Original Article

Nuclear Grading of Primary Pulmonary Adenocarcinomas

Correlation Between Nuclear Size and Prognosis

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BACKGROUND: According to the World Health Organization Classification of Tumors, the prognostic value of morphometric cytologic atypia has not been assessed in pulmonary adenocarcinoma. **METHODS:** Primary tumors of 133 pulmonary adenocarcinomas ≤2 cm were analyzed using an image processor for analytical pathology. The results were evaluated using receiver operator characteristic curve analysis, and survival curves were drawn by the Kaplan-Meier method. Furthermore, the results were applied to routine histological diagnosis. Four pathologists evaluated the nuclear factors relative to the size of small lymphocytes as a standard. **RESULTS:** By using the nuclear area and nuclear major axis dimension, lung adenocarcinomas were divisible into 2 groups showing extremely favorable prognosis and fairly favorable prognosis, without considering histological features or classification. A nuclear area level of $<67\ \mu m^2$ was correlated with longer survival ($P < .0001$), and the 5-year survival rate was 90.4%. Similarly, a nuclear diameter level of $<0.7\ \mu m$ was correlated with longer survival ($P = .0002$), and the 5-year survival rate was 88.6%. The mean (±standard deviation [SD]) value of the kappa statistic for the 4 pathologists who evaluated the cases using the size of small lymphocytes as a standard was 0.58 ± 0.10, and the mean (±SD) value of the accuracy metric was 0.66 ± 0.10. **CONCLUSIONS:** Nuclear area and nuclear major dimension are 2 useful independent markers for evaluating the prognosis of lung adenocarcinoma. ***Cancer*** **2010;116:2011–9.** © *2010 American Cancer Society.*

KEYWORDS: nuclear grading, prognosis, pulmonary adenocarcinoma, nuclear area, nuclear diameter.

In continuously dividing normal cells, the cell constituents increase in a progressive and precise manner during the cell cycle phases to avoid any progressive reduction of daughter cell size. Therefore, cell growth and proliferation are tightly coordinated and subjected to organized biological processes to ensure the generation of normal cells.[1] In cancer cells, however, these tightly coordinated processes are perturbed, and the nuclei of most cells in solid tumors vary in size, shape, and chromatin pattern, both in comparison with normal nuclei and also among cancer cells.[2] The features of such morphologic changes in the nucleus have not been explained in terms of conventional concepts of nuclear structure and theories of carcinogenesis. However, in various cancers such as breast cancer, nuclear atypia has been used clinicopathologically to evaluate malignancy.

Lung cancer is the most common cancer worldwide (12.6% of all new cancers, 17.8% of cancer deaths).[3] Among the histologic types of nonsmall cell carcinoma of the lung, adenocarcinoma has a poor prognosis.[4] Recently, surgical treatment of small-sized peripheral lung carcinomas, especially adenocarcinoma, has increased in parallel with improvements in diagnostic radiology.[4] Noguchi et al[5] examined many surgically resected adenocarcinomas of the lung at an early stage, and proved that some adenocarcinomas have a very favorable prognosis. According to their criteria, localized bronchioloalveolar carcinoma (BAC, type A) and localized BAC with alveolar collapse (type B) are defined as in situ adenocarcinoma, and localized BAC with foci of active fibroblastic proliferation includes minimally invasive adenocarcinoma (type

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DOI: 10.1002/cncr.24948, **Received:** May 20, 2009; **Revised:** July 23, 2009; **Accepted:** August 4, 2009, **Published online** February 11, 2010 in Wiley InterScience (www.interscience.wiley.com)

C). Type C tumors include adenocarcinomas showing various prognoses, and there are no useful criteria that can be used to distinguish minimally invasive carcinomas from type C tumors.

Conversely, the World Health Organization (WHO) Classification of Tumors of the Lung, Pleura, Thymus, and Heart states that for evaluation of malignancy, "Grading of pulmonary adenocarcinomas is based on conventional histological criteria, including the extent to which the architectural pattern of the tumor resembles normal lung tissue, and cytologic atypia."[3] In other words, malignant grading depends on the degree of differentiation, including variations in histological architecture and cell atypia. The judgment of histological differentiation is difficult. Although the WHO classifies 4 major histological subtypes on the basis of tumor differentiation, it does not define histological differentiation itself. The evaluation of cell atypia is also difficult, and there are no objective definitions of cell atypia in tumor cells of lung adenocarcinoma. Furthermore, using the WHO classification, it is not possible to distinguish minimally invasive adenocarcinomas from invasive cancers. Nuclear morphometry is a method for quantitative measurement of histopathologic changes in the appearance of stained cell nuclei. Some studies have indicated that such assessments may provide clinically relevant information related to the degree of progression and malignant potential of various cancers.[6-10] In the present study, we performed nuclear morphometry and tried to use the results for extracting minimally invasive adenocarcinomas.

