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## Chapter IX

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# Topoisomerase II-Alpha Index Predicts the Efficacy of Anthracycline-Based Chemotherapy for Breast Cancers

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## Abstract

DNA topoisomerase II-alpha (TOPOII $\alpha$ ) has been reported that its gene copy number or protein expression may be predictive of Anthracycline-based chemotherapy or patient's prognosis in breast cancers. Our data indicated the breast cancers with TOPOII $\alpha$  index  $\geq 25$  % regressed more effectively than those with TOPOII $\alpha$  index  $< 10$  % on histology by Anthracycline-based chemotherapy. TOPOII $\alpha$  index of TOPOII $\alpha$  gene-deleted tumors did not differ from that of TOPOII $\alpha$  gene-amplificated or normal tumors. TOPOII $\alpha$  index, not its gene amplification, is a useful marker of Anthracycline-based chemotherapy in breast cancers.

**Keywords:** DNA Topoisomerase II $\alpha$ , Index, Anthracycline, Effect prediction, Ki67

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## DNA Topoisomerase II $\alpha$

DNA topoisomerase is the generic name for an enzyme that severs and reconnects one or both strands of a double-stranded DNA. The enzyme that severs only one of the two strands of a double-stranded DNA is classified as type I, while that which severs both strands is classified as type II. Type II enzyme forms a severing complex by binding to a double-stranded DNA after forming a homodimer. It becomes stable in the presence of  $Mg^{++}$  and ATP [1, 2]. Type II enzyme exists in the nucleus and can be classified into  $\alpha$  and  $\beta$ , which are expressed from a different gene. Although on the N-terminal side 3/4 of TOPOII $\beta$  is highly homologous with TOPOII $\alpha$ , it has a different C-terminal domain, and the activity control mechanism exists in this domain.

DNA topoisomerase II $\alpha$  (TOPOII $\alpha$ ) gene (molecular weight: 170 kDa) is located in the domain 17q21–22, which is near the human epidermal growth factor receptor type 2 (HER2/neu) gene (17q12–21; molecular weight: 185 kDa).

## TOPOII $\alpha$ Index

TOPOII $\alpha$  protein is a cell proliferation-related antigen, which expresses in the S and G2/M phases during the normal cell cycle. TOPOII $\alpha$  protein increases by a factor of two to three times in the G2/M phase. Particularly, it expresses most in highly proliferative cells. TOPOII $\beta$ , which has a molecular weight of 180 kDa, constantly exists in any cell and at any phase of the cell cycle; however, its correlation with the therapy-related secondary carcinogenesis has been reported [2, 3]. Although the TOPOII $\alpha$  index relates to the Caspase-3 index ( $p < 0.05$ ) and the Ki67 index ( $p < 0.01$ ), it is not related to the PCNA index (Figures. 1, 2) [4]. The Ki67 nuclear antigen is not expressed at the silent period (G0); however, it is expressed throughout the cell cycle (i.e., G1, S, G2, and M phases). Ki67 serves as a proliferation marker and a tumor prognostic factor. Although PCNA is also a proliferation-related antigen, its synthesis level begins to increase inside the nucleus at the end of the G1 phase just before the start of DNA synthesis, it maximizes in the S phase and decreases in the G2/M phase. Thus, the expression index of the TOPOII $\alpha$  protein, which expresses in the S and G2/M phases, only relates to the Ki67 index and not to the PCNA index, even though they are the same cell proliferation-related antigens. Although it is not totally clear why the protein index of Caspase-3, which is a protease that works in the final stage of apoptosis, loosely relates to that of TOPOII $\alpha$ , one must pay careful attention when evaluating apoptosis for the evaluation of the therapeutic effects of Anthracycline-based chemotherapy, which induces apoptosis from TOPO II inhibition [5–8]. Moreover, the TOPOII $\alpha$  index not only indicates the proliferation activity but may also relate to the quantitative changes of cancer cells [8, 9].

## Ki67 Index

TOPOII $\alpha$  and Ki67 are often compared as prediction makers for the therapeutic effects of Anthracycline-based chemotherapy. However, because the methods of positive/negative

classification used in the reports vary to a small extent, it is necessary to be careful when comparing the positive rates or evaluating the significance of the measurements. According to Nakopoulou et al., 25% of the invasive breast cancer subjects experienced the TOPOII $\alpha$  protein expression in more than 10% of the tumor cells.

