Table 3 Univariate analysis of predictive markers in pathological response and tumor reduction

Parameter	pCR		Tumor reduction rate >30%	
	n (%)	*p* value	*n* (%)	*p* value
Age (years)				
≥51	42[a] (22)	0.022	61 (52)	N.S.
≤50	22 (13)		85 (56)	
Invasive ductal carcinoma				
Solid-tubular type	35[a] (27)	0.0006	60[a] (67)	0.005
Scirrhous type	12[a] (8)	0.0006	50 (52)	N.S.
Papillotubular type	8 (12)	N.S.	29 (54)	N.S.
ER-negative	53[a] (24)	<0.0001	96 (59)	N.S.
ER-positive	11 (7)		50 (48)	
PgR-negative	50[a] (23)	0.0005	92 (58)	N.S.
PgR-positive	14 (9)		54 (50)	
HER2 3+	19[a] (33)	0.004	24 (55)	N.S.
HER2 2+	6 (11)		27 (66)	
HER2 <1+	39 (15)		95 (52)	
Histological grade G3	45[a] (25)	0.001	89[a] (70)	<0.0001
G2	17 (10)		49 (39)	
G1	2 (11)		7 (58)	
Clinical response				
CR + PR	62[a] (20)	0.0017	138[a] (60)	<0.0001
NC + PD	2 (3)		8 (22)	
CT tumor type				
Localized type	16 (24)	0.063	48[a] (74)	0.0003
Nonlocalized type	29 (14)		98 (49)	

[a] $p < 0.05$

CT, computed tomography; *ER*, estrogen receptor; *PgR*, progesterone receptor; *CR*, complete response; *PR*, partial response; *NC*, neoadjuvant chemotherapy

NC and the clinicopathological examination results. Significantly higher percentages of patients achieved pCR if they were aged 50 years or older, had solid-tubular type disease, were negative for ER or PgR, were positive for HER-2, had histological grade 3 disease, demonstrated positive clinical sensitivity (CR [complete response] + PR [partial response]), or were classified as having localized disease using pretreatment CT imaging. Conversely, significantly lower percentages of patients experienced pCR if their tumors were histologically classified as scirrhous. When the pretreatment maximum tumor size and the postoperative pathological maximum tumor size were compared, the clinicopathological factors that were significantly associated with 30% or more reductions in tumor size were having solid tubular-type disease, testing negative for ER, classification of histological grade 3, positive clinical sensitivity (CR + PR), and classification as localized tumors based on pretreatment CT imaging. Table 4 shows the results of multivariate analysis of these factors. In this analysis, the factor that was significantly associated with reduced rates of pCR was tumors classified as scirrhous. Other factors did not significantly influence the pathological response. Histological grade 3, positive clinical sensitivity (CR + PR), and classification as localized tumors were significantly associated with tumor size reduction.

Discussion

In recent years, NC has been used not only for locally advanced breast cancer but also for relatively early-stage breast cancer. This type of therapy is used to (1) achieve pCR; (2) enable breast conservation by reducing the size of the tumor; and (3) evaluate the sensitivity of the breast cancer to anticancer drugs.

The primary purpose of NC is to achieve pCR, which is based on the understanding that patients who experience pCR after NC have better prognoses relative to other patients [8]. To accomplish this purpose, it is necessary to characterize the cases of breast cancer that are more likely to achieve pCR and to select anticancer drugs that are appropriate for each case. Immunohistological examinations, including analyses of hormone receptors, HER-2 and

Table 4 Multivariate analysis

Parameter	pCR		Tumor reduction rate >30%	
	p value	Odds ratio	*p* value	Odds ratio
Age >51 years	NS		NS	
Solid-tubular type	NS		NS	
Scirrhous type	0.008	0.2 (−1.441 to −0.239)	NS	
ER-negative	NS		NS	
PgR-negative	NS		NS	
HER2 3+	NS		NS	
Histological grade G3	NS		<0.0001	3.76 (0.349–0.989)
CR + PR	NS		0.0003	5.28 (0.405–1.309)
Localized type	NS		0.012	2.42 (0.104–0.796)

CR, complete response; *PR*, partial response; *NS*, not significant

Ki-67, have been reported to relate to the efficacy of PST [9–12]. In our study, we examined the characteristics of breast cancer tumors that made it easier to achieve pCR with NC. In univariate analysis, histological grade 3 and solid-tubular type tumors as well as lack of ER and PgR overexpression and the presence of HER-2 overexpression were shown to be significantly associated with improved treatment efficacy. However, multivariate analysis revealed that cases classified as scirrhous type were significantly less likely to achieve pCR. Interestingly, PST has been reported to be less effective for ILC [13–15]. In this study ILC had few effect of tumor size reduction of NC and there was no pCR case in ILCs (data not shown). However, ILC was rare in Japan formerly and there were few ILC patients in this study. One of the reasons for this low efficacy may be that tumor cells from ILCs are relatively isolated and are distributed among the fibrous stroma, leading to less blood flow to the tumor and less drug accessibility. Scirrhous-type tumors, which were associated with less NC efficacy, are histologically similar to ILCs growing as the stroma grows with relatively isolated tumor cells. Therefore, these histological features may be related to the efficacy of NC for these tumors.

It has been reported that NC is useful for breast conservation after a reduction of tumor size [16–18]. In the EORTC10902 study, NC enabled breast conservation in 57 of 246 (23%) patients who were scheduled to undergo total mastectomies [16]. In the present study, we characterized the tumor sizes, which tended to be reduced by NC, using pretreatment CT imaging as well as clinicopathological examinations. Magnetic resonance imaging (MRI) is more widely used to plan adequate surgical treatment for early breast cancer than CT probably because of the risk of radiation exposure. However, CT scan has an important advantage compared with MRI because CT breast images are obtained in the supine position used during surgery, thus providing precise information about the tumor extent; in contrast, in most previous studies of MRI, patients were examined in the prone position to minimize motion of the breast during breathing. There are helical CT scanners in many medium and small Japanese hospitals. Therefore, we can use CT without circumstance. As a result, a significant reduction of tumor size was observed in cases classified as localized tumors, as well as those categorized as histological grade 3 disease and those that achieved CR or PR in terms of clinical efficacy. There are previous reports about NC reducing the sizes of tumors and the safety of breast-conserving therapy, including one from our institution [18–20]. When the tumors show sporadic shrinkage, they need to be resected carefully after NC because the remaining tumor cells can be diffusely distributed. In contrast, when the shrinkage pattern is concentric, NC is thought to be more effective for reducing the tumor size, making breast-conserving therapy safer. Therefore, localized tumors may achieve a favorable degree of reduction because they often shrink in a concentric manner. In evaluation of the tumor reduction rate, we classified the tumor shape, measured the pretreatment tumor size, and compared it with the post-operative pathological tumor size. The classification of tumors into localized or nonlocalized types using CT imaging provides a basis for making this determination. Localized tumors responded well to NC and were reduced into smaller, concentric tumors that could be safely treated by wide excision, giving a negative margin status. However, nonlocalized tumors diminished into a mosaic pattern of residual tumor cells, giving a positive margin status when treated with breast conserving therapy and tumor reduction rate were low. Multivariate analysis demonstrated that classification by CT was a powerful predictor of the tumor reduction rate by NC in this study. To the best of our knowledge, this is the first report to show that the tumor shape is useful as a predictive criterion for the efficacy of NC.

Breast cancer therapy with anticancer drugs is thought to result in equivalent survival rates when performed before or after surgery [8, 16]. Currently, both anthracyclines and taxanes are sufficiently used to increase the percentage of patients achieving pCR; however, there are no definitive criteria that detail the proper indications of various anticancer drugs for different types of tumors. Therefore, unnecessary drugs may be administered to patients in excessive doses. The postoperative adjuvant therapy for primary breast cancer is provided in accordance with the recommendations from the St. Gallen consensus meeting [21]. Although adjuvant chemotherapy is considered to be standard for node-positive patients, many aspects concerning the administration of anticancer drugs to node-negative patients have not been clarified. In particular, whether the anthracyclines and taxanes used for NC are necessary for these node-negative patients is not clear, and thus, these drugs may be used excessively for these patients. We believe that it is critical to predict the efficacy of drugs used for different purposes to determine which drugs and doses should be for each patient. In the NSABPB-27 study, the addition of a taxane to an anthracycline did not result in a significantly improved survival rate, which suggested that more specific criteria are needed to identify the cases in which taxanes produce an additive effect [1]. In recently published studies, the sensitivity of a certain drug was evaluated and then therapy was continued only for patients who experienced efficacy by adding the drug, whereas surgeries were performed for those who did not benefit from the medication. In fact, there are patients who do not benefit from widely used anticancer drugs, including anthracyclines and taxanes [21, 22]. Performing NC aggressively in these patients is disadvantageous. Thus,

it is important to identify tumors resistant to NC before the treatment and to exclude such cases from NC.

