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Survival Analysis of Patients With Duodenal Gastrointestinal Stromal Tumors

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Goals: To evaluate the survival characteristics of patients with duodenal gastrointestinal stromal tumors (GISTs).

Background: GISTs represent the most common mesenchymal neoplasms. However, duodenal GISTs are relatively rare, and few studies have been performed with a focus on duodenal GISTs.

Study: We collected the data of 41 GIST patients including 7 duodenal cases. Clinicopathologic findings and recurrence-free survival (RFS) of duodenal GIST patients were analyzed.

Results: The proportion of having any symptoms was 86% in duodenum, 32% in stomach, and 56% in other GISTs ($P = 0.034$), and the most common symptoms of duodenal GISTs were melena and anemia. The 2-year RFS rates were 51.4% in duodenal GISTs, 78.4% in stomach GISTs, and 100% in other GISTs, and duodenal GISTs showed poorer RFS than nonduodenal GISTs (hazard ratio, 5.1; log-rank $P = 0.019$). Particularly, in low-risk and intermediate-risk group, the hazard ratio of recurrence was 12.3 (log-rank $P = 0.010$). Multivariate Cox analysis showed symptom ($P = 0.007$), mitotic index ($P = 0.011$), and tumor location ($P = 0.043$) were significant prognostic factors of recurrence.

Conclusions: RFS of duodenal GISTs was worse than nonduodenal GISTs.

Key Words: duodenum, GIST, RFS, survival

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Gastrointestinal stromal tumors (GISTs) represent the most common mesenchymal neoplasms arising within the gastrointestinal tract. These tumors are thought to share a common progenitor cell with the interstitial cells of Cajal, and usually have activating mutations in either c-kit (75% to 80%) or platelet-derived growth factor receptor α (PDGFRA) (5% to 10%), 2 closely related receptor tyrosine kinases.[1] These mutations lead to ligand-independent activation and signal transduction mediated by constitutively activated KIT or PDGFRA. This theory was first proposed by Kindblom et al[2] and Hirota et al[3] revealed an association between the presence of c-kit mutation and tumor development.

GISTs can arise anywhere in the gastrointestinal tract, but their most frequent locations are the stomach (60%) and the small intestine (25%). Duodenal GISTs are relatively rare and comprise about 5% of surgically resected GIST cases.[4,5] Earlier studies have reported that duodenal GISTs were larger than stomach GISTs, and that their most frequent locations were the second and third portions of the duodenum.[4] Owing to the unique and complex anatomy of the duodenum, complete resection of duodenal GISTs sometimes requires wide resection methods such as pancreaticoduodenectomy,[6] which is rarely the case for GISTs in other locations. Only few reports about the characteristics of duodenal GISTs have been published earlier,[7–9] and few studies have been performed a survival analysis of patients with duodenal GISTs. From August 1993 to January 2008, we encountered 41 GIST cases of which 7 were duodenal GISTs. Here we conduct a retrospective cohort study to evaluate the survival characteristics of duodenal GISTs.

PATIENTS AND METHODS

Patients

We retrospectively reviewed the records of all patients with GISTs treated at the Osaka National Hospital between August 1993 and January 2008. The diagnosis of GISTs was conducted by histologic examination, immunohistochemical staining for KIT and CD34, and detection of c-kit or PDGFRA mutations.

Data on patients' age, sex, tumor location, symptoms, pathologic findings, c-kit and PDGFRA mutations, treatment, and survival outcome were collected. Tumor size was defined as the largest diameter of the primary tumor in any dimension. Pathologic data included mitotic index and results of immunohistochemical staining for KIT and CD34. Treatment data included type of resection and adjuvant treatment. Tumor size and mitotic index were used for risk classification according to the Fletcher score.[10] However, in this study, we combined low-risk and intermediate-risk patients in the survival analysis because "low-risk" has not been defined for duodenal GISTs.

Statistical Analysis

Associations between tumor location and clinicopathologic variables were analyzed using the χ^2 test. Recurrence-free survival (RFS) was defined as the time

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TABLE 1. Characteristics of 7 Patients With Duodenal GISTs

Age (y)	Sex	Clinical Symptom	Location of Duodenal GIST	Size (mm)	Operation	Adjuvant Therapy	KIT	CD34	Mitotic Index (per 50 HPF)	*C-Kit* Mutation	Risk Classification
64	F	Melena	Second part	60	Gastrojejunostomy	−	−	−	< 5	Exon 11	Intermediate
58	F	Melena	Second part	70	Pancreaticoduodenectomy	−	+	+	< 5	NA	Intermediate
70	F	Abdominal mass	Fourth part	150	Partial duodenal resection	+	+	+	< 5	Exon 13	High
67	F	Absent	First part	60	Partial duodenal resection	−	+	+	< 5	Exon 11	Intermediate
39	F	Melena	First part	120	Partial duodenal resection	−	+	+	5-10	Exon 11	High
65	M	Anemia	Second part	30	Partial duodenal resection	−	+	NA	5-10	Exon 9	Intermediate
75	F	Anemia	Second part	40	Pancreaticoduodenectomy	−	+	−	> 10	Exon 11	High

GISTs indicates gastrointestinal stromal tumors; HPF, high-power field; F, female; M, male; NA, not analyzed.

from surgery to either the first recurrence or death from any cause. RFS curves were estimated by the Kaplan-Meier method and compared using the log-rank test. Multivariate Cox regression analyses were performed to adjust for the potential confounding factors whose *P* values were under 0.2 in univariate analyses. All statistical analyses were performed with SPSS software, version 15.0J. *P* values less than 0.05 were considered statistically significant, and all tests were 2-sided.

