Changes in identification of Her2 status according to 2018 College of American Pathologists/American Society of Clinical Oncology (CAP/ASCO) guideline recommendations for human epidermal growth factor receptor 2 (HER2) fluorescent in situ hybridization (FISH) testing: A real-world study based on consecutive hospital cohort of patients with Her2 IHC 2+ in China (2007-2017)

Ying Xu
Peking Union Medical College Hospital
Changjun Wang
Peking Union Medical College Hospital
Yi-Dong Zhou
Peking Union Medical College Hospital
Feng Mao
Peking Union Medical College Hospital
Yan Lin
Peking Union Medical College Hospital
Song-jie Shen
Peking Union Medical College Hospital
Xiao-hui Zhang
Peking Union Medical College Hospital
Qiang Sun (sunqiangpumch@yeah.net)
Chinese Academy of Medical Sciences & Peking Union Medical College Hospital

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Changes in identification of Her2 status according to 2018 College of American Pathologists/American Society of Clinical Oncology (CAP/ASCO) guideline recommendations for human epidermal growth factor receptor 2 (HER2) fluorescent in situ hybridization (FISH) testing: A real-world study based on consecutive hospital cohort of patients with Her2 IHC 2+ in China (2007-2017)

Ying Xu[1],* Changjun Wang[1],* Yi-Dong Zhou[1], Feng Mao[1], Yan Lin[1], Song-Jie Shen[1], Xiao-Hui Zhang[1], Qiang Sun[1]

[1]Department of Breast Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, P. R. China (100730)

* These authors have contributed equally to this work.
Correspondence to: Qiang Sun, **e-mail:** sunqiangpumch@yeah.net
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Abstract:

Background: Trastuzumab has been proved to reduce recurrence and death of Her-2 positive early breast cancer patients. However, the definition of Her-2 positive remains controversial and mutable. The recommendations promoted by ASCO/CAP changed frequently during decades and clinicians are confused when choosing treatment for BC patients, especially Her2 IHC 2+ patients. Although information is available regarding Her2 status and trastuzumab use in the western countries, no related research have been conducted in China to explore the Her-2 status and the utilization of HER2-targeted therapies.

Methods: We analysed data from 1,227 patients with histologically proven breast cancer operated in PUMCH from June 2007 to July 2017. From 2007 to 2017, there were 1,227 patients with histologically proven Her 2 IHC 2+ breast cancer enrolled in our study. The clinicopathological features, recurrence-free survival (RFS), distant recurrence free survival (DRFS), disease free survival (DFS) and overall survival (OS) were compared among subgroups. Prognostic factors of RFS, DRFS, DFS and OS were identified.

Results: Among groups, there was no significant difference in Tumor histology, pT, pTNM stage, Histological grade, Focality, Lymphovascular invasion (LVI), ER, PR, Her2 and Ki67 high (defined as ≥14%) ，surgery of breast，surgery of axilla，percentage of patients who needed chemotherapy, radiation therapy and endocrine therapy. There were significant difference both in the mean age of diagnosis (P=0.005) and different age groups (P=0.003). There was no significant difference in RFS or OS among five groups and between any two groups. There was no significant difference in DRFS and DFS between Group 1 and Group 2 (P=0.011 and P=0.008). According to univariate analyses and Cox multivariate analyses, RFS prognostic factor included pT, LVI and surgery of axilla, pT, pN and ER status were DRFS factors. DFS prognostic factor included pT, pN and PR status. Age at diagnosis, histological type, pT, pN and ER status were prognostic factors of OS.

Conclusions: Our study revealed that, according to ASCO/CAP guideline in 2018, compared to patients with HER2-to-CEP17 ratio＜2.0 and average Her2 copy number ＜4.0/tumor cell, the patients with HER2-to-CEP17 ratio＜2.0 and average Her2 copy number ≥4.0 and ＜6/tumor cell showed worse DFS and DRFS. Changing Her2 status of patients with HER2-to-CEP17 ratio＜2.0 and average Her2 copy number ≥4.0 and ＜6/tumor cell from Her2 equivocal to negative seemed not so reasonable. More clinical prognosis data

was necessary to prove the correctness of this change in the definition of Her2 status and whether the treatment of trastuzumab could improve DFS and DRFS in this group of patients.

