Histologic grade is an independent prognostic factor for survival in non-small cell lung cancer: An analysis of 5018 hospital- and 712 population-based cases

Zhifu Sun, MD, MS,^a Marie-Christine Aubry, MD,^b Claude Deschamps, MD,^c Randolph S. Marks, MD,^d Scott H. Okuno, MD,^d Brent A. Williams, MS,^e Hiroshi Sugimura, MD,^a V. Shane Pankratz, PhD,^e and Ping Yang, MD, PhD^a

Objective: Our objective was to determine whether histologic grade independently contributes to the prognosis of non-small cell lung cancer.

Methods: A total of 5018 hospital-based patients diagnosed from 1997 to 2003 and 712 population-based patients diagnosed from 1984 to 2003 were followed up through the end of 2004. The effect of histologic grade on postdiagnosis survival or postresection recurrence was evaluated by Cox proportional hazards models. Relative risks (RR) were estimated by comparing undifferentiated, poorly differentiated, and moderately differentiated carcinoma with well-differentiated carcinoma.

Results: Histologic grade was significantly associated with survival after adjustment for the effects of age, gender, smoking history, tumor stage, histologic cell type, and treatment modality. Patients with undifferentiated carcinoma had an 80% elevated risk of death (RR = 1.83; 95% confidence interval [CI], 1.4-2.4) compared with those with well-differentiated carcinoma; 70% and 40% elevated risks were observed for patients with poorly and moderately differentiated carcinoma, respectively (RR, 1.7 [1.5-2.0] and 1.4 [1.2-1.6]). Similar results were observed for 718 incidence cases in which the relative risks were 1.6 (1.1-2.2) and 1.4 (1.0-1.9) for poorly/undifferentiated carcinoma and moderately differentiated carcinoma, respectively. Patients with less-differentiated carcinoma after tumor resection had a higher risk of recurrence, with adjusted hazard ratios of 2.1 (95% CI: 1.4-2.9) and 1.4 (1.0-1.9) for poorly/undifferentiated and moderately differentiated carcinoma compared with well-differentiated carcinoma.

Conclusions: Histologic grade has significant prognostic value for survival of patients with non-small cell lung cancer. Histologic grade may provide useful information in defining the aggressiveness of tumors and should be considered as an independent factor affecting survival beyond TNM staging.

From the Divisions of Epidemiology,^a Anatomic Pathology,^b Thoracic Surgery,^c Medical Oncology,^d and Biostatistics,^e Mayo Clinic College of Medicine, Rochester, Minn.

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Address for reprints: Ping Yang, MD, PhD, Mayo Clinic, Department of Health Sciences Research, 200 First Street SW, Rochester, MN 55905 (E-mail: yang.ping@mayo.edu).

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Lung cancer is the leading cause of cancer death for both men and women in the United States and many regions of the world.¹ Only a few prognostic factors have been established for assisting patient management and predicting clinical outcome. For non-small cell lung cancer (NSCLC), histologic grading is routinely based on the degree of tumor cell differentiation and reported along with the histologic subtype and other information (tumor size, margin, and lymph node involvement). However, the role of histologic grade as a prognostic factor has rarely been systematically evaluated. The correlation of histologic grade with cancer prognosis was first described for squamous cell carcinoma of the skin by Broders² in 1920 and later was found in carcinomas of many other organs, such as the rectum, breast, kidney, and prostate. Patients with high-grade disease more often had a higher stage at diagnosis, a greater chance of recurrence or metastasis, and a shorter survival time after diagnosis. The predictive value of histologic grade prompted the development of specific grading systems for some tumors, such as Gleason's^{2a} grading for prostate carcinoma,

Abbreviations and Acronyms

CI	= confidence interval
NSCLC	= non-small cell lung cancer
RR	= relative risk

Furhman's^{2b} grading for renal cell carcinoma, and Elston's^{2c} grading for breast carcinoma. A similar phenomenon has not been observed with lung cancer, mostly due to the controversial results of earlier studies, likely as a result of tumor heterogeneity, short and variable survival, and small sample sizes, which precluded multivariate analysis. To answer the question whether histologic grade has an independent impact and to what extent, if any, on the prognosis of NSCLC, we have analyzed data from a large patient population diagnosed with NSCLC from Mayo Clinic in Rochester, Minnesota.

