의학석사 학위논문

Association of survival outcome and HER2/CEP17 ratio in advanced HER2-positive breast cancer treated with pertuzumab, trastuzumab, and docetaxel

Pertuzumab, trastuzumab, docetaxel을 투약한 진행성 HER2 양성 유방암 환자에서 HER2/CEP17 비율과 생존 성적의 연관성 분석

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의학과 중개의학 전공

서 정 민

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Abstract

Association of survival outcome and HER2/CEP17 ratio in advanced HER2-positive breast cancer treated with pertuzumab, trastuzumab, and docetaxel

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Background: HER2 positivity is a well-known predictive biomarker for HER2 targeted therapy. However, the duration of response to HER2-targeting agents varies from patient to patient. This study investigated the association between HER2/CEP17 ratio and treatment outcome in patients with advanced HER2-positive breast cancer.

Methods: This is a single center retrospective study. Patients with advanced HER2-positive breast cancer treated with first-line pertuzumab, trastuzumab, and docetaxel were included. The association between HER2/CEP17 ratio and treatment outcome was assessed.

Results: A total of 165 patients were included in this study with a median follow-up duration of 29.0 months. The correlation between HER2/CEP17 ratio and treatment outcome was assessed in 88 patients. Thirty-five patients had archival HER2 ISH result. Additional ISH test was performed in 53 patients with IHC 3+ who did not have previous ISH results. Cox proportional hazard

analysis revealed that HER2/CEP17 ratio is correlated with PFS (HR 0.23, $p < 0.001$). When dichotomized by the median HER2/CEP17 ratio, patients with higher HER2/CEP17 ratio had significantly longer PFS (median PFS, 37.5 vs. 17.4 months, HR 0.40, $p = 0.003$) and numerically higher ORR (54.5% vs. 34.1%, $p = 0.085$). Multivariate analysis revealed that HER2/CEP17 ratio is an independent prognostic factor for PFS (HR 0.72, $p = 0.001$). HER2/CEP17 ratio was associated with PFS in both HER2 IHC 1+/2+ patients (HR 0.12, $p = 0.037$) and IHC 3+ patients (HR 0.18, $p = 0.001$).

Conclusion: This is one of the first studies to report that higher HER2/CEP17 ratio is associated with longer PFS in HER2-positive advanced breast cancer patients treated with dual HER2 blockade. In addition, this study identified the prognostic role of ISH even in patients with HER2 IHC 3+. It would be helpful to perform ISH in patients with HER2 IHC 3+ to make better prediction of treatment outcome.

Keywords: HER2 positive breast cancer, HER2/CEP17 ratio, HER2 ISH, dual HER2 blockade

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Abbreviations

CEP17: Chromosome 17 centromere enumeration probe

CI: Confidence interval

ER: Estrogen receptor

FISH: Fluorescence in situ hybridization

HER2: Human epidermal growth factor receptor 2

HR: Hormone receptor; Hazard ratio

IHC: Immunohistochemistry

ISH: In situ hybridization

OR: Odds ratio

ORR: Objective response rate

OS: Overall survival

pCR: Pathologic complete response

PFS: Progression-free survival

PR: Progesterone receptor

RECIST: Response Evaluation Criteria in Solid Tumors

SISH: Silver in situ hybridization

Chapter 1. Introduction

Human epidermal growth factor receptor 2 (HER2) overexpression or amplification is found in 18–20% of invasive breast cancer and is associated with aggressive nature [1, 2]. The development of HER2 targeting agents over the last decades has dramatically prolonged the survival outcome of patients with HER2-positive breast cancer [3, 4]. In the metastatic setting, dual HER2 blockade with trastuzumab and pertuzumab combined with docetaxel led to a greater survival benefit compared to single HER2 blockade with trastuzumab and docetaxel [1]. In the CLEOPATRA trial, pertuzumab, trastuzumab, and docetaxel showed a median overall survival (OS) of 56.5 months and have become the standard first-line treatment for HER2-positive metastatic breast cancer [6, 7].

HER2 positivity is defined as HER2 immunohistochemistry (IHC) 3+ or the presence of HER2 amplification by in-situ hybridization (ISH). HER2 IHC 3+ is defined as a circumferential membrane staining that is complete, intense, and within >10% of tumor cells. If there is a circumferential membrane staining that is incomplete and/or weak/moderate and within >10% or tumor cells, or if there is a complete and circumferential membrane staining that is intense but within ≤10% of tumor cells, it is defined as IHC 2+. IHC 2+ requires a reflex test (same specimen using ISH) or a new test (new specimen using IHC or ISH). Dual-probe ISH assay is

considered positive if HER2/CEP17 ratio ≥2.0, or if HER2/CEP17 ratio <2.0 but average HER2 copy number ≥6.0 signals/cell [8, 9].

While HER2 positivity is a strong predictive biomarker for HER2 targeting agents [10], it often fails to predict the response to HER2 targeted therapy [11, 12]. More importantly, the duration of response to HER2-targeting agents varies from patient to patient [13]. Also, HER2 positivity comprises all intrinsic molecular subtypes, not always corresponding to HER2-enriched subtype. HER2-enriched subtype is significantly related to better treatment outcomes to anti-HER2 therapy compared to other subtypes [14]. However, in a combined analysis of 5 studies with 802 HER2-positive patients, only 47% of them were classified as HER2-enriched [15]. This indicates that additional factors other than HER2 positivity may be helpful in predicting the prognosis or the response to anti-HER2 therapies.

Evidence shows that HER2/CEP17 ratio could be a useful predictive and prognostic factor in HER2-positive disease [16-18]. In the neoadjuvant setting, higher HER2/CEP17 ratio was significantly associated with higher pathologic complete response (pCR) rate with both single and dual HER2 blockade regimens [19-22]. In addition, higher HER2/CEP17 ratio was associated with higher objective response rate (ORR) and progression-free survival (PFS) in metastatic HER2-positive breast cancer treated with trastuzumab based regimen [18, 23]. However, to our knowledge, there is no existing literature on the association

between HER2/CEP17 ratio and treatment outcome in HER2-positive metastatic breast cancer treated with dual HER2 blockade, especially in those with HER2 IHC 3+.

The purpose of this study is to evaluate the association between HER2/CEP17 ratio and treatment outcomes in advanced HER2-positive breast cancer patients treated with first-line pertuzumab, trastuzumab, and docetaxel.

Chapter 2. Materials and Methods

2.1. Study design and Population

In this retrospective cohort study, patients with locally advanced or metastatic HER2-positive breast cancer who had started palliative first-line pertuzumab, trastuzumab, and docetaxel at Seoul National University Hospital (SNUH, Seoul, South Korea) between August 2008 and January 2021 were included. Patients were eligible if they met the following criteria: age over 18, pathologically proven breast cancer, had archival tumor tissue taken prior to pertuzumab, trastuzumab, and docetaxel treatment, at least one measurable lesion, and at least two outpatient clinic visits after pertuzumab, trastuzumab, and docetaxel treatment. Eligible patients were identified from the electronic database and their medical charts were reviewed using the electronic medical record system of SNUH. Additional HER2 ISH staining of archival tissue was performed in patients without previous HER2 ISH results.

Each cycle of pertuzumab, trastuzumab, and docetaxel regimen consists of tri-weekly administration of docetaxel 75mg/m^2 body surface area on day 1 of each cycle, trastuzumab 8mg/kg administered on day 1 of cycle 1 followed by 6mg/kg on day 1 of the remaining cycles, and pertuzumab 840mg on day 1 of cycle 1 followed by 420 mg on day 1 of each subsequent cycle. Docetaxel was administered at the discretion of the treating physician with no

limitation on the number of cycles.

