trials are now being performed in preoperative or postoperative settings (10). These drugs will most likely play an important role in the future treatment of breast cancer. The benefits of oral 5-fluorouracil derivatives would be further enhanced by the ability to predict response, thereby identifying patients most likely to benefit from treatment and increasing the benefit-risk ratio.

Various approaches have been proposed to predict the response to oral 5-fluorouracil derivatives. Experimental and clinical evidence has suggested that tumor levels of enzymes involved in nucleoside metabolism, such as thymidylate synthase (TS), thymidine phosphorylase (TP), and dihydropyrimidine dehydrogenase (DPD), may be useful for predicting the response to oral 5-fluorouracil derivatives. Predictive accuracy may be further enhanced by using these enzymes in conjunction with other molecular markers.

We retrospectively examined whether the expression of the 3 enzymes TS, DPD, and TP and that of the oncogene HER2 and the tumor-suppressor gene p53 in breast cancer tissue could be used to predict the response to treatment with tamoxifen plus UFT. Resected tissue specimens were obtained from women with breast cancer who were enrolled in the 3rd Adjuvant Chemo-Endocrine Therapy for Breast Cancer (ACETBC) trials, randomized controlled studies comparing tamoxifen alone with tamoxifen plus UFT after surgery.

Patients and methods

Combined analysis of three randomized trials. A meta-analysis of 5 randomized controlled trials (n=1987) performed by the ACETBC study group in Japan has shown that the reduction in the risk of recurrence after treatment with UFT was 21±11% (P=0.06) in women with stage I to IIIA breast cancer who underwent mastectomy (5).

Three of these trials examined the effect of adding UFT (300-400 mg/day) to tamoxifen (20-30 mg/day) in women with estrogen-receptor (ER)-positive tumors who postoperatively received adjuvant chemotherapy for 2 years. ER status was determined at each center. Either biochemical (enzyme immunoassay) or immunohistochemical techniques were used. In 2 of these trials, mitomycin C (10 mg/m^2) was given intravenously on the day of surgery. Combined analysis of these 3 trials (n=1225; median follow up, 5.7 years) was performed according to the method of Peto (Fig. 1). The reduction in the risk of recurrence after treatment with UFT plus tamoxifen was found to be 26±12% (p=0.037). Subset analyses of pooled data in the 3 trials showed that UFT was most effective in premenopausal women with metastases to the axillary lymph nodes (reduction in odds of recurrence, 35±17%).We retrospectively studied the predictive values of biomarkers of response in this patient subset.

Immunohistochemically studied biomarkers

Collection of tumor samples. A list of subjects was submitted to centers that had agreed to participate in this biomarker study and had registered at least 5 patients to the 3rd ACETBC study. All available paraffin-embedded samples were sent from the centers to the operational office by mail. The samples were stored at room temperature until predictive markers were evaluated.

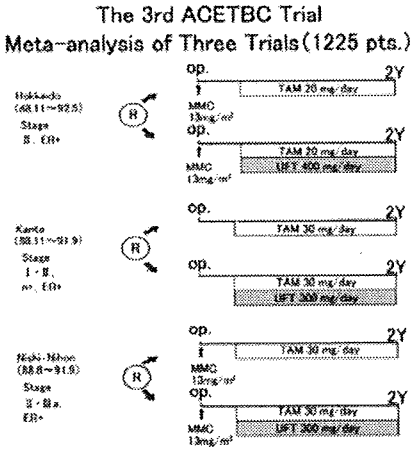

Figure 1. Protocols of the 3rd ACETBC trial.

Immunohistochemical labeling

Antibodies. TS polyclonal antibody RTSSA (dilution, 1:100; Taiho Pharmaceutical Co., Ltd., Tokyo, Japan), TP monoclonal antibody TMA-1 (dilution, 1:100; Taiho Pharmaceutical Co., Ltd.), DPD polyclonal antibody RDPDPA (dilution, 1:100; Taiho Pharmaceutical Co., Ltd.), HER2 polyclonal antibody A0485 (Dako, Carpinteria, CA, USA; dilution, 1:100), and p53 (DO7) monoclonal antibody (Novo-castra, Newcastle, UK; dilution, 1:40) were used for immunohistochemical analyses.

Immunohistochemical analyses. Immunohistochemical analyses were performed at a single central laboratory using the antibodies described above and mouse IgG (Dako) as negative control. An indirect avidin-biotin-peroxidase method was used. Briefly, deparaffinized tissue sections were treated with 0.3% hydrogen peroxide in methanol to block endogenous peroxidase activity. After washing with phosphate buffered-saline (PBS) containing 0.05% Tween-20, the sections were treated with 1.5% normal horse serum in PBS and incubated with each of the antibodies or with mouse IgG for 1 h at room temperature. The sections were washed again with PBS, incubated with biotinylated anti-mouse IgG (Dako) for 30 min, washed again with Tween-20-PBS, incubated with an elite ABC kit (Vector, Burlingame, CA, USA) for 30 min, and visualized with the use of 3,3'-diaminobenzidine tetrahydrochloride-hydrogen peroxide as chromogen. The sections were then counterstained with hematoxylin, dehydrated, and mounted.

Evaluation of staining. The slides were evaluated independently by 3 experienced pathologists (A.F., K.M., T.H.) blinded with regard to treatment group and outcome. Each pathologist evaluated TS, TP, and DPD on the basis of staining intensity of the cytoplasm, scored according to a 4-grade scale (0 to 3), and staining rate, also scored according to a 4-grade scale (≤25%, 0; >25% to ≤50%, 1; >50% to ≤75%, 2; and >75%, 3). The scores agreed on by 2 or more of the pathologists were adopted. Concordance rates of the evaluations among 2 or more pathologists were as follows: TS, staining intensity 95%, staining rate 80%; TP, staining intensity 92%, staining rate

Table I. Patients' characteristics in the biomarker study.

	TAM group (n=97)	UFT group (n=95)	p-value
Age			
≤50	89	89	0.78
>51	8	6	
Number of nodes involved			
1-3	65	73	0.15
≥4	32	22	
Tumor size			
<2 cm	23	24	0.87
≥2 cm	74	71	
TS expression			
Positive	57	48	0.31
Negative	40	47	
TP expression			
Positive	36	39	0.86
Negative	61	56	
DPD expression			
Positive	57	66	0.13
Negative	40	29	
HER2 expression			
Positive	14	14	1.00
Negative	83	81	
p53 expression			
Positive	30	33	0.85
Negative	67	62	

All patients had estrogen receptor-positive tumors and were pre-menopausal.

87%; and DPD, staining intensity 94%, staining rate 89%. The median score was adopted if all 3 pathologists disagreed on the score. Cases were considered positive if the staining intensity was ≥2, and the staining rate was 3 (staining rate, >75%).

HER2 was evaluated on the basis of staining of the membrane, and p53 was evaluated on the basis of staining of nuclei. The results were considered positive if the staining rate was ≥1%. The evaluation agreed on by 2 or more pathologists was adopted (concordance rates among the evaluations of the 3 pathologists were as follows: HER2, 89%; and p53, 72%).

Statistical analysis. Relapse-free survival was the outcome used to assess treatment efficacy and was defined as the interval elapsed between the date of surgery and the date of documented disease relapse or death. Relapse-free survival was calculated by the Kaplan-Meier method. Differences between groups in Kaplan-Meier estimates of relapse-free survival were evaluated with the log-rank test and generalized Wilcoxon test. Risk

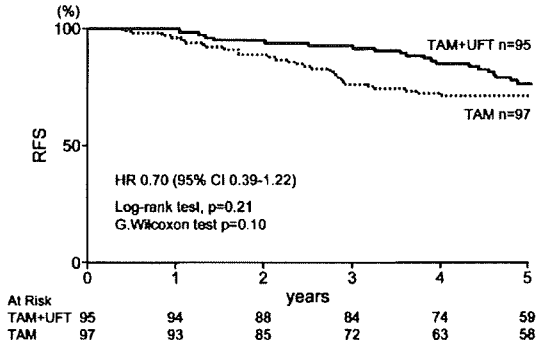

Figure 2. Relapse-free survival (RFS) according to study group (n=192).

ratios (RR) were estimated from Cox proportional-hazards regression models. No overall survival analysis was performed in the subgroups of patients identified by the evaluated biological markers because of the small numbers of events in each treatment group. Cox proportional-hazards regression models were also used to test for interactions between biomarkers and treatment.

Relative predictive values (RPV) were determined with use of the following equation, modified from the method described by Hayes (11): RPV for events in the tamoxifen + UFT group was compared with those in the tamoxifen alone group = Log (RR when tumors stained negatively for biomarkers/RR when tumors stained positively for biomarkers). Differences in distributions between groups were compared with the use of the χ^2 test. Differences were considered statistically significant when p-values were <0.05, and all reported p-values are two-tailed. All analyses were carried out with SAS software (version 6.12).

Results

Collection of samples. Samples collected from 192 (97 given tamoxifen and 95 given tamoxifen plus UFT) of the 204 women at the centers were assessable. There were no significant differences between the groups in demographic characteristics (age, tumor size, number of lymph node metastases) (Table I). The hazard ratio of the effect of adding UFT to tamoxifen was 0.70 (95% confidence interval, 0.39 to 1.22) (log-rank test, p=0.21; Wilcoxon test, p=0.10) (Fig. 2).

Expression of biomarkers. The rates of positive staining were as follows: TS, 55% (105/192); TP, 39% (75/192); DPD, 64% (123/192); HER2, 15% (28/192); and p53, 33% (63/192). The expression rates of these biomarkers were similar in the tamoxifen group and the tamoxifen plus UFT group (Table I).

