Theillet, C., Adelaide, J., Louason, G., Bonnet-Dorion, F., Jacquemier, J., Adnane, J., Longy, M., Katsaros, D., Sismondi, P., Gaudray, P., et al., 1993. FGFRI and PLAT genes and DNA amplification at 8p12 in breast and ovarian cancers. Genes Chromosom. Cancer 7, 219–226.

Valve, E.M., Tasanen, M.J., Ruohola, J.K., Harkonen, P.L., 1998. Activation of Fgf8 in S115 mouse mammary tumor cells is associated with genomic integration of mouse mammary tumor virus. Biochem. Biophys. Res. Commun. 250, 805–808.

Warren, S.M., Brunet, L.J., Harland, R.M., Economides, A.N., Longaker, M.T., 2003. The BMP antagonist noggin regulates cranial suture fusion. Nature 422, 625–629.

Watson, C.S., Campbell, C.H., Gametchu, B., 2002. The dynamic and elusive membrane estrogen receptor-alpha. Steroids 67, 429–437.

Yamamoto, T., Saatcioglu, F., Matsuda, T., 2002. Cross-talk between bone morphogenic proteins and estrogen receptor signaling. Endocrinology 143, 2635–2642.

Ye, L., Bokobza, S.M., Jiang, W.G., 2009. Bone morphogenetic proteins in development and progression of breast cancer and therapeutic potential (review). Int. J. Mol. Med. 24, 591–597.

Yoshimura, N., Sano, H., Hashiramoto, A., Yamada, R., Nakajima, H., Kondo, M., Oka, T., 1998. The expression and localization of fibroblast growth factor-1 (FGF-1) and FGF receptor-1 (FGFR-1) in human breast cancer. Clin. Immunol. Immunopathol. 89, 28–34.

Zammit, C., Coope, R., Gomm, J.J., Shousha, S., Johnston, C.L., Coombes, R.C., 2002. Fibroblast growth factor 8 is expressed at higher levels in lactating human breast and in breast cancer. Br. J. Cancer 86, 1097–1103.

Breast Cancer Res Treat (2011) 128:775–781
DOI 10.1007/s10549-011-1608-x

CLINICAL TRIAL

Effects of toremifene and anastrozole on serum lipids and bone metabolism in postmenopausal females with estrogen receptor–positive breast cancer: the results of a 2-year multicenter open randomized study

Keisei Anan · Shoshu Mitsuyama · Yasuhiro Yanagita · Morihiko Kimura · Hiroyoshi Doihara · Kansei Komaki · Mikihiro Kusama · Tadashi Ikeda

Received: 7 March 2011 / Accepted: 23 May 2011 / Published online: 3 June 2011

Abstract The potential long-term adverse effects on quality of life have to be considered when selecting agents for adjuvant hormonal treatment for postmenopausal patients with estrogen receptor–positive breast cancer. We performed a 2-year multicenter randomized study to assess the differences in the time course effects between toremifene (TOR) and anastrozole (ANA) on serum lipid profiles and bone metabolism. This study assessed the serum levels of triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), apolipoprotein A-1 (Apo A1), and apolipoprotein B (Apo B) as lipid profiles and bone-specific alkaline phosphatase (BAP) and the N-telopeptide of type-I collagen (NTX) as bone turnover markers in patients who received daily doses of 40 mg and 1 mg for TOR and ANA, respectively. A decreased serum level of TC, LDL-C, and Apo B was, respectively, observed at 6 months in 6.2, 12.9, and 13.8% of the patients who received TOR compared with the baseline. These decreases were maintained for at least 24 months. These lipid levels were not changed in those who received ANA. In the TOR patients, there was an increase in the serum level of HDL-C and Apo A1 at 6 months in 17.1 and 16.3%, respectively, which was maintained for at least 24 months, whereas these levels were almost stable in the patients who received ANA. Serum BAP decreased by 12.1% at 12 months and further decreased at 24 months and the serum NTX decreased by 22.0% at 6 months, which was maintained for at least 24 months in the patients who received TOR. In contrast, the serum BAP was increased by 26.0% at 6 months and by 29.2% at 12 months and the serum NTX increased by 21.3% at 24 months compared with baseline in those received ANA. However, the serum BAP increase was not significant at 24 months. TOR provides better effects than ANA in terms of lipid profiles and bone metabolism in postmenopausal females with early breast cancer.

Keywords Adjuvant therapy · High-density lipoprotein cholesterol · Low-density lipoprotein cholesterol · Bone-specific alkaline phosphatase · *N*-telopeptide of type-I collagen · Postmenopausal breast cancer

Abbreviations

TOR	Toremifene
ANA	Anastrozole
CHD	Coronary heart disease
TC	Total cholesterol
TG	Triglyceride
HDL-C	High-density lipoprotein cholesterol
LDL-C	Low-density lipoprotein cholesterol

K. Anan (✉) · S. Mitsuyama
Department of Surgery, Kitakyushu Municipal Medical Center, Kokurakita-ku, 2-1-1, Bashaku, Kitakyushu, Fukuoka 802-0077, Japan
e-mail: a.keisei@jcom.home.ne.jp

Y. Yanagita
Gunma Prefectural Cancer Center, Gunma, Japan

M. Kimura
Ota General Hospital, Gunma, Japan

H. Doihara
Okayama University Hospital, Okayama, Japan

K. Komaki
Breastopia Namba Hospital, Miyazaki, Japan

M. Kusama
Shinjuku Breast Center, Tokyo, Japan

T. Ikeda
Teikyo University Hospital, Tokyo, Japan

ApoA1	Apolipoprotein A-1
ApoB	Apolipoprotein B
BAP	Bone-specific alkaline phosphatase
NTX	*N*-telopeptide of type-I collagen

Introduction

Coronary heart disease (CHD) and osteoporosis-related fractures have become competing causes of death as females with breast cancer continue to live longer because of more effective therapies [1]. Although CHD accounts for one third of all deaths in postmenopausal females [2], the risk of recurrence among postmenopausal females with node-negative estrogen receptor–positive breast cancer is calculated to be less than 20% for 10 years, with 5 years of tamoxifen (TAM) therapy [3, 4]. The potential impact of adjuvant therapies on the competing risks is becoming an important consideration especially for breast cancer patients with low risk of recurrence.

Epidemiological studies in Europe, the United States, and Japan have shown that hypercholosterolemia is an important risk factor for CHD [5–7]. A high low-density lipoprotein cholesterol (LDL-C) level is an established important risk factor for CHD [8, 9]. In contrast, a high high-density lipoprotein cholesterol (HDL-C) level is associated with a decreased incidence of CHD [10]. Selective estrogen receptor modulator (SERM) therapy, including TAM, has been shown to induce beneficial effects on serum lipids profiles [11].

In addition to CHD, osteoporosis and its related fractures are common in older individuals and lead to premature mortality, loss of function and independence, and a reduced quality of life. Estimates indicate that half of Americans age 50 years or older are at risk for osteoporotic fractures, and the rates for females are higher than for males [12]. SERMs have also been reported to have beneficial effect in bone metabolism [11].

Toremifene (TOR) is a SERM, and its efficacy has been demonstrated to be similar to that of TAM with regard to disease-free survival and overall survival in adjuvant therapy [13, 14] for breast cancer, as well as efficacy for metastatic disease. We have previously shown that TOR is superior to TAM in terms of the serum lipid profile [15]. TOR has also been suggested to have a preventive effect on CHD.

Anastrozole (ANA) is a non-steroidal third-generation aromatase inhibitor being widely used for the treatment of postmenopausal patients with estrogen receptor–positive diseases [16]. Several randomized clinical trials in adjuvant settings have shown the superiority of ANA to TAM with regard to relapse-free survival, with similar overall survival [16, 17]. However, increased risks of osteoporosis and fracture have been reported in patients who received ANA.

Because there is increasing evidence that aromatase inhibitors, such as ANA, play an important role as a component of adjuvant therapy for postmenopausal females with breast cancer [18, 19], the long-term hazards of the impact of such agents on lipid and bone metabolism and the resulting differences in quality of life need to be considered when individualizing adjuvant treatment. We performed a randomized 2-year study to assess the time course effect of TOR and ANA on serum lipid profiles and bone metabolism in postmenopausal patients with early breast cancer.

Patients and methods

Study design and drug administration

This study was a multicenter open randomized study to assess serum lipid profiles and bone turnover parameters in patients receiving the approved clinical doses of TOR and ANA. The protocol was approved by the research ethics committee of each participating institution. Patients were provided written information about the study and gave written informed consent before participation. The block randomization method was used for randomization.

The study medication was commenced by everyday oral administration at doses of 40 mg and 1 mg for TOR and ANA, respectively, which are the standard doses used for adjuvant treatment. During the study, other treatments for breast cancer were prohibited except for radiotherapy after breast-conserving surgery.

Eligibility

The study subjects were postmenopausal patients with primary breast cancer who received adjuvant hormonal therapy in one of six hospitals. *Postmenopausal status* was defined as a more than 1-year duration of amenorrhea and both a serum E2 level of 20 pg/ml or less and a serum FSH level of 20 mIU/ml or more. In these patients, the tumor diameter was 5 cm or less, and a mastectomy or breast-conserving surgery was performed for curative intent. Histological examination confirmed breast cancer with no lymph node metastasis or with three of fewer metastatic lymph nodes. Immunohistological reactions were positive for the estrogen receptor or progesterone receptor in at least 10% of the cancer cells in the specimens. The performance status established by the World Health Organization was evaluated as 0 or 1. We excluded patients with bilateral breast cancer, inflammatory breast cancer, double cancers, heart disease, liver dysfunction, renal dysfunction, and

bone marrow dysfunction. Patients receiving agents for hyperlipidemia or osteoporosis were also excluded from the study.

Lipid analysis

The first blood sample was collected from patients before breakfast. In the afternoon, blood was collected before lunch, with an interval of at least 3 h from breakfast until blood collection. Immediately after blood collection, serum was isolated. The levels of triglyceride (TG), total cholesterol (TC), HDL-C, LDL-C, apolipoprotein A-1 (Apo A1), and apolipoprotein B (Apo B) were measured before the administration of a hormonal agent and 6, 12, and 24 months after the commencement of the study medication. The assays were done at BML Inc. (Tokyo). The TG, TC, and LDL-C levels were measured by enzymatic assays, HDL-C was measured by a direct method, and the Apo A1 and Apo B levels were determined by a turbidimetric immunoassay [20]. The abnormal levels of TC, LDL-C, HDL-C, and TG were established as $\geq$220, $\geq$140, $\leq$40, and $\geq$150 mg/dl based on the reference values described in the guidelines for hyperlipidemia treatment in Japan [21]. Patients persistently showing a cholesterol level of 350 mg/dl or more and an LDL-C level of 200 mg/dl or more for 1 year or more during treatment were regarded as dropout cases, and agents for hyperlipidemia were administered. Diet/exercise therapies were prescribed and explained if necessary.

