inducing the contact between the epithelium and the vaginal mesenchyme.[11] The persistent embryonic Müllerian epithelium develops into tuboendometrial-type adenosis.[11] About 25% of the DES-exposed offspring suffer from gross structural malformations of the cervix, including cervical hypoplasia, transverse vaginal septa and obliteration of the vaginal fornices.[3] Adenosis is thought to be the precursor of CCA, because adenosis is detected around the CCA in more than 90 % of the cases.[12] However, because the vaginal CCA is also rare in DES-exposed women, DES is supposed to be a teratogenic factor rather than a carcinogen.[12] Some other susceptible factors are thought to be necessary for the oncogenic transformation, such as genetic factors and hormonal factors.[12] Namely, DES might behave like a teratogen and induces the persistence of embryonic Müllerian epithelium, cervical and vaginal structural abnormalities and adenosis formation. Afterward, with some other factors the adenosis develops into CCA.

In DES-unexposed women, adenosis may arise congenitally[3] in correlation with genitourinary tract anomalies, or postnatally. Smith *et al.* reported eight adenosis in vaginal septum out of 23 patients with obstructed hemivagina with ipsilateral renal anomaly.[13] On the contrary, Goodman *et al.* reported postnatal adenosis appeared after trauma to the vagina.[14] Microscopically, adenosis of both DES-exposed and DES-unexposed are identical.[15] In addition, in DES-unexposed women, adenosis is thought to develop into CCA due to the presence of some other susceptible factors the same as in DES-exposed women.

In our case, when the congenital anomalies developed due to some mechanisms other than DES-exposure, the persistence of the embryonic Müllerian epithelium might occur and adenosis might form in the vaginal septum. Thereafter, adenosis transformed to vaginal CCA due to some other factors. In addition, the rare metanephric duct remained instead of the ureter and mesonephric remnant also remained in the specimen. Although there is a case report of adenocarcinoma originated from metanephric remnant,[8] it is an extremely rare case. It is unlikely for our case, because we could not detect any evidence suggesting the transition from the metanephric remnant to CCA. As for mesonephric remnant, it is unlikely an origin of the CCA because of the topographical disagreement, similar to the cases Kaminski *et al.* reported.[16] We speculate that the vaginal CCA was developed as a result of congenital anomalies of the genitourinary tract without prenatal DES-exposure. However, Ott *et al.* concluded that congenital malformations and a CCA might be a fortuitous occurrence and other mechanisms should be considered.[4] Our speculation is just a hypothesis, and further accumulation of the similar cases is therefore necessary to investigate whether vaginal or cervical adenocarcinoma coexists with such anomalies by chance or as a result of such anomalies.

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Predictors of recurrence in breast cancer patients with a pathologic complete response after neoadjuvant chemotherapy

M Tanioka*[,1,2], C Shimizu[1], K Yonemori[1], K Yoshimura[3], K Tamura[1], T Kouno[1], M Ando[1], N Katsumata[1], H Tsuda[4], T Kinoshita[5] and Y Fujiwara[1]

[1]Breast and Medical Oncology Division, National Cancer Center Hospital, Tokyo, Japan; [2]Medical Oncology Division, Hyogo Cancer Center, Hyogo, Japan; [3]Department of Clinical Trial Design and Management, Translational Research Center, Kyoto University Hospital, Kyoto, Japan; [4]Division of Diagnostic Pathology, National Cancer Center Hospital, Tokyo, Japan; [5]Division of Breast Surgery, National Cancer Center Hospital, Tokyo, Japan

BACKGROUND: Although a pathologic complete response (pCR) after neoadjuvant chemotherapy is associated with favourable outcomes, a small proportion of patients with pCR have recurrence. This study was designed to identify factors predictive of recurrence in patients with pCR.

METHODS: A total of 449 breast cancer patients received neoadjuvant chemotherapy, and 88 evaluable patients had a pCR, defined as no evidence of invasive carcinoma in the breast at surgery. The clinical stage was II in 61 patients (69%), III in 27 (31%). All patients received taxanes and 92% received anthracyclines. Among 43 patients with HER2-positive tumours, 27 received trastuzumab. Cox regression analyses were performed to identify predictors of recurrence.

RESULTS: Median follow-up was 46.0 months. There were 12 recurrences, including 8 distant metastases. The rate of locoregional recurrence was 10.4% after breast-conserving surgery, as compared with 2.5% after mastectomy. Multivariate analysis revealed that axillary metastases (hazard ratio (HR), 13.6; $P < 0.0001$) and HER2-positive disease (HR, 5.0; $P < 0.019$) were significant predictors of recurrence. Five of six patients with both factors had recurrence. Inclusion of trastuzumab was not an independent predictor among patients with HER2-positive breast cancer.

CONCLUSION: Our study results suggest that HER2 status and axillary metastases are independent predictors of recurrence in patients with pCR.

Neoadjuvant chemotherapy is a widely accepted treatment not only for locally advanced breast cancer, but also for earlier-stage operable disease (van der Hage et al, 2001; Bonadonna et al, 1998; Bear et al, 2003). Mauri et al (2005) performed a meta-analysis of clinical trials comparing patients who received preoperative chemotherapy with those who received postoperative chemotherapy. Death, disease progression, and distant recurrence were equivalent in both the arms. The main advantages of neoadjuvant chemotherapy included the evaluation of the in vivo chemosensitivity of tumours in individual patients; minimisation of micrometastases; and surgical downstaging of tumours, allowing breast-conserving surgery (BCS) to be performed in patients who might have otherwise required a mastectomy. However, the survival advantage of neoadjuvant chemotherapy appears to be negligible (Fisher et al, 1997; Bonadonna et al, 1998; Kuerer et al, 2001; Wolmark et al, 2001).

In several studies, a pathologic complete response (pCR), defined as the absence of invasive tumour in the breast only or in the breast and axilla, correlates with a far lower risk of subsequent recurrence, as well as with improved overall survival (Fisher et al, 1997, 1998; Bonadonna et al, 1998; Morrell et al, 1998;

Kuerer et al, 1999; Chollet et al, 2002). Thus, efforts have been made to increase pCR rates by using more effective drugs and treatment regimens (Smith et al, 2002; Buzdar et al, 2005); the achievement of pCR has become the primary end point of many clinical studies.