RESULTS

Patient Characteristics

Forty-one patients with GISTs were admitted for treatment to Osaka National Hospital between August 1993 and January 2008, and of these 7 patients (17%) were diagnosed with duodenal GISTs (Table 1). Six of the 7 duodenal GIST patients were female. The second portion of the duodenum was most frequently affected, which is of significance because of the need for pancreaticoduodenectomy if the tumor is located on the same side of intestine as the Papilla Vater. For 1 duodenal GIST patient, we could not perform radical surgery because of severe patient's general condition, whereas the other duodenal GIST patients received complete gross resection. Postoperative complications occurred in 3 of 7 duodenal GIST patients. These complications included pancreatic fistula, and intra-abdominal abscess, but none of the patients died within 1 month after surgery. Only 1 patient received adjuvant chemotherapy after surgery. One patient showed immunohistochemical staining of neither c-kit nor CD34. Six cases had c-kit mutations; 4 for exon 11, 1 for exon 9, and 1 for exon 13. The numbers of intermediate-risk and high-risk patients were 4 (57%) and 3 (43%), respectively.

We compared patients with duodenal GISTs to those with stomach GISTs and other GISTs (Table 2). Among 9 patients with other GISTs, 4 were found in rectum and in small intestine, and 1 in omentum. There were no statistical differences in clinicopathologic factors except for clinical symptoms and CD34 positivity. With regard to immunohistochemical findings, the KIT-positive rate was similar in duodenal and other GISTs, whereas the CD34-positive rate was lower in duodenal GISTs ($P = 0.049$). Although over 30% patients with stomach GISTs were classified as low-risk, there were no low-risk patients among the duodenal and other GIST groups.

Of patients with duodenal GISTs, 86% had symptoms, whereas 32% of patients with stomach GISTs, and 56% of those with other GISTs were affected; this difference was statistically significant ($P = 0.034$). Five of 6 symptomatic patients with duodenal GISTs had melena or anemia, whereas a half of symptomatic patients with stomach GISTs complained of epigastralgia (Table 3).

TABLE 2. Comparison of Characteristics Among Duodenal GISTs, Stomach GISTs, and GISTs in Other Locations

	Duodenum (n = 7)	Stomach (n = 25)	Other (n = 9)	*P*
Age (y)				0.50
Median (range)	65 (39-75)	67 (48-82)	59 (45-86)	
Sex				0.10
Male	1 (14%)	13 (52%)	2 (22%)	
Female	6 (86%)	12 (48%)	7 (78%)	
Clinical Symptom				0.034
Absent	1 (14%)	17 (68%)	4 (44%)	
Present	6 (86%)	8 (32%)	5 (56%)	
Immunohistochemistry				
KIT				0.53
Positive	6 (86%)	22 (88%)	9 (100%)	
Negative	1 (14%)	3 (12%)	0 (0%)	
CD34*				0.049
Positive	4 (67%)	23 (96%)	6 (67%)	
Negative	2 (33%)	1 (4%)	3 (33%)	
Tumor size (cm)†				0.40
Median (range)	6.0 (3.0-15)	5.0 (1.7-24)	6.0 (2.5-12)	
Mitotic Index (per 50 HPF)				0.59
< 5	4 (57%)	16 (64%)	3 (33%)	
5-10	2 (29%)	5 (20%)	3 (33%)	
> 10	1 (14%)	4 (16%)	3 (33%)	
Risk Classification				0.13
Low	0 (0%)	8 (32%)	0 (0%)	
Intermediate	4 (57%)	7 (28%)	5 (56%)	
High	3 (43%)	10 (40%)	4 (44%)	
C-kit mutation‡				0.33
Exon 9	1 (17%)	0 (0%)	1 (50%)	
Exon 11	4 (66%)	12 (86%)	1 (50%)	
Exon 13	1 (17%)	1 (7%)	0 (0%)	
Exon 17	0 (0%)	1 (7%)	0 (0%)	

*One duodenal GIST case and 1 stomach GIST case were not analyzed.
†One stomach GIST case was not analyzed.
‡One duodenal GIST case, 11 stomach GIST cases, and 7 other GIST cases were not analyzed.
GISTs indicates gastrointestinal stromal tumors; HPF, high-power field.

TABLE 3. Symptoms Among Duodenal GISTs, Stomach GISTs, and GISTs in Other Locations

	Duodenum (n = 6)	Stomach (n = 8)	Other (n = 5)
Anemia	2 (33%)	2 (25%)	0
Melena	3 (50%)	0	1 (20%)
Epigastralgia	0	4 (50%)	0
Abdominal mass	1 (17%)	1 (13%)	1 (20%)
Nausea	0	1 (13%)	1 (20%)
Others	0	0	2 (40%)

GISTs indicates gastrointestinal stromal tumors.

