Table 3. FcγR polymorphisms and pathological responses to trastuzumab in an N setting

Polymorphism	Patients	Pathological response (grade)							
		1a		1b		2		3 (pCR)	
		No.	%	No.	%	No.	%	No.	%
FcγR2A									
H/H	7	0	0	0	0	2	29	5	71
H/R	6	1	17	3	50	2	33	0	0
R/R	2	1	50	0	0	1	50	0	0
Fisher's exact test: *P*				*0.015*					
Chi-square test (pCR versus others): *P*[a]				*0.007*					
Linear correlation test: *P*				*0.0076*					
ANOVA test: *P*				*0.0088*					
FcγR3A									
V/V	7	0	0	1	14	2	29	4	57
F/V	6	1	17	2	33	2	33	1	17
F/F	2	1	1	0	0	1	1	0	0
Fisher's exact test: *P*				*0.45*					
Chi-square test (pCR versus others): *P*[a]				*0.12*					
Linear correlation test: *P*				*0.069*					
ANOVA test: *P*				*0.16*					

[a]Comparison of H/H versus R carrier (H/R + R/R) or V/V versus F carrier (F/V + F/F).

N, neoadjuvant; FcγR, fragment C γ receptor; pCR, pathological complete response; ANOVA, analysis of variance.

Table 4. FcγR polymorphisms and tumor responses to trastuzumab in an M setting

Polymorphism	Patients	Response					
		CR/PR		SD		PD	
		No.	%	No.	%	No.	%
FcγR2A							
H/H	15	6	40	7	47	2	13
H/R	18	2	12	8	44	8	44
R/R	2	0	0	0	0	2	100
Fisher's exact test: *P*				*0.043*			
Chi-square test (CR/PR versus SD/PD): *P*[a]				*0.051*			
Linear correlation test: *P*				*0.0077*			
ANOVA test: *P*				*0.029*			
FcγR3A							
V/V	15	6	40	5	33	4	27
F/V	17	1	6	10	59	6	35
F/F	3	1	33	0	0	2	67
Fisher's exact test: *P*				*0.053*			
Chi-square test (CR/PR versus SD/PD): *P*[a]				*0.051*			
Linear correlation test: *P*				*0.12*			
ANOVA test: *P*				*0.16*			

[a]Comparison between H/H versus R carrier (H/R + R/R) or V/V versus F carrier (F/V + F/F).

M, metastatic; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; FcγR, fragment C γ receptor; ANOVA, analysis of variance.

The median PFS time was 6.4 months (95% CI, 3.9–8.6 months). The PFS of patients with FcγR2A-131 H/H was significantly longer than that of patients with 131 H/R or R/R. (Figure 1A: 9.2 versus 3.5 months, $P = 0.034$). In contrast, no statistical difference in the PFS of patients with FcγR3A-158 V/V and that of patients with 158 F/V or F/F was observed (Figure 1B: 8.5 versus 5.3 months, $P = 0.37$). Linkage disequilibrium analyses were conducted among the two FcγR polymorphisms (Table 5). The incidence of the FcγR2A-131 genotype was associated with that of the FcγR3A-158 genotype according to a Fisher's exact test, a chi-square test, and a linear correlation test.

discussion

The overexpression of HER2 protein is observed in ~20–30% of patients with breast cancer and is correlated with a poor clinical outcome. Trastuzumab is an IgG1-type humanized HER2 mAb that has been shown to exhibit significant clinical efficacy as a treatment of MBC [27] and as an adjuvant treatment of operable breast cancer [28]. However, the clinical effectiveness of trastuzumab is somewhat limited: the response rate to single-agent trastuzumab as a first-line treatment is 20–30%; the pCR rate to neoadjuvant therapy including trastuzumab is ~30%. A substantial numbers of HER2-positive tumors exhibit *de novo* resistance to trastuzumab; therefore, the development of biomarkers to select patients who might benefit from trastuzumab is warranted, as a way of decreasing toxicity and reducing unnecessary cost.

The principal mechanism of action of trastuzumab is HER2 blockade with the inactivation of the signal transduction pathway, leading to apoptosis. ADCC is another not insignificant and generally accepted mechanism of trastuzumab action. In ADCC, the cytotoxicity of mAbs that target tumor cells, is mediated by immune effector cells that express FcγR. Recently, two FcγR gene polymorphisms have been identified that affect the binding affinity of IgG, thus changing the effectiveness of ADCC and affecting tumor response. FcγR3A-158 V/V, either alone or in combination with the FcγR2A-131 H/H genotype, was significantly associated with a better response and PFS among patients with follicular lyoma [5, 6] and among MBC patients [14] treated with rituximab- or trastuzumab-based therapy, respectively. Inconsistent data have been reported in metastatic colorectal cancer patients who had not responded to previous irinotecan- or oxaliplatin-based therapy and were subsequently treated with single-agent cetuximab [29]. The

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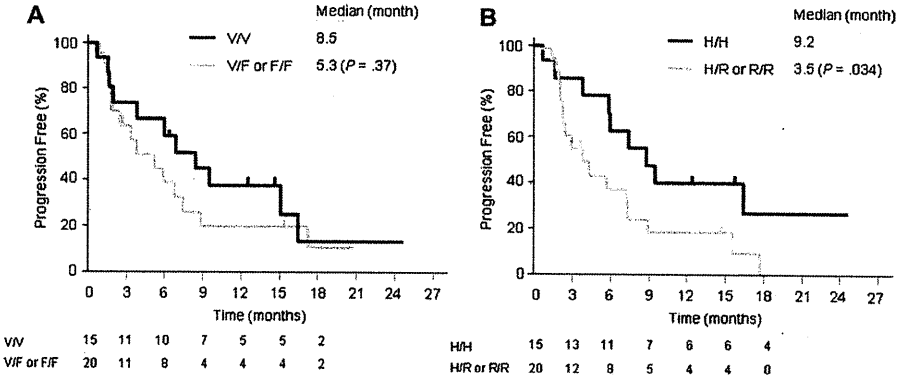

Figure 1. Progression-free survival for patients with metastatic breast cancer receiving single-agent trastuzumab categorized according to fragment C γ receptor (FcγR) polymorphisms. (A) Progression-free survival (PFS) curves were plotted for FcγR2A-131 H/H and H/R or R/R carriers. (B) PFS curves were plotted for FcγR3A-158 V/V and F/V or F/F carriers. V, valine allele; F, phenylalanine allele; H, histidine allele; R, arginine allele.

Table 5. Linkage analysis between FcγR2A and 3A alleles

	Patients	FcγR3A					
		V/V		F/V		F/F	
		No.	%	No.	%	No.	%
FcγR2A							
H/H	22	13	59	9	41	0	0
H/R	24	8	33	13	54	3	13
R/R	4	1	25	1	25	2	50
Total	50	22	44	23	46	5	10
Fisher's exact test: *P*				*0.030*			
Chi-square test (V/V versus F carrier) *P*[a]				*0.020*			
Linear correlation test: *P*				*0.0067*			

[a]Comparison between H/H versus R carrier.
FcγR, fragment C γ receptor.

influence of cytotoxic agents in combination with antibody therapy or the retrospective natures of these analyses might explain the previous inconsistencies. In the present prospective study, the FcγR2A-131 H/H genotype was significantly associated with a stronger tumor response and a longer PFS, and the FcγR3A-158 V/V genotype tended to be correlated with the tumor response after single-agent trastuzumab therapy.

Metastatic cancer patients mostly have suppressed immune function. Thus, early-stage breast patients treated with trastuzumab might be more sensitive to ADCC activity. In the current study, we have demonstrated for the first time that the FCγR2A-131 H/H genotype was significantly correlated with the pathological response after neoadjuvant trastuzumab-based treatment. Our data suggest that this genotype was correlated with not only the pCR rate but also the gradation of the response based on a precise assessment of pathological responses using established histopathological criteria (grade 1a–3; Table 3). A recent large adjuvant trial with trastuzumab-based therapy [15] has raised questions regarding the usefulness of the two FcγR SNPs as predictive biomarkers for recurrence. The sample size of this trial was relatively large; thus, the results seemed to be confirmatory. However, one possible explanation for this difference is that ADCC might be influenced by the existence of a target tumor volume. Another possible explanation is that the cytotoxic agents might influence outcome. Theoretically, the clinical efficacy of trastuzumab is based on both the direct blockade of signal transduction and its indirect effect, ADCC. On the other hand, the efficacy of cytotoxic agents is based on the direct DNA damaging effect and not on ADCC. Thus, the change in ADCC induced by different SNPs might be diluted in cases where trastuzumab and cytotoxic agents are combined, with cases in trastuzumab is used singly.

