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New Aspects of Photodynamic Therapy for Central Type Early Stage Lung Cancer

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Background: and Objective Photodynamic therapy (PDT) has come to be considered as the first choice of treatment for central type early stage lung cancer (CELC). Recent advances in the ability to diagnose CELC, and in photosensitizers, as well as sophisticated clinical management, may improve the therapeutic outcome and expand the indications of PDT.

Materials and Methods: We made the search for papers on PDT for lung cancer to select the most relevant articles. Based on this review and our recent data, we discussed the best available evidence for the diagnosis, the definition of indications, photosensitizers, and clinical management with regard to PDT.

Results: To obtain complete response (CR) by PDT, the selection of the indications is extremely important, including the extent of the tumor on the bronchial surface and the depth of invasion in the bronchial wall. The development of autofluorescence bronchoscopy (AFB) and endobronchial ultrasonography (EBUS) have had a large impact on diagnostic bronchoscopy for CELC. CELCs less than 1 cm in diameter showed a favorable cure rate by PDT, thus this is a good indication for PDT. The relatively newer photosensitizer NPe6, which has a stronger antitumor effect than Photofrin, showed similar treatment outcome even for large tumors >1.0 cm in diameter. Furthermore, comprehensive management including photodynamic diagnosis before and after PDT should be effective to minimize the possibility of local recurrence after PDT.

Conclusion: The present guidelines of PDT for CELC were established based on the data obtained from studies in the 1980's. We postulate that comprehensive diagnosis and the new generation of photosensitizers may increase the CR rate and expand the indications of PDT for larger tumors. Lasers Surg. Med. 43:749–754, 2011.

Key words: autofluorescence bronchoscopy; central type early stage lung cancer; endobronchial ultrasonography; photodynamic therapy

INTRODUCTION

Lung cancer, the leading cause of cancer-related death worldwide, is divided into four major histological types, each of which has distinct biological characteristics. It is also classified into two categories based on its location; central type, and peripheral type. The former originates from large bronchi, while the latter originates from lung parenchyma.

It is generally assumed that squamous cell carcinoma originating in the bronchial tree develops in a gradual and stepwise process where the epithelial changes from normal to preneoplastic lesions, then carcinoma *in situ* and microinvasive squamous cell carcinoma [1,2]. The histological classification by the World Health Organization includes precise guidelines for grading of preneoplastic lesions: grading squamous dysplasia as mild, moderate, and severe dysplasia, and defining carcinoma *in situ*. These precise criteria allowed better interobserver reproductibility [3]. Breuer et al. [1] reported that 9% of squamous metaplasia, 9% of mild/moderate dysplasia, and 32% of severe dysplasia progressed to carcinoma *in situ* or squamous cell carcinoma. Also, the progression rate of carcinoma *in situ* to invasive cancer (87%) is reported to be significantly higher than that of severe dysplasia (37%; $P < 0.05$) [4].

As smoking is by far the greatest cause of central type lung cancer, most patients with this disease also suffer from poor cardio-pulmonary function due to chronic

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obstructive pulmonary disease (COPD) and ischemic heart disease, thus, surgical resection can be difficult in such cases. Also, 20–30% of central type early stage lung cancer (CELC) are multicentric [5] and treatment preserving pulmonary function should be considered.

An annual mass screening program for lung cancer has been conducted since 1982 in Japan, including annual chest X-ray examinations for those aged 40 or over, and added sputum cytology for individuals aged 50 or over with a smoking history of over 30 pack-years and those aged 40 or over with a history of bloody sputum within the previous 6 months. For individuals suspected to have lung cancer on chest X-ray or sputum cytology, further examinations were performed to confirm the diagnosis [6–8].

Advances in bronchoscopy and the increasing prevalence of sputum cytology have helped to increase the detection of CELC [9]. Surgery is considered to be a curative treatment method for CELC, but it might be wasteful by sacrificing a large volume of lung parenchyma to treat only a 1–2 cm superficial lesion of a large bronchus. The first clinical endoscopic photodynamic therapy (PDT) for CELC was performed in 1980 and PDT has been employed to treat CELC with curative intent up to the present [10–13].

The objective selection of the lesions for PDT is important in terms of the tumor extent on the bronchial mucosa as well as the depth of invasion to the bronchial wall [13]. The penetration of laser light used for PDT is limited, therefore, the lesions should be limited to within the cartilagenous layer of the bronchial wall for curative PDT. For this reason, the meticulous observation of bronchial mucosa by a combination of conventional bronchoscopy and newly developed imaging techniques is necessary to determine the indications of PDT [14]. PDT has become the first treatment for selected CELC since the criteria for the indications of PDT were established.

MATERIALS AND METHODS

PubMed was searched using the medical subject headings (MeSH) for "lung cancer" and "PDT" (through February 2011). A total of 293 articles were found and clinical papers with analysis of outcomes of survival or response rate were selected and carefully reviewed.

RESULTS

Selection of CELC Suitable for PDT

The Japan Lung Cancer Society defined the bronchoscopic criteria of CELC as follows [15];

- Location in subsegmental or more proximal bronchi.
- The peripheral margin of the tumor is recognizable bronchoscopically.
- The tumor size is less than 2 cm in greatest dimension.
- Squamous cell carcinoma is proven histologically.

CELC is classified into three categories according to the endoscopic apperances, early polypoid type, nodular type, and flat type.

Previous studies showed that there was a correlation between the tumor features and the depth of bronchial invasion. Protruding type tumors tended to invade deeper than superficially spreading type tumors. In particular, over 90% of tumors classified as flat type and under 10 mm in maximum dimension were carcinoma *in situ*. These results demonstrated that a flat lesion, when the diameter is less than 10 mm and the distal margin is visible, could be an excellent indication for endoscopic treatment [16,17]. These judgements require expertise, however, advances in medical science may also contribute to better performance in the diagnosis and management of CELC.

Autofluorescence bronchoscopy (AFB) has been widely employed to capture the subtle abnormal findings of bronchial mucosa which sometimes cannot be detected by conventional bronchoscopy [18–24]. AFB is based on the principle that the normal bronchial tissue emits green autofluorescence (500–600 nm) excited by blue light, while malignant tissue lacks green autofluorescence. Abnormal sites can be discriminated from normal areas by enhancing the difference in intensity of green autofluorescence [18,22]. AFB has been reported to show higher sensitivity for carcinoma *in situ* and dysplasia and to contribute to the objective diagnosis of tumor extent on the bronchial surface [18–24].

The accurate evaluation of the depth of bronchial invasion of the tumor is another important issue to decide the indications of PDT because if the tumor invades beyond the cartilaginous layer, PDT should not be applied due to the limited light penetration, and surgery should be selected instead [14,25]. Endobronchial ultrasonography (EBUS) has been reported to be useful in the precise observation of the layered structure of the bronchial wall, and previous reports showed that the evaluation of degree of intrabronchial invasion by EBUS is superior compared to speculation based on the interpretation of the endoscopic appearance of tumor [25–29]. Kurimoto et al. demonstrated that EBUS was useful to determine the depth of tumor invasion into the bronchial wall, and the accuracy of EBUS from the histopathologic findings was 95.8%. The 20 MHz EBUS image shows five layers in the cartilaginous portion of bronchial wall. The third to fifth layers are images of cartilage. Therefore, it is feasible to evaluate the depth of invasion using EBUS and determine whether or not the tumor invades into or beyond the cartilaginous layer [26]. In lesions with an intact third layer on EBUS, complete response (CR) can be achieved with PDT. Takahashi et al. [29] performed EBUS to evaluate the degree of carcinoma invasion into the bronchial wall in 22 lesions suspected of CELC before treatment. Fourteen lesions were diagnosed to be intracartilagenous lesions, and the remaining 8 lesions, extracartilagenous lesions, respectively. Among the 14 intracartilagenous lesions, 10 lesions were treated by PDT and complete remission was obtained in 9. The remaining 4 lesions were surgically resected and pathological examination of resected lungs revealed that invasion did not reach the cartilaginous layer in 3. Of 8 lesions diagnosed to

be extracartilagenous, 4 cases received surgery and pathology revealed that EBUS diagnosis was correct in 3 and overestimated in 1.

PDT for CELC

PDT with photofrin. Phase II clinical trials using Photofrin for early stage lung cancer were conducted from June of 1989 to March of 1992. In the studies, a total of 61 CELCs (51 patients) were registered and treated using Photofrin (2.0 mg/kg) and 630-nm laser light illumination of 100–200 J/cm^2 [30]. Of 59 assessable CELCs, CR was obtained in 50 (84.8%), 6 had partial response, and 3 had no response. This study demonstrated that the length of longitudinal tumor extension on the bronchial surface was strongly related to the therapeutic outcome. Out of 45 CELCs 1 cm or less, CR was obtained in 44 (97.8%), on the contrary, among 14 CELCs that had a longitudinal extension greater than 1 cm, only 6 (42.9%) showed CR after PDT. The Japanese government approved PDT for lung cancer using Photofrin in October 1994, and authorized reimbursement through the National Health Insurance began in 1996. In 1995, the U.S. Food and Drug Administration (FDA) approved Photofrin for esophageal cancer, and in 1998, it was approved for the treatment of early lung cancer [31,32]. Kato reported the consecutive clinical data of PDT with Photofrin for CELC from1980 to 2005 in a single institute (Tokyo Medical University). This showed that CR was obtained in 224 (84.8%) out of the 264 lesions. The treatment outcome was analyzed based on the size of the lesions. The lesions were classified into four groups according to the maximum dimension on the longitudinal axis, as follows: <0.5 cm (56 lesions); 0.5–0.9 cm (124 lesions); 1.0–2.0 cm (50 lesions); and >2.0 cm (34 lesions). The CR rates of the first two groups were 94.6% and 93.5%, respectively. However, the CR rates of lesions from 1.0 to 2.0 cm was 80%, >2.0 cm, and 44.1%, respectively. CELCs less than 1 cm in diameter showed a favorable cure rate by PDT, thus this is a good standard to decide the indications of PDT [31].

