悪性骨軟部腫瘍に対する術前治療

——高い治療成績と機能再建を求めた治療法の確立

Neoadjuvant chemoradiotherapy for high-grade bone and soft-part tumors

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◎四肢原発の悪性骨・軟部腫瘍に対する術前・術後補助化学療法・放射線治療を併用する集学的治療を行うことで，全身の微小転移巣や原発巣周辺の微小浸潤巣を撲滅し，5年生存率10～20%の根治率と，きわめて予後不良であった治療成績を60～70%までに改善することができた．骨肉腫では広範切除で，Ewing肉腫，横紋筋肉腫では放射線療法または手術療法との併用で原発巣を根絶し，骨・関節を人工関節や血管柄付き骨移植で再建することで80%の症例で患肢温存術が行われている．温存された患肢機能評価，社会復帰状況，晩期障害，二次発癌の発生について10年以上の追跡が行われ，術前・術後化学療法の有用性と安全性が確認されている．

Key word：術前治療，骨肉腫，Ewing肉腫ファミリー，横紋筋肉腫，高悪性度軟部腫瘍

高悪性度骨軟部腫瘍は，初診時，限局性の腫瘍であると考えられても，多くの症例で微小遠隔転移や病巣周囲のスキップ転移，腫瘍周辺への微小浸潤が存在する．そのため，1970年代以前の患肢切断を中心とする手術治療単独では，代表的な高悪性度骨腫瘍である，骨肉腫，Ewing肉腫で約10%，悪性軟部腫瘍のうち，深部発生で直径10 cmを超えるような悪性度の高い腫瘍では約30%の5年生存率しか得られず，きわめて予後不良であった．多発肺転移や局所再発が顕在化する前に，化学療法や放射線治療を術後補助的に追加することで全身の微小転移や腫瘍周辺の微小浸潤巣を撲滅できることが明らかとなった．さらに，1980年代からの術前化学療法の導入により，病巣を術前にできるだけ沈静化し，CT，MRIの開発など，画像診断技術の発展も相まって，より高い機能を保った患肢温存手術が可能となった．その結果，現在では高悪性度骨軟部腫瘍の治療成績はきわめて改善され，約80%の症例で患肢の温存が可能となり，全体の約8割を占める限局症例では約

サイドメモ　悪性骨軟部腫瘍

分類される組織型・組織亜型が多く，悪性骨腫瘍では骨肉腫，Ewing肉腫ファミリー，骨悪性線維性組織球腫の高悪性骨腫瘍と通常型軟骨肉腫，傍骨性骨肉腫，脊索腫などの低悪性骨腫瘍が代表的である．悪性軟部腫瘍は，小児に多い横紋筋肉腫，軟部Ewing肉腫，滑膜肉腫，成人では粘液線維肉腫，脂肪肉腫，滑膜肉腫，平滑筋肉腫，悪性末梢神経鞘腫，悪性線維性組織球腫が多い組織亜型である．骨肉腫，骨悪性線維性組織球腫，Ewing肉腫ファミリー，横紋筋肉腫は化学療法，放射線治療，手術治療を駆使した治療体系(集学的治療)が確立され，四肢発生例では患肢温存手術が標準的治療となっている．低悪性度の骨・軟部腫瘍は広範切除縁での外科治療で四肢・体表発生では90%の治癒が可能であり，広範切除縁を確保しにくい骨盤，後腹膜，頭頸部発生の骨・軟部腫瘍は成績不良である．

