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Tumor histology in lymph vessels and lymph nodes for the accurate prediction of outcome among breast cancer patients treated with neoadjuvant chemotherapy

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The present study investigated fibrotic foci (FFs), the grading system for lymph vessel tumor emboli (LVTEs), and the histological characteristics of nodal metastatic tumors that were significantly associated with the outcomes of 115 patients with invasive ductal carcinoma (IDC) who had received neoadjuvant chemotherapy. We compared the outcome predictive power of FFs, the grading system for LVTEs, and the histological characteristics of metastatic tumors in lymph nodes with the well-known clinicopathological characteristics of tumor recurrence and tumor-related death in multivariate analyses. The presence of FFs, as assessed by a biopsy performed before neoadjuvant chemotherapy, significantly increased the hazard rates (HRs) for tumor-related death in all the cases and in cases with nodal metastasis. The grading system for LVTEs, which was assessed using surgical specimens obtained after neoadjuvant chemotherapy, was significantly associated with increasing hazard rates (HRs) for tumor recurrence and tumor-related death in all the cases and in cases with nodal metastasis. Moderate to severe stroma in nodal metastatic tumors and five or more mitotic figures in nodal metastatic tumors were significantly associated with elevated HRs for tumor recurrence and tumor-related death among all the cases. These results indicated that FFs, the grading system for LVTEs, and the histological characteristics of tumor cells in lymph nodes play important roles in predicting the tumor progression of IDCs of the breast in patients treated with neoadjuvant chemotherapy. (*Cancer Sci* 2009; 100: 1823–1833)

Traditionally, neoadjuvant chemotherapy has been used for the treatment of locally advanced or inoperable breast cancer.[1,2] More recently, neoadjuvant chemotherapy has been used for the treatment of patients with smaller tumors that would have previously been considered operable at the patient's initial presentation.[3] The purpose of neoadjuvant chemotherapy is to reduce the size of the primary tumor in the breast, so as to facilitate breast conservation surgery, and also to abolish or reduce the disease burden associated with micro-metastatic disease with the intention of prolonging the patient's overall survival.

Gene or protein expression profiles have recently been reported to be significant predictors of the outcome of patients receiving neoadjuvant chemotherapy.[4–6] However, identifying histological predictors of prognosis is very important because histopathological examinations of invasive ductal carcinomas (IDCs) can be routinely performed at any hospital and also are a very useful method for following IDC patients who received neoadjuvant chemotherapy clinically. Clinicopathological factors including age, residual invasive tumor size, histologic grade of the primary invasive tumors, axillary node status, and pathological response have been reported to be good predictors of prognosis among patients with IDC who have received neoadjuvant chemotherapy,[7–10] and we recently demonstrated that a grading system for lymph vessel tumor emboli (LVTEs) and the histological characteristics of tumor cells in lymph nodes are very important histological predictors of prognosis among IDC patients who did not receive neoadjuvant therapy.[11,12] These findings strongly suggest that a grading system for LVTEs or the histological characteristics of tumor cells in lymph node might also be very important histological predictors of prognosis among IDC patients who received neoadjuvant chemotherapy.

The purpose of this study was to investigate the histological characteristics of primary invasive tumors, the grading system for LVTEs, and the histological characteristics of nodal metastatic tumors that were significantly associated with the outcomes of IDC patients who received neoadjuvant chemotherapy. We found that the presence of fibrotic foci (FFs), as assessed using biopsy materials obtained before neoadjuvant chemotherapy; the grading system for LVTEs, as assessed using surgical specimens obtained after neoadjuvant chemotherapy; and several histological characteristics of tumor cells in lymph nodes, as assessed using surgical specimens obtained after neoadjuvant chemotherapy, had significant effects on outcome among IDC patients who received neoadjuvant chemotherapy.

Materials and Methods

Patients. The subjects of this study comprised 115 consecutive patients with IDC of the breast who had been surgically treated at the National Cancer Center Hospital between January 1997 and December 2002. The IDCs were diagnosed preoperatively by aspiration cytology, mammography, or ultrasonography. Clinical information was obtained from the patients' medical records after a complete histological examination of all the IDCs. All the patients were Japanese women, ranging in age from 30 to 71 years (median, 50 years). All the patients had a solitary lesion; 49 patients were premenopausal, and 57 were postmenopausal. A partial mastectomy had been performed in 14 patients, and a

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Table 1. Criteria used in the grading systems for lymph vessel tumor emboli in invasive ductal carcinoma (IDC)

Grading system for lymph vessel tumor emboli according to the number of mitotic and apoptotic figures in tumor cells of lymph vessel tumor emboli		
Grade 0	IDCs with no lymph vessel tumor emboli	
Grades 1, 2, and 3	IDCs with one or more lymph vessel tumor emboli	
	No. of mitotic figures	No. of apoptotic figures
Grade 1	Low-proliferative type	
1a	0	0
1b	0	>0
1c	>0	0
Grade 2	Intermediate-proliferative type	
2a	1 to 4	>0
2b	>0	1 to 6
Grade 3	High-proliferative type	
3a	>4	>6

modified radical mastectomy had been performed in 101. A level I and II axillary lymph node dissection had been performed in all the patients, and a level III axillary lymph node dissection had been performed in some of the IDC patients.

Of the 115 patients, 17 (12%) had only residual ductal carcinoma *in situ*, while 98 (88%) patients had residual IDC; none of the patients exhibited a pathological complete response (no tumor) to neoadjuvant chemotherapy. All the neoadjuvant chemotherapy regimens were anthracycline-based with or without taxane. No cases of inflammatory breast cancer were encountered in this series. All the tumors were classified according to the pathological International Union Against Cancer (UICC)-TNM (pTNM) classification.[13]

For the pathological examination, biopsy specimens obtained before neoadjuvant chemotherapy and surgically resected specimens obtained after neoadjuvant chemotherapy were fixed in 10% formalin and subsequently examined. The size and gross appearance of the surgically resected tumor specimens were recorded as the residual invasive tumor size. The tumor size of the surgically resected specimens was confirmed by comparison with the tumor size on histological slides; if more than one invasive focus was present, the size of the largest invasive focus was recorded as the residual invasive tumor size in this study.

Histological examination. Serial sections of the biopsy materials obtained before neoadjuvant chemotherapy, and serial sections of the tumor area in the surgically resected materials obtained after neoadjuvant chemotherapy were cut from paraffin blocks. One section of each biopsy or surgical specimen was stained with hematoxylin and eosin (H&E) and examined histologically to confirm the diagnosis, and another section was subjected to immunohistochemistry. The following eight histological features of the primary invasive tumors were evaluated in the biopsy materials obtained before neoadjuvant chemotherapy and the surgical materials obtained after neoadjuvant chemotherapy: (1) clinical invasive tumor size or residual invasive tumor size (≤20, >20 to ≤0, >50 mm); (2) histologic grade (1, 2, 3);[14] (3) tumor necrosis (absent, present);[15] (4) FF (absent, present) (Fig. 1a,b);[16,17] (5) blood vessel invasion (absent, present); (6) adipose tissue invasion (absent, present); and (7) skin invasion (absent, present). We also evaluated a grading system for LVTEs, as assessed using biopsy materials obtained before neoadjuvant chemotherapy and surgical materials obtained after neoadjuvant chemotherapy (Table 1, Fig. 1c,d).[11] Briefly, the number of tumor cell mitotic figures and the number of apoptotic figures in the lymph vessels were counted in 20 high-power fields of the surgical materials. In practice, for the surgical materials, we first examined all the slides of the IDCs containing both tumor areas and non-tumor areas to identify the LVTEs. Next, we selected the LVTEs, e.g. large LVTEs located far from the stroma–invasive tumor margin, and recorded the number of mitotic figures and the number of apoptotic figures in the tumor cells composing the LVTEs of the IDC. The mitotic and apoptotic figures were counted under a high-power field, and the largest number of mitotic figures and/or the largest number of apoptotic figures were recorded as the number of mitotic figures and apoptotic figures in the LVTEs of the IDC, respectively. The cumulative numbers of tumor cell mitotic figures and apoptotic figures in the LVTEs in all 20 high-power fields were not used. In IDCs containing a small number of LVTEs, the mitotic figures and apoptotic figures were counted in less than 20 high-power fields. For the biopsy materials, we examined the presence or absence of LVTE or LVTEs; when LVTE or LVTEs were observed in the biopsy material, an assessment similar to that described above was performed. We also evaluated the prognostic predictive power of the location of lymph vessel invasion,[18] the Fisher's neoadjuvant-chemotherapy-effect classification,[19,20] and the Japanese Breast Cancer Society (JBCS) neoadjuvant-chemotherapy-effect classification for surgical materials obtained after neoadjuvant chemotherapy.[21] Cases with non-invasive ductal carcinoma (NIDC) after neoadjuvant chemotherapy were classified as belonging to grade 3 of the JBCS neoadjuvant-chemotherapy effect classification.[21] None of the IDC cases exhibited the disappearance of all the tumor cells (invasive tumor cells and non-invasive tumor cells) after neoadjuvant chemotherapy in this series.

The following histological features of metastatic tumors in lymph nodes dissected at the time of surgery (after neoadjuvant chemotherapy) were examined:[12] (1) the maximum dimension of nodal metastatic tumors; (2) lymph nodes with extra-nodal invasion (absent, present); (3) extra-nodal blood vessel tumor emboli (absent, present); (4) number of mitotic figures in tumors in the lymph node (≤5, >5); (5) histologic grade of tumors in the lymph node (1, 2, 3); and (6) grade of stromal fibrosis of tumors in the lymph node (none, mild, moderate, severe) (Fig. 1e,f). Extra-nodal invasion was defined as the extension of tumor cells through the capsule of at least one lymph node into the perinodal adipose tissue. Nuclear atypia, structural atypia, and the number of mitotic figures were evaluated in the same manner as for the primary invasive tumors. The presence of metastases in the lymph nodes was evaluated using single sections of each node or half of each node stained with H&E.

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

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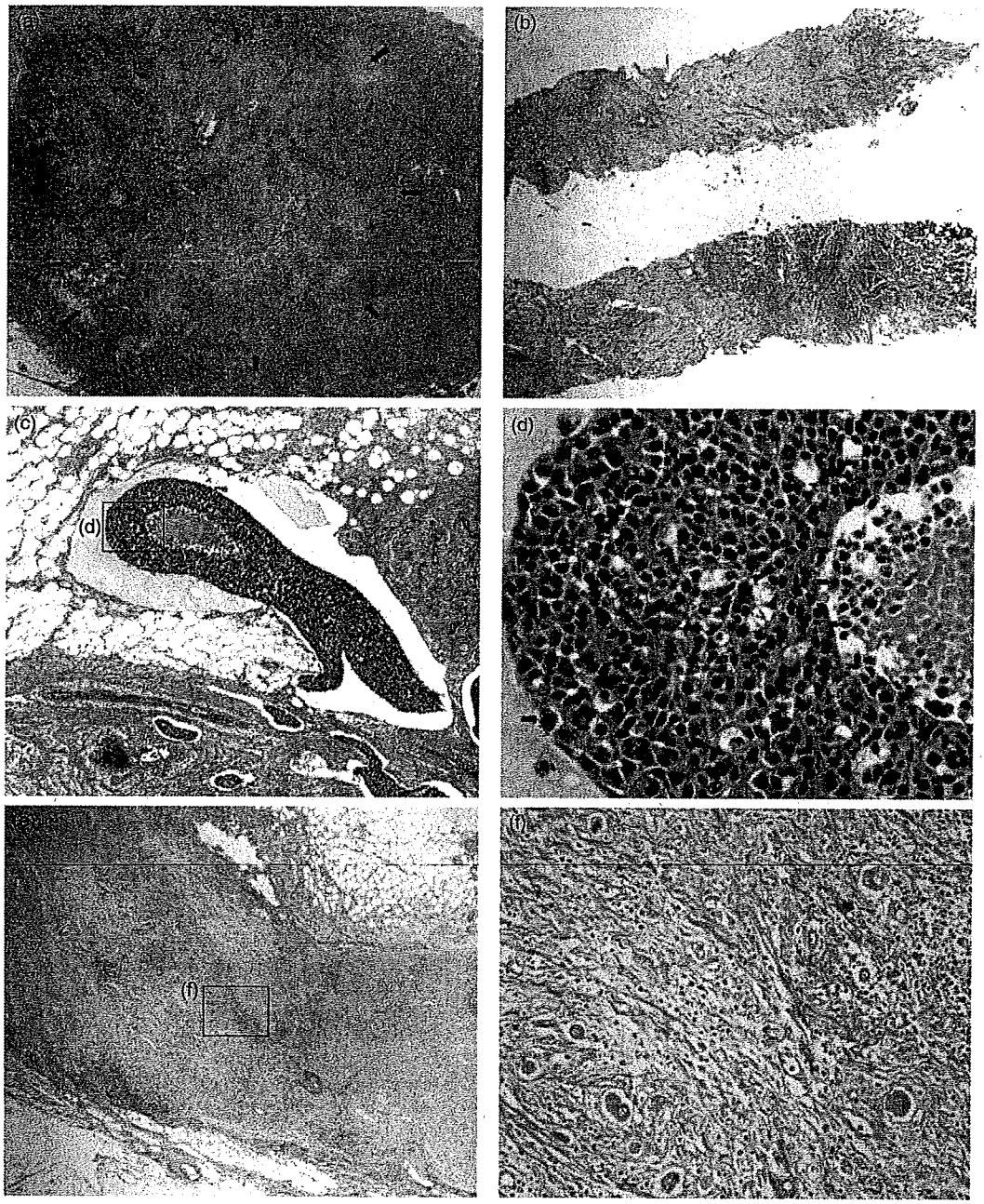

