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Cyclin-dependent kinase-specific activity predicts the prognosis of stage I and stage II non-small cell lung cancer

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Abstract

Background: Lung cancer is one of the leading causes of cancer death worldwide. Even with complete resection, the prognosis of early-stage non-small cell lung cancer is poor due to local and distant recurrence, and it remains unclear which biomarkers are clinically useful for predicting recurrence or for determining the efficacy of chemotherapy. Recently, several lines of evidence have indicated that the enzymatic activity of cyclin-dependent kinases could be a clinically relevant prognostic marker for some cancers. We investigated whether the specific activity of cyclin-dependent kinases 1 and 2 could predict recurrence or death in early non-small cell lung cancer patients.

Methods: Patients with newly diagnosed, pathologically confirmed non-small cell lung cancer were entered into this blinded cohort study. The activity of cyclin-dependent kinases was determined in 171 samples by the C2P® assay, and the results were subjected to statistical analysis with recurrence or death as a clinical outcome.

Results: The Cox proportional hazards model revealed that the activity of cyclin-dependent kinase 1, but not 2, was a predictor of recurrence, independent of sex, age, and stage. By contrast, cyclin-dependent kinase 2 activity was a predictor of death, independent of sex and stage.

Conclusion: This study suggested the possible clinical use of cyclin-dependent kinase 1 as a predictor of recurrence and cyclin-dependent kinase 2 as a predictor of overall survival in early-stage non-small cell lung cancer. Thus, a combination of activity of cyclin-dependent kinases 1 and 2 is useful in decision-making regarding treatment strategies for non-small cell lung cancer after surgery.

Keywords: Non-small cell lung cancer, Cyclin-dependent kinase, Surgical resection, Recurrence, Mortality

Background

Lung cancer is one of the leading causes of cancer death worldwide. Despite recent advances in cancer treatment, the prognosis of lung cancer is not sufficient compared with that of other solid organ tumors [1]. Even after complete surgical resection, the 5-year survival of early-stage non-small cell lung cancer (NSCLC) patients is only approximately 65% [2-4]. This poor prognosis is due to the high recurrence after resection [5,6], which supports the presence of occult metastases. The survival benefit of adjuvant platinum-based chemotherapy has been established in stage II–III NSCLC [7-9]; however, there is no data supporting the use of adjuvant treatments for stage IA NSCLC, and the use of adjuvant chemotherapy for stage IB NSCLC is controversial [10]. Recently developed molecular biomarkers predict only non-squamous NSCLC [11,12], and no biomarkers for squamous cell carcinoma (SCC) have reached the validation stage. Therefore, in the setting of lung cancer, the identification of biomarkers for predicting the outcome after surgery and selecting patients who could benefit from adjuvant chemotherapy is crucial.

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A breakdown in the cell cycle machinery induces the uncontrolled proliferation of tumors. This process is initiated by a variety of molecules in a cascade that activates the cyclin-dependent kinases (CDKs), which play a role in the progression of the cell cycle. On the molecular level, the activity of CDKs is regulated by subunits known as cyclins, and by phosphorylation and dephosphorylation of key residues, for example, Thr14, Tyr15, and Thr160 in CDK2 [13]. A series of pathological investigations of the molecules that stimulate CDKs have clearly demonstrated their clinical significance for cancer diagnosis and treatment. For example, clinical evidence has indicated that the overexpression of cyclin E and cyclin B, which bind to and activate CDK2 and CDK1, respectively, correlates with tumorigenesis, prognosis, and sensitivity to chemotherapy in a variety of malignancies, as does the inactivation of CDK inhibitors, such as p21WAF1 and p27Kip1 [14-20]. The pairing between the CDK and cyclin isotypes is specific. However, the amount of cyclin protein did not correspond perfectly with CDK activity in our investigation (data not shown). Similar results regarding the association between cyclin E and the activity of its associated kinase were reported by another group [21]. Therefore, we hypothesized that the direct measurement of CDK activity might produce relevant clinical indications for cancer diagnosis and treatment. Previously, we reported that the CDK-based risk score (C2P® assay, Sysmex, Japan) predicted the risk of distant recurrence in early breast cancer patients [22]. The C2P® assay is determined using a combination of the specific activity of CDK1 (CDK1SA) and CDK2 (CDK2SA). The feasibility of this assay was confirmed in a cohort study in Caucasian breast cancer patients [23] and in colon cancer patients [24]. CDKSAs were also significantly associated with a pathologically complete response (pCR) after weekly administration of paclitaxel followed by 5-fluorouracil, epirubicin, and cyclophosphamide in breast cancer [25].

In lung cancer, many cell cycle-related molecules have been reported to be correlated with prognosis [26-31]. Here, we investigated whether the activity of CDK1 and CDK2 could predict the recurrence of NSCLC or the death of stage I and II NSCLC patients.

Methods

Study design

This blinded cohort study was approved by the local ethics committees of Tohoku University and Hyogo Cancer Centre. All patients provided written informed consent. A total of 213 patients who were newly diagnosed with pathologically confirmed NSCLC at the two centers were enrolled in this study. The eligibility criteria were as follows: SCC, adenocarcinoma, and stage I–II disease. All patients underwent complete resection, and none received adjuvant or neoadjuvant chemotherapy. CDK1SA and CDK2SA were determined in 171 samples using a C2P® assay (Sysmex, Kobe, Japan), and the results were subjected to statistical analysis to evaluate recurrence or death as a clinical outcome. Tumor tissue was dissected immediately after resection, snap-frozen and stored at −80°C at each facility. Then, the samples were sent to the Sysmex Corporation (Kobe, Japan) and subjected to the C2P® assay. Tissues with extreme blood contamination were excluded from this study, because the expression level of CDKs is underestimated in the presence of more than 1600 ng/μL of hemoglobin. The histologic types were centrally reviewed at Tohoku University.

Patients

A total of 213 patients with primary NSCLC who had undergone surgery between July 2000 and September 2009 were recruited for this study. Twenty-four cases were excluded due to extreme blood contamination of the samples. C2P® assay measurements were performed on 189 frozen samples; in 18 cases, the CDK expression levels were below the detection threshold, and these samples were excluded from the analysis. Finally, 171 cases were subjected to statistical analyses, including 53 SCCs and 118 adenocarcinomas. The median follow-up period was 43.9 months (70–2820 days).

Measurement of CDK1SA and CDK2SA

The C2P® assay [15,22] was used to measure the specific activity of CDKs. In brief, lysates of freshly frozen samples were applied to the wells of a 96-well PVDF filter plate (Millipore, Billerica, MD, USA). The expression of CDK protein was detected quantitatively by sequential reactions with primary anti-CDK antibodies, biotinylated anti-rabbit antibodies and fluorescein-labeled streptavidin. To measure the kinase activity, CDK molecules were immunoprecipitated from the lysate using protein beads. CDK SA activity (maU/eU) was calculated as CDK activity units (maU/μL lysate), which were divided by their corresponding CDK expression units (eU/μL lysate). maU (CDK activity unit) and eU (CDK expression unit) were defined as the enzyme activity and expression equivalent to 1 ng of recombinant kinase, respectively. When the expression level was lower than the detection limit of the assay, the case was excluded from the analysis. The detection limits for the expression level of CDK1 and CDK2 are 0.1 and 0.003 eU/μL lysate, respectively.

Statistical analysis

Recurrence-free survival (RFS) was calculated from the date of surgery to the date of first local or distant recurrence; patients who were alive without recurrence at the time of data collection and those who died without any evidence of the disease on the date of death were censored. The overall

survival (OS) was calculated from the date of surgery to the date of death; patients who were alive were censored.

The data were analyzed using MedCalc version 12.3 (MedCalc Software, Ostend, Belgium), and survival between the groups was compared using the Kaplan-Meier method and an unstratified Cox proportional hazards model or the log-rank test. Correlation tables were analyzed using the chi-square test. Receiver operating characteristic (ROC) curves and the corresponding area under the curve (AUC) for the compared models were computed to simulate predictive accuracy.

Possible prognostic variables that were analyzed in this study included age (≥70 *vs* <70 years), sex (male or female), tumor size (>3 *vs* ≤3 cm), nodal status (negative or positive), pathological stage (≥IB *vs* IA), histological type (SCC or adenocarcinoma), CDK1SA (≥12.6 *vs* <12.6) and CDK2SA (≥222 *vs* <222). A value of p <0.05 was considered significant.

Results

We obtained fresh-frozen samples from 213 cases from two centers: Tohoku University Hospital and Hyogo Cancer Centre. Twenty-four cases were excluded due to extreme blood contamination. The C2P® assay was performed on 189 frozen samples (see Additional file 1); in 18 cases, the CDK expression levels were below the detection threshold, and these cases were excluded from the analysis (assay success rate =90%). Finally, 171 samples were subjected to statistical analysis (Figure 1). The patients who were analyzed included 106 (62%) males and 65 (38%) females, with a median age of 70 years (38–86) (Table 1). The median tumor size was 3.0 cm (0.9–10.0). A total of 150 cases (88%) were node-negative, and 21 cases (12%) were positive. The histologic type was centrally confirmed as SCC in 53 cases (31%) and adenocarcinoma in 118 cases (69%). The overall recurrence rate and overall survival rate at final follow-up were 22% (local, 8%; distant, 14%) and 78% (133/171), respectively. Thirty-five out of 37 recurrent cases received platinum-based chemotherapy. The distribution of CDK1SA and CDK2SA did not vary significantly between the two independent cohorts based on the chi-square test (p =0.2102 and p =0.3557, respectively; Table 2). To examine the prognostic significance of the CDK1SA and CDK2SA results, ROC analysis was performed with overall recurrence as a clinical outcome.