Bone turnover analysis

The levels of bone-specific alkaline phosphatase (BAP) and *N*-telopeptide of type-I collagen (NTX) as bone turnover markers were measured before administration of the hormonal agent and 6, 12, and 24 months after the commencement of the study medication. BAP was measured by an enzyme immunoassay, and NTX was measured by an ELISA at BML Inc., Tokyo.

Sample size and statistical analysis

Based on the data obtained in our previous study [15] showing that the serum HDL-C level was increased by 13.4 $\pm$ 11.3 (mean $\pm$ SD) after 1-year administration of TOR, and on an assumption that the serum HDL-C level is not changed by ANA administration, it was determined that having at least 12 subjects in each group would give 80% power at the 5% significance level to detect a difference between the groups. To allow for dropouts, the sample size was increased to 30 in each treatment arm.

Because the values of TG, TC, HDL-C, LDL-C, Apo A1, and Apo B were approximately normal, the mean was the preferred summary statistic. Serial changes in these variables in each group were analyzed using a paired *t*-test. The serial changes of BAP and NTX were analyzed by Wilcoxon signed ranks test. The SAS software program was used for all analyses.

Results

Sixty-nine patients enrolled in this study were randomly assigned to the TOR group ($n = 36$) and the ANA group ($n = 33$) for adjuvant therapy (Fig. 1). None of the patients received adjuvant chemotherapy. There were no significant differences in background factors between the two groups (Table 1). The median age of the patients in the TOR group was 62.5 years old and that in the ANA group was 60.0 years old. The median body mass index was 23.2 and 24.0 in the two groups, respectively. Changes in serum lipid profiles and in bone metabolic markers were observed in both groups after commencement of the hormonal agents (Table 2, Fig. 2).

Effects on lipid metabolism

The serum TC, TG, LDL-C, and Apo B levels significantly decreased in patients who received TOR, whereas these levels were not significantly changed in those who received ANA. A decrease in the TC, LDL-C, and Apo B levels at 6 months of 6.2% ($P < 0.005$), 12.9% ($P < 0.005$), and 13.8% ($P < 0.005$), respectively, was noted. These decreases were maintained for at least 24 months, with the values being 7.2% lower for TC ($P < 0.005$), 11.3% lower for LDL-C ($P < 0.005$), and 15.7% lower in Apo B ($P < 0.005$) compared with the values at baseline. The serum HDL-C and Apo A1 levels significantly increased in those who received TOR. The increase started at 6 months with a 17.1% increase ($P < 0.005$) in HDL-C and a 16.3% increase ($P < 0.005$) in Apo A1, and the increases were maintained at 24 months, with a 22.0% ($P < 0.005$) and 18.4% ($P < 0.005$) increase, respectively, compared with the baseline values. In contrast, the level of HDL-C was almost stable, and there was only a transient increase in

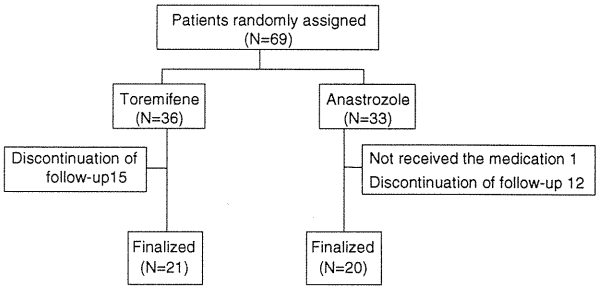

Fig. 1 Patient enrollment, randomization, and treatment

Table 1 The baseline characteristics of the study participants, according to the treatment group

	TOR		ANA	
	n	Average (range)	*n*	Average (range)
Age (year)	36	62.5 (44–87)	33	60.0 (50–86)
Height (cm)	35	153.5 (143–164)	33	151.0 (141–164)
Weight (kg)	35	55.0 (39–73)	33	43.3 (39–70)
BMI (kg/m^2)	35	23.2 (16.6–32.0)	33	24.0 (18.1–31.0)

TOR toremifene, *ANA* anastrozole, *BMI* body mass index

Table 2 Percentage changes from baseline for markers of lipid and bone metabolism at 6, 12, and 24 months after treatment with TOR and ANA

		6 months			12 months			24 months		
		n	Mean (95% CI)	*P* value	*n*	Mean (95% CI)	*P* value	*n*	Mean (95% CI)	*P* value
TC	TOR	28	−6.2 (−10.4, −2.1)	<0.005	32	−8.3 (−11.7, −4.8)	<0.005	21	−7.2 (−11.2, −3.1)	<0.005
	ANA	29	0.1 (−5.3, 5.6)	0.95	29	1.9 (−2.7, 6.6)	0.40	20	−1.2 (−8.5, 6.0)	0.75
HDL-C	TOR	28	17.1 (8.5, 25.6)	<0.005	32	17.0 (6.8, 27.3)	<0.005	21	22.0 (9.5, 34.5)	<0.005
	ANA	29	0.8 (−4.1, 5.8)	0.72	29	3.2 (−1.1, 7.5)	0.14	20	2.7 (−3.4, 8.9)	0.36
LDL-C	TOR	28	−12.9 (−19.7, −6.2)	<0.005	32	−10.2 (−14.7, −5.7)	<0.005	21	−11.3 (−17.8, −4.8)	<0.005
	ANA	29	1.4 (−6.7, 9.6)	0.89	29	2.8 (−5.0, 10.7)	0.46	20	−1.8 (−13.2, 9.4)	0.73
TG	TOR	28	10.2 (−11.9, 32.4)	0.35	32	−8.6 (−21.5, 4.3)	0.18	21	−12.3 (−28.7, 4.0)	0.13
	ANA	29	11.1 (−11.4, 33.7)	0.32	29	6.3 (−16.4, 29.0)	0.57	20	24.9 (−9.8, 59.6)	0.14
ApoA1	TOR	28	16.3 (11.3, 21.2)	<0.005	32	15.2 (8.8, 21.6)	<0.005	21	18.4 (9.5, 27.3)	<0.005
	ANA	29	1.7 (−1.5, 5.0)	0.29	29	4.4 (1.4, 7.5)	<0.05	20	−0.9 (−12.3, 10.5)	0.87
ApoB	TOR	28	−13.8 (−19.0, −8.5)	<0.005	32	−13.7 (−18.3, −9.1)	<0.005	21	−15.7 (−20.6, −10.8)	<0.005
	ANA	29	−1.3 (−6.8, 4.2)	0.63	29	−1.2 (−7.5, 4.9)	0.68	20	−4.6 (−13.5, 4.1)	0.28
BAP	TOR	28	1.0 (−6.3, 8.5)	0.72	32	−12.1 (−21.5, −2.7)	<0.05	21	−37.4 (−46.9, −27.8)	<0.005
	ANA	29	26.0 (15.9, 36.1)	<0.005	29	29.2 (15.1, 43.2)	<0.005	20	10.3 (−10.1, 30.7)	0.43
NTX	TOR	28	−22.0 (−31.5, −12.5)	<0.005	32	−21.5 (−32.4, −10.6)	<0.005	21	−15.9 (−27.0, −4.8)	<0.05
	ANA	29	9.3 (−3.0, 21.8)	0.30	29	8.4 (−5.2, 22.1)	0.32	20	21.3 (3.6, 39.0)	<0.05

CI confidence interval, *TOR* toremifene, *ANA* anastrozole, *TC* total cholesterol, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *TG* triglyceride, *ApoA1* apolipoprotein A-1, *ApoB* apolipoprotein B, *BAP* bone-specific alkaline phosphatase, *NTX* *N*-telopeptide of type-I collagen, *P* value compared with "baseline"

serum Apo A1 by 4.4% ($P < 0.05$) at 12 months in those who received ANA.

Effects on bone turnover

The serum BAP significantly decreased by 12.1% ($P < 0.05$) at 12 months and further decreased by 37.4% ($P < 0.005$) at 24 months in patients who received TOR. Similarly, the serum NTX level was significantly decreased by 22.0% ($P < 0.005$) at 6 months and the decreased level was maintained at 24 months. In contrast to the effects of TOR, the serum BAP was significantly increased by 26.0% ($P < 0.005$) at 6 months and by 29.2% ($P < 0.005$) at 12 months in those who received ANA, however, this increase was not significant at 24 months. The serum NTX level was significantly increased by 21.3% ($P < 0.05$) at 24 months in those who received ANA.

Discussion

CHD and osteoporosis induced by bone loss are major problems for postmenopausal females. The long-term risks and effects of hormonal agents on patient quality of life have to be considered when prescribing the adjuvant treatment for patients with early breast cancer with a low risk of recurrence. In this head-to-head analysis, we showed superior time course effects of TOR compared with ANA with regard to serum lipid profiles and bone metabolism for up to 2 years in postmenopausal patients with

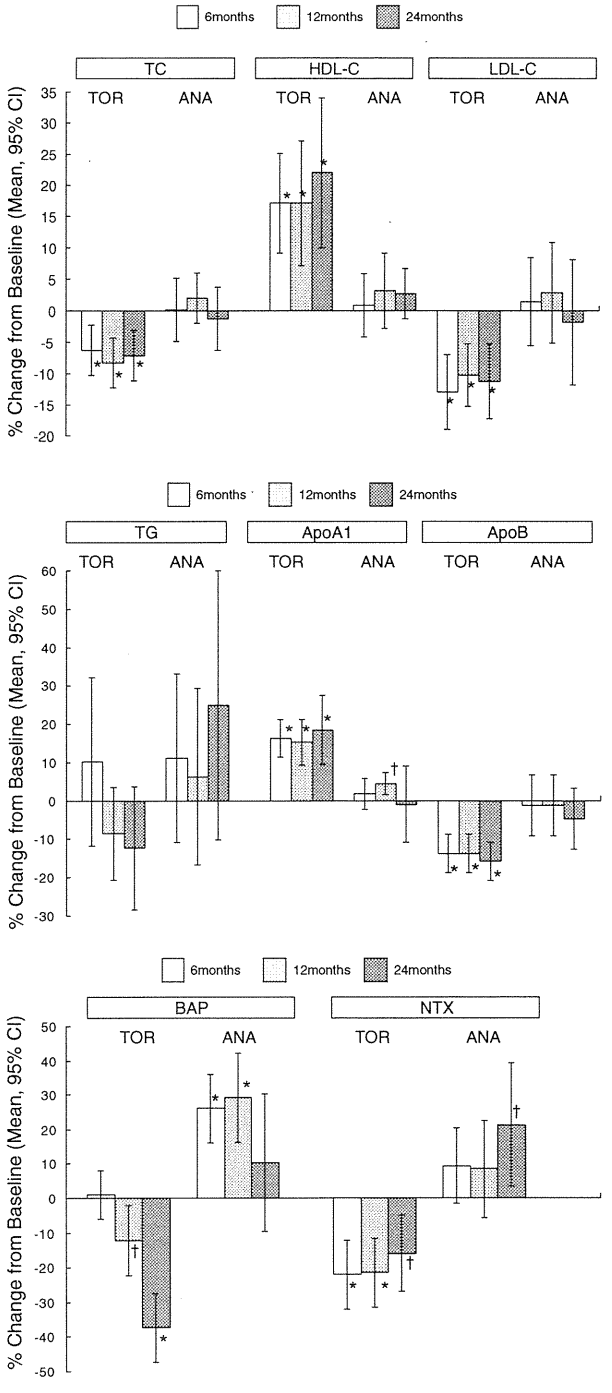

Fig. 2 Percentage changes presented by bar graphs from baseline for markers of lipid and bone metabolism. *CI* confidence interval, *TOR* toremifene, *ANA* anastrozole, *TC* total cholesterol, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *TG* triglyceride, *ApoA1* apolipoprotein A-1, *ApoB* apolipoprotein B, *BAP* bone-specific alkaline phosphatase, *NTX N*-telopeptide of type-I collagen, *P* value compared with "baseline" $*P < 0.005$, $\dagger P < 0.05$

hormone receptor–positive breast cancer who received adjuvant treatment.