PDT with the NPe6 second-generation photosensitizer. Among the second-generation photosensitizers, NPe6 is considered to be a promising photosensitizer and has shown both antitumor efficacy in a murine tumor model and rapid clearance from skin [33]. We postulated that since NPe6 has a longer absorption band (664 nm) than that of Photofrin (630 nm), PDT using NPe6 would have greater photodynamic efficacy due to a slight gain in the penetration depth of light than that with Photofrin. A phase II clinical study was conducted from 1997 to 2000 to investigate antitumor effects on CELC as well as the safety of the new generartion photosensitizer mono-L-aspartyl chlorine e6 (taraporfin sodium, NPe6) in combination with the diode laser [33]. Laser irradiation (100 J/cm^2) using a diode laser was performed 4 hours after administration of NPe6 (40 mg/m^2). A total of 45 CELCs (40 patients) were collected. CR was obtained in 84.6% of lesions (82.9% of patients). Skin photosensitivity was reported to be much lower than with photofrin and the disappearance of skin photosensitivity was recognized in 28 of 33 patients (84.8%) within 2 weeks after administration. NPe6 was approved for the treatment of CELC by Japanese Ministry of Health, Labor, and Welfare in 2004 and also for advanced lung cancer in 2010 [34].

Between June 2004 and December 2008, a total of 91 consecutive CELCs (75 patients) were treated by PDT using NPe6 in Tokyo Medical University and CR was obtained in 85 lesions (CR rate 93.4%). Of the 91 lesions examined in this study, 70 had a diameter of ≤1.0 cm and the rest of the 21 cancer lesions were >1.0 cm in size. The CR rate of CELC ≤1.0 cm in diameter was 94% (66/70) and for those >1.0 cm in diameter, 90.4% (19/21), respectively. This early result suggests that PDT with NPe6 has a stronger antitumor effect than Photofrin, therefore, similar treatment outcome even for large tumors >1.0 cm in diameter should be possible [34]. The CR ratio is shown according to the tumor size in Table 1.

State-of-the-art. In addition to advances in photosensitizer, precise evaluation and sophisticated treatment technique for CELC might improve the treatment outcome of PDT [35].

Our routine clinical process for PDT in CELC is divided into four phases: evaluation phase, photodynamic diagnosis (PDD) phase, PDT phase, and confirmation phase.

Evaluation phase. As already described, AFB and EBUS are routinely performed to evaluate the extent and the depth of CELC lesions to decide the indications for PDT.

PDD phase. Just before PDT, we perform PDD using an AFB system (SAFE-3000, Hoya Co., Tokyo, Japan). SAFE-3000, equipped with a diode laser (408 nm) can excite NPe6 and capture the red fluorescence emitted from the tumor enhanced by NPe6. The PDD technique is useful in the final determination of the area of laser illumination because it clearly reveals the tumor margin.

PDT phase. During PDT, the shutter speed of the image capture of the videoendoscope (SAFE 3000) is

TABLE 1. CR Rate According to Tumor Diameter

	Furuse et al. [30]	Kato [33]	Usuda [34]
No. of cases	59	264	91
Photosensitizer	Photofrin	Photofrin (NPe6 in 16)	Npe6
CR rate and tumor size			
1.0 cm≤	97.8%	93.9%	94.0%
1.0–2.0 cm	50.0%	80.0%	90.4%
>2.0 cm	37.5%	44.1%	NA

changed to the fastest mode to reduce capture of scattered laser light, which helps to observe the real time monitoring confirming that the laser light from the tip of the probe is correctly illuminating the target.

Confirmation phase. Immediately after the PDT phase, PDD using the SAFE-3000 system is again performed to confirm the loss of red fluorescence emitted from the tumor and that a sufficient dose of laser illumination is given. This procedure confirms that all the NPe6 in the tumor had been excited by the laser irradiation, resulting in the red fluorescence of the tumor no longer being able to be observed. If red fluorescence is observed, additional laser irradiation is necessary. The representative treatment process is shown in Figure 1.

These four processes enable us to determine the indication of PDT and provide the correct information of the area to treat as well as determine the optimal dose of laser illumination. The present procedure should be effective to minimize the possibility of local recurrence after PDT.

DISCUSSION

PDT is not indicated in all CELCs. Precise evaluation of the lesion for the indication of PDT is one of the important key issues successful treatment. PDT for CELC was initiated in 1980 in our institution, and the indications of PDT have become increasingly reliable based on the accumulation of clinical experiences. Lesions less than 1 cm in maximal dimension are considered to be good indications for PDT and favorable treatment outcome has been obtained [10–13,30–34]. Konaka et al. [16] analyzed resected cases of CELC and reported that the greatest tumor dimension strongly correlated with the depth of intrabronchial invasion of the tumor, and that polypoid type or nodular type tumors tended to invade deeper than superficially spreading type tumors.

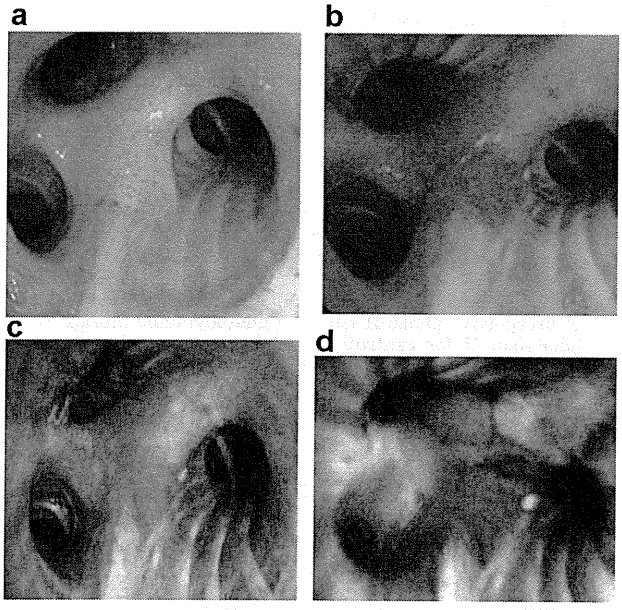

Fig. 1. Central type early stage lung cancer (Flat type) in right upper lobe bronchus. (a) Videoendoscopy, (b) autofluorescence bronchoscopy, and (c) photodynamic diagnosis before PDT (PDD phase). The red fluorescence emitted from the tumor enhanced by NPe6 can be observed. (d) Confirmation phase. All the NPe6 in the tumor was excited by the laser irradiation, resulting in the red fluorescence of the tumor no longer being able to be observed.

Akaogi et al. [17] analyzed the endoscopic and pathology features of CELC and reported that polypoid or nodular lesions less than 10 mm and flat lesions up to 15 mm were all within the cartilaginous layer and showed no nodal metastasis. In particular, over 90% of their tumors classified as the flat type and under 10 mm in maximum dimension were carcinoma *in situ*. Comprehensive evaluation for CELC using AFB and EBUS enables us to evaluate the extent and depth of tumor invasion and to select the optimal treatment modality. Bronchoscopic optical coherence tomography (OCT) will be widely applied in the near future [14,36,37]. Our early experience has revealed that endoscopic OCT examination provides high-resolution images of the bronchial surface, making possible detailed examination of intraepithelial lesions. Since the structures observed in this system are the boundaries between tissue layers, layers between epithelium and basement membrane were clearly demonstrated, which is helpful to evaluate the depth of invasion of bronchial tumors [36].

In addition to new diagnostic modalities, recent advances in PDT-related procedures, especially in the PDD phase and dose evaluation phase may contribute to more sophisticated endoscopic PDT for CELC [35].

The most recent generation photosensitizer, NPe6 seems to have stronger antitumor effects than Photofrin, but with reduced adverse reactions [33,34]. Another reason for the superior antitumor effect of NPe6 might be explained by molecular analysis. The expression of Breast Cancer Resistant Protein (BCRP) significantly negatively affected the efficacy of Photofrin but NPe6 exhibited antitumor effect, regardless of the expression status of BCRP [38]. Usuda et al. [34] reported that there was no significant difference in local control after CR between CELCs $\leq$1.0 cm in diameter and those >1.0 cm. These results suggest that PDT with NPe6 may have a similar treatment outcome regardless of tumor size, as long sufficient laser illumination of the entire tumor is possible.