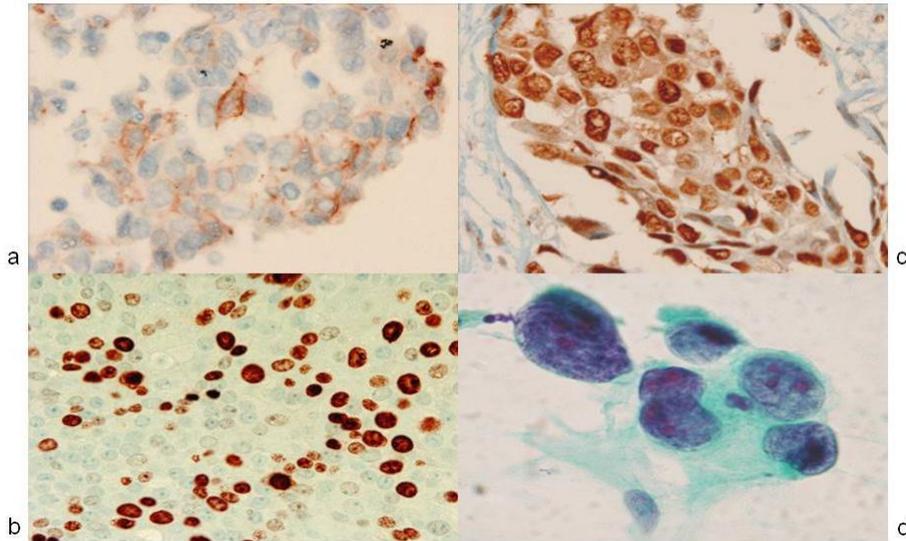


Figure 1. Immunohistochemical findings and cytological atypia of breast cancer cells. (a: Caspase 3 index- 44.8%, b: Ki67 index-18.8%, c: PCNA index-88.7%, d: nuclear atypia grade 3 by Robinson's classification).

The expression of TOPOII $\alpha$  protein had correlations with nuclear atypia, the Ki67 index, and the p53 index of the tumor cells, as well as hormone receptor non-expression and HER2 protein overexpression in the tumor cells [10]. We investigated the correlation of the positive rate using the 203 infiltrating duct carcinomas as subjects, and reported that the TOPOII $\alpha$  index had correlations with nuclear atypia (Robinson grade) [11] as well as with the Ki67 index, Caspase-3 index, hormone receptor non-expression, and HER2 gene amplification (Table 1) [4]. However, according to the study conducted by Petit et al., the following facts have been discovered: Of all the factors (nuclear atypia, hormone receptor, Ki67 index, HER2 protein expression, TOPOII $\alpha$  protein expression, HER2 gene amplification, and TOPOII $\alpha$  gene amplification), hormone receptor non-expression and the Ki67 index of 20% or greater are effective for the clinical CR (complete response) prediction for neoadjuvant chemotherapy or primary systemic therapy for breast cancer using an anthracycline; and while nuclear atypia has a correlation with the pathological CR prediction, neither protein expressions nor gene amplifications of HER2 and TOPOII $\alpha$  had any correlation with the therapeutic effect [12]. Meanwhile, Tinari et al. have reported that in the breast cancer cases treated with neoadjuvant chemotherapy or primary systemic therapy using an anthracycline, while HER2 protein and TOPOII $\alpha$  protein expressions had correlations with the therapeutic effect, neither nuclear atypia nor the Ki67 index had any correlation with the therapeutic effect [13].