Fig. 1. Histological characteristics of fibrotic foci (FFs), lymph vessel tumor emboli, and nodal metastatic tumors. (a) An FF measuring 8.4 × 6.2 mm is visible within the tumor (arrows) in a surgical specimen. The FF has the appearance of a scar-like feature, and it is surrounded by invasive ductal carcinoma cells. The FF area consists of fibroblasts and collagen fibers arranged in a storiform pattern with tumor cell nests. (b) A core-needle biopsy specimen shows an FF consisting of fibroblasts and collagen fibers in a storiform arrangement intermingled with invasive tumor cells (fibrosis grade 3). (c) One large lymph vessel tumor embolus and five lymph vessel tumor emboli are shown. A necrotic tumor focus is visible in the large lymph vessel tumor embolus. (d) Several apoptotic bodies and apoptotic tumor cells are visible (arrowheads), and six mitotic tumor cells (arrows) can be seen in the lymph vessel tumor embolus. The apoptotic bodies are small, variously shaped pyknotic bodies that resemble sesame seeds, and the apoptotic tumor cells were identified as tumor cells containing eosinophilic or amphophilic cytoplasm and irregularly shaped pyknotic nuclei. (e) Metastatic tumor in the lymph node exhibiting dense stromal fibrosis. (f) Tumor cells with light eosinophilic cytoplasm and irregularly shaped nuclei exhibiting scattered growth in dense fibrous stroma of a metastatic tumor in a lymph node.

anti-ER mouse monoclonal antibody (mAb), ER88 (BioGenex); an anti-PR mAb, PR88 (BioGenex); and an anti-HER2 mAb, CB11 (BioGnex). ER88, PR88, and CB11 were already diluted. After immunostaining, the sections were counterstained with hematoxylin. Sections of IDCs positive for ER, PR, and HER2 were used each time as a positive control. As a negative control, the primary antibody was replaced with normal mouse immunoglobin. An IDC with nuclear staining for ER or PR in 10% or more of its tumor cells was assessed as ER-positive or PR-positive. The HER2 status of the tumor cells was semi-quantitatively scored on a 0 to 3 scale according to the level of HER2 protein expression.[22]

One author (N.T.) assessed all the characteristics of the primary tumors, the tumors in the lymph vessels, and the nodal

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Factors	Cases	Number of patients (%)			
		Tumor recurrence		Tumor-related death	
	115	Present (n = 32)	P-values	Present (n = 16)	P-values
Clinical invasive tumor size (mm)					
≤20	0		0.004		0.088
>20 to ≤50	72	14 (19)		8 (11)	
>50	43	18 (42)		8 (19)	
Histologic grade of primary invasive tumor					
1	25	8 (32)	0.352	2 (8)	0.890
2	70	21 (30)		13 (19)	
3	20	3 (15)		1 (5)	
Fibrotic focus					
Absent	96	25 (26)	0.285	10 (11)	0.010
Present	19	7 (37)		6 (32)	
Tumor necrosis					
Absent	80	23 (29)	0.769	10 (13)	0.499
Present	35	9 (26)		6 (17)	
Grading system for lymph vessel tumor emboli					
Grade 0	106	28 (26)	0.079	12 (11)	0.009
Grade 1	5	1 (20)		1 (20)	
Grade 2	4	3 (75)		3 (75)	
Grade 3	0				
ER and PR status (n = 106)					
Negative	42	12 (29)	0.707	7 (17)	0.590
Positive	64	17 (27)		7 (11)	
HER2 status (n = 109)					
0 to 2	82	21 (26)	0.422	8 (10)	0.155
3	27	9 (33)		7 (26)	

ER and PR status negative, ER and PR both negative; ER and/or PR status positive, ER positive or PR positive, or both positive. ER, estrogen receptor; PR, progesterone receptor.

Table 2. Association of clinicopathological factors assessed using biopsy materials obtained before neoadjuvant chemotherapy with tumor recurrence and tumor-related death in all patients with invasive ductal carcinoma who received neoadjuvant chemotherapy

metastatic tumors as well as the immunohistochemical parameters of the biopsy and surgical materials, and another author (T.H.) identified the characteristics of all the IDCs or the immunohistochemical parameters to confirm the tumor cell characteristics in these tumor components and the immunohistochemical characteristics recorded by N.T. Whenever a discrepancy occurred, the authors re-examined the slides to reach a consensus.

Patient outcome and statistical analysis. Survival was evaluated using a median follow-up period of 52.3 months (range, 4.9 to 84.6 months) until February 2007. At that time, 83 of the 115 patients who had received neoadjuvant chemotherapy were alive and well, 32 had developed tumor recurrences, and 16 had died of their disease. The recurrence-free and overall survival periods were determined beginning at the time of surgery. Tumor relapse was considered to have occurred whenever evidence of metastasis was first observed.

We analyzed the outcome predictive power of a grading system for LVTEs assessed using biopsy or surgical materials, the seven histological factors of primary invasive tumors assessed using biopsy or surgical materials, six histological factors of metastatic tumors in lymph nodes assessed using surgical materials, ER and PR expression in primary invasive tumor cells assessed using biopsy or surgical materials, the category of HER2 expression in primary invasive tumor cells using biopsy or surgical materials, the Fisher's classification for neoadjuvant chemotherapy,[19,20] the classification of the JBCS for neoadjuvant chemotherapy,[21] age (≤39 years and >39 years), the UICC-pathological nodal status (UICC pN: no nodal metastasis, N0; 1 to 3 nodal metastases, N1; 4 to 9 nodal metastases, N2; and 10 or more nodal metastases, N3), the UICC-pTNM stage classification[13] for tumor recurrence, and tumor-related death using univariate analyses with the Cox proportional hazard regression model.[23] Factors significantly associated with outcome in the univariate analyses were then entered together into the multivariate analyses using the Cox proportional hazard regression model[23] according to nodal status. The step-down method was applied until all of the remaining factors were significant at a P-value of less than 0.05. Since the following factors were examined using both biopsy materials obtained before neoadjuvant therapy and surgical materials obtained after neoadjuvant chemotherapy, to be able to accurately assess the prognostic value of each of these factors using multivariate analyses, their mutual influence on the outcome was avoided by analyzing the prognostic predictive power of the biopsy materials obtained before neoadjuvant chemotherapy and that of the surgical materials obtained after neoadjuvant chemotherapy separately (model 1, factors examined using biopsy materials; model 2, factors examined using surgical materials): (1) invasive tumor size; (2) histologic grade; (3) FF; (4) tumor necrosis; (5) grading system for LVTEs; (6) blood vessel invasion; (7) ER and PR status; and (8) HER2 status. In IDC patients without nodal metastasis, since tumor recurrence was observed in three patients, and tumor-related death was observed in only two patients, we were unable to perform multivariate analyses for tumor recurrence or tumor-related death. The survival curves were drawn using the Kaplan–Meier method.[24] All analyses were performed with Statistica/Windows software (StatSoft, Tulsa, OK, USA).

Results

Factors significantly associated with tumor recurrence and tumor-related death. The univariate analyses of data for biopsy materials obtained before neoadjuvant chemotherapy showed that the clinical invasive tumor size and skin invasions were significantly associated with tumor recurrence, while the presence of FF (Fig. 2a) and the grading system for LVTEs were significantly associated with tumor-related death (Table 2). None of the

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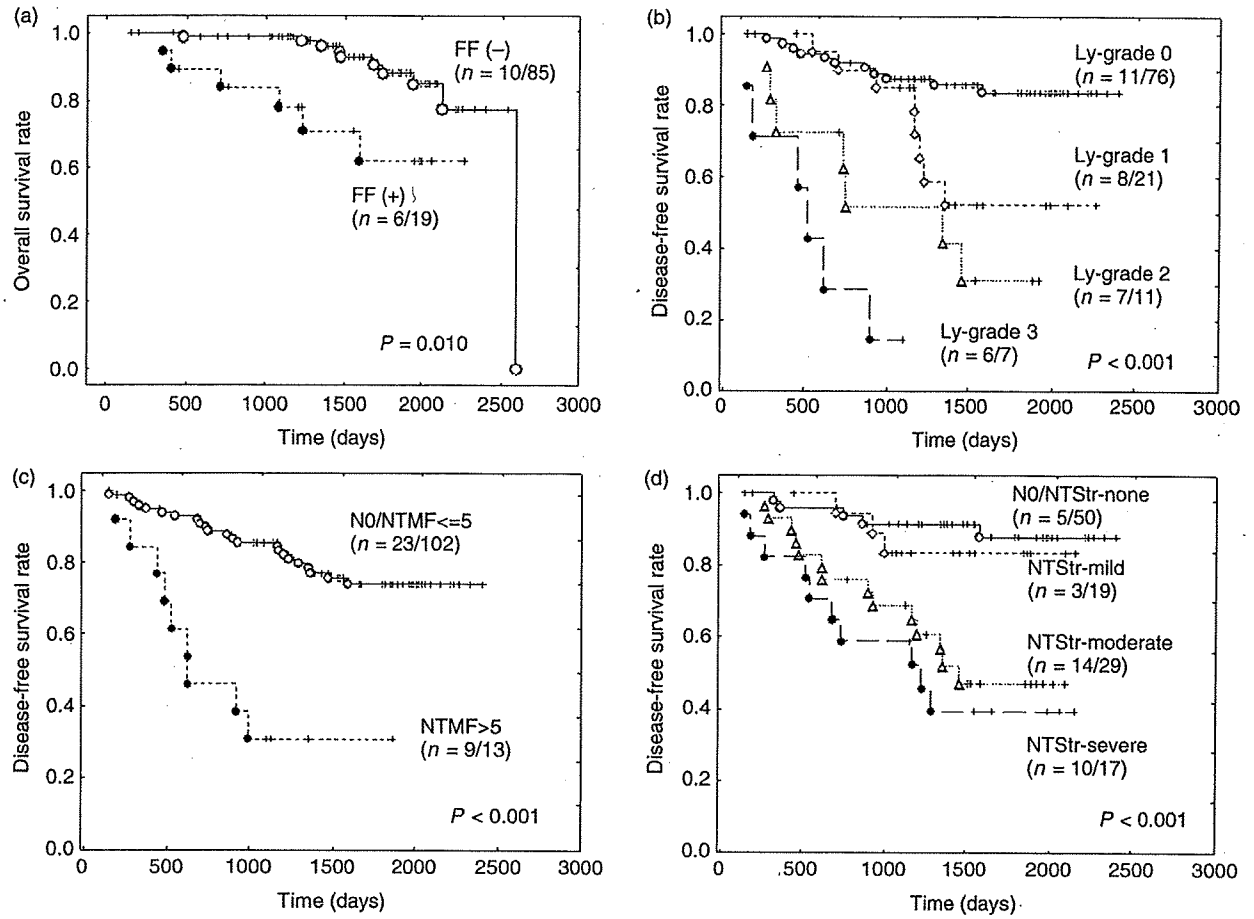

Fig. 2. (a–d) Overall survival curves and disease-free survival curves of invasive ductal carcinoma (IDC) patients who received neoadjuvant chemotherapy. (a) Patients with IDCs exhibiting fibrotic foci (FFs) assessed using biopsy specimens obtained before neoadjuvant chemotherapy have a significantly shorter overall survival period than patients with IDCs that do not exhibit FFs, as assessed using biopsy specimens obtained before neoadjuvant chemotherapy. (b) The disease-free survival of IDC patients classified according to a grading system for lymph vessel tumor emboli assessed using surgical materials obtained after neoadjuvant chemotherapy decreases significantly according to the grade. Ly, lymph vessel tumor embolus or emboli. (c) The disease-free survival of IDC patients with nodal metastatic tumors containing five or more mitotic figures is significantly shorter than that of IDC patients without nodal metastasis or those with nodal metastatic tumors containing less than five mitotic figures. N0, no nodal metastasis; NTMF, mitotic figures in nodal metastatic tumor. (d) The disease-free survival of IDC patients classified according to the tumor stroma of nodal metastatic tumors decreases significantly according to the degree of fibrosis in the nodal metastatic tumors. NTStr, nodal metastatic tumor stroma.

biopsy materials obtained before neoadjuvant chemotherapy exhibited blood vessel invasion.

