

Important Histologic Outcome Predictors for Patients With Invasive Ductal Carcinoma of the Breast

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Abstract: The pathologic diagnosis is regarded as the final diagnosis of a disease, and pathologic examination based on tumor histology is very important for the accurate assessment of the biological characteristics of tumors. The purpose of this study was to investigate the histologic factors that accurately predict patient outcome among 1042 patients with invasive ductal carcinoma of the breast. Both well-known histologic factors and our proposed histologic factors were examined according to several tumor statuses using multivariate analysis. This study clearly demonstrated that type 4 invasive ductal carcinomas having fibrotic foci and atypical tumor-stromal fibroblasts within the fibrotic foci are significant outcome predictors for lymph node-negative and lymph node-positive, the pathologic UICC-TNM stage II and III, luminal A-subtype, luminal B-subtype, and equivocal HER2 subtype invasive ductal carcinoma patients. Lymph vessel tumor embolus grades 2 and 3 were significant outcome predictors for lymph node-positive, UICC pTNM stages II and III, luminal A-subtype, and triple-negative invasive ductal carcinoma patients (except lymph vessel tumor embolus grade 2 in luminal A-subtype patients). More than 5 mitotic figures in metastatic carcinoma to the lymph nodes was a significant outcome predictor for lymph node-positive, UICC pTNM stage II, and luminal A-subtype invasive ductal carcinoma patients. A fibrotic focus diameter > 8 mm was a significant outcome predictor for UICC pTNM stages I and III invasive ductal carcinoma patients. These findings

strongly suggest that these histologic factors are very useful for accurately predicting the outcomes of patients with invasive ductal carcinoma of the breast.

Key Words: fibroblast, fibrotic focus, lymph vessel, lymph node, mitotic figure

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Pathologic examination is performed in all hospitals worldwide, and the pathologic diagnosis is regarded as the final diagnosis of a disease. Thus, pathologic examination based on the histology of tumors obtained as biopsy or surgical specimens is very important for the accurate assessment of the biological characteristics of tumors. For patients with invasive ductal carcinoma of the breast, the invasive tumor size, histologic grade, and presence of vessel invasion or nodal metastasis are well-known histologic outcome predictors.^{2,5,7,9,21,22,25,29} We and other researchers have previously reported that the presence of a fibrotic focus is a very useful histologic tumor-stromal factor for accurately predicting the outcome of patients with invasive ductal carcinoma.^{3,6,12,13,14,23} In a different patient series, we also reported that the grading system for lymph vessel tumor emboli and the presence of > 5 number of mitotic figures in metastatic carcinoma to the lymph nodes are very useful histologic factors for accurately predicting the outcome of patients with invasive ductal carcinoma.^{15,16,18,20} Furthermore, we recently reported that the presence of atypical tumor-stromal fibroblasts in invasive ductal carcinomas with or without a fibrotic focus is a very important histologic outcome predictor for patients with invasive ductal carcinoma of the breast.¹⁹

The purpose of this study was to investigate which histologic factors, including factors that we have proposed, were most capable of accurately predicting the outcome of patients with invasive ductal carcinoma of the breast. The results of this study clearly demonstrated that the histologic factors proposed by us, such as the fibrotic focus diameter,^{13,14} the grading system for lymph vessel tumor emboli,^{16,18} the number of mitotic figures in metastatic carcinoma to the lymph nodes,^{15,20} and the types of invasive ductal carcinoma,¹⁹ are very useful histologic outcome predictors for invasive ductal carcinoma patients with several tumor statuses.

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METHODS

Cases

The participants of this study were 1042 consecutive patients with invasive ductal carcinoma of the breast who did not receive neoadjuvant therapy and were surgically treated at the National Cancer Center Hospital between January 2000 and December 2005 (almost the same case series as that used in our previous study^{18,19}). The invasive ductal carcinomas were diagnosed preoperatively using needle biopsy, aspiration cytology, mammography, or ultrasonography. All the patients were Japanese women, ranging in age from 23 to 72 years (median, 55 y). All patients had a solitary lesion; 498 patients were premenopausal, and 544 were postmenopausal. A partial mastectomy had been performed in 458 patients, and a modified radical mastectomy had been performed in 584 patients. Levels I and II axillary lymph node dissection was performed in all patients, and Level III axillary lymph node dissection had been performed in some of the patients.

Of the 1042 patients, 873 received adjuvant therapy, consisting of chemotherapy in 217 patients, endocrine therapy in 281 patients, and chemoendocrine therapy in 375 patients. The chemotherapy regimens used were anthracycline based with or without taxane and non-anthracycline based. The endocrine therapy regimens consisted of tamoxifen with or without a gonadotropin-releasing hormone agonist, tamoxifen, with or without an aromatase inhibitor, an aromatase inhibitor alone, or a gonadotropin-releasing hormone agonist alone. No cases of inflammatory breast cancer were included in this series. All the tumors were classified according to the pathologic UICC-TNM (pTNM) classification.²⁷ The protocol for this study (20 to 112) was reviewed by the Institutional Review Board of the National Cancer Center. For the pathologic examination, the surgically resected specimens were fixed in 10% formalin, and the size and gross appearance of the tumors were recorded. The tumor size was confirmed by comparison with the tumor size on the histologic slides.

Histologic Examination

Serial sections of each tumor area were cut from paraffin blocks. One section from each tumor was stained with hematoxylin and eosin and was examined histologically to confirm the diagnosis, and the other sections were subjected to immunohistochemistry. The following 7 well-known histologic factors were evaluated: (1) invasive tumor size (≤ 20 mm, > 20 to ≤ 50 mm, > 50 mm), (2) histologic grade (1, 2, 3),^{5,7} (3) tumor necrosis (absent, present),¹⁰ (4) blood vessel invasion (absent, present), (5) adipose tissue invasion (absent, present), (6) skin invasion (absent, present), and (7) muscle invasion (absent, present). In addition, the mitotic activity index was evaluated in primary invasive tumors.^{4,7,27} The mitotic activity index was evaluated at a high-power magnification in 10 consecutive neighboring fields of view in the

most cell-dense area and was determined based on the total number of mitotic structures counted in the 10 fields of views. We analyzed different prognostic thresholds as follows: (1) 0 to 9, 10 to 19, and 20 or higher; (2) 0 to 5, 6 to 10, and 10 or higher; (3) 0 to 2, 3 to 9, and 10 or higher; and (4) 0 to 9 and 10 or higher. Among these thresholds, as the first thresholds (0 to 9, 10 to 19, and 20 or higher) were the only thresholds to increase the hazard ratios for tumor recurrence ($P = 0.004$) and tumor-related death ($P = 0.022$) significantly, we selected the first thresholds for evaluating the mitotic activity index in this study. Next, the following 4 histologic factors that we proposed were evaluated: (1) fibrotic focus (absent, fibrotic focus diameter ≤ 8 mm, fibrotic focus diameter > 8 mm) (Fig. 1A, B),^{13,14} (2) grading system for lymph vessel tumor emboli (Fig. 1C–E),^{16,18} (3) number of mitotic figures in metastatic carcinoma to the lymph nodes (no nodal metastasis, ≤ 5 , > 5) (Fig. 2A, B),^{15,20} and (4) the type of invasive ductal carcinoma (types 1, 2, 3, and 4) (Fig. 2C–F).¹⁹ In brief,¹⁹ we examined the presence or absence of atypical tumor-stromal fibroblasts in the tumor stroma inside and outside of fibrotic foci in the invasive ductal carcinoma and classified the invasive ductal carcinomas into the following 4 types according to the presence or absence of fibrotic foci and the presence or absence of atypical tumor-stromal fibroblasts: (1) type 1 invasive ductal carcinoma not having fibrotic foci and atypical tumor-stromal fibroblasts; (2) type 2 invasive ductal carcinoma not having fibrotic foci but having atypical tumor-stromal fibroblasts; (3) type 3 invasive ductal carcinoma having fibrotic foci but not having atypical tumor-stromal fibroblasts within the fibrotic foci; and (4) type 4 invasive ductal carcinoma having fibrotic foci and atypical tumor-stromal fibroblasts within the fibrotic foci. Types 2 and 4 invasive ductal carcinomas were then immunohistochemically studied using monoclonal antibodies to keratins (AE1/3) and α -smooth muscle actin (Fig. 2C, D, Fig. 2F) to confirm that the atypical tumor-stromal fibroblasts were not modified invasive tumor cells. In addition, some invasive ductal carcinomas contained large lymph vessel tumor emboli, and it was difficult to determine whether these components were true lymph vessel tumor emboli or a noninvasive ductal carcinoma component based on hematoxylin and eosin staining alone. We therefore performed immunohistochemical staining using D2-40 antibody (monoclonal mouse antibody, diluted 1:200; Signet, Dedham, MA) to confirm that the lymph vessel tumor emboli identified using hematoxylin and eosin staining were true tumor emboli (Fig. 1D).¹⁸