Methods**Study Population**

From January 1, 1997, to December 31, 2003, we prospectively enrolled 5018 patients with pathologically proven NSCLC (excluding carcinoid and salivary gland tumors) at Mayo Clinic, Rochester, Minnesota. Detailed procedures of patient enrollment, diagnosis, data collection, and follow-up have been described in previous publications.^{3,4} Histologic classification was made according to the World Health Organization's International Histological Classification of Tumors current at the time of diagnosis and TNM stage as proposed by Mountain⁵ in 1997. Histologic grade was evaluated by the pathologists at Mayo Clinic and categorized as follows: well differentiated, moderately differentiated, poorly differentiated, and undifferentiated. A full medical record abstraction was conducted to obtain demographics, history of tobacco exposure, lung cancer pathologic type, clinical staging, and treatment. All patients were actively followed up beginning 6 months after diagnosis, with subsequent annual follow-up by mailed questionnaires. Timely verification of patients' vital status (as of December 31, 2004) was accomplished through the Mayo Clinic's electronic clinical notes and registration database, next-of-kin reports, death certificates, and obituary documents filed in the patients' medical records, as well as through the Mayo Clinic Tumor Registry and Social Security Death Index website.

Our second study population was patients diagnosed with NSCLC from Olmsted County, Minnesota, where Mayo Clinic is located. Virtually all Olmsted County residents seek medical care at Mayo Clinic, where each lung cancer case has been identified and followed since 1984. A total of 712 NSCLC patients were enrolled by the end of 2003. We used this cohort to evaluate the potential referral bias of histologic grade effect on survival. All patients in this study were authorized for research and contact materials were reviewed and approved by the Mayo Foundation Institutional Review Board.

Statistical Analysis

The primary outcome in this analysis was survival after a lung cancer diagnosis. Survival was defined as the years from lung cancer diagnosis to death or the last known date alive. Patients

known to be alive at last contact were censored. Tumor distribution among well-differentiated, moderately differentiated, poorly differentiated, and undifferentiated carcinoma was tabulated by age, gender, histologic type, stage, smoking history, and treatment. The association of each variable with histologic grade was assessed by a chi-square test for categorical variables and an analysis of variance for continuous variables. Univariate association of age at diagnosis, gender, smoking history, stage, histologic cell type, histologic grade, and treatment with survival was evaluated by the Kaplan-Meier method.⁶ To evaluate the independent role of histologic grade on survival, a multivariate Cox proportional hazards model⁶ was applied to include all above-mentioned variables and adjusted relative risks (RR) were estimated. Because treatments were not assigned at random, we also adjusted for propensity scores that reflected the probabilities that an individual would be assigned a given treatment modality. These scores were generated by logistic regression models⁷ that used all available covariates to predict the probability of each possible treatment combination. This resulted in 8 different propensity scores, which we classified into tertiles to evaluate differences within treatment propensities.⁸ Indicator variables for these propensity score groupings were included in the Cox proportional hazards regression models, and separate Cox models were fit to the data, stratified by each of the treatment-specific propensity categorizations.

Since no significant difference between undifferentiated carcinoma and poorly differentiated carcinoma was observed in the overall regression model, we combined the two grades into a single group (poorly/undifferentiated carcinoma) in the downstream stratified and subset analyses. The impact of histologic grade on postdiagnosis survival was further assessed by estimating the RR of death stratified on gender, stage, histology, and treatment. Interactions between histologic grade and each of these variables were tested. The same analytical approaches were applied to the Olmsted County patients, and the same adjustment variables were included in the Cox model.

Because the main cause of cancer death after curative surgery is tumor recurrence, we evaluated what role histologic grade had on tumor recurrence by analyzing data of 1302 NSCLC patients diagnosed from 1997 to 2001 who received curative resection and had complete recurrence information.⁹ We used the Cox proportional hazards model with the time to recurrence after surgery as an event. Risk of recurrence was evaluated among three histologic grades after adjustment for age at diagnosis, gender, histologic cell type, tumor stage, and adjuvant therapies (chemotherapy and/or radiation therapy).

