

歩に加えて、抗がん剤においても経口フッ化ピリミジンの普及など比較的高齢者においても投与しやすい副作用が少なく効果のある薬剤が増加している。

今回われわれは、国立がんセンター中央病院(NCCH)においてこれまで経験した80歳以上の超高齢者乳癌の治療をretrospectiveに検討して、これらの症例に対する今後の治療方針決定に参考となるよう比較検討した。

### 1. 対象と方法

1996年6月から2006年5月までにNCCHにおいて手術を伴う治療を行った初診時80歳以上の原発性乳癌117症例を対象としてretrospectiveな検討を行った。これらの症例において、手術より得られた検体の病理組織学的検査結果を検討し高齢者乳癌の臨床的特徴を明らかにするとともに、臨床病理学的予後因子を検討した。さらにこれらの症例に対して行われた手術方法、術後ホルモン療法について予後との相関を検討した。治療方針は今回検討した症例の治療期間が40年と長く、また院内でも確固たる治療方針が決定していない症例群のため、それぞれの担当医の独自の判断で行われていた。エストロゲンレセプター(ER)はEIA法において10fmol/mgP以上またはIHC法において10%以上陽性の場合を陽性と判定した。また、生存率はKaplan-Meier法を使用してLog-rank testにより検定した。

### 2. 結果

今回検討した初診時80歳以上の原発性乳癌117例の臨床病理学的特徴を表1に示した。年齢中央値は82歳(範囲80~94歳)。80例(68%)の症例が既往歴を持ちうち19例(16%)が他がんの既往を持っていた。主訴はしこりを触知したものが103例(93%)と多く、検診発見や乳頭の症状などは少なかった。初診時の臨床病期はcStage I, II, III, IVがそれぞれ40例(34%), 68例(58%), 6例(5%), 3例(3%)であった。cStage IVの3例について転移部位はいずれも骨転移であり、初期治療として原発巣の切除が3例とも行われていた。手術検体における病理組織学的検査結果も表1に

表1 患者背景 (n=117)

Parameter	No. of patients (%)
年齢中央値 (範囲)	82 (80~94)
既往歴	
あり	80 (68)
なし	37 (32)
他がんの既往	
あり	19 (16)
主訴	
しこり	109 (93)
検診発見	6 (5)
乳頭分泌	1 (1)
乳頭びらん	1 (1)
臨床病期	
cStage I	40 (34)
cStage II	68 (58)
cStage III	6 (5)
cStage IV	3 (3)
病理腫瘍径cm (範囲)	2 (0.2~5)
病理組織型	
浸潤性乳管癌	71 (61)
浸潤性小葉癌	5 (4)
非浸潤性乳管癌	10 (9)
粘液癌	16 (14)
リンパ節転移個数	
0	61 (52)
1 < n < 3	16 (14)
4 < n < 9	4 (3)
n > 10	8 (7)
不明	28 (24)
ER陽性	67 (57)
PgR陽性	45 (38)
手術	
Bt+Ax	71 (60)
Bp+Ax	5 (4)
Bt+SLNB	10 (9)
Bp+SLNB	1 (1)
Bt	4 (3)
Bp	24 (20)
Biopsy	1 (1)
術後治療	
Hormone therapy	42 (63)
Chemotherapy	4 (3)

表2 センチネルリンパ節生検 (n=11)

結果	
方法	全例色素法
リンパ節個数 (範囲)	平均 2.2個 (1~4)
迅速病理結果	全例陰性
同定率	100%
再発・転移症例	0

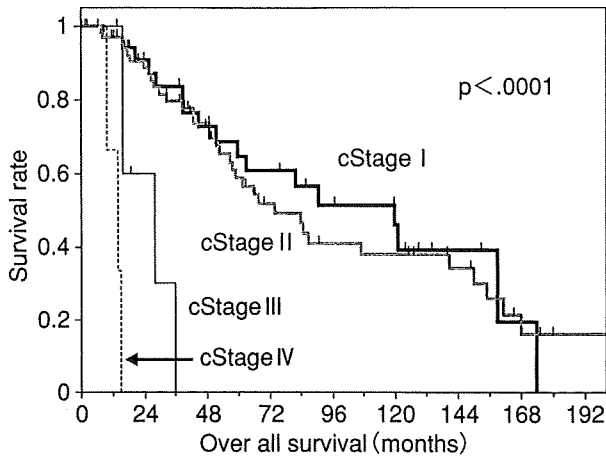


図1 cStage分類による全生存率の比較

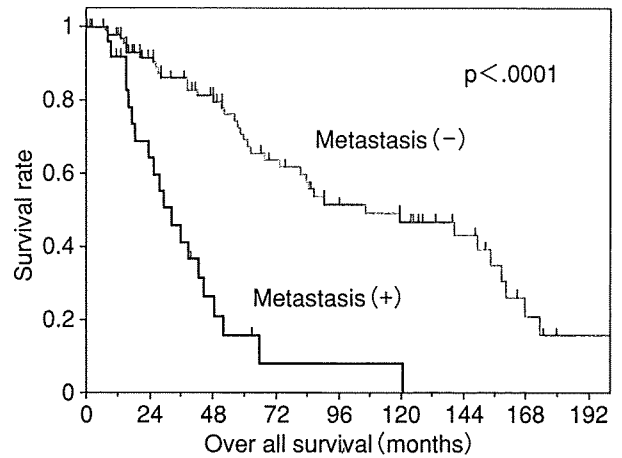


図2 遠隔転移・再発の有無による全生存率の比較  
Metastasis : distant and/or local metastasis

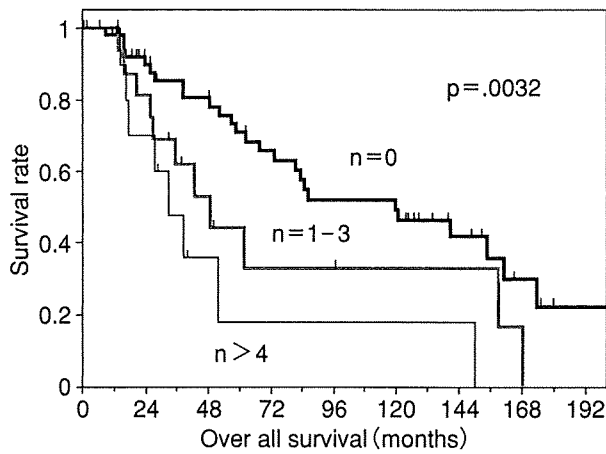


図3 腋窩リンパ節転移個数による全生存率の比較 (n=89)