We have examined the predictability of NC efficacy, which has no current definitive indication. Regarding the prediction of efficacy to achieve pCR, high degrees of responsiveness is reportedly obtained with the concomitant use of trastuzumab in patients who have HER-2 overexpression [2]. At our institution, trastuzumab has been administered to these patients in recent years, leading to a markedly high pCR rate, which surpassed that achieved using NC with anthracyclines and taxanes. These patients, however, were not included in this study because we only recently started routinely using trastuzumab and many patients who showed HER-2 expression did not receive this agent early in the study. The examination of both pCR and tumor size reduction in the present study identified several factors that are useful to determine the indications of NC. This study indicated that pCR of scirrhous type for NC was difficult and the primary tumor with localized tumor type in CT imaging or histological grade 3 will be fairly reduced by NC. However, these features could not predict the response completely and terminate the NC premature in nonresponders. Additional cases and prospective studies that are focused on particular types of cases are necessary.

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Med Oncol (2009) 26:344–349
DOI 10.1007/s12032-008-9127-2

ORIGINAL PAPER

Immunohistochemical expression of PTEN and phosphorylated Akt are not correlated with clinical outcome in breast cancer patients treated with trastuzumab-containing neo-adjuvant chemotherapy

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Received: 31 March 2008 / Accepted: 29 October 2008 / Published online: 18 November 2008

Abstract The loss of PTEN and phosphorylated Akt (pAkt) expression is thought to be involved in the mechanism leading to trastuzumab resistance in patients with HER2-positive breast cancer. We retrospectively performed immunohistochemical analyses for estrogen receptor, progesterone receptor, HER2/neu, PTEN, pAkt, and p53 expression in tumor specimens obtained before and after trastuzumab-containing neo-adjuvant chemotherapy. The intensity of staining was evaluated for each biomarker, and the correlations between the immunohistochemical profiles and the clinical outcome were analyzed. The changes in the immunohistochemical profiles between specimens obtained before and after trastuzumab-containing neo-adjuvant chemotherapy were evaluated for patients with residual tumors. The present study included 44 patients with breast cancer who received trastuzumab-containing neo-adjuvant chemotherapy. Seventeen patients achieved a pathological complete response. The patients were positive for PTEN and pAkt (PTEN = 14%, $N = 6/44$; pAkt, 80%, $N = 35/44$). The expression of both PTEN and pAkt were not correlated with pathological complete response. Persistent HER2/neu over-expression after neo-adjuvant chemotherapy was significantly associated with recurrence. Among 27 patients with residual cancer, the percentages of patients with HER2/neu-positive or pAkt-positive tumors were low, but PTEN expression was elevated. The present study suggested that neither the immunohistochemical expression of PTEN nor the expression of pAkt was associated with the clinical outcome of trastuzumab-containing neo-adjuvant chemotherapy. Except among patients with pathological complete remission, the persistent over-expression of HER2/neu may be a poor prognostic factor.

Keywords Breast cancer · Neoadjuvant chemotherapy · pAkt · PTEN · Trastuzumab

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Introduction

Breast cancer remains the major cause of death from cancer among woman throughout the world. Most breast cancers are thought to be systemic diseases at the time of diagnosis, and recurrence as a result of sub-clinical micro-metastasis is common. Recent advances in multidisciplinary approaches for treating breast cancer, including both neo-adjuvant chemotherapy and adjuvant chemotherapy, have played important roles in improving the survival rate [1]. A previous study revealed that patients who achieved a pathological complete response (pCR) had longer relapse-free survival periods than patients without pCR after neo-adjuvant

chemotherapy [2]. This study suggested that the chem otherapeutic response at the primary lesion may be correlated with the chemotherapeutic response of micrometastases; therefore, the selection of a chemotherapeutic regimen that best enables pCR may improve both the relapse-free and overall survival rates.

The epidermal growth factor receptor/HER family is involved in cell proliferation, differentiation, and survival. HER2/neu over-expression and *HER2/neu* amplification are widely known markers of aggressive tumor behavior, and a poor clinical outcome in breast cancer patients are observed in approximately 20–30% of breast cancer patients [3]. Trastuzumab, a monoclonal antibody against HER2/neu, has been shown to have a significant efficacy in both adjuvant and metastatic settings [4, 5]. Recently, a randomized phase II trial revealed that trastuzumab-containing neo-adjuvant chemotherapy significantly improved the pCR rate, compared with neo-adjuvant chemotherapy alone, in patients with HER2/neu-positive breast cancer [6].

Immunohistochemistry and fluorescence in situ hybridization (FISH) are currently available methods for identifying patients who are likely to benefit from trastuzumab; however, trastuzumab is ineffective in some patients and progression may still occur. Currently, the possible mechanisms of trastuzumab-resistance include the down-regulation of p27; the activation of insulin-like growth factor receptor (IGF-1R); the loss of expression of PTEN, pAkt, or the interaction HER receptor family; the masking of HER2/neu by membrane-associated glycoprotein mucin-4; angiogenesis; or antibody-dependent cellular toxicity [7]. These hypotheses remain controversial, and some studies that have assessed IGF-1R and p53 levels in clinical samples have reported negative results [8, 9].

PTEN is a dual phosphatase that mainly dephosphorylates position D3 of membrane phosphatidylinositol-3, 4, 5 triphosphate (PI3, 4, 5P3) and is a negative regulator of pAkt. Recently, Nagata et al. suggested that PTEN activation contributes to trastuzumab-induced tumor inhibition and that the loss of PTEN predicts trastuzumab resistance in patients with breast cancer [10]. The authors also validated their hypothesis in 47 metastatic breast cancer patients treated with a taxane-plus-trastuzumab therapy, revealing a statistically significant difference in the clinical response [10]. To test this hypothesis in patients with HER2-positive primary breast cancer, we compared the relationship between clinical outcome and the expression of immunohistochemical profiles, including those of p53, hormone receptors, PTEN, and pAkt, in patients receiving trastuzumab-containing neo-adjuvant chemotherapy. In addition, we investigated the changes in these profiles using specimens obtained before and after trastuzumab-containing neo-adjuvant chemotherapy.

Patients and methods

Patients

A total of 229 patients with breast cancer were treated with neo-adjuvant chemotherapy between January 1999 and January 2006 at the National Cancer Center Hospital. There were 49 patients who were classified as having HER2/neu-positive breast cancer (HER2/neu 3+ or HER2/neu 2+ and FISH-positive) and who received trastuzumab-containing neo-adjuvant chemotherapy. Of these, we identified 44 patients with adequate tumor tissue samples available for retrospective research. Trastuzumab was administered initially using an intravenous loading dose of 4 mg/kg, followed by weekly infusions of trastuzumab (2 mg/kg) in combination with weekly paclitaxel therapy. The dosages of the neo-adjuvant chemotherapy regimens were followed as: CEF therapy (cyclophosfamide, 500 mg/m^2, i.v. on day 1; epirubicin, 100 mg/m^2, i.v. on day 1; 5FU, 500 mg/m^2, i.v. on day 1; 21-day cycles), AC therapy (doxorubicin, 60 mg/m^2, i.v. on day 1; cyclophosphamide, 600 mg/m^2, i.v. on day 1; 21-day cycles), AT therapy (doxorubicin, 50 mg/m^2, i.v. on day 1; docetaxel, 60 mg/m^2, i.v. on day 1; 21-day cycles), and weekly paclitaxel therapy (80 mg/m^2, i.v. on day 1; 7-day cycles).

Tissue samples and microscopic and immunohistochemical analysis

Tissue samples were obtained from core-needle biopsy specimens before neo-adjuvant chemotherapy and from surgical specimens (mastectomy or lumpectomy) after neo-adjuvant chemotherapy. All hematoxylin-eosin stained core-needle biopsy specimens were reviewed by a pathologist (K. T.), and tissue sample were confirmed to contain adequate amounts of cancer tissue for use in the present study.

