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Original Article

Clinicopathologic characteristics of HER2-positive pure mucinous breast carcinoma: a systematic investigation into an unusual tumor

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Abstract: Pure mucinous breast carcinoma (PMBC) accounts for approximately 2% of all breast carcinoma. Overexpression or amplification of human epidermal growth factor receptor 2 (HER2) is rarely observed in PMBC. We retrieved 119 PMBCs, which included 12 HER2-positive PMBCs and 107 HER2-negative PMBCs, to compare the clinicopathologic features between HER2-positive and HER2-negative neoplasms. The assessed parameters included patient age, menstruation, laterality, tumor size, lymph node status, tumor-node-metastasis (TNM) stage, nuclear grade, receptor status, treatment and prognostic features. HER2-positive PMBCs represented approximately 10.1% of the PMBCs examined. HER2-positive PMBCs showed more frequent lymph node metastasis (P=0.038), a significantly higher clinical TNM stage (P<0.001) and nuclear grade (P<0.001), lower estrogen receptor (ER) and progesterone receptor (PR) expression and higher Ki67 expression than the HER2-negative group (P=0.011, P=0.005, and P=0.001, respectively). HER2-positive PMBCs (untreated with HER2-targeted therapy) had a significantly lower overall survival (OS) rate than HER2-negative PMBCs (P=0.005). Nodal metastasis, higher TNM stage and nuclear grade were identified as factors that result in poorer OS of patients with PMBCs (P<0.001, P=0.016, P<0.001, and P<0.001, respectively). Univariate and multivariate Cox analyses confirmed that HER2 status was an independent prognostic factor for PMBCs (P=0.003 and P=0.012, respectively). HER2-positive PMBC is a rare subtype of breast carcinoma with aggressive biological behavior. It is important to identify tumors with these aggressive clinical behaviors and manage them differently. To the best of our knowledge, this study represents the first systematic investigation of the clinicopathologic features of HER2-positive PMBCs.

Keywords: Breast cancer, pure mucinous carcinoma, HER2, prognosis

Introduction

Mucinous breast carcinoma (MBC), also known as colloid carcinoma, is characterized by nests of cells floating in lakes of partitioned mucin and accounts for approximately 1-6% of all breast carcinoma. Generally, MBC is associated with infrequent lymph node metastasis, low rates of local and distant recurrence, and high 5-year disease-free survival rates [1-5]. Most MBCs are positive for estrogen receptor (ER) and progesterone receptor (PR) expression, whereas androgen receptor (AR) is expressed at a low level and human epidermal growth factor receptor 2 (HER2) is not amplified [2].

At present, MBCs are classified in numerous ways and the clinicopathologic features of each subtype need to be investigated. MBCs are divided into two types according to the tumor components: pure mucinous breast carcinoma (PMBC), with a mucinous component of more than 90%, and mixed mucinous breast carcinoma (MMBC), with a 51-90% mucinous component [2]. Hypocellular MBC (type A) and hypercellular MBC (type B) have been proposed based on cell cluster density. A study of the transcriptomic features showed that the transcriptome of type A is distinct from that of type B [6]. Moreover, some studies have suggested that MMBC and invasive ductal carcinoma (IDC) patients exhibit significantly poorer prognosis than those with PMBC [1, 7]. Other special subtypes of MBC such as MBC with a micropapillary pattern have been reported, and most studies have confirmed that the micropapillary

intraepithelial component is associated with aggressive behavior and poor prognosis [8-10].

In recent years, molecular classification and gene expression profiling studies have established a widely applied molecular classification of breast cancers and have distinguished 5 subtypes: luminal A, luminal B, normal breast-like, HER2-enriched and basal-like types [11-13]. These subtypes differ in their clinical outcomes, responses to neoadjuvant chemotherapy [14] and risk factors [15]. A transcriptomic study confirmed that MBCs are of the luminal A molecular subtype [6], and the HER2 status assessed by immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH) was predominantly negative (HER2-positive rates ranged from 5.8-9.5%) [8, 16, 17]. The HER2 gene and its protein product, which can be detected simultaneously, have been the subject of some investigations [17-19], even in the subtypes with an indolent course, such as PMBC [8, 17, 20-22]. However, the association between alterations in HER2 overexpression and/or amplification and histologic breast cancer subtypes has not been extensively studied. Few studies on HER2-positive PMBC have been conducted [20], and the receptor status, clinicomorphologic and prognostic features of this type of breast cancer remain largely unknown.

In our study, we present a comparison of 12 HER2-positive and 107 HER2-negative PMBCs to illustrate their clinicopathologic features and to highlight important differences between HER2-positive and HER2-negative PMBCs.