The area under the ROC curves (AUC) of CDK1SA (p =0.0498) and CDK2SA (p =0.4206) were 0.607 and 0.545, respectively, which indicated that CDK1SA, but not CDK2SA, was likely to be predictive of recurrence. The analysis revealed that the Youden Indexes for CDK1SA and CDK2SA were maximized at 12.6 maU/eU and 222 maU/eU, respectively. Therefore, these values were tentatively set as the cut-off points in this study. Coincidentally, the optimal cut-off value of lung cancer approximated that of our colon study (11 maU/eU) [24]. The correlation analyses between CDKSA and the clinicopathologic parameters revealed that none of the parameters was associated with CDK1SA, while CDK2SA was significantly correlated with stage (p =0.0267) and histology (p <0.0001) (Table 2).

With a cut-off value of 12.6 maU/eU, the cases were classified as low CDK1SA (54%, 92 cases) or high CDK1SA (46%, 79 cases). In the Kaplan-Meier analysis, patients with low CDK1SA tumors showed significantly higher RFS than those with high CDK1SA tumors based on a log-rank test (Figure 2A, HR 2.26, 95% CI 1.18–4.32; p =0.0147); however, no prognostic value was observed (Figure 2B, p =0.0921).

CDK1SA and conventional clinicopathologic parameters, including sex (male *vs* female), age (<70 *vs* ≥70), tumor size (≤3 cm *vs* >3 cm), pathological lymph node status (positive *vs* negative), clinical stage (IA *vs* IB-IIB), and histology (SCC *vs* adenocarcinoma) were analyzed using a Cox proportional hazards model with recurrence or death as a clinical outcome (Tables 3 and 4). Univariate analysis for recurrence revealed that sex (HR 2.53, 95% CI 1.16–5.52; p =0.0200), age (HR 2.80, 95% CI 1.36–5.77; p =0.0054), tumor size (HR 1.99, 95% CI 1.04–3.81; p =0.0380), pathological lymph node status (HR 3.20, 95% CI 1.51–6.77; p =0.0025), stage (HR 2.54, 95% CI 1.30–4.99; p =0.0070) and CDK1SA (HR 2.26, 95% CI 1.16–4.43; p =0.0177) were statistically significant (p <0.05). Age, stage and CDK1SA remained significant by multivariate analysis for recurrence (Table 3, age

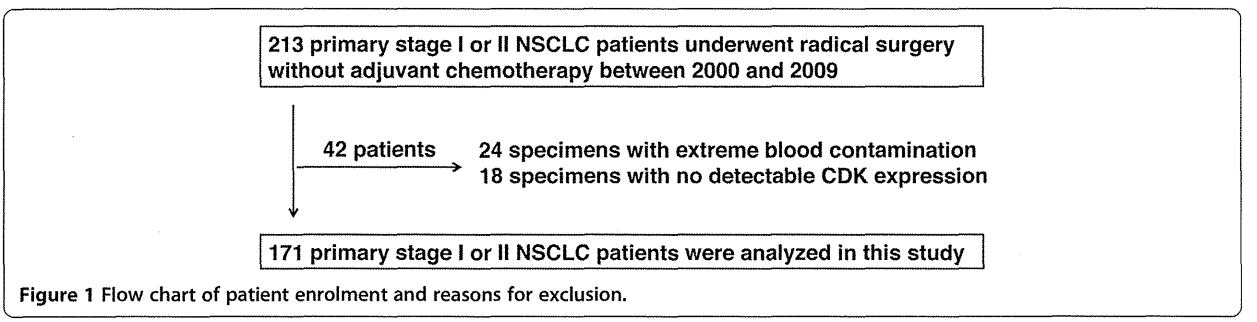

Figure 1 Flow chart of patient enrolment and reasons for exclusion.

Table 1 Clinical and pathological characteristics of patients

		Total collective	Tohoku University	Hyogo Cancer Centre
Frozen blocks available		213	111	102
Successful CDK assay		171	90	81
Sex	Male	106 (62%)	57 (63%)	49 (60%)
	Female	65 (38%)	33 (37%)	32 (40%)
Age	< 70 years	83 (49%)	43 (48%)	40 (49%)
	≥ 70 years	88 (52%)	47 (52%)	41 (51%)
Tumor size	≤ 3 cm	95 (56%)	52 (58%)	43 (53%)
	> 3 cm	76 (44%)	38 (42%)	38 (47%)
pN	–	150 (88%)	83 (92%)	67 (83%)
	+	21 (12%)	7 (8%)	14 (17%)
Stage	IA	89 (52%)	48 (53%)	41 (51%)
	IB	45 (26%)	28 (31%)	17 (21%)
	IIA	31 (18%)	11 (12%)	20 (25%)
	IIB	6 (4%)	3 (3%)	3 (4%)
Histology	Adenocarcinoma	118 (69%)	58 (64%)	60 (74%)
	SCC	53 (31%)	32 (36%)	21 (26%)
Recurrence	None	134 (78%)	81 (90%)	53 (65%)
	Local	14 (8%)	3 (3%)	11 (14%)
	Distant	23 (14%)	6 (7%)	17 (21%)
Survival information	Alive	133 (78%)	75 (83%)	58 (72%)
	Dead	38 (22%)	15 (17%)	23 (28%)

Table 2 Association between CDK-based risk groups and clinicopathological parameters

		CDK1SA (cut-off = 12.6)			CDK2SA (cut-off = 222)		
		Low	High	Significance (Chi-square)	Low	High	Significance (Chi-square)
Sex	Male	57	49		76	30	
	Female	35	30	p =0.8817	55	10	p =0.0800
Age	< 70 years	43	40		66	17	
	≥ 70 years	49	39	p =0.7230	65	23	p =0.4888
Tumor size	≤ 3 cm	55	40		76	19	
	> 3 cm	37	39	p =0.2955	55	21	p =0.3223
pN	–	85	65		118	32	
	+	7	14	p =0.0759	13	8	p =0.1544
Stage	IA	53	36		73	16	
	IB	25	20		37	8	
	IIA	12	19		18	13	
	IIB	2	4	p =0.1652	3	3	p =0.0267
Histology	Adenocarcinoma	64	54		101	17	
	SCC	28	25	p =0.9961	30	23	p <0.0001
Facility	Tohoku Univ	53	37		72	18	
	Hyogo CC	39	42	p =0.2102	59	22	p =0.3557

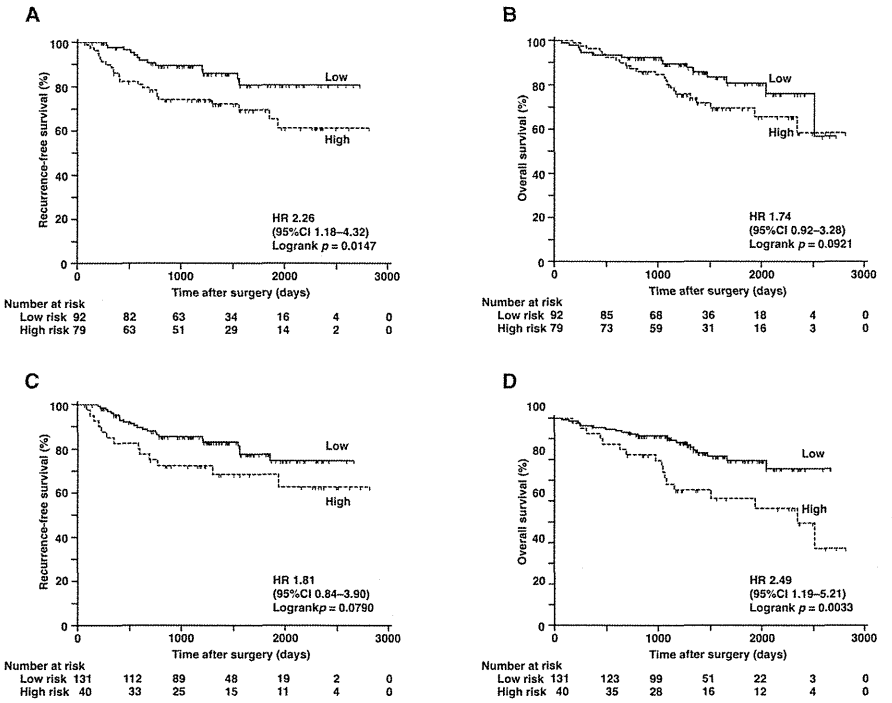

Figure 2 Analysis of recurrence and survival by risk category. (A) Recurrence-free survival and **(B)** overall survival according to CDK1SA-based risk with a cut-off value of 12.6 maU/eU. **(C)** Recurrence- free survival and **(D)** overall survival according to CDK2SA-based risk with a cut-off value of 222 maU/eU.

HR 3.06, p =0.0028; stage HR 2.16, p =0.0306; CDK1SA HR 2.25, p =0.0195). Even in the subgroup of 134 patients with stage IA and IB disease, CDK1SA but not CDK2SA had the prognostic power (Figures 3A and B, CDK1SA HR 2.56, p =0.0273; CDK2SA HR 1.18, p =0.7375). The Cox regression analysis revealed that CDK1SA was an independent predictor of recurrence (Table 5, HR 2.57, 95% CI 1.08–6.09; p =0.0335).

Regarding CDK2SA, which had a cut-off value of 222 maU/eU, the cases were classified as low CDK2SA (77%, 131 cases) or high CDK2SA (23%, 40 cases). Patients with low CDK2SA tumors showed significantly higher OS than those with high CDK2SA tumors based on a log-rank test (Figure 2D, HR 2.49, 95% CI 1.19–5.21; p =0.0033).

According to the univariate analysis for death, CDK2SA (HR 2.56, 95% CI 1.34–4.87; p =0.0045) was statistically significant along with sex (HR 5.51, 95% CI 1.96–15.5; p =0.0013), tumor size (HR 2.36, 95% CI 1.22–4.55, p =0.0111), pathological lymph node status (HR 2.82, 95% CI 1.34–5.96; p =0.0069) and stage (HR 2.75, 95% CI 1.39–5.44; p =0.0039). By multivariate analysis, sex, stage, and CDK2SA remained significant (sex HR 4.14, p =0.0081; stage HR 2.09, p =0.0421; CDK2SA HR 1.97, p =0.0500).

