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*Original Article*

# HER2 status in molecular apocrine breast cancer: associations with clinical, pathological, and molecular features

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**Abstract:** Molecular apocrine breast cancer (MABC) is a distinct subtype of breast cancer. The purpose of this study was to investigate the relationship between HER2 status and clinicopathologic characteristics of MABCs from Chinese Han cohort. A cohort of 90 MABC patients were enrolled. Immunohistochemical method was performed to analyze the molecular expression, and the human epidermal growth factor receptor 2 (HER2) amplification was verified by fluorescence in situ hybridization (FISH). By studying these 90 MABC cases, the majority of studied patients were premenopausal young women (median age 48 yr) with high grade tumors. We also found that MABCs had high positive expression rates of HER2, CK8, CD44, CD166, p53 and BRCA1, the elevated Ki-67 labeling index, and favorable prognosis. There was a significantly higher incidence of lymph node metastasis and lower CD166 positive rate in HER2-negative patients compared to HER2-positive patients (54.5% vs. 37.0%, $P = 0.044$ and 72.7% vs. 91.3%, $P = 0.021$, respectively). The CK5/6 and EGFR expression rates were significant higher in HER2-negative cases than in HER2-positive cases, suggesting that there is overlap between MABC with HER2-negative phenotype and basal-like breast cancer. In addition, HER2 positive was found to be significantly associated a poor overall survival in MABCs. In conclusion, HER2 are highly expressed, and HER2 positivity could be considered as a significant biomarker of poor prognosis in MABC. The results also suggest that a subtype tumor with distinct patterns of molecule expression depending on HER2 status presented in MABC.

**Keywords:** HER2, molecular apocrine breast cancer, FISH, basal-like breast cancer, biomarker

## Introduction

Breast cancer is one of the most common invasive cancers, accounting for 22.8% of all cancers in women, an estimated 1.38 million new cases diagnosed per year [1]. Recently, breast cancer incidence rate had been increasing, and ranking second among all cancers [2]. Breast cancer is a highly heterogeneous disease with distinct biological behavior, which can be classified into many different subtypes according to histopathological types as well as molecular profiles [3, 4]. Thus, it is necessary for the classification of breast cancer in clinical practice, because the tumor subtypes can help indicate correct treatment, clinical behavior and prognosis [5-10]. Farmer et al. reported that a class of molecular apocrine breast cancers (MABC) have been related to increased androgen signaling and characteristic molecular expression profile [11]. It is one of the subtypes of breast cancer, and constitutes 8%-14% of all breast cancer cases [12]. It is characterized by the apocrine histology, positive staining for androgen receptor (AR), and absence of immunostaining for estrogen receptor (ER) and progesterone receptor (PR) outside the basal-like group.

The human epidermal growth factor receptor 2 gene (HER2, also referred to as c-ErbB2) encodes a 185-kDa transmembrane tyrosine kinase (TK) receptor [13]. It belongs to the epi-

dermal growth factor receptor (EGFR) family. The receptors are expressed in a variety of tissues of epithelial, mesenchymal, and neuronal origin, and sensitive to signals that tell the cell to grow [13]. The activation of the HER2 under physiological conditions is controlled by spatial and temporal expression of their ligands, members of the epidermal growth factor family [14]. The HER2 over-expression results in constitutive activation of growth factor signaling in cells, and this becomes oncogenic driver in breast cancer [13, 15]. Approximately 20% to 30% of human breast cancer is HER2-positivity because of overexpression and/or amplification of the HER2 gene [15, 16]. The patients with HER2-positive are associated with a poor clinical prognosis [15, 17, 18].

However, HER2 amplification is frequently found in MABC tumors [12]. It is not clear the correlation of HER2 status with histological and immunologic features, clinical outcome, and molecular expression profile of the MABC patients. We here investigated the correlation between HER2 status and histological and immunologic features, and prognosis of MABC patients.

