Analysis of Histological Grade in Resected Lung-invasive Adenocarcinoma

MOTOAKI YASUKAWA¹, CHIHO OHBAYASHI², TAKESHI KAWAGUCHI¹, NORIKAZU KAWAI¹,
TOKIKO NAKAI², NORIYOSHI SAWABATA¹ and SHIGEKI TANIGUCHI¹

¹Department of Thoracic and Cardiovascular Surgery,

Nara Medical University School of Medicine, Kashihara, Japan;

²Department of Diagnostic Pathology, Nara Medical University School of Medicine, Kashihara, Japan

Abstract. *Background/Aim:* Although the histological grading systems (Grading) with respect to lung adenocarcinoma are defined using architectural approaches in the eighth cancer Tumor, Node, and Metastasis (TNM) Classification, the prognostic value of the architectural subtypes in advanced stages of the disease remains unclear. We aimed to assess which Grading was more suitable as a prognostic factor: i) (conventional) Grading based on differentiation or ii) (new) Grading based on architectural subtypes. *Patients and Methods:* We analyzed the outcomes and Gradings of resected 449 patients with adenocarcinoma using receiver operating characteristic (ROC) curves. *Results:* The tumor histological grade of 147 out of 449 patients changed using the results from the revised Grading. In these 147 patients, ROC curves showed that the area under the curve was 0.710 using the conventional Grading and 0.567 using the new Grading. *Conclusion:* The conventional Grading might be more suitable for lung adenocarcinoma recurrence compared to the new Grading.

Lung cancer is one of the most frequent types of cancer and one of the most lethal ones worldwide (1). Although there is rapid progress in medical treatment, non-small cell lung cancer (NSCLC) has a poor prognosis with a 5-year relative survival rate in about 15% of patients (2). To manage the prognosis of NSCLC patients and predict the benefits of adjuvant chemotherapy, it is important to identify accurate and practical biomarkers (2).

Histological grading may be helpful for lung cancer management, however, no well-established histological

grading system with clearly defined criteria exists for lung adenocarcinoma (3, 4). In 2011, the International Association for the Study of Lung Cancer, the American Thoracic Society, and the European Respiratory Society (IASLC/ATS/ERS) proposed a new classification of lung adenocarcinoma, simply based on histological subtypes and a revised histological grading system (4). Previous studies have indicated that this new histological grading based on architectural subtypes has been associated with a better prognosis in stage I lung adenocarcinoma (5-7). On the other hand, an association between a better prognosis of more advanced stages of lung adenocarcinoma, and the new histological grading remains unclear (8). Furthermore, the invasive adenocarcinoma subtypes are classified predominantly according to the greatest percentage of histological component subtype (4). This means that the predominant subtype classification might not reflect the progressive phase of invasive adenocarcinoma.

Here, we assessed which grading system is more suitable for a better prognosis in all stages of completely resected lung invasive adenocarcinoma: i) the histological grading system, based on tumor cell differentiation (the conventional histological grading system) or ii) the histological grading, based on architectural predominant subtypes (the new histological grading system). In this study, we retrospectively reviewed and analyzed the outcomes of 449 patients who underwent complete resection of lung invasive adenocarcinoma of all stages, with a focus on the type of histological grading system.

Patients and Methods

Patients. We enrolled 449 patients with complete resected pathologic lung invasive adenocarcinoma who underwent lobectomy or pneumonectomy with systematic lymphadenectomy at the Nara Medical University Hospital from January 2010 to December 2016. Patients with additional malignant diseases were excluded from this study. We used video-assisted thoracoscopic surgery using a standardized three-port approach to resect the tumors. The medical

Correspondence to: Motoaki Yasukawa, Department of Thoracic and Cardiovascular Surgery, Nara Medical University School of Medicine, Kashihara, Nara 634-0822, Japan. Tel: +81 744223051, Fax: +81 744248040, e-mail: myasukawa@naramed-u.ac.jp

Key Words: Lung adenocarcinoma, histological grade, prognosis.

record of each patient was reviewed, and the pathological (p) T descriptor was reclassified, based on the 8th edition of the Tumor, Node, and Metastasis (TNM) staging (9). In addition, the histological grade was reclassified, based on the 8th edition of the TNM staging and was defined as new grade: i) G1 for lepidic adenocarcinoma, ii) G2 for acinar adenocarcinoma and Papillary adenocarcinoma, and iii) G3 for micropapillary adenocarcinoma and Solid adenocarcinoma (9). According to the classification based on the 7th edition of the TNM staging (10), conventional grade is as follows: i) G1 for well differentiated adenocarcinoma, ii) G2 for moderately differentiated adenocarcinoma, and iii) G3 for poorly differentiated adenocarcinoma. In this study, the new grading system classifications G1, G2, G3 were defined as nG1, nG2, nG3, respectively, and the conventional grading system classifications G1, G2, G3 were defined as cG1, cG2, cG3, respectively.