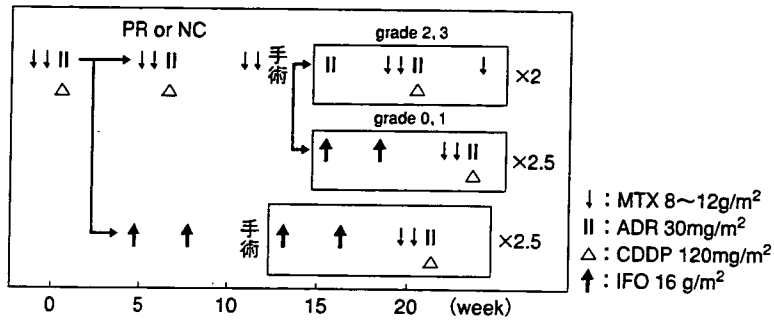

図 1　大量MTX, CDDP, ADR併用療法と，IFOの救済を目的としたNECO95-J (1995)による骨肉腫の治療プロトコール

70%，遠隔転移症例でも約 20%の 5 年生存率が達成されつつある．

本稿では，現在標準治療として全世界的に行われている，高悪性度骨腫瘍(骨肉腫，Ewing 肉腫)，円形細胞軟部肉腫(軟部 Ewing 肉腫，横紋筋肉腫)に対する術前化学療法について概説し，現在，術前化学療法の導入が模索されている高齢者悪性骨腫瘍，高悪性度非円形軟部腫瘍の現状についても紹介する．

骨肉腫の術前化学療法

1970 年代から術後化学療法の効果を確かめる臨床試験が行われ，術後補助化学療法の有用性が多くの臨床試験で確認された[1-3]．その結果，強力な化学療法が可能な若年者限局性骨肉腫の生存率が著明に改善し，1980 年代には骨肉腫の術前化学療法の検討が開始された．術前化学療法を行うことによって病巣の縮小や硬化を認め，より安全に縮小手術が行えることが明らかになった．すなわち，術前化学療法によって四肢原発骨肉腫の 70～80%の症例で腫瘍周辺の反応層は沈静化し，骨外病変が縮小するなどの臨床的奏効を得ることができたのである．また，術後に組織学的奏効性評価を最大割面で行い，術前化学療法によって 90%以上の壊死が得られた著効群では，壊死率 90%に満たない群に比べ統計学的に有意な予後改善し，80%以上の 5 年無病生存率が観察された[4,5]．また，腫瘍用の人工関節，処理骨の再利用，複合組織移植術などさまざまな患肢再建術の開発も相まって，より高い機能の温存術が達成されつつある[6,7]．

現在，40 歳以下のいわゆる若年者骨肉腫に対する標準治療薬はメトトレキセート(8～12 g/m²)，シスプラチン(100～120 mg/m²)，アドリアマイシン(60～80 mg/m²)，イホスファミド(12～16 g/m²)の 4 剤であり，初診時転移例も含んだ四肢骨肉腫全体では 2 剤併用で 40～50%，3 剤併用で 50～60%の 10 年生存率が達成されている[8,9]．わが国でも初診時非転移例の四肢原発骨肉腫に対する多施設共同プロトコール NECO-95J(図 1)が施行され，5 年無病生存率が約 75%という優れた治療成績が得られている．さらに高い機能の下肢を温存する努力も模索され，シスプラチンやアドリアマイシンの四肢動注療法のほか，上記 4 剤を併用し，術前化学療法を強化する研究も行われているが[10,11]，いまだに薬剤の最適な組合せおよび投与法について，はっきりとしたエビデンスが得られていない．

また，標準的術前化学療法の無効な症例が約 20%存在し，今後，検討すべき問題である．現状ではこのような症例はむやみに術前化学療法を継続せず，早期の切断や治癒的広範切除が勧められる．術前化学療法無効例でも手術によって局所制御し，術後補助化学療法と系統的治療を行うことで 40%の完治が期待されるのに対して，一度，局所再発を起こすときわめて予後不良である[12]．

Ewing肉腫の術前化学療法

Ewing 肉腫は若年者に好発する高悪性度の骨軟部腫瘍である．鑑別診断として骨肉腫のほか，骨髄炎，ヒスチオサイトーシス，悪性リンパ腫などがあげられる．1983 年に Ewing 肉腫において第

	VAdC	IE	VAdC	IE	局所治療	VAdC	IE	VAdC	IE
週	0	3	6	9	12	13	16	19	22
	VAdC	IE	VAC	IE	VAC	IE	VAC	IE	VAC
週	25	28	31	34	37	40	43	46	49

図 2　VAdCA＋IE交互療法によるCCG/POG(1988～1992)のEwing肉腫治療プロトコール
V：VCR 1.5 mg/m^2，Ad：ADR 75 mg/m^2/48 h，C：CYC 1,200 mg/m^2，I：IFM 1.8 g/m^2/day×5 days，E：VP-16 100 mg/day×5 days，A：Act-D 1.25 mg/m^2.

