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Research

Potential prognostic value of HER2/CEP17 FISH ratio in HER2-positive non-metastatic breast cancer: a real-world study

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Abstract

Background HER2-positive breast cancer (BC) requires anti-HER2 therapy. We aimed to determine whether the expression of the HER2/centromeric probe for chromosome 17 (CEP17) ratio was associated with prognosis in patients with HER2-positive non-metastatic BC.

Methods 267 HER2-positive BC were enrolled between January 2010 and December 2011. Stabilized inverse probability treatment weighting (sIPTW) was used to balance baseline characteristics. Real-world disease-free survival (DFS) and overall survival (OS) was analyzed.

Results The median follow-up time was 10.3 years (interquartile range: 9.4–10.8 years). HER2/CEP17 ratio of > 7.0 was defined as the HER2 ultra-positive group; a HER2/CEP17 ratio of ≤ 7.0 was defined as the HER2 normal-positive group. After sIPTW adjustment, no differences were observed in DFS and OS when anti-HER2 therapy was unknown, and similarly in the patients who were recorded as not receive trastuzumab (all $p > 0.05$). Interestingly, HER2 ultra-positive group had a worse DFS than the normal-positive group (hazard ratio [HR] = 2.72, $p = 0.02$), but there was no difference in OS ($p = 0.30$) in patients did receive trastuzumab. The multivariate Cox models also showed that the HER2 ultra-positive had worse DFS than HER2 normal-positive patients (HR = 3.71; $p < 0.01$).

Conclusion For non-metastatic HER2-positive BC with or without trastuzumab treatment, the HER2/CEP17 ratio did not predict DFS and OS. However, our study supported that HER2 ultra-positive group had a worse DFS than the normal-positive group among non-metastatic HER2-positive BC patients receiving trastuzumab; therefore, this could be a potential predictor of DFS in these patients.

Highlights

1. The HER2/CEP17 ratio cannot be used as a marker to predict DFS and OS in non-metastatic HER2-positive BC patients in a mixed population of patients, some of whom received trastuzumab and others of whom did not

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2. A higher HER2/CEP17 ratio was associated with a worse DFS in patients with non-metastatic HER2-positive breast cancer patients receiving trastuzumab.
3. The HER2/CEP17 ratio may be considered a potential biomarker to predict prognosis in patients with non-metastatic HER2-positive breast cancer receiving trastuzumab.

Keywords Breast cancer · HER2 · HER2/CEP17 ratio · Prognosis · Trastuzumab

Abbreviations

BC	Breast cancer
HER2	Human epidermal growth factor receptor 2
CEP17	Centromeric probe for chromosome 17
FISH	Fluorescence in situ hybridization
sIPTW	Stabilized inverse probability treatment weighting
pCR	Pathologic complete response
TTM	Time to first metastasis
DFS	Disease-free survival
OS	Overall survival

1 Introduction

Breast cancer (BC) is the most common malignant tumor and the leading cause of cancer-related deaths annually in women worldwide [1]. Among females, its incidence may be as high as 29.56 per 100,000 population in China [2]. Human epidermal growth factor receptor 2 (HER2) positive BC, is a particularly aggressive subtype, defined by amplified and/or overexpressed HER2, and accounts between 15 and 20% of BCs [3].

HER2, is a form of a transmembrane tyrosine kinase receptor, which is encoded by the ERBB2 gene [4]. Evidence indicates that anti-HER2 drugs reduce BC recurrence and improve long-term outcomes in patients with HER2-amplified or overexpressed. Such drugs include trastuzumab, pertuzumab, neratinib, lapatinib, trastuzumab deruxtecan and so on [5–9]. Trastuzumab, being the first anti-HER2 drug, has been widely used in neoadjuvant or adjuvant therapy in non-metastatic BC and maintenance treatment in HER2-positive metastatic BC; this is considered to be the standard therapy for BC patients with HER2-positive BC [8].

The American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines firstly consolidated and recommends that HER2 status should be determined for all invasive BC [3]. The following expressions of HER2 were regarded as positive: immunohistochemistry (IHC) staining of 3 + (membrane staining of > 30% of invasive tumor cells) or fluorescence in situ hybridization (FISH) positive (average HER2 gene copies > 6 per signals/nucleus or HER2/centromeric probe for chromosome 17 [CEP17] ratio > 2.2) [3]. The 2013 version of ASCO/CAP guidelines reported that HER2 expression was defined as positive based on IHC 3 +, average HER2 copy number ≥ 6.0 with Single-probe, or dual-probe HER2/CEP17 ratio ≥ 2.0 with an average HER2 copy number ≥ 4.0 [10]. The newly ASCO/CAP guidelines showed that HER2 diagnosed as positive, including IHC 3 +; IHC 2 + with HER2/CEP17 ratio < 2.0 with ≥ 6.0 HER2 signals/tumor cell (Dual-probe); HER2/CEP17 ratio ≥ 2.0 and average HER2 copy number ≥ 4.0 [11].