Immunohistochemical staining for estrogen receptors, progesterone receptors, and HER2 products was performed using an autoimmunostainer (Optimax Plus; BioGenex, San Ramon, CA). The antigen retrieval device for Optimax Plus was an autoclave, and each specimen was immersed in a citrate buffer and incubated at 121°C for 10 minutes. Immunoperoxidase staining was performed using a labeled streptavidin biotin staining kit (BioGenex) according to the manufacturer's instructions.

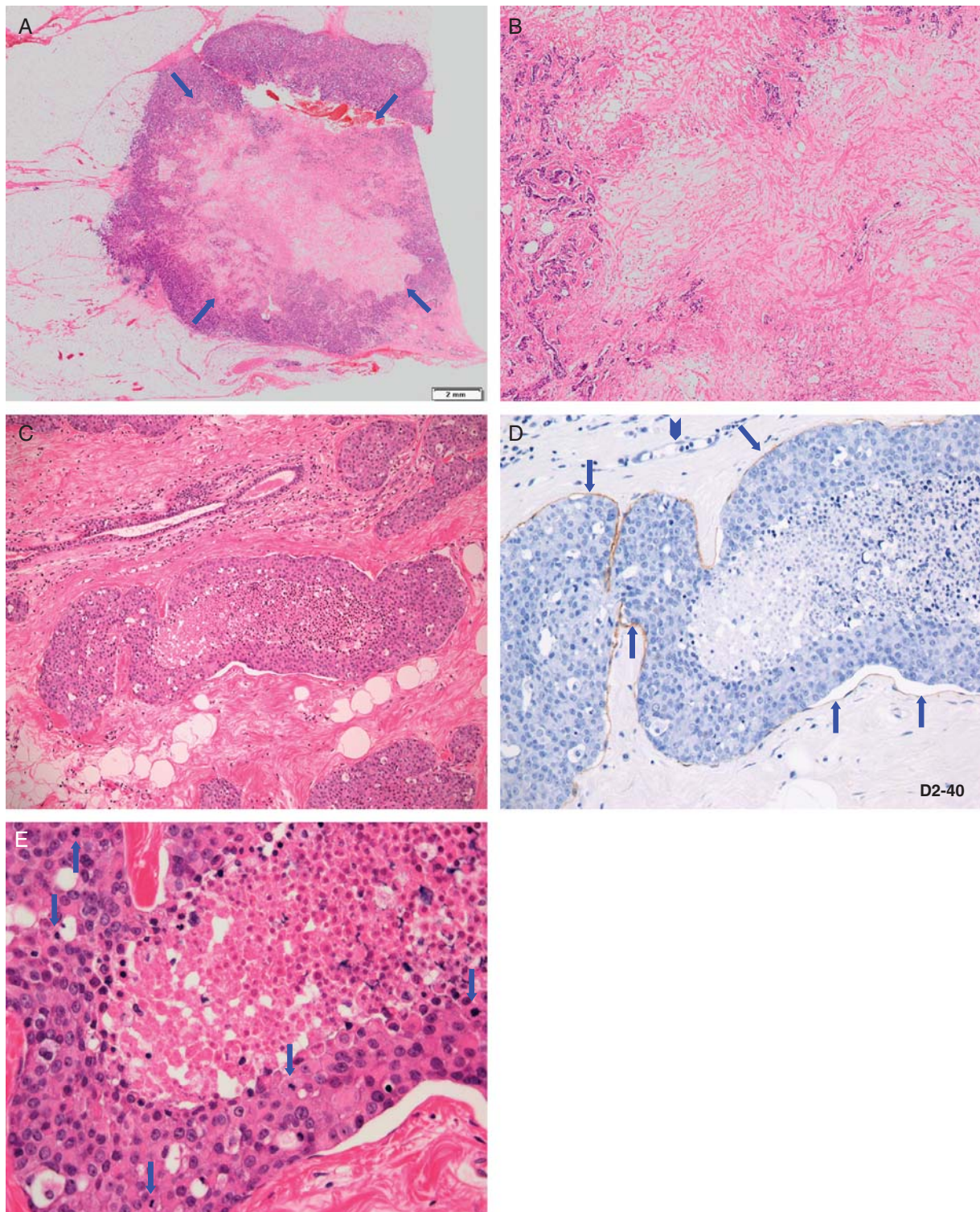


FIGURE 1. A and B, Invasive ductal carcinoma with a fibrotic focus. A, A fibrotic focus measuring 10.5×6.7 mm is visible within the tumor (panoramic view, arrows). The fibrotic focus has a scar-like appearance and is surrounded by invasive ductal carcinoma cells. B, The fibrotic focus area consists mainly of fibroblasts and collagen fibers arranged in a storiform pattern. C to E, Grade 3 lymph vessel tumor emboli. C, One very large lymph vessel tumor embolus located adjacent to one duct is present, and stroma-invasive carcinoma cell nests can be seen in the area surrounding the tumor embolus. D, The wall of the tumor lymph vessel containing the embolus is positive for D2-40 (arrows), and a small D2-40-negative artery is seen in the vicinity of the tumor embolus (arrowhead). E, Five mitotic tumor cells (arrows) and a nest consisting of many apoptotic tumor cells and apoptotic bodies are visible within the tumor embolus.

