Changes in the detection of human epidermal growth factor receptor 2 gene (*Her-2*) status for *Her-2* fluorescent *in situ* hybridization testing

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Breast cancer (BC) is among the commonly diagnosed malignancies, which displaced lung cancer to become the most leading diagnosed cancer worldwide in 2020.[1] Approximately 15% to 20% of BC patients are positive for the human epidermal growth factor receptor 2 gene (*Her-2*) and exhibits a high degree of malignancy. However, the definition of *Her-2* positivity remains controversial and fluctuating. The reasons for the uncertainty include different technical issues and laboratory experience, different accuracy of testing and cut-off point determination, tumor heterogeneity, preliminary clinical data and differential interpretation among pathologists.[2] To make *Her-2* assessment better suitable for selecting patients for anti-*Her-2* receptor treatment, the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) proposed recommendations to improve the analytic validity of Her-2 for the first time in 2007 and updated these in 2013 and 2018.[3] For clinicians, it was important to reveal the relationship among *Her-2* status, anti-*Her-2* therapy and prognosis and to further focus on the exact group of patients who would benefit from anti-*Her-2* therapy.

We have collected data from 1227 patients with histologically confirmed BC who underwent surgery in Peking Union Medical College Hospital (PUMCH) from June 2007 to July 2017. All the patients we chose had pathologically proven Her-2 IHC (++). We divided these patients into five groups according to *Her-2* fluorescence *in situ* hybridization (FISH) ratio and average Her-2 gene copy number per tumor cell according to the ASCO/CAP guidelines: group 1: Her-2-to-CEP17 (17p11.1-q11.1) ratio < 2.0, average Her-2 copies < 4.0; group 2: Her-2-to-CEP17 ratio < 2.0, $4.0 \leq$ average Her-2 copies < 6.0; group 3: Her-2-to-CEP17 ratio < 2.0, average Her-2 copies ≥ 6.0; group 4: Her-2-to-CEP17 ratio ≥ 2.0, average Her-2 copies < 4.0; group 5: Her-2-to-CEP17 ratio ≥ 2.0, average Her-2 copies ≥ 4.0. Information on the patient's clinicopathological factors and therapeutic regimen were obtained from the hospital records. All patients were followed up by telephone calls or outpatient clinic records. After excluding 19 patients lost to follow-up, 1208 patients were included in the analysis. [Supplementary Digital Content, Figure 1, http://links.lww.com/CM9/A742].

The comparisons of clinicopathologic variables were performed with the Kruskal-Wallis test for continuous variables and x^2 test for discrete variables. The Kaplan-Meier method was used to calculate the cumulative survival rate, and the difference among groups was assessed by using the log-rank test. *P* value of multiple comparisons was corrected by Benjamini-Hochberg false discovery rate. A two-tailed $P < 0.05$ was considered statistically significant. All data analysis was performed using the IBM SPSS Statistics (version 22.0; IBM Corp., Chicago, IL, USA) and R software (version 4.0.3; R Foundation for Statistical Computing, Vienna, Austria).

All patients underwent standard treatment in accordance with the diagnostic guidelines. There were significant differences both in the mean age at diagnosis ($P = 0.005$) and of different age groups ($P = 0.003$). Patients in group 4 were younger than those in the other groups when diagnosed with BC. There was no significant difference in other clinicopathological factors. [Supplementary Digital Content, Table 1 http://links.lww.com/CM9/A742].

Fifty-two patients developed local recurrence, and 118 patients developed distant metastasis. The majority of patients with distant metastasis developed bone metastasis, with a total of 54 patients. Other distant metastasis included 37 patients with multiple metastases, 18 patients with lung metastases, six patients with brain metastases, five patients with liver metastases and 1 patient with spinal canal metastases. Fifty-eight patients passed away, with 54 breast-related deaths and four non-BC-related deaths. The 5-year Kaplan-Meier estimated recurrence-free

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000001733

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Chinese Medical Journal 2022;135(7)

Received: 04-05-2021; **Online:** 18-10-2021 **Edited by:** Yanjie Yin and Xiuyuan Hao

Table 1: BC-related events in the five groups ($n = 1208$).