Although a pCR is associated with favourable outcomes in most patients, some patients with pCR have disease recurrence. Previous studies have reported 5-year recurrence rates of 13–25% (Fisher et al, 1998; Morrell et al, 1998; Kuerer et al, 2001; Wolmark et al, 2001). Only a few studies have examined predictors of recurrence in patients who have a pCR to neoadjuvant treatment (Ring et al, 2004; Gonzalez-Angulo et al, 2005; Guarneri et al, 2006). We therefore retrospectively analysed predictive factors of recurrence in patients with breast cancer who achieved a pCR after neoadjuvant chemotherapy.

PATIENTS AND METHODS

Patients

This was a retrospective study of 88 evaluable patients with primary breast carcinoma who had a pCR after receiving neoadjuvant chemotherapy at National Cancer Center Hospital, Tokyo between 1996 and 2006. The follow-up period was completed

in December 2008. The locoregional or distant recurrences were evaluated on physical examination, or by radiological imaging.

Histopathology

All patients were confirmed to have invasive carcinoma histologically by core needle biopsy. Surgical specimens were sectioned at 7- to 10-mm thick slices, and the pathological response was evaluated by pathologists specialised in breast pathology. The histologic type of the primary tumour was classified according to the *General Rules for Clinical and Pathological Recording of Breast Cancer*, The Japanese Breast Cancer Society (2004). The histologic grade of the tumours was classified according to the Elston–Ellis classification system (Elston and Ellis, 1991). The patients' levels of oestrogen receptor (ER, 1D5; Dako, Glostrup, Denmark), progesterone receptor (PgR, 1A6; Novocastra, Newcastle Upon Tyne, UK), and HER2 (HercepTest, Dako) were measured by immunohistochemical (IHC) analysis of paraffin-embedded tissue specimens. Oestrogen receptor and PgR were classified as positive if more than 10% of cancer cell nuclei were stained, regardless of the staining intensity. HER2-positive status was defined as IHC (3 +); more than 10% of cancer cells markedly positive, or positive results of fluorescence *in situ* hybridisation (FISH) for HER2 amplification, that is, a HER2/CEP17 signal ratio of 2.0 (Vysis Pathvysion; Abbott, Chicago, IL, USA). IHC (2 +) tumours, in which more than 10% of cancer cells were moderately positive, were excluded from the analysis without performing FISH test.

A wide range of criteria have been used to define pCR, and a consensus has yet to be reached. In this study, pCR was defined as no evidence of invasive carcinoma in the breast at the time of surgery in line with the criteria of the National Surgical Adjuvant Breast and Bowel Project B-18 (Wolmark *et al*, 2001) and the recommendations of Sataloff *et al* (1995). Because the presence or absence of residual ductal carcinoma *in situ* (DCIS) after preoperative therapy does not influence long-term rate of local recurrence or overall survival (Mazouni *et al*, 2007), we included patients with residual DCIS in the category of pCR.

Treatment

Neoadjuvant chemotherapy was indicated in patients with clinical stage II or III primary breast cancer whose tumours were larger than 3 cm. Although the potential benefits of adding taxanes to anthracycline-based regimens remain controversial in terms of long-term outcomes (Bear *et al*, 2006), regimens combining anthracyclines with taxanes, either sequentially or concomitantly, are widely used. In this study, neoadjuvant chemotherapy regimens included (1) four cycles of doxorubicin (DOX, $50\,mg\,m^{-2}$) and docetaxel (DTX, $60\,mg\,m^{-2}$) (AT), followed by additional adjuvant treatment with two cycles of AT or four cycles of intravenous cyclophosphamide, methotrexate, and 5-fluorouracil (CMF); (2) four cycles of fluorouracil ($500\,mg\,m^{-2}$)/epirubicin ($100\,mg\,m^{-2}$)/cyclophosphamide ($600\,mg\,m^{-2}$) (FEC) along with 12 weekly cycles of paclitaxel ($80\,mg\,m^{-2}$); (3) four cycles of doxorubicin ($60\,mg\,m^{-2}$)/cyclophosphamide ($600\,mg\,m^{-2}$) (AC) along with 12 weekly cycles of paclitaxel ($80\,mg\,m^{-2}$); (4) twelve weekly cycles of paclitaxel ($80\,mg\,m^{-2}$) only; and (5) four cycles of AC along with four cycles of DTX ($60\,mg\,m^{-2}$). After November 2002, patients with HER2-positive tumours received trastuzumab (initially $4\,mg\,kg^{-1}$ followed by $2\,mg\,kg^{-1}$ weekly) in combination with paclitaxel for 12 weeks. Trastuzumab was not administered post-operatively because it had not been approved for use in an adjuvant setting in Japan until 2007.

As for breast surgery, patients underwent either mastectomy ($n = 40$) or BCS ($n = 48$). Axillary lymph node dissection or sentinel lymph node biopsy alone was additionally performed. The decision to perform BCS was based on the ability to remove residual disease completely with optimal cosmetic results; patient

preference was also considered. Twenty-one patients (24%) received adjuvant endocrine therapy including tamoxifen, anastrozole, or both drugs for 5 years if either the pre-treatment biopsy specimen or the surgical specimen obtained after chemotherapy was positive for ER or PgR. We defined surgical margin positive if the tumour cells were directly exposed to the margin.

Postoperative radiotherapy was administered to 60 patients (68%) who had either undergone BCS or had locally advanced disease. The radiotherapy protocol was as follows: after mastectomy, patients with clinical stage III disease received radiotherapy, delivered in 2 Gy fractions to chest wall and axilla (total dose 50 Gy). After BCS, all patients received radiotherapy, delivered in 2 Gy fractions to the breast (total dose 50 Gy). A booster dose was delivered to the tumorectomy bed if the surgical margin was positive. Regardless of the surgical methods, patients with four or more positive axillary lymph nodes received radiotherapy, delivered in 2 Gy fractions to subclavicular region (total dose 50 Gy).

Clinical significance of locoregional recurrence after neoadjuvant chemotherapy

The impact of locoregional recurrence (LRR) survival after neoadjuvant chemotherapy on survival remains poorly understood. However, patients with LRR after adjuvant chemotherapy, especially those with ER-negative tumours, have substantially worse outcomes regardless of axillary node status (Wapnir *et al*, 2006; Anderson *et al*, 2009). Among patients who achieved a pCR in neoadjuvant setting in our study, the ER-negative rate was 73% and higher than that of patients in adjuvant settings. This suggests the LRR after neoadjuvant chemotherapy might be a negative prognostic factor.