Each patient received medical checkups and chest X-ray examinations at least twice a year and whole-body computed tomography (CT) scans every six to twelve months during the first two years following surgical intervention and yearly thereafter. The observation period was terminated on the 30th of November 2018. This retrospective study was approved by our institute Ethics Review Board (No. 1774).

Statistical analyses. The chi-square test or Fisher's exact test were used, as appropriate, to examine the association between the two groups. Overall survival and recurrence-free survival were defined as the time from the operation to death from any cause and the first cancer relapse, respectively. Overall survival curves and recurrence-free survival curves were plotted using the Kaplan-Meier method, and the statistically significant differences between groups were assessed using the log-rank test. Univariate and multivariate Cox regression analyses were used to evaluate the hazard ratio with the endpoint of overall survival or recurrence-free survival. Age, sex, pathological diameter of tumor infiltration, ground glass nodules (GGN) on CT, pT descriptor, pN descriptor, histological type, histological grade (according to the conventional or the new classification), pleural invasion factor (PL factor), vascular invasion factor (V factor), lymphatic vessel invasion factor (LY factor), pStage, and preoperative carcinoembryonic antigen (CEA) concentration were included in the univariate analysis. Covariates found to be significant on the univariate analyses were included in the multivariate analysis. A two-sided *p*-value of less than 0.05 was considered statistically significant. All analyses were performed using EZR on R commander version 1.33 (Saitama Medical Center, Jichi Medical University, Japan) (11).

Results

During the follow-up (median 46 months, range 0-102 months), lung adenocarcinoma recurrence was identified in 23.8% of patients (107/449) and death occurred in 8.7% (39/449) of patients. The overall 5-year survival rate was 89.1% (95% confidence interval (CI): 85.1-92.1%), and the 5-year recurrence-free survival rate was 73.6% (95% CI: 68.1-78.4%). Table I shows the clinicopathological characteristics of all patients.

Based on the conventional grading system, there were 209 patients (46.5%) in the well-differentiated adenocarcinoma group (cG1), 174 patients (38.8%) in the moderately

differentiated adenocarcinoma group (cG2), and 66 patients (14.7%) in the poorly differentiated adenocarcinoma group (cG3). Using the new grading system, a total of 147 patients (32.7%) were in the lepidic adenocarcinoma group (nG1), 226 patients (50.3%) were in the acinar or papillary adenocarcinoma group (nG2), and 76 patients (16.9%) in the solid or micropapillary adenocarcinoma group (nG3). The tumor histological grade changed in 32.7% (147/449) of patients using the revised grading system during the observation period (Table II).

In a total of 449 patients, the 5-year overall survival rates in the cG1, cG2, and cG3 groups (using the conventional grading system) were 97.0% (95% CI: 92.0%-98.9%), 85.9% (95% CI: 78.3%-91.0%), and 70.0% (95% CI: 53.3%-81.7%), respectively ($p < 0.001$) (Figure 1A). The 5-year recurrence-free survival rates in the cG1, cG2, and cG3 groups were 94.0% (95% CI: 88.1%-97.0%), 63.7% (95% CI: 53.8%-72.1%), and 34.2% (95% CI: 20.4%-48.5%), respectively ($p < 0.001$) (Figure 1B). On the other hand, using the new grading system, the 5-year overall survival rates in the nG1, nG2, and nG3 groups were 98.6% (95% CI: 94.3%-99.6%), 88.5% (95% CI: 82.2%-92.7%), and 69.7% (95% CI: 54.1%-80.9%), respectively ($p < 0.001$) (Figure 2A). The 5-year recurrence-free survival rates in the nG1, nG2, and nG3 groups were 95.0% (95% CI: 88.0%-98.0%), 69.1% (95% CI: 61.0%-75.8%), and 45.1% (95% CI: 30.1%-59.0%), respectively ($p < 0.001$) (Figure 2B).

Analysis of relative risks showed that the age, the sex, the pathological diameter of tumor infiltration, pure GGN on CT, the histological grade (using both new and conventional grading systems), the PL, V and LY factors, the pT and pN descriptors, the pStage, and the preoperative CEA concentration were indicators of overall survival (Table III). Multivariate analysis showed that the histological grade (conventional grade), the pT and pN descriptors were independent predictors of overall survival in cases classified using the conventional grading system (Table IV). Regarding the new grading system, the multivariate analysis also showed that the histological grade (new grade), the pT and pN descriptors were independent predictors of overall survival (Table V).