11 染色体と第 22 染色体の相互転座が発見され，それまで，成人神経芽腫(pPNET)，胸壁の Askin tumor とよばれていた腫瘍群にも同様な相互転座が検出されるために，現在では一連の腫瘍を Ewing's sarcoma family of tumor(EFT)と総称するようになりつつある．手術治療単独での 5 年生存率は 10%以下ときわめて予後不良の疾患であったが，1970 年代よりビンクリスチン，アクチノマイシン D，シクロホスファミドの 3 剤併用の VAC 療法が開始された．その後，シクロホスファミドの投与量を 1,400 mg/m^{2}に増加させ，アドレアマイシンを加えた VACA 療法により，初診時転移のない EFT の 5 年無病生存率は 50%を確実に超えることが明らかとなった．

さらに，1988～1992 年の期間にアメリカにて 530 例の Ewing 肉腫患者を集積し，POG(Pediatric Oncology Group)-CCG(Children's Cancer Group)共同の臨床試験が行われた．これは，当時の VACA 標準療法に対してビンクリスチン，アドリアマイシン，エンドキサン(VDC 療法)にイホスファミド，エトポシド(IE 療法)を加えた VDC-IE 交互療法の優位性を検証する臨床試験であった．結果，5 年無病生存率は 5 剤交互療法群 69%，VACA 群 53%，局所再発率もそれぞれ 7%と 20%と，VDC-IE 療法の優位性が示された[13]．またこの試験では 5 剤併用群の術前臨床奏効率が約 70%となり，VACD 併用療法の約 40%より向上したことで，多くの症例で縮小手術が可能となった．

Ewing 肉腫は放射線感受性も非常に高い腫瘍であるが，その後，切除可能であれば，外科的切除を行ったほうが局所再発率は低い．放射線治療後局所再発が 20～40%観察され，放射線治療による晩期障害，二次発癌のリスクから外科的切除術の比重が大きくなりつつある[14]．現時点での EFT に対する標準的治療は，ビンクリスチン 1.5 mg/m^2，アドリアマイシン 60～75 mg/m^2，エンドキサン 1,200 mg/m^2(VDC 療法)とイホスファミド 1,800 mg/m^2，エトポシド 100 mg/m^2(IE 療法)を 3 週ごとに 2 クール繰り返した後，手術療法を行うスケジュールで，術後はさらに 4～5 クールの交互療法を繰り返す治療方法である(図 2)．スケジュールに沿って確実に治療遂行することの重要性が報告されている[15]．

横紋筋肉腫の術前化学療法

横紋筋肉腫(rhabdomyosarcoma：RMS)は小児期に発生する頻度の高い円形細胞軟部肉腫のひとつである．小児悪性軟部腫瘍の半数を占め，その発生部位は，眼窩，頭頸部，泌尿生殖器，四肢と多岐にわたり，初診時遠隔転移例として診断されることも多い腫瘍である．組織型は胞巣状型，胎児型，多形型に分類される．胞巣状 RMS では第 2 染色体上の PAX3 遺伝子または第 1 染色体上の PAX7 遺伝子が第 13 染色体上の FKHR 遺伝子と