After surgical treatment, the pathologist evaluated the pathological responses of all the specimens using hematoxylin-eosin staining slides. pCR was defined as the complete disappearance of invasive cancer cells in the primary tumor and the axilla. We also evaluated the immunohistochemical changes in biomarker expression in the 27 patients who did not achieve pCR.

The pathological and immunohistochemical examinations were conducted by the same pathologists (K. T. and K. S.), who were blinded to the clinical statuses of the patients. Formalin-fixed, paraffin-embedded tissue samples were sectioned 4-μm thick and mounted on charged slides. Immunochistochemical staining of p53 (clone DO7; Dako, Glostrup, Denmark), ER (clone 1D5; Dako), and PgR (clone PgR636; Dako), were performed using the streptoavidin–biotin method, and were considered to be positive if

10% or more of the nuclei in the invasive component of the tumor was stained [8, 11]. The HER2/neu status, as assessed using Herceptest (Dako), was scored on a scale of 0–3+, according to the Dako scoring system. Clone 6H2.1 (Dako) and clone 14-5 (Dako) were used for immunohistochemical staining of PTEN and pAkt, respectively. For PTEN and pAkt, the slides were pretreated using heat-induced epitope retrieval and target retrieval solution, pH 9.0 (S2368; Dako), at 95–99°C for 40 min and then cooled for 20 min at room temperature. Immunohistochemistry was performed using the specified detection systems (ChemMate; Dako). Finally, the slides were incubated in DAB + substrate/chromogen solution (K3468; Dako) for 10 min, rinsed in diluted water, counterstained with hematoxylin, and mounted. Negative controls, in which the primary antibody was omitted, were also included in each run. Primary lung cancers and the stroma of endometria with a strong staining pattern (3+) were used as positive controls for PTEN, while primary lung cancer with a strong staining pattern (3+) was used as a positive control for pAkt in each run. The positive staining for PTEN was defined as cytoplasmic staining, which was same as internal control such as peripheral nerve. Positive staining for pAkt was defined as distinct cytoplasmic staining, which was recognized by low power fields. We defined cases with a score of 0 as being negative for PTEN and pAKT in the statistical analysis.

Statistical analysis

The Kaplan–Meier method was used to estimate the recurrence-free survival and the overall survival. Recurrence-free survival was measured from the first day of treatment until recurrence or the final day of the follow-up period without recurrence; Overall survival was measured from the first day of treatment until death or the final day of the follow-up period. The relationships between the expression of the biomarkers (p53, estrogen receptor, progesterone receptor, HER2/neu, PTEN, and pAkt), and the clinical outcomes of the patients were compared using the Chi-square test, the Fisher-exact test, and the log-rank test. All the statistical analyses were performed using SPSS 12.0J (SPSS Inc., Chicago, IL, USA), and the significance level for the results was set at 0.05 (two-sided).

Results

The present study included 44 patients. The patient's clinical characteristics are summarized in Table 1. Eighteen patients received CEF therapy followed by weekly paclitaxel/trastuzumab therapy, 11 patients received AC therapy followed by weekly paclitaxel/trastuzumab therapy, 8 patients received AT therapy followed by weekly paclitaxel/trastuzuab therapy, and 7 patients received weekly paclitaxel/trastuzumab therapy. None of the patients had progressive disease during neo-adjuvant chemotherapy. The median time between the last administration of neo-adjuvant chemotherapy until surgery was 5 weeks. Among 28 patients who had undergone a mastectomy, 9 patients received adjuvant radiotherapy. Sixteen patients received breast-conserving surgery followed by adjuvant radiotherapy. Nine patients received adjuvant hormone therapy.

Table 1 Patient characteristics

Characteristics	Value
Median age (range)	57 (33–78)
Side (right/left)	21/23
Median ECOG performance status	0
Menopausal status	
Pre-menopause	15 (34%)
Post-menopause	29 (66%)
Median clinical tumor size (range)	50 mm (20–120)
Number of patients with clinical lymph node swelling	23 (52%)
UICC-TNM staging	
IIA	15 (34%)
IIB	13 (30%)
IIIA	10 (23%)
IIIB	6 (13%)

After primary treatment, 12 patients developed recurrent disease: 3 loco-regional recurrences and 9 systemic recurrences. The sites of first relapse included six lung metastases, five liver metastases, three brain metastases, three loco-regional metastases, one bone metastasis, and one lymph node metastasis. Five patients died as a result of disease progression. The 5-year survival rate was 84%, and the 5-year recurrence-free survival rate was 65%.

Of the 44 patients, 17 patients achieved pCR. The median pathological tumor size of the patients with non-pCR was 15 mm (range, 0–100 mm). Significant differences in recurrence-free survival but overall survival were seen between patients with or without pCR (log-rank test, $P = 0.016$, 5-year recurrence-free survival rate; 86% vs. 52%, $p = 0.086$, 5-year survival rate; 100% vs. 77%, respectively). The relationship between pCR and the results of immunohistochemical staining of the biomarkers before neo-adjuvant chemotherapy are summarized in Table 2. None of the biomarkers examined in the specimens obtained at the time of diagnosis were significantly associated with either pCR or recurrence (Chi-square test, Fisher-exact test, $P > 0.1$).

Table 2 Relationship between pCR and immunohistochemical profiles in specimens at the time of diagnosis (Chi-square test and Fisher-exact test)

Variables	Total (%) $n = 44$	pCR		*P*-value
		pCR ($n = 17$)	Non-pCR ($n = 27$)	
Grade[a]				0.99
1	4 (9)	1	3	
2–3	40 (91)	16	24	
p53				0.680
Negative	25 (57)	9	16	
Positive	19 (43)	8	11	
ER				0.065
Negative	39 (89)	13	26	
Positive	5 (11)	4	1	
PgR				0.273
Negative	41 (93)	17	24	
Positive	3 (7)	0	3	
ER and/or PgR				0.402
Negative	37 (85)	13	24	
Positive	7 (15)	4	3	
PTEN				0.186
Negative	38 (86)	13	25	
Positive	6 (14)	4	2	
pAkt				0.275
Negative	9 (20)	5	4	
Positive	35 (80)	12	23	

Abbreviations: *pCR* pathological complete response

[a] Grade was defined using hematoxylin-eosin staining

Table 3 Relationship between recurrence and immunohistochemical profiles in residual tumor specimens obtained after neoadjuvant chemotherapy (Chi-square test and Fisher exact test)

Variables	Recurrence		*P*-value
	Rec[b] ($n = 11$)	Non-rec ($n = 16$)	
Grade[a]			0.618
1	1	4	
2–3	10	12	
p53			0.411
Negative	6	12	
Positive	5	4	
ER			0.99
Negative	10	14	
Positive	1	2	
PgR			0.99
Negative	11	15	
Positive	0	1	
ER and/or PgR			0.624
Negative	10	13	
Positive	1	3	
HER2/neu			0.008
Negative	0	8	
Positive	11	8	
PTEN			0.391
Negative	7	13	
Positive	4	3	
pAkt			0.453
Negative	5	5	
Positive	6	11	

Abbreviations: *Rec* recurrence

[a] Grade was defined using hematoxylin-eosin staining

[b] Twelve patients had recurrences in the present study. One patient with pCR was excluded in this statistical analysis

The relationship between recurrence and the results of immunohistochemical staining in the surgical specimens obtained after neo-adjuvant chemotherapy are summarized in Table 3. Only a persistent HER2/neu-positive status in the surgical specimens obtained after neo-adjuvant chemotherapy was significantly associated with recurrence (Fisher-exact test, $P = 0.008$), and the expressions of the other biomarkers were not associated with recurrence.

The proportions of changes in the immunohistochemical profiles before and after neo-adjuvant chemotherapy are summarized in Fig. 1. The changes in the immunohistochemical profiles were not correlated with clinical outcome (data not shown).

Discussion

This study demonstrated that clinical outcome, including pCR, recurrence, was not correlated with the immunohistochemical profiles of p53, estrogen receptor, progesterone receptor, PTEN, or pAkt in specimens obtained before trastuzumab-containing neo-adjuvant chemotherapy.

