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A Proposal for a New Histological Classification Scheme for Predicting Short-term Tumor Recurrence and Death in Patients with Invasive Ductal Carcinoma of the Breast

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Tumor recurrence rate (TRR) and mortality rate (MR) of invasive ductal carcinoma (IDC) of the breast in short-term follow-up are relatively low. Nevertheless, it is extremely important to identify patients at risk of early recurrence or death after surgery. The aim of this study was to establish a new histological prognostic classification scheme for IDC in order accurately to predict the short-term outcome. The following histological parameters were analyzed in 201 IDCs: 1) tumor size, 2) structural atypia, 3) nuclear atypia, 4) number of mitotic figures, 5) fibrotic focus (FF), 6) vascular invasion, 7) tumor necrosis, 8) skin invasion, 9) muscle invasion, 10) nodal status and 11) extramammary fat invasion. Multivariate analysis showed that nuclear atypia, presence of FF, and the invasive length of fat invasion (ILFI) were the most important histological parameters correlated with TRR or MR of IDCs. Accordingly, a new histological classification based on nuclear atypia, FF and ILFI (Nucleus-Fibrotic focus-Fat invasion, NFF) was devised. Comparative studies were performed with the following existing prognostic classifications: 1) histological grade, 2) modified Scarff-Bloom-Richardson histological grade, 3) prognostic index and 4) pathological TNM (pTNM) stage classifications. Patient grouping defined by NFF classification significantly correlated with tumor recurrence or death of IDCs in all cases, cases at stages I and II, those without lymph node metastasis and those who were estrogen receptor (ER)-positive after adjustment for the other four classifications, using multivariate analysis. NFF classification appeared superior to existing prognostic classifications for the accurate prediction of the short-term outcome for patients with IDCs in low risk groups.

Key words: Invasive ductal carcinoma — Breast — Prognosis — Fibrotic focus — Staging

Tumor size, histologic grade, nodal status, DNA ploidy, proliferative activity of the tumor cells and gene abnormalities are important prognostic parameters for patients with invasive ductal carcinoma (IDC) of the breast.[1–13] Most previous studies of prognostic factors have focused on the long-term survival of patients with IDCs, and not on the short-term survival. Recently, we demonstrated that the presence of a fibrotic focus (FF) within the tumor is an important histological factor for the prediction of short-term as well as long-term survival of patients with IDCs.[14,15] However, the frequency of tumor recurrence and the mortality rate of IDCs in short-term follow-up are relatively low, which makes it difficult to predict the patients' outcomes accurately using a single prognostic parameter. Therefore, it seems better to combine powerful histological parameters in order to predict short-term surival.

Greenough first reported a histological grading system for breast cancer, which consisted of the following parameters: tubular formation, secretory activity of the tumor cells, the overall size of the tumor cells and their nuclei, variation in the size of both tumor cells and their nuclei, nuclear hyperchromatism, and mitotic counts.[16] Patey and Scarff followed Greenough's method, but only used tubular formation, variation in nuclear size and shape, and nuclear hyperchromatism as parameters.[17] In 1957, Bloom and Richardson proposed a histological grading system for breast cancer, making it more reproducible by introducing a scoring system based on three histological features: 1) tubular formation, 2) nuclear features and 3) number of mitotic figures.[3] This grading system was modified by Le Doussal *et al.* in 1989 and was called the modified Scarff-Bloom-Richardson (MSBR) histologic classification.[18] Elston and Ellis have refined the definition and the method of assessing the parameters which constitute the Bloom and Richardson grading system, and it is currently the most widely used histological grading (HG) system for breast cancer.[19] Besides these grading systems, Todd *et al.* proposed a prognostic index (PI) classification consisting of tumor size, lymph node stage and HG.[20] HG, MSBR and PI classifications, in addition to the pathological TNM (pTNM) stage classification,[21]

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are the major histological prognostic classifications which are clinically used for the analysis of the outcome for patients with IDCs.

The aim of this study is to establish a new histological prognostic classification for IDCs, which is composed of independently significant histological parameters. Studies were performed with HG, MSBR, PI and pTNM stage classifications in order to compare the predictive power of the new classification with that of the existing systems.

MATERIALS AND METHODS

Cases Two hundred and one (of which 152 were the same patients as in our previous report[14]) consecutive patients with IDC of the breast surgically treated between July 1992 and June 1994 at the National Cancer Center Hospital East constitute the basis of this study. Clinical information was obtained from the patients' medical records. All of the patients were Japanese women ranging in age from 28 to 87 years (average, 54 years), and all had a solitary lesion. One hundred patients were pre-menopausal and 101 post-menopausal. Modified radical mastectomy was performed in 166 patients, standard radical mastectomy in 16, extended radical mastectomy in 3 and partial mastectomy in 16. Axillary lymph node dissection was carried out in 198 patients, and 95 patients had no lymph node metastasis of the tumor. The number of patients at stages I, IIA, IIB, IIIA and IIIB were 47, 62, 41, 20 and 28, respectively. None of the patients had received radiotherapy or chemotherapy before surgery. Estrogen receptor (ER) assay was performed on 170 of the 201 tumors, and 107 tumors were positive. Postoperative adjuvant chemotherapy was performd in 113 patients, and 122 patients were treated with postoperative hormone therapy.

For histological examination, the surgically resected specimens were fixed in 10% formalin overnight at 4°C, and the entire tumor was cut into slices at intervals of 0.5