The univariate analyses of data for surgical materials obtained after neoadjuvant chemotherapy showed that skin invasion, the histologic grade of the primary invasive tumors, tumor necrosis, the grading system for LVTEs (Fig. 2b), the UICC pN category, nodal metastatic tumor stroma (Fig. 2d), five or more mitotic figures in nodal metastatic tumors (Fig. 2c), the histologic grade of the nodal metastatic tumors, the presence of a node with extranodal blood vessel tumor emboli, the presence of a node with extranodal invasion, and the UICC pTNM stage classification were significantly associated with tumor recurrence and tumor-related death (Table 3). Residual invasive tumor size, the presence of lymph vessel tumor emboli in the advanced area of primary invasive tumors, and the presence of lymph vessel tumor emboli in the non-tumor areas of primary invasive tumors were significantly associated with tumor recurrence but not tumor-related death, while the other factors were not significantly associated with tumor recurrence or tumor-related death in the univariate analyses (Table 3).

Overall, five or more mitotic figures in nodal metastatic tumors, and nodes with extranodal invasion were significantly associated with elevated hazard rates (HRs) for tumor recurrence and tumor-related death (Table 4, model 1). Clinical invasive tumor size, the presence of tumor necrosis (assessed using surgical materials), and severe nodal metastatic tumor stroma were significantly associated with elevated HRs for tumor recurrence (Table 4, model 1). Grade 2 LVTEs (assessed using biopsy materials) and the presence of FF (assessed using biopsy materials) were significantly associated with elevated HRs for tumor-related death in the multivariate analyses (Table 4, model 1). In model 2, the grading system for LVTEs (assessed using surgical materials), five or more mitotic figures in nodal metastatic tumors, moderate to severe stroma in nodal metastatic tumors, and the presence of tumor necrosis (assessed using surgical materials) were significantly associated with elevated HRs for tumor recurrence; among these factors, grade 2 LVTEs (assessed using surgical materials), five or more mitotic figures in nodal metastatic tumors, and severe stroma in nodal metastatic tumors were also significantly associated with elevated HRs for tumor-related death in the multivariate analyses (Table 4).

In patients with nodal metastasis, five or more mitotic figures in the nodal metastatic tumors was significantly associated with elevated HRs for tumor recurrence and tumor-related death,

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Table 3. Association of clinicopathological factors using surgical materials obtained after neoadjuvant therapy with tumor recurrence and tumor-related death in all patients with invasive ductal carcinoma (IDC) who received neoadjuvant chemotherapy

Factors	Cases	Number of patients (%)			
		Tumor recurrence		Tumor-related death	
	115	Present (n = 32)	P-values	Present (n = 16)	P-values
Age, years					
≤39	20	4 (20)	0.332	2 (10)	0.622
>39	95	28 (30)		14 (15)	
Adjuvant therapy					
No	10	1 (10)	0.234	0	0.195
Yes	105	31 (30)		16 (15)	
Fisher's classification					
NIDC cases	17	2 (12)	0.095	1 (6)	0.162
IDC cases	98	30 (31)		15 (15)	
Grade classification of neoadjuvant chemotherapy according to the Japan Breast Cancer Society classification					
Grade 0	3	0	0.364	0	0.368
Grade 1a	48	14 (29)		6 (13)	
Grade 1b	31	10 (32)		6 (19)	
Grade 2	14	4 (29)		3 (21)	
Grade 3	17	2 (12)		1 (6)	
Residual invasive tumor size (mm)					
NIDC cases	17	2 (12)	0.014	1 (6)	0.163
≤20	33	9 (27)		7 (21)	
>20 to ≤50	45	11 (24)		4 (9)	
>50	20	10 (50)		4 (20)	
Skin invasion					
Absent	86	20 (24)	0.030	8 (9)	0.006
Present	29	12 (41)		8 (28)	
Histologic grade of primary invasive tumor					
NIDC cases	17	2 (12)	0.012	1 (6)	0.003
1	27	5 (19)		0	
2	48	17 (35)		11 (23)	
3	23	8 (35)		4 (17)	
Fibrotic focus					
Absent	88	23 (26)	0.417	12 (14)	0.687
Present	27	9 (33)		4 (15)	
Tumor necrosis					
Absent	88	20 (23)	0.010	10 (11)	0.038
Present	27	12 (44)		6 (22)	
Grading system for lymph vessel tumor emboli					
Grade 0	76	11 (14)	<0.001	7 (9)	<0.001
Grade 1	21	8 (38)		2 (10)	
Grade 2	11	7 (64)		5 (45)	
Grade 3	7	6 (86)		2 (30)	
Lymph vessel tumor emboli in the advance area					
Absent	91	20 (22)	0.003	10 (11)	0.328
Present	24	12 (50)		6 (30)	
Lymph vessel tumor emboli in the non-tumor stroma area					
Absent	88	18 (20)	<0.001	10 (11)	0.051
Present	27	14 (52)		6 (22)	
Blood vessel invasion					
Absent	6	1 (17)	0.510	1 (17)	0.757
Present	109	31 (28)		15 (14)	
UICC pN category					
N0	41	3 (7)	<0.001	2 (5)	0.032
N1	39	11 (28)		6 (15)	
N2	24	10 (42)		6 (25)	
N3	11	8 (73)		2 (18)	
Nodal metastatic tumor stroma					
N0/none	50	5 (10)	<0.001	4 (8)	0.009
Mild	19	3 (16)		1 (5)	
Moderate	29	14 (48)		6 (21)	
Severe	17	10 (59)		5 (29)	
Number of mitotic figures in nodal metastatic tumor (/1-high power field)					
N0/≤5	102	23 (23)	<0.001	12 (12)	0.002
>5	13	9 (69)		4 (31)	

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Table 3. *Continued*

Factors	Cases	Number of patients (%)			
		Tumor recurrence		Tumor-related death	
	115	Present (n = 32)	P-values	Present (n = 16)	P-values
Histologic grade of nodal metastatic tumor					
N0	41	3 (7)	<0.001	2 (5)	0.022
1	10	5 (50)		1 (10)	
2	45	15 (33)		10 (22)	
3	19	9 (47)		3 (16)	
Nodes with extranodal blood vessel invasion					
N0	41	3 (7)	<0.001	2 (5)	0.004
Absent	45	13 (29)		5 (11)	
Present	29	16 (55)		9 (31)	
Nodes with extranodal invasion					
N0	41	3 (7)	<0.001	2 (5)	0.039
Absent	30	8 (27)		6 (20)	
Present	44	21 (48)		8 (18)	
UICC pTNM stage classification					
0	15	0	<0.001	0	0.014
I	1	0		0	
IIA	19	5 (26)		3 (16)	
IIB	30	4 (13)		3 (10)	
IIIA	23	9 (39)		4 (17)	
IIIB	16	6 (38)		4 (25)	
IIIC	11	8 (73)		2 (18)	
ER and PR status (n = 107)					
Negative	42	12 (29)	0.667	7 (17)	0.549
Positive	65	17 (26)		7 (11)	
HER2 status (n = 101)					
0 to 2	84	24 (29)	0.923	10 (11)	0.087
3	17	6 (35)		5 (29)	

ER and PR status negative, ER and PR both negative; ER and PR status positive, ER positive or PR positive, or both positive. N0, no nodal metastasis; N1, one to three nodal metastases; N2, four to nine nodal metastases; N3, 10 or more nodal metastases. ER, estrogen receptor; NIDC, non-invasive ductal carcinoma; pN, pathological regional lymph node; PR, progesterone receptor; UICC, International Union Against Cancer.

while clinical invasive tumor size and the UICC pN3 category were significantly associated with elevated HRs for tumor recurrence (Table 5, model 1). The presence of FF (assessed using biopsy materials) and the presence of nodes with extranodal invasion were significantly associated with elevated HRs for tumor-related death in the multivariate analyses (Table 5, model 1). In model 2, the grading system for LVTEs (assessed using surgical materials), severe stroma in nodal metastatic tumors, and the presence of tumor necrosis (assessed using surgical materials) were significantly associated with elevated HRs for tumor recurrence in the multivariate analysis (Table 5). Grade 2 LVTEs (assessed using surgical materials) and five or more mitotic figures in nodal metastatic tumors were significantly associated with elevated HRs for tumor-related death in the multivariate analysis (Table 5).

Discussion

The results of this study clearly showed that a grading system for LVTEs (assessed using surgical materials) can be used to classify IDC patients with lymph vessel invasion who received neoadjuvant chemotherapy into low-risk, intermediate-risk, and high-risk groups; furthermore, this grading system for LVTEs was significantly associated with the HRs for tumor recurrence and tumor-related death in patients with IDC both overall and in patients with nodal metastasis, and the outcome predictive power of the grading system for LVTEs assessed using surgical materials was superior to that of the grading system for LVTE assessed using biopsy materials obtained before neoadjuvant chemotherapy. Although there have been many studies showing the prognostic usefulness of the presence of lymphatic invasion,[25–27] we previously demonstrated that the biological histological characteristics, especially mitotic figures and/or apoptotic figures, of tumor cells in lymph vessels are a more significant outcome predictor than the presence or absence of lymph vessel invasion or the number of lymph vessels that have been invaded.[28] We have also demonstrated that the location of lymph vessel invasion is an important outcome predictor for IDC patients,[18] but the result of this study clearly demonstrated that the grading system for LVTEs assessed using surgical materials is significantly superior to the location of lymph vessel invasion for accurately predicting the outcomes of IDC patients who have received neoadjuvant chemotherapy. Thus, this grading system for LVTEs assessed using surgical materials, but not biopsy materials, appears to be an excellent histological system for accurately predicting the outcome of IDC patients who do or do not receive neoadjuvant chemotherapy. Although we could not examine the outcome predictive power of the grading system for LVTEs in IDC patients without nodal metastasis in this study, we previously reported that this grading system for LVTEs assessed using surgical materials was a very important histological predictor of the prognosis of patients with IDC who did not receive neoadjuvant therapy independent of their nodal status.[11] Thus, the grading system for LVTE might be an important outcome predictor for IDC patients who have received neoadjuvant chemotherapy and do not have nodal metastasis, although the outcome predictive power of the grading system for LVTEs should be investigated in this patient

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Table 4. Multivariate analyses for tumor recurrence and tumor-related death in all patients with invasive ductal carcinoma (IDC) who received neoadjuvant chemotherapy

Factors	Tumor recurrence			Tumor-related death		
	HRs	95% CI	*P*-values	HRs	95% CI	*P*-values
Model 1						
Clinical invasive tumor size (mm) before neoadjuvant chemotherapy						
>20 to ≤50	Referent			Referent		
>50	2.2	1.1–4.4	0.034	–	–	
Grading system for lymph vessel tumor emboli assessed using biopsy materials obtained before neoadjuvant chemotherapy						
Grade 0	Referent			Referent		
Grade 1	–	–		0.7	0.03–14.2	0.796
Grade 2	–	–		5.9	1.3–27.9	0.025
Fibrotic focus assessed using biopsy materials obtained before neoadjuvant chemotherapy						
Absent	Referent			Referent		
Present	–	–		6.2	1.9–19.6	0.002
Tumor necrosis assessed using surgical materials obtained after neoadjuvant chemotherapy						
Absent	Referent			Referent		
Present	2.9	1.1–8.0	0.034	1.2	0.2–9.1	0.868
No. of mitotic figures in nodal metastatic tumors assessed using surgical materials obtained after neoadjuvant chemotherapy						
N0 and ≤5	Referent			Referent		
>5	4.5	1.7–11.9	0.003	7.5	1.7–31.5	0.006
Nodes with extranodal invasion assessed using surgical materials obtained after neoadjuvant chemotherapy						
N0 and absent	Referent			Referent		
Present	4.8	2.3–10.6	<0.001	5.0	1.7–14.7	0.003
Nodal metastatic tumor stroma assessed using surgical materials obtained after neoadjuvant chemotherapy						
N0 and none	Referent			Referent		
Mild	0.7	0.1–5.3	0.719	1.1	0.04–28.3	0.967
Moderate	1.3	0.2–7.8	0.771	4.5	0.3–76.9	0.302
Severe	3.9	1.6–9.2	0.002	7.6	0.3–183.9	0.214
Model 2						
Grading system for lymph vessel tumor emboli assessed using surgical materials obtained after neoadjuvant chemotherapy						
Grade 0	Referent			Referent		
Grade 1	3.2	1.2–8.6	0.020	0.2	0.01–3.8	0.302
Grade 2	9.5	3.3–27.3	<0.001	5.9	1.9–18.8	0.002
Grade 3	5.5	1.7–17.4	0.004	5.3	0.5–61.6	0.183
Tumor necrosis assessed using surgical materials obtained after neoadjuvant chemotherapy						
Absent	Referent			Referent		
Present	3.1	1.1–8.8	0.038	2.4	0.8–13.3	0.300
No. of mitotic figures in nodal metastatic tumors assessed using surgical materials obtained after neoadjuvant chemotherapy						
N0 and ≤5	Referent			Referent		
>5	3.7	1.2–11.7	0.027	12.6	3.2–48.5	<0.001
Nodal metastatic tumor stroma assessed using surgical materials obtained after neoadjuvant chemotherapy						
N0 and none	Referent			Referent		
Mild	0.4	0.05–3.1	0.366	0.2	0.01–6.9	0.395
Moderate	2.9	1.2–7.1	0.017	1.3	0.1–17.9	0.856
Severe	10.0	3.3–20.9	<0.001	3.5	1.1–10.8	0.034

–/–, not significant in univariate analysis; CI, confidence interval; HR, hazard rate; N0, no nodal metastasis.

population. Since the presently described grading system for LVTEs is based on assessments of mitotic figures and apoptotic figures in tumor cells located in lymph vessels, tumor cells with a high turnover rate in lymph vessels are more likely to be capable of spreading tumor nests throughout the lymph vessels than tumor cells with a low turnover rate. Thus, factors that accelerate the turnover rate of tumor cells in lymph vessels are probably very important for explaining the significant outcome of the predictive power of this grading system for LVTEs.