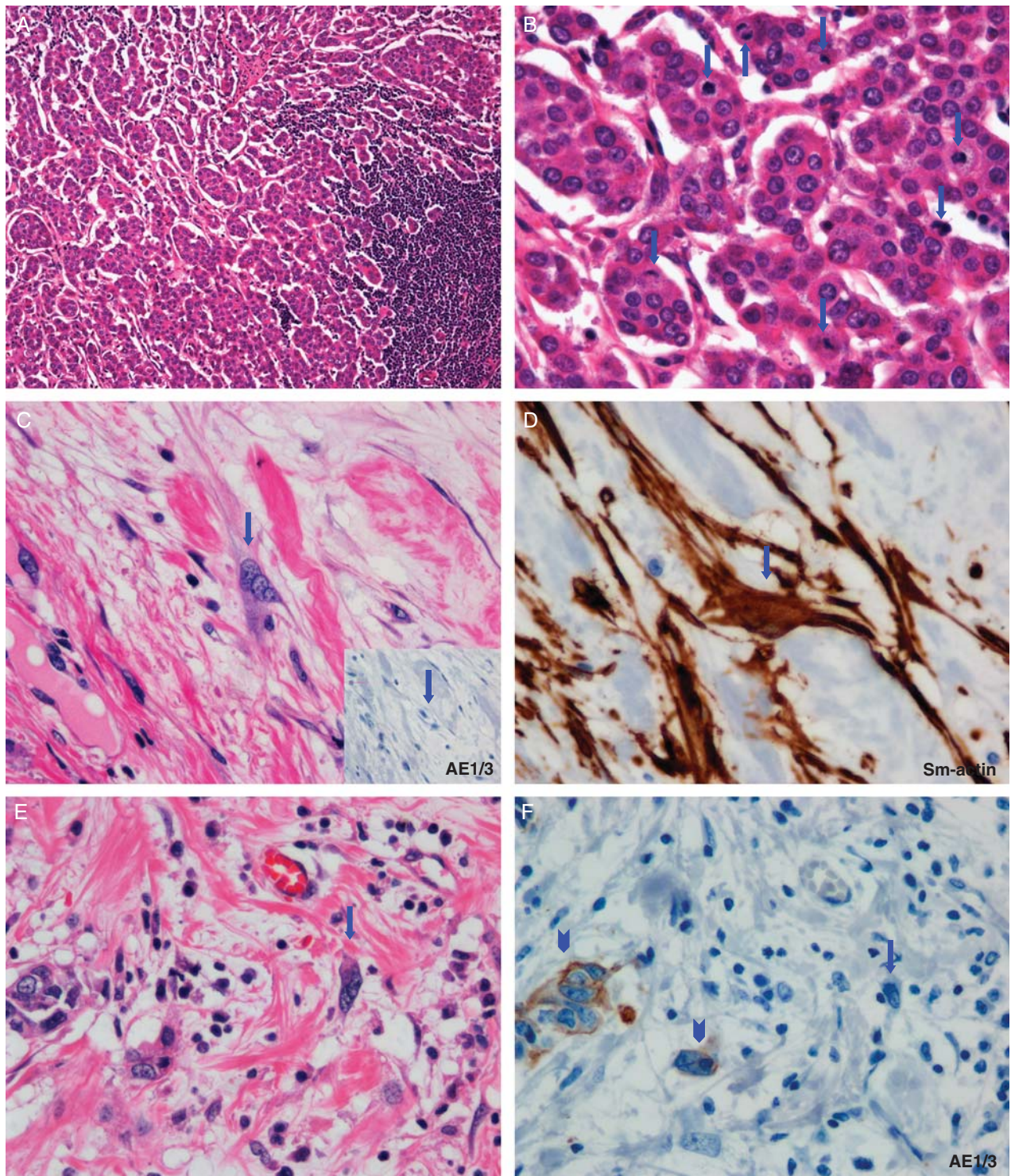


FIGURE 2. A and B, Metastasis of carcinoma to the lymph node. A, Metastatic carcinoma cells in the lymph node. B, Seven mitotic figures are visible in the tumor cells (arrows). C to F, Histologic features of atypical tumor-stromal fibroblasts. C, One atypical tumor-stromal fibroblast with a bizarre and convoluted large nucleus is visible (arrows), and the fibroblast is negative for keratins (AE1/3, arrow in the insert). D, Positive cytoplasmic staining for α -smooth muscle actin (arrow) in a fibroblast. E and F, One atypical tumor-stromal fibroblast with a large bizarre nucleus with obvious large nucleoli and coarsely granulated nuclear chromatin is visible in the vicinity of the tumor cells (arrow); the fibroblast exhibits negative staining for keratin (arrow), but tumor cells adjacent to the fibroblast are positive for keratins (AE1/3, arrowheads).

TABLE 1. Multivariate Analyses for Tumor Recurrence and Tumor-Related Death in All the Invasive Ductal Carcinoma Patients in this Series (n = 1042)

	Tumor Recurrence				Tumor-Related Death		
	Cases	Cases (%)	HR 95% CI	P	Cases (%)	HR 95% CI	P
Allred scores for progesterone receptors in tumor cells							
0 or 2	183	48 (26)	Referent		24 (13)	Referent	
3 to 6	303	58 (19)	0.7	0.097	36 (12)	0.9	0.863
			0.5-1.1			0.5-1.7	
7 or 8	556	78 (14)	0.6	0.037	29 (5)	0.5	0.004
			0.4-0.9			0.3-0.8	
Blood vessel invasion							
Absent	891	139 (16)	Referent		62 (7)	Referent	
Present	149	45 (30)	1.6	0.010	27 (18)	2.0	0.004
			1.1-2.4			1.2-3.3	
Grading system for lymph vessel tumor emboli							
Grade 0	666	74 (11)	Referent		30 (5)	Referent	
Grade 1	250	43 (17)	1.4	0.087	18 (7)	1.4	0.263
			0.9-2.1			0.8-2.7	
Grade 2	97	46 (47)	3.0	< 0.001	24 (25)	3.2	< 0.001
			1.9-4.7			1.9-5.2	
Grade 3	29	21 (72)	4.9	< 0.001	17 (59)	4.3	< 0.001
			2.7-9.0			2.2-8.5	
Fibrotic focus, diameter (mm)							
Absent	667	95 (14)	Referent		42 (6)	Referent	
≤ 8	221	37 (17)	Referent		15 (7)	Referent	
> 8	154	52 (34)	1.7	0.025	32 (21)	1.9	0.016
			1.1-2.6			1.1-7.9	
Histologic grade							
Grade 1	262	15 (5)	Referent		2 (0.7)	Referent	
Grade 2	439	61 (14)	1.7	0.081	27 (6)	5.2	0.026
			0.9-3.3			1.2-22.0	
Grade 3	341	108 (31)	2.4	0.030	60 (18)	5.7	0.019
			1.1-5.4			1.3-24.4	
No. mitotic figures in metastatic carcinoma to lymph nodes							
n0	591	54 (9)	Referent		17 (3)	Referent	
≤ 5	283	46 (16)	Referent		17 (6)	Referent	
> 5	165	84 (55)	1.9	0.006	55 (33)	3.8	< 0.001
			1.2-3.0			2.3-6.3	
Types of invasive ductal carcinoma							
Type 1	627	78 (12)	Referent		34 (5)	Referent	
Type 2	40	17 (43)	2.2	0.008	8 (20)	2.0	0.126
			1.2-3.9			0.8-5.0	
Type 3	346	72 (21)	1.6	0.364	34 (10)	1.5	0.322
			0.8-2.1			0.7-3.2	
Type 4	29	17 (59)	3.2	0.001	13 (45)	3.2	0.001
			1.9-10.3			1.6-6.5	

CI indicates confidence interval; HR, hazard ratio; n0, no nodal metastasis.

The antibodies used were the antiestrogen receptor mouse monoclonal antibody ER88 (BioGenex), the anti-progesterone receptor mouse monoclonal antibody PR88 (BioGenex), and the anti-HER2 mouse monoclonal antibody CB11 (BioGenex). ER88, PR88, and CB11 were previously diluted. After immunostaining, the sections were counterstained with hematoxylin. Sections of the invasive ductal carcinomas that were positive for estrogen receptor, progesterone receptor, and HER2 were used each time as a positive control. As a negative control, the primary antibody was replaced with normal mouse immunoglobulin. Slides of the tumor cells immunostained for estrogen and progesterone receptors were scored using the Allred scoring system, as described previously.^{1,11,26} The Allred scores for estrogen and progesterone receptors

in the tumor cells were classified as follows¹⁷: (1) Allred score for estrogen receptor in tumor cells (0 or 2, 3 to 6, and 7 or 8) and (2) Allred score for progesterone receptor in tumor cells (0 or 2, 3 to 6, and 7 or 8). The HER2 status of the tumor cells was semiquantitatively scored on a scale of 0 to 3 according to the level of HER2 protein expression³⁰ and was classified into 3 categories: 0 or 1, 2 and 3.