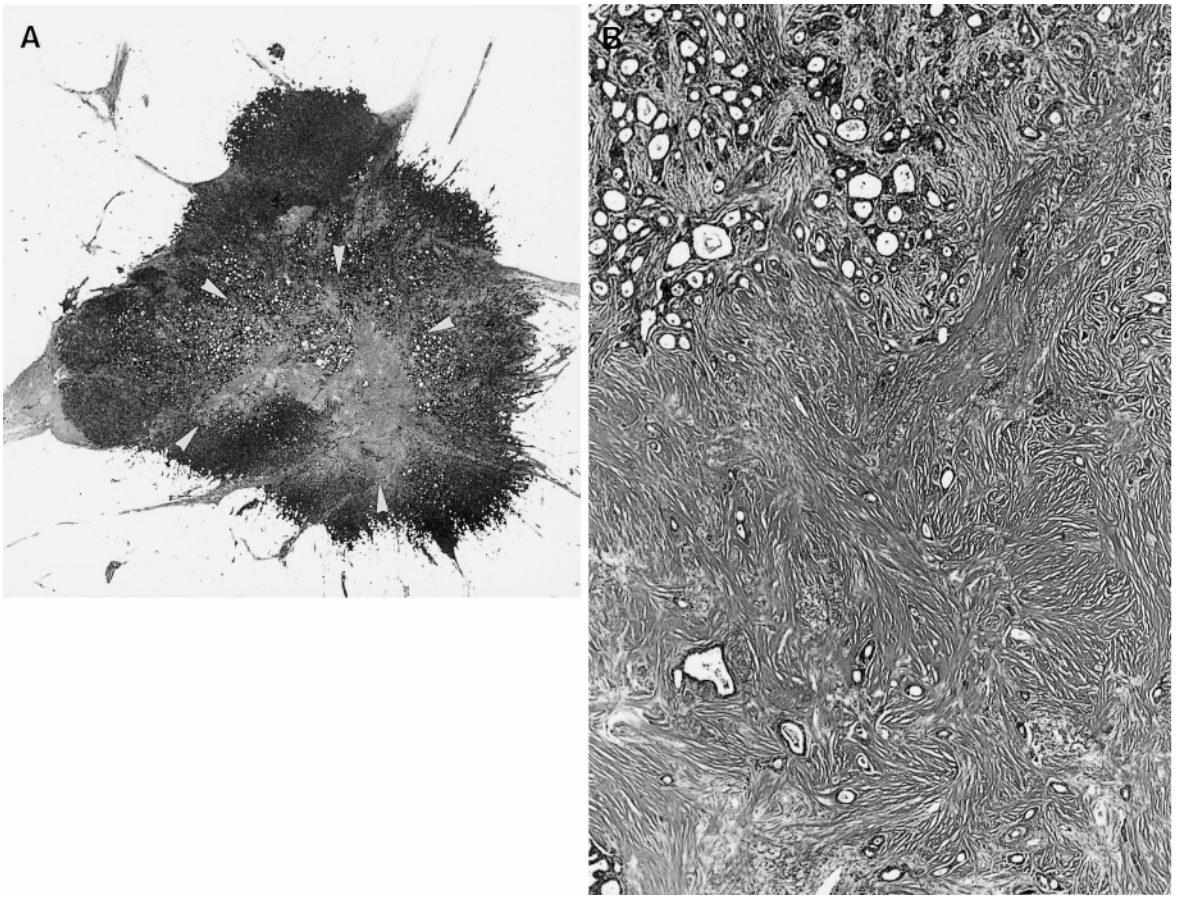

Fig. 1. IDC with FF. A. An FF measuring 7×8 mm in size is observed within the tumor (arrowheads). The FF is irregular in shape and surrounded by invasive ductal carcinoma cells. Residual tumor islands of various sizes are observed within the FF (HE, panoramic view). B. The fibroblast and collagen fibers composing FF show a storiform-like arrangement (HE, original magnification ×40).

to 0.7 cm. The size and gross appearance of the cancers were recorded, and the former was validated by comparison with tumor size on histologic slides. Multiple histological sections were taken from each tumor in order to measure the maximum tumor diameter. The sections were processed routinely and embedded in paraffin.

Histological examination Two sections of each tumor were cut from the paraffin blocks. One section was stained with hematoxylin and eosin and examined pathologically to confirm the diagnosis. The remaining sections were used for elastica staining to confirm the presence or absence of vascular invasion by the tumor cells.

The following histological parameters were examined: 1) tumor size, 2) structural atypia, 3) nuclear atypia, 4) the number of mitotic figures, 5) FF[14, 15, 22] (Figs. 1, 2 and 3), 6) tumor necrosis, 7) vascular invasion, 8) nodal status, 9) skin invasion, 10) muscle invasion and 11) extramammary fat invasion. All tumors were classified according to the guidelines of the World Health Organization,[23] and their structural and nuclear atypias, and the number of mitotic figures were used for HG.[19] The maximum diameter of the tumor was defined as the maximum cut surface of the tumor, and all tumors were classified according to the pTNM classification.[21]

When the tumor had coagulation necrosis in more than 10% of the whole tumor area, the tumor was regarded as having coagulation necrosis. Skin invasion was considered positive if the tumor cells had invaded the dermis or epidermis of the breast skin, and muscle invasion implied tumor cell invasion to the pectoralis muscle. When the tumor cells invaded surrounding fat tissue, extra-mammary fat invasion was considered to be present. Furthermore, we measured the invasive width and length of fat invasion of the tumor cells using a microscope equipped with a ×10 eyepiece enclosing a graticule, which consisted of a grid lattice of 9 vertical and 9 horizontal intersecting lines, resulting in a total of 81 points per field. Most counts were made with a ×2 or ×4 objective. If several foci of fat invasion were observed in one IDC, the longest length and the widest width of fat invasion were considered as the length and width of fat invasion, respectively (Fig. 4). When we measured the length or width of

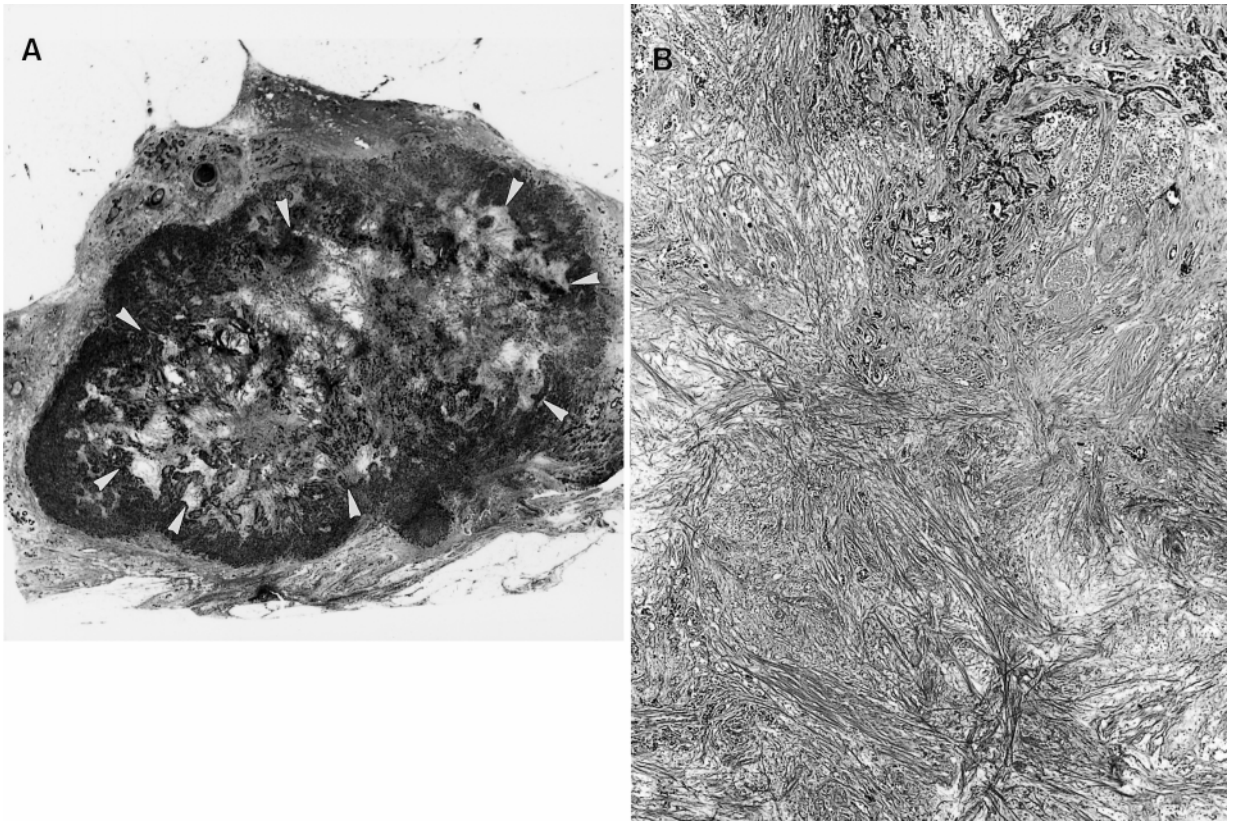

Fig. 2. IDC with FF. A. An FF measuring 19×10 mm in size is observed within the tumor (arrowheads). The FF appears as a radiating fibrosclerotic core or scar. B. The FF contains residual tumor cells arranged in small solid nests or strands admixed with fibroblast or collagen fibers showing a storiform-like arrangement (HE, original magnification ×40).

fat invasion, we drew a straight line between both ends of fat invasion adjacent to the breast tissue. We regarded this line as the baseline for the measurement of the length of fat invasion, then measured the longitudinal length from the top of the fat invasion to the baseline. The width of fat invasion was the distance between the two ends of the baseline of fat invasion. Besides the width and length of fat invasion, the invasive pattern of tumor cells was classified into large solid, small solid and septal (Fig. 5, A, B and C).

