in terms of less invasiveness compared to TBLB, which is the standard method, because the procedure is rarely associated with complications including bleeding and pneumothorax. We estimated that 30 patients would

be required, calculated under the following conditions: alternative diagnostic yield 85% [8–12], null diagnostic yield 65% [25] and a statistical power of 90% at a one-sided significance level of 0.1. We arranged to enroll 33 patients to compensate for a dropout rate of 10%.

Statistical analyses were performed using a statistical software program (PASW Statistics 18; SPSS Inc., Chicago, Ill., USA). Means and percentages were presented as appropriate.

Results

Patients and Procedures

Characteristics of patients and lesions are shown in table 1. Among the 33 patients enrolled in this study, 18 patients had suspected stage I and 15 patients suspected stage II sarcoidosis. EUS-B-FNA was performed for 62 lymph nodes (mean shortest diameter on chest computed tomography 13.6 mm) with a mean of 3.3 punctures per lesion in 32 patients (97%). EUS-B-FNA was not performed in the remaining patient because a puncturable lymph node could not be detected by ultrasound. Temporary supplemental oxygen was administered in 2 patients (6%). All patients tolerated the procedure well and no events such as cough, vomiting or dyspnea prevented continuation of the procedure. The median procedure time was 22.5 min (range 12.8–40.5).

A diagnostic flow chart is shown in figure 1. EUS-B-FNA showed noncaseating epithelioid cell granulomas in 25 patients, and all patients were judged to have sarcoidosis from the clinicoradiological compatibility (fig. 2). Three patients without definitive pathological findings by EUS-B-FNA underwent EBUS-TBNA afterwards. EBUS-TBNA provided a positive result in 2 patients and a suspicious result in 1 patient. One patient with suspicious pathological findings for sarcoidosis by EUS-B-FNA with clinicoradiological compatibility was judged to have sarcoidosis. The remaining 4 patients had other diseases – tuberculosis in 1 (fig. 3), lung cancer in 1 and nonspecific lymphadenitis in 2 subjects. Thus, a total of 29 patients were given a final diagnosis of sarcoidosis. Overall, EUS-B-FNA provided diagnostic materials in 27 of 33 patients (82%).

Diagnostic Yield in Patients with Sarcoidosis

EUS-B-FNA confirmed a diagnosis of sarcoidosis by providing specimens with noncaseating epithelioid cell granulomas in 14 of 17 patients (82%) with stage I sarcoidosis, in 11 of 12 patients (92%) with stage II sarcoidosis and overall in 25 of 29 patients (86%; 95% confidence interval 73–100). There was no significant difference in the diagnostic yield of EUS-B-FNA between stage I and stage II sarcoidosis ($p = 0.44$). The sensitivity, negative predictive value and accuracy of EUS-B-FNA for pathological diagnosis of sarcoidosis was 86, 50 and 88%, respectively. Cytologic and histologic specimens contained diagnostic material in 23 of 29 patients (79%) and in 24 of 29 patients (83%), respectively. Among 55 nodal lesions examined in the 29 patients with a final diagnosis of sarcoidosis, noncaseating epithelioid cell granulomas were demonstrated in 44 lesions (80%) by EUS-B-FNA.

No complications were associated with EUS-B-FNA.

Table 1. Characteristics of patients and lesions

Characteristics	Data
Patients	33
Mean age ± SD, years	51.5 ± 18.5 (25–82)
Males/females	18/15
Chest radiographic staging	
Stage I/II	18/15
Serum angiotensin-converting enzyme	
>21.4/≤21.4 U/l	8/25
Bronchoscopy	
Initial bronchoscopy/previous non-diagnostic bronchoscopy	29/4
Total LN number evaluated by EUS-B-FNA	62
Shortest LN diameter, mm	13.6 (6.8–28.7)
Mean number of punctures per lesion	3.3 (1–7)
LN location for EUS-B-FNA	
2L	1
3p	1
4L	22
4R	1
7	30
8	3
10L	4
Median follow-up duration, months	17 (1–26)

Figures in parentheses are ranges. LN = Lymph node.

Discussion

Our study demonstrated a high diagnostic yield (86%) of EUS-B-FNA demonstrating noncaseating epithelioid granulomas in patients with suspected stage I/II sarcoidosis. In addition, mediastinal observation through the esophagus using an ultrasound bronchoscope could be performed in all 33 patients and specimens could be sampled by pulmonologists in 32 patients without any complications. To our knowledge, this is the first study

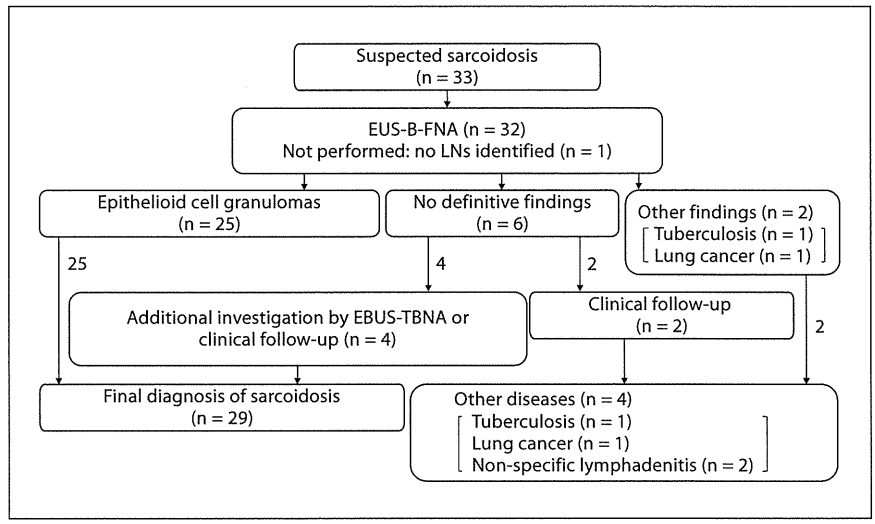

Fig. 1. Diagnostic flow chart. LNs = Lymph nodes.

to evaluate the utility of EUS-B-FNA for the pathological diagnosis of sarcoidosis.

For this kind of diagnosis, the specimens must be obtained from the most accessible organ by means of the least invasive procedure. Because the lungs are involved in most cases, the most common procedure so far has been TBLB through a flexible bronchoscope, which is a widespread procedure for sampling specimens from the lungs. However, the diagnostic yield of TBLB, which was reportedly 58% for stage I and 75% for stage II sarcoidosis [25], is not satisfactorily high. Additionally, the procedure is occasionally associated with pneumothorax (incidence of 1–5%) and bleeding (incidence of 9%) [25]. EUS-FNA and EBUS-TBNA, which are newer methods for diagnosing sarcoidosis, surpass TBLB in accuracy and safety [2, 4, 5]. Although sarcoidosis is a benign disease, cytologic diagnosis is highly reliable [26]. Thus, needle aspiration procedures, which can sample both histologic and cytologic specimens, seem to be favorable. Although our study was not designed to be compared with TBLB directly, the diagnostic yield of EUS-B-FNA (86%; 95% confidence interval 73–100) in detection of noncaseating epithelioid cell granulomas seems to be higher than that of TBLB. Furthermore, EUS-B-FNA seems advantageous in terms of less invasiveness.

EUS-FNA introduced in the early 1990s [27] has made it possible to explore lesions around the esophagus. The procedure has been reported to be a safe and accurate method for the diagnosis of hilar/mediastinal benign as well as malignant lesions [6]. Although EUS-FNA is undoubtedly useful, it has some limitations in that it requires a skilled endoscopist and dedicated equipment including an ultrasound endoscope and needles. Ultrasound bronchoscopes have a mechanism similar to ultrasound endoscopes. Thus, the needle aspiration procedure through the esophagus can be performed with ultrasound bronchoscopes as well as with ultrasound endoscopes. EUS-B-FNA overcomes the limitations of EUS-FNA which require a skilled endoscopist and an ultrasound endoscope; several investigators have demonstrated the feasibility, safety and effectiveness of the procedure for diagnosing benign [21] and malignant diseases [17–20, 22]. Hwangbo et al. [19] reported the effectiveness of adding EUS-B-FNA to EBUS-TBNA in the mediastinal staging of lung cancer. The sensitivity, negative predictive value and accuracy increased from 84 to 91, 93 to 96 and 95 to 97% by adding EUS-B-FNA to EBUS-TBNA, respectively. Furthermore, no complication associated with EUS-B-FNA was observed in their study. Herth et al. [20] also demonstrated that the combination of EUS-B-FNA and EBUS-TBNA increased the diagnostic accuracy compared with each method alone, without any complications in the mediastinal staging of lung cancer.