The patients were classified into the following 4 subtypes according to their hormone receptor status and HER2 category^{8,24}: (1) luminal A subtype, comprised of estrogen receptor positive and/or progesterone receptor positive and HER2 category 0 or 1; (2) luminal B subtype, comprised of estrogen receptor positive and/or progesterone receptor positive and HER2 category 3; (3) HER2 subtype, comprised of estrogen receptor negative,

progesterone receptor negative, and HER2 category 3; and (4) triple negative subtype, comprised of estrogen receptor negative, progesterone receptor negative, and HER2 category 0 or 1. Invasive ductal carcinomas with an Allred score 0 or 2 for estrogen receptor and progesterone receptor were considered negative for estrogen receptor and progesterone receptor, respectively. As only HER2 samples scored as category 3 were considered positive,³⁰ a total of 182 patients with HER2 category 2 invasive ductal carcinoma were classified as equivocal HER2 subtype (without taking their hormone receptor status into account) in this study.

Patient Outcome and Statistical Analysis

Survival was evaluated using a median follow-up period of 98 months (range, 63 to 134 mo) until March 2011. Of the 1042 invasive ductal carcinoma patients, 858 patients were alive and well, 184 had developed tumor recurrences, and 89 had died of their disease. The tumor recurrence-free survival and overall survival periods were calculated using the time of surgery as the starting point. Tumor relapse was

considered to have occurred whenever evidence of distant-organ metastasis or local recurrence was found.

We analyzed the outcome predictive power of the 7 well-known histologic factors, the 4 histologic factors that we proposed (fibrotic focus, type of invasive ductal carcinoma, grading system for lymph vessel tumor emboli, No. of mitotic figures in metastatic carcinoma to the lymph nodes), the Allred scores for estrogen and progesterone receptors and the category of HER2 expression in the tumor cells, the use of adjuvant therapy (yes or no), patient age (< 39 y and > 39 y), and the UICC-pathologic nodal status²⁸ for tumor recurrence and tumor-related death in univariate analyses using the Cox proportional hazard regression model. The factors that were significantly associated with outcome in the univariate analyses were then entered together into a multivariate analysis. Univariate analysis and multivariate analysis were performed using the Cox proportional hazard regression model. The case-wise and step-down methods were applied until all the remaining factors were significant at a P value below 0.05. All the analyses were performed using Statistica/Windows software (StatSoft, Tulsa, OK).

TABLE 2. Multivariate Analyses for Tumor Recurrence and Tumor-Related Death in Invasive Ductal Carcinoma Patients With or Without Nodal Metastases

Cases	Tumor Recurrence			Tumor-Related Death		
	Cases (%)	HR 95% CI	P	Cases (%)	HR 95% CI	P
Patients Without Nodal Metastasis (n = 591)						
Types of invasive ductal carcinoma						
Type 1	393	27 (7)	Referent	9 (2)	Referent	
Type 2	22	6 (27)	2.9	2 (9)	2.3	0.314
			1.2-6.9		0.5-11.0	
Type 3	163	15 (9)	0.6	4 (3)	0.5	0.414
			0.2-1.7		0.1-3.1	
Type 4	13	6 (46)	6.8	2 (15)	5.2	0.036
			2.8-16.3		1.1-24.7	
Patients With Nodal Metastases (n = 451)						
			Tumor Recurrence			Tumor-Related Death
Blood vessel invasion						
Absent	364	93 (26)	Referent	48 (13)	Referent	
Present	87	37 (43)	2.0	24 (28)	2.0	0.025
			1.3-2.9		1.1-3.6	
Grading system for lymph vessel tumor emboli						
Grade 0	201	36 (18)	Referent	19 (10)	Referent	
Grade 1	139	34 (25)	1.6	14 (10)	1.5	0.319
			0.9-2.6		0.7-3.1	
Grade 2	83	40 (48)	3.3	23 (28)	2.7	0.005
			1.1-5.4		1.4-5.3	
Grade 3	28	20 (71)	5.2	16 (57)	4.0	< 0.001
			3.3-9.3		1.8-9.0	
No. mitotic figures in metastatic carcinoma to lymph nodes						
≤ 5	286	46 (16)	Referent	17 (6)	Referent	
> 5	165	84 (51)	3.0	55 (33)	3.3	< 0.001
			2.1-4.5		1.8-6.4	
Types of invasive ductal carcinoma						
Type 1	234	51 (22)	Referent	25 (11)	Referent	
Type 2	18	11 (61)	1.9	6 (33)	1.6	0.346
			1.0-3.4		0.6-4.5	
Type 3	183	57 (31)	1.9	30 (16)	1.0	0.932
			0.9-4.0		0.4-2.0	
Type 4	16	11 (69)	3.1	11 (69)	3.5	0.021
			1.6-6.0		1.2-10.1	

CI indicates confidence interval; HR, hazard ratio.

RESULTS

Among all the patients with invasive ductal carcinoma, the presence of blood vessel invasion, lymph vessel tumor embolus grades 2 and 3, a fibrotic focus diameter >8mm, histologic grade 3, >5 mitotic figures in metastatic carcinoma to the lymph nodes, and type 4 invasive ductal carcinoma had significantly higher hazard ratios for tumor recurrence and tumor-related death in multivariate analyses (Table 1). Type 2 invasive ductal carcinoma had a significantly higher hazard ratio for tumor recurrence, and histologic grade 2 had a significantly higher hazard ratio for tumor-related death in a multivariate analysis (Table 1). An Allred score of 7 or 8 for progesterone receptor in the tumor cells had significantly lower hazard ratios for tumor recurrence and tumor-related death in multivariate analyses (Table 1).

Among patients with invasive ductal carcinoma without nodal metastasis, type 4 invasive ductal carcinoma had significantly higher hazard ratios for tumor recurrence and tumor-related death in multivariate analyses (Table 2). A fibrotic focus >8mm ($P = 0.009$), histologic grades 2 ($P = 0.030$) and 3 ($P = 0.011$), type 2 invasive ductal carcinoma (Table 2), lymph vessel tumor embolus grade 2 or 3 ($P < 0.001$), and HER2 category 3 ($P = 0.028$) had significantly higher hazard ratios for tumor recurrence in a multivariate analysis. A mitotic

activity index of >20 in primary invasive tumors had a significantly higher hazard ratio for tumor-related death in a multivariate analysis ($P = 0.011$).

Among patients with invasive ductal carcinoma with nodal metastases, the presence of blood vessel invasion, lymph vessel tumor grades 2 and 3, >5 mitotic figures in metastatic carcinoma to the lymph nodes, and type 4 invasive ductal carcinoma had significantly higher hazard ratios for tumor recurrence and tumor-related death in multivariate analyses (Table 2). Type 2 invasive ductal carcinoma had a significantly higher hazard ratio for tumor recurrence in a multivariate analysis (Table 2).