Recently, previous studies have demonstrated that a higher HER2/CEP17 ratio is a predictor of better pathologic complete response (pCR) in HER2-positive patients with early and locally advanced BC [12–16]. However, E-M.F. et al. found that a higher HER2/CEP17 ratio (> 6) was also associated with worse time to first metastasis (TTM) in patients who developed metastatic BC [12]. Another study showed that a higher HER2/CEP17 ratio was significantly associated with longer overall survival (OS) in patients with noninflammatory HER2-positive nonmetastatic BC [15]. To date, no studies have evaluated the association between the HER2/CEP17 ratio and survival in patients with HER2-positive non-metastatic BC treated with or without adjuvant trastuzumab. We sought to explore whether a higher HER2/CEP17 ratio is a prognostic predictor in patients with HER2-positive non-metastatic BC.

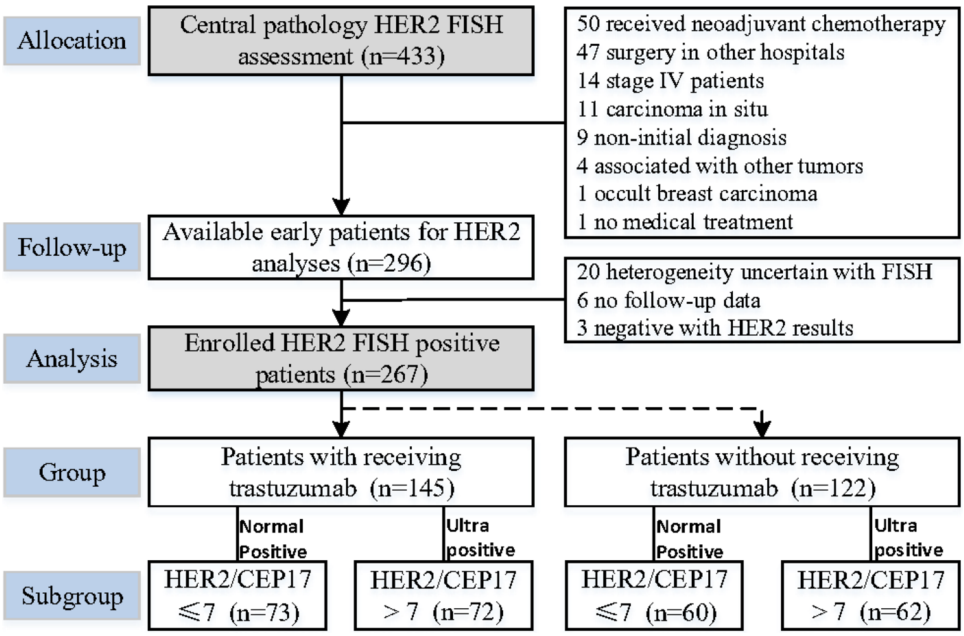

Fig. 1 Patients flow chart diagraming

2 Patients and methods

2.1 Patient selection

The study was carried out in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines and was approved by the ethics committees of the Cancer Hospital, Chinese Academy of Medical Sciences. Informed consent was obtained from all patients enrolled in the study.

In the real-world study, we retrospectively enrolled patients diagnosed with non-metastatic BC between January 2010 and December 2011, and the inclusion criteria were: (1) undergoing radical surgery or breast-conserving, (2) HER2-positive BC by FISH testing, (3) stage I-III BC. The exclusion criteria were as follows: (1) receiving neoadjuvant chemotherapy (n = 50), (2) surgery in other hospitals (n = 47), (3) stage IV patients (n = 14), (4) heterogeneity uncertain with FISH (n = 20), (5) carcinoma in situ (n = 11) or occult breast carcinoma (n = 1), (6) non-primary breast cancer (n = 9) or associated with other tumors (n = 4), and (7) no follow-up data (n = 6) or no medical treatment (n = 1). All enrolled patients were followed up via an outpatient service system or telephone including patients and relatives. The detailed information is shown in Fig. 1.

2.2 Pathologic evaluation

All paraffin-embedded samples were obtained from breast cancer surgery or biopsy specimens. HER2 FISH staining was performed using the PathVysion HER2 DNA Probe Kit (PathVysion, Abbott Molecular, Des Plaines, Illinois, USA), and the 20 nuclear signals of *HER2* and *CEP17* were counted at least two different tumor areas. To reduced heterogeneity, dedicated breast pathologists evaluated HER2 expression and chromosome 17 status at Cancer Hospital, Chinese Academy of Medical Sciences. Tumor receptor status was assessed using previous diagnostic biopsy specimen. HER2 and CEP17 signals of ≥ 20 nuclei of tumor cells within invasive tumor areas were measured to determine the HER2/CEP17 ratio. The positive expression of HER2 FISH amplification was defined as HER2/CEP17 ≥ 2 and HER2 average copy number per cell ≥ 4 [3, 10]. HER2 positivity was defined as a IHC positive (3 + score) and/or a HER2 FISH positive. HER2/CEP17 testing was performed in most patients with a 2 + score on IHC and some patients with a 3 + score on IHC.

2.3 Efficacy assessments and endpoints

Disease-free survival (DFS) was defined as the period from the date of pathological diagnosis until the date of the first relapse (both local and regional), appearance of non-breast primary cancer, death with no signs or symptoms of

recurrence or metastasis, or the last recorded follow-up. OS was defined as the period from the date of pathological diagnosis until the date of death from any cause or the last recorded follow-up.