Introduction:

Breast cancer (BC) is one of the most commonly diagnosed malignancies with an increased incidence among females. [1] 15-20% of human breast cancer has been reported human epidermal growth factor receptor 2 gene (Her-2) positive. [2] Breast cancer with Her-2 gene amplification performed high degree of malignancy, easily to develop resistance to chemotherapy and showed insensitive to endocrine therapy and radiotherapy. [3] Trastuzumab has been proved to reduce recurrence and death of Her-2 positive early breast cancer patients in four large randomised trials. [4-7] However, the definition of Her-2 positive remains controversial and mutable. The reason of the uncertainty including different technical issues and laboratory experience, different accuracy of testing and cut point determination, tumor heterogeneity, preliminary clinical data and differential interpretation among pathologists. [8-9] To make HER-2 assessment better suitable for selecting patients for anti-HER2 receptor treatment, the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) promoted recommendations to improve the analytic validity of HER2 at the first time in 2007.[10] The panel recommended that a positive Her2 result is IHC staining of 3+ (uniform, intense membrane staining of > 30% of invasive tumor cells), a fluorescent in situ hybridization (FISH) result of more than six HER2 gene copies per nucleus or a FISH ratio (Her2 gene signals to chromosome 17 signals) of more than 2.2; a negative result is an IHC staining of 0 or 1+, a FISH result of less than 4.0 Her2 gene copies per nucleus, or FISH ratio of less than 1.8. Equivocal results require additional action for final determination. [10] However the recommendations updated in 2013. The panel recommended that the Her2 test result as positive for HER2 if IHC 3+ based on circumferential membrane staining that is complete or FISH positive based on Single-probe average Her2 copy number≥6.0 signals/cell or dual-probe HER2/CEP17 ratio≥2.0, or dual-probe HER2/CEP17 ratio<2.0 with an average HER2 copy number≥6.0 signals/cell.FISH equivocal was defined as Dual-probe HER2/CEP17 ratio<2.0 with an average HER2 copy number ≥4.0 and <6.0 signals/cell.[11]While the latest update was in 2018. The panel recommended that if a case has a HER2/CEP17 ratio of ≥2.0 but the average HER2 signals/cell is ＜4.0 or a case has an average HER2 signals/cell ≥6 with a HER2/CEP17 ratio ＜2.0 or the case has an average HER2 signals/tumor cell of ≥4.0 and ＜6.0 and the HER2/CEP17 ratio is ＜2.0, a definitive diagnosis will be rendered based on additional work-up. If the counts remained the same, diagnosis were HER2 negative, positive or negative respectively with a comment.[12] Since the recommendations changed frequently during decades, clinicians always changed the treatment of anti-Her2 therapy for BC patients, especially Her2 IHC 2+ patients. We expect that new recommendations reflect new and better evidence.

Compared to Caucasian counterparts, Chinese women have younger age at onset of breast cancer, delayed in diagnosis that result in more advanced stage of disease at presentation and inadequate resources to finish standard treatment.[13] However, due to the high cost of trastuzumab, the use of trastuzumab has been restrained in China.[14] Although information is available regarding Her2 status and trastuzumab use in the western countries, no related research have been conducted in China to explore the Her-2 status and the utilization of HER2-targeted therapies.[15] It was important to reveal the relationship among Her2 status, anti-Her2 therapy and prognosis and further focus the exact group of patient who would benefit form trastuzumab. This real world study was performed based on consecutive hospital cohort to investigate if Her2 FISH status would affect the prognosis of Her2 IHC 2+ BC patients and which subgroup of BC patients with Her2 IHC 2+ would benefit from anti-Her2 therapy.

Materials and Methods:

Ethics statement

This study was a retrospective observational study which 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 permission of PUMCH to collect data for this retrospective study. Our study was carried out in accordance with the relevant guidelines and regulations.