## Materials and methods

### *Patient selection and data collection*

All patients who had been diagnosed with primary breast cancer between January 2002 and December 2013 were enrolled into the study from the Second Affiliated Hospital of Nanjing Medical University (Nanjing, China), Jiangsu Hospital of Traditional Chinese Medicine and Western Medicine (Nanjing, China) and Xuzhou Central Hospital (Xuzhou, China). The study was approved by the local Institutional Review Board (IRB), and written, informed consents were obtained from all of the patients. We reviewed medical record from the breast cancer patients, and the patients who underwent pre-operative neoadjuvant treatment as hormonal therapy, chemotherapy, and radiotherapy would be excluded from this study. Hematoxylin and eosin-stained (H&E) slide of each patient was reviewed by two pathologists (XZ and ZS), and the apocrine histology was evaluated. The ER and PR status was evaluated, and a cut-off value of 1% or more positively stained nuclei was used to define the ER and PR positivity [19]. The information of age at diagnosis, ethnicity, family cancer history, menopausal status, and tumor histological grade data were collected from medical records and questionnaires. Pathological specimens were collected from 239 Chinese Han patients with ER-, PR-negative breast cancer.

### *Tissue microarray*

The pathological tissues were obtained by operation, and routinely fixed in neutral formalin and then embedded in paraffin. After reviewing H&E-stained slides, the suitable formalin-fixed, paraffin-embedded (FFPE) tumor tissue specimens were retrospectively selected. The most representative tumor area on the specimens was then marked and a 3-mm tissue core was punched out by a tissue extractor and planted onto a 6 × 5 recipient block. More than two tissue cores were extracted for specimen to minimize the extraction bias, and each tissue core was assigned with a unique number of tissue microarray locations.

### *Immunohistochemical staining (IHC) analysis*

Immunohistochemical staining (IHC) was performed by an avidin-biotin peroxidase system. The primary antibodies were used as follows: AR (clone AR441, Dako), HER2 (clone CB11, Novocastra), epidermal growth factor receptor (EGFR) (clone E30, Dako), CK5/6 (D5/16B4, Zymed), CK8 (clone TS1, Dako), CD44 (clone DF1485, Dako), CD166 (clone MOG/07, Novocastra), p53 (clone DO-7, Novocastra), Bcl-2 (clone 124, Dako), BRCA1 (clone GLK-2, Dako) and Ki-67 (clone GM001, Dako). The biotinylated universal secondary antibody was used. The slides were developed using DAB chromogen and counterstained with Mayer's hematoxylin, and then the slides were mounted and scored. The slides were reviewed and scored by two pathologist (TZ and YW) blind to patients' clinical characteristics.

HER2 staining was scored as 0-3+ based on the maximum area of staining intensity, according to the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines [20] as followed: no staining (0); weak incomplete membranous staining (1+); complete membranous staining, either uniform or weak in at least 10% of tumor cells (2+); and uniform intense membranous staining in at

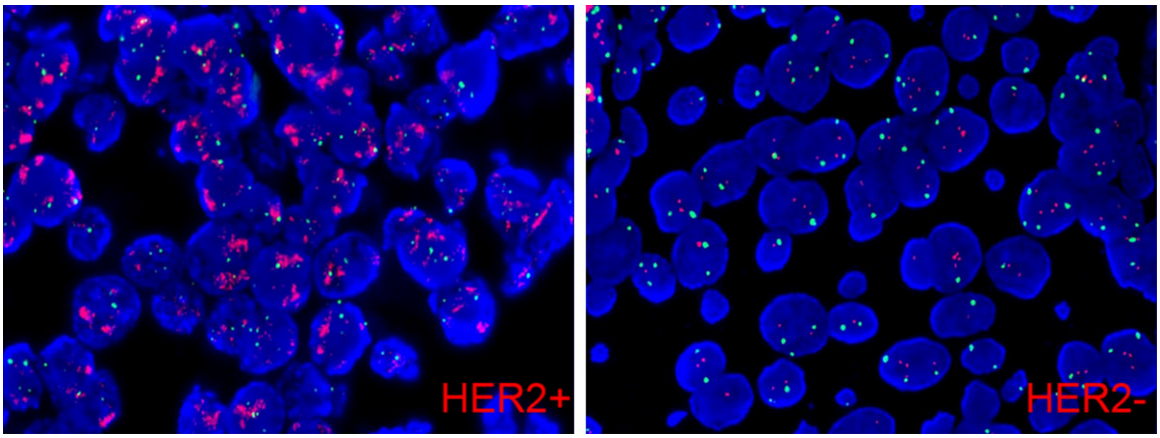


**Figure 1.** Representative image of dual color HER2 fluorescence in situ hybridization (FISH) for the MABC tissues. The left image is positive for amplified HER2 gene (HER2+), and the right image is negative for HER2 gene amplification (HER2-). HER2 genes are seen as red-orange dots and CEN 17 targets are seen as green dots. The blue nuclei counterstained with DAPI.