Survival

In survival analysis of all GIST patients, the 2-year RFS rates of duodenal, stomach, and other GISTs were 51.4%, 78.4%, and 100%, respectively ($P = 0.058$) (Fig. 1A). As the survival curves of stomach and other GISTs were similar, we combined the stomach GISTs with other GISTs as a nonduodenal group, and compared RFS of duodenal GIST patients with those of nonduodenal GIST patients. As the result, the hazard ratio (HR) of recurrence was 5.1 [95% confidence interval (CI), 1.1-23.2] in the duodenal GIST patients, and the log-rank test showed statistical significance ($P = 0.019$) (Fig. 1B). In the low-risk and intermediate-risk groups specifically, the 2-year RFS rates of patients with duodenal and nonduodenal GISTs were 50% and 100%, respectively, showing a statistical difference (log-rank $P = 0.010$) and the HR of recurrence was 12.3 (95% CI, 1.1-142.9) (Fig. 2A). However, in the high-risk group there was no significant difference in RFS between duodenal GIST patients and nonduodenal GIST patients (log-rank $P = 0.60$) (Fig. 2B), and the HR of recurrence was 1.8 (95% CI, 0.19-17.9).

Univariate analyses revealed that symptom ($P = 0.009$), mitotic index ($P = 0.038$), and tumor location ($P = 0.035$) were the statistically significant prognostic factors of RFS (Table 4). These 3 factors were significantly associated with RFS even in multivariate analysis.

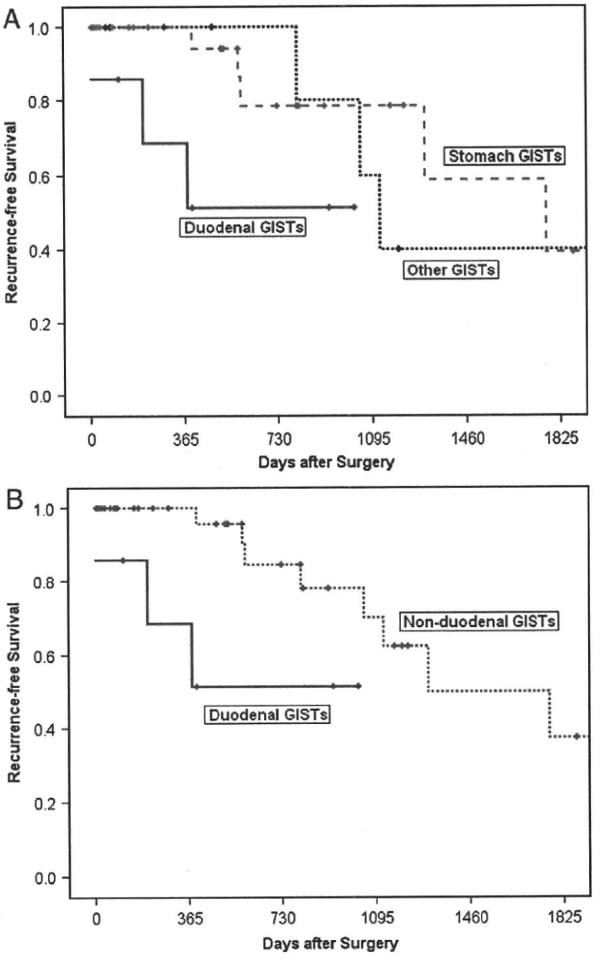

FIGURE 1. Recurrence-free survival of patients with gastrointestinal stromal tumors (GISTs) on the basis of tumor location. A, Duodenal versus stomach versus other GISTs. B, Duodenal versus nonduodenal GISTs.

DISCUSSION

GISTs are often discovered in the stomach and small intestine, but duodenal GISTs comprise only about 5% of these. Although 2 case series have studied duodenal GISTs,[7,8] neither conducted a survival analysis. This study showed that the RFS of duodenal GIST patients was worse than that of patients with stomach GISTs or GISTs in other locations, and the poor prognosis of duodenal GISTs was more remarkable in low-risk and intermediate-risk patient groups. Several earlier studies have reported that patients with GISTs of the small intestine have an unfavorable prognosis, compared with stomach GISTs.[11–13] In this study, we combined small intestine cases with stomach cases, because the survival curves of stomach and other GISTs were similar. Multivariate Cox analyses performed after adjusting for other prognostic factors revealed that tumor location was

TABLE 4. Association of Clinicopathological Factors With Recurrence-free Survival

	Univariate		Multivariate	
	Hazard Ratio (95% CI)	P	Hazard Ratio (95% CI)	P
Age (>65 y)	2.4 (0.64-9.2)	0.20	1.6 (0.34-7.3)	0.56
Sex (male)	1.4 (0.43-4.8)	0.55	—	—
Symptom (present)	17.4 (2.0-150.1)	0.009	158.1 (3.9-6374.0)	0.007
Tumor size (>5 cm)	1.6 (0.45-5.7)	0.47	—	—
Mitotic index (≥5/50 HPF)	5.1 (1.1-23.8)	0.038	37.0 (2.3-596.7)	0.011
Location (duodenum)	5.1 (1.1-23.3)	0.035	10.9 (1.1-111.1)	0.043

CI indicates confidence interval; HPF, high-power field.