Conversely, the analysis of relative risk with the endpoint of cancer recurrence showed that the pathological diameter of tumor infiltration, the pure GGN on CT, the histological grade (using both new and conventional grading systems), the PL, V and LY factors, the micropapillary, the pStage, the pT and pN descriptors, and the preoperative CEA concentration were indicators of cancer recurrence (Table VI). The multivariate analysis showed that the pathological diameter of tumor infiltration, the pN descriptor, the histological grade (conventional grading), and the V factor were independent predictors of recurrence using the conventional grading system (Table VII). Regarding the new grading system, multivariate analysis showed that the pathological diameter

Table I. Clinicopathological characteristics of the study population (n=449).

Characteristics	N (%)
Age	
<70 Years	239 (53.2)
≥70 Years	210 (46.8)
Gender	
Male	240 (53.5)
Female	209 (46.5)
CT findings	
Pure GGN	96 (21.4)
Other	353 (78.6)
Location	
Peripheral	366 (81.5)
Other	83 (18.5)
pStage	
I	342 (76.2)
≥II	107 (23.8)
pT descriptor	
Tmin	13 (2.9)
T1a	30 (6.7)
T1b	136 (30.3)
T1c	105 (23.4)
T2a	115 (25.6)
T2b	22 (4.9)
≥T3	28 (6.2)
Invasive size of tumor	
≤30 mm	337 (75.1)
>30 mm	112 (24.9)
pN descriptor	
pN0	375 (83.5)
pN1	42 (9.4)
≥pN2	32 (7.1)
Histological grade (conventional)	
cG1	209 (72.1)
cG2	174 (38.8)
cG3	66 (14.7)
Histological grade (new)	
nG1	147 (32.7)
nG2	226 (50.3)
nG3	76 (16.9)
PL	
Present	98 (21.8)
Absent	351 (78.2)
LY	
Present	165 (36.7)
Absent	284 (63.3)
V	
Present	140 (31.2)
Absent	309 (68.8)
EGFR mutation	
Present	75 (22.4)
Absent	260 (77.6)
CEA	
<5 ng/ml	328 (73.1)
≥5 ng/ml	121 (26.9)
Recurrence	
Yes	107 (23.8)
No	342 (76.2)
Survival	
Yes	410 (91.3)
No	39 (8.7)

CEA: Carcinoembryonic antigen, CT: computed tomography; GGN: ground glass nodule; LY: lymphatic vessel invasion factor; PL: pleural invasion factor, V: vascular invasion factor.

Table II. Univariate analysis of factors associated with the revised grading system considering all patients.

Characteristics	Without change (n=302)	With change (n=147)	p-Value
Age			
<70 Years	154 (51.0)	85 (57.8)	0.191
≥70 Years	148 (49.0)	62 (42.2)	
Gender			
Male	158 (52.3)	82 (55.8)	0.545
Female	144 (47.7)	65 (44.2)	
CT findings			
Pure GGN	72 (23.8)	24 (16.3)	0.086
Other	230 (76.2)	123 (83.7)	
Location			
Peripheral	240 (79.5)	126 (85.7)	0.121
Other	62 (20.5)	21 (14.3)	
pStage			
I	232 (76.8)	110 (74.8)	0.639
≥II	70 (23.2)	37 (25.2)	
pT descriptor			
Tmin	12 (4.0)	1 (0.7)	NA
T1a	21 (7.0)	9 (6.1)	
T1b	89 (29.5)	47 (32.0)	
T1c	67 (22.2)	38 (25.9)	
T2a	75 (24.8)	40 (27.2)	
T2b	18 (6.0)	4 (2.7)	
≥T3	20 (6.6)	8 (5.4)	
Invasive size of tumor			
≤30 mm	222 (73.5)	115 (78.2)	0.297
>30 mm	80 (26.5)	32 (21.8)	
pN descriptor			
pN0	256 (84.8)	119 (81.0)	0.562
pN1	26 (8.6)	16 (10.9)	
≥pN2	20 (6.6)	12 (8.2)	
Histological grade (conventional)			
cG1	128 (42.4)	81 (55.1)	0.028
cG2	129 (42.7)	45 (30.6)	
cG3	45 (14.9)	21 (14.3)	
Histological grade (new)			
nG1	129 (42.7)	18 (12.2)	<0.001
nG2	128 (42.4)	98 (66.7)	
nG3	45 (14.9)	31 (21.1)	
PL			
Present	61 (20.2)	37 (25.2)	0.273
Absent	241 (79.8)	110 (74.8)	
LY			
Present	97 (32.1)	68 (46.3)	0.005
Absent	205 (67.9)	79 (53.7)	
V			
Present	85 (28.1)	55 (37.4)	0.051
Absent	217 (71.9)	92 (62.6)	
EGFR mutation			
Present	41 (18.7)	34 (29.3)	0.038
Absent	178 (81.3)	82 (70.7)	
CEA			
<5 ng/ml	229 (75.8)	99 (67.3)	0.070
≥5 ng/ml	73 (24.2)	48 (32.7)	
Recurrence			
Yes	74 (24.5)	33 (22.4)	0.723
No	228 (75.5)	114 (77.6)	
Survival			
Yes	276 (91.4)	134 (91.2)	1.000
No	26 (8.6)	13 (8.8)	

CEA: Carcinoembryonic antigen; CT: computed tomography; GGN: ground glass nodule; LY: lymphatic vessel invasion factor; NA: not applicable; PL: pleural invasion factor, V: vascular invasion factor.

