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# Review Article

## Sentinel Lymph Node Biopsy is Feasible for Breast Cancer Patients after Neoadjuvant Chemotherapy

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***Background***: Despite the increasing use of both sentinel lymph node (SLN) biopsy and neoadjuvant chemotherapy (NAC) in patients with operable breast cancer, information on the feasibility and accuracy of sentinel node biopsy following neoadjuvant chemotherapy is still quite limited. Therefore, we investigated the feasibility and accuracy of sentinel lymph node biopsy for breast cancer patients after NAC.

***Methods***: A total of 104 patients with Stage II and III breast cancers, previously treated by NAC, were enrolled in the study. All patients were clinically node-negative after NAC. The patients underwent SLN biopsy, which involved a combination of an intradermal injection of radiocolloid and a subareolar injection of blue dye over the tumor. This was followed by completion axillary lymph node dissection (ALND).

***Results***: SLN could be identified in 97 of 104 patients (identification rate, 93.3%). In 93 of the 97 patients (95.9%), the SLN accurately predicted the axillary status. Four patients' SLN biopsies were false negative, resulting in a false-negative rate of 10.0%. The SLN identification rate tended to be lower among patients with T4 primary tumors prior to NAC (62.5%).

***Conclusion***: The SLN identification and false-negative rates were similar to rates in non-neoadjuvant studies. The SLN accurately predicted metastatic disease in the axilla of patients with tumor response following NAC.

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Key words: Sentinel node biopsy, Neoadjuvant chemotherapy, Breast cancer, Intradermal injection

## Introduction

Currently, the status of the axillary lymph nodes is the most important prognostic indicator for breast cancer and helps guide the physician in adjuvant therapy. More than 40 peer-reviewed pilot studies, published between 1993 and 1999, have established the validity of the SLN biopsy technique for clinically node-negative breast cancer[1] and SLN biopsy has become the standard of care for axillary staging in such patients.

Recent studies report identification rates greater than 90% and false-negative rates ranging from 2 to 10%[2,3]. To ensure a high SLN identification rate and a low false-negative rate, some relative contraindications for SLN biopsy have been established, including T3 or T4 tumors, multicentric or multifocal lesions, a large biopsy cavity, previous axillary surgery, previous chest-wall irradiation, and NAC[4,5].

The application of SLN biopsy in NAC patients may identify, as in non-neoadjuvant chemotherapy groups, patients who do not necessarily require an ALND. Several studies have evaluated the use of SLN biopsy in patients with breast cancer after NAC, but the results have been varied and inconclusive[6-14].

Recently, the American Society of Clinical Oncology panel concluded that there are insufficient data to recommend SLN biopsy for patients receiving preoperative therapy, although SLN biopsy after preoperative systemic chemotherapy is technically feasible[15]. It is possible that the tumor response to chemotherapy may alter or

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Abbreviations:
SLN, Sentinel lymph node; NAC, Neoadjuvant chemotherapy; ALND, Axillary lymph node dissection

interrupt the lymphatic drainage, thus causing lower SLN identification rates and higher false-negative rates than in non-neoadjuvant studies. We hypothesize that the lymphatic flow within the skin lesion overlying the tumor is less damaged by chemotherapy than that in the parenchyma surrounding the tumor, except in T4 tumors. Thus, the usefulness of SLN biopsy with intradermal radiocolloid injection for patients with NAC-treated breast cancer has yet to be established.

The objective of this study was to determine the feasibility and accuracy of SLN biopsy using intradermal radiocolloid injection over the tumor in clinically node-negative, NAC-treated breast cancer patients.

## Patients and Methods

Between May 2003 and October 2005, 104 patients with T2-4N0-2 breast cancer underwent NAC with SLN biopsy plus ALND performed by a single surgeon. The pathologic diagnosis was established by core needle biopsy in all patients prior to NAC.

Patients under 65 of age received four cycles of 5FU (500mg/m$^2$) / epirubicin (100mg/m$^2$) / cyclophosphamide (500mg/m$^2$) (FEC), plus twelve weekly cycles of paclitaxel (80mg/m$^2$). Patients over 65 years of age received twelve weekly cycles of paclitaxel (80mg/m$^2$) alone. After NAC, we enrolled the 104 clinically node-negative patients into this study.

Lymphatic mapping was performed using a 3 ml combination of blue dye (Patent blue V®, TOC Ltd., Tokyo, Japan) and 30-80 megabecquerels of technetium-99m-labeled Phytate (Daiichi RI Laboratory, Tokyo, Japan). One day prior to surgery, the radiotracer was intradermally injected into the area overlying the tumor, while blue dye was intraoperatively injected into the subareolar site. For nonpalpable lesions, injections were performed using mammographic or ultrasonic needle localization. Sentinel lymph nodes were identified as blue stained, radioactive, or both. SLN biopsy was then followed by a standard level I/II ALND. For 32 patients, lymphscintigraphy was also performed prior to NAC, and was compared to lymphatic mapping after NAC.

All sentinel nodes were histologically evaluated by creating 3-5 mm serial sections and staining with hematoxylin and eosin (H&E). Lymph nodes submitted as part of the axillary dissection were submitted in their entirety and evaluated using standard H&E staining.

**Table 1. Patient demographics**

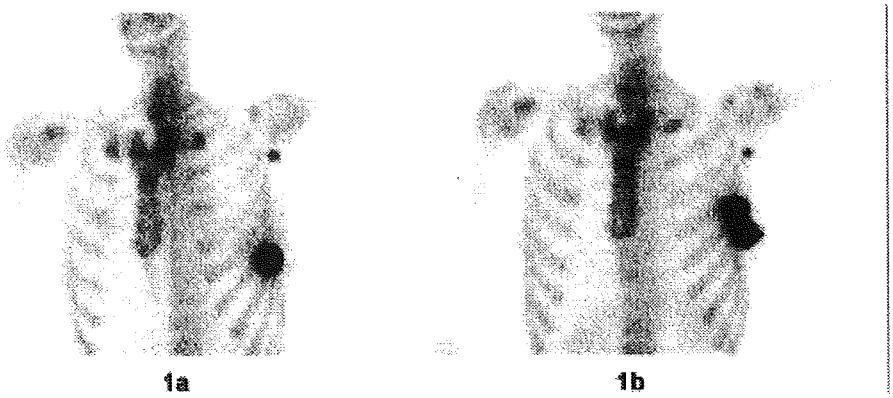
| | Number of patients |
|---|---|
| Age (years) | |
| Mean | 50.2 |
| Range | 27-77 |
| Clinical tumor size (cm)* | |
| Mean | 4.89 |
| Range | 2.5-12 |
| Tumor classification* | |
| T2 | 61 (58.7%) |
| T3 | 35 (33.6%) |
| T4 | 8 ( 7.7%) |
| Lymph node status* | |
| N0 | 54 (52.0%) |
| N1 | 40 (38.5%) |
| N2 | 10 ( 9.5%) |
| Tumor type | |
| Invasive ductal | 102 (98.1%) |
| Invasive lobular | 2 ( 1.9%) |
| Type of NAC | |
| FEC plus paclitaxel | 100 (96.2%) |
| paclitaxel alone | 4 ( 3.8%) |
| Clinical response of the tumor | |
| CR | 55 (52.9%) |
| PR | 41 (39.4%) |
| SD | 8 ( 7.7%) |
| Pathological response of the tumor | |
| pCR | 23 (22.1%) |
| pINV | 81 (77.9%) |
| Pathological nodal status | |
| Negative | 60 (57.7%) |
| Positive | 44 (42.3%) |

*Before NAC.
pCR = pathological complete response; pINV = pathological invasive.
CR = Complete response; PR = Partial response; SD= Stable disease

## Results

The patient characteristics, type of chemotherapy, clinical response of the tumor, and pathological findings are summarized in Table 1. All patients underwent breast-conserving therapy or mastectomy and were clinically node-negative at the time of operation.

Based on lymphscintigraphy studies before and after NAC, the results of lymphatic mapping were quite similar in 30/32 patients, as shown in Fig 1. SLN were not detected in two cases with a



1a 1b

**Fig 1.** Lymphscintigraphy before and after NAC (1a and 1b, respectively) revealed one sentinel node at the axilla. The bone scintigram was performed simultaneously to detect bone metastasis.