Materials and methods

Cases and collection of clinicopathologic data

In all, 188 consecutive cases diagnosed as MBC were retrieved between January 2009 and December 2016 from the surgical pathology files of West China Hospital, Sichuan University. Of these patients, we selected 119 cases of PMBC and divided them into two groups: 12 in the HER2-positive PMBC group and 107 in the HER2-negative PMBC group. Of these tumors, 11 were screen-detected breast cancers, and 103 were spontaneous. The detection methods for 5 tumors were not available.

All available clinicopathologic data, including age, tumor size, menstrual status, laterality, lymph node status, metastasis, tumor-node-metastasis (TNM) stage, nuclear grade, hormone receptor expression, Ki67 expression, treatment and follow-up information, were collected from the system of West China Hospital. Pathologic tumor stage (TNM stage) was assessed according to the 8th edition of the American Joint Committee on Cancer (AJCC) staging manual. Exemption from informed consent after the identification of information was approved by the Institutional Review Board of West China Hospital, Sichuan University.

Histopathological review

Hematoxylin and eosin (H&E)-stained slides of the 119 PMBCs were reviewed independently by two pathologists according to the 2012 WHO classification of breast tumors [2]. Slides were histopathologically assessed for ER, PR, Ki67, and HER2 status, histologic grade, tumor size, pT stage and pN stage. Discrepancies were resolved by synchronous analysis on a multiheaded microscope. Parameter evaluations were all based on resection specimens.

IHC and FISH

Immunohistochemical staining was performed on tissue microarray sections using the following antibodies: ER (sp1, no dilution, Roche, China); PR (1E2, no dilution, Roche, China); HER2 (4B5, no dilution, Roche, China); and Ki67 (30-9, no dilution, Roche, China). The expression of ER and PR was assessed according to the 2010 ASCO/CAP Guideline Recommendations [23]. For Ki67, nuclear staining of any intensity was evaluated. We assessed Ki67 status according to the 2011 recommendations [24]. The Ki67 labeling index was evaluated as a percentage of positive cells among more than 500 cancer cells in 5 high-powered fields (40× objective) of the deepest portion and the superficial margin of the tumor. HER2 was scored according to the recommended criteria and cases were given a staining intensity score of 0 to 3 [25]. The stricter criteria of the new IHC recommendation [26] had no effect on prior cases for the HER2-positive group.

FISH analyses were performed on 4-μm-thick, formalin-fixed, paraffin-embedded (FFPE) sections of HER2 2+ PMBCs with HER2/neu and chromosome 17 centromere (CEP17) probes (PathVysion HER2 DNA Probe Kit, Abbott, IL, USA). The hybridized slides were examined

Table 1. Comparison of the clinicopathologic features in HER2-negative and HER2-positive PMBC patients (n=119)

	HER2 negative PMBC (%)	HER2 positive PMBC (%)	*P* Value
Cases/n (%)	107 (89.9)	12 (10.1)	
Mean age (y ± SD)	52.1±12.8	50.6±14.0	0.698
Mean size (cm ± SD)	2.6±1.2	3.3±2.2	0.150
Age			1.000
≤50 y	63 (52.9)	7 (5.9)	
>50 y	44 (37.0)	5 (4.2)	
Sex			0.899
Female	106 (99.1)	12 (100)	
Male	1 (0.9)	0 (0)	
Menstruation			1.000
Premenopausal	65 (60.7)	7 (58.3)	
Postmenopausal	26 (24.3)	2 (16.7)	
Unknown	16 (15.0)	3 (25.0)	
Laterality			1.000
Left	56 (52.3)	6 (50.0)	
Right	51 (47.7)	6 (50.0)	
Lymph node status			0.038
pN0	94 (87.9)	8 (66.7)	
pN1-3	10 (9.3)	4 (33.3)	
Unknown	3 (2.8)	0	
Metastasis			0.083
M0	100 (93.5)	8 (66.7)	
M1	0	1 (8.3)	
Unknown	7 (6.5)	3 (25.0)	
TNM stage			0.000
Stage I and II	95 (88.8)	5 (41.7)	
Stage III and IV	2 (1.9)	4 (33.3)	
Unknown	10 (9.3)	3 (25.0)	
Nuclear grade			0.000
1, 2	105 (98.1)	1 (8.3)	
3	2 (1.9)	11 (91.7)	
ER			0.011
Positive	101 (94.4)	8 (66.7)	
Negative	3 (2.8)	3 (25.0)	
Unknown	3 (2.8)	1 (8.3)	
PR			0.005
Positive	99 (92.5)	7 (58.3)	
Negative	5 (4.7)	4 (33.4)	
Unknown	3 (2.8)	1 (8.3)	
Ki67			0.001
<20%	88 (82.2)	5 (41.7)	
≥20%	13 (12.1)	7 (58.3)	
Unknown	6 (5.7)	0	
Radiotherapy			0.611
Yes	12 (11.2)	3 (25.0)	

using a Leica DM6000 BX51 fluorescence microscope with a ×100 objective and the following filter sets: triple bandpass Spectrum Green-/Spectrum Green/Spectrum Orange), dual bandpass (FITC/Texas Red) and single bandpass (Spectrum Green or Spectrum Orange) filters. One hundred tumor cells were selected for signal examination, and the FISH results were evaluated by the new criteria [26].