The histological characteristics of the nodal metastatic tumors were also significantly associated with tumor recurrence or tumor-related death in the patients with IDCs who received neoadjuvant chemotherapy in the current study. Among these histological characteristics, the degree of nodal tumor stroma and the number of mitotic figures in the nodal metastatic tumors were the most accurate predictors of outcome among the IDC patients who received neoadjuvant chemotherapy. We previously reported that severe tumor stroma and the number of mitotic figures in nodal metastatic tumors are significant predictors of outcome among IDC patients with nodal metastasis who did not receive neoadjuvant chemotherapy.[12,29] Thus, this study clearly confirmed that these two factors are also significant histological predictors of outcome among IDC patients with nodal metastasis who received neoadjuvant chemotherapy. We previously reported that the proliferative activity of tumor–stromal fibroblasts plays a very important role in nodal metastasis and distant organ metastasis by IDCs,[30,31] and that growth factors produced by tumor cells and tumor stromal cells play a very important role in tumor progression by IDC.[32] These findings strongly suggest that the tumor stroma plays a significant role in tumor progression in IDC. Furthermore, the gene expression profile and the protein expression profile of the tumor stroma have recently

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Table 5. Multivariate analyses for tumor recurrence and tumor-related death in lymph node–metastasis-positive invasive ductal carcinoma (IDC) patients who received neoadjuvant chemotherapy

Factors	Cases	Number of patients (%)			
		Tumor recurrence		Tumor-related death	
	74	Present (n = 29)	HRs/95% CI P-values	Present (n = 14)	HRs/95% CI P-values
Model 1					
Clinical invasive tumor size (mm) before neoadjuvant chemotherapy					
>20 to ≤50	41	11 (27)	Referent	6 (15)	Referent
>50	33	18 (54)	2.7/1.2–5.7 0.013	8 (24)	–/–
Fibrotic focus assessed using biopsy materials obtained before neoadjuvant chemotherapy					
Absent	60	22 (37)	Referent	8 (13)	Referent
Present	13	7 (54)	–/–	6 (46)	7.0/2.2–22.3 <0.001
UICC pN category					
N1	39	11 (28)	Referent	6 (15)	Referent
N2	24	10 (42)	2.3/0.6–8.0 0.211	6 (25)	–/–
N3	11	8 (73)	3.4/1.4–8.1 0.005	2 (18)	–/–
No. of mitotic figures in nodal metastatic tumors assessed using surgical materials obtained after neoadjuvant therapy					
≤5	61	20 (33)	Referent	10 (16)	Referent
>5	13	9 (69)	3.9/1.6–9.1 0.002	4 (31)	8.6/2.0–37.0 0.004
Nodes with extranodal invasion assessed using surgical materials obtained after neoadjuvant chemotherapy					
Absent	45	13 (29)	Referent	5 (11)	Referent
Present	29	16 (55)	2.4/0.7–7.7 0.143	9 (31)	5.3/1.5–18.3 0.007
Model 2					
Grading system for lymph vessel tumor emboli assessed using surgical materials obtained after neoadjuvant chemotherapy					
Grade 0	39	9 (23)	Referent	6 (15)	Referent
Grade 1	19	8 (42)	2.7/1.0–7.4 0.047	2 (11)	1.2/0.7–7.0 0.872
Grade 2	9	6 (67)	8.5/2.6–27.8 <0.001	4 (44)	3.9/1.1–13.7 0.035
Grade 3	7	6 (86)	8.0/2.5–26.0 <0.001	2 (29)	3.4/0.6–19.2 0.172
Nodal metastatic tumor stroma assessed using surgical materials obtained after neoadjuvant therapy					
N0/none	9	2 (22)	Referent	2 (22)	Referent
Mild	19	3 (16)	0.8/0.1–6.9 0.826	1 (5)	–/–
Moderate	29	14 (48)	3.7/0.6–23.9 0.168	6 (21)	–/–
Severe	17	10 (59)	5.3/2.0–14.2 <0.001	5 (29)	–/–
Tumor necrosis assessed using surgical materials obtained after neoadjuvant chemotherapy					
Absent	54	18 (33)	Referent	9 (17)	Referent
Present	20	11 (55)	5.3/1.7–16.4 0.004	5 (25)	–/–
No. of mitotic figures in nodal metastatic tumors assessed using surgical materials obtained after neoadjuvant chemotherapy					
≤5	61	20 (33)	Referent	10 (16)	Referent
>5	13	9 (69)	2.0/0.4–9.5 0.376	4 (31)	6.1/1.6–22.9 0.008

–/–, not significant in univariate analysis; CI, confidence interval; HR, hazard rate; NIDC, non-invasive ductal carcinoma; pN, pathological regional lymph node; UICC, International Union Against Cancer.

Model 1

Tumor recurrence: adjusted for clinical invasive tumor size before neoadjuvant chemotherapy, tumor necrosis assessed using surgical materials obtained after neoadjuvant chemotherapy, nodal metastatic tumor stroma assessed using surgical materials obtained after neoadjuvant chemotherapy, no. of mitotic figures in nodal metastatic tumors assessed using surgical materials obtained after neoadjuvant chemotherapy, UICC pTNM-pN category assessed using surgical materials obtained after neoadjuvant chemotherapy, nodes with extranodal invasion assessed using surgical materials obtained after neoadjuvant chemotherapy, and histologic grade of primary invasive tumors assessed using surgical materials obtained after neoadjuvant chemotherapy.

Tumor-related death: adjusted for fibrotic focus assessed using biopsy materials obtained before neoadjuvant chemotherapy, no. of mitotic figures in nodal metastatic tumors assessed using surgical materials obtained after neoadjuvant chemotherapy, and nodes with extranodal blood vessel invasion assessed using surgical materials obtained after neoadjuvant chemotherapy.

Model 2

Tumor recurrence: adjusted for grading system for lymph vessel tumor emboli assessed using surgical materials obtained after neoadjuvant chemotherapy, tumor necrosis assessed using surgical materials obtained after neoadjuvant chemotherapy, nodal metastatic tumor stroma assessed using surgical materials obtained after neoadjuvant chemotherapy, no. of mitotic figures in nodal metastatic tumors assessed using surgical materials obtained after neoadjuvant chemotherapy, UICC pTNM-pN category assessed using surgical materials obtained after neoadjuvant chemotherapy, nodes with extranodal invasion assessed using surgical materials obtained after neoadjuvant chemotherapy, and histologic grade of nodal metastatic tumors assessed using surgical materials obtained after neoadjuvant chemotherapy.

Tumor-related death: adjusted for grading system for lymph vessel tumor emboli assessed using surgical materials obtained after neoadjuvant chemotherapy, no. of mitotic figures in nodal metastatic tumors assessed using surgical materials obtained after neoadjuvant chemotherapy, and nodes with extranodal blood vessel invasion assessed using surgical materials obtained after neoadjuvant chemotherapy.

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been reported to play a very important roles in tumor progression in carcinoma,[33–35] and the interaction between tumor cells and stromal cells also plays a very important role in tumor progression in carcinoma.[36–38] Thus, tumor cell–stromal cell interactions probably heighten the malignant potential of nodal metastatic tumors with moderate to severe tumor stroma. Furthermore, in previous studies we and others have reported that a characteristic histological feature of the tumor stroma in primary invasive tumors, an FF, is a very useful prognostic histological tumor–stromal indicator for accurately predicting the outcome of IDC patients who did not receive neoadjuvant therapy;[16,17,39,40] the present study clearly demonstrated that the presence of FFs (assessed using biopsy materials obtained before neoadjuvant chemotherapy, but not using surgical materials obtained after neoadjuvant chemotherapy) was a significant tumor-death-related factor. Thus, tumor cell–stromal cell interactions in nodal metastatic tumors as well as in primary invasive tumors probably play very important roles in the progression of IDCs that have been treated with neoadjuvant chemotherapy, and in IDC patients who have received neoadjuvant chemotherapy, the outcome predictive power of FFs should be assessed using biopsy materials obtained before neoadjuvant chemotherapy.

The grading system for LVTEs assessed using surgical materials and the histological features of the nodal metastatic tumors mentioned above were superior to Fisher's classification or the classification of the JBCS for neoadjuvant chemotherapy for predicting the outcome of IDC patients who had received neoadjuvant chemotherapy in this study. The classification of the JBCS for neoadjuvant chemotherapy assesses the degree of fibrosis or the presence or absence of tumor necrosis in primary invasive tumors and tumors metastasizing to the lymph node, and a severe degree of fibrosis and the presence of tumor necrosis are considered as histological findings predicting a good response to neoadjuvant chemotherapy.[21] In the classification of JBCS for neoadjuvant chemotherapy, a complete response (grade 3) is regarded as necrosis or the disappearance of all tumor cells, with all carcinoma cells being replaced by granuloma-like and/or fibrous tissue. However, this study clearly demonstrated that the presence of tumor necrosis in primary invasive tumors and a moderate to severe degree of fibrosis in nodal metastatic tumors were important histological predictors of a poor prognosis among IDC patients who have received neoadjuvant chemotherapy. Therefore, determining whether the presence of tumor necrosis or the presence of tumor–stromal dense fibrosis in IDCs treated with neoadjuvant chemotherapy have truly been produced by neoadjuvant chemotherapy or not is of great importance, and the latter finding strongly suggests that the presence of tumor necrosis or the presence of tumor–stromal dense fibrosis may reflect biological tumor characteristics that are closely associated with a poor outcome among patients with IDCs. The tumor-related predictive ability of the presence of FF assessed using biopsy materials obtained before neoadjuvant chemotherapy was lost when the presence of FF was assessed using surgical materials obtained after neoadjuvant chemotherapy. This strongly suggests that FF-like stromal changes produced by neoadjuvant chemotherapy probably occurred in the IDCs treated with neoadjuvant chemotherapy, and the true FFs could not be differentiated from the FF-like stromal changes in IDCs. Thus, when the presence of tumor necrosis in primary invasive tumors or the presence of moderate to severe fibrosis in nodal metastatic tumors is observed during the pathological examination of IDCs treated with neoadjuvant therapy, the pathological assessment of the response to neoadjuvant chemotherapy should be carefully assessed as to whether the presence of tumor necrosis in primary invasive tumors or moderate to severe fibrosis in nodal metastatic tumors truly demonstrates a response to neoadjuvant chemotherapy. Although the outcome predictive power of FFs among patients with IDC was lost after neoadjuvant chemotherapy, the histological factors maintained their significant outcome predictive power among IDC patients who received neoadjuvant chemotherapy. Thus, pathologists carefully assess the response to neoadjuvant chemotherapy based on the presence of tumor necrosis in primary invasive tumors or the degree of fibrosis in nodal metastatic tumors, since pathologists might misjudge IDC patients who have received neoadjuvant chemotherapy and whose primary invasive tumors exhibited tumor necrosis or whose nodal metastatic tumors exhibited dense fibrosis as having attained a good response to neoadjuvant chemotherapy.

The results of this study clearly demonstrated that many histological factors of tumors assessed using biopsy materials, such as histologic grade and tumor necrosis, failed to show a significant association with tumor recurrence or tumor-related death. These findings strongly suggest that biopsy materials containing small amounts of primary invasive tumors do not accurately reflect the true biological malignant potential of IDCs. Thus, with the exception of evaluating the presence of FF, histological evaluations of the malignant potential of IDCs treated using neoadjuvant chemotherapy should be performed using surgical materials obtained after neoadjuvant chemotherapy.