**Table 2. Results of sentinel node biopsy**

| | Number of patients |
|---|---|
| Total no. of patients | 104 |
| SLN identified | 97 (93.4%) |
| SLN positive | 36 (34.6%) |
| SLN was only positive lymph node | 16 (44.4%) |
| SLN identification method | |
| Radiocolloid and blue dye | 91 (87.5%) |
| Blue dye only | 13 (12.5%) |

**Table 3. Comparison of lymph node status of SLNs and non-SLNs (n=97)**

| | Non-SLN status | |
|---|---|---|
| SLN status | Positive | Negative |
| Positive | 20 | 16 |
| Negative | 4 | 57 |

False-negative rate, 10%; overall accuracy, 96%; negative predictive value, 93%; positive predictive value, 100%

T4d primary tumor.

As seen in Table 2, the overall SLN identification rate was 93.4% (97 of 104). Of the 97 patients in whom an SLN could be identified, 36 (34.6%) had positive SLNs. In 16 of these patients (44.4%), the SLN was the only positive node. SLNs were identified by both radiocolloid and blue dye in 91 patients (87.5%) and by blue dye alone in 13 patients (12.5%).

The pathological status of the SLNs and non-SLNs is outlined in Table 3.

The SLNs accurately predicted axillary status in 93/97 patients (95.9%). Four patients had false-negative SLN biopsies, a false-negative rate of 10.0% (4/40). Fifty-seven patients had pathologically negative SLN or non-SLN.

**Table 4. Comparison of lymph node status of SLNs and non- SLNs among tumor classifications before NAC**

| | T2 (n=59) | | T3/T4 (n=38) | |
|---|---|---|---|---|
| | Non- SLN status | | | |
| SLN status | Positive | Negative | Positive | Negative |
| Positive | 7 | 7 | 13 | 9 |
| Negative | 2 | 43 | 2 | 14 |
| | SLN identified, 59/61 (97%) False-negative rate, 13% | | SLN identified, 38/43 (88%) False-negative rate, 8% | |

The pathological status of the SLNs and non-SLNs was analyzed according to tumor classifications before NAC, clinical lymph node status before NAC, and the response of the tumor after NAC.

In T2 tumors before NAC, the SLN identification rate was 97% (59 of 61), and 2 patients had false-negative SLN biopsies, or a false-negative rate of 13%. In T3 and T4 tumors, the results were 88.4% (38 of 43) and 8%, respectively (Table 4). The SLN identification rate tended to be higher in patients with a T2 primary tumor before NAC than in those with T3/T4 primary tumor before NAC, but the difference was not statistically significant.

In the SLN biopsy results, there was no significant difference between nodal status prior to NAC.

**Table 5. Comparison of lymph node status of SLNs and non- SLNs among nodal status before NAC**

| | N0 (n=52) | | N1/N2 (n=45) | |
|---|---|---|---|---|
| | Non- SLN status | | | |
| SLN status | Positive | Negative | Positive | Negative |
| Positive | 4 | 8 | 16 | 8 |
| Negative | 2 | 38 | 2 | 19 |
| | SLN identified, 52/54 (96%) False-negative rate, 14% | | SLN identified, 45/50 (90%) False-negative rate, 7% | |

**Table 6. Comparison of lymph node status of SLNs and non- SLNs among clinical response after NAC**

| | CR (n=50) | | PR/SD (n=47) | |
|---|---|---|---|---|
| | Non- SLN status | | | |
| SLN status | Positive | Negative | Positive | Negative |
| Positive | 6 | 5 | 14 | 11 |
| Negative | 2 | 37 | 2 | 20 |
| | SLN identified, 50/55 (91%) False-negative rate, 15% | | SLN identified, 47/49 (96%) False-negative rate, 7% | |

**Table 7. Success rate of sentinel node identification according to tumor characteristics**

| | No. of Attempted | Success Rate (%) | P |
|---|---|---|---|
| Tumor classification | | | |
| T2 | 61 | 97 % | *N.S.* |
| T3 | 35 | 94 % | |
| T4 | 8 | 63 % | |
| Clinical nodal status | | | |
| Negative | 54 | 96 % | *N.S.* |
| Positive | 50 | 90 % | |
| Clinical tumor response | | | |
| CR | 55 | 91 % | *N.S.* |
| PR/SD | 49 | 96 % | |
| Pathological tumor response | | | |
| pCR | 23 | 91% | *N.S.* |
| pINV | 81 | 94 % | |

In the patients with clinically negative lymph nodes (N0) before NAC, the SLN identification rate was 96.3% (52 of 54), and two patients had a false-negative SLN biopsy, a false-negative rate of 14%. In the patients with clinically positive lymph nodes (N1/N2), the results were 90% (45 of 50) and 7%, respectively (Table 5). In the SLN biopsy results, there was no significant difference between nodal status prior to NAC.

For patients with complete tumor response (CR) after NAC, the SLN identification rate was 91.0% (50/55) and two patients had false-negative SLN biopsies, resulting in a false-negative rate of 15%. For patients with partial tumor response (PR) and stable disease (SD), the results were 96.0% (47/49) and 7%, respectively (Table 6). The SLN identification rate tended to be lower, although the difference was not statistically significant, after NAC in patients with CR after NAC as compared to those with PR and SD.

There was no significant difference in the false-negative rate according to the tumor classification before NAC, the clinical lymph node status before NAC, or the tumor responses after NAC.

There was also no significant difference in the success rate of SLN identification according to tumor classifications before NAC, the clinical lymph node status before NAC, the clinical response of the tumor after NAC, or the pathological response of the tumor after NAC, although the success rate tended to be lower in patients with a T4 primary tumor (Table 7).

## Discussion

Although the use of SLN biopsy has dramatically increased over the past several years, and some experienced surgeons are performing this procedure without completing axillary dissection, it is unlikely that SLN biopsy will become the generally accepted standard of care in axillary staging until results from ongoing randomized trials

**Table 8. Studies of SLN biopsy after NAC**

| | No. of patients | Stage | Tumor size (cm) | No (%) of successful SLN biopsies | False negative (%) |
|---|---|---|---|---|---|
| Breslin et al.,2000 [6] | 51 | II or III | 5.0 | 43 (84.3) | 3 (12) |
| Miller et al., 2002 [7] | 35 | T1-3N0 | 3.5 | 30 (86.0) | 0 ( 0) |
| Stearns et al.,2000 [8] | 34 | T3-4, any N | 5.0 | 29 (85.0) | 3 (14) |
| Haid et al.,2001 [9] | 33 | T1-3, any N | 3.3 | 29 (88.0) | 0 ( 0) |
| Julian et al.,2002 [10] | 31 | I or II | NS | 29 (93.5) | 0 ( 0) |
| Tafra et al.,2001 [12] | 29 | Any T, N0 | NS | 27 (93.0) | 0 ( 0) |
| Nason et al.,2000 [13] | 15 | T2-4, N0 | NS | 13 (87.0) | 3 (33) |
| Shimazu et al.,2004 [14] | 47 | II or III | 4.5 | 44 (93.6) | 4 (12) |
| Current study | 104 | T2-4, any N | 4.9 | 97 (93.0) | 4 (10) |

demonstrate the equivalence of this procedure with axillary dissection in terms of axillary recurrence and overall survival. At the same time, it is unlikely that the value of sentinel node biopsy following NAC will be established[1]. The main reason for this is that only a small proportion of operable breast cancer patients currently receive NAC, making a randomized trial quite difficult. Another reason is that when the results from the ongoing randomized trials are disclosed, if they are favorable towards the SLN biopsy procedure, the majority of surgeons will extrapolate the applicability of these results to patients who have received NAC. Thus, it is quite possible that demonstrating the feasibility and efficacy of SLN biopsy after NAC will depend on the retrospective data of single-institution experiences.

