the HRs and 95% CIs in the lower row were obtained by the multivariate analyses in model 2 (FF fibrosis grade).

FF diameter greater than 8 mm, FF fibrosis grade 1 (Fig. 2A), age 39 years or younger, histologic grade 2, and histologic grade 3 significantly increased the HRs for distant-organ metastasis in the N0 IDC group and the stages I and II IDC group (Table 3). Muscle invasion and FF fibrosis grade 3 significantly increased the HRs for distant-organ metastasis in the N0 IDC group and the stages I and II IDC group, respectively.

FF diameter greater than 8 mm, FF fibrosis grade 1, and histologic grade 3 significantly increased the HRs for bone metastasis in the N0 IDC group and the stages I and II IDC group (Table 3). Muscle invasion significantly increased the HRs for bone metastasis in the N0 IDC group, and FF fibrosis grade 3 significantly increased the HR for bone metastasis in the stages I and II IDC group.

Many factors that were significantly associated with organ metastasis in the multivariate analyses were associated with an 18- to 24-month interval between surgery and detection of the initial organ metastasis, and presence of muscle invasion was associated with the shortest median interval between surgery and detection of the initial distant-organ metastasis and bone metastasis, independent of nodal status and pTNM stage, and it was followed by histologic grade 3 and characteristics of FF (Table 4).

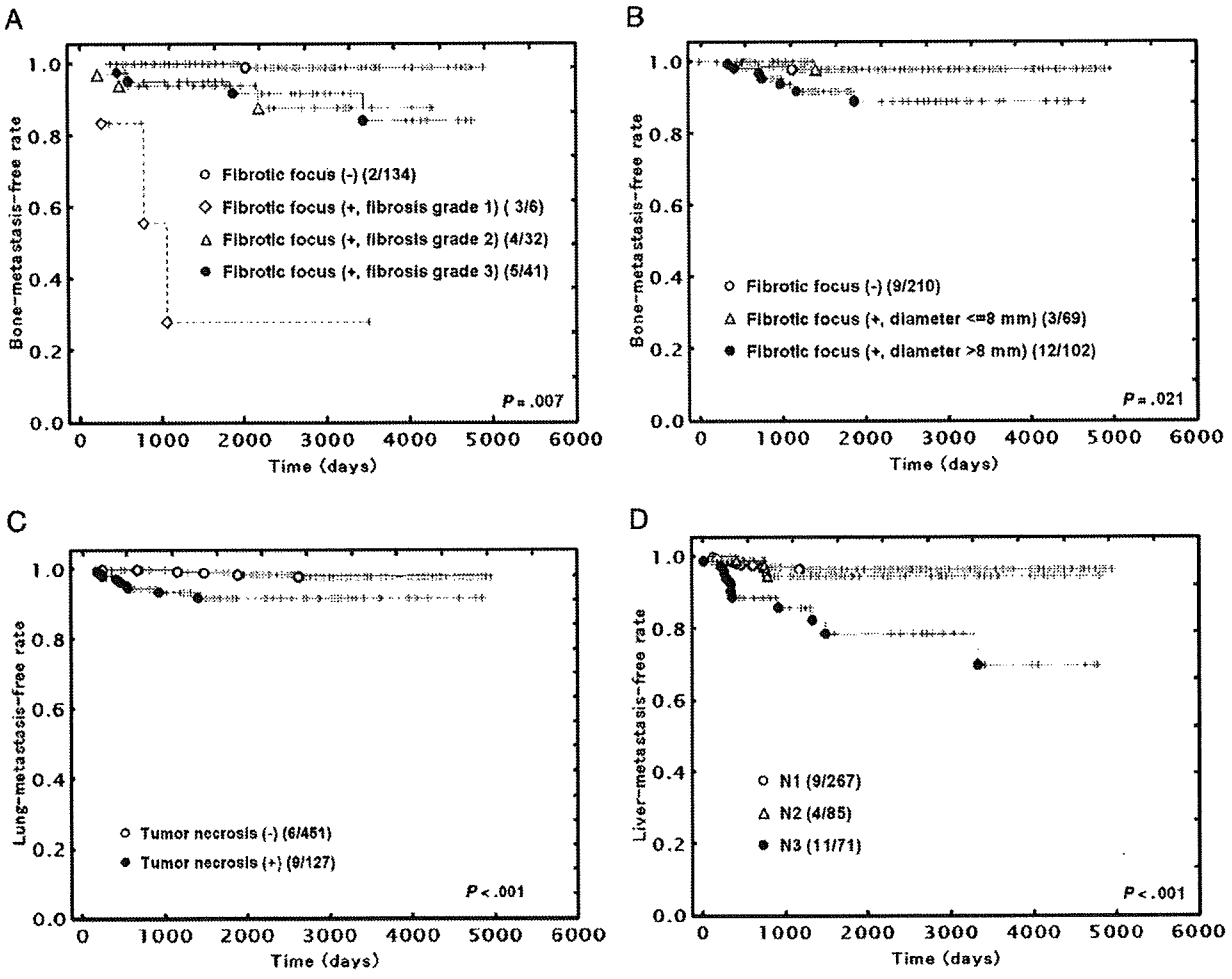

Fig. 2 Survival curves of patients with IDC with bone metastasis (A and B), lung metastasis (C), and liver metastasis (D). A, Among the patients with IDC who did not receive adjuvant therapy and had no nodal metastasis, the patients with FF fibrosis grade 1 IDC have a significantly shorter bone metastasis–free survival curve than any of the other groups of patients. B, Among the patients with IDC who received adjuvant therapy and had no nodal metastasis, the patients with IDC with an FF greater than 8 mm in diameter have a significantly shorter bone metastasis–free curve than the patients with IDC without an FF and the patients with IDC with an FF 8 mm or less in diameter. C, Among the patients with stages I and II IDC who received adjuvant therapy, the patients with tumor necrosis had a significantly shorter lung metastasis–free curve than those without tumor necrosis. E, Among the patients with IDC who received adjuvant therapy and have nodal metastasis, the patients with IDC with 10 or more nodal metastases (N3) have a significantly shorter liver metastasis–free curve than the patients with IDC classified as N1 (1-3 nodal metastases) or N2 (4-9 nodal metastases).

Table 3 Factors in the multivariate analyses that significantly increased the HR for organ-specific metastasis according to nodal status and pTNM stage in patients with IDC who did not receive adjuvant therapy

Groups of patients with IDC according to nodal status and pTNM stage	N0	Stages I and II
Distant-organ metastasis		
FF, diameter >8 mm (*vs* FF, absent)	13.9/4.5-43.2	20.4/4.4-92.6
FF, fibrosis grade 1 (*vs* FF, absent)	22.3/4.1-119.8	43.7/8.0-250.8
Age, ≤39 y	–/–	–/–
(*vs* age, >39 y)	11.1/2.2-56.6	10.0/2.0-48.8
Histologic grade 2	–/–	–/–
(*vs* histologic grade 1)	13.5/1.6-115.2	6.7/1.3-35.4
Histologic grade 3	–/–	–/–
(*vs* histologic grade 1)	16.0/1.9-142.0	7.0/1.2-39.0
Muscle invasion,	17.2/2.0-149.3	NA
present (*vs* muscle invasion, absent)	–/–	NA
FF, fibrosis grade 3 (*vs* FF, absent)	–/–	4.3/1.3-14.5
Bone metastasis		
FF, diameter >8 mm (*vs* FF, absent)	8.0/1.9-32.5	27.6/3.3-225.2
FF, fibrosis grade 1 (*vs* FF, absent)	13.3/2.9-60.3	32.2/5.8-189.5
Histologic grade 3	4.0/1.0-15.4	–/–
(*vs* histologic grade 1)	6.6/1.7-25.1	4.3/1.1-16.7
Muscle invasion, present	22.4/2.2-228.7	NA
(*vs* muscle invasion, absent)	24.4/2.3-220.3	NA
FF, fibrosis grade 3 (*vs* FF, absent)	–/–	5.3/1.1-24.0

Note: Models 1 and 2: values are shown as HR/95% CI.
Abbreviations: –/–, not significant; NA, not available.

3.3. Multivariate analyses for distant organ metastasis, bone metastasis, lung metastasis, and liver metastasis according to nodal status and pTNM stage in the IDC group that received adjuvant therapy

FF diameter greater than 8 mm significantly increased the HRs for distant organ metastasis in all IDC groups in the multivariate analyses, and N3 significantly increased the HRs for distant organ metastasis in the N+ group and the stage III IDC group (Table 5). Tumor necrosis significantly increased the HRs for distant organ metastasis in the N0, N+, and stages I and II IDC groups in the multivariate analyses. FF fibrosis grades 2 and 3 significantly increased the HRs for distant organ metastasis in the N+ group and stages I and II IDC group in the multivariate analyses. Age 39 years or younger significantly increased the HRs for distant organ metastasis in the N+ group and stage III IDC group.

FF diameter greater than 8 mm significantly increased the HRs for bone metastasis in all IDC groups in the multivariate analyses (Table 5) (Fig. 2B). Age 39 years or younger and FF fibrosis grade 3 significantly increased the HRs for bone metastasis in the N+ group and the stage III IDC group in the multivariate analyses.

Tumor necrosis significantly increased the HRs for lung metastasis in the N+ group and stages I and II IDC group (Table 5) (Fig. 2C). ER–/PR– significantly increased the HRs for lung metastasis in the N+ group and the stage III IDC group.

N3 significantly increased the HRs for liver metastasis in the N+ group and the stage III IDC group in the multivariate analyses (Table 5) (Fig. 2D). ER–/PR– and FF fibrosis grade 3 significantly increased the HRs for liver metastasis in the multivariate analyses.