Table 3 Cox proportional hazards models for recurrence

		Univariate analysis		Multivariate analysis		Multivariate analysis	
		HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value
Sex	Male	2.53 (1.16–5.52)	0.0200	1.99 (0.90–4.42)	0.0913	2.00 (0.91–4.40)	0.0876
Age	≥ 70 years	2.80 (1.36–5.77)	0.0054	3.06 (1.48–6.34)	0.0028	2.79 (1.35–5.75)	0.0056
Tumor size	> 3 cm	1.99 (1.04–3.81)	0.0380				
pN	+	3.20 (1.51–6.77)	0.0025				
Stage	≥ IB	2.54 (1.30–4.99)	0.0070	2.16 (1.08–4.31)	0.0306	2.23 (1.13–4.43)	0.0222
Histology	SCC	1.23 (0.62–2.44)	0.5567				
CDK1SA	≥ 12.6	2.26 (1.16–4.43)	0.0177	2.25 (1.14–4.42)	0.0195		
CDK2SA	≥ 222	1.82 (0.93–3.56)	0.0837			1.53 (0.79–3.01)	0.2165

Table 4 Cox proportional hazards models for death

		Univariate analysis		Multivariate analysis		Multivariate analysis	
		HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Sex	Male	5.51 (1.96–15.46)	0.0013	4.29 (1.50–12.30)	0.0070	4.14 (1.45–11.78)	0.0081
Age	≥ 70 years	1.24 (0.66–2.35)	0.5088				
Tumor size	> 3 cm	2.36 (1.22–4.55)	0.0111				
pN	+	2.82 (1.34–5.96)	0.0069				
Stage	≥ IB	2.75 (1.39–5.44)	0.0039	1.79 (0.87–3.71)	0.1174	2.09 (1.03–4.24)	0.0421
Histology	SCC	2.49 (1.31–4.72)	0.0053	1.53 (0.78–2.97)	0.2156	1.23 (0.62–2.47)	0.5555
CDK1SA	≥ 12.6	1.74 (0.91–3.32)	0.0962	1.53 (0.79–2.97)	0.2122		
CDK2SA	≥ 222	2.56 (1.34–4.87)	0.0045			1.97 (1.00–3.87)	0.0500

Subanalysis by histology revealed that the predictive value of CDK1SA for recurrence was stronger in adenocarcinoma than in SCC (Figure 4A and B, adenocarcinoma HR 2.26, p =0.0439; SCC HR 2.09, p =0.2182). On the contrary, in the SCC cases, the Cox regression analysis for recurrence revealed that only CDK2SA was statistically significant (Figure 4C and D, Table 6, HR 3.86, 95% CI 1.05–14.2; p =0.0428).

In the distribution of 35 recurrent cases on a scatter diagram with logarithmic scales according to CDK1SA and CDK2SA, we observed that the distribution of the non-survivors slightly shifted to the higher CDKSAs area (data not shown). This observation let us to perform Kaplan-Meier analyses, and it was found that the prognostic power of CDK2SA, but not of CDK1SA (Figure 5A), was significant in 35 patients who were treated with chemotherapy after recurrence (Figure 5B, HR for death 4.30, 95% CI 1.56–11.8; p <0.0001).

Discussion

In this study, we demonstrated that CDK1SA and CDK2SA could identify individuals that were at high risk for recurrence and death among early-stage NSCLC patients after surgical resection. Even with complete resection, the prognosis of early-stage NSCLC is not good due to local and distant recurrence [6], and it remains unclear which biomarkers are clinically useful for predicting recurrence, although some single molecules and gene signatures of non-squamous cell carcinomas are being validated in a large number of cohorts [11,32]. Previously, cyclin expression and prognosis were reported to be correlated in lung cancer patients [33]; however, this is the first study revealing that CDK activity is a promising predictor for early-stage NSCLCs. Furthermore, CDK1SA and CDK2SA are the first biomarkers that can be used to predict the prognosis of both adenocarcinoma and SCC. Because CDKs are the targets of new anti-cancer drugs and the development of many CDK inhibitors is underway [34], this study will provide a basis for future personalized medicine using CDK inhibitors in NSCLC patients.

CDK1SA and CDK2SA have different implications in lung cancer. CDK1SA predicted recurrence after surgery, whereas CDK2SA predicted the overall survival of stage I and II NSCLC patients (Figure 2). Sub-analysis of 134 patients with stage IA and IB disease showed that CDK1SA was an independent predictor only for recurrence, but the power of prediction was much better than conventional criteria such as tumor size and stage (Figure 3 and Table 5). The clinical relevance of

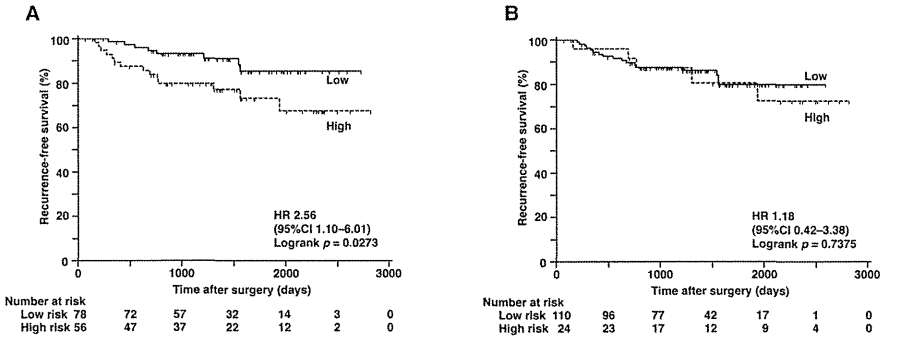

Figure 3 Analysis of recurrence by risk category in stage I NSCLC. (A) Recurrence-free survival according to CDK1SA-based risk with a cut-off value of 12.6 maU/eU. **(B)** Recurrence-free survival according to CDK2SA-based risk with a cut-off value of 222 maU/eU.

Table 5 Cox proportional hazards models for recurrence (Stage I)

		Univariate analysis	
		HR (95% CI)	*p* value
Sex	Male	2.17 (0.85–5.53)	0.1050
Age	≥ 70 years	1.79 (0.75–4.24)	0.1908
Tumor size	> 3 cm	1.41 (0.59–3.35)	0.4413
Stage	≥ IB	1.67 (0.71–3.90)	0.2390
Histology	SCC	1.10 (0.41–2.96)	0.8568
CDK1SA	≥ 12.6	2.57 (1.08–6.09)	0.0335
CDK2SA	≥ 222	1.19 (0.44–3.21)	0.7381

Table 6 Cox proportional hazards models for recurrence (SCC)

		Univariate analysis	
		HR (95% CI)	*p* value
Sex	Male	NA	
Age	≥70 years	1.48 (0.47–4.65)	0.5014
Tumor size	>3 cm	1.77 (0.48–6.52)	0.3905
pN	+	1.94 (0.59–6.44)	0.2793
Stage	≥IB	2.42 (0.54–10.99)	0.2530
CDK1SA	≥12.6	2.09 (0.63–6.91)	0.2286
CDK2SA	≥222	3.86 (1.05–14.17)	0.0428

NA, not analyzed due to bias (89% of SCC patients were male).

CDK1SA as a marker for recurrence prediction is in agreement with the conclusion of the colon study [24]. In this colon study, CDK1SA was significantly elevated in microsatellite-stable tumors. Because most of colorectal cancers with stable microsatellites demonstrate chromosomal instability [35], CDK1SA may have value as a marker of genomic instability. Genomic instability has been reported to predict clinical outcomes in multiple cancer types, including lung cancer [36-39]; therefore, prediction of recurrence demonstrated in this study may reflect the genomic instability of the tumors. According to sub-analysis by histology, the predictive value of CDK1SA for recurrence was greater in adenocarcinoma than in SCC (Figure 4A and B). Since adenocarcinoma is the most common histologic type of colon cancer, there is a similarity in the role of CDK1SA in colon and lung adenocarcinoma. Interestingly, Cox regression analysis for recurrence revealed that CDK2SA expression was statistically significant in SCC but not in adenocarcinoma (Figure 4C, D and Table 6). This result suggests that CDK1 and CDK2 have different roles in adenocarcinoma and SCC.

In contrast to CDK1SA, CDK2SA predicted overall survival after surgery. This finding may be related to the

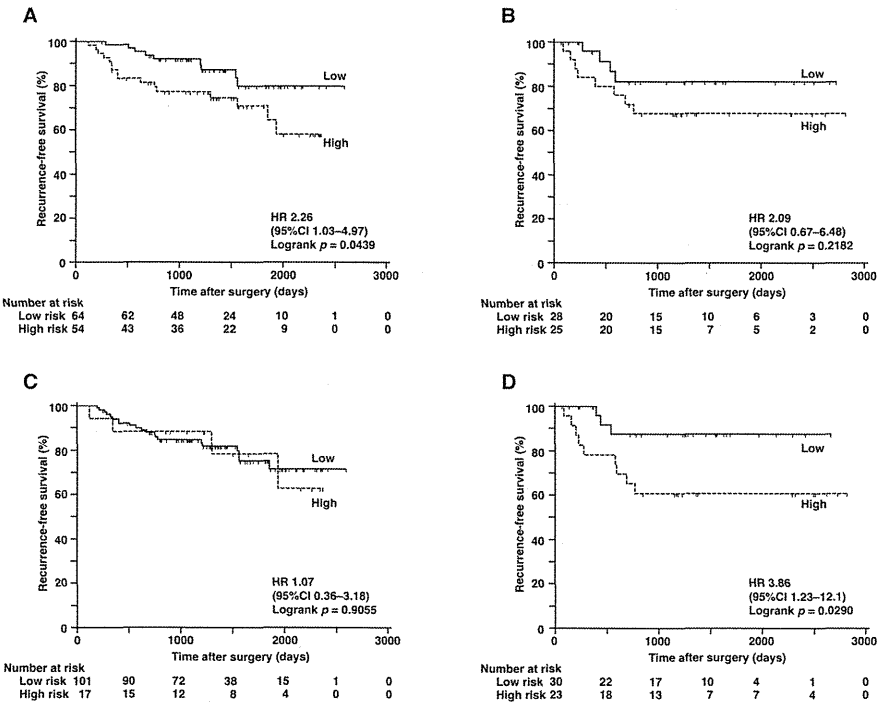

Figure 4 Analysis of recurrence by risk category in adenocarcinoma (A) (C) and SCC (B) (D). (A)(B) Recurrence-free survival according to CDK1SA-based risk with a cut-off value of 12.6 maU/eU. **(C)(D)** Recurrence-free survival according to CDK2SA-based risk with a cut-off value of 222 maU/eU.