Relation between relapse-free survival and expression of biomarkers in tumors. Demographic characteristics were similar in women whose tumors stained positively for each biomarker (TS, TP, or DPD) and those whose tumors stained negatively for each biomarker. Univariate analyses showed no significant differences in relapse-free survival between women whose tumors stained positively for TS, TP, or DPD and those whose tumors stained negatively for these 3

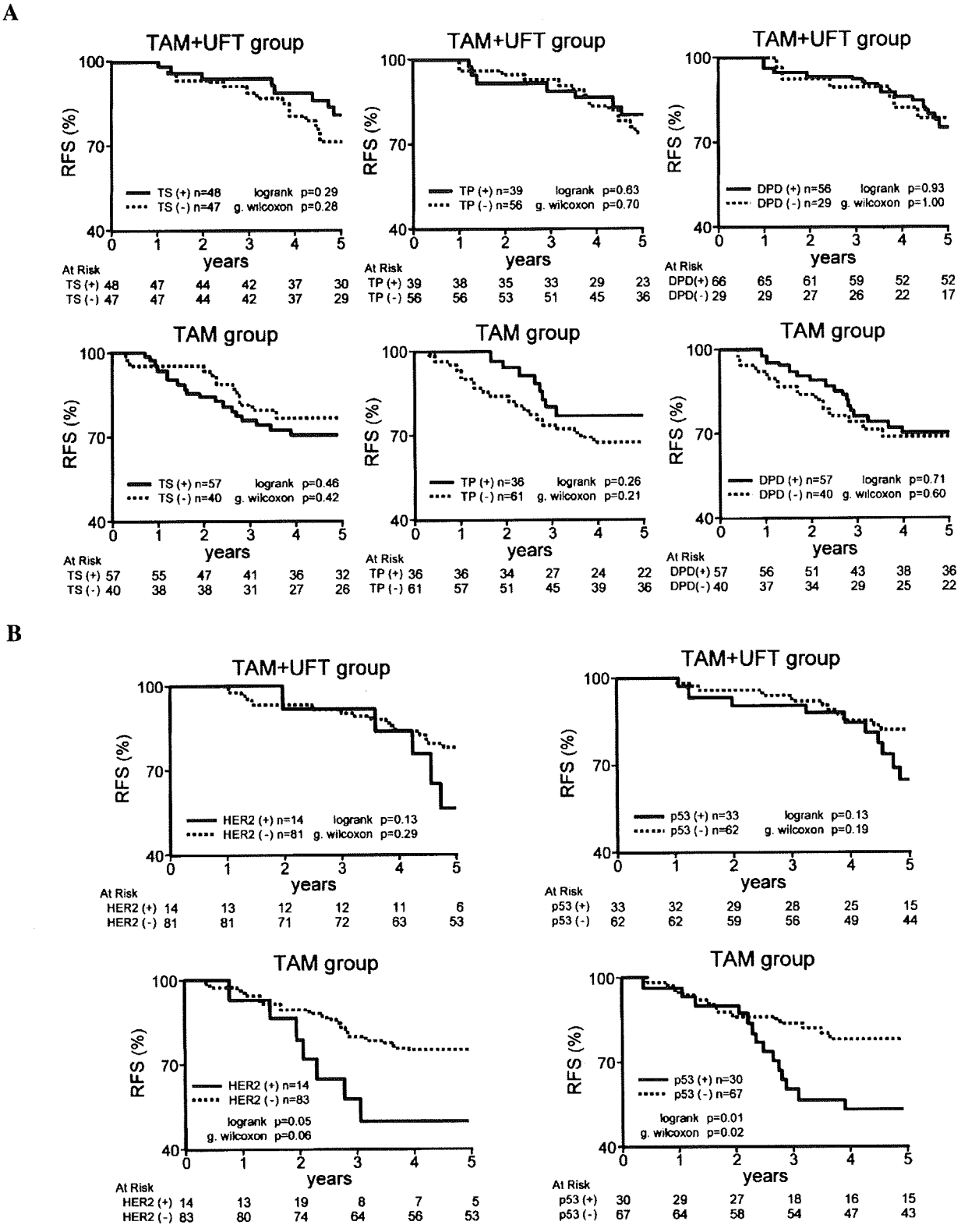

Figure 3. (A) Relation between relapse-free survival (RFS) and tumor expression of thymidylate synthase (TS), thymidine phosphorylase (TP), and dihydropyrimidine dehydrogenase (DPD) according to treatment. (B) Relation between relapse-free survival and tumor expression of HER2 and p53 according to treatment.

biomarkers in either treatment group. Women whose tumors stained positively for HER2 or p53 in the tamoxifen alone group had significantly poorer outcomes than those whose tumors stained negatively for these biomarkers. HER2 and p53 were not significant prognostic factors in the tamoxifen plus UFT group (Fig. 3).

Table II. Relative risk (TAM+UFT vs. TAM) according to biomarker expression.

Biomarker	Biomarker positive			Biomarker negative			Interaction
	RR	95% CI	p-value (G. Wilcoxon test)	RR	95% CI	p-value (G. Wilcoxon test)	p-value
TS	0.48	0.20-1.07	0.04	1.00	0.44-2.36	1.00	0.22
TP	0.80	0.28-2.23	0.60	0.66	0.33-1.30	0.124	0.76
DPD	0.75	0.37-1.52	0.29	0.61	0.21-1.56	0.222	0.73
HER2	0.59	0.17-1.86	0.19	0.72	0.37-1.37	0.220	0.77
p53	0.57	0.25-1.28	0.09	0.78	0.35-1.72	0.418	0.59

RR, relative risk by addition of UFT to TAM; TS, thymidylate synthase; TP, thymidine phosphorylase; DPD, dihydropyrimidine dehydrogenase.

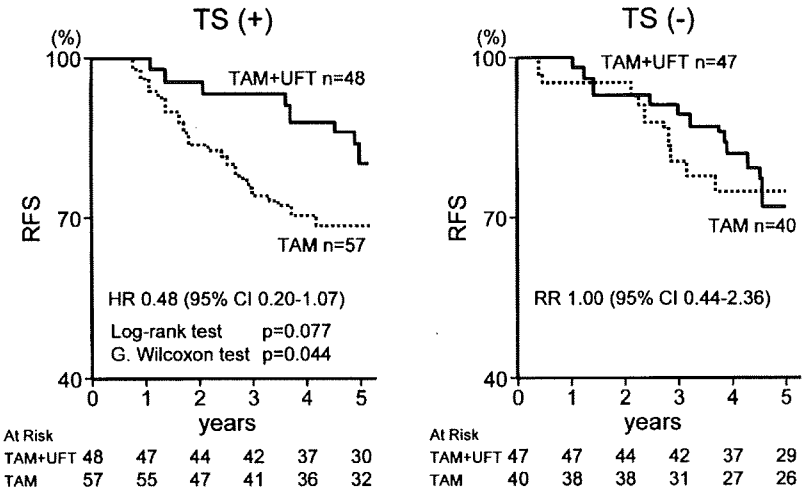

Figure 4. Comparison of relapse-free survival (RFS) between TAM and TAM+UFT treatment according to thymidylate synthase (TS) status.

Relation between expression of biomarkers in tumors and effect of adding UFT to tamoxifen

TS. In women with TS-positive tumors, the risk ratio of the effect of adding UFT to tamoxifen was 0.48 (95% confidence interval, 0.20 to 1.07), and response differed significantly between women given tamoxifen alone and those given tamoxifen plus UFT (p=0.04 by the generalized Wilcoxon test, p=0.08 by the log-rank test). In women with TS-negative tumors, however, there was no significant difference in response (hazard ratio, 1.00; 95% confidence interval, 0.44-2.36). Interaction testing showed that the expression of TS was not significantly related to the effect of UFT (p=0.22) (Fig. 3, Table II).

TP. The risk ratio of the effect of adding UFT to tamoxifen was 0.80 (95% confidence interval, 0.28-2.23) in women with TP-positive tumors and 0.66 (95% confidence interval, 0.33-1.30) in women with TP-negative tumors. There were no significant differences in response between the treatment groups. Interaction testing showed no significant relation between the expression of TP and the effect of UFT (p=0.76) (Table II).

DPD. The risk ratio of the effect of adding UFT to tamoxifen was 0.75 (95% confidence interval, 0.37-1.52) in women with DPD-positive tumors and 0.61 (95% confidence interval, 0.21-1.56) in those with DPD-negative tumors. There were no significant differences between the treatment groups. Interaction testing showed that the expression of DPD was not significantly related to the effect of UFT (p=0.73) (Table II).

HER2. The risk ratio of the effect of adding UFT to tamoxifen was 0.59 (95% confidence interval, 0.17-1.86) in women with HER2-positive tumors and 0.72 (95% confidence interval, 0.37-1.37) in those with HER2-negative tumors. There were no significant differences between the treatment groups. Interaction testing showed that the expression of HER2 was not significantly related to the effect of UFT (p=0.77) (Table II).

p53. The hazard ratio of the effect of adding UFT to tamoxifen was 0.57 (95% confidence interval, 0.25-1.28) in women with p53-positive tumors and 0.78 (95% confidence interval, 0.35-1.72) in women with p53-negative tumors. There were no significant differences between the treatment groups.

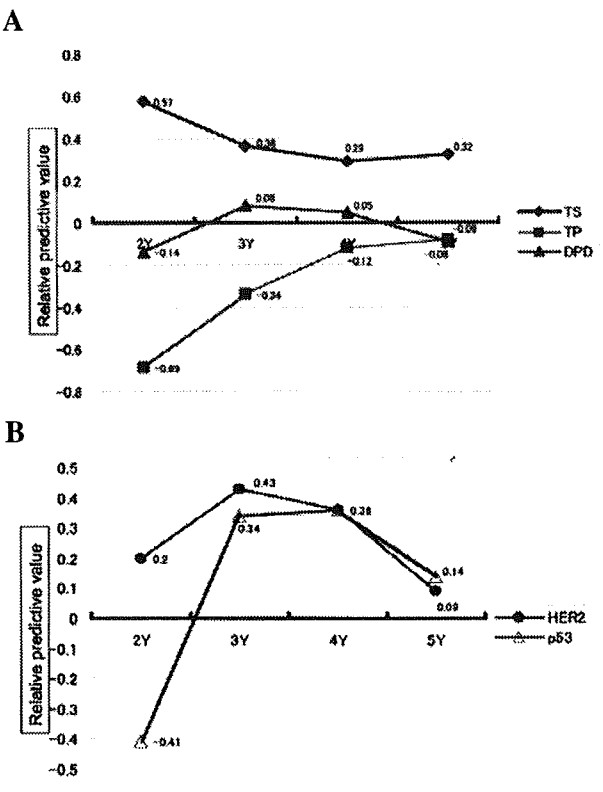

Figure 5. (A) Change in relative predictive values (TS, TP, and DPD). (B) Change in relative predictive values (HER2 and p53). Relative predictive value: log [RR of marker (-)/RR of marker (+)]. RR: risk ratio (TAM vs. TAM+UFT).

Interaction testing demonstrated no relation between the expression of p53 and the effect of UFT (p=0.58) (Table II).

Changes in RPV. Changes in the RPV of each biomarker over time are shown in Fig. 5. (The RPV at 1 year could not be determined because some subgroups of patients had no events at 1 year.) The RPV of TS gradually decreased with time for up to 4 years (0.57 at 2 years, 0.34 at 3 years, and 0.29 at 4 years), and was 0.32 at 5 years. The absolute value for the RPV of TP gradually decreased over time (-0.67 at 2 years, -0.34 at 3 years, -0.12 at 4 years, and -0.08 at 5 years). The RPV of DPD was approximately 0 for up to 5 years (-0.14 at 2 years, 0.08 at 3 years, 0.05 at 4 years, and -0.09 at 5 years). The RPV of HER2 was 0.20 at 2 years, 0.43 at 3 years, 0.36 at 4 years, and 0.09 at 5 years. The RPV of p53 was -0.41 at 2 years, 0.34 at 3 years, 0.36 at 4 years, and 0.14 at 5 years.