EUS-FNA has been reported useful in the diagnosis of sarcoidosis. The reported diagnostic yield for sarcoidosis by detecting noncaseating epithelioid cell granulomas ranged from 82 to 100% [8–12]. The diagnostic yield of 86% with EUS-B-FNA in our study seems comparable to that of EUS-FNA. EUS-B-FNA may have another advantage over EUS-FNA in that the primary physician, the pulmonologist (but not the gastroenterologist), who

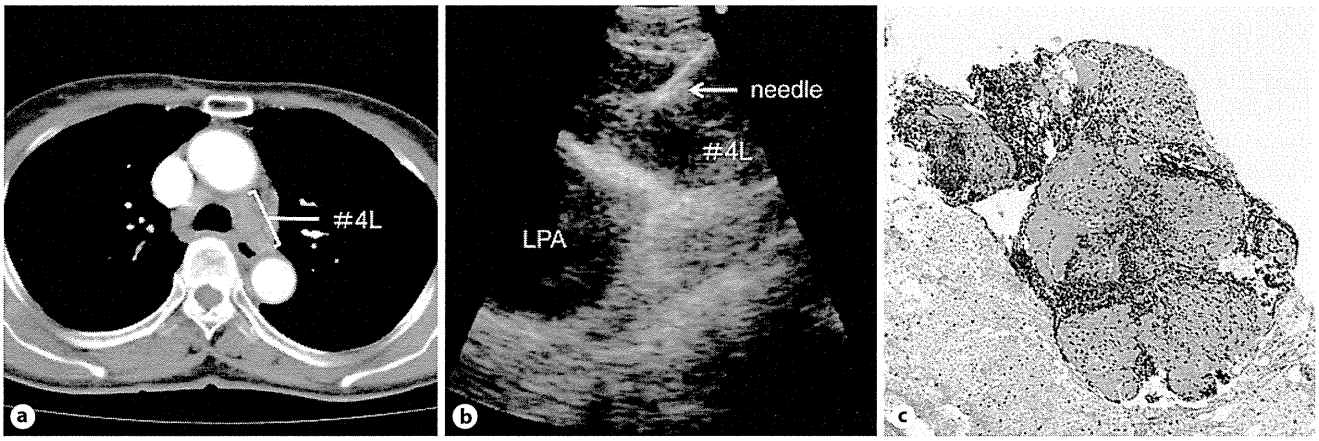

Fig. 2. Transesophageal EUS-B-FNA for the left lower paratracheal lymph node. **a** Enhanced computed tomographic image. **b** Ultrasound image. **c** Specimen obtained by EUS-B-FNA from the left lower paratracheal lymph node (#4L) containing noncaseating epithelioid cell granulomas suggestive of sarcoidosis. Hematoxylin and eosin. ×100. LPA = Left pulmonary artery.

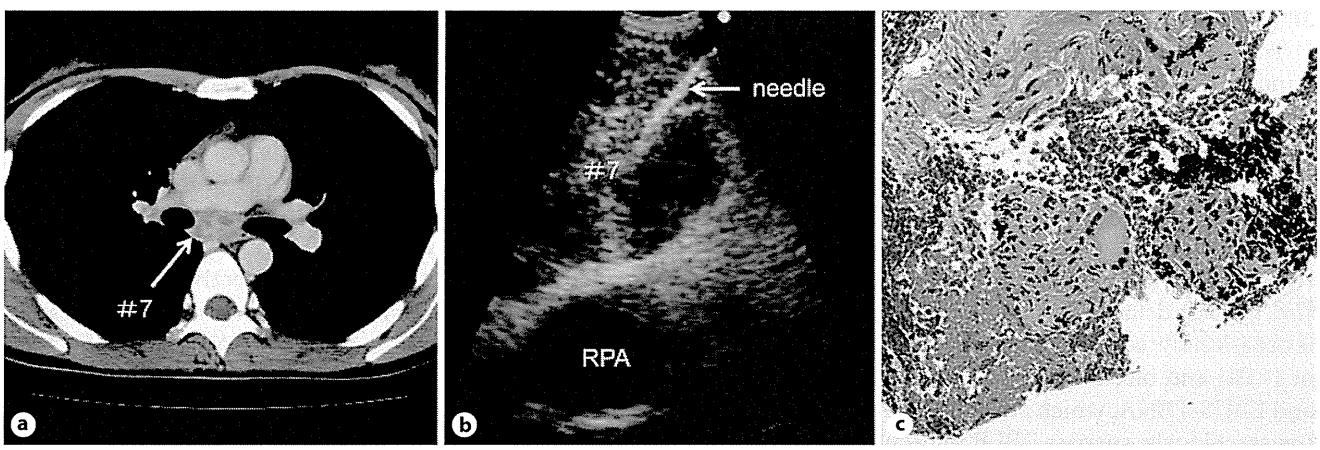

Fig. 3. Transesophageal EUS-B-FNA for subcarinal lymph node. **a** Enhanced computed tomographic image. **b** Ultrasound image. **c** Specimen obtained by EUS-B-FNA for subcarinal lymph node (#7) showing necrotizing epithelioid cell granulomas with a Langhans-type giant cell suggestive of tuberculosis. Hematoxylin and eosin. ×200. RPA = Right pulmonary artery.

makes the management decision for patients with sarcoidosis as well as many other diseases involving mediastinal lymph nodes, may be able to perform the procedure [28]. In addition, the procedure using an ultrasound bronchoscope, which has only half the outer diameter of an ultrasound endoscope, is likely more tolerable for patients. This procedure cannot replace EUS-FNA completely because ultrasound endoscopes have distinct advantages over ultrasound bronchoscopes, such as a larger working channel which enables the use of needles of various size and length, a wider ultrasonic scanning range, as well as better visibility and adjustability of the protruding needle angle, all of which may improve the diagnostic accuracy. However, EUS-B-FNA, which provided satisfactory results in our study with a simple procedure that can be performed by pulmonologists for diagnosing sarcoidosis, seems particularly promising.

EBUS-TBNA is another useful method for the diagnosis of sarcoidosis [2, 4, 5, 12–16, 29]. The yield of the procedure for the detection of noncaseating epithelioid cell granulomas of sarcoidosis has been reported to range from 71 to 94% [2, 4, 5, 12–16], and several researchers have indicated that the diagnostic yield of EBUS-TBNA was higher than the yield of conventional methods such as TBLB [2, 4, 5] and TBNA without ultrasound guidance [16]. The advantage of EBUS-TBNA over EUS-B-FNA or EUS-FNA in the diagnosis of sarcoidois is the higher accessibility to the mediastinal and hilar lymph nodes [19]. Lymph nodes that are located at the right paratracheal and hilar lesions, which are commonly involved with sarcoidosis and difficult to be biopsied by the esophageal approach, can be readily sampled by EBUS-TBNA. However, most patients with stage I/II sarcoidosis have multiple lymph node involvement, including subcarinal lymph nodes and left paratracheal lymph nodes which can be sampled easily with the transesophageal approach. EUS-B-FNA may have the advantage of greater tolerability for patients. Some specialists [30, 31] have insisted that EUS-FNA has better tolerability than EBUS-TBNA. In clinical practice, we occasionally encounter patients with severe cough during the EBUS-TBNA [5], which is likely to occur less frequently during EUS-B-FNA. Furthermore, oxygen desaturation in 6% of the cases during EUS-B-FNA in the current study appears to be less frequent than during bronchoscopy [32]. Although a certain experience and skill may be necessary to obtain satisfactory specimens, the EUS-B-FNA procedure does not seem so difficult for pulmonologists who are familiar with handling ultrasound bronchoscopes. Our study indicates that EUS-B-FNA is sensitive enough for use as the sole diagnostic tool in the pathological diagnosis of sarcoidosis. Thus, this procedure, as well as EUS-FNA or EBUS-TBNA, may be the procedure of choice for experienced examiners. Further comparative study with EBUS-TBNA or EUS-FNA with the focus on diagnostic accuracy and patient tolerability may be required.

In conclusion, our findings suggest that EUS-B-FNA is feasible, safe and accurate for the diagnosis of stage I/II sarcoidosis in detecting noncaseating epitheloid cell granulomas. This procedure may be a useful alternative to conventional EUS-FNA or EBUS-TBNA.

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Financial Disclosure and Conflicts of Interest

The authors have no conflicts of interest to disclose.

