Int J Clin Oncol (2008) 13:314–319
DOI 10.1007/s10147-007-0753-z

ORIGINAL ARTICLE

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Evaluation of axillary status in patients with breast cancer using thin-section CT

Received: November 1, 2007 / Accepted: December 11, 2007

Abstract
Background. In recent years, the surgical management of patients with breast cancer has shifted to a locoregional approach, and evaluating the patient's axillary lymph node status is of the greatest importance in determining the appropriate treatment strategy. We evaluated on the efficacy of preoperative axillary staging using contrast-enhanced computed tomography (CE-CT).
Methods. Between 2000 and 2003, 235 patients with operable breast cancer who underwent CE-CT before surgery and 137 patients who received neoadjuvant chemotherapy (NAC) and underwent CE-CT before NAC and surgery were enrolled in this study. The axillary status was evaluated based on three criteria (short-axis diameter, shape, and enhancement type), and the diagnosis was correlated with the histopathological results.
Results. In patients who did not receive NAC, the size criterion of a short-axis diameter of more than 5 mm provided a sensitivity of 78%, a specificity of 75%, and an accuracy of 76% in predicting node-positive status. According to the size criterion of a short-axis diameter of more than 5 mm and the shape criterion of the absence of intranodal fat density, the specificity and accuracy were 90% and 81%, respectively, and according to the enhancement type criterion of early enhancement, the corresponding values were 89% and 78%. Evaluation was more difficult in patients who received NAC and the sensitivity of the size-based criterion in the patients who received NAC was lower than in those who did not.
Conclusion. These findings suggest that CE-CT based on size criteria is useful for evaluating the preoperative axillary status of breast cancer patients, but that evaluation is more difficult and the sensitivity is reduced in patients who have received NAC.

Key words Breast · CT · Axillary status · Neoadjuvant · Chemotherapy

Introduction

There have been remarkable advances in the treatment of breast cancer in recent years. Diagnostic techniques and methods are improving and more and more new devices are being introduced. However, their usefulness has not been established, and it is necessary to develop more detailed and accurate diagnostic methods.

In the surgical management of patients with breast cancer, conservative treatment and sentinel lymph node biopsy (SLNB) are now widely employed. For these procedures, accurate preoperative evaluation of the lesion is required. In such preoperative evaluation, the extent of the main lesion should be assessed in order to determine the range of tumor excision; also, axillary lymph node metastases should be evaluated in order to perform an SLNB procedure safely. There have been many reports on the evaluation of tumor extent, and we have published several articles on the usefulness of mammary gland computed tomography (CT) in this regard.[1-3] For the evaluation of axillary lymph node metastases, various approaches and examination techniques have been proposed to improve the diagnostic accuracy of ultrasound (US), CT, positron emission tomography (PET), and other diagnostic modalities.[4-15] However, no definitive criteria have yet been established. Mammary gland CT can be performed with the patient in nearly the same position as that during surgery, and it is

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beginning to attract attention as a modality that permits the extent of the primary lesion and axillary lymph node metastases to be evaluated simultaneously.

In chemotherapy, molecular-targeted therapeutic agents, including anthracyclines, taxanes, and trastuzumab (Herceptin; Roche, Nutley, NJ, USA) are now employed, and the antitumor effect of chemotherapy is increasing remarkably. Given this background, neoadjuvant chemotherapy (NAC) is now widely employed not only for locally advanced breast cancers but also for primary breast cancers, and excellent results have been reported.[16–18] However, new problems have begun to emerge as NAC has become more common. Because NAC is highly effective, it is difficult to visualize the residual lesions after chemotherapy. Even more accurate evaluation is therefore needed to determine the excision range. We have previously reported the usefulness of CT in the visualization of residual lesions after NAC.[1,3] In addition, several reports have suggested that the number of residual lymph node metastases after NAC is a strong prognostic factor.[16–18] The evaluation of axillary lymph node metastases after NAC is therefore of great importance. Furthermore, identifying the presence or absence of axillary lymph node metastases before and after NAC may be very important in determining whether or not SLNB is indicated after NAC.

In this study, we evaluated the usefulness of multislice CT in the evaluation of axillary lymph node metastases in order to establish suitable criteria for evaluating axillary lymph node metastases using this modality. We also examined the effectiveness of NAC for axillary lymph node metastases, using multislice CT.

Patients and methods

Patients

This study group included 235 women with operable breast cancers measuring less than or more than 30 mm in diameter who refused NAC and 137 women who received NAC. NAC was indicated in patients with clinical stage II breast cancer with a tumor larger than 3 cm, or in patients with stage III breast cancer. All patients were treated at the National Cancer Center Hospital (NCCH), Tokyo, between January 2000 and December 2003. The patients were evaluated by contrast-enhanced (CE)-CT before surgery and before NAC.

The surgical method was mastectomy or breast-conserving surgery with axillary lymph node dissection. The NAC protocol consisted of four cycles of doxorubicin (50 mg/m^2)/docetaxel (60 mg/m^2) with a 21-day cycle length (AT protocol), or four cycles of doxorubicin (60 mg/m^2)/cyclophosphamide (600 mg/m^2) plus 12 weekly cycles of paclitaxel (80 mg/m^2) (ACT protocol) followed by surgery. The initial pathologic confirmation of breast cancer was based on the findings of needle biopsy. All patients gave their informed consent to participate in the study, which was approved by the institutional review board of the NCCH.

Imaging examinations

All CT examinations were performed with the patient in the prone position; from January to June 2000, a helical CT scanner (X-Vigor; Toshiba, Tokyo, Japan) was used, and from July 2000 onwards a multislice (four-row) CT scanner (Aquilion; Toshiba) was used. The first noncontrast-enhanced CT scan served as the baseline, with images acquired from the cranial end of the sternum to the inframammary fold. Subsequently, an enhanced zoomed scan was performed to visualize the entire breast. A 100-ml bolus of nonionic contrast material (300 mg I/ml of iohexol [Omnipaque' Daiichisankyo Pharmaceutical, Tokyo, Japan]) was injected intravenously at a rate of 3 ml/s, using an automated injector, via an antecubital vein on the side opposite the affected breast. Image acquisition was started at 40 s after the start of bolus injection of the contrast material. The reconstruction interval was 5 mm. Metastatic lymph nodes were evaluated based on the short-axis diameter, internal fat density indicating absence of a center image, and early strong enhancement, compared with the late phase of the axillary lymph node on CT images. Benign lymph node enlargement such as hyperplasia has internal fat at the normal hilum of the lymph node and does not show early strong enhancement. We evaluated the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy compared with presurgical lymph node status diagnosed by CT imaging, according to several criteria of pathological lymph node status after operation. Two authors (T. S. and K. M.) retrospectively interpreted the CT images together, and reached their conclusions by consensus.

Histopathological examinations

All nodes obtained by axillary dissection were cut into single sections and stained with hematoxylin-eosin (H&E) for analysis by breast pathologists. The pathological response of the primary tumors to NAC was classified according to the *General rules for clinical and pathological recording of breast cancer* of the Japanese Breast Cancer Society (JBCS).[19] In grade 0 tumors, no response was observed; in grade 1a tumors, degenerative changes or severe degenerative changes were observed in fewer than one-third of the cancerous cells; in grade 1b tumors, severe degenerative changes were observed in one-third to two-thirds of the cancerous cells; in grade 2 tumors, degeneration was observed in more than two-thirds of the cancerous cells; and in grade 3 tumors, a complete response was observed, with no cancerous cells remaining.

Results

Patient characteristics

Table 1 shows the clinicopathological features of the 235 patients who did not receive NAC (without NAC) and the

Table 1. Clinicopathologic features

Variable	Without NAC (n = 235) Data	NAC (n = 137) Data
Age, years, median (range)	51 (22–83)	51 (26–68)
Primary tumor size, mm, median (range)	21 (2–110)	40 (15–80)
T1a	3 (1%)	
T1b	8 (3%)	
T1c	88 (37%)	3 (2%)
T2	105 (45%)	89 (65%)
T3 and T4	31 (13%)	45 (33%)
Histology		
Invasive ductal carcinoma	218 (93%)	128 (93%)
Invasive lobular carcinoma	14 (6%)	6 (4%)
Mucinous carcinoma	1 (0.4%)	3 (2%)
Undifferentiated adenocarcinoma	2 (1%)	
Pathological lymph node status		
Negative	142 (60%)	55 (40%)
Positive	93 (40%)	82 (60%)
Pathological response to NAC		
Grade 0		1 (0.7%)
Grade 1a		66 (48%)
Grade 1b		27 (20%)
Grade 2		32 (23%)
Grade 3		9 (7%)

Table 2. Results for CT imaging of axillary lymph nodes in patients without NAC, obtained using each diagnostic criterion

Parameter	Short-axis diameter of LN		Short-axis diameter >5 mm and	
	>5 mm	>7 mm	Early enhancement	Without absence of center image
Sensitivity	78%	35%	62%	67%
Specificity	75%	94%	89%	90%
PPV	67%	80%	78%	81%
NPV	84%	69%	78%	82%
Accuracy	76%	71%	78%	81%

LN, lymph node; PPV, positive predictive value; NPV, negative predictive value

137 patients who received NAC (NAC). The size of the primary tumor was measured on the pretreatment CT images. In the patients without NAC, the median age was 51 years (range, 22–83 years). The median tumor size was 21 mm (range, 2–110 mm). In the 2 patients with undifferentiated adenocarcinomas, 1 had a matrix-producing carcinoma and 1 had stromal sarcoma. Ninety-three patients (40%) had node-positive pathology.