The present guidelines of PDT for CELC were established based on the data in the previous era; bronchoscopists decided on the indications of PDT solely using white light bronchoscopy and performed PDT with Photofrin. We postulate that more comprehensive diagnostic techniques and new generation photosensitizers may increase the CR rate and expand the indications of PDT for larger tumors.

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A Prospective Radiological Study of Thin-Section Computed Tomography to Predict Pathological Noninvasiveness in Peripheral Clinical IA Lung Cancer (Japan Clinical Oncology Group 0201)

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Purpose: Pathological noninvasiveness needs to be precisely predicted in preoperative radiological examinations of patients with early lung cancer for the application of limited surgery.

Patients and Methods: Patients with clinical T1N0M0 peripheral lung cancer were recruited. Radiological findings of the main tumor were evaluated as to ground-glass opacity with thin-section computed tomography. The primary end point was specificity, i.e., the proportion of patients with radiologically diagnosed invasive lung cancer to patients with pathologically diagnosed invasive lung cancer. The precision-based planned sample size was 450. We expected that the lower limit of the 95% confidence interval (CI) for specificity should be satisfied in ≥97% of patients.

Results: We enrolled 811 patients from 31 institutions between December 2002 and May 2004. The primary end point was evaluated in 545 patients. The specificity and sensitivity for the diagnosis of pathologically diagnosed invasive cancer were 96.4% (161/167, 95% CI: 92.3–98.7%) and 30.4% (115/378, 95% CI: 25.8–35.3%), respectively, i.e., a negative result. Nevertheless, the specificity for lung adenocarcinoma ≤2.0 cm with ≤0.25 consolidation to the maximum tumor diameter was 98.7% (95% CI: 93.2–100.0%), and this criterion could be used to radiologically define early adenocarcinoma of the lung.

Conclusions: Although our predetermined criterion for specificity was not statistically confirmed, radiological diagnosis of noninvasive lung cancer with a thin-section computed tomography scan corresponded well with pathological invasiveness. Radiological noninvasive peripheral lung adenocarcinoma could be defined as an adenocarcinoma ≤2.0 cm with ≤0.25 consolidation.

Key Words: Ground-glass opacity, Bronchioloalveolar carcinoma, Thin-section, Computed tomography, Limited resection.

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Lung cancer is the leading cause of cancer death worldwide.[1] Occult lymph node metastasis in hilum and mediastinum are found in approximately 15 to 20% in the literature[2,3]; however, a conventional preoperative workup cannot detect these metastases. Thus, a major lung resection with lymphadenectomy is recommended even for small-sized lung cancer.

There are two indications for the use of limited surgical resection. Some authors insist that only the size of the main tumor is an indication for limited surgical resection.[4–6] This strategy is supported by segmentectomy as the limited surgery and an intraoperative evaluation of the hilar lymph node. If there is lymph node involvement, then the surgery is converted from segmentectomy to major lung resection. Thus, diagnosis from intraoperative frozen sections of several lymph nodes is mandatory for this strategy, and a wide wedge resection, another limited surgical resection technique, is not suitable because it is impossible to evaluate the status of the hilar nodes using this approach. Conversely, a wide wedge resection can be used as a limited surgical resection for peripheral lung cancer.[7–9] This strategy should be adopted on the supposition that the lung cancer has not metastasized to the nodes. As the intraoperative nodal status cannot be estimated using a wide wedge resection, a preoperative evaluation of the primary tumor is vital. Preoperative predictors for the lack of metastasis to the lymph node are necessary for this strategy. The findings from thin-section computed tomogra-

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phy (CT) are reported to be the best predictors for the invasiveness and nodal status of lung cancers.[10–17] It has been proposed that lung cancer with a consolidation less than 50% of the maximum tumor diameter could be one of the most promising definitions to predict "early" lung cancer; however, this definition was derived from retrospective studies, and it should be confirmed in a prospective study.

Therefore, we performed a multiinstitutional prospective study for the radiological diagnosis of early lung cancer (Japan Clinical Oncology Group [JCOG] 0201) to assess these retrospective findings. If the validity of the criteria to radiologically diagnose "early" lung cancer is confirmed by this study, then a limited surgical resection could be used instead of a major lung resection.

PATIENTS AND METHODS

Patient Eligibility Criteria

The eligibility criteria were as follows: (1) a suspected or diagnosed lung cancer based on the findings from a plain x-ray and/or CT scan; (2) clinical stage IA, i.e., T1N0M0, by thoracic enhanced CT; (3) the center of the tumor was located peripherally, i.e., the outer half of the lung field on CT; (4) measurable at least in one dimension in thin section CT; (5) age range from 20 to 75 years, (6) no prior thoracotomy; (7) feasible for pulmonary lobectomy; and (8) obtained written informed consent.

The exclusion criteria included (1) synchronous or metachronous (within 5 years) malignancy other than carcinoma in situ and (2) interstitial pneumonitis, lung fibrosis, or severe pulmonary emphysema.

All patients underwent a preoperative CT scan, and hilar or mediastinal nodes less than 1.0 cm in the shortest diameter were regarded as clinical N0. Disease stages were determined based on the tumor node metastasis classification of the International Union Against Cancer, 6th edition.[18] The study protocol was approved by the JCOG Clinical Trial Review Committee and by the institutional review board of each participating center. The JCOG Data Center conducted the central registration, data management, central monitoring, and statistical analysis.

Radiological Evaluation of the Primary Tumor

A contrast-enhanced CT scan was performed to evaluate the entire lung for preoperative staging. In addition, the main tumor was evaluated preoperatively to estimate the extent of ground-glass opacity (GGO) with thin-section helical CT scan with 1 to 3 mm collimation. Images were reconstructed with a field of view of 15 to 20 cm. The lung was photographed with a window level of −500 to −700 H and a window width of 1000 to 2000 H as a lung window setting, and with a window level of 30 to 60 H and a window width of 350 to 600 H as a mediastinal window setting. The evaluated factors on the lung window were the maximum diameters of the tumor and consolidation; the presence of a pleural tail; air bronchogram; the homogeneity of consolidation; and the sharpness of the tumor margin. The maximum tumor diameter was also evaluated from the mediastinal window. The consolidation component was defined as an area of increased opacification that completely obscured the underlying vascular markings. GGO was defined as an area of a slight, homogenous increase in density that did not obscure the underlying vascular markings.

Surgical Intervention

A preoperative needle biopsy or cytology was not required. When the diagnosis of lung adenocarcinoma was preoperatively made, a lobectomy and lymph node dissection were recommended; otherwise, an intraoperative frozen section diagnosis was performed, and if the tumor had histology other than adenocarcinoma, the protocol treatment was terminated, and the patients were excluded from the analysis. If the tumor was intraoperatively diagnosed as an adenocarcinoma, major lung resection and lymph node dissection were recommended. For some adenocarcinomas with large GGO areas, such as "pure GGO," a limited surgical resection was allowed, but this population was excluded from the primary end point analysis.

Pathological Diagnosis

The resected specimen was sectioned at intervals of 5 to 10 mm throughout the whole lung. The main tumor was sectioned into 2 to 4 mm slices, and the following pathological factors were evaluated by means of hematoxylin and eosin staining, and elastic fiber staining: histological typing; grade of differentiation; Noguchi's classification[19]; the maximum diameter of the main tumor and central fibrosis; pleural involvement; vascular invasion; lymphatic invasion; and intrapulmonary metastasis. Histological typing was determined according to the classification system of the World Health Organization.[20]

Study Design

Surgical resection was performed after the radiological evaluation of the peripherally located adenocarcinoma. The mode of surgery was basically a pulmonary lobectomy and lymph node dissection, and the postoperative pathological diagnosis was compared with the preoperative radiological diagnosis of early lung cancer. If the postoperative pathological diagnosis of "noninvasive adenocarcinoma" of the lung was predicted by the preoperative radiological diagnosis, a limited surgical resection or other nonsurgical local therapy was indicated.

Definition of Radiological Noninvasive and Invasive Lung Cancer

On the basis of retrospective findings,[10–15] radiological noninvasive lung cancer was tentatively defined as a tumor with a maximum diameter of consolidation of the maximum tumor diameter (consolidation/tumor ratio, C/T ratio) less than 0.5, indicating a tumor with a wide GGO area (Figure 1). Additionally, we adopted other criteria for radiological noninvasive lung cancer. One was the tumor shadow disappearance rate (TDR),[17] and the other was the visual estimation (VE) of the consolidation component.[11] TDR was evaluated from the maximum tumor diameter on the lung and mediastinum windows. TDR was calculated using the following formula: TDR = tumor size on mediastinal window/tumor

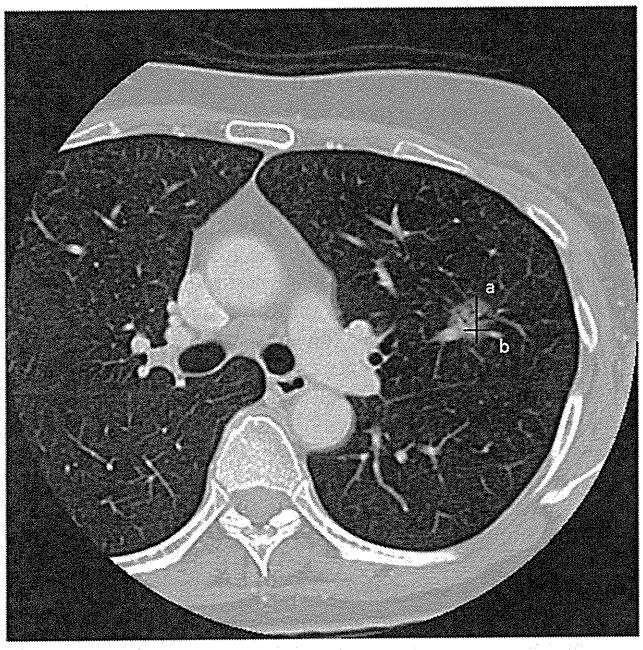

FIGURE 1. Example of radiological noninvasive lung cancer. The maximum diameter of consolidation (*B*) is less than the half of the maximum tumor diameter (*A*), which means tumor with wide area of ground glass opacity.