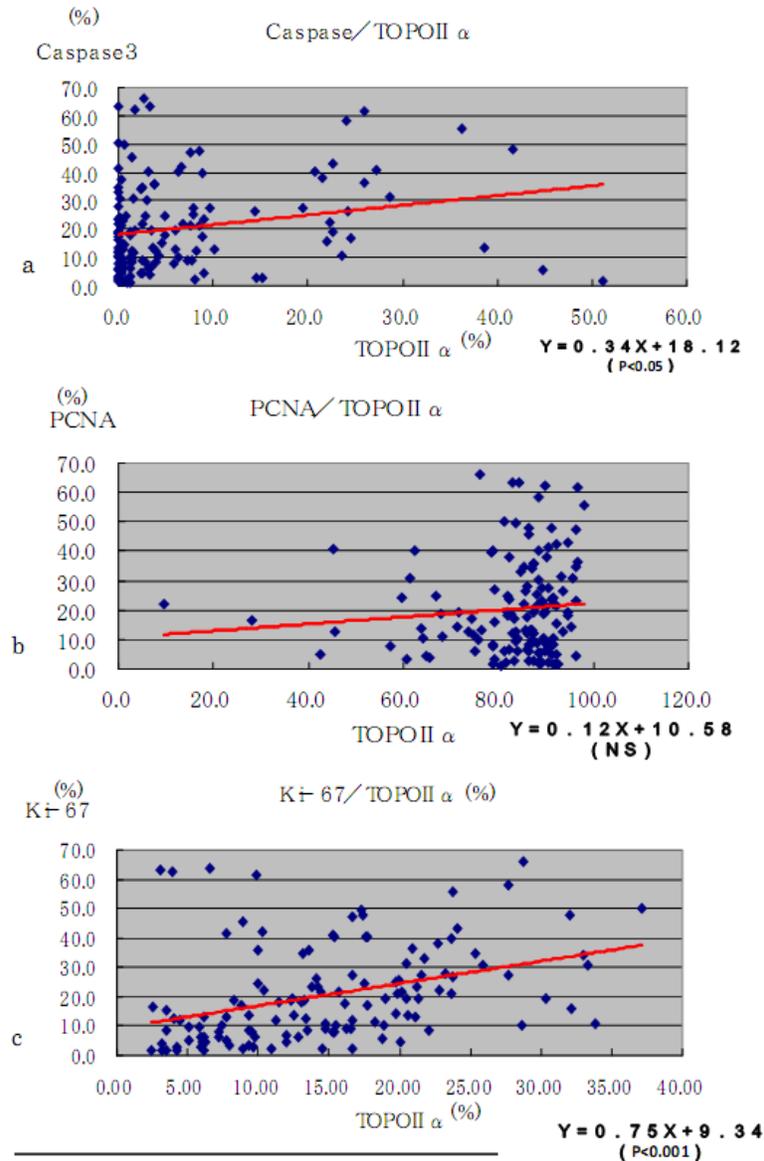


Figure 2. Relations of TOPOII $\alpha$  index to Caspase 3 index (A), PCNA index (B), and Ki67 index (C), with statistical significance in A and C.

## TOPOII $\alpha$ and HER2 Genes

HER2 genes are the cancer genes that amplify most frequently in breast cancer. One must also be careful when interpreting the results of gene amplification evaluation on these genes because the standards used for evaluation in the previous reports vary. The HER2 gene amplification measured through the fluorescence in situ hybridization (FISH) method can be indicated in the form of signal comparison with CEP17 (Chromosome 17 centromere) (Figure 3). It has been reported that the frequency of occurrence of the signal rate of  $\geq 2.0$  is 18%–

29.4% [14–17]. Although monoclonal antibody trastuzumab (Herceptin) is effective in only 23%–26% of the breast cancers having overexpressed HER2 proteins during the simple substance treatment against HER2 proteins, when combined with other anticancer agents the effect will increase up to 50% [17].

**Table 1. Relationships among clinicopathological characteristics, topoisomerase II alpha, HER2, Ki67, Caspase 3, and hormon receptors in 203 invasive ductal breast carcinomas of females**

Clinico-pathological characteristics	Number of cases	Age	Tumor size (cm)	Imunohistochemistry						FISH	
				ER score (0–3)	PgR score (0–3)	Herc score (0–3)	Ki67(%)	TOPOII(%)	Caspase3(%)	HER2/C EP index	TOPOIIα/ CEP index
All	203	60, 2	2,3	1,7	1,5	1,0	19,0	18,5	6,3	2,9	1,5
Tumor size											
pT1	112	60, 7	1,4a	1,8	1,5	0,9	18,4	18,1	5,6	2,8	1,4
pT2	79	58, 9	2,9	1,7	1,5	1,0	20,0	19,2	7,1	2,9	1,6
pT3,4	12	63, 3	6,7	1,8	1,3	1,4	17,9	17,7	7,6	4,3	1,7
Pathological stage											
I	76	61, 0	1,3a	1,8	1,5	0,8	17,5	16,8	5,9	2,5	1,5
IIA	68	60, 3	2,3	1,8	1,4	1,2	18,9	20,0	5,8	3,4	1,6
IIB	44	58, 9	3,0	1,7	1,7	0,9	20,3	19,3	7,4	2,7	1,4
III, IV	12	60, 3	6,1	1,7	1,1	1,5	18,1	19,4	8,7	4,3	1,7
LN meta (pT1&2)											
absent	110	60, 7	1,8a	1,8	1,5	0,9	17,9	17,4	6,2	2,8	1,5
present	78	59, 2	2,3	1,7	1,5	1,1	19,8	20,3	6,3	3,0	1,5
Robinson grade											
1	92	62, 5b	2,1	2,1a	1,8a	0,8a	14,5b	14,9a	3,4c	1,9b	1,4
2	82	59, 0	2,4	1,6	1,4	1,1	20,8	20,5	7,7	3,3	1,6
3	24	54, 2	2,5	0,7	0,6	1,6	30	30,6	12,6	6,1	1,7

Positive cells at ER, PgR score: none, 0; <10%, 1; 10–50%, 2; ≥50%, 3.

a, P<0.001 ; b, P<0.01 ; c, P<0.05.