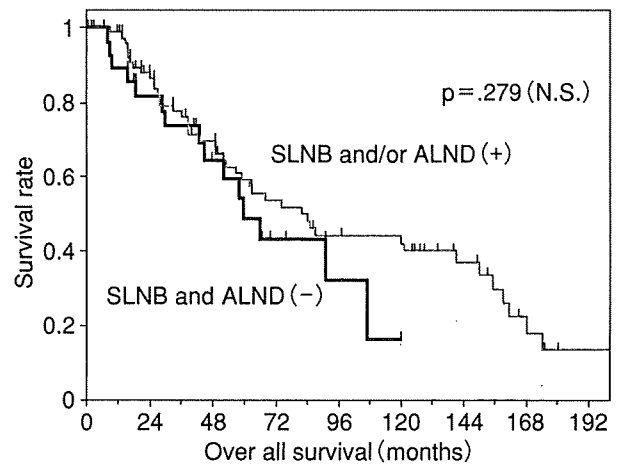


図4 腋窩手術の有無による全生存率の比較  
SLNB : sentinel lymph node biopsy, ALND : axillary lymph node dissection

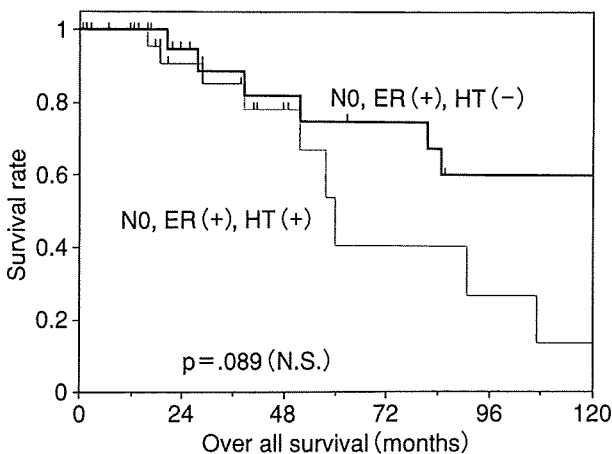


図5 N0症例における術後ホルモン療法の有無による全生存率の比較 (n=54)

N0 : clinical lymph node negative, ER (+) : estrogen receptor positive, HT : adjuvant hormone therapy.

示した。腫瘍径は中央値 2 cm (範囲0.2~5 cm) で病理組織型は浸潤性乳管癌71例(61%), 浸潤性小葉癌5例(4%), 非浸潤性乳管癌10例(9%) に対して粘液癌が16例(14%)とやや多かった。リンパ節転移個数は1~3個陽性16例(14%), 4~9個陽性4例(3%), 10個以上8例(7%)で転移なしが61例(52%)であった。28例(24%)は腋窩手術を行っておらず不明であった。免疫病理検査結果ではER陽性67例(57%), PgR陽性45例(38%)だった。手術は郭清を伴う全乳房切除71例(60%), 郭清を伴う乳房温存療法は5例(4%), 原発巣のみの切除は部分切除のみ24例(20%), 全乳房切除のみ4例(3%)でセンチネルリンパ節生検(SLNB)を伴う手術は11例(10%)に行われた。術後のホルモン療法はER陽性だった67例中42

例に行われ、術後補助化学療法としてCMF療法が3例に行われていた。また、当院フォローアップ中に再発をきたした症例は初診時遠隔転移を認めた3例も含めて26例(22%)であった。うち2例がanthracyclineを含む抗がん剤治療を施行されていたが、他はホルモン療法また放射線療法のみであった。抗がん剤を投与された症例はいずれも全身状態が良好な症例で重篤な副作用はなかった。SLNBを伴う手術が行われた11例の生検結果を表2に示した。1%パテントブルーまたは1%メチレンブルー約3mlを使用した色素法で行われていた。摘出されたセンチネルリンパ節(SLN)の個数は平均2.2個で迅速病理検査の結果は全例陰性であった。SLNの同定率は100%で、これらの症例に今のところ腋窩再発を含む再発症例は認められていない。

続いて予後における検討を示す。全症例の全生存期間(OS)の中央値は70カ月であった(data not shown)。OSにおいて臨床病期は有意な予後因子であった( $p < .0001$ )。とくにcStage III以上の症例で予後が悪かった(図1)。また、再発の有無とリンパ節転移個数はOSにおける非常に有意な予後因子であった( $p < .0001$ ,  $p = .0032$ ) (図2, 3)。

手術方法とくに腋窩操作(郭清またはSLNB)の有無によってOSに差はなかった(図4)。最後に術後補助ホルモン療法の有無のOSにおける検討を図5に示した。今回の対象症例においては、前述の通り治療方針は担当医の判断に任されておりとくに追加治療の有無にはばらつきがあるため、ER陽性の症例のうち臨床的にリンパ節転移の認められなかったN0症例(54例)に絞って術後補助ホルモン療法を行った24例(44%)と行わなかった30例(51%)についてOSとの相関を検討した。結果、補助ホルモン療法の有無はOSにおいて有意な因子ではなかった。

### 3. 考 察

80歳を越える超高齢者の乳癌の臨床病理学的特徴は今回の検討においては、しこりを主訴とし組織学的には粘液癌の割合が比較的多いというものであった。文献的にも粘液癌やアポクリン癌の割

が多いとされ、またホルモンレセプター陽性率が高いとされている<sup>1,2)</sup>。アポクリン癌に関しては今回の症例では認められなかったが、ホルモンレセプターは58%が陽性であった。さらに腫瘍径は中央値2cmと比較的小さめで限局しているものが多い傾向にあった。

手術に関しては、多くの患者が治療中の既往歴を有していたが手術・麻酔による肺梗塞等致命的な合併症を起こした症例はなかった。術式をみると、まず乳房において術後放射線治療を行うことの煩雑さや局所再発を避ける患者・家族の希望から近年まで全乳房切除を行うことが多かった。しかし、乳房温存術が安全に行われる症例が増加したことより超高齢者に対しても徐々に温存を行うことが増えてきた。これには、70歳以上のホルモン陽性患者に対して温存療法後の残存乳房への放射線治療は不要といった報告<sup>3)</sup>も影響しており、今回の症例においても温存療法を行った患者のうち術後に放射線療法を行ったものは10例のみで他の症例は行っていない。温存療法後の局所のみ再発症例は今回の症例中1例のみであった。腋窩郭清においては、初期では標準的郭清を行っていた症例もあったが、近年センチネルリンパ節生検を行う症例が少しずつ増加してきた。高齢者ではセンチネルリンパ節の同定率が低下するため注意が必要であるが、今回われわれが行った11症例では同定できなかった症例はなかった。腋窩リンパ節郭清の意義は現在予後因子として補助療法を決定するリンパ節転移個数を確認することとされている。今回の検討においても腋窩郭清の有無は予後には影響していなかった。そのことから、術後に補助療法を何らかの理由で行わない症例においては郭清の意義が損なわれることになり腋窩郭清は必要がないと考えられる。