TABLE 1. Relationship Between Radiological and Pathological Features

	Pathological Diagnosis	
Radiological Diagnosis	**Noninvasive**	**Invasive**
Noninvasive[a]	A	C—undertreated
Invasive	B—overtreated	D

[a] Radiological noninvasive lung cancer was tentatively defined as a tumor with a maximum diameter of consolidation of the maximum tumor diameter <0.5 (see text).
Specificity = D/(C + D), sensitivity = A/(A + B), positive predictive value = A/(A + C), and negative predictive value =D/(B + D).

size on lung window. For VE, the consolidation component was defined as the proportion of the area of consolidation to that of the tumor visually estimated without measuring the diameter; a value less than 0.5 was diagnosed as noninvasive cancer. We compared the sensitivity and specificity of these three methods of radiological evaluation.

Definition of Pathological Noninvasive and Invasive Lung Cancer

The provisional pathological definition of noninvasive lung cancer was defined as a lung adenocarcinoma without nodal involvement, vascular invasion, or lymphatic invasion.

End Point and Planned Sample Size

The primary end point was the specificity based on the radiological diagnosis using the C/T ratio. The relationship between the radiological and pathological diagnoses is presented in Table 1. If limited surgical resection was performed on a patient with radiological noninvasive but pathological invasive cancer, the treatment was considered as "undertreatment" (group C, Table 1). Conversely, if major surgical resection was performed on a patient with radiological invasive but pathological noninvasive cancer, the treatment was defined as "overtreatment" (group B), and a limited surgical resection may be indicated. Patients with radiological and pathological noninvasive lung cancer belonged to group A; group D included patients with radiological and pathological invasive lung cancer. Considering that local recurrence of lung cancer results in a dismal prognosis, undertreatment should be avoided at any cost. Therefore, the number of patients belonging to "C" of Table 1 should be minimized, and the primary end point of specificity was defined as the proportion of patients with radiologically diagnosed invasive lung cancer in patients with pathologically diagnosed invasive lung cancer, i.e., D/(C + D). Conversely, patients with radiological invasive but pathological noninvasive lung cancer, who belong to category "B," may undergo overtreatment. The number of patients in the "B" category should be minimized, and sensitivity was selected as a secondary end point. Sensitivity was defined as the proportion of patients with radiologically diagnosed noninvasive cancer in patients with pathologically diagnosed noninvasive cancer, i.e., A/(A + B).

The primary end point was evaluated for the patients who were resected with a lobectomy and lymph node dissection, diagnosed with adenocarcinoma, and who were regarded as eligible in the radiological central review. We expected that the lower limit for the 95% confidence interval (CI) of specificity was satisfied in ≥97% of patients for an estimated sample size of 400 pathological invasive cancer cases. Assuming the sensitivity is 50% and the 95% CI range is ≤15%, the estimated sample size for pathological noninvasive cancer was 50 cases. The precision-based planned sample size was 450, i.e., ≥400 cases for pathological invasive cancers and ≥50 cases for pathological noninvasive cancers.

Central Review of Radiological Evaluation

To ensure the final diagnosis, radiological findings based on thin-section CT were reviewed by six reviewers. This radiological central review was indicated for patients who were preoperatively or intraoperatively diagnosed with adenocarcinoma. CT findings were evaluated coincidentally by the six reviewers, and the final results were decided in consensus.

Exploratory Analysis

We conducted additional exploratory analyses for patients with an adenocarcinoma ≤2.0 cm in size and evaluated the specificity and sensitivity. We also evaluated two other cutoff values for the C/T ratio on lung window, 0.25 and 0.75, to identify the optimal cutoff value to predict pathologically noninvasive adenocarcinoma of the lung.

RESULTS

Patients' Characteristics

Between December 2002 and May 2004, we enrolled 811 patients from 31 institutions. We expected that the number of pathological noninvasive and invasive cancers was 50 and 400, respectively; however, we recruited patients with

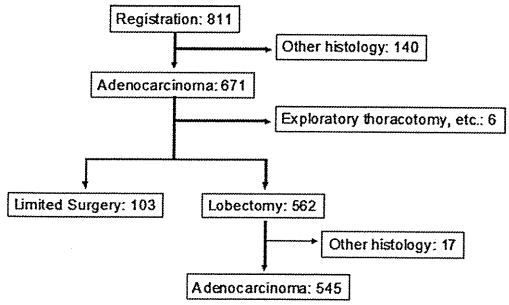

FIGURE 2. Scheme for study population. Finally, 545 patients with adenocarcinoma were the population for the primary analysis.

more pathological noninvasive and less invasive cancer than expected. Thus, we increased the total sample size to recruit more than 400 patients with pathological invasive cancer. Nevertheless, the primary end point proved to be lower than expected before sufficient numbers of pathological invasive cancer cases were recruited. Therefore, the accrual of patients was terminated before the planned period. We recruited 357 men and 454 women (age range, 27–75 years; median, 61 years). Among them, 671 (82.7%) patients were diagnosed with lung adenocarcinoma at the time of the surgical resection. The other cases included benign pathology or other type of cancers, such as pulmonary metastasis of colorectal cancer. Of the 671 patients with lung adenocarcinoma, 562 (83.8%) underwent major lung resection, 103 (15.3%) underwent limited resection, five (0.75%) underwent exploratory thoracotomy, and one underwent another procedure. Among the 562 patients, 17 (3.0%) patients were ineligible based on their postoperative pathological findings (Figure 2). Thus, the remaining 545 patients satisfied the inclusion criteria (described in the Patients and Methods section) and were taken into the primary analysis (Table 2).

Evaluation of the Primary End Point and Comparison among the Three Methods of Radiological Evaluation

The primary end point was evaluated among the 545 patients who met the inclusion criteria (Table 3). The specificity and sensitivity of the diagnosis for pathologically invasive cancer based on the C/T ratio from the lung window was 96.4% (161/167, 95% CI: 92.3–98.7%) and 30.4% (115/378, 95% CI: 25.8–35.3%), respectively. As a result, the lower 95% CI limit for specificity did not exceed the prespecified threshold of 97%. The specificity and sensitivity for the diagnosis of pathologically invasive cancer based on the TDR from the mediastinal window was 89.8% (150/167, 95% CI: 84.2–94.0%) and 44.4% (168/378, 95% CI: 39.4–49.6%), respectively. The most favorable specificity was obtained by the evaluation of the C/T ratio, and the lowest specificity was observed by the TDR method.

Radiological-Pathological Association in Lung Adenocarcinoma ≤2.0 cm in Size

Additional exploratory analysis was performed for lung adenocarcinoma ≤2.0 cm in size in the maximum tumor dimension to examine the appropriate tumor size for diagnosis of radiological early lung cancer. The specificity and sensitivity for the diagnosis of pathological invasive cancer based on the C/T ratio from the lung window was 97.5% (95% CI: 91.2–99.7%) and 31.0% (65/210, 95% CI: 24.8–37.7%), respectively. The point estimate of specificity was

TABLE 2. Characteristics of 545 Eligible Patients for the Investigation of the Primary Endpoint

Characteristics	Number of Patients
Clinical factors	
Gender	
Men	233
Women	312
Age range (median)	35–75 (62)
Maximum tumor dimension	
≤1.0cm	30
>1.0–2.0	270
>2.0–3.0	243
>3.0	2
Radiological factors	
Cons/Tumor ratio[a]	
Non-invasive (≤ 0.5)	137
The others (> 0.5)	381
TDR[b]	
Non-invasive (≤ 0.5)	234
The others (> 0.5)	311
Visual estimation of consolidation[c]	
Non-invasive (≤ 0.5)	200
The others (> 0.5)	345
Surgical factors	
Type of surgery	
Pneumonectomy	1
Lobectomy	544
Pathological factors	
Final histological diagnosis[d]	
Adenocarcinoma	529
Squamous cell carcinoma	7
Large cell carcinoma	4
Others	5
Lymph node metastasis	
Positive	47
Negative	498
Vascular invasion[e]	
Positive	100
Negative	443
Lymphatic invasion[f]	
Positive	113
Negative	428

[a] There were 27 cases of which tumors could not be evaluated the size of consolidation on lung window because of their unclear margin.
[b] TDR was calculated with the following formula: TDR = tumor size on mediastinal window/tumor size on lung window.
[c] The size of consolidation component was evaluated with visual estimation.
[d] Patients with adenocarcinoma which was diagnosed at the time of surgery were eligible and there were 16 patients with different final pathological diagnosis.
[e] There were one missing data and one unknown findings.
[f] There were one missing data and three unknown findings.
Cons, consolidation, TDR: tumor disappearance ratio.