表 1　術後グループ分類(IRSG clinical grouping classification, post-surgical)

clinical group	
Ⅰ	組織学的に全摘除された限局性腫瘍 A．原発臓器または筋に限局 B．原発臓器または筋を越えて(筋膜を越えて)周囲に浸潤 ただし,いずれの場合も領域リンパ節に転移は認めない(頭頸部を除いてサンプリングまたは郭清により組織学的確認を必要とする)
Ⅱ	肉眼的に全摘除された領域内進展腫瘍 A．切除断端に顕微鏡的腫瘍遺残あり，ただし領域リンパ節に転移を認めない B．領域リンパ節に転移を認めるが，完全摘除を行った．すなわち，もっとも遠位の廓清リンパ節に転移を認めない C．領域リンパ節に転移を認め，しかも切除断端に顕微鏡的腫瘍遺残を認めるか，もっとも遠位の廓清リンパ節に転移を認める
Ⅲ	肉眼的な腫瘍遺残 A．生検のみ施行 B．亜全摘除または 50%以上の部分摘除を施行
Ⅳ	1．遠隔転移(肺，肝，骨，骨髄，脳，遠隔筋組織，遠隔リンパ節など)を認める 2．脳脊髄液，胸水，腹水中に腫瘍細胞が存在 3．胸膜播種，腹膜(大網)播種を伴う

初回手術後(化学療法，放射線療法未施行)の病期分類．初回の術中所見および病理所見により分類され，以後の二期手術の結果には影響されない．

融合遺伝子を形成し，胎児型ではしばしば第 11 染色体の短腕からの特異的なゲノムの欠失が確認されるなど，遺伝子学的診断で鑑別が可能となった．

RMS は化学療法，放射線治療の感受性が高く，発生した部位によっては術後の機能障害も大きいため，骨肉腫，EFT のように，無理な手術的治療が行われないことも多い．国際横紋筋肉腫協会(International Rhabdomyosarcoma Study Group：IRSG)の検討により，手術可能であれば化学療法・放射線治療前にまず治癒的切除を行い，その後に臨床病期(表 1)により術後に化学療法・放射線治療を施行することが基本である．しかし，手術後，高度の機能障害を避けるために，縮小手術の可能性を追求したり，放射線治療の回避を目的とした術前化学療法の研究も症例を限定して行われている．膀胱原発横紋筋肉腫に対する術前化学療法を行って膀胱温存の試みの臨床試験や，若年者の放射線治療による晩期障害，二次発癌を回避するためにヨーロッパで行われた臨床試験(MMT89)では，術前導入化学療法を組み込んだ検討が行われた．現在のところ IRS-Ⅲの微小残存腫瘍を認める場合(臨床グループⅡ)に，すべて放射線治療追加する局所制御方法が勝っていて，臨床グループⅡに対する術前化学療法の救済効果は確認されていない[16,17]．

高齢者悪性骨腫瘍の術前化学療法

40 歳以上の高齢者に発生した骨肉腫，骨原発悪性線維性組織球種(malignant fibrous histiocytoma of bone：MFH of bone)は近年増加傾向にあり，若年者と比較すると，脊椎，骨盤などの体幹部発生が多い．また，放射線後の二次発癌や骨 Paget 病や線維性骨異形成，骨梗塞部に発生することもあり，病態・悪性度に大きな幅がある．治療方針もいまだ確立されていない．すなわち，手術治療単独でも生存根治可能な低悪性度例から，術前化学療法が必要で，若年者と同様に化学療法に奏効する高悪性度症例まで，悪性度の幅が広いことがその主因である．また，治療強度にもよるが，術前化学療法の完遂率が 50%との報告が多く，術前後化学療法の施行に懐疑的な意見もある．予後に関しては十分な化学療法を行なわなくても高齢者骨肉腫は若年者の予後と差を認めないとの報告がなされているが，この結果は若年者に発生した骨肉腫と比較して低悪性度骨肉腫の割合が高いセレクションバイアスの可能性があり，今後，多施設での慎

重な検討が必要と考えられる[18-20].