A high-LDL-C level is an established important risk factor for CHD [8, 9]. In contrast, a high-HDL-C level is associated with a decreased incidence of CHD [10]. Apo A1 activates lecithin cholesterol acryltransferase and leads to the maturation of HDL-C. Mature HDL-C returns cholesterol in the peripheral tissues to the liver. A high incidence of hypo-HDL-emia is known to be caused by hypertriglyceridemia. Apo B promotes the binding of LDL receptors to LDL. In this study, the serum TC, TG, LDL-C, and Apo B all significantly decreased and the serum HDL-C and Apo A1 significantly increased in patients who received TOR, suggesting that the agent has beneficial effects for preventing CHD in females.

The data from randomized clinical trials in Scotland [22] and Sweden [23] that investigated the efficacy of TAM in patients with breast cancer have shown that the agent also reduced the risk of CHD. In a Scottish adjuvant TAM trial, the incidence of myocardial infarction was reduced by 48% in the TAM group compared with that in the control group that did not receive TAM. In the data from Swedish trial, the mortality from CHD was reduced by 33% in the 5-year TAM treatment group compared with that in the 2-year treatment group. Of note, the reduction in mortality was even significant in the period beyond 7-years for diagnosis, whereas the reduction in mortality was smaller and not significant during the first 7 years. Death from CHD has been suggested as a late event leading to the need for a long observation. We previously reported the superiority of TOR over TAM with regard to the serum lipid profile during a 1-year treatment [15]. In this study, we showed that TOR maintained a preferable serum lipid profile over the 2-years treatment of postmenopausal females with early breast cancer, whereas ANA did not lead to any remarkable changes in the serum lipid profile.

Bone metabolism is of significance when making a decision about adjuvant treatment of postmenopausal females. In most postmenopausal females, both BAP (as a bone formation marker) and NTX (as a bone resorption marker) are increased compared with premenopausal females because of the decrease in serum estrogen after menopause. In this study, the bone turnover decreased after administration of TOR and increased after administration of ANA in postmenopausal females with early breast cancer, suggesting the beneficial effects of TOR for preventing osteoporosis.

The effects of raloxifene (RAL), another SERM that is being prescribed for the prevention and treatment of postmenopausal osteoporosis, on bone are well established [24–27]. In postmenopausal females with osteoporosis,

treatment with RAL decreased bone turnover markers by 30–40% after 1 year, and decreased the incidence of vertebral fractures by 30–50%. In our study, treatment with TOR at 40 mg significantly decreased the serum BAP by 37% and NTX by 22%. The effects of TOR at this dose are significant, but it may be a milder bone agonist compared with RAL. However, RAL was reported to be associated with an increased risk of fatal stroke and venous thromboembolism in clinical trials, such as the RUTH trial [25, 28, 29] as in the treatments with TAM, which has been shown to be associated with an increased incidence of these adverse effects in the patients receiving TAM as adjuvant therapy for breast cancer. From the limited available data [30, 31], the incidence of stroke and venous thromboembolism with TOR appears to be lower than that of patients treated with TAM, and the incidence of pulmonary embolism and deep vein thrombosis appears to be lower than that of patients treated with RAL. It is possible that the balance between a drug's prothrombotic and antiatherosclerotic effects is determined by such variables as the type of drugs, dosage, and possible durations of SERM treatment. In fact, lasofoxifen, another SERM tested in postmenopausal females with osteoporosis, was associated with a reduced risk of stroke but with an increased risk of venous thromboembolic events [32]. This issue should therefore be further studied to determine the agent(s) providing the best balance between anti-cancer, anti-osteoporotic, and anti-thrombotic effects.

In conclusion, both TOR and ANA are effective hormonal agents in the treatment of postmenopausal females with early breast cancer. However, TOR provides better effects than ANA in terms of the lipid profile and bone metabolism for at least 2 years after initiating treatment.

Acknowledgments This study received financial support from Nihonkayaku Co., Ltd. Japan. The following investigators and institutions also participated in the trial: Nobuaki Sato, Niigata Cancer Center Hospital, Niigata, Japan; Muneaki Sano, Niigata Breast Exam Center, Niigata, Japan; and Keisuke Miyauchi, Miyauchi Clinic, Hyogo, Japan

Conflict of interest None.

References

1. Chapman JA, Meng D, Shepherd L, Parulekar W, Ingle JN, Muss HB, Palmer M, Yu C, Goss PE (2008) Competing causes of death from a randomized trial of extended adjuvant endocrine therapy for breast cancer. J Natl Cancer Inst 100(4):252–260
2. Wenger NK (1997) Coronary heart disease: an older woman's major health risk. BMJ 315(7115):1085–1090
3. Kennecke H, McArthur H, Olivotto IA, Speers C, Bajdik C, Chia SK, Ellard S, Norris B, Hayes M, Barnett J, Gelmon KA (2008) Risk of early recurrence among postmenopausal women with estrogen receptor-positive early breast cancer treated with adjuvant tamoxifen. Cancer 112(7):1437–1444
4. Kennecke HF, Olivotto IA, Speers C, Norris B, Chia SK, Bryce C, Gelmon KA (2007) Late risk of relapse and mortality among postmenopausal women with estrogen responsive early breast cancer after 5 years of tamoxifen. Ann Oncol 18(1):45–51
5. Kannel WB, Castelli WP, Gordon T, McNamara PM (1971) Serum cholesterol, lipoproteins, and the risk of coronary heart disease. The Framingham study. Ann Intern Med 74(1):1–12
6. Martin MJ, Hulley SB, Browner WS, Kuller LH, Wentworth D (1986) Serum cholesterol, blood pressure, and mortality: implications from a cohort of 361, 662 men. Lancet 2(8513):933–936
7. Matsuzawa Y, Tarui S (1989) Recent trend in the research of hyperlipidemia in Japan (1). Profiles of primary hyperlipidemia. Nippon Naika Gakkai Zasshi 78(10):1396–1399
8. National Cholesterol Education Program (1994) Second report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II). Circulation 89(3):1333–1445
9. Carleton RA, Dwyer J, Finberg L, Flora J, Goodman DS, Grundy SM, Havas S, Hunter GT, Kritchevsky D, Lauer RM et al (1991) Report of the Expert Panel on Population Strategies for Blood Cholesterol Reduction. A statement from the National Cholesterol Education Program, National Heart, Lung, and Blood Institute, National Institutes of Health. Circulation 83(6):2154–2232
10. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR (1977) High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. Am J Med 62(5): 707–714
11. Riggs BL, Hartmann LC (2003) Selective estrogen-receptor modulators: mechanisms of action and application to clinical practice. N Engl J Med 348(7):618–629
12. Jones G, Nguyen T, Sambrook PN, Kelly PJ, Gilbert C, Eisman JA (1994) Symptomatic fracture incidence in elderly men and women: the Dubbo Osteoporosis Epidemiology Study (DOES). Osteoporos Int 4(5):277–282
13. Lewis JD, Chagpar AB, Shaughnessy EA, Nurko J, McMasters K, Edwards MJ (2010) Excellent outcomes with adjuvant toremifene or tamoxifen in early stage breast cancer. Cancer 116(10): 2307–2315
14. Pagani O, Gelber S, Price K, Zahrieh D, Gelber R, Simoncini E, Castiglione-Gertsch M, Coates AS, Goldhirsch A (2004) Toremifene and tamoxifen are equally effective for early-stage breast cancer: first results of International Breast Cancer Study Group Trials 12–93 and 14–93. Ann Oncol 15(12):1749–1759
15. Kusama M, Miyauchi K, Aoyama H, Sano M, Kimura M, Mitsuyama S, Komaki K, Doihara H (2004) Effects of toremifene (TOR) and tamoxifen (TAM) on serum lipids in postmenopausal patients with breast cancer. Breast Cancer Res Treat 88(1):1–8
16. Cuzick J, Sestak I, Baum M, Buzdar A, Howell A, Dowsett M, Forbes JF (2010) Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 10-year analysis of the ATAC trial. Lancet Oncol 11(12):1135–1141
17. Sanford M, Plosker GL (2008) Anastrozole: a review of its use in postmenopausal women with early-stage breast cancer. Drugs 68(9):1319–1340
18. Gradishar WJ (2010) Adjuvant endocrine therapy for early breast cancer: the story so far. Cancer Invest 28(4):433–442
19. Dent SF, Gaspo R, Kissner M, Pritchard KI (2011) Aromatase inhibitor therapy: toxicities and management strategies in the treatment of postmenopausal women with hormone-sensitive early breast cancer. Breast Cancer Res Treat 126(2):295–310
20. Labeur C, Shepherd J, Rosseneu M (1990) Immunological assays of apolipoproteins in plasma: methods and instrumentation. Clin Chem 36(4):591–597
21. Japan Atherosclerosis Society (2007) Japan atherosclerosis society guidelines for prevention of atherosclerotic cardiovascular diseases. Japan Athelosclerosis Society