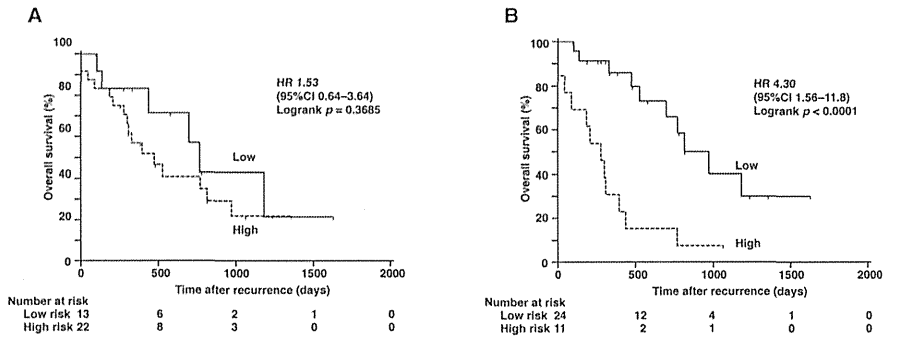

Figure 5 Analysis of survival by risk category in recurrent cases. *The zero timepoint indicates the diagnosis of recurrence.* **(A)** Survival according to CDK1SA-based risk with a cut-off value of 12.6 maU/eU. **(B)** Survival according to CDK2SA-based risk with a cut-off value of 222 maU/eU.

chemo-sensitivity of the patients. Kaplan-Meier analysis indicated that the prognostic power of CDK2SA was significant in patients treated primarily with platinum-based chemotherapy after recurrence (Figure 5), suggesting that CDK2SA can predict platinum sensitivity/resistance. A similar trend was observed in our ovary study: tumors with high CDK2SA were more platinum-resistant in patients who underwent incomplete resection and subsequent platinum-based chemotherapy (unpublished data). The ability of cyclin E-associated kinase activity to predict the response to platinum-based chemotherapy in ovarian cancer patients was also reported by another group [21]. In addition, inhibition of CDK2, but not CDK1, induced growth arrest in lung cancer cell lines through anaphase catastrophe [40]. Taken together, these data indicate that CDK2 would be a good target for lung cancer treatment, and the measurement of CDK2SA could be useful for identifying patients who would receive the full benefit of CDK2 inhibitors.

A limitation of this study was that the number of cases in the sub-analysis for the outcome after platinum-based chemotherapy was low; however, the significant difference was quite clear (Figure 5). Prospective studies should be performed to clarify the predictive capacity of CDK2SA in platinum sensitivity/resistance in early-stage NSCLC patients after surgical resection.

In summary, this study suggested the possible clinical use of CDK1SA for recurrence prediction and CDK2SA for the prognosis of stage I and II NSCLC. Moreover, CDK2SA might be a predictor of platinum-based chemotherapy sensitivity/resistance. To the best of our knowledge, this is the first report that suggests a relationship between chemosensitivity and CDK activity in lung cancer. Thus, a combination of CDK1SA and CDK2SA might be helpful in decision-making regarding NSCLC treatment strategies.

Conclusions

CDK1SA is a predictor of recurrence and CDK2SA is a predictor of overall survival in early-stage NSCLC after surgery.

Additional file

Additional file 1: Distribution of lung tumors according to CDK1SA and CDK2SA. Adenocarcinoma cases and SCC cases are plotted on a scatter diagram with logarithmic scales according to CDK1SA and CDK2SA. Black square; the specific activity was defined as 0.5 when the activity of CDK is lower than the detection limit of the assay. The detection limits for the activity of CDK1 and CDK2 are 10 and 2 maU/μL lysate, respectively.

Abbreviations

NSCLC: Non-small cell lung cancer; SCC: Squamous cell carcinoma; CDKs: Cyclin-dependent kinases; CDK1SA: Specific activity of CDK1; CDK2SA: Specific activity of CDK2.

Competing interests

This study was supported by the Sysmex Corporation (Kobe, Japan). The sponsor was involved in the study design as well as the data collection, analysis, and interpretation. ST and TM of Sysmex Corporation had access to the full raw data. HI was previously affilaited with the Sysmex Corporation, was authorized to access to the primary raw data, and performed the initial analysis of this study. However, HI is now affilaited with the other company, Nittobo Medical Co. Ltd., which has no relation with the Sysmex Corporation; and has no access to the full raw data. Otherwise, the authors declare that they have no competing interests.

Authors' contributions

HK: Conception and design, collection and assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; TS: Collection and assembly of data, pathological analysis and interpretation, final approval of manuscript; TM and ST: Collection and assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; HI: Collection and assembly of data, data analysis and interpretation, final approval of manuscript; KU, SS, MY, and TK: Provision of study material and patients, obtain informed content from the patients, final approval of manuscript. All authors read and approved the final manuscript.

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ORIGINAL ARTICLE

THORACIC

Outcomes of segmentectomy for cT1bN0M0 lung adenocarcinoma and squamous cell carcinoma: a possible association with pathological invasion

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Abstract

OBJECTIVES: We evaluated the clinical benefit of segmentectomy for patients with cT1bN0M0 lung cancer.

METHODS: We retrospectively reviewed the medical records of 178 patients who underwent lobectomy or segmentectomy for cT1bN0M0 lung adenocarcinoma and squamous cell carcinoma from January 1995 to December 2005. We investigated the association of surgical outcomes with the presence of pathological invasion.

RESULTS: The median follow-up period was 93.4 months. Of 178 patients, 37 were scheduled for segmentectomy, but 6 of these patients were switched to receive lobectomy due to surgical N1 or N2 in 3 patients and insufficient surgical margins in 3 patients. In total, 31 patients underwent segmentectomy, and 147 underwent lobectomy. The 5-year overall survival (OS) was similar between the patients who underwent lobectomy (5-year OS, 81.6%) and segmentectomy (5-year OS, 77.4%, $P = 0.73$). Among pN0 patients without pathological invasion, there was no difference in OS between patients who underwent lobectomy (5-year OS, 89.9%) and segmentectomy (5-year OS, 88.9%, $P = 0.80$). In contrast, among pN0 patients with pathological invasion, OS was greater in patients who underwent lobectomy (5-year OS, 80.9%) than in those who underwent segmentectomy (5-year OS, 54.6%; $P = 0.19$). Moreover, a significantly higher rate of local and local + distant recurrence was observed in patients who underwent segmentectomy (45%) than in those who underwent lobectomy (15%) in this group ($P = 0.02$).

CONCLUSIONS: The results of our study suggest that segmentectomy may not be recommended for cT1b tumours if pathological invasion is suspected before surgery.

Keywords: Lobectomy • Segmentectomy • Wedge resection • Lung cancer surgery • Lung cancer • Diagnosis

INTRODUCTION

Treatment of early-stage lung cancer primarily involves surgical approaches, with patients undergoing either lobectomy or segmentectomy. The relative benefit of lobectomy versus segmentectomy, or sub-lobar resection, as a standard surgical approach to treating non-small-cell lung cancer is yet to be established. In the only prospective randomized, controlled trial comparing sub-lobar resection versus lobectomy for clinical stage T1N0M0 non-small-cell lung cancer, the Lung Cancer Study Group reported that a significantly higher local recurrence rate was observed in the sub-lobar resection group than in the lobectomy group [1]. These results established lobectomy with systemic lymph node dissection as the standard surgical procedure for early-stage lung cancer. However, recent advances in radiology and application of computed tomography (CT) to patient screening have now made it possible to detect small-sized lung cancers [2], allowing for segmentectomy to again be considered as an alternative surgical procedure in these instances. Some researchers have reported a favourable outcome with segmentectomy over lobectomy for small-sized peripheral lung cancers in retrospective studies [3–10], but others have reported inferior oncological outcomes of segmentectomy to those of lobectomy [11–13]. Therefore, the issue of whether to treat small-sized peripheral cancers with lobectomy or segmentectomy remains controversial. To address this controversy, a phase III clinical trial comparing lobectomy and segmentectomy for patients with (cT1a) peripheral lung cancer, with tumour sizes up to 20 mm, is in progress in the United States (CALGB 140503) and Japan (JCOG0802/WJOG4607L). Contrary to the abundant data on segmentectomy for patients with cT1aN0M0 lung cancer, there are few reports of segmentectomy for patients with cT1bN0M0 lung cancer. In this study, we

evaluated the benefit of patients with cT1bN0M0 lung adenocarcinoma and squamous cell carcinoma by comparing oncological outcomes of segmentectomy versus lobectomy. Moreover, we investigated the association between surgical outcomes and the presence of pathological invasion. The purpose of this study was to review our experience with and evaluate the clinical benefit of segmentectomy for patients with cT1bN0M0 lung cancer.