NAC can reduce tumor size and significantly increase the ability to perform breast-conserving therapy[16-18]. After NAC, axillary downstaging is similarly affected. NAC with anthracycline/cyclophosphamide-containing regimens has been shown to neutralize the involved axillary nodes in about 30% of patients[16]. The addition of taxanes to anthracycline/cyclophosphamide-containing regimens has increased the conversion rate to around 40%[19, 20]. With the number of patients receiving NAC increasing, the question arises as to whether SLN biopsy is an option for these patients. We summarize the studies regarding SLN biopsy after NAC in Table 8, but they are inconclusive[6-14]. Breslin *et al.*[6] reported a study of 51 patients who underwent SLN biopsy after NAC and concluded that SLN biopsy following NAC is accurate. They had an identification rate of 84.3% and a false-negative rate of 12.0%. Nason *et al.*[13] reported a smaller number of patients who hard received NAC, and their identification and false-negative rates were 87.0% and 33.3%, respectively. They concluded that SLN biopsy resulted in an unacceptably high false-positive rate. However, in these small series, even 1 or 2 patients with false-negative SLNs can greatly affect the conclusions in a different direction. We report here a study of 104 patients who received NAC and had an identification rate of 93.4% and false-negative rate of 10.0%. We conclude in our study that SLN biopsy after NAC is accurate and feasible even for large tumors and patients with positive axillary nodal status before NAC without inflammatory breast cancer.

It has been speculated that among patients who have had their axillary lymph node status downstaged by NAC, tumors also typically respond to NAC and shrink so that damage to and alteration of the lymphatic flow from tumor tissues to the axillary basin are more likely to occur. This might then cause an increased false-negative rate for SLN biopsy and a decreased identification rate of SLN biopsy. However the hypothesis of the present study is that the lymphatic flow around skin lesions is rich and less influenced by the effects of chemotherapy and tumor size than that in the parenchyma surrounding the tumor. The lymphscintigraphy in this study results before and after NAC demonstrated that the effect of NAC did not at all change the lymphatic flow of the breast.

The results of our study suggest that SLN biopsy after NAC using intradermal injection of radiocolloid is feasible and can accurately predict axillary lymph node status for patients with clinically negative lymph node status following NAC. This procedure could help patients who have had their

axillary lymph node status downstaged from positive to negative and patients with large tumors qualify as appropriate candidates for SLN biopsy.

Further, multicenter studies, involving a larger number of patients from a variety of clinical locations, will be required to fully establish the feasibility and accuracy of SLN biopsy for patients with breast cancer who have been treated with NAC.

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# Case Report

## Brain Metastases after Achieving Local Pathological Complete Responses with Neoadjuvant Chemotherapy

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***Background*:** We encountered two patients with inflammatory breast carcinoma who developed symptomatic brain metastases after achieving local pathological complete responses (pCR) with neoadjuvant chemotherapy (NAC).

***Case presentations*:** The first patient is a 39-year-old woman (Case 1), who underwent NAC with AC (doxorubicin + cyclophosphamide) followed by weekly paclitaxel. After achieving a clinical CR (cCR), we conducted a modified radical mastectomy. Pathological evaluation confirmed no residual malignant cells within the breast tissue or lymph nodes. However, she developed neurological symptoms from brain metastases one month postoperatively. The second patient is a 44-year-old woman (Case 2). Again, no residual malignant cells were detected within the breast tissue or lymph nodes following NAC, but the patient developed symptomatic brain metastases eight months postoperatively. When primary breast tumors are locally advanced, it may be worthwhile to rule out brain metastases even if pCR is obtained after NAC.

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Key words: Brain metastasis, Pathological complete response, Breast cancer

### Introduction

Neoadjuvant chemotherapy (NAC) is a standard treatment option for patients with locally advanced and/or inflammatory breast cancers. The outcomes of patients achieving pCR of their primary tumors are significantly better than those with residual disease[1-3]. Here, we introduce two patients who developed symptomatic brain metastases shortly after documented pCRs following NAC and surgery.

### Case Report

#### *Case 1*

A 39-year-old premenopausal woman sought medical attention for erythematous induration of her left breast. With a working diagnosis of inflammatory breast cancer, fine needle aspiration cytology revealed adenocarcinoma. The patient was referred to the National Cancer Center Hospital for further treatment in February 2005. Physical examination revealed an indistinct 12 cm mass in the upper area of the left breast, and the surface of this lesion exhibited a peau d'orange appearance. Axillary and supraclavicular lymph nodes were palpable and measured 4 and 2 cm in diameter, respectively. The axillary lymph node was fixed to the surrounding tissue. Ultrasonography (US) revealed a 7 cm breast mass with dermal thickening, edematous subcutaneous tissue, and enlarged lymph nodes (Fig 1a). These findings were also observed on computed tomography (CT) and magnetic resonance imaging (MRI).

Core needle biopsy led to a pathological diagnosis of invasive ductal carcinoma (grade 3, nuclear grade 3, and HER-2 negative) (Fig 2a). The tumor was negative for both estrogen and progesterone receptors. Chest X-ray, bone scintigraphy, abdominal US, and chest and abdominal CT revealed no distant metastases. Due to the presumed low incidence of brain metastases at this clinical stage, brain imaging was not done at

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Abbreviations:
pCR, Pathological complete response; NAC, neoadjuvant chemotherapy; US, ultrasonography; CT, Computed tomography; MRI, Magnetic resonance imaging

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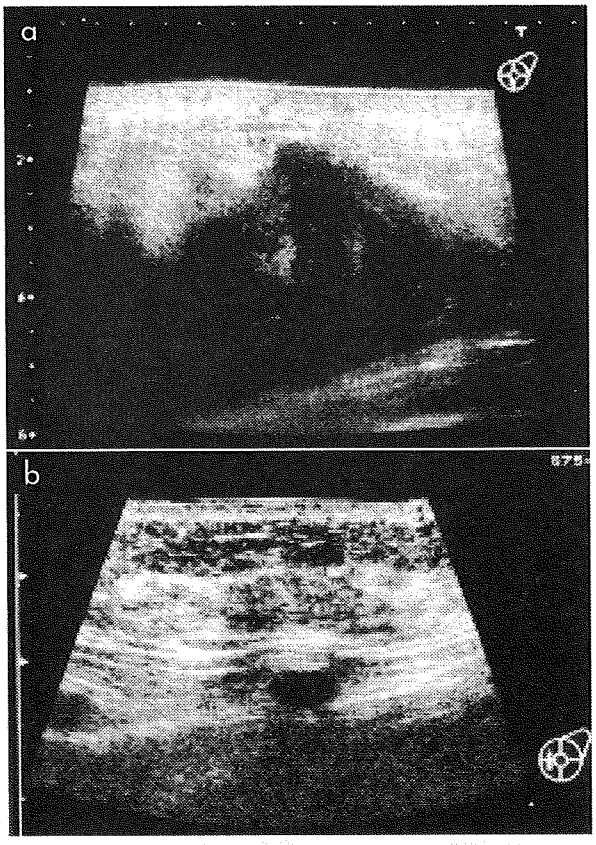


**Fig 1.** (a) US reveals a 7 cm breast mass with overlying skin thickening, edematous subcutaneous tissue. (b) US reveals no residual tumor following neoadjuvant chemotherapy.