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Histology and Smoking Status Predict Survival of Patients with Advanced Non–Small-Cell Lung Cancer

Results of West Japan Oncology Group (WJOG) Study 3906L

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Introduction: Smoking status is one of the prognostic factors in advanced non–small-cell lung cancer (NSCLC). Currently, adenocarcinoma (Ad) histology is considered a predictive factor in advanced NSCLC. We investigated the correlation between histology or smoking status and survival of NSCLC patients receiving chemotherapy.

Methods: We retrospectively reviewed clinical data from stage IIIB or IV NSCLC patients who started first-line chemotherapy at affiliated institutions of West Japan Oncology Group from 2004 to 2005. We also collected information on pack-years of cigarette smoking and years since cessation. Overall survival was compared using log-rank test, and Cox regression analysis was used to identify independent prognostic factors.

Results: In total, 2542 consecutive patients were enrolled at 40 institutions. Of those, 71 were excluded because of unknown smoking history. The median overall survival of nonsmoking Ad patients (593 days) was longer than that of smoking Ad, nonsmoking non-Ad, and smoking non-Ad patients (384, 374, and 319 days, respectively; $p < 0.001$). In Cox regression with sex, age, stage, performance, and treatment as covariates, we found significant interaction ($p = 0.039$) between histology (Ad/non-Ad) and smoking status (smoker/nonsmoker); smoking conferred a hazard ratio of 1.34 (95% confidence interval, 1.15–1.55) in Ad, but only 0.99 (0.75–1.31) in non-Ad. Higher pack-years and shorter period since cessation were significantly associated with poorer survival in Ad ($p < 0.001$), but not in non-Ad ($p \geq 0.434$).

Conclusion: Ad histology is associated with better prognosis, and only smoking status had a prognostic impact in Ad.

Key Words: Non–small-cell lung cancer, Histology, Adenocarcinoma, Smoking status.

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This study is registered with University Hospital Medical Information Network-Clinical Trial Registry (UMIN-CTR) (http://www.umin.ac.jp/ctr/index.htm umin.ac.jp/ctr; identification number UMIN000001263).

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Lung cancer is the leading cause of cancer-related mortality in Japan, and the rest of the world, with more than one million people dying from it each year. Non–small-cell lung cancer (NSCLC), which accounts for nearly 80% of all lung cancers, comprises several histological types, including adenocarcinoma (Ad), squamous cell carcinoma (Sq), and large-cell carcinoma (La). NSCLC had been treated as a single disease because of similar therapeutic effects of conventional chemotherapeutic agents. In the last few decades, however, treatment with new drugs, such as epidermal

growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs), bevacizumab, and pemetrexed revealed that tumor histology has profound impact on the benefits of a variety of chemotherapy or targeted-therapy regimens for advanced NSCLC.[1–4] Thus, histology came to be considered a predictive factor for the effectiveness of specific chemotherapy in patients with advanced NSCLC. However, there is no previous report on histology as a prognostic factor, that is, a variable determining survival irrespective of the chemotherapy regimen administered.

Previous studies showed that cigarette smoking is an independent prognostic factor in patients with NSCLC,[2,5–7] but a dose–response relationship between the quantity of smoking and survival has not been established. Although Yelena et al.[6] noted that patients who had smoked up to 15 pack-years had a longer survival than those with more than a 15 pack-year history, other cutoff points for the amount of cigarette smoking have not been considered. In addition, the relationship between smoking and survival was not investigated with respect to differences in NSCLC histological subtypes, and the studies that did evaluate survival in Sq versus non-Sq patients did not reach a firm conclusion.[7,8] However, Kawaguchi et al.[8] showed that Ad had better prognosis than Sq in never-smokers, but not in ever-smokers, suggesting that the prognostic impact of cigarette smoking may differ among histologic subtypes in NSCLC.

We hypothesized that Ad histology and lower smoking status would result in better overall survival (OS) in advanced NSCLC. To test this hypothesis, we investigated the impact and possible interaction of histology and smoking status on survival of advanced NSCLC patients receiving chemotherapy in the clinic.

PATIENTS AND METHODS

Study Patients

We sent case report forms to 40 affiliated institutions of West Japan Oncology Group, and requested them to provide demographic and clinical data from medical records for all patients with stage IIIB or IV NSCLC, who started first-line systemic chemotherapy between January 1, 2004 and December 31, 2005. Patients who had a relapse after surgery or radiotherapy were excluded. The case report forms were submitted by the participating institutions during the period from September 2008 to January 2009. This study was approved by the institutional review board of each participating institution.

Demographic and Clinical Variables

We obtained the following baseline demographic and clinical information from the case report forms: age, sex, histology, disease stage, Eastern Cooperative Oncology Group performance status (PS), smoking status, type of first-line chemotherapy, number of treatment regimens, and the year in which first-line chemotherapy was started. Disease stage was determined according to the tumor, node, metastasis system.[9] Staging classification was performed by physical examination, chest–abdominal computed tomography, brain magnetic resonance imaging, bone scan, and positron emission tomography if necessary. Patients were categorized into nonsmokers and smokers according to smoking status. Nonsmokers were defined as those who had smoked less than 100 cigarettes. Among smokers, exsmokers were defined as those who had quit smoking 1 year or more before diagnosis, and current smokers as those who continued their smoking habit at diagnosis. Pack-years of smoking were calculated by multiplying the number of packs (20 cigarettes in one pack) smoked per day by the number of years smoked, and categorized as less than 10, 10 to 19, 20 to 29, 30 to 39, 40 to 49, 50 to 59, and 60 or more. Years of smoking cessation were categorized as 1 to 4, 5 to 9, 10 to 14, 15 to 19, and 20 or more. Type of first-line chemotherapy was categorized into platinum-based combination, nonplatinum combination, and single-agent chemotherapy. Because the only approved EGFR-TKI for the treatment of inoperable or recurrent NSCLC in Japan before October 2007 was gefitinib, we collected information on gefitinib usage during the observation period and noted the starting day of gefitinib treatment. OS was calculated from the start of first-line chemotherapy to the date of death. Patients still alive were censored as of the last known follow-up.

TABLE 1. Patient Characteristics

Parameter	Ad (n = 1731)	Non-Ad (n = 740)	p
Men/women	1056/675	641/99	<0.001
Smoking status			<0.001
Nonsmoker	659	79	
Exsmoker	300	165	
Current smoker	772	496	
Stage IIIB/IV	444/1287	271/469	<0.001
PS			0.002
0	546	206	
1	873	402	
2	191	96	
3	90	25	
4	31	11	
Histology			—
Sq	—	516	
La	—	71	
Others	—	153	
Chemotherapy			0.181
Single-agent	354	137	
P doublet	1306	571	
Non-P doublet	71	32	
Regimen			<0.001
1	536	285	
2	445	201	
3	322	115	
≥4	428	139	
Gefitinib Y/N	959/772	146/594	<0.001

Ad, adenocarcinoma; PS, performance status; Sq, squamous cell; La, large cell; P, platinum; Y, yes; N, no.

Statistical Analysis

Demographic and clinical variables were compared among groups according to lung cancer histology, using the χ^2 test. The primary endpoint of this study was OS. Survival curves were calculated by the Kaplan–Meier method and compared using the log-rank test. Prognostic importance of histology and smoking status were analyzed using the Cox regression analysis adjusted for sex, age, disease stage, PS, type of first-line chemotherapy, and the year in which first-line chemotherapy was started. For detection of possible interaction between histology and smoking status, the terms of interaction of the two variables were evaluated by the likelihood ratio test. Because gefitinib was the preferred choice in patients with Ad, another Cox regression analysis was performed, in which patients were censored at the start of gefitinib administration, and the results were compared with the original Cox analysis. Significance level was set at a p value of 0.05. Statistical analyses were performed with SAS version 9.2 software (SAS Institute, Cary, NC).