MATERIALS AND METHODS

Patients

Primary tumors were obtained from 139 patients with pulmonary adenocarcinomas $\leq$2 cm in maximum dimension who were treated surgically during the period between January 1993 and December 2000. These patients underwent surgical resection of their tumors along with mediastinal and pulmonary hilar lymph node dissection at the National Cancer Center Hospital, Tokyo, Japan. Informed consent for specimen collection was obtained from all patients. Moreover, none of the patients selected had received neoadjuvant or adjuvant chemotherapy or radiotherapy before or after surgery. Six patients subsequently died of causes other than lung carcinoma. The study focused on a series of 133 patients, excluding these 6 patients.

Tissue Specimens and Pathologic Information

The resected specimens were fixed with 10% to 15% neutral buffered formalin at room temperature, and then embedded in paraffin for histologic examination. All of the sections (4 μm thick), including the largest cut surface of the tumor, were stained with hematoxylin and eosin and elastica van Gieson and examined by light microscopy. Tumors were classified according to the criteria of the WHO International Histological Classification of Tumors and also the histological criteria proposed by Noguchi et al.[5] Microscopically, the diagnosis was performed by 3 pathologists (Y.N., Y.M., M.N.). If 2 or more opinions coincided, the diagnosis was considered to be firm. All patients gave informed consent for specimen collection. The small-sized lung adenocarcinomas were classified histologically as described previously (Table 1).[5] Lung tumors of types A, B, and C show replacement growth of the pulmonary alveolar structure, whereas those of types D, E, and F show nonreplacement growth. This staging was evaluated according to the International Union Against Cancer TNM Classification of Malignant Tumors (fifth edition).

Morphometric Procedure

An Image Processor for Analytical Pathology (Sumitomo Technoservice Co., Osaka, Japan) was used for morphometric analysis of nuclear size (nuclear area, nuclear major axis diameter [nuclear diameter], and nuclear roundness). The system was connected to a BX50 microscope (Olympus, Japan). The instrument was calibrated with a micrometer slide before each measurement. All measurements were performed on the monitor screen using a $\times$40 objective and a $\times$10 video ocular. We chose tumor areas with the largest available nuclei for morphometric investigation. On examining the sections for selection of fields, tumor cells from the most cellular area at the center of the tumor were selected. Necrotic and inflammatory areas were avoided, and overlapping nuclei were omitted. Five microscopic fields were screened, 10 cells per field were selected, and 50 cells per tumor were measured. The nuclear profile area measurements were assessed by tracing the nuclear membrane using the computer mouse. Fifty nuclei of the tumor cells in each specimen were measured using a computer software package (IPAP-WIN Version 3.0, Sumika Technoservice Co., Osaka, Japan). In each case, the mean nuclear size (nuclear area, nuclear diameter, nuclear perimeter, and nuclear roundness) was used for evaluation. The picture on the computer monitor captured from histologic specimens was manipulated. As a

Table 1. Patient Characteristics

Characteristics	No. of Patients
No. of patients	133
Sex (men/women)	64/69
Mean age±SD, y (range)	60.4±9.8 (38-82)
Mean tumor size±SD, mm (range)	15.9±3.4 (6-20)
Tumor classification	
T1	112
T2	7
T3	4
T4	10
Lymph node status	
N0	90
N1	18
N2	24
N3	1
Pleural invasion	
P0	98
P1	24
P2	8
P3	3
Stage	
I (IA/IB)	86 (83/3)
II (IIA/IIB)	20 (16/4)
III (IIIA/IIIB)	26 (17/9)
IV	1
WHO histological classification	
BAC	25
Mixed subtypes	86
Acinar	1
Papillary	5
Solid	16
Noguchi classification	
Type A/B/C	12/14/66
Type D/E/F	27/8/6
Type of resection	
Lobectomy	126
Pneumonectomy	2
Segmentectomy	4
Wedge resection	1

SD indicates standard deviation; WHO, World Health Organization; BAC, bronchioloalveolar carcinoma.

result, the nuclei were identified and measured using the computer software (Fig. 1a-f).