2.4 Statistical analysis

The study's primary endpoint was DFS, and the secondary endpoint was OS. Associations between the positive group and ultra-positive group were assessed using the chi-square test, Fisher's exact test, or the exact Mann–Whitney U test. Patients with HER2 FISH positive patients were divided into two groups (≤ 7.0 and > 7.0 expression groups; the cut-off was 7.0) using the average value (average value = 7.2) of the HER/CEP17 ratio. An HER2/CEP17 ratio of ≤ 7.0 defined as the HER2 normal-positive group, with a higher HER2/CEP17 ratio of > 7.0, was defined as HER2 ultra-positive group. Notely, the cut off value needs to be further explored and verified in future studies.

Stabilized inverse probability of treatment weighting (sIPTW) based on propensity scores was used to balance the potential confounding baseline characteristics between the two groups[17]. The survival analysis was performed using the product-limit method according to the Kaplan–Meier method, and any differences between survival curves were evaluated by the logrank test [18, 19].To estimate hazard ratios of survival, we used multivariate Cox proportional hazards regression models. Variables with a p-value < 0.1 in the univariate models were evaluated in a multivariate Cox model. All p values are the results of two-sided tests. A p value < 0.05 was considered statistically significant.

All calculations were performed using the SPSS software (version 26.0, IBM, Chicago, USA), or R software (version 4.3.2).

3 Result

3.1 Patients

Between January 2010 and December 2011, 433 patients were evaluated using central pathology to test the expression of HER2 FISH. One hundred and sixty-six patients did not meet the inclusion criteria or exclusion criteria; therefore, 267 patients with HER2-positive status by FISH testing were enrolled for eligibility (Fig. 1). The median follow-up time was 10.3 years (interquartile range: 9.4–10.8 years), and the follow-up cutoff date was July 26, 2021. The number of events for the DFS and OS analyses were respectively 41 and 22 patients. The anthracyclin and/or taxane based chemotherapy combined with trastuzumab were used in patients. After sIPTW adjustment, the patient demographic and clinical characteristics were generally well balanced, including the following baseline characteristics: age, blood type, tumor location, histological grade, Ki67, pT, pN, pTNM stage, surgery type, ER status, PR status, endocrine therapy status, and radiotherapy status (Tables 1 and 2). After sIPTW adjustment, 133 patients were included in the normal-positive group and 132 patients in the ultra-positive group (Table 1); 72 patients were included in the normal-positive group and 71 patients in the ultra-positive group of HER2-positive breast cancer patients who received trastuzumab (Table 2).

3.2 Efficacy in the full HER2 positive patients: HER2/CEP17 ratio and survival time

In the unadjusted analysis of the full HER2 positive patients (n = 267), DFS (hazard ratio [HR] = 1.28; 95%CI = 0.69–2.37, p = 0.40; Fig. 2A) and OS (HR = 1.58; 95%CI = 0.67–3.70, p = 0.30; Fig. 2C) had no differences among patients in the normal-positive group versus the ultra-positive group. After sIPTW analysis, DFS (HR = 1.29; 95%CI = 0.67–2.46, p = 0.45; Fig. 2B) and OS (HR = 1.46; 95%CI = 0.60–3.55, p = 0.42; Fig. 2D) were not significantly different between the two groups (n = 265; normal-positive n = 133 versus ultra-positive group n = 132).

3.3 Efficacy in patients with receiving trastuzumab: HER2/CEP17 ratio and survival time

Several studies have previously demonstrated that the use of trastuzumab improves the survival of patients with HER2 FISH positive, and related guidelines for the treatment of non-metastatic BC have been widely recognized [7, 20–22]. Hence, patients receiving trastuzumab were also considered in the analyses.

In unadjusted patients who received trastuzumab (n = 145), Kaplan–Meier curves showed that the HER2 ultra-positive group had a worse DFS (p = 0.05; Fig. 3A) than the HER2 normal-positive group in patients receiving trastuzumab, but there was no difference in OS (HR = 1.54; 95%CI = 0.49–4.85; p = 0.50; Fig. 3C). After sIPTW analysis, Kaplan–Meier curves

Table 1 Baseline characteristics of HER2 positive breast cancer patients with and without receiving trastuzumab (%)