In the NAC patients, the median age was 51 years (range, 26–68 years). The median tumor size was 40 mm (range, 15–80 mm). Of these patients, 128 were histologically diagnosed as having invasive ductal carcinoma. Invasive lobular carcinomas and mucinous carcinomas were found in 6 and 3 patients, respectively. Eighty-two patients were node-positive after operations (60%) and there was a pathological response of the primary tumor according to the JBCS classification (Table 1).

Evaluation of axillary status in patients who did not receive NAC

Pathologically, 93 patients (40%) were diagnosed as node-positive and 142 (60%) as node-negative, based on the criterion that an axillary lymph node greater than 5 mm in short-axis diameter on the CT images was node-positive; the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were 78%, 75%, 67%, and 84%, respectively (Table 2). Based on the criterion that a node greater than 7 mm in short-axis diameter was node-positive, the specificity and PPV increased while the sensitivity and accuracy decreased. When the other CT criteria were used in addition, the diagnostic accuracy was higher than that obtained using the size criteria alone. When the lymph nodes greater than 5 mm in short-axis diameter with early enhancement and those without the absence of a center image (absence of a center image means that the lymph node contains fatty tissue) were diagnosed as node-positive, the accuracy rates were 78% and 81% and false negative rates were 22% and 18%. In patients with false-negative results using the 5-mm criterion, the number of metastases was one to three nodes (78%) and in those with false-negative results using the 7-mm criterion, the number of metastases was more than four nodes (22%).

Evaluation of axillary status in patients who received NAC

Of the 137 patients who received NAC before surgery, lymph node metastases were confirmed by postoperative pathological examination in 82 (60%). The clinical stage of the patients with NAC was relatively advanced. Therefore, the pathological lymph node status was worse than that in patients without NAC. Based on the criterion that a lymph node greater than 5 mm in short-axis diameter on preoperative CT images was node-positive, the sensitivity, specificity, NPV, PPV, and accuracy were 60%, 95%, 61%, 94%, and 74%, respectively. Based on the criterion that a lymph node greater than 7 mm in short-axis diameter was node-positive, the specificity and PPV were both 100%, while the sensitivity, NPV, and accuracy were 20%, 55%, and 52%, respectively–values that were significantly lower than the values obtained using the 5-mm criterion (Table 3).

On CT imaging before NAC, a lymph node enlarged to greater than 5 mm in short-axis diameter and diagnosed as node-positive was observed in 120 patients (88%), but on comparison with preoperative CT imaging, it was found that the lymph node had become smaller after NAC in 113 (94%) of these 120 patients (Table 4). On the postoperative pathological examination, lymph node metastasis was confirmed in 82 patients. If it is assumed that the diagnosis in all 120 patients who were determined to be node-positive before NAC was correct, the pathological complete response (pCR) rate for NAC in axillary lymph node metastases can be considered to be 32%.

Table 3. Results for CT imaging of axillary LN in patients with NAC

Parameter	Short-axis diameter of LN	
	>5 mm	>7 mm
Sensitivity	60%	20%
Specificity	95%	100%
PPV	94%	100%
NPV	61%	55%
Accuracy	74%	52%

LN, lymph node; PPV, positive predictive value; NPV, negative predictive value

Discussion

In a number of studies, authors have described the usefulness of preoperative diagnostic imaging of the axillary lymph nodes in patients with breast cancer and have discussed the diagnostic criteria,[4–15] but none of these studies can be considered to be definitive. We conducted a study of the diagnosis of axillary lymph node metastases, using mammary gland CT to evaluate the extent of primary breast cancer. Unlike other modalities, mammary gland CT can be performed with the patient in almost the same position as that during surgery, and it is therefore possible to perform lymph node evaluation under the same imaging conditions as those used to evaluate the extent of the primary lesion. In most of the previous reports, the lymph nodes were evaluated in terms of their size and shape.[7–11] However, the assessment of node shape is prone to error, and this method is too complicated for routine use. In the present study, we selected size, which is relatively easy to assess, and we also added evaluation of the contrast enhancement effect to investigate the accuracy of the evaluation of metastases. When a lymph node greater than 5 mm in short-axis diameter was considered to be node-positive, the sensitivity, specificity, and accuracy were 78%, 75%, and 76%, respectively. These values are slightly lower than those in previous reports. This is mainly because, in our study, the subjects were limited to patients in whom no lymph nodes were found by palpation and in whom the size of the primary breast cancer was determined to be 3 cm or less by palpation. The clinical stage of the patients without NAC was a relatively early stage. The patients with advanced breast cancer received NAC. If palpably enlarged lymph nodes were to be included in the evaluation, the sensitivity would theoretically increase and the diagnostic accuracy would undoubtedly be improved. With regard to the lymph node size cutoff value, many recent reports have used a lymph node short-axis diameter of 5 mm as the criterion for determining it to be node-positive.[4–11] However, the number of patients in these reports was small, and detailed studies of lymph node size were not performed. In many reports on the diagnosis of mediastinal lymph node metastases, a short-axis diameter of 1 cm was used as the criterion for determining a lymph node to be node-positive, and the accuracy was reported to be 78% to 85%.[20–22] In the present study, in the patients without NAC, when it was assumed

Table 4. Efficacy of NAC for axillary LN metastases (n = 137)

Variable	Data
No. of patients with axillary lymph node metastases before NAC[a]	120 (88%)
No. of patients with residual axillary lymph node metastases after NAC[b]	82 (60%)
Size change of axillary lymph nodes after NAC in the patients with lymph node metastases before NAC (n = 120)	
Smaller	113 (94%)
Same	5 (4%)
Larger	2 (2%)

[a]Short-axis diameter >5 mm on CT images before NAC
[b]Pathologically proven metastases after axillary lymph node dissection

that a lymph node greater than 7 mm in short-axis diameter was node-positive, the sensitivity, specificity, and accuracy were 35%, 94%, and 71%, respectively. The specificity was increased to nearly 100%, but the sensitivity and accuracy were decreased compared with values for the 5-mm criterion. This result indicates that a short-axis diameter of 7 mm is not a suitable cutoff value for preoperative examination. If a short-axis diameter of greater than 3 mm is used as the criterion, visual measurement is quite difficult and the measurement may have a large degree of error. Considering the fact that a slice width of 5 mm is generally employed in CT examinations, the use of 3 mm as the criterion is not practical. Based on these results, we decided that lymph nodes with a short-axis diameter of greater than 5 mm should be considered to be node-positive.

In order to improve accuracy, we included early enhancement and the absence of fat within the lymph node, which are characteristics of lymph node metastases, in the evaluation criteria. Most previous reports also included the characteristics of the lymph node and the contrast-enhancement effect in their diagnostic criteria, in addition to node size, in order to improve diagnostic accuracy. In the present study, when a lymph node with a short-axis diameter of greater than 5 mm and with the absence of internal fat was considered to be node-positive, the diagnostic accuracy was 81%. When a lymph node with a short-axis diameter of greater than 5 mm and with early enhancement was considered to be node-positive, the accuracy was 78%. The results were improved slightly by the addition of extra criteria.

Many studies are currently under way on the preoperative evaluation of lymph node metastases in patients who have received NAC. It is much more difficult to evaluate the axillary lymph nodes in patients who have received NAC than in those who have not.[2,3] In our examinations for the preoperative evaluation of lymph node metastases in patients who had received NAC, the sensitivity, specificity, and accuracy were 60%, 95%, and 74%, respectively, when a node greater than 5 mm in short-axis diameter was considered to be node-positive. When a node greater than 7 mm in short-axis diameter was considered to be node-positive, the corresponding values were 20%, 100%, and 52%, respectively. In the patients who had received NAC, the size of the metastatic lymph nodes was reduced due to the administration of anticancer agents. However, even when a lymph node becomes smaller, it may still contain malignant cells. We decided that this was the reason that the sensitivity was reduced in the patients with NAC compared with that in the patients without NAC. On the other hand, the number of reactively enlarged lymph nodes after NAC was smaller than that before NAC, and this is the reason that the specificity was higher when lymph nodes were evaluated by size alone. In the present study, based on the criterion of a short-axis diameter greater than 7 mm, the specificity was 100%; however, when the criterion of a short-axis diameter greater than 5 mm was used, the accuracy was higher than that for the 7-mm criterion.

SLNB is starting to be widely employed. Candidates for an SLNB procedure are said to be patients in whom no metastatic axillary lymph nodes are found on preoperative examination. It is therefore necessary, during the preoperative examination of patients who may be candidates for SLNB, to correctly identify patients with nodal metastases and exclude them as SLNB candidates. When SLNB is being considered, methods that can provide high specificity and PPV, rather than high accuracy or sensitivity, are more useful for the preoperative diagnosis of axillary lymph node metastases. CT studies can be very useful in this regard. As for SLNB after NAC, however, it is difficult to apply the primary theory of SLNB even if the specificity and PPV are 100% in a CT study, because pathological diagnosis is difficult and the effects of NAC appear to be relatively nonuniform. In our study, in the patients who did not receive NAC and who showed false-negative findings, the number of pathological lymph node metastases was low, and we need to diagnose these metastases in patients who are to have SLNB. Although the lymph node size had become small in the patients with NAC, malignant tumor cells existed sparsely in these lymph nodes. These findings indicate that SLNB after NAC is very difficult. Further investigations are required.[23–26]

We conducted this study to determine suitable criteria for the diagnosis of axillary lymph node metastases, in terms of node size, using mammary CT, the objective being to have criteria that are easy to understand and can be employed at any institution. Our results suggest that the most suitable criterion is to consider an axillary lymph node to be node-positive if it is greater than 5 mm in short-axis diameter, and the accuracy of this criterion was found to be favorable. More accurate diagnosis was possible by adding evaluation of the absence or presence of internal fat and the contrast enhancement effect in each patient. It was also found that the evaluation of axillary lymph node metastases was more difficult after NAC, and further advances in imaging and diagnostic methods will be necessary for evaluation in these patients. At the present time, it is recommended that comprehensive evaluation be performed using a combination of mammary CT and another modality. The results of this study have shown that mammary CT, as employed to diagnose the extent of the primary lesion, is also useful for the preoperative evaluation of axillary lymph node metastases.