In this study, we examined 384 SNPs at FcγRI, RII, RIII, HER2 and FUT8 loci. Our findings demonstrated that only two SNP hot spots were correlated with the clinical efficacy of trastuzumab, indicating a high specificity. Our finding that the incidences of the two FCγR2A-131 H/H and FcγR3A-158 V/V genotypes were moderately linked with each other is inconsistent with a previous report [14]. One possible explanation for this discrepancy might be ethnical differences in SNP frequency. Zhang et al. [29] showed that the FCγR2A-131 H/H and FcγR3A-158 V/V genotypes were more frequent among Asian populations than among Western populations. Statistical approaches, including a linear correlation or ANOVA test, suggested that heterozygosity for the two SNPs might have a minimal effect on ADCC activity. Additional studies evaluating the relationship between ADCC activity and the SNP status are needed.

In conclusion, this study supports the hypothesis that FcγR polymorphisms play a role in trastuzumab-mediated ADCC and can predict the clinical outcome of patients with both early and MBC in Asian populations.

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disclosure

The authors declare no conflict of interest.

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Prognostic Factors in Young Japanese Women with Breast Cancer: Prognostic Value of Age at Diagnosis

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Objective: The primary objective of this study was to verify whether breast cancer patients aged <35 at diagnosis have poorer prognoses than those aged 35–39, in other words, to identify the prognostic value of age in younger premenopausal patients under 40 years old. The secondary objective was to assess prognostic factors specific for younger premenopausal patients.

Methods: We identified 242 consecutive patients who were diagnosed with stage I–III breast cancer before the age of 40 and underwent surgery between 1990 and 2004. We compared disease-free survival and overall survival in patients aged <35 years and those aged 35–39 years, and evaluated clinicopathological factors associated with disease-free survival or overall survival in each age group and in all patients under the age of 40.

Results: Ninety-nine (41%) patients were younger than 35 years and 143 (59%) were between 35 and 39 years. No significant difference in disease-free survival or overall survival was found between the two groups. In our cohort of patients under the age of 40, the independent factors associated with poor disease-free survival and overall survival included positive axillary lymph nodes and triple-negative status, but not age at diagnosis. Adverse prognostic factors also did not differ considerably between the two age groups.

Conclusions: Age at diagnosis was not an independent prognostic factor in our study. Our findings suggest that other clinicopathological features rather than age should be used to determine individualized treatment courses for breast cancer patients younger than 40 years.

Key words: breast cancer – young – disease-free survival – overall survival

INTRODUCTION

Many studies have reported that younger women with primary breast cancer have poorer prognoses than older women. The St Gallen international expert consensus reports from 1998 to 2007 concluded the age of <35 years was a high-risk factor for relapse in node-negative breast cancer patients and recommended adjuvant chemotherapy for most young women with breast cancer (1–5). However, the decision regarding chemotherapy in young patients must be made after taking into consideration not only the risk of relapse but also the age-specific problems caused by chemotherapy such as infertility, bone loss and changes in sexual function and appearance.

The cutoff value for classifying a patient as 'young' varies among studies and it is unclear whether the age of <35 years at diagnosis was an appropriate threshold to identify patients with primary breast cancer at high risk of relapse. It also remains to be determined whether Japanese patients aged <35 years at diagnosis have poorer prognoses since there have been few reports focusing on young Japanese women with breast cancer.

Prognostic factors in younger patients with primary breast cancer have been recently identified, but are not yet well

understood. A recent study showed that gene expression profile was a powerful predictor of disease outcome in young patients with breast cancer, but age was not an independent prognostic factor (6).

Gene expression profiling has identified intrinsic breast cancer subtypes that predict distinct clinical outcomes (7,8). In particular, triple-negative breast cancer, defined by the lack of expression of estrogen receptor (ER), progesterone receptor (PgR) and human epidermal growth factor receptor 2 (HER2), is known to be a subtype associated with poor clinical outcome. A high prevalence of triple-negative breast cancer has been reported to contribute to the poor prognosis of young African American women with breast cancer (9).

The primary objective of this study was to verify whether breast cancer patients aged <35 at diagnosis have poorer prognoses than those aged 35–39, in other words, to identify the prognostic value of age in younger premenopausal patients under 40 years old. The secondary objective was to assess the prognostic factors specific for younger premenopausal patients.

PATIENTS AND METHODS

Patients and Treatment

From the database of the National Cancer Center Hospital, Tokyo, Japan, we identified consecutive patients who were diagnosed with breast cancer before the age of 40 years and underwent surgery between January 1990 and December 2004. Only patients with stage I–III disease who underwent definitive surgery were included. Patients who had undergone preoperative adjuvant therapy or had excisional biopsy in a local clinic were also excluded because it is difficult to determine pathological factors influencing prognoses.

The complete medical records of patients enrolled in the study were reviewed. Information derived from the database and medical records included clinical and histological variables such as age; family history; pT (primary tumor) and pN (regional lymph node) status; histological type; histological grade; peritumoral vessel invasion (PVI) [including lymphatic vessel invasion (LVI) and blood vessel invasion (BVI)]; ER, PgR and HER2; tumor subtype stratified by hormone receptor (HR) and HER2 status; operative procedure; radiation therapy; adjuvant systemic therapy (chemotherapy and endocrine therapy).

Familial breast cancer (that does not fit hereditary breast cancer definition) was defined as breast cancer with a family history of one or more first- or second-degree relatives with breast cancer prior to or at the time of the patient's initial diagnosis (10,11). In all cases, pT and pN status were assessed according to the UICC TNM classification (6th edition) (12). Histological grade was evaluated according to Elston and Ellis (13). ER and PgR expression were determined by enzyme immunoassay or immunohistochemistry (IHC) (threshold for positivity: staining in more than 10% of tumor cells) (14). The definition of HER2 positive was a score 3+ by IHC (uniform, intense membrane staining in more than 10% of invasive cancer cells) and/or a 2.0 or higher of HER2/CEP17 (centromere probe chromosome 17) ratio by fluorescence *in situ* hybridization (15). On the basis of the expression profile of HR and HER2, all tumors were categorized into one of the four subtypes: HR+HER2−, HR+HER2+, HR−HER2+, HR−HER2− (triple-negative). HR-positive status (HR+) was defined as ER and/or PgR positivity, and HR-negative status (HR−) was defined as ER and PgR negativity. PVI was determined by the presence of tumor emboli within peritumoral endothelial-lined spaces and was assessed on hematoxylin and eosin-stained slides by making a distinction between lymphatic and blood vessels. LVI was graded as absent, focal to moderate (one to five foci of tumor thrombi in all the tumor specimens examined) or extensive (more than five foci of tumor thrombi in all the tumor specimens examined) (16). BVI was classified as either absent or present.

All patients received clinically necessary local treatment (breast-conserving surgery or mastectomy) in addition to sentinel node biopsy or complete axillary dissection. Postoperative breast irradiation was indicated for all patients who underwent breast-conserving surgery. After 1999, patients with pT3 presentation who had undergone mastectomy received postoperative radiation to the chest wall. Patients with four or more metastatic axillary lymph nodes received postoperative radiation to the axillary and supraclavicular regions. Adjuvant chemotherapy was followed by radiotherapy for all indicated patients. The adjuvant chemotherapy regimen widely used prior to 1993 comprised doxorubicin, cyclophosphamide (AC), methotrexate and 5-fluorouracil. After 1993, patients generally received four cycles of intravenous doxorubicin and AC. After 1999, high-risk patients received AC followed by taxane (docetaxel or paclitaxel). For women with endocrine-responsive disease aged <40 years, adjuvant endocrine therapy was indicated, such as tamoxifen for 2–5 years or the combination of tamoxifen for 5 years plus gonadotropin-releasing hormone analogues for at least 2 years. Patients who received adjuvant chemotherapy for endocrine-responsive disease were treated with tamoxifen immediately after the completion of chemotherapy.

Patients were followed up every 3–6 months during the first 5 years and every 6–12 months from 5 to 10 years. In addition to physical examination, annual mammography with or without breast ultrasound was performed for 10 years. Blood tests including two tumor markers (carcinoembryonic antigen and cancer antigen 15-3), chest X-ray, abdominal ultrasonography and bone scintigraphy were performed when the patients complained of any symptoms and/or tumor recurrence was suspected.

The study was conducted with support from the Health and Science Grants for Clinical Research in Cancer, as part of the investigations directed by the Ministry of Health, Labor and Welfare of Japan. The data on which the study was based were obtained in the course of daily clinical

practice and no additional burdens were imposed on patients. Hence, ethical approval was not required.