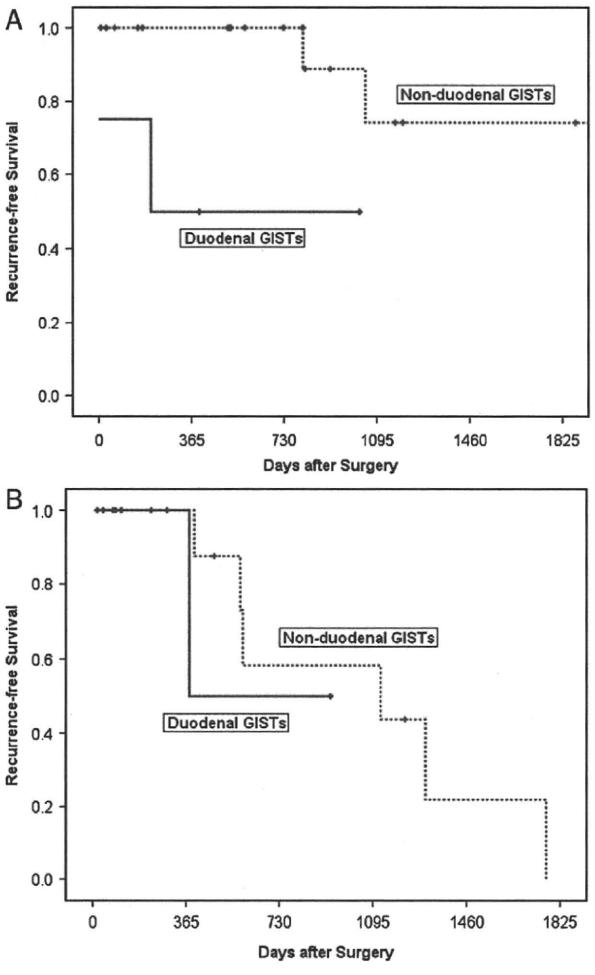

FIGURE 2. Recurrence-free survival of patients with duodenal gastrointestinal stromal tumors (GISTs) and nonduodenal GISTs in (A) low-risk and intermediate-risk group and (B) high-risk group.

an independent prognostic factor for GISTs. This result may indicate that the duodenal GISTs are biologically different from other GISTs.

Over the past several years, site-specific differences in appearance, morphology, and clinical outcome have been identified in GISTs. It has been reported that the proportion of CD34-positive tumors and the frequency of c-kit mutations are different depending on location.[14,15] An earlier study reported that CD34 positivity was more frequent in malignant tumors than in borderline or benign tumors.[16] Another study reported that CD34 positivity in patients with recurrence is higher than those without recurrence, although the difference was not statistically significant.[17] In this study, the proportion of CD34-positive patients with duodenal GISTs was even lower than that in patients with stomach GISTs. Thus, we cannot explain the poor survival of patients with duodenal GISTs by CD34 positivity alone. In contrast, earlier studies showed that mutations of exon 9 were more common in patients with small intestinal GISTs than in those with stomach GISTs.[18,19] GISTs with exon 9 mutations are often clinically and pathologically malignant, and this subgroup of patients is often resistant to imatinib. In our population, a duodenal GIST patient with an exon 9 mutation showed early metastases to the liver after surgery. The positivity rate of c-kit exon 9 mutations may contribute to the poor survival of patients with duodenal GISTs. In this study, however, we did not analyze c-kit mutation sites for about half of all GIST cases, so we could not evaluate the association between survival and the location of c-kit mutation.

In comparison of clinicopathologic characteristics among 3 location types of GISTs, clinical symptom was the most significant finding. Many duodenal GIST patients had symptomatic complaints that were mainly associated with bleeding from tumor, whereas the proportion of stomach GIST patients who had any clinical symptoms in diagnosis was low (28%). Most of asymptomatic patients with stomach GISTs were diagnosed in medical screening or follow-up of other diseases. In Japan, medical screening with upper gastrointestinal endoscopy or x-ray has been widespread because of high prevalence of gastric cancer, and it may contribute to early detection of asymptomatic stomach GISTs. These features may induce the survival difference between the duodenal and nonduodenal GISTs. However, the tumor location was an independent prognostic factor after adjusting for the presence of clinical symptoms in the multivariate Cox analyses.

Surgery remains the mainstay of treatment for patients with primary GISTs without distant metastasis. A recent retrospective study to compare the survivals of duodenal GIST patients after pancreaticoduodenectomy with those after limited resection reported that the disease-free survivals were similar between 2 surgical procedures.[6] In this study, both the 2 cases who received pancreaticoduodenectomy are alive without recurrence, whereas 2 of 4 patients who received limited duodenal resection had recurrence after surgery. Complete gross resection with an intact pseudocapsule may be the most important thing to treat duodenal GISTs, and so we should not hesitate to perform combined resection such as pancreaticoduodenectomy to achieve gross resection, even though the surgical procedure is highly invasive.

Limitations of this study include its retrospective design and small sample size. As survival analyses with small number of patients sometimes mislead the results, we should therefore be careful in evaluating its results. However, to our knowledge, this is the first study to focus on the survival of patients with duodenal GISTs, and the difference of RFS between duodenal and nonduodenal GISTs was remarkable. In the future, prospective studies using larger numbers of patients will be needed.

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Chapter IX

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

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Abstract

DNA topoisomerase II-alpha (TOPOIIα) 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 TOPOIIα index ≧ 25 % regressed more effectively than those with TOPOIIα index < 10 % on histology by Anthracycline-based chemotherapy. TOPOIIα index of TOPOIIα gene-deleted tumors did not differ from that of TOPOIIα gene-amplificated or normal tumors. TOPOIIα index, not its gene amplification, is a useful marker of Anthracycline-based chemotherapy in breast cancers.

Keyterms: *DNA Topoisomerase IIα, Index, Anthracycline, Effect prediction, Ki67*

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DNA Topoisomerase IIα

DNA topoisomerase is the gèneric 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 α and β, which are expressed from a different gene. Although on the N-terminal side 3/4 of TOPOIIβ is highly homologous with TOPOIIα, it has a different C-terminal domain, and the activity control mechanism exists in this domain.