Histological prognostic classifications for comparative study The following existing histological classifications were compared with our proposed new classification for predicting disease-free survival (DFS) and overall survival (OS): 1) HG, 2) MSBR, 3) PI and 4) pTNM stage classifications. HG and pTNM stage classifications are well-known histopathological prognostic classifications.[19, 21] The MSBR histological grading system is based only on nuclear pleomorphism and the number of mitotic figures, without assessment of tubular formation.[18] Nuclear pleomorphism is divided into minimal, moderate and marked, and they are each scored as 1, 2 and 3 points, respectively. The number of mitotic figures was calculated in one high power field (HPF, ×400) having the largest number of mitotic figures. One or less mitosis/HPF was scored as 1 point, two mitoses/HPF as 2 points and three or more mitoses/HPF as 3 points. The scores for the nuclear features and the mitotic figures were added together, and groups with values of the sum of 2, 3, 4, 5 and 6 points were classified as MSBR groups 1, 2, 3, 4 and 5, respectively.

PI classification[20] consisted of tumor size, lymph node stage and HG.[19] Lymph node stage was classified as follows: 1) stage A: tumor absent from all lymph nodes sampled, 2) stage B: tumor in low axillary node only and 3) stage C: tumor in apical axillary and/or internal mammary nodes. Stages A, B and C were scored as 1, 2 and 3. PI classification was calculated according to the following formula: PI = 0.2 × tumor size (cm) + stage score + HG. The PI was computed for each of the IDCs, and the cases were

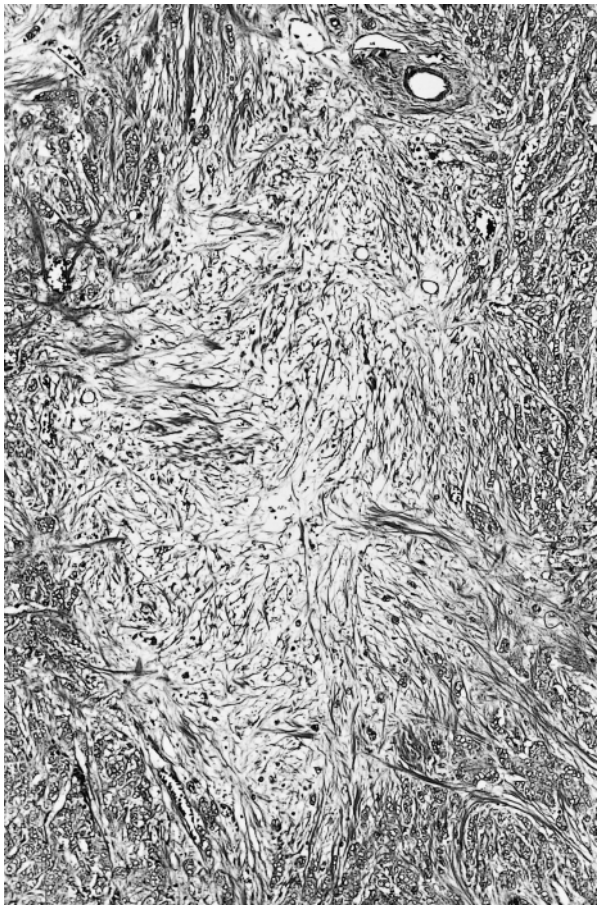

Fig. 3. IDC with FF. This tumor has a small FF measuring 1×1 mm in size. The fibroblasts forming the FF show a storiform-like arrangement (HE, original magnification ×100).

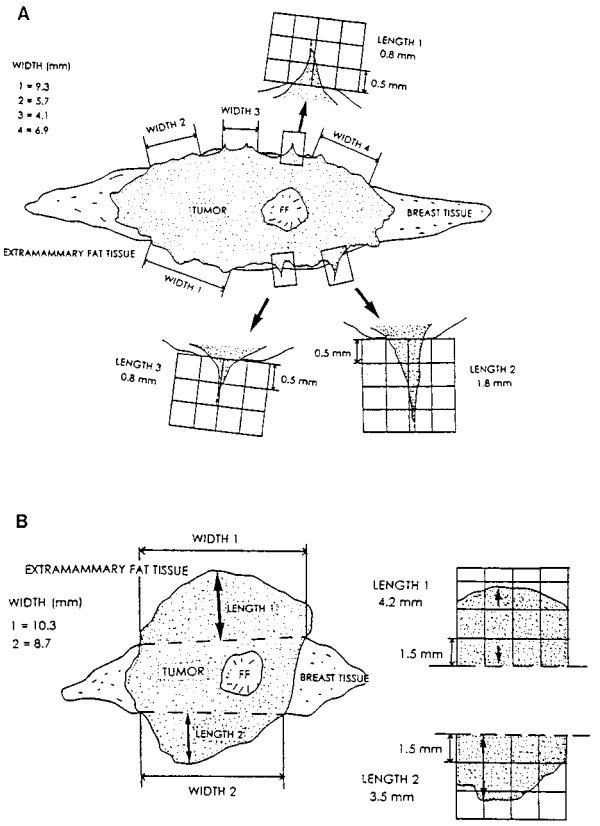

Fig. 4. A schematic illustration of the measuring method of length and width of extra-mammary fat invasion (A and B).

assigned to one of three prognostic groups: low (PI≤3.4), medium (3.4<PI≤5.4) and high (>5.4).

Outcome The survival of the patients was determined by follow-up through July, 1997 with a median period of 30 months. One hundred and seventy-four patients were alive and well, 36 had tumor recurrence, and 18 had died of their disease. Measurement of DFS and OS began at the time of surgery. Tumor relapse was defined as any evidence of metastasis or local recurrence. Death due to IDC was the only endpoint considered for the purpose of this study.

Statistical analysis The mutual relationships among histological factors were analyzed by linear regression analysis. DFS and OS curves of the patients were drawn by the Kaplan-Meier method,[24] and the differences between curves were compared using the log-rank tests.[25] The Cox proportional hazards regression model was used to estimate the univariate and multivariate hazard risk (HR) of tumor death and recurrence (with their 95% confidence intervals (CI)).[26] The following variables were examined as potential prognostic factors: 1) menopausal status (pre- or post-), 2) age (≤50 or >50 yrs.), 3) tumor size (≤20 or