当院におけるこれまでの超高齢者に対する治療方針は年代ごとにやや異なりそのつど詳細に決められているものではないが、術後は他の年代の患者とは異なってリスクにあわせた術後補助化学療法は行われずホルモン療法剤が担当医の判断で追加されるかまたは何もせずに経過のみ観察するというものであった。そのために、手術によりリンパ節転移が陽性であった症例ではホルモン療法剤

が投与されている症例が多く、これらの症例でホルモン療法剤がOSに寄与しているかどうかは検討できなかった。しかし、逆にN0症例に対しては必ずしも投与されておらず、今回検討を行ったところホルモン療法剤の有無はOS延長に有意な因子ではなかった。これらのことから、再発リスクの少ないリンパ節転移陰性の症例では術後のホルモン療法は不要ではないかと考えられた。しかし、リンパ節転移の個数は他の年代同様に80歳以上の高齢者においても重要な予後因子であり、リンパ節転移があるハイリスクの症例については術後ホルモン療法の効果がある可能性は十分にあり、また投与を安全に行ってよい効果を得たといった報告<sup>4-6)</sup>も認められることから、今後詳細な検討が必要と考える。

術後補助療法としての抗がん剤の投与の有用性に関しては今回の検討では明らかにはならなかった。しかし、経口フッ化ピリミジンなどのコンプライアンスの非常に高く、しかも効果のある薬剤が報告されるようになってきている<sup>7,8)</sup>。これらの薬剤の術後補助療法としてのエビデンスはいまだ少ないが、今後高齢者の治療に有用される可能性は十分にあると考える。また、現在広く使用されているタキサンにおいても高齢者に対して安全に使用したという報告も多い<sup>9)</sup>。それに対して、高齢者においては他の年代よりも副作用の出現率が高いとする報告もある<sup>10)</sup>。80歳以上の超高齢者においても他の年代同様にリンパ節転移個数や遠隔転移の有無がOSを左右することは今回の検討でも明らかであり、今後これらの症例に対して安全にしかも有効に抗がん剤を使用することを目標とする検討が必要と考える。

#### 4. 結 語

今回われわれは80歳以上の超高齢者乳癌の治療方針についてretrospectiveな検討を行った。これらの症例におけるエビデンスはまだ少なく、確固

たる治療方針は明らかとなっていないが平均寿命の延長に伴って、これらの症例は増加しこれまで以上に適切な治療が求められてくるのは明らかであり、ホルモン療法剤や抗がん剤の進歩に伴うコンプライアンスの上昇、および乳癌手術の低侵襲化にあわせた高齢者に対する適切な治療方針の決定が必要であると考えた。

#### 文 献

- 1) 新井富生, 沢辺元司, 笠原一郎, 他: 高齢者がんの病理学的特徴. 高齢者に多い組織型(高齢者がんの転移の特徴を含む). 癌の臨床 52: 767-774, 2006
- 2) Honma N, Sakamoto G, Akiyama F, et al: Breast carcinoma in women over the age of 85: distinct histological pattern and androgen, oestrogen and progesterone receptor status. *Histopathology* 42: 120-127, 2003
- 3) Hughes KS, Schnaper LA, Berry D, et al: Lumpectomy plus tamoxifen with or without irradiation in women 70 years of age or older with early breast cancer. *New Engl J Med* 51: 971-977, 2004
- 4) 大佐古智文, 井上克彦, 永本展英, 他: ホルモン療法が著効した高齢者の局所進行乳癌の1例. 癌と化療 34: 73-75, 2007
- 5) 雷 哲明, 安藤充嶽, 相良吉昭, 他: 術前内分泌療法 Anastrozoleを用い切除が可能となった高齢者局所進行乳癌の1症例. 癌と化療 32: 1301-1305, 2005
- 6) 内藤哲也, 佐野宗明, 佐藤信昭, 他: 高齢者(75歳以上)乳癌治療の検討. 日臨外会誌 64: 1038-1042, 2003
- 7) 大崎昭彦, 佐伯俊昭: 高齢者癌薬物療法の進歩乳癌. 癌と化療 34: 374-379, 2007
- 8) 本間英之: 超高齢者の多剤耐性再発乳癌に低用量 Capecitabineが奏効した1例. 癌と化療 33: 2045-2048, 2006
- 9) 田代英哉, 増野浩二郎, 芝原幸太郎, 他: 高齢(75歳以上)の乳癌患者に対するDocetaxelの効果と安全性. 癌と化療 34: 453-455, 2007
- 10) Cassidy J, Twelves C, Van Cutsem E, et al: First-line oral capecitabine therapy in metastatic colorectal cancer: A favorable safety profile compared with intravenous 5-fluorouracil/leucovorin. *Ann Oncol* 13: 566-575, 2002

## 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<sup>1)</sup> 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%<sup>2,3)</sup>. 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<sup>4,5)</sup>.

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<sup>6-10)</sup>.

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<sup>15)</sup>. 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<sup>2</sup>) / epirubicin (100mg/m<sup>2</sup>) / cyclophosphamide (500mg/m<sup>2</sup>) (FEC), plus twelve weekly cycles of paclitaxel (80mg/m<sup>2</sup>). Patients over 65 years of age received twelve weekly cycles of paclitaxel (80mg/m<sup>2</sup>) 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, lymphoscintigraphy 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

**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

submitted in their entirety and evaluated using standard H&E staining.

### 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 lymphoscintigraphy 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

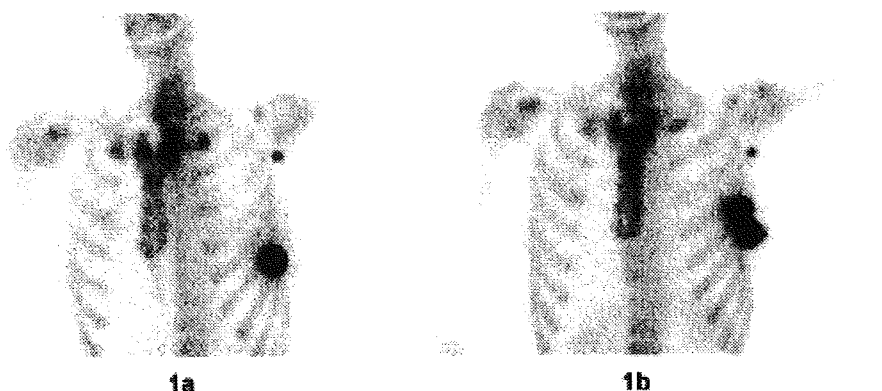


Fig 1. Lymphoscintigraphy before and after NAC (1a and 1b, respectively) revealed one sentinel node at the axilla. The bone scintigram was performed simultaneously to detect bone metastasis.