Many factors that were significantly associated with organ metastasis in the multivariate analyses were associated with an 18- to 24-month interval between surgery and detection of the initial metastasis, and some factors were associated with an interval of more than 10 years (Table 6). When the median interval between surgery and the detection of the initial metastasis in the cases as a whole was used as the standard median interval, the presence of muscle invasion, the presence of tumor necrosis, histologic grade

Table 4 Intervals between surgery and detection of the initial tumor metastasis according to factors that were significantly associated with organ-specific metastasis in patients with IDC who did not receive adjuvant therapy, according to nodal status and pTNM stage

Factors	N0	Stages I and II
Median interval between surgery and detection of the initial distant-organ metastasis (mo)		
Cases as a whole	30 (7-112)	30 (9-112)
Muscle invasion, present	7	NA
Histologic grade 3	19	25
FF, fibrosis grade 1	25	25
FF, diameter >8 mm	27	27
Age, ≤39 y	30	30
Histologic grade 2	32	32
FF, fibrosis grade 3	–/–	26
Median intervals between surgery and detection of the initial bone metastasis (mo)		
Cases as a whole	25 (7-112)	29 (9-112)
Muscle invasion, present	7	N/A
FF, diameter >8 mm	18	27
Histologic grade 3	19	25
FF, fibrosis grade 1	25	25
FF, fibrosis grade 3	–/–	40

Note: Values in parentheses are ranges.

3, N3, and age 39 years or younger tended to be associated with shorter median intervals (Table 6), whereas the characteristics of FF and ER/PR status tended to be associated with longer median intervals independent of nodal status or pTNM stage.

4. Discussion

The data for the factors that significantly increased the HRs for distant-organ metastasis and bone metastasis in the IDC group that did not receive adjuvant therapy and in

Table 5 Factors in the multivariate analyses that significantly increased the HR for organ-specific metastasis according to nodal status and pTNM stage in patients with IDC who received adjuvant therapy

	Groups of patients with IDC according to nodal status and pTNM stage			
	N0	N+	Stages I and II	Stage III
Distant-organ metastasis				
FF, diameter >8 mm (*vs* FF, absent)	2.8/1.2-6.3	2.1/1.3-3.2	2.6/1.3-4.9	2.0/1.3-3.1
N3 (*vs* nodal status, N0,	NA	4.4/2.8-6.9	NA	2.8/1.8-4.5
in stage III status;	NA	4.2/2.6-6.8	NA	2.9/1.8-4.6
vs nodal status, N1, in N+ status)				
Tumor necrosis, present	–/–	1.6/1.0-2.6	2.6/1.4-5.0	–/–
(*vs* tumor necrosis, absent)	3.2/1.4-7.2	–/–	3.0/1.6-5.7	–/–
FF, fibrosis grade 2 (*vs* FF, absent)	–/–	1.9/1.1-3.3	2.2/1.0-4.7	–/–
FF, fibrosis grade 3 (*vs* FF, absent)	–/–	2.5/1.4-4.3	3.0/1.3-6.7	–/–
Age, ≤39 y (*vs* age, >39 y)	–/–	2.5/1.3-4.7	–/–	2.7/1.4-5.4
	–/–	2.5/1.3-4.7	–/–	2.5/1.3-5.0
Other factors	Muscle invasion, present	ER+/PR-		
		ER-/PR-		
		Skin invasion, present		
		Histologic grade 3		
Bone metastasis				
FF, diameter >8 mm (*vs* FF, absent)	3.7/1.1-12.0	3.1/1.5-6.3	4.9/1.5-16.0	2.4/1.2-5.0
Age, ≤39 y (*vs* age, >39 y)	–/–	5.1/2.1-12.6	–/–	6.8/2.8-16.3
	–/–	5.3/2.2-13.1	–/–	6.9/2.9-16.6
FF, fibrosis grade 3 (*vs* FF, absent)	–/–	2.8/1.4-5.6	–/–	2.5/1.2-5.1
Other factors	Muscle invasion, present	Invasive tumor size, >50 mm		
	FF, fibrosis grade 2	Nodal status, N2		
		Nodal status, N3		
Lung metastasis				
Tumor necrosis, present		2.2/1.1-4.7	5.3/1.8-15.3	–/–
(*vs* tumor necrosis, absent)		2.2/1.1-4.7	6.0/2.1-17.1	–/–
ER-/PR- (*vs* ER+/PR+)		2.8/1.2-6.4	–/–	2.8/1.1-7.0
		2.8/1.2-6.4	–/–	2.8/1.1-7.0
Other factors		Skin invasion, present		Lymph vessel invasion, present
		N3		
Liver metastasis				
N3 (*vs* nodal status, N0,		5.4/2.4-12.5	NA	7.6/1.6-34.4
in stage III status;		5.9/2.6-14.1	NA	7.6/1.6-34.4
vs nodal status, N1, in N+ status)				
ER-/PR- (*vs* ER+/PR+)		3.8/1.5-9.6	8.2/1.0-65.2	–/–
		3.3/1.3-7.7	11.0/1.4-86.3	–/–
FF, fibrosis grade 3 (*vs* FF, absent)		2.6/1.2-6.1	4.3/1.3-14.1	–/–
Other factors		ER+/PR-		
		FF, diameter >8 mm		
		Histologic grade 3		

Note: Models 1 and 2: values are shown as HR/95% CI.

Table 6 Interval periods for initial tumor metastasis from operation of factors that were significantly associated with organ-specific metastasis according to nodal status and pTNM stage in patients with IDC who received adjuvant therapy

Factors	N0	N+	Stages I/II	Stage III
Median intervals between surgery and detection of the initial distant-organ metastasis (mo)				
Cases as a whole	25 (7-86)	18 (5-122)	24 (4-122)	18 (5-110)
Muscle invasion, present	17	–/–	NA	–/–
Tumor necrosis, present	19	11	17	–/–
Histologic grade 3	–/–	11	–/–	–/–
N3	NA	14	NA	14
Skin invasion, present	–/–	14	NA	–/–
Age, ≤39 y	–/–	15	–/–	15
FF, fibrosis grade 2	–/–	18	38	–/–
FF, diameter >8 mm	24	20	25	17
FF, fibrosis grade 3	–/–	25	15	–/–
ER+/PR–	–/–	27	–/–	–/–
ER–/PR–	–/–	37	–/–	–/–
Median intervals between surgery and detection of the initial bone metastasis (mo)				
Cases as a whole	24 (11-61)	26 (8-122)	31 (8-122)	24 (9-90)
Muscle invasion, present	17	–/–	NA	–/–
Age, ≤39 y	–/–	17	–/–	19
N2	NA	25	NA	–/–
N3	NA	26	NA	–/–
FF, fibrosis grade 2	24	–/–	–/–	–/–
FF, diameter >8 mm	25	30	36	26
Invasive tumor size, >50 mm	–/–	36	NA	–/–
FF, fibrosis grade 3	–/–	39	–/–	30
Median intervals between surgery and detection of the initial lung metastasis (mo)				
Cases as a whole		27 (6-110)	17 (6-88)	28 (6-110)
Tumor necrosis, present		10	27	–/–
N3		16	NA	–/–
Skin invasion, present		17	NA	–/–
ER–/PR–		44	–/–	20
Lymph vessel invasion, present		–/–	–/–	26
Median intervals between surgery and detection of the initial liver metastasis (mo)				
Cases as a whole		13 (5-109)	18 (4-51)	13 (5-109)
Histologic grade 3		11	–/–	–/–
N3		11	NA	11
FF, diameter >8 mm		14	–/–	–/–
FF, fibrosis grade 3		14	10	–/–
ER–/PR–		25	16	–/–
ER+/PR–		33	–/–	–/–

Note: Values in parentheses are ranges.

the IDC group that received adjuvant therapy are shown in Figs. 3 and 4, respectively. The factors are ranked in decreasing order of contribution to accurate prediction of metastasis to each of the organs according to nodal status and pTNM stage. Factors that significantly increased the HRs for metastasis to each distant organ in models 1 and 2 in all the tumor status categories are marked by an asterisk.

Fig. 3 shows that FF diameter greater than 8 mm and FF fibrosis grade 1 were the most important of the 7 distant-organ metastasis predictive factors and of the 5 bone-metastasis predictive factors, and that FF fibrosis grade 3 ranked third. Thus, the diameter and fibrosis grade of an FF can be concluded to be very important histologic factors of primary invasive IDCs for accurate prediction of distant-organ metastasis and bone metastasis by the IDCs of patients who did not receive adjuvant therapy. FF diameter also had strong organ-metastasis predictive power for distant-organ metastasis and bone metastasis in patients with IDC who received adjuvant therapy, and it was more accurate than N3 in predicting bone metastasis among patients with IDC who received adjuvant therapy, independent of nodal status or pTNM stage (Fig. 4A). FF fibrosis grades 2 and 3 ranked within the 3 most accurate predictive factors for distant-organ metastasis and bone metastasis. It can therefore be

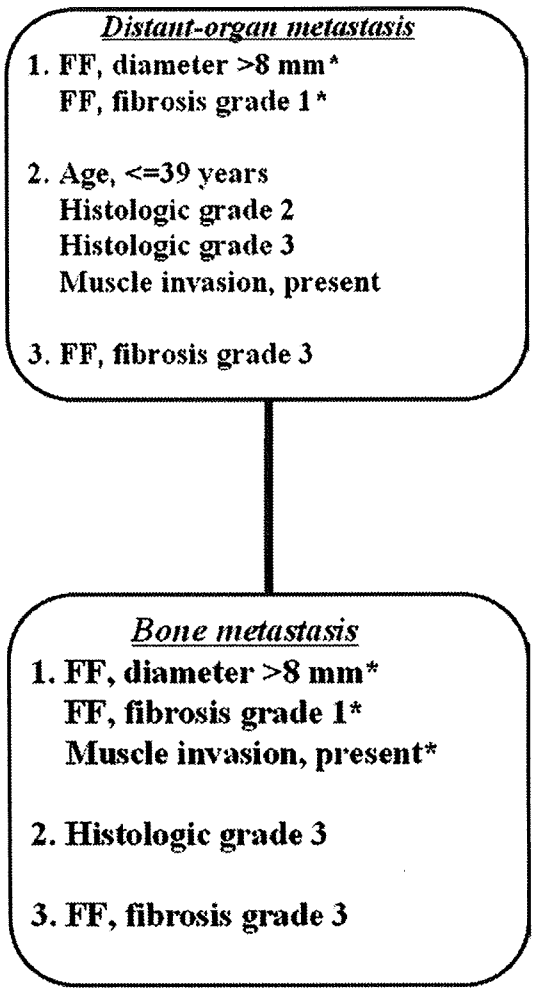

Fig. 3 Factors significantly associated with distant-organ metastasis and bone metastasis according to nodal status and pTNM stage in the IDC group that did not receive adjuvant therapy. The factors are separately ranked in decreasing order of their contribution to accurate prediction of metastasis according to nodal status and pTNM stage. Factors marked with an asterisk significantly increased the HRs for distant-organ metastasis and bone metastasis independent of nodal status and pTNM stage.

concluded that FF characteristics are closely correlated with distant-organ metastasis by IDC, especially with bone metastasis, independent of adjuvant therapy status, nodal status, and pTNM stage status. Fig. 4A clearly shows that N3 and N2 rank low among bone-metastasis predictive factors and that ER/PR status has no predictive power for bone metastasis. Thus, the results of the study clearly show that important prognostic factors do not always accurately predict bone metastasis.