MATERIALS AND METHODS

Patient selection

We retrospectively reviewed the medical records of 1695 consecutive patients who underwent resection for lung cancer at Hyogo Cancer Center between 1 January 1994 and 31 December 2005. We included the patients who satisfied the following criteria: (i) CT tumour size was between 21 and 30 mm with no sign of lymph node metastasis or distant metastasis (cT1bN0M0); (ii) tumour was pathologically confirmed to be adenocarcinoma or squamous cell carcinoma; (iii) tumour was not located in the right middle lobe; (iv) there was no synchronous or metachronous lung cancer; (v) lobectomy or segmentectomy with mediastinal lymphadenectomy was performed (sleeve lobectomy was excluded); (vi) complete resection was confirmed pathologically; (vii) general medical condition and lung function were able to tolerate lobectomy. We analysed 178 eligible patients. Contrast-enhanced CT was performed to evaluate the entire lung for preoperative staging. Only a few patients underwent positron emission tomography CT (PET-CT) scanning because the majority of these procedures were performed before PET-CT scan was introduced in our institution. Staging was designated by tumour, node, metastasis (TNM) classification according to the 7th edition of the American Joint Committee on Cancer Staging Manual and the Revised International System for staging lung cancer. Two expert pathologists evaluated the histopathology of all cases. After fixing the surgical specimens with 10% formalin and embedding them in paraffin, the serial sections were stained with haematoxylin and eosin and Elastica van Gieson to identify elastic tissue. The presence of vascular invasion was determined by identifying intravascular tumour cells surrounded by elastic fibres. The presence of lymphatic permeation was determined by identifying tumour cells floating in lymphatic vessels with no supporting smooth muscles or elastic fibres. We defined pathological invasion as the presence of vascular invasion or lymphatic permeation of the tumour.

This was a non-randomized retrospective study, and candidates for segmentectomy were selected according to the responsible surgeons' judgement with regard to resectability and adequate surgical margins. After receiving a thorough explanation of both surgical procedures, patients selected their preferred surgical method. Our institutional review board approved the database used in this retrospective analysis, and written informed consent was obtained from all patients.

Surgical procedure of segmentectomy

The surgical procedure of segmentectomy was performed as follows. After the corresponding segmental bronchus was isolated, every lobe was temporarily inflated. The corresponding segmental bronchus was then tied to keep the segment inflated, and the other segment was deflated naturally. The line between the inflated lung and the collapsed lung indicated the intersegmental plane. The segment was cut along the line using stapling or electrocautery. This type of operation was reported as 'extended segmentectomy' by Tsubota *et al.* [3, 14]. During surgery, the surgeon was obliged to corroborate that the tumour and required lymph nodes had been completely removed and proved to be negative for involvement by frozen-section examination. Surgical margins were routinely confirmed by dividing the resected specimens during the surgery. We defined that adequate surgical margins for segmentectomy were greater than the size of tumour. If there were signs of lymph node metastasis, or adequate surgical margins were not confirmed, the planned segmentectomy was then converted to lobectomy.

Patient follow-up

All patients were evaluated postoperatively at 3-month intervals for 2 years, at 6-month intervals for the subsequent 3 years and then once a year thereafter. Follow-up examinations included chest radiographs, CT scans, haematological and biochemical analyses, including tumour markers, along with periodic clinical follow-up.

End-points and statistical analyses

The end-points of this retrospective study were overall survival (OS) and recurrence-free survival (RFS). Local recurrence was defined as any recurrence within the same lung, ipsilateral lymph nodes or pulmonary hilum. Distant recurrence was defined as any recurrence other than local recurrence. Statistical analyses were carried out using JMP 9 software (SAS Institute, Cary, NC). Student's *t*-test and the χ^2 test were performed to assess the significance of the differences in age, sex, smoking status, surgical procedure and pathological stage (p-stage) between patients who underwent lobectomy and segmentectomy. Survival was calculated according to the Kaplan–Meier method, and differences in the distributions were evaluated by the log-rank test. The threshold for statistical significance was set at $P < 0.05$.

RESULTS

Segmentectomy was scheduled in 37 patients according to the responsible surgeons' judgement regarding resectability and adequate surgical margins using a segmental approach. Of these patients, 6 patients' procedures were converted to lobectomy during surgery. The reasons for conversion were surgical N1 or N2 in 3 patients and insufficient surgical margins in 3 patients. In total, segmentectomy was performed in 31 patients and lobectomy was performed in 147 patients (Fig. 1). There were no perioperative deaths. The median follow-up period was 90.4 and 100.4 months in patients who underwent lobectomy and segmentectomy. Clinicopathological characteristics of the 178 patients are given in Table 1. There were no significant differences in age, gender, smoking status and lung function. Adjuvant chemotherapy was administered to 26 (18%) of the 147 patients who underwent lobectomy, and 0 (0%) of the 31 patients who underwent segmentectomy ($P = 0.01$). There were no significant differences in pathological tumour size and pathological stage. Adenocarcinoma histology was more common among the patients who underwent lobectomy ($P = 0.10$). The rates of pleural invasion and vascular

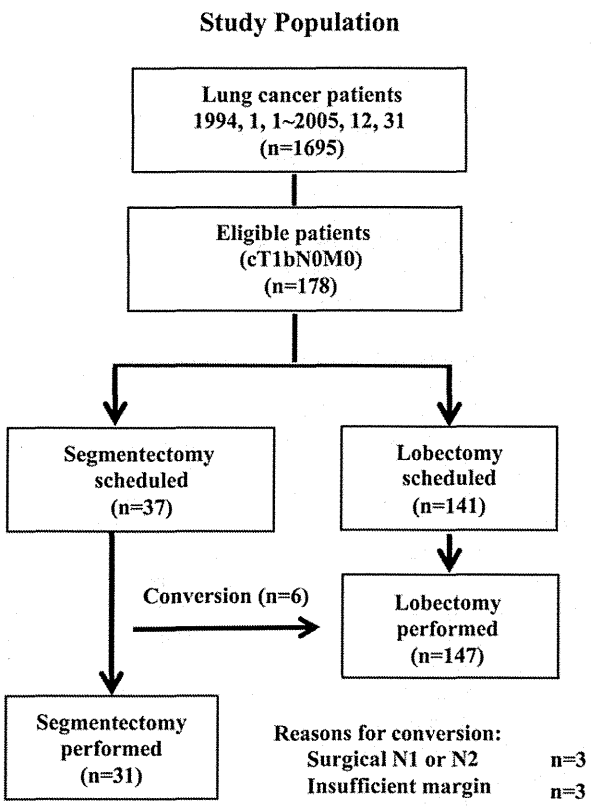

Figure 1: Scheme for the study population. In total, 147 patients underwent lobectomy and 31 patients underwent segmentectomy.

invasion were similar between the patients who underwent lobectomy and segmentectomy; however, the rate of lymphatic permeation was higher in the patients who underwent lobectomy ($P = 0.05$).

All resected lobe and segments are given in Table 2. In patients who underwent segmentectomy, more than half (54.8%) underwent left s1 + 2 + 3 segmentectomy. Figures 2A and B show the OS and RFS of the patients who underwent lobectomy and segmentectomy. There was no significant difference in either OS or RFS in patients who underwent lobectomy versus segmentectomy (5-year OS: 81.6 and 77.4%, respectively; $P = 0.73$; 5-year RFS: 71.4 and 74.2%, respectively; $P = 0.79$).

To more strictly adjust for patients' pathological background in the analysis, we then selected only pathologically confirmed pN0 (no lymph node metastasis) patients and divided them into two groups according to pathological invasiveness. Among pN0 patients without pathological invasion, there was no difference in OS or RFS between patients who underwent lobectomy and segmentectomy (5-year OS: 89.9 and 88.9%, respectively; $P = 0.80$; Fig. 3A; 5-year RFS: 84.1 and 88.9%, respectively; $P = 0.93$; Fig. 3B). In contrast, among pN0 patients with pathological invasion, greater OS and RFS were observed in patients who underwent lobectomy rather than in those who underwent segmentectomy (5-year OS: 80.9 and 54.6%, respectively; $P = 0.19$; Fig. 3C; 5-year RFS: 70.2 and 45.5%, respectively; $P = 0.28$; Fig. 3D).

Initial recurrence sites are given in Table 3. Among pN0 patients without pathological invasion, there were no differences in the recurrence rate or initial recurrence site between lobectomy and segmentectomy. However, among pN0 patients with pathological invasion, a significantly higher rate of local and local + distant

Table 1: Patient characteristics ($n = 178$)

Factors	Lobectomy ($n = 147$)	Segmentectomy ($n = 31$)	*P*-values
Mean age, range (years)	63.9 (36–87)	65.2 (51–79)	0.45
Sex			
Male	85 (58%)	19 (61%)	0.72
Female	62 (42%)	12 (39%)	
Smoking status			
Former or current	66 (55%)	17 (55%)	0.98
Never-smokers	81 (45%)	14 (45%)	
$\%FEV_{1.0}$			
Mean	98.5	101.1	0.48
Adjuvant chemotherapy			
Performed	26 (18%)	0 (0%)	0.01
Pathological tumour size			
Mean (mm)	26.6	25.2	0.30
Pathological stage			
IA	75 (51%)	17 (55%)	0.31
IB	39 (27%)	12 (39%)	
IIA	19 (13%)	1 (3%)	
IIB	2 (1%)	0 (0%)	
IIIA	12 (18%)	1 (3%)	
Histology			
Adenocarcinoma	123 (83.7%)	22 (71%)	0.10
Squamous cell	24 (16.3%)	9 (29%)	
Pleural invasion			
Absent	103 (70%)	21 (71%)	0.97
Present	44 (30%)	9 (29%)	
Vascular invasion			
Absent	94 (64%)	21 (68%)	0.69
Present	53 (36%)	10 (32%)	
Lymphatic permeation			
Absent	103 (70%)	27 (87%)	0.05
Present	44 (33%)	4 (13%)	

recurrence was observed in patients who underwent segmentectomy rather than lobectomy (45 and 15%, respectively; $P = 0.02$).