高悪性度非円形細胞肉腫の術前化学療法

軟部肉腫のなかで，骨外性骨肉腫，横紋筋肉腫，骨外性 Ewing 肉腫は化学療法に感受性が高く，多剤併用化学療法が標準的治療として確立している．しかし，平滑筋肉腫，悪性線維性組織球腫，線維肉腫などの非円形細胞肉腫は化学療法に対する感受性がそれほど高くなく，従来は手術を中心とした治療が行われてきた．しかし，メモリアルスローンケンタリングがんセンターの 2,000 例を超える臨床検討の結果，高悪性度非円形細胞肉腫軟部腫瘍のなかで深部発生，5 cm 以上の最大径をもつ高悪性度の軟部肉腫は，手術のみによる 5 年無病生存率は 30%前後であり，きわめて予後不良である．

さらに，これらの予後不良の原因は多発肺転移を中心とした遠隔転移であり，化学療法の必要性が検討されてきた．まず，遠隔転移をもつ進行例を対象とした臨床研究によって高悪性度非円形細胞肉腫に対してイホスファミドとアドリアマイシンがもっとも優れた奏効性を示すことが報告された．しかし，これら 2 剤の含むさまざまなプロトコール治療によっても，進行例において優位に生存率を上昇させる知見は得られていない．その後，初診時肺転移のない非進行症例に対する術前化学療法の検討が海外を中心に開始され，日本においても，JCOG(Japan Clinical Oncology Group)骨軟部腫瘍グループによってイホスファミド 9 g/m^2 とアドリアマイシン 60 mg/m^2 の術前 3 回・術後 2 回の化学療法を行うスケジュールの臨床研究がはじまっている[21].

おわりに

悪性骨軟部腫瘍は比較的まれな疾患であり，その組織型・発生部位も多彩で，標準的治療の開発が非常に困難な分野である．しかし，骨肉腫，EFT などでは術前化学療法の有用性が多くの多施設共同研究で確認され，その治療成績は飛躍的に改善した．いまだ予後不良な高悪性軟部腫瘍では，より有効な治療薬の開発が必要で，さらなる機能温存をめざすことに加え，治療薬の組合せ，投与法を最適化し，患者個々に応じたカスタムメード治療など，新規コンセプトの治療開発が不可欠である．また，初診時転移例や術前化学療法に反応しない無効例などに対する，分子標的治療薬なども含めた新規治療薬の開発も急務であると考えられる．そのためには，骨軟部腫瘍の希少性を考えると，骨軟部腫瘍臨床および研究にかかわる，世界的規模の協力体制が必要である．

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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

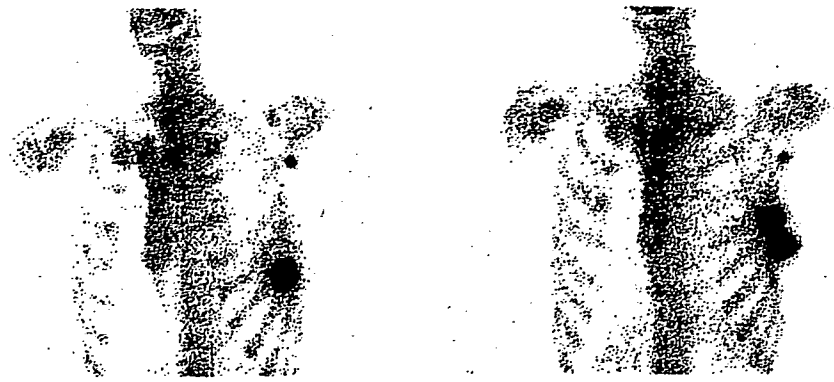
	Number of patients
Age (years)	
Mean	50.2
Range	27-77
Clinical tumor size (cm)*	
Mean	4.89
Range	2.5-12
Tumor classification*	
T2	61 (58.7%)
T3	35 (33.6%)
T4	8 (7.7%)
Lymph node status*	
N0	54 (52.0%)
N1	40 (38.5%)
N2	10 (9.5%)
Tumor type	
Invasive ductal	102 (98.1%)
Invasive lobular	2 (1.9%)
Type of 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%

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%	

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.