All analyses were performed with SAS software, version 8.2 (SAS Institute, Cary, NC). Adjusted survival curves were created by the optimal corrected group prognosis method as described by Ghali and associates.¹⁰

Results**Basic Characteristics and Univariate Analysis**

Age, gender, smoking history including pack-years smoked and time after smoking cessation, tumor stage, histologic cell type, and treatment modality varied significantly with histologic grade (Table 1). Younger patients were more likely to have poorly or undifferentiated carcinoma. Men

TABLE 1. Demographic and clinical characteristics of 5018 NSCLC patients

Characteristics	Well-differentiated (n = 668)	Moderately differentiated (n = 2111)	Poorly differentiated (n = 1410)	Undifferentiated (n = 829)	P value
Age, y (mean)	67	66	65	65	<.001
≤50	56, 8.4%	168, 8.0%	168, 11.9%	99, 11.9%	<.001
50< and ≤65	208, 31.1%	762, 36.1%	496, 35.2%	289, 34.9%	
65< and ≤80	350, 52.4%	1053, 49.9%	661, 46.9%	373, 45.0%	
>80	54, 8.1%	128, 6.1%	85, 6.0%	68, 8.2%	
Gender					
Male	331, 49.6%	1245, 59.0%	857, 60.8%	498, 60.1%	<.001
Female	337, 50.5%	866, 41.0%	553, 39.2%	331, 40.0%	
Smoking history					
Never	139, 20.9%	228, 11.0%	151, 10.9%	74, 9.2%	<.001
Past	367, 55.3%	1191, 57.2%	717, 51.7%	408, 50.7%	
Current	158, 23.8%	664, 31.9%	518, 37.4%	323, 40.1%	
Unknown	4	28	24	24	
Smoking pack-years					
None	139, 23.5%	228, 13.0%	152, 13.2%	74, 10.9%	<.001
0-20	93, 15.7%	201, 11.4%	127, 11.0%	86, 12.6%	
20-40	133, 22.5%	408, 23.2%	267, 23.2%	178, 26.1%	
40-60	113, 19.1%	452, 25.7%	297, 25.8%	171, 25.1%	
>60	113, 19.1%	470, 26.7%	309, 26.8%	173, 25.4%	
Unknown	77	352	258	147	
Quit years (former smokers)					
0-10	128, 39.9%	469, 48.2%	308, 53.8%	196, 57.7%	<.001
10-20	80, 24.9%	248, 25.5%	135, 23.6%	64, 18.8%	
20-30	59, 18.4%	159, 16.3%	78, 13.6%	52, 15.3%	
>30	54, 16.8%	98, 10.1%	52, 9.1%	28, 8.2%	
Stage					
I	372, 55.9%	641, 30.7%	204, 14.6%	91, 11.3%	<.001
II	39, 5.9%	225, 10.8%	120, 8.6%	42, 5.2%	
III	113, 17.0%	618, 29.6%	514, 36.9%	243, 30.0%	
IV	141, 21.2%	603, 28.9%	555, 39.8%	433, 53.5%	
Unknown	3	24	17	20	
Histologic cell type					
Adenocarcinoma	571, 85.5%	1204, 57.0%	823, 58.4%	0	<.001
Squamous	96, 14.4%	876, 41.5%	439, 31.1%	0	
Large cell	0	0	23, 1.6%	149, 18.0%	
Adenosquamous	1, 0.2%	31, 1.5%	117, 8.3%	0	
Unspecified NSCLC	0	0	8, 0.6%	644, 77.7%	
Sarcomatoid	0	0	0	36, 4.3%	
Treatment					
Surgery only	417, 63.6%	886, 42.6%	344, 25.0%	98, 12.3%	<.001
Chemotherapy only	61, 9.3%	202, 9.7%	216, 15.7%	168, 21.0%	
Radiation only	17, 2.6%	151, 7.3%	141, 10.2%	126, 15.8%	
Surgery + radiation	10, 1.5%	94, 4.5%	47, 3.4%	15, 1.9%	
Surgery + chemotherapy	21, 3.2%	81, 3.9%	48, 3.5%	26, 3.3%	
Chemotherapy + radiation	39, 6.0%	284, 13.7%	277, 20.1%	182, 22.8%	
Surgery + chemotherapy + radiation	19, 2.9%	126, 6.1%	57, 4.1%	28, 3.5%	
None	72, 11.0%	257, 12.4%	248, 18.0%	156, 19.5%	
Unknown	12	30	32	30	