The study protocol was reviewed and approved by the institutional review board of SNUH (H-2103-218-1210). This study was carried out in accordance with the recommendations of the Declaration of Helsinki for biomedical research involving human subjects. Informed patient consent was waived by the institutional review board of SNUH for posing minimal risk to the patients as this is a retrospective study.

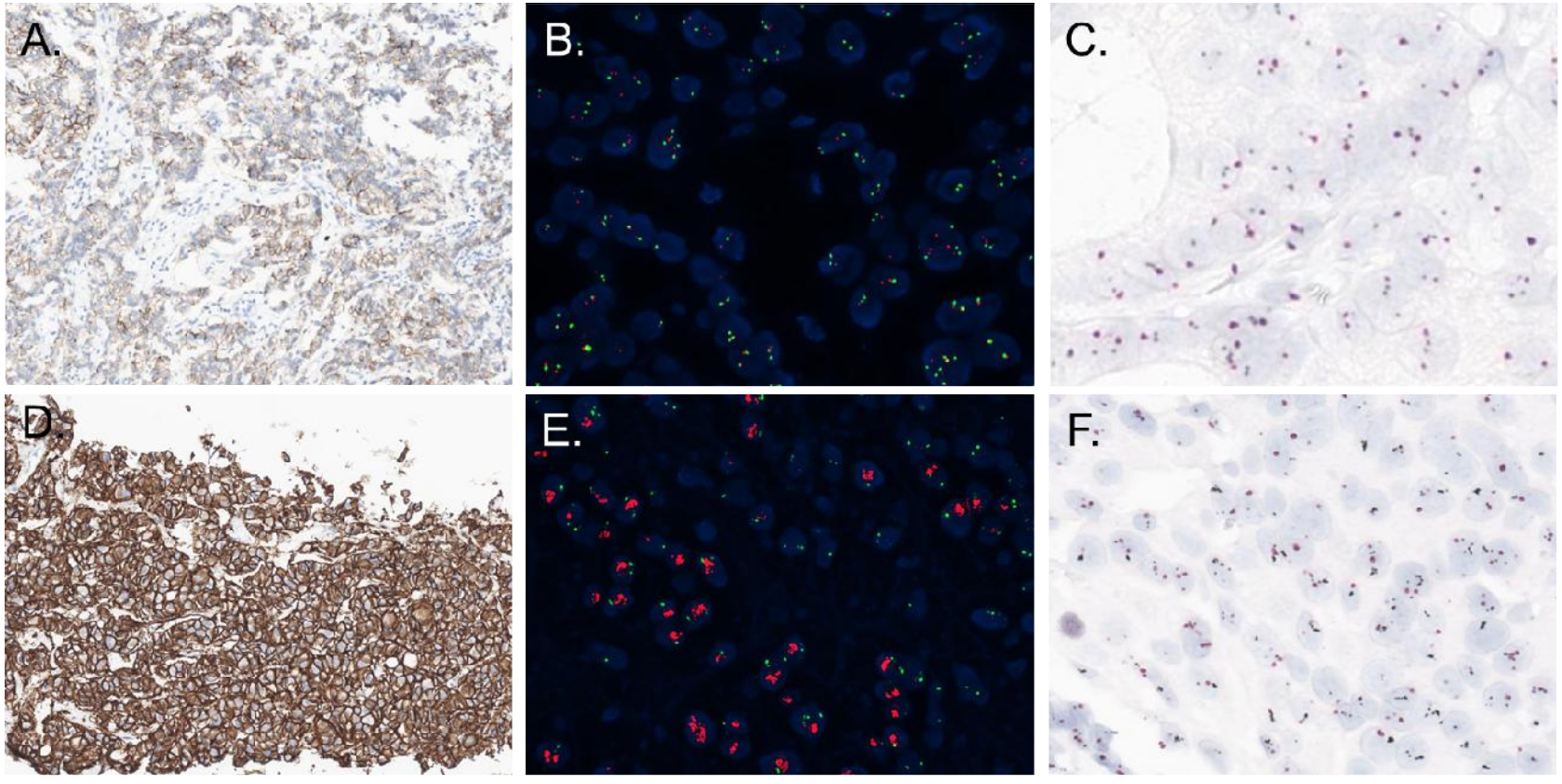
2.2. Analysis of tumor subtype and HER2 ISH

IHC staining of estrogen receptor (ER; clone 6F11; Novocastra Laboratories, Newcastle, UK), progesterone receptor (PR; clone 16; Novocastra Laboratories), HER2 (clone 4B5; Ventana Medical Systems, Tucson, AZ, USA), and Ki-67 (clone MIB-1; Dako/Agilent, Glostrup, Denmark) was performed on formalin-fixed, paraffin-embedded tissue. Hormone receptor (HR) status was considered positive if either ER or PR was positive (IHC ≥1%).

The data including HER2/CEP17 ratio was collected from the pathology reports of the patients who had archival HER2 ISH result. HER2 FISH was performed at the time of diagnosis using PathVysion HER-2 DNA Probe Kit (Abbott Molecular, Downers Grove, IL). Additional SISH tests were performed using INFORM Her2 Dual ISH probe (Ventana Medical Systems) for HER2 IHC 3+ patients who did not have reflex HER2 ISH results.

The examples of HER2 IHC, FISH, and SISH are shown in Figure 1.

Figure 1. The examples of HER2 IHC, FISH, and SISH.



(A) IHC 2+ (B) FISH negative (C) SISH negative (D) IHC 3+ (E) FISH positive (F) SISH positive

2.3. Statistical analysis

The primary objective of this study is to investigate the prognostic and predictive role of HER2/CEP17 ratio on the PFS to pertuzumab, trastuzumab, and docetaxel. The clinical database was last updated in March 2021. PFS was calculated from the first day of pertuzumab, trastuzumab, and docetaxel administration to disease progression or death, whichever occurred first. OS was calculated from the first day of pertuzumab, trastuzumab, and docetaxel administration to death. Treatment response was evaluated by RECIST criteria version 1.1.

Categorical variables were compared by chi-square test and continuous variables were compared using the independent-samples T test. Correlation between HER2/CEP17 ratio and PFS was examined by Cox proportional hazard analysis. As HER2/CEP17 ratio did not show a normal distribution, statistics were performed through a log transformation. PFS was estimated using the Kaplan–Meier method and comparisons were made using log-rank tests. Response rates were compared using chi-square test. Multivariate analysis was performed with Cox proportional hazard regression model using a backward-stepwise method. To adjust for baseline characteristics, Cox proportional hazard analysis of PFS included the following covariates: age, HR status, metastatic site, HER2 IHC, HER2/CEP17 ratio, and Ki-67 IHC. Two-sided *P* values of less than 0.05 were considered statistically significant.

Statistical analysis was performed with R version 4.0.4

(www.r-project.org) using *survival*, *ggkm*, and *MatchIt* packages and SPSS Statistics for Windows version 25.0 (IBM, Armonk, NY).

Chapter 3. Results

3.1. Patient characteristics

In total, 165 patients were included in this retrospective study. All patients were female with a median age of 52 years. Baseline characteristics are summarized in Table 1. Seventy-six patients (46.1%) had de novo metastatic disease and 89 (53.9%) had recurred disease. Visceral metastasis comprised 69.7%, while locally advanced tumor and bone only metastasis were found in 13.9% and 16.4%, respectively. Liver, lung, and brain metastases were found in 30.9%, 37.0%, and 5.5% of patients, respectively. Seventy-five (45.5%) patients had HR-positive HER2-positive disease, and 90 (54.5%) patients had HR-negative HER2-positive disease. Fifty percent of patients received prior adjuvant or neoadjuvant therapy. Among them, anthracycline was administered in 36.4%, taxane in 31.5%, anti-HER2 therapy in 33.3%, and hormonal therapy in 28.5%. Additionally, 37 patients (21.8%) received surgery after the initiation of palliative pertuzumab, trastuzumab, and docetaxel. Among them, pCR was reported in 12 patients (32.4% among surgery recipients).