Participants

We analysed data from 1,227 patients with histologically proven breast cancer operated in PUMCH from June 2007 to July 2017. All the patients we choosen were pathological proved Her2 IHC(++).We divided these patients into 5 groups according to HER2 FISH ratio and average HER2 gene copy number per tumor cell according to the ASCO-CAP guidelines: Group 1 : ratio ＜ 2.0, average HER2 copies ＜ 4.0; Group 2 : ratio ＜2.0, 4.0≤ average HER2 copies ＜6.0; Group 3 : ratio＜2.0, average HER2 copies ≥6.0; Group 4: ratio ≥2.0，average HER2 copies＜4.0；Group 5: ratio ≥2.0，average HER2 copies≥4.0. Information on the patient's pT stage, pN stage, ER level, PR level, Ki-67 level, Histology and therapeutic regimen were obtained from the hospital records. All patients were followed by telephone call, by out-patient clinics records or by both measures. After excluding 19 patients lost to follow-up, 1,208 patients were included in the analysis and we compared clinicopathological features and prognosis among five groups of patients.(Figure 1)

Statistical analysis

The comparisons of clinicopathologic variables were performed with the Kruskal-Wallis test for continuous variable and x^2 test for discrete variables (Table 1). The KaplaneMeier method was used for calculating cumulative survival rate, and the difference among groups was assessed by using the log-rank test (Figure 2, Table 2 and 3). RFS, DRFS, DFS and OS related prognostic factors were identified respectively by Kaplan-Meier univariate analyses and Cox multivariate analyses (Table 4 and 5). The accepted level of significance was $P < 0.05$. All data analysis was performed using the IBM SPSS Statistics.

Results:

Descriptive information

There were 1,227 pathologically proved breast cancer patients with HER2 IHC(2+) enrolled in our study. All patients were conducted breast-conserving surgery or mastectomy. After excluding 19 patients lost to follow-up, we divided the 1,208 patients into 5 groups according to HER2 FISH ratio and average HER2 gene copy number per tumor cell according to the ASCO-CAP guidelines. The number of patients of 5 groups was 791, 167, 29, 20, 201 respectively (Figure 1). The median follow-up time was 57 months. 52 patients developed local recurrence and 118 patients distance metastasis. The majority of patients with distance metastasis developed bone metastasis, with a total of 54 patients. Other distance metastasis included 37 patients with multiple metastases, 18 patients with lung metastases, 6 patients with brain metastases, 5 patients with liver metastases and 1 patient with spinal canal metastases. 58 patients passed away with 54 breast-related deaths and 4 no-breast-related deaths.(Table 2)

Comparison of clinicopathological characteristics

There was no significant difference in Tumor histology, pT, pTNM stage, Histological grade, Focality, Lymphovascular invasion (LVI), ER, PR, Her2 and Ki67 high (defined as ≥14%). There were significant difference both in the mean age of diagnosis (P=0.005) and different age groups (P=0.003). Patients in group 4 were younger than other groups when diagnosed with BC. There was significant difference in the mean number of positive LN with a P value of 0.017 while there was no significant difference in LN status and pN Refered to therapeutic regimen, there was no significant difference in surgery of breast and surgery of axilla. Patients of five groups showed similar percentage of women who needed chemotherapy, radiation therapy and endocrine therapy. As the information of Her2 status was used to define different groups, there were significant difference in subtype and anti-her2 therapy.(Table 1).

Survival outcomes and prognostic factors

The 5-year Kaplan-Meier estimated RFS of these five groups of BC patients were 96.1%, 95.0%, 96.6%, 88.7% and 95.0%, the DRFS were 91.5%, 86.5%, 82.8%, 85.0% and 89.9%, the DFS were 89.2%, 82.9%, 82.8%, 73.8% and 85.4% and 5-year OS were 96.2%, 93.3%, 89.5%, 100.0% and 94.6% respectively (Table 3). There was no significant difference in RFS or OS among five groups and between any two groups. There was no significant difference in DRFS, while comparison between two groups showed significant difference between Group 1 and Group 2 (P=0.011) (Figure 2, Table 2). There was significant difference in DFS among five groups with a P value of 0.038(Figure 2). When compared between groups, there was significant difference between Group 1 and Group 2 (P=0.008) (Table 2). According to univariate analyses and Cox multivariate analyses, RFS prognostic factor included pT, LVI and surgery of axilla, pT, pN and ER status were DRFS factors (Table 4). DFS prognostic factor included pT, pN and PR status. Age at diagnosis, histological type, pT, pN and ER status were prognostic factors of OS (Table 5).