**Table 1.** Baseline Characteristics of the 90 MABC patients

| Characteristic | Median (range) | Number (%) |
|---|---|---|
| Age, yr | 48 (30-82) | |
| Gender | | |
| Female | | 90 (100) |
| Tumor diameter, cm | 3.0 (0.5-10) | |
| Histological grade | | |
| In situ breast carcinoma | | 17 (18.9) |
| I | | 8 (8.9) |
| II | | 27 (30.0) |
| III | | 38 (42.2) |
| Lymph node metastasis | | |
| No | | 46 (51.1) |
| Yes | | 41 (45.6) |
| NA | | 3 (3.3) |
| HER2 | | |
| Negative | | 44 (48.9) |
| Positive | | 46 (51.1) |
| EGFR | | |
| Negative | | 36 (40.0) |
| Positive | | 54 (60.0) |
| CK5/6 | | |
| Negative | | 61 (67.8) |
| Positive | | 29 (32.2) |
| CK8 | | |
| Negative | | 6 (6.7) |
| Positive | | 84 (93.3) |
| CD44 | | |
| Negative | | 29 (32.2) |
| Positive | | 61 (67.8) |
| CD166 | | |
| Negative | | 16 (17.8) |

least 30% of tumor cells (3+). HER2 immunostaining was considered positive when strong membranous staining (2+ and 3+ grades) was observed, whereas tissues with 0 to 1+ grades were regarded as negative expression. If the degree of expression of HER2 was positive staining, a fluorescent in situ hybridization (FISH) for HER2 amplification was applied. For Ki67 expression, this protein was determined by the percentage of positively stained cells with a 14% cutoff. The cases are considered positive for p53 immunostaining if more than 10% of the tumor cells showed nuclear staining.

*FISH*

The HER2 amplification in breast cancer was evaluated by a PathVysion HER2 DNA Probe Kit (Vysis) according to the manufacturer's instructions. The HER2 gene copy number was indicated, and at least 60 nuclei of the tumor cells were examined to count the number of HER2 and chromosome 17 (Chr17) signals. A HER2/Chr17 ratio of 2.2 was used as the cut-off point, defining more than 2.2 as HER2 amplified (**Figure 1**).

*Statistical analysis*

Continue data were expressed as the mean ± sd. Categorical variables,

| | | |
|---|---|---|
| Positive | | 74 (82.2) |
| p53 | | |
| Negative | | 37 (41.1) |
| Positive | | 53 (58.9) |
| Bcl-2 | | |
| Negative | | 68 (75.6) |
| Positive | | 22 (24.4) |
| BRCA1 | | |
| Negative | | 26 (28.9) |
| Positive | | 64 (71.1) |
| Ki-67 | | |
| < 14% | | 39 (43.3) |
| > 14% | | 51 (56.7) |
| Duration of follow-up (mo)[1] | 33 (9-106) | |
| Tumor recurrence[1] | | 1 (1.4) |
| Distant metastasis[1] | | 4 (5.6) |
| Patient death[1] | | 7 (7.8) |

Note: Values are presented as median (range) or number (%). [1]A follow-up information of 72 cases was available in current study. Abbreviation: MABC, molecular apocrine breast cancer; NA, not available; HER2, human epidermal growth factor receptor 2; EGFR, epidermal growth factor receptor; CK5/6, cytokeratin 5/6; CK8, cytokeratin 8; CD166, cluster of differentiation 166; Bcl-2, B-cell lymphoma-2; BRCA1, breast cancer 1, early onset; mo, months.

expressed as percentages, were evaluated by $\chi^2$ test or Fisher's exact test. *P*-values were two-sided and a significance level of less than 0.05 was considered statistically significant. The Kaplan-Meier estimates were calculated by the log-rank test for overall survival, the values stratified by categorical variables. All statistical analyses were carried out using the statistical software program package SPSS for Microsoft Windows version 16.0 (SPSS Inc., Chicago, IL, USA).