In the biopsy taken before the initiation of pertuzumab, trastuzumab, and docetaxel, 140 patients (84.8%) showed HER2 IHC of 3+, 23 (13.9%) had 2+, and 2 (1.2%) had 1+ disease. HER2 ISH result from archival tissue was available in 35 patients (2 patients with HER2 IHC 1+, 23 patients with 2+, and 10 patients

with 3+ disease) with a median HER2/CEP17 ratio of 3.45 (ranged 1.6−11.6).

Among the 130 patients with HER2 IHC 3+ without previous ISH results, archival tumor tissue was available in 73 patients. Excluding 20 samples with insufficient tumor cells or inadequate quality, we performed ISH testing in 53 patients, all with HER2 IHC 3+. Eventually, HER2/CEP17 ratio was analyzed in 88 patients. In this population, the median HER2/CEP17 ratio was 4.17 (ranged 1.6−12.25). Among them, 25 patients had HER2 IHC 1+ or 2+ and 63 patients had HER2 IHC 3+ (**Figure 2**). Only one patient out of 63 HER2 3+ patients had HER2/CEP17 ratio below 2.0.

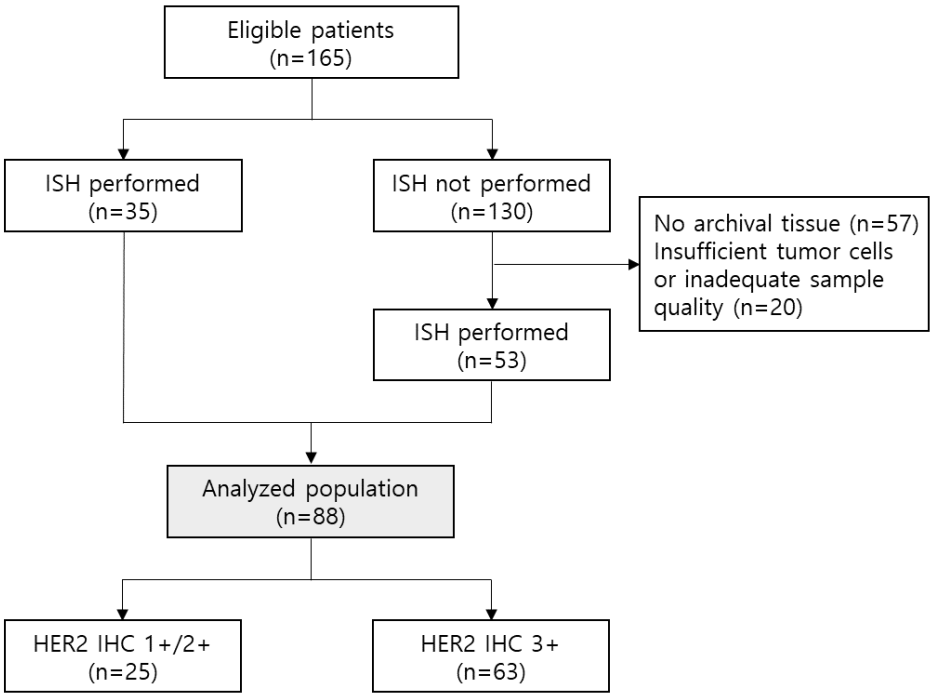
Sequential biopsies taken during or after progression to pertuzumab, trastuzumab, and docetaxel were available in 25 patients. HER2 was persistent in 19 patients, while HER2 loss was observed in 6 patients.

Table 1. Baseline characteristics.

Characteristic	n=165
Female sex – no. (%)	165 (100.0%)
Age – year	
Median	52
Range	22 – 79
Disease status – no. (%)	
De novo metastatic disease	76 (46.1%)
Recurrence	89 (53.9%)
Metastatic site – no. (%)	
Locally advanced	23 (13.9%)
Bone only	27 (16.4%)
Visceral metastasis	115 (69.7%)
Specific metastatic sites – no. (%)	
Liver	51 (30.9%)
Lung	61 (37.0%)
Brain	9 (5.5%)
Hormone receptor status – no. (%)	
HR+ (ER+ or PR+)	75 (45.5%)
HR− (ER− and PR−)	90 (54.5%)
HER2 IHC – no. (%)	
1+	2 (1.2%)
2+	23 (13.9%)
3+	140 (84.8%)
HER2/CEP17 ratio by ISH (n=35)	
Median	3.45
Range	1.6 – 11.6
Prior adjuvant or neoadjuvant therapy – no. (%)	
No	82 (49.7%)
Yes	83 (50.3%)

Anthracycline	60 (36.4%)
Taxane	52 (31.5%)
Anti-HER2 therapy	55 (33.3%)
Hormonal therapy	47 (28.5%)

Figure 2. Study flow chart.



3.2. Predictive implication of HER2 ISH

After a median follow-up duration of 29.0 months, 79 patients had disease progression and 8 patients had death events. Among the 88 patients with HER2 ISH results, 45 patients had disease progression and 5 patients had death events. When dichotomized by the median HER2 ISH ratio of 4.17, the general characteristics of the two groups with lower and higher HER2/CEP17 ratio did not differ significantly (Table 2).

HER2/CEP17 ratio was significantly associated with PFS. Cox proportional hazard analysis revealed that HER2/CEP17 ratio (in log transformation) is correlated with PFS (HR 0.23, 95% CI 0.11-0.49, $p < 0.001$) (Figure 3). When dichotomized by the median ISH ratio of 4.17, patients with higher HER2/CEP17 ratio had longer PFS (median 37.5 vs. 17.4 months, HR 0.40, 95% CI 0.22-0.75, $p = 0.003$) compared to those with lower HER2/CEP17 ratio (Figure 4).

Objective response rate was numerically higher in patients with higher HER2/CEP17 ratio (ORR 54.5% vs 34.1%, $p = 0.086$) with an odds ratio of 2.32 (95% CI, 0.98-5.49) (Table 3).

Table 2. General characteristics according to HER2/CEP17 ratio.

Characteristic	HER2/CEP17 <4.17 (n=44)	HER2/CEP17 ≥4.17 (n=44)	*P* value
Age – yr			0.177
Median	59	55.5	
Range	23 – 82	39 – 79	
Disease status – no. (%)			1.000
De novo metastatic disease	17 (38.6%)	18 (40.9%)	
Recurrence	27 (61.4%)	26 (59.1%)	
Metastatic site – no. (%)			0.626
Locally advanced	6 (13.6%)	4 (9.1%)	
Bone only	8 (18.2%)	6 (13.6%)	
Visceral metastasis	30 (68.2%)	34 (77.3%)	
Specific metastatic sites – no. (%)			
Liver	13 (29.5%)	16 (36.4%)	0.650
Lung	14 (31.8%)	21 (47.7%)	0.191
Brain	3 (6.8%)	1 (2.3%)	0.609

Hormone receptor status – no. (%)			1.000
HR+ (ER+ or PR+)	19 (43.2%)	19 (43.2%)	
HR− (ER− and PR−)	25 (56.8%)	25 (56.8%)	
HER2 IHC – no. (%)			<0.001
1+	1 (2.3%)	1 (2.3%)	
2+	20 (45.5%)	3 (6.8%)	
3+	23 (52.3%)	40 (90.9%)	
HER2/CEP17 ratio by ISH			<0.001
Median	3.18	6.34	
Range	1.60 – 4.15	4.18 – 12.25	

Figure 3. Progression-free survival according to continuous HER2/CEP17 ratio (n=88).