Statistical Methods

The χ^2 test (Pearson statistic) was used to determine the differences in clinical and pathological factors between two groups of patients. A P value of <0.01 was considered statistically significant.

The follow-up duration was calculated as the length of time between the date of diagnosis and the date of death or last contact. Disease-free survival (DFS) was defined as the time from surgical resection to the first of any of the following events: locoregional relapse, distant relapse, second primary breast cancer, any second (non-breast) malignancy or death from any cause. Locoregional relapse was defined as the reappearance of cancer in the ipsilateral breast, chest wall or regional lymph nodes. We classified distant relapse into two categories depending on metastatic sites: non-visceral (soft-tissue and/or bone) or visceral (including lung, liver, brain and other organs). Overall survival (OS) was defined as the time from surgical resection to death due to any cause, regardless of recurrence. DFS and OS curves were drawn by the Kaplan–Meier method and were compared among patient subsets using the log-rank test.

In univariate analyses, the following prognostic factors were evaluated for their potential associations with DFS and OS: age at the time of diagnosis, familial breast cancer, pT, pN, histological type, histological grade, LVI, BVI, tumor subtype stratified by HR and HER2 status, operative procedure, administration of radiation therapy and adjuvant systemic therapy. ER, PgR and HER2 were excluded from the prognostic analyses for DFS and OS because these factors are closely related to tumor subtype. Multivariate analysis of potential prognostic factors was performed to generate a Cox proportional hazards model. Multivariate models were created using age at diagnosis and other variables that showed significant association ($P < 0.01$) with DFS or OS on univariate analysis. All tests were two-tailed, with $P < 0.01$ being taken as an indicator of statistical significance. The statistical software SPSS version 12.0 (SPSS, Inc., Chicago, IL, USA) was used for all statistical analyses.

RESULTS

Patient Characteristics

Out of a total of 3944 patients who underwent surgery at the National Cancer Center Hospital, Tokyo, Japan, between January 1990 and December 2004, 242 patients were eligible for this study. Of which 99 (41.0%) were aged <35 years at diagnosis, and 143 (59.0%) were aged between 35 and 39 years (Table 1). The median age at diagnosis was 36 years (range 22–39 years). The distribution of various clinicopathological factors did not differ significantly between the two age groups. PgR positivity was observed in a higher percentage of patients aged 35–39 years than in those aged <35 years, but the proportion of patients falling into each of these four tumor subtypes did not differ significantly between the two groups. Sixty-nine percent of the 242 patients were classified as HR+HER2−, 10.3% were HR+HER2+, 5.8% were HR−HER2+ and 14.9% were HR−HER2− (triple-negative).

During a median follow-up of 80 months (range 5–186 months), 86 patients (35.5%) experienced DFS events [second primary breast cancer 3.7%; locoregional relapse 7.4%; distant relapse 24.4% (non-visceral 8.7%; visceral 15.7%)] and 51 patients (21.1%) died. No significant difference was found in DFS and OS between patients aged <35 years and those aged 35–39 years (Fig. 1). We did not also find a significant difference in frequency of occurrence of various DFS events between the two age groups (Table 1).

Univariate Analyses

For breast cancer patients under 40 years old, univariate analyses showed that significant adverse factors associated with both DFS and OS included higher T stage (pT3–4), positive lymph nodes (pN1–3), grade 3, extensive LVI, BVI, triple-negative status and adjuvant chemotherapy (Tables 2 and 3). With regard to adjuvant chemotherapy, patients who were treated with chemotherapy had significantly worse DFS and OS. No significant difference in survival was observed between the familial breast cancer group and the non-familial group.

Multivariate Analyses

For all patients under the age of 40, multivariate analyses identified positive axillary lymph nodes (pN1–pN3) and triple-negative status as independent factors associated with poor DFS and OS (Tables 2 and 3, and Fig. 2). Age, represented as either a categorical or a continuous variable, was not an independent prognostic factor in multivariate analyses. The independent factors negatively influencing DFS included pN1 (hazard ratio 3.69, 95% CI 1.61–8.47), pN2–pN3 (hazard ratio 6.55, 95% CI 2.72–15.75) and triple-negative status (hazard ratio 2.45, 95% CI 1.37–4.36). The independent adverse factors affecting OS included pN1 (hazard ratio 6.00, 95% CI 1.77–20.35), pN2–pN3 (hazard ratio 7.95, 95% CI 2.31–27.37), the presence of BVI (hazard ratio 2.88, 95% CI 1.35–6.13) and triple-negative status (hazard ratio 4.25, 95% CI 2.08–8.72).

For patients aged <35, multivariate analyses indicated that positive axillary lymph nodes (pN1–pN3) and triple-negative status were the independent factors associated with poor DFS and OS (Table 4). For those aged 35–39, triple-negative status was the only independent adverse prognostic factor identified. Axillary lymph node status was not found to be an independent factor, probably due to the

Table 1. Clinicopathological characteristics of breast cancer patients under 40 years old ($n = 242$)

Variable	All patients		Aged <35		Aged 35–39		P^a
	($n = 242$)	(%)	($n = 99$)	(%)	($n = 143$)	(%)	
Familial breast cancer							
No	192	79.3	78	78.8	114	79.7	
Yes	50	20.7	21	21.2	29	20.3	0.492
Primary tumor							
pT1	25	10.3	11	11.1	14	9.8	
pT2	78	32.2	37	37.4	41	28.7	
pT3	112	46.3	40	40.4	72	50.3	
pT4	27	11.1	11	11.1	16	11.2	0.436
Regional lymph node							
pN0	127	52.5	55	55.5	72	50.3	
pN1	65	26.9	21	21.2	44	30.8	
pN2	34	14.0	16	16.2	18	12.6	
pN3	16	6.6	7	7.1	9	6.3	0.411
Histological type							
Invasive ductal carcinoma	221	91.3	95	96.0	126	88.1	
Invasive lobular carcinoma	5	2.1	1	1.0	4	2.8	
Others	16	6.6	3	3.0	13	9.0	0.103
Histological grade							
Grade 1	14	5.8	3	3.0	11	7.7	
Grade 2	84	34.8	31	31.3	53	37.1	
Grade 3	144	59.5	65	65.7	79	55.2	0.148
Lymph vessel invasion							
Absent	98	40.5	46	46.5	52	36.4	
Focal–moderate	135	55.8	50	50.5	85	59.4	
Extensive	9	3.7	3	3.0	6	4.2	0.283
Blood vessel invasion							
Absent	222	91.7	89	89.9	133	93.0	
Present	20	8.3	10	10.1	10	7.0	0.264
Estrogen receptor							
Negative	78	32.2	35	35.4	43	30.1	
Positive	164	67.8	64	64.6	100	69.9	0.234
Progesterone receptor							
Negative	63	26.0	34	34.3	29	20.3	
Positive	179	74.0	65	65.7	114	79.7	0.014
HER2 receptor							
Negative	203	83.9	81	81.8	122	85.3	
Positive	39	16.1	18	18.2	21	14.7	0.290
Subtype							
HR+HER2−	167	69.0	61	61.6	106	74.1	
HR+HER2+	25	10.3	11	11.1	14	9.8	
HR−HER2+	14	5.8	7	7.0	7	4.9	

Continued

Table 1. *Continued*

Variable	All patients		Aged <35		Aged 35–39		P^a
	($n = 242$)	(%)	($n = 99$)	(%)	($n = 143$)	(%)	
HR−HER2− (triple-negative)	36	14.9	20	20.3	16	11.2	0.165
Operative procedure							
Breast-conserving surgery	87	36.0	40	40.4	47	32.9	
Mastectomy	155	64.0	59	59.6	96	67.1	0.230
Radiation							
No	163	67.4	66	66.6	97	67.8	
Yes	79	32.6	33	33.3	46	32.2	0.479
Adjuvant endocrine therapy							
No	84	34.7	38	38.4	46	32.2	
Yes	158	65.3	61	61.6	97	67.8	0.318
Adjuvant chemotherapy							
No	89	36.8	35	35.4	54	37.8	
Yes	153	63.2	64	64.6	89	62.2	0.702
DFS event							
None	156	64.5	68	68.7	88	61.5	
Second primary breast cancer	9	3.7	4	4.0	5	3.5	
Locoregional relapse	18	7.4	8	8.1	10	7.0	
Distant relapse—non-visceral	21	8.7	5	5.1	16	11.2	
Distant relapse—visceral	38	15.7	14	14.1	24	16.8	0.493

[a]χ^2 test.
HR, hormone receptor; DFS, disease-free survival.

subtraction of LVI and BVI, which significantly correlate with positive axillary lymph nodes (Table 4).