Fig. 4A, however, clearly shows that the characteristics of an FF has no predictive power for lung metastasis, but that both tumor necrosis and ER−/PR− status are very important predictive factors for lung metastasis. Fig. 4A also clearly shows that some factors generally recognized as important prognostic factors, for example, age 39 years or younger, histologic grade 3, and invasive tumor size greater than 50 mm, do not have predictive power for lung metastasis. On the other hand, Fig. 4A clearly shows that N3, FF fibrosis grade 3, and FF diameter greater than 8 mm, which were found to be important predictive factors for distant-organ metastasis, also have predictive power for liver metastasis. Thus, the mechanism responsible for the establishment of lung metastases is probably quite different from the mechanism responsible for distant-organ metastasis, bone metastasis, or liver metastasis.

Fig. 4B shows the predictive factors for organ metastasis to individual organs in the IDC group that received adjuvant therapy. Although N3 is listed as a bone-, lung-, and liver-metastasis predictive factor, its predictive power for metastasis to each of these organs is different. Patients with N3-IDC are probably at significantly greater risk of metastasis to liver than to lung or bone, because N3 was the most accurate predictive factor for liver metastasis. However, because Fig. 4A clearly shows that FF diameter greater than 8 mm is a significant predictive factor for bone metastasis in patients with IDC with nodal metastasis, patients with N3-IDC with an FF diameter greater than 8 mm are probably at higher initial risk for metastasis to bone than patients with N3-IDC without FF or with an FF diameter 8 mm or less. Fig. 4B shows the 5 organ-metastasis predictive factors for bone, the 3 for lung, and the 2 for liver, and they can be concluded to be specific single-organ metastasis-predictive factors for patients with IDC who received adjuvant therapy.

This study clearly demonstrated that the initial organ metastases generally occurred between 18 and 24 months after the operation in patients with IDC who had not received adjuvant therapy, and in patients with IDC who had received adjuvant therapy, and that the presence of muscle invasion, the presence of tumor necrosis, histologic grade 3, N3, age 39 years or younger, and the presence of skin invasion tended to be associated with shorter median intervals between surgery and the detection of initial organ metastasis, independent of nodal status, pTNM stage, adjuvant therapy status, and site of metastasis. By contrast, the characteristics of FF, ER/PR status, and the presence of lymph vessel invasion tended to be associated with longer median intervals between surgery and the detection of the initial organ metastasis. The findings in this study strongly suggest that the site of initial metastasis and interval until detection of the initial metastasis are dependent on the histologic features of the primary tumor.

The results of this study clearly demonstrated that FF diameter and fibrosis grade are very important predictive factors for distant-organ metastasis and that they are far superior to N3 for predicting bone metastasis. We have already reported that the biological characteristics of the fibroblasts forming an FF play a very important role in tumor progression by IDCs with an FF, and that the tumor cells in IDCs with an FF exhibit significantly greater proliferative activity than those in IDCs without an FF [14].

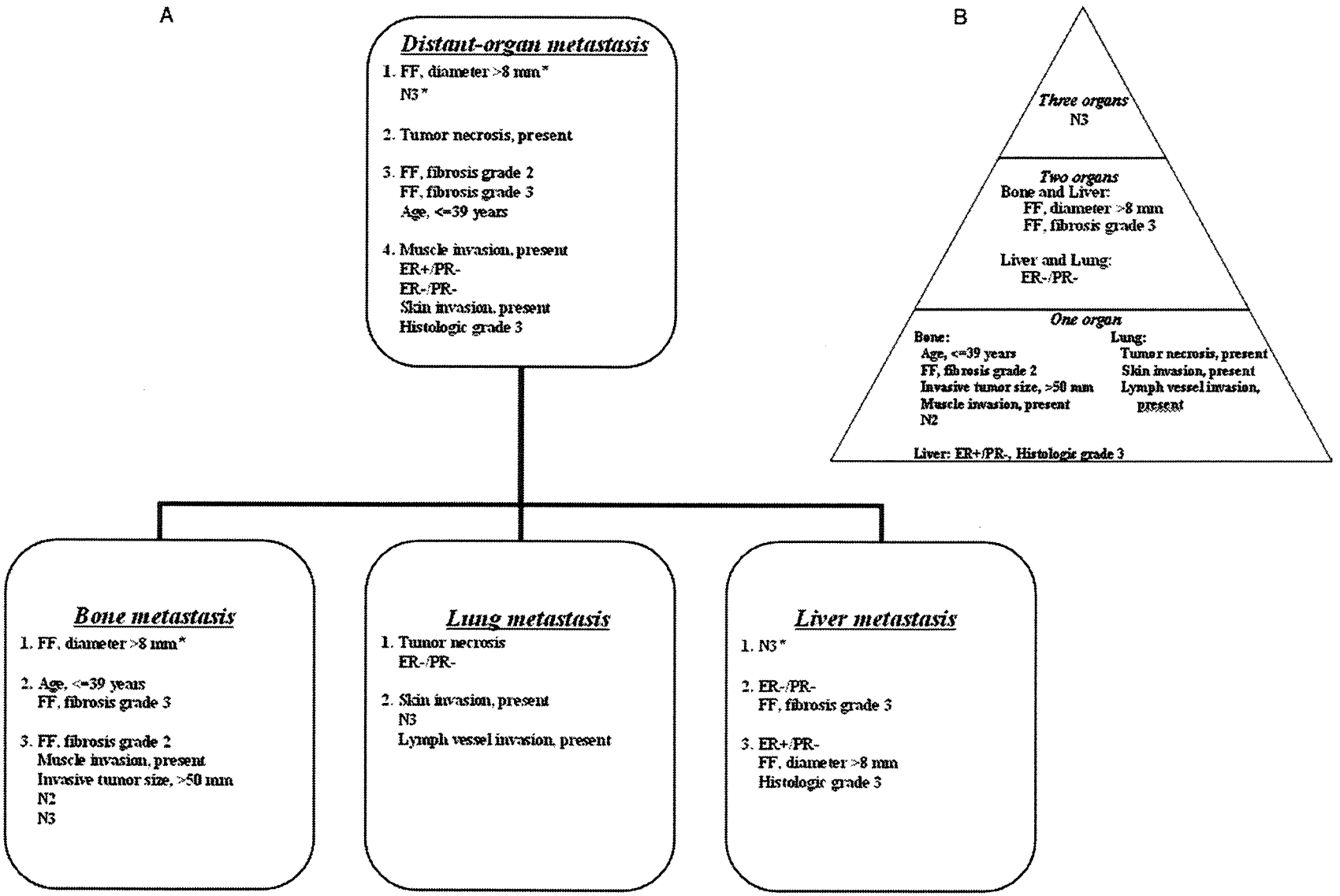

Fig. 4 A, Factors significantly associated with distant-organ metastasis, bone metastasis, lung metastasis, and liver metastasis according to nodal status and pTNM stage in the IDC group that received adjuvant therapy. The factors are separately ranked in decreasing order of their contribution to accurate prediction of metastasis according to nodal status and pTNM stage. Factors marked with an asterisk significantly increased the HRs for distant-organ metastasis, bone metastasis, lung metastasis, and liver metastasis independent of nodal status and pTNM stage. B, Factors significantly associated with metastasis to 1 to 3 organs (within the triangle).

Significantly greater tumor angiogenesis was also found to occur in IDCs with an FF than in IDCs without an FF [25], a finding confirmed by others [16,17]. We previously demonstrated a significant correlation between fibroblast growth factor receptor expression by fibroblasts forming an FF and basic fibroblast growth factor expression by tumor cells, suggesting a paracrine action of basic fibroblast growth factor and fibroblast growth factor receptor between the tumor cells and the fibroblasts [26], and Colpaert et al [16,27] reported a significant association between the presence of an FF and tumor hypoxia. As several biologically important interactions between tumor cells and tumor stromal cells probably occur more frequently in IDCs with an FF than in IDCs without an FF, the characteristics of FFs are likely to be closely associated with bone metastasis in patients with IDC, independent of adjuvant therapy, nodal status, and pTNM stage.

In conclusion, this is the first prospective study to clearly demonstrate the important predictive factors for metastasis to specific organs in patients with IDC of the breast who received and who did not receive adjuvant therapy. Although several biological studies have attempted to identify factors significantly associated with organ-specific metastasis by IDCs [28-30], it is very difficult to assess the biological characteristics that promote organ-specific metastasis by IDC in routine examinations. As pathologists are able to assess the histologic factors that predict organ-specific metastasis in routine examinations of IDCs, they should assess them carefully to accurately estimate the degree of biological malignancy of IDCs of the breast.