DNA topoisomerase IIα (TOPOIIα) 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).

TOPOIIα Index

TOPOIIα protein is a cell proliferation-related antigen, which expresses in the S and G2/M phases during the normal cell cycle. TOPOIIα protein increases by a factor of two to three times in the G2/M phase. Particularly, it expresses most in highly proliferative cells. TOPOIIβ, 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 TOPOIIα 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 TOPOIIα 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 TOPOIIα, 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 TOPOIIα index not only indicates the proliferation activity but may also relate to the quantitative changes of cancer cells [8, 9].

Ki67 Index

TOPOIIα 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 TOPOIIα protein expression in more than 10% of the tumor cells.

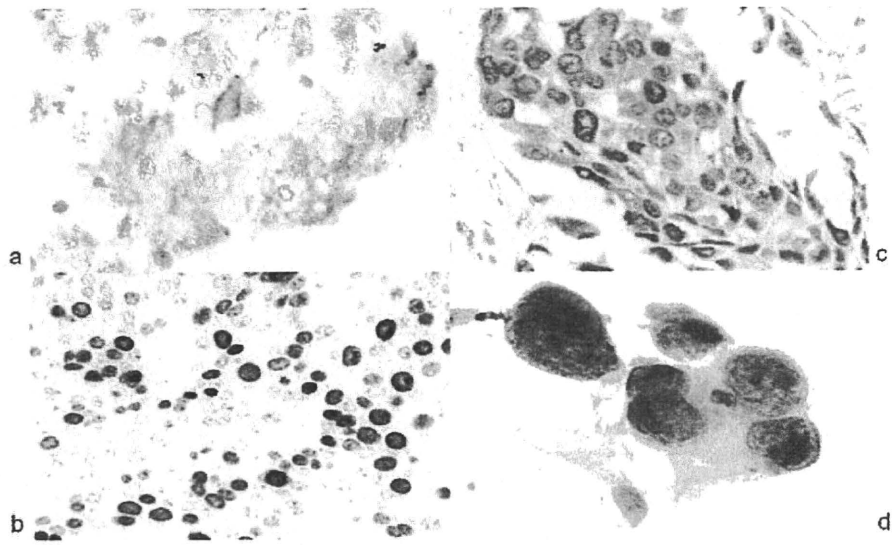

Figure 1. Immunochistochemical findings and cytological atypia of breast cancer cells. (a: Caspase 3 index- 44.8%, b: Ki67 index-18.8%, c: PCNA index-88.7%, d: nuclar atypia grade 3 by Robinson's classification).

The expression of TOPOIIα 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 TOPOIIα 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, TOPOIIα protein expression, HER2 gene amplification, and TOPOIIα 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 TOPOIIα 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 TOPOIIα protein expressions had correlations with the therapeutic effect, neither nuclear atypia nor the Ki67 index had any correlation with the therapeutic effect [13].

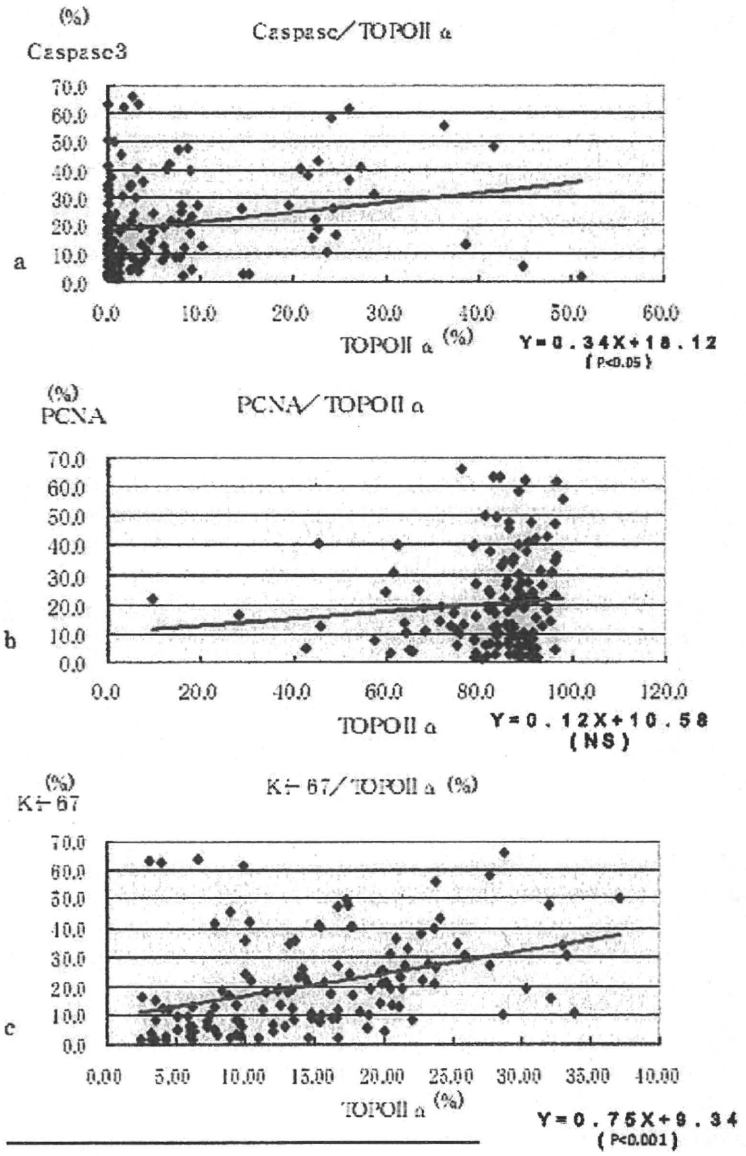

Figure 2. Relations of TOPOIIα index to Caspase 3 index (A), PCNA index (B), and Ki67 index (C), with statistical significance in A and C.