Characteristics	unadjusted			sIPTW		
	Normal-positive(n = 133)	Ultra-positive(n = 134)	*p*	Normal-positive(n = 133)	Ultra-positive(n = 132)	*p*
Ages (mean (SD))	50.82 (9.48)	49.49 (10.06)	0.27	50.63 (9.77)	50.41 (9.78)	0.86
Blood type			0.90			0.99
A	31 (23.3)	38 (28.4)		38 (28.7)	34 (26.0)	
B	42 (31.6)	41 (30.6)		40 (29.7)	41 (30.8)	
AB	12 (9.0)	10 (7.5)		12 (8.9)	11 (8.6)	
O	32 (24.1)	31 (23.1)		30 (22.1)	30 (23.0)	
Missing or unkown	16 (12.0)	14 (10.4)		14 (10.6)	15 (11.6)	
Tumor location			0.41			0.98
Left breast	81 (60.9)	74 (55.2)		75(56.4)	75 (56.6)	
Right breast	52 (39.1)	60 (44.8)		58(43.6)	57 (43.4)	
Histological grade			0.54			0.87
G1	1 (0.8)	3 (2.2)		4 (3.3)	2 (1.7)	
G2	75 (56.4)	73 (54.5)		74 (55.2)	74 (55.8)	
G3	51 (38.3)	55 (41.0)		52 (38.7)	53 (40.3)	
Missing or unkown	6 (4.5)	3 (2.2)		4 (2.8)	3 (2.3)	
ER			0.03			0.51
Negative (< 1%)	35 (26.3)	52 (38.8)		43 (32.1)	44 (33.2)	
Positive (≥ 1%)	98 (73.7)	80 (59.7)		90 (67.9)	87 (66.0)	
Missing or unkown	0 (0.0)	2 (1.5)		0.0 (0.0)	1.0 (0.8)	
PR			0.10			0.69
Negative (< 1%)	32 (24.1)	46 (34.3)		38 (28.3)	39 (29.2)	
Positive (≥ 1%)	101 (75.9)	87 (64.9)		96 (71.7)	93 (70.5)	
Missing or unkown	0 (0.0)	1 (0.7)		0 (0.0)	1 (0.4)	
Ki67			0.77			1.00
< 30%	57 (42.9)	56 (41.8)		56 (42.1)	56 (42.7)	
≥ 30%	70 (52.6)	74 (55.2)		73 (54.6)	71 (54.0)	
Missing or unkown	6 (4.5)	4 (3.0)		4 (3.3)	4 (3.3)	
pT			0.30			0.90
pT1	78 (58.6)	67 (50.0)		74 (55.3)	72 (54.5)	
pT2	53 (39.8)	61 (45.5)		56 (42.0)	56 (42.3)	
pT3	2 (1.5)	5 (3.7)		4 (2.7)	4 (2.7)	
Missing or unkown	0 (0.0)	1 (0.7)		0.0 (0.0)	1 (0.4)	
pN			0.67			0.95
pN0	73 (54.9)	77 (57.5)		76 (57.4)	76 (57.8)	
pN1	38 (28.6)	31 (23.1)		35 (26.0)	33 (24.6)	
pN2	13 (9.8)	16 (11.9)		12 (9.3)	14 (10.6)	
pN3	8 (6.0)	10 (7.5)		9 (7.0)	9 (7.0)	
Missing or unkown	1 (0.8)	0 (0.0)		1 (0.4)	0 (0.0)	
pTNM stage			0.77			1.00
I	54 (40.6)	63 (47.0)		60 (45.0)	59 (45.1)	
II	65 (48.9)	59 (44.0)		61 (46.1)	60 (45.6)	
III	13 (9.8)	11 (8.2)		12 (8.6)	12 (8.9)	
Missing or unkown	1 (0.8)	1 (0.7)		1 (0.4)	1 (0.4)	
Surgery			0.34			0.78
Radical surgery	114 (85.7)	108 (80.6)		108 (80.9)	109 (82.5)	
Breast conserving	19 (14.3)	26 (19.4)		25 (19.1)	23 (17.5)	
Endocrinotherapy			0.13			0.94
Yes	29 (21.8)	44 (32.8)		36 (27.0)	38 (28.5)	
NO	96 (72.2)	84 (62.7)		91 (68.3)	88 (66.3)	
Missing or unkown	8 (6.0)	6 (4.5)		6 (4.6)	7 (5.3)	

Table 1 (continued)

sIPTW, stabilized inverse probability treatment weighting

showed that the HER2 ultra-positive group had a worse DFS (HR = 2.72; 95%CI = 1.11–6.68, $p = 0.02$; Fig. 3B) than the HER2 normal-positive group of patients receiving trastuzumab, but there was still no difference in OS (HR = 2.15; 95%CI = 0.63–7.28; $p = 0.30$; Fig. 3D).

Using the HER2 normal-positive as the reference for HER2 by FISH testing, the multivariate Cox models by sIPTW analysis showed that the HER2 ultra-positive had worse DFS (HR = 3.71; 95%CI = 1.63–8.46; $p < 0.01$; Table 3) and was no different from OS (HR = 2.21; $p = 0.92$; Table 4). With pN0 as a reference for lymph node metastasis, pN1 was associated with a shorter DFS (HR = 9.76; 95%CI = 2.95–32.29; $p < 0.01$; Table 3) and OS (HR = 12.06; 95%CI = 1.49–97.84; $p < 0.05$; Table 4); pN3 also showed worse DFS (HR = 5.95; 95%CI = 1.1–32.17; $p < 0.05$; Table 3) and OS (HR = 40.34; 95%CI = 1.67–975.1; $p < 0.05$; Table 4) after sIPTW analysis.

4 Discussion

Our real-world study of HER2 + patients some of whom were treated with trastuzumab and others not, showed that the HER2 ultra-positive group and the HER2 normal-positive group showed no difference in DFS or OS; and DFS and OS of the two groups also showed no difference in the HER2-positive BC patients did receive trastuzumab. Interestingly, for these patients some of whom were treated with trastuzumab, we found that the HER2 ultra-positive group had worse DFS than the HER2 normal-positive group.