Discussion:

Breast cancer incidence has increased dramatically while the mortality rates have decreased in China.[16] Humanized anti-HER2 monoclonal antibodies have improved the prognosis of BC with Her2 gene amplification and overexpression.[6,17-18] However, it was not easy to identify the status of Her2 exactly. Actually, the recommendations promoted by ASCO/CAP changed frequently during last decades.[10-12] The changes and controversies focused on BC patients with Her2 IHC 2+. Compared to Caucasian counterparts, the use of trastuzumab has been restrained in China, due to the high cost of trastuzumab.[14] There was no related research which been conducted in China to explore the Her-2 status and the utilization of HER2-targeted therapies. Thus we performed this real world study based on hospital population and focused on the BC patients with Her2 IHC 2+.

We divided the 1,208 Her2 IHC 2+ BC patients into 5 groups according to HER2 FISH ratio and average HER2 gene copy number per tumor cell according to the ASCO-CAP guidelines: Group 1 : ratio ＜ 2.0, average HER2 copies ＜ 4.0; Group 2 : ratio ＜2.0, 4.0≤ average HER2 copies ＜6.0; Group 3 : ratio＜2.0, average HER2 copies ≥6.0; Group 4: ratio ≥2.0, average HER2 copies＜4.0； Group 5: ratio ≥2.0, average HER2 copies≥4.0. The number of patients of 5 groups was 791(65.5%), 167(13.8%), 29(2.4%), 20(1.6%), 201(16.6) respectively (Figure 1). According to ASCO-CAP guidlelines in 2013, 791(65.5%) patients were identified as Her2 negative, 250(20.7%) patients were identified as Her2 positive and 167(13.8%) patients were Her2 equivocal. Howerver, according to ASCO/CAP guidelines in 2018, after additional work-up, 978(80.9%) patients were identified as Her2 negative and 230(19.1%) patients were identified as Her2 positive. Changes happened in Group 2 and Group 4. In each of the two groups，based on the guidelines and the economic status of patients，there were 4.2% and 60% of patients received treatment of trastuzumab respectively. Comepared to the re-evaluated Her2 status of patients in BCIRG-005/006/007 trails，our study showed higher Her2 negative rate.[15] 592 out of 10,468 BC patients in BCIRG-005/006/007 trails performed Her2 IHC 2+ and there were 96.5% of patients performed Her2 FISH positive while the positive rate in our study was 19.1%. [15] This may due to the stricter inclusion criteria of three RCT studies. While compared to another study in USA, which enrolled 373 Her2 IHC 2+ patients, the rate of Her2 positive (52.3%) or negative (47.7%) was similar to our study.[19] In a study in India, researchers evaluated 242 Her IHC 2+ patients. According to ASCO/CAP 2013 guidelines, 168(69.5%) patients were identified as Her2 negative, 68(28.0%) patients were identified as Her2 positive and 6(2.5%) patients were Her2 equivocal.[20] This results were also close to our study.

According to 2018 guideline，the identification of Her2 status changed in Group 2 and Group 4. Based on patients economic situation and the guideline at the time of diagnosis，there are 4.2% and 60% BC patients received treatment of trastuzumab respectively in the 2 groups. Since there was no change in the definition of Her 2 status in Group 1, no patient received anti-Her2 therapy. Patients in group 3 and group 5 have been identified Her2 positive in 2018 guidelines and 44.8% and 93.5% of patients received anti-Her2 therapy respectively. It is known that Her2 gene amplification and overexpression predict worse DFS and OS.[21]While anti-Her2 therapy significantly improved DFS and OS.[4-7,22-23]The different percentage of the use of trastuzumab