In conclusion, this is the first study to clearly demonstrate that the presence of FF in biopsy materials obtained before neoadjuvant chemotherapy, the grading system for LVTEs in surgical materials obtained after neoadjuvant chemotherapy, and the histological characteristics of nodal metastatic tumors in surgical materials obtained after neoadjuvant chemotherapy were strongly associated with the outcome of IDC patients who received neoadjuvant chemotherapy. In the future, the following topics should be examined to clarify the tumor progression of IDCs treated with neoadjuvant chemotherapy based on the data in this study: (1) the functions of tumor cells in lymph vessels and nodal metastatic tumor cells should be determined; (2) the factors that accelerate the proliferative activity of tumor cells in lymph vessels or lymph nodes should be identified; and (3) the factors that accelerate tumor cell–stromal cell interactions in nodal metastatic tumors should be discerned.

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References

1 Ragaz J, Baird R, Rebbeck P, Goldie A, Coldman A, Spinelli J. Preoperative adjuvant chemotherapy (neoadjuvant) for carcinoma of the breast: rationale and safety report. *Recent Results Cancer Res* 1985; **98**: 99–105.
2 Ragaz J. Preoperative (neoadjuvant) chemotherapy for breast cancer: outline of the British Columbia Trial. *Recent Results Cancer Res* 1986; **103**: 85–94.
3 Ferriere JP, Assier I, Cure H *et al.* Primary chemotherapy in breast cancer: correlation between tumor response and patient outcome. *Am J Clin Oncol* 1998; **21**: 117–20.
4 Daidone MG, Silvestrini R, Luisi A *et al.* Changes in biological markers after primary chemotherapy for breast cancers. *Int J Cancer* 1995; **61**: 301–5.
5 Cavailles V, Gompel A, Portois MC *et al.* Comparative activity of pulsed or continuous estradiol exposure on gene expression and proliferation of normal and tumoral human breast cells. *J Mol Endocrinol* 2002; **28**: 165–75.
6 Koukourakis MI, Simopoulos C, Polychronidis A *et al.* The effect of trastuzumab/docatexel combination on breast cancer angiogenesis: dichotomus effect predictable by the HIFI alpha/VEGF pre-treatment status? *Anticancer Res* 2003; **23**: 1673–80.
7 Petit T, Borel C, Ghnassia JP *et al.* Chemotherapy response of breast cancer depends on HER-2 status and anthracycline dose intensity in the neoadjuvant setting. *Clin Cancer Res* 2001; **7**: 1577–81.
8 Chollet P, Amat S, Cure H *et al.* Prognostic significance of a complete

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pathological response after induction chemotherapy in operable breast cancer. *Br J Cancer* 2002; **86**: 1041–6.
9 Penault-Llorca F, Vincent-Salomon A. Roles of the pathologist in neoadjuvant chemotherapy: evaluation of response, prognostic and predictive factors. *Ann Pathol* 2003; **23**: 555–63.
10 Bollet MA, Sigal-Zafrani B, Gambotti L *et al.* Pathological response to preoperative concurrent chemo-radiotherapy for breast cancer: results of a phase II study. *Eur J Cancer* 2006; **42**: 2286–95.
11 Hasebe T, Yamauchi C, Iwasaki M *et al.* Grading system for lymph vessel tumor emboli for prediction of the outcome of invasive ductal carcinoma of the breast. *Hum Pathol* 2008; **39**: 427–36.
12 Hasebe T, Sasaki S, Imoto S *et al.* Histological characteristics of tumor in vessels and lymph nodes are significant predictors of progression of invasive ductal carcinoma of the breast: a prospective study. *Hum Pathol* 2004; **35**: 298–308.
13 Sobin LH, Wittekind CH, eds. *TNM Classification of Malignant Tumors*, Geneva: Wiley-Liss, 2002; 131–141.
14 Bloom HJG, Richardson WW. Histological grading and prognosis in breast cancer. *Br J Cancer* 1957; **11**: 359–77.
15 Gilchrist KW, Gray R, Fowble B *et al.* Tumor necrosis is a prognostic predictor for early recurrence and death in lymph node-positive breast cancer: a 10-year follow-up study of 728 eastern cooperative oncology group patients. *J Clin Oncol* 1993; **11**: 1929–35.
16 Hasebe T, Tsuda H, Hirohashi S *et al.* Fibrotic focus in infiltrating ductal carcinoma of the breast: a significant histopathological prognostic parameter for predicting the long-term survival of the patients. *Breast Cancer Res Treat* 1998; **49**: 195–208.
17 Hasebe T, Sasaki S, Imoto S *et al.* Prognostic significance of fibrotic focus in invasive ductal carcinoma of the breast: a prospective observational study. *Mod Pathol* 2002; **15**: 502–16.
18 Yamauchi C, Hasebe T, Iwasaki M *et al.* Accurate assessment of lymph vessel tumor emboli in invasive ductal carcinoma of the breast according to tumor areas, and their prognostic significance. *Hum Pathol* 2007; **38**: 247–59.
19 Fisher B. Biological and clinical considerations regarding the use of surgery and chemotherapy in the treatment of primary breast cancer. *Cancer* 1977; **40**: 574–87.
20 Fisher B. Adjuvant chemotherapy in the primary management of breast cancer. *Med Clin North Am* 1977; **61**: 953–65.
21 Kurosumi M. Significance of histopathological evaluation in primary therapy for breast cancer – recent trends in primary modality with pathological complete response (pCR) as endpoint. *Breast Cancer* 2004; **11**: 139–47.
22 Wolff AC, Hammond ME, Schwartz JN *et al.* American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *Arch Pathol Lab Med* 2007; **131**: 18–43.
23 Cox DR. Regression models and life-tables. *J R Stat Soc* 1972; **34**: 187–220.
24 Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958; **53**: 457–81.
25 Lee AHS, Pinder SE, Macmillan RD *et al.* Prognostic value of lymphovascular invasion in women with lymph node negative invasive breast carcinoma. *Eur J Cancer* 2006; **42**: 357–62.
26 El-Gohary YM, Metwally G, Saad RS *et al.* Prognostic significance of intratumoral and peritumoral lymphatic density and blood vessel density in invasive breast carcinomas. *Am J Clin Pathol* 2008; **129**: 578–86.
27 Amaout-Alkarain A, Kahn HJ, Narod SA *et al.* Significance of lymph vessel invasion identified by the endothelial lymphatic marker D2-40 in node negative breast cancer. *Mod Pathol* 2007; **20**: 183–91.
28 Hasebe T, Sasaki S, Imoto S *et al.* Characteristics of tumors in lymph vessels play an important role in the tumor progression of invasive ductal carcinoma of the breast: a prospective study. *Mod Pathol* 2002; **15**: 904–13.
29 Hasebe T, Sasaki S, Imoto S *et al.* Significance of nodal metastatic tumor characteristics in nodal metastasis and prognosis of patients with invasive ductal carcinoma of the breast. *Cancer Sci* 2003; **94**: 181–7.
30 Hasebe T, Sasaki S, Imoto S *et al.* Proliferative activity of intratumoral fibroblasts is closely correlated with lymph node and distant organ metastases of invasive ductal carcinoma of the breast. *Am J Pathol* 2000; **156**: 1701–10.
31 Hasebe T, Sasaki S, Imoto S *et al.* Highly proliferative fibroblasts forming fibrotic focus govern metastasis of invasive ductal carcinoma of the breast. *Mod Pathol* 2001; **14**: 325–37.
32 Hasebe T, Imoto S, Ogura T *et al.* Significance of basic fibroblast growth factor and fibroblast growth factor receptor protein expression in the formation of fibrotic focus in invasive ductal carcinoma of the breast. *Jpn J Cancer Res* 1997; **88**: 877–85.
33 Finak G, Bertos N, Pepin F *et al.* Stromal gene expression predicts clinical outcome in breast cancer. *Nature Med* 2008; **14**: 518–27.
34 Singer CF, Gschwantler-Kaulich D, Fink-Retter A *et al.* Differential gene expression profile in breast cancer-derived stromal fibroblasts. *Breast Cancer Res Treat* 2008; **110**: 273–81.
35 Sheehan KM, Gulmann C, Eichler GS *et al.* Signal pathway profiling of epithelial and stromal compartments of colonic carcinoma reveals epithelial-mesenchymal transition. *Oncogene* 2008; **27**: 323–31.
36 Hasegawa M, Furuya M, Kasuya Y *et al.* CD151 dyanmicx in carcinoma-stroma interaction: integrin expression, adhesion strength and proteolytic activity. *Lab Invest* 2007; **87**: 882–92.
37 Loussouarn D, Campion L, Sagan C *et al.* Prognostic impact of syndecan-1 expression in invasive ductal carcinomas. *Br J Cancer* 2008; **98**: 1993–8.
38 Studebaker AW, Storci G, Werbeck JL *et al.* Fibroblasts isolated from common sites of breast cancer metastasis enhance cancer cell growth rates and invasiveness in an interleukin-6-dependent manner. *Cancer Res* 2008; **68**: 9087–95.
39 Baak JP, Colpaert CG, van Diest PJ *et al.* Multivariate prognostic evaluation of the mitotic activity index and fibrotic focus in node-negative invasive breast cancers. *Eur J Cancer* 2005; **41**: 2093–101.
40 Colpaert C, Vermeulen PB, van Beest P *et al.* Intratumoral hypoxia resulting in the presence of a fibrotic focus is an independent predictor of early distant relapse in lymph node-negative breast cancer patients. *Histopathology* 2001; **39**: 416–25.

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Phase II randomised trial

A randomized phase II study of cisplatin/5-FU concurrent chemoradiotherapy for esophageal cancer: Short-term infusion versus protracted infusion chemotherapy (KROSG0101/JROSG021)

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ABSTRACT

Purpose: A randomized phase II study was conducted to compare the toxicity and efficacy of combining short-term chemotherapy (CT) or protracted CT with radiotherapy (RT) for esophageal cancer.
Materials and methods: Eligible patients were <75 years and with performance status (PS) of 0–2, and had stages II–IVA esophageal cancer. Two cycles of cisplatin 70 mg/m^2 for 1 day and 5FU 700 mg/m^2 for 5 days (arm A) or cisplatin 7 mg/m^2 for 10 days and 5FU 250 mg/m^2 for 14 days (arm B) were given with RT of 60 Gy/30 fractions/7 weeks (1-week split).
Results: Of 91 patients enrolled, 46 were randomized to arm A and 45 to arm B. Two cycles of CT were given concurrently with RT for 89% in arm A and for 71% in arm B with significant difference ($P = .031$). The 2- and 5-year overall survival rates for arm A were 46% and 35%, while those for arm B were 44% and 24%, respectively, without significant difference. The 2- and 5-year progression-free survival rates for arm A were 30% and 30%, while those for arm B were 29% and 12%, respectively.
Conclusions: Protracted infusion CT with RT provides no advantage over standard short-term infusion CT with RT for esophageal cancer.
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For locally advanced esophageal cancer, a significant improvement in local control and overall survival was achieved with concurrent chemoradiotherapy (CRT) as compared with radiotherapy (RT) alone [1–3]. In the phase III randomized trial (RTOG-8501), four cycles of full-dose 5-FU/cisplatin were concurrently combined with 50 Gy of RT [1,2]. However, the incidence of local failure was still as high as 44–54%. To improve these results, a phase III trial comparing standard dose RT (50.4 Gy) and high-dose RT (64.8 Gy) concurrently combined with 5-FU/cisplatin was conducted [4]. In the INT0123 trial, the high-dose RT arm did not offer a survival benefit compared with the standard RT dose arm [4]. Thus, at present, four cycles of full-dose 5-FU/cisplatin combined with 50 Gy of RT are a standard CRT regimen for advanced esophageal cancer in the USA.

In Japan, surgical resection is preferably performed for esophageal cancer with the T1-3N0, 1M0 disease, staged according to the 1997 International Union Against Cancer TNM classification (UICC 1997). Thus, many patients to be treated with CRT in Japan have T4 squamous cell carcinomas. Our previous study of concurrent CRT with the protracted infusion of cisplatin and 5-FU for T4 esophageal cancer with or without a fistula showed a 2-year survival rate of 27% for patients with stage III disease [5]. Several investigators also showed promising clinical results of low-dose protracted infusion CT combined with full-dose RT of 60–66 Gy for locally advanced esophageal squamous cell carcinomas [5–10]. Low-dose protracted infusion of 5-FU or 5-FU plus cisplatin was proposed to decrease the acute toxicities of concurrent CRT [8,10]. In addition, to obtain maximum radiosensitization by CT, daily administration of low-dose protracted CT combined with RT may be better than full-dose short-term CT plus RT.

To test the hypothesis, a randomized phase II study was conducted to compare the relative toxicity and efficacy of combining

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full-dose short-term CT or low-dose protracted CT with RT for esophageal cancer.

Patients and methods

Investigational design

This randomized phase II multicenter study was started by the Kyoto Radiation Oncology Study Group (KROSG), and joined subsequently by the Japanese Radiation Oncology Study Group (JROSG). The protocol (KROSG0101/JROSG021) was approved by the institutional review boards or ethics committees of all participating institutions, and written informed consent was obtained before entry into the study.