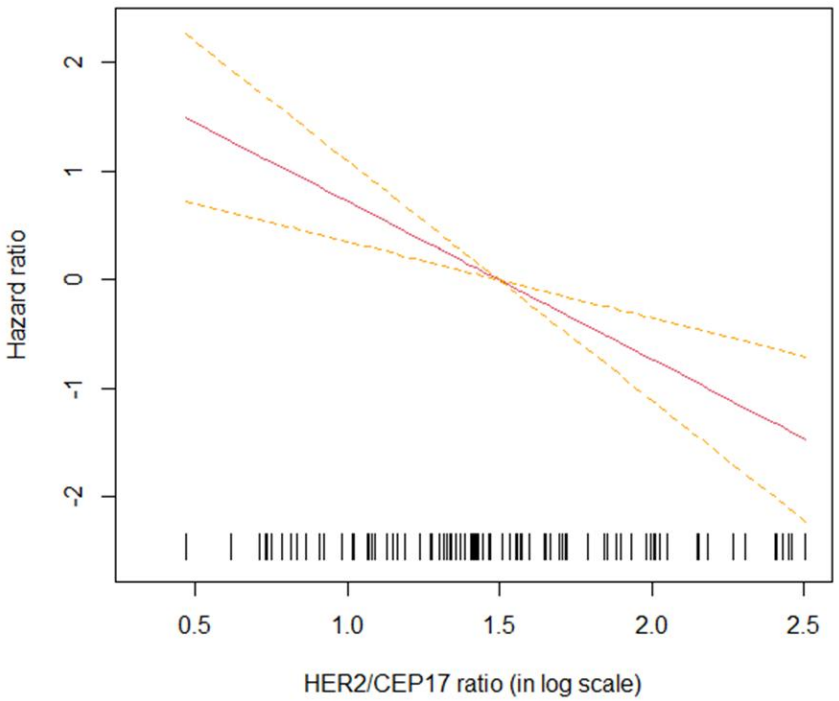


Figure 4. Progression-free survival according to dichotomized HER2/CEP17 ratio (n=88).

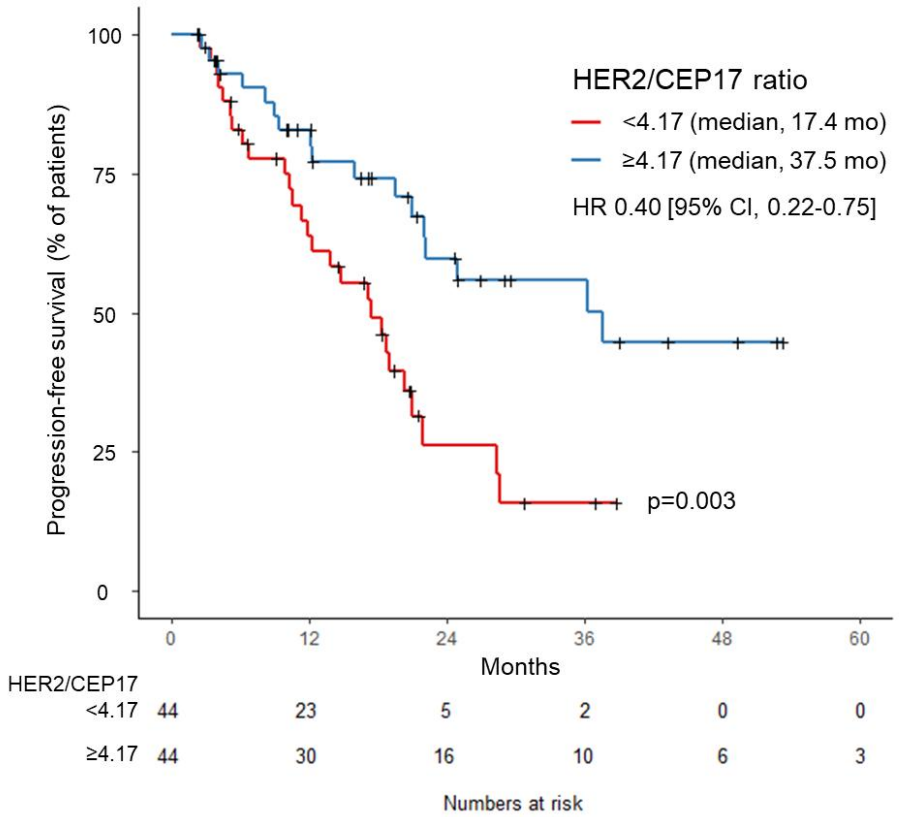


Table 3. Objective response rate and survival outcomes according to HER2/CEP17 ratio.

	HER2/CEP17 <4.17	HER2/CEP17 ≥4.17	*P* value
CR	1 (2.3%)	3 (6.8%)	
PR	14 (31.8%)	21 (47.7%)	
SD	2 (4.5%)	2 (4.5%)	
PD	27 (61.4%)	18 (40.9%)	
ORR	34.1%	54.5%	0.086
	Odds ratio 2.32 (95% CI, 0.98−5.49)		

3.3. Predictive implication of HER2 ISH according to HER2 IHC

HER2/CEP17 ratio was associated with PFS in both HER2 IHC 1+/2+ patients (HR 0.12, 95% CI 0.02−0.88, p = 0.037) and HER2 IHC 3+ patients (HR 0.18, 95% CI 0.07−0.49, p = 0.001) (Figure 5). When dichotomized by the median HER2/CEP17 ratio (2.95 for IHC 1+/2+, 4.75 for IHC 3+), patients with higher HER2/CEP17 ratio had longer PFS in both HER2 IHC 1+/2+ patients (median 28.6 vs. 12.9 months, HR 0.17, 95% CI 0.04−0.63, p = 0.003) and HER2 IHC 3+ patients (median not reached vs. 18.3 months, HR 0.35, 95% CI 0.17−0.76, p = 0.005) (Figure 6).

Figure 5. Progression-free survival according to continuous HER2/CEP17 ratio in (A) HER2 IHC 1+/2+ (n=25) and (B) HER2 IHC 3+ (n=63).