DISCUSSION

The present study demonstrated similar long-term outcomes of lobectomy and segmentectomy for cT1bN0M0 lung adenocarcinoma and squamous cell carcinoma. This study had a strong selection bias because the candidates for segmentectomy were selected according to the responsible surgeons' judgement, and patients who were scheduled for segmentectomy may have been switched to receive lobectomy if lymph node metastasis was proved by intraoperative rapid diagnosis. To minimize these kinds of biases, we selected only pN0 patients and divided them into two groups according to the presence of pathological invasion. As shown in Fig. 3C and D, there was a tendency that the outcomes of segmentectomy were inferior to those of lobectomy, and significantly higher rates of local and local + distant recurrence were observed in the patients with pathological invasion who underwent segmentectomy. From these results, we considered that the presence of pathological invasion had a strong relationship with the outcomes of lobectomy and segmentectomy. Koike *et al.* reported that pathological invasion and wedge resection were closely associated with the loco-regional recurrence of cT1aN0M0 lung non-small-cell lung cancer [15]. These results showed that even in cT1a cases, pathological invasion itself was associated with loco-regional recurrence. Therefore, patients with pathological invasion may need greater surgical margins and lymph node dissection than patients without pathological invasion. As it was obvious that lobectomy permitted better surgical margins and easier lymph node dissection than sub-lobar resection, segmentectomy for patients with cT1b tumours with pathological invasion may be inadequate to eradicate tumour cells, and these facts may have affected the oncological outcomes in this study.

On the other hand, segmentectomy may be performed for patients with T1b tumours with the same potential for cure as with lobectomy if pathological non-invasiveness could be accurately predicted before surgery. Several researchers reported the relationships between pathological invasiveness and radiological examinations. Suzuki *et al.* reported that the pathological invasiveness of cT1a lung adenocarcinoma was accurately predicted by consolidation to tumour size ratio measured by high-resolution computed tomography (HRCT) [16]. They reported that radiological non-invasive peripheral lung adenocarcinoma could be defined as an adenocarcinoma with tumour size less than 20 mm and the consolidation to a tumour size ratio less than 0.25, because cT1a adenocarcinomas that satisfy these criteria were diagnosed as pathologically non-invasive adenocarcinoma with a specificity of 98.7% [16]. Several researchers reported an association between maximum standardized uptake value (SUV_{max}) on PET-CT and the presence of pathological invasion of lung adenocarcinoma [17–19]. In a multicentre analysis, Okada *et al.* reported that stage 1A adenocarcinoma patients with revised SUV_{max} 1.5 or less rarely had pathological invasion. They also suggested that these patients might be good candidates for sub-lobar resection [19]. Several recent studies reported that solid tumour size was a more important predictive factor of pathological invasiveness and prognosis than whole tumour size [20–22]. Tsutani *et al.* reported that the presence of pathological invasion could be predicted with considerable accuracy by the combination of solid tumour size on HRCT and SUV_{max} on PET-CT [21]. In cases of T1b lung adenocarcinoma, patients with solid tumour size of less than 8 mm on HRCT or a SUV_{max} of less than 1.5 on

Table 2: Resected site of this study ($n = 178$)

Resected site	Number of patients
Lobectomy	
RUL	63
RLL	31
LUL	25
LLL	28
Segmentectomy	
Ls1 + 2 + 3	17
Ls6	5
Ls4 + 5	3
Rs7 + 8 + 9 + 10	2
Ls8 + 9 + 10	1
Rs2	1
Rs3	1
Rs2 + 6	1

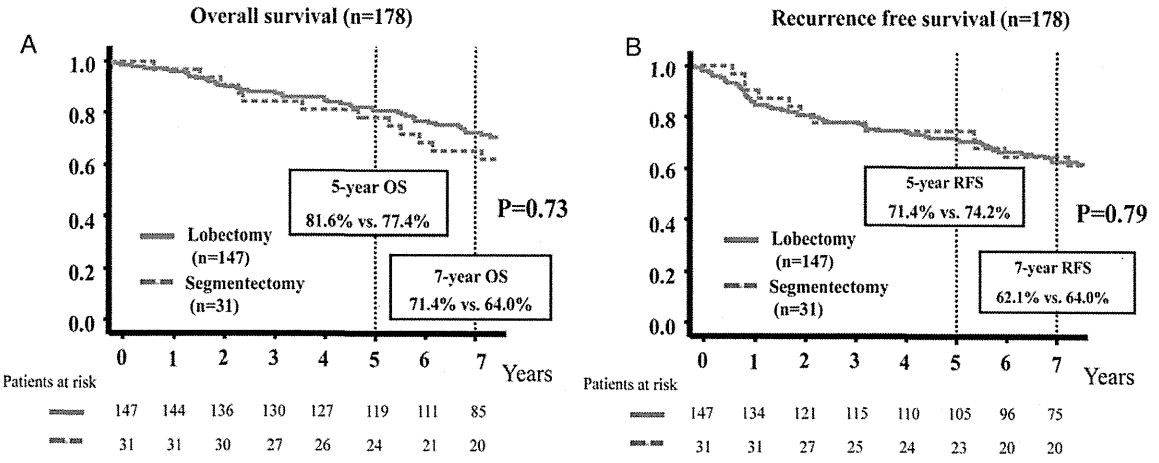

Figure 2: (**A**) Comparison of overall survival of patients who underwent lobectomy and segmentectomy. (**B**) Comparison of recurrence-free survival of patients who underwent lobectomy and segmentectomy.

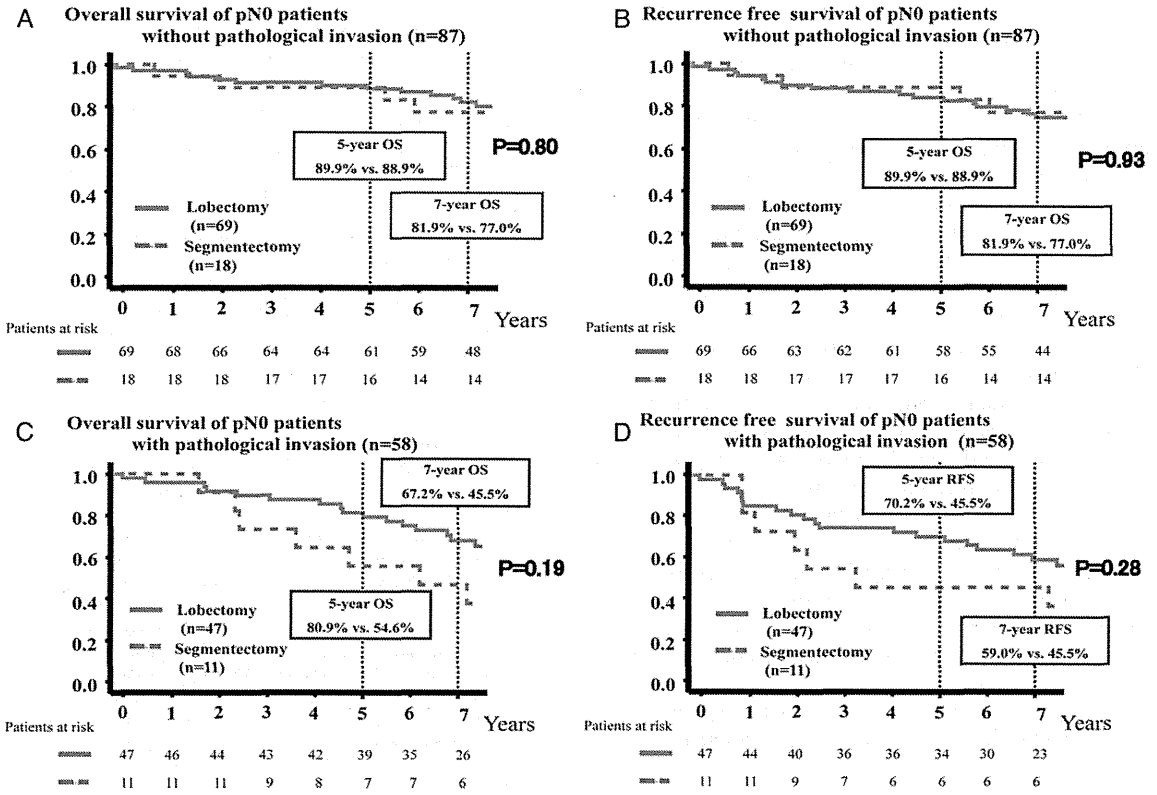

Figure 3: (**A**) Comparison of OS of pN0 patients without pathological invasion who underwent lobectomy and segmentectomy. (**B**) Comparison of RFS of pN0 patients without pathological invasion who underwent lobectomy and segmentectomy. (**C**) Comparison of the OS of pN0 patients with pathological invasion who underwent lobectomy and segmentectomy. (**D**) Comparison of the RFS of pN0 patients without pathological invasion who underwent lobectomy and segmentectomy. OS: overall survival; RFS: recurrence-free survival.

Table 3: Comparison of initial recurrence site of pN0 patients ($n = 145$)

Initial recurrence site	Pathological invasion (+)			Pathological invasion (−)		
	Lobectomy ($n = 47$)	Segmentectomy ($n = 11$)	*P* value	Lobectomy ($n = 69$)	Segmentectomy ($n = 18$)	*P* value
Patient with recurrence (%)	14 (30%)	6 (54%)	0.11	7 (10%)	2 (11%)	0.90
Local related recurrence	7 (15%)	5 (45%)	0.02	5 (7%)	1 (5.5%)	0.80
Local recurrence only	6 (13%)	3 (27%)		3 (4%)	0 (0%)	
Local + distant	1 (2%)	2 (18%)		2 (3%)	1 (5.5%)	
Distant recurrence only	7 (15%)	1 (9%)	0.61	2 (3%)	1 (5.5%)	0.58

[18F]-fluoro-2-deoxy-D-glucose (FDG) PET-CT were diagnosed as pathologically non-invasive with a specificity of 98.8% [21]. Though these studies include only adenocarcinoma cases, in contrast to the present study, which includes adenocarcinoma and squamous cell carcinoma cases, based on the results of these radiological studies as well as those of the present study, patients who satisfy these radiological criteria at screening may be candidates for segmentectomy.