Statistical analysis

Statistical analyses were performed using SAS, version 9.2 (SAS Institute Inc., Cary, NC, USA). The log-rank test was used to identify predictive factors associated with recurrence after the achievement of pCR. Then, variables with *P*-values of ≤0.20 on univariate analysis were included in the multivariate models. Multivariate analysis with a Cox proportional-hazards model was used to identify independent predictors in all 88 patients. Models were selected by stepwise forward analysis, retaining variables significant at the $\alpha = 0.05$ level for the final model. The Kaplan–Meier product-limit method was used to compute recurrence-free survival according to the number of predictive factors. Recurrence-free survival was measured from the date of initial diagnosis to the date of recurrence (including LRR) or the last follow-up visit. In addition, the relations of recurrence to clinicopathological factors in the 43 patients with HER2-positive tumours were also evaluated. A Cox proportional-hazards model including variables with *P*-values of ≤0.05 on univariate analysis was used to identify independent predictors of recurrence.

RESULTS

Characteristics of patients with relapse

Of 449 patients with breast cancer who received neoadjuvant chemotherapy, 88 (20%) evaluable patients were identified as having a pCR. The median follow-up was 46 months (range, 8–115). Table 1 shows the patient and tumour characteristics. The median age was 54.5 years (range, 29–78). The median diameter of the primary breast tumour was 45.0 mm (range, 25–130). All patients received taxane-based chemotherapy, and 92% also received anthracycline-based therapy.

A total of 12 patients (13.6%) had tumour recurrence (Table 2). All recurrences were diagnosed within 32 months after initial diagnosis. Seven patients died of breast cancer within the follow-up

period. Among the six patients who had LRR, five had received BCS as primary surgery, and four had DCIS after neoadjuvant chemotherapy. LRR occurred in 5 of 48 patients (10.4%) after BCS, as compared with only 1 of 40 patients (2.5%) after mastectomy.

Predictive factors for recurrence in all 88 patients with pCR

The results of univariate analysis of predictive factors for recurrence are shown in Table 3. Variables tested for inclusion in the multivariate model were axillary lymph node metastasis at surgery, HER2 status (positive vs negative) and stage (III vs II). After controlling for these factors, axillary lymph node metastasis

Table I Patient characteristics

Characteristic	All patients (N = 88) No. of patients
Age, years	
≤50/>50	33/55
Clinical stage	
II/IIIA/IIIB,IIIC	61/18/9
Pre-treatment pathology	
Invasive ductal/lobular/mucinous/others	85/1/1/1
Nuclear grade	
1/2/3/unknown	2/24/61/1
Hormone receptor status	
ER or PgR positive/both negative	23/65
HER2 status	
Positive/Negative	43/45
Neoadjuvant chemotherapy	
FEC→weekly paclitaxel (± trastuzumab)	31 (16 with trastuzumab)
AC→weekly paclitaxel (± trastuzumab)	30 (8 with trastuzumab)
AT (doxorubicin + docetaxel)	19
Weekly paclitaxel (± trastuzumab)	7 (3 with trastuzumab)
AC→docetaxel	1
Surgery	
Mastectomy/Breast-conserving surgery	40/48

Abbreviations: FEC = fluorouracil + epirubicin + cyclophosphamide; AC = doxorubicin + cyclophosphamide; PgR = progesterone receptor.

(hazard ratio (HR), 13.6; 95% CI, 4.6–63.3; $P<0.0001$) and HER2-positive disease (HR, 5.0; 95% CI, 1.3–19.3; $P<0.019$) remained significant independent predictors of recurrence (Table 4). According to the number of independent risk factors (HER2-positive disease and axillary lymph node metastasis) for recurrence, the 5-year recurrence-free rate varied between 94.4% for no factor ($n=36$), 89.1% for 1 factor ($n=46$), and 0% for 2 factors ($n=6$).

Predictive factors for recurrence among 43 patients with HER2-positive disease

Among 43 patients with HER2-positive breast cancer who had a pCR, 27 received trastuzumab. The results of the univariate analysis of predictive factors for recurrence are shown in Table 3. Variables tested for inclusion in the multivariate model were axillary lymph node metastasis at surgery, inclusion of trastuzumab, and stage (III). After controlling for these factors, only axillary lymph node metastasis (HR, 74.6 (8.0–692.9); $P<0.0001$) remained a significant independent predictor of recurrence.

DISCUSSION

Because a small proportion of patients with breast cancer have recurrence after achievement of a pCR, prediction of the risk of recurrence has an important role in postoperative management. Our multivariate analysis of all 88 patients with a pCR showed that axillary lymph node metastasis and HER2-positive disease were independent predictors of recurrence. Five of the six patients with both of these factors had recurrence after achieving a pCR in our study. Such patients may benefit from additional postoperative therapy and not be optimal candidates for clinical trials with pCR as the primary end point.

Although pCR in this study was defined as no evidence of invasive carcinoma only in the breast, the trial of the University of Texas MD Anderson Cancer Center pCR criteria requires not only complete response of the primary lesion but also the disappearance of axillary metastasis (Green et al, 2005). We also performed Cox regression model analysis of 73 patients who satisfied the MD Anderson pCR criteria (results not shown). On univariate analysis, tumour diameter (>50 mm) and grade (3) had P-values of ≤0.20. However, no factor was independently significant in the multivariate analysis. The reasons for the differences in the results according to the definitions of pCR were the smaller sample size, the smaller number of recurrences (only five recurrences), and the elimination of the large influence of axillary lymph nodes on recurrence.