## Results

### *Baseline characteristics*

A total of 239 cases with ER-, PR-negative breast cancer were enrolled in our study. In total, 149 out of 239 breast cancer samples (62.3%) were AR negative staining, the remaining 90 breast cancer samples (37.7%) were AR positivity. We analyzed the association between the AR expression and HER2 status in the cases with ER-, PR-negative breast cancer. Interestingly, the cases with AR positivity showed significant higher rate of HER2 overexpression than AR-negative cases (51.1% vs. 26.2%, $P < 0.001$).

There were 90 cases (37.7%) of MABC subtype in our enrolled patients (N = 239) because they demonstrated the representative MABC markers, apocrine histology, AR positivity and ER-/PR-negative staining. Baseline characteristics of MABC patients are presented in **Table 1**. A total of 90 MABC patients in our study were 100% female. Patients range from 30 to 82 years old, with a median of 48. The tumor diameter in MABC patients varied from 0.5 to 10 centimeter, with a median tumor diameter of 3.0 centimeter. Seventeen (18.9%) cases were carcinoma in situ of breast. The remaining 73 (81.1%) cases were invasive carcinoma, with 8 (8.9%) at grade I, 27 (30.0%) at grade II, and 38 (42.2%) at grade III. Lymph node metastasis could be detected in almost half of the MABC cases (41/90), and 46 cases (51.1%) have not observed the lymph node metastasis. The HER2 status was assessed and checked by IHC staining and FISH, more than half of the MABC cases (51.1%) have been found positive for HER2. The expression of EGFR, CK5/6, CK8, CD166, p53, Bcl-2, CD44 and BRCA1 were evaluated by IHC staining (**Figure 2**) with positive rates of 60.0%, 32.2%, 93.3%, 82.2%, 58.9%, 24.4%, 67.8% and 71.1%, respectively, in these 90 MABC cases. The cases with Ki-67 labeling index more than 14% account for 56.7% of the MABCs. Data of follow-up information were available for 72 patients, these patients were followed up for an average of 39.6 months (range from 9 to 106 months).

### *Comparison of clinicopathologic features according to HER2 status in cases of MABC*

The clinicopathologic features of the MABCs, stratified for HER2 positive and HER2 negative, are provided in **Table 2**. Of the 90 eligible MABC patients constitute the study samples, 44 had HER2-negative and 46 showed HER2-positive. The HER2-negative and HER2-positive MABC patients are aged 49.6 ± 12.9 and 48.8 ± 9.5, respectively. To investigate whether the HER2 status was related to various patient characteristics, the patients were grouped according to the status of HER2 status (HER2-negative and positive). We have not observed the associa-