Discussion

We immunohistochemically studied whether the biomarkers TS, TP, DPD, HER2, and p53 could be used to predict the effect of adding UFT to tamoxifen in women with breast cancer who underwent mastectomy. In women with TS-positive tumors, relapse-free survival was significantly better in the tamoxifen plus UFT group than in the tamoxifen group, whereas there was no significant difference between the treatment groups in women with TS-negative tumors. These results suggest that TS can be used to predict the response to UFT plus tamoxifen, although interaction testing showed no significant interaction between TS expression and treatment response.

Several studies have reported that TS can be used to predict the response to 5-fluorouracil-based adjuvant chemotherapy in patients with colorectal cancer (12-15). These studies consistently found that 5-fluorouracil-based chemotherapy was ineffective for patients with TS-negative tumors, but effective for patients with TS-positive tumors. Pestalozzi *et al* (16) examined whether TS could be used to predict treatment response in women with breast cancer who were enrolled in a randomized controlled trial (the International Breast Cancer Study Group-V) comparing 1 course of CMF given perioperatively with 6 courses of CMF given postoperatively. Their results showed that suppression of recurrence after 6 courses of postoperative CMF was superior to that after 1 course of perioperative CMF only among women who had TS-positive tumors. Our results are in accordance with their findings. TS, an enzyme involved in DNA synthesis, catalyzes the methylation of deoxyuridine monophosphate to produce deoxythymidine monophosphate. TS is targeted by 5-fluorouracil.

Most experimental studies using cell lines and studies of metastatic cancers (17) have shown that high TS expression is associated with a low antitumor response to 5-fluorouracil, a finding that conflicts with the results of studies in an adjuvant setting. Recent experimental studies by Rahman *et al* (18) have reported that TS has oncogene-like properties. Overexpression of TS under the condition of serum deprivation was clearly demonstrated to induce apoptosis. Therefore, overexpression of TS due to tumor-related or environmental factors may alter the response to 5-fluorouracil-based chemotherapy. In addition, a recent investigation found that tamoxifen up-regulates TS (19). This phenomenon may have a part in the enhanced response to adjuvant chemotherapy with tamoxifen plus UFT.

TP expression was not significantly related to the effect of adding UFT to tamoxifen. TP is an enzyme involved in nucleoside metabolism, antiapoptosis activity, and the promotion of neovascularization. It also converts capecitabine, a prodrug of 5-fluorouracil, and 5'-deoxy-5-fluorouridine (5'-DFUR), an intermediate metabolite of capecitabine, to 5-fluorouracil. Many basic and clinical trials have reported the relation between TP expression and the effects of capecitabine and 5'-DFUR (10). Tominaga *et al* (20) immunohistochemically studied the relation between TP expression and the response to 5'-DFUR in women with early breast cancer who were enrolled in a randomized controlled trial comparing surgery alone with postoperative adjuvant chemotherapy with 5'-DFUR. They concluded that TP expression can be used to predict the response to 5'-DFUR. UFT is a prodrug of 5-fluorouracil, combining tegafur with uracil. Tegafur is converted to 5-fluorouracil principally by liver cytochrome CYP2A6 (21). This mechanism may account for the lack of a relation between TP expression and the effect of adding UFT to tamoxifen in this study.

DPD expression in tumors was also not significantly related to the effect of adding UFT to tamoxifen. DPD, present mainly in the liver, is a rate-limiting enzyme that inactivates 5-fluorouracil. DPD activity in tumors is related

to sensitivity to 5-fluorouracil. Tumors with high DPD expression are thought to respond poorly to 5-fluorouracil derivatives. Indeed, some studies have reported that sensitivity to capecitabine or doxifluridine is governed by DPD (22-24). UFT contains uracil, an inhibitor of DPD, and may be effective against tumors with high expression levels of DPD (25). This characteristic may account for the fact that the effect of adding UFT to tamoxifen was unrelated to tumor DPD expression.

The expression of HER2 and of p53 was also unrelated to the effect of adding UFT to tamoxifen. Previous studies have reported that the expression of HER2 and p53 is related to the response to anthracycline-based chemotherapy (26,27). However, our study suggests that these factors do not influence the response to UFT. HER2 and p53 were significant prognostic factors in the tamoxifen alone group. Because we did not evaluate these factors in the groups not given tamoxifen, we cannot be certain, but our results suggest that HER2 and p53 are predictive markers of the response to treatment with tamoxifen alone. This notion is supported by the findings of Carlomagno *et al* (28), who reported that overexpression of HER2 is related to the response to tamoxifen in women with breast cancer.

Hayes described a method for quantifying the pure predictive values of biomarkers for forecasting treatment response (11). He used risk ratio (RR) in a treated group relative to that in a control group for subgroups of patients whose tumors were positive or negative for a given biomarker. The RR was used in the following equation to derive the RPV of the biomarker: RPV = [1 - RR (biomarker-positive tumors)]/[1 - RR (biomarker-negative tumors)]. Because RR was often >1 for patients with either biomarker-positive or -negative tumors, we modified Hayes' method and used the following equation: RPV = log [RR (biomarker-negative tumors)]/[RR (biomarker-positive tumors)]. The RPV scores were calculated and plotted over time to examine the time course of the RPV (Fig. 4). The RPV was positive if the treatment response was greater when tumors were biomarker positive. Conversely, the RPV was negative if the treatment response was greater when tumors were biomarker negative.

The higher the absolute value of the RPV, the stronger was the power to predict treatment response. Because the natural logarithm was used, the predictive power can be considered weak if the absolute value was <0.3 and strong if the absolute value was ≥0.5. The RPV of TS was 0.57 at 2 years and was then gradually decreased with time, but remained at >0.3 at 5 years. These data suggest that TS is a pure predictive factor of the response to UFT.

A likely explanation for the reduction in the RPV of TS with time is that the magnitude of the effect of adding UFT to tamoxifen decreased from year 2 onward. A recent overview of randomized trials of adjuvant therapy compiled by the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) (29) showed that the response to poly-chemotherapy, including regimens such as AC and CMF, diminishes with time, suggesting that this phenomenon is commonly associated with chemotherapy. In the studies analyzed, both tamoxifen and UFT were given for 2 years. Treatment response may persist if UFT is continued for more than 2 years. However, these data should be interpreted with caution because specific subgroups of patients were studied retrospectively.

The RPV of TP was -0.69 at 2 years, and the absolute values were low at 4 and 5 years (-0.12 and -0.08, respectively). As mentioned previously, TP was not a statistically significant predictive factor in our study, but there was a trend toward a higher additive effect of UFT when TP was negative. The RPV of DPD consistently remained at approximately 0, suggesting that the value of DPD for predicting the response to UFT was low.

The RPVs of HER2 and p53 were >0.3 at 3 and 4 years, but neither of these biomarkers were significant predictive factors in our study. This is attributed to the fact that positive rates for HER2 and p53 were low in our study, thereby diminishing statistical power. Interestingly, the time courses of the RPVs of these markers differed from those of TS and TP.

Our results suggested that the expression of TP and DPD, factors related to the response to capecitabine, do not influence the response to UFT. Therefore, different types of oral fluorouracil derivatives may be most effective in distinct subgroups of patients. In the future, expression of TS, DPD, and TP might be useful for selecting patients most likely to respond to tegafur-based oral fluorouracil derivatives, such as UFT and S-1, and those more likely to respond to capecitabine.

At present, however, breast cancer is often treated by a multidisciplinary approach. Care should be exercised when using oral fluorouracil derivatives in combination with other anticancer drugs because the latter may modify nucleoside-metabolizing enzymes, thereby affecting the metabolism of fluorouracil (30). The measurement of biomarkers before and after treatment may also have an important role in the selection of preoperative chemotherapy.

An important limitation of our study was the retrospective design and the inclusion of only a subset of patients (node-positive premenopausal women) who were enrolled in randomized controlled trials. Our results must therefore be verified in prospective randomized controlled studies in which women with breast cancer are assigned to adjuvant treatment on the basis of the prior determination of biomarker levels.

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Original article

Feasibility study on radiofrequency ablation followed by partial mastectomy for stage I breast cancer patients

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ABSTRACT

To evaluate the safety and reliability of thermal ablation therapy instead of breast-conserving surgery (BCS), we performed radiofrequency ablation (RFA) for clinical stage I breast cancer patients. Subjects were T1N0 breast cancer patients with no extensive intraductal components. Under general anesthesia, sentinel node biopsy was performed, followed by RFA and BCS. Resected specimens were examined at 5-mm intervals by hematoxylin–eosin (H&E) staining and nicotinamide adenine dinucleotide (NADH) diaphorase staining. Thirty of the 34 eligible patients were enrolled. RFA-related adverse events were observed in nine patients: two with skin burn and seven with muscle burn. Twenty-six patients (87%) showed pathological degenerative changes in tumor specimens with H&E staining. In 24 of the 26 cases (92%) examined by NADH diaphorase staining, tumor cell viability was diagnosed as negative. RFA proved to be reliable and feasible in clinical stage I breast cancer, with no extensive intraductal components. Randomized clinical trials are needed to compare RFA with BCS.

Introduction

Recent progress in multi-disciplinary treatment has changed the rationale for the surgical management of breast cancer. Breast-conserving surgery (BCS) followed by breast irradiation has been proven to be as effective as radical mastectomy for local and systemic control of early breast cancer.[1,2] Axillary lymph node dissection (ALND) for clinically node-negative breast cancer patients is decided upon according to the results of sentinel node biopsy (SNB).[3,4] In addition to such advanced surgical techniques, a meta-analysis of randomized clinical trials demonstrated that tamoxifen reduced the risk of in-breast recurrence after BCS for patients with hormone-sensitive breast cancer.[5] On the other hand, 20–50% of patients with breast cancer with a specific molecular signature had a complete pathological response to primary poly-chemotherapy or primary chemo-antibody therapy. In the near future, these cases should be considered good candidates for not needing surgical resection.[6,7]

The most important issue in breast surgery is currently the local control of malignancy. Despite a small number of randomized trials, radiofrequency ablation (RFA), a type of thermal ablation therapy, is used worldwide for the local control of hepatocellular carcinoma because this disease often relapses in virus-infected liver.[8,9] The RFA technique is also used for the control of pain in metastatic bone disease.[10] If RFA could be used to control primary breast tumors, it may constitute an alternative to BCS. Some investigators have attempted feasibility studies on RFA in early breast cancer, but the indications, standard techniques, and local control of RFA in breast cancer are all still unclear.[11–18] To evaluate the safety and reliability of thermal ablation therapy in breast cancer, we performed RFA immediately before BCS in clinical stage I breast cancer patients.