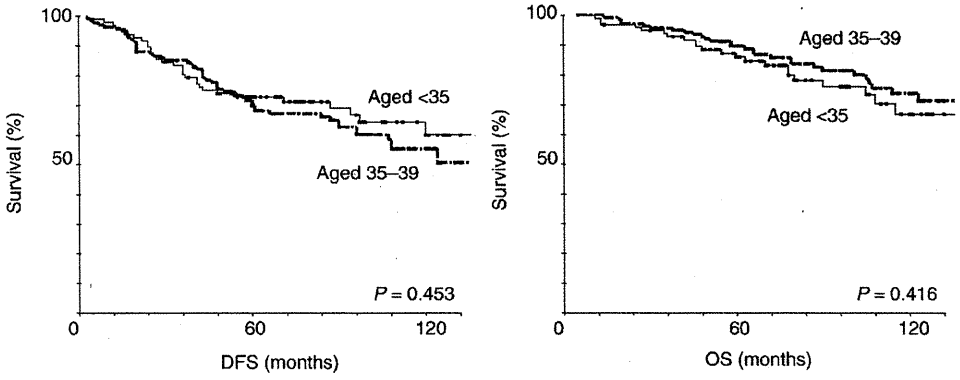
DISCUSSION

Although being 'young' has been reported to be a predictor of poor prognosis independent of other known factors (17–21), the definition of 'young' has varied across studies. The age of 35 years has been used as a cutoff age based on consensus in the international guidelines for treatment of primary breast cancer (1–5). However, the St Gallen international expert consensus panel discontinued the use of the threshold of 35 years of age as a risk category in 2009 (22).

The primary objective of this study was to verify whether breast cancer patients aged <35 at diagnosis have poorer prognoses than those aged 35–39 or to identify the prognostic value of age in younger premenopausal patients under 40 years old. Our results did not indicate any significant differences between patients aged <35 years and those aged 35–39 years in either DFS or OS, and age at diagnosis was not an independent factor associated with DFS or OS in our cohort of breast cancer patients younger than 40 years. We believe that these observations are reliable because the distribution of various clinical and pathological factors did not differ significantly between the two age groups.

A population-based study in Switzerland found no effect of young age on survival when accounting for breast tumor characteristics and treatment (23). A study by van de Vijver et al. (6) also demonstrated that, whereas gene-expression profile was a powerful predictor of disease outcome in younger women with breast cancer, age was not an independent prognostic factor. Younger premenopausal women have been reported to more frequently present with breast cancer marked by poor prognostic features such as higher T stage, positive lymph nodes, endocrine non-responsiveness, high grade, extensive PVI and high proliferating fraction than older premenopausal women (24–29). Kollias et al. (25) concluded that age itself had no influence on the prognosis of individuals because the association of poor prognosis with young age at diagnosis could be explained by a higher proportion of aggressive tumors.

Our present study of breast cancer patients under the age of 40 supports these observations and we consider that the age of <35 years at diagnosis is an unreasonable threshold to identify patients with primary breast cancer at high risk of relapse.



	5-year survival	10-year survival
Aged <35	72.9%	60.2%
Aged 35–39	70.1%	55.3%

	5-year survival	10-year survival
Aged <35	86.1%	66.9%
Aged 35–39	89.7%	73.9%

Figure 1. Kaplan–Meier curves of disease-free survival (DFS) and overall survival (OS) compared between breast cancer patients aged <35 years ($n = 99$) and aged 35–39 years ($n = 143$).

Table 2. Univariate and multivariate analyses of clinicopathological factors associated with disease-free survival in breast cancer patients under 40 years old ($n = 242$)

Variable	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	P^a	Hazard ratio	95% CI	P^a
Age						
<35	1	–	–	1	–	–
35–39	1.18	0.76–1.84	0.455	1.27	0.80–2.02	0.320
Regional lymph node						
pN0	1	–	–	1	–	–
pN1	2.93	1.69–5.10	<0.001	3.69	1.61–8.47	0.002
pN2–3	6.23	3.67–10.57	<0.001	6.55	2.72–15.75	<0.001
Lymph vessel invasion						
Absent	1	–	–	1	–	–
Focal–moderate	3.32	1.86–5.90	<0.001	2.29	1.19–4.38	0.013
Extensive	4.90	2.64–9.11	<0.001	2.10	0.95–4.65	0.066
Blood vessel invasion						
Absent	1	–	–	1	–	–
Present	3.90	2.23–6.84	<0.001	1.99	1.05–3.78	0.034
Subtype						
HR+HER2–	1	–	–	1	–	–
HR+HER2+	1.22	0.62–2.40	0.559	1.12	0.53–2.36	0.768
HR–HER2+	0.89	0.32–2.46	0.822	1.11	0.39–3.15	0.847
HR–HER2– (triple-negative)	2.16	1.25–3.73	0.006	2.45	1.37–4.36	0.002

95% CI, 95% confidence interval; HR, hormone receptor.
[a]Cox proportional hazards model.

Table 3. Univariate and multivariate analyses of clinicopathological factors associated with overall survival in breast cancer patients under 40 years old ($n = 242$)

Variable	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	P^a	Hazard ratio	95% CI	P^a
Age						
<35	1	–	–	1	–	–
35–39	0.80	0.46–1.34	0.418	0.86	0.47–1.57	0.617
Regional lymph node						
pN0	1	–	–	1	–	–
pN1	4.90	2.13–11.28	<0.001	6.00	1.77–20.35	0.004
pN2–3	10.47	4.72–23.24	<0.001	7.95	2.31–27.37	0.001
Lymph vessel invasion						
Absent	1	–	–	1	–	–
Focal–moderate	4.22	1.82–9.77	0.001	2.41	0.98–5.98	0.057
Extensive	7.71	3.23–18.41	<0.001	2.80	0.95– 8.26	0.063
Blood vessel invasion						
Absent	1	–	–	1	–	–
Present	5.69	3.02–10.73	0.077	2.88	1.35–6.13	0.006
Subtype						
HR+HER2–	1	–	–	1	–	–
HR+HER2+	0.92	0.32–2.62	0.876	0.73	0.24–2.22	0.584
HR–HER2+	1.33	0.41–4.38	0.636	1.64	0.46–5.85	0.445
HR–HER2– (triple-negative)	3.65	1.92–6.95	<0.001	4.25	2.08–8.72	<0.001

95% CI, 95% confidence interval; HR, hormone receptor.
[a]Cox proportional hazards model.

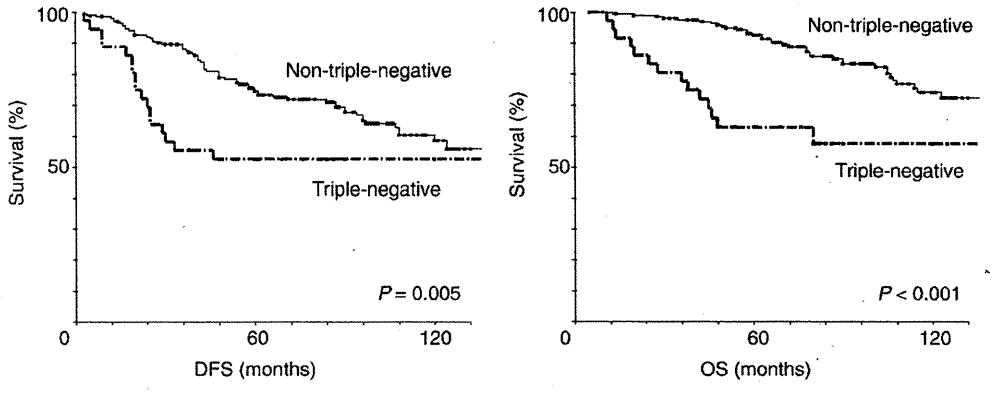
In contrast to our findings, de la Rochefordiere et al. (19) reported that, in a series of 1703 patients from a single institution, the relationship between recurrence hazard and age was best fitted by a log-linear function that indicated a 4% decrease in recurrence and a 2% decrease in death for every year of age in premenopausal women. Han and Kang also recently reported that in patients younger than 35 years, the risk of death rose by 5% for every year of decrease in age, whereas death risk did not vary significantly with age in patients aged 35 years or older (30).

What is more, our unpublished data confirms that breast cancer patients aged <40 years have poorer DFS than those aged 41–49 years (5-year DFS: 79 vs. 86%, $P = 0.04$), while no significant difference was found in OS (5-year OS: 86 vs. 90%, $P = 0.2$). However, there were a much greater number of patients aged 41–49 years compared with those aged <40 years, and the difference in sample number between the two groups was beyond the allowed limit. Therefore, we limited ourselves only to calculating DFS and OS for patients between 40 and 49 years of age. Anders et al. (31) documented similar findings that survival rate in patients who were diagnosed before the age of 40 years was worse when compared with that in older women.