22. McDonald CC, Alexander FE, Whyte BW, Forrest AP, Stewart HJ (1995) Cardiac and vascular morbidity in women receiving adjuvant tamoxifen for breast cancer in a randomised trial. The Scottish Cancer Trials Breast Group. BMJ 311(7011):977–980
23. Nordenskjold B, Rosell J, Rutqvist LE, Malmstrom PO, Bergh J, Bengtsson NO, Hatschek T, Wallgren A, Carstensen J (2005) Coronary heart disease mortality after 5 years of adjuvant tamoxifen therapy: results from a randomized trial. J Natl Cancer Inst 97(21):1609–1610
24. Delmas PD, Bjarnason NH, Mitlak BH, Ravoux AC, Shah AS, Huster WJ, Draper M, Christiansen C (1997) Effects of raloxifene on bone mineral density, serum cholesterol concentrations, and uterine endometrium in postmenopausal women. N Engl J Med 337(23):1641–1647
25. Ettinger B, Black DM, Mitlak BH, Knickerbocker RK, Nickelsen T, Genant HK, Christiansen C, Delmas PD, Zanchetta JR, Stakkestad J, Gluer CC, Krueger K, Cohen FJ, Eckert S, Ensrud KE, Avioli LV, Lips P, Cummings SR (1999) Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with raloxifene: results from a 3-year randomized clinical trial Multiple Outcomes of Raloxifene Evaluation (MORE) Investigators. JAMA 282(7):637–645
26. Johnston CC Jr, Bjarnason NH, Cohen FJ, Shah A, Lindsay R, Mitlak BH, Huster W, Draper MW, Harper KD, Heath H 3rd, Gennari C, Christiansen C, Arnaud CD, Delmas PD (2000) Long-term effects of raloxifene on bone mineral density, bone turnover, and serum lipid levels in early postmenopausal women: three-year data from 2 double-blind, randomized, placebo-controlled trials. Arch Intern Med 160(22):3444–3450
27. Lufkin EG, Whitaker MD, Nickelsen T, Argueta R, Caplan RH, Knickerbocker RK, Riggs BL (1998) Treatment of established postmenopausal osteoporosis with raloxifene: a randomized trial. J Bone Miner Res 13(11):1747–1754
28. Barrett-Connor E, Cox DA, Song J, Mitlak B, Mosca L, Grady D (2009) Raloxifene and risk for stroke based on the framingham stroke risk score. Am J Med 122(8):754–761
29. Barrett-Connor E, Mosca L, Collins P, Geiger MJ, Grady D, Kornitzer M, McNabb MA, Wenger NK (2006) Effects of raloxifene on cardiovascular events and breast cancer in postmenopausal women. N Engl J Med 355(2):125–137
30. Harvey HA, Kimura M, Hajba A (2006) Toremifene: an evaluation of its safety profile. Breast 15(2):142–157
31. Holli K, Valavaara R, Blanco G, Kataja V, Hietanen P, Flander M, Pukkala E, Joensuu H (2000) Safety and efficacy results of a randomized trial comparing adjuvant toremifene and tamoxifen in postmenopausal patients with node-positive breast cancer Finnish Breast Cancer Group. J Clin Oncol 18(20):3487–3494
32. Cummings SR, Ensrud K, Delmas PD, LaCroix AZ, Vukicevic S, Reid DM, Goldstein S, Sriram U, Lee A, Thompson J, Armstrong RA, Thompson DD, Powles T, Zanchetta J, Kendler D, Neven P, Eastell R (2010) Lasofoxifene in postmenopausal women with osteoporosis. N Engl J Med 362(8):686–696

Cancer Chemother Pharmacol (2011) 67:911–917
DOI 10.1007/s00280-010-1371-4

ORIGINAL ARTICLE

Predictive factors for the effectiveness of neoadjuvant chemotherapy and prognosis in triple-negative breast cancer patients

Hiroko Masuda · Norikazu Masuda · Yoshinori Kodama · Masami Ogawa · Michiko Karita · Jun Yamamura · Kazunori Tsukuda · Hiroyoshi Doihara · Shinichiro Miyoshi · Masayuki Mano · Shoji Nakamori · Toshimasa Tsujinaka

Received: 24 January 2010 / Accepted: 14 May 2010 / Published online: 1 July 2010

Abstract
Purpose Triple-negative breast cancers (TNBCs) do not derive benefit from molecular-targeted treatments such as endocrine therapy or anti-HER2 therapy because they lack those molecular targets. On the other hand, TNBCs have been shown to respond to neoadjuvant chemotherapy (NAC). In this study, we analyzed TNBC patients who were treated with NAC at Osaka National Hospital over a recent 5-year period to clarify the predictive factors for NAC and prognostic factors.
Patients and methods Thirty-three TNBC patients underwent sequential NAC with anthracycline (FEC100: 5FU 500 mg/m^2, epirubicin 100 mg/m^2, and cyclophosphamide 500 mg/m^2/q3w, 4 courses) and taxanes (paclitaxel 80 mg/m^2/qw, 12 courses or docetaxel 75 mg/m^2/q3w, 4 courses) from May 2003 to July 2008. Pre-therapeutical and surgical specimens were studied for expressions of ER, PgR, HER-2, EGFR, cytokeratin 5/6, Ki-67, p53 and androgen receptor by immunohistochemistry (IHC). We analyzed clinicopathological factors and molecular markers in regard to the response to NAC and prognosis.
Results Pathological complete response (pCR) was achieved in 12 TNBC patients (36%). The pCR rate in the basal-like phenotype was significantly lower than in the non-basal-like phenotype (23 vs. 64%, respectively: $P = 0.02$). High pre-operative expressions of Ki-67 (≥50%) and HER-2 (2+) were considered as predictive factors for a better response from NAC. Pre-operative Ki-67 expression showed a significant correlation with disease-free survival (DFS) and a lower expression of Ki-67 (<50%) after NAC was favorable for DFS among non-pCR patients.
Conclusions A non-basal-like phenotype and higher expressions of Ki-67 and HER-2 (2+) were favorable factors for NAC. However, a higher expression of Ki-67 on the surgical specimen after NAC was also a poor prognostic factor.

Keywords Triple-negative breast cancer · Neoadjuvant chemotherapy · Pathological complete response · Ki-67 · Basal-like phenotype

Abbreviations

TNBC	Triple negative breast cancer
NAC	Neoadjuvant chemotherapy
pCR	Pathological complete response
ER	Estrogen receptor
PgR	Progesterone receptor
AR	Androgen receptor
EGFR	Epidermal growth factor receptor
CK	Cytokeratin

H. Masuda · N. Masuda · M. Ogawa · M. Karita · J. Yamamura · S. Nakamori · T. Tsujinaka
Department of Surgery,
National Hospital Organization Osaka National Hospital,
Osaka, Japan

Y. Kodama · M. Mano
Department of Pathology,
National Hospital Organization Osaka National Hospital,
Osaka, Japan

H. Masuda (✉) · K. Tsukuda · H. Doihara · S. Miyoshi
Department of Cancer and Thoracic Surgery,
Okayama University Graduate School of Medicine,
Dentistry and Pharmaceutical Sciences,
2-5-1 Shikatacho Kitaku,
Okayama 700-8558, Japan
e-mail: masuhiro123@hotmail.com

Introduction

Triple-negative breast cancers (TNBCs) are characterized by the lack of expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER-2). These cancers occur in ~20–25% of all breast cancers and are associated with an unfavorable prognosis. They derive no benefit from molecularly targeted treatments such as endocrine therapy or trastuzumab [1]. Therefore, identifying appropriate treatments for TNBC is an important issue.

Recent precise gene expression analysis revealed that TNBC is a heterogeneous group of tumors. One of the subgroups is a basal-like subtype, which is characterized by similar gene expression as the basal/myoepithelial cells of the normal breast [1–5]. Basal-like breast cancer has also been identified with immunohistochemical (IHC) staining of basal markers, such as cytokeratins (CKs) and epithelial growth factor receptor (EGFR). TNBCs without these basal markers are classified as non-basal-like subtypes, which are rare breast cancers, and classifications based on gene expression have not been clarified yet. Non-basal-like tumors are also reported to have a better prognosis than basal-like phenotypes [6, 7]. Because of the lack of targeted therapies and their aggressive clinical behaviors, TNBCs are relevant groups to be investigated for their characteristics. Though TNBCs are considered to have poor prognosis generally, TNBCs have been shown to be chemosensitive.

Neoadjuvant chemotherapy (NAC) in primary breast cancers has been shown to produce an outcome equivalent to that of adjuvant chemotherapy [8, 9]. Patients who show a pathological complete response (pCR) in the primary tumors after NAC have a better prognosis [10]. The pathological responses are important prognostic parameters and can be used as surrogate parameters for clinical outcome, so we analyzed the effects of clinicopathological factors as well as immunohistochemical factors on pathological responses after NAC. However, the paradox that TNBC and HER-2 positive subtypes showed higher chemosensitivity but worse survival due to higher relapse after chemotherapy is also known well [10, 11].

Several biological markers have been proposed as prognostic characteristics in breast cancers. ER, PR and HER-2 are such biological markers as well as being therapeutic markers and Ki-67, p53 and androgen receptor (AR) are shown to be associated with prognosis [12–16]. AR is known to be present in the majority of primary and metastatic invasive breast tumors and is often co-expressed with ER and PR in these tumors. Though little is known about the role of AR in hormonal response, AR expression has been shown to be associated with a better outcome for untreated breast cancer patients [14]. Ki-67 is a nuclear antigen expressed in the G1, S, and G2 phases but not in the G0 or resting phase of the cell cycle. Ki-67 has been established as a proliferation marker in breast cancers and high proliferation activity has been found to have predictive value for the response to NAC [17]. Also p53 expression status has been used as a predictive factor for response to systemic therapy, because tumor cells with non-functional p53 do not respond to systemic therapy due to a failure in apoptosis [13, 15].

Because chemotherapy is the only treatment other than surgery for TNBC, the definition of clinical markers in regard to chemotherapeutic response and prognosis is very important. However, there are still few studies focusing on TNBC. In this study, we analyzed clinicopathological factors, phenotypes, and molecular markers of TNBC in regard to the response to NAC and prognosis.

Patients and methods

Patients and neoadjuvant chemotherapy

One hundred and 63 breast cancer patients underwent NAC with a sequential regimen containing anthracycline (FEC100: 5FU 500 mg/m^2, epirubicin 100 mg/m^2, cyclophosphamide 500 mg/m^2/q3w, 4 courses) and taxanes (paclitaxel 80 mg/m^2/qw, 12 courses or docetaxel 75 mg/m^2/q3w, 4 courses) at Osaka National Hospital (Osaka, Japan) from May 2003 to July 2008. The criteria for entry were invasive breast cancer patients from 20 to 70 years old with any T and N0-2 disease, who were diagnosed histologically, were absent from distant metastasis and with normal organ functions. Thirty-three patients (20%) among 163 breast cancer patients were identified as TNBCs. The clinical evaluation of the response to NAC was determined by clinical findings, CT and MRI examinations according to RECIST. All patients were included in clinical trials approved by an institutional review board and asked for written informed consent.

Immunohistochemistry

Pre-therapeutical specimens were obtained by the 14G-needle biopsy in all cases and pathological examinations using standard hematoxylin and eosin staining were carried out. Immunohistochemical evaluation for ER, PgR, HER-2, EGFR, CK5/6, Ki-67, p53 and AR in tissue sections were detected using antibodies (ER:Cat.No. 760-2596I, PgR: 760-2816, HER-2:760-2901, EGFR:790-2988, CK5/6:960-4253, Ki-67:760-2910, p53:760-2912, Ventana Japan, Yokohama, Japan, AR:M3562, Dako Japan, Tokyo, Japan). Visualization of the bound antibodies was performed using a DAKO EnvisionTM + System (Dako Japan Inc., Tokyo, Japan) according to the manufacturer's instructions. Positive

cell rates (%) of ER and PgR were determined as a ratio of positive cells to total cancer cells and a value of 10% or higher were rated as positive [18, 19]. HER-2 expression was defined as (0) to (3+) based on positive cell rates and the intensity of IHC staining. Tumors showing weak overexpression (2+) of HER-2 were also tested by the fluorescence in situ hybridization (FISH) method to clarify the gene amplification of the *HER-2* gene. The *HER-2* gene is visualized as green fluorescent grains and a control of centromere 17 is visualized as orange fluorescent grains (Path Vysion, Abbott, IL, USA). Thus, HER-2 positives were either strong positives (3+) from IHC or positive for gene amplification from FISH analysis.