RESULTS

Between January 1, 2004 and December 31, 2005, 2542 consecutively treated patients were enrolled at 40 institutions. Of these, 71 were excluded because of unknown smoking history. The characteristics of the study population, categorized into Ad and non-Ad, are listed in Table 1. There were 1731 Ad and 740 non-Ad patients (29.9% and 70.1%, respectively). Among them, we confirmed 1346 and 599 deaths in Ad and non-Ad patients, respectively. There were significantly more women (39.0% in Ad versus 13.4% in non-Ad) and nonsmokers (38.1% in Ad versus 10.7% in non-Ad) in the Ad group than in the non-Ad group. Patients who received single-agent chemotherapy accounted for approximately 20% of the study population. Compared with combination regimens, single-agent chemotherapy was associated with old age (63.6 years for combination regimens versus 71.1 years for single-agent chemotherapy), high proportions of female patients (29.3% versus 40.0%), nonsmokers (27.8% versus 34.0%), stage IV (69.4% versus 78.3%), and PS 0 to 1 (60.9% versus 87.1%). The proportion of Ad histology was not significantly different between single-agent and combination regimens (72.1% and 69.5%, respectively). The OS was 464 days in Ad compared with 326 days in non-Ad ($p < 0.001$; Fig. 1*A*). Between Ad and non-Ad, which was divided into Sq and La, Ad had significantly better survival than the other two histological groups (Sq, 341 days; La, 254 days; $p < 0.0001$; Fig. 1*B*). With regard

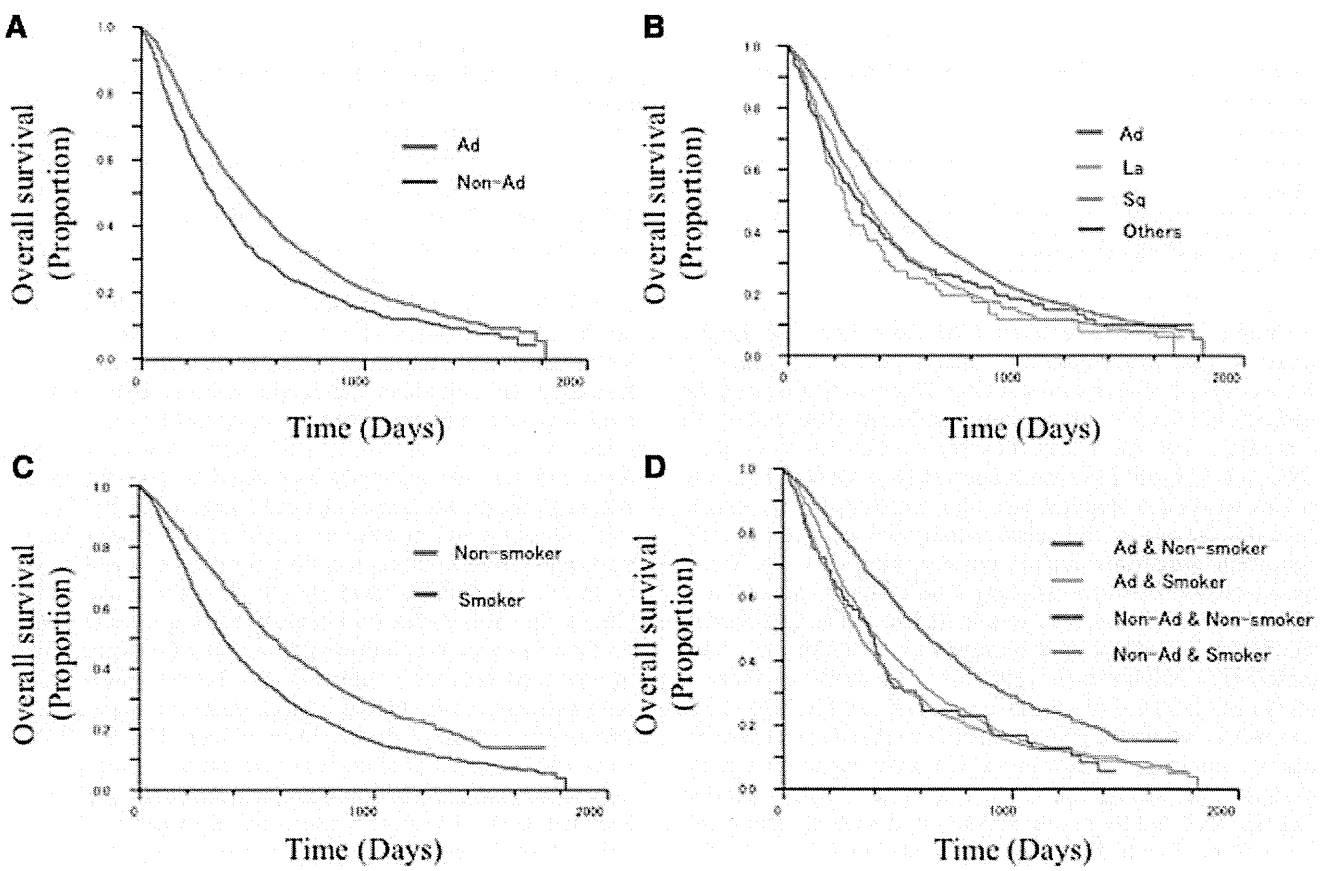

FIGURE 1. Kaplan–Meier plots of overall survival for patients classified according to histology type as (*A*) Ad and Non-Ad; histologic subtype as (*B*) Ad, La, Sq, and others; smoking status as (*C*) smokers and nonsmokers; and combination of smoking status and histology as (*D*) Ad and nonsmoker, Ad and smoker, Non-Ad and nonsmoker, and Non-Ad and smoker. Ad, adenocarcinoma; La, large cell; Sq, squamous cell.

TABLE 2. Survival Analysis by Cox Proportional Hazards Model (n = 2471)

Parameter	HR	95% CI	p
Sex			
Women	1		
Men	1.342	1.168–1.541	<0.001
Age yrs	1.007	1.002–1.012	0.005
Smoking status			
Nonsmoker	1		
Exsmoker	1.178	0.997–1.391	0.054
Current smoker	1.335	1.155–1.543	<0.001
Clinical stage			
Stage IIIB	1		
Stage IV	1.505	1.358–1.669	<0.001
PS			
0	1		
1	1.609	1.446–1.790	<0.001
2	2.229	1.910–2.601	<0.001
3	3.048	2.455–3.785	<0.001
4	5.487	3.864–7.790	<0.001
Histology			
Ad	1		
Sq	1.143	1.015–1.286	0.028
La	1.542	1.182–2.011	0.001
Others	1.397	1.159–1.683	<0.001
Chemotherapy			
Single-agent	1		
Non-P doublet	0.842	0.657–1.080	0.175
P doublet	0.793	0.699–0.899	<0.001

HR, hazard ratio, CI, confidence interval; PS, performance status; Ad, adenocarcinoma; Sq, squamous cell; La, large cell; P, platinum.

to smoking status, nonsmokers (568 days) had significantly longer survival than smokers (358 days; $p < 0.0001$; Fig. 1*C*). In a combined analysis of smoking status and histology, the median OS of Ad in nonsmokers was longer than that of Ad in smokers, non-Ad in nonsmokers, and non-Ad in smokers (593, 384, 374, and 319 days, respectively; $p < 0.001$; Fig. 1*D*). In Cox regression analysis, sex, age, smoking status, disease stage, PS, histology, and chemotherapy showed a statistically significant prognostic impact on survival (Table 2). When the interaction between histology (Ad/non-Ad) and smoking status (smoker/nonsmoker) was included in the Cox model, significant interaction was observed ($p = 0.039$); smoking conferred a hazard ratio (HR) of 1.34 (95% confidence interval [CI], 1.15–1.55) in Ad, in contrast to 0.99 (0.75–1.31) in non-Ad. In detailed analyses that excluded the 104 patients (current smokers, 89; unknown, 15) with unknown amount of cigarette smoking, shorter period since cessation showed a significant trend for poorer survival in the whole population ($p < 0.001$). This trend was also observed in Ad ($p < 0.001$; Table 3), but not in non-Ad ($p \geq 0.434$; Table 3). When non-Ad patients were divided into Sq and La or others, the trend p was 0.534 in Sq and 0.165 in La or others. The prognosis became significantly worse with higher pack-years of cigarette smoking in the whole population and Ad ($p < 0.001$; Table 3), but no significance was not achieved for the non-Ad group ($p = 0.519$; Table 3). When non-Ad patients were divided into Sq and La or others, the trend p was 0.798 in Sq and 0.380 in La or others. The prognostic impact of histology and smoking status remained significant in the Cox regression analysis, in which patients were censored at the start of gefitinib administration; positive smoking history, Sq histology, and La or other histology conferred an HR of 1.51 (95% CI, 1.21–1.88), 1.22 (95% CI, 1.06–1.41), and 1.59 (95% CI, 1.32–1.93), respectively. The negative prognostic impact of shorter period since cessation and pack-years of cigarette smoking was also essentially unchanged ($p < 0.001$ in both).