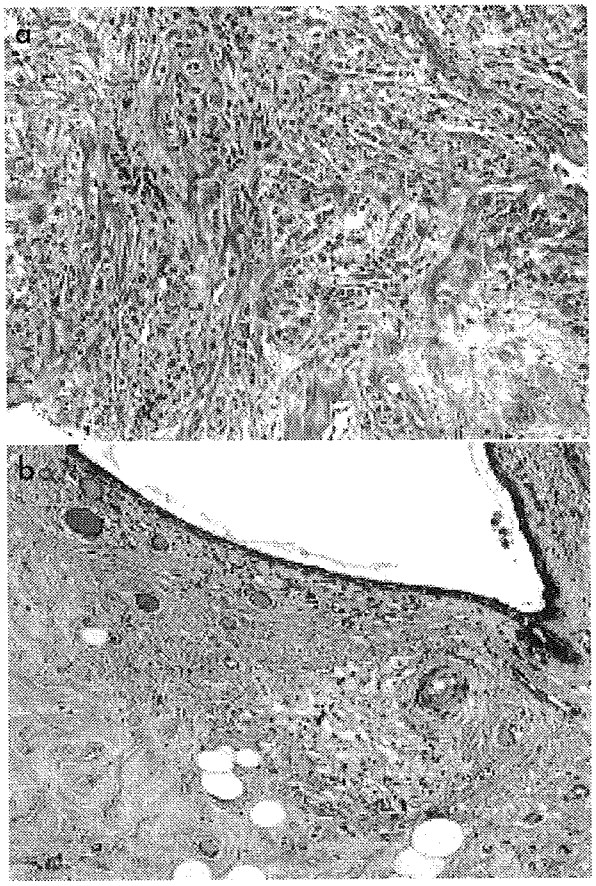


**Fig 2.** (a) Core needle biopsy reveals invasive ductal carcinoma, grade 3, nuclear grade 3. (b) No residual tumor is detected. The presence of inflammatory cells surrounding a duct with an increased number of enlarged capillary vessels, typical after tumor disappearance, is observed. (hematoxylin-eosin staining, ×100).

this point. Inflammatory breast cancer of the left breast was initially diagnosed, T4dN3M0, Stage IIIC, according to the general rules for clinical and pathological grading of breast cancers[4]. She received NAC from February to July consisting of doxorubicin and cyclophosphamide (60/600 mg/m$^2$) 4 times every 3 weeks, followed by paclitaxel (80 mg/m$^2$) weekly for 12 weeks. Following NAC, only induration of her left breast was apparent upon physical examination, and no breast masses or axillary lymph nodes were detected by US (Fig 1b) and CT. Additionally, serum levels of tumor markers (CEA, CA 15-3, ST 439) remained within normal limits before and after chemotherapy. We subsequently conducted a modified radical mastectomy in August, and no malignant cells were detected in the resected breast tissue and dissected axillary lymph nodes (Fig 2b). However, the patient presented with vertigo and severe headache prior to the initiation of radiotherapy to the left chest wall in September. Brain MRI revealed multiple metastatic lesions in her right frontal lobe, temporal lobe, and bilateral cerebellum (Fig 3). To control her symptoms, whole-brain radiotherapy with a total dose of 30 Gy/10 fractions was incorporated in October. However, her condition deteriorated, and she expired in December.

### *Case 2*

A 44-year-old premenopausal woman was seen at a nearby hospital with a chief complaint of an erythematous enlarged right breast. Inflammatory breast cancer was suspected, so she was referred to our institution in December 2004.

On initial examination, the right breast was firm, erythematous, and edematous with a thickened dermis. Axillary and supraclavicular lymph nodes were palpable and measured 5 cm and 1 cm

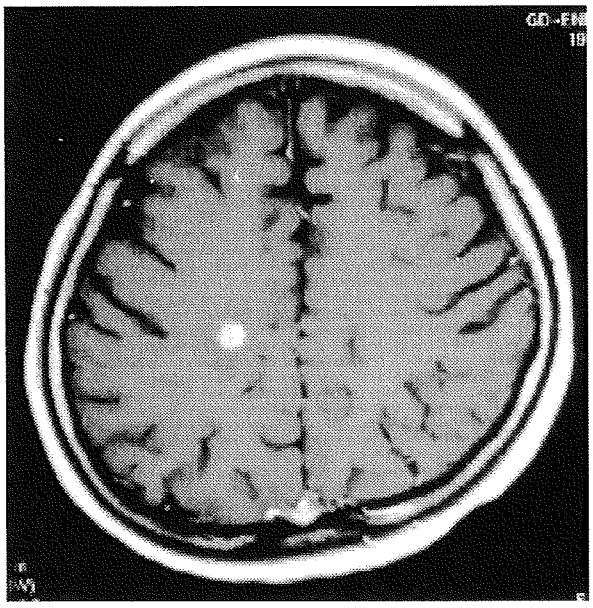

**Fig 3.** The metastatic lesions exhibited high signal intensity in the right temporal lobe by T1 weighted MRI.

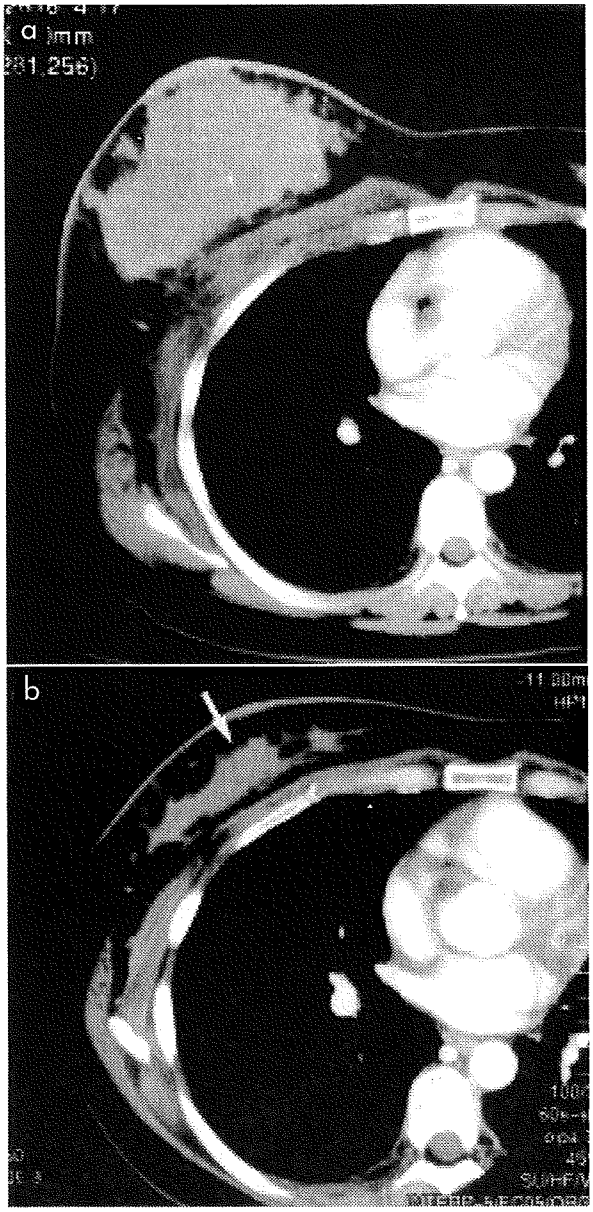


**Fig 4.** (a) CT shows a large right breast mass with overlying edematous subcutaneous tissue and thickened skin. This is not the early phase but late phase scan of breast CT, because only chest CT without an early phase scan was performed to detect distant metastasis instead of breast CT. (b) CT scan reveals a mass-like lesion measuring 3 cm, without enhancement, in the right breast.

in diameter, respectively. CT showed a large right breast mass with an edematous dermis and subcutaneous tissue.Additionally, the axillary and supraclavicular lymph nodes were enlarged (Fig 4a). The specimen obtained by the core needle biopsy was consistent with an invasive ductal carcinoma (solid tubular type, grade 3, nuclear grade 3, HER-2 negative, estrogen and progesterone receptor negative) (Fig 5a). No metastatic lesions were detected by bone scintigraphy, chest X-ray, chest CT, or abdominal US, though diagnostic brain imaging was not performed at that time. Serum tumor markers were elevated, with a CEA of 52.4 ng/ml, CA 15-3 of 279 U/ml, and NCC-ST 439 of 910 U/ml. Inflammatory breast cancer, T4dN3M0, Stage IIIC[4] was diagnosed. She underwent NAC from December to May 2005, using the same treatment regimen as Patient 1. Following NAC, physical examination revealed only induration of the right breast with slight thickening of the overlying skin. CT revealed a slightly enhanced, 3-cm lesion in the breast (Fig 4b) without enlarged lymph nodes. All tumor markers were within normal limits after chemotherapy. We performed a modified radical mastectomy in July, and no tumor cells were pathologically detected in the breast tissue and axillary lymph nodes (Fig 5b). Following surgery, we performed local radiotherapy with a total dose of 60 Gy/30 fractions from August through October. However, the patient developed headache and ambulatory disturbance in early December. Brain CT and MRI scans performed in March 2006 detected a tumor measuring 5 cm in diameter in her right temporal lobe with surrounding edema (Fig 6). A right frontotemporal craniotomy followed by whole-brain radiotherapy of 37.5 Gy/15 fractions was carried out from