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)

SLN status	Non-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-

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

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

negative SLN biopsies, a false-negative rate of 10.0% (4/40). Fifty-seven patients had pathologically negative SLN or non-SLN.

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**

SLN status	N0 (n=52)		N1/N2 (n=45)	
	Non- 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**

SLN status	CR (n=50)		PR/SD (n=47)	
	Non- 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 %	<i>N.S.</i>
T3	35	94 %	
T4	8	63 %	
Clinical nodal status			
Negative	54	96 %	<i>N.S.</i>
Positive	50	90 %	
Clinical tumor response			
CR	55	91 %	<i>N.S.</i>
PR/SD	49	96 %	
Pathological tumor response			
pCR	23	91%	<i>N.S.</i>
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 <sup>6</sup>	51	II or III	5.0	43 (84.3)	3 (12)
Miller et al., 2002 <sup>7</sup>	35	T1-3N0	3.5	30 (86.0)	0 ( 0)
Stearns et al.,2000 <sup>8</sup>	34	T3-4, any N	5.0	29 (85.0)	3 (14)
Haid et al.,2001 <sup>9</sup>	33	T1-3, any N	3.3	29 (88.0)	0 ( 0)
Julian et al.,2002 <sup>10</sup>	31	I or II	NS	29 (93.5)	0 ( 0)
Tafra et al.,2001 <sup>12</sup>	29	Any T, N0	NS	27 (93.0)	0 ( 0)
Nason et al.,2000 <sup>13</sup>	15	T2-4, N0	NS	13 (87.0)	3 (33)
Shimazu et al.,2004 <sup>14</sup>	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<sup>11</sup>. 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<sup>16-18</sup>. 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<sup>16</sup>. The addition of taxanes to anthracycline/cyclophosphamide-containing regimens has increased the conversion rate to around 40%<sup>19,20</sup>. 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<sup>6-14</sup>. Breslin *et al.*<sup>6</sup> 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.*<sup>13</sup> reported a smaller

number of patients who had 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 lymphoscintigraphy 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.

## References

- 1) Cody HS 3rd: Clinical aspects of sentinel node biopsy. *Breast Cancer Res* 3:104-1088. 1B, 2001.
- 2) Cody HS, Borgen PI: State-of-the-art approaches to sentinel node biopsy for breast cancer: study design, patient selection, technique and quality control at Memorial Sloan-Kettering Cancer Center. *Surg Oncol* 8:85-91, 1999.
- 3) Krag D, Weaver D, Ashikaga T, Moffat F, Klimberg VS, Shriver C, Feldman S, Kusminsky R, Gadd M, Kuhn J, Harlow S, Beitsch P: The sentinel node in breast cancer—a multicenter validation study. *N Engl J Med* 339:941-946, 1998.
- 4) Anderson BO: Sentinel lymphadenectomy in breast cancer: an update on NCCN Clinical Practice Guidelines. *J Natl Compr Cancer Network* 1 Suppl 1:S64-70, 2003.
- 5) Reintgen D, Giuliano R, Cox C: Lymphatic mapping and sentinel lymph node biopsy for breast cancer. *Cancer J* 8 Suppl 1:S15-21, 2002.
- 6) Breslin TM, Cohen L, Sahin A, Fleming JB, Kuerer HM, Newman LA, Delpassand ES, House T, Ames FC, Feig BW, Ross MI, Singletary SE, Buzdar AU, Hortobagyi GN, Hunt KK: Sentinel lymph node biopsy in accurate after neoadjuvant chemotherapy for breast cancer. *J Clin Oncol* 18:3480-3486, 2000.
- 7) Miller AR, Thompson VE, Yeh IT, Alrakwan A, Sharkey FE, Stauffer J, Otto PM, McKay C, Kahlenberg MS, Phillips WT, Cruz AB Jr: Analysis of sentinel lymph node mapping with immediate pathologic review in patients receiving preoperative chemotherapy for breast carcinoma. *Ann Surg Oncol* 9:243-247, 2002.
- 8) Stearns V, Ewing CA, Slake R, Panannen MF, Hayes DF, Tsangaris TN: Sentinel lymphadenectomy after neoadjuvant chemotherapy for breast cancer may reliably represent the axilla except for inflammatory breast cancer. *Ann Surg Oncol* 9: 235-242, 2000.
- 9) Haid A, Tausch C, Lang A, Lutz J, Fritzsche H, Prschina W, Breitfellner G, Sege W, Aufschnaiter M, Sturn H, Zimmermann G: Is sentinel lymph node biopsy reliable and indicated after preoperative chemotherapy in patients with breast carcinoma? *Cancer* 92:1080-1084, 2001.
- 10) Julian TB, Patel N, Dusi D, Olson P, Nathan G, Jansozs K, Isaacs G, Wolmark N: Sentinel node biopsy after neoadjuvant chemotherapy for breast cancer. *Am J Surg* 182:407-410, 2001.
- 11) Julian TB, Dusi D, Wolmark N: Sentinel node biopsy after neoadjuvant chemotherapy for breast cancer. *Am J Surg* 184:315-317, 2002.
- 12) Tafra L, Verbanac KM, Lannin DR: Preoperative chemotherapy and sentinel lymphadenectomy for breast cancer. *Am J Surg* 182:312-315, 2001.
- 13) Nason KS, Anderson BO, Byrd DR, Dunnwald LK, Eary JF, Mankoff DA, Livingston R, Schimidt RA, Jewell KD, Yeung RS, Moe RE: Increased false negative sentinel node biopsy rates after preoperative chemotherapy for invasive breast carcinoma. *Cancer* 89:2187-2194, 2000.
- 14) Shimazu K, Tamaki Y, Taguchi T, Akazawa K, Inoue T, Noguchi S: Sentinel lymph node biopsy using periareolar injection of radiocolloid for patients with neoadjuvant chemotherapy-treated breast carcinoma. *Cancer* 100:2555-2561, 2004.
- 15) Lyman GH, Giuliano AE, Somerfield MR, Bensen AB, Bodurka DC, Burstein HJ, Cochran AJ, Cody III HS, Edge SB, Galper S, Hayman JA, Kim TY, Perkins CL, Podoloff DA, Sivausbramaniam VH, Turner RR, Waki R, Weaver RW, Wolff CA, Winer EP: American Society of Clinical Oncology guideline recommendations for sentinel lymph node biopsy in early-stage breast cancer. *JCO* 23:2540-2545, 2005.
- 16) Fisher B, Brown A, Mamounas E, Wieand S, Robidoux A, Margolese RG, Cruz AB Jr, Fisher ER, Wicferham DL, Wolmark N, DeCillis A, Hoehn JL, Lees AW, Dimitrov NV: Effect of preoperative chemotherapy on local-regional disease in women with operable breast cancer: findings from the National Surgical Adjuvant Breast and Bowel Project B-18. *J Clin Oncol* 15:2483-2493, 1997.
- 17) Hutcheon AW, Heys SD, Miller ID: Improvements in survival in patients receiving primary chemotherapy with docetaxel for breast cancer: a randomized control trial. Presented at the 24th Annual San Antonio Breast Cancer Symposium. San Antonio, Texas, December 2001.
- 18) O'Hea BJ, Hill AD, El-Shirbiny AM, Yeh SD, Rosen PP, Coit DG, Borgen PI, Cody HS 3rd: Sentinel lymph node biopsy in breast cancer: Initial experience at Memorial Sloan-Kettering Cancer Center. *J Am Coll Surg* 186:423-427, 1998.
- 19) Mamounas E, Brown A, Smith R: Accuracy of sentinel node biopsy after neoadjuvant chemotherapy in breast cancer: update results from NSABP B-27. *Proc Am Soc Clin Oncol* 21:36a, 2002.
- 20) Gianni L, Baselga H, Eiermann W: First report of European Cooperative Trial in operable breast cancer (ECTO): effect of primary systemic therapy (PST) on local-regional disease. *Proc Am Soc Clin Oncol* 21:34a, 2002.