Jarvinen et al. conducted a study deeming the TOPOIIα / CEP17 signal rate of ≥1.5 as amplification and that of ≤0.7 as deletion, and reported that the TOPOIIα genes were either amplified or deleted in 90% of the breast cancers, in which HER2 genes had been amplified [8, 9]. According to the report by Coon et al. that deemed the signal rate of ≥2.5 as amplification, 23% of the invasive breast cancers showed HER2 gene amplification. Of these

23% tumors, TOPOII $\alpha$  gene amplification was evident in 67.7%. However, no deletion was observed in either the HER2 genes or TOPOII $\alpha$  genes [18]. Knoop et al. reported that when deeming the signal rate of  $\geq 2.0$  as amplification and that of  $< 0.8$  as deletion, the HER2 genes amplified in 29.4% of the breast cancers. In 32.5% of such tumors, the TOPOII $\alpha$  genes also amplified. Moreover, deletion was observed in 23.6% of the HER2 gene-amplified breast cancers.

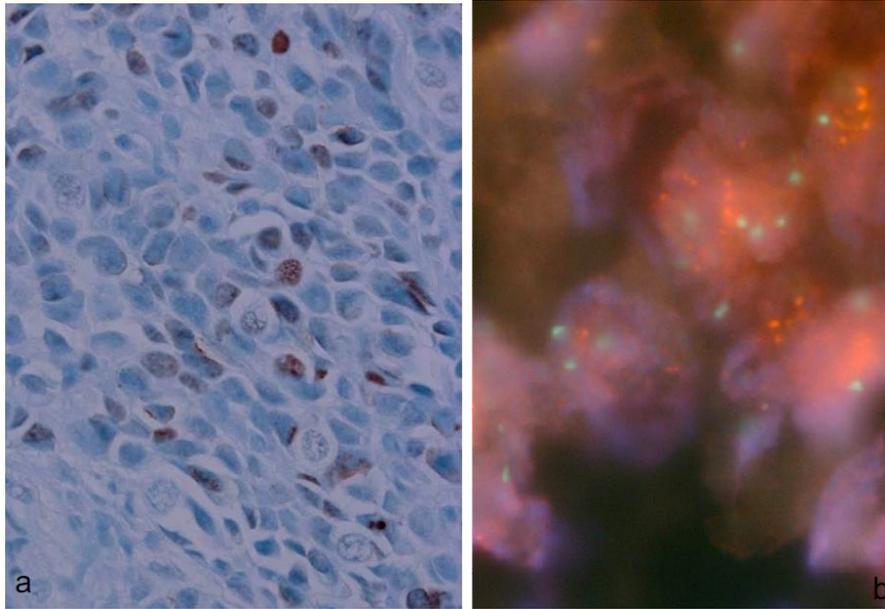


Figure 3. TOPOII $\alpha$  index of 16.3% (a) and aneuploidy of TOPOII $\alpha$  gene (b: TOPOII $\alpha$  / CEP17 = 1.14). Index was evaluated under 40 $\times$  objective with respect to the positive cell ratio calculated based on the selected five visual fields that are most frequently stained.

In our study, when deeming the signal rate of  $\geq 2.0$  as amplification and that of  $< 0.8$  as deletion, HER2 gene amplification was observed in 26.6% of the infiltrating duct carcinomas. Of these tumors, TOPOII $\alpha$  gene amplification and deletion were observed in 25.9% and 11.1% of the tumors, respectively. In addition, when deeming the signal rate of  $\geq 1.5$  as amplification, the HER2 gene amplification was observed in 39.9% of the tumors. Of these tumors, TOPOII $\alpha$  gene amplification and deletion were observed in 51.9% and 8.6% of the tumors, respectively. Moreover, when the HER2 genes were normal (signal rate ranging from 0.8 to 1.5), the TOPOII $\alpha$  gene amplification was observed in 1.7% at the signal rate of  $\geq 2.0$  and in 10.1% at the signal rate of  $\geq 1.5$ , while deletion was observed in 0.8% [4].

At present, in Japan, the classification of gene amplification is being unified into the HER2 Gene Amplification Standards published by the American Society of Clinical Oncology (ASCO) in 2007. According to these standards, the signal rate of  $\geq 2.2$  is deemed as amplification, ranging from 0.8 to 1.8 is deemed as normal, and that of  $< 0.8$  is deemed as deletion. The signal rate ranging from 1.8 to 2.2 is classified under the new concept of “equivocal” (borderline region) [15]. Table 1 shows the classification of the results of our study previously described based on these standards. Compared to the data having the signal rate of  $\geq 2.0$ , the amplification positive rate decreased slightly.

## Topoisomerase Inhibition

There are three types of topoisomerase inhibitors: type I inhibitor, type II inhibitor, and dual inhibitor (which inhibits both types). There are two classifications of type II inhibitors: One inhibits the reconnection of the severed double-stranded DNA caused by TOPO II, in doing so it stabilizes the severing complex; and the other inhibits DNA synthesis through the intercalation (bonding) of the double-stranded DNA. Those that display both actions are called intercalators, and those that only show the TOPO II inhibitor action are called non-intercalators [1]. TOPOII inhibitors include anthracyclines (doxorubicin, epirubicin, mitoxantrone, amsacrine, and actinomycin D) as intercalators and epipodophyllotoxins (etoposide and teniposide) as non-intercalators [19].