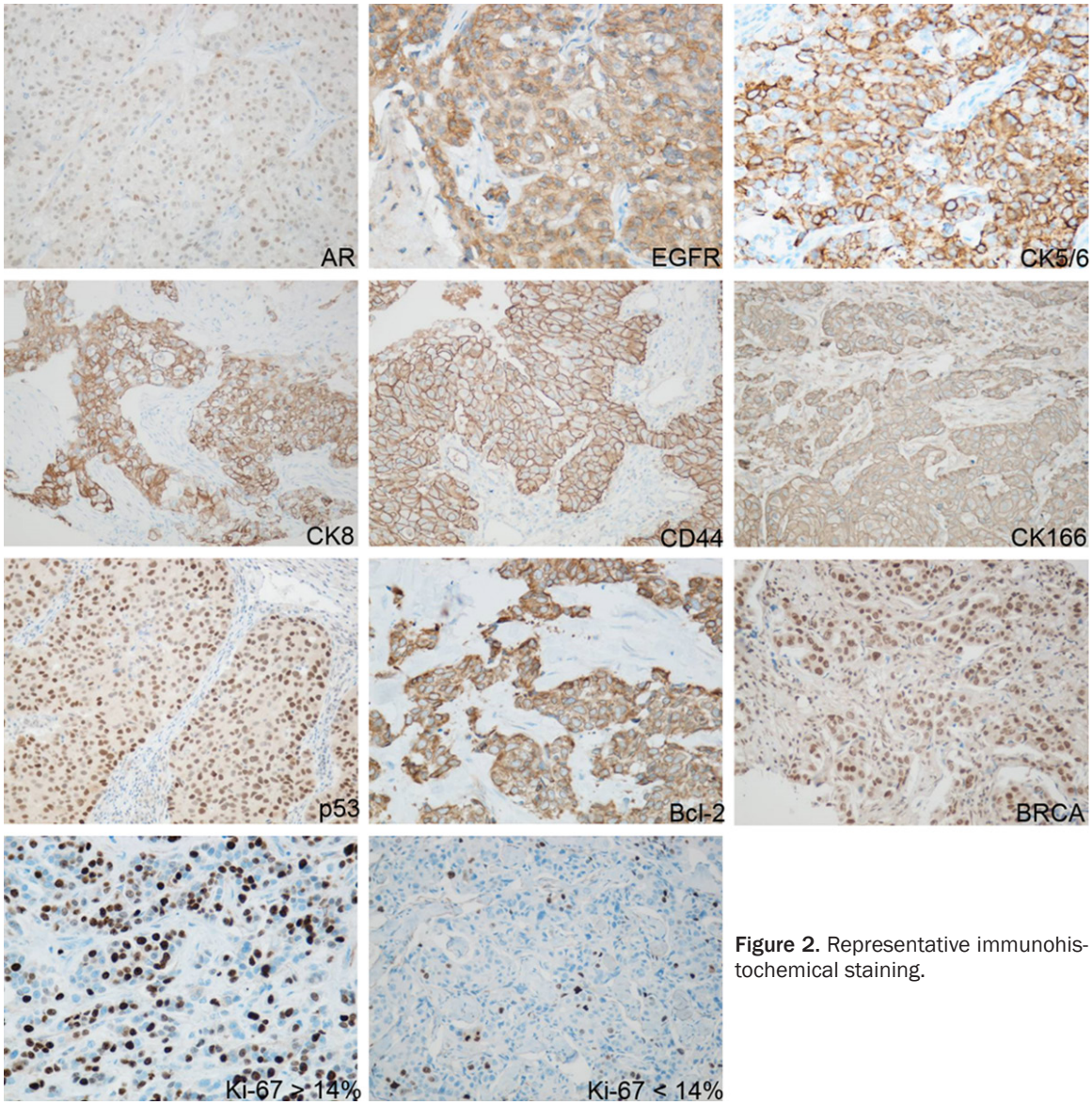


**Figure 2.** Representative immunohistochemical staining.

tion between the HER2 expression status and clinicopathologic features (age, tumor diameter, histological grade, and expression of CK8, p53, Bcl-2, CD44 and BRCA1) in the MABC patients (**Table 2**). However, we observed that HER2-negative MABCs has a higher lymph node metastasis rate than HER2-positive MABCs (54.5% vs. 37.0%, $P$ = 0.044), more than half of HER2-negative MABCs (54.5%) are lymph node metastasis. The patients with HER2 negative have a higher rate of EGFR and CK5/6 positive expression when compared to patients with HER2 positive (70.5% vs. 50.0%, $P$ = 0.048 for EGFR, and 43.2% vs. 21.7%, $P$ = 0.030 for CK5/6). In contrast, the lower rate of CD166 positive expression was observed in the HER2-negative MABCs, when compared with HER2-positive MABCs (72.7% vs. 91.3%, $P$ = 0.021). Comparisons of Ki-67 expression between HER2-negative and positive cases showed a borderline significant higher in the HER2-negative cases (65.9% vs. 47.8%), with $P$ values of 0.081.

*Association of overall survival by basic clinical characteristics and molecular expression features in MABC patients*

Kaplan-Meier analyses revealed a tendency of poor overall survival (OS) in patients with HER2-positive MABCs was significant increased than with HER2-negative MABC ($P$ = 0.034, **Figure**