## 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<sup>1-3</sup>. 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).

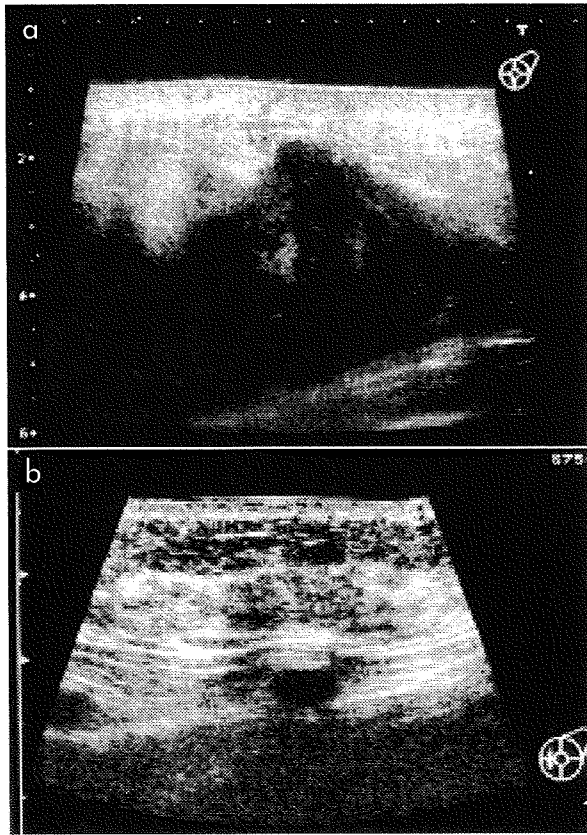
Core needle biopsy led to a pathological diagnosis of invasive ductal carcinoma (grade 3, nuclear grade 3, and HER-2 negative) (Fig 2a). The tumor was negative for both estrogen and progesterone receptors. Chest X-ray, bone scintigraphy, abdominal US, and chest and abdominal CT revealed no distant metastases.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

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#### Abbreviations:

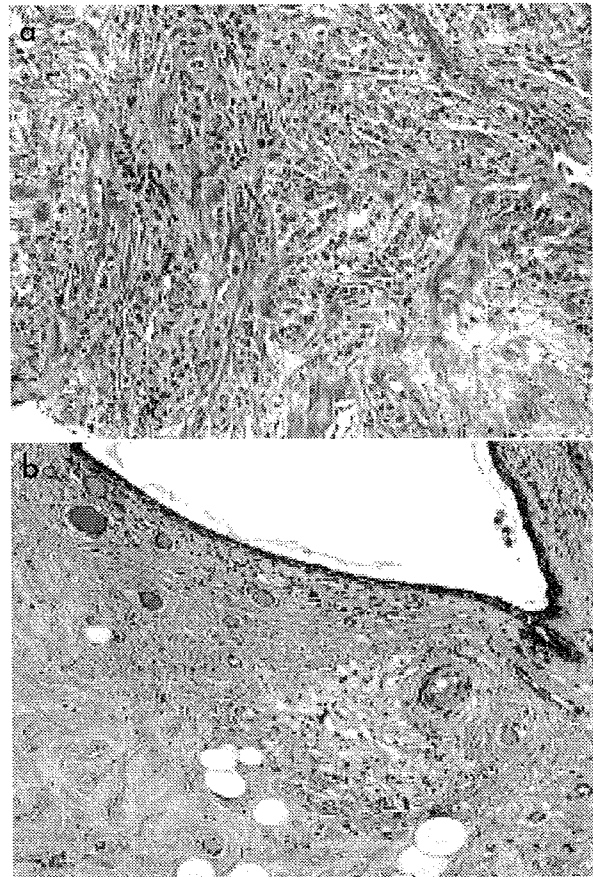
pCR, Pathological complete response; NAC, neoadjuvant chemotherapy; US, ultrasonography; CT, Computed tomography; MRI, Magnetic resonance imaging

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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.

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<sup>4</sup>. She received NAC from February to July consisting of doxorubicin and cyclophosphamide (60/600 mg/m<sup>2</sup>) 4 times every 3 weeks, followed by paclitaxel (80 mg/m<sup>2</sup>) 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



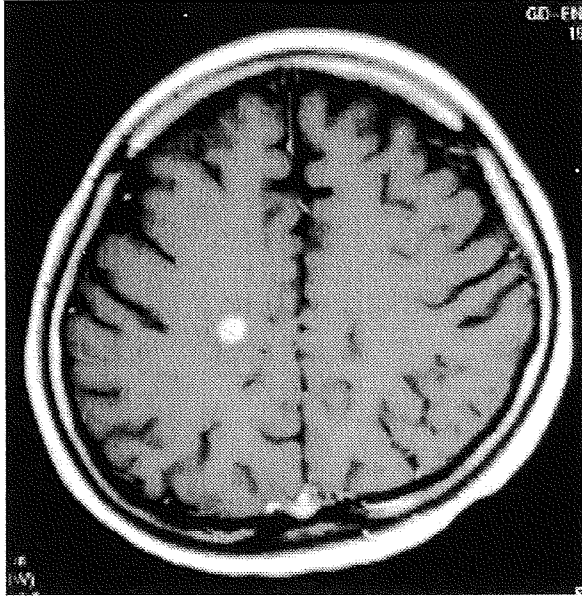
**Fig 2.** (a) Core needle biopsy reveals invasive ductal carcinoma, grade 3, nuclear grade 3. (b) No residual tumor is detected. The presence of inflammatory cells surrounding a duct with an increased number of enlarged capillary vessels, typical after tumor disappearance, is observed. (hematoxylin-eosin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