Interobserver Variability and Accuracy of the Nuclear Factors

We applied the morphological results to routine histological diagnosis using the size of small lymphocytes as a standard. Sixty patients were randomly selected from this series of 133 patients. A tumor cell was judged to be positive if its nuclear area and nuclear diameter were 5× and 3× larger than the corresponding values for small lymphocytes, respectively. A field with ≥5 positive cells was considered to be a positive field. If there were ≥3 positive fields, we considered the case to be positive. Any case that did not meet all of these requirements was judged to be negative. In general, cases with critical nuclear area levels of ≥67 μm^2 tended to be positive, and cases with critical nuclear area levels <67 μm^2 tended to be negative. Four pathologists (M.N., Y.M., H.K., and K.S.) evaluated all 60 cases independently and divided the specimens into 2 groups (positive cases and negative cases). The kappa statistic value was used for nuclear grading among the 2 groups (positive cases and negative cases) between the 4 pathologists.

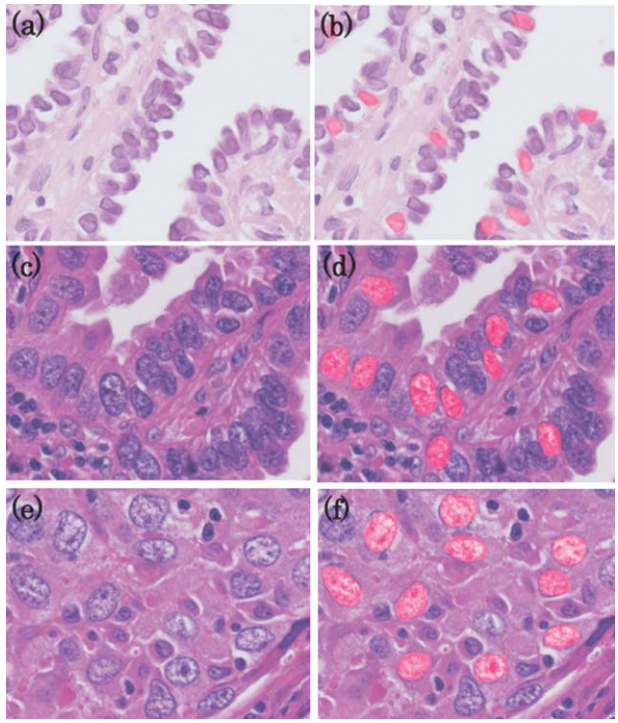
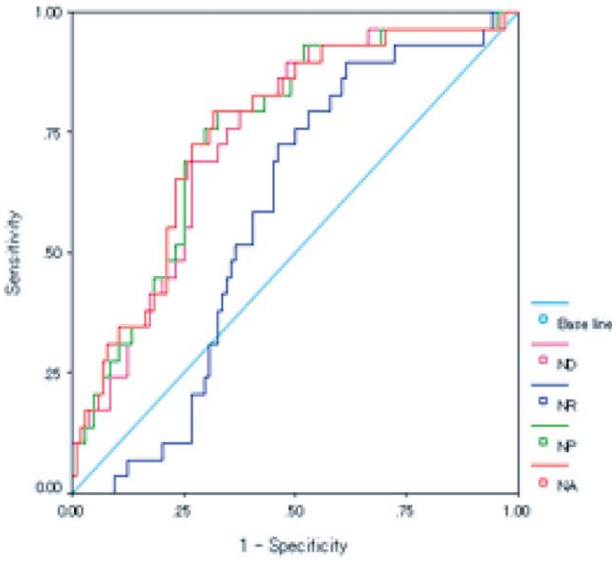


Figure 1. (a, c, e) Histology of small-sized adenocarcinoma of the lung is shown (H & E; original magnification, ×400). (b, d, f) Karyometric analysis using an Image Processor for Analytical Pathology is shown. The nucleus in the carcinoma cell was picked up from the field in each panel. The red area represents the nucleus. Morphometry was performed on each area. (a, b) A type A tumor using Noguchi classification is shown. (c, d) A type C tumor using the Noguchi classification is shown. (e, f) A type D tumor using the Noguchi classification is shown.