DISCUSSION

The consensus report of prognostic factors in NSCLC at the 1990 International Association for the Study of Lung Cancer Workshop showed that histology was not a prognostic factor for advanced NSCLC.[10] Our study is the first report to reveal that histology is a significant prognostic factor for advanced NSCLC. Importantly, we showed that Ad patients have the longest survival of all three histological groups (Ad, Sq, and La). Ad is the most common histological subtype of lung cancer in nonsmokers,[11] who have been reported to have a better prognosis than smokers.[12–14]

Smoking has been described as a prognostic factor in lung cancer. Although multiple studies have demonstrated the negative effects of smoking in patients with NSCLC, most included a heterogeneous population comprising patients with all stages and types of lung cancer.[5] In contrast, our study cohort consisted exclusively of patients with advanced NSCLC treated with first-line chemotherapy. We showed that smoking status is an independent prognostic factor for survival in those patients. Similar data have been shown in former studies.[2,5] However, those reports did not show whether smoking conferred any survival impact for advanced NSCLC irrespective of histological subtypes. In our study, only Ad histology had significant interaction with smoking status or smoking index and prognosis. A higher level of smoking was related to shorter survival in Ad patients, whereas smoking level and survival were not associated in non-Ad patients. Although the proportion of non-Ad patients was 29.9% of the total, the observed number of deaths in this study yielded a statistical power of more than 80% for detecting an HR of 1.5 at the 5% significance level in both Ad and non-Ad patients. Others have found that Ad histology is a significant prognostic factor in separate multivariate analysis for never-smokers in advanced NSCLC.[8] Yelena et al.[6] showed that high cigarette smoking, as measured in pack-years, is associated with decreased survival after diagnosis of stage IIIB/IV NSCLC. However, the patients of that study received a wide variety of therapies, raising the possibility that the outcomes might have been the result of distinct therapeutic responses. Although we only assessed the prognostic value of smoking status at diagnosis, assessment of smoking status at a later point, that is, at the time of treatment, would also have been of interest to determine whether cessation at the time of diagnosis leads to improved survival.

TABLE 3. Hazard Ratios According to Quantitative Aspects of Smoking

	Ad			Non-Ad		
	HR	95% CI	*p*	HR	95% CI	*p*
Years after cessation	(*n* = 1731)			(*n* = 740)		
Current	1.492	1.271–1.750	<0.001	1.204	0.849–1.707	0.297
Exsmoker 1–4 yr	1.438	1.114–1.857	0.005	1.101	0.733–1.653	0.643
Exsmoker 5–9 yr	1.549	1.101–2.180	0.012	1.228	0.700–2.155	0.474
Exsmoker 10–14 yr	1.127	0.783–1.621	0.520	1.235	0.680–2.245	0.488
Exsmoker 15–19 yr	1.199	0.761–1.890	0.433	1.410	0.712–2.794	0.325
Exsmoker ≥20 yr	0.873	0.834–1.203	0.407	1.103	0.662–1.837	0.706
Trend *p*	<0.001			0.434		
Pack-yr	(*n* = 1665)			(*n* = 702)		
<10	1.267	0.899–1.785	0.176	1.196	0.535–2.672	0.662
10–19	1.118	0.801–1.561	0.513	0.963	0.512–1.812	0.908
20–29	1.346	1.048–1.729	0.020	1.368	0.887–2.109	0.157
30–39	1.345	1.071–1.689	0.011	0.954	0.624–1.458	0.827
40–49	1.370	1.096–1.712	0.006	1.128	0.763–1.669	0.546
50–59	1.483	1.164–1.890	0.001	1.238	0.828–1.851	0.298
≥60	1.595	1.312–1.939	<0.001	1.135	0.791–1.628	0.491
Trend *p*	<0.001			0.519		

[a] Nonsmokers were set as the reference category.
Ad, adenocarcinoma; HR, hazard ratio; CI, confidence interval.

In agreement with the findings of another study,[15] we also found that a large proportion of Ad patients were nonsmoking. The prognostic difference between Ad in never-smokers and smokers may suggest that both are different disease entities. Of note, tumor-mutational frequencies and spectra suggest differences between smokers and nonsmokers.[16,17] However, significant differences in the frequency of somatic mutations in oncogenes such as *EGFR* and *KRAS* have been observed between smoking and nonsmoking lung cancer patients.[11] *EGFR* mutations, clinical predictors of EGFR-TKI therapeutic benefits, are more frequently found in nonsmoking Ad patients.[11] In another study, *EGFR* mutations were identified in nonsmokers (51%), former smokers (19%), and current smokers (4%).[18] Moreover, the incidence of *EGFR* mutations decreased with increasing number of pack-years of cigarette smoking.[18] However, *KRAS* mutations, predicting poor survival and resistance to EGFR-TKI, are more frequently found in smoking Ad patients. Interestingly, *EGFR* and *KRAS* mutations are mutually exclusive.[11]

Currently, therapeutic options other than EGFR-TKIs (e.g., bevacizumab and pemetrexed) are available in Japan. Still, NSCLC subtypes have been showing variable response rates and adverse events.[2,4,19,20] Non-Sq histology, especially Ad, is currently the NSCLC subtype with broader and more efficacious treatment options. At the time of this study, however, the only approved therapeutic agent for NSCLC in Japan was gefitinib. Unfortunately, we did not investigate *EGFR* mutation status. However, genetic background could possibly predict response to gefitinib. Along with its retrospective nature, this was a limitation of our study. However, we found that the treatment choice was made on the basis of clinical background, and we were unable to conclude whether or not gefitinib contributed to better survival under unknown *EGFR* mutation status. Hence, we suggest that decision-making based on clinical information alone is inappropriate. Both the V15-32 study[21] and the Iressa Survival Evaluation in Lung Cancer (ISEL) study[22], support our observations. Furthermore, the IRESSA Pan-Asia Study (IPASS) study,[23] conducted under the hypothesis that EGFR-TKI would be effective in clinically selected patients, confirmed the strong predictive value of *EGFR* mutations for the response of Ad to gefitinib.

This retrospective study has a few other limitations as well. First, information on smoking was not obtained from the interview or the self-administered questionnaire. Smoking data can be inaccurate, particularly when collected retrospectively. Second, we did not collect data on the procedures for histological diagnosis. The basis for pathological diagnosis is important because cytological assessment alone may lead to underdiagnosis of specific histologic types.

In conclusion, this survey demonstrated that Ad histology is associated with better prognosis, and that smoking status has a prognostic impact only in patients with Ad.

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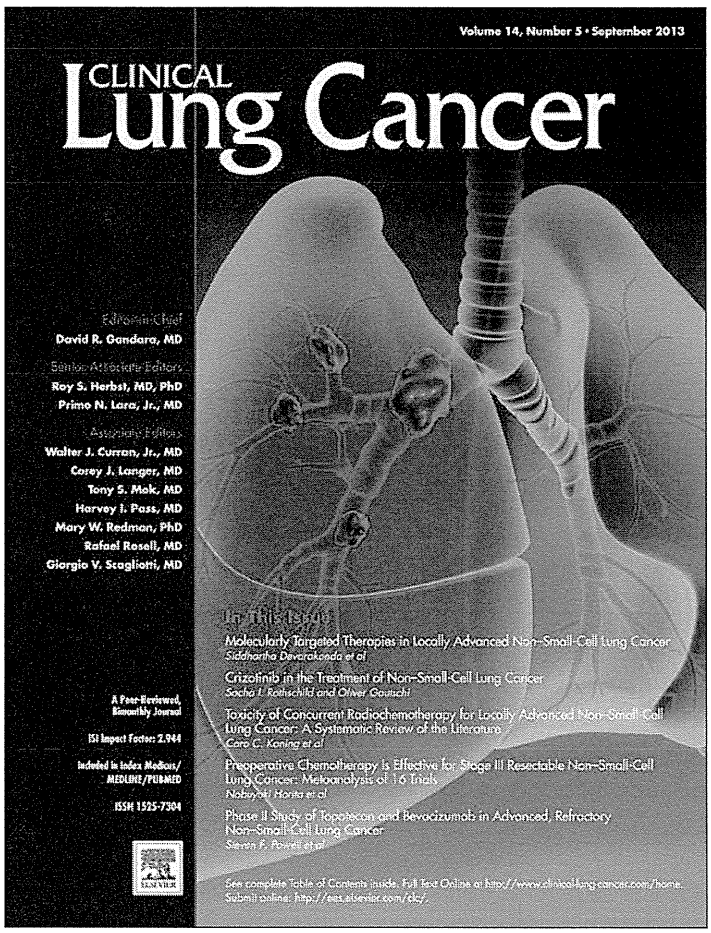

Current Trial Report

Rationale and Design of the Japan Molecular Epidemiology for Lung Cancer Study

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Abstract

We present the rationale for the Japan Molecular Epidemiology for Lung Cancer study designed to elucidate molecular mechanisms of carcinogenesis in smokers and never-smokers with non–small-cell lung cancer. This prospective, ongoing, multicenter study is being conducted nationwide in Japan. Although there is no doubt that active smoking is the major cause of lung cancer, the contribution of other possible factors, including environmental tobacco or wood smoke, human papilloma virus, radon, occupational exposures, and genetic susceptibility, is highly likely, based on studies of never-smokers with non–small-cell lung cancer. Because of the predominance of women in the never-smoker subgroup, the role of female hormones in lung cancer development has also been considered. We hypothesize that driver mutations, which are critical for the development of lung cancer, are triggered by the environmental factors with or without the influence of the hormone. The SWOG-led intergroup molecular epidemiology study S0424 was conducted to focus on these issues by using a detailed questionnaire and specimen collection in statistically significant cohorts of smokers and never-smokers from both sexes. The Japan Molecular Epidemiology for Lung Cancer study follows and extends the S0424 molecular epidemiology concept in principle by using a similar approach that will facilitate future comparisons between the studies but with a greater focus on more recently defined driver mutations and broad genomic sequencing.