Interestingly, there were 38 patients with negative PTEN expression and 13 patients who achieved a pCR in the present study. Although we had hypothesized that the loss of PTEN and pAkt expression would influence the clinical outcome of trastuzumab-containing therapy, such as the rate of pCR, the results of the present study did not support our hypothesis, which had been based on the results of Nagata et al's study [10]. One explanation for this contradiction is that the neo-adjuvant chemotherapy used in the present study contained cytotoxic agents plus trastuzumab. HER2/neu over-expression is reportedly associated with sensitivity to anthracycline-containing chemotherapy [12]. Therefore, cytotoxic neo-adjuvant chemotherapy including anthracycline may be a confounding factor in evaluations of the impact of the loss of PTEN. There are also multiple

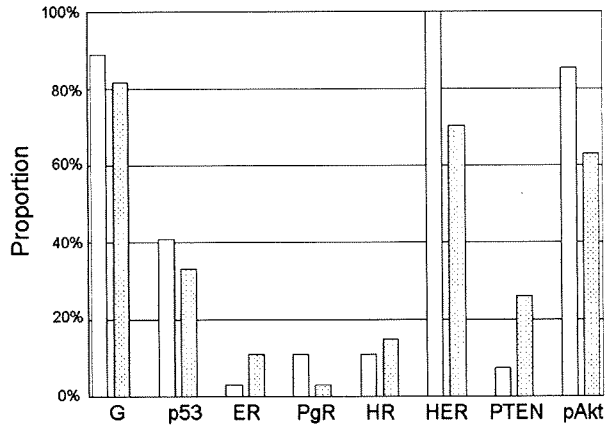

Fig. 1 Proportions of changes in the immunohistochemical profiles of specimens obtained before and after neo-adjuvant chemotherapy in patients with non-pCR ($n = 27$). Abbreviations: G, grade; HR, hormone receptor positive for ER and/or PgR; HER, HER2/neu

hypotheses regarding the mechanism(s) of primary or acquired resistance to trastuzumab [13]. Thus, the mechanism of trastuzumab-resistance may be difficult to explain using only one hypothesis.

The limitations of this study included the small sample size and the considerable heterogeneity in the chemotherapy treatment regimens. However, the majority of the patients had received chemotherapy that had included both an anthracycline and a taxane, and the treatment outcome among the patients was considered representative.

More essentially, the negative results might be attributable to the antibodies used for PTEN and pAkt detection or to a technical problem related to the immunohistochemistry studies. The loss of PTEN and pAkt expression has been reported to occur at various frequencies, [14–16] and the high frequency of the loss of PTEN and pAkt expression observed in the present study was the same as that in previous studies [14, 16]. The monoclonal antibody used in this study, 6H2.1, has been reported to be the only antibody correlated with the presence of molecular alterations in PTEN and to be associated significantly with immunostaining for pAkt; thus, we considered 6H2.1 to be the most suitable antibody for detecting the inactivation of PTEN, compared with the three other known antibodies [17]. In addition, 6H2.1 has been shown to have the same reactivity as the antibody that was used to analyze the loss of PTEN expression in a previous study by Nagata et al [10]. The immunohistochemical staining experiments in the present study were prudentially performed using both positive and negative controls, according to the methods described in a previous study [18], and we believe that any technical problems that may have occurred were of a limited nature.

Another recent study has described a patient cohort that received single-agent trastuzumab in a neo-adjuvant setting; in this previous study, no relationship between tumor response and pAkt expression was reported [19]. Although the different definition of response among patients with operable breast cancer and those with metastatic disease, that is clinical tumor regression and pathological eradication of the tumor, may be one possible explanation for the conflicting results among the studies. At any rate, it is difficult to make any conclusions regarding the role of the loss of PTEN and pAkt expression based on these limited data sets [14, 19], and the exploration of the PI3 K signaling pathway may not lead to a simple breakthrough in our understanding of trastuzumab resistance.

Although changes in the immunohistochemical profiles were frequently observed, such changes may not be useful for predicting clinical outcome. A previous study reported that neo-adjuvant chemotherapy had a minor, but significant (8%), effect on hormone status [20]. However, the HER2/neu status reportedly remained unchanged after neo-adjuvant chemotherapy; thus, HER2/neu expression was regarded as a stable phenotype [20, 21]. Conversely, other studies have reported that the HER2/neu status changed in 15–25% of patients, and the hormone receptor status changed in 26–42% of patients, after neo-adjuvant chemotherapy [22, 23]. Most HER2/neu status changes tend to represent down-regulation [23]. The frequency of changes in immunohistochemical profiles and their influences on clinical outcome are still controversial in neo-adjuvant chemotherapy settings. In addition, these studies included only patients treated without trastuzumab, so the influence of trastuzumab on HER2/neu expression is uncertain. The results of the present study suggest that the HER2/neu status may be altered and down-regulated by the addition of trastuzumab therapy to neo-adjuvant chemotherapy. Further studies on the effects of treatment on immunohistochemical profile changes, tumor cell biology, and/or trastuzumab resistance are needed.

In present study, persistent HER2/neu over-expression in the surgical specimen after trastuzumab-containing neo-adjuvant chemotherapy was associated with recurrence in patients without pCR; this observation may hold a clue to solving the mechanism of resistance to trastuzumab therapy. Previous studies have demonstrated that the down-regulation of target molecules, as shown by immunohistochemical staining in specimens obtained before and after neo-adjuvant chemotherapy, was significantly associated with both the tumor response and RFS, while persistent expression was associated with relapse [24, 25]. In addition, a recent study revealed that a short period of administration, such as 3 weeks of trastuzumab therapy, did not lead to the down-regulation of HER2/neu and did not result in any changes in expression level [19]. Concerning these results, the cancer cells with persistent HER2/neu over-expression after 3 months of trastuzumab treatment may be truly resistant to trastuzumab.

In the coming era of molecular-targeted drugs, multiple options of targeted therapy is becoming an increasingly important problem [7, 13]. However, the selection of appropriate candidates for trastuzumab therapy, which has now become a "classic" targeted therapy, still requires more investigation.

Acknowledgments This study was supported by grants from the Ministry of Health, Labour, and Welfare. The authors thank Miwa Yoshida, Tadahiko Shien, Takashi Hojo, Sadako Tanaka-Akashi (Breast Surgery Division, National Cancer Center Hospital), Eriko Nakano, Mayu Yunokawa, (Breast and Medical Oncology Division, National Cancer Center Hospital), and Kunihiko Seki (Pathology Division, JR Tokyo Hospital) for their assistance.

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The Breast 18 (2009) 171–174

Contents lists available at ScienceDirect

The Breast

journal homepage: www.elsevier.com/brst

Original article

21-Gene expression profile assay on core needle biopsies predicts responses to neoadjuvant endocrine therapy in breast cancer patients

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ARTICLE INFO

Article history:
Received 5 January 2009
Received in revised form
12 March 2009
Accepted 14 March 2009

Keywords:
Breast cancer
Multigene assay
Recurrence score
Neoadjuvant endocrine therapy
Predictive factor

ABSTRACT

This study examined postmenopausal estrogen receptor-positive breast cancer patients who received prospective neoadjuvant endocrine therapy (NAET) with tamoxifen or anastrozole to determine if the 21-gene recurrence score (RS) predicts NAET responses. RS scores were determined from pretreatment core biopsy specimens. Although half of the specimens yielded insufficient RNA, the remaining samples were highly representative. Patients with a low RS tended to respond better than those with an intermediate or high RS ($n = 43$). Response rates by RS were similar between the tamoxifen and anastrozole groups. Patients with a low RS tended to have better relapse-free survival (RFS) than those with an intermediate or high RS (5y-RFS; 100% vs. 84% and 73%, respectively). These results suggest that RS predicts responses to NAET with tamoxifen or anastrozole. Because this pilot study examined a small sample size, these results should be validated in larger studies.

Introduction

Compared to postoperative chemotherapy, preoperative systemic therapy in women with early breast cancer is a standard treatment that allows more breast-conserving surgery without negatively affecting patient survival. Several studies have shown that administering chemotherapy before definitive local surgery can result in overall tumor response rates over 70% with complete clinical response rates over 10%.[1–3] Perhaps less appreciated is the potential role for preoperative endocrine therapy in women with locally advanced estrogen receptor-positive breast cancer. For these patients, multiple studies have shown overall response rates to hormone manipulation over 50% in a neoadjuvant setting, while the rate of complete clinical responses is less.[4–7]

The 21-gene expression profile assay is a genomic classifier validated for women with lymph node-negative, estrogen receptor-positive breast cancer.8. The assay is based on RT-PCR analysis of the expression of 21 genes and yields a "Recurrence Score (RS)" that is reported as a value from 0 to 100. This value corresponds to the risk of recurrence at 10 years for node-negative, estrogen receptor (ER)-positive breast cancer and the benefit of adjuvant tamoxifen treatment. The 21-gene expression profile assay has been validated in several data sets[9] and is the current genomic risk classifier used in the large TAILORx trial sponsored by the Breast Intergroup.