Statistical Analysis

Analysis of the correlation between clinicopathologic features and nuclear size was performed using F test, Student *t* test, and Tukey test. Evaluation of the cutoff point for nuclear size was performed using receiver operating



	AUC	SE	Significance Probability	95% CI
NA	0.756	0.049	0.000	0.66-0.85
NP	0.750	0.048	0.000	0.66-0.85
NR	0.578	0.052	0.200	0.48-0.68
ND	0.739	0.048	0.000	0.64-0.83

Figure 2. Receiver operating characteristic curves of mean nuclear size (nuclear area [NA], nuclear major axis dimension [ND], nuclear roundness [NR], and nuclear perimeter [NP]) are shown for the diagnosis of malignant stricture. AUC indicates the area under the curve; SE, standard error; CI, confidence interval.

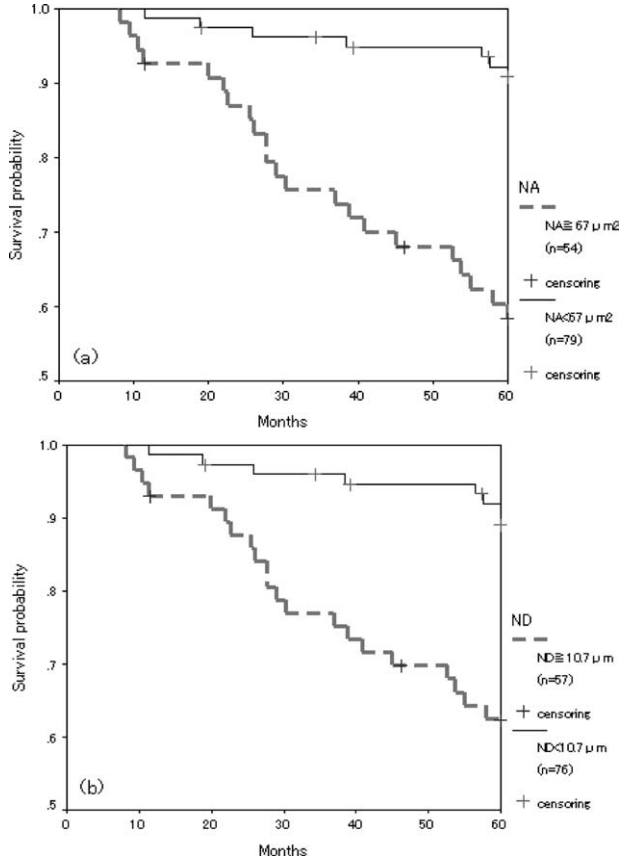


Figure 3. Five-year recurrence-free survival rates are shown for all patients, classified using the critical nuclear size. (a) A nuclear area (NA) of 67 μm^2 was used as a cutoff value ($P <$ .0001). (b) A nuclear major axis dimension (ND) of 10.7 μm was used as a cutoff value ($P = .0002$).

characteristic (ROC) curve analysis. The survival curves were drawn by the Kaplan-Meier method. Overall survival was calculated from the date of primary surgery for lung tumors to the date of death or last follow-up. The curves were evaluated by the log-rank test ($P = .05$). The independent staging factors for pulmonary adenocarcinomas were evaluated by multivariate analysis for nuclear size. Interobserver variability and accuracy were evaluated using kappa statistics. Data were censored when patients were lost to follow-up. All analyses were performed using SPSS statistical software (version 12.0; SPSS, Chicago, Ill).

RESULTS

Clinical and Histological Findings

The most relevant clinicopathologic features are listed in Table 1. The tumors were classified according to the histological criteria proposed by Noguchi et al.[5] Follow-up was complete for all patients up to January 2005 and ranged from 8 to 150 months (mean, 79.8; median, 84.1). The overall 5-year survival rates for stages I, II, and III were 91.9%, 75.0%, and 38.5%, respectively.

Morphometric Analysis and Outcome

Mean (±standard deviation [SD]) values of nuclear size parameters were: nuclear area 64 ± 17 μm^2 (range, 34-130), nuclear diameter 10.3 ± 1.3 μm (range, 7.4-14.6), and nuclear roundness 0.860 ± 0.016 (range, 0.812-0.893). The ROC curve analysis showed that a cutoff nuclear area level of 67 μm^2 had a sensitivity and specificity of 75% and 70%, respectively (area under the curve [AUC], 0.756; 95% confidence interval [CI], 0.66-0.85) (Fig. 2). The nuclear dimension level of 10.7 μm had a sensitivity and specificity of 75% and 65%, respectively (AUC, 0.739; 95% CI, 0.64-0.83) (Fig. 2). The mean nuclear area and nuclear diameter were significantly higher in patients with malignant stricture. However, the AUC for nuclear roundness was