Table 2 Characteristics of patients with recurrence

		Initial diagnosis			Operative information			State at recurrence			
No.	Age	Tumour diameter	HER2	ER or PgR	Ax. M.	DCIS	BCS	LRR	Distant M.	Brain M.	RFS
1	39	90	−	−	−	−	−	−	+	+	8
2	33	52	−	+	−	−	+	−	+	−	26
3	62	55	+	−	−	−	+	+	+	−	26
4	29	35	+	+	−	+	+	+	+	−	30
5	58	42	+	−	−	−	+	+	+	−	32
6	55	65	+	−	+	+	−	−	+	−	32
7	63	49	+	−	+	+	−	+	−	−	18
8	36	34	−	+	+	-	−	−	+	−	20
9	49	30	+	−	+	+	−	−	+	−	21
10	56	25	+	−	+	+	+	+	−	−	21
11	50	55	+	−	+	-	+	−	+	+	29
12	71	60	−	−	+	+	+	+	−	−	32

Abbreviations: Ax. M. = axillary lymph node metastasis; M. = metastasis; BCS = breast-conserving surgery; RFS = recurrence-free survival (months); LRR = locoregional recurrence; ER = oestrogen receptor; PgR = progesterone receptor; HER2 = human epidermal growth factor 2; DCIS = ductal carcinoma in situ.

As expected, histopathological lymph node status was a strong predictor of recurrence in patients who had a pCR of their primary tumours. In contrast, HER2 status was found to be a predictor of recurrence for the first time. Gonzalez-Angulo et al (2005) studied predictive factors for distant metastasis in 226 patients with pCR. Although HER2 positivity was not a significant predictor of distant metastasis, HER2 status was unknown in 58% of the patients, and only 5% received taxane-based chemotherapy. Interactions between HER2 status and paclitaxel have been reported in an adjuvant setting, especially among patients with ER-negative tumours (Hayes et al, 2007). In our exploratory study, HER2 status was assessed by IHC or FISH analyses in all patients, the ER- or PgR-positive rate was low (26%), and all the patients received taxane-based therapy. The combination of these factors may have contributed to the identification of HER2 positivity as a significant independent predictor of recurrence after the achievement of a pCR.

Buzdar et al (2005, 2007) and Gianni (2008) reported the results of randomised trials of trastuzumab given with neoadjuvant chemotherapy to patients with HER2-positive breast cancer, and the pCR rate was significantly higher than that in the control arm. However, there are only a few, small randomised trials

Table 4 Multivariate analysis of predictors of recurrence (all 88 patients)

Characteristic	HR	P-value	95% CI
Axillary lymph node metastasis	13.6	<0.0001	4.6–63.3
HER2-positive disease	5.0	0.019	1.3–19.3

Abbreviations: HR = hazard ratio; CI = confidence interval; HER2 = human epidermal growth factor receptor 2.

Table 3 Univariate analysis of predictive factors for recurrence

Characteristic	All patients (N = 88)			HER2 positive (N = 43)		
	No.	Patients with recurrence (%)	P-value	No.	Patients with recurrence (%)	P-value
Age						
> 50 years old	55	10.9		28	17.9	
⩽ 50 years old	33	18.2	0.28	15	20	0.83
Tumour diameter						
> 50 mm	30	20.0		12	25.0	
⩽ 50 mm	58	10.3	0.22	31	16.1	0.44
Clinical stage						
II	61	9.8		30	13.3	
III	27	22.2	0.09	13	30.8	0.11
ER or PgR						
Positive	23	13.0		9	11.1	
Negative	65	13.8	0.87	34	20.6	0.45
HER2						
Positive	43	18.6				
Negative	45	9.1	0.19			
Nuclear grade						
3	61	14.5		28	21.4	
1–2	26	11.5	0.71	15	13.3	0.49
Type of chemotherapy						
Anthracycline + taxane	81	13.4		39	18.0	
Taxane based	7	28.6	0.38	4	25.0	0.91
Type of chemotherapy						
With trastuzumab	27	7.4		27	7.4	
Without trastuzumab	61	16.4	0.28	16	37.5	0.015
Surgery						
Mastectomy	40	12.5		21	23.8	
BCS	48	14.6	0.84	23	13.6	0.48
Residual DCIS						
Present	39	15.4		23	21.7	
None	49	12.2	0.65	20	15.0	0.50
No. of LNs examined						
⩽ 10	15	14.7		7	14.3	
> 10	73	13.7	0.93	36	19.4	0.79
Axillary LN status						
Node positive	15	46.7		6	83.3	
Node negative	73	6.9	<0.001	37	8.1	<0.001

Abbreviations: ER = oestrogen receptor; PgR = progesterone receptor; HER2 = human epidermal growth factor receptor 2; pCR = pathological complete response; BCS = breast-conserving surgery; DCIS = ductal carcinoma in situ; LN = lymph node.

of neoadjuvant trastuzumab, and so far no study has shown that neoadjuvant trastuzumab can improve overall survival (Rowan, 2009). Indeed, in our study, the pCR rate in patients with HER2-positive breast cancer who received neoadjuvant chemotherapy with trastuzumab was 50% (27 out of 54), which was much higher than that for the study group as a whole (20%, 88 out of 449). However, the inclusion of trastuzumab was not a significant predictor of recurrence on multivariate analysis. This is partly because trastuzumab was not administered post-operatively. The optimal duration of trastuzumab in neoadjuvant and adjuvant setting should be confirmed prospectively in randomised trials.

The demand for BCS is expected to rise as the reported rate of pCR after BCS increases. However, LRR rates after BCS in patients who received neoadjuvant chemotherapy in previous studies have varied from 2.6 to 22.6% (Mauriac *et al*, 1999; Rouzier *et al*, 2001; Peintinger *et al*, 2006). This wide variability has led to uncertainty, and the benefits of BCS have been questioned. Objective evaluation of the safety and effectiveness of BCS has been precluded by the small numbers of patients who have achieved a pCR, different criteria for determining whether BCS is indicated, and different treatment regimens. Mauri *et al* (2005) performed a meta-analysis of clinical trials comparing preoperative with postoperative chemotherapy. Although the proportion of patients with distant recurrence was equivalent in both arms, LRR was more frequent in the preoperative chemotherapy arm, with an HR of about 1.2. In our study, most cases of LRR occurred after BCS, and the proportion of patients with LRR was 10.4% after BCS, as compared with only 2.5% after mastectomy. Our study results suggest that even after achieving a pCR, patients should be carefully followed up for LRR after BCS.