One of the strengths of this study was that we performed long-term follow-up with short intervals. The median follow-up period was 93.4 months, and we performed systemic work-up more than once in a year. On the other hand, this study had certain limitations. Firstly, because this study was a retrospectively analysed, small-sized and non-randomized study, there was a strong selection bias, as mentioned above. Prospective or randomized trials are required for a definitive conclusion. Secondly, this study included old cases; some of these cases were not examined by HRCT, and only a few cases were examined by PET-CT; hence, there may be differences in accuracy between old and recent cases. Thirdly, we did not have the data of surgical margins, and could not evaluate how surgical margins may have affected the oncological outcomes. Fourthly, adjuvant chemotherapy was not administered to patients who underwent segmentectomy, whereas chemotherapy was administered to 18% of the patients who underwent lobectomy. Actually, among the patients who underwent segmentectomy, there were two patients who had lymph node metastasis ; however, they refused to receive adjuvant chemotherapy. Despite these limitations, this study provided important information in selecting surgical procedures for this group of patients.

In summary, segmentectomy can be performed for cT1bN0M0 lung adenocarcinoma and squamous cell carcinoma with the

same curability as lobectomy if the patients are carefully selected. We also found a possible association between oncological outcomes of surgical procedures and the presence of pathological invasion. If pathological non-invasiveness can be accurately predicted prior to surgery, we are confident that segmentectomy may be performed with the same oncological outcomes as lobectomy. As a previous study showed, cT1bN0M0 lung adenocarcinoma with a solid tumour size of less than 8 mm on HRCT or a SUV max of less than 1.5 on FDG PET-CT were considered to be pathologically non-invasive; therefore, these patients might be good candidates for segmentectomy. In addition, International Association for the Study of Lung Cancer (IASLC)/American Thoracic Society (ATS)/European Respiratory Society (ERS) have proposed the international multidisciplinary lung adenocarcinoma classification providing consistent terms and diagnostic criteria for adenocarcinoma subtypes [23]. This classification takes the degree of invasion as a diagnostic criterion and is reported to have a close association with clinical outcomes after surgery [24]. This may be useful in selecting candidates for segmentectomy, and so further study is needed to clarify this. Indeed, segmentectomy has many advantages over lobectomy in terms of preserving lung function and the quality of life after surgery [4, 6, 9, 25]; hence, segmentectomy is beneficial if similar oncological outcomes are expected. However, based on previous studies and the data presented in this study, rigorous patient screening must be done to select cT1bN0M0 lung cancer patients for segmentectomy so as to achieve similar clinical benefits as with lobectomy.

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Clinical significance of the 'not otherwise specified' subtype in candidates for resectable non-small cell lung cancer

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Abstract. The histological subtype of non-small-cell lung cancer (NSCLC) is a significant factor when selecting treatment strategies. However, cases are occasionally encountered that are diagnosed as 'not otherwise specified' (NOS) prior to surgery, due to an uncertain histological subtype. The present study investigated the prognostic significance of the NOS subtype for patients with resectable NSCLC. Between 2001 and 2011, 1,913 patients were diagnosed with NSCLC using transbronchial biopsy and underwent surgical resection at two facilities in Japan. Of these patients, 151 (7.9%) were pre-operatively diagnosed with NSCLC-NOS (NOS group) and the remainder had confirmed histological subtypes (confirmed group). The present study compared the clinicopathological features and prognoses of these groups. Analyses of resected specimens revealed that pleomorphic cell carcinoma, large cell neuroendocrine cell carcinoma, large cell carcinoma and adenosquamous carcinoma were significantly more common in the NOS group than in the confirmed group (P<0.001, P=0.002, P=0.019 and P=0.014, respectively). The five-year survival rate was significantly poorer in the NOS group (60.5 vs. 67.1%; P=0.010), particularly for stage I disease (70.8 vs. 80.7%; P=0.007). The results of a multivariate analysis of overall survival indicated that NOS was a significant independent prognostic factor (hazard ratio, 1.40; 95% confidence interval, 1.02-1.86; P=0.041). These results indicated that pre-operative NOS was significantly associated with poorer survival, including for stage I disease. In conjunction with other clinicopathological parameters, NOS can be a useful prognostic factor when deciding on a treatment strategy for NSCLC.

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Key words: non-small cell lung cancer, not otherwise specified, transbronchial biopsy, histological subtypes, prognostic factor, surgery

Introduction

Lung cancer is a leading cause of cancer-related mortality worldwide. From a histological perspective, the field of lung cancer treatment has been relatively static for several decades. Yet, several studies have shown that the histological subtyping of non-small cell lung cancer (NSCLC) is extremely important in predicting response rates, progression-free survival and specific drugs toxicities (1-3). For example, NSCLC subtypes differ significantly with respect to the prevalence of specific molecular alterations, including the epidermal growth factor receptor (EGFR) gene (3). Consequently, treatments are selected according to the histological subtypes of NSCLC on a daily basis.

Although novel diagnostic procedures, improved imaging modalities and new immunostaining techniques have improved histological accuracy, pathological examination occasionally fails to subtype NSCLC, leading to the rather non-specific diagnosis of NSCLC not otherwise specified (NOS). NOS diagnoses are often the consequence of small sample sizes and highly heterogeneous tumors, which limit the consistency and accuracy of subtyping using bronchoscopic biopsies. It has been reported that NOS is an unfavorable independent prognostic factor among stage IV NSCLCs, as NOS is associated with an aggressive tumor biology (4). However, the prognostic value of NOS in resectable NSCLC has not been studied.

Thoracic surgeons typically select from among the available surgical procedures according to the lung tumor type. For instance, limited resection has recently been recommended for early lung cancer that is peripherally located and exhibits a glass ground opacity (associated with minimum invasive adenocarcinoma) on thin-section computed tomography (CT) (5). Conversely, limited resection may be inappropriate as a curative surgery for certain aggressive tumors, even those that are small (6). The NOS subtype is occasionally

encountered pre-operatively, yet no surgical consensus has been established for NOS tumors.

Therefore, the present study sought to assess the association between a pre-operative NOS subtype and the prognosis of candidates for resectable NSCLC. Accordingly, this study aimed to retrospectively determine whether pre-operative NOS can provide prognostic information for patients who undergo surgical resection for NSCLC. Additionally, the study sought to clarify the association between a pre-operative NOS classification and the pathological features of the resected specimen.

Materials and methods

Patients. The clinical data of 2,519 patients with primary NSCLC who underwent complete surgical resection at the Kobe University Hospital and Hyogo Cancer Center (Kobe, Hyogo, Japan) between January 2001 and December 2011 was retrospectively examined. In total, 20 patients were excluded due to incomplete data. Of the 2,499 remaining patients, 2,309 had undergone a pre-operative bronchoscopy to establish the tumor malignancy, and 1,913 of these were diagnosed with NSCLC (396 patients were excluded due to pre-operative biopsy results that were 'negative' or 'suspicious' for malignancy). The 1,913 patients included in the present study were divided into two groups: Cases diagnosed as NOS (the NOS group) and cases with confirmed specific histological subtypes (the confirmed group), and their clinical features and outcomes were compared. The Kobe University Hospital and Hyogo Cancer Center institutional review boards approved the study and each participant provided informed consent. All patients were operated on with curative intent. The candidates for limited resection, such as segmentectomy and wedge resection, were selected by the judgment of the surgeon responsible, who considered resectability and the ability to obtain enough surgical margins from the tumor. Patients with salivary gland-type tumors, carcinoids and small cell carcinoma were excluded. All patients treated with induction therapy were also excluded. Medical records provided data on patient age, gender, body mass index (BMI), smoking status, respiratory function, stage, surgery, pathological findings, adjuvant therapy and prognosis. Contrast-enhanced CT scans of the chest, abdomen and head, bone scintigraphy and positron emission-CT since 2006 were executed routinely for pre-operative evaluation. Staging was determined according to the new International Union Against Cancer Staging System (7).

Diagnostic techniques. During the bronchoscopy sessions, cytological and histological diagnostic procedures were performed whenever feasible. The diagnostic results of cytological materials (transbronchial needle aspiration or transbronchial brushing cytology) were obtained for all cases. Histological diagnosis (bronchoscopic biopsy) was performed wherever sufficient tumor tissue material was available. If necessary, immunostaining was also performed to maximize the diagnostic accuracy using biopsy material.

Sample analysis. All samples were reviewed by two expert pathologists. Carcinomas diagnosed using pre-operative transbronchial samples were classified as adenocarcinomas, squamous cell carcinomas or NSCLC-NOS depending on the cytological diagnosis. These carcinomas were also classified as adenocarcinomas, squamous cell carcinomas, large cell carcinomas, combined tumors, adenosquamous cell carcinomas or NSCLC-NOS by histological examination. Surgical specimens were morphologically classified according to the 2004 World Health Organization classification criteria (8).