Table 2. Distribution of Clinicopathologic Features and Nuclear Size

Factor	NA			ND		
	<67 μm²	≥67 μm²	*P*	<10.7 μm	≥10.7 μm	*P*
Pathologic stage						
Stage I	65	21	<.0001	60	26	<.0001
Stage ≥II	14	33		16	31	
Tumor classification						
T1	74	38	<.0001	71	41	<.0001
≥T2	5	16		5	16	
Lymph node status						
N0	67	23	<.0001	62	28	<.0001
≥N1	12	31		14	29	
Pleural invasion						
P0	68	30	<.0001	64	34	.001
≥P1	11	24		12	23	
WHO histological classification						
BAC	25	0	<.0001	24	1	<.0001
Mixed subtypes	46	40	.254	45	41	.149
Solid, acinar, papillary	8	14		7	15	
Noguchi classification[a]						
Types A and B	25	1	.001	25	1	<.0001
Type C	38	28	.104	36	30	.119
Types D, E, and F	16	25		15	26	

NA indicates nuclear area; ND, nuclear major axis dimension; WHO, World Health Organization; BAC, bronchioloalveolar carcinoma.
[a]Table adapted from Noguchi et al.[5]

<0.6 (95% CI, 0.48-0.68) (Fig. 2). The Kaplan-Meier survival curves showed that the 5-year survival rate of patients whose tumor cells had a mean nuclear area of <67 μm² was 90.4% (Fig. 3a). Conversely, the corresponding survival rate of those with tumor cells having a mean nuclear area of ≥67 μm² was 57.7%. A nuclear area of ≥67 μm² was correlated with shorter survival ($P < .0001$). Similarly, the 5-year survival rate of patients whose tumor cells had a mean nuclear diameter of <10.7 μm was 88.6% (Fig. 3b). The corresponding survival rate of patients with tumor cells having a mean nuclear diameter of ≥10.7 μm was 61.8%. A nuclear diameter of ≥10.7 μm was correlated with shorter survival ($P = .0002$). The clinicopathological characteristics and the nuclear size (nuclear area and nuclear diameter) were compared in Table 2. All prognostic factors reported before, such as pathological stage, tumor classification (T stage), lymph node metastasis, pleural invasion, WHO histological classification, and Noguchi's classification, were significantly associated with the nuclear size (nuclear area and nuclear diameter). Then, we performed multivariate analysis to determine the factors contributing most significantly to the 5-year recurrence-free survival rate using Cox regression analysis. It demonstrated that nuclear area was 1 of the 4 significant prognostic factors including pleural invasion, tumor classification, and lymph node status ($P = .037$) (Table 3).

The data from morphometric analysis were then compared with the WHO classification (Table 4, Fig. 4). The mean (±SD) value of nuclear area was 48 ± 9 μm² in BAC, 68 ± 8 μm² in the papillary subtype, 82 ± 20 μm² in the solid subtype, and 65 ± 15 μm² in the mixed subtype. The nuclear areas of BAC tumor cells were significantly smaller than those of the other subtypes except for the acinar subtype, and the nuclear areas of solid tumor cells were significantly larger than those of other subtypes except for the acinar subtype (Fig. 4). The mean (±SD) value of nuclear diameter was 9.1 ± 0.9 μm in BAC, 10.8 ± 0.8 μm in the papillary subtype, 11.4 ± 1.3 μm in the solid subtype, and 10.5 ± 1.2 μm in the mixed subtype. The mean nuclear diameter of BAC tumor cells was significantly smaller than that of the other subtypes except for the acinar subtype, and the nuclear diameter of solid tumor cells was larger than that of the other subtypes except for the acinar subtype. The 5-year survival rate for all 133 patients was 78.2%. Conversely, the corresponding rates for patients with BAC (n = 25), the solid subtype (n = 16), and the mixed subtype (n = 86) were 100%, 75%, and 70.9%, respectively (Table 4).

Table 3. Multivariate Cox Regression Analysis of Pathological Staging Factors

Variable	*P*	Relative Risk	95% CI
Nuclear area: ≥67 μm^2 vs <67 μμm^2	.037	0.35	0.13-0.94
Pleural invasion: P0 vs P1-3	.046	2.49	1.02-6.12
Tumor classification: T1 vs ≥T2	.010	0.31	0.12-0.76
Lymph node status: N0 vs ≥N1	.001	0.20	0.08-0.50

CI indicates confidence interval.