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[15]. 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 lymph-scintigraphy 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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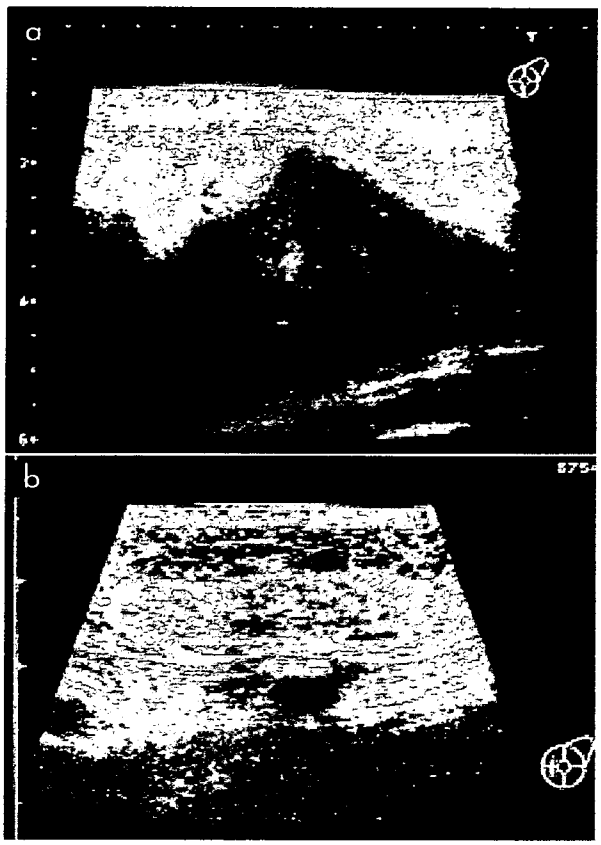

Fig 1. (a) US reveals a 7 cm breast mass with overlying skin thickening, edematous subcutaneous tissue. (b) US reveals no residual tumor following neoadjuvant chemotherapy.

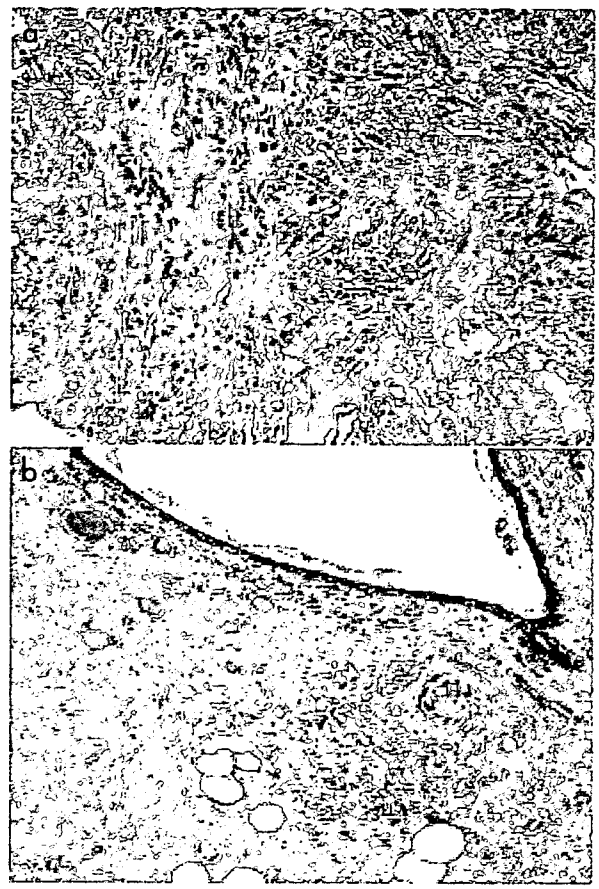

Fig 2. (a) Core needle biopsy reveals invasive ductal carcinoma, grade 3, nuclear grade 3. (b) No residual tumor is detected. The presence of inflammatory cells surrounding a duct with an increased number of enlarged capillary vessels, typical after tumor disappearance, is observed. (hematoxylin-eosin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