There was accumulating evidence confirming that trastuzumab was beneficial in HER2-positive BC and should be considered for both non-metastatic and advanced stages of BC [7, 20, 22–24]. Several previous studies have shown that a higher HER2/CEP17 ratio is a predictor of pCR in BC patients [12–16]. One retrospective single-center study demonstrated that a higher HER2/CEP17 ratio (≥ 7) was a predictor of longer OS in patients with HER2 noninflammatory positive locally advanced BC (only stage III) who received neoadjuvant chemotherapy with or without trastuzumab, and the study population was Caucasian [25]. Another prospectively study observed that a higher HER2/CEP17 ratio (of > 6) independently predicted a significantly shorter TTM in a cohort of 120 HER2-positive patients with metastatic BC [12]. Meanwhile, s Korean study reported that a higher HER2/CEP17 ratio was associated with improved PFS and OS in advanced HER2-positive breast cancer treated with trastuzumab and pertuzumab [26]. In addition, Rönnlund C et al. found that the patients with tumors with the lower levels of HER2 copy number (< 7.03 signals/cell) had a worse recurrence-free survival than those with intermediate levels (7.03 to 14.03 signals/cell) [24]. Our data suggest that a higher HER2/CEP17 ratio is associated with worse DFS, at least in Chinese stage I-III patients receiving adjuvant trastuzumab. Based on that, we hypothesized that more vigorous treatment regimens might be necessary for patients with HER2 ultra-positive, and further clinical trials are needed. A potential bias of our study regarding analyses of DFS was that it included only HER2-positive patients defined by FISH testing. Notably, the cut-off value of the HER2/CEP17 ratio remains controversial in different studies.

Moreover, our study noted that pN1 and pN3 had worse DFS and OS than pN0 in patients who received trastuzumab. These findings are consistent with those of previous studies; adjuvant trastuzumab plus pertuzumab has been an option in the adjuvant treatment for HER2-positive patients with node-positive disease[23], and even that adjuvant extended neratinib should added [27]. Thus, adjuvant trastuzumab plus pertuzumab could be considered a standard adjuvant therapy for patients with node-positive HER2-positive non-metastatic BC.

Limitations of our present study include the following: (1) The study was retrospective; even so, we enrolled 267 patients from a single hospital that used standardized treatment and more than 10 years of follow-up. (2) We only enrolled HER2 FISH positive patients and did not include any patients who were IHC-positive and were not tested using the HER2/CEP17 assay. (3) Dual HER2 targeting was not used in this study. Combination with other HER2 targeted drugs, such as pertuzumab, is associated with a longer DFS in HER2-positive non-metastatic BC [21, 23]. The APHINITY study [21] showed a longer invasive-DFS in patients treated with adjuvant pertuzumab plus trastuzumab than with adjuvant trastuzumab alone. We also need to clarify whether HER2 ultra-positive patients and HER2 normal-positive group have difference with survival rates when they are treated with a combination of trastuzumab and pertuzumab. Despite these limitations, to our best knowledge, the present study is the first to reported that a higher

Table 2 Baseline characteristics of HER2-positive breast cancer patients with receiving trastuzumab (%)