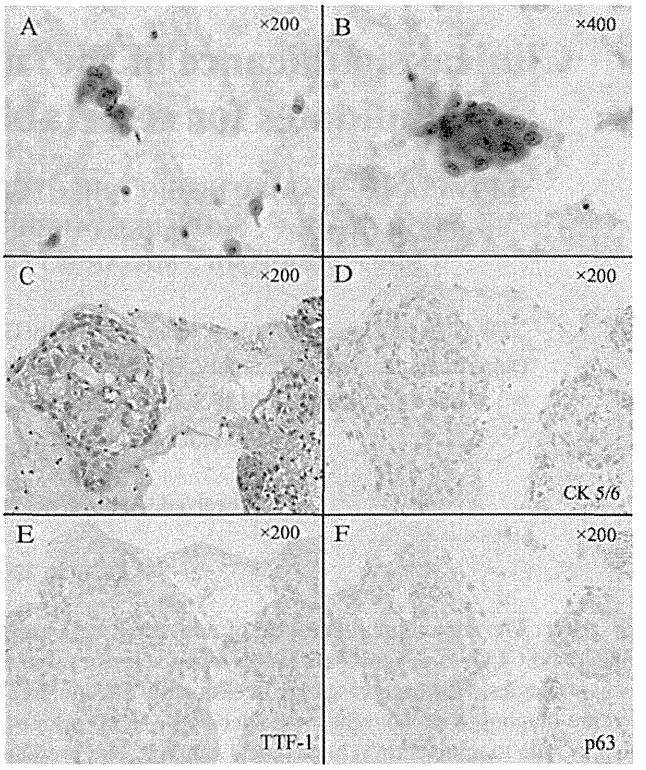

Figure 1. Representative case of diagnosed NOS. Cytological and histological examinations resulted in the classification of NSCLC-NOS due to a poorly-differentiated subtype (A and B) Papanicolaou staining of cytological specimens. (C) Hematoxylin and eosin staining of biopsy specimens. Immunohistochemistry was negative for (D) cytokeratin (CK) 5/6, (E) thyroid transcription factor-1 (TTF-1) and (F) p63, indicating that there was no differentiation toward adenocarcinoma or squamous cell carcinoma. NOS, not otherwise specified.

Representative case. A representative NOS case is shown in Fig. 1. As this case was subtyped as NSCLC-NOS according to bronchial smear and biopsy material, immunohistochemistory (IHC) was additionally performed. The IHC results were considered to indicate NSCLC-NOS if they included negative findings for thyroid transcription factor-1 (TTF-1), cytokeratin (CK)5/6 and p63 (9). The majority of pulmonary adenocarcinomas expressed TTF-1, whereas the majority of squamous cell carcinomas expressed CK5/6 and p63.

Follow-up. Post-operative follow-up generally proceeded as follows. During the 2 years after surgical intervention, systemic and local examinations were performed every six months, including blood tests, chest and abdominal CT, magnetic resonance imaging and bone scintigrams. Between three and five years post-surgery, these intensive examinations were performed every year. To check for tumor recurrence and

Table I. Clinicosurgical characteristics of the study population.

Factor	NOS group	Confirmed group	P-value
Total, n	151	1762	
Gender, n (M/F)	127/24	1194/568	<0.001
Age, years (mean ± SD)	69±9	68±9	0.453
BMI (mean ± SD)	22.4±2.8	22.3±3.0	0.521
Smoking status, n			<0.001
Smoker	130	1211	
Non-smoker	21	551	
FEV1.0, liters (mean ± SD)	2.22±0.60	2.19±0.61	0.090
FEV1.0/FVC, % (mean ± SD)	69.8±12.6	73.0±10.6	0.020
Size of tumor, mm (mean ± SD)	35.3±16.0	33.9±16.6	0.410
Procedure, n			0.159
Pneumonectomy	0	15	
Lobectomy	120	1353	
+ extended resection	14	116	
Segmentectomy	12	197	
Wedge resection	5	81	
Adjuvant chemotherapy, n (yes/no)	57/94	502/1260	0.003

FEV1.0, forced expiratory volume in 1 second; FVC, forced vital capacity; BMI, body mass index; NOS, not otherwise specified; SD, standard deviation.

determine survival, observational follow-up was continued indefinitely or for at least five years.

Statistical analysis. Statistical analyses were performed using JMP software, version 8 (SAS Institute, Cary, NC, USA). Differences between the NOS and confirmed groups were analyzed using Student's t-test and the χ^2 test with regard to gender, age, BMI, smoking status, respiratory function, size of tumor, surgical procedure, histological subtype and pathological stage between the NOS and confirmed groups. With respect to surgical procedures, segmentectomy and wedge resection were considered to be limited resection. The duration of overall survival was defined as the interval between the day of the surgery and the date of mortality (by any cause) or the last recorded follow-up. Disease-free survival was defined as the interval between resection and the proven detection of recurrence or metastases. Disease-free survival and overall survival were estimated using the Kaplan-Meier method, and differences in survival distributions were evaluated using the log-rank test. The Cox proportional hazards model was used to evaluate the association between the prognostic factors and survival rate following pulmonary resection, in terms of hazards ratios and 95% confidence intervals. $P<0.05$ was used to indicate a statistically significant difference.

Results

Included patients. Of 2,519 patients with primary NSCLC who underwent complete surgical resection between January 2001 and December 2011, 1,913 satisfied the inclusion criteria. Fig. 2 presents a flow chart of the inclusion and exclusion criteria and diagnostic procedures. The initial sample included 1,662 males and 837 females, with a median age of 69 years (range, 30-91 years). Resected tumors were 3-170 mm in size (median, 28 mm).

Diagnosis of NOS. Of the included cases, 151 (7.9%) were pre-operatively diagnosed as NOS. Table I presents the association between NOS findings and clinicosurgical factors. The NOS subtype was more frequently observed among male patients, smokers and patients with chronic obstructive pulmonary disease (COPD). In total, 57 (37.7%) patients received adjuvant chemotherapy in the NOS group, whereas 502 (28.4%) patients received adjuvant chemotherapy in the confirmed group.

A total of 88 NOS cases (58.3%) were diagnosed using cytomorphology alone. The remaining 63 NOS cases were evaluated histologically. IHC was performed in 24 of the histologically evaluated cases.

Tumor histology and staging. Table II presents the distribution of histologies and pathological stages in the NOS and confirmed groups. In the NOS group, the histopathological types were ultimately determined on the basis of the resected specimens; 60 (39.7%) adenocarcinomas, 42 (27.8%) squamous cell carcinomas, 19 (12.6%) pleomorphic cell carcinomas, 12 (7.9%) large cell neuroendocrine cell carcinomas, 8 (5.3%) adenosquamous carcinomas, 8(5.3%) large cell carcinomas and 2 (1.3%) sarcomatoid carcinomas. Pleomorphic cell carcinoma, large cell neuroendocrine cell carcinoma, large cell

Table II. Histopathological characteristics.

Factor	NOS group	Confirmed group	P-value
Total, n	151	1762	
Pathological stage, n (%)			
IA	39 (25.8)	604 (34.3)	0.127
IB	51 (33.8)	479 (27.1)	
IIA	24 (15.9)	270 (15.3)	
IIB	14 (9.3)	141 (8.0)	
IIIA	23 (15.2)	249 (14.1)	
IIIB	0 (0.0)	9 (0.5)	
IV	0 (0.0)	10 (0.6)	
Vessel invasion, n (Yes/no)	99/52	965/797	<0.001
Lymphatic invasion, n (Yes/no)	56/95	721/1041	0.350
Pleural invasion, n (P0/P1/P2/P3)	88/37/7/19	1149/330/135/148	0.053
Histology, n (%)			
Adenocarcinoma	60 (39.7)	1144 (64.9)	<0.001
Squamous cell carcinoma	42 (27.8)	481 (27.3)	0.969
Adenosquamous carcinoma	8 (5.3)	34 (1.9)	0.014
Large cell carcinoma	8 (5.3)	29 (1.6)	0.019
Large cell neuroendocrine carcinoma	12 (7.9)	47 (2.7)	0.002
Pleomorphic cell carcinoma	19 (12.6)	23 (1.3)	<0.001
Sarcomatoid carcinoma	2 (1.3)	4 (0.2)	0.074

NOS, not otherwise specified.

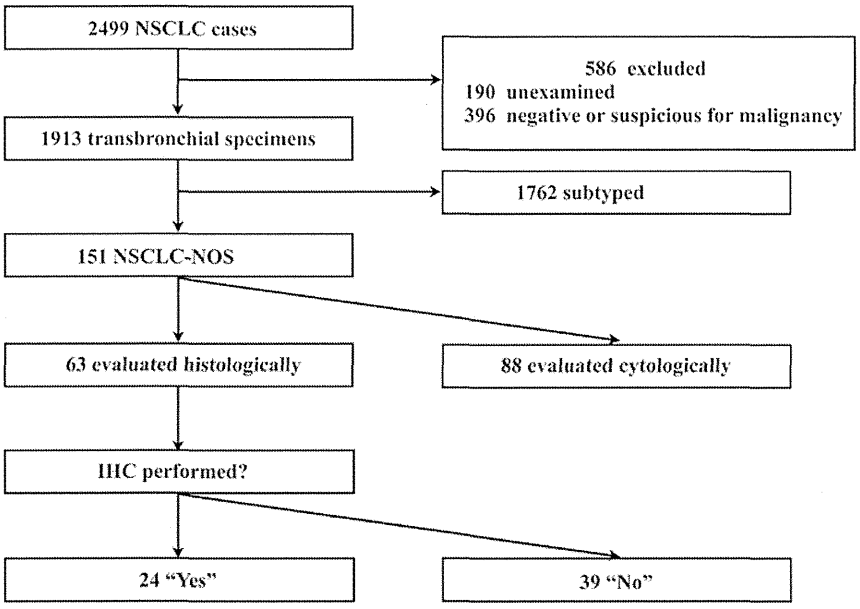

Figure 2. Flow chart of the inclusion of patients. NSCLC, non-small cell lung cancer; NOS, not otherwise specified; IHC, immunohistochemistry.

carcinoma and adenosquamous carcinoma were significantly more common in the NOS group than in the confirmed group (P<0.001, P=0.002, P=0.019 and P=0.014, respectively). The NOS group included 39 (25.8%) stage IA, 51 (33.8%) stage IB, 24 (15.9%) stage IIA, 14 (9.3%) stage IIB and 23 (15.2%) stage IIIA cases. No NOS cases were stages IIIB or IV. The pathological stage distribution did not differ significantly between the NOS and confirmed groups (P=0.127).