Figure 1. (A) Overall survival curve of all patients according to the conventional histological grading. The 5-year overall survival rates were 97.0% in the cG1 group, 85.9% in the cG2 group, and 70.0% in the cG3 group ($p < 0.001$). (B) Recurrence-free survival curve of all patients according to the conventional histological grading. The 5-year recurrence-free survival rates were 94.0% in the cG1 group, 63.7% in the cG2 group, and 34.2% in the cG3 group ($p < 0.001$).

Figure 2. (A) Overall survival curve of all patients according to the new histological grading. The 5-year overall survival rates were 98.6% in the nG1 group, 88.5% in the nG2 group, and 69.7% in the nG3 group ($p < 0.001$). (B) Recurrence-free survival curve of all patients according to the new histological grading. The 5-year recurrence-free survival rates were 95.0% in the nG1 group, 69.1% in the nG2 group, and 45.1% in the nG3 group ($p < 0.001$).

of tumor infiltration, the pN descriptor, and the V factor were independent predictors of cancer recurrence (Table VIII). Using the new histological grading system no independent predictors of cancer recurrence were identified.

Furthermore, due to evaluating and the revision of the histological grading system, all 449 patients were re-evaluated and were checked up on the changed grade (Table II). From these, we analyzed the 147 cases that changed

Figure 3. (A) Overall survival curve of 147 patients who changed histological grade according to conventional histological grading. The 5-year overall survival rates were 93.7% in the cG1 group, 83.3% in the cG2 group, and 82.9% in the cG3 group ($p=0.081$). (B) Recurrence-free survival curve of 147 patients who changed histological grade according to conventional histological grading. The 5-year recurrence-free survival rates were 88.3% in the cG1 group, 68.6% in the cG2 group, and 34.8% in the cG3 group ($p<0.001$).

Figure 4. (A) Overall survival curve of 147 patients who changed histological grade according to new histological grading. The 5-year overall survival rates were 93.8% in the nG1 group, 91.1% in the nG2 group, and 79.0% in the nG3 group ($p=0.208$). (B) Recurrence-free survival curve of 147 patients who changed histological grade according to new histological grading. The 5-year recurrence-free survival rates were 88.5% in the nG1 group, 75.9% in the nG2 group, and 63.1% in the nG3 group ($p=0.407$).

histological grade. Among the 147 patients, the 5-year overall survival rates in the cG1, cG2, and cG3 groups (using the conventional grading system) were 93.7% (95% CI: 81.4%-98.0%), 83.3% (95% CI: 65.7%-92.3%), and 82.9%

(95% CI: 54.5%-94.4%), respectively ($p=0.081$) (Figure 3A). The 5-year recurrence-free survival rates in the cG1, cG2, and cG3 groups were 88.3% (95% CI: 75.5%-94.7%), 68.6% (95% CI: 46.4%-83.1%), and 34.8% (95% CI: 12.7%-

Table III. Univariate analysis of factors influencing the survival of all patients after complete resection of the primary lung cancer.

Factors	Comparison	Hazard ratio	95% CI	p-Value
Age	≥70 vs. <70 years	2.291	1.188-4.417	0.013
Gender	Male vs. female	2.070	1.048-4.087	0.036
CT findings	Pure GGN vs. other	0.090	0.012-0.656	0.017
Location	Peripheral vs. other	0.717	0.349-1.474	0.366
Histological grade (conventional)	cG2+3 vs. cG1	2.936	1.930-4.467	<0.001
Histological grade (new)	nG2+3 vs. nG1	3.271	2.020-5.299	<0.001
pStage	≥II vs. I	9.488	4.796-18.77	<0.001
pT	≥2 vs. ≤1	4.362	2.208-8.616	<0.001
Invasive size of tumor	>30 vs. ≤30 mm	3.790	2.018-7.117	<0.001
pN	≥1 vs. 0	2.347	1.624-3.392	<0.001
PL	Present vs. absent	4.111	2.189-7.719	<0.001
V	Present vs. absent	6.942	3.450-13.97	<0.001
LY	Present vs. absent	5.355	2.609-10.99	<0.001
Micropapillary	Present vs. absent	1.906	0.744-4.881	0.1789
CEA	≥5 vs. <5 ng/ml	2.493	1.328-4.682	0.004

CEA: Carcinoembryonic antigen; CI: confidence interval; CT: computed tomography; GGN: ground glass nodule; LY: lymphatic vessel invasion factor; PL: pleural invasion factor; V: vascular invasion factor.