TOPOIIα 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 ≥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

More proper arrangement for this table is requested

Clinico-	Number		Tumor	Imunochistochemistry						FISH	
pathological	of	Age	size	ER score	PgR score	Herc ep score	Ki67(%)	TOPOII(%)	Caspase3 (%)	HER2/CEP	TOPOIIα/CEP
characteristics	cases		cm	0~3	0~3	0~3	%	%	%	index	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
Pathologic al 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, TOPOIIα gene amplification was evident in 67.7%. However, no deletion was observed in either the HER2 genes or TOPOIIα genes [18]. Knoop et al. reported that when deeming the signal rate of ≥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 TOPOIIα genes also

amplified. Moreover, deletion was observed in 23.6% of the HER2 gene-amplified breast cancers.

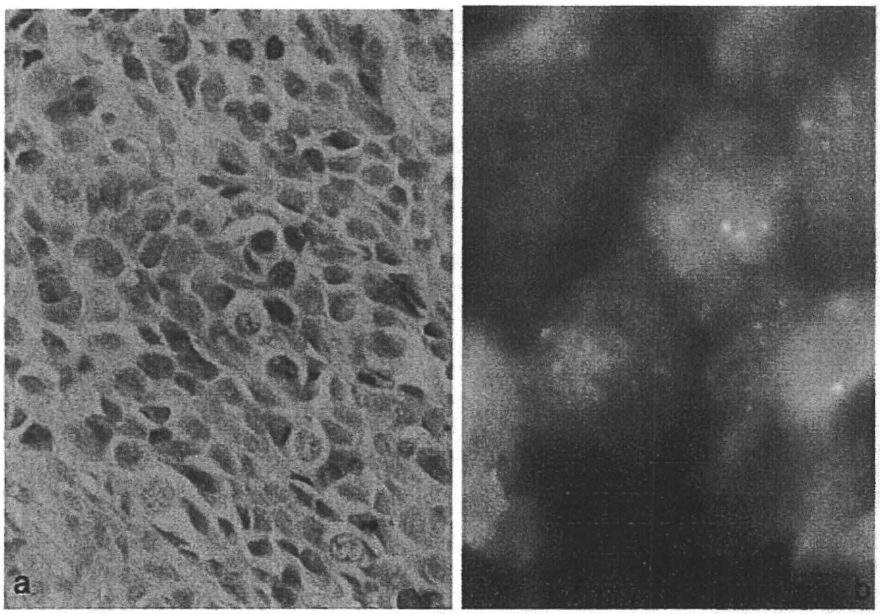

Figure 3. TOPOIIα index of 16.3% (a) and aneuploidy of TOPOIIα gene (b: TOPOIIα / CEP17 = 1.14). Index was evaluated under 40× objective with respect to the positive cell ratio calculated based on the selected five visual fields that are most frequently stained.

In our study, when deeming the signal rate of ≥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, TOPOIIα gene amplification and deletion were observed in 25.9% and 11.1% of the tumors, respectively. In addition, when deeming the signal rate of ≥1.5 as amplification, the HER2 gene amplification was observed in 39.9% of the tumors. Of these tumors, TOPOIIα 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 TOPOIIα gene amplification was observed in 1.7% at the signal rate of ≥2.0 and in 10.1% at the signal rate of ≥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 ≥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 ≥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].

TopoIIα Genes, Protein Expression, and TopoIIα Inhibitor Sensitivity

TOPOIIα is a molecular target of TOPO II inhibitors, which inhibit the function of TOPOIIα proteins. Thus, the sensitivity of TOPOIIα inhibitors depends on the level of TOPOIIα protein expression of the cancer cells. In other words, a tumor having a low TOPOIIα protein concentration has lower TOPOIIα inhibitor sensitivity than a tumor having a higher TOPOIIα protein concentration. Moreover, in the cancer cells having a high concentration TOPOIIα 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 TOPOIIα genes, Knoop et al. reported that both TOPOIIα 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 TOPOIIα 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 TOPOIIα 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 TOPOIIα 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 ≥1.5, deletion ≤0.7), and that the TOPO II inhibitor sensitivity decreases [8]. In our study, when deeming the signal rate of ≥1.5 as amplification, HER2 gene amplification was observed in 39.9% of all the infiltrating duct carcinomas. While TOPOIIα gene amplification occurred in 51.9% of the 39.9% infiltrating duct carcinomas, TOPOIIα gene deletion occurred in only 8.6% [4]. Park et al. also investigated breast cancers, deeming the signal rate of ≥1.5 as amplification and that of ≤0.75 as deletion. As a result, HER2 gene amplification was observed in 8.5% of the tumors. Of the 8.5% tumors, TOPOIIα gene amplification occurred in 18 tumors (75%) and deletion occurred in 25% of the tumors. Based on these findings, they reported that TOPOIIα gene deletion does not occur as frequently as amplification [23]. With regard to HER2 gene amplification, the signal rate of ≥2.0 is usually deemed as amplification in the conventional evaluation method. However, because it has been proposed that having the signal rate of ≥2.2 or more than six copies should be deemed as amplification from 2007 [15], it is necessary to organize the frequency of TOPOIIα genetic abnormalities according to the new classification.