**Table 2.** Clinical and IHC characteristics according to the HER2 status in MABCs

| Characteristics | | HER2 negative (N = 44) | HER2 positive (N = 46) | *P*-value |
|---|---|---|---|---|
| Age, yr | | 49.6 ± 12.9 | 48.8 ± 9.5 | 0.756 |
| Tumor diameter, cm | | 3.48 ± 1.63 | 3.77 ± 2.14 | 0.467 |
| Histological grade | in situ | 5 (11.4) | 12 (26.1) | 0.103 |
| | I | 6 (13.6) | 2 (4.3) | |
| | II | 16 (36.4) | 11 (23.9) | |
| | III | 17 (38.6) | 21 (45.7) | |
| Lymph node metastasis | No | 17 (38.6) | 29 (63.0) | 0.044 |
| | Yes | 24 (54.5) | 17 (37.0) | |
| EGFR | Negative | 13 (29.5) | 23 (50.0) | 0.048 |
| | Positive | 31 (70.5) | 23 (50.0) | |
| CK5/6 | Negative | 25 (56.8) | 36 (78.3) | 0.030 |
| | Positive | 19 (43.2) | 10 (21.7) | |
| CK8 | Negative | 3 (6.8) | 3 (6.5) | 0.955 |
| | Positive | 41 (93.2) | 43 (93.5) | |
| CD44 | Negative | 11 (25.0) | 18 (39.1) | 0.152 |
| | Positive | 33 (75.0) | 28 (60.9) | |
| CD166 | Negative | 12 (27.3) | 4 (8.7) | 0.021 |
| | Positive | 32 (72.7) | 42 (91.3) | |
| p53 | Negative | 17 (38.6) | 20 (43.5) | 0.641 |
| | Positive | 27 (61.4) | 26 (56.5) | |
| Bcl-2 | Negative | 31 (70.5) | 37 (80.4) | 0.271 |
| | Positive | 13 (29.5) | 9 (19.6) | |
| BRCA1 | Negative | 10 (22.7) | 16 (34.8) | 0.207 |
| | Positive | 34 (77.3) | 30 (65.2) | |
| Ki-67 | < 14% | 15 (34.1) | 24 (52.2) | 0.081 |
| | > 14% | 29 (65.9) | 22 (47.8) | |

Note: Values are presented as mean (sd) or number (%). Abbreviation: IHC, immunohistochemistry; MABC, molecular apocrine breast cancer; HER2, human epidermal growth factor receptor 2; EGFR, epidermal growth factor receptor; CK5/6, cytokeratin 5/6; CK8, cytokeratin 8; CD166, cluster of differentiation 166; Bcl-2, B-cell lymphoma 2; BRCA1, breast cancer 1, early onset.

3). No significant differences were observed in the overall survival rate for histological grade ($P$ = 0.574), lymph node metastasis ($P$ = 0.742), and EGFR ($P$ = 0.231), CK5/6 ($P$ = 0.259), CK8 ($P$ = 0.342), CD44 ($P$ = 0.238), CD166 ($P$ = 0.259), p53 ($P$ = 0.427), Bcl-2 ($P$ = 0.754), BRCA1 ($P$ = 0.980) and Ki-67 ($P$ = 0.118) expression statuses (**Figure 4**).

## Discussion

Breast cancer is a sex steroid-dependent tumor. Estrogens, progesterons and androgens have been associated with breast cancer risk and prognosis in women [21-23]. The sex steroids and their receptors play a significant role in the cell proliferation and tumor progression. The expressions of ER and PR have the better prognosis, and are widely used as biomarkers for hormone-based therapies [5, 10]. However, about 30% to 60% of breast cancer are ER- and/or PR- negative [24]. They have significantly higher risk of mortality compared with patients with tumors that are ER and/or PR positive [6]. AR is commonly expressed in breast cancers. Previous studies have reported that almost half of ER- and/or PR-negative breast cancer were AR positive [6, 25]. In current study, AR status was assessable in 239 women with ER-/PR-negative tumors. Among these, 90 tumors (37.7%) were AR positive and 149 tumors (62.3%) were AR negative. Recent study shows that HER2 has a greater prognostic value for recurrence in patients with ER- and PR-negative breast cancer [26]. In this study, we found that the significant association between the AR and HER2 expressions in the ER-, PR-negative breast cancer. The cases with AR positivity have a higher HER2 amplification rate than AR-negative cases (51.1% vs. 26.2%, $P$ < 0.001). The result is consistent with the previous study [27].

MABC has the characteristic features of histology and immunostaining status of sex steroid receptors which include apocrine histology, and ER-negative, PR-negative and AR-positive staining [12]. In the present study, 90 cases of ER- and PR-negative tumors have the representative histological hallmark and molecular expression status for sex steroid receptors of MABC tumors. We investigated the clinicopathological characteristics of MABCs in a Chinese Han cohort of patients with ER-negative expression.