Among patients with UICC pTNM stage I invasive ductal carcinoma, a fibrotic focus diameter >8mm (Fig. 3A, B) and histologic grade 3 had significantly higher hazard ratios for tumor recurrence and tumor-related death in multivariate analyses (Table 3). Histologic grade 2 (Table 3) and lymph vessel tumor embolus grade 2 or 3 had a significantly higher hazard ratio for tumor recurrence ($P < 0.001$), and an Allred score of 7 or 8 for estrogen receptors in tumor cells had a significantly lower hazard ratio for tumor recurrence ($P = 0.008$) in a multivariate analysis.

Among patients with UICC pTNM stage II invasive ductal carcinoma, the presence of blood vessel invasion, lymph vessel tumor embolus grades 2 and 3, >5 mitotic

TABLE 3. Multivariate Analyses for Tumor Recurrence and Tumor-Related Death in UICC pTNM Stages I, II, and III Invasive Ductal Carcinoma Patients

	Cases	Tumor Recurrence			Tumor-Related Death		
		Cases (%)	HR 95% CI	P	Cases (%)	HR 95% CI	P
UICC pTNM Stage I Patients (n = 363)							
Fibrotic focus, diameter (mm)							
Absent	273	18 (7)	Referent		5 (2)	Referent	
≤8	66	4 (6)	Referent		1 (2)	Referent	
>8	24	6 (25)	3.1 1.2-7.9	0.020	4 (17)	6.9 1.5-20.6	0.009
Histologic grade							
Grade 1	128	1 (0.8)	Referent		0	Referent	
Grade 2	160	11 (7)	8.4 1.1-65.2	0.041	2 (1)	Referent	
Grade 3	75	16 (21)	14.6 1.9-113.2	0.010	8 (11)	7.6 1.5-37.9	0.014
UICC pTNM Stage II Patients (n = 487)							
Blood vessel invasion							
Absent	404	64 (16)	Referent		24 (6)	Referent	
Present	83	23 (28)	1.8 1.1-2.9	0.027	11 (13)	2.4 1.1-5.5	0.046
Grading system for lymph vessel tumor emboli							
Grade 0	296	35 (12)	Referent		10 (3)	Referent	
Grade 1	136	25 (18)	1.5 0.9-2.6	0.132	12 (9)	2.7 1.2-6.3	0.022
Grade 2	46	22 (48)	3.3 1.9-5.5	< 0.001	8 (17)	3.9 1.5-10.3	0.006
Grade 3	9	5 (56)	4.1 1.6-11.0	0.005	5 (56)	8.5 2.7-27.0	< 0.001
No. mitotic figures in metastatic carcinoma to lymph nodes							
n0	228	26 (11)	Referent		7 (3)	Referent	
≤5	184	27 (15)	Referent		8 (4)	Referent	
>5	75	34 (45)	2.7 1.6-4.2	< 0.001	20 (27)	5.6 2.7-11.8	< 0.001

TABLE 3. (continued)

	Cases	Tumor Recurrence			Tumor-Related Death		
		Cases (%)	HR 95% CI	P	Cases (%)	HR 95% CI	P
Types of invasive ductal carcinoma							
Type 1	271	34 (13)	Referent		14 (5)	Referent	
Type 2	20	11 (55)	2.4 1.2-4.7	0.013	4 (20)	1.2 0.4-4.2	0.761
Type 3	184	35 (19)	1.3 0.7-2.4	0.391	13 (7)	0.8 0.4-1.9	0.695
Type 4	12	7 (58)	4.4 1.9-10.3	< 0.001	4 (33)	5.4 1.8-9.5	0.002
UICC pTNM Stage III Patients (n = 192)							
Fibrotic focus, diameter (mm)							
Absent	103	32 (31)	Referent		16 (27)	Referent	
≤ 8	47	16 (34)	Referent		8 (17)	Referent	
> 8	42	21 (50)	2.9 1.6-5.0	< 0.001	17 (41)	3.4 1.7-6.6	< 0.001
Grading system for lymph vessel tumor emboli							
Grade 0	79	19 (24)	Referent		13 (16)	Referent	
Grade 1	52	14 (27)	1.0 0.5-2.3	0.917	4 (8)	0.6 0.2-1.9	0.366
Grade 2	42	21 (50)	2.4 1.4-4.3	0.002	16 (38)	3.7 1.8-7.4	< 0.001
Grade 3	19	15 (79)	4.9 2.5-9.8	< 0.001	11 (58)	4.7 2.0-10.9	< 0.001
HER2 category							
0 or 1	126	41 (33)	Referent		24 (19)	Referent	
2	38	10 (26)	0.5 0.2-1.2	0.110	6 (16)	2.3 0.8-5.9	0.095
3	28	18 (64)	2.4 1.4-4.3	0.003	14 (50)	3.4 1.7-6.7	< 0.001
UICC pN category							
pN0	23	2 (9)	Referent		1 (4)	Referent	
pN1	36	8 (22)	1.5 0.3-8.1	0.617	6 (17)	2.7 0.3-29.2	0.411
pN2	85	30 (35)	1.6 0.3-7.9	0.539	16 (19)	2.5 0.3-24.8	0.435
pN3	48	29 (60)	2.1 1.3-3.5	0.004	21 (44)	1.9 1.1-3.5	0.037

CI indicates confidence interval; HR, hazard ratio; n0, no nodal metastasis; pN, pathologic regional lymph node; pN0, no nodal metastasis; pN1, 1 to 3 nodal metastases; pN2, 4 to 9 nodal metastases; pN3, 10 or more nodal metastases.

figures in metastatic carcinoma to the lymph nodes, and type 4 invasive ductal carcinoma had significantly higher hazard ratios for tumor recurrence and tumor-related death in multivariate analyses (Table 3). Type 2 invasive ductal carcinoma had a significantly higher hazard ratio for tumor recurrence (Table 3), and lymph vessel tumor embolus grade 1 had a significantly higher hazard ratio for tumor-related death in a multivariate analysis (Table 3).

Among patients with UICC pTNM stage III invasive ductal carcinoma, a fibrotic focus diameter > 8 mm, lymph vessel tumor embolus grades 2 and 3, HER2 category 3, and the UICC pN3 category had significantly higher hazard ratios for tumor recurrence and tumor-related death in multivariate analyses (Table 3). A mitotic activity index of 20 or higher in the primary invasive tumors had a significantly higher hazard ratio for tumor recurrence in a multivariate analysis ($P = 0.005$).

Among patients with luminal A-subtype invasive ductal carcinoma, the presence of blood vessel invasion,

lymph vessel tumor embolus grade 3 (Fig. 3C, D), > 5 mitotic figures in metastatic carcinoma to the lymph nodes (Fig. 3E, F), type 4 invasive ductal carcinoma (Fig. 3G, H), and the UICC pN3 category had significantly higher hazard ratios for tumor recurrence and tumor-related death in multivariate analyses (Table 4). Lymph vessel tumor embolus grade 2 (Table 4, Fig. 3C) and a fibrotic focus diameter > 8 mm ($P = 0.016$) had significantly higher hazard ratio for tumor recurrence, and the UICC pN1 category ($P = 0.019$) had a significantly higher hazard ratio for tumor-related death in a multivariate analysis.