Table 4. Nuclear Size of Histologic Typing in Patients With Small Adenocarcinoma of the Lung With 5-Year Survival Rate

Type	No. of Patients	NA, Mean±SD, μm^2	*P*	ND, Mean±SD, μm	*P*	5-Year Survival, %
Acinar	1	53		9.6		—
Papillary	5	68±8	.029	10.8±0.8	.014	100
BAC	25	48±9	<.0001	9.1±0.9	<.0001	100
Solid	16	82±20	<.0001; vs BAC, <.0001	11.4±1.3	<.0001; vs BAC, <.0001	75
Mixed subtypes	86	65±15		10.5±1.2		71

NA indicates nuclear area; SD, standard deviation; ND, nuclear major axis dimension; BAC, bronchioloalveolar carcinoma.
Adapted from World Health Organization Classification of Tumors.[3]

The data obtained by morphometric analysis were then compared with Noguchi's classification (Table 5, Fig. 5). The mean nuclear area of type A tumors (mean ± SD, 47 ± 7 μm^2) was similar to that of type B tumors (mean ± SD, 51 ± 15 μm^2), whereas the mean nuclear area of type C tumors (mean ± SD, 63 ± 15 μm^2) was significantly larger than that of types A and B tumors (mean ± SD, 49 ± 12 μm^2) ($P < .0001$). In addition, the nuclear area of type D tumors (mean ± SD, 77 ± 18 μm^2) was significantly larger than that of type C tumors ($P = .002$). The mean nuclear diameter of type A tumors (mean ± SD, 8.9 ± 0.6 μm) was similar to that of type B tumors (9.4 ± 1.4 μm), whereas the mean nuclear diameter of type C tumors (mean ± SD, 10.4 ± 1.2 μm) was significantly larger than that of types A and B tumors (9.2 ± 1.1 μm) ($P < .0001$). The 5-year survival rates of patients with type C tumors (n = 66) and nonlepidic-type tumors (types D, E, and F) (n = 41) were 72.3% and 73.2%, respectively (Fig. 6). The 5-year survival rate for patients with types A and B tumors (n = 26) was 100%. The results of morphometric analysis were compared between 2 different histological groups: lepidic-type tumors (types A, B, and C) and nonlepidic-type tumors (types D, E, and F). The nuclear area of nonlepidic-type tumors (mean ± SD, 73 ± 17 μm^2) was significantly larger than that of lepidic-type tumors (mean ± SD, 59 ± 16 μm^2) ($P < .0001$), and the nuclear diameter of nonlepidic-type tumors (mean ± SD, 11.0 ± 1.2 μm) was significantly larger than that of lepidic-type tumors (mean ± SD, 10.0 ± 1.2 μm) ($P < .0001$).

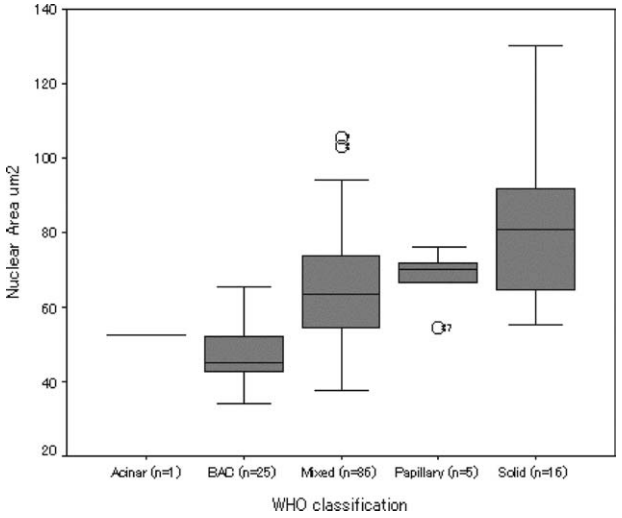


Figure 4. A box plot of the nuclear area in all patients is shown, classified according to the World Health Organization (WHO) classification. BAC indicates bronchioloalveolar carcinoma.