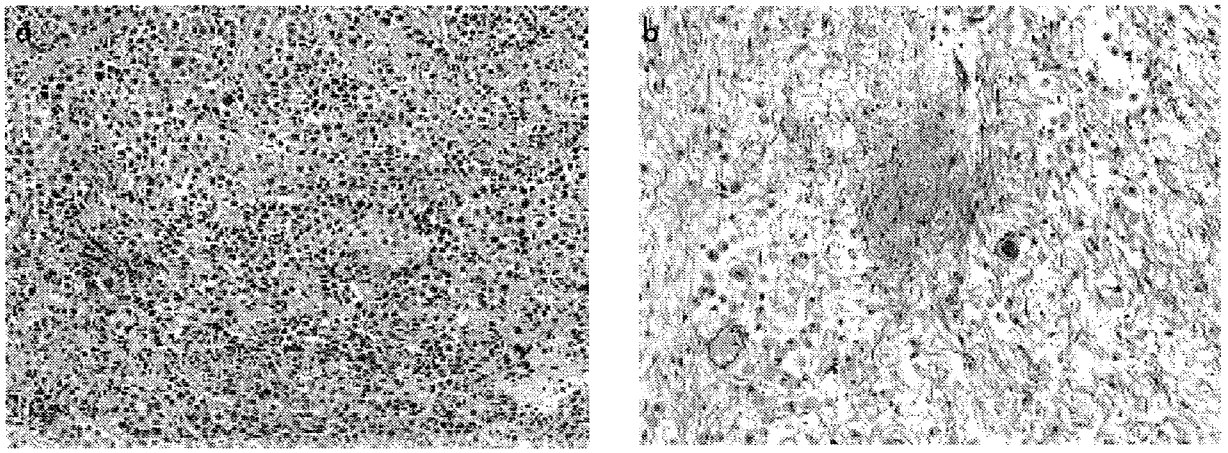

**Fig 5.** (a) Core needle biopsy reveals invasive ductal carcinoma, grade 3, nuclear grade 3. (b) No residual tumor is detected. Many foamy cells and a disturbance of the fiber rows after the disappearance of the tumor are observed (hematoxylin and eosin staining, ×100).

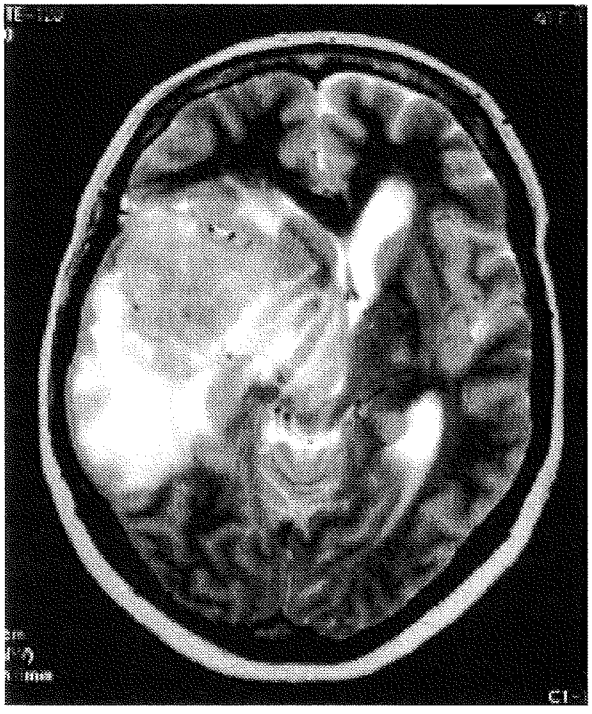

**Fig 6.** MRI demonstrates a tumor measuring 5 cm in diameter, with surrounding edema, in the right temporal lobe.

March through April. Intracranial recurrence is now controlled three months after radiotherapy.

## Discussion

Several studies have indicated that breast cancer patients with pCR following NAC have better overall survival and disease-free survival rates[1-3]. Moreover, pCR of axillary lymph nodes is an excellent prognostic factor for locally advanced breast cancers[5-8]. The two cases presented were first diagnosed with inflammatory breast cancer with axillary and supraclavicular lymph node metastases. The patients achieved pCR for both the main tumors and the axillary lymph nodes following NAC, and favorable prognoses were expected from the published literature. However, both patients developed symptomatic brain metastases soon after mastectomy. The interval between surgery and the occurrence of neurological signs was only one month for Patient 1 and five months for Patient 2. This led us to the theory that the blood brain barrier restricted access of the chemotherapeutic agents to the central nervous system. Therefore despite locally effective NAC, occult brain metastases may continue to progress into clinical significance. This theory may help us understand the progression of brain metastases in these patients[9]. There have been no reports examining the rates of brain metastasis following NAC. Yet there are reports of patients receiving adjuvant chemotherapy having an increased incidence of brain metastases as the site of first recurrence compared to control[10, 11]. In the present cases, we suspect that subclinical metastases were present in the brain before initiating NAC. It is likely that, because of inadequate delivery of cytotoxic agents to the brain, these metastases continued to grow despite effective tumor control elsewhere the body.

Several studies have identified risk factors for brain metastases in patients with breast cancer. Young age[12, 13], unresponsiveness to the hormonal

therapies, and HER-2 over expression are reported risk factors[14-17]. Intracranial metastases are also related to the use of trastuzumab[18]. In the two patients presented here, relatively young age and the absences of both estrogen and progesterone receptor were concordant risk factors for developing brain metastases.

The combination of NAC and surgery can lead to favorable outcomes in many cases of breast cancer, but effective control over the primary lesions and the extracranial micrometastases by the cytotoxic agents may not predict future intracranial event. The blood brain barrier would likely prevent chemotherapeutic agents from reaching the central nervous system. As a consequence, brain metastases may continue to grow and become symptomatic despite pCR of primary sites and lymph node metastases. This can be a concerning factor, especially in patients at risk for developing brain metastases. Further investigations are warranted to identify the mechanisms leading to intracranial metastases, as well as pretherapeutic risk factors.

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Original Article

# The prevalence of intrinsic subtypes and prognosis in breast cancer patients of different races

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**Abstract**

A recent report indicated that a high prevalence of basal-like breast tumors (estrogen receptor [ER]-negative, progesterone receptor [PR]-negative, human epidermal growth factor receptor [HER] 2-negative, and cytokeratin 5/6-positive and/or HER1-positive) could contribute to a poor prognosis in African American women with breast cancer. It has been reported that Japanese women with breast cancer have a significantly better survival rate than other races in the USA. These findings suggest that breast cancers in Japanese women have favorable biological characteristics. To clarify this hypothesis, we conducted a cohort study to investigate the prevalence of intrinsic subtypes and prognosis for each subtype in 793 Japanese patients. This study revealed a very low prevalence (only 8%) of basal-like breast tumors with aggressive biological characteristics in Japanese patients. Survival analysis showed a significantly poorer prognosis in patients with basal-like tumors than in those with luminal A tumors (ER- and/or PR-positive, and HER2-negative) with favorable biological characteristics. These findings support the hypothesis that breast cancers in Japanese women have more favorable biological characteristics and a better prognosis than those in other races. In conclusion, the prevalence of basal-like breast tumors could influence the prognosis of breast cancer patients of different races. The prevalence of intrinsic subtypes should be taken into account when analyzing survival data in a multi-racial/international clinical study.


*Keywords:* Breast cancer; Intrinsic subtype; Triple-negative tumor; Prevalence; Japanese; Prognosis

## Introduction

Although breast cancer survival has improved over the past 20 years in some developed countries,[1] significant differences in breast cancer stage, treatments, and mortality rates still exist in the world with regard to race and ethnicity.[2] The causes of survival difference are likely to be multifactorial including socio-economical factors, differences in access to insurance, screening and treatments, and biological differences among breast cancers themselves. These biological differences may reflect genetic influences and differences in lifestyle, nutrition or environmental exposure.