These results indicate that age does have some impact on long-term outcome of patients. Our report and unpublished data suggest that other clinicopathological features rather than age at diagnosis should be used to determine individualized treatment courses for breast cancer patients under 40 years old, but not across all age groups. Further analyses are needed in order to assess the prognostic value of age at diagnosis in women with primary breast cancer across all age groups. However, this can still be a significant finding given that women are now commonly bearing children at older ages in Japan.

Our secondary objective in this study was to assess prognostic factors specific for younger premenopausal women with primary breast cancer. We found that the most important factors associated with poor DFS and OS in patients under the age of 40 were positive axillary lymph nodes (pN1–pN3) and triple-negative status. Triple-negative status was also an independent factor associated with worse DFS and OS in both age groups.

Previous studies have identified axillary lymph node status, HR and HER2 status, tumor size, histological grade, operative procedure, radiation therapy, adjuvant systemic therapy, family history of ovarian cancer and age <35 or 40



	5-year survival	10-year survival
Non-triple-negative	74.5%	58.8%
Triple-negative	52.6%	52.6%

	5-year survival	10-year survival
Non-triple-negative	92.7%	74.3%
Triple-negative	63.0%	57.8%

Figure 2. Kaplan–Meier curves of DFS and OS compared between triple-negative breast cancer patients ($n = 36$) and breast cancer patients whose tumors fall into one of the other three subtypes (non-triple-negative; $n = 206$).

Table 4. Multivariate analyses of clinicopathological factors associated with disease-free survival and overall survival for the two age groups; aged <35 vs. aged 35–39

Variable	Disease-free survival						Overall survival					
	Aged <35 ($n = 99$)			Aged 35–39 ($n = 143$)			Aged <35 ($n = 99$)			Aged 35–39 ($n = 143$)		
	Hazard ratio	95% CI	P^a	Hazard ratio	95% CI	P^a	Hazard ratio	95% CI	P^a	Hazard ratio	95% CI	P^a
Regional lymph node												
pN0	1	–	–	1	–	–	1	–	–	1	–	–
pN1	18.64	3.36–103.30	0.001	1.43	0.52–3.94	0.489	56.57	7.74–413.30	<0.001	1.30	0.32–5.37	0.715
pN2–3	11.86	1.95–72.00	0.007	3.72	1.28–10.83	0.016	52.95	5.55–505.71	0.001	1.94	0.46–8.25	0.368
Lymph vessel invasion												
Absent	1	–	–	1	–	–	1	–	–	1	–	–
Focal–moderate	1.82	0.62–5.32	0.277	2.32	1.00–5.40	0.051	0.86	0.24–3.12	0.816	6.06	1.22–30.10	0.028
Extensive	3.36	0.76–14.83	0.110	2.04	0.75–5.51	0.162	2.69	0.50–14.55	0.250	4.49	0.83–24.42	0.082
Blood vessel invasion												
Absent	1	–	–	1	–	–	1	–	–	1	–	–
Present	2.57	0.90–7.29	0.077	1.32	0.53–3.32	0.555	2.75	0.81–9.32	0.104	3.28	1.07–10.06	0.037
Subtype												
HR+HER2–	1	–	–	1	–	–	1	–	–	1	–	–
HR+HER2+	1.04	0.31–3.43	0.951	1.00	0.33–3.02	0.996	0.49	0.09–2.67	0.407	0.66	0.12–3.69	0.640
HR–HER2+	1.86	0.38–9.17	0.447	0.74	0.16–3.42	0.703	1.17	0.11–12.66	0.899	1.35	0.22–8.25	0.745
HR–HER2–(triple-negative)	3.80	1.39–10.42	0.009	3.16	1.42–7.01	0.005	7.58	2.18–26.37	0.001	7.64	2.66–21.94	<0.001

95% CI, 95% confidence interval; HR, hormone receptor.
[a]Cox proportional hazards model.

years as independent prognostic factors in younger premenopausal patients (17,19–21,23,24,27,28).

Axillary lymph node status in particular has been highlighted as a powerful independent prognostic parameter in women with primary breast cancer across all age groups. However, in the present study, axillary lymph node status was not an independent prognostic factor in patients aged 35–39 years. This discrepancy with previous studies is likely the result of the subtraction effects of LVI and BVI, which significantly correlate with positive axillary lymph nodes. We also observed that, in univariate analyses, patients who were treated with chemotherapy had significantly worse DFS and OS. This finding reflects the significantly higher proportion of positive axillary lymph nodes in those patients. Taken together, these results support axillary lymph node status as an important prognostic factor.

The triple-negative subtype or the basal-like subtype (defined immunohistochemically as ER negative, HER2 negative and cytokeratin 5/6 and/or HER1 positive) (32) is associated with aggressive histology and poor clinical outcome. In our study, triple-negative status was confirmed as a prognostic factor for poorer long-term outcome. The triple-negative subtype accounts for ~15% of the four tumor subtypes in the general population and for a higher percentage of breast cancer arising in African-American women (33,34) which is a contributing factor to their poorer prognosis (9). According to surveillance data from the Registration Committee of the Japanese Breast Cancer Society, the triple negative subtype accounts for 15.5% of breast cancers, with no difference in mean age at diagnosis among the four tumor subtypes (35). In our study of breast cancer patients under age 40, the proportion of patients falling into each of these four tumor subtypes was approximately the same as that in a representative population of Japanese women with breast cancer, and did not differ significantly between patients aged <35 and those aged 35–39 years. Further studies are needed to clarify the associations between the factors involved in triple-negative status, younger onset and poorer prognosis in patients with breast cancer.

In conclusion, our results did not indicate any significant differences between patients aged <35 years and those aged 35–39 years in either DFS or OS. In our cohort of breast cancer patients under the age of 40, the independent factors associated with poor DFS and OS included positive axillary lymph nodes (pN1–pN3) and triple-negative status, but not age at diagnosis. Adverse prognostic factors also did not differ considerably between the two age groups. Our findings suggest that other clinicopathological features rather than age should be used to determine individualized treatment courses for breast cancer patients younger than 40 years.

Funding

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Conflict of interest statement

None declared.

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Radiofrequency ablation as local therapy for early breast carcinomas

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Abstract
Purpose To evaluate the safety and efficacy of radiofrequency ablation (RFA) as a local therapy for early breast carcinomas, we performed a phase I/II study at our institution.
Patients and methods Fifty patients with core-needle biopsy-proven breast carcinoma that was ≤3 cm in diameter on ultrasonography were enrolled in this study. Under ultrasound (US) guidance, the tumor and surrounding breast tissue were ablated with a saline-cooled RF electrode followed by immediate surgical resection. Resected specimens were examined by hematoxylin and eosin (H&E) staining and nicotinamide adenine dinucleotide (NADH) diaphorase staining to assess tumor viability.
Results Forty-nine patients completed the treatment. The mean tumor size was 1.70 cm. The mean ablation time was 8.7 min using a mean power of 48.5 W. Of the 49 treated patients, complete ablation was recognized in 30 patients (61%) by H&E staining and/or NADH diaphorase staining. The NADH viability staining was available for 38 patients, and in 29 (76.3%), there was no evidence of viable malignant cells. Of the 29 treated patients with breast carcinomas ≤2 cm in diameter examined by pathological examination, complete ablation was achieved in 24 patients (83%). Of the 26 treated patients with breast carcinomas without an extended intraductal component (EIC) according to pathological examination, complete ablation was determined in 22 patients (85%). RFA-related adverse events were observed in five cases: two with skin burn and three with muscle burns.
Conclusion RF ablation is a safe and promising minimally invasive treatment for small breast carcinomas with pathological tumor size ≤2 cm in diameter and without EIC.

Keywords Radiofrequency ablation · Local therapy · Early breast carcinomas · Phase I/II study

Introduction

There has been a change in the management of cancer patients with localized disease from total mastectomy to lumpectomy complemented by adjuvant radiotherapy and chemo-endocrine therapy, without significant outcome [1, 2]. Early detection of small breast lesions may further change the attitude toward less invasive and even non-invasive management [3].