Materials and methods

Patients with breast cancer, as proven by aspiration biopsy cytology or core needle biopsy, were recruited. The eligibility criteria were as follows: tumor diameter of 2 cm or less as measured by a caliber rule, no swelling of axillary lymph nodes as diagnosed by ultrasound, no diffuse calcification according to mammography, and no evidence of extensive intraductal components on magnetic resonance (MR) mammography. Subjects who were pregnant, had had a pacemaker implanted, or who had a hematological abnormality (platelet count of 50,000/ml or less or

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prothrombin time of 70% or less) to avoid needle-induced bleeding were excluded. Since BCS is a standard treatment for breast cancer, patients were informed that there would be no benefit from RFA before BCS in this study and they signed a written consent form. The study was approved by the ethics committee at the National Cancer Center Hospital.

Under general anesthesia, SNB was performed with a combination method of indigocarmine (Daiichi Pharmaceutical, Tokyo, Japan) and technetium-99m-labeled phytate (Daiichi Radioisotope Laboratories, Tokyo, Japan).[19] ALND up to levels I and II was determined according to the pathological diagnosis of the frozen sections of sentinel lymph nodes. We attempted RFA in breast cancer using a LeVeen electrode system (Boston Scientific Corporation, USA). The needle electrode or co-access needle electrode of a 10-tine multiple array was applied. The needle electrode was 14-gauge and had an array diameter of 3 cm when fully deployed. It was inserted under ultrasound guidance (EUB-5500, Toshiba, Japan): the tip was located at the center of the breast tumor and the correct location was confirmed by 2-dimensional ultrasound. About 20–40 ml of 5% glucose was injected into the subdermal tissues and retromammary spaces around the tumor to protect overlying skin and pectoralis major muscle from being burned by RFA-induced heat. After the ten-tine multiple array was deployed from the tip of the needle electrode, thermal ablation was performed using an RF-2000 generator (Boston Scientific Corporation, USA) with two grounding pads (Valleylab, USA) on each thigh. Izzo et al. reported a pilot study of RFA using a LeVeen electrode and a generator system, and we followed their ablation algorithm in most cases.[12] In brief, the first ablation was started at an initial electrical power level of 10 W for 2 min and then the power was increased in steps of 5 W every minute until 'roll-off', which is the level of maximum impedance due to complete tissue coagulation. If this phenomenon did not occur at 80 W for 1 min, the 80-W level was maintained until roll-off. During the ablation procedure, the skin surface was cooled with 100 ml of iced saline in a poly pack. After the first roll-off, we waited for 30 s. The second ablation was then started at 10 W for 1 min, and the power was increased in the same manner as in the first ablation.

All adverse events regarding RFA were recorded. When RFA was completed, the deployed array was closed and the needle electrode was gently removed from the breast. Partial mastectomy was then performed immediately. The intra-operative pathological diagnosis of breast specimens was not examined. Breast specimens were cut at 5-mm intervals and macroscopic findings of the ablated lesions were recorded. Before the slices were fixed with 10% buffered formalin, two or three representative slices including the largest tumor lesion and non-tumor lesion were fixed with OCT compound and stored at −70 °C until use.

Pathological and biological examinations of the specimens were performed using hematoxylin–eosin (H&E) staining and nicotinamide adenine dinucleotide (NADH) diaphorase staining. NADH diaphorase staining was performed as follows. First, a tissue specimen fixed with OCT compound was sliced at 5 μm, placed on the glass and covered with incubation media at room temperature. Incubation media consisted of 120 ml of 0.05 M Tris-buffered-saline with 60 mg of Tetranitro BT and 96 mg of β-NADH (Wako Junyaku, Tokyo, Japan). After incubation for 30 min under aerobic conditions at 37 °C, slides were incubated with 10% buffered formalin for 30 min and then washed in distilled water for 2 min. Cell viability was determined from NADH diaphorase staining by one pathologist (T.H.) and one laboratory technician (Y.M.) who examined whether viable cells with purple mitochondria were present (Fig. 1). Immunostaining for estrogen receptor (ER) and progesterone receptor (PR) was considered positive when distinct nuclear localization was found in 10% or more of the cells. The HER2 status was evaluated using the Herceptest (DakoCytomation, Carpinteria, CA, USA). Tumors were considered 3-positive if 10% or more of the tumor cells had distinct circumferential membrane staining.

The primary endpoint was the rate of adverse events and the secondary endpoint was the efficacy of ablation therapy. Previous studies reported that skin burn, the most frequent adverse event, occurred in 0–6% of breast cancer patients after RFA. If the rate of adverse events related to RFA were estimated to be 6% or less, a 37-patient sample size would be needed for 80% confidence. The statistical significance of differences was examined using the paired *t*-test.

Results

We expected that accrual would be completed within 1 year because 100 or more cases of stage I breast cancer are treated annually in our hospital. However, there were very few eligible cases of T1N0 breast cancer with no extensive intraductal components among all of the patients with stage I breast cancer. Finally, 34 eligible patients were informed of this study and 30 (88%) were enrolled between June 2005 and March 2007. They ranged in age from 38 to 76, and 21 of them (70%) were over 50 years old. Eighteen (60%) had a tumor in the upper outer region and none had a tumor in the lower inner region. Tumor size as measured by ultrasound ranged from 9 mm to 24 mm (median 17 mm) and the distance from the nipple to the tumor ranged from 10 mm to 100 mm (median 45 mm; Table 1). MR mammography revealed no findings of an extensive intraductal component.

In most cases, the needle electrode was successfully placed in the center of the tumor through the co-access sheath. The

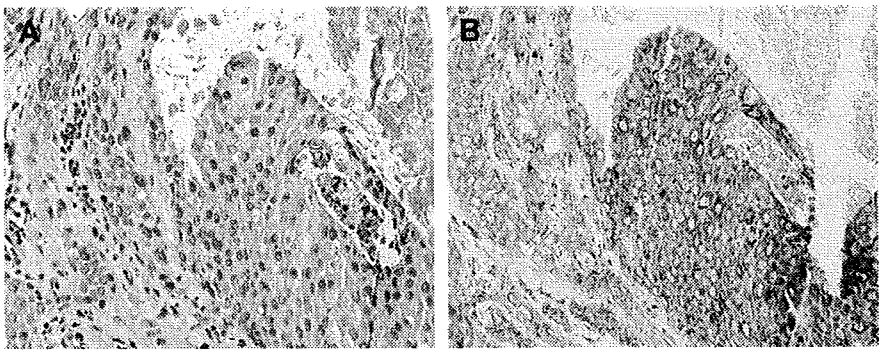

Fig. 1. Positive control of NADH diaphorase staining. (A) Tumor staining with H&E (×400). (B) Tumor staining with NADH diaphorase (×400).

Table 1
Patient and primary tumor characteristics.

	No. of cases
Histology	
IDC	26
NIDC	2
Others[a]	2
Distance from nipple to tumor on ultrasound (mm)	
10–19	3
20–29	1
31 or more	26
Tumor size on ultrasound (mm)	
10 or less	2
11–20	22
21 or more	6
Magnetic resonance mammography	
No finding of ductal spread	29
Suspected localized ductal spread	1

IDC, invasive ductal carcinoma; NIDC, non-invasive ductal carcinoma.
[a] Apocrine carcinoma and mucinous carcinoma in one case each.

completely ablated lesion was usually macroscopically discolored with some degree of carbonation and a red ring, which represented the border of thermal degenerative change, was seen (Fig. 2). NADH diaphorase staining was negative. However, one case showed skin burn under the nipple–areolar complex at the first ablation and we were unable to perform the second ablation. In addition, we performed incomplete ablation in another case because the tip of the needle electrode was carelessly placed outside the tumor (Fig. 3).

Table 2 shows an analysis of the parameters of the RFA generator. The initial impedance (median) for the first and second RFA was 136 and 114 Ω, the maximum electrical power (median) was 45 and 20 W, and time until 'roll-off' (median) was about 12 min and 5 min, respectively. The second RFA had significantly lower values for the initial impedance, maximum electrical power, and time until 'roll-off' ($p < 0.0001$). This suggests that breast tissue was almost completely ablated after the first RFA. The failed case shown in Fig. 3 showed impedance of 237 and 220 Ω at the first and second ablations, maximum electrical power of 15 and 10 W, and time until 'roll-off' of 2 min 45 s and 30 s, respectively. Overall, the total ablation procedure ranged from 4 min to 42 min (median and mean durations, 18 and 18 min).

Histological findings were listed in Table 1. Twenty-eight patients had a well- or moderately differentiated tumor, and two had a poorly differentiated tumor. With regard to hormone receptor status, 18 patients had positive staining for either ER or PR and 12 had negative staining for both hormone receptors. In addition, 27 patients had negative or 1-positive HER2 staining and 3 had 2- or 3-positive HER2 staining. We also examined the ER and HER2 status of tumor specimens from core needle biopsy and ablated tumor specimens in 11 cases. We found loss of ER expression in six of the nine cases and loss of HER2 overexpression in one patient after RFA.

Although it was difficult to determine tumor cell viability from H&E staining, degenerative changes such as swelling and picnosis of tumor cells were observed in 28 cases (Table 3). An extensive intraductal component beyond the ablated breast tissues, which was under-diagnosed by MR mammography, was found in two of these cases. Microscopic viable tumor cells on H&E staining were observed in two of the 30 patients: one had failure of needle electrode insertion (Fig. 3) and another received a modified ablation algorithm, in which the increase of 5 W every minute was changed to an increase of 5 W every 2 min. This modified RFA algorithm was tried in three patients, including one failure, to examine the possibility of thermal ablation by low-energy power. Overall, the complete ablation rate was 87% (26/30) based on the results of H&E staining.

Tumor specimens in all cases were stored for NADH diaphorase staining. However, four patients had no tumor lesions in the OCT compound, and thus tumor cell viability was examined by NADH diaphorase staining in 26 cases. Twenty-four of these patients had completely negative tumor staining. No viable tumor cells were observed in ablated breast tissues in 92% (24/26) based on NADH diaphorase staining.

Adverse events were observed in nine patients during partial mastectomy: two cases of skin burn including one patient with skin necrosis of the nipple–areolar region and seven cases of burns to the pectoralis major muscle. One patient had muscle burn over a wide and deep region and medication with non-steroidal anti-inflammatory drugs was required for 1 month to control pain in the chest wall.