Characteristics	Unadjusted			sIPTW		
	Normal-positive(n = 73)	Ultra-positive(n = 72)	*p*	Normal-positive(n = 72)	Ultra-positive(n = 71)	*p*
ages (mean (SD))	48.36 (8.72)	47.71 (9.03)	0.66	47.83 (8.65)	47.91 (8.67)	0.96
Blood type			0.89			0.99
A	20 (27.4)	21 (29.2)		24 (33.3)	21(29.5)	
B	19 (26.0)	21 (29.2)		17 (24.2)	18 (26.0)	
AB	6 (8.2)	5 (6.9)		5 (7.2)	5 (7.0)	
O	20 (27.4)	15 (20.8)		17 (23.6)	17 (23.8)	
Missing or unkown	8 (11.0)	10 (13.9)		8 (11.7)	10 (13.7)	
Tumor location			0.46			1.00
Left breast	47 (64.4)	41 (56.9)		42 (58.5)	41 (58.5)	
Right breast	26 (35.6)	31 (43.1)		30 (41.5)	30 (41.5)	
Histological grade			0.22			0.55
G1	1 (1.4)	2 (2.8)		3 (3.6)	3 (2.5)	
G2	41 (56.2)	43 (59.7)		41 (57.3)	41 (58.3)	
G3	27 (37.0)	27 (37.5)		26 (36.3)	28 (39.3)	
Missing or unkown	4 (5.5)	0 (0.0)		2 (2.8)	0 (0.0)	
ER			0.10			0.68
Negative (< 1%)	17 (23.3)	27 (37.5)		20 (27.3)	20 (28.9)	
Positive (≥ 1%)	56 (76.7)	44 (61.1)		52 (72.7)	50 (70.4)	
Missing or unkown	0 (0.0)	1 (1.4)		0 (0.0)	1 (0.7)	
PR			0.25			0.96
Negative (< 1%)	17 (23.3)	24 (33.3)		19 (26.7)	19 (27.1)	
Positive (≥ 1%)	56 (76.7)	48 (66.7)		53 (73.3)	512 (72.9)	
Ki67			0.60			0.97
< 30%	29 (39.7)	30 (41.7)		29 (39.9)	30 (42.1)	
≥ 30%	41 (56.2)	41 (56.9)		41 (57.4)	39 (55.5)	
Missing or unkown	3 (4.1)	1 (1.4)		2 (2.7)	2 (2.4)	
pT			0.28			0.99
pT1	43 (58.9)	33 (45.8)		38.9 (54.0)	39.0 (55.1)	
pT2	28 (38.4)	37 (51.4)		31.2 (43.4)	30.2 (42.7)	
pT3	2 (2.7)	2 (2.8)		1.8 (2.5)	1.6 (2.2)	
pN			0.68			0.96
pN0	40 (54.8)	38 (52.8)		40 (54.9)	39 (55.3)	
pN1	22 (30.1)	19 (26.4)		19 (26.9)	19 (26.8)	
pN2	7 (9.6)	10 (13.9)		8 (10.6)	9 (12.0)	
pN3	3 (4.1)	5 (6.9)		5 (6.9)	4 (5.8)	
Missing or unkown	1 (1.4)	0 (0.0)		1 (0.7)	0 (0.0)	
pTNM stage			0.67			0.90
I	29 (39.7)	31 (43.1)		31 (43.4)	31 (44.1)	
II	37 (50.7)	33 (45.8)		33 (45.8)	32 (45.3)	
III	6 (8.2)	8 (11.1)		7 (10.1)	8 (10.6)	
Missing or unkown	1 (1.4)	0 (0.0)		1 (0.7)	0 (0.0)	
Surgery			0.31			0.93
Radical surgery	66 (90.4)	60 (83.3)		62 (85.7)	61 (86.3)	
Breast conserving	7 (9.6)	12 (16.7)		10 (14.3)	10 (13.7)	
Endocrinotherapy			0.09			0.99
Yes	12 (16.4)	21 (29.2)		16 (21.8)	16 (22.9)	
NO	55 (75.3)	49 (68.1)		52 (72.9)	51 (72.2)	
Missing or unkown	6 (8.2)	2 (2.8)		4 (5.3)	4 (5.0)	

Table 2 (continued)