A major goal of breast-conserving treatment is the preservation of a cosmetically acceptable breast. Although a variety of patient and treatment factors have been reported to influence the cosmetic results, the amount of breast tissue resected appears to be a major factor [4]. Several investigators are studying the feasibility of percutaneous minimally invasive techniques to ablate breast tumors. Several modalities, such as cryosurgery, laser ablation, thermoablation, and high-intensity focused US, have been investigated [5]. By minimizing damage and

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disruption to normal surrounding tissue, the morbidity of local treatment, such as scarring and deformity, can be reduced, and the cosmetic results can potentially be improved. With the widespread application of screening mammography, the mean size of the breast tumors detected has continued to decrease, which further emphasizes the need for less invasive means for achieving local tumor destruction, such as RF ablation [6].

The aim of this phase I/II study was to determine the safety and efficacy of radiofrequency ablation (RFA) of early breast carcinomas using saline-cooled electrodes. Our secondary goals were to determine the size, configuration and pathological features of acute RF ablative treatment of breast carcinomas.

Patients and methods

Patients

All patients had a prior histological diagnosis of breast cancer established by stereotactic or US-guided core biopsy. Core biopsy had to be adequate for routine pathological evaluations (grade, estrogen receptor, progesterone receptor, Her 2-neu) because after RF ablation has been performed, viable tumor may not be available for these analyses.

Eligibility criteria included age between 20 and 90 years and tumor size $\leq$3.0 cm in diameter on US examination. Patients were excluded if there was evidence of diffuse calcification suggestive of extensive multifocal ductal carcinoma in situ of more than 3.0 cm in size.

MRI was performed on all patients to evaluate the lesions more precisely and compared with the results of RF ablation. Patients treated with preoperative chemotherapy were excluded. This study was approved by the National Cancer Center, Japan Institutional Review Board, and all patients provided written informed consent.

Treatment

All patients underwent breast US and MRI preoperatively to determine if the tumor was visible in order to facilitate US-guided RF ablation. The patient could elect to undergo either a lumpectomy or a mastectomy as in both situations the RF ablated tissue would be available for pathological review. Sentinel lymph node biopsy (SLNB) was performed for axillary staging. Tracers for SLNB were injected into the subareolar parenchyma to prevent the interference of air/fluid with intra-operative US imaging.

After general anesthesia was induced and SLNB was completed, the breast tumor was identified with intraoperative US using the Toshiba Aplio XG SSA-790A (Toshiba Medical Systems Corporation, Otawara, Japan) with a PLT-1204AT (2D, 12 MHz) and a PLT-1204MV (4D, 14 MHz) probe. Under US guidance, the 17-gauge Valleylab^{TM} RF Ablation System with Cool-tip^{TM} Technology (Covidien, Energy-Based Devices, Interventional Oncology, Boulder, CO) was inserted in the center of the tumor (Fig. 1). With US imaging in the two planes, we ensured that the electrode was located in the center of the lesions using a linear 2D probe for the vertical image and 4D probe for the coronary image (Fig. 2). In all cases, a 2-cm active tip electrode was used. Before ablation, we injected 20 to 40 ml of 5% glucose to avoid skin or muscle burn. The needle electrode was attached to a 500-kHz monopolar RF generator capable of producing 200-W power. Grounding was achieved by attaching two grounding pads to the patient's thighs before the producer. Tissue impedance was monitored continuously using circuitry incorporated into the generator. A peristaltic pump (Watson-Marlow, Medford, MA) was used to infuse 0°C normal saline solution into the lumen of the electrode at a rate sufficient to maintain a tip temperature of 15–25°C.

RF energy was applied to tissue with an initial power setting of 10 W and subsequently increased with increments of 5 W each minute to a maximum power of 55 W. Saline circulating internally within the electrode cools the adjacent tissue, maximizing energy deposition and reducing tissue charring. The power setting was left at this point until power 'rolloff' occurred. Power rolloff implies that there is an increase in the tissue impedance caused by loss of sodium chloride, which occurs with tissue coagulation around the monopolar electrode. When this occurs, the power generator will shut off, stopping the flow of current and further tissue coagulation. After waiting 30–60 s, the second phase was started at 75% of the last maximum power until a second rolloff occured. The appearance and progression of hyperechogenicity on US were used to guide the therapy. Radiofrequency was applied until the tumor was completely hyperechoic (Fig. 3). To minimize thermal injury to the skin, sterile ice packs were placed on the breast during the ablation procedure (Fig. 4). Following RF ablation, standard tumor resection was achieved with either a wide local excision or mastectomy according to the preference of the patient. The surgical specimen was obtained and immediately sent fresh to the pathology department.

Pathological evaluation

Frozen section

Specimens resected by the surgeons were submitted to the pathologists for nicotinamide adenine dinucleotide

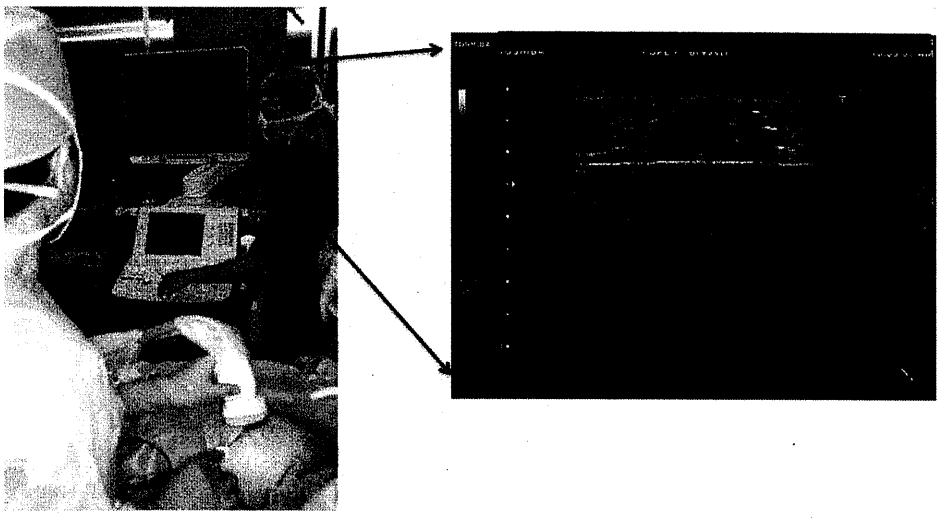

Fig. 1 Technique for performing breast radiofrequency ablation (RFA). Under ultrasound guidance the RF electrode is percutaneously inserted into the breast tumor. The needle is seen transversing the target tumor

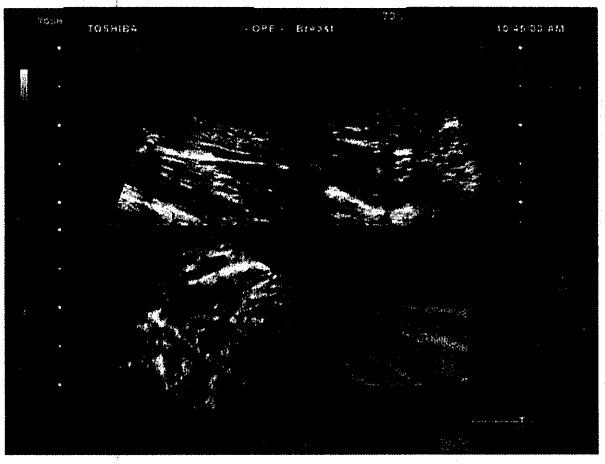

Fig. 2 Confirmation of the location of the needle using a 4D probe. With ultrasound imaging in the two planes, we ensured that the electrode was located in the center of the lesions using a linear 2D probe for the vertical image and 4D probe for the coronary image

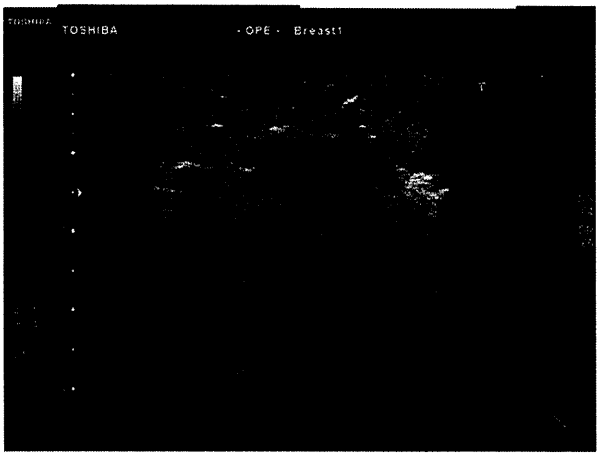

Fig. 3 Intraoperative breast ultrasound. Radiofrequency was applied until the tumor was completely hyperechoic

(NADH) diaphorase cell viability analysis and routine histopathological examination. A tissue slice including the representative cut surface of the tumor and non-ablated mammary glands was removed and subjected to frozen section preparation.