Table IV. Multivariate analysis of factors influencing the survival of all patients after complete resection of the primary lung cancer using the conventional grading system.

	Comparison	Hazard ratio	95% CI	p-Value
pT	≥2 vs. ≤1	2.560	1.240-5.288	0.011
pN	≥1 vs. 0	1.580	1.051-2.375	0.028
Histological grade (conventional)	cG2+3 vs. cG1	2.023	1.259-3.249	0.004

Table V. Multivariate analysis of factors influencing the survival of all patients after complete resection of the primary lung cancer using the new grading system.

	Comparison	Hazard ratio	95% CI	p-Value
pT	≥2 vs. ≤1	2.487	1.181-5.238	0.016
pN	≥1 vs. 0	1.585	1.041-2.413	0.032
Histological grade (new)	nG2+3 vs. nG1	2.176	1.270-3.726	0.005

58.3%), respectively ($p<0.001$) (Figure 3B). On the other hand, the 5-year overall survival rates in the new nG1, nG2, and nG3 groups (using the new grading system) were 93.8% (95% CI: 63.2%-99.1%), 91.1% (95% CI: 80.8%-96.0%), and 79.0% (95% CI: 55.3%-91.1%), respectively ($p=0.208$) (Figure 4A). The 5-year recurrence-free survival rates in the new nG1, nG2, and nG3 groups were 88.5% (95% CI: 61.4%-97.0%), 75.9% (95% CI: 63.6%-84.5%), and 63.1% (95% CI: 37.6%-80.5%), respectively ($p=0.407$) (Figure 4B).

Next, receiver operating characteristic (ROC) curves were drawn to compare the conventional with the new grading system, so as to assess which grading system is more suitable

for identifying prognostic factors in all stages of completely resected lung adenocarcinoma. The ROC curve of the 147 patients that changed histological grade with respect to overall survival, showed that the area under the curve (AUC) was 0.636 (95% CI: 0.489-0.783) when using the conventional grading system, and 0.603 (95% CI: 0.452-0.755) when using the new grading system ($p=0.738$) (Figure 5A). The ROC curve of 147 patients changed histological grade with respect to recurrence-free survival, showed that the AUC were 0.710 (95% CI: 0.608-0.811) when using the conventional grading system, and 0.567 (95% CI: 0.476-0.657) when using the new grading system ($p=0.035$) (Figure 5B).

Table VI. Univariate analysis of factors influencing the recurrence in all patients after complete resection of the primary lung cancer.

Factors	Comparison	Hazard ratio	95% CI	p-Value
Age	≥70 vs. <70 years	1.143	0.778-1.679	0.498
Gender	Male vs. female	1.118	0.764-1.635	0.567
CT findings	Pure GGN vs. other	0.162	0.066-0.400	<0.001
Location	Peripheral vs. other	0.794	0.512-1.231	0.303
Histological grade (conventional)	cG2+3 vs. cG1	3.058	2.382-3.926	<0.001
Histological grade (new)	nG2+3 vs. nG1	2.689	2.028-3.567	<0.001
pStage	≥II vs. I	7.982	5.363-11.88	<0.001
pT	≥2 vs. ≤1	3.670	2.462-5.472	<0.001
Invasive size of tumor	>30 vs. ≤30 mm	3.645	2.473-5.371	<0.001
pN	≥1 vs. 0	2.734	2.214-3.376	<0.001
PL	Present vs. absent	2.836	1.926-4.174	<0.001
V	Present vs. absent	5.014	3.379-7.441	<0.001
LY	Present vs. absent	5.744	3.667-8.996	<0.001
Micropapillary	Present vs. absent	2.318	1.317-4.080	0.004
CEA	≥5 vs. <5 ng/ml	2.471	1.688-3.618	<0.001

CEA: Carcinoembryonic antigen; CI: confidence interval; CT: computed tomography; GGN: ground glass nodule; LY: lymphatic vessel invasion factor; PL: pleural invasion factor; V: vascular invasion factor.

Table VII. Multivariate analysis of factors influencing the recurrence of all patients after complete resection of the primary lung cancer using the conventional grading system.

Factors	Comparison	Hazard ratio	95% CI	p-Value
Age	≥70 vs. <70 years	1.195	0.796-1.795	0.391
Gender	Male vs. female	1.161	0.768-1.758	0.479
CT findings	Pure GGN vs. other	0.665	0.250-1.770	0.414
Histological grade (conventional)	cG2+3 vs. cG1	1.826	1.354-2.464	<0.001
Invasive size of tumor	>30 vs. ≤30 mm	2.300	1.506-3.513	<0.001
pN	≥1 vs. 0	1.853	1.384-2.481	<0.001
PL	Present vs. absent	0.693	0.434-1.105	0.124
V	Present vs. absent	2.221	1.288-3.832	<0.001
LY	Present vs. absent	1.216	0.619-2.390	0.571
CEA	≥5 vs. <5 ng/ml	1.206	0.798-1.824	0.374

CEA: Carcinoembryonic antigen; CI: confidence interval; CT: computed tomography; GGN: ground glass nodule; LY: lymphatic vessel invasion factor; PL: pleural invasion factor; V: vascular invasion factor.