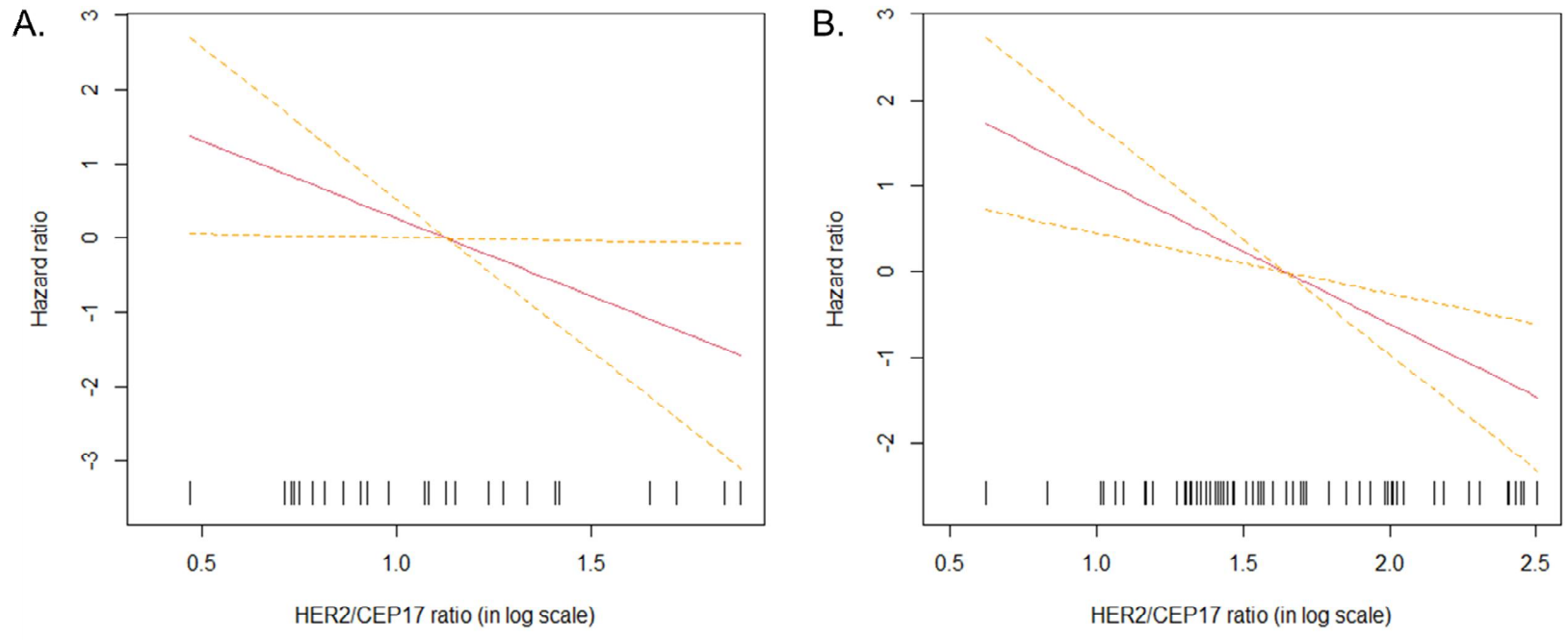
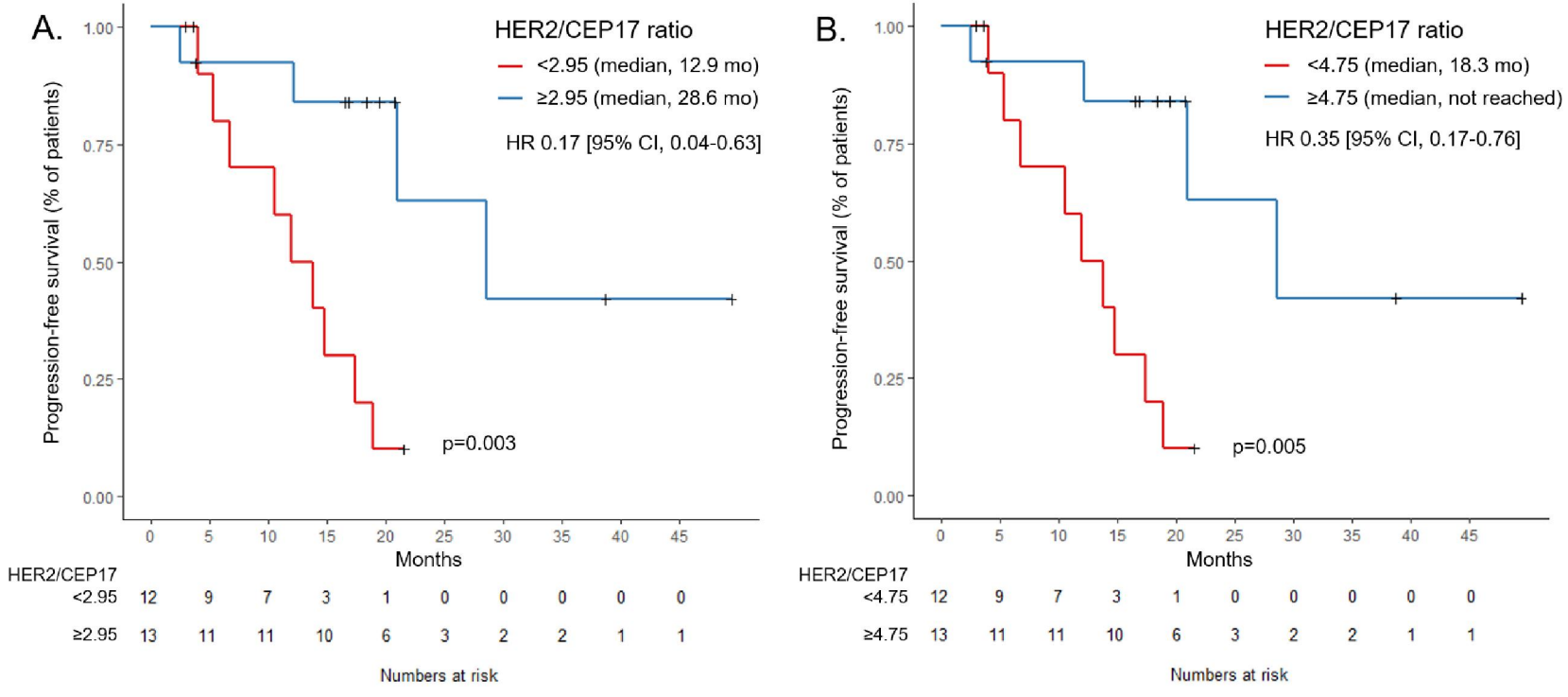


Figure 6. Progression-free survival according to dichotomized HER2/CEP17 ratio in (A) HER2 IHC 1+/2+ (n=25) and (B) HER2 IHC 3+ (n=63).



3.4. Predictive implication of HER2 ISH copy number

We performed additional analyses with HER2 ISH copy number, which is the other factor to define HER2 positivity. The median HER2 ISH copy number was 10.2 (ranged, 2.95–28.05). When dichotomized by the median HER2 copy number, patients with higher HER2 copy number had longer PFS (median 28.6 vs. 17.4 months, HR 0.46, 95% CI 0.24–0.85, p = 0.011) compared to those with lower HER2 copy number (Figure 7).

We also looked at this association according to HER2 IHC 1+/2+ and 3+. When dichotomized by the median HER2 ISH copy number (7.75 for IHC 1+/2+, 10.4 for IHC 3+), patients with higher HER2 copy number had significantly longer PFS in HER2 IHC 3+ patients (median not reached vs. 18.3 months, HR 0.34, 95% CI 0.16–0.73, p = 0.004). However, PFS did not differ significantly in patients with HER2 IHC 1+/2+ (median 20.9 vs. 14.3 months, HR 0.57, 95% CI 0.19–1.70, p = 0.303) (Figure 8).

Figure 7. Progression-free survival according to HER2 ISH copy number (n=88).