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Jpn J Clin Oncol 2008;38(3)172–175
doi:10.1093/jjco/hyn001

Clinical Efficacy of S-1 in Pretreated Metastatic Breast Cancer Patients

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Received August 10, 2007; accepted December 26, 2007; published online February 22, 2008

Background: S-1, an oral fluoropyrimidine carbamate, is an active and well-tolerated agent against solid cancer. However, the clinical efficacy of S-1 in patients with metastatic breast cancer has not been determined.
Methods: We retrospectively evaluated the efficacy of S-1 and identified its adverse effects in patients with metastatic breast cancer who had failed to respond to prior chemotherapy regimens. All the patients were treated at the National Cancer Center Hospital and received S-1 twice daily at a dose of 80 mg/m^2 for 4 weeks, followed by a 2-week rest interval.
Results: Between 2003 and 2007, 37 women with metastatic breast cancer received S-1 as a third line or greater chemotherapy regimen. All the patients had been previously treated with both anthracyclines and taxanes prior to S-1 chemotherapy. The median order of S-1 administration was as a fifth-line treatment, and 23 patients (62%) received S-1 as their final anticancer drug. One (3%) partial response and two (5%) stable diseases were observed. The median time to progression (TTP) was 84 days. Grade 2 adverse events, such as diarrhea, stomatitis and neutropenia occurred in 5 (16%), 1 (3%) and 1 (3%) patients, respectively.
Conclusions: S-1 was safety administered to heavily treated metastatic breast cancer patients with limited efficacy. Further evaluation of S-1 is necessary to elucidate its clinical role in breast cancer treatment.

Key words: S-1 – metastatic breast – cancer – chemotherapy

INTRODUCTION

Treatment of patients with metastatic breast cancer (MBC) aims to prolong survival while relieving symptoms and maintaining a good quality of life (QOL).

Capecitabine is an orally administered fluoropyrimidine that has been reported to be effective in both monotherapy and combination therapy regimens. Capecitabine as a single agent produced an overall response rate (RR) of 29% and a median time to disease progression of 4.6 months in large phase II trials in taxane-pretreated MBC patients (1–3). Since capecitabine can sustain the QOL of MBC patients, it has been widely used as a third-line or subsequent chemotherapy regimen for heavily treated patients.

On the other hand, S-1 is another orally administered fluorinated pyrimidine that has been reported to be a well-tolerated and active agent against solid cancers. In a phase II study of S-1, the RR was 41.7% and the median survival time was 872 days among taxane-pretreated patients with MBC; S-1 has been approved in Japan as a salvage chemotherapy for patients who have received anthracycline and taxane (4,5). In addition, S-1 has been used mainly for the treatment of cancers of the digestive tract (6–8), and its efficacy is well known. However, the clinical usefulness of S-1 in patients with MBC is uncertain. Here, we describe the efficacy and tolerability of S-1 in a clinical setting.

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PATIENTS AND METHODS

PATIENTS

A retrospective analysis was performed on patients with MBC who received S-1 monotherapy between January 2003 and December 2006 at the National Cancer Center Hospital (NCCH). The patient population was identified from a

database at the NCCH. All the patients had received chemotherapy previously. They were followed up until death or, if they were still alive, to their last visit prior to March 2007.

The best response for each patient was assessed according to the WHO criteria (8). A complete response (CR) was defined as the disappearance of all clinical and radiographic evidence during two observations performed at least 4 weeks apart. A partial response (PR) was defined as a decrease of 30% or more in the sum of the products of the biperpendicular diameters of measurable lesions. Stable disease (SD) was defined as a <30% decrease and a <25% increase in the sum of the products of the biperpendicular diameters of measurable lesions and no appearance of new lesions; these conditions had to be maintained for at least 12 weeks. Progressive disease was defined as a greater than 25% increase in the sum of the products of the biperpendicular diameters of measurable lesions or the appearance of new lesions. The clinical benefit rate was defined as the proportion of patients who achieved either a CR, PR or SD. The National Cancer Institute common toxicity criteria (9) were adopted to determine toxicity.

Treatment

S-1 was administered orally twice daily (80 mg/m^2) for 28 days followed by 14 days of rest. Treatment was continued until disease progression, unacceptable adverse effects or withdrawal of the patient's consent. In the case of Grade 2 or worse toxicity, S-1 administration was interrupted and not resumed until the toxicity had resolved or improved to Grade 1.

The time to progression (TTP) was calculated from the day of commencement of S-1 administration until the day of documented progression. Overall survival (OS) was calculated from the start date of S-1 to the date of death from any cause. TTP and OS were analysed according to the Kaplan–Meier estimates.

RESULTS

Thirty-seven patients received S-1 as a greater than second-line chemotherapy for MBC between January 2003 and December 2006 at NCCH. Table 1 shows the patient's characteristics. The median age was 49 (28–70) years. The Eastern Cooperative Oncology Group (ECOG) performance statuses of the patients were all <2. The sites of metastatic disease were the bone and/or soft tissue in only six patients (16%) and involved visceral sites in 31 patients (84%). Table 2 shows the chemotherapy regimens that were administered prior to S-1. The median number of chemotherapy regimens used before the administration of S-1 including adjuvant and neoadjuvant treatments, was 4, and 23 patients (62%) received S-1 as their final chemotherapy regimen. All the patients had previously received both anthracyclines and taxanes, 13 patients (35%) had received vinorelbine and 11 patients (30%) had received oral 5FU-derivatives prior to the administration of S-1. All the patients who had responded to treatment had exhibited adequate progression-free intervals from the prior taxane administration until the subsequent taxane administration. Three patients received the same taxane regimen twice, once as adjuvant chemotherapy and the second time in combination with Trastuzumab after recurrence. Prior oral 5FU-derivatives included in other regimens were CMF (five patients), UFT (five patients), 5′DFUR (five patients) and CPT-11 (one patient). Sixteen patients (43%) with ER-positive diseases had received hormone therapy, and 13 patients (35%) with HER2-positive diseases had received Trastuzumab as a monotherapy or in combination with taxane or vinorelbine.

Table 1. Patient characteristics

	No. of patients (n = 37)	% of patients
Median age (years; range)	49 (28–70)	
Metastatic sites involved		
Bone/Soft tissue	6	16
Visceral	31	84
Oestrogen receptor		
Positive	16	43
Negative	21	57
Progesteron receptor		
Positive	17	46
Negative	20	54
HER2/neu status		
Positive	13	35
Negative	24	65

Table 2. Prior chemotherapy

Prior chemotherapy	No. of patients (n = 37)	% of patients
No. of regimens used		
2/3/4/5/6/7/8	4/10/10/4/2/0/3	
Median (range)	4 (2–8)	
Neoadjuvant chemotherapy	6	16
Adjuvant chemotherapy	17	46
S-1 was the last regimen	23	62
Prior chemotherapy		
Anthracycline	37	100
Taxane	37	100
Vinorelbine	13	35
Capecitabine	1	3

The median number of administration days was 70 (6–415 days). The RR was 3%, with no cCR and 3% (1/37) PR. The overall clinical benefit rate (CR, PR and SD for more than 6 months) was 8% (3/37). The median TTP was 84 days (range, 6–415) (Fig. 1; note that a colour version of this figure is available as supplementary data at http://www.jjco.oxfordjournals.org). The median OS from the start of S-1 treatment was 284 days (range, 14–1511), and six patients (16%) were still alive at the last follow-up. Nine patients (24%) received S-1 for more than 100 days. Six out of these nine patients had visceral involvement. Two out of seven patients had oestrogen receptor-positive diseases and four of them were HER2-positive.

Overall, S-1 was well tolerated. Table 3 shows the adverse events in response to S-1 chemotherapy. Toxicities of Grade 3 or more were not reported. The most common toxicities arising from S-1 administration were diarrhea (33%) and nausea (30%). Most of the adverse events were Grade 1, and none of the S-1-related adverse events were fatal. The most frequent reasons for treatment discontinuation were disease progression (30 patients, 81%) and adverse event (seven patients, 19%). The adverse events that were encountered were Grade 2 diarrhea (five cases), Grade 2 stomatitis (one case) and Grade 2 neutropenic fever (one case).

DISCUSSION

The number of patients with MBC who have been pretreated with anthracyclines and/or taxanes are increasing. However, the optimal chemotherapy for patients with MBC who have been pretreated with both anthracyclines and taxanes has not been determined. These patients require palliative therapy that offers a chance of prolonging life with minimal toxicity according to the antitumor response and the alleviation of tumor-related symptoms.

In this study, S-1 chemotherapy produced a 3% RR and an 8% rate of clinical benefit in previously treated patients

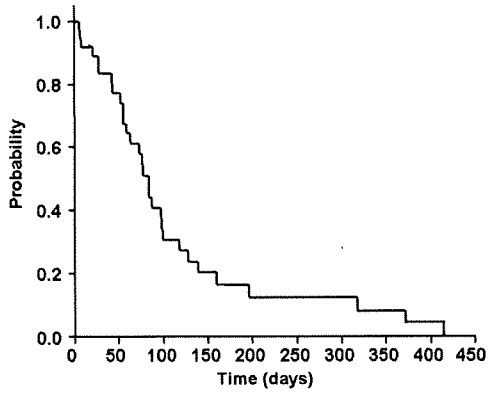

Figure 1. Kaplan–Meier curve for time to progression (TTP). Median TTP was 84 (range 6–415) days.