Table VIII. Multivariate analysis of factors influencing the recurrence of all patients after complete resection of the primary lung cancer using the new grading system.

Factor	Comparison	Hazard ratio	95% CI	p-Value
Age	≥70 vs. <70 years	1.182	0.787-1.776	0.420
Sex	Male vs. female	1.151	0.761-1.742	0.505
CT findings	Pure GGN vs. other	0.591	0.221-1.581	0.295
Histological grade (new)	nG2+3 vs. nG1	1.292	0.891-1.888	0.177
Invasive size of tumor	>30 vs. ≤30 mm	2.498	1.641-3.802	<0.001
pN	≥1 vs. 0	1.973	1.472-2.647	<0.001
PL	Present vs. absent	0.739	0.459-1.189	0.213
V	Present vs. absent	2.001	1.137-3.522	0.016
LY	Present vs. absent	1.510	0.771-2.956	0.229
CEA	≥5 vs. <5 ng/ml	1.202	0.796-1.816	0.382

CEA: Carcinoembryonic antigen; CI: confidence interval; CT: computed tomography; GGN: ground glass nodule; LY: lymphatic vessel invasion factor; PL: pleural invasion factor; V: vascular invasion factor.

Discussion

In this study, the conventional histological grade, which is based on tumor cell differentiation, was shown to be an independent predictive factor of survival and recurrence in patients at all stages of lung-invasive adenocarcinoma. The conventional histological grading system was more predictive for the prognosis of these patients compared to the new grading system. In general, the most significant predictor of recurrence and prognosis in NSCLC patients arises from using the TNM staging system. As each stage comprises a heterogeneous group of tumors with different responses to treatment and overall outcomes, a tumor-related biological prognostic factor would be very valuable for the clinicians treating these patients.

The histological grading could have important prognostic implications (4, 12). Histological grade are correlated with an unfavorable prognosis include cell differentiation and vascular invasion (12). The 2004 World Health Organization (WHO) classification (13) described the histological grade for lung adenocarcinoma as: "Grading is based on conventional histological criteria, including the extent to which the architectural pattern of the tumor resembles that of the normal lung tissue, and cytologic atypia grades 1, 2, and 3 refer to well-, moderately, and poorly differentiated tumors, respectively". Thus, the degree of tumor cell differentiation, including variations in histological architecture and cell atypia, is an important determinant of tumor biological malignancy in lung adenocarcinoma. The 2015 WHO classification simply defined grade 1 (well differentiated and lepidic predominant), grade 2 (moderately differentiated and acinar or papillary predominant), and grade 3 (poorly differentiated and solid or micropapillary predominant), based on the single most predominant architectural pattern of the adenocarcinoma (3). Sica *et al.* (5) proposed that the new histological grading system provided a reliable method for the classification of patients with stage I lung adenocarcinoma in terms of disease-free survival, and it was more objective than the conventional grading based on tumor differentiation. Furthermore, recent data suggest that the poorly differentiated solid and micropapillary histological subtypes are predictive of a survival benefit when adjuvant chemotherapy is offered to patients with surgically resected adenocarcinoma (14, 15). However, although previous studies have demonstrated that stage I adenocarcinoma can be prognostically stratified using the architectural subtypes, the prognostic value of architectural subtypes in more advanced stages of lung adenocarcinoma remains unclear (8).

Determination of accurate histological grading system might be difficult. Lung-invasive adenocarcinoma consists of a complex heterogeneous mixture of histological patterns. The invasive adenocarcinoma subtypes are now classified predominantly according to the greatest percentage of

Figure 5. (A) Receiver operatorating characteristic (ROC) curve of 147 patients who changed histological grade according to overall survival. The area under the curve (AUC) was 0.636 (95% CI: 0.489-0.783) in the conventional grading system, and 0.603 (95% CI: 0.452-0.755) in the new grading system ($p=0.738$). (B) ROC curve of 147 patients who changed histological grade according to recurrence-free survival. The AUC was 0.710 (95% CI: 0.608-0.811) in the conventional grading system, and 0.567 (95% CI: 0.476-0.657) in the new grading system ($p=0.035$).

histological component subtypes (4). This predominant subtype classification might not reflect the progressive phase of the invasive adenocarcinoma (16, 17). Ito *et al.* (16) reported that acinar or papillary predominant adenocarcinoma with no lepidic characteristics exhibits a significantly poor prognosis compared to acinar or papillary predominant adenocarcinoma with lepidic characteristics. It appears that this definition is still problematic for the grading of lung adenocarcinoma using separately architectural and nuclear approaches or their combination as there may be a divergence between the two grading systems in quite a large number of cases. For example, a tumor composed of the major components of a lepidic pattern with low cytological atypia and the minor components of a solid pattern with high cytological atypia is classified as G3 using the conventional system, while it is classified as G1 using the new system (4, 16, 17). In this study, several pathologists determined the histological grades of lung adenocarcinoma, focusing on cell differentiation in the period between 2010-2016. The conventional histological grade, based on tumor cell differentiation, was an independent predictor of prognosis and recurrence for all stages.