TNBCs are negative for ER, PgR and HER-2 as described earlier. Among TNBCs with 1–9% of ER and/or PgR expression were defined as hormone receptor (HR) weak and analyzed separately. TNBCs with HER-2 (2+) and that were FISH negative were also analyzed separately.

Proliferative activity was determined by IHC for the Ki-67 antibody. Ki-67 values were expressed as the percentage of positive cell counts among at least 100 tumor cells in each case. Patients with positive staining of Ki-67 at 50% or more were defined as high Ki-67 patients. AR and p53 were defined as positive if tumor cells showed positive staining regardless of rate. Basal-like subtype was defined as CK5/6 positive and/or EGFR positive in 5% or more cells.

Surgical treatment

All patients underwent surgical treatment after NAC. Breast conservative therapy or a mastectomy with or without axillary dissection was performed according to the decision of the surgeons' conference. Surgical specimens were histologically analyzed again, and the pathological response for NAC was evaluated. When no residual invasive tumor cells were found, tumors were identified as pathological complete response (pCR). Surgical specimens from non-pCR patients were analyzed for expressions of Ki-67, p53 and AR as described earlier.

Statistics

A univariate analysis of the pCR rate was carried out by the χ^2 test, and a multivariate analysis was done by multiple logistic regression analysis. The patients' survival was calculated from the first date of treatment until the date of death or the end of follow-up. A univariate analysis of disease-free survival (DFS) was done using the Kaplan–Meier method with a log-rank test, and a multivariate disease survival analysis was carried out under the Cox proportional hazards model. All data were analyzed with JMP for Windows (SAS Institute, Tokyo, Japan).

Results

Relationship between pCR and clinicopathological factors

Thirty-three patients were identified as TNBCs, and the patients' data are shown in Table 1. The age of the patients ranged from 30 to 68 years old (median 50.0) and 21 patients had clinically positive nodes. Clinical response after NAC was rated as clinical complete response for 14 patients (42%), a clinical partial response for 14 patients (42%), a clinical stable disease for 3 patients (9%), and as a clinical progress disease for 2 patients (6%). Also pCR was achieved in only 12 patients (36%).

The correlations between clinicopathological factors such as tumor size, lymph nodal metastasis, age, histological grade, and pCR rate were analyzed (Table 2). However,

Table 1 Patients' characteristics

Variables	No (%)
Total	33
Age: years-old	30–68 (50 ± 11.1)
Histology	
Papillo-tubular	4 (12)
Solid tubular	14 (42)
Schirrous	11 (33)
Special type	4 (13)
T	
1	1 (3)
2	24 (72)
3	6 (18)
4	2 (6)
N	
0	12 (36)
1	17 (52)
2	4 (12)
Histological grade	
1	1 (3)
2	4 (12)
3	27 (81)
Unknown	1 (3)
HER-2	
0	18 (55)
1+	11 (33)
2+	4 (12)
HR (hormone receptor)	
Negative	26 (79)
Weak	7 (21)

T and *N* were defined by the criteria of UICC-breast

HR weak is a tumor with low levels of ER and/or PgR determined by IHC (1–9% weakly positive cells)

Table 2 pCR ratio based on clinicopathologic and immunohistochemistrical factors

Variables	Number (%)	pCR (%)	*P* volume	Odd
Age (years old)				
<50	18 (55)	6 (33)	0.69	
50≤	15 (45)	6 (40)		
Size (cm)				
<5	25 (76)	11 (44)	0.09	5.5
5≤	8 (24)	1 (13)		
N				
Positive	21 (64)	8 (38)	0.78	
Negative	12 (36)	4 (33)		
Histological grade				
1–2	5 (15)	3 (60)	0.26	
3	27 (84)	9 (33)		
HR				
Negative	26 (79)	10 (38)	0.95	
Weak	7 (21)	2 (28)		
HER-2				
0, 1+	29 (88)	9 (31)	0.08	6.67
2+	4 (12)	3 (75)		
p53				
Positive	21 (64)	8 (38)	0.78	
Negative	12 (36)	4 (33)		
Ki-67				
50≤ (high)	20 (61)	10 (50)	*0.04	5.5
<50 (low)	13 (39)	2 (15)		
AR				
Positive	6 (18)	3 (50)	0.45	
Negative	27 (82)	9 (33)		
Basal-like[#]				
Positive	22 (67)	5 (23)	*0.02	5.9
Negative	11 (33)	7 (64)		
CK5/6				
Positive	14 (42)	2 (14)	*0.02	
Negative	19 (58)	10 (53)		
EGFR				
Positive	18 (55)	4 (22)	0.06	
Negative	15 (45)	8 (53)		

* Statistically significant

[#] Basal-like subtype is defined as CK5/4 positive and/or EGFR positive. Thus, CK5/6 was not used for multivariate analysis

these clinicopathological factors did not show any correlation with the pCR rate.

Relationship between pCR, and molecular markers

Next, the correlation between molecular markers and the pCR rate was also analyzed. HER-2 (2+) tended to show a higher pCR rate than HER-2 negative (0 or 1+; 75 and 31%, respectively). In this study, basal markers of CK5/6 and EGFR were evaluated with 22 of 33 patients (67%) diagnosed with basal-like phenotype, and eleven patients (33%) diagnosed with the non-basal-like phenotype. The pCR rate for the basal-like phenotype was significantly lower than in the non-basal-like phenotype (23 and 64%, respectively: $P = 0.02$; Table 2). Ki-67 was also considered as a predictive factor for NAC response, because the pCR rate reaches 50% among high Ki-67 (≥50%) patients, while it was 15% in low Ki-67 patients ($P = 0.04$). The expressions of HR, p53 and AR were not correlated with pCR in this study. Multivariate analysis showed that only high Ki-67 was a significant factor for the prediction of pCR (Table 3). The classification of basal-like or non-basal-like phenotypes was negative for multivariate analysis, probably because high Ki-67 and non-basal-like were strongly correlated with each other; high Ki-67 accounted for 33% in the basal-like and 75% in the non-basal-like phenotype.

Relationship between pCR and disease-free survival

All patients underwent surgical resection after NAC and non-pCR patients were histologically evaluated. The average observation period after surgery was 2 years and eight patients (24%) showed distant metastasis during the observation period. Seven out of 8 patients had been defined as non-pCR and only one patient obtained pCR after NAC. Non-pCR patients showed a worse DFS compared with pCR patients, but it was not statistically significant (Fig. 1a). Basal-like phenotype and other clinicopathological factors such as age, tumor size and lymph nodal involvement failed to show a correlation with DFS (Table 4). Ki-67 before NAC showed a significant correlation with DFS and high Ki-67 patients showed a poor prognosis (Fig. 1b).

Disease-free survival among non-pCR patients

Among non-pCR patients, only 7 patients (29%) showed a recurrence. We analyzed clinicopathological and IHC factors for better prognosis among non-pCR patients. The immunohistological changes of tumors after NAC were

Table 3 Multivariate analysis of pCR and immunopathological factors

Variables	Odds	*P* value
Non-basal-like	3.9	0.13
HER2 (2+)	10.2	0.12
High Ki-67	8.4	0.03*

* Statistically significant

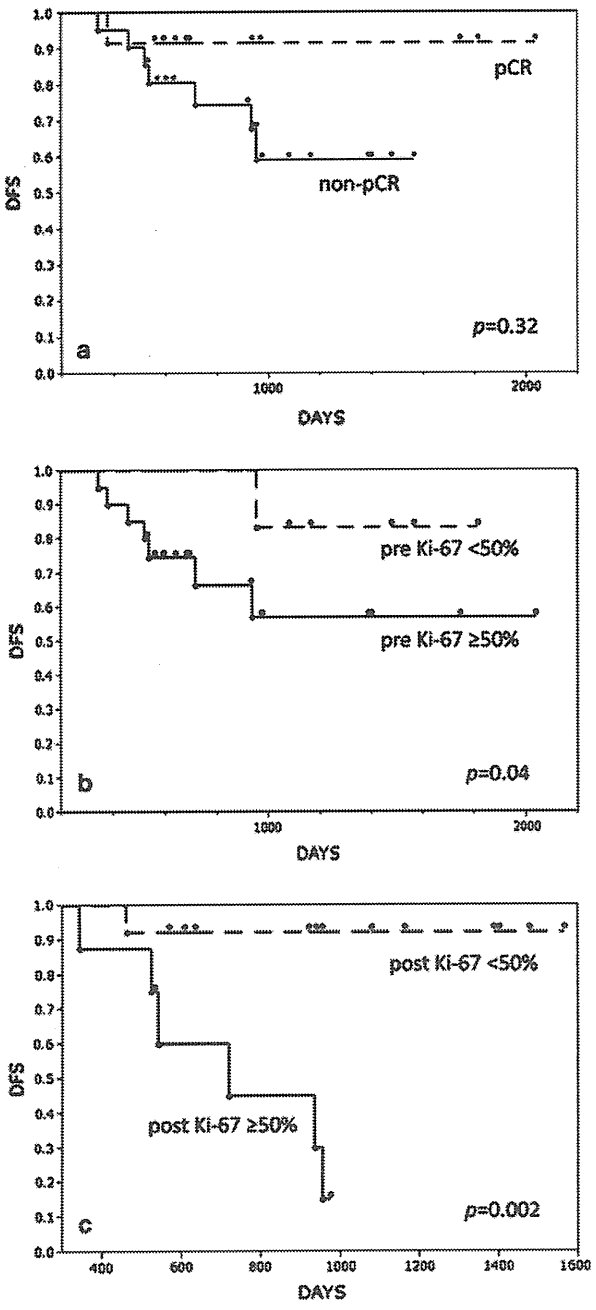

Fig. 1 Disease-free survival (DFS). **a** DFS of pCR and non-pCR patients after NAC. Non-pCR patients showed worse disease-free survival compared with pCR patients, but it was not statistically significant ($P = 0.32$). **b** DFS based on Ki-67 expression of pre-chemotherapy. High Ki-67 (≥50%) patients showed significantly worse disease-free survival than low Ki-67 (<50%) patients ($P = 0.04$). **c** DFS based on Ki-67 expression of post-NAC among non-pCR patients. Non-pCR patients who had high Ki-67 expression after NAC showed a poor prognosis ($P = 0.002$)

evaluated. Among non-pCR patients, 10 patients showed high Ki-67 before chemotherapy and 7 patients still showed high Ki-67 after NAC (Table 5). Among these patients, 6 showed a recurrence and Ki-67 values after NAC were significantly correlated with DFS (Fig. 1c). The expressions of p53 and AR after NAC were not correlated with DFS (data not shown).