Table 3. Treatment-related adverse event of TS-1

	Gradre 1 (%)	Grade 2 (%)
Diarrhea	7 (19)	5 (14)
Stomatitis	5 (14)	1 (3)
Nausea/vomitting	11 (30)	0 (0)
Neutropenia	1 (3)	1 (3)
Disorder of liver function	2 (6)	0 (0)

with MBC who were refractory to both anthracyclines and taxanes. The median TTP was 84 days, and 24% of the patients received S-1 for more than 100 days. These results were worse than those reported in clinical trials. This discrepancy is probably because 11 patients had received other 5FU-derivatives prior to S1, the median order of S-1 administration was fifth line (most of the patients received S-1 chemotherapy as their final treatment), and most of the patients had multiple metastatic sites (84% had visceral metastases). The toxicity of S-1, however, was mild in these heavily treated patients, and S-1 is considered to be a feasible palliative chemotherapy in heavily treated MBC patients.

Several oral 5FU-derivatives have been used to treat MBC, but only S-1 and capecitabine have been tested in taxane-refractory MBC patients (10). The treatments were administered based upon physicians' decisions, but the reason why S-1, and not capecitabine, was selected in this study population is unclear. S-1 is a fluoropyrimidine that consists of 1-(2-tetrahydrofuryl)-5-fluorouracil (FTO), a pro-drug of 5-FU, and two other compounds, 5-chloro-2, 4-dihydroxypyrimidine (CDHP; gimestat) and potassium oxonate (OXO; otastat), in molar proportions of 1:0.4:1. CDHP is an inhibitor of dihydropirimidine dehydrogenase (DPD), which degenerates 80% of 5-FU in the liver and maintains the 5-FU level above a minimal effective concentration level. On the other hand, capecitabine is converted to 5'-DFUR either by human carboxyesterase (CE) or cytidine deaminase (CD), which is mainly localized in the human liver. 5'-DFUR is converted to the active form of 5-FU by thymidine phosphorylase (dThdPase) in human tumors. Low CE and CD activity levels are thought to protect the digestive wall and bone marrow from capecitabine toxicity.

Clinically, the reported RRs of capecitabine and S-1 in taxane-pretreated MBC patients are similar, but the toxicity profile seems to be different. Relatively severe diarrhea (14%, Grade 3) and hand-foot syndrome (10%, Grade 3) were observed in a phase II study for capecitabine (2,3), whereas the incidence of Grade 3 or severe diarrhea was relatively low (0.9%) and no hand-foot syndrome was observed in a phase II study of S-1 for MBC (4). A direct comparison of capecitabine and S-1 monotherapy is surely necessary, and since the antitumor activity of capecitabine might be relatively low in tumor cells with high DPD levels, an evaluation of the efficacy of S-1 after progression with

capecitabine or in tumors with high DPD expression levels is warranted.

Moreover, while the efficacy of capecitabine in combination therapy with other cytotoxics (11–16) or as first-line chemotherapy (17) has already been reported, few evidence of the efficacy of S-1 in combination therapy or first-line chemotherapy is available (18,19). The efficacy and safety of S-1 in combination with molecular-targeted drugs, such as antibodies and small molecule tyrosine kinase inhibitors, are also unknown. Further studies are thus required to elucidate the clinical role of S-1 in the management of breast cancer patients.

Conflict of interest statement

None declared.

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Int J Clin Oncol (2008) 13:447–451
DOI 10.1007/s10147-008-0773-3

ORIGINAL ARTICLE

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Long-term prognostic study of carcinoembryonic antigen (CEA) and carbohydrate antigen 15-3 (CA 15-3) in breast cancer

Received: July 19, 2007 / Accepted: February 24, 2008

Abstract
Background. Tumor markers are frequently used for screening and monitoring in oncology. We investigated the use of preoperative tumor marker (carcinoembryonic antigen [CEA] and carbohydrate antigen [CA] 15-3) levels in estimating the prognosis of breast cancer patients.
Methods. We conducted a retrospective study in patients who underwent breast cancer surgery at National Cancer Center Hospital between 1975 and 1994 and whose serum CEA ($n = 1663$) and CA 15-3 ($n = 1500$) levels were measured prior to operation. When we excluded patients with stage IV disease from the study, the CEA level was within the normal range in 1470 patients, while 150 patients had an elevated CEA level. For CA 15-3, 1395 patients were within the normal range, while 70 patients exhibited an elevated level.
Results. The 5-year and 10-year survival rates for patients with normal CEA levels were 87% and 76%, respectively. However, the 5-year and 10-year survival rates for patients with elevated CEA levels were 76% and 65%, respectively. At both time points, patients with normal CEA levels had higher survival rates ($P < 0.05$). The 5-year and 10-year survival rates for the patients with normal CA 15-3 levels were 86% and 76%, respectively, while only 71% and 52% patients with elevated CA 15-3 levels survived at 5 and 10 years, respectively. These differences were also significant ($P < 0.05$). However, there were no significant differences in disease-free survival (DFS) according to CEA or CA 15-3 levels.
Conclusion. There was a positive correlation between CEA levels and CA 15-3 levels and patient prognosis. Thus, the levels of these tumor markers may help to determine prognosis in breast cancer patients.

Key words Breast cancer · Long-term survival · CEA · CA 15-3 · Retrospective study

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Introduction

Tumor markers, which are proteins or enzymes produced by tumor cells or generated by host cells in response to tumorigenesis, are frequently used for screening and monitoring in oncology. The expression of tumor-specific antigens varies, however, and, in general, tumor cells express several different unique antigens. Therefore, the most effective cancer screening protocols would combine multiple markers for increased specificity.

A number of tumor markers (e.g., carcinoembryonic antigen [CEA] and carbohydrate antigen 15-3 [CA 15-3]) are used clinically in the treatment of breast cancer, but the sensitivity of these markers is low, so that they are not useful as screening tools.[1] However, abnormally elevated levels of tumor markers prior to surgery in a patient with primary breast cancer suggest the presence of undetectable metastatic foci, and this is a negative prognostic factor. In addition, tumor marker levels tend to increase as tumor progression occurs; therefore, tumor markers, while of limited diagnostic use, are important for determining the prognosis of breast cancer.[1] In this study, we investigated the use of preoperative tumor marker (CEA and CA 15-3) levels in estimating the prognosis of breast cancer patients.

Patients and methods

We conducted a retrospective study in patients who underwent breast cancer surgery at National Cancer Center Hos-

Table 1. Characteristics of the patients

Stage	No. of patients							
	0	I	IIA	IIB	IIIA	IIIB	IV	Not known
CEA normal (n = 1495)	19	431	774	2	90	57	25	97
CEA high (n = 168)	1	29	69	1	24	22	18	4
CA15-3 normal (n = 1418)	18	413	744	3	80	49	23	88
CA15-3 high (n = 82)	0	4	32	0	13	20	12	1

pital between 1975 and 1994 and whose serum CEA and CA 15-3 levels were measured prior to operation. For serum CEA measurement, an enzyme immunoassay (EIA) was used until 1989 (n = 462), while a latex photometric immunoassay (LPIA) was used between 1990 and 1992 (n = 706). Until 1993, serum CA 15-3 was measured with a quantitative sandwich radioimmunoassay (RIA) utilizing two monoclonal antibodies (115D8, DF3; n = 1017). However, since 1993, a chemiluminescent enzyme immunoassay (CLEIA) has been used to measure both CEA and CA 15-3 (CEA, n = 495; CA 15-3, n = 483).

We set the criteria as follows. Normal values (thresholds) for CEA and CA 15-3 were set at less than 5.0 ng/ml and less than 28 U/ml, respectively. In this study, the CEA level was within the normal range in 1495 patients, while 168 patients had an elevated CEA level. As for CA 15-3, 1418 patients were within the normal range, while 82 patients exhibited an elevated level. When we excluded stage IV patients from the study, CEA level was within the normal range in 1470 patients, while 150 patients had an elevated CEA level. As for CA 15-3, 1395 patients were within the normal range, while 70 patients exhibited an elevated level. The clinical stages of the patients in each group are listed in Table 1. We used the Japanese Breast Cancer Society classification of breast cancer[2] for the stage classification.

Statistical analyses

The Kaplan-Meier method was used to calculate the cumulative survival rates for the different groups: CEA (normal and elevated levels), and CA 15-3 (normal and elevated levels). Statistical significance was tested using the log-rank test. P values of less than 0.05 were considered as significant.