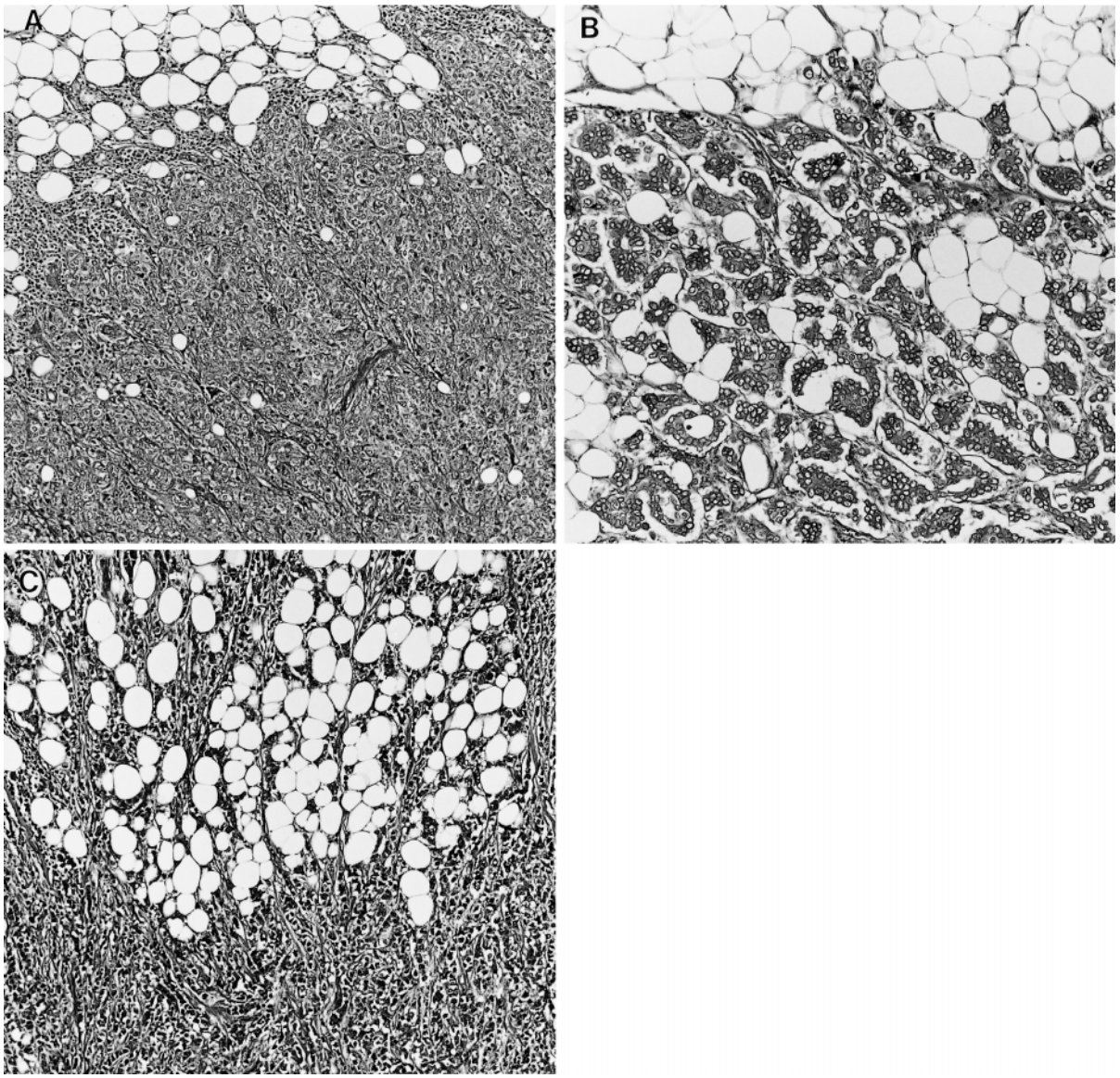

Fig. 5. Invasive pattern of the extra-mammary fat invasion of IDCs. A. Large solid nests (HE, original magnification ×100). B. Small solid nests (HE, original magnification ×100). C. Septal invasion (HE, original magnification ×100).

>20 mm), 4) structural atypia (grade 1, 2 or 3), 5) nuclear atypia (grade 1, 2 or 3), 6) mitotic figure (≤10 or >10 per 10 high-power fields), 7) FF (absent or present), 8) tumor necrosis (absent or present), 9) vascular invasion (absent or present), 10) nodal status (0, 1–3, or >3), 11) skin invasion (absent or present), 12) muscle invasion (absent or present) and 13) extra-mammary fat invasion (absent or present). Then, the variables showing a significant increase in HRs of tumor recurrence or death in the univariate analysis were entered into the multiple regression analysis using the step-down method until all the remaining factors were significant at a *P*-level below 0.05.

Among these factors, those which significantly increased HR of tumor recurrence or death were selected in order to establish a new histological prognostic classification for IDC. Then, multivariate analyses for tumor recurrence or death between the new histological prognostic classification and the other four existing histological prognostic classifications were conducted in 4 populations: all cases, those at stages I and II, those without lymph node metastasis, and those with tumors positive for ER. In order to consider the influence of chemotherapy or hormone therapy on the patient's outcome, multivariate analyses were also adjusted with these factors.

MSBR is a modification of HG. PI depends on tumor size, nodal status and HG. Tumor size and nodal status are also components of the pTNM stage classification. These four prognostic classifications are closely correlated with each other. In order to clarify the prognostic value of each classification, it was necessary to avoid their mutual influence.[27] Therefore, in order to analyze whether the new histological classification was superior to the existing classifications, multivariate analyses were performed between the new histological classification and HG (model 1), MSBR (model 2), pTNM (model 3) and PI (model 4). The specificity and sensitivity of each classification for all cases, cases at stage I and II, those without lymph node metastasis, and those that were ER-positive were also calculated. All analyses were conducted with Statistica/Windows software (StatSoft, Tulsa, OK).

RESULTS

Multivariate analyses for tumor recurrence and death of IDCs High-risk factors for tumor recurrence were large tumor size (>20 mm), nuclear atypia 3, the presence of FF, more than three lymph node metastases and the presence of skin invasion, according to the results of the Cox proportional hazard regression model (data not shown).

As for tumor death, in addition to tumor size, nuclear atypia, FF and nodal status, IDCs with mitotic figures higher than 10 showed a significant increase in HR of tumor death over those with mitotic figures of 10 or less (data not shown). Therefore, multivariate analysis was conducted with adjustment for tumor size, nuclear atypia, mitotic figures, FF, vascular invasion, nodal status, and skin invasion. Since there was no significant difference in HRs of tumor recurrence or death between IDCs with nuclear atypia 1 and those with nuclear atypia 2, or IDCs without lymph node metastasis and those with three or less lymph node metastases in the univariate analysis, these groups were combined in the multivariate analyses.

Table I. Multivariate Analysis for Tumor Recurrence and Tumor Death due to Invasive Ductal Carcinoma within 5 Years after the Initial Operation

Parameters	Cases	TRR (%)	HR	95% CI	MR (%)	HR	95% CI
All cases (*n*=201)		36			18		
Tumor size (mm)							
≤20	72	4 (6)	1.0*		2 (3)	1.0*	
>20	129	32 (25)	3.1*c)*	1.0–8.7	16 (12)	—	
Nuclear atypia							
Grades 1 and 2	122	8 (7)	1.0*		2 (2)	1.0*	
Grade 3	79	28 (35)	4.2*a)*	1.9–9.1	16 (20)	11.7*b)*	2.7–50.9
Fibrotic focus							
Absent	100	12 (12)	1.0*		3 (3)	1.0*	
Present	101	24 (24)	3.2*b)*	1.5–6.9	15 (15)	6.2*b)*	1.7–22.4
Nodal status							
0 and 1–3	134	14 (10)	1.0*		4 (4)	1.0*	
>3	64	22 (34)	2.8*b)*	1.4–5.7	11 (17)	3.0*c)*	1.2–7.9

TRR, tumor recurrence rate; MR, mortality rate; HR, hazard rate; CI, confidence interval; ∗, referent category. *P*-value: *a*) <0.001; *b*) <0.01; *c*) <0.05.
Multivariate analysis using the step-down method adjusted for tumor size, nuclear atypia, mitotic figure, fibrotic focus, vascular invasion, nodal status, and skin invasion.

Table II. Effect of Extra-mammary Fat Invasion on Tumor Recurrence and Tumor Death due to Invasive Ductal Carcinoma within 5 Years after the Initial Operation

Parameters	Cases	TRR (%)	HR	95% CI	MR (%)	HR	95% CI
Univariate analyses							
All cases	201	36 (18)			18 (9)		
Invasive length (mm)							
None	32	2 (6)	1.0*		2 (6)	1.0*	
≤2	121	16 (13)	2.0	0.6–11.0	7 (6)	1	0.2–4.8
>2	48	18 (38)	5.9a)	1.4–25.5	9 (19)	3.1	0.7–14.3
		P for trend: 0.020			*P* for trend: 0.039		
Invasive width (mm)							
None	32	2 (6)	1.0*		2 (6)	1.0*	
≤10	103	19 (18)	3.1	0.7–13.4	8 (8)	1.3	0.3–6.0
>10	66	15 (23)	4.7b)	1.1–20.9	8 (12)	2.3	0.5–10.8
		P for trend: 0.026			*P* for trend: 0.205		
Invasive pattern							
None	32	2 (6)	1.0*		2 (6)	1.0*	
Large solid	66	16 (24)	4.8b)	1.1–20.9	6 (9)	1.6	0.3–7.8
Small solid	87	11 (13)	2.2	0.5–10.0	5 (6)	1.0	0.2–5.0
Septal	16	7 (48)	7.2b)	1.5–34.7	5 (31)	5.2b)	1.0–26.8
		P for trend: 0.011			*P* for trend: 0.142		
Multivariate analysis							
Invasive length (mm)							
None and ≤2	153	18 (12)	1.0*		9 (6)	1.0*	
>2	48	18 (38)	2.4a)	1.3–4.8	9 (19)	2.8b)	1.1–7.3
Invasive pattern							
None/small solid	119	13 (11)	1.0*		7 (6)	1.0*	
Large solid/septal	82	23 (28)	2.7a)	1.4–5.2	11 (13)	2.1	0.8–5.5

TRR, tumor recurrence rate; MR, mortality rate; HR, hazard rate; CI, confidence interval. *P*-value: *a*) <0.01; *b*) <0.05.
Multivariate analysis adjusted for invasive length and invasive pattern.