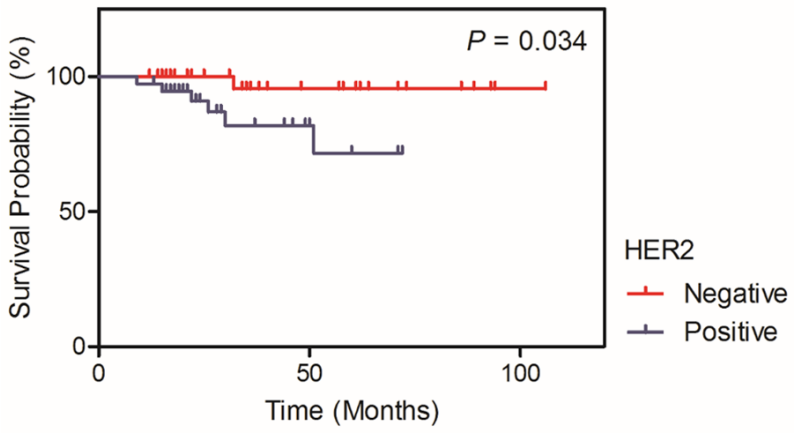


**Figure 3.** Kaplan-Meier plot of overall survival according to HER2 status. The HER2 positive was found to be significantly associated with poor overall survival.

The majority of the studied patients were premenopausal young women (median age 48 yr, range 30-82) with high grade (of those 72.2% cases with II and III grades) tumors. By studying basic pathological characteristics of these cases, we found that MABCs had the clinicopathological characteristics including high lymph node metastasis rate, high histological grade of tumor cells, increased expressions of p53, CD44 and BRCA1, elevated Ki-67 labeling index, and favorable prognosis. It is interesting that more than 50% of MABC cases were HER2 positive.

HER2 is one member of the ErbB receptor tyrosine kinase (TK) family [13]. It plays a major role in the proliferation, growth and survival of tumor cells, and is overexpressed in up to 30% of primary breast cancer [16]. In this paper we investigated the relationship between HER2 and various clinicopathological characteristics of MABCs. Our study has found that the HER2 positivity is associated with CD166 expression in the MABCs. CD166, also called activated leukocyte cell adhesion molecule (ALCAM), is a transmembrane glycoprotein. It is involved in cell migration and development. Hein [28] and Ihnen [7] reported that CD166 protein expression was significantly associated with an ER-positive phenotype in human breast carcinoma. CD166 overexpression was also found in ER-negative and AR-positive human breast carcinoma tissue [11, 29]. It has been reported that the breast carcinoma tissue have a higher CD166 expression when compared with adjacent normal tissue [30, 31]. However, the role of CD166 expression in human breast carcinoma cells is still being debated. Numerous studies have shown that CD166 expression seems to be significantly correlated with cancer progression and prognosis [28, 31]. The findings shown that high CD166 expression was associated with cancer cell invasion, tumor growth, metastasis, shorter recurrence-free interval and overall survival in breast cancer [7, 28, 30, 31]. Instead, the opposite results have been reported [7, 28]. Therefore, those findings indicate that the biologic role of CD166 in breast cancer is complex. The specific mechanism needs to be further explored.

Our findings suggested that the HER2 negative is associated with CK5/6 and EGFR positive immunostaining in the cases with AR-positive, ER-/PR-negative phenotype. In most studies the triple-negative breast cancer (TNBC) is defined by immunohistochemical profile of negative expression for hormone receptors (ER, PR) and HER2 in paraffin-embedded tissue [32]. Other studies, however, shown that ER-, PR- and HER2-negative combined with positive expressions of CK5/6 and EGFR can improve the sensitivity and specificity in definition of TNBC [33]. CK5/6 and EGFR are characteristic markers to distinguishing basal-like subtype of breast cancer from TNBC [33, 34]. The basal-like breast cancer is a clinically distinct subgroup within TNBC, and accounts for three-fourths. In the current study, the cases with molecular classification for ER-, PR-, HER2-, EGFR+ and CK5/6+ were 16 (17.8%). Thus, our results indicate that there is overlap between MABC and basal-like breast cancer, which could explain the negative correlation between the expression levels of HER2 and basal-like related markers (CK5/6 and EGFR).