Table 4 Multivariate analysis of disease-free survival and patients' characteristics

Variables	Hazard ratio	*P* value
≥50 years-old	0.39	0.2
≥5 cm	2.2	0.3
N positive	4.2	0.11
HR positive	3.2	0.1
HER-2 (2+)	3.2	0.56
Non-basal	1.4	0.6
High Ki-67	5.95	0.04*
p53 positive	0.48	0.3
AR positive	0.000	0.054
Non-pCR	3.7	0.16
High Ki-67 post-NAC[#]	13.2	0.0029*

[#] Data among non-pCR patients

* Statistically significant

Table 5 The correlation between Ki-67 expression, pCR and the change of Ki-67 expression among non-pCR patients

TNBC ($n = 33$)	Non-pCR		pCR
	Post-NAC Ki-67		
	High	Low	
Pre-NAC Ki-67			
High	7	3	10
Low	1	10	2

Discussion

TNBC is defined by the lack of ER, PgR and HER-2 expression. Because targeted therapies are not useful, chemotherapy is the only systemic treatment option for TNBC [1–5]. Thus, a comprehensive examination of the clinical phenotypes of TNBCs which respond to chemotherapy is important. TNBCs are a heterogeneous group and generally divided into two subtypes; basal-like phenotype and non-basal-like phenotype [6]. The basal-like phenotype is characterized as having a high expression of keratins, laminin, and EGFR.

Many data indicated that the pCR rate is higher in TNBC compared with other phenotypes [10]. A pathological evaluation after NAC is very important because pCR after NAC indicates better survival [8, 9]. Our data showed the pCR rate in TNBCs was 36%, which is consistent with previous

reports which stated 22–45% [10, 20]. This study hypothesized that non-basal-like phenotype, HER-2 (2+), and high Ki-67 could be predictive factors for pCR achievement, but multivariate analysis revealed that only Ki-67 was a significant factor for the prediction of pCR. This is probably because the non-basal-like phenotype showed a significantly higher Ki-67 expression compared with the basal-like phenotype. This study is consistent with previous studies which showed that Ki-67 indicates proliferation and high level of proliferation activity are associated with chemosensitivity [14]. Additionally, there are many reports that showed that the basal-like phenotype has a positive correlation with pCR [20]. Rouzier et al. reported that basal-like subtypes were more sensitive to NAC than luminal and normal-like cancers, but normal-like subtypes classified based on gene expression profiles are quite different from non-basal-like phenotypes based on IHC, because normal-like subtypes involved 60% of ER positive samples. Because classification based on gene expression is difficult for clinical use, our data based on IHC classification are quite useful. There are some reports that non-basal-like tumors showed better prognosis than basal-like phenotypes [6, 7]. Though the pCR rate was significantly higher in non-basal-like tumors, there was no difference in DFS between the two groups in this study.

Our study failed to show the significant benefit of pCR on DFS. That is probably because of the small number of the patients included or the short duration after surgical treatment in this study. Most cases which showed a recurrence in such a short period were non-pCR patients, and the only recurrent case in the pCR group was a patient with an intraductal residual after NAC and who showed brain metastasis within a year. In this study, Ki-67 was the only significant factor which was proved to affect DFS. Pre-NAC high Ki-67 was a poor prognostic factor in spite of the positive correlation with pCR. The post-NAC status of Ki-67 was also correlated with recurrence. High Ki-67 expression post-NAC showed a very poor prognosis and low Ki-67 post-NAC showed better survival even in the non-pCR group. The contradiction of high Ki-67 tumors, which showed a high chemosensitivity and high pCR rate but poor prognosis, may indicate the diversity of these tumors. As shown in Table 4, most high Ki-67 patients who could not achieve pCR kept a high expression of Ki-67 after NAC. Tumors which maintained high Ki-67 expression may indicate that the cellular activity is not suppressed by NAC. All of these facts showed that high Ki-67 tumors should be divided into two groups: tumors which show a high sensitivity to current chemo-drugs and a good prognosis and the tumors which continue to have high cellular activity after NAC and show a poor prognosis. Further study is needed to find other treatments for the latter.

Though many reports defined 20–30% of Ki-67 labeling index as a threshold [21], 50% was used for categorization in this study because most TNBCs are positive for Ki-67 and a 50% threshold at 50% was shown to be useful to predict both chemosensitivity and prognosis in TNBC patients.

The prognosis of HER-2 positive breast cancer has been proved by the usage of trastuzumab. The criteria of HER-2 positive are defined as a strong positive IHC or gene amplification in FISH [22]. HER-2 (2+) breast cancers without gene amplification are generally included in TNBC but HER-2 (2+) breast cancers showed higher chemosensitivity in this study and HER-2 (3+) breast cancers have been reported to be chemosensitive. The criteria of HER-2 positivity might be a moot point if TNBCs with HER-2 (2+) show a different cancer biology from TNBCs with negative HER-2.

Less than 10% of hormone receptor positivity had been considered as uncertain endocrine responsiveness or potential resistance [18, 19]. Though tumors with less than 10% hormone receptor positivity were included in TNBCs, we classified those with 0% staining both ER and PgR as HR negative and those with 1–9% as HR weak in this study. But the expressions of HR were not correlated with pCR. Moreover, tumors with any ER positive staining of at least 1% are recommended to be treated with endocrine therapy in latest reports [21, 23]. The categories of highly endocrine responsive and incompletely endocrine responsive are not relevant to the decision for endocrine therapy, but those categories are still important for the decision of chemotherapy.

In this study, we found that the pCR rate for the non-basal-like phenotype was significantly higher than that in the basal-like phenotype, though that difference was negative for multivariate analysis. This is because the positivity of Ki-67 was higher in the non-basal-like phenotype tumors. These data based on classification by IHC are very interesting and informative in a clinical setting because there are some discrepancy between criteria by gene expression profiling and those by IHC. Some previous papers were confused about classification by gene expression and by IHC. Non-basal-like subtype is a term correlated with IHC classification and difficult to adapt to criteria of gene expression. There are few reports focused on the non-basal-like phenotype. Our data may insinuate that non-basal-like subtypes are well adaptive to current chemotherapy and basal-like subtypes need another therapeutic agent. Because our data was based on a small number of patients, further examinations based on IHC classification are needed.

Our study indicated that TNBCs which were found to be non-pCR with high Ki-67 expression after NAC had a poor prognosis. How to treat these TNBCs will be a most important subject for future study. Only chemotherapy is a

proven treatment for TNBCs, but chemotherapy based on anthracyclins and taxanes has not been shown to be enough. There are several studies which showed the efficacy of new chemotherapeutic agents such as carboplatin, bavasituzumab and poly (ADP-ribose) polymerase-1 (PARP-1) inhibitor in TNBCs [24–26]. Studies of NAC with these agents are expected to improve the treatment of TNBCs.

References

1. Dawson SJ, Provenzano E, Caldas C (2009) Triple negative breast cancers: clinical and prognostic implications. Eur J Cancer 45(Suppl 1):27–40
2. Rakha EA, El-Sayed ME, Green AR et al (2007) Prognostic markers in triple-negative breast cancer. Cancer 109:25–32
3. Kreike B, van Kouwenhove M, Horlings H et al (2007) Gene expression profiling and histopathological characterization of triple-negative/basal-like breast carcinomas. Breast Cancer Res 9:R65
4. Kuroda N, Ohara M, Inoue K et al (2009) The majority of triple-negative breast cancer may correspond to basal-like carcinoma, but triple-negative breast cancer is not identical to basal-like carcinoma. Med Mol Morphol 42:128–131
5. Pintens S, Neven P, Drijkoningen M et al (2009) Triple negative breast cancer: a study from the point of view of basal CK5/6 and HER-1. J Clin Pathol 62:624–628
6. Rakha EA, Elsheikh SE, Aleskandarany MA et al (2009) Triple negative Breast cancer: distinguishing between basal and nonbasal subtypes. Clin Cancer Res 15:2301–2310
7. Gluz O, Liedtke C, Gottschalk N et al (2009) Triple-negative breast cancer-current status and future directions. Ann Oncol 20:1913–1927
8. Fisher B, Bryant J, Wolmark N et al (1998) Effect of preoperative chemotherapy on the outcome of women with operable breast cancer. J Clin Oncol 16:2672–2685
9. Scholl SM, Fourquet A, Asselain B et al (1994) Neoadjuvant versus adjuvant chemotherapy in premenopausal patients with tumors considered too large for breast conserving surgery: preliminary results of a randomized trial:S6. Eur J Cancer 30A:645–652
10. Liedtke C, Mazouni C, Hess KR et al (2008) Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer. J Clin Oncol 26:1275–1281
11. Carey LA, Dees EC, Sawyer L et al (2007) The triple negative paradox: Primary tumor chemosensitivity of breast cancer subtypes. Clin Cancer Res 13:2329–2334
12. Abrial SC, Penault-Llorca F, Delva R et al (2005) High prognostic significance of residual disease after neoadjuvant chemotherapy: a retrospective study in 710 patients with operable breast cancer. Breast Cancer Res Treat 94:255–263
13. von Minckwitz G, Sinn HP, Raab G et al (2008) Clinical response after two cycles compared to HER2, Ki-67, p53, and bcl-2 in independently predicting a pathological complete response after preoperative chemotherapy in patients with operable carcinoma of the breast. Breast Cancer Res 10:R30
14. OgawaY Hai E, Matsumoto K et al (2008) Androgen receptor expression in breast cancer: relationship with clinicopathological factors and biomarkers. Int J Clin Oncol 13:431–435
15. Berns EM, Foekens JA, Vossen R et al (2000) Complete sequencing of TP53 predicts poor response to systemic therapy of advanced breast cancer. Cancer Res 60:2155–2162
16. Assersohn L, Salter J, Powles TJ et al (2003) Studies of the potential utility of Ki67 as a predictive molecular marker of clinical response in primary breast cancer. Breast Cancer Res Treat 82:113–123
17. Burcombe RJ, Makris A, Richman PI et al (2005) Evaluation of ER, PgR, HER2 and Ki-67 as predictors of response to neoadjuvant anthracycline chemotherapy for operable breast cancer. Br J Cancer 92:147–155
18. Harvey JM, Clark GM, Osborne K et al (1999) Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. J Clin Oncol 17:1474–1481
19. Goldhirsh A, Glick JH, Gelber RD et al (2005) Meeting highlights: international expert consensus on the primary therapy of early breast cancer 2005. Ann Oncol 16:1569–1583
20. Rouzier R, Perou CM, Symmans WF et al (2005) Breast cancer molecular subtypes respond differently to preoperative chemotherapy. Clin Cancer Res 11:5678–5685
21. Goldhirsch A, Ingle JN, Gelber RD et al (2009) Thresholds for therapies: highlights of the St Gallen International expert consensus on the primary therapy of early breast cancer. Ann Oncol 20:1319–1329
22. Wolff AC, Hammond MEH, Schwartz JN et al (2007) American society of clinical oncology/College of American pathologists guideline recommendations of human epidermal growth factor receptors 2 testing in breast cancer. J Clin Oncol 25:118–145
23. Hammond ME, Hayes DF, Dowsett M et al (2010) American society of clinical oncology/College of American pathologists guideline recommendations for immunohistochemical testing and estrogen and progesterone receptors in breast cancer. J Clin Oncol 2010 (Epub)
24. Pal SK, Mortimer J (2009) Triple-negative breast cancer: novel therapies and new directions. Maturitas 63:269–274
25. Corkery B, Crown J, Clynes M et al (2009) Epidermal growth factor receptor as a potential therapeutic target in triple-negative breast cancer. Ann Oncol 20:862–867
26. Inbar-Rozensal D, Castiel A, Visochek L et al (2009) A selective eradication of human nonhereditary breast cancer cells by phenanthridine-derived polyADP-ribose polymerase inhibitors. Breast Cancer res 11:R78