Table III. Nucleus, Fibrotic Focus, and Fat Invasion (NFF) Classification for Invasive Ductal Carcinoma

NFF classification factors	Point of score	
1) Nuclear atypia		
Grade 1/2 vs. 3	0 vs. 1	
2) Fibrotic focus		
Absent vs. present	0 vs. 1	
3) Extramammary fat invasion		
Invasive length (mm)		
None/≤2 mm vs. >2 mm	0 vs. 1	Total: 0–3

Multivariate analyses showed that IDCs with tumor size larger than 20 mm ($P<0.05$), those with nuclear atypia grade 3 ($P<0.001$), those with FF ($P<0.01$) and those with more than 3 nodal metastases ($P<0.01$) had significantly higher HRs of tumor recurrence than those with tumor size 20 mm or smaller, those with nuclear atypia grades 1 and 2, those without FF and those with 3 or fewer nodal metastases, respectively (Table I). Among these cases of tumor recurrence, those with nuclear atypia of grade 3 ($P<0.01$), those with FF ($P<0.01$) and those with nodal status greater than 3 ($P<0.05$) showed significantly higher HRs of tumor death than those with nuclear atypia of grades 1 and 2, those without FF and those with nodal status of 3 or less, respectively (Table I).

Subclassification of extra-mammary fat invasion of IDCs The presence or absence of extra-mammary fat invasion failed to show a significant correlation with tumor recurrence or death in the univariate analysis. However, we hypothesized that the invasive ability of tumor cells is reflected in the presence of extra-mammary fat invasion. Therefore, HRs of tumor recurrence or death by the width, length or invasive pattern of extra-mammary fat invasion was analyzed using the Cox proportional hazard regression model, and the results were entered into the multivariate analysis.

In the univariate analysis, invasive length and invasive pattern of the tumor cells significantly correlated with the HR of tumor recurrence and death, respectively, whereas invasive width failed to show a significant correlation with HR of tumor death (Table II). Therefore, multivariate analyses were performed using invasive length and invasive pattern of the tumor.

IDCs with invasive length longer than 2 mm showed a significantly higher HR of tumor recurrence or death than those without fat invasion or with an invasive length of 2 mm or shorter ($P<0.01$). Although IDCs growing in a large solid nest or in the septum also showed a significantly higher HR of tumor recurrence than those without fat invasion or growing in small solid nests ($P<0.01$), there was no significant difference in HRs of tumor death between the former and the latter (Table II).

Proposal of a new histological prognostic classification We attempted to establish a new histological prognostic classification based on the nuclear atypia, FF and invasive length of fat invasion (ILFI), and named it the NFF (Nucleus-Fibrotic focus-Fat invasion) classification. There was no significant correlation among these factors (data not shown). A score of 1 was given to IDCs with nuclear atypia 3, those with FF, or those with ILFI of >2 mm. If any of the above factors were absent, a score of 0 was given. Then, the total score of NFF was calculated. The total score for each patient ranged from 0 to 3 (Table III).

The number of patients with scores of 0, 1, 2 and 3 among all cases was 50, 92, 41 and 18, respectively. Since there was no significant difference in HRs of tumor recurrence or death between NFF scores of 0 and 1 in the univariate analysis using the Cox proportional hazard regression model (NFF0, a referent; NFF1, HR=1.24, 95% CI=0.4–3.7, $P=0.693$), these cases were added together. Fig. 6, A and B show the DFS and OS curves, respectively. For both DFS and OS, the differences in the DFS and OS curves among IDCs with score 0/1, those with score 2 and those with score 3 were significant ($P<0.05$).

Comparative studies between NFF classification and other prognostic classifications The NFF, HG, MSBR, pTNM and PI classifications mostly showed significant *P*-values for linear trend in tumor recurrence (*P* for trend: NFF, HG, MSBR and PI, $P<0.001$, respectively; pTNM, $P=0.018$) or death (*P* for trend: NFF, HG, MSBR and PI, $P<0.001$, respectively; pTNM, $P=0.034$) in univariate analysis using the Cox proportional hazards regression.

Multivariate analyses showed that HRs of tumor recurrence or death for IDCs with NFF scores 2 and 3, compared to those with NFF score 0/1 as reference, were statistically significant and increased in order of increasing NFF in all cases, and in cases of stage I and II, even after the NFF classification was adjusted for HG, MSBR, pTNM stage, chemotherapy or hormone therapy (Tables IV, V and VI). On the other hand, IDCs with HG II or III, those with MSBR score 3 or 4/5 and those with pTNM stage II or III failed to show a significantly higher HR of tumor recurrence or death than the referent group (Tables IV, V and VI).

As for multivariate analyses between NFF and PI classifications, in all cases and cases of stages I and II, significantly higher HRs of tumor recurrence were observed in IDCs with NFF score 3. Significantly higher HRs of tumor recurrence or death were also observed in IDCs with high PI, but not in those with medium PI (Table VII).

In cases without lymph node metastasis, IDCs with NFF scores 2 and 3 showed significantly higher HRs of tumor recurrence than those with NFF scores 0 and 1 ($P<0.05$). In contrast, the HG, MSBR, pTNM and PI classifications failed to show any significant increase of HRs of tumor recurrence or death (Tables IV–VII). Similarly, in ER-positive cases, HR of tumor recurrence was statistically increased in order of increasing NFF classification,

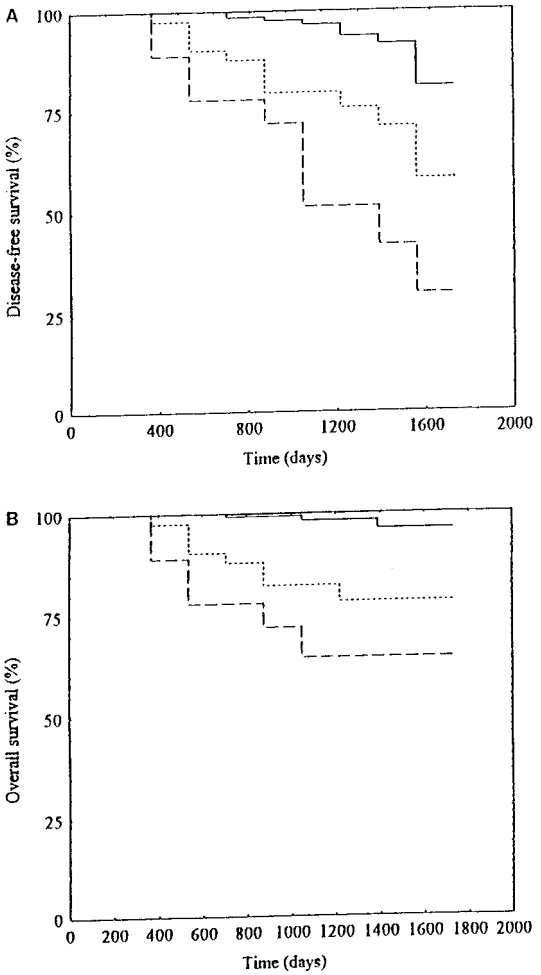

Fig. 6. Disease-free survival and overall survival curves by NFF classification. IDCs with NFF classification scores 0 and 1 show the longest disease-free and overall survival, and those with NFF classification score 3 have the shortest disease-free and overall survival. IDCs with NFF classification score 2 show intermediate disease-free survival or overall survival. —— NFF 0/1 ($n=142$), ····· NFF 2 ($n=41$), ---- NFF 3 ($n=18$).