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ORIGINAL ARTICLE

Invasive Apocrine Carcinoma of the Breast: Clinicopathologic Features of 57 Patients

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■ **Abstract:** Apocrine carcinoma is a rare, unique, and morphologically distinctive type of invasive ductal carcinoma (IDC). The features of invasive apocrine carcinoma (IAC) and their possible prognostic implications have not been fully investigated. To this end, we examined the clinicopathologic characteristics and outcome of patients with IAC and compared these factors with those of patients with IDC. Out of 2,055 breast cancer patients who had undergone breast surgery between 1995 and 2005, 57 patients of IAC and 1,583 patients of IDC were analyzed. The mean ages of the patients with IAC and of those with IDC were 58.5 ± 10.9 years and 54.4 ± 11 years, respectively ($p = 0.006$). The percentages of patients with axillary nodal metastasis and lymphatic invasion were significantly lower in the IAC group than in the IDC group ($p = 0.03$ and 0.02, respectively). The percentage of estrogen and progesterone receptor negativity was higher in the IAC group than in the IDC group ($p < 0.001$). After a median follow-up period of 49 months (range, 1–133 months), seven (12%) patients with IAC and 244 (15%) patients with IDC had experienced recurrences. Three (5%) patients with IAC and 125 (8%) patients with IDC died of recurrent breast cancer. No significant differences in the relapse-free survival ($p = 0.83$) and overall survival ($p = 0.75$) rates were observed between the two groups. Although IAC and IDC have different clinicopathologic characteristics, the prognoses of patients with these diseases are similar. ■

Key Words: apocrine carcinoma, axillary nodal metastasis, breast cancer, hormone receptor

Apocrine carcinoma is a rare tumor accounting for 0.4 to 4% of all breast cancers (1–6). Despite an increasing recognition of this tumor in recent years, the morphologic diagnostic criteria remain unclear. Rosen stipulated that the term should be reserved for "neoplasms in which all or nearly all the epithelium has apocrine cytologic features" (7).

Since Krompecher first described apocrine carcinoma in 1916 (8), its clinicopathologic characteristics and prognosis have been described in several reports (1–6,9–17). Apocrine carcinoma cells tend to exhibit a loss of hormone receptors. With regard to prognosis, invasive apocrine carcinoma (IAC) had the same prognosis as invasive ductal carcinoma (IDC) in some reports, but these studies were based on small numbers of patients with IAC. In this report, we investigated the clinicopathologic characteristics and outcomes of patients with IAC whose clinical records had been included in our database to clarify the biological differentiation between IAC and IDC.

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MATERIALS AND METHODS

From January 1995 to August 2005, a total of 2,055 primary breast cancer patients underwent breast surgery in the Breast Surgery Division of the National Cancer Center Hospital East. The records of all these patients were reviewed, and 61 patients (3%) were diagnosed as having had apocrine carcinoma. Among the patients with apocrine carcinomas, four did not have an invasive component (intraductal apocrine carcinoma). Thus 57 patients with IAC were retrospectively compared with 1,583 patients with IDC who had been treated during the same time. The clinicopathologic features and the outcomes of these patients were retrospectively examined.

In our institution, apocrine carcinoma was defined as a neoplasm composed of 80% or more tumor cells with abundant, granular eosinophilic cytoplasm, and

round centrally to the eccentrically located malignant nuclei containing prominent nucleoli. On the other hand, neoplasms in which less than 80% of the tumor cells exhibited apocrine cytologic features were defined as IDC with apocrine features. Histologic grading was performed according to the modified Bloom–Richardson histologic grading scale for infiltrating carcinomas. The estrogen receptor (ER) and progesterone receptor (PgR) status was assessed using an enzyme immunoassay or immunohistochemistry. For the enzyme immunoassays, the upper cutoff values for ER and PgR in the cytosol fraction were set at 13 fmol/mg and 10 fmol/mg of protein, respectively. Immunostaining for ER and PgR was considered positive when a distinct nuclear localization was found in 10% or more of the cells. The human epidermal growth factor receptor type 2 (HER2) status was evaluated using the Herceptest (DakoCytomation, Carpinteria, CA). Tumors were considered positive if 10% or more tumor cells had distinct circumferential membrane staining.

Statistical Analysis

Relapse-free survival was defined as the time until the first occurrence of disease recurrence at any site. Overall survival was defined as the time until death from any cause. Statistical differences between the two groups were analyzed using the chi-squared test (or the Fisher's exact probability test). Survival curves were produced according to the Kaplan–Meier method and were compared using the log-rank test. statmate III (for Macintosh) was used for the statistical analysis. A p-value of <0.05 was considered significant.

RESULTS

Clinicopatholgic Characteristics

The clinicopathologic characteristics of the patients with IAC and of those with IDC are shown in Table 1. The patients with IAC were older ($p = 0.006$) and had a lower frequency of axillary nodal metastasis ($p = 0.03$), a lower frequency of lymphatic invasion ($p = 0.02$), and a lower frequency of ER and PgR positivity ($p < 0.001$) than those with IDC. On the other hand, no significant differences with regard to menopausal status, pathologic tumor size, stage, histologic grade, vascular invasion, or HER2 status were observed between the two groups.

Table 1. Comparison of Clinicopathologic Characteristics between Invasive Apocrine Carcinoma and Invasive Ductal Carcinoma

	Invasive apocrine caricnoma ($n = 57$)	Invasive ductal carcinoma ($n = 1583$)	p
Mean age (years)	58.5 ± 10.9	54.4 ± 11.0	0.006
Menopausal status			
Pre	19	712	0.08
Post	38	871	
Stage			
I	20	532	0.35
II	34	871	
III	3	180	
Tumor size (cm)			
–2.0	20	564	0.55
2.1–5.0	34	876	
5.1–25.0	3	143	
Pathologic nodal metastasis			
Negative	41	925	0.03
Positive	15	646	
Unknown*	1	12	
Lymphatic invasion			
Negative	47	1075	0.02
Positive	10	502	
Unknown*	0	6	
Vascular invasion			
Negative	33	825	0.41
Positive	24	750	
Unknown*	0	6	
Histologic grade			
1	9	207	0.23
2	32	751	
3	16	619	
Unknown*	0	6	
ER			
Negative	48	580	<0.001
Positive	9	913	
Unknown*	0	90	
PgR			
Negative	45	701	<0.001
Positive	12	792	
Unknown*	0	90	
HER2			
0–2+	35	514	0.74
3+	6	102	
Unknown*	16	967	

ER, estrogen receptor; PgR, progesterone receptor.
*Unknown cases were excluded from statistical analysis.

Survival

The relapse-free survival and overall survival curves are shown in Figure 1. The median follow-up time was 49 months (range, 1–133 months). Seven (12%) patients in the IAC group and 244 (15%) patients in the IDC group developed recurrences (Table 2). The numbers of patients with loco-regional recurrence, distant metastasis, and distant metastasis with loco-regional recurrence were 5 (9%), 2 (3%), and 0 (0%) in the IAC group and 76 (5%), 133 (8%), and 35 (2%) in the IDC group, respectively. Three (5%) patients

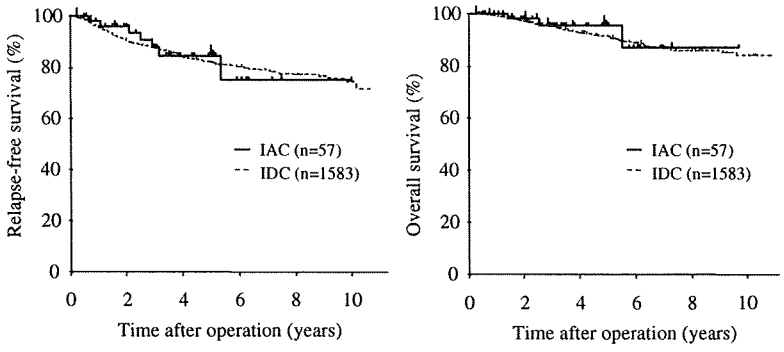

Figure 1. Relapse-free survival (p = 0.83) and overall survival curves (p = 0.75) of patients with invasive apocrine carcinoma (full line) and invasive ductal carcinoma (dotted line).

Table 2. Comparison of Recurrence and Survival between Invasive Apocrine Carcinoma and Invasive Ductal Carcinoma

	Invasive apocrine caricnoma (*n* = 57)	Invasive ductal carcinoma (*n* = 1583)	p
Recurrence	7	244	0.83
Loco-regional	5	76	
Distant	2	133	
Distant with loco-regional	0	35	
Survival			
Death because of breast cancer	3	125	0.75

with IAC and 125 (8%) patients with IDC died of breast cancer. No significant differences in the relapse-free survival (p = 0.83) and overall survival (p = 0.75) periods of the two groups were observed. We also examined the overall survival of patients stratified according to nodal status and ER status. No significant differences in overall survival were observed between the two groups among patients without axillary nodal metastases (p = 0.29), with axillary nodal metastases (p = 0.49), with ER negativity (p = 0.27), or with ER positivity (p = 0.74).

DISCUSSION

We reviewed several previously reported patients of apocrine carcinoma (Table 3). Apocrine carcinoma accounted for 3% of all breast cancers treated at our institution. The incidence tended to be higher than those mentioned in other reports. However, the incidence of apocrine carcinoma varied considerably from one report to another. Differences in the pathologic criteria of apocrine carcinoma may result in the variety in the reported incidences.