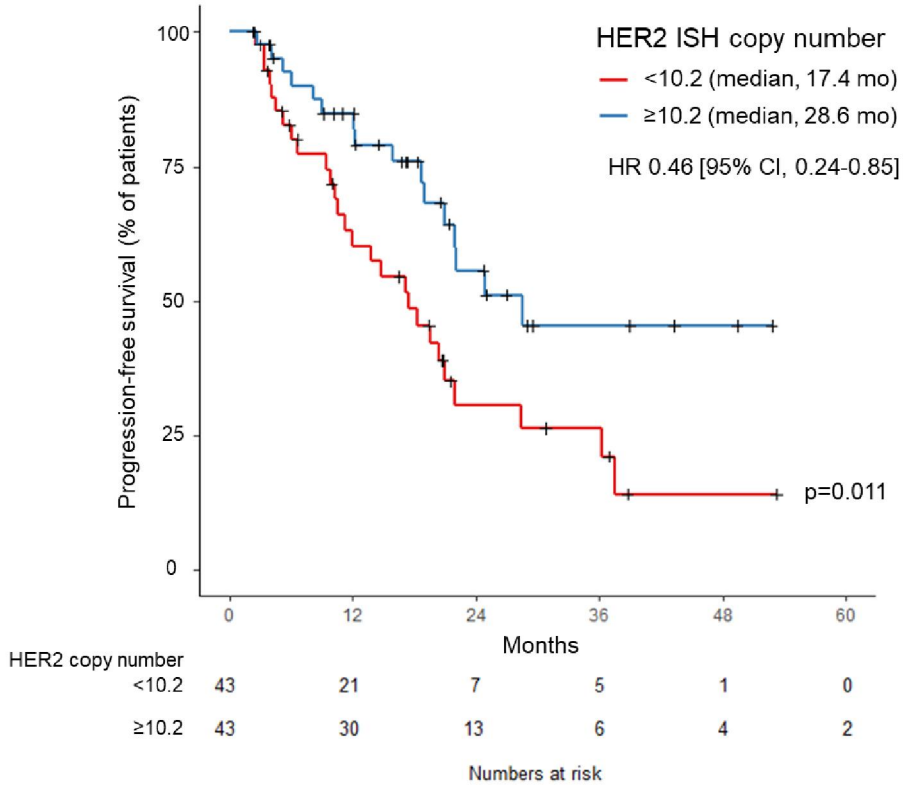
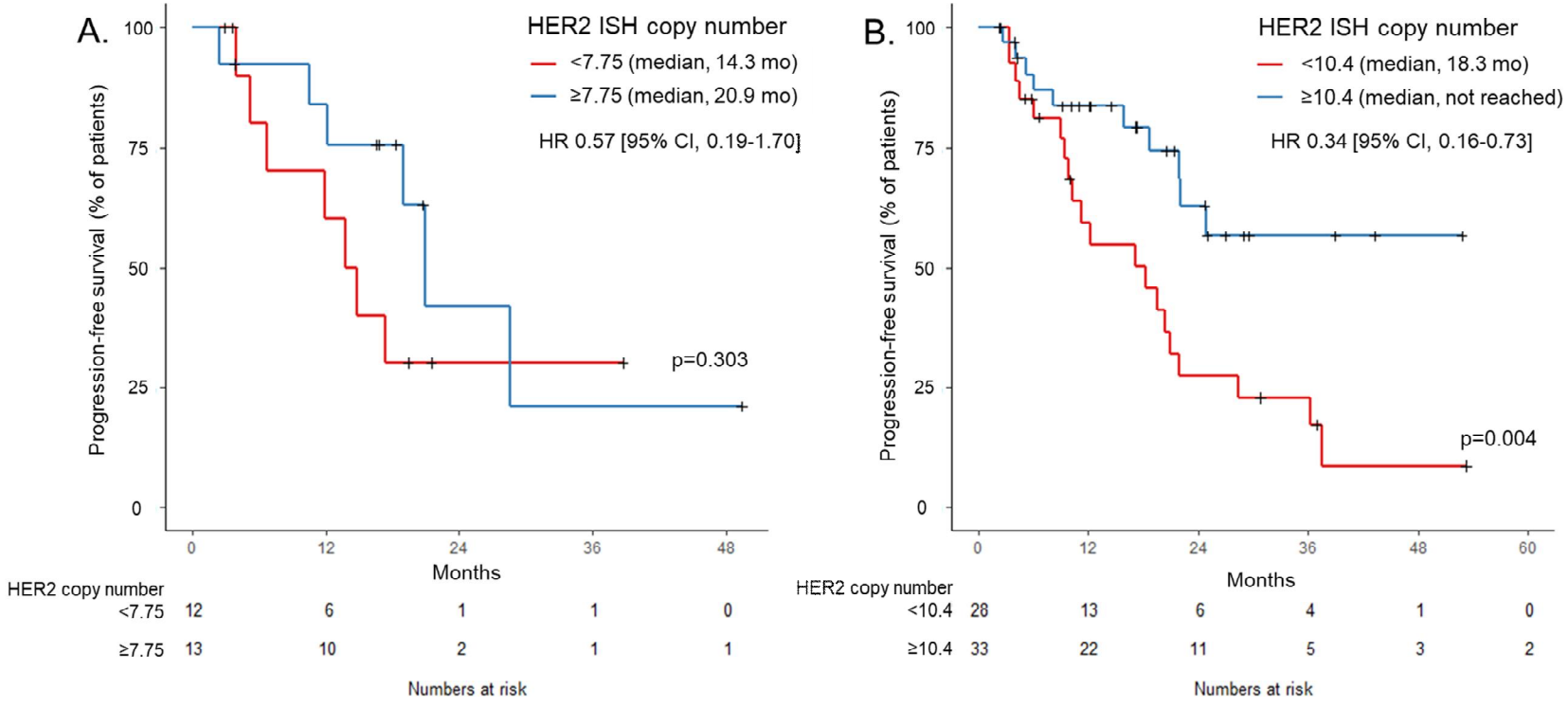


Figure 8. Progression-free survival according to HER2 ISH copy number in (A) HER2 IHC 1+/2+ (n=25) and (B) HER2 IHC 3+ (n=63).



3.5. Analyses on other clinicopathologic factors

Metastatic site was divided into locally advanced disease, bone only metastasis, and visceral metastasis. Among them, bone only metastasis showed better treatment outcome than locally advanced or visceral metastasis. The median PFS of locally advanced disease, bone only metastasis, and visceral metastasis were 17.4 months, 37.5 months, and 21.0 months, respectively (Figure 9A). If plotted between bone only metastasis and the others (locally advanced and visceral metastasis combined), bone only metastasis still showed better PFS (37.5 vs. 19.6 months) with a HR of 0.46 but was not statistically significant (p = 0.074) (Figure 9B).

The association between the number of cycles of docetaxel and PFS was assessed with propensity score matching using optimal matching method. In this analysis, patients who never responded to pertuzumab, trastuzumab, and docetaxel was excluded by omitting the patients whose PFS were shorter than 3 months. Patients were matched by age and the number of total cycles of chemotherapy. Patients who received more than 9 cycles of docetaxel showed numerically longer PFS yet statistically insignificant (median PFS, 17.2 vs. 14.8 months, HR 0.65, 95% CI 0.84−2.81, p = 0.166) (Figure 10).

The level of p53 and Ki−67 are known to be adverse prognostic factors. In this study, Ki−67 IHC ≥50% was significantly associated with shorter PFS (median PFS, 11.9 vs. 20.4 months, HR 3.27, 95% CI 0.96−11.15, p = 0.045). However, p53 IHC was not

associated with PFS using cutoffs of either 1% or 50% (data not shown). Also, HR status did not show a significant association with PFS (data not shown). Furthermore, there was no definite correlation between HER2/CEP17 ratio and ER status (Figure 11).

Figure 9. Progression-free survival according to metastatic site.