Table IV. Multivariate Analyses of NFF and HG Classifications for Tumor Recurrence and Tumor Death of IDC in All Cases, Cases at Stages I/II, Those without Lymph Node Metastasis and Those that are ER-Positive

	Cases	TRR (%)	HR	95% CI	MR (%)	HR	95% CI
Model 1							
All cases							
NFF	201	36			18		
Score 0/1	142	12 (8)	1*		3 (2)	1*	
Score 2	41	13 (32)	2.2	0.2–5.0	8 (20)	6.3[b)]	1.5–26.9
Score 3	18	11 (61)	4.9[a)]	1.9–12.8	7 (39)	12.7[a)]	2.5–63.3
		P for trend: <0.001			*P* for trend: 0.002		
HG	201	36			18		
Grade I	48	2 (4)	1*		0	1*	
Grade II	98	12 (12)	1.5	0.3–7.2	5 (5)	1*	
Grade III	55	22 (40)	3.5	0.7–16.6	13 (24)	2.2	0.7–7.7
		P for trend: 0.063			*P* for trend: 0.142		
Chemotherapy	201	36			18		
No	88	8 (9)	1*		5 (6)	1*	
Yes	113	28 (25)	4.2[a)]	1.7–10.7	13 (12)	2.7	0.8–8.6
Hormone	201	36			18		
No	79	12 (15)	1*		9 (11)	1*	
Yes	122	24 (20)	0.5	0.2–1.0	9 (7)	0.3[b)]	0.1–0.9
Cases at stages I and II							
NFF	150	21			10		
Score 0/1	114	8 (7)	1*		1 (1)	1*	
Score 2	27	8 (30)	3.6[b)]	1.2–11.2	5 (19)	10.4[b)]	1.1–100.8
Score 3	9	5 (56)	4.5[b)]	1.2–18.7	4 (44)	13.1[b)]	1.3–134.6
		P for trend: 0.008			*P* for trend: 0.021		
HG	150	21			10		
Grade I	40	2 (5)	1*		0	1*	
Grade II	72	6 (8)	0.9	0.2–4.9	1 (1)	1*	
Grade III	38	13 (34)	2.3	0.4–12.5	9 (24)	8.3	0.9–76.3
		P for trend: 0.211			*P* for trend: 0.059		
Chemotherapy	150	21			10		
No	76	7 (9)	1*		4 (5)	1*	
Yes	74	14 (19)	3.0[b)]	1.1–8.6	6 (8)	2.3	0.6–9.3
Hormone	150	21			10		
No	67	8 (12)	1*		6 (9)	1*	
Yes	83	13 (16)	0.6	0.2–1.6	4 (5)	0.5	0.1–1.9
Cases without lymph node metastasis							
NFF	93	8					
Scores 0/1	76	2 (3)	1*				
Scores 2/3	17	6 (35)	10.8[b)]	1.3–91.5			
HG	93	8					
Grades I/II	70	3 (4)	1*				
Grade III	23	5 (22)	2.2	0.2–22.0			
Chemotherapy	93	8					
No	67	3 (5)	1*				
Yes	26	5 (19)	8.7[b)]	1.3–58.0			
Hormone	93	8					
No	58	5 (9)	1*				
Yes	35	3 (9)	0.6	0.1–3.6			
ER-Positive cases							
NFF	107	12					
Score 0/1	83	4 (5)	1*				
Score 2	14	3 (21)	2.6	0.4–15.5			
Score 3	10	5 (50)	8.0[c)]	1.0–68.5			
		P for trend: 0.021					

Table IV. (Continued)

	Cases	TRR (%)	HR	95% CI	MR (%)	HR	95% CI
HG	107	12					
Grade I	36	2 (6)	1*				
Grade II	49	4 (8)	0.8	0.1–5.3			
Grade III	22	6 (27)	2.4	0.2–26.9			
		P for trend: 0.497					
Chemotherapy	107	12					
No	48	2 (4)	1*				
Yes	59	10 (17)	15.9	0.6–433.7			
Hormone	107	12					
No	37	2 (5)	1*				
Yes	70	10 (14)	0.08	0.003–2.3			

NFF, nucleus-fibrotic focus-fat invasion; HG, histological grade; ER, estrogen receptor; TRR, tumor recurrence rate; MR, mortality rate; HR, hazard rate; CI, confidence interval; Hormone, hormone therapy; *, referent category (referent category of MR of HG, grade I/II). *P*-value: *a*) <0.01; *b*) <0.05.
Model 1: Each multivariate analysis adjusted for all variables listed on the table.

and NFF score 3 showed significantly higher HRs of tumor recurrence than NFF scores 0/1.

Sensitivities and specificities of each classification In cases without lymph node metastasis, NFF classification showed the highest sensitivity and specificity for predicting tumor recurrence (Table VIII). NFF classification also showed the highest sensitivity for tumor recurrence rate (TR) in ER-positive cases. In all cases, and cases at stages I and II, NFF, HG and MSBR showed almost equivalent sensitivity and specificity for tumor recurrence or death (Table VIII). The PI classification showed higher specificity for tumor recurrence or death than the NFF, HG and MSBR classifications in cases at stages I and II (Table VIII). The pTNM classification showed the lowest sensitivity for tumor recurrence or death in all cases, and the lowest specificity for tumor recurrence or death in cases of stages I and II (Table VIII).

DISCUSSION

This study clearly demonstrated that the NFF classification is superior to the HG, MSBR, pTNM and PI classifications in the prediction of the outcome for patients with IDCs. This suggests that each factor used in this classification has a strong effect on tumor progression or invasion.

It has been reported that nuclear atypia of IDC closely correlates with a patient's outcome.[28–30] Nuclear features probably reflect abnormality of genes or of their expression.[31–33] Therefore, the malignant potential of tumor cells may be reflected in their nuclear features.

The interaction between tumor cells and stromal cells is probably important for the formation of FF.[34] Tumor cell and stromal cell interaction has been shown to play a major role in tumor invasion and metastasis, and several factors are believed to participate in this interaction.[35–39] We have already demonstrated that a paracrine mechanism exists between basic fibroblast growth factor and fibroblast growth factor receptors on the tumor cells and the fibroblasts in FF, and this mechanism probably plays a key role in the formation of FF within IDCs.[34] Therefore, the formation of FF appears to be evidence of active tumor cell and stromal cell interaction, which also affects the malignant potential of IDCs, probably through growth stimulation of the tumor cells.

This study showed that ILFI is an important prognostic factor for tumor recurrence and death of patients with IDC. ILFI is probably determined by the degree of proliferative and/or invasive abilities of the tumor cells. Therefore, it is plausible that ILFI reflects the aggressive characteristics of tumor cells in IDC.

Considering the above circumstances, each factor of the NFF classification appears to represent not only the biological characteristics of the tumor cells themselves, but also the tumor and stromal cell interaction. This probably enhances the value of the NFF classification as a histological prognostic classification.