Apocrine carcinoma has a unique hormone receptor profile. The percentages of IAC patients with ER and PgR positivity were significantly low (16% and 21%, respectively). The reason for the low percentage of ER

Table 3. The Comparison of Present Study with Other Studies

Author [year] (ref.)	No. of patients	Incidence reported	Age mean	Tumor size (cm)	Nodal metastases positivity	ER positivity	PgR positivity
Honma et al. [2005] (15)	52	–	–	–	37% (14/38)	4% (2/52)	6% (3/52)
Japeze et al. [2005] (11)	37	–	52.9	2.5	42% (14/33)	61% (22/36)	58% (21/36)
Takeuchi et al. [2004] (6)	33	1.6%	57.1	3.1	30% (10/33)	33% (8/24)	27% (6/22)
Sapp et al. [2003] (10)	15	–	–	–	–	13% (2/15)	7% (1/15)
Matsuo et al. [2002] (13)	24	–	61	2.6	14% (3/22)	17% (4/24)	17% (4/24)
Leal et al. [2001] (17)	35	–	59	2.3	0% (0/9)	6% (2/35)	3% (1/34)
Tavassoli et al. [1994] (16)	37	–	–	–	0% (0/23)	–	–
Abati et al. [1990] (12)	72	–	54.2	1.8	11% (5/45)	40% (4/10)	50% (2/4)
Aoyagi et al. [1990] (5)	10	0.65%	58.1	3.5	50% (5/10)	–	–
d'Amore et al. [1988] (9)	34	–	59	2.1	53% (18/34)	–	–
Gadaleanu et al. [1986] (4)	6	0.4%	48.6	2.6	0% (0/6)	–	–
Eusebi et al. [1986] (3)	4	4.0%	70.3	2.4	50% (2/4)	75% (3/4)	50% (2/4)
Mossler et al. [1980] (2)	6	0.4%	66.5	2.4	67% (4/6)	0% (0/6)	0% (0/6)
Frable et al. [1968] (1)	19	1.0%	–	–	41% (7/17)	–	–
Present study	57	3.0%	58.5	2.6	27% (15/56)	16% (9/57)	21% (12/57)
Total	441	1.2%	57.1	2.4	29% (97/336)	21% (56/263)	20% (52/254)

and PgR positivity among patients with IAC is unclear. Bratthauer et al. demonstrated that 9 out of 11 ER-negative lesions contained ER mRNA (18). This result suggests that the mechanism for the production of ER through the first transcriptional splice region is intact and functional in most cases. Therefore, the immunohistochemical absence of ER in apocrine cells cannot be explained by an abnormal message at these common sites and should be sought at a downstream position in the pathway. Sap et al. reported that the grade of IAC differentiation was correlated with the loss of ER and PgR and that apocrine differentiation was linked to the overexpression of androgen receptors (AR) (10). Gatalica also demonstrated the increased expression of AR in patients with apocrine-differentiated tumors, compared with that in nonapocrine tumors (19). Although we did not investigate the AR status in the present study, these results suggest that in addition to the loss of ER and PgR, AR positivity may be a distinctive feature of apocrine carcinoma.

In our study, the positive rates of axillary nodal metastasis and lymphatic invasion among patients with IAC were 27% and 18%, respectively. In patients with IDC, axillary nodal metastasis, lymphatic invasion, and vascular invasion were more strongly associated with tumor recurrence or death (20–23). IAC was characterized by a lower frequency of lymph node metastasis and lymphatic invasion. These findings suggest that IAC may be less aggressive. Japeze et al. reported that patients with IAC had a significantly better outcome (11). On the other hand, the patients with IAC tended to show a loss of hormone receptors on their cancer cells. Breast cancer patients with ER and PgR negativity generally have a poor prognosis. In the present study, no significant differences in the relapse-free survival and the overall survival periods were seen between the IAC group and the IDC group. These findings were consistent with the results reported by other investigators (1,6,9,12). Taken together, patients with IAC may have the same prognosis as those with IDC.

In conclusion, IAC is thought to be a clinicopathologically distinctive entity with different characteristics from those of other breast lesions. Although we were able to clarify some of the characteristics of apocrine carcinoma, the prognosis of patients with IAC was similar to that of patients with IDC. Further molecular biological study will be needed to understand the clinicophathologic features of IAC.

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Original contribution

Grading system for lymph vessel tumor emboli for prediction of the outcome of invasive ductal carcinoma of the breast

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Summary There are no suitable histologic diagnostic clues for determining the true biological malignancy of invasive ductal carcinomas associated with lymph vessel tumor emboli. The purpose of this study was to devise a grading system for lymph vessel tumor emboli in invasive ductal carcinomas that would allow accurate prediction of the outcome of invasive ductal carcinoma patients with lymph vessel invasion. We classified 393 invasive ductal carcinomas into the following 4 grades according to the number of mitotic and apoptotic figures in tumor cells in lymph vessels at 1 high-power field: grade 0, no lymph vessel invasion; grade 1, absence of mitotic and apoptotic figures, presence of any number of mitotic figures and absence of apoptotic figures, or absence of mitotic figures and presence of any number of apoptotic figures; grade 2, 1 to 4 mitotic figures and 1 or more of apoptotic figures, or 1 or more of mitotic figures and 1 to 6 apoptotic figures; and grade 3, more than 4 mitotic figures and more than 6 apoptotic figures. The mortality rate increased with the grade, and the mortality rate of patients with grade 3 lymph vessel tumor emboli was more than 70%. Multivariate analyses with well-known prognostic factors demonstrated that grade 3 lymph vessel tumor emboli significantly increased the hazard rates for tumor recurrence, and tumor death independent of adjuvant therapy status, nodal status, or invasive tumor size. The grading system for lymph vessel tumor emboli is the best histologic grading system for accurately predicting the outcome of patients with invasive ductal carcinoma of the breast.

1. Introduction

Lymphatic invasion in breast cancer patients with invasive ductal carcinoma (IDC) has been reported to have

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prognostic significance [1-5] because the presence of lymph vessel invasion is most likely responsible for the lymph node metastasis that is the most important prognostic parameter in patients with IDC. However, some IDCs are unassociated with nodal metastasis despite the presence of several invasive lymphatic foci, and not all IDC patients with lymph vessel invasion die of their disease. In addition, some IDCs are characterized by numerous lymph vessel tumor emboli (LVTE) that extend throughout the entire breast, and many pathologists have probably occasionally diagnosed such IDCs based on routine histologic examination of surgically resected breast cancers. However, there are no suitable histodiagnostic clues for determining the true biological malignancy of IDCs associated with LVTE. We recently clearly demonstrated that the numbers of mitotic and apoptotic figures in tumor cells in lymph vessels are important histologic parameters because they are associated with higher numbers of invaded lymph vessels [3], strongly suggesting that a grading system based on the numbers of mitotic and apoptotic figures in LVTE in IDCs would provide an important basis for accurately assessing the true biological malignancy of IDCs with lymph vessel invasion.

The purpose of this study was to devise a grading system for LVTE in IDCs that would allow accurate prediction of the outcome of IDC patients with lymph vessel invasion. The grading system that was devised allowed classification of IDC patients with lymph vessel invasion into low-, intermediate-, and high-risk groups.

Table 1 Criteria used in the grading systems for LVTE in IDC

Grading system for LVTE according to the number of mitotic and apoptotic figures in tumor cells of LVTE		
Grade 0	IDCs with no LVTE	
Grades 1, 2, and 3	IDCs with ≥1 LVTE	
	No. of mitotic figures	No. of apoptotic figures
Grade 1	Low-proliferative type	
1a	0	0
1b	0	Any
1c	Any	0
Grade 2	Intermediate-proliferative type	
2a	1 to 4	>0
2b	>0	1 to 6
Grade 3	High-proliferative type	
3a	>4	>6
Grading system for LVTE according to the percentage of mitotic and apoptotic figures in tumor cells of LVTE		
Grade 0	IDCs with no LVTE	
Grades 1, 2, 3, and 4	IDCs with one or more LVTE	
	% of mitotic figures	% of apoptotic figures
Grade 1	Low-proliferative type	
1	0	0
Grade 2	Intermediate-proliferative type	
2a	0	Any
2b	Any	0
Grade 3	High-proliferative type	
3a	>0 to ≤0.06	>0
3b	>0	>0 to ≤0.4
Grade 4	Very high-proliferative type	
4	>0.06	>0.4

NOTE. 0.06 is the median value of mitotic figure in 1 high-power field; 0.4, median value of apoptotic figures in 1 high-power field.

2. Materials and methods

2.1. Cases

The study included 393 consecutive cases (the same cases studied in reference [3]) of IDC of the breast surgically treated at the National Cancer Center Hospital East (Kashiwa, Chiba, Japan) between July 1992 and November 1998. Clinical information was obtained from the patients' medical records after complete histologic examination of all IDCs. All patients were Japanese women, and they ranged in age from 28 to 78 years (mean, 51 years). All had a solitary lesion. Partial mastectomy had been performed in 55, modified radical mastectomy in 314, and standard radical mastectomy in 24. Levels I, II, and +/−III axillary lymph node dissection had been performed in all patients. None of the patients had received radiotherapy or chemotherapy before surgery, but 294 patients had received adjuvant therapy. Among them, 99 received chemotherapy, 59 received endocrine therapy, and 136 received combined chemoendocrine therapy. The chemotherapy regimens used were anthracycline-based with or without taxane in 89 patients and non–anthracycline-based in 146 patients. The endocrine therapy regimens used were tamoxifen with or without aromatase inhibitor in 195 patients. There were no cases of inflammatory breast cancer in this series. All tumors were classified according to the pathologic TNM classification [6]. Estrogen receptors (ERs) and progesterone receptors (PRs) in the cytosol fractions were determined by enzyme immunoassay (Otsuka Assay Laboratory, Tokushima, Japan).