Anthracyclines, which are intercalators, serve as substrates of P-glycoprotein, and therefore, transfer into cells through passive diffusion [1], where they cause DNA damage due to the TOPO II inhibition and thereby induce apoptosis. They are secreted by P-glycoprotein existing in the brain-vascular barrier. P-glycoprotein high expression, TOPO II protein low expression, and gene mutation are all related to the resistance against the Anthracycline-based chemotherapy.

In addition, because the cardiotoxicity of anthracyclines is irreversible [20], if anthracyclines are used in combination with trastuzumab, cardiac disturbance occurs with high frequency [21]. When administering an anthracyclines, the left ventricular ejection function should be measured using echography and scintigraphy on a regular basis. If the ejection fraction deteriorates, it is important to immediately stop administering the anthracyclines [22].

## TopoII $\alpha$ Genes, Protein Expression, and TopoII $\alpha$ Inhibitor Sensitivity

TOPOII $\alpha$  is a molecular target of TOPO II inhibitors, which inhibit the function of TOPOII $\alpha$  proteins. Thus, the sensitivity of TOPOII $\alpha$  inhibitors depends on the level of TOPOII $\alpha$  protein expression of the cancer cells. In other words, a tumor having a low TOPOII $\alpha$  protein concentration has lower TOPOII $\alpha$  inhibitor sensitivity than a tumor having a higher TOPOII $\alpha$  protein concentration. Moreover, in the cancer cells having a high concentration TOPOII $\alpha$  protein level, hormone receptor non-expression, HER2 protein overexpression, p53 genetic abnormality, DNA aneuploidy, and poor differentiation are observed [9]. Meanwhile, with regard to the TOPOII $\alpha$  genes, Knoop et al. reported that both TOPOII $\alpha$  gene amplification and deletion were effective markers for the prediction of anticancer agents, including epirubicin [14]. Epirubicin is one of anthracyclines in which the cardiotoxicity has been reduced [1]. Knoop et al. investigated the HER2 genes and TOPOII $\alpha$  genes of breast cancers that had been surgically removed prior to the implementation of the CMF (cyclophosphamide, methotrexate, fluorouracil) and CEF (cyclophosphamide, epirubicin, fluorouracil) treatments, using the FISH method. The researchers then investigated the HER2 protein expression immunohistochemically. As a result, it was found that although the state of the HER2 genes had no correlation with the therapeutic effect, the abnormality of

the TOPOII $\alpha$  genes (i.e., amplification and deletion) correlated with the patient's disease-free survival and the overall increase in the survival rate.

With regard to the TOPOII $\alpha$  gene deletion, Jarvinen et al. have reported that under the condition of HER2 gene amplification, it occurs with frequency similar to that of amplification (amplification  $\geq 1.5$ , deletion  $\leq 0.7$ ), and that the TOPO II inhibitor sensitivity decreases [8]. In our study, when deeming the signal rate of  $\geq 1.5$  as amplification, HER2 gene amplification was observed in 39.9% of all the infiltrating duct carcinomas. While TOPOII $\alpha$  gene amplification occurred in 51.9% of the 39.9% infiltrating duct carcinomas, TOPOII $\alpha$  gene deletion occurred in only 8.6% [4]. Park et al. also investigated breast cancers, deeming the signal rate of  $\geq 1.5$  as amplification and that of  $\leq 0.75$  as deletion. As a result, HER2 gene amplification was observed in 8.5% of the tumors. Of the 8.5% tumors, TOPOII $\alpha$  gene amplification occurred in 18 tumors (75%) and deletion occurred in 25% of the tumors. Based on these findings, they reported that TOPOII $\alpha$  gene deletion does not occur as frequently as amplification [23]. With regard to HER2 gene amplification, the signal rate of  $\geq 2.0$  is usually deemed as amplification in the conventional evaluation method. However, because it has been proposed that having the signal rate of  $\geq 2.2$  or more than six copies should be deemed as amplification from 2007 [15], it is necessary to organize the frequency of TOPOII $\alpha$  genetic abnormalities according to the new classification.