In this study, IDCs with an NFF score 2, an intermediate group, had significantly higher HRs of tumor recurrence or death than those with NFF score 1. In the HG, MSBR or PI classification, the cases were divided into three groups, but the intermediate group behaved similarly to the low risk group. Therefore, only the NFF classification was able to separate the intermediate group from the low-risk group. In addition, stratification of IDCs without lymph node metastasis or those that were ER-positive into low- and high-risk groups was possible only by using the NFF classification. The NFF classification also showed

Table V. Multivariate Analyses of NFF and MSBR Classifications for Tumor Recurrence and Tumor Death of IDC of All Cases, Cases at Stages I/II, Those without Lymph Node Metastasis and Those that are ER-Positive

	Cases	TRR (%)	HR	95% CI	MR (%)	HR	95% CI
Model 2							
All cases							
NFF	201	36			18		
Score 0/1	142	12 (8)	1*		3 (2)	1*	
Score 2	41	13 (32)	2.2	0.9–4.9	8 (20)	6.3[a)]	1.5–25.8
Score 3	18	11 (61)	4.8[a)]	1.9–11.7	7 (39)	11.8[b)]	2.5–56.7
		P for trend: <0.001			*P* for trend: 0.001		
MSBR	201	36			18		
Score 1/2	80	3 (4)	1*		0	1*	
Score 3	55	9 (16)	3.0	0.8–11.4	4 (7)	1*	
Score 4/5	66	24 (36)	5.0[c)]	1.4–17.7	14 (21)	2.6	0.7–9.2
		P for trend: 0.058			*P* for trend: 0.091		
Chemotherapy		28 (25)	3.9[b)]	1.7–9.6	13 (12)	2.7	0.8–8.5
Hormone		24 (20)	0.5	0.2–1.0	9 (7)	0.3[c)]	0.1–0.9
Cases at stage I and II							
NFF	150	21			10		
Score 0/1	114	8 (7)	1*		1 (1)	1*	
Score 2	27	8 (30)	3.6[c)]	1.3–10.2	5 (19)	13.1[c)]	1.4–123.3
Score 3	9	5 (56)	4.2[c)]	1.2–14.4	4 (44)	15.7[c)]	1.6–161.2
		P for trend: 0.011			*P* for trend: 0.012		
MSBR	150	21			10		
Score 1/2	61	2 (4)	1*		0	1*	
Score 3	41	4 (10)	2.3	0.4–12.9	1 (2)	1*	
Score 4/5	48	15 (31)	4.6	0.9–22.2	9 (19)	6.2	0.7–55.4
		P for trend: 0.078			*P* for trend: 0.046		
Chemotherapy		14 (19)	2.9[c)]	1.1–7.8	6 (8)	2.3	0.6–9.6
Hormone		13 (16)	0.6	0.2–1.6	4 (5)	0.4	0.1–1.6
Cases without lymph node metastasis							
NFF	93	8					
Scores 0/1	76	2 (3)	1*				
Scores 2/3	17	6 (35)	10.9[c)]	1.2–96.1			
MSBR	93	8					
Scores 1–3	65	3 (5)	1*				
Scores 4/5	28	5 (18)	1.4	0.2–9.2			
Chemotherapy		28 (19)	7.2[c)]	1.2–40.6			
Hormone		3 (9)	0.7	0.1–4.0			
ER-Positive cases							
NFF	107	12					
Score 0/1	83	4 (5)	1*				
Score 2	14	3 (21)	2.4	0.5–12.5			
Score 3	10	5 (50)	7.0[c)]	1.2–39.4			
		P for trend: 0.022					
MSBR	107	12					
Score 1/2	53	3 (6)	1*				
Score 3	28	2 (7)	1.2	0.1–5.3			
Score 4/5	26	7 (27)	4.5	0.7–30.6			
		P for trend: 0.342					
Chemotherapy		10 (17)	17.2	0.9–317.5			
Hormone		10 (14)	0.06	0.003–1.3			

NFF, nucleus-fibrotic focus-fat invasion; MSBR, modified Scarff-Bloom-Richardson; ER, estrogen receptor; TRR, tumor recurrence rate; MR, mortality rate; HR, hazard rate; CI, confidence interval; Hormone, hormone therapy; ∗, referent (referent category of MR of MSBR, score of 1–3); referent category of chemotherapy or hormone therapy, no. *P*-value: *a*) <0.001; *b*) <0.01; *c*) <0.05.
Model 2: Each multivariate analysis adjusted for all variables listed on the table.

Table VI. Multivariate Analyses of NFF and pTNM Classifications for Tumor Recurrence and Tumor Death of IDC of All Cases, Cases at Stages I/II, Those without Lymph Node Metastasis and Those that are ER-Positive

	Cases	TRR (%)	HR	95% CI	MR (%)	HR	95% CI
Model 3							
All cases							
NFF	201	36			18		
Score 0/1	142	12 (8)	1*		3 (2)	1*	
Score 2	41	13 (32)	2.6[c)]	1.1–5.8	8 (20)	7.9[b)]	2.0–30.8
Score 3	18	11 (61)	8.7[a)]	3.8–20.1	7 (39)	20.6[a)]	5.1–79.2
		P for trend: <0.001			*P* for trend: <0.001		
pTNM	198	36			18		
Stage I	47	3 (7)	1*		2 (4)	1*	
Stage II	103	18 (17)	2.9	0.7–12.7	8 (8)	2	0.3–13.9
Stage III	48	15 (31)	3.4	0.7–16.6	8 (17)	3.2	0.4–24.0
		P for trend: 0.818			*P* for trend: 0.849		
Chemotherapy		28 (25)	2.8[c)]	1.0–7.4	13 (12)	1.8	0.5–6.6
Hormone		24 (20)	0.3[b)]	0.2–0.8	9 (7)	0.2[b)]	0.1–0.7
Cases at stages I and II							
NFF	150	21			10		
Score 0/1	114	8 (7)	1*		1 (1)	1*	
Score 2	27	8 (30)	4.3[b)]	1.5–12.1	5 (19)	21.5[b)]	2.5–186.1
Score 3	9	5 (56)	7.7[a)]	2.3–23.5	4 (44)	41.6[a)]	4.3–385.3
		P for trend: 0.002			*P* for trend: 0.001		
pTNM	150	21			10		
Stage I	47	3 (6)	1*		2 (4)	1*	
Stage II	103	18 (18)	2.5	0.5–11.8	8 (8)	2.2	0.2–22.9
Chemotherapy		14 (19)	2.0	0.7–6.0	6 (8)	1.4	0.3–7.4
Hormone		13 (16)	0.5	0.2–1.3	4 (5)	0.3	0.08–1.3
Cases without lymph node metastasis							
NFF	93	8					
Score 0/1	76	2 (3)	1*				
Score 2/3	17	6 (35)	10.6[c)]	1.5–69.5			
pTNM	93	8					
Stage I	47	3 (6)	1*				
Stage II	46	5 (11)	3.7	0.2–60.5			
Chemotherapy		5 (19)	6.2[c)]	1.2–32.7			
Hormone		3 (9)	0.5	0.1–3.8			
ER-Positive cases							
NFF	107	12					
Score 0/1	83	4 (5)	1*				
Score 2	14	3 (21)	3.2	0.5–18.8			
Score 3	10	5 (50)	22.6[a)]	3.6–137.4			
		P for trend: 0.022					
pTNM	107	12					
Stage I	24	1 (4)	1*				
Stage II	57	6 (11)	2.0	0.1–33.2			
Stage III	25	5 (20)	1.0	0.1–21.3			
		P for trend: 0.873					
Chemotherapy		10 (17)	24.4	0.5–1143.2			
Hormone		10 (14)	0.04	0.0007–1.8			

NFF, nucleus-fibrotic focus-fat invasion; ER, estrogen receptor; TRR, tumor recurrence rate; MR, mortality rate; HR, hazard rate; CI, confidence interval; Hormone, hormone therapy; referent category of chemotherapy or hormone therapy, no; *, referent category. *P*-value: *a*) <0.001; *b*) <0.01; *c*) <0.05.
Model 3: Each multivariate analysis adjusted for all variables listed on the table.