Recently published data have shown that RS correlates with chemotherapy responses in the adjuvant situation.[10] The NSABP B-20 study showed that the addition of CMF or MF chemotherapy resulted in a modest 4.4% benefit for the 10-year distant recurrence risk in estrogen receptor-positive, lymph node-negative patients. However, a recent analysis showed that most of this benefit was restricted to patients with a high RS who collectively had a 28% absolute and 74% relative risk reduction from chemotherapy. Similarly, two studies in patients treated with neoadjuvant chemotherapy showed that a higher RS predicted clinical responses to chemotherapy with either a taxane- or anthracycline-based regimen.[11,12]

Conversely, data from the NSABP B-14 study population have shown that the benefit of tamoxifen in the adjuvant population was related to a lower RS.[13] The purpose of this pilot study is to explore if the clinical response rate to neoadjuvant endocrine therapy is likewise associated with a lower RS.

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doi:10.1016/j.breast.2009.03.005

Patients and methods

This study examined tumors from 87 postmenopausal women who had operable estrogen and progesterone receptor (ER and PgR)-positive breast tumors larger than 3 cm and had received neoadjuvant endocrine therapy (NAET) at the National Cancer Center Hospital, Tokyo. Between February 1999 and July 2002, 37 patients were enrolled in a neoadjuvant tamoxifen study (neo TAM), in which they received tamoxifen for four months preoperatively. Between November 2002 and 2007, 50 patients were enrolled in a neoadjuvant anastrozole study (neo ANZ), in which they received anastrozole for four months preoperatively. Patients who responded to NAET continued the same endocrine therapy postoperatively for five years. When tolerable, patients who showed clinically progressive disease, stable disease, or pathological lymph node involvement after NAET received adjuvant chemotherapy with a regimen containing anthracycline or classical CMF following surgery. All patients provided written informed consent for their core needle biopsy specimens to be examined in this study. The study protocol was approved by the institutional review board of the National Cancer Center Hospital, Tokyo.

Sample preparation for RT-PCR

Ten 3-μm unstained sections and two hematoxylin and eosin sections from each core needle biopsy (CNB) paraffin block were shipped to Genomic Health Inc. (Redwood City, CA) anonymously. Quantitative gene expression was determined by a multianalyte TaqMan RT-PCR assay with standardized operating procedure as reported previously.[8] The RS was calculated on a scale from 0 to 100. Cutoff values were predefined in order to classify patients into the following categories: low-risk (RS less than 18), intermediate-risk (RS higher than 18 but less than 31), and high-risk (RS higher than 31).

Tumor response

Primary tumors were clinically assessed by measuring their size in two dimensions with calipers. A clinical complete response (cCR) was defined according to WHO criteria as the clinical disappearance of the tumor at the end of NAET, and a clinical partial response (cPR) was defined as a ≥50% decrease in tumor area from baseline. Clinical stable disease (cSD) was defined as a decrease of less than 50% in tumor area from baseline or an increase of less than 25% in tumor area from the most reduced size. Clinical progressive disease (cPD) was defined as a ≥25% increase in tumor area from the most reduced size.

Outcome measures

Relapse-free survival (RFS) was defined as the time from the initiation of treatment to local, regional, or distant treatment failure.

Statistical analysis

The chi-squared test was used to compare tumor characteristics among groups. The Kaplan–Meier method was used to estimate RFS curves. An order-restricted version of the log-rank test (a log-rank trend test) was used to test ordered differences between the estimated RFS curves. All p-values were two-sided. Differences with $p < 0.05$ were considered significant.

Results

Of the 87 patients who received neoadjuvant endocrine therapy, we could not obtain informed consent for nine patients because of lost follow up[6] or patients refused to include their specimens.[3] Five tumors were inadequate for this analysis because they contained insufficient invasive cancers (mostly intraductal cancers) in the CNB. For three patients, the remaining specimen was insufficient to generate unstained slides. The total RNA yields were insufficient to assay (<500 ng) in 29 patients. Therefore, a RS was determined in the remaining 43 patients.

The patient characteristics at the time of diagnosis for the 43 assessable patients and all 87 patients who were treated by NAET are summarized in Table 1. One patient in the assessable 21-gene expression profile group did not undergo surgery because of progressive disease during NAET. Fourteen patients received neoadjuvant tamoxifen and 29 received neoadjuvant anastrozole.

The relationship between the RS and the clinical response is shown in Fig. 1. All four tumors with progressive disease had a high-risk RS. On the contrary, one tumor with a complete response had a low-risk RS.

Response rates (cCR + cPR) to NAET for low-risk (<18), intermediate-risk, and high-risk RS were 64%, 31%, and 31%, respectively ($p = 0.11$, by trend test) as shown in Table 2. When divided into the neo Tam and neo ANZ groups, the clinical response rates for the low-risk RS group were about two-thirds in both treatment groups, whereas those in the intermediate- and high-risk RS were approximately one-third. The association between response rates and RS risk categories does not appear to differ between the

Table 1
Characteristics of patients and tumors with an assessable 21-gene expression profile and non-analyzed patients.

	21-Gene expression profile assessable patients ($n = 43$)	Non-analyzed patients ($n = 44$)	
Mean age (range)	61 (48–79)	62.5 (51–87)	
Tumor			
before NAET			
T2	27 (52%)	25 (48%)	$p = 0.35$
T3	14 (52%)	13 (48%)	
T4	2 (25%)	6 (75%)	
NAET			
Tamoxifen	13 (33%)	26 (67%)	$p = 0.01$
Anastrozole	30 (63%)	18 (37%)	
Clinical response			
CR	1 (17%)	5 (83%)	$p = 0.34$
PR	16 (52%)	15 (48%)	
SD	22 (50%)	22 (50%)	
PD	4 (67%)	2 (33%)	
Axillary nodal status			
Negative	12 (40%)	18 (60%)	$p = 0.03$
1–3	14 (47%)	16 (53%)	
4–9	15 (75%)	5 (25%)	
>10	1 (17%)	5 (83%)	
NA	1		
Adjuvant therapy			
Endocrine only	25 (50%)	25 (50%)	$p = 0.90$
Chemotherapy added	18 (49%)	19 (51%)	
21-gene RS			
Low-risk	11		
Intermediate-risk	16		
High-risk	16		

NAET, neoadjuvant endocrine treatment; NA, not applicable because one patient did not undergo surgery.

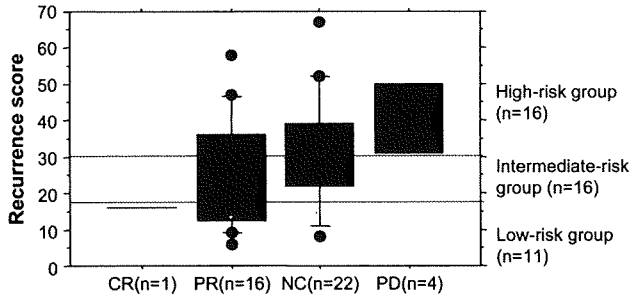

Fig. 1. Recurrence score classified by clinical response.

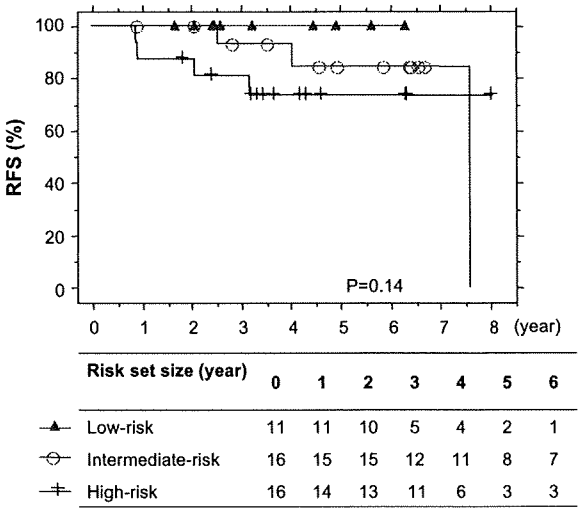

Fig. 2. Relapse-free survival curves by RS risk groups.