TABLE 3. Relationship Between Radiological and Pathological Features in the 545 Eligible Cases

	Pathological Diagnosis[b]	
Radiology (Cutoff: 0.5)[a]	**Noninvasive**	**Invasive**
Consolidation/tumor ratio on lung window		
Noninvasive[a]	115	6
Invasive	263	161
Sensitivity		30.4% (95% CI: 25.8–35.3)
Specificity		96.4% (95% CI: 92.3–98.7)
TDR		
Noninvasive[a]	168	17
Invasive	210	150
Sensitivity		44.4% (95% CI: 39.4–49.6)
Specificity		89.8% (95% CI: 84.2–94.0)
Visual estimation of consolidation		
Noninvasive[a]	140	11
Invasive	238	156
Sensitivity		37.0% (95% CI: 32.2–42.1)
Specificity		93.4% (95% CI: 88.5–96.7)

[a] Radiological noninvasive lung cancer was tentatively defined as a tumor with a maximum diameter of consolidation of the maximum tumor diameter <0.5, indicating a tumor with a wide GGO area (see text).

[b] Pathological diagnosis was based on the criteria using nodal status, lymphatic invasion, and vascular invasion.

TDR, tumor disappearance ratio; CI, confidence interval; GGO, ground-glass opacity.

TABLE 4. Radiologic-Pathologic Correlation in Lung Cancer 2.0 cm or Less in Size (Cutoff: 0.25)

	Pathological Diagnosis[b]	
Radiology (Cutoff: 0.25)[a]	**Noninvasive**	**Invasive**
Consolidation/tumor ratio on lung window		
Noninvasive[a]	34	1
Invasive	176	78
Sensitivity	16.2% (95% CI: 11.5–21.9)	
Specificity	98.7% (95% CI: 93.2–100.0)	
TDR		
Noninvasive[a]	58	3
Invasive	152	76
Sensitivity	27.6% (95% CI: 21.7–34.2)	
Specificity	96.2% (95% CI: 89.3–99.2)	
Visual estimation of consolidation		
Noninvasive[a]	26	0
Invasive	184	79
Sensitivity	12.4% (95% CI: 8.3–17.6)	
Specificity	100.0% (95% CI: 95.4–100.0)	

[a] Radiological noninvasive lung cancer was tentatively defined as a tumor with a maximum diameter of consolidation of the maximum tumor diameter <0.25, indicating a tumor with a wide GGO area (see text).

[b] Pathological noninvasive is defined as adenocarcinoma with no nodal involvement, lymphatic invasion, or vascular invasion.

TDR, tumor disappearance ratio; GGO, ground-glass opacity; CI, confidence interval.

higher than observed in the primary analysis, but the lowest limit of the 95% CI for specificity was still lower than 97%.

Evaluation of the Optimal Cutoff Value for the C/T Ratio

Radiologically noninvasive lung cancer was primarily defined in this study as a C/T ratio less than 0.5 on thin-section CT; however, the specificity for this criterion was lower than expected, so we examined two other cutoff values, 0.25 and 0.75, for the C/T ratio in patients with lung adenocarcinoma ≤2.0 cm in size. As a result, the 0.25 cutoff value showed the highest specificity, although its sensitivity was relatively low (Table 4).

DISCUSSION

This is the first multiinstitutional prospective study on the definition of radiological early lung cancer. Several radiological criteria for early lung cancer have been reported, but these reports were based on retrospective and single institute analysis.[10–15] The majority of these reports supported the hypothesis that lung cancer with a consolidation less than 0.5 of the maximum tumor diameter and a wide GGO could be regarded as early lung cancer. If this hypothesis was correct, then a limited surgical resection, instead of lobectomy, should be sufficient to treat this population. Nevertheless, before generalizing the strategy, we had to confirm this hypothesis obtained from retrospective findings on a multiinstitutional basis. On the basis of our results, although the radiological findings of GGO and consolidation were well correlated with the pathologically invasive nature of the tumor, the radiological criteria for early lung cancer using the 50% cutoff value were not valid to predict pathological noninvasiveness. Thus, based on this exploratory analysis, lung carcinoma ≤2.0 cm in size and with a consolidation ≤25% of the maximum tumor diameter was considered to be radiological early lung cancer. We have just started a clinical trial to evaluate the validity of limited resection for lung cancer based on these criteria.

There has not been a general consensus formed on the optimal method to evaluate the extent of GGO. Three methods have been mainly reported: the C/T ratio from the lung window; the TDR from the mediastinal window; and the VE of the extent of GGO from the lung window. Each method has been reported as an optimal method based on a single institute retrospective analysis.[10–15] This study is the first prospective study to compare the three methods. The highest specificity was obtained from the C/T ratio and was the lowest for the TDR method. Conversely, the highest sensitivity was found with the TDR method, and the lowest was for the C/T ratio. Therefore, if the TDR method was used to determine radiological early lung cancer, more invasive cancers would be misdiagnosed as radiologically noninvasive. This situation should be avoided as much as possible because an invasive cancer would be resected using a limited resection that is ill suited for such cancers. Conversely, the C/T ratio provided clinically safe criteria to identify noninvasive cancers. On the basis of the primary analyses, the C/T ratio was the best criterion for the highest specificity. In this trial,

mode of surgery is not controlled for GGO lesions. Such GGO lesions were not included in the primary analysis because of limited surgery which was indicated for these. If these lesions were included for the analysis, sensitivity may increase with a slight decrease of specificity. The point estimate of specificity was much higher for lung cancer ≤2.0 cm in size. When the cutoff value was set as 0.25, the specificity was the highest. In short, a pathological noninvasive cancer can be predicted by a C/T ratio with a cutoff value of 0.25 and a specificity of 98.7% (95% CI: 93.2–100.0%) for lung cancer ≤2.0 cm in size. Thus, we prefer to use the criteria derived from the lung window to select candidates to undergo a limited resection.

Major lung resection has been recommended as a standard procedure, even for small-sized lung cancer, because lymph node metastasis can be found in approximately 15% of lung cancers ≤2.0 cm size.[2] Nevertheless, our radiological criteria could be used to predict pathological noninvasiveness, and such patients would be candidates to undergo a limited surgical resection. Limited pulmonary resection consists of wide wedge resection or segmentectomy. As for surgical invasiveness, a wedge resection can be performed with a smaller skin incision, reduced blood loss, and a shorter operation time. On the other hand, segmentectomy offers a sufficient surgical margin. To select the optimal limited resection, the key note is the status of lymph node metastasis. A wide wedge resection should be indicated for lung cancer without lymph node involvement.

In conclusion, although our predetermined criterion for specificity was not statistically confirmed, the radiological diagnosis of noninvasive lung cancer using a thin-section CT scan correlated well with pathological invasiveness based on the exploratory investigation. We are planning to perform a study of the efficacy of limited surgical resection for lung cancers selected by the criterion using a cutoff value of 0.25 and a maximum tumor diameter ≤2.0 cm in size. We will use a wide wedge resection as the limited surgical procedure because these cases have a limited potential for nodal involvement or lymphatic/vascular invasion. We are also planning to perform a phase III trial to compare pulmonary lobectomy and segmentectomy for lung cancer ≤2.0 cm in size, excluding patients with radiological noninvasive cancer. If we obtain positive results in these future clinical trials, it will present a good opportunity to change the standard treatment for early-stage lung cancer.

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Postoperative complications and respiratory function following segmentectomy of the lung – comparison of the methods of making an inter-segmental plane☆

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Abstract

Segmentectomy could be one of the standard modes of surgery for the treatment of early lung cancer. However, segmentectomy could be more difficult than lobectomy as to the management of inter-segmental plane. The relationship between methods of dividing an inter-segmental plane and postoperative complication/pulmonary function was investigated in this study. A retrospective study was conducted on 49 patients who underwent segmentectomy of the lung between February 2008 and April 2009 at our institute. Eighteen (36.7%) were male and 31 (63.3%) were female. The inter-segmental plane was divided with only a mechanical stapler in 18 patients, and electrocautery was used in the other 31 patients. There were no significant relationships between clinicopathological features and both procedures, except gender, operative time, and pleurodesis ($P<0.05$). Preserved forced expiratory volume in one second (FEV_1) was not affected by the procedures. Patients who underwent left upper division segmentectomy had significantly more complications. On multivariate analysis, resected segment and intraoperative blood loss were found to be significant predictors for postoperative complications. There were no significant relationships between the methods of making inter-segmental planes and postoperative complications and/or lung functions. Resected segment and intraoperative blood loss were predictors for postoperative complication in segmental resection of the lung.