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

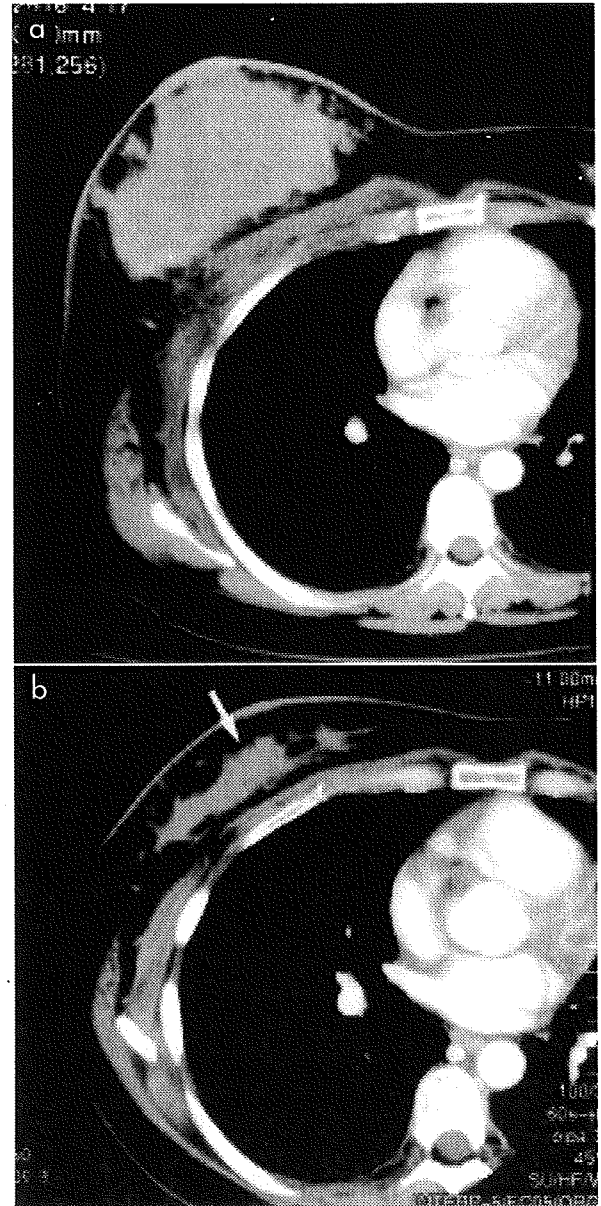
A 44-year-old premenopausal woman was seen at a nearby hospital with a chief complaint of an erythematous enlarged right breast. Inflammatory breast cancer was suspected, so she was referred to our institution in December 2004.

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



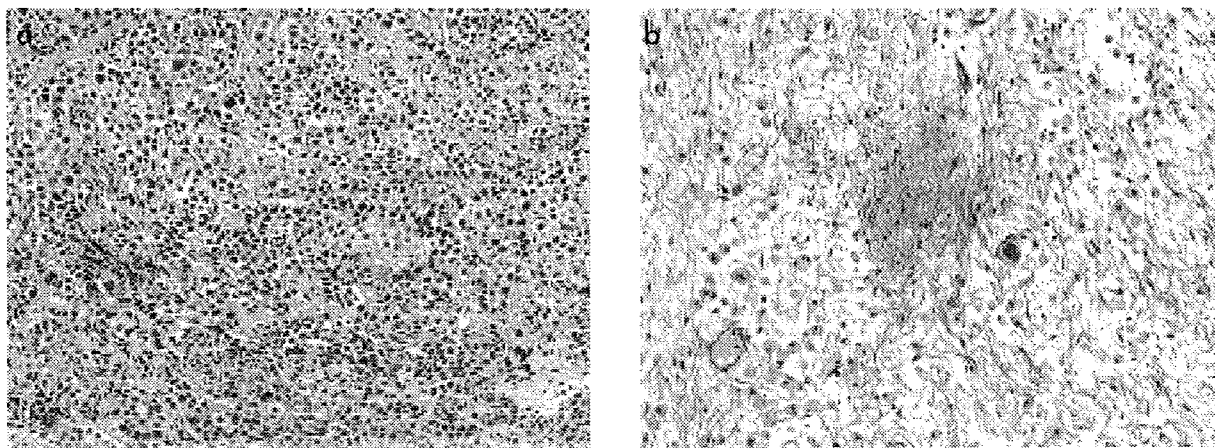
**Fig 3.** The metastatic lesions exhibited high signal intensity in the right temporal lobe by T1 weighted MRI.

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<sup>4</sup> 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

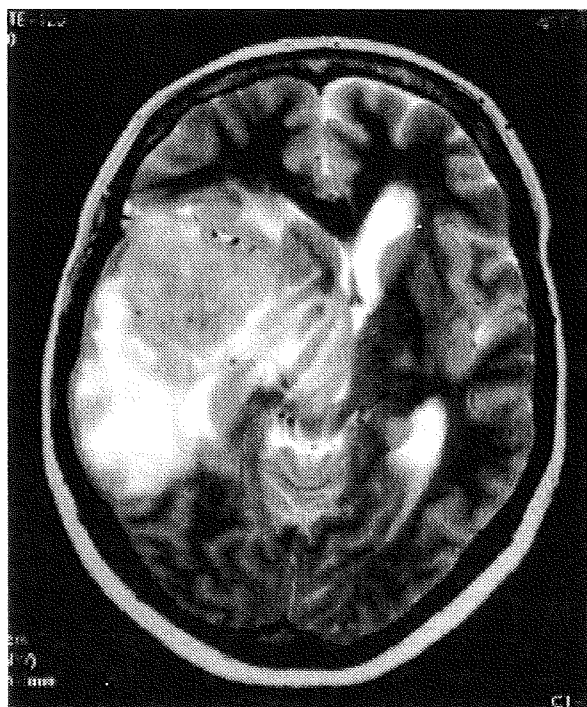


**Fig 4.** (a) CT shows a large right breast mass with overlying edematous subcutaneous tissue and thickened skin. This is not the early phase but late phase scan of breast CT, because only chest CT without an early phase scan was performed to detect distant metastasis instead of breast CT. (b) CT scan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

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,  $\times 100$ ).