Results

We found that the 5-year and 10-year survival rates for patients with normal CEA levels (n = 1470) were 87% and 76%, respectively. However, the 5-year and 10-year survival rates for patients with elevated CEA levels (n = 150) were 76% and 65%, respectively. At both time points, patients with normal CEA levels had higher survival rates (P < 0.05; Fig. 1). The 5-year and 10-year survival rates for the patients with normal CA 15-3 levels (n = 1395)

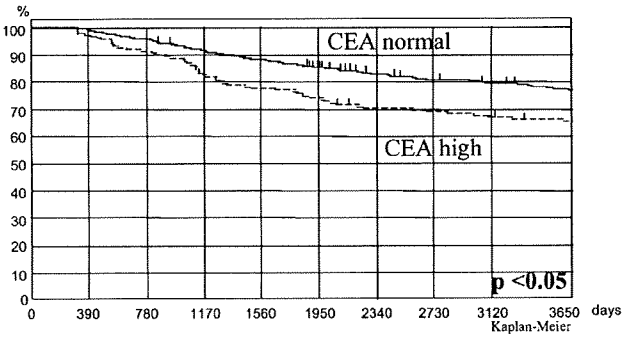

Fig. 1. The 5-year and 10-year survival rates for patients in relation to carcinoembryonic antigen (*CEA*) levels. *CEA normal* denotes normal CEA levels, and *CEA high* denotes elevated CEA levels

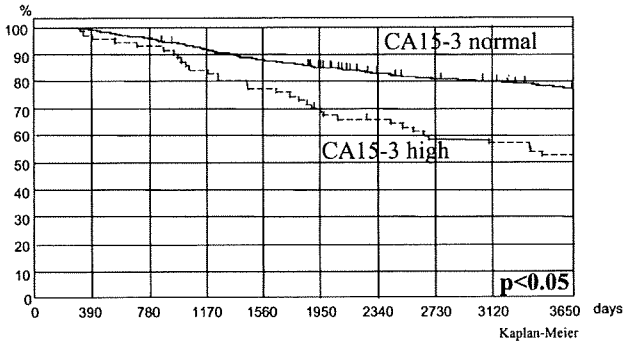

Fig. 2. The 5-year and 10-year survival rates for patients in relation to carbohydrate antigen 15-3 (*CA 15-3*) levels. *CA 15-3 normal* denotes normal CA 15-3 levels, and *CA 15-3 high* denotes elevated CA 15-3 levels

were 86% and 76%, respectively, and only 71% and 52% patients with elevated CA 15-3 levels (n = 70) survived at 5 and 10 years, respectively. These differences were also significant (P < 0.05; Fig. 2). However, there were no significant differences in disease-free survival (DFS) according to either CEA or CA15- levels. The 5-year DFS rate in patients with normal CEA levels was 82%, and the rate in patients with elevated CEA levels was 73% (Fig. 3). The 5-year DFS rate in patients with normal CA 15-3 levels was 83%, and the rate in those with elevated CA 15-3 levels was 67% (Fig. 4).

We also performed a prognostic analysis of the levels of these tumor markers in relation to disease stage. In patients with stage II disease, those with normal CA 15-3 levels had

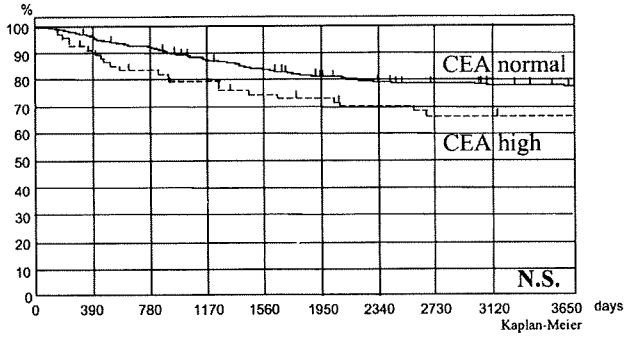

Fig. 3. Disease-free survival rates for patients in relation to CEA levels. *N.S.*, Not significant

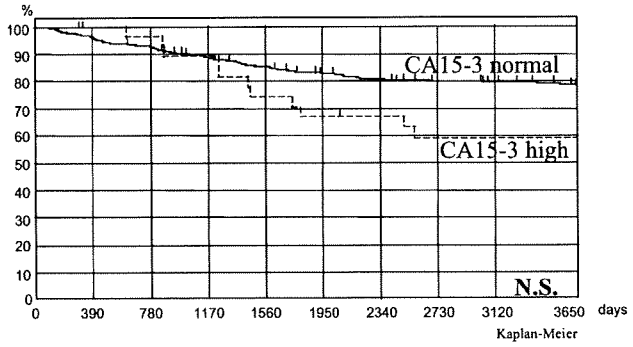

Fig. 4. Disease-free survival rates for patients in relation to CA 15-3 levels

a significantly better prognosis than those with elevated CA 15-3 levels. However, in patients with other disease stages, there was no significant difference in prognosis between those with normal levels and those with elevated levels of either tumor marker.

Discussion

During cellular transformation and progression to cancer, cancer cells release unique enzymes or proteins. Additionally, host cells can produce proteins in response to cancer. Such proteins are termed tumor markers and can be used to screen and monitor disease progression in oncology. Different tumor cells typically produce several unique tumor makers, and, while the specificity of any one marker may be low, the combination of several appropriate tumor markers is a powerful clinical tool.

Carcinoembryonic antigen (CEA) and carbohydrate antigen 15-3 (CA 15-3) are tumor markers commonly used in the screening for breast cancers. CEA is a glycoprotein that is overexpressed in various adenocarcinomas, while CA 15-3 is a mucin-like glycoprotein that is produced in stem cells in response to tumorigenesis.[1]

We conducted a retrospective study in breast cancer patients (excluding stage IV patients) treated at our hospital between 1975 and 1994, and investigated the relationship between CEA and CA 15-3 levels (measured at the time of first medical examination) and patient survival. Interestingly, the 5-year and 10-year survival rates of patients with normal CEA levels were 87% and 76%, while those of patients with elevated CEA levels were 76% and 65%, respectively. Thus, the prognosis of patients whose CEA level was within the normal range at the time of diagnosis was significantly better than the prognosis of those with elevated CEA levels (log-rank; $P < 0.05$). In addition, the 5-year and 10-year survival rates of patients with normal CA 15-3 levels were 86% and 76%, while these rates in the patients with elevated CA 15-3 levels were 71% and 52%, respectively. As with the CEA levels, the long-term survival of breast cancer patients with CA 15-3 levels within the normal range at the time of diagnosis was significantly better than the survival of patients with elevated CA 15-3 levels (log-rank; $P < 0.05$). Previous studies have identified an inverse relationship between tumor marker levels and prognosis when comparing patients with tumors of the same clinical stage.[3] Our results further demonstrate a relationship between preoperative tumor marker levels and long-term survival. Further work is needed to clarify which marker is superior for predicting prognosis, but both may be suitable. However, CA 15-3 may be more sensitive than CEA. The American Society of Clinical Oncology (ASCO) has reported that CA27-29, a MUC-1 marker, is better at tracking tumor recurrence than CA 15-3 (also a MUC-1 marker). Regardless of which marker is better, measuring the levels of MUC-1 markers is likely to be highly effective for monitoring tumor progression or recurrence.[1] Some studies have reported that tumor markers are effective for the early screening of recurrence,[4–7] but the sensitivity varies in these reports. Similar variation was observed when tumor markers were used in the diagnosis of primary breast cancer.[3,8,9] Of note, ASCO has reported that: (i) CEA is not recommended for screening, diagnosis, staging, or routine surveillance of breast cancer patients after primary therapy (1997, 2000, 2007 recommendation); (ii) routine use of CEA for monitoring the response of metastatic disease to treatment is not recommended; and (iii) in the absence of readily measureable disease, elevated levels of MUC-1 markers (CA 15-3 and/or CA27-29) or a rising CEA level may suggest treatment failure.[1]

Significant differences in CA 15-3 expression levels between those in benign tumors and those in stage III and IV disease have been reported.[10] However, Gion et al.[11] reported that no differences were seen in CA 15-3 expression between benign tumors and stage I and II disease. Tumor marker levels tend to rise as disease progresses; therefore, tumor markers may be important prognostic factors.[1] Detecting elevated levels of preoperative tumor markers in patients with primary breast cancer may suggest the presence of undetectable metastatic foci; therefore, increased tumor marker levels are one factor that may predict a poor prognosis. Molina et al.[3,8] conducted a study of locoregional breast cancer and reported that the preoperative sensitivities of CEA and CA 15-3 were 11.7%–13% and 12.7%–18.8%, respectively. In addition, the sensitivities of CEA and CA 15-3 after recurrence were 30%–70%

Table 2. Past reports

Author	Year	No. of patients	Tumor marker	Sensitivity (%)	Patients state
Present study	2007	1620/1465	CEA, CA 15-3	CEA=9.11; CA 15-3=5.36	Preoperative
Soletormos[15]	2004	406	CEA, CA 15-3, TPA	CEA=or CA 15-3 or TPA=44–69	Recurrence
Molina[8]	2003	503	CEA, CA 15-3	CEA=11.7; CA 15-3=12.7	Locoregional breast cancer
Molina[3]	2003	1057	CEA, CA 15-3	CEA=13; CA 15-3=18.8	Locoregional breast cancer
Guadagni[9]	2001	2191	CEA, CA 15-3	CEA=16.7, CA 15-3=33.0, CEA+CA 15-3=39	Stage I–IV or metastatic disease
				CEA=38.5, CA 15-3=60, CA 15-3 and/or CEA=60	Recurrence
Lumachi[16]	2000	62	CEA, CA 15-3	CEA=40.3, CA 15-3=41.9, CEA+CA 15-3=59.7	Recurrence
Lumachi[17]	1999	103	CEA, CA 15-3	CEA=38.1, CA 15-3=61.1	Recurrence
Sutterlin[10]	1999	664	CEA, CA 15-3	CEA=30.3, CA 15-3=48.7	Recurrence
Molina[18]	1999	250	CEA, CA 15-3	CEA=31.6,CA 15-3=46.3, CEA+CA 15-3=59	Recurrence
Sutterlin[19]	1999	76	CEA, CA 15-3	CEA=30, CA 15-3=49,	Recurrence
Lauro[20]	1999	70	CEA, CA 15-3	CEA=35, CA 15-3=79, CEA+CA 15-3=79	Recurrence
Pectasides[21]	1996	68	CEA, CA 15-3	CEA=34, CA 15-3=68, CEA+CA 15-3=68	Recurrence
Jezersek[22]	1996	56	CEA, CA 15-3	CEA=70, CA 15-3=75	Recurrence

and 41.9%–79%, respectively (Table 2). In addition to well-known prognostic factors such as T-factor, N-factor, and hormone receptors, several references have acknowledged the relevance of tumor markers and prognosis.