Keywords: Lung function; Cautery; Dividing device; Stapler

1. Introduction

Pulmonary segmentectomy is one of the options for resectable lung cancer, especially for compromised patients having severe preoperative complications, such as unstable angina, and/or chronic obstructive pulmonary disease (COPD) [1, 2]. Recently, this mode of surgery has been applied for small-sized peripheral lung cancers, as such lung cancers frequently have a minimally-invasive pathological nature [3–9], although phase III trials on the comparison between limited surgery and pulmonary lobectomy have failed to show the superiority of limited resection [10]. We have already started randomized controlled trials for the feasibility of segmentectomy for small-sized lung carcinoma [11]. There is a possibility that in the future, segmentectomy of the lung will be selected as the standard procedure for early lung cancer.

However, controversies still remain as to technical aspects of pulmonary segmentectomy. One of the most difficult points in segmentectomy may be how to make an inter-segmental plane. Some prefer electrocautery for dividing the plane, and others prefer to use a stapler. This preference should be decided based on the following factors: postoperative complication, postoperative pulmonary function, local control for lung cancer and prognosis. However, there have been few reports on these controversies. Thus, we tried to investigate the relationship between the methods for making an inter-segmental plane and postoperative lung function and/or complications.

2. Materials and methods

Between February 2008 and April 2009, lung resection was performed in 378 patients at our institute. Among them segmental resection of the lung was performed in 49 patients and this retrospective study was performed on this population. Segmentectomy was performed for peripherally located lung cancer or suspected lung cancer in the following patients: 1) compromised patients who had severe preoperative complications, such as severe angina pectoris, severe diabetes mellitus with systemic disorder, etc; 2) patients with poor lung function which is defined as postoperative predictive forced expiratory volume in one second (FEV_1) <1000 ml; 3) any patients having minimally-invasive lung cancer 2.0 cm or less in maximum tumor dimension without suspected nodal involvement. Minimally-invasive lung cancer was defined based on the findings of

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thin-section computed tomography (CT) [11]. A contrast-enhanced CT-scan was performed to evaluate the entire lung for preoperative staging. In addition, the main tumor was evaluated preoperatively to estimate the extent of ground-glass opacity (GGO) with thin-section helical CT-scan with 1–3 mm collimation. Images were reconstructed with a field of view of 15–20 cm. The lung was photographed with a window level of −500 to −700 H and a window width of 1000–2000 H as a 'lung window,' and with a window level of 30–60 H and a window width of 350–600 H as a 'mediastinal window.' The consolidation component was defined as an area of increased opacification that completely obscured the underlying vascular markings. GGO was defined as an area of a slight, homogenous increase in density that did not obscure the underlying vascular markings. Minimally-invasive lung cancer was tentatively defined as a tumor with a maximum diameter of consolidation of the maximum tumor diameter (consolidation/tumor ratio, C/T ratio) <0.5, indicating a tumor with a wide GGO area. All patients underwent posterolateral thoracotomy or anterior thoracotomy with the incision ranging from 8 to 12 cm. Eighteen (36.7%) were males and 31 (63.3%) were females (Table 1). Malignant tumors were found in 40 (81.2%). No patient underwent blood transfusion. The mean operative time was 167.3 min, with a range of 65–315 min. Resected regions were the following; right S1a+S3b in one patient, S2 in one, S3 in two, S6 in six, S8 in four, left upper division in 20, lingular division in five and S6 in six patients (Table 2).

When performing segmentectomy, the inter-segmental plane was developed with mechanical stapling and/or electrocautery. The inter-segmental plane was divided with only mechanical staplers in 18 patients, and with electrocautery in the other 31 patients. Among these 31 patients electrocarutery and staplers were used in 28 patients, i.e. the combination method. In the combination method, staplers were used mainly for the hilar side of the inter-segmental plane. We believe this technique results in decreased postoperative alveolar air leakage. When using electrocautery, the output was 60 joules for developing an inter-segmental plane.

The following factors were analyzed to investigate the relationship between these methods and the clinicopathological features; age, gender, histological diagnoses, the length of postoperative thoracic drainage, operative time, intraoperative blood loss, tumor size, postoperative pleurodesis, preoperative FEV_1, and preserved FEV_1. Preserved FEV_1 is calculated as preoperative FEV_1 divided by postoperative FEV_1 (%). Postoperative complications were investigated by the procedures. Statistical analysis was performed with uni- and multivariate analysis using logistic regression analysis. A P-value <0.05 is considered to be significant.

Table 1. Overall patient characteristics

Clinicopathological features	Number of patients
Overall	49
Gender	
Male/female	18/31
Age	
Range (mean)	24–81 (65.5)
Disease	
Primary lung cancer	33 (67.3%)
Metastatic tumor	6 (12.2%)
Benign tumor	3 (6.1%)
Others[a]	7 (14.3%)
Resected segments	
Right S1a+S3	1 (2.0%)
Right S2	1 (2.0%)
Right S3	2 (4.1%)
Right S6	9 (18.4%)
Right S8	4 (8.2%)
Left upper division	20 (40.8%)
Left lingular division	5 (10.2%)
Left S6	7 (14.3%)
Operative time (min)	
Range (mean)	65–315 (167.3)
Intraoperative blood loss (ml)	
Range (mean)	3–330 (46.6)
Methods of making an inter-segmental plain	
Stapling	18 (36.7%)
Electrocautery[b]	31 (63.3%)

[a]Others include inflammation, mucosa-associated lymphoid tissue lymphoma, giant bulla, sarcoidosis. [b]This category includes not only electrocautery but also both procedures (see text in detail).

3. Results

Stapler method and the above electrocautery method were used in 18 and 31 patients, respectively. There were no significant relationships between clinicopathological features and both procedures, except gender, operative time, and pleurodesis (Table 3). Women tended to undergo segmentectomy using electrocautery. As to operative time, segmentectomy using stapler needed more time than when using electrocautery. There were no patients who needed postoperative pleurodesis in the stapler group. However, preoperative FEV_1 was independent of the procedures, which meant that both procedures were used equally for patients having COPD. Preserved FEV_1 was not affected by the procedures.

Postoperative complications were found in 12 (29.3%) patients. The following complications occured: air leak resulting from treatments in four patients (8.2%), residual pulmonary torsion in one (2.0%), atelectasis in two (4.1%), hypoxemia in one (2.0%), atrial fibrillation in one (2.0%), liver dysfunction in one (2.0%) and infected wound in two patients (4.1%). Three of four patients with an air leak underwent chemical pleurodesis performed by the use of OK-432. Moreover, one of those three patients with chemical pleurodesis had surgical treatment to close the air leak. These three cases were as postoperative early air

Table 2. Relationship between resected segments and procedures

Segments	Stapling	Electrocautery
Overall	18	31
Right S1a+S3	0	1
Right S2	0	1
Right S3	1	1
Right S6	3	6
Right S8	2	2
Left upper division	9	11
Left lingular division	2	3
Left S6	1	6

Table 3. Relationship between clinicopathological features and procedures used for dividing inter-segmental planes

Variables	Stapling	Electrocautery[a]	*P*-value
Age	67.8 (56–81)	64.3 (24–84)	0.27
Gender (male/female)	12/6	9/22	0.013
Disease (primary lung cancer/others)	11/7	22/9	0.185
Thoracic drainage (days)	2.7	4.7	0.185
Operative time (min)	187.2	155.8	0.018
Intraoperative blood loss (ml)	66.7	34.9	0.12
Tumor size (mm)	20.6	15.6	0.092
Pleurodesis (+/−)	0/18	3/28	0.005
Preoperative FEV_1 (ml)	2.36	2.17	0.289
Preserved FEV_1[b]	90.0%	87.7%	0.652

[a]This category includes not only electrocautery but also both procedures (see text in detail). [b]Preserved FEV_1 is calculated as preoperative FEV_1 divided by postoperative FEV_1 (%).
FEV_1, forced expiratory volume in one second.

Table 4. Relationship between postoperative complications and procedures used for dividing inter-segmental planes

Variables	Stapling	Electrocautery[a]	*P*-value
Complications (+/−)	4/14	9/22	0.603

[a]This category includes not only electrocautery but also both procedures (see text in detail).

leak. Meanwhile one of four patients with an air leak experienced empyema and underwent fenestration. This case was a postoperative air leak which occurred seven months after a left upper division segmentectomy. Residual pulmonary torsion occurred in the residual left upper division after segmentectomy of the left lingular division. This case required surgical treatment. Atrial fibrillation and hypoxemia were found in the left upper division segmentectomy. The patient with postoperative hypoxemia required temporary home oxygen therapy. However, home oxygen therapy was discontinued two months after the operation.

Postoperative complications were independent of the method of dividing inter-segmental plane (Table 4). However, patients who underwent left upper division segmentectomy had significantly more complications (Table 5). Intraoperative blood loss was found to be a significant predictor for complications. On multivariate analysis, the resected segment and intraoperative blood loss were found to be significant predictors for postoperative complications (Table 6).