Eligibility criteria

Inclusion criterion was histologically confirmed esophageal squamous cell carcinoma or adenocarcinoma with stages II–IVA (UICC 1997). Only patients with no prior therapy, age <75 years, performance status (PS) of 0–2, and adequate bone marrow, hepatic and renal functions were eligible. Patients with a serum creatinine level <1.5 mg/dl, creatinine clearance value ≧60 ml/min, white blood cell count (WBC) ≧4000/mm^3, hemoglobin (Hb) ≧10 g/dl, and platelet count ≧100,000/mm^3 were eligible. Patients treated with thoracotomy alone for unresectable tumors were eligible, but patients after complete or incomplete resection of tumors were ineligible. Multiple esophageal tumors were also eligible, but tumors with fistula were excluded. Exclusion criteria were patients with serious infection, uncontrolled heart disease, uncontrolled diabetes mellitus, suffering from other cancers within 3 years, and esophageal stent.

Staging work-up included clinical examination, plain chest XP, upper gastrointestinal fiberscope with biopsies, an upper gastrointestinal series, and thoraco-abdominal computed tomography scan. Computed tomography was performed with contrast enhancement whenever possible. Endoscopic ultrasonography (EUS), bronchoscopy, brain MRI or computed tomography, or bone scintigraphy was performed optionally when available. Although EUS was performed to determine the depth of tumors for most T1 or T2 tumors, EUS was not done for more advanced tumors due to stenosis of the esophageal lumen. Positron emission tomography (PET) was not performed for most patients because availability of PET was limited in most participating institutions during the study period.

Design and random assignment

All eligible patients were registered at the office of the primary investigator. The patients were randomly assigned either to arm A (full-dose short-term CT) or to arm B (low-dose protracted CT) by customized randomization software; patients were stratified according to tumor length (≤6 cm vs. >6 cm), clinical stage (stages IIA, IIB vs. stages III, IVA), and institution. Because the Common Toxicity Criteria for Adverse Events (CTCAE) version 3.0 was not available in 2001, acute toxicity was graded according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) (version 2.0), and late toxicity was graded according to the RTOG/EORTC Late Radiation Morbidity Scoring Scheme.

Treatment: radiation and chemotherapy

Two courses of concurrent CT were combined with RT of 60 Gy/30 fractions/7 weeks (1-week split at the 4th week) (Fig. 1). A 6–15 MV X-ray was used. The daily fractional dose of RT was 2 Gy administered 5 days a week. The primary tumor and the involved

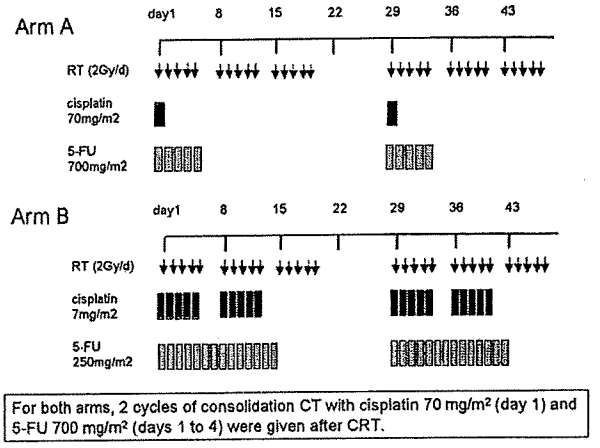

Fig. 1. Treatment design of the KROSG-0101/JROSG-021. CT, chemotherapy; RT, radiation therapy. Arm A full-dose short-term CT with RT. Arm B low-dose protracted CT with RT.

lymph nodes of ≧0.5 cm in the shortest diameter on computed tomography were gross tumor volume (GTV). The initial 40 Gy was delivered to clinical target volume 1 (CTV1), and the final 20 Gy was delivered to a reduced volume defined as clinical target volume 2 (CTV2), including the GTV with a margin (lateral and anterior/posterior directions 0.5 cm; cranio-caudal direction 1 cm). CTV1 for cervical, upper, and middle thoracic esophageal cancers included the GTV with a margin plus the supraclavicular and mediastinal lymph nodal areas (T-shaped field). For cervical esophageal cancer, lower mediastinal lymph nodal areas were excluded from CTV1. For tumors originating in the lower thoracic esophagus, CTV1 included the GTV with a margin plus the mediastinal and perigastric/celiac lymph nodal areas (I-shaped field).

For both CTV1 and CTV2, a margin (lateral and anterior/posterior directions 0.5 cm; cranio-caudal direction 1 cm) was added for planning target volumes 1 and 2 (PTV1, 2). In addition, leaf margins for PTV1, 2 of 0.5–0.8 mm were added. RT doses were specified in the center of the target volume and calculated with lung inhomogenous correction.

At 40 Gy, the RT field was reduced to the PTV2. The total RT dose delivered to the spinal cord was limited to 40 Gy, usually by using oblique opposed fields. RT was stopped if grade-4 leukocytopenia, thrombocytopenia of <20,000/mm^3, grade-4 esophagitis, or a fever of >38 °C was observed.

Two cycles of CT were delivered concurrently with RT for both arms (Fig. 1). For arm A, cisplatin 70 mg/m^2 (day 1) was delivered during 2-h intravenous infusion (IV), and 5-FU 700 mg/m^2/day was administered as a continuous IV (days 1–5). For arm B, cisplatin 7 mg/m^2 (days 1–5, and days 8–12) was delivered 1-h IV, and 5-FU 250 mg/m^2/day was administered as continuous IV (days 1–14). For arm B, RT was administered within 1 h after the administration of cisplatin. The total dose of CT was the same for the two arms. This schedule was repeated twice every 4 weeks concurrently with RT. For both arms, 2 cycles of consolidation CT with cisplatin 70 mg/m^2 (day 1) and 5-FU 700 mg/m^2/day (days 1–4) were given after CRT as protocol.

For both arms, the second to fourth cycles of CT were started when WBC count of ≧3000/mm^3, a platelet count of ≧75,000/mm^3, and a creatinine level of <1.5 mg/dl were confirmed. CT was postponed if grade-3 leukocytopenia or thrombocytopenia was noted. When grade-4 hematological toxicities or grade-3 non-hematological toxicities excluding nausea, vomiting, and esophagitis were observed in the first course of CT, 80% dose for both 5-FU and cisplatin was used in the second course of CT.

Follow-up

The local response was evaluated 2–4 weeks after the CRT by barium swallow, esophageal endoscopy with biopsy, and thoracoabdominal computed tomography scan with contrast enhancement. Esophagography or endoscopy was performed every 2–4 months for asymptomatic patients, and any clinically suspected tumor recurrence required biopsy and histopathological confirmation. Computed tomography scans were obtained at 3- to 6-month intervals, and used to evaluate the recurrence of primary tumors and regional lymph nodes. When tumor progression or recurrence was noted, salvage treatment was mandatory for the attending physicians.

Endpoints

The primary endpoint of the study was the 2-year overall survival rate. Secondary endpoints were overall survival curves, progression-free survival (PFS) curves, acute and late toxicities, and compliance rate of the protocol. When four cycles of CT and 60 Gy of RT could be given as protocol, the patient was regarded to be in full compliance with the protocol. When two cycles of CT and 60 Gy of RT could be given concurrently, the patient was regarded to be in partial compliance with the protocol. Other patients were regarded as non-compliant. As the concurrent phase of CRT is a major part of the protocol, when at least two cycles of CT and 60 Gy of RT could be given concurrently (full compliance and partial compliance), patients were regarded as per protocol set.

Statistical analysis

In the RTOG-8501 trial, in which T4 tumors were not included, the 2-year survival rate of patients treated with 50 Gy CRT was 36% [1,2]. In one Japanese phase II trial for advanced esophageal cancer with T4 or distant lymph node metastasis, the 2-year survival rate of patients treated with 60 Gy CRT was 31.5% [11]. As our protocol included T4 tumors, the baseline 2-year survival rate was expected to be 35%. In this protocol, two arms were studied. The sample size for a randomized phase II trial was calculated as 35 patients per arm, with a probability of 0.80 of selecting the treatment schedule that had a 2-year survival rate of 35% + 10% = 45% [12]. In the protocol, the sample size was estimated as 45 patients per arm supposing several ineligible or dropped cases.

The probability of survival was estimated using the Kaplan–Meier method with statistical significance assessed by the log-rank test. Data were analyzed according to the intent-to-treat principle. Survival was calculated from the date of randomization. For overall survival, deaths due to any cause were considered. For progression-free survival, any local or distant tumor progression and deaths due to any causes were considered as an event. The difference in compliance rates was assessed by the chi-squared test.

Results

From December 2001 to June 2006, 91 patients were registered. Forty-six patients were randomly assigned to arm A, and 45 were assigned to arm B (Fig. 2). Although all the 91 patients were eligible at registration, bone metastasis was detected 13 days after registration by bone scintigraphy in one patient in arm A. This patient was also included according to the intent-to-treat analysis. Table 1 shows the characteristics of the 91 patients and treatment parameters according to treatment arm. There were no significant differences in patient characteristics and treatment parameters between the two arms.

Table 2 shows the compliance rates according to treatment arm. The planned dose of 60 Gy was delivered to 88 patients (97%), while RT was terminated at 30 Gy for three patients. Two patients

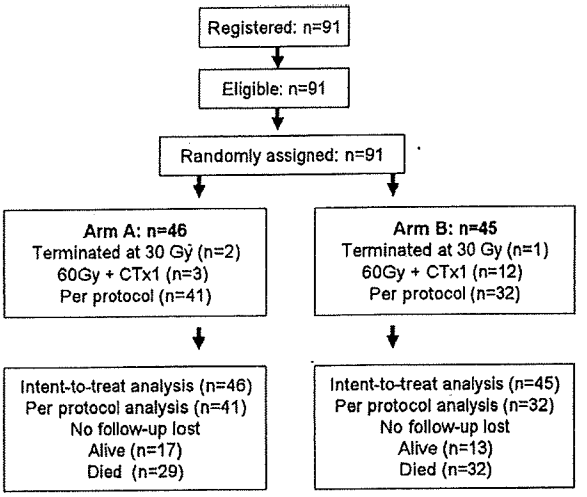

Fig. 2. The flow diagram of the patients registered.

Table 1
Characteristics of patients and treatment parameters according to treatment arm (intention-to-treat analysis).

Arm	A (n = 46)	B (n = 45)
Age (median)	45–74 (63)	48–74 (63)
Male/female	41/5	41/4
PS: 0/1/2	23/20/3	22/21/2
Body surface of patients		
Range (median)	1.20–1.97 m^2 (1.59)	1.15–1.90 m^2 (1.52)
Comorbidity	11	11
Double cancer	2 (1)[a]	5 (3)[a]
Histology		
Sq/Ade	45/1	45/0
Primary site		
Ce/Ut/Mt/Lt	6/13/15/12	4/15/19/7
Length of the tumor		
≤6 cm/>6 cm	23/23 (2–12 cm)	21/24 (1–19 cm)
TNM stage (UICC 1997)		
T1/2/3/4	4/7/14/21	4/9/13/19
N0/1	8/38	9/36
St 2/3/4a	11/30/5	11/27/7
Shape of initial RT field		
T-field	38	38
I-field	8	7
Length of initial RT field		
Range (median)	18–35 cm (26)	19–33 cm (26)

Note: There was no significant difference between arms for any of the characteristics. Sq, squamous cell carcinoma; Ade, adenocarcinoma; Ce, cervical esophagus; Ut, upper thoracic esophagus; Mt, middle thoracic esophagus; Lt, lower thoracic esophagus.

[a] Detected other cancers in the follow-up period of CRT for esophageal cancer.

in each arm underwent surgery due to poor tumor regression by 30 Gy of CRT. The remaining one patient in arm A refused further treatment due to grade-3 acute toxicities and worsening of depression, and committed suicide at the 67th day of the protocol treatment.

Although the full compliance rate was higher in arm A (54%) than in arm B (36%), there was no significant difference. When patients with full and partial compliance were combined as per the protocol set, the rate of per protocol in arm A (89%) was significantly higher than that in arm B (71%) (P = 0.031). Because of prolonged leukopenia (<3000/mm^3), second CT could not be started during RT as a protocol for eight patients in arm B, while there was only one such patient in arm A (Table 2). As the patients in

Table 2
Compliance of protocol according to treatment arm.

Arm	A (n = 46)	B (n = 45)
RT stopped at 30 Gy	2	1
Full compliance (60 Gy + CT × 4)	25 (54%)	16 (36%)
Partial compliance (60 Gy + CT × 2)	16 (35%)	16 (36%)
Reasons for partial compliance		
Non-CR, PD	13	8
Renal toxicity G1/2	1	3
Comorbidity, toxicities	2	3
Refusal of further CT	0	2
Non-compliance	5 (11%)	13 (29%)
Reasons for non-compliance		
Leukopenia	1	8
Renal toxicity G1/2	2	1
NC, PD, fistula	1	2
Other toxicities	1	2

CR, complete response; NC, no response; PD, progressive disease.

arm A recovered quickly from leukopenia, the compliance rate was better in arm A than in arm B.