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Keywords: Driver mutations, Molecular epidemiology, Never-smokers, Non–small-cell lung cancer, Smokers

Introduction

Lung cancer is a leading cause of cancer-related morbidity and mortality in the world. Although the disease is predominantly caused by tobacco smoke, approximately 25% of all lung cancers worldwide are not attributable to this etiology. In fact, approximately 30% of Japanese patients with non–small-cell lung cancer (NSCLC) are never-smokers, as observed in a study that consisted of more than 20,000 patients.[1] Lung cancer in never-smokers differs significantly from that of smokers in clinical characteristics and in the distribution of oncogenic abnormalities, and it has been suggested to be a distinct disease.[2]

Although several possible explanations have been proposed, the cause of lung cancer in never-smokers remains unclear. Explanations include environmental tobacco smoke (ETS) exposure,[3] radon,[4] wood smoke,[5] occupational exposure,[6] oncogenic virus,[7,8] genetic change,[9] and sex hormone.[10,11] A Japan Public Health Center–based prospective study showed that, in Japan, second-hand smoke exposure is clearly related to the development of lung adenocarcinoma in never-smokers.[3] The study identified a statistically significant dose-response relationship between the quantity and the intensity of husbands' smoking and their wives' incidence of lung cancer. Our previous study with a detailed questionnaire in a prospective way enhances this finding that the development of epidermal growth factor receptor (*EGFR*) mutations is significantly associated with the dose of ETS exposure in never-smokers.[12] However, there are con-

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flicting data published on the relationship between ETS and *EGFR* mutations in never-smokers with NSCLC. A study from Korea showed opposite results, in which the development of the mutation was inversely proportional to the ETS[13]; although, in the United States, there was no association between them.[14] A study with a well-designed and standardized questionnaire in a larger sample size is required to conclude this issue.

An oncogenic role for the HPV has been widely investigated in NSCLC.[7] Although all the published reports were retrospective analyses with potentially significant limitations and bias, the systematic review nevertheless suggested that the development of lung cancer in Asia can be attributed to some extent to HPV. Moreover, a different detection rate was observed geographically even within east Asia, with a higher rate in the southern area than in the northern regions. There is a substantial need to confirm these findings by using a standardized HPV detection methodology in a prospective study in Japanese patients.

The association between sex and lung cancer carcinogenesis is also an important consideration. Although studies provide conflicting results on the strength of this association, it has been postulated that women are more vulnerable to tobacco smoke–associated carcinogens than men. The large SWOG study S0424 was originally designed to address this issue by using a detailed questionnaire and NSCLC tissue specimens from smoker and never-smoker men and women with newly diagnosed stage I, stage II, or stage III NSCLC,[15] in which polycyclic aromatic hydrocarbons and aromatic amines of DNA adducts are measured to quantitate levels of DNA damage stratified by sex and the smoking status. Cigarette smoke contains a large number of carcinogens, and polycyclic aromatic hydrocarbons and aromatic amines are among the most important contributors to the carcinogenic process. The Japan Molecular Epidemiology for lung cancer (JME) study follows and extends the concept of S0424 by using a similar approach that will allow for direct comparison of data in the future.

Sex hormones, including estrogen and progesterone, have been suggested to play an important role in lung carcinogenesis. Results of epidemiologic studies showed that women were predominant in number in the never-smoking subpopulation. Further, results of large randomized studies suggest that estrogen plus progestin therapy is associated with an increased risk of lung cancer. The prospective Vitamins and Lifestyle Study followed a cohort of more than 36,000 peri- and postmenopausal women during 6 years of follow-up.[16] After adjusting for smoking and other confounding factors, the incidence of lung cancer was increased for those who used estrogen plus progestin. The risk was proportional to the duration of hormone exposure (hazard ratio 1.48 [95% CI, 1.03-2.12] for those with ≥10 years of exposure to estrogen plus progestin).

In terms of biologic function, estrogen receptors (ER) are expressed in diverse normal and neoplastic tissues, and mediate growth and maturation of normal tissue. A number of studies have noted expression of ERs in a large portion of lung tumors. In a couple of studies, the development of *EGFR* mutations was significantly associated with expression of ER β in NSCLC surgical specimens.[10,11] There have been no studies that systematically evaluated ER expression in lung cancer and its relationship with genetic mutations or environmental and reproductive risk factors.

Identification of driver mutations in NSCLC has been instrumental in improving treatment strategies. *ALK* (anaplastic lymphoma kinase) gene translocations have been demonstrated to be critical targets and biomarkers for crizotinib efficacy,[17] similar to *EGFR* mutations for gefitinib and erlotinib, and the discovery of other mutations for treatment is ongoing. The Lung Cancer Mutational Consortium in the United States[18] and the Lungscape project in the European Union[19] are currently exploring new molecular targets for treatment in lung cancer. Powerful tools for genome-wide characterization have been developed, including next-generation sequencing, which enables comprehensive examination of somatic mutations associated with carcinogenesis. The Cancer Genome Atlas is an ongoing global project that uses this technology to distill essential driver abnormalities from the background noise.[20] A focus of the JME study is to explore new driver mutations by using advanced technologies and approaches now available with regard to sex of the patient and tobacco smoke exposure. The association between oncogenic abnormality profiles and drug sensitivity and prognosis will also be examined.

In addition, the JME study is designed to investigate the relationship between ethnicity and NSCLC carcinogenesis. It is clear that NSCLCs are different in tumor biology between Caucasian and Asian patients. Gandara et al[21] showed that there was a significant difference in survival and toxicities between the US and the Japanese patients treated with carboplatin and paclitaxel in a "common arm" trial, in which the study design, eligibility criteria, and staging were similar. The median overall survival in the metastatic disease was 12 and 14 months for Japanese patients vs. 9 months for US patients ($P = .0006$).[21] As for *EGFR* mutations, the frequencies appear to be highly distinct; the high detection rate in Asia was reported consistently across publications. Different influences of smoking status on the development of NSCLC also was observed between the United States and Japan in population-based prospective studies. In a comparison of the Japanese cohort with US Cancer Prevention Study II during the same period,[22] Japanese never-smokers had an increased risk of lung cancer, whereas Japanese current smokers were at a lower risk of the cancer compared with those in the United States. To elucidate the mechanistic contributions of ethnic differences, there is a need to collaborate in comprehensive and global approaches for examining development of NSCLC as well as the clinical behavior and outcome.

Objectives

The primary objective of this study is to assess surgical lung specimens from patients with stage I, stage II, stage IIIA, or stage IIIB NSCLC for driver mutations, expression of HER2 and ER α and ER β, the presence of smoking-associated DNA adducts, and evidence of HPV, and to explore new molecular markers by using next-generation sequencing. By using information collected before surgery on patient demographics, smoking history and occupational exposures, carcinogenic mechanisms will be elucidated in never-smokers and ever-smokers. Secondary objectives are to examine whether the relapse rate, disease-free survival, and overall survival time differ among the patients with different mutational

spectrums, and whether mutational profiles differ between Japanese and Caucasian populations.

Patients and Methods

Eligible patients are those with pathologically proven NSCLC with stage I and II, IIIA, or IIIB disease[23] who underwent surgery with a curative intent (Figure 1). Patients with prior chemotherapy and/or radiotherapy are excluded, as are patients with other prior malignancies except for adequately treated basal cell or squamous cell skin cancer or in situ cervical cancer. Patients are stratified according to smoking status. Never-smokers are defined as those who smoked fewer than 100 cigarettes during their lifetime, and ever-smoker who smoked 100 or more cigarettes during their lifetime.