4. Discussion

One of the merits of segmentectomy of the lung is the preservation of postoperative pulmonary function [12]. However, segmentectomy could be associated with more postoperative complications. Segmentectomy of the lung is recognized as a difficult procedure for surgeons compared with lobectomy of the lung, as the division of inter-segmental planes is frequently troublesome. It is facile and convenient for thoracic surgeons to divide inter-segmental planes with mechanical staplers. Some surgeons prefer to use electrocautery for the division. Division with a stapler could lead to less postoperative complications and division with electrocautery can result in better postoperative lung

Table 5. Univariate analysis for the predictive factors for postoperative complications

Variables	Hazard ratio	95% CI	*P*-value[a]
Age	1.065	0.977–1.161	0.151
Gender, female	0.781	0.214–2.857	0.709
Disease, primary lung cancer	0.449	0.121–1.666	0.231
Side, left	3.929	0.757–20.375	0.103
Procedure, left upper division segmentectomy	8.667	1.968–38.157	0.004
Procedure, right/left S6 segmentectomy	0.117	0.014–0.997	0.05
Tumor size[b] (mm)	0.960	0.895–1.013	0.882
Intraoperative blood loss[b] (ml)	1.014	1.001–1.026	0.027
Operative time[b] (min)	1.432	0.369–5.552	0.603
Methods of making an inter-segmental plain (stapler vs. electrocautery[c])	1.432	0.369–5.552	0.603
Preoperative FEV_1	0.504	0.167–1.527	0.226

[a]*P*-value in logistic regression analysis. [b]Continuous valuable. [c]This category includes not only electrocautery but also both procedures (see text in detail).
CI, confidence interval; FEV_1, forced expiratory volume in one second.

Table 6. Multivariate analysis for the predictors for postoperative complications

Variables	Hazard ratio	95% CI	*P*-value[a]
Procedure, left upper division segmentectomy	9.783	1.834–52.178	0.008
Intraoperative blood loss	1.014	1.001–1.028	0.036

[a]*P*-value in logistic regression analysis.
CI, confidence interval.

function. However, there have been few reports on the relationship between the methods of dividing inter-segmental planes and postoperative complications and/or lung function.

When performing segmentectomy we prefer to use electrocautery because of the following reasons; 1) better postoperative lung function can be obtained; 2) better local control can be expected. This investigation is focused on the former point of view. In this study the decision as to which procedures were used depended on surgeons preference. However, preoperative clinical parameters were not associated with the procedures. Thus, both procedures were used equally for patients with severe complications, such as angina pectoris, diabetes mellitus, and/or COPD. Female patients tended to undergo segmentectomy using electrocautery. This observation was associated with the fact that earlier lung cancers showing GGO were found in women, though this should be investigated in the near future. Pleurodesis were not performed at all in patients who underwent segmentectomy using a stapler. This may mean segmentectomy using electrocautery resulted in more prolonged air leakage.

Patients who underwent left upper division segmentectomy had significantly more postoperative complications. Left upper division segmentectomy is considered to be equivalent to right upper lobectomy in terms of resected lung volume. This could mean resected lung volume was associated with the frequency of postoperative complications. Another significant predictor for complications was intraoperative blood loss. Prolonged air leakage may be observed in patients having pleural adhesion and this may be the reason for it. The limitation of this study may be the small number of patients investigated. Further investigations are warranted.

In conclusion, our limited investigation fails to show the significant relationship between the methods of making inter-segmental planes and postoperative complications and/or lung functions. As to the efficacy of segmental resection of the lung, a final decision should be made based on the results of the phase III trials conducted by JCOG [11].

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Clonality status of multifocal lung adenocarcinomas based on the mutation patterns of *EGFR* and *K-ras*

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ABSTRACT

Purpose: The purpose of this study is to clarify the clonality status of multifocal lung adenocarcinomas based on the mutation patterns of *epidermal growth factor receptor* (*EGFR*) and *K-ras*.
Methods: We analyzed 82 multifocal lung adenocarcinomas from 36 patients who underwent surgical resection. Genomic DNA was extracted from formalin-fixed, paraffin-embedded tissue and analyzed for *EGFR* and *K-ras* mutations. We determined the clonality status of multifocal lung adenocarcinomas based on the mutation patterns of *EGFR* and *K-ras*. The actuarial survival time was estimated and the prognostic factors were evaluated for 31 patients with synchronous multifocal lung adenocarcinomas.
Results: *EGFR* and *K-ras* mutations were detected in 36 (44%) and 19 (23%) of the 82 tumors, respectively. *EGFR* mutations had occurred randomly in 20 (91%) of the 22 patients with at least one *EGFR* mutated tumor. *K-ras* mutations had occurred randomly in 14 (93%) of the 15 patients with at least one *K-ras* mutated tumor. Combining the results for the *EGFR* and *K-ras* mutation patterns, the clonality status of multifocal lung adenocarcinomas could be determined in 30 (83%) of the 36 patients. No statistically significant difference in the actuarial survival of the patient subgroups stratified according to the clonality status, which was based on the presence of *EGFR* and *K-ras* mutations, was observed.
Conclusions: Both *EGFR* and *K-ras* mutations frequently occur randomly in multifocal lung adenocarcinomas. Combined mutation pattern analyses of *EGFR* and *K-ras* may be useful for making decisions regarding treatment strategies for patients with multifocal lung adenocarcinomas.

1. Introduction

Adenocarcinoma is now the most common histological type of lung cancer, followed by squamous cell carcinoma and small cell carcinoma. Bronchioloalveolar carcinoma (BAC) is a specific subtype of adenocarcinoma that disproportionately affects women, Asians, and non-smokers [1]. Adenocarcinomas, including BACs, frequently develop as synchronous and/or metachronous multifocal disease [2]. Although surgical resection is considered to be the best means of obtaining a definitive diagnosis and curative treatment, resecting all the lesions completely is sometimes difficult in patients with a poor cardiopulmonary function or those with numerous pulmonary lesions.

Epidermal growth factor receptor (*EGFR*) mutation is the most important predictor of the efficacy of EGFR tyrosine kinase inhibitors (TKIs) such as gefitinib and erlotinib [3–7]. In contrast, *K-ras* mutations are a useful biomarker of resistance to EGFR-TKIs [5]. Therefore, if multifocal lung adenocarcinomas simultaneously harbor *EGFR* mutations, they can likely be managed successfully using EGFR-TKIs. But, if they simultaneously harbor *K-ras* mutations, the use of EGFR-TKIs is not preferred. If *EGFR* and *K-ras* mutations are random events in multifocal lung adenocarcinomas, the efficacy of EGFR-TKIs would be limited to only the tumors carrying *EGFR* mutations, and not to those carrying *K-ras* mutations.

The purpose of this study was to clarify the clonality status of multifocal lung adenocarcinomas. The present study, to our knowledge, is the largest investigation of the clonality status of multifocal lung adenocarcinomas based on the mutation patterns of *EGFR* and *K-ras*.

2. Materials and methods

This retrospective review was performed under a waiver of authorization approved by the institutional review board of Juntendo University School of Medicine.

2.1. Patients

Between September 1996 and December 2008, 1047 patients with primary lung cancers underwent pulmonary resection. Among

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them, 57 patients had synchronous or metachronous multifocal lung cancers. Patients with pneumonic-type mucinous BAC were excluded. Patients whose tumor tissues were not available for molecular analyses were also excluded. Therefore, we conducted a retrospective review of a total of 82 multifocal lung adenocarcinomas in 36 patients.

2.2. Histological examination

The differential diagnosis of multiple primary lung cancer (MPLC) or pulmonary metastasis (PM) was clinicopathologically performed according to the criteria proposed by Martini and Melamed [8]. The proportion of the BAC component was evaluated microscopically on all the slides, including the largest cut surface of the tumor, using hematoxylin and eosin staining and elastica van Gieson staining. The BAC component was defined as the component of lepidic growth patterns of tumor cells.

2.3. Molecular analyses

DNA extraction and mutation analyses for *EGFR* and *K-ras* were conducted at Mitsubishi Chemical Medience Corporation (Tokyo, Japan). Genomic DNA was extracted from formalin-fixed, paraffin-embedded tissue. Serial slices at 5 μm were made from each block for tumor cell dissection. After deparaffinization with xylene, the tissue sections were stained with hematoxylin and eosin, and the target tumor lesions were macroscopically dissected to minimize contamination with normal tissue. The peptide nucleic acid-locked nucleic acid (PNA-LNA) polymerase chain reaction (PCR) clamp method [9] was used for *EGFR* mutation analysis, while the peptide nucleic acid (PNA)-mediated PCR clamping method [10] was used for the *K-ras* mutation analysis.

2.4. Clonality assessment

In synchronous multifocal tumors, the largest tumor was defined as the "primary tumor" and the remaining tumors were defined as "secondary tumors". In metachronous multifocal tumors, the first tumor was defined as the "primary tumor" and the tumors that developed after the surgical resection of the first tumor were defined as "secondary tumors".

First, we separately compared *EGFR* and *K-ras* mutation statuses between each primary and secondary tumor and classified the results as belonging to one of six different patterns of multifocal tumors (Table 1): pattern A, mutation in only the primary tumor; pattern B, different mutations in the primary and secondary tumors; pattern C, mutation in only the secondary tumor; pattern D, identical mutations in the primary and secondary tumors; pattern E, no mutations both in the primary and secondary tumors; and pattern F, undetermined mutation status in either the primary or secondary tumor regardless of the mutation status in the other tumor. Patterns A, B and C were regarded as indicating different clonal origins, whereas pattern D was regarded as indicating the same clonal origin. Patterns E and F were regarded as indicating an undetermined clonality status.