Tam- and ANZ-treated groups. This tendency was consistent for different T categories and axillary nodal status.

The median follow-up time was 45 months. There were seven events including four distant metastasis and three locoregional recurrences. Patients with a low-risk RS tended to have a longer relapse-free survival (RFS) than those with an intermediate- and high-risk RS (5y-RFS; 100% vs. 84%, 73%, respectively, $p = 0.14$ by logrank trend test) as shown in Fig. 2.

Discussion

This is the first report to evaluate the predictive value of the 21-gene recurrence score in the NAET setting. A good clinical response to NAET was associated with a low-risk RS, while disease progression during NAET was associated with a high-risk RS. Previous reports suggested that a higher RS was positively associated with obtaining pCR following neoadjuvant chemotherapy.[11,12] Therefore, a hypothesis of a new neoadjuvant treatment strategy for endocrine-sensitive tumors with operable but large breast cancers may be recommended. For patients with a low-risk RS, NAET may be recommended. For patients with a high-risk RS, neoadjuvant chemotherapy is recommended because these patients are more likely to have progressive disease during NAET and to benefit from chemotherapy. For those with an intermediate-risk RS, since the response rate was as low as the high-risk group, neoadjuvant chemotherapy may be adequate. However, the data were not statistically significant, potentially because of the small sample size. This strategy must be validated in a large prospective study.

RS consists of proliferation-, estrogen-, HER2- and invasion-related gene groups. It has been reported that a high RS is correlated with low tubule formation, high nuclear grade, high mitotic count, ER negativity, PgR negativity, and HER2 positivity.[14] It is well known that tumors associated with more aggressive characteristics (poor histologic grade, high proliferation, low ER expression, etc.) respond well to chemotherapy.[15,16] By contrast, tumors with lower proliferation and higher ER expression have been reported to be most likely to respond to endocrine therapy.[17] We speculate that patients with a low RS respond better to endocrine therapy than patients with a high RS, because this score integrates genes related to known predictors and assigns them a quantitative value from 0 to 100.

Table 2
Clinical response rates and 21-gene recurrence score by neoadjuvant treatment.

Neoadjuvant Treatment	n	Low (RS < 18)	Intermediate (18 < RS ≤ 30)	High (RS ≥ 31)	p-Value by trend test
Tamoxifen	14	67% (2/3)	33% (2/6)	40% (2/5)	0.53
Anastrozole	29	63% (5/8)	30% (3/10)	27% (3/11)	0.13
All	43	64% (7/11)	31% (5/16)	31% (5/16)	0.11

The response rates were consistently the same in the neo TAM and neo ANZ groups. The predictive value of the 21-gene RS assay for tamoxifen response has been proven in previous studies.[13] For postmenopausal women with hormone-sensitive breast cancer, the superiority of the aromatase inhibitor to tamoxifen was proven in adjuvant, neoadjuvant and metastatic settings in many randomized studies.[4–6,18,19] This study is the first report that describes a positive association between RS and ANZ, which is noteworthy.

Of the 87 patients who received NAET, an RS was generated for only 43 patients, mainly because the paraffin embedded sections from the core needle biopsies were insufficient to determine an RS. On the other hand, only four (4.3%) of 95 core needle biopsy specimens and one (1.2%) of 81 samples had insufficient RNA levels for testing in previous reports.[11,12] In our study, most of the blocks, which were taken before the end of 2004 and consisted mainly of patients in the neo Tam group, had been sliced to make unstained glass slides several years ago for another study on Ki-67,[7] leaving only small fragments of tumor samples for the RS assay. If we had not used the samples to perform a previous study, we could have determined the RS in more patients.

A significantly larger number of patients in the assessable group were treated with ANZ and had less lymph node involvement compared with the non-analyzed group (Table 1). However, whether patients were treated with ANZ or Tam in this study did not affect the response rate to NAET for the various RS risk groups as shown in Table 2. RS was a prognostic factor regardless of the number of lymph node metastases.[20] Therefore, we think the RS assessable group is the representativeness of all the NAET patients. It is important to note that future personalized treatments will require that sufficient samples be obtained at diagnosis.

Patients with a low-risk RS tended to have a more favorable prognosis than patients with an intermediate- and high-risk RS after NAET. Approximately 40% of the patients received adjuvant chemotherapy with a regimen containing anthracycline or classical CMF after surgery because of poor responses to NAET or lymph node involvement. Although these patients received heterogeneous treatment, RS remained a prognostic factor.

In conclusion, the results of this study indicate that RS may predict clinical responses to neoadjuvant endocrine therapy not only with tamoxifen but also with anastrozole. Because this study

examined a small sample size, these results should be validated in studies with a larger patient population.

Conflict of interest statement

Employment: none.

Consultancies: none.

Stock Ownership: none.

Honoraria: Sadako Akashi-Tanaka, Genomic Health Inc.

Research Funding: Sadako Akashi-Tanaka, Genomic Health Inc.

Patent applications: none.

Ethical approval

All patients provided written informed consent for their core needle biopsy specimens to be examined in this study. The study protocol was approved by the institutional review board of the National Cancer Center Hospital, Tokyo.

Acknowledgements

This study was supported in part by a Grant-in Aid for research of cancer treatment (17-7) from the Ministry of Health, labor and welfare of Japan and Genomic Health. Genomic Health paid the costs of shipping the paraffin-embedded tissue sections and performing all RT-PCR assays.

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Breast Cancer (2009) 16:62–67
DOI 10.1007/s12282-008-0076-6

ORIGINAL ARTICLE

Whole-breast volume perfusion images using 256-row multislice computed tomography: visualization of lesions with ductal spread

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Received: 17 February 2008 / Accepted: 13 August 2008 / Published online: 26 September 2008

Abstract
Background The aim of this study was to apply perfusion techniques to breast tumors using a prototype 256-row multislice computed tomography (CT) scanner (which allows a wide range of 128 mm to be scanned and can provide whole-breast perfusion maps without any dead angles) to improve contrast and assess the possibility of precisely depicting the extent of breast cancer.
Patients and methods The study group included seven patients with breast cancer who were scheduled to undergo radical surgery and radiotherapy. Dynamic scanning was performed using a 256-row multislice CT scanner during normal respiration. Volume perfusion images of the entire breast were obtained using the maximum slope method. Perfusion map images and early-phase breast CT images at 54 s were compared by means of pathological examination.
Results All breast cancers could be distinguished from normal mammary glands based on the perfusion value. The extent of cancer depicted in perfusion images showed excellent agreement with the pathology findings for invasive ductal carcinoma and ductal carcinoma in situ. In three patients, all ductal spread, parts of which were not visualized by early-phase CT, were depicted in volume perfusion images. Simulation analysis suggested that perfusion maps could be generated with fewer scanning points.
Conclusion The results of the present study suggest that volume perfusion imaging may be useful for depicting the extent of breast cancer, with excellent sensitivity. Further research is needed to determine the clinical relevance of these findings.

Keywords Breast cancer · Breast-conserving surgery · Ductal spread · Multislice CT · Perfusion map

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Introduction

At our institution, a prototype 256-row multislice computed tomography (MSCT) scanner was developed, and short-term operation for system evaluation has been performed. In this scanner, four of the same detectors used in the currently used 64-row MSCT scanner were arranged longitudinally to permit scanning over a wide range of 128 mm (256 rows × 0.5 mm), at a scanning speed of up to 0.5 s per rotation. As a result, 256-row CT allows isophasic data to be acquired for almost the entire breast, without the need to employ the helical scanning method.

We have previously reported that CT of the breast is useful for clinical decision making regarding the extent of breast surgery [1–4]. Due to the increase in the number of detector rows in CT scanners, the scan speed and scan range have been increased. However, increasing the number of detector rows does not lead to a significant improvement in the contrast between breast cancer and normal mammary gland tissue. Because CT numbers (Hounsfield Unit) are evaluated in conventional CT scanning, there is no significant difference between 256-row CT and single-slice helical CT in the depiction of the extent of breast cancer. With regard to sensitivity, it remains at 80–90% [1, 3–7]. It is therefore considered necessary to employ image processing techniques, such as the perfusion technique, which permits depiction of blood flow. Perfusion CT results in quantitative visualization of blood flow in parenchymal organs, while maintaining high spatial resolution [8]. By dividing the rate of tissue enhancement by the blood flow, contrast medium is employed as a physiological indicator. The functional images obtained using this technique may provide higher contrast.