Among patients with luminal B-subtype invasive ductal carcinoma, types 2 and 4 invasive ductal carcinoma had significantly higher hazard ratios for tumor recurrence and tumor-related death in multivariate analyses (Table 4). Type 3 invasive ductal carcinoma (Table 4), lymph vessel tumor embolus grades 2 ($P = 0.030$) and 3 ($P < 0.001$), and the UICC pN3 category ($P < 0.001$) had significantly higher hazard ratio

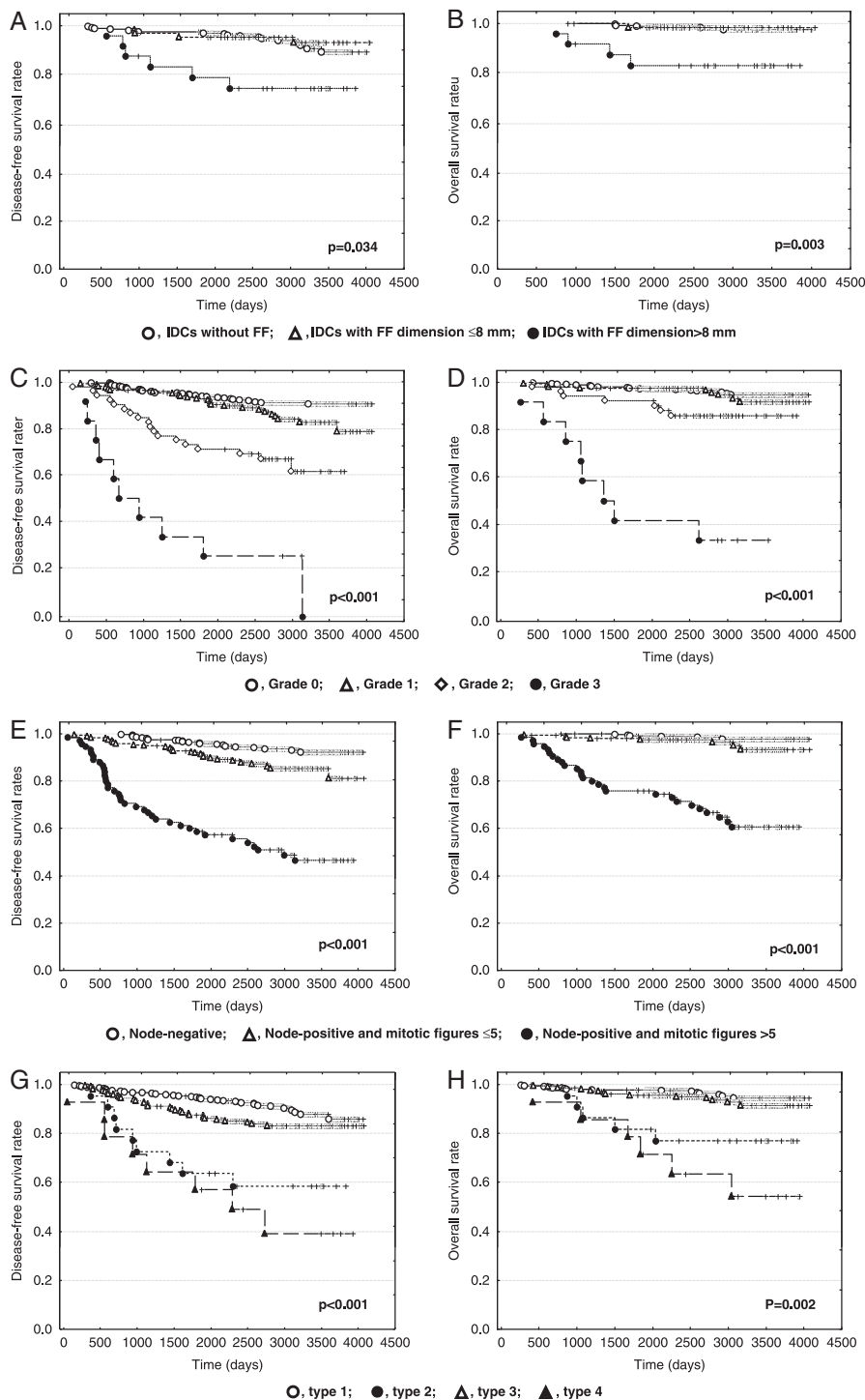


FIGURE 3. Disease-free survival curves and overall survival curves of invasive ductal carcinoma patients with the UICC pTNM stage I (A and B) and luminal-A subtype invasive ductal carcinoma patients (C–H). A and B, Patients with invasive ductal carcinoma with fibrotic foci dimension >8 mm have shorter disease-free and overall survival times than patients without fibrotic foci and those with fibrotic foci <8 mm. IDC, invasive ductal carcinoma; FF, fibrotic foci. C and D, The disease-free and overall survival curves decreased significantly according to the grade of lymph vessel tumor embolus. Grade, lymph vessel tumor embolus grade. E and F, The disease-free and overall survival curves decreased significantly according to the number of mitotic figures in metastatic carcinoma to the lymph nodes. Node negative, no nodal metastasis; node positive, nodal metastases; mitotic figures, number of mitotic figures in metastatic carcinoma to the lymph nodes. G and H, Patients with types 2, 3, and 4 invasive ductal carcinoma have shorter disease-free and overall survival times than patients with type 1 invasive ductal carcinoma.

TABLE 4. Multivariate Analyses for Tumor Recurrence and Tumor-Related Death in Patients With Invasive Ductal Carcinoma According to Biological Subtype

	Tumor Recurrence				Tumor-Related Death		
	Cases	Cases (%)	HR 95% CI	P	Cases (%)	HR 95% CI	P
Luminal A-subtype Patients (n = 658)							
Blood vessel invasion							
Absent	552	62 (11)	Referent		25 (5)	Referent	
Present	104	27 (26)	2.2 1.4-3.6	0.002	16 (15)	2.3 1.2-4.4	0.015
Grading system for lymph vessel tumor emboli							
Grade 0	425	36 (9)	Referent		17 (4)	Referent	
Grade 1	169	25 (15)	1.5 0.9-2.5	0.141	9 (5)	1.1 0.5-2.5	0.885
Grade 2	52	18 (35)	3.4 1.9-6.0	< 0.001	7 (14)	0.9 0.3-3.3	0.898
Grade 3	12	10 (83)	6.5 3.0-14.0	< 0.001	8 (66)	4.7 2.0-11.4	< 0.001
No. mitotic figures in metastatic carcinoma to lymph nodes							
n0	383	24 (6)	Referent		6 (2)	Referent	
≤ 5	200	27 (14)	Referent		8 (4)	Referent	
> 5	75	38 (51)	3.5 2.1-5.8	< 0.001	27 (36)	6.6 3.1-14.2	< 0.001
Types of invasive ductal carcinoma							
Type 1	409	38 (9)	Referent		16 (4)	Referent	
Type 2	22	9 (41)	1.9 0.8-4.3	0.152	5 (23)	1.6 0.4-5.7	0.470
Type 3	213	34 (16)	0.9 0.5-1.7	0.679	14 (7)	0.8 0.3-2.1	0.583
Type 4	14	8 (57)	2.4 1.0-5.6	0.045	6 (43)	3.9 1.5-9.9	0.005
UICC pN category							
pN0	383	24 (6)	Referent		6 (2)	Referent	
pN1	199	38 (19)	1.7 0.9-3.1	0.104	21 (11)	2.6 1.2-5.8	0.019
pN2	50	11 (22)	1.1 0.4-2.7	0.864	4 (8)	1.1 0.2-5.3	0.879
pN3	26	16 (62)	2.3 1.2-4.2	0.008	10 (39)	3.9 1.4-10.5	0.008
Luminal B-subtype Patients (n = 88)							
Types of invasive ductal carcinoma							
Type 1	51	8 (16)	Referent		3 (6)	Referent	
Type 2	6	4 (67)	8.6 2.4-30.7	0.001	3 (50)	21.2 2.5-184.7	0.006
Type 3	28	13 (46)	4.3 1.7-11.0	0.002	7 (25)	1.4 0.1-15.4	0.780
Type 4	3	2 (67)	13.2 2.5-68.2	0.002	2 (67)	150.9 1.1-2048.8	0.046
Equivocal HER2-subtype Patients (n = 182)							
Grading system for lymph vessel tumor emboli							
Grade 0	109	10 (9)	Referent		2 (2)	Referent	
Grade 1	49	10 (20)	1.7 0.6-4.5	0.299	5 (10)	7.7 1.4-42.9	0.020
Grade 2	18	10 (56)	6.8 2.1-22.6	0.002	5 (28)	14.3 2.6-77.9	0.002
Grade 3	6	2 (33)	3.7 0.9-16.1	0.079	2 (33)	16.8 2.2-127.6	0.006
Invasive tumor size (mm)							
≤ 20	84	6 (7)	Referent		2 (2)	Referent	
> 20- < 50	93	24 (26)	4.5 0.7-28.4	0.114	10 (11)	1.8 0.3-9.7	0.516
> 50	5	2 (40)	3.2 1.1-9.5	0.036	2 (40)	4.8 1.2-36.8	0.029
Types of invasive ductal carcinoma							
Type 1	95	13 (14)	Referent		6 (6)	Referent	
Type 2	6	2 (33)	2.7 0.5-15.7	0.265	0	Referent	
Type 3	75	13 (17)	0.8 0.4-2.0	0.697	6 (7)	1.3 0.4-4.5	0.692