sIPTW, stabilized inverse probability treatment weighting

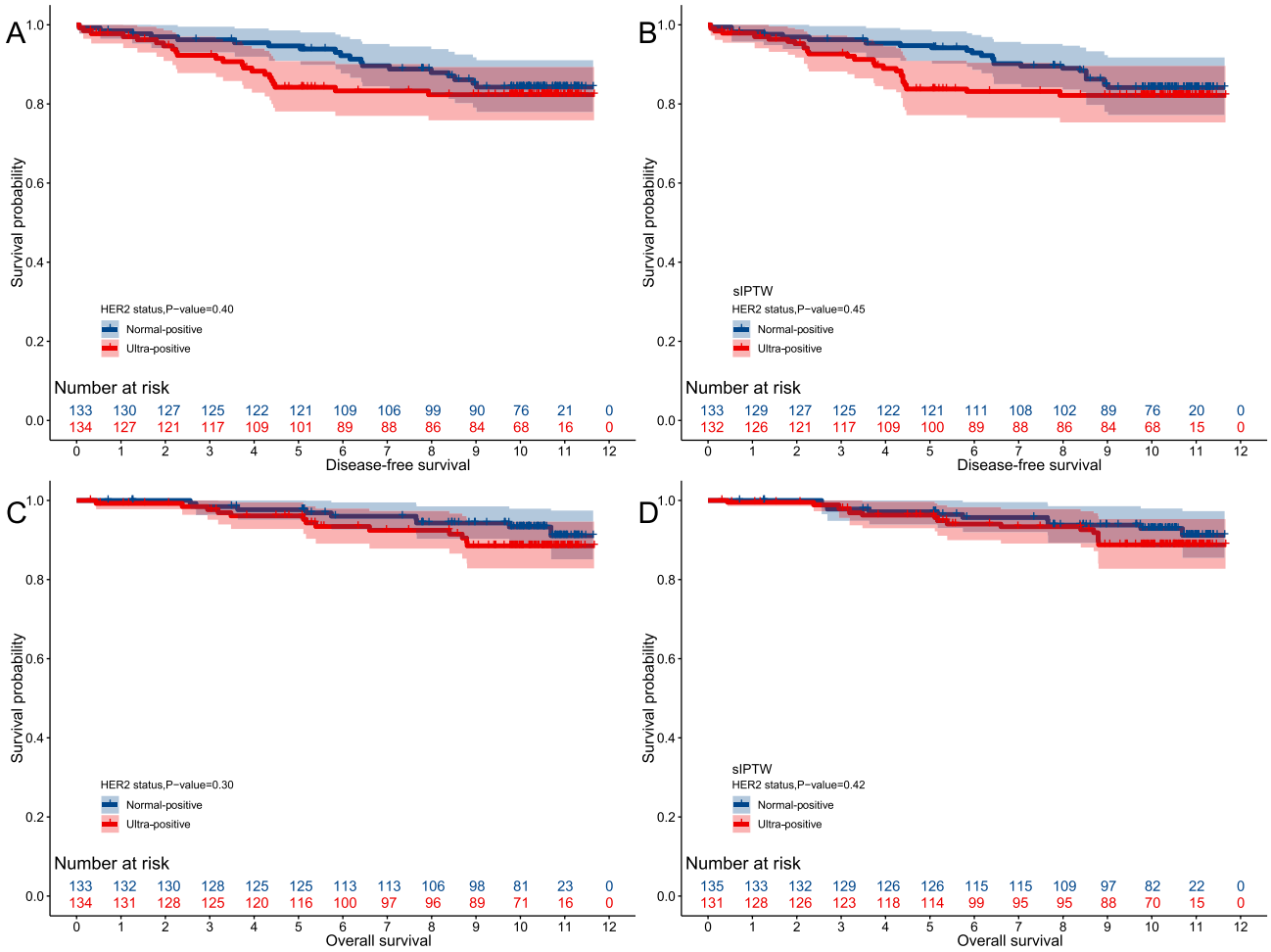

Fig. 2 Kaplan–Meier plots show DFS (**A**) and OS (**C**) for all enrolled patients with unadjusted analysis; Kaplan–Meier plots show DFS (**B**) and OS (**D**) for all enrolled patients after sIPTW adjustment. DFS, disease-free survival; OS, overall survival; sIPTW, stabilized inverse probability treatment weighting

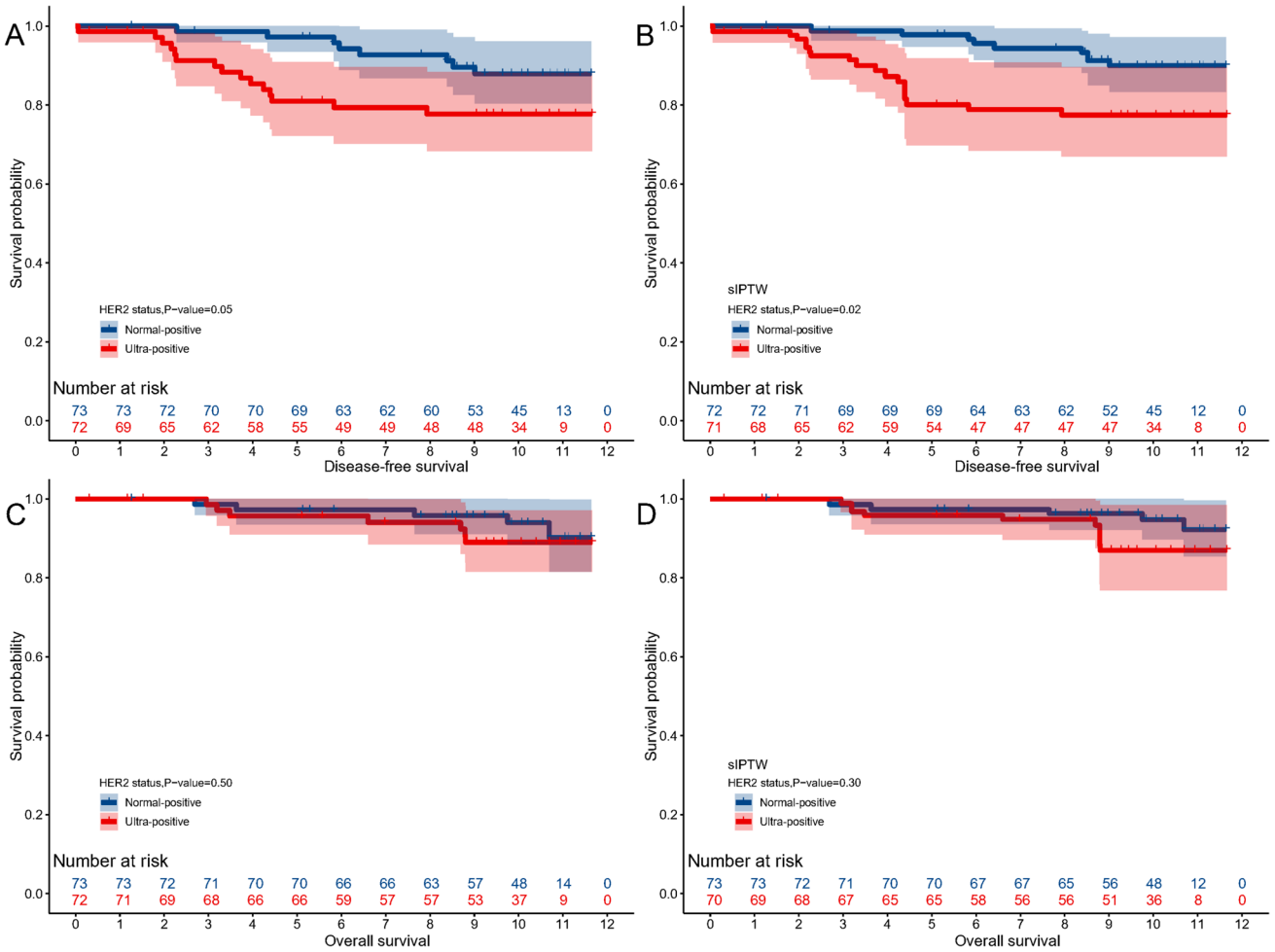

Fig. 3 Kaplan–Meier plots show DFS (**A**) and OS (**C**) for patients with receiving trastuzumab by unadjusted analysis; Kaplan–Meier plots show DFS (**B**) and OS (**D**) for patients with receiving trastuzumab after sIPTW adjustment. DFS, disease-free survival; OS, overall survival; sIPTW, stabilized inverse probability treatment weighting