This study was retrospective and lacked a sufficient number of patients with recurrence after the achievement of a pCR to allow us to make firm recommendations for a given treatment option. Despite these limitations, some tentative conclusions can be drawn. First, our retrospective analysis showed that HER2-positive disease and axillary metastasis were independent predictors of recurrence after the achievement of a pCR at the primary site in response to neoadjuvant chemotherapy. This finding suggests that patients with HER2-positive disease and axillary metastasis may be candidates for more aggressive adjuvant therapy even after the achievement of a pCR, but this assumption must be confirmed in future clinical trials. Second, the inclusion of trastuzumab in regimens for neoadjuvant chemotherapy might not be predictive of recurrence, even though the rate of pCR among patients who received trastuzumab was much higher than that among all patients who received neoadjuvant chemotherapy. Third, the rate of LRR was higher after BCS than after mastectomy. Patients who undergo BCS should thus be closely followed up for LRR.

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Clinical Studies

Original Article

Nuclear Grading of Primary Pulmonary Adenocarcinomas

Correlation Between Nuclear Size and Prognosis

Yoshimasa Nakazato, MD[1,2,3]; Yuko Minami, MD[2]; Hiromi Kobayashi, MD[2]; Kaishi Satomi, MD[2]; Yoichi Anami, MD[2]; Koji Tsuta, MD[4]; Ryota Tanaka, MD[5]; Masafumi Okada, MD[6]; Tomoyuki Goya, MD[1]; and Masayuki Noguchi, MD[2]

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 μm^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 μm was correlated with longer survival ($P = .0002$), and the 5-year survival rate was 88.6%. The mean ($\pm$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 ($\pm$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

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 ≤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 ×40 objective and a ×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 lym-
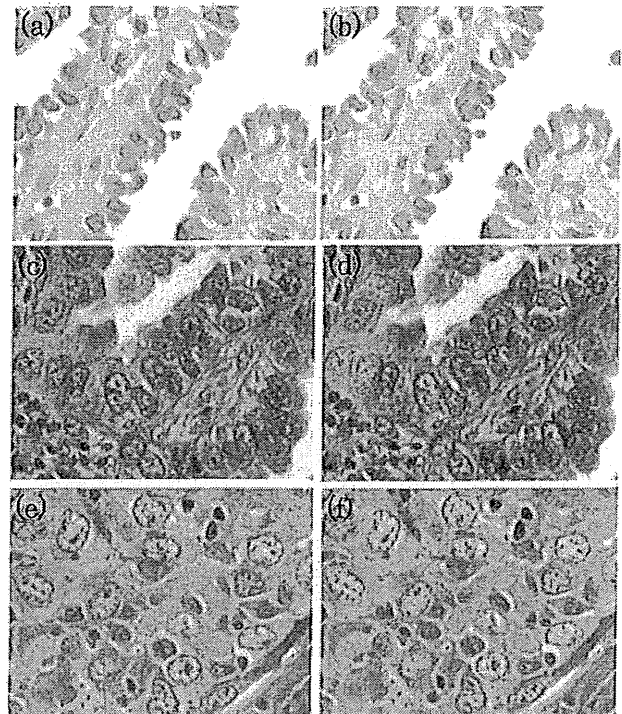

Figure 1. (a, c, e) Histology of small-sized adenocarcinoma of the lung is shown (H & E; original magnification, ×400). (b, d, f) Karyometric analysis using an Image Processor for Analytical Pathology is shown. The nucleus in the carcinoma cell was picked up from the field in each panel. The red area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

phocytes, 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.

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

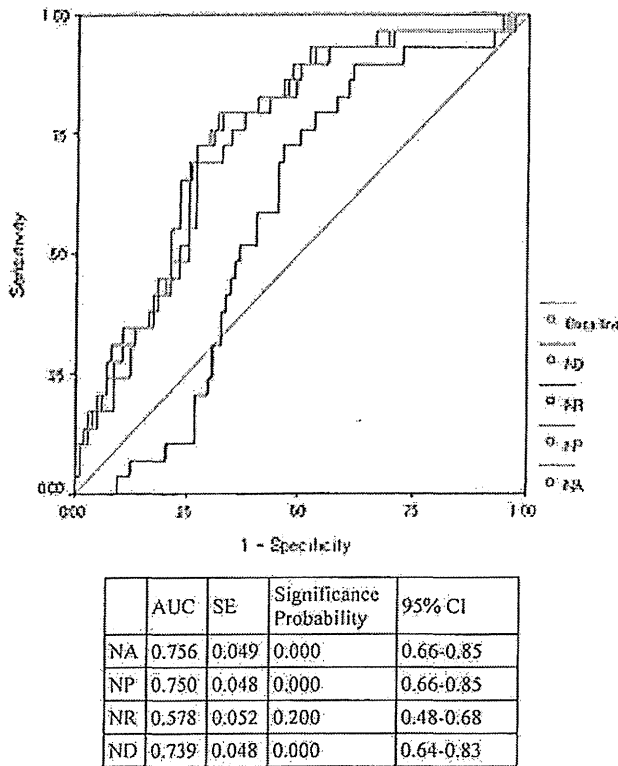|	AUC	SE	Significance Probability	95% CI
NA	0.756	0.049	0.000	0.66-0.85
NP	0.750	0.048	0.000	0.66-0.85
NR	0.578	0.052	0.200	0.48-0.68
ND	0.739	0.048	0.000	0.64-0.83

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.
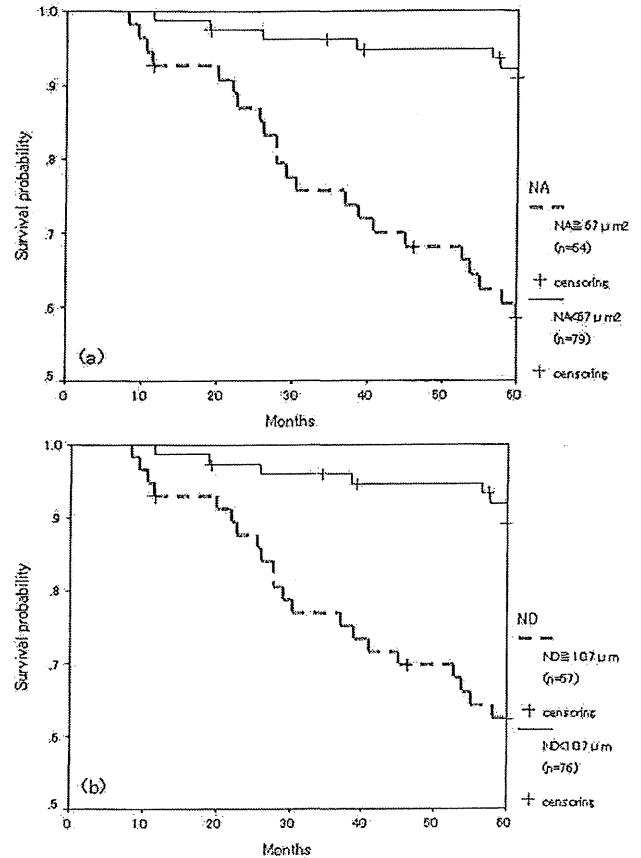