TABLE 4. (continued)

	Tumor Recurrence				Tumor-Related Death		
	Cases	Cases (%)	HR 95% CI	P	Cases (%)	HR 95% CI	P
Type 4	6	4 (67)	11.9 3.1-44.8	< 0.001	2 (33)	7.6 1.5-38.0	0.013
Triple-negative Patients (n = 75)							
Grading system for lymph vessel tumor emboli							
Grade 0	51	6 (12)	Referent		2 (4)	Referent	
Grade 1	8	1 (13)	0.8 0.1-6.7	0.830	0	Referent	
Grade 2	11	10 (90)	19.1 3.6-101.2	< 0.001	7 (64)	24.1 4.7-122.8	< 0.001
Grade 3	5	4 (80)	65.3 5.9-722.6	< 0.001	3 (60)	32.6 5.2-209.0	< 0.001

CI indicates confidence interval; HR, hazard ratio; n0, no nodal metastasis; pN, pathologic regional lymph node; pN0, no nodal metastasis; pN1, 1 to 3 nodal metastases; pN2, 4 to 9 nodal metastases; pN3, 10 or more nodal metastases.

for tumor recurrence, and the UICC pN2 category ($P = 0.019$), a fibrotic focus diameter 8 mm ($P < 0.001$), and an invasive tumor size > 50 mm ($P = 0.047$) had significantly higher hazard ratio for tumor-related death in a multivariate analysis.

Among patients with HER2-subtype invasive ductal carcinoma (n = 39), the presence of blood vessel invasion ($P = 0.009$) and the UICC pN3 category ($P = 0.007$) had significantly higher hazard ratio for tumor recurrence in a multivariate analysis. As only 7 patients died as a result of their disease, a multivariate analysis for tumor-related death could not be performed in this patient series.

Among the patients with equivocal HER2 invasive ductal carcinoma, lymph vessel tumor embolus grade 2, invasive tumor size > 50 mm, and type 4 invasive ductal carcinoma had significantly higher hazard ratios for tumor recurrence and tumor-related death in multivariate analyses (Table 4). Lymph vessel embolus grades 1 and 3 (Table 4) and histologic grade 3 ($P = 0.010$) had significantly higher hazard ratio for tumor-related death in a multivariate analysis. Among the patients with triple-negative invasive ductal carcinoma, lymph vessel tumor embolus grades 2 and 3 had significantly higher hazard ratios for tumor recurrence and tumor-related death in multivariate analyses (Table 4).

DISCUSSION

The histologic factors that significantly increased the hazard ratios for tumor recurrence or tumor-related death are shown in Table 5. Histologic factors that significantly increased hazard ratios for both tumor recurrence and tumor-related death were evaluated as A rank predictors, those that significantly increased the hazard ratio only for tumor-related death were evaluated as B rank predictors, those that significantly increased the hazard ratio only for tumor recurrence were evaluated as C rank predictors, and those that failed to significantly increase the hazard ratio for either tumor recurrence or tumor-related death were evaluated as D rank predictors.

The 20 histologic factors were then ranked in decreasing order of their contribution to the accurate prediction of tumor recurrence or tumor-related death according to various tumor statuses (Table 5). Among them, type 4 invasive ductal carcinoma was evaluated as A rank predictors in 7 of the tumor status classifications (A7), followed by lymph vessel tumor embolus grades 3 and 2 (A6), blood vessel invasion (A4), > 5 mitotic figures in metastatic carcinoma to the lymph nodes (A4), a fibrotic focus diameter > 8 mm (A3), histologic grade 3 (A2), UICC pN3 category (A2), invasive tumor size > 50 mm (A1), type 2 invasive ductal carcinoma (A1), HER2 category 3 (A1), and an Allred score of 7 or 8 for progesterone receptors in tumor cells (A1). These 12 histologic factors were also evaluated as B or C rank predictors in other tumor status classifications in which they were not identified as A rank predictors. Thus, these 12 histologic factors are likely to be very important histologic outcome predictors for patients with invasive ductal carcinoma of the breast.

Of note, only C rank predictors (blood vessel invasion and UICC pN3 category) were identified among these 12 histologic factors for patients with HER2-subtype invasive ductal carcinoma. This result may reflect the relatively small number of patients with HER2-subtype invasive ductal carcinoma in this study (39 patients, 4%), although this study included 1042 patients with invasive ductal carcinoma of the breast. Thus, a multi-institutional case study may be needed to clarify the optimal histologic predictors of outcome for patients with HER2-subtype invasive ductal carcinoma.

Although an absolute histologic predictor of outcome applicable to all patients with invasive ductal carcinoma was not identified in this study, appropriate histologic predictors of outcome were identified for various tumor statuses. We listed only the A rank predictors for each tumor status in Table 6. These factors are the core histologic predictors of outcome for patients with specific invasive ductal carcinoma tumor statuses