Interobserver Variability and Accuracy

We then attempted to apply our results to routine histological diagnosis. As the mean (±SD) values of nuclear

Table 5. Nuclear Size of Histologic Typing in Patients With Small Adenocarcinoma of the Lung With 5-Year Survival Rate

Type	No. of Patients	NA, Mean±SD, μm^2	*P*	ND, Mean±SD, μm	*P*	5-Year Survival, %	Log-Rank *P*
Lepidic type							
A	12	47±7	.008 (A-C)	8.9±0.6	.001 (A-C)	100	
B	14	51±15		9.4±1.4		100	
C	66	63±15		10.4±1.2		73	
Nonlepidic type							
D	27	77±18	.002 (vs C)	11.2±1.2	.034 (vs C)	70	
E	8	67±10		10.7±0.9		75	
F	6	64±15		10.3±1.3		83	
Types A and B	26	49±12	<.0001	9.2±1.1	<.0001	100	.018
Type C	66	63±15	.005	10.4±1.2	.035	73	
Types D, E, and F	41	73±17		11.0±1.2		73	
Lepidic type	92	59±16	<.0001	10.0±1.2	<.0001	80	.288
Nonlepidic type	41	73±17		11.0±1.2		73	

NA indicate nuclear area; SD, standard deviation; ND, nuclear major axis dimension.
Adapted from Noguchi et al.[5]

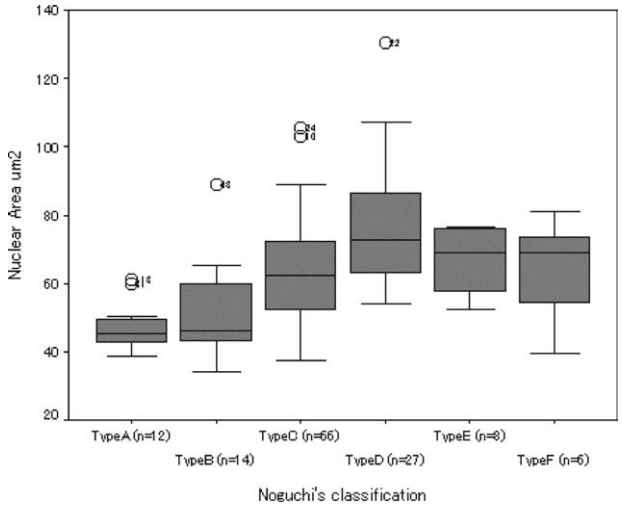


Figure 5. A box plot of the nuclear area in all patients is shown, classified according to the Noguchi classification.

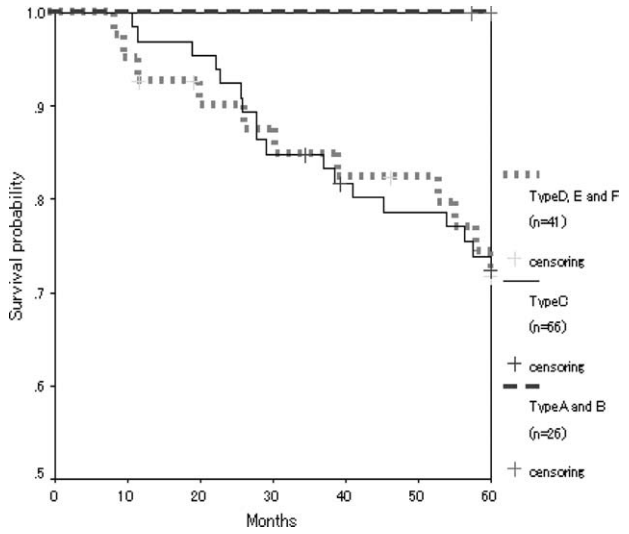


Figure 6. The 5-year recurrence-free survival rates of all patients is shown, classified according to the modified Noguchi classification with 3 subtypes: types A and B (n = 26), type C only (n = 66), and nonlepidic type (types D, E, and F; n = 41).

size parameters of small lymphocytes were nuclear area 14 ± 4 μm^2 and nuclear diameter 3.9 ± 0.03 μm, the critical nuclear area level of 67 μm^2 was approximately 5× larger than that of lymphocytes,[6] and the critical nuclear diameter level of 10.7 μm was approximately 3× larger. The mean (±SD) value of the kappa statistic for the 4 pathologists was 0.58 ± 0.10 (range, 0.47-0.76), and the mean (±SD) value of the accuracy metric was 0.66 ± 0.10 (range, 0.56-0.80).