**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<sup>1,3</sup>. Moreover, pCR of axillary lymph nodes is an

excellent prognostic factor for locally advanced breast cancers<sup>5,8</sup>. 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<sup>9</sup>. 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<sup>10,11</sup>. 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<sup>12,13</sup>, unresponsiveness to the hormonal

therapies, and HER-2 over expression are reported risk factors<sup>14,17</sup>. Intracranial metastases are also related to the use of trastuzumab<sup>18</sup>. 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.

## References

- 1) Fisher B, Bryant J, Wolmark N, Mamounas E, Brown A, Fisher R, Wickerham L, Begovic M, DeCillis A, Robidoux A, Margolese G, Cruz B, Hoehn L, Lees W, Dimitrov V, Bear D: Effect of preoperative chemotherapy on the outcome of woman with operable breast cancer. *J Clin Oncol* 16: 2672-2685, 1998.
- 2) Norman W, Jiping W, Eleftherios M, John B, Bernard F: Preoperative chemotherapy in patients with operable breast cancer: nine-year results from national surgical adjuvant breast and bowel project B-18. *J Natl Cancer Inst Monogr* 30: 96-102, 2001.
- 3) Sataloff D, Mason B, Prestipino A, Seinige U, Lieber C, Baloch Z: Pathologic response to induction chemotherapy in locally advanced carcinoma of the breast: A determinant of outcome. *J Am Coll Surg* 180: 297-306, 1995.
- 4) Sakamoto G, Inaji H, Akiyama F, Haga S, Hiraoka M, Inai K, Iwase T, Kobayashi S, Sakamoto G, Sano M, Sato T, Sonoo H, Tsuchiya S, Watanabe T; The Japanese Breast Cancer Society: General rules for clinical and pathological recording of breast cancer 2005. *Breast cancer* 12 (Suppl): S1-27, 2005.
- 5) Hennessy T, Hortobagyi N, Rouzier R, Kuerer H, Sneige N, Buzdar U, Kau W, Fornage B, Sahin A, Broglio K, Singletary E, Valero V: Outcome after pathologic complete eradication of cytologically proven breast cancer axillary node metastases following primary chemotherapy. *J Clin Oncol* 23: 9304-9311, 2005.
- 6) Rouzier R, Extra M, Klijanienko J, Falcou C, Asselain B, Salomon V, Vielh P, Bourstyn E: Incidence and prognostic significance of complete axillary downstaging after primary chemotherapy in breast cancer patients with T1 to T3 tumors and cytologically proven axillary metastatic lymph nodes. *J Clin Oncol* 20: 1304-1310, 2002.
- 7) Hennessy T, Gonzalez-Angulo M, Hortobagyi N, Cristofanilli M, Kau W, Broglio K, Fornage B, Singletary E, Sahin A, Buzdar A, Valero V: Disease-free and overall survival after pathologic complete disease remission of cytologically proven inflammatory breast carcinoma axillary lymph node metastases after primary systemic chemotherapy. *Cancer* 106: 1000-1006, 2006.
- 8) Kuerer M, Newman A, Smith L, Ames C, Hunt K, Dhingra K, Theriault L, Singh G, Binkley M, Sneige N, Buchholz A, Ross I, McNeese D, Buzdar U, Hortobagyi N, Singletary E: Clinical course of breast cancer patients with complete pathologic primary tumor and axillary lymph node response to doxorubicin-based neoadjuvant chemotherapy. *J Clin Oncol* 17: 460-469, 1999.
- 9) Freilich J, Seidman D, DeAngelis M: Central nervous system progression of metastatic breast cancer in patients treated with paclitaxel. *Cancer* 76: 232-236, 1995.
- 10) Paterson G, Agarwal M, Lees A, Hanson J, Szafran O: Brain metastases in breast cancer patients receiving adjuvant chemotherapy. *Cancer* 49: 651-654, 1982.
- 11) Crivellari D, Pagani O, Veronesi A, Lombardi D, Nole F, Thurlimann B, Hess D, Borner M, Bauer J, Martinelli G, Graffeo R, Sessa C, Goldhirsch A: High incidence of central nervous system involvement in patients with metastatic or locally advanced breast cancer treated with epirubicin and docetaxel. *Ann Oncol* 12: 353-356, 2001.
- 12) DiStefano A, Yap Y, Hortobagyi N, Blumenschein R: The natural history of breast cancer patients with brain metastases. *Cancer* 44: 1913-1918, 1979.
- 13) Tsukada Y, Fouad A, Pickren W, Lane W: Central nervous system metastasis from breast carcinoma: autopsy study. *Cancer* 52: 2349-2354, 1983.
- 14) Slimane K, Andre F, Delalage S, Dunant A, Perez A, Grenier J, Massard C, Spielmann M: Risk factors for brain relapse in patients with metastatic breast cancer. *Ann Oncol* 15: 1640-1644, 2004.
- 15) Clark M, Sledge W, Osborne K, McGuire L: Survival from first recurrence: relative importance of prognostic factors in 1,015 breast cancer patients. *J Clin Oncol* 5: 55-61, 1987.
- 16) Miller D, Weathers T, Haney G, Timmerman R, Dickler M, Shen J, Sledge W: Occult central nervous system involvement in patients with metastatic breast cancer: prevalence, predictive factors and impact on overall survival. *Ann Oncol* 14: 1072-1077, 2003.
- 17) Bendell C, Domchek M, Burstein J, Harris L, Younger J, Kuter I, Bunnell C, Rue M, Gelman R, Winer E: Central nervous system metastases in women who receive trastuzumab-based therapy for metastatic breast carcinoma. *Cancer* 97: 2972-2977, 2003.
- 18) Matsumoto K, Shimizu C, Fujiwara Y: The next step to approaching central nervous system metastasis in HER-2-positive metastatic breast cancer patients. *Asia-Pac J Clin Oncol* 2: 6-8, 2006.



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.

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**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,<sup>1</sup> significant differences in breast cancer stage, treatments, and mortality

rates still exist in the world with regard to race and ethnicity.<sup>2</sup> 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,<sup>3–5</sup> such as high-grade and estrogen receptor (ER)-negative (ER–), patient characteristics,<sup>6,7</sup> such as obesity and a higher rate of comorbidity, inadequate mammographic screening,<sup>8,9</sup> delay of diagnosis leading to advanced stage,<sup>10,11</sup> and inadequate treatment,<sup>12–14</sup> 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.<sup>15</sup>

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.<sup>16</sup> 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).<sup>17</sup> 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.<sup>18</sup> Blood vessel invasion (BVI) was evaluated using elastic 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.<sup>18</sup>

### 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.<sup>19</sup> 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.,<sup>15</sup> 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  $P \leq 0.05$ .