had a higher proportion of poorly or undifferentiated carcinoma than women did. Never smokers had a higher proportion of well-differentiated carcinoma than of moderately, poorly, or undifferentiated carcinoma. Conversely, heavy

smokers or smokers who quit within a short time of cancer diagnosis had more poorly or undifferentiated carcinoma. Most well-differentiated carcinomas were seen in earlier stages (I and II), and the majority of poorly or undifferen-

Figure 1. A, Crude survival curves by histologic grade for all patients. B, Survival curves by histologic grade for all patients after adjustment for age, gender, smoking history, tumor stage, histologic cell type, and treatment. RR, Relative risk, compared with well-differentiated carcinoma; *significantly different from 1 ($P < .05$); **significantly different from moderately differentiated carcinoma ($P < .05$).

tiated carcinomas were found in the later stages (III and IV). Well-differentiated carcinomas were mostly adenocarcinoma. Patients with well-differentiated carcinoma had a much higher chance of being treated surgically than patients with poorly or undifferentiated carcinoma.

Stage, histologic cell type, histologic grade, treatment, age, and gender were all significantly associated with post diagnosis survival ($P < .001$). However, smoking history was not significant ($P = .2$). For histologic grade, the estimated crude survival at 1 and 5 years in well-differentiated carcinoma was 80.5% (95% confidence interval [CI]: 77.5-83.6) and 43.5% (95% CI: 39.0-48.6), respectively, with a median survival time of 3.7 years. The survival for moderately differentiated carcinoma was 65.5% (95% CI: 63.5-67.6) and 23.4% (95% CI: 21.2- 25.8) at 1 and 5 years, respectively, with a median survival time of 1.7 years. The survival for poorly differentiated carcinoma was 47.7% (95% CI: 45.1-50.4) and 13.4% (95% CI: 11.5-15.7) at 1 and 5 years, respectively, with a median survival time of 0.9 year; and the survival for undifferentiated carcinoma was 40.0% (95% CI: 36.7-43.6) and 8.3% (95% CI: 6.2-11.2) at 1 and 5 years, respectively, with a median survival time of 0.8 year. The RR of post-diagnosis mortality among patients with undifferentiated carcinoma versus those with well-differentiated carcinoma was 3.5 (95% CI: 3.1-4.0); between poorly differentiated carcinoma and well-differentiated carcinoma and between moderately differentiated carcinoma and well-differentiated carcinoma, the RRs were 2.7 (95% CI: 2.4-3.1) and 1.8 (95% CI: 1.6-2.0), respectively (Figure 1, A).

Multivariate Analysis

After adjustment for age at diagnosis, tumor stage, gender, histologic cell type, smoking history, and treatment with the

use of a multivariate Cox proportional hazard model, survival still differed significantly among the four histologic grades (Table 2, Figure 1, B). Compared with well-differentiated carcinoma, undifferentiated, poorly, and moderately differentiated carcinomas had RRs of 1.8- (95% CI: 1.4-2.4), 1.7- (95% CI: 1.5-2.0) and 1.4-fold (95% CI: 1.2-1.6), respectively. The RRs of undifferentiated and poorly differentiated carcinoma over moderately differentiated carcinoma were 1.3- (95% CI: 1.0-1.6) and 1.2-fold (95% CI: 1.1-1.3), respectively. However, the increased risk of mortality for undifferentiated carcinomas was not significantly different from that of poorly differentiated carcinomas (RR: 1.1, 95% CI: 0.9-1.3). The two grades were combined in the following subset analyses to reduce the data sparsity. In addition, we found that histologic grade was the third most significant prognostic factor, following tumor stage and treatment modality. Tumor histologic cell type and patient gender were also confirmed to be predictors of survival.