TABLE 5. Ranking of 18 Histologic Factors According to Outcome Predictive Power

	Rank	Overall (n = 1042)	N0 (n = 591)	N+ (n = 451)	Stage I (n = 363)	Stage II (n = 487)	Stage III (n = 192)	Luminal A (n = 658)	Luminal B (n = 88)	HER2 (n = 39)	eHER2 (n = 182)	TN (n = 75)
Type 4	A7; B0 C0; D4	A	A	A	D	A	D	A	A	D	A	D
Ly grade 3	A6; B1 C3; D1	A	C	A	C	A	A	A	C	D	B	A
Ly grade 2	A6; B0 C4; D1	A	C	A	C	A	A	C	C	D	A	A
BV invasion	A4; B0 C1; D6	A	D	A	D	A	D	A	D	C	D	D
> 5 MF, met ca to LN	A4; B0 C0; D6	A	NA	A	D	A	D	A	D	D	D	D
FF diameter > 8 mm	A3; B1 C2; D5	A	C	D	A	D	A	C	B	D	D	D
Histologic grade 3	A2; B1 C0; D7	A	C	D	A	D	D	D	D	D	B	D
pN3	A2; B0 C2; D4	D	NA	D	NA	NA	A	A	C	C	D	D
Tumor size > 50 mm	A1; B1 C0; D8	D	D	D	NA	D	D	D	B	D	A	D
Type 2	A1; B0 C4; D6	C	C	C	D	C	D	D	A	D	D	D
HER2 category 3	A1; B0 C2; D3	C	C	D	D	D	A	NA	NA	NA	NA	NA
PR Allred 7 or 8	A1; B0 C0; D6	A	D	D	D	D	D	NA	NA	NA	D	NA
Ly grade 1	A0; B2 C0; D9	D	D	D	D	B	D	D	D	D	B	D
MAI of > 20 in PIT	A0; B2 C0; D9	D	B	D	D	D	B	D	D	D	D	D
Histologic grade 2	A0; B1 C2; D8	B	C	D	C	D	D	D	D	D	D	D
pN2	A0; B1 C0; D7	D	NA	D	NA	NA	D	D	B	D	D	D
Skin invasion	A0; B1 C0; D8	B	D	D	NA	NA	D	D	D	D	D	D
pN1	A0; B1 C0; D9	D	NA	D	D	D	D	B	D	D	D	D
ER Allred 7 or 8	A0; B0 C1; D6	D	D	D	C	D	D	NA	NA	NA	D	NA
Type 3	A0; B0 C1; D10	D	D	D	D	D	D	D	C	D	D	D

A rank of A was given for factors significantly associated with tumor recurrence and tumor-related death in multivariate analyses; a rank of B was given for factors that were significantly associated with tumor recurrence in a multivariate analysis; a rank of C was given for factors that were significantly associated with tumor recurrence in a multivariate analysis; a rank of D was given for factors that were not associated with either tumor recurrence or tumor-related death in multivariate analyses or in univariate analyses; Overall, all the patients in this study; N0, patients without nodal metastasis; N+, patients with nodal metastasis; Stages I, II, and III, UICC pTNM stages I, II and III, respectively; Luminal A, luminal-A subtype invasive ductal carcinoma patients; Luminal B, luminal-B subtype invasive ductal carcinoma patients; HER2, HER2-subtype invasive ductal carcinoma patients; eHER2, equivocal HER2 subtype; TN, triple-negative invasive ductal carcinoma patients; Type 4, type 4 invasive ductal carcinoma; Ly grade, grading system for lymph vessel tumor emboli; Ly grade 3, grade 3 lymph vessel tumor emboli; Ly grade 2, grade 2 lymph vessel tumor emboli; BV, blood vessel; MF, mitotic figures; met, metastatic; ca, carcinoma; LN, lymph nodes; FF, fibrotic focus; pN3, UICC pN3 category; Tumor size, primary invasive tumor size; Type 2, type 2 invasive ductal carcinoma; PR, progesterone receptor; Allred, Allred score; Ly grade 1, grade 1 lymph vessel tumor emboli; MAI, mitotic activity index; PIT, primary invasive tumor; pN2, UICC pN2 category; pN1, UICC pN1 category; ER, estrogen receptor; Type 3, type 3 invasive ductal carcinoma; NA, not available.

and may enable pathologists or clinicians to predict the outcomes of many patients with invasive ductal carcinoma accurately. Among these factors, a fibrotic focus diameter > 8 mm, lymph vessel tumor embolus grades 2 and 3, types 2 and 4 invasive ductal carcinoma, and > 5 mitotic figures in metastatic carcinoma to the lymph nodes were histologic factors that we proposed.^{12-16,18-20} Thus, many readers may believe that the reliabilities of these factors as outcome predictors are inferior to those of well-known histologic factors, such as the presence of blood vessel invasion, histologic grade 3, HER2

category 3, and UICC pN3 category. However, outcome predictive power of a fibrotic focus among patients with invasive ductal carcinoma without nodal metastasis or patients with early invasive ductal carcinoma has also been confirmed by other investigators.^{3,6,23} We have also confirmed the outcome predictive powers of the grading system for lymph vessel tumor emboli and the presence of > 5 mitotic figures in metastatic carcinoma to the lymph nodes in different invasive ductal carcinoma patient groups.^{15,16,18,20} Thus, the proposed histologic factors seem to be very useful as predictors of outcome

TABLE 6. Best histologic Factors for Predicting Outcome Among Patients With Invasive Ductal Carcinoma According to Tumor status

Lymph Node Status	
Lymph node-negative invasive ductal carcinoma	
Type 4 invasive ductal carcinoma	
Lymph node-positive invasive ductal carcinoma	
Blood vessel invasion	Lymph vessel tumor embolus grades 2 and 3
> 5 mitotic figures in metastatic carcinoma to lymph nodes	Type 4 invasive ductal carcinoma
	UICC pTNM stage
UICC pTNM stage I invasive ductal carcinoma	
Fibrotic foci diameter > 8 mm	Histologic grade 3
UICC pTNM stage II invasive ductal carcinoma	
Blood vessel invasion	Lymph vessel tumor embolus grades 2 and 3
> 5 mitotic figures in metastatic carcinoma to lymph nodes	Type 4 invasive ductal carcinoma
UICC pTNM stage III invasive ductal carcinoma	
Fibrotic foci diameter > 8 mm	HER2 category 3
Lymph vessel tumor embolus grades 2 and 3 and UICC pN3	Type 4 invasive ductal carcinoma
	Carcinoma subtype
Luminal A invasive ductal carcinoma	
Blood vessel invasion	Lymph vessel tumor embolus grade 3
> 5 mitotic figures in metastatic carcinoma to lymph nodes and UICC pN3	Type 4 invasive ductal carcinoma
Luminal B invasive ductal carcinoma	
Types 2 and 4 invasive ductal carcinoma	
Equivocal HER2 invasive ductal carcinoma	
Invasive tumor size > 50 mm	Lymph vessel tumor embolus grade 2
Type 4 invasive ductal carcinoma	
Triple-negative invasive ductal carcinoma	
Lymph vessel tumor embolus grades 2 and 3	

among patients with invasive ductal carcinoma of the breast.

This study clearly demonstrated that the outcome predictive power of the invasive tumor size is inferior to that of a fibrotic focus, blood vessel invasion, the grading system for lymph vessel tumor emboli, histologic grade, or the type of invasive ductal carcinoma. The outcome predictive power of the number of nodal metastases was also inferior to the number of mitotic figures in metastatic carcinoma to the lymph nodes in this study. The number of nodal metastases and the invasive tumor size reflect the quantity of invasive ductal carcinoma cells, whereas the presence of a fibrotic focus, blood vessel invasion, grading system for lymph vessel tumor emboli, histologic grade, type of invasive ductal carcinoma, and the number of mitotic figures in metastatic carcinoma to the lymph nodes reflect the tumor characteristics of invasive ductal carcinomas. Furthermore, we observed that 1 UICC stage I patient with 1 micrometastasis with > 5 mitotic figures died of her diseases in this study (data not shown). Thus, histologic factors reflecting tumor characteristics are most likely superior to histologic factors reflecting tumor quantity as outcome predictors.

In conclusion, this study clearly demonstrated that our proposed histologic factors, such as type 4 invasive ductal carcinoma, lymph vessel tumor embolus grades 2 and 3, presence of > 5 mitotic figures in metastatic carcinoma to the lymph nodes, and a fibrotic focus diameter > 8 mm, are very important histologic factors for accurately predicting the outcomes of patients with

invasive ductal carcinoma. Combined pathologic examinations based on these histologic factors and A-ranked, well-known histologic factors (blood vessel invasion, histologic grade, HER2 category, and UICC pN category) would most likely enable pathologists to assess the true malignant potential of invasive ductal carcinomas of the breast accurately.

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