In the present study, there were no significant differences in DFS according to preoperative levels of the tumor markers CEA and CA 15-3. Many studies have examined the relationship between recurrence and rising tumor marker expression levels. However, there is a delay between increases in marker levels and the confirmation of clinical recurrence, and this time period differs for each patient. In current practice, although a rise in marker expression may be detected, a patient will not be treated unless clinical recurrence is confirmed. A recent study compared the 7-year survival rates of patients undergoing surveillance treatment upon the detection of a rise in marker expression (n = 36) and those who were treated after the recurrence was confirmed by imaging (n = 32). Interestingly, tumor marker-guided salvage treatment prolonged the survival of the breast cancer patients.[12] However, a large-scale study conducted in 1320 postoperative breast cancer patients by the GIVIO investigators[13] found no significant differences in time to detection of recurrence between an intensive surveillance group and a control group. Furthermore, another study of postoperative breast cancer patients (n = 1243) found no difference in 5-year overall mortality between an intensive surveillance group and a control group.[14] Despite differences in the accuracy of current test methods, most studies have not found any differences in survival between groups undergoing intensive surveillance treatment for recurrence at an early stage and control groups; therefore, we believe that tumor marker-guided salvage treatment may not improve patient prognosis.

While tumor marker monitoring may not be useful for the detection of disease recurrence, our data support a role for CEA and CA 15-3 levels at least in helping to determine preoperative prognosis. We found that patients with elevated preoperative tumor marker levels had a significantly worse long-term prognosis than those patients with levels in the normal range. In relation to disease stage, stage II patients with normal CA 15-3 levels had a significantly better prognosis than those with elevated CA 15-3 levels. However, there were no significant differences in prognoses according to either CEA or CA 15-3 levels in patients with any other disease stage. This result suggested that the tumor marker level at the time of diagnosis was an independent prognostic factor. The early detection of recurrent foci may be accomplished using highly sensitive tumor markers, together with modern imaging technologies. Although the results of randomized controlled trials have demonstrated that the timing to initiate treatment for recurrence does not affect the overall survival rate, advances in imaging technology and improved treatment regimens may allow the early detection of recurrent foci and lead to improved patient survival. As diagnostic and treatment techniques improve, tumor markers will likely become more important in cancer therapy.

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Cross-sectional analysis of germline *BRCA1* and *BRCA2* mutations in Japanese patients suspected to have hereditary breast/ovarian cancer

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(Received January 18, 2008/Revised May 11, 2008; June 16, 2008/Accepted June 17, 2008/Online publication October 9, 2008)

The prevalence of *BRCA*1/2 germline mutations in Japanese patients suspected to have hereditary breast/ovarian cancer was examined by a multi-institutional study, aiming at the clinical application of total sequencing analysis and validation of assay sensitivity in Japanese people using a cross-sectional approach based on genetic factors estimated from personal and family histories. One hundred and thirty-five subjects were referred to the genetic counseling clinics and enrolled in the study. Full sequencing analysis of the *BRCA*1/2 gene showed 28 types of deleterious mutations in 36 subjects (26.7%), including 13 types of *BRCA*1 mutations in 17 subjects (12.6%) and 15 types of *BRCA*2 mutations in 19 subjects (14.1%). Subjects were classified into five groups and 22 subgroups according to their personal and family history of breast and/or ovarian cancer, and the prevalence of deleterious mutations was compared with previously reported data in non-Ashkenazi individuals. Statistical analysis using the Mantel-Haenszel test for groups I through IV revealed that the prevalence of Japanese subjects was significantly higher than that of non-Ashkenazi individuals ($P = 0.005$, odds ratio 1.87, 95% confidence interval 1.22–2.88). Family history of the probands suffering from breast cancer indicated risk factors for the presence of deleterious mutations of *BRCA*1/2 as follows: (1) families with breast cancer before age 40 within second degree relatives ($P = 0.0265$, odds ratio 2.833, 95% confidence interval 1.165–7.136) and (2) families with bilateral breast cancer and/or ovarian cancer within second degree relatives ($P = 0.0151$, odds ratio 2.88, 95% confidence interval 1.25–6.64). (*Cancer Sci* 2008; 99: 1967–1976)

In Japan, breast cancer is the most frequent malignancy in women and estimates of new cases and deaths in 2002 were 32 245 and 9178, respectively.[1] The standardized incidence ratio of breast cancer in Japan was approximately one-third that of the US (32.7 *vs* 101.7 per 100 000 women).[1] The incidence of breast cancer in Japanese women shows a steady increase; however, it is still much lower than in Western countries. In breast cancer, family history is the strongest risk factor for cancer predisposition. Epidemiological studies showed that 12% of women with breast cancer have one affected family member and 1% have two or more affected relatives.[2] Women with one, two, and three or more first-degree affected relatives have an increased breast cancer risk when compared with women who do not have an affected relative (risk ratios 1.8, 2.9, and 3.9, respectively).[2] Recent advances in molecular genetics elucidated *BRCA*1 and *BRCA*2 (*BRCA*1/2) as two major susceptibility genes for breast cancer predisposition.[3,4] Gene testing of *BRCA*1/2 is available as a routine clinical test for diagnosing hereditary breast/ovarian cancer (HBOC) in the US and other Western countries,[5,6] while only a few reports have been published concerning the prevalence of *BRCA*1/2 mutations among Japanese people.[7–12] The methods of genetic analysis employed in these studies varied, such as polymerase chain reaction (PCR)/ single strand conformational polymorphisms (SSCP), protein truncation test, and PCR/direct sequencing, but they were performed as preliminary in-house tests in the research setting. In the US, commercial *BRCA*1/2 gene testing was initiated by Myriad Genetic Laboratories in 1996. The test is based on the exon-by-exon PCR/direct sequencing approach comprising sequence analysis of over 17 500 base pairs of the protein-coding and adjacent non-coding regions of *BRCA*1 and *BRCA*2 genes of which test results performed in more than 10 000 individuals were reported.[5,6] Myriad Genetic Laboratories released some of the results to a public database, the Breast Cancer Information Core (BIC) and this test is now utilized as the de facto standard in the US.[13] Examinees with positive test results or pathogenic mutations may undergo close surveillance of breast and ovarian cancers or there might be other options such as risk-reducing surgeries including prophylactic salpingo-oophorectomy and/or mastectomy.[14,15] Chemoprevention using tamoxifen or oral contraceptives were also reported to reduce the risk for contralateral breast cancer or ovarian cancers.[16,17] Clinical application of *BRCA*1/2 gene testing brought a paradigm shift in cancer prevention strategies targeted to at-risk mutation carriers. The aim of the present study was to clarify the prevalence of germline *BRCA*1/2 mutations among Japanese patients suspected to have

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doi: 10.1111/j.1349-7006.2008.00944.x

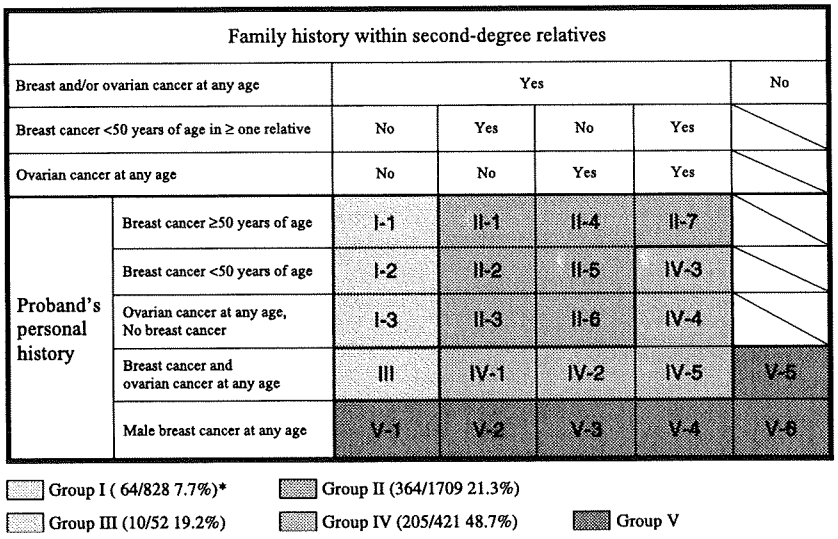

Fig. 1. Classification and grouping of the enrolled subjects. *Numbers in parentheses indicate prevalence of *BRCA*1/2 mutations reported in non-Ashkenazi individuals in the US.[6]

HBOC using the standardized method available in the clinical setting and to establish the genetic basis required for the clinical application of *BRCA*1/2 gene testing for Japanese.

Materials and Methods

The study was designed to validate the sensitivity of *BRCA*1/2 gene testing among Japanese with relevance to personal and family histories of breast and/or ovarian cancer in comparison with the previously reported data of non-Ashkenazi individuals in the US.[6] The study was performed as a contract research by FALCO biosystems. The study protocol was approved by the institutional review board of each participating institution before study initiation. All patients gave written informed consent before registration.

Recruitment of subjects. Candidates were recruited from surgical or gynecologic clinics in five major hospitals in the Tokyo metropolitan area (The Cancer Institute Hospital of Japanese Foundation for Cancer Research, National Cancer Center Hospital, Keio University Hospital, St. Lukes' International Hospital, and Tochigi Cancer Center Hospital) or recruited through newspaper advertisements in the Tokyo metropolitan area. All participants were referred to genetic counseling clinics in these hospitals and checked for eligibility for enrollment in the study. As genetic counseling was mandatory in this study, they underwent genetic counseling before and after gene testing. All expenses for genetic counseling were covered by FALCO biosystems. From August 2003 through May 2006, 135 patients were enrolled in the study, among which 101 subjects were treated for breast and/or ovarian cancers in these hospitals, and 34 subjects were recruited through newspaper advertising. Patient records of their personal history of cancer, including diagnosis, treatment, and clinicopathological parameters, were obtained through a survey completed by their attending surgeons or physicians.