精密検査・診断・モニタリング

MRI/CT（広がり診断と病期診断）

土井原博義* 枝園忠彦
岡山大学病院乳腺・内分泌外科 *教授

プライマリ・ケアにおけるポイント

乳がんの手術は，乳房全摘術または乳房温存術（部分切除），腋窩リンパ節に対してはセンチネルリンパ節生検または腋窩リンパ節郭清がされる．乳房の術式選択においては病変の広がりの範囲診断が非常に重要であり，そのためには術前のMRIやCT検査が有用である．とくに，超音波やマンモグラフィと比較して主腫瘍から乳管内をがんが広がる乳管内進展の診断はMRI/CTが行われることが多い．ただ，乳腺症などの良性変化が強い乳腺では偽陽性となることに注意が必要である．

リンパ節の手術においては，超音波による検査に加えてMRI/CTを追加することで術前転移診断の精度が向上し，術式選択に有用である．また，術前薬物療法においては薬物療法前後にMRI/CTを行っておくことは薬剤の腫瘍縮小効果をみるだけでなく，薬物療法後残存した腫瘍の範囲を診断し必要最小限の正確な手術を可能にする．

Ⅰ 治療前診断におけるMRI/CTの意義

① 正確な術式選択を可能にする

乳がん画像診断におけるMRI/CTの意義は，存在診断や質的診断を行うことを目的に使用される超音波やマンモグラフィとは異なり，主に乳がんと診断された病変がどの程度乳房内に広がっているか，乳管内を進展しているかを調べる広がり診断をすることにある（図1）．乳がんはその発生部位が乳腺内に樹枝状に広がる乳管の上皮であることから，主病巣から乳管に沿って広がる乳管内進展を伴うことが多い．広がり診断とは，それらの乳管内進展を含めた病巣の広がりを正確に評価することであり，治療前病期診断を正確にするためだけでなく，手術の術式選択においても重要な情報である．

一方，乳がんに対する乳房温存療法は，わが国では約60％の症例に行われている標準手術である．ただ，安易な乳房温存療法は腫瘍の残存や乳房内再発につながり，温存できないのであればむしろ乳腺全摘を行って乳房再建を行うほうが術後の整容性もよく，局所再発も少ない．こういった背景から，術前に正確に病変の進展範囲を診断することの重要性は明らかであり，マンモグラフィ，超音波だけでなく，MRI/CTも加えた総合診断が重要である．

② 術前薬物療法の効果判定および残存腫瘍の診断

術前薬物療法はリンパ節転移のある症例や腫瘍径の大きな乳がんに対して最近よく施行されている治療法であるが，その治療効果は非常に高く，術前容易に触知されていた病変が終了時には，不明瞭になっていることが多い．治療前にMRI/CTを行って正確な病変の場所や範囲を把握しておき，治療後の再検査にて残存腫瘍の有無や広がり診断を行う．術式選択においても，わずかに残った病変の部位を正確に評価することは非常に重要

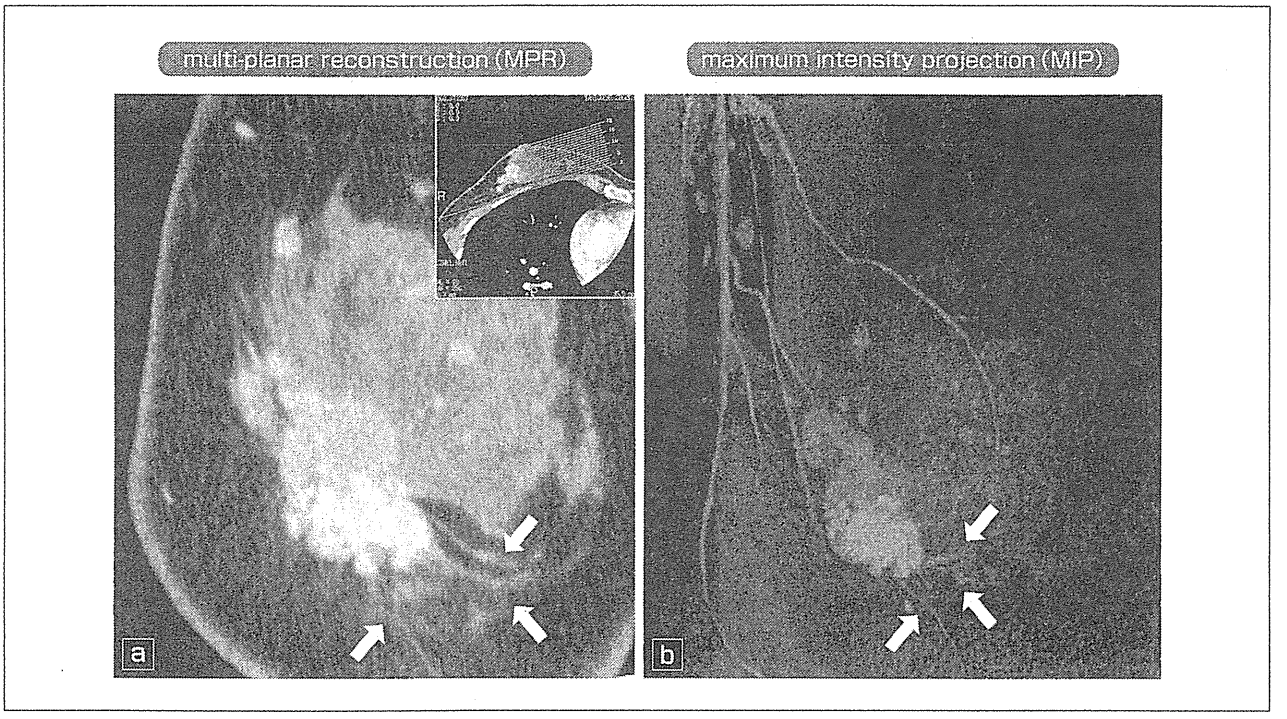

図1　MRI/CTによる乳管内進展
矢印は乳管内進展を示す．

であり，通常の広がり診断よりもさらに高度な診断が求められる．

③多発病変，対側乳腺病変の診断

広がり診断に加えてMRI/CTを術前に行う意義として多発病変の描出がある．乳がんの診断時，患側乳房に多発病変を認めることはまれではなく，また健側乳房にも同時性に乳がんをもつ可能性は約3～5%といわれている．したがって，術前にMRI/CTを両側撮影することは両側乳がんや多発乳がんの発見においても有用である．

押さえておきたいキーワード

MDCT (multidetector-row computed tomography)

従来のSingledetector-row CTでは検出器が体軸方向に1列しか測定チャンネルを有しておらず，管球1回転につき1断面しか得られないのに対してMDCTでは体軸方向に複数の検出器を有し，1回の走査で複数の断面が得られる．したがって，従来のヘリカル装置に比べ3～8倍の高速で広範囲スキャンが可能である．また，その高速性から薄いスライス厚の画像を大量に得ることが可能になったことや，1回のスキャンから得られる生データから異なるスライス厚の画像を再構成できる点で三次元画像の作成も容易になった．MDCTではどの方向でも解像度が落ちることがないのでMPR (multi-planar reconstruction)，MIP (maximum intensity projection)などの三次元画像は診断に大きく貢献した．とくに乳腺におけるMDCTでは，検査体位が手術時と同様の仰臥位で行うので乳房温存手術の切除範囲のシミュレーションが可能であり，また肺・肝転移とともに腋窩や縦隔リンパ節転移の検索も同時に行うことができるというメリットがある．

II MRIとCTの相違点(それぞれの特徴と問題点)

MRIとCTの選択において，その優劣や適応を決める決定的なエビデンスはない．ただ，それぞれの特徴によるいくつかの相違点がある．

①撮影体位・範囲の違い

現在，MRIが乳がん領域では広く利用されているが，MRIと比較した際のmultidetector-row computed tomography (MDCT)の相違についていくつかあげてみる．まず撮影範囲・体位や撮影時間の違いであるが，MRIの撮影範囲は通常の乳房撮影の場合，構成次第では腋窩病変，肺・肝転移病巣の有無も観察可能であるが，乳房専用のコイルを使用するので乳房に限局したものとなる．また，撮影体位は手術体位と同様の仰臥位もしくは斜位で撮影を行う施設もあるが，腹臥位で施行しているのが一般的と思われる．一方，MDCTの場合，通常の乳房撮影のときに胸部から肝まできわめて短時間で撮影可能であり，1回の検査で詳細な対側乳房・肺・肝・腋窩リンパ節病変の観察が可能である．またほとんどの場合，仰臥位もしくは半斜位といった実際の手術体位に近い姿勢で検査をすることが多く3次元構成することで手術に即した立体的位置関係を把握するのに優れている[1,2]．

②被曝，造影剤など

通常，CTにおける被曝量は約10 mSvで腹部骨盤CTを加えることでさらに被曝量は増加するが，MRIによるX線被曝はない．また，造影剤による副作用はMRIに比べてCTに多い．ただ，CTは撮影がMRIに比較して短時間で行うことが可能であり，体内金属(ペースメーカーなど)がある場合もCT検査は可能であるなど両者に若干の相違はみられる[1,2]．

III 広がり診断の実際の読影

①感度と特異度

乳がん手術，とくに乳房温存療法の際にとくに問題となる乳管内進展の描出の相違に関して，一般的にMRIはCTと比較して高い感度がある一方で，特異度においてやや劣る傾向があるといわれている[1,2]．MDCTの乳管内進展の存在診断感度は71.8～88.2%，特異度は67.8～85.7%と報告されている[3]．

②乳管内進展の評価と偽陽性

MRI/CTでは，乳管内進展を腫瘍から連続して造影される線状～棍棒状の陰影として捉える．乳管内進展が高度な症例では末梢の主病変から乳頭直下まで乳管が造影され進展を認めることがある(**図2**)．ただ，このような乳管の造影効果は良性の乳腺症様変化でも認められることがある(**図3**)．乳がんが発生した背景乳腺に乳腺症の変化が強い場合，乳管内進展を実際よりも広く，偽陽性病変として診断してしまうことがある．若年者や生理周期(開始直前)などが偽陽性になりやすい因子としてあげられる．病変以外の背景乳腺を対側乳腺と比較することが重要である．