The TNM classification does not always accurately reflect the tumor cell biological malignancy because of the clinical, pathological, and molecular heterogeneity. Lung adenocarcinoma is the most prevalent pathologic type of NSCLC, and it comprises a heterogeneous mixture of histologic patterns with a broad spectrum of malignant behaviors. Alas, the histological grade, especially when using the conventional grading system, might reflect not only the prognosis, but also the therapeutic efficacy following recurrence. It is true that new grading system for lung invasive adenocarcinoma could be one of the prognostic factors (5-7). However, the conventional grading system might be more suitable for the prognosis of lung adenocarcinoma and cancer recurrence. The new grading system can uniformly offer diagnosis based on the architectural subtypes of lung adenocarcinoma, while the conventional histological grading system can reflect the exact biological malignancy of invasive lung adenocarcinoma based on fitting diagnoses from experienced pathologists. We have previously reported that the conventional histological grade could reflect the biological malignancy of NSCLC and could provide as a simple biomarker for NSCLC in all stages (18). Therefore, we speculate that the conventional histological grading system provides acceptable prognosis and beneficial prediction for adjuvant chemotherapy and for all the stages of resectable NSCLC.

This study has two main limitations: its retrospective design and its small sample size. More validation studies are needed before an appropriated grading system can be recommended for lung invasive adenocarcinoma.

In summary, we found that conventional histological grading is a prognostic factor for completely resected lung-invasive adenocarcinoma, despite the limitations of this study. Future studies should perform additional investigation of histological grading to determine a simple and clear-cut classification that offers optimal prognosis or treatment.

Conflicts of Interest

The Authors declare that they have no conflict of interest in regard to this study.

Authors' Contributions

MY is the corresponding author and wrote the manuscript. TN and CO performed the pathological diagnosis. TK, NK, NS, and MY participated in the surgery and postoperative management. CO and ST supervised the writing of the manuscript. All authors read and approved the final manuscript.

References

- 1 Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 68: 394-424, 2018. PMID: 30207593. DOI: 10.3322/caac.21492.
- 2 Ettinger DS, Wood DE, Aisner DL, Akerley W, Bauman J, Chirieac LR, D'Amico TA, DeCamp MM, Dilling TJ, Dobelbower M, Doebele RC, Govindan R, Gubens MA, Hennon M, Horn L and Komaki R: Non-small cell lung cancer, Version 5 2017, NCCN clinical practice guidelines in oncology. *J Natl Compr Netw* 15: 504-535, 2017. PMID: 28404761.
- 3 Geisinger K, Rami-Porta R, Moreira AL, Travis WD and Nicholson AG: Lung cancer staging and grading. *In: World Health Organization Classification of Tumors. Pathology and Genetics of the Lung, Pleura, Thymus and Heart* (Travis WD, Brambilla E, Burke AP, Marx A and Nicholson AG (eds.). Lyon: IARC Press, pp. 14-15, 2015.
- 4 Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger KR, Yatabe Y, Beer DG, Powell CA, Riely GJ, Van Schil PE, Garg K, Austin JH, Asamura H, Rusch VW, Hirsch FR, Scagliotti G, Mitsudomi T, Huber RM, Ishikawa Y, Jett J, Sanchez-Cespedes M, Sculier JP, Takahashi T, Tsuboi M, Vansteenkiste J, Wistuba I, Yang PC, Aberle D, Brambilla C, Flieder D, Franklin W, Gazdar A, Gould M, Hasleton P, Henderson D, Johnson B, Johnson D, Kerr K, Kuriyama K, Lee JS, Miller VA, Peterson I, Roggli V, Rosell R, Saijo N, Thunnissen E, Tsao M and Yankelewitz D: International association for the study lung cancer/american thoracic society/european respiratory society international multidisciplinary classification of lung adenocarcinoma. *J Thorac Oncol* 6: 244-285, 2011. PMID: 21252716. DOI: 10.1097/JTO.0b013e318206a221.
- 5 Sica G, Yoshizawa A, Sima CS, Azzoli CG, Downey RJ, Rusch VW, Travis WD and Moreira AL: A grading system of lung adenocarcinoma based on histologic pattern is predictive of disease recurrence in stage I tumors. *Am J Surg Pathol* 34: 1155-1162, 2010. PMID: 20551825. DOI: 10.1097/PAS.0b013e3181e4ee32.