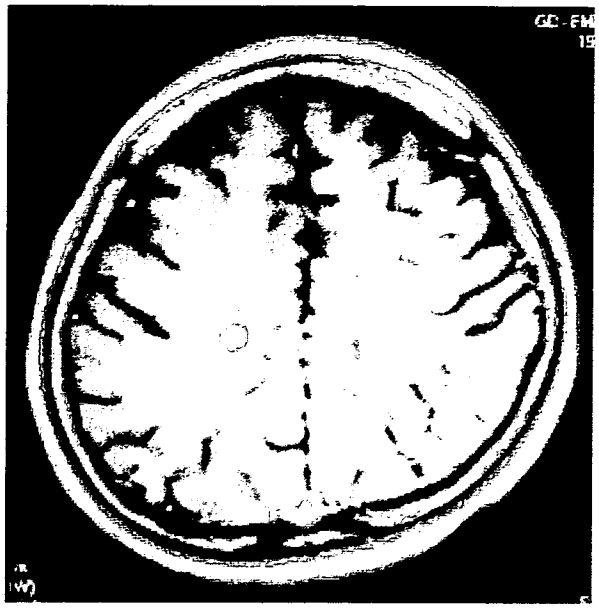

Fig 3. The metastatic lesions exhibited high signal intensity in the right temporal lobe by T1 weighted MRI.

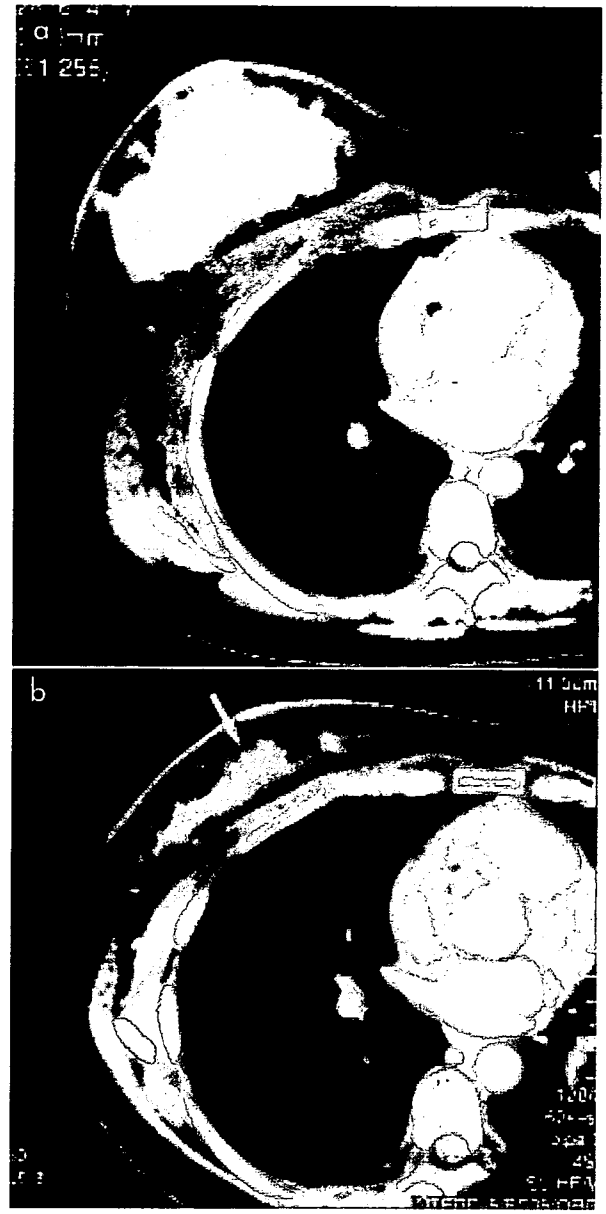

Fig 4. (a) CT shows a large right breast mass with overlying edematous subcutaneous tissue and thickened skin. This is not the early phase but late phase scan of breast CT, because only chest CT without an early phase scan was performed to detect distant metastasis instead of breast CT. (b) CT scan reveals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