**Table 2. HER2 gene and TOPOII $\alpha$  gene in 203 breast cancers (ASCO 2007)**

HER2/CEP			TOPOII $\alpha$ /CEP		
	Amplification $\geq 2.2$	Equivocal 1.8-2.2	Normal 0.8 - 1.8	Deletion $<0.8$	All
Amplification $\geq 2.2$	12 (23.5%)	4 (7.8%)	29 (56.9%)	6 (11.8%)	51 (25.1%)
Equivocal 1.8-2.2	1 (6.7%)	3 (20.0%)	10 (66.7%)	1 (6.7%)	15 (7.4%)
Normal 0.8 - 1.8	3 (2.2%)	6 (4.5%)	124 (92.5%)	1 (0.7%)	134 (66.6%)
Deletion $<0.8$	0	0	1 (33.3%)	2 (66.7%)	3 (1.5%)
All	16 (7.9%)	13 (6.4%)	164 (80.8%)	10 (4.9%)	203

**Table 3. Relationships between HER2 gene and TOPOII $\alpha$  gene status**

HER2/CEP			TOPOII $\alpha$ /CEP		
	Amplification $\geq 2.0$	Gain 1.5-2.0	Normal 0.8 - 1.5	Deletion $<0.8$	All
Amplification $\geq 2.0$	14 (25.9%)	13 (24.1%)	21 (38.9%)	6 (11.1%)	54 (26.6%)
Gain 1.5-2.0	5 (18.5%)	10 (37.0%)	11 (40.7%)	1 (7.4%)	27 (13.3%)
Normal 0.8 - 1.5	2 (1.7%)	10 (8.4%)	106 (89.1%)	1 (0.8%)	119 (58.6%)
Deletion $<0.8$	0	0	1 (33.3%)	2 (66.7%)	3 (1.5%)
All	21 (10.3%)	33 (16.3%)	139 (68.5%)	10 (4.9%)	203

In our study, when deeming the signal rate of  $\geq 2.0$  as amplification, TOPOII $\alpha$  genetic abnormalities (i.e., amplification and deletion) occurred in 37.0% of the HER2-amplified breast cancers (Table 3). Moreover, when deeming the signal rate of  $\geq 2.2$  as amplification, the same phenomena occurred in 35.3% of the same cancers (Table 2). In addition, although it had been shown in previous reports that TOPOII $\alpha$  genetic abnormalities could be observed only in HER2-amplified breast cancer<sup>(24)</sup>, it was subsequently reported that such genetic abnormalities can also be observed in HER2 non-amplified tumors. Knoop et al. has observed TOPOII $\alpha$  genetic abnormalities in 56.9% of the HER2-amplified tumors (with the signal rate of  $\geq 2.0$ ) and in 7.6% of the HER2 non-amplified tumors<sup>(14)</sup>. Park et al. also found TOPOII $\alpha$  genetic abnormalities in 0.95% of the entire group of HER2 non-amplified tumors [23]. In our study, when deeming the HER2 genetic normality as 0.8–1.8, amplification was observed in 2.2% of the tumors and deletion was observed in 0.7% of the tumors (Table 2).