## 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) <sup>†</sup>	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 <sup>‡</sup>
5-year OS	92.8%	96.9%	86.6%	86.9%	86.2%	83.3%	<0.001 <sup>‡</sup>

\*Comparing five subtypes using  $\chi^2$  test or Fisher's exact test.

<sup>†</sup>In %.

<sup>‡</sup>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

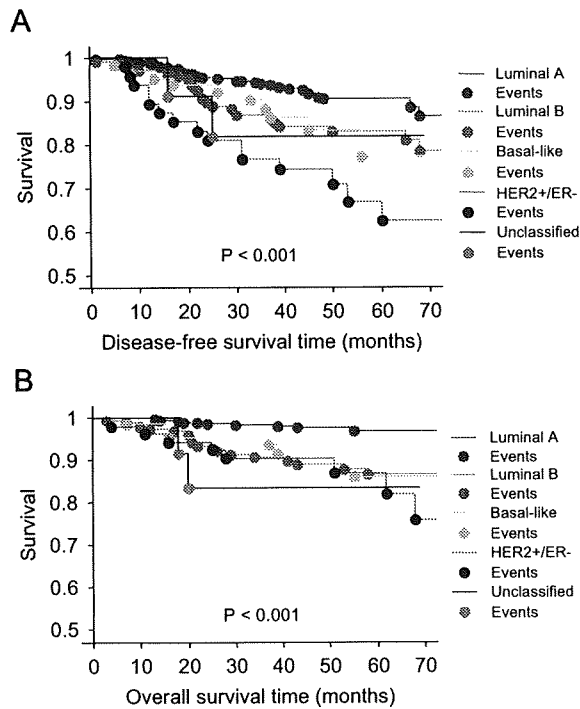


Fig. 1. DFS (A) and OS (B) curves in breast cancer patient groups divided by IHC intrinsic subtypes.

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.<sup>15</sup> This IHC-based assay has been verified against

gene expression profiles to estimate the prevalence of intrinsic subtypes.<sup>15,20</sup> 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.,<sup>15</sup> 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.<sup>15</sup> 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<sup>15</sup>; it was 78% in the present study. The prevalence of triple-negative tumors was 22% in the Carolina Breast Cancer Study,<sup>15</sup> 16% in a large series of patients in the UK,<sup>21</sup> 26% in conservatively managed patients in the USA,<sup>22</sup> and 31% in consecutive patients in Korea.<sup>23</sup> 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 cancer incidence rates among different races.<sup>5</sup> 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,<sup>15,20</sup> 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.<sup>24,25</sup> 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.<sup>26</sup> 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.<sup>27</sup> 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.<sup>28–30</sup> 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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### References

- Boyle P. Breast cancer control: signs of progress, but more work required. *Breast* 2005;14:429–38.
- Blackman DJ, Masi CM. Racial and ethnic disparities in breast cancer mortality: are we doing enough to address the root causes? *J Clin Oncol* 2006;24:2170–8.
- Li CI, Malone KE, Daling JR. Differences in breast cancer hormone receptor status and histology by race and ethnicity among women 50 years of age and older. *Cancer Epidemiol Biomarkers Prev* 2002;11:601–7.
- Porter PL, Lund MJ, Lin MG, et al. Racial differences in the expression of cell cycle-regulatory proteins in breast carcinoma. *Cancer* 2004;100:2533–42.
- Chlebowski RT, Chen Z, Anderson GL, et al. Ethnicity and breast cancer: factors influencing differences in incidence and outcome. *J Natl Cancer Inst* 2005;97:439–48.
- Polednak AP. Racial differences in mortality from obesity-related chronic diseases in US women diagnosed with breast cancer. *Ethn Dis* 2004;14:463–8.
- Tammemagi CM, Nerenz D, Neslund-Dudas C, Feldkamp C, Nathanson D. Comorbidity and survival disparities among black and white patients with breast cancer. *JAMA* 2005;294:1765–72.
- Smith-Bindman R, Miglioretti DL, Lurie N, et al. Does utilization of screening mammography explain racial and ethnic differences in breast cancer? *Ann Intern Med* 2006;144:541–53.
- Sassi F, Luft HS, Guadagnoli E. Reducing racial/ethnic disparities in female breast cancer: screening rates and stage at diagnosis. *Am J Public Health* 2006;96:2165–72.
- Gwyn K, Bondy ML, Cohen DS, et al. Racial differences in diagnosis, treatment, and clinical delays in a population-based study of patients with newly diagnosed breast carcinoma. *Cancer* 2004;100:1595–604.
- Gorin SS, Heck JE, Cheng B, Smith SJ. Delays in breast cancer diagnosis and treatment by racial/ethnic group. *Arch Intern Med* 2006;166:2244–52.
- Joslyn SA. Racial differences in treatment and survival from early-stage breast carcinoma. *Cancer* 2002;95:1759–66.
- Li CI, Malone KE, Daling JR. Differences in breast cancer stage, treatment, and survival by race and ethnicity. *Arch Intern Med* 2003;163:49–56.
- Bickell NA, Wang JJ, Oluwole S, et al. Missed opportunities: racial disparities in adjuvant breast cancer treatment. *J Clin Oncol* 2006;24:1357–62.
- Carey LA, Perou CM, Livasy CA, et al. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. *JAMA* 2006;295:2492–502.
- Braun KL, Fong M, Gotay C, Pagano IS, Chong C. Ethnicity and breast cancer in Hawaii: increased survival but continued disparity. *Ethn Dis* 2005;15:453–60.
- Elston EW, Ellis IO. Method for grading breast cancer. *J Clin Pathol* 1993;46:189–90.
- Rosen PP. *Rosen's breast pathology*. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 325–64.
- Jacobs TW, Gown AM, Yaziji H, Barnes MJ, Schnitt SJ. Specificity of HercepTest in determining HER-2/neu status of breast cancers using the United States Food and Drug Administration-approved scoring system. *J Clin Oncol* 1999;17:1983–7.
- Nielsen TO, Hsu FD, Jensen K, et al. Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. *Clin Cancer Res* 2004;10:5367–74.
- Rakha EA, El-Sayed ME, Green AR, Lee AH, Robertson JF, Ellis IO. Prognostic markers in triple-negative breast cancer. *Cancer* 2007;109:25–32.
- Haffty BG, Yang Q, Reiss M, et al. Locoregional relapse and distant metastasis in conservatively managed triple negative early-stage breast cancer. *J Clin Oncol* 2006;24:5652–7.
- Kim MJ, Ro JY, Ahn SH, Kim HH, Kim SB, Gong G. Clinicopathologic significance of the basal-like subtype of breast cancer: a comparison with hormone receptor and Her2/neu-over-expressing phenotypes. *Hum Pathol* 2006;37:1217–26.