The factors used in this regression model were associated with treatment modality, with concordance between predicted and observed treatments ranging from 70% to 90% for the 8 treatment combinations. Adding indicator variables representing tertiles of these propensity scores for all 8 treatment modalities to the proportional hazards model made little difference in the hazard ratio estimates. On average, however, they slightly increased the risk estimates of higher histologic grades, with hazard ratios of 1.91 (95% CI: 1.40-2.60), 1.82 (95% CI: 1.51-2.20), and 1.46 (95% CI: 1.21-1.77) for undifferentiated, poorly differentiated, and moderately differentiated carcinoma, respectively, compared with well-differentiated carcinoma.

TABLE 2. Histologic grade effect on survival of NSCLC adjusted for age, gender, smoking status, stage, histologic cell type, and treatment with comparison of their relative significance

Factors	Hazard ratio	95% CI	P value
Stage			
Stage I	1	—	—
Stage II	1.66	1.42, 1.94	<.001
Stage III	2.21	1.94, 2.53	<.001
Stage IV	3.28	2.86, 3.76	<.001
Treatment			
Surgery	1	—	—
Surgery + chemotherapy	1.20	0.97, 1.49	.09
Surgery + chemotherapy + radiation	1.25	1.04, 1.51	.02
Surgery + radiation	1.66	1.35, 2.01	<.001
Chemotherapy	2.60	2.25, 3.00	<.001
Radiation	3.58	3.07, 4.17	<.001
No treatment	4.02	3.51, 4.59	<.001
Histologic grade			
Well differentiated	1	—	—
Moderately differentiated	1.41	1.24, 1.61	<.001
Poorly differentiated	1.71	1.49, 1.95	<.001*
Undifferentiated	1.83	1.42, 2.36	<.001*†
Histologic cell type			
Squamous	1	—	—
Large cell	0.85	0.63, 1.16	.30
Adenosquamous	0.93	0.66, 1.32	.68
Unspecified NSCLC	0.93	0.74, 1.15	.49
Adenocarcinoma	0.92	0.84, 1.00	.04
Sarcomatoid	1.56	1.03, 2.34	.04
Age			
≤50	1	—	—
50 < and ≤65	0.92	0.81, 1.04	.20
65 < and ≤80	1.25	1.11, 1.41	<.001
>80	1.44	1.21, 1.71	<.001
Gender			
Female	1	—	—
Male	1.25	1.17, 1.35	<.001
Smoking history			
Nonsmokers	1	—	—
Former smokers	1.09	0.97, 1.23	.13
Current smokers	1.13	1.00, 1.28	.05

*Also significant when compared with moderately differentiated carcinoma.

†Not significantly different from poorly differentiated carcinoma.

Stratified Analysis (Table 3)

When stratified by tumor stage, the unfavorable impact of less-differentiated carcinoma on survival was observed in all stages except for stage II, where the effect size (RR 1.7 and 1.5) was similar to other stages but had wider 95% CIs overlapping with unity. Poorly/undifferentiated and moderately differentiated stage I carcinomas had a 51% and 34% increased risk of death, respectively, compared with well-differentiated

TABLE 3. Stratified analyses of histologic grade on survival by gender, stage, histologic cell type, and treatment

	Moderately differentiated* RR,† P value (95% CI)	Poorly/undifferentiated* RR,† P value (95% CI)
Gender		
Male (n = 2931)	1.48, <.001 (1.25, 1.76)	1.78, <.001‡ (1.49, 2.13)
Female (n = 2087)	1.35, .003 (1.11, 1.65)	1.65, <.001‡ (1.34, 2.04)
Stage		
Stage I (n = 1308)	1.34, .02 (1.05, 1.73)	1.51, .01 (1.11, 2.06)
Stage II (n = 426)	1.45, .17 (0.85, 2.45)	1.72, .06 (0.99, 2.98)
Stage III (n = 1488)	1.24, .09 (0.97, 1.59)	1.45, .004‡ (1.13, 1.86)
Stage IV (n = 1732)	1.48, .001 (1.19, 1.84)	1.90, <.001‡ (1.53, 2.37)
Histologic cell type		
Squamous (n = 1411)	1.34, .04 (1.01, 1.76)	1.53, .004 (1.15, 2.05)
Adenocarcinoma (n = 2598)	1.41, <.001 (1.21, 1.64)	1.70, <.001‡ (1.45, 1.99)
Treatment		
Surgery only (n = 1745)	1.43, .002 (1.14, 1.79)	1.63, <.001 (1.26, 2.11)
Chemotherapy and/or radiation (n = 1864)	1.50, <.001 (1.20, 1.88)	1.83, <.001‡ (1.46, 2.30)
No treatment (n = 733)	1.35, .04 (1.01, 1.80)	1.51, .01 (1.13, 2.03)