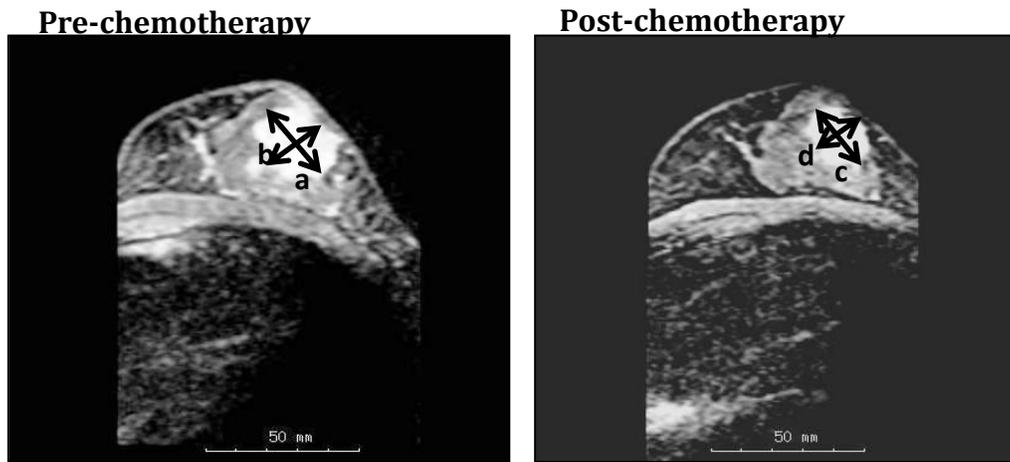
The TOPO II inhibitor sensitivity depends on the level of TOPOII $\alpha$  protein expression of the cancer cells. Table 4 indicates the results of comparison among the TOPOII $\alpha$  gene, the TOPOII $\alpha$  index, the Ki67 index, and the HER2 gene in 172 infiltrating duct carcinomas. There was no difference between the TOPOII $\alpha$  index of the TOPOII $\alpha$  gene-deleted tumors and that of other groups. Moreover, there was no difference between the Ki67 index of the TOPOII $\alpha$  gene-deleted tumor and that of the amplified tumors. Furthermore, the Ki67 index of the TOPOII $\alpha$  gene amplification cases (signal rate of  $\geq 2.2$ ) significantly increased in comparison to the normal cases (signal rate ranging 0.8–1.8). We performed the neoadjuvant chemotherapy with anthracyclines for 12 infiltrating ductal carcinomas of the breast and evaluated the tumor reduction rates (Figure 4) after the chemotherapy. As a result, we found a statistically significant ( $p = 0.01$ ) correlation of the tumor reduction rate with the TOPOII $\alpha$  index, but not with TOPOII $\alpha$  or HER2 gene amplification (Figure 5). Moreover, among the 28 tumors in which the neoadjuvant chemotherapy with anthracyclines was performed, the correlation between the TOPOII $\alpha$  index and the tumor regression rates had been evaluated. Consequently, it was observed that the tumor regression rate increased more significantly in the breast cancers in which the TOPOII $\alpha$  index was evaluated  $\geq 25\%$  than the tumors in which the TOPOII $\alpha$  index was evaluated  $< 10\%$  (Figure 6). Although these 28 cases included no case of TOPOII $\alpha$  gene deletion, PR (partial response) with the regression rate of 22% was observed in a single TOPOII $\alpha$  gene-deletion case (TOPOII $\alpha$  gene signal rate: 0.69, TOPOII $\alpha$  protein index: 17.0%, and Ki67 index: 53.5%), on which the clinical image evaluation was conducted after administering the anthracyclines and taxane. These facts suggest the possibility that the TOPOII $\alpha$  index, immunohistochemically calculated on the tissue obtained prior to the Anthracycline-based chemotherapy, may serve as a prediction factor for the effects of TOPO II inhibitors. In addition, Tinari et al. conducted a study on the breast cancer cases in which neoadjuvant chemotherapy or primary systemic therapy was performed using the anthracyclines. They reported that the HER2 protein expression and TOPOII $\alpha$  protein expression correlated to the therapeutic effects, and the cases in which the level of TOPOII $\alpha$  protein expression had increased after the therapy showed significantly low survival rates [13]. Considering this data, when using the TOPO II inhibitor for chemotherapy, it is important to calculate the immunohistochemical TOPOII $\alpha$  index in the tissues obtained before and after chemotherapy, particularly in terms of prediction for the therapeutic effect of the anthracyclines as well as the prediction of patient prognosis.

**Table 4. Comparison of TOPOII $\alpha$  gene, TOPOII $\alpha$  Index, Ki67 index and HER2 gene in 172 infiltrating ductal carcinomas of the breast**

TOPOII $\alpha$	Number	TOPOII $\alpha$	Ki67	Number of HER2			
				A	E	N	D
Amplification							
$\geq 2.2$	15 (8.7%)	19.7%	26.6%	11	1	3	0
Equivocal							
1.8-2.2	9 (5.2%)	20.8%	15.9%	2	2	5	0
Normal							
0.8-1.8	140 (81.4%)	16.9%	18.1%	19	9	111	1
Deletion							
$< 0.8$	6 (3.5%)	22.9%	29.7%	4	1	1	0

A, Amplification; E, Equivocal; N, Normal; D, Deletion

<sup>a</sup> $P = 0.038$  (Welch),  $P = 0^b$ .022 (student  $t$ )



$$\text{Reduction rate} = \frac{(a + b) - (c + d)}{a + b}$$

Figure 4. Tumor reduction rate calculating the tumor diameter before and after the chemotherapy.