A number of studies have investigated the causative factors leading to racial disparity in breast cancer survival

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between African American (AA) and white American patients in the USA. Possible explanations include aggressive phenotypes of breast tumors,[3–5] such as high-grade and estrogen receptor (ER)-negative (ER−), patient characteristics,[6,7] such as obesity and a higher rate of co-morbidity, inadequate mammmographic screening,[8,9] delay of diagnosis leading to advanced stage,[10,11] and inadequate treatment,[12–14] such as not meeting treatment guidelines in AA women; however, these factors are unable to totally elucidate the disparity. Interestingly, a recent report indicated that a higher prevalence of basal-like breast tumors (ER−, progesterone receptor negative [PR−], human epidermal growth factor receptor 2-negative [HER2−], cytokeratin [CK] 5/6-positive, and/or HER1-positive [HER1+]), which have aggressive biological phenotypes and a poor outcome, and a lower prevalence of luminal A tumors (ER+ and/or PR+, and HER2−), which have an estrogen-responsive phenotype and a favorable outcome, could contribute to a poorer prognosis in young AA women with breast cancer.[15]

In contrast to AA patients, according to the Hawaii Tumor Registry of the Surveillance, Epidemiology, and End Results Program in the USA, Japanese patients with breast cancer have a significantly better survival rate than patients of other races after controlling for age, stage, and ER/PR status. There are no differences, however, in the survival rates of Chinese, Filipino, and Caucasian women.[16] These findings suggest that breast cancers in Japanese women have favorable biological characteristics, such as a lower prevalence of basal-like breast tumors. To clarify this hypothesis, we conducted a retrospective cohort study to investigate the prevalence of intrinsic subtypes of breast tumors and prognosis for each subtype in Japanese breast cancer patients.

## Patients and methods

### *Study patients*

The goal of the present study was to estimate the prevalence of breast cancer subtypes in Japanese breast cancer patients, and to examine correlations between clinico-pathologic variables and survival. Clinico-pathologic data of a cohort of consecutive Japanese patients with invasive breast cancer treated between January 2000 and December 2003 were collected from three different institutes, Kawasaki Medical School Hospital, Tohoku University Hospital, and Tohoku Kousai Hospital in Japan. The study procedures were approved by the institutional review board of each hospital.

Based on the histologic records, tumors were classified into two categories: invasive ductal carcinomas not otherwise specified (NOS) and others. The American Joint Committee on Cancer (AJCC, 5th edition) stage and lymph node status were collected from the medical records. Histologic grading was according to the modified Bloom and Richardson method by Elston and Ellis (Nottingham's grading system).[17] Lymph vessel invasion (LVI) was assessed using hematoxylin–eosin-stained glass slides. Vascular channels lined by thin endothelial cells, especially close to the small arteries and veins, were considered as lymph vessels, and tumor emboli were floating in the lumen in LVI-positive cases. Most LVI were seen at the periphery of the invasive tumors.[18] Blood vessel invasion (BVI) was evaluated using elastica Masson stain or immunostaining for CD34. Tumor cell nests surrounded by elastic fibers and the wall of smooth muscle, next to the small arteries (but not mammary ducts with multilayered elastic fibers) were considered as positive.[18]

### *Immunohistochemical (IHC) subtypes*

ER and PR status were determined by IHC performed at each institute. The cutoffs for receptor positivity were 10%. The HER2 status was also determined by IHC at each institute. According to the criteria of the HecepTest, scores 0 and 1 were considered negative, and scores 2 and 3 were considered positive.[19] Triple-negative (ER−, PR−, and HER2−) breast cancer samples were examined by IHC for CK 5/6 and HER1. CK 5/6 and HER1 were considered positive when more than 10% of the tumor cells were labeled. First antibodies and IHC procedures are presented in Table 1.

According to Carey et al.,[15] IHC intrinsic subtypes were defined as follows: luminal A (ER+ and/or PR+, HER2−), luminal B (ER+ and/or PR+, HER2+), basal-like (ER−, PR−, HER2−, CK 5/6-positive, and/or HER1+), HER2+/ER−, and unclassified (negative for all five markers).

### *Statistical analysis*

Differences between breast cancer subtypes with regard to clinico-pathologic characteristics were examined using analysis of variance, $\chi^2$ tests or Fisher's exact test. Survival curves were generated using the Kaplan–Meier method, and the log-rank test was used to compare mean survival across IHC subtypes. StatView statistical software was used to manage and analyze data. Statistical differences were considered significant at $P \leqslant 0.05$.

## Results

### *IHC subtypes and characteristics of patients*

Clinico-pathologic data on 793 Japanese patients with invasive breast cancer were collected from three hospitals in Japan. The characteristics of the patients with IHC data, overall and according to IHC subtypes, are presented in Table 2. IHC subtypes differed significantly by age ($P = 0.025$), AJCC stage ($P < 0.001$), histologic grade ($P < 0.001$), LVI ($P = 0.018$), and BVI ($P = 0.026$). Patients with the basal-like subtype were younger than patients with the HER2+/ER− subtype. Patients with basal-like tumors were more likely to be in the more advanced stage, and to have tumors with a higher histologic grade or BVI than patients with luminal A tumors.

Table 1
Source, dilution, pretreatment and cutoff values of antibodies used

| Antibody, clone | Dilution | Source | Pretreatment | Cutoff values |
|---|---|---|---|---|
| ER [1D5] | 1:400 | IMMUNOTECH | Autoclaved | ⩾10% (positive) |
| PR [636] | 1:2000 | DAKO | Autoclaved | ⩾10% (positive) |
| HER2 [HercepTest] | NA* | DAKO | None | NA |
| HER1 [2-18C9] | NA | DAKO | Proteinase K | ⩾10% (positive) |
| CK 5/6 [D5/16134] | 1:100 | DAKO | Autoclaved | ⩾10% (positive) |

*Not assessable.

Table 2
Prevalence of intrinsic subtypes and clinico-pathological characteristics in Japanese breast cancer patients

| | All cases | Luminal A | Luminal B | HER2+/ER− | Basal-like | Unclassified | *P* value* |
|---|---|---|---|---|---|---|---|
| No. of cases | 793 | 502 (63)† | 155 (20) | 55 (7) | 67 (8) | 14 (2) | |
| Age, median (range), years-old | 54 (19–88) | 53 (27–88) | 53 (19–85) | 60 (31–84) | 54 (30–79) | 50 (36–66) | 0.025 |
| AJCC stage | | | | | | | <0.001 |
| I | 289 | 213 | 48 | 4 | 18 | 6 | |
| II | 360 | 208 | 70 | 39 | 38 | 5 | |
| III | 68 | 36 | 17 | 4 | 8 | 3 | |
| IV | 40 | 19 | 15 | 4 | 2 | 0 | |
| Missing | 36 | 26 | 5 | 4 | 1 | 0 | |
| Histology | | | | | | | 0.142 |
| Invasive ductal carcinoma NOS | 721 | 447 | 149 | 53 | 60 | 12 | |
| Specific types | 70 | 54 | 5 | 2 | 7 | 2 | |
| Missing | 2 | 1 | 1 | 0 | 0 | 0 | |
| Histologic grade | | | | | | | <0.001 |
| I | 156 | 131 | 23 | 0 | 1 | 1 | |
| II | 320 | 235 | 56 | 15 | 11 | 3 | |
| III | 197 | 61 | 48 | 33 | 49 | 6 | |
| Missing | 120 | 75 | 28 | 7 | 6 | 4 | |
| LVI | | | | | | | 0.018 |
| Positive | 345 | 212 | 69 | 32 | 27 | 5 | |
| Negative | 373 | 249 | 62 | 20 | 36 | 6 | |
| Missing | 75 | 41 | 24 | 3 | 4 | 3 | |
| BVI | | | | | | | 0.026 |
| Positive | 126 | 82 | 18 | 10 | 14 | 2 | |
| Negative | 570 | 267 | 105 | 40 | 49 | 9 | |
| Missing | 97 | 53 | 32 | 5 | 4 | 3 | |
| Nodal status | | | | | | | 0.572 |
| Positive | 303 | 184 | 62 | 25 | 27 | 5 | |
| Negative | 437 | 286 | 78 | 25 | 29 | 9 | |
| Not applicable or missing | 53 | 32 | 15 | 5 | 1 | 0 | |
| Outcome | | | | | | | |
| Follow-up, median (range), months | 46.5 (1–84) | | | | | | |
| 5-year DFS | 85.5% | 90.3% | 82.9% | 62.1% | 77.1% | 81.8% | <0.001‡ |
| 5-year OS | 92.8% | 96.9% | 86.6% | 86.9% | 86.2% | 83.3% | <0.001‡ |

*Comparing five subtypes using $\chi^2$ test or Fisher's exact test.
†In %.
‡Log-rank test.