DISCUSSION

In 1987, the potential role of morphometry in surgical pathology was reported by Paplanus et al,[7] who indicated that morphometry could be specifically helpful for 1) identifying malignant cells in lesions that are largely composed of apparently benign cells (eg, follicular thyroid neoplasms), 2) defining reference points in apparent continua (eg, in the progression from normal colon tissue to adenoma to adenocarcinoma), 3) distinguishing between benign and malignant lesions with similar appearances (eg, fibromatosis and soft tissue fibrosarcoma), and 4) distinguishing between malignant neoplasms of a similar appearance (eg, small-cell carcinoma of the lung and

small-cell lymphoma). Many studies have performed quantitative assessment of nuclear morphometry in pulmonary malignant tumors as an adjunct to the diagnostic and prognostic work of pathologists.[6,10-13] However, no study has established prognostic cutoff points based on nuclear morphology. Of course, a small fraction of tumor cells in S-G2 phase may show a larger nuclear size, and some nuclei may not be sectioned through the largest dimension. Therefore, the data obtained in these experiments did not necessarily reflect the accurate size of the nuclei. However, we focused on estimating the malignancy of the tumors based on nuclear morphometry, and not on the accurate nuclear size.

In the present study, ROC curve analysis showed that a cutoff nuclear area of 67 μm^2 had 75% sensitivity and 70% specificity, and that a nuclear diameter of 10.7 μm had 75% sensitivity and 65% specificity for detecting malignant strictures, respectively. Furthermore, it was proved that the 5-year survival rate of both groups was significantly different by log-rank test ($P < .001$) (Fig. 3). Table 2 shows that the most significant prognostic and staging factors for all the subtypes of small-sized pulmonary adenocarcinoma were significantly associated with nuclear area and nuclear diameter. Furthermore, multivariate analysis demonstrated that nuclear area was a significant prognostic determinant ($P = .037$). These results indicated that small-sized adenocarcinomas can be divided into 2 groups: those showing an extremely favorable prognosis (5-year survival rate around 90%) and those showing a fairly favorable prognosis (5-year survival rate around 60%-70%). The former group showing a 90% 5-year survival rate may be regarded as having minimally invasive carcinoma; members are candidates for reduction or limited surgery, similarly to early stage gastric carcinoma, which is treatable by endoscopic surgery.

It is of considerable practical interest that pathologists can extract cases showing an extremely favorable prognosis using only morphometric calculation of nuclear area or nuclear diameter for each tumor. To select patients eligible for limited surgery, it is not necessary to examine histological structures such as those of the papillary, acinar, and solid subtypes. Of course, nuclear area and nuclear diameter status are associated with the ratio of the lepidic growth area and Noguchi's classification, which are purely structural classifications. For example, Noguchi's classification reflects the prognosis of small-sized adenocarcinomas of the lung. Figure 5 indicates that the nuclear area of type C tumors was significantly larger than that of type A tumors ($P < .0001$). Conversely, the nuclear area of type D tumors was significantly larger than that of type C tumors ($P < .002$). As the 5-year survival rate of patients with type A tumors was better than that of patients with type C tumors, and that of patients with type C tumors was better than that of patients with type D tumors, the prognostic significance of the mean nuclear areas of these tumors coincides with Noguchi's classification. By using small biopsy specimens, it is sometimes very difficult to make an accurate histological diagnosis. However, if oncologists can obtain information from thin-slice computed tomography examinations that allow calculation of the lepidic growth component ratio of the tumor, together with nuclear morphometry data from biopsy specimens, it would be very practical to extract candidate patients who would benefit from limited treatment before carrying out surgery. In practical terms, we cannot use the Image Processor for Analytical Pathology in routine pathology examinations. We recommend that the size of intermingled small lymphocytes be used as an internal control. Tumor cells with a nuclear area of ≥ 67 μm^2 and a nuclear diameter of 10.7 μm are 5× and 3× larger than small lymphocytes, respectively.

Grading of nuclear structure has already been used to assess the malignancy of various carcinomas, such as breast carcinoma, urinary bladder carcinoma, and renal cell carcinoma. For example, after Zajdela et al[8] reported the relationship between the outcome of mammary cancer and morphological characteristics using cytological materials, several studies demonstrated the prognostic value of nuclear morphometry in invasive ductal carcinoma of the breast. Nuclear morphology is now applied for histological grading of invasive breast carcinomas in the WHO Classification of Tumors of the Breast.[14] The WHO recommends that nuclear grade be included in the surgical reports of cases of invasive ducal carcinoma of the breast. In the present study, we demonstrated that nuclear area and nuclear diameter can also be used to estimate the malignant potential of small-sized adenocarcinomas of the lung.

We stress the importance of nuclear area and nuclear diameter for estimating the malignancy of small-sized adenocarcinomas of the lung. If nuclear grading can be applied along with a pure histological classification such as the WHO or Noguchi classifications, then it may be possible to predict the biological behavior of small-sized adenocarcinomas more precisely than on the basis of histological classification.

CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

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