*The reference is well-differentiated carcinoma. †RR, Relative risk adjusted for age, gender, smoking history, histologic cell type, stage, and treatment. ‡Also significant at $P < .05$ level compared to moderately differentiated carcinoma.

carcinoma. The excess risk of mortality was 90% and 48% for stage IV carcinomas, respectively.

When stratified by histologic cell type, the patients with poorly/undifferentiated and moderately differentiated adenocarcinoma had a 70% and 41% increased risk of death compared with patients with well-differentiated adenocarcinoma, respectively. The elevated risk of mortality for patients with squamous cell carcinoma was 53% and 34% for poorly/undifferentiated carcinoma and moderately differentiated carcinoma, respectively.

Regardless of treatment modalities, moderately differentiated or poorly/undifferentiated carcinoma added a 35% to 83% risk of mortality compared with well-differentiated carcinoma.

Subgroup Analysis for Olmsted County Patients

When compared with well differentiated carcinoma, the unadjusted RRs for poorly/undifferentiated carcinoma and moderately differentiated carcinoma were 3.0 (95% CI: 2.2-4.0) and

1.9 (95% CI: 1.4-2.5), respectively. The adjusted RR for poorly/undifferentiated carcinoma was 1.6 (95% CI: 1.2-2.2) and for moderately differentiated carcinoma, 1.4 (95% CI: 1.0-1.9), suggesting that the independent role of histologic grade on survival in NSCLC was less likely inflated by patient referral in the tertiary hospital setting.

Histologic Grade Is Associated With Tumor Recurrence After Surgery

Among 1302 surgically treated patients with complete resection, those with poorly/undifferentiated, moderately differentiated, and well-differentiated carcinoma had recurrence rates of 47.3%, 32.7%, and 20.8%, respectively. After adjustment for age at diagnosis, gender, histologic cell type, tumor stage, and adjuvant therapies, patients with poorly/undifferentiated carcinoma had a 2.1-fold increased risk (hazard ratio: 2.1; 95% CI: 1.4-2.9) of cancer recurrence compared with well-differentiated carcinoma. For patients with moderately differentiated carcinoma, the increased risk of recurrence was 1.4-fold (95% CI: 1.0-1.9). These results provided additional evidence that less-differentiated tumors were independently associated with a higher risk of recurrence and offered a possible explanation for a shortened overall survival.

Conclusions

In our study, we focused on the role of histologic grade on survival of NSCLC. Histologic grade was shown to be a significant prognostic factor. Similar results were observed in a cohort of patients from a single county, suggesting that the results obtained from the large cohort were unlikely to be biased by patient referral. No significant difference on survival was observed between undifferentiated carcinoma and poorly differentiated carcinoma. Less-differentiated carcinoma was associated with a higher risk of tumor recurrence after complete tumor resection.

In the literature, the role of histologic grade as a prognostic factor in lung cancer is controversial. Similar to our findings, in an analysis of 96 patients with lung cancer, Chung and associates¹¹ found that poorly differentiated tumors had higher rates of lymph node metastasis and local recurrence as well as shorter survival than did well- or moderately differentiated tumors. However, the results were obtained mostly by univariate analyses, and the independent role of histologic grade was not assessed because of the limited sample size. In one of a few studies using multivariate adjustment, Ichinose and coworkers¹² found histologic grade was a significant predictor for resected stage I tumors but did not have any impact on stage II and stage IIIA tumors. Noted in their study, the numbers of patients in stage II and IIIA were only 63 and 108, respectively, which were far less than the 243 patients in stage I.¹² Disparate survival was also reported in early-stage resected adenocarcinoma¹³ or in late-stage nonresectable adenocarcinoma treated with chemotherapy and/or radiation therapy.¹⁴