among 5 groups affected prognosis. As for other Clinicopathological Characteristics, there was no significant difference in Tumor histology, pT, LN status，pN，pTNM stage, Histological grade, Focality, Lymphovascular invasion (LVI), ER, PR, Her2 and Ki67，surgery of breast，surgery of axilla，percentage of patients who needed chemotherapy, radiation therapy and endocrine therapy. There were significant difference both in the mean age of diagnosis (P=0.005) and different age groups (P=0.003). Patients in group 4 were younger than other groups when diagnosed with BC. And there was significant difference in the mean number of positive LN with a P value of 0.017. (Table 1) There was no significant difference in RFS or OS among five groups and between any two groups. While comparison between two groups showed significant difference of DRFS and DFS between Group 1 and Group 2 (Table 2). Patients in Group 2 was identified as Her2 equivocal in 2013 guidelines but negative with comment after additional work in 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 when compared to patients of Group 1. However, there was no difference in RFS and OS between two groups. With more patients treated with trastuzumab in other three groups, there was no significant difference in RFS, DRFS, DFS and OS when compared with Group 1 and between each other. We wondered whether it was appropriate to identify the Her2 status of Group 2 patients as negative and whether the treatment of trastuzumab could improve DFS and DRFS in patients of Group 2. In BCIRG-005/006/007 trails，these group of patients were accrued to the BCIRG-005 trial of sequential or concurrent chemotherapy.[24] Outcomes among these 176 patients did not differ significantly from outcomes in patients with HER2-to-CEP17 ratio＜2.0 and average Her2 copy number ＜ 4.0/tumor cell.[15] However, there were only 7 patients performed IHC 2+ in this group and 126 patients performed IHC 1+ or 0.[15]

According to univariate analyses and Cox multivariate analyses, Factors suggesting disease nature such as pT and pN were identified as prognosis predictors. Patients with LVI and sentinel lymph node biopsy showed a higher local recurrence rate. ER status were both DRFS and OS predict factors while DFS prognostic factor included PR status. (Table 5). The results of univariate and multivariate analysis were consistent with previous cognition.[3-9]

In this study, we focused on the Her2 IHC 2+ BC patients in China and re-evaluated Her2 gene status by FISH according to the ASCO/CAP guideline in 2018 at the fist time. This real world study provided the data of Her2 status and current situation of anti-Her2 therapy in China. As trastuzumab entered China's Medical Insurance List in 2017, the restrictions on the use of anti-Her2 therapy caused by economic reasons were significantly reduced. Pertuzumab was marketed in China in March 2019，bringing new anti-Her2 therapy choices for Her2 positive BC patients. 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. It was difficult to reveal the difference in prognosis among groups statistically. Third, since the guidelines have changed in the last decade and for economic reasons, the use of anti-Her2 therapy was confusing，which was a real-world phenomenon in China.

Conclusion:

In this real world study, we focused on the Her2 IHC 2+ BC patients in China and provided the data of Her2 status and current situation of anti-Her2 therapy . Our study revealed that, according to ASCO/CAP guideline in 2018, compared to patients with HER2-to-CEP17 ratio＜2.0 and average Her2 copy number ＜4.0/tumor cell, the patients with HER2-to-CEP17 ratio＜2.0 and average Her2 copy number ≥4.0 and ＜6/tumor cell showed worse DFS and DRFS. Other groups showed similar prognosis compared to patients with HER2-to-CEP17 ratio＜2.0 and average Her2 copy number ＜4.0/tumor cell and between each other. The clinical pathological characteristics and the adjuvant treatment were similar among five groups. Taken together, changing Her2 status of patients with HER2-to-CEP17 ratio＜2.0 and average Her2 copy number ≥4.0 and ＜6/tumor cell from Her2 equivocal to negative wasn't so reasonable. More clinical prognosis data was necessary to prove the correctness of this change in the definition of Her2 status and whether the treatment of trastuzumab could improve DFS and DRFS in this group of patients.

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Data availability statement

We confirmed the data availability.

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None.

Conflicts of Interest Disclosure

The authors have declared that no competing interests exist.

Author Contributions

Conception and design: Q. Sun, Y. Xu and C. Wang. Provision of study materials and patients: Y. Zhou, F. Mao, Y. Lin, S. Shen, X. Zhang, Q. Sun. Collection and assembly of data: Y. Xu and C. Wang. Data analysis: Y. Xu, C. Wang and S. Shen. Manuscript draft written：Y. Xu and C. Wang. Manuscript revision：Y. Zhou, F. Mao, Y. Lin, S. Shen, X. Zhang, Q. Sun. Approval of manuscript submission: All authors.