Table 3 shows the acute toxic effects associated with CRT. Although grade-3 leukopenia and esophagitis were noted frequently in both arms, there was no significant difference in the incidence of acute toxicities. In arm A grade-4 leukopenia was noted in four patients, but there was no grade-4 leukopenia in arm B. However, grade-2 or -3 leukopenia was prolonged in arm B. As rare grade-4 toxicities, consciousness loss due to hyperammoniemia in arm A and esophageal bleeding due to Mallory–Weiss syndrome in arm B were noted in one patient each. Both patients recovered quickly with appropriate treatment.

Late toxicities associated with CRT were scored for 87 patients excluding four patients who died within 4 months (Table 3). The follow-up period ranged from 4.5 months to 73 months (median; 19.5 months). There were no significant differences in late toxicities between the two arms. In total, 22 patients (25%) showed grade-2 or higher late toxicities, and 12 patients (14%) had toxicities of grade-3 or higher. Grade-4 heart toxicities were noted in three patients.

Table 3
Acute and late toxicities according to treatment arm (NCI-CTC version 2.0, RTOG/EORTC late radiation morbidity scoring scheme).

Arm	A (n = 46)	B (n = 45)
Acute toxicities		
WBC G3/4	16/4	25/0
Hb G3/4	2/0	0/3
Plt G3/4	2/0	2/0
PS G3/4	5/3	5/0
Vomit G3/4	3/0	1/0
Esophagitis G3/4	11/0	7/1
Infection G3/4	5/0	4/0
Consciousness G3/4	0/1	0/0
Cardiac ischemia G3/4	0/0	1/0
Kidney: CRN G1/2/3	4/1/0	5/1/0
Liver G1/2/3	0/1/1	0/1/1
Arm	**A (n = 42)**	**B (n = 45)**
Late toxicities		
Esophagus G2/3/4	1/1/0	2/1/0
Heart G2/3/4	2/2/1	2/1/2
Lung G2/3/4	2/0/0	0/1/0
Pleura[a] G2/3/4	0/2/0	4/2/0
Hypothyroid[a] G2/3/4	3/0/0	3/1/0
Kidney[a] G2/3/4	0/0/0	0/1/0
Patient max G2/3/4	4/4/1	6/5/2
Patient max ≥G2	9 (21%)	13 (29%)

Note: Four patients who died within 4 months were excluded from the analysis of late toxicities.

[a] Late toxicities graded according to the NCI-CTC version 2.0.

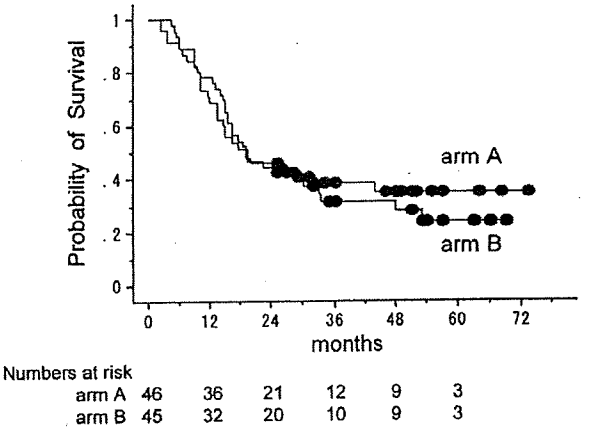

Fig. 3. Intent-to-treat analysis of overall survival curves for arm A and arm B.

All 91 patients were evaluated in terms of survival based on the intent-to-treat principle. As of August 2008, all 91 patients could be followed-up, and 30 patients (arm A, 17 patients; arm B, 13 patients) are alive with a median follow-up period of 48 months, ranging from 25 months to 73 months. Fig. 3 shows the overall survival curves for both arms. The 2-year and 5-year survival rates for arm A were 46% (95% confidence interval (CI); 31–60%) and 35% (95% CI; 20–49%), respectively. Those for arm B were 44% (95% CI; 30–59%) and 24% (95% CI; 10–38%), respectively. There was no significant difference in both the 2-year survival rates as the primary endpoint, and in the overall survival curves (P = 0.536).

Fig. 4 shows the PFS curves for both arms. The 2- and 5-year PFS rates for arm A were 30% (95% CI; 17–44%) and 30% (95% CI; 17–44%), while those for arm B were 29% (95% CI; 16–42%) and 12% (95% CI; 2–22%), respectively. Although there was also no significant difference between the two curves (P = 0.430), late recurrences after 2 years were noted only in arm B. In arm A, 13 patients (28%) were progression-free at 24 months, whereas 10 patients (22%) were progression-free at 24 months in arm B. Six patients in arm B showed recurrences after 2 years, and all of the recurrences were loco-regional. As per protocol rate was significantly higher in arm A than in arm B, PFS was analyzed only for patients with per protocol (Fig. 5). Although there was also no significant difference between the two curves (P = 0.476), a similar trend of late recurrences was noted only in arm B.

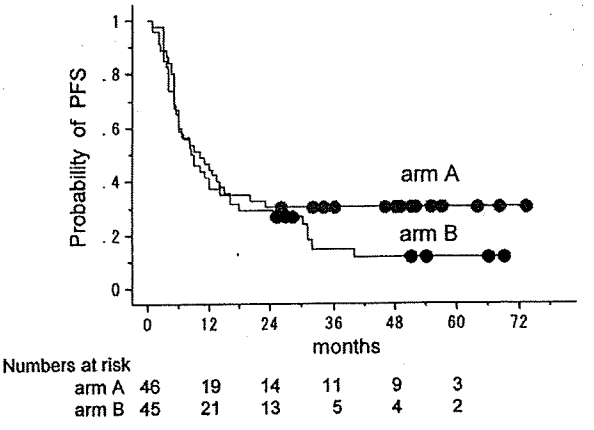

Fig. 4. Intent-to-treat analysis of progression-free survival (PFS) for arm A and arm B.

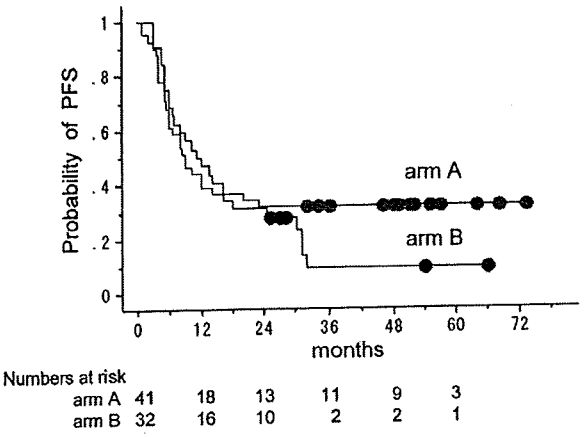

Fig. 5. Per-protocol set analysis of progression-free survival (PFS) for arm A and arm B.

When residual or recurrent tumors were detected after 60 Gy of CRT, appropriate treatment was chosen by the attending physicians, and salvage surgery was performed for 15 patients. For 11 patients (six patients in arm A and five in arm B), potentially curative resection was achieved, while non-curative resection was achieved in four patients (two patients in arm A and two in arm B).

Discussion

This study is the first randomized clinical trial comparing the type of infusion CT in definitive CRT for esophageal cancer. In the present study, both arms used the same total RT dose of 60 Gy and the same total dose of cisplatin and 5-FU to evaluate the effect of type of infusion CT. The 2-year survival rate as the primary endpoint was not different between full-dose short-term infusion CT (arm A) and low-dose protracted infusion CT (arm B). There was also no significant difference in acute and late toxicities between the two arms (Table 3). However, the compliance rate of the protocol as a secondary endpoint was significantly higher in arm A than in arm B, and the late recurrences after 2 years occurred only in arm B. Thus, our hypothesis that daily administration of low-dose protracted CT is better than full-dose short-term CT in reducing acute toxicities and in enhancing radio-sensitization effects was not proved.

In Japan, low-dose protracted infusion CT combined with full-dose RT of 60–66 Gy is a popular regimen for locally advanced esophageal squamous cell carcinomas [5–9]. A main reason for the preference of protracted infusion CT is weak acute toxicities. As expected, low-dose protracted infusion CT was associated with slightly lower incidences of high grade nausea and vomiting and grade-4 leukopenia in the present study. However, there was no significant difference in the rate of acute and late toxicities between the arms (Table 3). Sai et al. [9] reported that modification or reduction of CT dose was frequently necessary for low-dose protracted infusion CT due to leukopenia or decreased renal function. In fact, compliance with the protocol was significantly worse in arm B, mostly due to prolonged leukopenia (Table 2).

Cisplatin is known not only as a cytotoxic agent but also as a radiosensitizer [13]. For unresectable non-small cell lung cancer, a randomized clinical trial comparing RT alone of 55 Gy/20 fractions, same RT dose with daily administration of cisplatin of 6 mg/m^2, and same RT dose with weekly administration of cisplatin of 30 mg/m^2 combined with RT has been reported [14]. In that study, overall survival was significantly improved in the daily-cisplatin group as compared with the RT alone group. The daily-cisplatin group showed a slightly longer median PFS time than the weekly-cisplatin group without significance. Thus, it was postulated that daily protracted infusion CT has the advantage of maximum radiosensitizing effect compared with weekly or intermittent CT. Unfortunately, this rationale was not proved for esophageal cancer. In the per protocol analysis, there were still many late loco-regional recurrences in arm B (Fig. 5). It is suggested that the poor long-term control in arm B is not related to the low compliance with protocol in arm B, but that low-dose protracted CT has a lower sensitizing effect than full-dose short-term CT.

Another potential advantage of the protracted infusion CT is to avoid a rapid depopulation of massive T4 tumors by full-dose CT [13]. Ahmed et al. [15] reported that malignant fistulae disappeared completely in four of five patients treated with 5-FU (400–600 mg/m^2) by protracted continuous infusion and RT of 60 Gy. Koike et al. [6] reported that malignant esophageal fistulae were closed in seven (44%) of 16 tumors with fistulae by low-dose protracted CT of similar regimen in arm B. As T4 tumors with fistulae were excluded in the present analysis, protracted infusion CT may still have some advantage for T4 tumors with fistula.

In arm A, the 2-year and 5-year overall survival rates were as good as 46% and 35%, respectively, even though 46% of the tumors had T4 disease. In the RTOG-8501 trial, the 2-year survival rate of patients treated with 50 Gy CRT was 36% [1,2]. In this trial, T4 tumors were not included. In the INT-0123, T4 tumors comprised 9%, and the 2-year survival rates for the 50.4 Gy arm and 64.8 Gy arm were 40% and 31%, respectively [4]. In our protocol, the total dose of RT was 60 Gy with 1-week split. This split may be attributable to the high compliance rate of 89% in arm A. In terms of late toxicities, grade 3 and grade 4 late toxicities were noted in 14% of the patients. This rate is much lower than 37% in the 50.4 Gy arm of the INT-0123 or 29% in the CRT arm of RTOG-8501 [2,4]. Thus, our arm-A protocol is promising in overall survival rate and in the incidence of late toxicities.

In conclusion, our results suggest that low-dose protracted infusion CT with RT is not superior to full-dose short-term infusion CT with RT for esophageal cancer.

Acknowledgments

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References

[1] Al-Sarraf M, Herskovic A, Martz K, et al. Progress report of combined chemoradiotherapy versus radiotherapy alone in patients with esophageal cancer: an Intergroup study. J Clin Oncol 1997;15:277–84.
[2] Cooper JS, Guo MD, Herskovic A, Radiation Therapy Oncology Group. Chemoradiotherapy of locally advanced esophageal cancer: long-term follow-up of a prospective randomized trial (RTOG 85-01). JAMA 1999;281:1623–7.
[3] Smith TJ, Ryan LM, Douglass Jr HO, A study of the Eastern Cooperative Oncology Group. Combined chemoradiotherapy vs. radiotherapy alone for early stage squamous cell carcinoma of the esophagus. Int J Radiat Oncol Biol Phys 1998;42:269–76.
[4] Minsky BD, Pajak TF, Ginsberg RJ, et al. INT 0123 (Radiation Therapy Oncology Group 94-05) phase III trial of combined-modality therapy for esophageal cancer: high-dose versus standard-dose radiation therapy. J Clin Oncol 2002;20:1167–74.
[5] Nishimura Y, Suzuki M, Nakamatsu K, et al. Prospective trial of concurrent chemoradiotherapy with protracted infusion of 5-fluorouracil and cisplatin for T4 esophageal cancer with or without fistula. Int J Radiat Oncol Biol Phys 2002;53:134–9.
[6] Koike R, Nishimura Y, Nakamatsu K, et al. Concurrent chemoradiotherapy for esophageal cancer with malignant fistula. Int J Radiat Oncol Biol Phys 2008;70:1418–22.
[7] Ohtsu A, Boku N, Muro K, et al. Definitive chemoradiotherapy for T4 and/or M1 lymph node squamous cell carcinoma of the esophagus. J Clin Oncol 1999;17:2915–21.