Event/Survival rate	Group 1 ($n = 791$)	2 ($n = 167$)	3 ($n = 29$)	4 ($n = 20$)	5 ($n = 201$)
Local recurrence	31 (3.9)	8 (4.8)	1 (3.5)	2 (10.0)	10 (5.0)
Distance metastasis	65 (8.2)	24 (14.4)	5 (17.2)	3 (15.0)	21 (10.4)
Disease progression	86 (10.9)	30 (18.0)	5 (17.2)	5 (25.0)	30 (14.9)
Death	34 (4.3)	9 (5.4)	3 (10.3)	0	12 (6.0)
5-year RFS	96.1	95.0	96.6	88.7	95.0
5-year DRFS	91.5	86.5	82.8	85.0	89.9
5-year DFS	89.2	82.9	82.8	73.8	85.4
5-year OS	96.2	93.3	89.5	100.0	94.6

Data are presented as *n* (%) or %. BC: Breast cancer; DFS: Disease-free survival; DRFS: Distance recurrence-free survival; OS: Overall survival; RFS: Recurrence-free survival.

survival (RFS) of BC patients from group 1–5 was 96.1%, 95.0%, 96.6%, 88.7% and 95.0%, the distance recurrence-free survival (DRFS) rates were 91.5%, 86.5%, 82.8%, 85.0% and 89.9%, the disease-free survival (DFS) rates were 89.2%, 82.9%, 82.8%, 73.8% and 85.4% and the 5-year overall survival (OS) rates were 96.2%, 93.3%, 89.5%, 100.0% and 94.6% respectively [Table 1]. There was no significant difference in RFS or OS among the five groups or between any two groups. There was a significant difference in DFS among the five groups ($P = 0.038$), while the comparisons between two groups of DRFS and DFS showed significant difference between group 1 and group 2 ($P = 0.011$ and $P = 0.008$) [Supplementary Digital Content, Figure 2 and Table 2, http://links.lww.com/CM9/A742].

Anti-*Her-2* therapy has improved the prognosis of BC with *Her-2* gene amplification and over-expression.[4] However, the recommendations promoted by ASCO/CAP have changed frequently during recent decades. The changes and controversies focused on BC patients with Her-2 IHC 2+. According to the 2013 and 2018 guidelines, the identification of Her-2 status changed in group 2 and group 4. Based on the patients' economic situation and the guidelines at the time of diagnosis, 4.2% and 60.0% of BC patients received anti-*Her-2* therapy, respectively, in the two groups. The different percentages of trastuzumab use among the 5 groups affected prognosis. Comparisons between the two groups showed significant difference in DRFS and DFS between group 1 and group 2. Patients in group 2 were identified as *Her-2* equivocal in 2013 guidelines but negative with comments after additional work by 2018 guidelines. In our study, only 4.2% of the patients in group 2 were treated with trastuzumab. This group of patients showed worse DFS and DRFS than patients in group 1. However, there was no difference in RFS and OS between the two groups. With more patients treated with trastuzumab in the other three groups, there was no significant difference in RFS, DRFS, DFS and OS when compared with group 1 and between each other. In the BC International Research group (BCIRG)-005/006/007 trails, outcomes among these 176 patients did not differ significantly from outcomes in patients with *Her-2*-to-CEP17 ratio < 2.0 and average *Her-2* copy number < 4.0/tumor cell.[5]

There are several limitations in our study. Firstly, it was a retrospective single-center study based on hospital population. Secondly, the total sample size was limited and the sample size of each group varies greatly.

Our study revealed that, according to ASCO/CAP guidelines in 2018, compared to patients with *Her-2*-to-CEP17 ratio< 2.0 and average *Her-2* copy number < 4.0/tumor cell, the patients with *Her-2*-to-CEP17 ratio< 2.0 and average *Her-2* copy number ≥ 4.0 and < 6/tumor cell showed worse DFS and DRFS. The clinical pathological characteristics and the adjuvant treatment were similar among the five groups. Changing the *Her-2* status of patients with *Her-2*-to-CEP17 ratio< 2.0 and average *Her-2* copy number ≥ 4.0 and < 6/tumor cell from *Her-2* equivocal to negative might not be reasonable. More clinical prognosis data are necessary to prove the correctness of this change in the definition of Her-2 status and ascertain whether the treatment of trastuzumab could improve DFS and DRFS in this group of patients.

Declaration of patient consent

This study was a retrospective observational study that was approved by the Ethics Committee of the Peking Union Medical College Hospital (PUMCH), Chinese Academy of Medical Sciences. All participants signed informed consent when admitted to PUMCH, and we obtained the permission of PUMCH to collect data for this retrospective study.

Conflicts of interest

None.

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How to cite this article: Xu Y, Wang C, Xu Y, Pan B, Sun Q. Changes in the detection of human epidermal growth factor receptor 2 gene (*Her-2*) status for *Her-2* fluorescent *in situ* hybridization testing. Chin Med J 2022;135:849–850. doi: 10.1097/CM9.0000000000001733