Patients are required to complete the questionnaire before surgery for detailed assessment of the following: exposure to active and passive smoke, occupational exposures, reproductive and hormonal risk factors, weight loss, family history of cancer, medication use, and diet and exercise. DNA is extracted from all formalin-fixed paraffin-embedded surgical tissues. *EGFR* and *KRAS* (v-Ki-ras2 Kirsten rat sarcoma) mutations are examined by using real-time polymerase chain reaction and *ALK* by immunohistochemical staining and fluorescence in situ hybridization. HPV genotyping is performed by using a polymerase chain reaction–based microarray system for detection of 23 HPV types, including high-risk (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and low-risk or risk-unknown types (HPV types 6, 11, 30, 34, 40, 42, 53, 54, 61, and 66). In addition, multiplexed targeted deep sequencing is applied to the tumors, including 48 cancer-associated genes, such as *ABL1, AKT1, CSF1R, CTNNB1, IDH1, MET, MLH1, PIC3CA, RET, STK11,* and *TP53*.

Surgical samples are examined for DNA adducts levels, and polycyclic aromatic hydrocarbons and/or aromatic amines–induced DNA damage is assessed by immunohistochemical staining and immunofluorescence. ER α and ER β are assessed by immunohistochemistry. Patients will be followed up annually for up to 4 years to capture relapse rate, disease-free survival, and overall survival time. Whether mutational profiles differ between Japanese and Americans will be determined after adjusting for sex, smoking status, and other clinical backgrounds.

Statistical Consideration

Sample size in this study is 900 patients, which consists of 450 ever-smokers and 450 never-smokers, which was calculated to ensure >80% power for testing all individual hypotheses at the 2-sided .05 significance level. Based on the review article including the published data,[24] the assumed proportion of patients with *EGFR* mutation is expected to be approximately 7% and 45% in smokers and never-smokers, respectively. Mutation of *KRAS* is expected to be 30% to 43% in smokers and 0% to 7% in never-smokers. When several examples are given to detect differences of 30% to 50% in mutation-positive frequency between smokers and never-smokers, the power is >90% in most cases. Less common driver mutations are also considered and calculated based on published data[17]; the assumed proportion of patients with *ALK* fusion is expected to be approximately 3.5% and 9.9% in smokers and never-smokers, respectively. The power is >90% in most cases to detect differences of 5% to 7% in fusion-positive frequency between the 2 groups.

According to our study in never-smokers,[12] more *EGFR* mutations were observed in those who had longer ETS exposure. When the length of ETS is divided by the median of the value, if the *EGFR* detection rate differs by more than 15% between the 2 groups overall, then the power is >90% in most cases.

The meta-analysis on HPV and lung cancer showed that, when using polymerase chain reaction, there were 22% of cases (95% CI, 18%-27%) possibly associated with the virus.[8] The presence of HPV is expected to be observed at least in approximately 160 patients in smokers and never-smokers, and the geographic distribution is also examined.

Based on our previous study, which included approximately 20,000 Japanese patients,[1] it is assumed that 350 female never-smokers, 100 male never-smokers, 120 female ever-smokers and 330 male ever-smokers will be accrued in this study. A possible fluctuation in accrual on sex is expected to be with a range of ±20%. If the detection rate of *EGFR* mutation differs by more than 15% between male and female subjects, then the power is >90% in most cases overall and >80% in most cases within smokers and never-smokers.

The prognostic value of each unique *EGFR* and *KRAS* mutation, along with other abnormalities, will initially be assessed by using multivariable proportional hazards regression when adjusting for strata. Relationships will be graphically displayed for each prognostic group by using Kaplan-Meier curves. The classification and regression tree method will be used to identify prognostic risk groups based on these measures of the mutations combined with other patient demographic and correlative data.

Conclusion

The JME study is a prospective project sponsored by an independent administrative agency in Japan to use advanced molecular technologies to improve our understanding of the underlying biology of NSCLC in Japanese patients nationwide. The primary focus of this study is on the relationships among tumor carcinogenesis; patterns of biomarkers, including driver mutations; and detailed demographic information. This study is currently ongoing, and successful accrual to date supports the feasibility of the study design. The outcomes of the JME study will have clinical implication with respect to establishing a model for lung cancer carcinogenesis and will provide a wealth of information on driver mutations to better understand the tumor carcinogenic process and to improve therapeutic options for patients with NSCLC.

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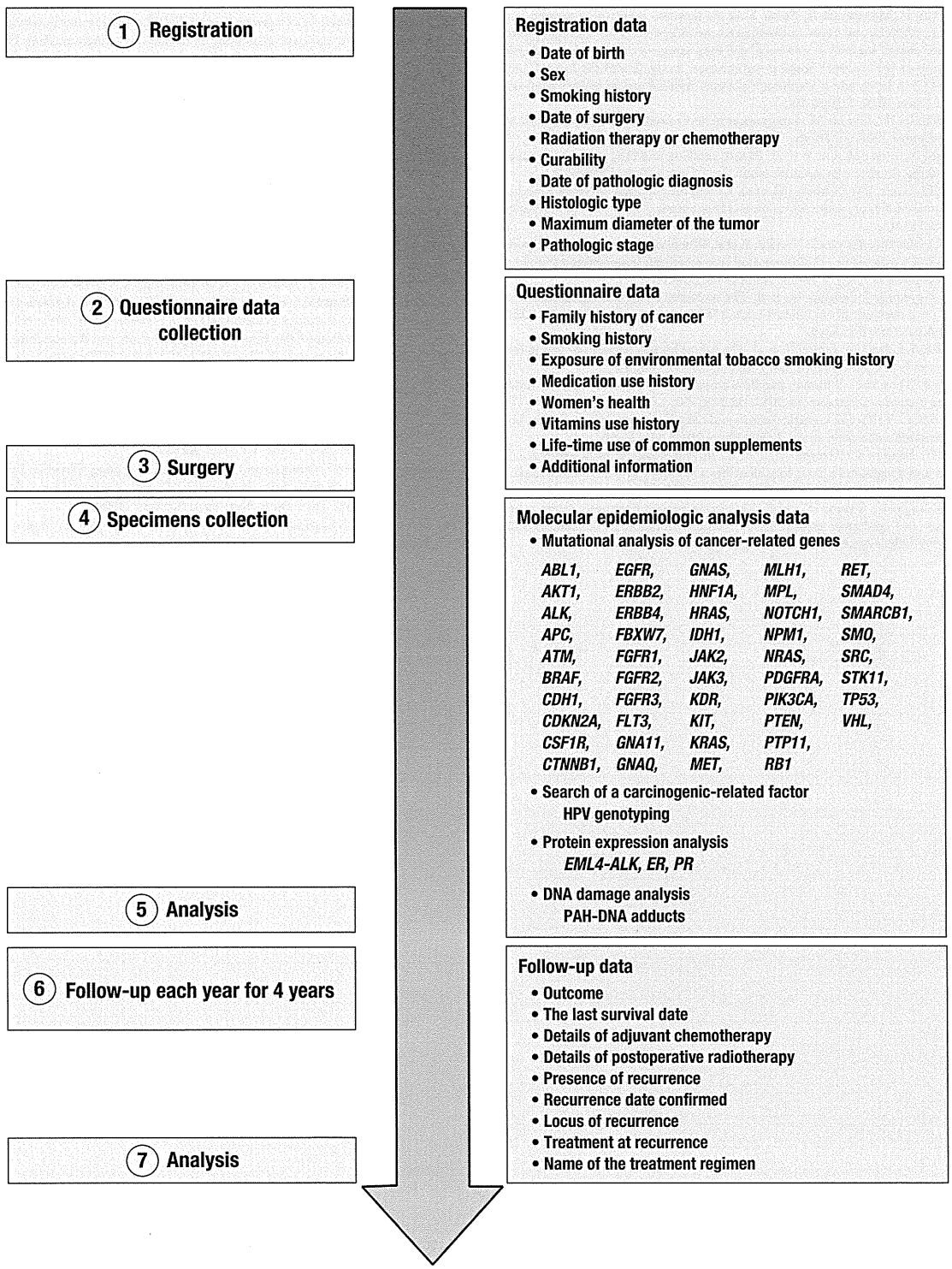
Figure 1 Scheme of the Japan Molecular Epidemiology for Lung Cancer



Disclosure

The authors have stated that they have no conflicts of interest.