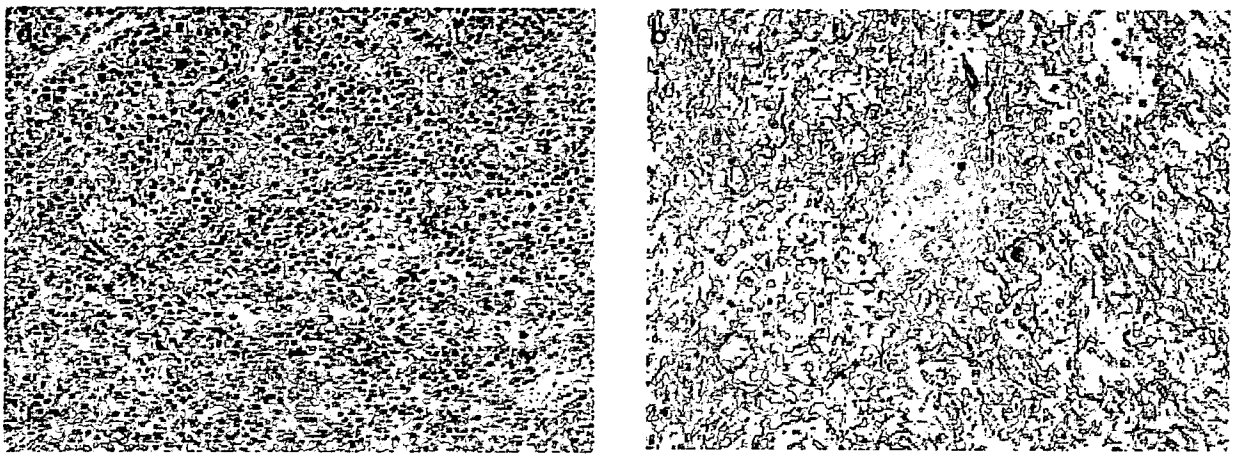

Fig 5. (a) Core needle biopsy reveals invasive ductal carcinoma, grade 3, nuclear grade 3. (b) No residual tumor is detected. Many foamy cells and a disturbance of the fiber rows after the disappearance of the tumor are observed (hematoxylin and eosin staining, ×100).

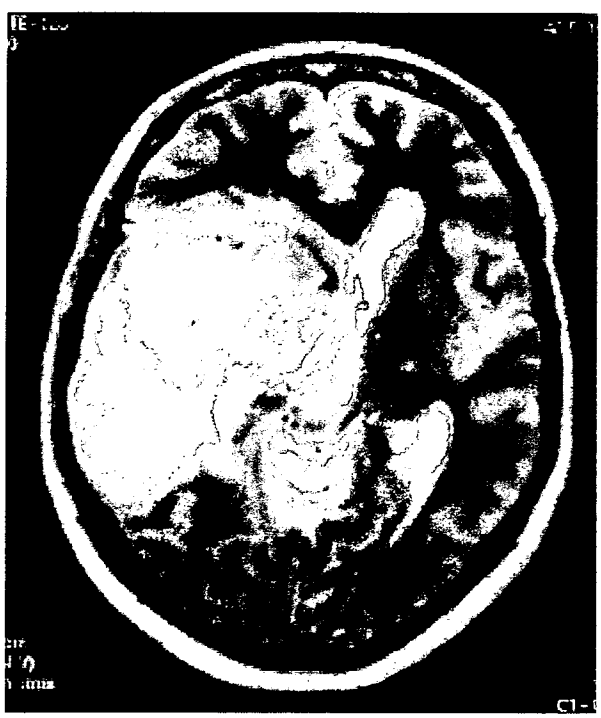

Fig 6. MRI demonstrates a tumor measuring 5 cm in diameter, with surrounding edema, in the right temporal lobe.

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, χ^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 χ^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