For pathologic examination, the surgically resected specimens were fixed in 10% formalin, and the entire tumor was cut into slices at 0.5-cm intervals. The size and gross appearance of the tumors were recorded, and their size was confirmed by comparison with tumor size on histologic slides by taking multiple histologic sections from each tumor. In IDCs of 50 mm or less, all tumor areas and the nontumor breast tissue area surrounding the largest tumor area and the second largest tumor area were sampled for histologic examination. In IDCs of more than 50 mm, the largest tumor area, second largest tumor area, third largest tumor area, bilateral tumor end areas, and nontumor breast tissue

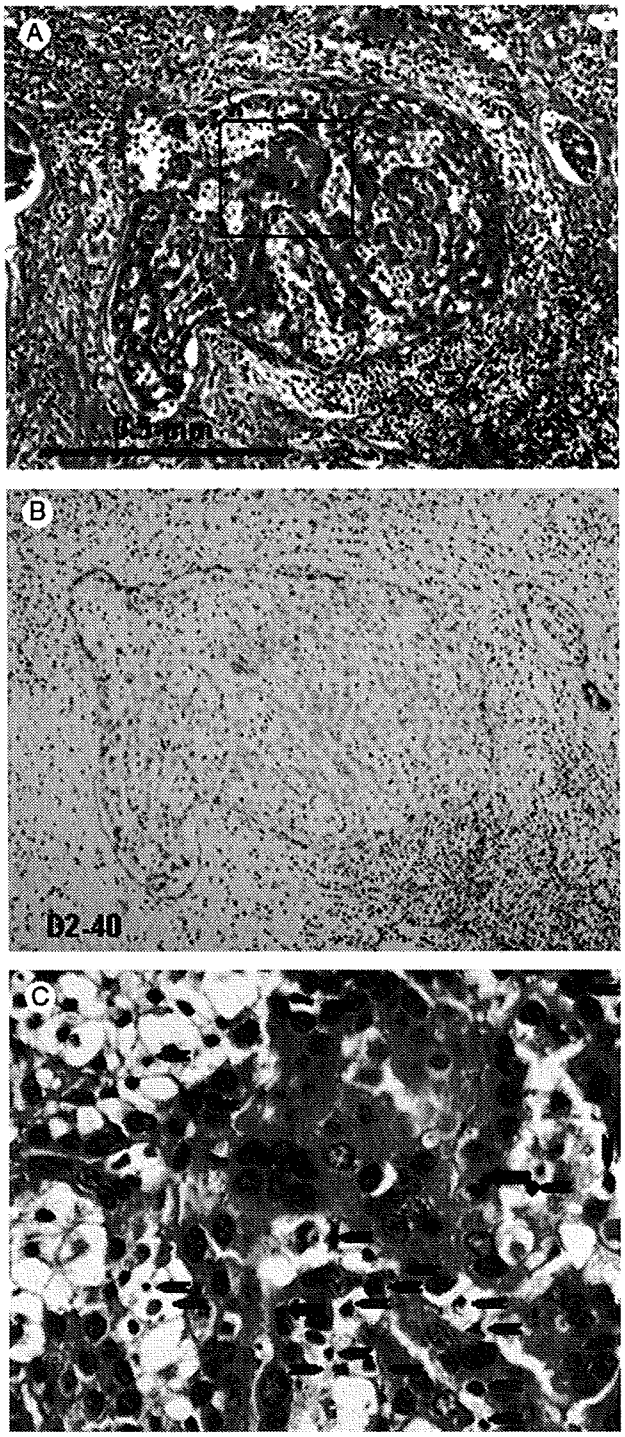


Fig. 1 A and B, One large lymph vessel tumor embolus and one small lymph vessel tumor embolus whose D2-40–positive vessel walls can be seen. C, Several apoptotic bodies and apoptotic tumor cells are observed (arrowheads), and there are 2 mitotic tumor cells (arrows) in the lymph vessel tumor embolus. Apoptotic bodies are small, variously shaped pyknotic bodies that resemble sesame seeds, and apoptotic tumor cells were identified as tumor cells that contained eosinophilic or amphophilic cytoplasm and an irregularly shaped pyknotic nucleus.

surrounding each of the above tumor areas were sampled for histologic examination. The sections were routinely processed and embedded in paraffin. Serial sections of each tumor area were cut from the paraffin blocks. One section was stained with hematoxylin-eosin and examined histologically to confirm the diagnosis. Elastica staining was performed on another section to assess blood vessel invasion in all cases. All tumors were classified according to the guidelines of the World Health Organization [7].

2.2. Grading system for LVTE in IDCs

We devised the following 2 grading systems for LVTE in IDCs based on the number and percentage of mitotic and apoptotic figures in tumor cells comprising the emboli (Table 1) [3]. The cases with LVTE containing at least one mitotic figure but no apoptotic figures and the cases with LVTE containing at least one apoptotic figure but no mitotic figures were lumped together with the cases with LVTE that contained neither mitotic nor apoptotic figures because there were no significant differences between their disease-free survival curves and their overall survival curves (data not shown). The cutoff values of 4 mitotic figures and 6 apoptotic figures were based on data obtained in our previous study [3]. Next, we devised a grading system for LVTE in IDCs based on the percentage of cells that contained mitotic or apoptotic figures among the cells comprising the emboli (Table 1). The percentage of cells that contained a mitotic figure and percentage of cells that contained an apoptotic figure among the cells comprising LVTE were calculated from counts of mitotic and apoptotic tumor cells among all the tumor cells in a single high-power field (×400) because there were cases in which there was only one lymph vessel tumor embolus. The median percentage containing a tumor cell mitotic figure in a single high-power field was 0.06%, and the median percentage containing a tumor cell apoptotic figure was 0.4%. We therefore used these percentages as the cutoff values for tumor cells containing mitotic and apoptotic figures in the emboli, and the IDCs with LVTE were classified into the 4 groups.

Tumor cell apoptotic figures consist of apoptotic bodies of tumor cells and apoptotic tumor cells (Fig. 1) [8]. The numbers of tumor cell mitotic and apoptotic figures in lymph vessels were counted in 20 high-power fields. In practice, we first examined all slides of IDCs that contained both tumor areas and nontumor areas to identify LVTE. Next, we selected the LVTE, for example, large LVTE far from the stroma-invasive tumor margin to record mitotic and apoptotic figures of LVTE in an IDC. We selected the LVTE in which to count the mitotic and apoptotic figures and then recorded the numbers and the percentages of mitotic and apoptotic figures in tumor cells making up the lymph vessel tumor embolus or emboli of the IDC in the high-power field that contained the largest number or percentage of mitotic figures; and/or the largest number or percentage of apoptotic figures were recorded as the number and percentage of

mitotic and apoptotic figures in the LVTE of the IDC. The cumulative numbers of tumor cell mitotic and apoptotic figures in LVTE in all 20 high-power fields were not used in this study. In IDCs containing a small number of LVTE, the mitotic and apoptotic figures were counted in fewer than 20 high-power fields.

We evaluated lymph vessels invaded by tumor cells at or beyond the border of the stroma-invasion area of the tumor. However, some IDCs contained large LVTE, especially in IDCs containing a grade 2 or grade 3 lymph vessel tumor embolus, and it was difficult to determine whether they were true LVTE or a noninvasive ductal carcinoma component by hematoxylin-eosin staining alone. We therefore performed immunohistochemical staining with D2-40 antibody (monoclonal mouse antibody, Signet, Dedham, MA; 1:200) to confirm that LVTE identified by hematoxylin-eosin staining were true tumor emboli in the 254 of the IDCs. The D2-40 antibody was generated against an O-linked sialoglycoprotein having a molecular weight of 40 kd and had been demonstrated to be a selective marker of lymphatic endothelium (Fig. 1B) [9,10]. Immunostaining for D2-40 was performed on paraffin-embedded sections of formalin-fixed tissue by the DAKO EnVision+ system (DakoCytomation, Glostrup, Denmark). Sections of breast tissue containing D2-40–positive lymph vessels were used each time as positive controls. Normal mouse immunoglobulin was substituted for the primary antibody to provide negative controls. Two investigators (T. H. and C. Y., or T. H. and G. I.) used hematoxylin-eosin and D2-40 staining to identify the LVTE in each tumor area of all IDCs; and whenever there was a discrepancy, they reexamined the slides together to reach a consensus.

2.3. Patient outcome and statistical analysis

Patient survival was evaluated by follow-up for a median period of 106 months (range, 88-174 months) until March 2006. At that time, 273 patients were alive and well, 120 had developed tumor recurrence, and 84 had died of their disease. The measurements of intervals until tumor relapse started at the time of surgery. Tumor relapse was considered to have occurred whenever there was evidence of metastasis, and only deaths due to breast cancer were considered for the purposes of this study.

Analysis of variance was used to perform the statistical analyses for associations between the grade of LVTE and number of lymph node metastases.

The log-rank test [11] was used to analyze the predictive power of the grading systems of LVTE for tumor death in the IDC cases as a whole. We used the Cox proportional hazard regression model [12] to compare the prognostic predictive power of the grading system of LVTE based on the number of tumor cells containing mitotic figures and the number of tumor cells containing apoptotic figures in LVTE with the prognostic predictive power of the grading system for tumor cells in LVTE based on the percentage of tumor cells containing mitotic and apoptotic figures in LVTE.