Discussion

Early breast cancer is well controlled by BCS followed by breast irradiation, and shows about a 10% probability of local recurrence.[1,2] Breast cancer tends to spread through the mammary ducts, and an important prognostic factor for local recurrence is a positive margin of removed breast tissue. BCS is an appropriate technique for breast resection that is based on the precise diagnosis of extensive intraductal components by ultrasound and MR mammography. If RFA is to be used for the treatment of breast cancer, the indications should

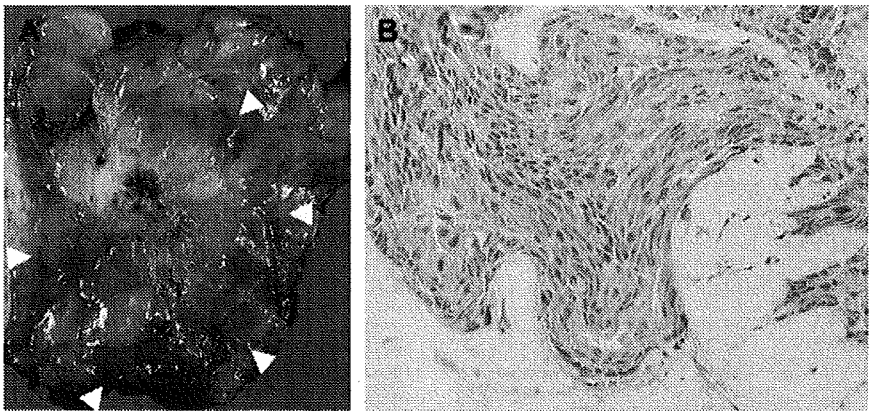

Fig. 2. Tumor cell viability after radiofrequency ablation. (A) Ablated breast tissues with a red ring (arrowheads). (B) Negative tumor staining with NADH diaphorase (×200).

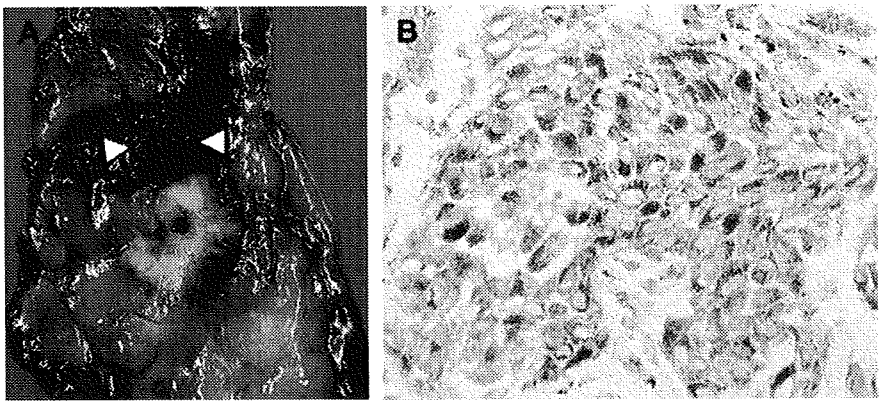

Fig. 3. Case of failure of radiofrequency ablation. (A) Macroscopic findings revealed partial tumor carbonization (arrowheads) due to unsuccessful electrode insertion. (B) Positive tumor staining with NADH diaphorase (×400).

be carefully selected so that intraductal components do not exist outside the ablated lesion after RFA.

Various thermal ablation therapies have been attempted for breast cancer: RFA,[11–18] laser therapy,[20] cryoablation,[21] and MR-guided focused ultrasound ablation.[22] Many investigators have attempted feasibility studies on RFA followed by breast surgery (Table 4). These studies have been small because of the limited number of eligible cases. In all of these studies, a needle electrode was inserted into the center of the breast tumor under ultrasound guidance. However, the ablation procedure varied. The RFA algorithms with LeVeen and Elektrotom electrodes and their generators depend on the initial setting and the step-up of electric power, e.g., 10 W for the initial 2 min and 5-W steps every 2 min. On the other hand, the algorithms with RITA and Cool-Tip electrodes and their generators are based on the maximum temperature of the needle tip and its duration, e.g., 95 °C for 15 min. We used the RFA algorithm described by Izzo et al.,[12] but seemed to require more time to complete ablation than they did (Table 4). However, the mean duration of RFA and the complete ablation rate were not so different (18 min and 87% vs. 16 min and 96%), which suggests that this algorithm is feasible and reliable.

Examination of biomarkers such as ER and HER2 status is essential when choosing adjuvant treatment for breast cancer. However, the expression of biomarkers may be changed after tumor ablation.[17] We found loss of ER expression and HER2 overexpression in ablated tumor specimens examined in 11 cases. According to our results, ER and HER2 status should be evaluated in tumor specimens before the RFA procedure.

We experienced two cases of skin burn and seven cases of muscle burn. The most plausible cause of such burning was thought to be hooking of the needle electrode. To avoid this, sterile 5% glucose was injected around the tumor. Careful tissue preparation before deploying the tines should be carried out under ultrasound guidance.

There is no consensus on the pathological evaluation of the cell viability of ablated breast tissues, in comparison to the criteria for the pathological evaluation of breast specimens after primary chemotherapy.[6] Our pathologist (T.H.) and cytologist (Y.M.), who are specialists in breast pathology and cytology, became accustomed to diagnosing ablated specimens with H&E staining based on discussion of their initial experience in several cases. It might be easier to examine pathological degeneration in ablated specimens obtained by delayed breast surgery than in those obtained by immediate breast surgery, since the degenerative changes caused by RFA continue to progress in ablated breast tissues.[13,15] NADH diaphorase staining is often used to examine the activity of NADH dehydrogenase in cells. We examined whether or not breast tissues were ablated completely by NADH diaphorase staining.

Despite the use of various RFA procedures, the overall complete ablation rate in the literature has been 85% (146/172) (Table 4). RFA followed by breast irradiation may be promising for the local control of breast carcinoma. To confirm that RFA is an alternative to BCS, a randomized controlled trial is needed to compare the two treatments. However, there are some difficulties in planning such a study. First, there are few eligible cases of breast cancer with no extensive intraductal components. Second, MR mammography or other detailed breast imaging may be essential for examining the extent of intraductal components. Third, the investigators must become skilled at the insertion of a needle electrode under ultrasound guidance. Fortunately, many surgical oncologists have an opportunity to learn basic ultrasound skills in Japan.

In conclusion, RFA was shown to be reliable and feasible in clinical stage I breast cancer with no extensive intraductal components. We are planning a phase II trial of RFA for the non-surgical treatment of breast cancer.

Table 2
Parameters of radiofrequency ablation.

Parameters	Range, Median	Paired *t*-test (29 cases)
Initial impedance (Ω)		
First ablation (30 cases)	85–237, 136	<0.0001
Second ablation (29 cases)	83–220, 114	
Maximum electric power (W)		
First ablation (30 cases)	15–89, 45	<0.0001
Second ablation (29 cases)	7–50, 20	
Time until 'roll-off'		
First ablation (30 cases)	2′45″–41′52″, 12′02″	<0.0001
Second ablation (29 cases)	0′15″–11′30″, 5′15″	
Total duration of ablation procedure	4′15″–41′52″, 17′54″	

Table 3
Pathological findings of ablated breast tissue specimens.

	No. of cases
Degenerative changes according to H&E staining	28
No ductal components beyond ablated breast tissues	26
Ductal components beyond ablated breast tissues	2
Viable tumor cells according to H&E staining	2
Tumor cell viability with NADH diaphorase staining	
Negative (not viable)	24
Positive (viable)	2

H&E, hematoxylin–eosin; NADH, nicotinamide adenine dinucleotide.

Table 4
Feasibility studies on radiofrequency ablation followed by surgical resection.

First author (year)[Ref.]	No. of cases	T	Electrode	Power (W)	Time (min)	Complete ablation	Complications
Jeffrey (1999)[11]	5	T2–T3	LeVeen	20–60	12–28	80%	None
Izzo (2001)[12]	26	T1–T2	LeVeen	25–80	7–25	96%	Skin burn (1 case)
Burak (2003)[13]	10	T1	LeVeen	–	7–21	90%	None
Singletary (2003)[14]	29	T1–T2	RITA	–	30–45	86%	Skin burn (1 case)
Hayashi (2003)[15]	22	T1	RITA	–	–	64%	Skin burn (1 case) Wound infection (4 cases)
Noguchi (2006)[16]	10	T1	RITA	–	–	100%	None
Khatri (2007)[17]	15	T1	Cool-Tip	14–53	7–36	93%	Skin puckering (2 cases) Wound infection (1 case)
Medina-Franco (2008)[18]	25	T1–2	Elektrotom	30–55	–	76%	Skin burn (3 cases) Wound infection (1 case)
Present study	30	T1	LeVeen	7–89	5–42	87%	Skin burn (2 cases) Muscle burn (7 cases)
Total	172	T1–3	Various	–	–	85%	Skin burn (8 cases) Miscellaneous (15 cases)

Conflict of interest statement

None declared.

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センチネルリンパ節

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はじめに

センチネルリンパ節とは、悪性腫瘍の原発巣からのリンパ流を直接受けるリンパ節のことであり、乳癌をはじめとする多くの悪性腫瘍においてリンパ行性転移がこのリンパ節に初発すること(センチネルリンパ節の概念とよばれている)が報告されている。これらの腫瘍に対しては、最初にセンチネルリンパ節の生検を行い、その転移状態を確認することにより、所属リンパ節全体の転移状態を予測することができるため、センチネルリンパ節転移が陰性の症例に対しては、所属リンパ節の郭清を省略した低侵襲治療が可能となる。このセンチネルリンパ節の転移状態に応じて所属リンパ節の郭清の有無を判断する術式は、Sentinel Node Navigation Surgery(SNNS)とよばれ、乳癌では標準的な治療法として定着しつつある(図1)。この個別化治療の成功にはセンチネルリンパ節の確実な同定が重要である。

センチネルリンパ節マッピング

センチネルリンパ節の同定は、原発巣周囲など原発巣からのリンパ流の流れる領域にリンパ移行性の良好なトレーサーを投与し、その動態を追跡することにより可能である。センチネルリンパ節検索のためのトレーサーとしては、視認性に優れる青色色素や検出感度が良好な放射性薬剤が用いられている。適当な^{99m}Tc標識コロイド製剤を用いれば、センチネルリンパ節への移行した後の停滞が良好であるため、術前にリンパシンチグラフィを行い、センチネルリンパ節の解剖学的な局在を把握することができる。

乳癌では、原発巣からのリンパ流が主として周囲乳腺組織内から直上の皮下に向かい、乳輪部に収束し、そこから腋窩に向かうことが知られているので、必ずしも原発巣周囲乳腺にトレーサーを投与する必要はなく、組織密度が高く、トレーサーのリンパ管への移行が良好な腫瘍直上の皮下や乳輪付近にトレーサーを投与している施設も多い。腋窩領域のセンチネルリンパ節の局在範囲は限られているので、色素単独でもセンチネルリンパ節の同定は比較的容易である。しかし、皮下投与や乳輪周囲投与では胸骨傍領域へのリンパ流を捉えることは難しく、色素による胸骨傍領域のセンチネルリンパ節の同定は困難である。