Table 3 The multivariate Cox models (unadjusted and sIPTW) of disease-free survival in patients with receiving trastuzumab

Characteristics	Unadjusted		sIPTW	
	Hazard ratio	*p*	Hazard ratio	*p*
Age ≤ 50	1.00 (reference)		1.00 (reference)	
Age > 50	0.98 (0.93–1.03)	0.48	0.99 (0.93–1.06)	0.16
Type-A	1.00 (reference)		1.00 (reference)	
Type-B	0.59 (0.17–2.1)	0.42	0.73 (0.19–2.84)	0.45
Type-AB	NA	NA	NA	NA
Type-O	1.26 (0.4–3.94)	0.69	1.52 (0.59–3.93)	0.86
HER2 normal-positive	1.00 (reference)		1.00 (reference)	
HER2 ultra-positive	**3.09 (1.19–8.03)**	**< 0.05**	**3.71 (1.63–8.46)**	**< 0.01**
Ki67 < 30%	1.00 (reference)		1.00 (reference)	
Ki67 ≥ 30%	0.82 (0.32–2.08)	0.67	0.65 (0.25–1.69)	0.89
pN0	1.00 (reference)		1.00 (reference)	
pN1	**7.92 (2.68–23.42)**	**< 0.01**	**9.76 (2.95–32.29)**	**< 0.01**
pN2	1.88 (0.34–10.23)	0.47	2.00 (0.35–11.29)	0.78
pN3	**5.9 (1.28–27.14)**	**< 0.05**	**5.95 (1.1–32.17)**	**< 0.05**

Bold indicates statistically significant values ($p < 0.05$)

NA: no appliable; sIPTW, stabilized inverse probability treatment weighting

Table 4 The multivariate Cox models (unadjusted and sIPTW) of overall survival in patients with receiving trastuzumab

Characteristics	unadjusted		sIPTW	
	Hazard ratio	*p*	Hazard ratio	*p*
Age ≤ 50	1.00 (reference)		1.00 (reference)	
Age > 50	0.96 (0.89–1.03)	0.27	0.96 (0.89–1.04)	1.03
Type-A	1.00 (reference)		1.00 (reference)	
Type-B	3.7 (0.27–50.19)	0.33	3.53 (0.32–38.5)	1.03
Type-AB	6.47 (0.28–151.1)	0.25	5.61 (0.12–257.99)	0.88
Type-O	8.65 (0.81–92.5)	0.07	**12.55 (1.92–82)**	**< 0.05**
HER2 normal-positive	1.00 (reference)		1.00 (reference)	
HER2 ultra-positive	1.81 (0.41–7.93)	0.43	2.21 (0.41–11.97)	0.92
Ki67 < 30%	1.00 (reference)		1.00 (reference)	
Ki67 ≥ 30%	0.75 (0.18–3.2)	0.7	0.48 (0.14–1.67)	1.15
pN0	1.00 (reference)		1.00 (reference)	
pN1	**8.93 (1.46–54.65)**	**< 0.05**	**12.06 (1.49–97.84)**	**< 0.05**
pN2	3.08 (0.23–40.69)	0.39	2.83 (0.15–51.93)	0.70
pN3	**45.21 (3.8–537.99)**	**< 0.01**	**40.34 (1.67–975.1)**	**< 0.05**

Bold indicates statistically significant values ($p < 0.05$)

NA: no appliable; sIPTW, stabilized inverse probability treatment weighting

HER2/CEP17 ratio is associated with a worse DFS in patients with HER2-positive non-metastatic BC who received only trastuzumab.

5 Conclusion

Our real-world study defined the a HER2 normal-positive group and a HER2 ultra-positive group in non-metastatic HER2-positive BC based on the HER2/CEP17 ratio. The HER2/CEP17 ratio does not predict the survival of HER2-positive patients in a mixed population of patients, some of whom received trastuzumab and others of whom did not. It should be noted that the HER2 ultra-positive group had a significantly worse DFS than HER2 normal-positive group in non-metastatic HER2-positive BC patients receiving trastuzumab. Thus, the HER2/CEP17 ratio may be a biomarker which helps define a population of patients who will benefit from more intensive treatment.

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Data availability All data generated or analyzed during this study are included in this published article, and detailed data are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate The study was carried out in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines and was also approved by the ethics committees of Cancer Hospital, Chinese Academy of Medical Sciences. Informed consent was obtained from all patients enrolled in the study.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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