Figure 3. Five-year recurrence-free survival rates are shown for all patients, classified using the critical nuclear size. (a) A nuclear area (NA) of 67 μm² was used as a cutoff value ($P <$ .0001). (b) A nuclear major axis dimension (ND) of 10.7 μm was used as a cutoff value ($P = $.0002).

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 μm² (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 μm² 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		
	$<67\ \mu m^2$	$\geq 67\ \mu m^2$	P	$<10.7\ \mu m$	$\geq 10.7\ \mu m$	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 $<67\ \mu m^2$ was 90.4% (Fig. 3a). Conversely, the corresponding survival rate of those with tumor cells having a mean nuclear area of $\geq 67\ \mu m^2$ was 57.7%. A nuclear area of $\geq 67\ \mu m^2$ was correlated with shorter survival ($P < .0001$). Similarly, the 5-year survival rate of patients whose tumor cells had a mean nuclear diameter of $<10.7\ \mu m$ was 88.6% (Fig. 3b). The corresponding survival rate of patients with tumor cells having a mean nuclear diameter of $\geq 10.7\ \mu m$ was 61.8%. A nuclear diameter of $\geq 10.7\ \mu m$ 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 fac-

tors 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 $48 \pm 9\ \mu m^2$ in BAC, $68 \pm 8\ \mu m^2$ in the papillary subtype, $82 \pm 20\ \mu m^2$ in the solid subtype, and $65 \pm 15\ \mu m^2$ 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 $9.1 \pm 0.9\ \mu m$ in BAC, $10.8 \pm 0.8\ \mu m$ in the papillary subtype, $11.4 \pm 1.3\ \mu m$ in the solid subtype, and $10.5 \pm 1.2\ \mu m$ 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 μm² vs <67 μμm²	.037	0.35	0.13-0.94
Pleural invasion: P0 vs P1-3	.046	2.49	1.02-6.12
Tumor classification: T1 vs $\geq$T2	.010	0.31	0.12-0.76
Lymph node status: N0 vs $\geq$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, μm²	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 μm²) was similar to that of type B tumors (mean ± SD, 51 ± 15 μm²), whereas the mean nuclear area of type C tumors (mean ± SD, 63 ± 15 μm²) was significantly larger than that of types A and B tumors (mean ± SD, 49 ± 12 μm²) ($P < .0001$). In addition, the nuclear area of type D tumors (mean ± SD, 77 ± 18 μm²) 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 μm²) was significantly larger than that of lepidic-type tumors (mean ± SD, 59 ± 16 μm²) ($P < .0001$), and the nuclear diameter of nonle-
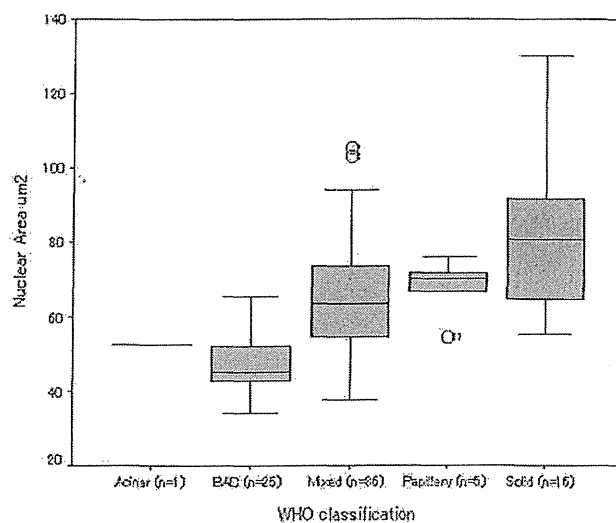

Figure 4. A box plot of the nuclear area in all patients is shown, classified according to the World Health Organization (WHO) classification. BAC indicates bronchioloalveolar carcinoma.

pidic-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) ($P < .0001$).

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.
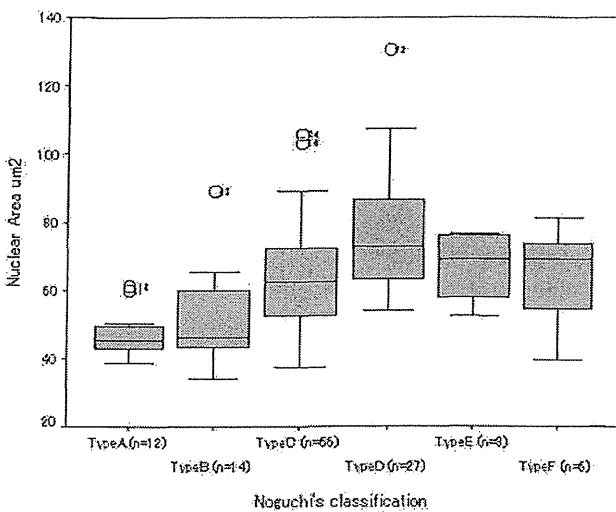Adapted from Noguchi et al.[5]

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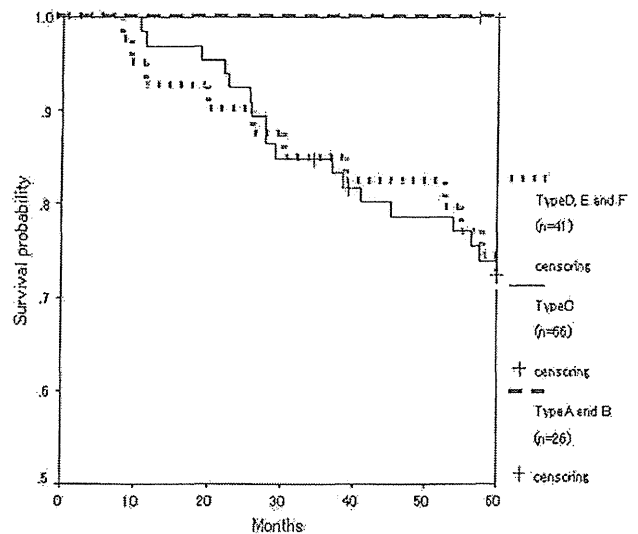

Figure 6. The 5-year recurrence-free survival rates of all patients is shown, classified according to the modified Noguchi classification with 3 subtypes: types A and B (n = 26), type C only (n = 66), and nonlepidic type (types D, E, and F; n = 41).