③術前薬物療法後の画像評価

術前薬物療法は乳房温存率の向上に大きな意義があり，90%近くの症例で縮小が認められる．そのため，薬物療法後に必要最小限の乳房温存療法を行うためには残存する病巣の評価が必須であり，より正確な広がり診断が要求される．術前薬物療法奏功例では病巣の縮小形式として求心性に縮小

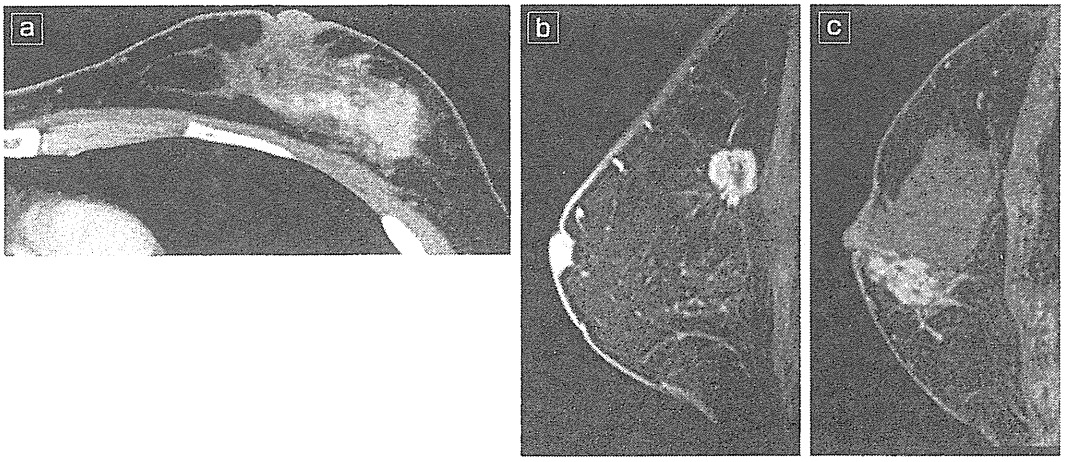

図2　CTおよびMRIによる広がり診断
a：造影マルチスライスCT（主腫瘍と乳管内進展），b：MRI限局性腫瘤，c：MRI区域性に広がる非浸潤がん．

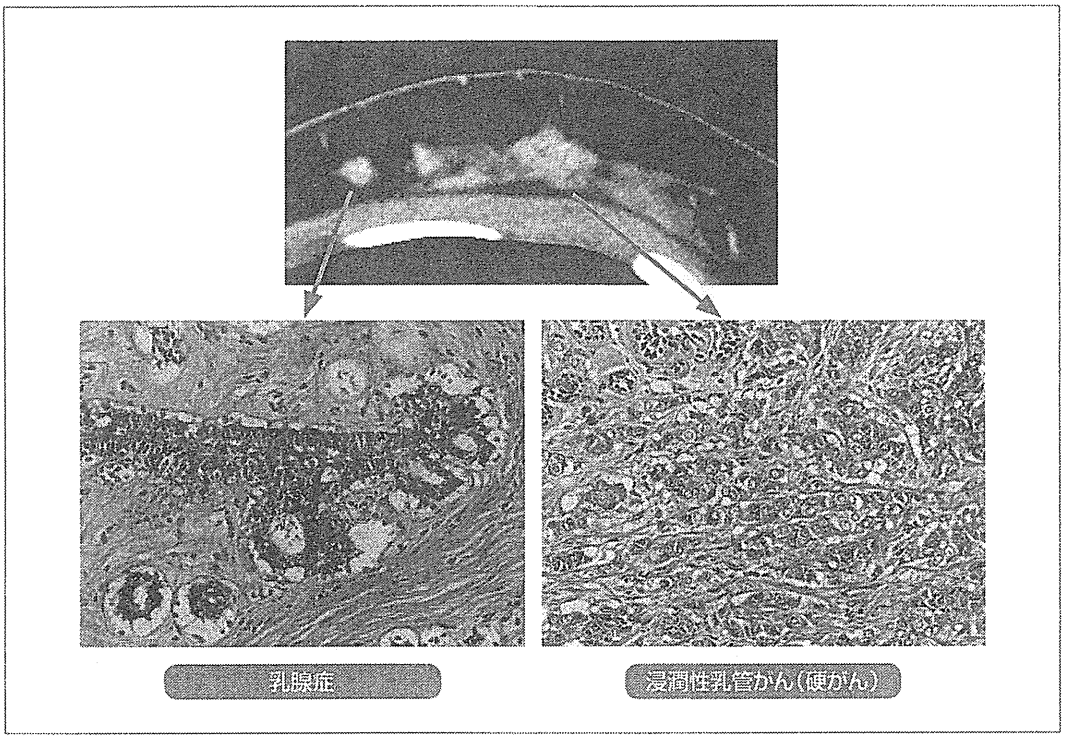

図3　MDCTによる偽陽性例

するconcentric typeと，まばらに点状に残存するmulti-focal typeに大別される．前者における範囲診断では，術後の病理検査における腫瘍の範囲との一致率が85％程度と高い相関が示されている一方で，multi-focal typeにおいては術前診断よりも実際の範囲が広いことが多く注意が必要である[4]．術前薬物療法後の範囲診断においてはMRIを用いた報告が中心であり，とくに微小残存病変に対して高い感度が示されている（図4）．

④ リンパ節転移診断

乳がんはリンパ節転を認めることが多く，その診断は病期の診断のみならず治療方針や生命予後を予測する因子として非常に重要である．治療前に腋窩リンパ節転移の有無を診断することは，センチネルリンパ節生検を行うか標準的腋窩郭清を行うかを決定するために非常に重要である．腋窩リンパ節転移における診断基準は決定的なものはないが，これまでの報告によるとMDCTによる造

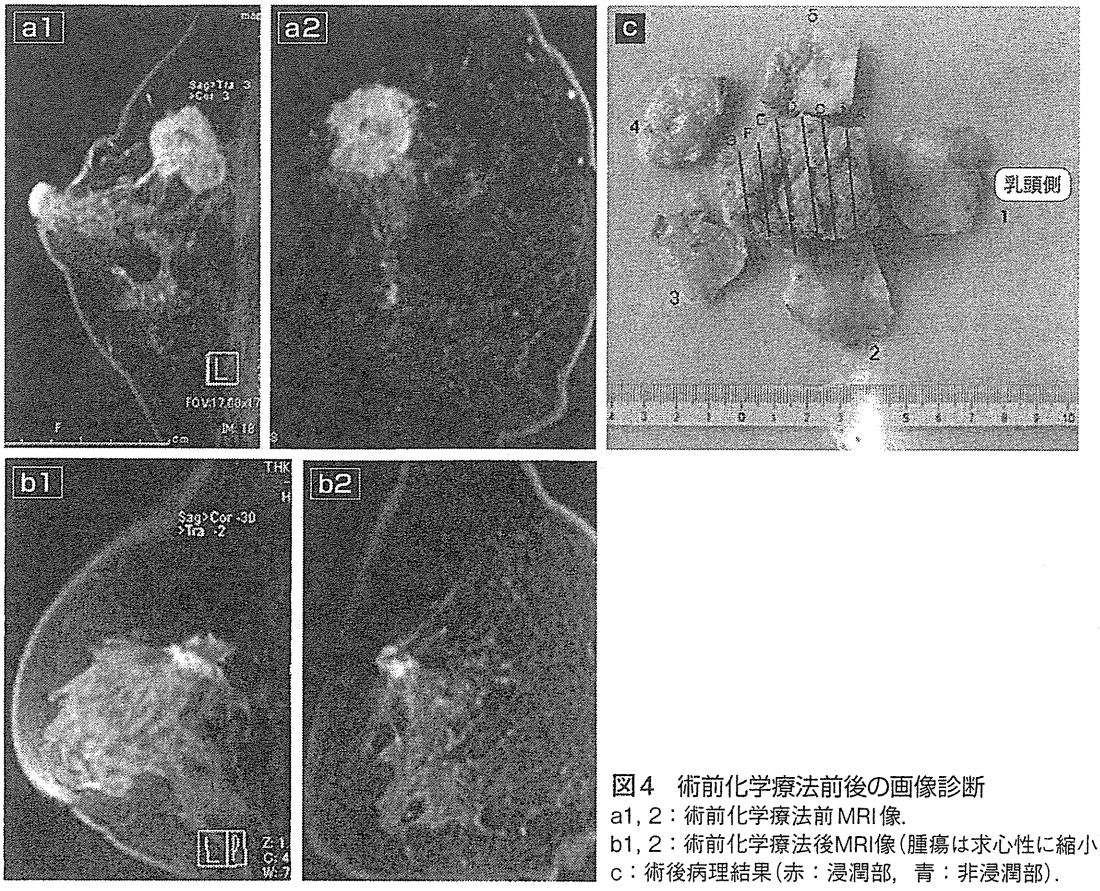

図4　術前化学療法前後の画像診断
a1, 2：術前化学療法前MRI像.
b1, 2：術前化学療法後MRI像（腫瘍は求心性に縮小）.
c：術後病理結果（赤：浸潤部，青：非浸潤部）.

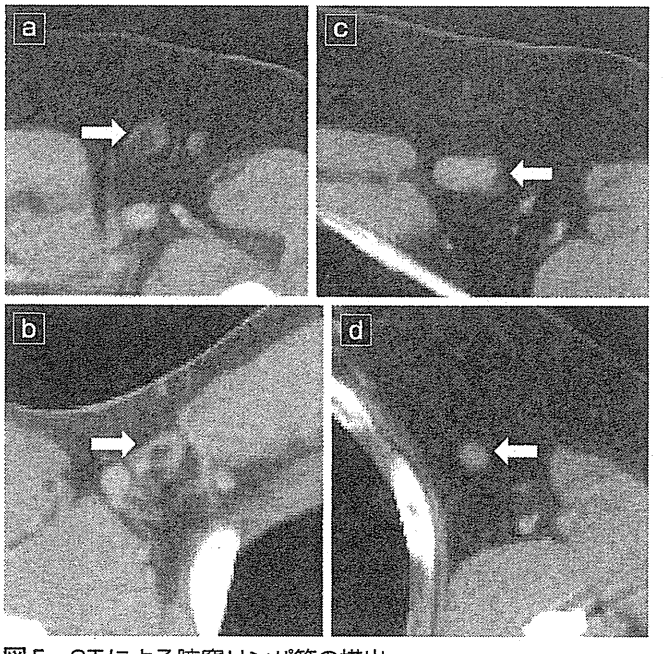

図5　CTによる腋窩リンパ節の描出
a：「C字形」，b：「リング状」，c：「卵円形」，d：「円形」.

影パターンから一般的に「円形」，「卵円形」，「リング状」，「C字形」と分類されることが多く，術前の画像上転移陽性と考えられるものは大きさも加味した「円形」，「卵円形」病変が中心となる（**図5**）．これらの診断基準を踏まえたMDCTを用いた評価におけるその組織学的転移との一致率は，おおむ