Figures

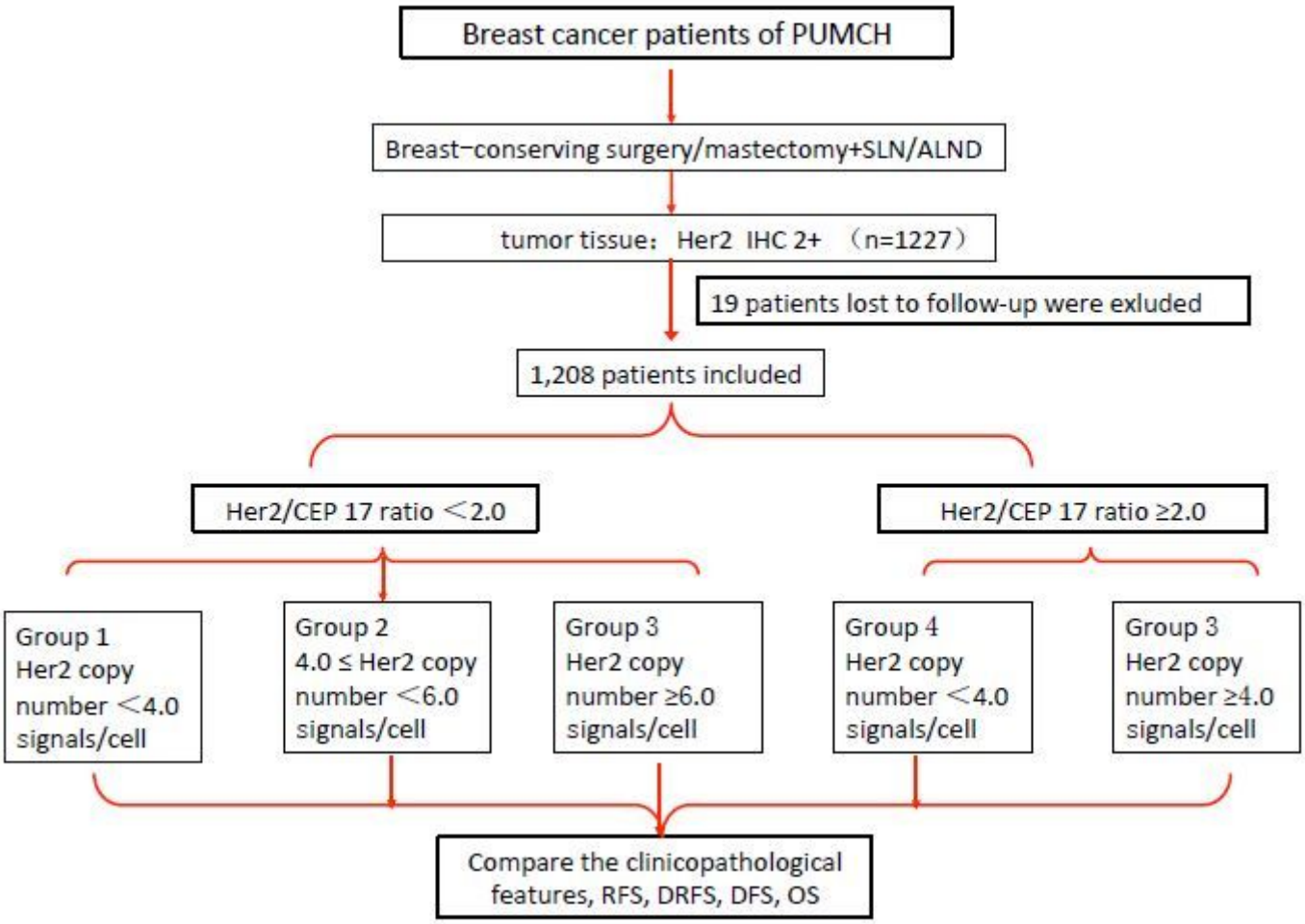

Figure 1

Diagram of the research design

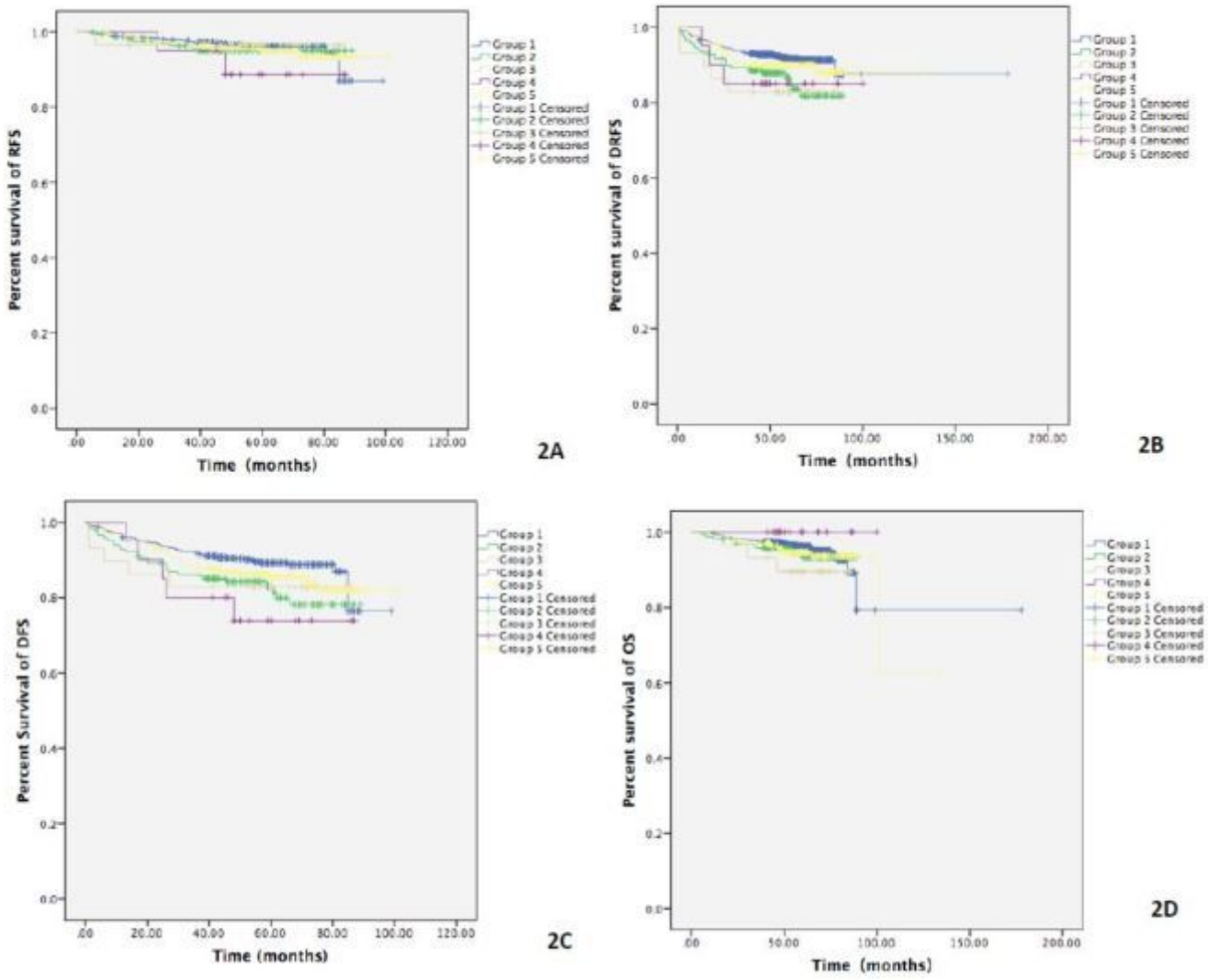

Figure 2

Kaplan-Meier estimated long-term prognosis of breast cancerpatients. There was significant difference in DFS(2C,P=0.038) among five groups. While there was no significant difference in RFS (2A) , DRFS (2B) and OS (2D) among five groups.

Supplementary Files

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- Her2tables.pdf