*Survival by IHC subtypes*

Survival data on 786 of 793 patients with invasive breast cancer were available from three hospitals. The duration of follow-up was 1–84 months (median, 46.5). During this period, recurrence was observed in 91 patients, and 48 patients died of any causes.

Breast cancer subtypes significantly differed in 5-year disease-free survival (DFS, $P<0.001$): luminal A (90.3%), luminal B (82.9%), HER2+/ER− (62.1%), basal-like

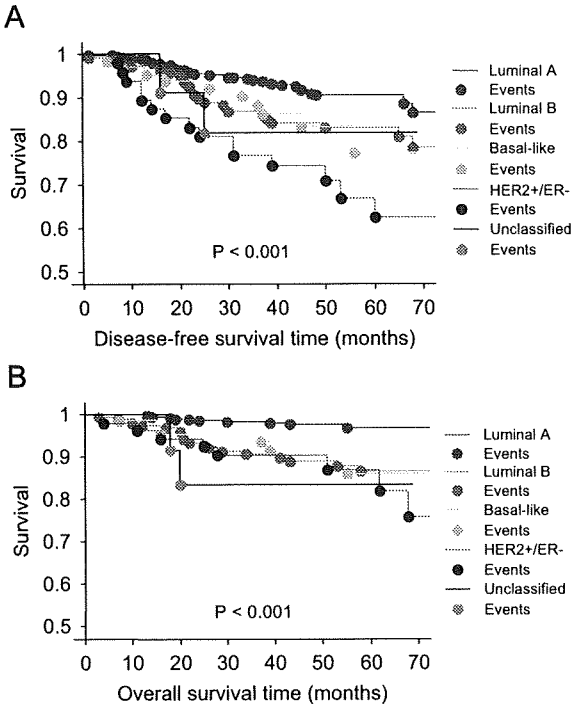


Fig. 1. DFS (A) and OS (B) curves in breast cancer patient groups divided by IHC intrinsic subtypes.

subtype (77.1%), and unclassified (81.8%). They also differed in 5-year overall survival (OS, $P<0.001$): luminal A (96.9%), luminal B (86.6%), HER2+/ER− (86.9%), basal-like subtype (86.2%), and unclassified (83.3%). Kaplan–Meier survival curves are presented in Fig. 1. Both DFS and OS were significantly worse among basal-like and HER2+/ER− breast cancer patients compared with luminal A patients.

Differences in DFS and OS by IHC subtypes were seen among lymph node-positive patients ($P = 0.006$ for DFS and $P<0.001$ for OS) but not lymph node-negative patients; however, the number of patients after stratifying by lymph node status was limited and these data should be interpreted with caution. Five-year DFS within lymph node-positive patients by subtype was as follows: luminal A (79.3%), luminal B (71.2%), HER2+/ER− (35.2%), basal-like subtype (68.1%), and unclassified (50.0%). Five-year OS within lymph node-positive patients was as follows: luminal A (96.3%), luminal B (75.6%), HER2+/ER− (84.1%), basal-like subtype (83.9%), and unclassified (60.0%).

## Discussion

Carey et al. have recently reported for the first time the population-based prevalence of intrinsic subtypes of breast tumors. They refined an IHC-based assay to identify breast tumor intrinsic subtypes instead of gene expression profiling.[15] This IHC-based assay has been verified against gene expression profiles to estimate the prevalence of intrinsic subtypes.[15,20] Additionally, large-scale subtyping using gene expression profiling from formalin-fixed, paraffin-embedded samples is not currently feasible; therefore, we conducted this cohort study to investigate the prevalence of intrinsic subtypes using the IHC-based assay in Japanese breast cancer patients.

According to Carey et al.,[15] the prevalence of basal-like and luminal A tumors in the Carolina Breast Cancer Study was 27% and 47% in AA patients and 16% and 54% in non-AA patients, respectively. Since breast cancer-specific survival was significantly worse in patients with basal-like tumors than with luminal A tumors, the higher prevalence of a basal-like subtype could contribute to a worse prognosis in AA patients. Moreover, the prevalence of basal-like and luminal A tumors was 39% and 36% in premenopausal AA patients, respectively. In contrast, the prevalence of basal-like and luminal A tumors was 8% and 63% in Japanese breast cancer patients, respectively, in the present study. The prevalence of basal-like tumors was 2–3 times lower in Japanese patients than in non-AA patients or AA patients. In addition, the prevalence of luminal A tumors was 9–16% higher in Japanese patients than in non-AA patients or AA patients. Breast cancer patients with basal-like tumors had a poorer prognosis in terms of DFS and OS than those with luminal A tumors in the present study (Fig. 1) as previously indicated in the report by Carey et al.[15] These findings have suggested that the lower prevalence of basal-like tumors and higher prevalence of luminal A tumors in Japanese patients could contribute to their better prognosis.

A limited number of studies have investigated the prevalence of intrinsic subtypes by the IHC-based assay in different races. On the other hand, the prevalence of triple-negative breast tumors has recently become available. Triple-negative tumors include both basal-like and unclassified tumors. The prevalence of basal-like tumors was reported to be approximately 70% in triple-negative tumors[15]; it was 78% in the present study. The prevalence of triple-negative tumors was 22% in the Carolina Breast Cancer Study,[15] 16% in a large series of patients in the UK,[21] 26% in conservatively managed patients in the USA,[22] and 31% in consecutive patients in Korea.[23] In the present study, the prevalence of triple-negative tumors was only 10%, 1.6–3 times lower in Japanese patients than in patients of other races. These findings also support the lower prevalence of basal-like tumors in Japanese patients.

Differences in genetic influences or lifestyle may explain the prevalence of intrinsic subtypes among different races. Differences in the distribution of breast cancer risk factors, such as breast cancer family history, age at menarch, age at first birth, body mass index, and hormone replacement therapy, have been extensively investigated, and these differences may explain differences in breast caner incidence rates among different races.[5] However, the investigation of causative factors leading to differences in the prevalence of intrinsic subtypes in different races remains

to be investigated. Because of a close correlation between the prevalence of intrinsic subtypes and the prognosis of breast cancer patients indicated by us and others,[15,20] nutritional or environmental factors influencing the prevalence may provide hints for developing new intervention strategies to reduce breast cancer mortality rates. It has been indicated that the intake of green tea or soy beans relates to a reduction in breast cancer incidence rates.[24,25] Furthermore, the consumption of green tea was suggested to correlate with not only a reduction in breast cancer incidence but also improved outcome of breast cancer patients in Japanese women.[26] In addition, it is suggested that breast cancer patients with a high intake of green tea tend to have less aggressive and hormone-responsive breast tumors.[27] Interestingly, recent experimental studies have revealed that green tea extracts such as (−)-epigallocatechin gallate have significant anti-tumor activity in breast cancer cells with basal-like phenotypes.[28–30] These findings suggest that green tea intake may modify the biological characteristics of breast tumors and the prevalence of intrinsic subtypes. Further epidemiologic and experimental studies are warranted to investigate the role of green tea intake in breast cancer development and progression.

In conclusion, the present study suggests for the first time that a lower prevalence of basal-like breast tumors and a higher prevalence of luminal A breast tumors could contribute to a favorable prognosis of Japanese breast cancer patients. Taken together with the worse prognosis of AA patients having a higher prevalence of basal-like tumors and a lower prevalence of luminal A tumors, it could be concluded that the prevalence of intrinsic subtypes differs among different races and such a difference may explain differences in the prognosis of breast cancer patients of different races. From the clinical point of view, the prevalence of intrinsic subtypes should be taken into account when analyzing survival data in a multi-racial/international clinical study. In addition, causative factors influencing the prevalence of intrinsic subtypes should be explored to develop intervention strategies to reduce breast cancer incidence and the mortality rate.