We prospectively analyzed the predictive power of the following histologic factors separately in patients with IDC who received and who did not receive adjuvant therapy: (1) invasive tumor size (≤20, >20 to ≤50, >50 mm), (2) histologic grade (1, 2, 3) [13], (3) tumor necrosis (absent, present) [14], (4) fibrotic focus (FF) diameter [15,16] (absent, FF diameter ≤8 mm, FF diameter >8 mm), (5) blood vessel invasion (absent, present), (6) adipose tissue invasion (absent, present), (7) skin invasion (absent, present), and (8) muscle invasion (absent, present); and we also analyzed the predictive power of the grade of LVTE, age (≤39 years and >39 years), and ER/PR status (ER or PR positive or ER/PR both positive, and ER/PR both negative) for tumor recurrence, and for tumor death according to nodal status and according to invasive tumor size. Type of adjuvant therapy (endocrine therapy, chemotherapy, and chemoendocrine therapy) was analyzed as a prognostic predictive factor for patients with IDC who received adjuvant therapy. The factors that were significantly associated with outcome in the univariate analyses were then entered together into the multivariate analyses

Table 2 Tumor recurrence rate and mortality rate of patients with IDC and multivariate analyses for tumor recurrence and tumor death in patients as a whole (N = 393)

Outcomes	Grading system for LVTE according to the number of mitotic and apoptotic figures in tumor cells of LVTE				
	G0	G1	G2	G3	
	183	103	88	19	
Tumor recurrence	31 (17)	26 (25)	48 (55)	15 (79)	
Tumor death	21 (12)	13 (15)	36 (41)	14 (74)	
	Grading system for LVTE according to the percentage of mitotic and apoptotic figures in tumor cells of LVTE				
	G0	G1	G2	G3	G4
	183	69	36	41	64
Tumor recurrence	31 (17)	13 (19)	14 (39)	26 (63)	36 (56)
Tumor death	21 (12)	5 (7)	9 (25)	20 (49)	29 (45)
	Multivariate analyses				
Grading systems	Disease-free survival		Overall survival		
	Trend HRs/95% CIs/*P*		Trend HRs/95% CIs/*P*		
No. of MF/AF	1.8/1.1-3.1/.017		2.2/1.3-3.7/.004		
% of MF/AF	1.2/0.8-1.7/.435		1.1/0.7-1.7/.629		

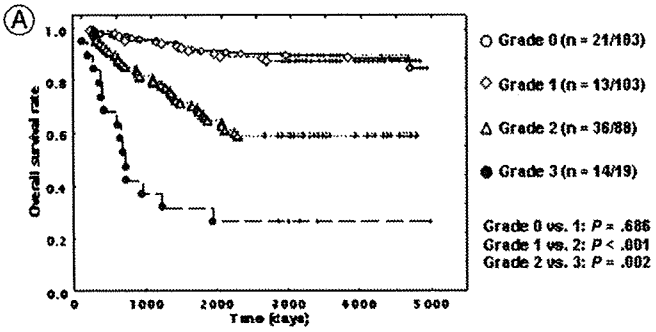
Abbreviations: G0, grade 0; G1, grade 1; G2, grade 2; G3, grade 3; G4, grade 4; no. of MF/AF, grading system for LVTE according to the number of mitotic and apoptotic figures in tumor cells of LVTE; % of MF/AF, grading system for LVTE according to the percentage of mitotic and apoptotic figures in tumor cells of LVTE.

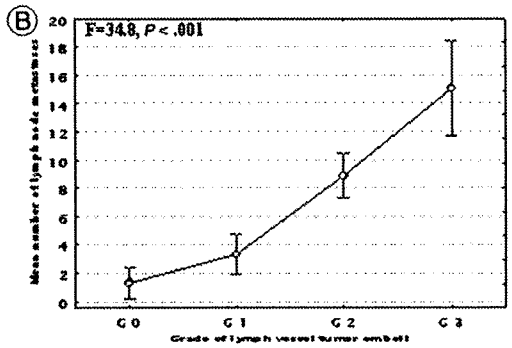
using the Cox proportional hazard regression model [12], and the step-down method was applied until all of the remaining factors were significant at a P value of less than .05. Because fewer than 10 of the IDC patients with nodal metastasis who did not receive adjuvant therapy had tumor recurrence and fewer than 10 of the IDC patients of an invasive tumor size of 20 mm or less who did not receive adjuvant therapy had tumor recurrence (Table 3), we could not perform multivariate analyses for tumor recurrence in these groups. Similarly, because there were fewer than 10 tumor deaths among the cases without nodal metastasis, the cases with nodal metastasis, the cases with an invasive size of 20 mm or less, or the cases with an invasive tumor size of more than 20 mm (Table 3), multivariate analyses for tumor death could not be performed in these groups. Survival curves were drawn using the Kaplan-Meier method [17]. All P values reported were 2-sided, and the significance level was set at $P < .05$. All analyses were performed with Statistica/Windows software (StatSoft, Tulsa, OK).

3. Results

3.1. Multivariate analyses for tumor recurrence and tumor death to determine which of the grading systems of LVTE were suitable for predicting the outcome of patients with IDC

Because the multivariate analyses showed that the grading system of LVTE based on the number of mitotic and





Grade	No. of cases	Maximum	Minimum	Median	Mean +/- SD
0	183	25.0	0	0	1.3 +/- 3.2
1	103	57.0	0	1.0	3.3 +/- 8.1
2	88	43.0	0	5.5	8.9 +/- 10.5
3	19	50.0	0	13.0	15.1 +/- 14.1

C

Number of lymph vessels invaded		
	Cases	MR (%)
0	183	21 (11)
1-5	101	15 (15)
6-20	52	22 (42)
>20	57	26 (46)
Total	393	84

MR, mortality rate

D

	Grades of lymph vessel tumor emboli								
	Grade 0		Grade 1		Grade 2		Grade 3		
No. of LVTE	Cases	MR (%)	Cases	MR (%)	Cases	MR (%)	Cases	MR (%)	Total
0 to 5	183	21 (11)	82	8 (9)	18	6 (33)	1	1 (100)	284
6 to 20	0		18	5 (28)	33	16 (48)	1	1 (100)	52
>20	0		3	0	37	14 (38)	17	12 (71)	57
Total	183	21 (11)	103	13 (13)	88	36 (41)	19	14 (74)	393

LVTE, lymph vessel tumor embolus; MR, mortality rate

Fig. 2 A, Overall survival curve of all IDC patients with grade 0, 1, 2, and 3 tumor emboli. IDC patients with grade 0 and grade 1 have almost the same overall survival curves, and they compose the low-risk IDC group. IDCs with grade 2 and grade 3 LVTE compose the intermediate-risk and high-risk group, respectively. B, Graph showing association between mean nodal metastasis values and the grades of LVTE, and table showing the maximum, minimum, median, and mean values of nodal metastasis according to grade. The mean number of nodal metastasis increases significantly with the grade of the LVTE. C, Table showing the mortality rates of patients according to grade of LVTE and number of lymph vessels invaded: 0 to 5, 6 to 20, and more than 20. D, Table showing the mortality rates of patients according to grade of LVTE and number of lymph vessels invaded: 0 to 5, 6 to 20, and more than 20.

Table 3 Tumor recurrence rate and mortality rate of patients with IDC who did not receive adjuvant therapy classified by the grading system for LVTE according to nodal status and tumor size

Outcomes	Grading system for LVTE							
	G0	G1		G2		G3		
	Cases as a whole (%) (n = 99)							
	63	22		12		2		
Tumor recurrence	6 (10)	6 (27)		5 (42)		1 (50)		
Tumor death	2 (3)	3 (14)		3 (25)		1 (50)		
	G0	G1	G2	G3	G0	G1	G2	G3
	Cases without nodal metastasis (%) (n = 85)				Cases with an invasive tumor size ≤20 mm (%) (n = 54)			
	61	18	6	0	43	8	3	0
Tumor recurrence	6 (10)	4 (22)	2 (33)		2 (5)	1 (13)	2 (67)	
Tumor death	2 (3)	3 (17)	1 (17)		1 (2)	0	1 (33)	
	Cases with nodal metastasis (%) (n = 14)				Cases with an invasive tumor size >20 mm (%) (n = 45)			
	2	4	6	2	20	14	9	2
Tumor recurrence	0	2 (50)	3 (50)	1 (50)	4 (20)	5 (36)	3 (33)	1 (50)
Tumor death	0	0	2 (33)	1 (50)	1 (5)	3 (21)	2 (22)	1 (50)

apoptotic figures in tumor cells comprising the emboli is significantly superior to the grading system of LVTE based on the percentages of tumor cells that contained mitotic and apoptotic figures among the tumor cells comprising the emboli (Table 2 and Fig. 2A), we adopted the former to accurately analyze the prognostic predictive power of the grading system of LVTE in this study.

3.2. Association between grade of LVTE and nodal metastasis

The number of nodal metastases significantly increased with the grade, and the median number of grade 3 nodal metastases was more than 10 (Fig. 2B).

3.3. Prognostic power of the grading system of LVTE according to the number of lymph vessels invaded by tumor cells

Patients with IDC classified by the number of lymph vessels invaded could be divided into low- and high-risk groups (Fig. 2C). However, patients with IDCs in which more than 20 lymph vessels had been invaded had almost the same mortality rate as patients with IDC in which 6 to 20 lymph vessels had been invaded. On the contrary, the grading system of LVTE increased the mortality rate according to the order of the grades, and the mortality rate of patients with grade 3 LVTE was more than 70% (Fig. 2D). Almost 80% of IDCs with grade 1 LVTE were in the IDC group in which 0 to 5 lymph vessels had been invaded, and the mortality rates of IDC patients with grade 0 LVTE and those with grade 1 LVTE were almost the same in the IDC group in which 0 to 5 lymph vessels had been invaded. Only 3 of IDC patients with grade 1 LVTE were in the IDC group in which more than 20 lymph vessels had been invaded, and they were all alive and well without tumor recurrence. By contrast, 90% of the IDCs with grade 3 LVTE were in the IDC group in which more than 20 lymph vessels had been invaded. The number of IDCs with grade 2 LVTE was almost the same in each IDC group according to number of lymph vessels invaded, and their number gradually increased with the number of lymph vessels invaded.