悪性黒色腫は、我が国では症例が少ないが、白人の多い欧米諸国では一般的な皮膚悪性腫瘍である。この疾患でも早期症例はセンチネルリンパ節生検のよい適応となっている。腫瘍周囲の皮内にトレ

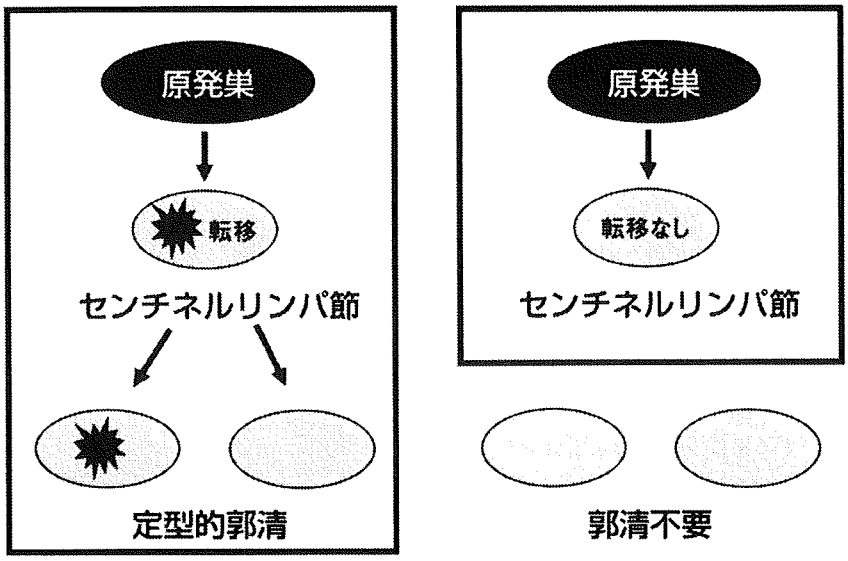


図1 Sentinel Node Navigation Surgery

ーサーを投与し、その流れを追跡することにより、センチネルリンパ節を同定できる。皮膚のリンパ流、特に体幹部の皮膚のリンパ流はしばしば複雑であり、色素法では追跡が困難な症例もあり、放射性薬剤の利用が有用である。

頭頸部癌、消化管癌でも、直視下にあるいは経内視鏡的にトレーサーを腫瘍周囲組織あるいは腫瘍周囲粘膜下層に投与すれば、センチネルリンパ節の検索が可能である。頭頸部、消化管もリンパ流が複雑な場合が多く、病変が存在する側と対側のリンパ節領域あるいは遠隔部位にセンチネルリンパ節が存在することもまれではない。このため、頭頸部や消化管の悪性腫瘍に対しては、放射性薬剤を用いたリンパ流の追跡が確実なセンチネルリンパ節の同定に重要な役割を果たす。

センチネルリンパ節マッピングに用いられる放射性薬剤

センチネルリンパ節マッピングのための放射性薬剤としては、リンパ移行性があり、流入したリンパ節内で、マクロファージに貪食され、そこに停滞する性質を有する^{99m}Tc標識コロイド製剤が一般的に利用されている。現時点では、センチネルリンパ節マッピングの目的で開発された製剤は医薬品として供給されておらず、各国で入手が可能なコロイド製剤を利用している。我が国では、^{99m}Tc標識フチン酸と^{99m}Tc標識スズコロイドが肝シンチグラフィ製剤として販売されており、これらがセンチネルリンパ節マッピングに用いられている。

乳癌のセンチネルリンパ節マッピングに関しては、これらの2種類のコロイド製剤が保険適応となった。来春にはセンチネルリンパ節生検という手術手技に対する保険承認も見込まれている。^{99m}Tc標識フチン酸は投与後体内でカルシウムイオンと反応し、径200nm程度のコロイドを形成する。^{99m}Tc標識スズコロイドはフチン酸コロイドより大きな径500nmを超えるコロイド粒子を多く含んでいる。^{99m}Tc標識フチン酸の方がリンパ管への移行は良好で、センチネルリンパ節への移行量も多く、センチネルリンパ節の同定が容易であると言われているが、センチネルリンパ節での停滞が不良で2次リンパ節に流出しやすく、真のセンチネルリンパ節の同定が困難となる場合がある。

腫瘍の発生臓器によって、腫瘍周囲組織のリンパ網の発達の程度が異なるため、対象とする腫瘍により使用するコロイド製剤を選択した方がよい。例えば、胃癌では粒子径の大きな^{99m}Tc標識スズコロイドを用いた方がセンチネルリンパ節からの放射性核種の流出を抑え、より正確にセンチネルリンパ節を同定することができる。

最近、センチネルリンパ節マッピングを目的に、リンパ節中のマクロファージに発現するマンノース受容体に親和性を示す物質を標識した製剤が開発された。この製剤を用いると、リンパ管への移行率、センチネルリンパ節への停滞性の両方が改善することが報告されている。

センチネルリンパ節マッピングのためのシンチグラフィ

放射性薬剤を用いたセンチネルリンパ節マッピングの特長は、術前にシンチグラフィを行い、センチネルリンパ節の解剖学的な局在を確認できることである。

センチネルリンパシンチグラフィにおいて、単純な撮像では投与部位とセンチネルリンパ節しか描出されないため、センチネルリンパ節の局在を知るには、撮像法の工夫や収集画像の画像処理が必須である。

体輪郭を描出させると、センチネルリンパ節の局在が明瞭となる。体輪郭の描出には、外部線源を用いて透過像を併せて撮像する方法が一般的である(図2)。しかし、この方法は外部線源を加えた撮像を追加するために、検査時間が延長する。患者および検査に立ち会う医療従事者の被曝線量が増加するという問題がある。また、手術時の術者の視野に近い画像が得られる斜位像の撮像では、透過線源の固定が難しいため、この方法での体輪郭の描出は難しい。

これらの問題を解決する方法として、低エネルギーの散乱線成分を、投与部位やセンチネルリンパ節に集積した^{99m}Tcから放出される一次線成分と同時に収集してやる方法がある。体内に存在する^{99m}Tcから放出されるガンマ線は、体内でコンプトン散乱を起こし、低エネルギーのガンマ線に変換される。このため、低エネルギーの散乱線成分の分布を確認すれば、人体の形状すなわち体輪郭を描出することができる。この方法は、散乱線成分の収集を通常の一次線成分の撮像と同時に行うことができるため、検査時間の延長や被曝線量の増加といった問題もない。また、斜位像に対しても体輪郭

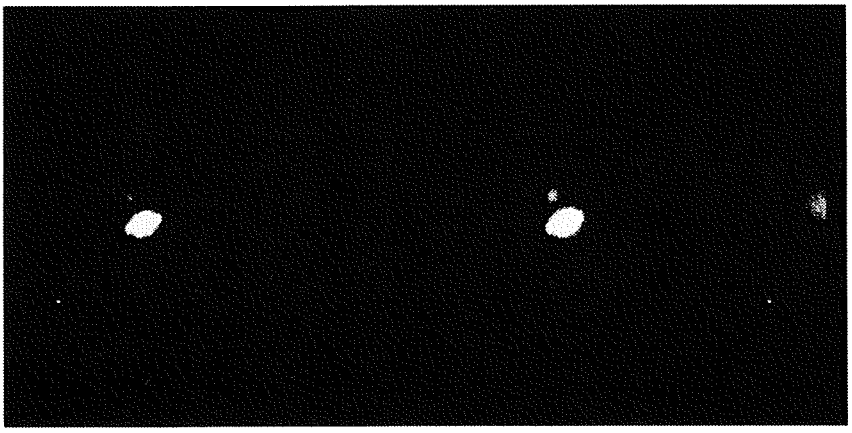


図2 リンパシンチグラフィにおける体輪郭の描出
a 単純な撮像による画像。
b 外部線源をおいた透過像の追加により体輪郭が描出される。

の描出は可能である。外部線源を用いて透過像を撮像する方法と比較して、部位により体輪郭の描出の明瞭さにばらつきがあるなどの問題点はあるが、簡便であり、臨床的有用性は高い。また、散乱線成分はセンチネルリンパ節の付近でもなだらかなカウント分布を示すため、一次線成分を散乱線成分で除算すると、センチネルリンパ節のコントラストを強調し、センチネルリンパ節の描出を明瞭化させることができる(図3)。さらに、フィルター処理により、ノイズ成分を除去することで、体輪郭のコントラストを強調させることもできる(図4)。これは後述のSPECT/CT検査で、SPECT画像を正確にCT画像に重ね合わせる際に役立つと考えられる。

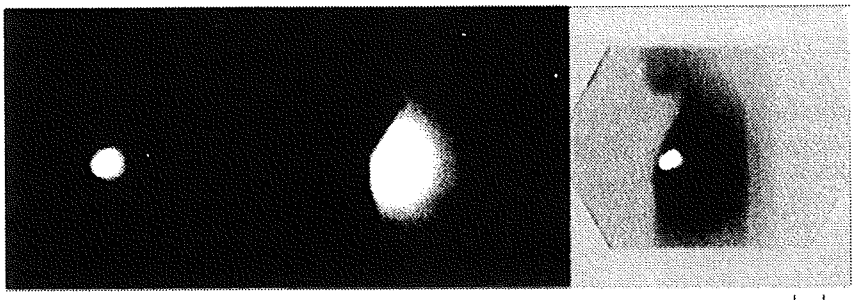


図3　左乳癌のセンチネルリンパ節　a | b | c
一次線成分の画像(a)を散乱線成分の画像(b)で除算すると、体輪郭が描出され、センチネルリンパ節のコントラストが改善する(c)。