The aims of the present study were to apply perfusion techniques to breast tumors using a 256-row MSCT scanner and, using volume perfusion maps generated from the resulting data, evaluate the possibility of precisely depicting the extent of breast cancer—especially in comparison with CT scanning in the early-enhancement phase.

Patients and methods

Subjects

The study protocol was approved by our institutional review board, and written informed consent was obtained from all patients. Due to the limited period of operation of the 256-row MSCT scanner at our institution, seven patients with breast cancer were enrolled in the study between July and September 2006. Their mean age was 62 years (range 44–83 years). Five invasive ductal cancers and two ductal carcinomas in situ were evaluated.

Scan conditions

The CT system employed was a prototype 256-row MSCT scanner (Toshiba Medical Systems Corporation, Tochigi, Japan). The scan conditions were 256 rows × 0.5 mm, 120 kV, 150–250 mA (depending on patient size), 0.5 s/rotation, reconstruction kernel FC13 (standard abdominal reconstruction function), scan field of view (FOV) 400 mm, and display FOV 200 mm. A total of 100 ml of nonionic contrast medium (300 mg I/ml; Omnipaque 300, Daiichi Sankyo Co., Tokyo, Japan) was injected at a rate of 3 ml/s. Intermittent dynamic scanning was performed at 16 time points (before contrast medium injection and at 2, 4, 6.5, 9, 12, 15, 18.5, 22, 26, 30, 34.5, 39, 44, 49, and 54 s after the start of contrast medium injection) during normal respiration, and time–density curves (TDCs) were obtained. No delayed-phase scanning was performed in this study. Simulation analysis was conducted to determine the optimal scan timing.

Generation of perfusion maps

CT images were acquired and transferred to an image processing workstation. The acquired images showed a small amount of displacement due to respiratory motion. Therefore, the image data were shifted in the *X*–*Y*–*Z* planes for each time sequence to perform position-matching.

Perfusion analysis was performed using the maximum slope method. First, a region of interest (ROI) was placed in an artery near the mammary gland tissues, and the maximum CT value (peak arterial enhancement) was measured. The TDCs were then generated for each pixel from time-sequential images, and perfusion values were calculated for all pixels using Eq. 1 [8].

$$\text{Perfusion value} = \frac{\text{maximum rate of tissue enhancement}}{\text{peak arterial enhancement}} \quad (1)$$

The perfusion values were converted to a 256-level color scale to display perfusion maps with a slice thickness of 0.5 mm in the window.

Early-enhancement-phase CT

In conventional CT studies of the breast, scanning is performed 50–60 s after the start of contrast medium injection to depict the early-enhancement phase [1]. Therefore, the CT images acquired at 54 s using the 256-row MSCT scanner were considered to be early-enhancement-phase CT images.

Results

Evaluation based on perfusion values

The TDCs for the main tumors were generated. A steady increase was observed in cases 1–4, and an irregular gradual increase was observed in cases 5–7. The perfusion values for each case are shown in Table 1. The tumors could be distinguished based on the perfusion values in all cases. The perfusion values were 20 or less in normal mammary gland tissue and greater than 40 in tumors, including both the invasive and intraductal components.

Table 1 Perfusion values [ml/min/ml]

Case no.	Pathology	Normal mammary gland	Tumor		
			Invasive component	Intraductal component	LN
1	IDC	5–10	111	NA	150
2	DCIS	1–5	NA	42	
3	IDC with DS	5	95–106	80	
4	IDC with DS	10–20	194	NA	
5	IDC with DS	5	52	37–43	
6	IDC	10	48	NA	
7	DCIS	10–20	NA	140	

DS ductal spread, *NA* not applicable, *LN* lymph node

In general, the perfusion values tended to be slightly lower in the intraductal components than in invasive tumors.

Pathology findings and relationships with CT findings

In three patients with a pathologically demonstrated ductal spread, its extent was clearly depicted in the perfusion map images, parts of which could not be depicted by early-enhancement-phase CT. These images showed good agreement with the pathology findings (Figs. 1, 2, 3). In the four patients with localized tumors, including two with ductal carcinoma in situ, the tumor regions were the same in the perfusion map images, early-phase CT images, and pathology specimens. These results suggest that volume perfusion map imaging using a 256-row MSCT scanner can more precisely depict the extent of the ductal spread than conventional breast CT imaging.

Simulation for reduction of scan time points

The exposure dose was 43.7–45 mSv. To reduce the number of scan time points and the exposure dose, we calculated the variation rate of the TDC gradient to determine the perfusion value. The time points at 4, 6.5, 9.0, and 12.0 s were used as the start points of the TDC gradient, and those at 39.0, 44.0, and 49.0 s were used as the end points. The time point at 0 s was defined as the base value, and the data obtained at these eight points was used to calculate the TDC gradient. The variation rates from the original TDC gradients in each case were 0.7, 2.8, 1.65, 1.5, 2.3, 6.4, and 12.9%, respectively. The variation rates for evaluation of the intraductal component in cases three and four were 2.5 and 3.3%, respectively. The consistency of the graphs in patients showing a steady increase in the TDCs was ensured by performing position matching of the image data. However, in cases 6 and 7, which showed an irregular gradual increase in the TDCs, the variation rates were greater than 6%, which was probably attributable to shifting of the ROIs due to respiratory motion that could not be completely corrected. We speculate that breath-holding may reduce the variation rates, and the results suggest that comparable results could be obtained by performing scanning eight times with breath-holding.

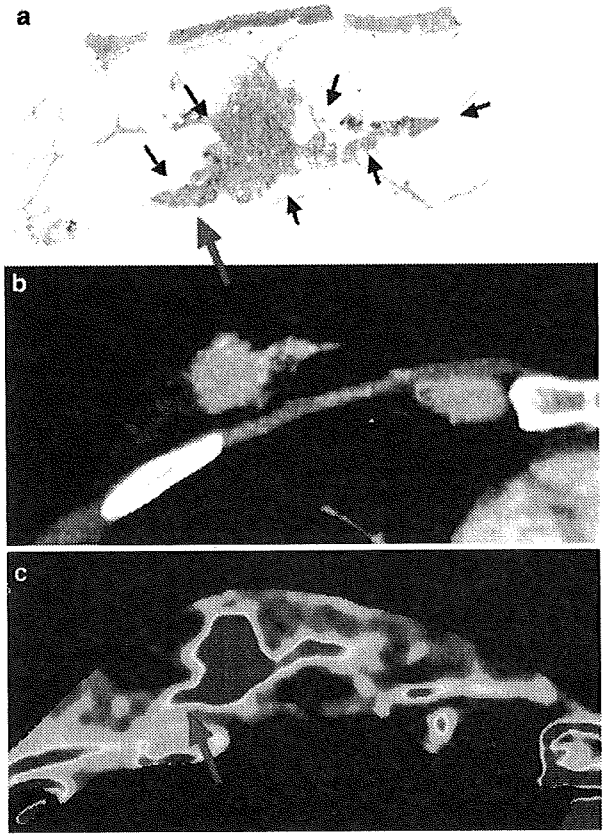

Fig. 1 A 44-year-old woman with invasive ductal carcinoma of the breast. **a** Panoramic view of the tumor in the resected tissues. **b** CT image in the early-enhancement phase. Tumor tissues extending to the left from the main tumor are not depicted. **c** Perfusion map image. The tumor tissues (including the tumor on the left side that could not be visualized in the early-enhancement phase) are clearly depicted (*pink arrow*), showing good agreement with the pathology findings