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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SPECIAL FEATURE

Strategies of radiotherapy for recurrent and metastatic breast cancer

Management of locoregional recurrence of breast cancer

Naoto Shikama · Kenji Sekiguchi · Naoki Nakamura

Abstract The locoregional recurrence of breast cancer is not a sign of distant metastases, and a substantial proportion of cases are cured by salvage therapy. Patients with locoregional recurrence should not be treated with palliative intent as if they have visceral metastases. The recommended treatment for ipsilateral breast recurrence after breast conservative therapy is a mastectomy. For patients who suffer from isolated chest wall recurrence after mastectomy, a surgical approach is recommended. Neoadjuvant chemotherapy is considered for patients with unresectable disease in order to render the disease resectable. For patients with isolated chest wall recurrence who have received no prior radiotherapy, postoperative radiotherapy involving the chest wall and regional lymph nodes is recommended. Patients with isolated axillary lymph node recurrence should be treated with axillary dissection or resection. Although the effectiveness of systemic therapy for patients with locoregional recurrence is unclear, there is a trend toward treating patients with supraclavicular lymph node recurrence with radiotherapy plus systemic therapy. Pain relief and the eradication of other distressing symptoms resulting from inoperable disease are achieved in two-thirds to three-quarters of patients by radiotherapy with or without systemic therapy. New anticancer agents and molecular target therapies should be evaluated with the objective of improving the treatment outcome of patients with locoregional recurrence. A combination of approaches is required for treatment of patients with locoregional recurrence, and a multidisciplinary tumor board should be organized at each institute.

Keywords Local recurrence · Lymph node recurrence · Radiotherapy · Chemotherapy · Mastectomy

Introduction

Ten to thirteen percent of patients who receive breast conservative therapy develop locoregional recurrence within 10 years of their initial treatment, and three to eight percent of patients who receive mastectomy plus postoperative radiotherapy will also develop locoregional recurrence [1]. The omission of postoperative radiotherapy increases the risk of ipsilateral breast recurrence or chest wall recurrence threefold. Ipsilateral breast recurrence after breast conservative therapy sometimes occurs after more than 10 years; however, approximately 80% of locoregional recurrences after mastectomy arise within the first 5 years [1–3]. The standard of care for locoregional recurrence has not been clarified because of its heterogeneous biological characteristics and a lack of well-designed prospective clinical trials. The authors have strived to assess the effectiveness of treatment strategies developed in previous studies.

Diagnosis and re-staging

The first step for choosing an appropriate treatment is pathological evaluation of the recurrent disease, and fine needle biopsy, core needle biopsy, and/or open biopsy can

N. Shikama (✉)
Department of Radiation Oncology, Saku General Hospital,
197 Usuda-machi, Saku, Nagano 384-0301, Japan
e-mail: nshikama0525@gmail.com

N. Shikama · K. Sekiguchi · N. Nakamura
Department of Radiation Oncology,
St Luke's International Hospital, Tokyo, Japan

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be used for this. The pathological subtype, histological grade, expression of hormonal receptors, and human epidermal growth factor receptor type2 (HER-2) overexpression should be evaluated when choosing appropriate treatment strategies for patients with recurrent disease. Radiation-induced sarcomas in the chest wall appear at a median of 10 years after postoperative treatment, but the latency period varies. The next step is a staging evaluation. Systemic disease can be carefully evaluated by using blood tests, chest computed tomography (CT), abdominal CT, pelvic CT, and radionuclide bone scans. Magnetic resonance imaging (MRI), CT, and color Doppler ultrasonography are useful for evaluating the extent of supraclavicular and infraclavicular lymph node recurrence. Positron emission tomography (PET) scans are performed increasingly in clinical practice and are more sensitive than CT and bone scans; however, meta-analysis of evaluation of breast cancer recurrence demonstrated that the false positive rate of PET scans was relatively high (11%) [4]. The clinical value of PET scans alone is not satisfactory, so addition of other conventional imaging modalities is required.

Prognostic factors

For patients with locoregional recurrence after breast conservative therapy, disease-free interval (DFI) from the initial treatment to recurrence is the most powerful predictive factor. The 5-year survival rate of patients who developed recurrence within 2 years of the initial treatment was 65% and that of the patients who developed recurrence after 2 years was over 80% [5]. Other poor prognostic factors of mortality have been reported, for example age ($\geq$60 years), the number of positive lymph nodes at the initial treatment (four or more), primary tumor size ($\geq$2 cm), histology (invasive cancer), and estrogen receptor expression (negative) [6]. For patients with locoregional recurrence after mastectomy, some tumor characteristics at the diagnosis of recurrence, for example an operable tumor, the absence of tumor necrosis, the recurrent site (chest wall or axillary lymph node), a pT1-2N0 primary tumor, and a long DFI, are associated with a good treatment outcome [7–9].

Schmoor et al. [9] reviewed 337 patients with locoregional recurrence among the 2,746 patients who received conservative therapy or mastectomy in four prospective studies of the German Breast Cancer Study Group. Multivariate analysis demonstrated that number of positive lymph nodes, tumor grade, estrogen receptor, and DFI were independent prognostic factors for progression-free survival after locoregional recurrence. They simplified the risk strata and defined three risk groups:

- low risk: primary node-negative status and a DFI of more than 2 years;
- intermediate risk: primary node-positive status or a DFI of more than 2 years; and
- high risk: primary node-positive status and a DFI of less than 2 years (Table 1).