## Conflict of Interest Statement

None declared.

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ORIGINAL ARTICLE

# Favorable outcome in patients with breast cancer in the presence of pathological response after neoadjuvant endocrine therapy ☆

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**Summary** Neoadjuvant endocrine therapy (NAET) can expand the number of breast cancer patients who can be treated with breast-conserving surgery and can predict benefit from adjuvant endocrine therapy. Because no validated surrogate markers for long-term outcome have been established, we conducted prospective trials to evaluate pathological response and Ki-67 index following treatment with tamoxifen or anastrozole. The study population included postmenopausal women with operable breast tumors that were both estrogen and progesterone receptor-positive and larger than 3 cm. Response was classified as pathological response (minimal response or better) and non-response. Non-responding (25.5%, vs. response 85.9%, $p = 0.002$), axillary node-positive (58.4% vs. node negative 100%, $p = 0.045$), and high pretreatment Ki-67 index (41.4% vs. low Ki-67 87.1%, $p = 0.03$) patients were significantly associated with poor 5-year relapse-free survival. Multivariate analysis of relapse-free survival indicated that pathological response was independent. Therefore, pathological response may be a favorable prognostic factor after NAET.


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## Introduction

With the recent development of aromatase inhibitors, neoadjuvant endocrine therapy (NAET) has attracted attention as a potentially effective therapy that might allow breast conservation even in women with large breast tumors[1–4]. In addition, NAET offers the possibility of testing therapeutic efficacy *in vivo*, which is of great importance for optimal adjuvant treatment. However, the short history of NAET leaves several questions to be answered. First, short-term surrogate markers of subsequent risk of relapse and death from breast cancer have not been established for NAET[5]. Recently, early changes in Ki-67 have been reported to be possible predictors of long-term outcome[6–8]. The short-term reduction in Ki-67 levels in NAET (in the IMPACT trial) paralleled that observed in patients who received the same endocrine therapy in the adjuvant setting (ATAC); this suggested that the changes in Ki-67 in NAET might be predictive of long-term outcome[7]. However, these data were not obtained in direct long-term follow-up studies of NAET. Second, classifications of pathological therapeutic response, which have been mainly produced based on pathological changes following chemotherapy or radiotherapy, have not been validated for tumors treated by NAET. We conducted a small study to clarify the significance of the classification of pathological therapeutic response and the Ki-67 index as prognostic factors of long-term outcome in response to NAET.

## Patients and methods

This analysis includes 45 postmenopausal women with operable estrogen and progesterone receptor (ER and PgR)-positive breast tumors that were larger than 3 cm as confirmed by core needle biopsy. These women were enrolled in two-phase II studies on NAET at the National Cancer Center Hospital (NCCH), Tokyo. Between February 1999 and July 2002, 31 patients were enrolled in a neoadjuvant tamoxifen study (neo TAM), in which they received tamoxifen for 4 months preoperatively. Between November 2002 and 2004, 17 patients were enrolled in a neoadjuvant anastrozole study (neo ANZ), in which they received anastrozole for 5 months preoperatively. Three patients in the neo TAM group were excluded from this analysis because they received preoperative chemotherapy following NAET and their tumors could not be evaluated for pathological response to endocrine therapy; two of these patients rejected mastectomy when there was no reduction of their tumors by NAET. These patients received chemotherapy with the hope that their tumors might shrink enough to allow breast-conserving surgery. Unfortunately, their tumors remained widespread in a mosaic pattern and they finally agreed to mastectomies. The third patient showed progressive disease, which led to skin invasion, and received chemotherapy before surgery. All patients provided written informed consent for study participation as approved by the institutional review board of the NCCH. Patients who responded to NAET continued the same endocrine therapy postoperatively for 5 years. Patients who showed clinically progressive disease or stable disease and pathological lymph node involvement after NAET received adjuvant chemotherapy, if tolerable, with a regimen containing anthracycline or classical CMF (cyclophosphamide, methotrexate, and 5-fluorouracil) following surgery. All patients who underwent breast-conserving surgery received postoperative radiotherapy to the ipsilateral breast.

### Tumor response

Primary tumors were clinically assessed every month. Clinical complete response (cCR) was defined as the clinical disappearance of the tumor at the end of NAET, and clinical partial response (cPR) was defined as a $\geqslant$70% decrease from baseline of the largest diameter[9]. Clinical progressive disease was defined as a $\geqslant$20% increase from the most reduced size of the largest diameter. If progressive disease was observed, patients immediately underwent radical mastectomy.

### Outcome measures

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

### Histological examination

Evaluation of ER and PgR status was by immunohistochemical studies using antibodies 1D5 and PgR636 (DAKO, Glostrup, Denmark), and tumors with more than 10% strongly stained nuclei were described as ER- or PgR-positive. Tumors obtained by core needle biopsy judged as positive for both receptors before treatment were eligible for this study. HER2 status was evaluated immunohistochemically using HercepTest (Dako), and 3+: strong complete membrane staining in $>$10% of tumor cells was defined as positive.

Ki-67 was stained using the MIB-1 antibody (DAKO) according to previously described methodology[10]. Ki-67 was scored as the percentage of positively stained cells among 1000 malignant cells in specimens obtained by either core needle biopsy before treatment (baseline) or by surgery after NAET. The cut-off value for Ki-67 positivity was defined as the median value of the Ki-67 index in this study population. The proportional change in Ki-67 expression from baseline was calculated as (residual Ki-67 index—pretreatment Ki-67 index) × 1/pretreatment Ki-67 index[7].

Histopathological therapeutic response was classified according to the General Rules for the Clinical and Pathological Recording of Breast Cancer 2005[11]. For Grade 0, no response was observed; Grade 1a comprised those tumors with mild changes in cancer cells regardless of the area, or marked changes seen in less than one-third of cancer cells; Grade 1b comprised tumors with marked changes seen in more than one-third but less than two-thirds of tumor cells; Grade 2 tumors contained marked changes in more than two-thirds of tumor cells; and Grade 3 tumors demonstrated a complete response, with no cancerous cells remaining. Mild changes include slight degenerative changes in cancer cells not suggestive of cancer cell death (including cancer cells with vacuolation of the cytoplasm, eosinophilic cytoplasm, swelling of the nucleus, etc). Marked changes include marked degenerative changes in cancer cells suggestive of cancer cell death (including liquefaction, necrosis, and disappearance of cancer cells). The pathological response group was defined as tumors with Grade1a, 1b, and 2 responses. The non-response group was defined as tumors with Grade 0 response.

## Statistical analysis

The $\chi^2$ test was used for comparisons of tumor characteristics and responses among groups. The Kaplan–Meier methods were used to generate RFS curves. The log rank test was used for the comparison of RFS between two groups. Differences with $p<0.05$ were considered to be significant.

**Table 1** Characteristics of patients and tumors treated with tamoxifen (neo TAM group) and anastrozole (neo ANZ group).

| | Neo TAM group ($n = 28$) | Neo ANZ group ($n = 17$) | |
|---|---|---|---|
| Age | 60 (51–75) | 61 (54–87) | |
| Tumor before NAET | | | |
| T2 | 18 | 11 | |
| T3 | 7 | 4 | NS |
| T4 | 3 | 2 | |
| Clinical response | | | |
| CR | 1 | 3 | |
| PR | 12 | 10 | |
| NC | 15 | 4 | $p = 0.05$ |
| PD | 0 | 0 | |
| Surgery | | | |
| Mastectomy | 17 | 13 | |
| BCS | 11 | 4 | NS |
| Pathological response | | | |
| Grade 2 | 3 | 3 | |
| Grade 1b | 4 | 2 | |
| Grade 1a | 11 | 11 | $p = 0.02$ |
| Grade 0 | 10 | 1 | |
| Axillary nodal status | | | |
| Negative | 7 | 6 | |
| 1–3 | 12 | 7 | NS |
| 4–9 | 7 | 3 | |
| >10 | 2 | 1 | |

NAET: neoadjuvant endocrine treatment; CR: complete response; PR: partial response; NC: no change; PD: progressive disease; NS: not significant; BCS: breast-conserving surgery.