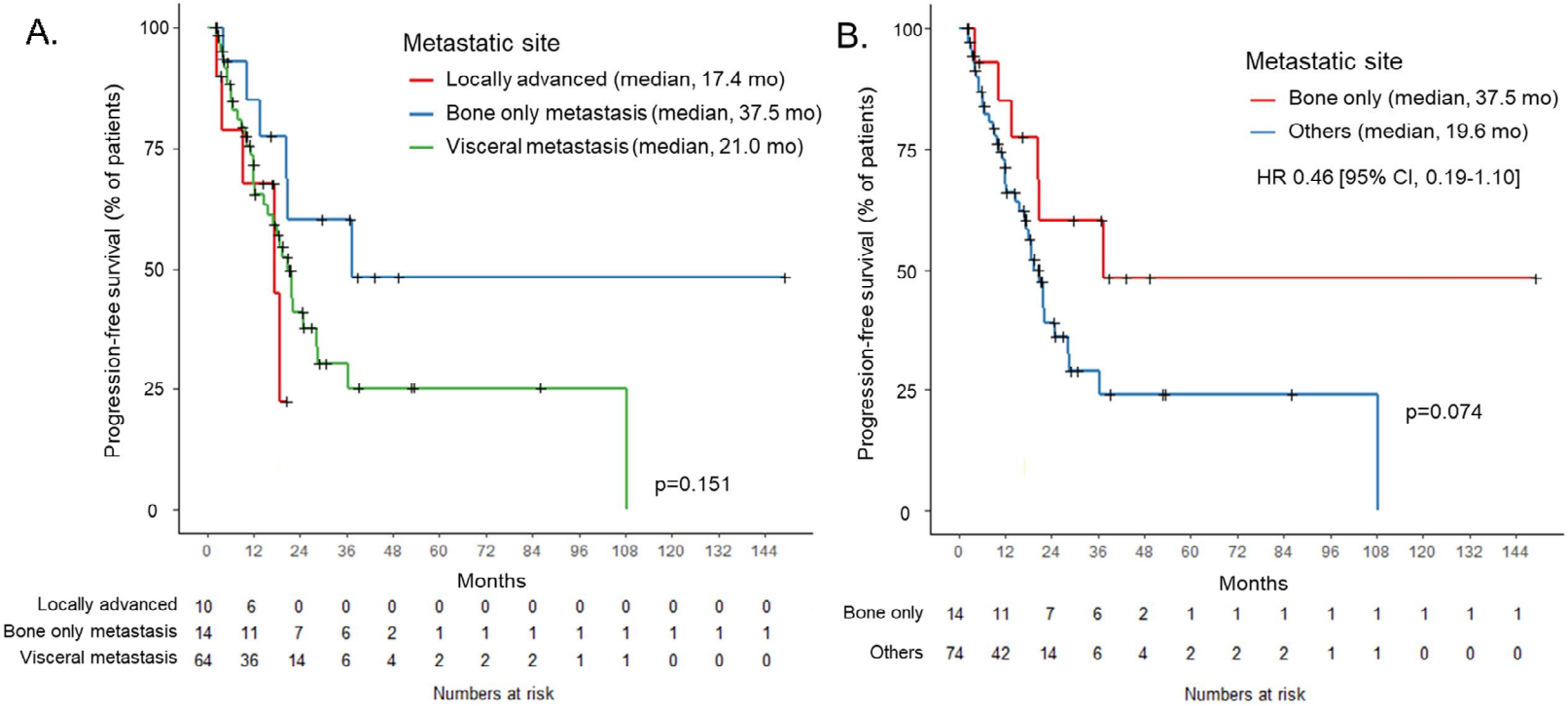


Figure 10. Progression-free survival according to the number of cycles of docetaxel.

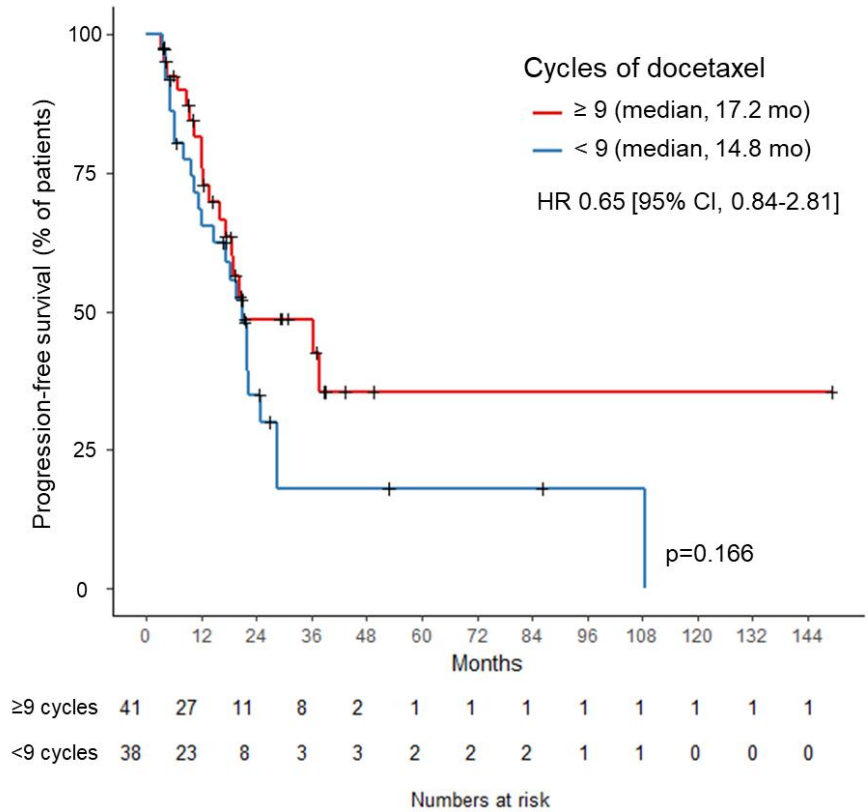
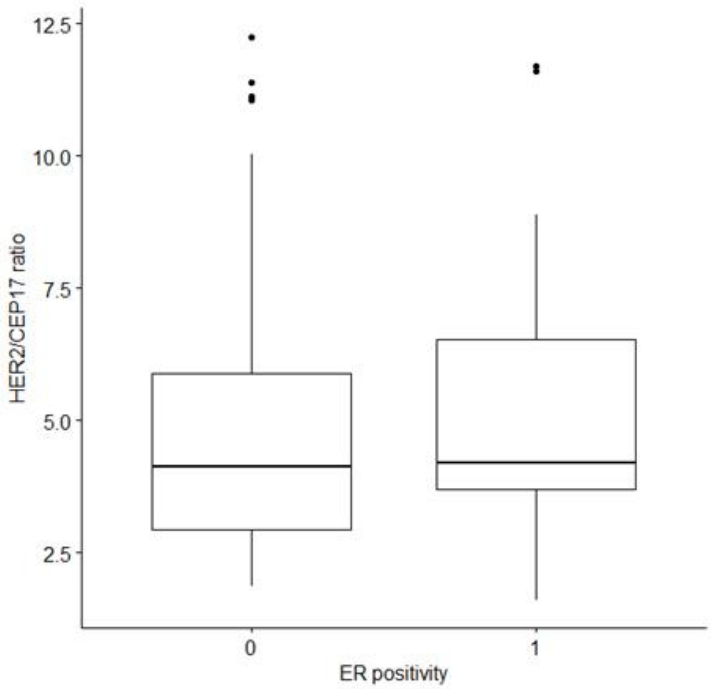


Figure 11. The distribution of HER2/CEP17 ratio according to ER positivity.



3.6. Multivariate analysis

The impact of various factors on PFS was assessed with Cox proportional hazard regression as in Table 4. The factors analyzed were as follows: age, HR status, metastatic site, HER2 IHC, HER2/CEP17 ratio, and Ki-67 IHC.

In the univariate analysis, age ≥50 years (HR 2.15, 95% CI 1.09-4.21, p = 0.027) and HER2/CEP17 ratio (HR 0.75, 95% CI 0.63-0.89, p = 0.001) were found to be statistically significant. HR positivity and bone-only metastasis showed a tendency toward longer PFS, while Ki-67 IHC ≥50% had a tendency of shorter PFS. In the multivariate analysis, only HER2/CEP17 ratio was associated with longer PFS (HR 0.72, 95% CI 0.60-0.87, p = 0.001).

Table 4. Proportional hazard regression of progression-free survival.