It is worth mentioning that HER2 negativity is significantly associated with lymph node metastasis. We also found an association between HER2 negativity and Ki-67 expression level of cell proliferative marker, although that

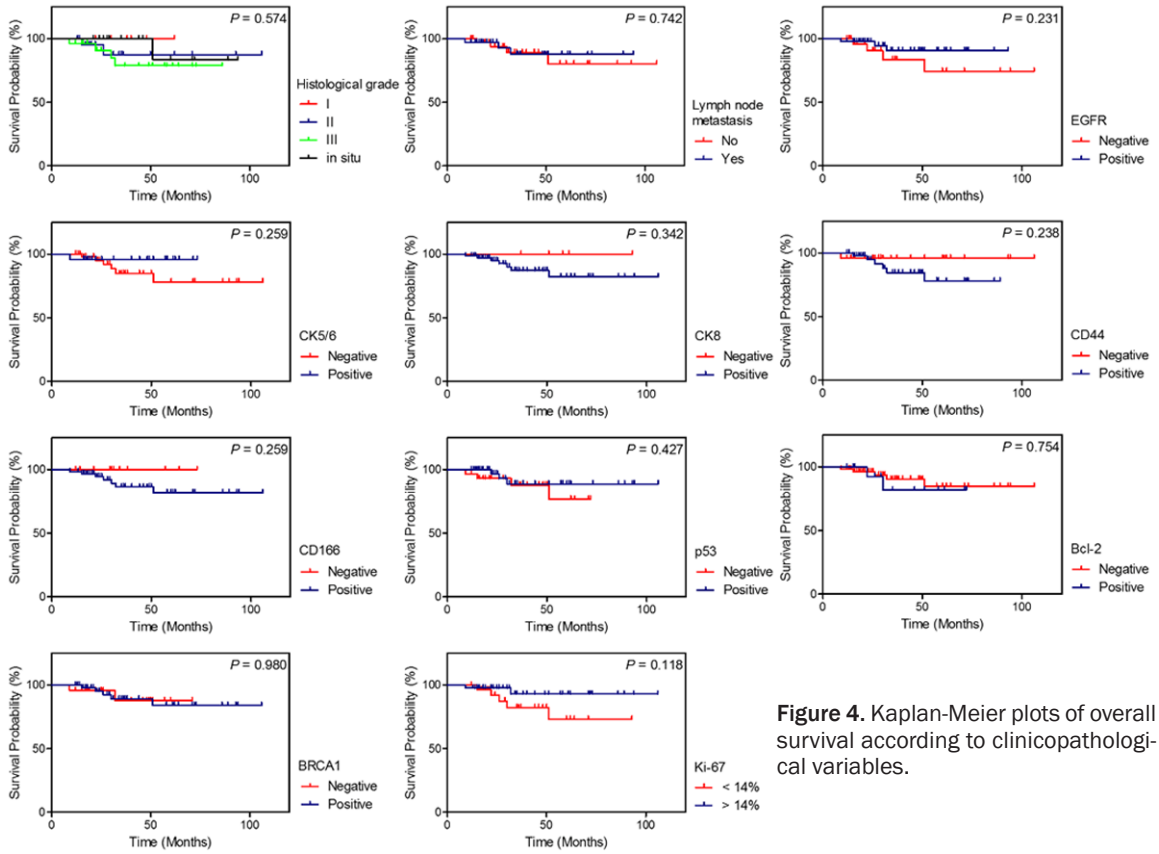


**Figure 4.** Kaplan-Meier plots of overall survival according to clinicopathological variables.

was at the brink of significance ($P$ = 0.081). However, by Kaplan-Meier analysis, the HER2 positivity is related to poor overall survival in patients with MABC. This is to be expected given that the HER2 positivity is a significant marker of poor prognosis in breast cancer [18]. Thus, these data suggest that a distinct subgroup depending on HER2 status is present in MABC. The additional study to investigate a molecularly distinct subgroup by a gene expression profiling in MABC is needed.

In summary, these results from the present study indicate that HER2 diagnostic is a significant prognostic indicator and would be valuable for distinguishing breast cancer subtypes in MABC.

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## Disclosure of conflict of interest

None.

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