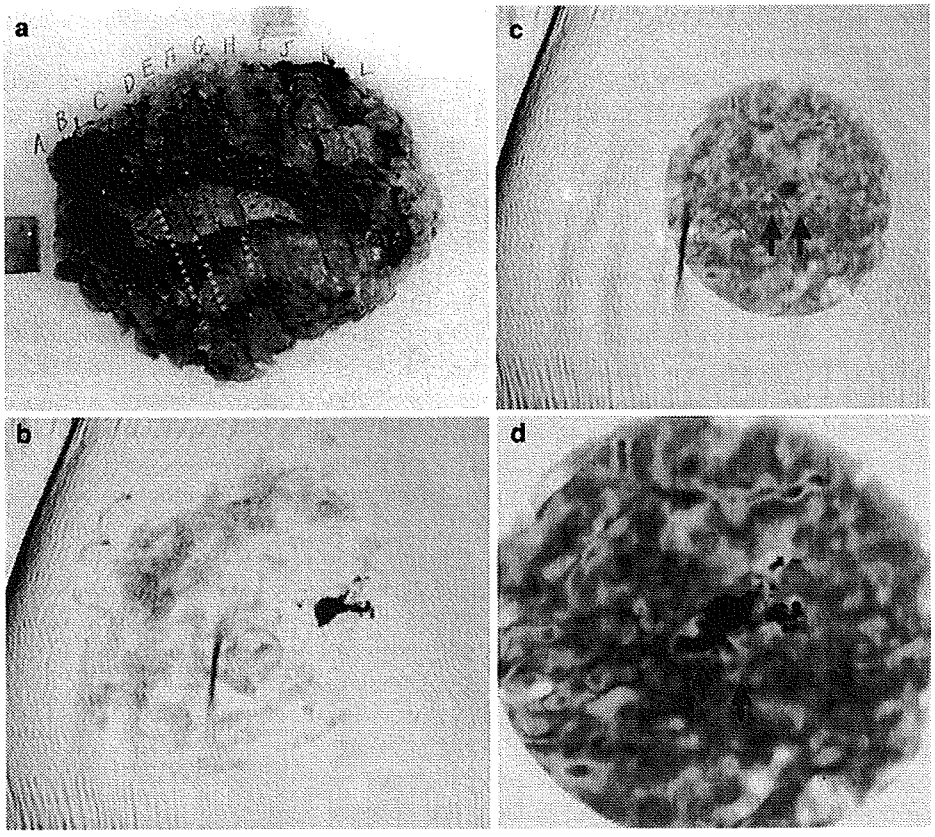

Fig. 2 A 60-year-old woman with invasive ductal carcinoma of the breast. **a** Image showing resected tissues only. *Pink lines* invasive ductal carcinoma. *Green lines* lesion with ductal spread. **b** Three-dimensional CT image in the early-enhancement phase. **c** Perfusion image. **d** Fusion image combining the images shown in **b** and **c**. The lesion with ductal spread located on the side toward the nipple from the main tumor could be depicted only in the perfusion map image (*pink arrows*)

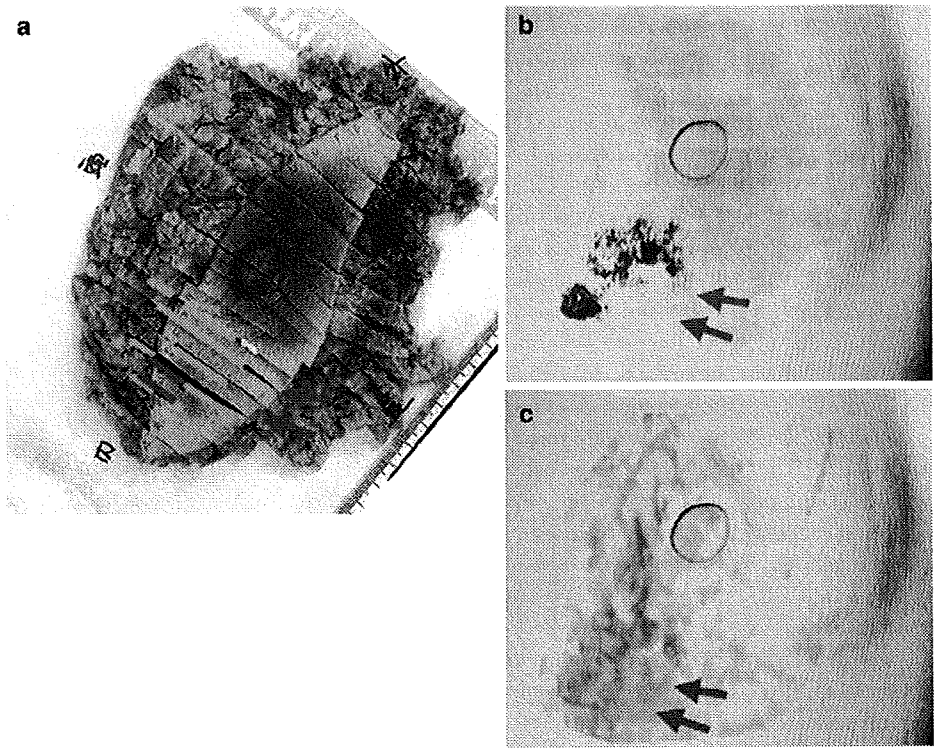

Fig. 3 A 45-year-old woman with invasive ductal carcinoma of the breast. **a** Image showing resected tissues only. *Red lines* tumor invasion. *Green lines* lesion with ductal spread. **b** Three-dimensional CT image in the early-enhancement phase (generated by the volume-rendering method). The intraductal component extending from the main tumor toward the nipple is depicted; the extent of tumor tissue on the right side of the tumor mass is not depicted (*pink arrows*). **c** Perfusion map image. The tumor tissues (including the ductal spread extending to the right of the main tumor that could not be depicted in the early-enhancement phase) are clearly depicted (*pink arrows*), showing good agreement with the pathology findings

Discussion

The results of the present study suggest that perfusion CT can depict the extent of breast cancer more precisely than conventional breast CT in the early-enhancement phase, especially in patients with ductal spread. Perfusion processing was performed for the data acquired by 256-row CT to obtain functional images. As a result, the tissue resolution was increased, making it possible to visualize small lesions, as shown in Figs. 1, 2, and 3. One reason for being able to visualize such small lesions was the integration effect of aggregate time. Then the total amount of information became big. These findings suggest that CT images can be improved not only in terms of spatial resolution by employing a larger number of detector rows, but also in terms of contrast levels by performing image processing, which may lead to higher sensitivity.

Precisely determining the extent of primary breast cancer before surgery is essential for achieving local control and an acceptable cosmetic result [9–11]. While breast MRI is known to be useful for assessing the extent of cancer, it requires both a dedicated breast coil and radiologists who are experts in breast imaging and familiar with the optimal imaging sequences and other technical details related to image interpretation [12]. Moreover, the shape of the breast in MRI images obtained in the prone position differs from that in the supine position during surgery. Breast CT images acquired in the supine position therefore provide more accurate information for surgical planning [1, 9]. Some studies have reported that MSCT images can be used to assess the extent of breast cancer with a high degree of accuracy [5–7, 13]. In another study, CT and MRI examinations were performed to assess the extent of cancer in the same patient, and the results showed that the MR images were superior or equal to the CT images in terms of sensitivity, that the CT images were superior in terms of specificity, and that the MR images and the CT images were equal in terms of diagnostic accuracy [14–16]. Recent improvements in MRI systems have led to an increase in the specificity of MRI images. Determining whether CT perfusion maps are superior to MR images for evaluating the extent of cancer is a subject for future research.

Only one recent report has described the usefulness of perfusion CT in patients with breast cancer [17]. The patients were examined using a 16-row MSCT scanner, and perfusion maps were obtained over a range of 8 mm. That study suggested the feasibility of breast cancer perfusion and reported differences in the perfusion values among histological subtypes.

One disadvantage of breast CT is radiation exposure. Scanning with the 256-row MSCT scanner was performed in patients at 16 time points from 0 to 54 s at 43.7–45 mSv. Simulation analysis suggested that the exposure dose could be reduced by half (to 20 mSv) if scanning were performed eight times with breath-holding. In addition, it is thought that the linear high-perfusion areas observed at the borders represented artifacts due to respiratory motion. It is therefore expected that clearer images could be obtained with breath-holding. At time points of 12.0 s or earlier and at 39.0 s or later, which were used in the simulation, if scanning were performed with breath-holding in the inspiratory phase, it might be possible to reduce the fluctuation in TDCs, permitting perfusion maps of equivalent level to be generated and images with fewer artifacts to be obtained. In the future, it should become possible to obtain clearer images with reduced exposure dose by taking these scan conditions into consideration.

This pilot study with 256-row MSCT is the world's first trial. We synchronized the timing for scan, injection speed, and perfusion analysis algorithm (maximum slope method), all of which have been used for liver cases. It remains a matter of discussion whether to evaluate an optimum model for outflow of contrast medium, injection speed, and scan timing.

Conclusion

The results of this pilot study suggest that volume perfusion images (as functional images) acquired using 256-row CT may be useful for depicting, with higher sensitivity, the extent of breast cancer. Further research is needed to validate these results.

Acknowledgments This work was supported in part by Health and Labour Sciences Research Grants for Third Term Comprehensive Control Research for Cancer.

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