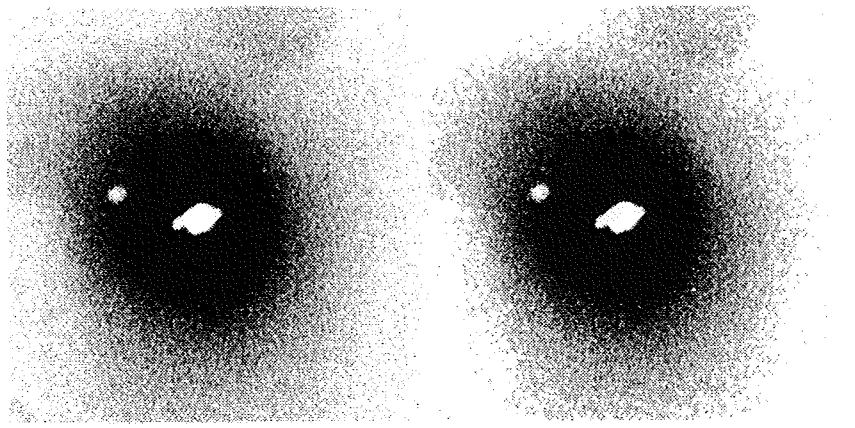


図4　リンパシンチグラフィの画像処理　a | b
ノイズ除去フィルターを用いることにより、体輪郭部のコントラストが強調される(a　処理前画像、b　処理後画像)。

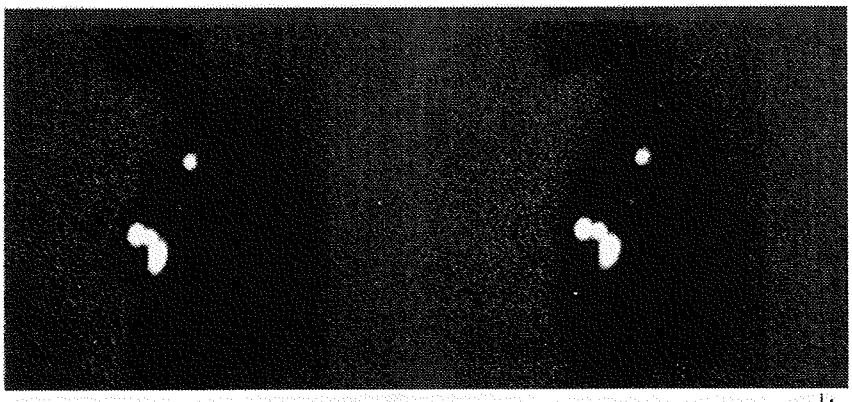


図5　左乳癌のセンチネルリンパ節の立体視　a | b
左側面像(a)を右目で観察し、左側面より10度腹側から観察した画像(b)を左目で観察し、焦点を合わせると画像が立体的に見える。頭側の強い集積を示すリンパ節の方が、尾側の弱い集積を示すリンパ節より手前にある。

センチネルリンパ節の3次元表示

リンパシンチグラフィを実際のセンチネルリンパ節生検で役立てるには、センチネルリンパ節の局在を平面像として示すだけでなく、深さに関する情報も提供できるとよい。

大まかな深さに関する情報を得るだけであれば、収集方向より10度程度斜めの方向からの斜位撮像を追加し、得られた2枚の画像を立体視することにより、センチネルリンパ節の立体的位置関係を知ることができる(図5)。肉眼での立体視には多少の慣れが必要であるが、立体視用の眼鏡も市販されているので、それを用いれば容易に観察することができる。

センチネルリンパ節の深さに関する情報をより正確に知るには、断層撮像法であるSPECTを施行する必要がある。複数方向から撮像した平面画像のデータを基に断層像を再構成する手法である。原理的には、撮像方向を増やすことにより、センチネルリンパ節の位置情報の精度が改善することになるが、検査時間全体の制約から撮像方向数の増加はそれぞれの原画像の撮像時間の短縮につながる。撮像時間の短縮により各原画像のカウント数が減少するため、信号雑音比が低下し、画質が低下する。このため、センチネルリンパ節マッピングにおいては、SPECT収集のステップ角度をあまり小さくしない方がよい。

PET/CTの開発で機能形態融合画像の有用性が示されたことから、SPECTに関してもSPECT/CTの導入が進められている。SPECT/CT装置の導入により、センチネルリンパ節の位置情報を得るこ

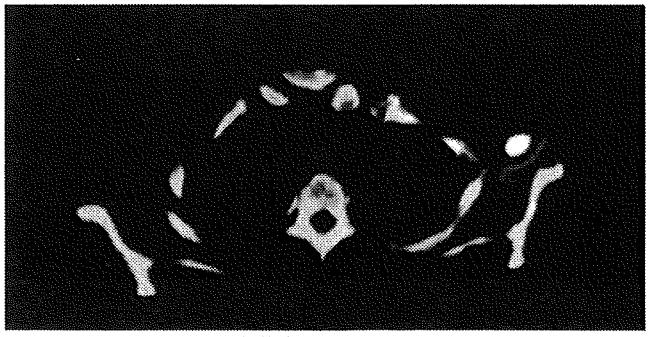
とが容易となってきたが(図6)、SPECT/CT装置でのSPECT撮像とCT撮像は同時撮像ではなく、別々の撮像であるため収集時間に差があり、体動により両画像にずれが生じる可能性がある。したがって、SPECT/CT装置において、正確な画像重ね合わせを行うためにはSPECT画像上での体輪郭の描出が有用と考えられる。この場合、上記のように外部線源の利用は難しく、散乱線成分の収集による方法が役立つであろう。

術中センチネルリンパ節イメージング

常温で作動するテルル化カドミウム系の半導体放射線検出器が実用化したことで、術中に撮像が可能な小型ガンマカメラが開発された。このような装置を用いると、手術室での術中シンチグラフィも可能であるが、リアルタイムでの走査が可能な携帯型小型放射線計測器ガンマプローブよりもはるかに感度が低く、リアルタイムでのイメージングは難しく、一時的に手術を中断する必要がある。現時点では、術中のセンチネルリンパ節検索は、ガンマプローブにより行い、生検後の確認の目的でこのような小型ガンマカメラでシンチグラフィを撮像するのが合理的であろう。

まとめ

センチネルリンパ節生検は、悪性腫瘍の低侵襲個別化治療を実現する重要な手法である。センチネルリンパ節の確実な同定には、放射性薬剤を用いる方法がしばしば有用である。放射性薬剤を用いる方法では、シンチグラフィによりセンチネルリンパ節の解剖学的な局在を術前に知ることができ便利である。撮像装置の進歩などにより、センチネルリンパ節の3次元的な位置情報の取得や術中イメージングも可能となっており、最新の核医学技術が先進医療であるセンチネルリンパ節生検の精度を高めるために大きく貢献している。



巻頭カラー参照

図6 SPECT/CTによる左乳癌センチネルリンパ節マッピング
^{99m}Tc標識コロイド製剤の集積を示すSPECT画像とCT画像の重ね合わせ画像(John Wayne癌研究所 Glass EC博士の厚意による)。

NEWS!!

日本磁気共鳴医学会大会、AZEがイブニングセミナーを開催

10月1～3日、パンパシフィック横浜ベイホテル東急(神奈川県横浜市)において開催された第37回日本磁気共鳴医学会大会では、イブニングセミナーも開催された。第1日目には(株)AZEの共催で、佐久間肇氏(三重大学医学部附属病院)を座長に迎え「MRIによる3D,4D volume reading活用術—Thin client systemでisotropic dataを使いこなす—」と題した講演を片平和博氏(熊本中央病院)が行った。

CTの多列化が進む中で近年注目されているthin clint system。多列CTやvolume MRI時代では必須のシステムである。従来ではgradient echo系の撮像法ではvolume data取得は比較的容易であったが、最近ではspin echo系やSSFP系のvolume dataも容易に取得可能となってきた。このため、thin client systemは不可欠なものになってきている。

Thin client systemを用いた任意断面のMPR、MIP画像による有用性は大きい。3D読影において、端末状で任意断面が選択可能となることで、MR angiographyでは時相を加えた4D読影も可能となった。VR画像を用いた有用性も大きい。MRIにおけるVR画像はCTほど普及していないが、造影剤を用いない画像でもVR画像が容易に作成可能。腎機能障害・造影剤アレルギーによって造影剤が使用不可の場合に有効である。同氏は「AZE Virtual Placeは元々MRI対応で定評がある。しかし、今回はnetwork対応型のRaijinを用いて作成した有効なVR画像を紹介する」と症例を呈示した。

片平和博氏

冠動脈撮像と遅延造影MRIの組み合わせや、異なるmodalityを用いたvolume fusionなどの画像に、来場者は熱心に見入っていた。

臨床放射線 54：1435-1445，2009

【8. センチネルリンパ節生検】

診 断

乳腺のリンパ系の解剖と画像診断

藤井博史*1 山口雅之*1 梅田 泉*1 和田徳昭*2

はじめに

乳癌の最大の予後因子は所属リンパ節の転移状態であるため，それを正確に評価することは乳癌患者の治療方針の決定や予後予測に重要である[1]。所属リンパ節への転移は，原発巣周囲のリンパ管内に浸潤した癌細胞がリンパ行性にリンパ節に流入することにより生じるが，乳腺からのリンパ液は主に同側の腋窩領域や胸骨傍領域に流れるため，乳癌のリンパ節転移もこれらの領域に好発する。腋窩領域は，触診でもリンパ節の腫大がある程度判断できるが詳細な評価は難しい。また，胸骨傍領域は触診が困難である。このため，乳癌のリンパ節転移診断に画像診断検査が果たす役割は大きい[2]。

これまでの研究から，癌の原発巣からのリンパ流の大半はほぼ一定の方向に流れ，特定のリンパ節に注ぐことが分かってきた。この原発巣からのリンパ流を他のリンパ節を介することなく直接的に受けるリンパ節のことをセンチネルリンパ節といい，リンパ行性転移はこのリンパ節に初発する。したがって，このリンパ節を生検して（センチネルリンパ節生検）転移状態を調べれば，少ない侵襲で所属リンパ節の転移状態を知ることができる。乳癌ではセンチネルリンパ節生検の有用性が認められ，普及してきたため，乳癌の所属リンパ節転移の進行期分類もセンチネルリンパ節生検の結果を取り入れたものが提案されている。

以上のことを踏まえて，本稿では，乳腺のリンパ系の解剖，最近の乳癌の所属リンパ節転移の分類，乳癌のリンパ節転移の画像診断およびセンチネルリンパ節イメージングについて解説する。

① 乳腺のリンパ系と所属リンパ節転移

乳腺組織内には，リンパ管がネットワークを形成しているが，その中を流れるリンパ流は，いくつかの系統に分けられることが古くから報告されている[3-5]。最も重要な流れは，乳腺内から皮下に向い，それらが乳輪部に集合し，そこから同側の腋窩領域に向かう流れである（図1）。この他に，乳腺内の深部から大胸筋を貫き胸骨傍領域に向かう流れなどがある。乳腺の底面から腋窩領域に向かうリンパ流も報告されている（図2）[6]。原発巣からのリンパ流を直接受けるリンパ節であるセンチネルリンパ節は，乳癌の場合，腋窩領域や胸骨傍領域に存在することが多く，乳癌のリンパ行性転移は，センチネルリンパ節に始まった後，リンパ管が連絡している鎖骨上リンパ節などに広がっていく。

乳癌の所属リンパ節は，最新の乳癌取扱い規約（第16版，2008年）では表1のように定義されている（図3）[7][8]。この分類は，国際的な分類であるInternational Union Against Cancer（UICC）のTNM分類（第6版，2002年）の定義にほぼ準拠しているが[9][10]，TNM分類で区別している腋窩リンパ節レベルIIIと鎖骨下リンパ節の境界の定義が明確ではなく混乱が生じていることから，乳癌取扱い規約ではこれらは腋窩リンパ節レベルIIIで統一されている[7]。