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Risk Factors for Drug-Resistant Pathogens in Community-acquired and Healthcare-associated Pneumonia

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Rationale: **Identification of patients with drug-resistant pathogens at initial diagnosis is essential for treatment of pneumonia.**

Objectives: **To elucidate clinical features of community-acquired pneumonia (CAP) and healthcare-associated pneumonia (HCAP), and to clarify risk factors for drug-resistant pathogens in patients with CAP and HCAP.**

Methods: **A prospective observational study was conducted in hospitalized patients with pneumonia at 10 institutions in Japan. Pathogens identified as not susceptible to ceftriaxone, ampicillin-sulbactam, macrolides, and respiratory fluoroquinolones were defined as CAP drug-resistant pathogens (CAP-DRPs).**

Measurements and Main Results: **In total, 1,413 patients (887 CAP and 526 HCAP) were analyzed. CAP-DRPs were more frequently found in patients with HCAP (26.6%) than in patients with CAP (8.6%). Independent risk factors for CAP-DRPs were almost identical in patients with CAP and HCAP. These included prior hospitalization (adjusted odds ratio [AOR], 2.06; 95% confidence interval [CI], 1.23–3.43), immunosuppression (AOR, 2.31; 95% CI, 1.05–5.11), previous antibiotic use (AOR, 2.45; 95% CI, 1.51–3.98), use of gastric acid–suppressive agents (AOR, 2.22; 95% CI, 1.39–3.57), tube feeding (AOR, 2.43; 95% CI, 1.18–5.00), and nonambulatory status (AOR, 2.45; 95% CI, 1.40–4.30) in the combined patients with CAP and HCAP. The area under the receiver operating characteristic curve for counting the number of risk factors was 0.79 (95% CI, 0.74–0.84).**

Conclusions: **The clinical profile of HCAP was different from that of CAP. However, physicians can predict drug resistance in patients with either CAP or HCAP by taking account of the cumulative number of the risk factors.**

Clinical trial registered with https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr.cgi?function=brows&action=brows&type=summary&recptno=R000004001&language=E; number UMIN000003306.

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

The optimal prediction method of the occurrence of drug-resistant pathogens at diagnosis of pneumonia needs to be developed. From this perspective, the necessity of distinguishing community-acquired pneumonia (CAP) and healthcare-associated pneumonia (HCAP) has been debated, and multicenter studies that clarify the risk factors for drug-resistant pathogens are needed.

What This Study Adds to the Field

This multicenter prospective study elucidated six independent risk factors for resistance to commonly used antibiotics for pneumonia, and revealed the risk factors were similar in patients with CAP and HCAP. We suggest that a simple clinical prediction rule comprised of counting the number of risk factors for drug resistance may be used by physicians to predict risk of drug-resistant pathogens in patients with either CAP or HCAP.

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Pneumonia is a common disease and one of the world's leading causes of death (1). To achieve appropriate initial antibiotic

treatment, accurate assessment and classification of patients with pneumonia at initial diagnosis is essential. The optimal method of achieving this goal has been greatly debated (2–6).

The 2005 and 2007 guidelines for the management of pneumonia provided by the American Thoracic Society and the Infectious Diseases Society of America recommend that pneumonia should be classified into one of three categories at diagnosis: (*1*) community-acquired pneumonia (CAP), (*2*) healthcare-associated pneumonia (HCAP), and (*3*) hospital-acquired pneumonia (HAP) (7, 8). These three types of pneumonia have different clinical features (7, 8). In the last decade, several studies have argued that HCAP should be distinguished from CAP because of the higher prevalence of drug-resistant pathogens (DRPs), such as *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* (MRSA), in patients with HCAP (9–12). Other studies showed that patients with HCAP received inappropriate initial antibiotic treatment (IIAT) more often than patients with CAP (11–14). However, administration of a broad-spectrum multidrug antibiotic regimen is not necessary in all patients with HCAP because of the wide regional variation of the frequency of multidrug-resistant pathogens in this type of pneumonia (2). The necessity of distinguishing HCAP and CAP to predict the risk of drug resistance has also been debated (3, 15, 16).

IIAT has been clearly associated with poor outcomes (12, 17). To ensure that appropriate initial antibiotic treatment is administered, more accurate information is needed regarding risk factors for drug resistance and an improved method of quantifying those factors (2, 18–20). Recently, two single-center studies proposed two separate scoring systems to predict drug resistance in pneumonia arising in communities (21, 22). Shorr and colleagues (22) proposed a prediction model using the following weighted point assignments: 4, recent hospitalization; 3, nursing home; 2, chronic hemodialysis; and 1, critically ill. However, simpler indicators for drug resistance would be helpful for physicians who prescribe antibiotics in clinical settings.

Therefore, a multicenter, prospective, observational study including hospitalized adult patients with pneumonia was conducted. The objectives of this study were to identify the clinical and microbiologic features of CAP and HCAP, both of which occur in communities, and to clarify the risk factors for drug resistance to common antibiotics.

Some of the results of this study have been previously reported in the form of an abstract (23), and the revised version was distributed to meeting attendees.

METHODS

Supplemental information on methods is provided in the online supplement.

Study Design and Setting

This observational study was performed prospectively from March 15, 2010 through December 22, 2010 at 10 medical institutions (a 1,000-bed university hospital and nine major community hospitals, each equipped with more than 500 beds), all of which are members of the Central Japan Lung Study Group. This study was approved by the institutional review boards of these institutions. The protocol in this study adhered to the Japanese Ethical Guidelines for Epidemiological Studies. This study is registered with University Hospital Medical Information Network in Japan (number UMIN000003306).

Participants and Categories of Pneumonia

All adult patients (age ≥ 20 yr) in whom pneumonia had developed during daily community living and to whom in-hospital treatment was subsequently administered in the participating institutions were included in the study. Pneumonia was diagnosed according to previously published international guidelines (7, 8). The details of diagnostic criteria and exclusion criteria are provided in the online supplement.

Further details associated with the different categories of pneumonia are as follows (7, 8, 12):

1. HAP: pneumonia occurring 48 hours or more after hospital admission, including ventilator-associated pneumonia
2. HCAP: pneumonia co-occurring with any of the following conditions:
   a. Hospitalization for 2 days or more during the preceding 90 days
   b. Residence in a nursing home or extended care facility
   c. Home intravenous therapy (including antibiotics and chemotherapy)
   d. Chronic dialysis (including hemodialysis and peritoneal dialysis) during the preceding 30 days
   e. Home wound care during the preceding 30 days
3. CAP: pneumonia not matching the criteria for HAP and HCAP

In this study, patients with CAP and HCAP were enrolled, and those with HAP were not included in the current analysis because the data on HAP were collected in limited two institutions.

Procedure and Data Collection

The procedure of this study is provided in the online supplement. The following data were collected at diagnosis (Day 0): demographic information, including past medical history and living conditions; comorbidities; use of antibiotics within the previous 90 days; use of gastric acid–suppressive agents (histamine H_2-receptor blockers or proton pump inhibitors) at the time of diagnosis; tube feeding, functional status, and positive MRSA history within the previous 90 days; symptoms; physical, laboratory, and radiologic findings; indexes of disease severity (including Pneumonia Severity Index and the age, dehydration, respiratory failure, orientation disturbance, and low blood pressure [A-DROP] score) (24, 25); microbiologic characteristics; and initial empirical antibiotic therapy. Additional details of the collected data and definitions of comorbidities are provided in the online supplement. Information regarding outcomes was obtained after Day 30.

Microbiologic Evaluation

Microbiologic laboratories in all study institutions provided possible causative pathogens, which were cultured in a semiquantitative manner from samples of sputum, tracheobronchial aspirates, bronchoalveolar lavage fluid, pleural fluid, and blood. Serologic tests were performed to detect antibodies against *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* (26, 27). *Legionella pneumophila* serogroup 1 antigen in urine was tested by immunochromatography. Microbiologic test results were independently reviewed by two investigators (Y. Shindo and I.Y.). Pathogens provided by the 10 institutions were recultured and antibiotic susceptibility tests were performed at a central laboratory (SRL, Inc., Tokyo, Japan). Viruses, acid-fast bacilli, fungus, and anaerobes were not recultured. The susceptibility tests focused on antibiotics frequently prescribed or recommended for the treatment of pneumonia (7, 8). Additional details including susceptibility tests are provided in the online supplement.

Endpoints

In this observational study, we set several clinical and microbiologic endpoints. In those, we focused on the following endpoints: (*1*) the drug resistance of identified pathogens, (*2*) the IIAT, (*3*) 30-day mortality and in-hospital mortality, and (*4*) receiving mechanical ventilation from Day 0 through Day 30.

The definition of multidrug-resistant pathogens from a recent international consensus statement was adopted to facilitate international comparison regarding the epidemiology of DRPs (28). In the initial empirical antibiotic treatment of CAP, two regimens (combination therapy with nonantipseudomonal β-lactams and macrolides or monotherapy with fluoroquinolones) have been recommended in the international