Nevertheless, many studies have not found an independent role of histologic grade in lung cancer survival or did not even include it in their survival analyses.¹⁵⁻¹⁸ The diverse results are likely due to several factors: (1) Sample sizes are generally small, which may not be able to detect moderate predictive effects. (2) Since NSCLC is a very heterogeneous group of tumors, the effect of histologic grade on survival may differ among different cell types or treatment modalities and results obtained from one subset of patients with lung cancer may not be same as others. (3) Inconsistent grading criteria among pathologists and different grouping systems among grades in analysis (eg, well- and moderately differentiated combined versus moderately and poorly differentiated combined) may also contribute to varied results. In our study, we have tried to address all the points listed above; however, inconsistent grading criteria may remain an issue.

A challenge of evaluating histologic grade is that the present grading system for lung cancer is not standardized. A four-tiered system of grading ranging from well-differentiated, moderately differentiated, poorly differentiated, and undifferentiated carcinoma is suggested for most cancers, including the lung, in the American Joint Committee on Cancer Staging Handbook¹⁹ and World Health Organization Histological Typing of Lung and Pleural Tumors,²⁰ but no specific criteria have been developed for a universal assessment in lung cancer. Usually, histologic grade is based on the percentage of tumor differentiation and other features, such as specific growth patterns, cytologic atypia, and mitotic rates. In this study, histologic grade was obtained mostly from the routine readings of the pathologists at Mayo Clinic following the general guidelines, but no centralized slide review was conducted owing to lack of a universally accepted “gold standard” to follow. With the findings from this study, further investigation and development of a systematic grading system for a unified assessment is warranted to help better define prognostic subgroups.

The results of this study have several implications: (1) Histologic grade has a significant impact on survival of patients with NSCLC; the impact is the third most important after tumor stage and treatment modality and is independent of other known predictors. However, similar results may not be obtained from a limited number or restricted subset of patients in the context of high complexity and heterogeneity of NSCLC, as reported in the literature. (2) With the development of new technologies, our current search for determinants of NSCLC survival has been focusing on the molecular level. For example, gene expression profiling using microarray analysis may provide more accurate classification of NSCLC^{21,22} and better prediction for survival.^{23,24} However, the markers or signatures identified from that approach have been rarely evaluated along with other key prognostic factors such as tumor stage and histologic grade, and it is not clear whether those markers are related to histologic grade. If histologic grade is proven prog-

nostic, our search for new promising markers needs to focus on those that can provide additional value beyond histologic grade or can provide accurate measurement of histologic grade. (3) Histologic grade is correlated with smoking amount and duration. Tumors in heavy smokers or current smokers tend to be less differentiated and more aggressive. It has been observed that smoking is associated with shortened lung cancer survival independent of smoking-related comorbidities and other factors,²⁵ and a beneficial effect from long-term cigarette smoking cessation for longer survival is observed for women patients with NSCLC.⁴ Smoking might affect lung cancer survival through modifying tumor genetic make-up and affecting tumor differentiation.

The variables included in the risk adjustment for histologic grade in the study are those more frequently implicated in the literature for NSCLC prognosis. Other factors such as performance status, comorbidities, or other malignant diseases may play a role in affecting lung cancer survival and modify the risk of histologic grade. To evaluate the potential effect, we added performance status, previous other cancer, chronic obstructive pulmonary disease, other lung disease, diabetes, cardiovascular disease, hypertension, and any other disease into our primary model and analyzed a subset of 2859 patients with complete information on these additional variables. The results from this expanded model were almost identical to our original model. Another concern is whether the traditional regression model is adequate for confounding adjustment in an observational study. To address the issue, we applied the preferred propensity score method to our data and obtained the similar results with the traditional model-based adjustment. The supplementary results further support an independent association of histologic grade with lung cancer survival, and it is less likely that histologic grade is a surrogate of other factors.

In summary, histologic grade has important prognostic value for NSCLC as determined in two cohorts of patients with extensive follow-up data. The inconsistent results reported previously may be due to underpowered sample sizes or varied patient populations. Histologic grade may provide useful information in defining the aggressiveness of tumors, should be considered as an independent factor affecting survival beyond TNM staging, and may offer guidance in patient management.

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