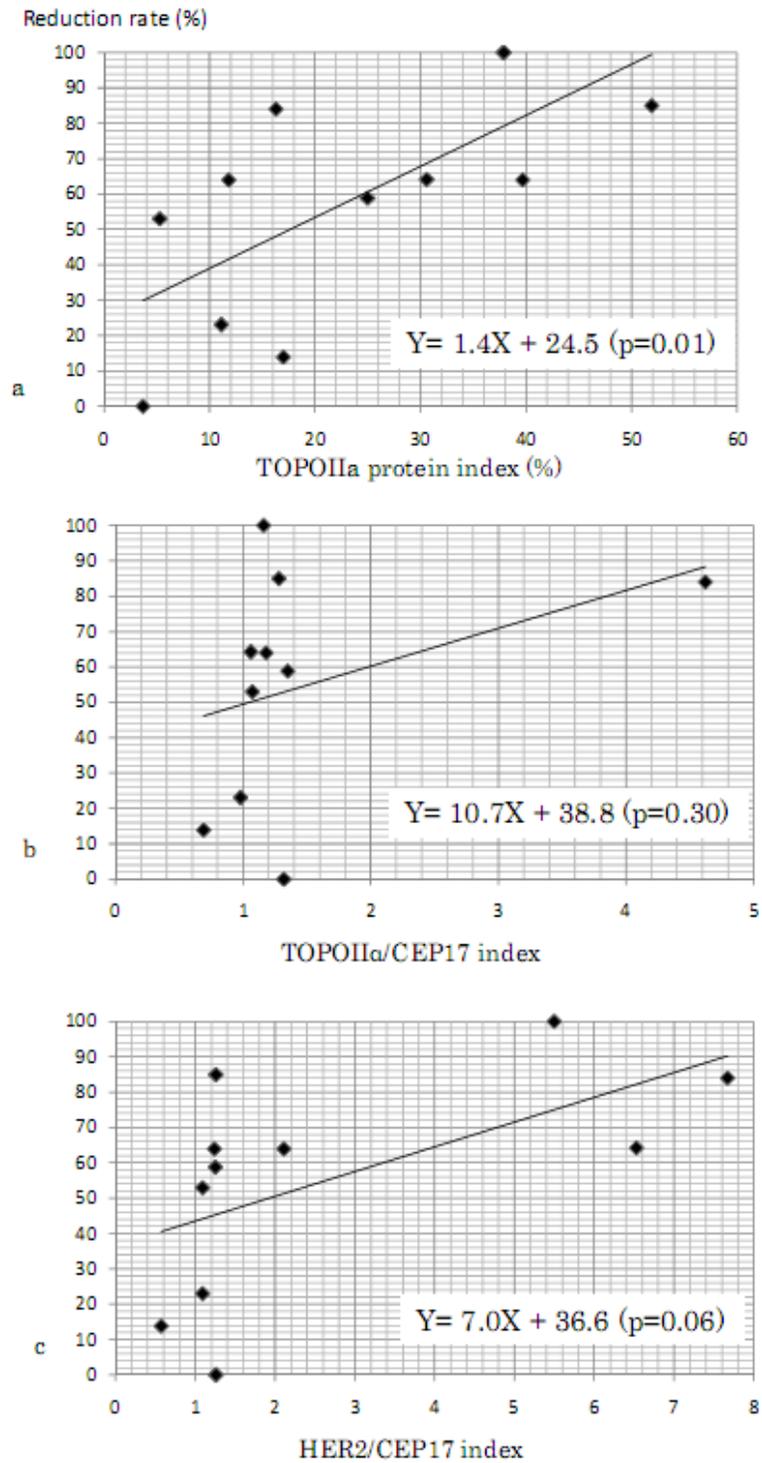


Figure 5. Relations of the tumor reduction rates to the TOPOII $\alpha$  index (a;  $p = 0.01$ ), TOPOII $\alpha$  gene (b;  $p = 0.30$ ) and HER2 gene (c;  $p = 0.06$ ).

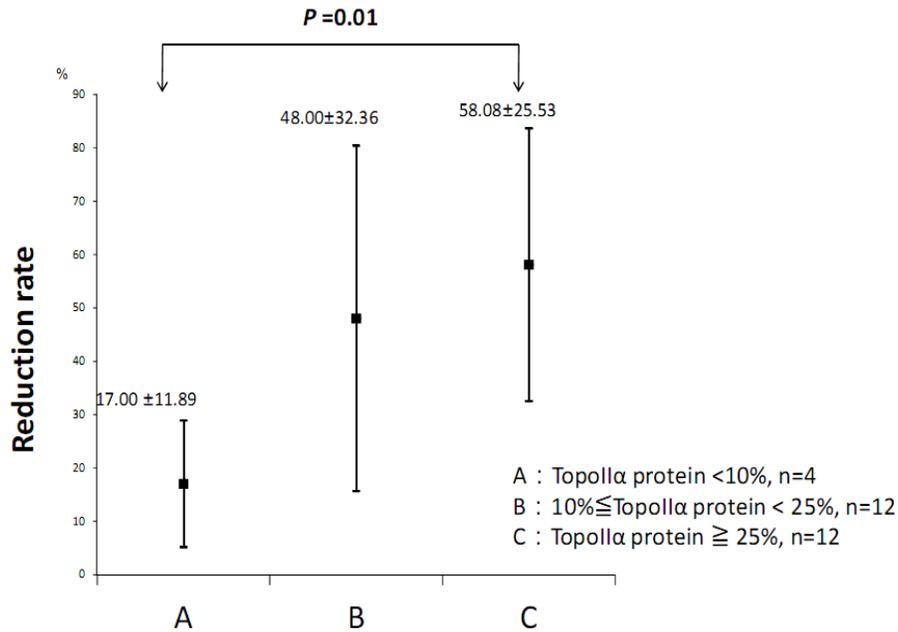


Figure 6. Correlation between the TOPOIIα index and the tumor regression rate.

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