One to two pieces of representative tumor tissue and another piece of non-ablated mammary gland were snap frozen in liquid nitrogen and cut into 5-μm-thick sections using a cryostat (Shiraimatsu, Tokyo, Japan). One of the sections was stained with H&E and was microscopically confirmed to contain the representative tumor tissue and non-ablated mammary gland tissue. Other sections were stored at −20°C until NADH diaphorase assay.

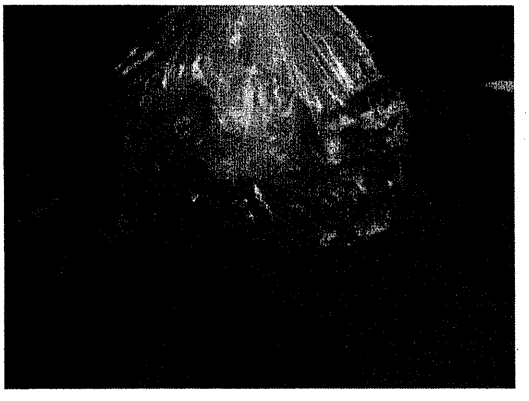

Fig. 4 Skin protection. Skin is protected by the placement of an ice pack during the RFA procedure

Histological analysis

From residual tissue specimens, an entire representative cut surfaces of the ablated tumor and surrounding tissue were taken as tissue blocks for histopathological examination. The blocks were formalin-fixed, paraffin-embedded and cut into 3- to 4-μm-thick sections. These sections were stained with HE. Histopathologically, the viability of tumors was evaluated in consideration of thermocautery artifacts. When the degeneration was marked in cancer cells, the effect of thermocautery was effective. The tumor area with marked degeneration was calculated for each case.

Immunohistochemically, expression of estrogen receptor (ER, clone ID5 clone, Dako, Glostrup, Denmark), progesterone receptor (PR, clone IA6, Dako) and HER2 (Herceptest, Dako) were examined. For ER and PgR, a tumor was judged as positive if 10% or more of the tumor cells showed positive nuclear immunoreactions irrespectively of the intensity of the immunoreactions. For HER2, judgment about immunoreactions was made according to the recommendations of the ASCO/CAP guidelines.

NADH diaphorase cell viability analysis

The enzyme histochemical analysis of cell viability was performed based on the reduction of nitroblue tetrazolium chloride, a redox indicator, by NADH diaphorase, resulting in an intense blue cytoplasmic pigmentation. The activity of this enzyme has been shown to subside immediately upon cell death. For this analysis, 5-μm cryostat-cut unfixed sections were placed in a coprin jar and incubated in 0.05 M tris-buffered saline (TBS, pH 7.4) containing 500 mg/l tetranitro blue tetazolium and 800 mg/l β-NADH (Sigma-Aldrich Corp., St. Louis, MO) for 30 min at 37°C. Thereafter, the sections were fixed in 10% formalin for 30 min, washed with distilled water for 2 min and mounted with a cover glass. Based on the area of cells with blue cytoplasmic staining, the viability of tumor cells and non-ablated mammary gland cells as control was evaluated.

Results

Fifty patients were enrolled in the study, and 49 completed RF ablation therapy. For one patient, the ablation system had some trouble, and we decided not to proceed with the therapy. The patient demographics of the 49 patients who received the proposed RF ablation therapy are shown in Table 1. The median age was 61 years (range 36–82). The median breast tumor size based on the ultrasonographic maximum dimension was 1.70 cm (range 0.5–3.0). The histology was invasive ductal carcinoma for 43 patients (88%).

Table 1 Patient demographics of 49 patients

	Number of patients
Age (years)	
Median	61
Range	36–82
Method of diagnosis	
Mammgoram screening	32 (65%)
Palpable mass ± mammogram	17 (35%)
Tumor classification	
Tis	1 (2%)
T1	34 (69%)
T2	14 (29%)
Tumor location	
Upper outer	18 (37%)
Lower outer	5 (10%)
Upper inner	18 (37%)
Lower inner	7 (14%)
Central	1 (2%)
Tumor size on US (cm)	
Median	1.70
Range	0.5–3.0
Tumor size on MRI (cm)	
Median	1.50
Range	0.7–4.5
Lymph node status	
N0	44 (90%)
N1	5 (10%)

RF ablation time ranged from 3–18 min (mean 8.7 min). Mean tumor impedance was 195.1 Ω, and for 4 of 49 patients, there was a reduction in the impedance during treatment by a mean of 53.4 Ω.

A median of one cycle and a mean power of 48.5 W (range 5–118 W) were used to achieve tumor ablation.

RFA of the breast tumor was monitored with ultrasonography every 3 min.

In 49 patients, as tumor heating around the multiple array electrodes developed, an ill-defined, hyperechoic zone developed. The size of the ablation measured by ultrasonography ranged from 15 to 50 mm (mean 27.3 mm).

RFA-related adverse events were observed in five cases (10%): two with skin burns and three with muscle burns. The entire skin burn area was excised during the breast tumor resection, and the patient had no further sequelae. These events occurred in initial cases. So, in order to avoid these burns, 10 ml of 5% glucose was injected between the skin and tumor, and also between the muscle and tumor. Since then, no skin burns have been observed.

There was no bleeding from the needle track upon removal of the RFA needle electrode in any of the 49 patients.

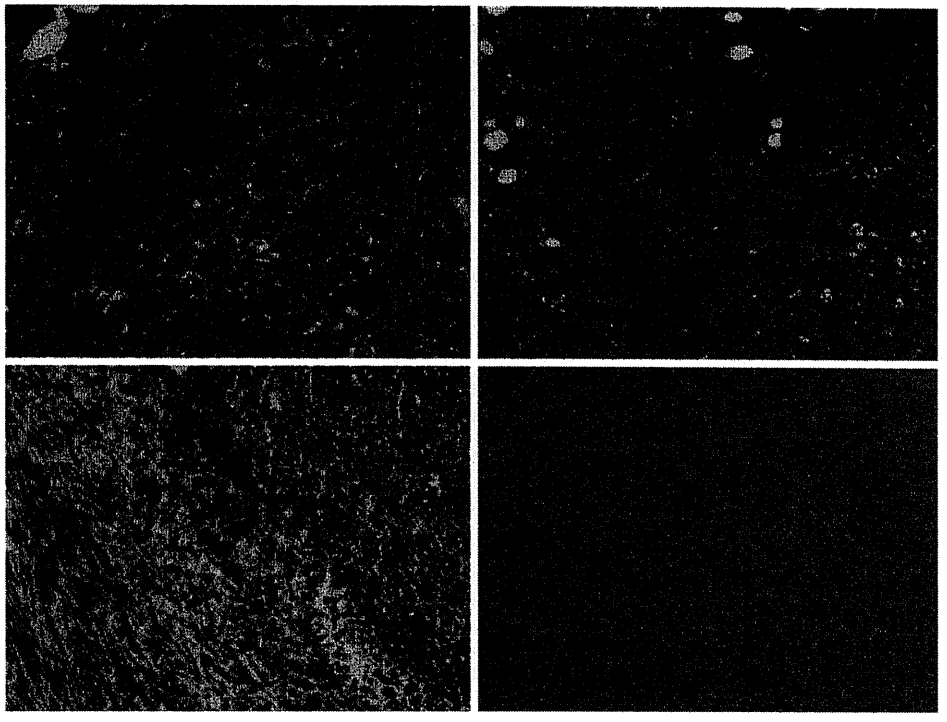

Fig. 5 NADH viability study. **a** H&E section of non-ablated breast tumor; **b** NADH viability study of non-ablated breast tumor T; **c** H&E section of ablated breast tumor; **d** NADH viability study demonstrating non-viable ablated tumor (magnification ×200)

Surgical resection consisted of total mastectomy in 27 patients, whereas 22 patients underwent wide local excision. In the early stages of this study, we selected the patients with small breast cancers who prefered to be treated by mastectomy.

On H&E examination, the tumor architecture was maintained despite ablation, which allowed the pathological size to be assessed accurately. The RFA-treated carcinomas showed a range of pathological findings. All of the treated tumors showed elongated nuclei with smudged chromatin (Fig. 5c). All cases showed extensive electrocautery changes with densely eosinophilic stromas.

In resected samples ablated with a 2.0-cm active tip of the electrode, the results of H&E and NADH examination showed that the mean diameter of the major axis was 3.0 cm (range 0–6.6 cm) and of the minor axis 2.2 cm (range 0–6.6 cm) (Table 2).

NADH viability staining was available for 26 patients, and in 20 (76.9%), there was no evidence of viable malignant cells (Fig. 5d).