- 6 Hung JJ, Jeng WJ, Chou TY, Hsu WH, Wu KJ, Huang BS and Wu YC: Prognostic value of the new International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society lung adenocarcinoma classification on death and recurrence in completely resected stage I lung adenocarcinoma. *Ann Surg* 258: 1079-1086, 2013. PMID: 23532112. DOI: 10.1097/SLA.0b013e31828920c0.
- 7 Yoshizawa A, Motoi N, Riely GJ, Sima CS, Gerald WL, Kris MG, Park BJ, Rusch VW and Travis WD: Impact of proposed IASLC/ATS/ERS classification of lung adenocarcinoma: prognostic subgroups and implications for further revision of staging based on analysis of 514 stage I cases. *Mod Pathol* 24: 653-664, 2011. PMID: 21252858. DOI: 10.1038/modpathol.2010.232.
- 8 Westaway DD, Toon CW, Farzin M, Sioson L, Watson N, Brady PW, Marshman D, Mathur MM and Gill AJ: The International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society grading system has limited prognostic significance in advanced resected pulmonary adenocarcinoma. *Pathology* 45: 553-558, 2013. PMID: 24018816. DOI: 10.1097/PAT.0b013e32836532ae.
- 9 Brierley JD, Gospodarowicz MK and Wittekind C: *TNM Classification of Malignant Tumours*. 8th ed. NJ, Wiley-Blackwell, 2016.
- 10 Sobin LH, Gospodarowicz MK and Wittekind C: *TNM Classification of Malignant Tumours*. 7th ed. NJ, Wiley-Blackwell, 2011.
- 11 Kanda Y: Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant* 48: 452-458, 2013. PMID: 23208313. PMCID: PMC3590441. DOI: 10.1038/bmt.2012.244.
- 12 Chung CK, Zaino R, Stryker JA, O'Neill M Jr and DeMuth WE Jr: Carcinoma of the lung: evaluation of histological grade and factors influencing prognosis. *Ann Thorac Surg* 33: 599-604, 1982. PMID: 7092385.
- 13 Colby TV, Ohori P, Jambhekar NA, Noguchi M, Rami-Porta R, Petersen I, Henschke C, Franks T, Takahashi T, Vazquez MF, Shimosato Y, Kawai T, Geisinger K, Matsuno Y, Meyerson M, Yokose T, Khor A, Hanash SM, Westra WH and Jen J: Adenocarcinoma. In: *World Health Organization Classification of Tumors. Pathology and Genetics of the Lung, Pleura, Thymus and Heart* (Travis WD, Brambilla E, Müller-Hermelink HK and Harris CC (eds.). Lyon, IARC Press, pp. 35-44, 2004.
- 14 Yoshiya T, Mima T, Tsutani Y, Tsubokawa N, Sasada S, Miyata Y, Kushitani K, Takeshima Y, Murakami S, Ito H, Nakayama H and Okada M: Prognostic role of subtype classification in small-sized pathologic N0 invasive lung carcinoma. *Ann Thorac Surg* 102: 1668-1673, 2016. PMID: 27344277. DOI: 10.1016/j.athoracsur.2016.04.087.
- 15 Russell PA and Wright GM: Predominant histologic subtype in lung adenocarcinoma predicts benefit from adjuvant chemotherapy in completely resected patients: discovery of a holy grail? *Ann Transl Med* 4: 16, 2016. PMID: 26855952. PMCID: PMC4716942. DOI: 10.3978/j.issn.2305-5839.2015.10.21.
- 16 Ito M, Miyata Y, Yoshiya T, Tsutani Y, Mimura T, Murakami S, Ito H, Nakayama H and Okada M: Second predominant subtype predicts outcomes of intermediate malignant invasive lung adenocarcinoma. *Eur J Cardiothorac Surg* 51: 218-222, 2017. PMID: 28186287. DOI: 10.1093/ejcts/ezw318.
- 17 Takahashi Y, Eguchi T, Kameda K, Lu S, Vaghjiani RG, Tan KS, Travis WD, Jones DR and Adusumilli PS: Histologic subtyping in pathologic stage I-IIA lung adenocarcinoma provides risk-based stratification for surveillance. *Oncotarget* 9: 35742-35751, 2018. PMID: 30515266. PMCID: PMC6254662. DOI: 10.18632/oncotarget.26285.
- 18 Yasukawa M, Sawabata N, Kawaguchi T, Kawai N, Nakai T, Ohbayashi C and Taniguchi S: Histological grade: analysis of prognosis of non-small cell lung cancer after complete resection. *In Vivo* 32: 1505-1512, 2018. PMID: 30348709. DOI: 10.21873/invivo.11407.

Received January 6, 2019

Revised January 30, 2019

Accepted January 31, 2019