[8] Sakai K, Inakoshi H, Sueyama H, et al. Concurrent radiotherapy and chemotherapy with protracted continuous infusion of 5-fluorouracil in inoperable esophageal squamous cell carcinoma. Int J Radiat Oncol Biol Phys 1995;31:921–7.
[9] Sai H, Mitsumori M, Yamauchi C, et al. Concurrent chemoradiotherapy for esophageal cancer: comparison between intermittent standard-dose cisplatin with 5-fluorouracil and daily low-dose cisplatin with continuous infusion of 5-fluorouracil. Int J Clin Oncol 2004;9:149–53.
[10] Hsu C, Yeh K, Lui L, et al. Concurrent chemoradiotherapy for locally advanced esophageal cancer; a pilot study by using daily low-dose cisplatin and continuous infusion of 5-fluorouracil. Anticancer Res 1999;19:4463–8.
[11] Ishida K, Iizuka T, Ando N, et al. Phase II study of chemoradiotherapy for advanced squamous cell carcinoma of the thoracic esophagus: Nine Japanese Institutions Trial. Jpn J Clin Oncol 1996;26:310–5.
[12] Simon R, Wittes RE, Ellenberg SS. Randomized phase II clinical trials. Cancer Treat Rep 1985;69:1375–81.
[13] Nishimura Y. Rationale for chemoradiotherapy. Int J Clin Oncol 2004;9:414–20.
[14] Schaake-Koning C, van den Bogaert W, Dalesio O, et al. Effects of concomitant cisplatin and radiotherapy on inoperable non-small-cell lung cancer. N Engl J Med 1992;326:524–30.
[15] Ahmed HF, Hussain MA, Grant CE, et al. Closure of tracheoesophageal fistulas with chemotherapy and radiotherapy. Am J Clin Oncol 1998;21:177–9.

Impact of radiation therapy on breast-conserving therapy for breast cancer in Japanese women: A retrospective analyses of multi-institutional experience. Kansai Breast Cancer Radiation Therapy Study Group

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Abstract. Whole breast radiation therapy (RT) after breast-conserving surgery is sometimes omitted in Japan; however, its impact on the outcome has not been properly evaluated. A multi-institutional retrospective study was conducted to clarify the impact of RT on local control after breast-conserving therapy (BCT). Data were collected from 3576 patients from 37 participating hospitals, of whom 1763 were eligible for analyses. Five hundred and five patients had ipsilateral breast tumor recurrence (IBTR) and 1258 patients did not. Details of IBTR were available for 245 of 505 patients who had IBTR, the location of IBTR was within or adjacent to the original tumor bed in 168 patients (68.6%). IBTR was salvaged with partial mastectomy in 119 patients (48.6%). Second recurrence in the ipsilateral breast was observed in 27 patients (11.0%). Univariate analyses demonstrated that administration of RT, the resection margin status, hormone responsiveness, T stage, N stage and stage were significantly related to IBTR. Multivariate analysis demonstrated that administration of RT, T stage and N stage were significantly correlated to IBTR. Among them, administration of RT had the largest impact on RT and it decreased the risk of IBTR by 77.3%. Omission of RT had the most significant impact on IBTR. RT should be given as a standard component of BCT.

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Introduction

The incidence of breast cancer in Japanese women has become the highest among various cancers and it was estimated that 40675 women were newly diagnosed with breast cancer in 2001. The ratio of patients who undergo breast-conserving surgery (BCS) is also increasing and BCS has become the most frequently employed method of initial surgery for breast cancer in Japan (1). According to the NIH consensus statement, breast-conserving therapy (BCT) comprises of BCS and adjuvant radiation therapy (RT). The role of RT in BCT has been well established as a result of at least 8 randomized controlled trials and meta-analyses of these trials (2-10). Moreover, the subgroup of patients who do not receive a benefit from RT after BCS has not been defined in spite of various attempts to find such a subgroup. In Japan, however, ~20% of patients who undergo BCS do not receive RT (1). This number is larger than in the USA (11). One reason for not receiving RT in Japan is that some surgeons believe that RT is not necessary if the tumor was resected with an ample pathologically negative margin and that RT is harmful and deteriorates the cosmetic outcome. To clarify the impact of RT on ipsilateral breast tumor recurrence (IBTR) in such practice in Japan, we collected data from participating institutions of the Kansai Breast Cancer Radiation Therapy Study Group (KBCRTSG) and analyzed them retrospectively.

Patients and methods

Study design. This study was conducted as a multi-institutional retrospective review. The primary endpoint was IBTR, including those preceded by any form of regional and distant recurrence.

Patients. Between August 2004 and February 2005, data from 3576 patients were collected from 37 participating hospitals in

Table I. Patient characteristics.

	Patients with IBTR (n=505)	Patients without IBTR (n=1258)	P-value
Age	49.8±12.2	49.8±9.9	N.S.
Method of surgery			P=0.082
Quadrantectomy	129	211	
Wide excision	362	572	
Tumorectomy	8	3	
Other	0	2	
Unknown	6	470	
T stage[a]			P=0.017
T0	4	0	
T1	169	402	
T2	153	256	
T3	3	1	
Unknown	176	599	
N stage[a]			P=0.000
N0	193	570	
N1	121	159	
N2	26	15	
N3	0	1	
Unknown	165	513	
Stage[a]			P=0.000
Stage 0	5	0	
Stage 1	142	349	
Stage 2a	119	233	
Stage 2b	73	71	
Stage 3a	27	7	
Unknown	139	658	
Margin status			P=0.000
>5 mm	302	750	
≤5 mm	139	219	
Unknown	63	289	
Hormone receptor stastus			P=0.000
Positive	236	715	
Negative	184	289	
Unknown	85	254	
Radiation therapy			P=0.000
Yes	356	1146	
No	148	69	
Unknown	1	43	

IBTR, Ipsilateral Breast Tumor Recurrence. [a]General rules for clinical and pathological recording of breast cancer. 14th edition, The Japanese Breast Cancer Society.

KBCRTSG. The data format was developed by the steering committee of KBCRTSG and includes patient characteristics, including clinicopathological findings, method of BCT and outcome.

Table II. Details of IBTR.

	Patients with detailed information of IBTR (n=245)	
Location of IBTR		
TR/MM[a]	168	68.6%
Other than TR/MM	65	26.5%
Unknown	12	4.9%
Type of IBTR		
Nodular	209	85.3%
Diffuse	32	13.1%
Nodular/diffuse	3	1.2%
Method of salvage		
Partial mastectomy	119	48.6%
With RT	36	14.7%
Total mastectomy	102	41.6%
With RT	3	1.2%
Unknown surgery	6	2.4%
With RT	2	0.8%
No surgery	18	7.3%
With RT	2	0.8%
Re-IBTR		
No	193	78.8%
Yes	27	11.0%
Unknown	25	10.2%

[a]True recurrence/marginal miss: Recurrence within or adjacent to original tumor bed.

Eligibility criteria for this study were as follows: i) Japanese female, ii) received BCS alone or BCT, including RT, at participating hospitals of KBCRTSG, iii) has outcome data regarding both local and systemic control and iv) longer than 5-year follow-up for patients without IBTR.

Thus, 1813 cases without IBTR were excluded due to shorter follow-up than 5 years. Consequently, 505 cases of IBTR and 1258 cases of no IBTR were subjected to further analyses. Of note, 173 of the former and 70 of the latter had distant metastasis in their disease course. Patient characteristics are shown in Table I.

Statistical analyses. Univariate and multivariate Cox regression analyses were used to evaluate the impact of patient and treatment factors on the endpoint. Pearson's Chi-square test was used to evaluate the distribution of the patients' background. A p-value of <0.05 was regarded as significant.

Results

Details of IBTR were available for 245 of 505 patients with IBTR (Table II), the location of IBTR was within or adjacent to original tumor bed in 168 patients (68.6%), in another location in 65 patients (26.5%) and unknown in 12 patients (4.9%). The type of IBTR was nodular in 209

Table III. Univariate analyses.

	No. of available patients	RR	95% C.I.	P-value
Age	1748	1.011	1.003-1.020	P=0.006
Radiation therapy	1722	0.276	0.229-0.333	P=0.000
T stage	986	1.391	1.121-1.725	P=0.003
N stage	1085	1.808	1.503-2.174	P=0.000
Stage	1032	1.328	1.178-1.498	P=0.000
Margin status	1390	1.471	1.194-1.812	P=0.000
Hormone receptor status	1424	0.593	0.487-0.721	P=0.000
Method of surgery	1309			
Method (1) quadrantectomy		90.410[a]	0.000-5.95x10^{17}	P=0.808
Method (2) wide excision		205.605[a]	0.000-1.35x10^{18}	P=0.774
Method (3) lumpectomy		612.053[a]	0.000-4.04x10^{18}	P=0.730

[a]Relative risk against method (4) 'other method'.

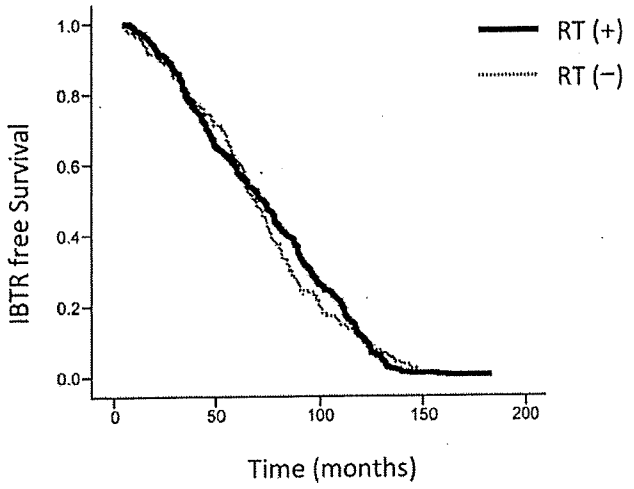

Figure 1. Kaplan-Meier estimate of ipsilateral breast tumor recurrence (IBTR)-free survival of the patients who eventually had IBTR. Note that the rate of IBTR is fairly consistent through 10 years.

patients (85.3%), diffuse/inflammatory in 32 patients (13.1%) and a combination of these in 3 patients (1.2%). IBTR was salvaged with partial mastectomy in 119 patients (48.6%), total mastectomy in 102 patients (41.6%), unknown surgery in 6 patients (2.4%) and no surgery in 18 patients (7.3%), of whom radiation therapy was used as a component of salvage therapy in 36 (14.7%), 3 (1.2%), 2 (0.8%) and 2 (0.8%). Second IBTR was observed in 27 patients (11.0%). Univariate analyses demonstrated that the administration of RT, resection margin status, hormone responsiveness, T stage, N stage and stage were significantly related to IBTR. Univariate analyses demonstrated that the administration of RT, resection margin status, hormone responsiveness, T stage, N stage and stage were significantly related to IBTR (Table III). The test for correlation among these variables demonstrated that several variables are dependent on each other (Table IV). Among them, stage was strongly correlated to T stage and N stage; therefore, RT, resection margin status, hormone responsive-ness, T stage and N stage were employed as variables for multivariate analysis using the Cox regression model. This demonstrated that RT, T stage and N stage were significantly correlated to IBTR. Among them, administration of RT had the largest impact on RT and decreased the risk of IBTR by 77.3% (Table V).

The IBTR-free survival curve was plotted for patients who eventually developed IBTR (Fig. 1). It revealed that the risk of IBTR is fairly constant over time both for patients who received RT and patients who did not.

Discussion

Several factors may influence the risk of local recurrence after BCT. Among them, administration of RT has been shown to have a large impact on local control, as shown in this study. According to a meta-analysis by EBCTCG, the effect of RT after BCS is highly consistent and reduces the risk of isolated IBTR by ~70% compared to those allocated to no RT (5). Other factors which are known to increase the risk of IBTR include young age, positive resection margin and existence of EIC.

There have been continuous efforts to identify a subgroup of patients for whom RT after BCS can be safely omitted. In the Joint Center for Radiation Therapy at Harvard Medical School, women considered to be at low risk for IBTR were prospectively observed without RT after BCS. The patients in this study had pT1N0 tumor, absence of both lympho-vascular invasion and extensive intraductal component and no cancer cells within 1 cm of resection margins. This study was terminated before it reached accrual goal because of an excessive number of IBTR. Of note, there were no eligibility limitations on patient age for this study and these patients did not receive any adjuvant chemo-endocrine therapy regardless of the status of hormone receptors (12). Considering that young age is a known risk factor for IBTR (13-19) and that systemic adjuvant therapy provides a benefit for local control (20,21), some patients in this study may not have been at low risk for IBTR. Previously, the CALGB C9343 trial demonstrated that it is a realistic choice for the treatment of