Patient selection. Patients were eligible for the study if they fulfilled the following conditions: (i) native Japanese over 20 years old; (ii) histological diagnoses of invasive breast cancer and/or ovarian cancer; and (iii) at least one first- or second-degree relative diagnosed with either or both cancers. Eligible patients were assigned to the matrix chart according to their personal and family history of cancer (Fig. 1). This matrix chart was formulated based on the reported prevalence of *BRCA*1/2 mutations in 3011 non-Ashkenazi individuals in the US.[6] Enrolled subjects were classified into five groups, i.e. Group I through Group V. All subjects assigned to Groups I through IV had some form of risk factor estimated from their personal and family history, while the risk of those in Groups I and III was personal rather than familial. The proportion of familial risk increased in Groups II and IV, and Group IV had a combination of the highest risk of both personal and familial history. Reportedly, the prevalence of *BRCA*1/2 mutations in Group I is relatively low, 7.7% (64/828 cases), while those in Groups II, III, and IV were 21.3% (364/1709), 19.2% (10/52), and 48.7% (205/421) respectively.[6] Group V included cases of male breast cancer at any age irrespective of their family history and sporadic cases of breast cancers with concomitant ovarian cancers. The prevalence of *BRCA*1/2 mutations in each matrix or group in the present study could be compared with those reported in the US, except for Group V that had no comparable counterpart.

Exclusion criteria for enrollment were as follows: (i) informed consent was provided not by a principal, but by a representative; (ii) a precise history of breast and/or ovarian cancers was not available; (iii) the patient was undergoing bilateral oophorectomy due to non-malignant disorders; (iv) correct diagnosis was not disclosed to the patient; (v) informed consent was not provided due to psychiatric problems; (vi) the patient had non-invasive breast cancer; (vii) borderline ovarian neoplasms; (viii) the patient was undergoing allogeneic bone marrow transplantation; (ix) another relative had undergone *BRCA*1/2 gene testing; and (x) when gene testing and disclosure of genetic information might cause serious sociopsychological problems.

***BRCA*1 and *BRCA*2 gene testing.** Analyses of *BRCA*1/2 were performed by direct sequencing, as described previously.[5,6] Briefly, 7 mL of anticoagulated blood was sent to FALCO biosystems for DNA extraction. Aliquots of patient DNA were sent to Myriad Genetic Laboratories (Salt Lake City, UT, USA) and subjected to PCR/direct sequencing analysis for *BRCA*1/2. This analysis also included the detection of the following five specific large genomic rearrangements of the *BRCA*1 gene (five-site rearrangement panel): 3.8-kb deletion of exon 13 and 510-bp deletion of exon 22 described in individuals of Dutch ancestry,[18] 6-kb duplication of exon 13 described in individuals of European (particularly British) ancestry,[19] 7.1-kb deletion of exons 8 and 9 described in individuals of European ancestry,[20] and 26-kb deletion of exons 14–20.[21] Nucleotide positions of mutations were expressed according to GenBank entries U14680 and U43746. All variants were interpreted according to the following criteria.[6]

Positive for deleterious mutation. Mutations were interpreted as positive deleterious mutations if they prematurely terminated (truncated) the protein product of *BRCA*1 at least 10 amino

doi: 10.1111/j.1349-7006.2008.00944.x

Table 1. Background of subjects enrolled in the study

Variables	All	Non-carrier	Deleterious mutations in		
			*BRCA*1/2	*BRCA*1	*BRCA*2
Number of patients	135	99	36	17	19
Age at enrollment	51.6 ± 12.4	52.5 ± 12.7*	49.1 ± 11.3*	48.9 ± 10.1	49.2 ± 12.5
Number of sessions for genetic counseling	3.36 ± 0.82 (1–8)‡	3.22 ± 0.56 (1–5)***	3.75 ± 1.20 (1–8)***	–	–
Sex					
Female	131	96	35	17	18
Male	4	3	1	0	1
Types of affected cancer					
Female					
Breast	113	83	30	12	18
Ovarian	9	8	1	1	0
Both	9	5	4	4	0
Male					
Breast	4	3	1	0	1
Genealogical information; number of relatives ascertained					
Relatives ≤ 1st degree	673 (4.99)†	487 (4.92)**	186 (5.16)**	92 (5.41)	94 (4.95)
Male	305 (2.26)	218 (2.20)	87 (2.42)	42 (2.47)	45 (2.37)
Female	368 (2.73)	269 (2.72)	99 (2.75)	50 (2.94)	49 (2.58)
1st < Relatives ≤ 2nd degree	1142 (8.46)	825 (8.33)**	317 (8.80)**	166 (9.76)	151 (7.95)
Male	526 (3.90)	383 (3.87)	143 (3.97)	72 (4.24)	71 (3.74)
Female	616 (4.56)	442 (4.46)	174 (4.83)	94 (5.53)	80 (4.21)
Relatives > 2nd degree	427 (3.16)	254 (2.57)**	173 (4.81)**	106 (6.24)	67 (3.53)
Male	192 (1.42)	112 (1.13)	80 (2.22)	55 (3.24)	25 (1.32)
Female	235 (1.74)	142 (1.43)	93 (2.58)	51 (3.00)	42 (2.21)

†Numbers in parentheses indicate average number of relatives in a family. ‡Minimal and maximal. ***$P = 0.0007$. *$P = 0.1594$, unpaired t-test. **χ^2 value 26.90, d.f. = 2, $P < 0.0001$.

acids from the C-terminus or the protein product of *BRCA*2 at least 110 amino acids from the C-terminus, based on the documentation of deleterious mutations in *BRCA*1/2. In addition, specific missense mutations and non-coding intervening sequence mutations were interpreted as deleterious on the basis of data derived from linkage analysis of high-risk families, functional assays, biochemical evidence, or demonstration of abnormal mRNA transcript processing.

Genetic variant of uncertain significance. This group includes missense mutations and mutations that occur in analyzed intronic regions whose clinical significance has not yet been determined, chain-terminating mutations that truncate *BRCA*1 and *BRCA*2 distal to amino acid positions 1853 and 3308, respectively, and mutations that eliminate the normal stop codons of these proteins.

Mutational types were defined according to the international nomenclature system reported by Antonarakis *et al.*[22] All of the detected mutations were searched for in the BIC database.[13] The description of mutational types previously reported in the BIC database is indicated along with those defined by the international nomenclature system, in order to facilitate the comparison of the data with those reported in the database or other publications.

Multiplex ligation-dependent probe amplification (MLPA) analysis. To search for unknown genomic rearrangements, we performed MLPA analysis for all samples in which no deleterious mutation was detected, using Salsa MLPA Kits P002 and P087, which are commercially available from MRC-Holland (Amsterdam, The Netherlands). MLPA is a quantitative multiplex PCR approach to determine the relative copy number of each *BRCA*1/2 exon.[23,24] Assay procedures were performed according to the manufacturer's instructions.

Statistical analysis. Statistical significance was analyzed by Fisher's exact test and unpaired t-test using Prism 4 (GraphPad Software, San Diego, CA, USA). Comparisons of the prevalence of *BRCA*1/2 germline mutations divided by subgroups between Japanese and non-Ashkenazi individuals were analyzed by Fisher's exact test or the Mantel-Haenszel test using R package (version 1.1.2), available from the Comprehensive R Archive Network (CRAN) (http://strimmerlab.org/software/genets/). Cumulative incidence was analyzed by Kaplan–Meier plot (log-rank test) using SAS software (SAS Institute Japan, Tokyo, Japan).

Results

Characteristics of the enrolled subjects. A total of 135 subjects were examined for *BRCA*1/2 germline mutations, and deleterious mutations were found in 36 subjects (17 for *BRCA*1 and 19 for *BRCA*2). Backgrounds of all subjects and those divided by carrier status are shown in Table 1. In the analysis of all subjects, average age at enrollment was 51.6 ± 12.4 years and the average number of counseling sessions per client was 3.36 ± 0.82. There was no significant difference as to the age at enrollment between non-carriers and carriers with deleterious mutations. As for genetic counseling, significantly more sessions were performed for those carrying deleterious mutations of *BRCA*1/2 as compared to non-carriers (3.75 ± 1.20 *vs* 3.22 ± 0.56, $P = 0.0007$). Of the 135 subjects examined, 131 were women and 4 were men. All of the male subjects developed breast cancer, while 113 women developed breast cancers (9 women developed ovarian cancers and 9 women developed both breast and ovarian cancers). There was no statistical significance in these variables between *BRCA*1/2 mutation carriers and non-carriers. In the study, patient accrual was determined by personal and family histories within second-degree relatives. Family history was precisely assessed in genetic counseling clinics and familial information was obtained for 2242 family members, including probands. There were no statistical differences in the numbers of ascertained relatives within the first- or second-degree between non-carriers and carriers. As for relatives beyond the second-degree, significantly more relatives were ascertained in pedigrees with deleterious *BRCA*1/2 mutations as compared to