Table 4 Multivariate analyses for tumor recurrence according to nodal status and invasive tumor size in patients with IDC who did not receive adjuvant therapy

Cases	Tumor recurrence		
	TRR (%)	HR/95% CI	*P*
IDCs, cases as a whole			
FF diameter >8 mm (*vs* FF absent)			
19	9 (47)	16.3/4.1-64.8	< .001
Invasive tumor size, >50 mm (*vs* invasive tumor size, ≤20 mm)			
4	3 (75)	16.3/3.2-85.3	< .001
Grade 3 lymph vessel tumor embolus (*vs* grade 0 lymph vessel tumor embolus)			
2	1 (50)	14.3/1.3-162.6	.029
Grade 2 lymph vessel tumor embolus (*vs* grade 0 lymph vessel tumor embolus)			
12	5 (42)	3.9/1.0-14.6	.048
IDCs without nodal metastasis			
FF diameter >8 mm (*vs* FF absent)			
16	7 (44)	10.3/2.8-36.4	< .001
IDCs, invasive tumor size, >20 mm			
Invasive tumor size, >50 mm (*vs* invasive tumor size, >20 to ≤50 mm)			
4	3 (75)	50.0/4.9-499.1	< .001
FF diameter >8 mm (*vs* FF absent)			
14	8 (57)	28.1/3.0-268.8	.004
Grade 3 lymph vessel tumor embolus (*vs* grade 0 lymph vessel tumor embolus)			
2	1 (50)	22.8/1.2-432.7	.036

Abbreviations: TRR, tumor recurrence rate; CI, confidence interval.

Table 5 Tumor recurrence rate and mortality rate of patients with IDC who received adjuvant therapy classified by grading system for LVTE according to nodal status and tumor size

Outcomes	Grading system for LVTE							
	G0		G1		G2		G3	
	Cases as a whole (%) (n = 294)							
	120		81		76		17	
Tumor recurrence	25 (21)		20 (25)		43 (57)		14 (82)	
Tumor death	19 (16)		10 (12)		33 (43)		13 (76)	
	G0	G1	G2	G3	G0	G1	G2	G3
	Cases without nodal metastasis (%) (n = 103)				Cases with an invasive tumor size ≤20 mm (%) (n = 101)			
	61	32	8	2	55	31	15	0
Tumor recurrence	10 (16)	6 (19)	2 (25)	2 (100)	5 (9)	4 (13)	6 (40)	
Tumor death	5 (8)	3 (9)	1 (13)	2 (100)	4 (7)	1 (3)	5 (33)	
	Cases with nodal metastasis (%) (n = 191)				Cases with an invasive tumor size >20 mm (%) (n = 193)			
	59	49	68	15	65	50	61	17
Tumor recurrence	15 (25)	14 (29)	41 (60)	12 (80)	20 (31)	16 (32)	37 (61)	14 (82)
Tumor death	14 (24)	7 (14)	32 (47)	11 (73)	15 (23)	9 (18)	28 (46)	13 (77)

3.4. Prognostic power of the grades of LVTE according to nodal status and according to invasive tumor size among patients with IDC who did not receive adjuvant therapy

The rate of tumor recurrence and rate of tumor death increased with the grade of LVTE independent of nodal status and of invasive tumor size (Table 3). Because there were no IDCs with grade 3 LVTE among IDCs without nodal metastasis or the IDCs whose invasive tumor size was 20 mm or less, IDCs with grade 2 LVTE accounted for most of the high-malignancy IDCs in these groups.

Among IDC cases as a whole, FF diameter of more than 8 mm, invasive tumor size of more than 50 mm, grade 3

Table 6 Multivariate analyses for tumor recurrence and tumor death according to nodal status in patients with IDC who received adjuvant therapy

Cases	Tumor recurrence			Tumor death		
	TRR (%)	HR/95% CI	*P*	MR (%)	HR/95% CI	*P*
IDCs without nodal metastasis						
Grade 3 lymph vessel tumor embolus (*vs* grade 0 lymph vessel tumor embolus)						
2	2 (100)	18.0/3.8-87.2	<.001	2 (100)	14.3/2.8-72.7	.001
FF diameter >8 mm (*vs* FF absent)						
26	10 (38)	4.2/1.6-10.6	.003	4 (15)	–/–	
IDCs with nodal metastasis						
Grade 3 lymph vessel tumor embolus (*vs* grade 0 lymph vessel tumor embolus)						
15	12 (80)	4.5/2.1-8.6	<.001	11 (73)	4.4/1.9-10.2	<.001
Grade 2 lymph vessel tumor embolus (*vs* grade 0 lymph vessel tumor embolus)						
68	41 (60)	2.3/1.4-3.7	.003	32 (47)	1.9/1.0-3.4	.037
N3 (*vs* N1)						
50	35 (70)	2.1/1.3-3.4	.004	32 (64)	3.4/2.0-5.9	<.001
ER/PR both negative (*vs* ER or PR is positive or are both positive)						
69	39 (57)	0.6/0.4-0.9	.011	35 (51)	0.4/0.2-0.6	<.001
Histologic grade 3 stroma-invasive tumor cells (*vs* histologic grade 1 stroma-invasive tumor cells)						
85	48 (56)	1.8/1.1-2.7	.019	41 (48)	1.8/1.1-3.1	.032
FF diameter >8 mm (*vs* FF absent)						
66	39 (59)	2.7/1.6-4.7	<.001	33 (50)	–/–	
FF diameter ≤8 mm (*vs* FF absent)						
53	24 (45)	1.9/1.1-3.6	.033	16 (30)	–/–	
Tumor necrosis present (*vs* tumor necrosis absent)						
42	27 (64)	–/–		23 (55)	2.3/1.4-4.0	.002

Abbreviations: MR, mortality rate; –/–, not significant.

Table 7 Multivariate analyses for tumor recurrence and tumor death according to invasive tumor size in patients with IDC who received adjuvant therapy

Cases	Tumor recurrence			Tumor death		
	TRR (%)	HR/95% CI	*P*	MR (%)	HR/95% CI	*P*
IDCs, invasive tumor size, ≤20 mm						
Grade 2 lymph vessel tumor embolus (*vs* grade 0 lymph vessel tumor embolus)						
15	6 (40)	6.0/2.2-20.4	<.001	5 (33)	11.3/2.7-45.2	<.001
Tumor necrosis present (*vs* tumor necrosis absent)						
13	4 (31)	5.1/1.5-17.5	.010	4 (31)	10.5/2.5-44.7	.001
IDCs, invasive tumor size, >20 mm						
Grade 3 lymph vessel tumor embolus (*vs* grade 0 lymph vessel tumor embolus)						
17	14 (82)	5.0/2.4-10.0	<.001	13 (76)	3.9/1.9-7.7	<.001
FF diameter >8 mm (*vs* FF absent)						
81	46 (57)	3.0/1.7-5.1	<.001	35 (43)	2.2/1.3-3.7	.004
N3 (*vs* N0)						
48	33 (69)	2.0/1.2-3.2	.005	30 (63)	8.3/3.4-19.3	<.001
ER/PR both negative (*vs* ER or PR is positive or are both positive)						
72	39 (54)	0.6/0.4-0.8	.011	34 (47)	0.4/0.2-0.6	<.001
Grade 2 lymph vessel tumor embolus (*vs* grade 0 lymph vessel tumor embolus)						
61	37 (61)	2.3/1.4-4.7	<.001	28 (46)	–/–	
N2 (*vs* N0)						
32	17 (53)	–/–		11 (34)	3.4/1.4-8.9	.012
Invasive tumor size, >50 mm (*vs* invasive tumor size, >20 to ≤50 mm)						
36	24 (67)	–/–		20 (55)	2.0/1.2-3.6	.016
Histologic grade 3 stroma-invasive tumor cells (*vs* histologic grade 1 stroma-invasive tumor cells)						
105	51 (49)	–/–		42 (40)	1.9/1.1-3.2	.018
N1 (*vs* N0)						
57	23 (40)	–/–		17 (30)	2.8/1.2-6.9	.024

LVTE, and grade 2 LVTE significantly increased the hazard rates (HRs) for tumor recurrence in the multivariate analyses (Table 4). Among the IDC cases without nodal metastasis, only FF diameter of more than 8 mm significantly increased the HR for tumor recurrence in the multivariate analyses (Table 4). Among the IDCs of invasive tumor size of more than 20 mm, invasive tumor size of more than 50 mm, FF diameter of more than 8 mm, and grade 3 LVTE significantly increased the HRs for tumor recurrence in the multivariate analyses (Table 4).

3.5. Prognostic power of the grades of LVTE according to nodal status and according to invasive tumor size among patients with IDC who received adjuvant therapy

Although there were no IDCs with grade 3 LVTE among IDCs whose invasive tumor size was 20 mm or less, the rate of tumor recurrence and rate of tumor death increased with the grade of LVTE independent of nodal status or invasive tumor size, but both rates were almost the same in the group of IDC patients with grade 0 LVTE and the group with grade 1 LVTE (Table 5).

Among the IDC cases without nodal metastasis, grade 3 LVTE and FF diameter of more than 8 mm significantly increased the HRs for tumor recurrence in the multivariate analyses, and the former also significantly increased the HR for tumor death (Table 6). Among the IDC cases with nodal metastasis, grade 3 LVTE, grade 2 LVTE, N3, ER/PR both negative, and histologic grade 3 stroma-invasive tumor cells significantly increased the HRs for tumor recurrence and tumor death in the multivariate analyses (Table 6). FF diameter of more than 8 mm and FF diameter of 8 mm or less significantly increased the HRs for tumor recurrence in the multivariate analyses, and the presence of tumor necrosis significantly increased the HR of tumor death.

Among the IDC cases with an invasive tumor size of 20 mm or less, grade 2 LVTE and the presence of tumor necrosis significantly increased the HRs for tumor recurrence and for tumor death in the multivariate analyses (Table 7). Among the IDC cases with an invasive tumor size of more than 20 mm, grade 3 LVTE, FF diameter of more than 8 mm, N3, and ER/PR both negative significantly increased the HRs for tumor recurrence and tumor death in the multivariate analyses (Table 7). Grade 2 LVTE significantly increased the HR for tumor recurrence in the multivariate analyses; and N2, invasive tumor size of more than 50 mm, histologic grade 3 stroma-invasive tumor cells, and N1 significantly increased the HRs of tumor death.