Table VII. Multivariate Analyses of NFF and PI Classifications for Tumor Recurrence and Tumor Death of IDC of All Cases, Cases at Stages I/II, Those without Lymph Node Metastasis and Those that are ER-Positive

	Cases	TRR (%)	HR	95% CI	MR (%)	HR	95% CI
Model 4							
All cases							
NFF	201	36			18		
Score 0/1	142	12 (8)	1*		3 (2)	1*	
Score 2	41	13 (32)	1.8	0.8–4.1	8 (20)	4.7[c)]	1.3–18.8
Score 3	18	11 (61)	4.4[b)]	1.7–11.1	7 (39)	6.7[b)]	1.5–29.7
		P for trend: 0.002			*P* for trend: 0.015		
PI	196	35			17		
Low	56	2 (4)	1*		0	1*	
Medium	91	10 (10)	1.9	0.4–9.7	3 (3)	1*	
High	49	23 (47)	7.1[c)]	1.2–42.3	14 (29)	13.4[b)]	2.9–61.5
		P for trend: 0.004			*P* for trend: <0.001		
Chemotherapy		28 (25)	2.6	0.9–7.2	13 (12)	1.1	0.3–4.7
Hormone		24 (20)	0.3[b)]	0.1–0.7	9 (7)	0.2[b)]	0.07–0.5
Cases at stages I and II							
NFF	150	21			10		
Score 0/1	114	8 (7)	1*		1 (1)	1*	
Score 2	27	8 (30)	3.4	0.9–12.7	5 (19)	6.7	0.6–79.8
Score 3	9	5 (56)	3.5[c)]	1.1–10.9	4 (44)	34.6[b)]	2.9–415.4
		P for trend: 0.030			*P* for trend: 0.049		
PI	149	21			10		
Low	55	2 (4)	1*		0	1*	
Medium	73	8 (11)	1.7	0.3–9.9	3 (4)	1*	
High	21	11 (52)	10.6[c)]	1.5–74.9	7 (33)	86.4[a)]	6.5–1149.6
		P for trend: 0.002			*P* for trend: <0.001		
Chemotherapy		14 (19)	1.6	0.5–4.9	6 (8)	0.6	0.1–3.1
Hormone		13 (16)	0.4[b)]	0.1–1.1	4 (5)	0.05[c)]	0.004–0.6
Cases without lymph node metastasis							
NFF	93	8					
Score 0/1	76	2 (3)	1*				
Score 2/3	17	6 (35)	4.0[c)]	1.2–13.3			
PI	93	8					
Low	50	2 (4)	1*				
Medium	43	6 (14)	0.2	0.01–3.5			
Chemotherapy		5 (19)	17.5[c)]	2.0–153.8			
Hormone		3 (9)	0.2	0.04–1.4			
ER-Positive cases							
NFF	107	12					
Score 0/1	83	4 (5)	1*				
Score 2	14	3 (21)	2.4	0.4–15.4			
Score 3	10	5 (50)	15.5[b)]	2.1–105.2			
		P for trend: 0.005					
PI	105	12					
Low	36	2 (6)	1*				
Medium	47	4 (9)	1.1	0.2–14.0			
High	22	6 (27)	1.1	0.1–13.2			
		P for trend: 0.905					
Chemotherapy		10 (17)	21.1	0.4–936.0			
Hormone		10 (14)	0.06	0.001–2.5			

NFF, nucleus-fibrotic focus-fat invasion; PI, prognostic index; ER, estrogen receptor; TRR, tumor recurrence rate; MR, mortality rate; HR, hazard rate; CI, confidence interval; Hormone, hormone therapy; *, referent category (referent category of PI, low/medium); referent category of chemotherapy or hormone therapy, no. *P*-value: *a*) <0.001; *b*) <0.01; *c*) <0.05.
Model 4: Each multivariate analysis adjusted for all variables listed on the table.

Table VIII. Sensitivities and Specificities of Each Prognostic Classification

Classification	Cases					
	All		Stages I/II		LN(–)	ER(+)
	TR	MR	TR	MR	TR	TR
NFF (0/1 vs. 2/3)						
Sensitivity (%)	68	83	62	90	75	67
Specificity (%)	78	76	82	81	87	83
HG (I/II vs. III)						
Sensitivity (%)	61	72	62	90	63	50
Specificity (%)	80	77	81	79	79	83
MSBR (1–3 vs. 4/5)						
Sensitivity (%)	67	78	71	90	63	58
Specificity (%)	73	72	76	72	79	80
pTNM (All, ER-positive, I/II vs. III; LN(–), I vs. II)						
Sensitivity (%)	42	44	86	80	63	42
Specificity (%)	80	78	34	32	52	79
PI (All, Stages I/II, ER-positive, L/M vs. H; LN(–), L vs. M)						
Sensitivity (%)	66	82	52	70	75	50
Specificity (%)	84	80	92	90	57	83

NFF, nucleus-fibrotic focus-fat invasion; HG, histological grade; MSBR, modified Scarff-Bloom-Richardson; PI, prognostic index; LN(–), negative lymph node metastasis; ER(+), estrogen receptor-positive; L, low; M, medium; H, high; TR, tumor recurrence rate; MR, mortality rate.

the highest sensitivity and specificity for tumor recurrence in IDCs without lymph node metastasis, and the highest sensitivity in those that were ER-positive. Thus, the NFF classification is probably most suitable for further separating IDCs with the lowest malignant potential in the conventional sense into low- and high-risk groups.

The PI classification consists of tumor size, nodal status and HG, which is almost identical to a combination of pTNM stage and HG or MSBR. Therefore, it is believed that the PI classification is the most powerful histological prognostic classification for IDC. However, this study showed that the PI classification failed to stratify IDCs without lymph node metastasis into low- and high-risk groups. This suggests that nodal status is more important than tumor size or HG for the estimation of malignant potential of IDCs. Therefore, unlike the NFF classification, the usefulness of the PI classification as a prognostic indicator seems limited to IDCs, in which outcome depends on the nodal status. In addition, the PI classification failed to show a significant increase of HR of tumor recurrence with ER-positive IDCs. Therefore, the PI classification is not a suitable classification for predicting the outcome for patients with IDCs in the low-risk group.

The HG classification is based on structural and nuclear features, and the mitotic figures of tumor cells. The MSBR classification depends on the nuclear features and the mitotic figures of the tumor cells. Therefore, both classifications concentrate only on the cytological characteristics of the tumor cells. The NFF classification evaluates not only the cytological characteristics of the tumor cells, but also the status of tumor-stromal cell interaction and the invasive ability of the tumor cells. Therefore, the NFF classification probably extracts more information regarding the biological characteristics of IDCs than either the HG or MSBR classification. This should make the NFF classification superior to the other two classifications as a prognostic indicator.

After adjusting for the NFF classification in the multivariate analyses, the pTNM stage classification failed to correlate with tumor recurrence or death in the 4 populations analyzed: all cases, those at stages I and II, those without lymph node metastasis and those that were ER-positive. In addition, fluctuations of sensitivity or specificity for tumor recurrence or death were most frequently observed in this classification. Thus, this classification may not be suitable for the prediction of the short-term outcome for patients with IDC. However, this classification is used widely to estimate the benefit of adjuvant chemotherapy, hormone therapy or radiotherapy in post-operative patients with IDCs. This study has demonstrated the clear superiority of the NFF classification for prediction of the outcome for patients treated with post-operative chemotherapy or hormone therapy, or those with ER-positive IDCs. Therefore, if the NFF were to be combined with pTNM stage classification, the predictive ability might increase dramatically. Such a study seems worthwhile.

The ability of the NFF classification to predict long-term outcome for patients with IDCs has not been tested. However, nuclear atypia of the tumor cells and the presence of FF or fat invasion have been shown to be important prognostic factors for long-term survival.[15, 28–30, 40, 41] Therefore, it seems likely that the NFF classification is also a useful classification for predicting the long-term survival of patients with IDCs.

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