Table 1
Patterns of *EGFR* and *K-ras* mutations.

Clonality status	Pattern	Primary tumor	Secondary tumor
Different clonality	A	●	○
	B	●	■
	C	○	●
Same clonality	D	●	●
Not determined	E	○	○
	F	Any/?	?/any

EGFR, epidermal growth factor receptor; ●/■, mutation positive; ○, mutation negative; ?, clonality status could not be determined.

Next, we determined the clonality status based on combining the results of the mutation patterns for *EGFR* and *K-ras* genes. A secondary tumor was classified as exhibiting a different clonality if either *EGFR* or *K-ras* mutation belonged to pattern A, B or C but both *EGFR* and *K-ras* mutations did not belong to pattern D. A secondary tumor was classified as exhibiting the same clonality whenever either the *EGFR* or *K-ras* mutation belonged to pattern D. The clonality status was regarded as undetermined for secondary tumors in which both *EGFR* and *K-ras* mutations belonged to either pattern E or F.

2.5. Statistical analysis

The relationships between *EGFR*/*K-ras* mutation status and the clinicopathological features were statistically evaluated using a chi-square test or a Fisher's exact test.

Survival analyses were performed only for the patients with synchronous multifocal adenocarcinomas, since most of the patients (31/36) had synchronous tumors. The length of survival was defined as the interval in days between the day of surgical intervention and the date of either death or the last follow-up. The survival rates were calculated using the Kaplan–Meier method, and the curve differences were tested using the log-rank test. Multivariate analyses of independent prognostic factors were performed using Cox's proportional hazards model. A *P*-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using the SPSS statistical software package (version 17.0, SPSS Inc., Chicago, IL).

3. Results

3.1. Clinicopathological characteristics of patients (Table 2)

The patients comprised 18 men and 18 women. The median age at the time of the first operation was 67 years (range 44–79 years). Twenty-two patients (61%) had a smoking history (either current or ex-smoker). Synchronous multifocal adenocarcinomas were noted in 31 patients (86%) and metachronous ones were noted in 5 patients (16%). The median size of the tumors was 16 mm (range 1–105 mm). Twenty secondary tumors (43%) were located in the same lobe as the primary tumor, and 26 (57%) were located in a different lobe. The number of patients according to the pathological nodal status was 29 with N0, 3 with N1, and 4 with N2, respectively. Therefore, the majority of patients in the present study did not have lymph node involvement.

3.2. Clonality assessment based on EGFR mutation status (Table 2)

EGFR mutations were detected in 36 (44%) of the 82 tumors in total: 20 (56%) of the 36 primary tumors, and 16 (35%) of the 46 secondary tumors. The point mutation L858R in exon 21 and a deletion in exon 19 were detected in 18 and 17 tumors, respectively. T790M in exon 20, which has been recognized as a mutation that confers resistance to EGFR-TKIs, was detected in one tumor.

Simultaneously, two tumors in one patient (No. 16) harbored double *EGFR* mutations and one tumor in one patient (No. 27) had three different types of *EGFR* mutations.

Patient No. 27 had a primary tumor with three different types of *EGFR* mutations and three secondary tumors with one *EGFR* mutation (L858R in exon 21) identical to one in the primary tumor. We classified this patient as having tumors exhibiting the same clonality (pattern D), which corresponds to PM according to the *EGFR* mutation status. Because lung adenocarcinoma is morphologically

Table 2
Clinicopathological characteristics and molecular findings of 82 multifocal lung adenocarcinomas from 36 patients.

Case	P/S	Sex	Age	Pack-year	CEA	M/S	Location of secondary tumors[a]	Tumor size	BAC (%)[b]	pN	PM or MPLC[c]	*EGFR* mutation	*EGFR* mutation pattern	*K-ras* mutation	*K-ras* mutation pattern	Clonality[d]
1	P	F	57	0	4.3	S		32	30	0	MPLC	Ex21: L858R				Same
	S						Same	32	80			Ex21: L858R	D	codon12 AGT	C	
2	P	F	67	0	1.4	S		10	100	0	MPLC			codon12 GCT		Different
	S						Same	2	100				F		A	
3	P	F	44	24	2	S		18	0	0	MPLC					ND
	S						Same	2	70				F		E	
4	P	M	75	55	10.3	S		32	30	0	MPLC	Ex19: L747-T751del				Different
	S						Same	6	60				A		E	
5	P	M	61	10	2.7	S		45	20	2	MPLC			codon12 GAT		Different
	S						Same	5	40				E		A	
6	P	F	78	0	2.9	S		25	40	0	MPLC	Ex19: E746-A750del				Different
	S						Same	1	100				A		E	
7	P	F	68	0	4.3	S		33	40	0	MPLC	Ex21: L858R				Same
	S						Different	16	60			Ex21: L858R	D		E	
8	P	F	60	0.2	3.8	S		50	60	1	MPLC	Ex21: L858R				Same/Different
	S1						Different	3	60				A	codon13 AGC	C	
	S2						Same	5	100			Ex21: L858R	D		E	
9	P	M	58	0	1.2	S		23	40	0	MPLC	Ex19: E746-A750del				Different
	S						Different	5	100			Ex19: del[e]	B		E	
10	P	F	66	20	1.8	S		25	60	0	MPLC	Ex19: L747-E749del Ex19: A750P				Same/Different
	S1						Same	10	60			Ex19: L747-E749del Ex19: A750P	D		E	
	S2						Same	13	60			Ex18: G719S	B		E	
11	P	M	68	86	5.2	M		42	0	0	MPLC			codon12 TGT		Different
	S						Different	18	60				E		A	
12	P	F	74	54	14.1	S		52	80	0	MPLC	Ex19: E746-A750del				Different
	S						Same	6	100				A	codon12 GCT	C	
13	P	F	73	0	3	S		15	50	0	MPLC	Ex21: L858R				Different/Different
	S1						Same	8	100				A		E	
	S2						Different	6	100				A		E	
14	P	M	63	40	3.2	M		20	0	0	MPLC			codon12 GCT		Different
	S						Different	12	10				E		A	
15	P	F	78	0	6.5	S		40	80	0	MPLC			codon12 GAT		Different
	S						Same	7	80			Ex21: L858R	C		A	

Table 2 (*Continued*)

Case	P/S	Sex	Age	Pack-year	CEA	M/S	Location of secondary tumors[a]	Tumor size	BAC (%)[b]	pN	PM or MPLC[c]	*EGFR* mutation	*EGFR* mutation pattern	*K-ras* mutation	*K-ras* mutation pattern	Clonality[d]
16	P	F	60	0	3.1	S		32	5	2	MPLC	Ex21: L858R Ex19: del[e]				Different
	S						Different	6	30			Ex18: G719S Ex19: del[e]	B		E	
17	P	M	78	32.5	1.2	S		29	10	0	MPLC			codon12 GCT		Different
	S						Same	15	100				E	codon12 GTT	B	
18	P	M	72	52	15.2	S		36	0	2	MPLC			codon12 GCT		Different
	S						Different	13	0				E	codon12 TGT	B	
19	P	M	58	0	1.8	S		27	60	0	MPLC	Ex21: L858R				Different/Different
	S1						Different	8	90			Ex19: E746-A750del	B		E	
	S2						Different	22	10				A	codon13 GAC	C	
20	P	M	70	50	0.5	S		20	100	0	MPLC	Ex21: L858R				Different
	S						Different	5	100			Ex19: del[e]	B		E	
21	P	M	66	45	1.8	S		18	20	2	PM					ND
	S						Same	6	10				F		E	
22	P	M	74	90	23.1	S		35	0	0	PM	Ex19: E746-A750del				Different/Different
	S1						Different	25	0				A		E	
	S2						Different	25	0				A		E	
23	P	M	61	21	6.2	S		25	0	1	PM					Different/ND
	S1						Different	7	0				E		E	
	S2						Same	12	0			Ex19: E746-A750del	C		E	
24	P	M	56	19	7.1	S		105	0	0	PM	Ex18: G719S				Different
	S						Different	22	0				A		E	
25	P	M	79	90	3.6	S		70	80	0	PM					ND
	S						Same	7	100				E		E	
26	P	M	49	60	6	M		27	0	0	MPLC					Different
	S						Different	57	0				E	codon12 GAT	C	
27	P	F	68	0	4.2	M		35	40	0	MPLC	Ex19: L747-S752del Ex19: E746V Ex21: L858R Ex20: T790M				Same/Same/Same
	S1						Different	19	70			Ex21: L858R	D		E	
	S2						Different	12	100			Ex21: L858R	D		E	
	S3						Different	12	100			Ex21: L858R	D		E	
28	P	F	78	0	2.4	S		22	60	0	MPLC	Ex18: G719S				Different
	S						Same	20	90				A		E	
29	P	M	63	60	21.3	M		31	70	0	MPLC					Different
	S						Different	15	0				E	codon12 AGT	C	
30	P	F	63	0	1.4	S		21	70	0	MPLC	Ex19: E746-A750del				Different
	S						Different	15	100				A		E	