乳癌の所属リンパ節への転移は，乳癌取扱い規約

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〔索引用語：乳癌，リンパ節，転移，センチネルリンパ節，リンパシンチグラフィ〕

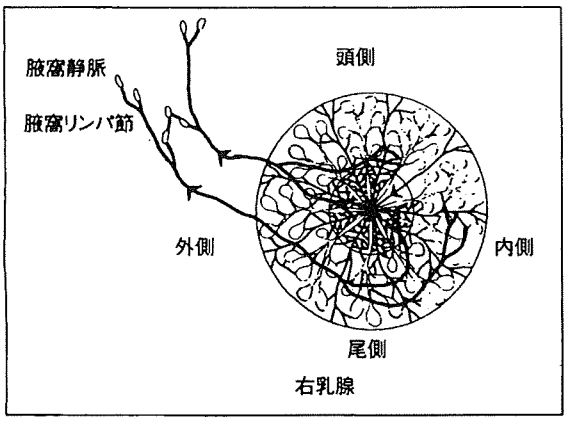

図1　乳腺の主要なリンパ流（文献3，図3を一部改変）

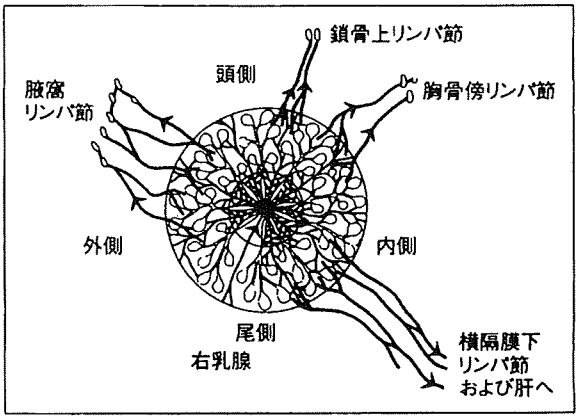

図2　乳腺の副次的なリンパ流（文献3，図4を一部改変）

表1　乳癌の所属リンパ節

乳癌取扱い規約（第16版，2008年）
1）腋窩リンパ節
　Level I, II, III に分類する
　Level I：小胸筋外側縁より外側
　　乳房内リンパ節はLevel Iとみなす
　Level II：小胸筋背側および胸筋間
　Level III：小胸筋内側縁より内側
2）胸骨傍リンパ節
3）鎖骨上リンパ節

UICC TNM分類（第6版，2002年）
1）腋窩リンパ節
　Level I, II, III に分類する
　Level I：小胸筋外側縁より外側
　　乳房内リンパ節はLevel Iとみなす
　Level II：小胸筋背側および胸筋間
　Level III：小胸筋内側縁より内側
2）鎖骨下リンパ節
3）胸骨傍リンパ節
4）鎖骨上リンパ節

では表2のように分類されており，リンパ節転移は触診および画像診断検査により臨床的に診断することになっている[7]。UICC TNM分類では，臨床診断に基づく分類に加えて，病理組織学的診断に基づく分類が記載されているが[9]，乳癌取扱い規約ではこれらは全面的には掲載されておらず，センチネルリンパ節の微小転移（径2mm以下の転移を指す）の表記法pN1mi (sn) が取り上げられているだけである[7]。

❷ 乳癌のリンパ節転移の画像診断

乳癌の所属リンパ節転移の診断には，複数の画像検査が利用されている。従来よりCT検査，超音波検査，MRI検査などが行われているが，これらの主として形態学的な判断による診断法に加えて，最近では，病巣の糖代謝活性の評価ができるFDG-PET検査による機能的な診断も積極的に行われるようになっている。特にCT装置と一体化したPET/CT装置の開発によりFDG-PET検査の有用性が増している。

乳癌のリンパ節転移の最好発部位は腋窩領域であるため，腋窩リンパ節転移に関する研究が多く報告されている。

CT検査における腋窩リンパ節転移の診断基準は報告により異なるが，短径が5mm以上で円形あるいは類円形の形状を示しているものを転移陽性とする報告が多い（図4）。Ogasawaraらは[11]，マルチディテクターCTで撮像された画像をこの基準で診断し，腋窩リンパ節転移の診断成績が，感度76.9%，特異度96.6%，正診率90.5%，陽性予測率90.9%であったと報告している。健側に比して患側の腋窩領域のリンパ節数が多い場合も転移の可能性がある[2]。N2aに分類されるリンパ節転移ではリンパ節と周囲組織あるいはリンパ節相互間に固定が認められるが，CT画像上はリンパ節の辺縁がぼけ，周囲脂肪織の濃度上昇が観察される[2]。

超音波検査における腋窩リンパ節転移の診断基

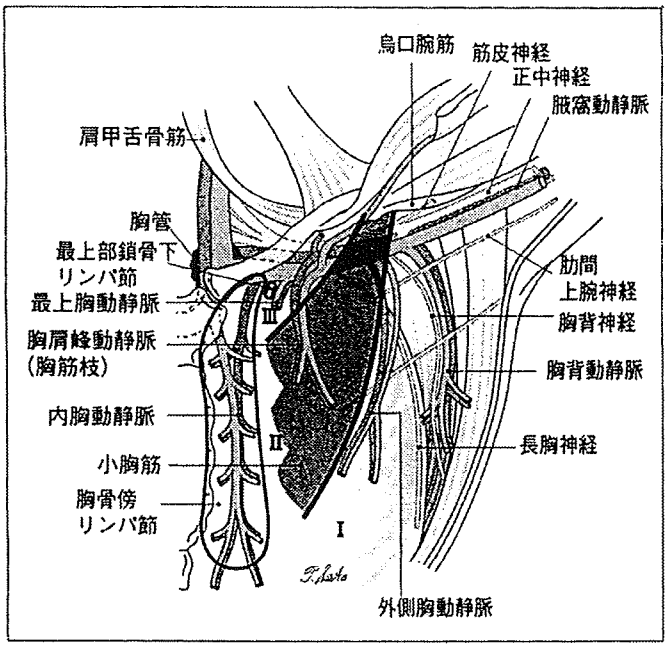

図3　乳癌の所属リンパ節（文献8）より

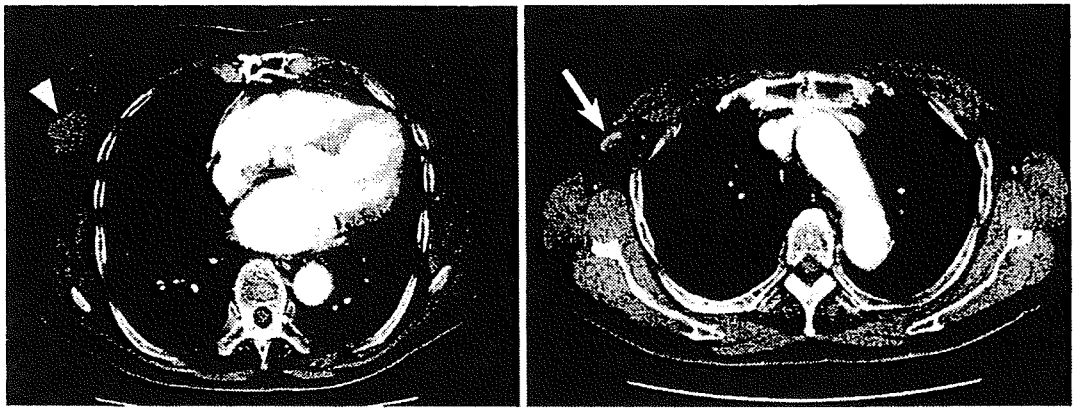

図4　右乳癌の右腋窩リンパ節転移症例のCT画像　80歳代，女性
原発巣：▲，右腋窩リンパ節転移：→

準も，短径5mm以上を転移陽性とするものが多い。形態学的な基準としては，円形，低エコー，分葉化などが採用されている（図5）。Alvarezら[12]が16の研究をまとめた総説論文によれば，腋窩リンパ節非触知症例に限ると，大きさの基準で診断した場合，感度が48.8～87.1%，特異度が55.6～97.3%であり，形態学的な基準で診断した場合，感度が26.4%～75.9%，特異度が88.4%～98.1%であった。彼らは，腋窩リンパ節転移診断における超音波検査を，中等度の感度を示し，かなり高い特異度を示す検査と位置づけている。超音波検査では，疑わしいリンパ節を見つけた場合，それを生検して組織学的に検証することも可能である。これにより特異度をほぼ100%にすることができる。超音波検査により転移陰性と診断された症例を対象としてセンチネルリンパ節生検を実施すると，センチネルリンパ節生検の陰性予測率が改

表2　所属リンパ節の転移診断の分類

乳癌取扱い規約（第16版）による分類

		同側腋窩リンパ節レベルI, II		同側胸骨傍リンパ節	同側腋窩リンパ節レベルIII	同側鎖骨上リンパ節
		可動	周囲組織への固定あるいはリンパ節癒合			
NX		評価不可能				
N0		−	−	−	−	−
N1		+	−	−	−	−
N2	a	−	+	−	−	−
	b	−	−	+	−	−
N3	a	+/−	+/−	+/−	+	−
	b	+	+	−	−	
	c	+/−	+/−	+/−	+/−	+

+/−：転移の有無を問わない

UICC　TNM分類（第6版）

臨床診断に基づく分類

TNM分類		同側腋窩リンパ節同側胸骨傍		その他のリンパ節	リンパ節
		可動	固定（周囲組織またはリンパ節相互間）		
NX		評価不能			
N0		−	−	−	−
N1		+	−	−	−
N2	a	−	+	−	−
	b	−	−	+	−
N3	a	+/−		+/−	同側鎖骨下リンパ節（+）
	b	+		+	−
	c	+/−		+/−	同側鎖骨上リンパ節（+）

病理組織学的診断に基づく分類

TNM分類		同側腋窩リンパ節転移	同側胸骨傍リンパ節転移	その他のリンパ節の転移
pNX		評価不能		
pN0		−（最大径0.2mm以下の小さな細胞の集塊はpN0とする）		
pN1mi		微小転移（最大径が0.2mm～2mmの転移病巣）		
pN1	a	1～3	−	−
	b	−	SLNBで見つかった微小転移	−
	c	1～3	SLNBで見つかった微小転移	−
pN2	a	4～9	−	−
	b	−	+	−
pN3	a	10～	−	−
	a	+/−	+/−	同側鎖骨下リンパ節転移
	b	+	+	
	b	4～	SLNBで見つかった微小転移	
	c	+/−	+/−	同側鎖骨上リンパ節転移

SLNB：センチネルリンパ節生検