Table 2. Clinical characteristics of the subjects with breast or ovarian cancer

Variables		Breast cancer†	Ovarian cancer‡
Number of subjects		126	18
Sex	Female	122	18
	Male	4	–
Age at diagnosis	Female	46.2 ± 12.1	50.2 ± 11.8
	Male	64.5 ± 3.7	–
Tumor size (T)	Tis	2¶	–
	T1	66	11
	T2	49	1
	T3	4	4
	Missing data (No.)	5	2
Nodal status (N)	Negative	95	10
	Positive	24	5
	Missing data (No.)	7	3
Stage	0	2	–
	I	71	9 (IA 4, IC 5)
	II	38	1 (IIA 1)
	III	4	4 (IIIB 1, IIIC 3)
	IV	1	1
	Missing data (No.)	13	3
Histology	Non-invasive	2	Serous 3, mucinous 3,
	Invasive	111	endmetrioid 2, clear cell 2,
	Missing data (No.)	13	undifferentiated 2, mixed-cell type 2,
			others 2, sex-cord stromal tumor 1, germ cell tumor 1
Estrogen receptor status	Positive	72	
	Negative	37	
	Missing data (No.)	17	
Progesterone receptor status	Positive	64	
	Negative	44	
	Missing data (No.)	18	
Histological grade	Grade 1	24	
	Grade 2	37	
	Grade 3	24	
	Missing data (No.)	41	
Laterality	Unilateral	104	
	Bilateral	22	
Mutational status	No mutation	91	13
	BRCA1/2	35	5
	BRCA1	16	5
	BRCA2	19	0

†Includes subjects with ovarian cancer ($n = 9$). ‡Includes subjects with breast cancer ($n = 9$). ¶Subjects with multiple primary breast cancer, in which histology of the first primary cancer was pTis. They were enrolled in the study as the histology of the second primary breast cancer was ascertained to be invasive cancer.

those of non-carriers ($P < 0.0001$) (Table 1). The clinical characteristics of breast and ovarian cancers that developed in enrolled subjects are listed in Table 2. In this protocol, non-invasive cases, including pTis or DCIS, did not fulfill the eligibility, but two cases were enrolled in the study due to the subsequent occurrence of invasive breast cancers in the contralateral breast.

Results of *BRCA1/2* gene testing. In the analysis of *BRCA1*, 13 types of deleterious mutations were detected in 17 subjects. One mutation (L63X, c.188T > A) was found in five subjects. Genetic variants of uncertain significance were detected in nine subjects, among which three subjects had the same mutational types (S1557P, c.4729T > C), substituting cytosine for thymine (Table 3).

In the analysis of *BRCA2*, 15 types of deleterious mutations were detected in 19 subjects. Each of four mutational types (c.1813delA, S1882X[c.5645C > A], c.5576_5579delTTAA, and R2318X[c.6952C > T]) was detected in two subjects. Eight types of genetic variants of uncertain significance were detected in 13 subjects, among which four types were detected in more than two subjects (Table 4). Of the deleterious mutations, five of 13 mutational types in *BRCA1* and four of 15 mutational types in *BRCA2* were not reported previously in the BIC database. As for genetic variants of uncertain significance, all of these variants were missense mutations, among which three in *BRCA1* and four in *BRCA2* were not reported in the BIC database. No genomic rearrangement of the *BRCA1* gene was detected in analysis using a 5′-site rearrangement panel. In analysis using MLPA, genomic rearrangements were not detected in *BRCA1/2* in all subjects.

Comparison of prevalence of *BRCA1/2* germline mutations between Japanese and non-Ashkenazi individuals. Deleterious mutations of *BRCA1/2* were detected in 26.7% (36/135) of the subjects enrolled in the study. The prevalence of deleterious mutations in non-Ashkenazi individuals in each matrix or group was calculated based on the data reported previously.[6] The prevalence of mutations in Groups I through IV in the Japanese cohort was 27.2% (34/125), while that in non-Ashkenazi individuals was 20.3% (590/2900), respectively (Table 5). The prevalence of *BRCA1/2* mutations in each matrix or subgroup was compared with that in non-Ashkenazi individuals. In the analysis of each subgroup, statistical difference was observed only in the subgroup I-1 between Japanese and non-Ashkenazi individuals,

doi: 10.1111/j.1349-7006.2008.00944.x

Table 3. Deleterious mutations and genetic variants of uncertain significance detected in *BRCA1*

BRCA1: Deleterious mutations Exon	Designation	No. detected	Subgroup assigned	dbSNP ID	(Breast Cancer Information Core [BIC] mutation database) BIC designation	Type	No. reported	Ethnicity
5	L63X(c.188T > A)	5	II-2, II-6, IV-3, V-5, III-1	NR	L63X	N	6	Asian: 5
5	c.190_193delTGTA	1	I-2	NR	(309del4)‡	F	0	
7	Y130X(c.390C > A)	1	II-7	NR	Y130X	N	1	Asian: 1
11	c.1112delC	1	II-2	NR	(1231delC)	F	0	
11	K503X(c.1507 A > G)	1	IV-2	NR	(K503X)	N	0	
11	E908X(c.2722G > T)	1	II-5	NR	E908X	N	58	Asian: 0
11	Q934X(c.2800C > T)	1	IV-5	NR	Q934X	N	4	Asian: 2
11	c.3442delG	1	II-2	NR	3561delG	F	2	Asian: 2
11	c.3505_3509delGACAT	1	IV-3	NR	(3624del5)	F	0	
11	c.4041_4042delAG	1	I-2	NR	4160delAG	F	7	Asian: 0
13	IVS13 + 1G > T†	1	II-5	NR	IVS13 +1G > T	S	1	NR
13	R1443X(c.4327C > T)	1	IV-3	NR	R1443X	N	126	Asian: 1
24	c.5533_5534insT	1	II-2	NR	(5652insT)	F	0	
BRCA1: Genetic variant of uncertain significance					(BIC Mutation Database)			
Exon	Designation	No. detected	Subgroup assigned	dbSNP ID	BIC Designation	Type	No. reported	Ethnicity
5	L52F(c.154C > T)	1	II-1	NR	L52F	M	5	Asian: 2
10	P209L(c.626C > T)	1	II-1	NR	(P209L)	M	0	
11	S1217P(c.3649T > C)	1	II-1	NR	(S1217P)	M	0	
16	S1577P(c.4729T > C)	3	I-2, II-2, II-5	NR	S1577P	M	1	Asian: 1
20	R1753T(c.5258G > C)	1	II-3	NR	(R1753T)	M	0	
21	F1761S(c.5282T > C)	1	II-2	NR	F1761S	M	1	Asian: 0
24	Y1853C(c.5558 A > G)	1	II-5	NR	Y1853C	M	1	Asian: 0

†Mutation in the donor site of intron 13, suspected to be deleterious, resulting in a splicing error. ‡Mutations in parentheses indicate mutational types of unreported cases represented according to the style of BIC nomenclature. F, frameshift mutation; M, missense mutation; N, nonsense mutation; NR, not reported; P, genetic polymorphism; S, splice site mutation; Syn, synonymous mutation.

Table 4. Deleterious mutations and genetic variants of uncertain significance detected in *BRCA2*

BRCA2: deleterious mutation Exon	Designation	No. detected	Subgroup assigned	dbSNP ID	(Breast Cancer Information Core [BIC] mutation database) BIC designation	Type	No. reported	Ethnicity
3	c.86_87delTT	1	I-1	NR	314delTT	F	1	NR
10	c.1813delA	2	II-2, II-2	NR	2041delA	F	16	Asian: 0
11	c.2612delCinsTTT	1	I-2	NR	(2840delC insTTT)‡	F	0	
11	c.3847_3848delGT	1	V-6	NR	4075delGT	F	61	Asian: 0
11	c.4021delT	1	II-2	NR	(4249delT)	F	0	
11	S1882X(c.5645C > A)	2	I-2, II-1	NR	S1882X	N	28	Asian: 2
11	c.5207_5208delAA	1	II-5	NR	(5435delAA)	F	0	
11	c.5576_5579delTTAA	2	I-1, I-1	NR	5804del4	F	29	Asian: 0
11	c.6445_6446delAT	1	II-2	NR	6673delAT	F	3	Asian: 0
13	R2318X(c.6952C > T)	2	II-1, II-5	NR	R2318X	N	5	Asian: 2
18	c.8064_8065delCT	1	II-2	NR	(8292delCT)	F	0	
20	S2835X(c.8504C > A)	1	II-2	NR	S2835X	N	1	Asian: 0
23	Q3026X(c.9076C > T)	1	II-2	NR	Q3026X	N	3	Asian: 1
23	P3039P (c.9117G > A)†	1	II-2	rs28897756	P3039P	Syn	14	Asian: 0
25	R3128X(c.9382C > T)	1	II-2	NR	R3128X	N	50	Asian: 0
BRCA2: Genetic variant of uncertain significance					(BIC Mutation Database)			
Exon	Designation	No. detected	Subgroup assigned	dbSNP ID	BIC designation	Type	No. reported	Ethnicity
10	K322Q(c.964 A > C)	2	I-1, II-1	rs11571640	K322Q	M	11	Asian: 7
10	M524I(c.1572G > C)	1	I-1	NR	(M524I)	M	0	
10	K610Q(c.1828 A > C)	1	II-2	NR	(K610Q)	M	0	
11	I770V(c.2308 A > G)	1	II-5	NR	(I770V)	M	0	
11	K1132R(c.3395 A > G)	1	I-2	rs1801406	K1132R	M	1	Asian: 1
11	G2044V(c.6131G > T)	3	II-2, II-2, V-5	NR	G2044V	M	10	Asian: 10
11	V2109I(c.6325G > A)	2	I-1, I-2	NR	V2109I	M	8	Asian: 5
17	S2616F(c.7847C > T)	2	I-1, II-1	NR	(S2616F)	M	0	

†Synonymous mutation in the exon–intron junction of exon 23, suspected to be deleterious, resulting in a splicing error. ‡Mutations in parentheses indicate mutational types of unreported cases represented according to the style of BIC nomenclature. F, frameshift mutation; M, missense mutation; N, nonsense mutation; NR, not reported; P, genetic polymorphism; S, splice site mutation; Syn, synonymous mutation.