Variables	Univariate		Multivariate	
	HR [95% CI]	P value	HR [95% CI]	P value
Age ≥50yr	2.15 [1.09−4.21]	0.027*	1.73 [0.89−3.35]	0.106
HR+ (ER+ or PR+)	0.70 [0.39−1.28]	0.252		
Bone metastasis only	0.49 [0.20−1.17]	0.107		
HER2 IHC 3+ vs 2+	0.84 [0.43−1.64]	0.601		
HER2/CEP17 ratio	0.75 [0.63−0.89]	0.001*	0.72 [0.60−0.87]	0.001*
Ki−67 IHC ≥50%	3.39 [0.99−11.62]	0.053		

Chapter 4. Discussion

This is one of the first studies to show that higher HER2/CEP17 ratio is associated with favorable treatment outcome in advanced HER2-positive breast cancer patients treated with first-line pertuzumab, trastuzumab, and docetaxel. Higher HER2/CEP17 ratio was associated with longer PFS in both HER2 IHC 1+/2+ and IHC 3+ patients.

HER2 positivity which is defined by HER2 IHC or ISH is a strong predictive biomarker for HER2-directed therapy [10]. However, the duration of response to HER2-directed therapy varies from patient to patient [13]. The magnitude of HER2/CEP17 ratio has been proposed as a potential predictive marker for HER2-directed therapy [16, 24]. In neoadjuvant setting, HER2/CEP17 ratio ≥4.5 (OR 2.11, p = 0.005) [22] or >6 (pCR rate 69.0% vs. 30.4%, p = 0.001) [21] was associated with higher pCR for trastuzumab-based single blockade. For dual blockade, high HER2/CEP17 ratio was also associated with higher pCR (pCR vs. non-pCR, median HER2/CEP17 ratio 7.08 vs. 4.70, p = 0.030) [20]. A linear relationship between HER2/CEP17 ratio and pCR was also reported (pCR rate according to HER2/CEP17 ratio in quartiles: 20%, 33%, 44%, and 56%) yet statistically insignificant [19]. In palliative setting, HER2/CEP17 ratio ≥3.0 was associated with longer PFS (17.2 vs. 7.4 months, p = 0.002) for single HER2 blockade [18]. To our knowledge, there is no report on the

predictive role of HER2/CEP17 ratio in HER2-positive metastatic breast cancer treated with dual HER2 blockade. This study is one of the first to report the association between HER2/CEP17 ratio and survival outcome in advanced HER2-positive breast cancer patients treated with first-line pertuzumab, trastuzumab, and docetaxel.

In this study, patients with high HER2/CEP17 ratio had longer PFS (37.5 vs. 17.4 months, HR 0.40, p = 0.003) and numerically higher ORR (54.5% vs. 34.1%, p = 0.086) compared to those with low HER2/CEP17 ratio. Higher HER2/CEP17 ratio was associated with longer PFS in both HER2 IHC 1+/2+ patients (28.6 vs. 12.9 months, HR 0.17, p = 0.003) and HER2 IHC 3+ patients (Not reached vs. 18.3 months, HR 0.35, p = 0.005). In the multivariate analysis, HER2/CEP17 ratio was an independent predictive marker of PFS (HR 0.72, p = 0.001) while age, HR status, metastatic site, or Ki-67 failed to show predictive implications.

The strength of our study is that we performed additional ISH in all HER2 IHC 3+ patients with available samples and thus were able to evaluate the predictive role of HER2/CEP17 ratio in patients of all HER2 IHC status. In the clinical practice, HER2 ISH is usually performed as a reflex testing for those with HER2 2+ by IHC and is seldom assessed in patients with HER2 IHC 3+, because these patients are regarded as HER2-positive irrespective of ISH results [9]. Therefore, retrospective studies on HER2/CEP17 ratio tend to involve more patients with HER2 IHC 2+ which do not represent the entire spectrum of HER2-positive disease [18]. In the present

study, we were able to perform additional ISH in 53 patients with HER2 IHC 3+, providing novel information regarding HER2/CEP17 ratio within this population.

We also evaluated the clinical implication of other clinicopathologic factors. Higher HER2 copy number was associated with longer PFS in all population and HER2 IHC 3+ patients. However, unlike HER2/CEP17 ratio, this association was not statistically significant in patients with HER2 IHC 1+/2+. HR positivity, bone-only metastasis, and greater number of docetaxel administrations showed a tendency toward longer PFS, while Ki-67 IHC ≥50% had a tendency of shorter PFS. However, multivariate analyses showed that only HER2/CEP17 ratio was an independent prognostic factor for PFS.

The limitation of this study is that we could not evaluate the impact of HER2/CEP17 ratio on overall survival because of insufficient follow-up duration and small number of death events. A follow-up analysis or future research with matured cohorts are warranted.

In conclusion, higher HER2/CEP17 ratio is associated with longer PFS in HER2-positive advanced breast cancer patients treated with first-line pertuzumab, trastuzumab, and docetaxel. It would be helpful to perform ISH not only in patients whose HER2 IHC is equivocal, but also in patients with HER2 IHC 3+ to make better prediction of treatment outcome.

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국문 초록

배경: HER2 양성 여부는 HER2 표적치료제의 효과에 대한 예측 인자로 잘 알려져 있다. 하지만 HER2 표적치료제에 대한 치료 반응이 유지되는 기간은 환자마다 다르다. 본 연구는 진행성 HER2 양성 유방암에서 HER2/CEP17 비율과 치료 성적과의 연관성에 대해 탐구하였다.

방법: 진행성 HER2 양성 유방암의 일차 치료로 pertuzumab, trastuzumab, docetaxel을 투약한 환자들을 분석한 단기관 후향적 연구이다. HER2/CEP17 비율과 치료성적의 관련성을 평가하였다.

결과: 총 165명의 환자가 등록되었고 중앙 추적관찰 기간 29.0개월 동안 관찰하였다. HER2/CEP17 비율과 치료성적 간의 관계는 총 88명에서 평가되었다. 35명에서는 HER2 ISH 결과가 존재하였고, 이전에 ISH를 시행하지 않은 IHC 3+ 환자 중 53명을 대상으로 ISH 검사를 추가 시행하였다. Cox 비례위험모형 분석 결과 HER2/CEP17 비율은 무진행 생존기간과 유의미한 연관성을 보였다 (위험비 0.23, 95% 신뢰구간 0.11−0.49). HER2/CEP17 비율의 중앙값으로 양분하여 분석하자, HER2/CEP17 비율이 더 높은 군에서 무진행 생존기간이 유의미하게 증가하였으며 (중앙 무진행 생존기간, 37.5 vs 17.4개월, p = 0.003), 객관적 반응률이 수치 상 높았다 (54.5% vs 34.1%, p = 0.085). 다변량분석 결과 HER2/CEP17 비율은 무진행 생존기간에 대한 독립적 예후인자였다 (위험비 0.72, p = 0.001). HER2/CEP17 비율은 HER2 IHC 1+/2+인 환자군과 3+인 환자군 모두에서 무진행 생존기간과 유의미한 연관성을 보였다 (각각 위험비 0.12, p = 0.037; 위험비 0.18, p = 0.001).

결론: 본 연구는 HER2 양성 진행성 유방암에서 HER2 표적치료제를 2종 사용할 때 HER2/CEP17 비율이 높으면 무진행 생존기간이 길다는 것을 보인 첫 연구들 중 하나이다. 아울러, HER2 IHC 3+인 환자에서도 ISH가 예후인자로 작용한다는 것을 보였다. HER2 표적치료의 성적을 더 잘 예측하기 위하여 HER2 IHC 3+ 환자에서도 ISH를 시행하는 것이 도움이 될 수 있음을 제안한다.

키워드 : HER2 양성 유방암, HER2/CEP17 비율, HER2 ISH, 이중 HER2 표적치료

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