Among cases of tumor diameter less than 2 cm in pathological examination, the NADH viability staining was available for 22 patients, and in 20 (90.9%), there was no evidence of viable malignant cells. The two viable cases were due to insufficient ablation; the reason for one case was a defective device and for another case was that the impedance was too high for the tumor to be ablated completely.

In H&E examinations, all tumors diagnosed with non-viability with NADH staining had confirmed changes with characteristics, i.e., amorphism, in the interstitial cells, linear form, rarefaction and inspissation in the nucleus of epithelial cells, etc.

Among most of the cases with viable malignant cells diagnosed with NADH staining, each tumor diameter was nearly 3 cm. Only one case had incomplete ablation of the index tumor because the tumor was eccentric within the RFA zone.

In total, on H&E and or NADH staining, 18 patients (37%) in 49 RFA cases had some viable invasive or in situ disease seen in the surgical excision specimen.

Table 3 shows treatment results depending on the pathological tumor size, including both invasive and intraductal lesions measured in surgical excision specimens. Out of 29 cases with tumor diameters $\leq$2 cm in pathological examination, 25 (86%) had confirmed complete ablation. In 20 cases of tumor diameter >2 cm, only 6 cases (30%) showed confirmed complete ablation. Table 4 indicates that the treatment result also depended on the existence of an extended intraductal component (EIC) of the tumor in the surgical excision specimen. In 26 cases of tumor without EIC in pathological

Table 2 Pathological findings for 49 patients

	Number of patients
Tumor type	
Invasive ductal	43 (88%)
Invasive lobular	1 (2%)
Mucinous	2 (4%)
Medullary	2 (4%)
DCIS	1 (2%)
Tumor grade	
1	22 (45%)
2	16 (33%)
3	11 (22%)
Pathological nodal status	
Negative	39 (80%)
Positive	10 (20%)
Pathologic tumor size (cm)	
Median	1.7
Range	0.1–8
Extended intraductal component (EIC)	
Present	23 (47%)
Absent	26 (53%)
Pathologic response to RFA	
Longest diameter of ablation zone (cm)	
Median	3.0
Range	0–6.6
Shortest diameter of ablation zone (cm)	
Median	2.2
Range	0–6.6
Incomplete tumor ablation	19 (39%)
Residual INV	8 (16%)
Residual DCIS	11 (23%)

RFA Radio frequency ablation

Table 3 Correlation between pathological tumor size and tumor ablation

Pathological tumor size (cm)[a]	No. of patients	Complete tumor ablation (%)	Incomplete tumor ablation (%)
≤2	29	25 (86)	4 (14)
>2	20	6 (30)	14 (70)

[a] Size of invasive and DCIS

Table 4 Correlation between existence of EIC and tumor ablation

	No. of patients	Complete tumor ablation (%)	Incomplete tumor ablation (%)
EIC present	23	9 (39)	14 (61)
EIC absent	26	22 (85)	4 (15)

EIC Extended intraductal component

examination, 22 (85%) had confirmed complete ablation. In 23 cases of tumor with EIC, only 9 (39%) had confirmed complete ablation.

According to these results, pre-RFA MRI detection with ultrasonography should be examined to detect the EIC of the tumor, and appropriate cases for RFA must be determined.

Discussion

Radiofrequency ablation is mainly used in clinical practice to treat unresectable hepatic tumors, and so far experience with breast carcinomas is limited [7–12].

RFA causes local tumor cell destruction by thermal coagulation and protein denaturation [5, 7, 13]. The higher the target temperature, the less exposure time is needed for cellular destruction [14, 15]. Cell death occurs above 45–50°C approximately. The target temperature mostly used at the tip of the prongs was 95°C and was maintained around 15 min [16]. It is conceivable that this setting could result in melting the fatty tissue, with bad cosmetic results. However, the lesions might be destroyed equally well with a lower target temperature and shorter ablation time [8].

Clearly, more research on the radiofrequency dose and effect is necessary to optimize RFA in breast carcinomas.

The shape, size and design of the RF electrode determined the shape of the ablation zone and, in the end, the success of the procedure. Because the size of the thermal lesion is limited using a single-needle electrode, multiarray electrodes have been developed that can produce thermal lesions of 3–5 cm in diameter.

In our trials, the ablation zone with a 2-cm active tip of the electrode had the following characteristics: the mean diameter of the major axis was 3.0 cm (range 0–6.6 cm) and that of the minor axis was 2.0 cm (range 0–6.6 cm).

However, some studies reported the distance between the tumor and the skin and the chest wall should be at least 1 cm because of possible burning of normal tissue. Lateral compression of the breast during the entire ablation procedure or ice cooling in cases of borderline distance to the skin is also essential to prevent possible skin burns [7, 8].

However, using a 5% glucose injection between the skin and tumor, and between the chest wall and tumor, and cooling skin with ice in order to avoid these burns, a tumor diameter less than 1 cm could be achieved with RFA.

The difficulty in assessing the margin of the ablated lesions is a limitation in all percutaneous ablation techniques. To minimize the risk of local recurrence and to make sure the whole tumor and safe margin are ablated, the lesions need to be excised with a rim of at least 1 cm.

After excision, tumor viability is tested by NADH diaphorase. Almost every study described immediate

excision of the ablated lesion [8, 10–12]. Burak et al. and Hayashi et al. [1, 9] had an interval of 1–3 weeks before excising the ablated zone. It was hypothesized that due to the effect of local vessel thrombosis and necrosis of surrounding tissue, the ablated zone expands in the period of time and provides a more accurate excision. In the end, the two trials did not have higher percentages of complete tumor ablation compared to the other studies, and it was concluded that an interval time between ablation and excision of the tumor might not be necessary [7, 9].

In cases of tumor diameter less than 2 cm, the NADH viability staining was available for 22 patients, and in 20 (90.9%), there was no evidence of viable malignant cells. The other two viable cases were due to insufficient ablation; the reason for one case was a defective device and for another case that impedance was too high for the tumor to be ablated completely. Breast cancer tissue is usually composed of tumor, normal tissue, fat, vessels, etc., and shows heterogeneity. The fat tissue has one of the highest electrical resistances. High resistance mean less effect from electrical power, such as radiofrequency. Therefore, we suspect that in our cases the component with high impedance against RFA might be fatty.

Up until now, only one pilot study has been performed that tested RFA in three elderly patients with breast cancer without excision of the ablated zone [17]. All three patients completed the treatment without complications, and after 18 months of follow-up, no recurrence had occurred. In the future, if RFA is to be used as a replacement for surgery, CNB might also be an option to confirm successful ablation. Fornage et al. suggested that multiple core-needle biopsies through the ablated lesion and its periphery should be obtained 3–4 weeks after the RFA procedure.

An indication for RFA can be early breast cancer ($T \leq$ 2 cm). In 29 cases of tumor diameter $\leq$2 cm, 25 (86%) were confirmed to have complete ablation (Table 3). In 26 cases of tumors without EIC in pathological examination, 22 (85%) were confirmed to have complete ablation. In 23 cases of tumor with EIC, only 9 (39%) were confirmed to have complete ablation. According to MRI detection, tumor diameter and the EIC could be evaluated more accurately. Appropriate cases for RFA should be selected deliberately after enough diagnosing with US and MRI detection concerning the diameter, type, EIC, multiple lesions, etc.

The optimal conditions for RFA correlate to results under the following conditions: (1) tumor diameter <2 cm diagnosed with US and (2) <2 cm except for multiple lesions and extended intraductal spread of lesions of more than 2 cm diagnosed with MRI detection.

Also, in two cases, the tumor body could not be ablated sufficiently. Effects of RFA depend on tissue resistivity, so fatty tissue and tumor components can affect these effects. Components of breast carcinoma are different for each patient, and further studies are needed. In cases in which the initial resistance is too high and rolloff occurs immediately, as our study showed, the target temperature cannot be reached, and procedures should be changed from RFA to lumpectomy for the patients' safety. The reasons for these incidents need to be examined with resected samples.

In Japan, RFA is a popular treatment method for liver cancer.

Half of liver cancer patients are treated by RFA. This system is familiar to many physicians even in local hospitals and clinics.

Although cryoablation and the HIFU have not been approved by the Japanese government, only RFA has been approved and has the possibility to be admitted as an option for local treatment.

RFA seems to be a promising new tool for a minimally invasive procedure for small breast carcinomas. However, follow-up data regarding the local effects on the surrounding breast tissue or recurrence rates are hardly available. Further research will be necessary to establish the optimal technique and to demonstrate the long-term oncological and cosmetic effects of RFA.

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