Although it excludes other prognostic factors, for example age, tumor grade, recurrent site, and estrogen receptor, this simplified prognostic index is a useful tool for choosing treatment strategies in clinical practice and clinical trials.

Recurrence after breast conservative therapy

Thirteen percent of patients who develop recurrence after conservative therapy have locoregional recurrence alone, 30% have locoregional recurrence with distant metastases, and another 57% have distant metastases alone [2]. Approximately 80% of patients with locoregional recurrence develop ipsilateral breast recurrence as the first site [10, 11]. Recurrence in the ipsilateral breast includes two different types of disease, true recurrence and second primary tumors. True recurrence occurs within the primary tumor site or its vicinity, and second primary tumors occur in other quadrants of the breast or have a different pathological subtype [10, 12, 13]. However, some second primary tumors may occur in the same quadrant, and others will have the same pathological subtype. Strict distinction between true recurrence and second primary tumors is difficult, and some investigators have distinguished between them by using pathological subtype, location, and deoxyribonucleic acid (DNA) flow cytometry [10, 12, 13]. True recurrence is associated with early development (median interval: 3.7 vs. 7.3 years) and poor treatment outcome (10-year overall survival: 55 vs. 75%) compared with second primary tumors [12].

Table 1 Prognostic index for patients with locoregional recurrence of breast cancer [9]

	5-year PFS (95%CI)	5-year OS (95%CI)
Low risk		
Node (−) and DFI $\leq$2 years	53% (41–64)	66% (55–77)
Intermediate risk		
Node (+) or DFI >2 years	40% (31–49)	53% (44–62)
High risk		
Node (+) and DFI >2 years	17% (9–25)	27% (17–36)

Node (−), primary node-negative status; DFI, disease-free interval from initial treatment to recurrence; Node (+), primary node-positive status; PFS, progression-free survival; OS, overall survival; 95%CI, 95% confidence interval

Ipsilateral breast recurrence after breast conservative therapy

More than 20% of evaluated mastectomy specimens of ipsilateral breast recurrence after conservative therapy revealed substantial residual disease in two or more quadrants of the breast [14]. The generally recommended treatment for ipsilateral breast recurrence after breast conservative therapy is salvage mastectomy with or without axillary dissection [5, 6, 14–17]. Approximately 90% of the patients have operable recurrent tumors, and other patients have inoperative tumors with diffuse infiltration or inflammatory changes [11, 14–16, 18]. Most patients who received salvage mastectomy achieved good local control, and the 5-year overall survival rates after recurrence ranged from 60 to 86% [5, 6, 12, 14, 18]. Patients who have inoperative tumors involving diffuse infiltration or inflammatory changes have a poor prognosis [19].

Less intensive salvage care for locoregional recurrence has also been investigated. Several investigators have reported the outcome of repeated conservative therapy including partial breast resection with or without radiotherapy after ipsilateral breast recurrence [16, 18, 20]. Salvadori et al. [18] reported the same overall survival in patients who underwent re-conservative therapy (85%) and patients who received salvage mastectomy (70%); however, second ipsilateral recurrence was more common in the patients who received re-conservative therapy (19 vs. 4%). Galper et al. [16] reviewed 341 patients with local recurrence after conservative therapy and reported that the time to distant failure, second malignancy, or death of the patients who received re-conservative therapy was worse than that of the patients who received salvage mastectomy (hazard ratio: 2.0, $p = 0.02$). Re-conservative therapy for ipsilateral breast recurrence is not recommended. Sentinel lymph node (SLN) biopsy is a less toxic tool, and the experience of the Memorial Sloan–Kettering Cancer Center demonstrated that SLN were identified in 55% of 117 patients who had undergone prior axillary dissection or biopsy. Although SLN biopsy is available for some patients who have undergone prior axillary dissection, further studies are required [21].

Postoperative radiotherapy after salvage mastectomy is used for patients with a positive surgical margin or macroscopic residual tumor who have no history of breast irradiation. Re-irradiation is associated with late adverse effects such as tissue necrosis, fibrosis, and rib fractures. There are no data supporting prophylactic regional lymph node irradiation after salvage mastectomy for patients with ipsilateral breast recurrence.

Only one randomized clinical trial has evaluated addition of tamoxifen (TAM) for patients who underwent complete resection and postoperative radiotherapy [22].

Although the addition of TAM prolonged relapse-free survival, 9-year overall survival did not improve. Le et al. [23] reported that systemic chemotherapy and hormonal therapy reduced the risk of death for premenopausal patients, but did not reduce it for postmenopausal patients. Cochran's systematic review concluded that there was little evidence to support the addition of systemic therapy for patients with locoregional recurrence of breast cancer [24]. However, the addition of hormonal therapies is considered to be reasonable in selected patients because of their limited toxicities [25].

Regional lymph nodes recurrence after breast conservative therapy

Regional lymph node recurrence after breast conservative therapy is relatively rare (0.5–6.3%) [6, 26, 27]. The most common sites of regional recurrence are the axillary area and supraclavicular fossa [28, 29]. The pooled analyses of the National Surgical Adjuvant Breast and Bowel Project studies demonstrated that the prognosis of patients with isolated axillary lymph node recurrence was more favorable than that of patients with supraclavicular lymph node recurrence, and the 5-year distant metastases-free survival of the former was 31.5% whereas that of the latter was only 12.1% [6].

The experience of the MD Anderson Cancer Center was that surgery for axillary recurrence achieved good local control; however, the absence of radiotherapy or systemic therapy from the multimodality treatment strategy did not correlate with disease control or the frequency of distant metastases [30]. Maximum axillary control is achieved with an axillary dissection whenever feasible. Limited data are available regarding postoperative regional lymph node irradiation [28]. Radiotherapy is indicated for patients who undergo incomplete resection of axillary disease and patients with supraclavicular lymph nodes metastases [29]. Although the role of systemic therapy has not been established, there is a trend towards administering systemic therapy to patients with supraclavicular lymph nodes recurrence [17].

Fowble et al. [27] reported that none of their six patients with isolated axillary recurrence subsequently developed breast recurrence. They also concluded that isolated axillary node recurrence without clinical or mammographic evidence of ipsilateral breast recurrence does not require a prophylactic mastectomy.

Recurrence after mastectomy

According to the pooled analysis of the Easton Cooperative Oncology Group, locoregional recurrence developed in 420