Table 2. HER2 gene and TOPOIIα gene in 203 breast cancers (ASCO 2007)

HER2/CEP			TOPOIIα/CEP		
	Amplification ≥2.2	Equivocal 1.8-2.2	Normal 0.8 - 1.8	Deletion <0.8	All
Amplification ≥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 TOPOIIα gene status

HER2/CEP			TOPOIIα/CEP		
	Amplification ≥2.0	Gain 1.5-2.0	Normal 0.8 - 1.5	Deletion <0.8	All
Amplification ≥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 ≥2.0 as amplification, TOPOIIα 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 ≥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 TOPOIIα genetic abnormalities could be observed only in HER2-amplified breast cancer [24], it was subsequently reported that such genetic abnormalities can also be observed in HER2 non-amplified tumors. Knoop et al. has observed TOPOIIα genetic abnormalities in 56.9% of the HER2-amplified tumors (with the signal rate of ≥2.0) and in 7.6% of the HER2 non-amplified tumors [14]. Park et al. also found TOPOIIα 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 TOPOIIα protein expression of the cancer cells. Table 4 indicates the results of comparison among the TOPOIIα gene, the TOPOIIα index, the Ki67 index, and the HER2 gene in 172 infiltrating duct carcinomas. There was no difference between the TOPOIIα index of the TOPOIIα gene-deleted tumors and that of other groups. Moreover, there was no difference between the Ki67 index of the TOPOIIα gene-deleted tumor and that of the amplified tumors. Furthermore, the Ki67 index of the TOPOIIα gene amplification cases (signal rate of ≥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 TOPOIIα index, but not with TOPOIIα or HER2 gene amplification (Figure 5). Moreover, among the 28 tumors in which the neoadjuvant chemotherapy with anthracyclines was performed, the correlation between the TOPOIIα 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 TOPOIIα index was evaluated ≥25% than the tumors in which the TOPOIIα index was evaluated <10% (Figure 6). Although these 28 cases included no case of TOPOIIα gene deletion, PR (partial response) with the regression rate of 22% was observed in a single TOPOIIα gene-deletion case (TOPOIIα gene signal rate: 0.69, TOPOIIα 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 TOPOIIα 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 TOPOIIα protein expression correlated to the therapeutic effects, and the cases in which the level of TOPOIIα 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 TOPOIIα 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 TOPOIIα gene, TOPOIIα Index, Ki67 index and HER2 gene in 172 infiltrating ductal carcinomas of the breast

TOPOIIα	Number	TOPOIIα	Ki67	Number of HER2			
				A	E	N	D
Amplification ≥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, b

A,Amplification; E,Equivocal; N, Normal; D, Deletion
[a]$P = 0.038$(Welch), $P = 0$[b].022(student t)

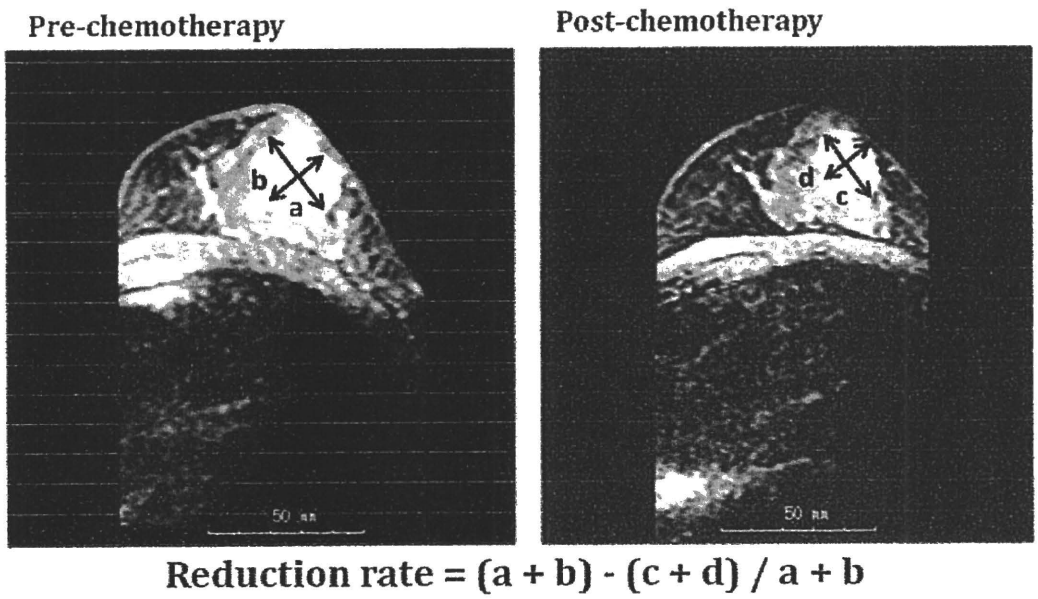

Figure 4. Tumor reduction rate calculating the tumor diameter before and after the chemotherapy.

~~(above "pre- and post-chemotherapy" spelled wrong above pictures-please edit)~~

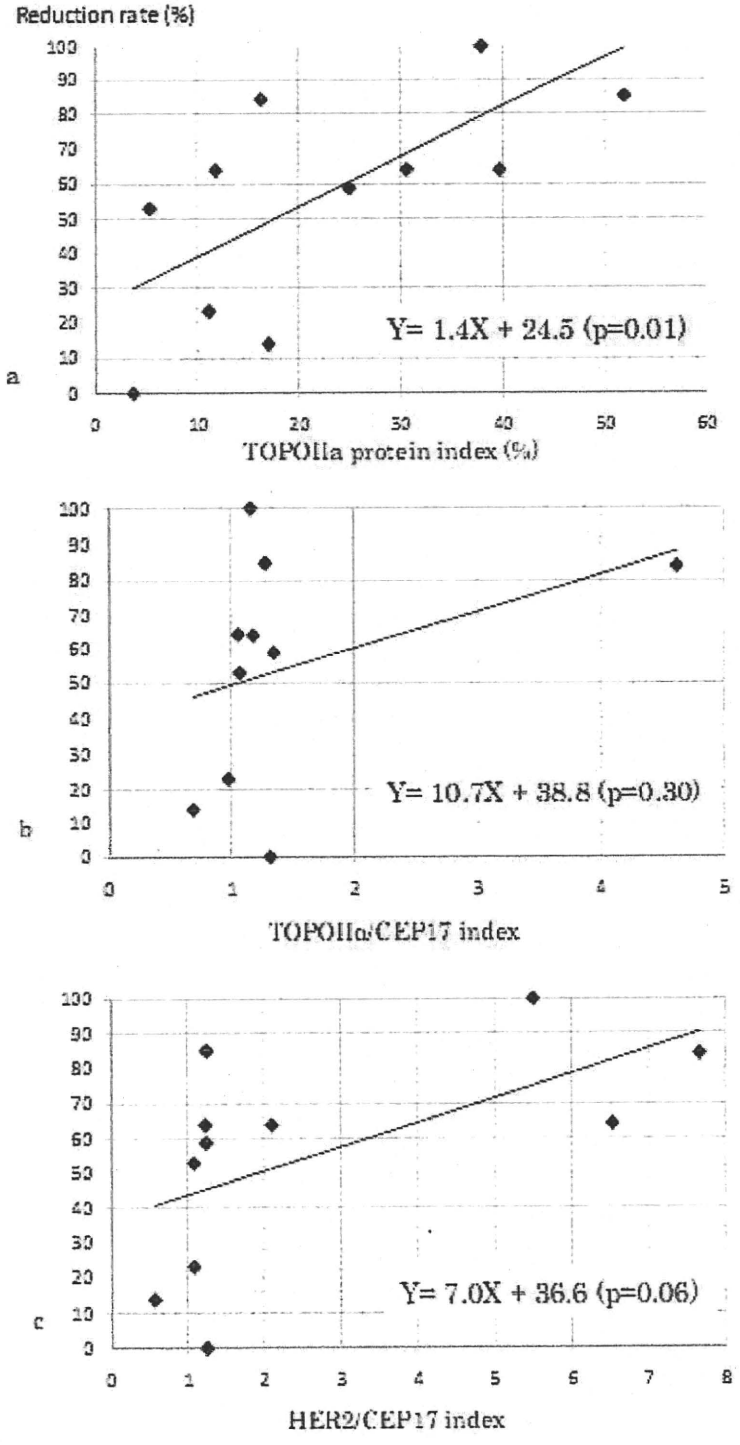

Figure 5. Relations of the tumor reduction rates to the TOPOIIα index (a; p = 0.01), TOPOIIα gene (b; p = 0.30) and HER2 gene (c; p = 0.06).

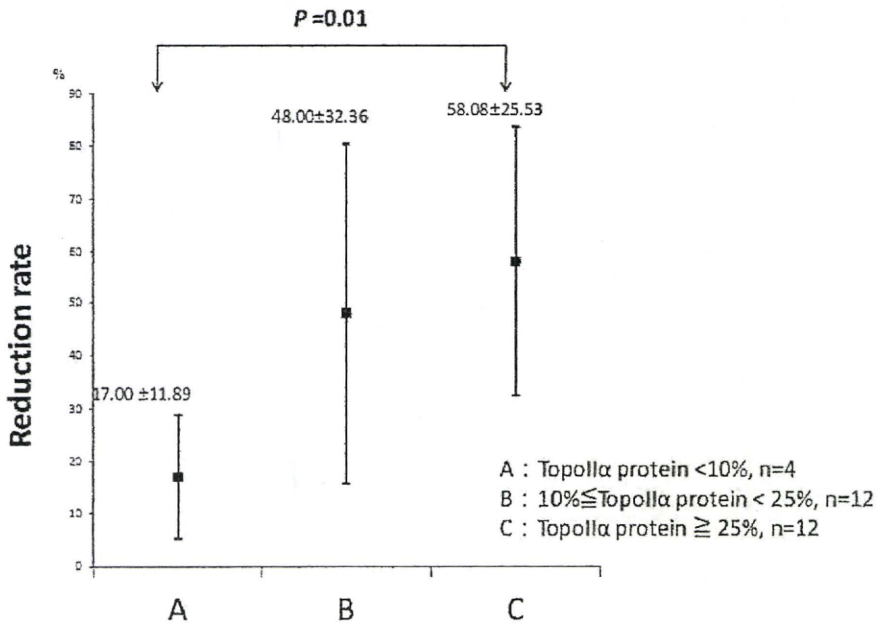

Figure 6. Correlation between the TOPOIIα index and the tumor regression rate.

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