Statistical analysis

Statistical analysis was performed using SPSS version 24.0 statistical software (SPSS, Chicago, IL, USA). Descriptive statistics were calculated for the demographic and clinicopathologic factors, and differences between the two groups were evaluated using the chi-square or Fisher exact test, as appropriate. Follow up months were assessed using Student's t-test.

The overall survival (OS) and disease-free survival (DFS) for each group was determined by Kaplan-Meier analysis. A univariate survival analysis was performed, and significance was assessed using the log-rank test. Relationships between the clinicopathologic factors and the clinical prognosis were estimated using the Cox proportional hazards regression model. Two-tailed *P* values less than 0.05 were considered significant.

Results

HER2 status of study cohort

We retrieved 119 PMBCs, which included 12 HER2-positive PMBCs and 107 HER2-negative PMBCs.

HER2-positive PMBCs represent approximately 10.1% of the PMBCs examined. In HER2-positive group, the HER2 IHC was interpreted as follows: 1 as HER2 (1+), 4 as HER2 (2+), and 7 as HER2 (3+) (8.3%, 33.3%, and 58.3%, respectively). The FISH results in the HER2-positive group are included in **Table 2**. For the HER2-negative group, the HER2 IHC was interpreted as follows: 58 as HER2 (0),

No	78 (72.9)	6 (50.0)	
Unknown	17 (15.9)	3 (25.0)	
Chemotherapy			0.656
Yes	63 (58.9)	7 (58.3)	
No	13 (12.1)	2 (16.7)	
Unknown	31 (29.0)	3 (25.0)	
Endocrine therapy			0.068
Yes	77 (72.0)	6 (50.0)	
No	8 (7.5)	3 (25.0)	
Unknown	22 (20.5)	3 (25.0)	
Surgical procedure			1.000
Mastectomy	98 (91.6)	11 (91.7)	
Lumpectomy	5 (4.6)	0	
Unknown	4 (3.8)	1 (8.3)	

PMBC, pure mucinous breast carcinoma; SD, standard deviation; ER, estrogen receptor; PR; progesterone receptor.

31 as HER2 (1+) and 18 as HER2 (2+) (54.2%, 29.0%, and 16.8%, respectively). FISH analysis in the HER2-negative group was performed on all cases of HER2 (2+) and 27 HER2 (1+), and the results showed that the HER2 gene was not amplified. The paraffin blocks of the remaining 4 HER2 (1+) cases in the HER2-negative group were not available.

Comparison of HER2-positive and HER2-negative PMBCs

The clinicopathologic features of HER2-positive and HER2-negative PMBCs are compared in **Table 1**.

The mean age at diagnosis in patients with HER2-positive and HER2-negative PMBCs was 50.6 years (range, 32-78) and 52.1 years (range, 34-86), respectively (P=0.698). The mean tumor size in HER2-positive and HER2-negative PMBCs was 3.3 cm (range, 1.5-9.0) and 2.6 cm (range, 0.7-6.0), respectively (P=0.150).

HER2-positive PMBCs had a higher frequency of lymph node metastasis (P=0.038), higher TNM stage (P<0.001), higher nuclear grade (P<0.001), lower expression of ER and PR, and higher Ki67 expression (P=0.011, P=0.005, and P=0.001, respectively) than HER2-negative PMBCs. The rates of other prognostic indicators, such as menstruation, tumor location, adjuvant therapy, and surgical procedure, were similar in the two groups.

The mean follow-up time at diagnosis in patients with HER2-positive and HER2-negative PMBCs was 50.2 months (range, 17-98) and 48.8 months (range, 9-88), respectively (P=0.864). HER2-positive and HER2-negative PMBC patients who were alive and disease-free at the time of analysis had a mean follow-up time of 54.0 months (range, 17-98) and 31.0 months (range, 9-88), respectively (P=0.311). In the HER2-positive group, distant metastases were found in 3 patients with high TNM stage, and of those, 2 (16.7%) died of bone metastases. In the HER2-negative group, metastasis occurred in 10 (9.3%) patients, and distant metastases were found in 1 patient (0.9%) with TNM stage III.

Clinicopathologic findings of HER2-positive PMBCs

Detailed clinicopathologic characteristics of each HER2-positive patient are presented in **Table 2**.

The mean age of the HER2-positive PMBC patients was 50.6 years (range, 32-78 years; median, 49.0 years), and the mean tumor diameter was 3.3 cm (range, 1.5-9.0 cm; median, 2.3 cm). The nuclear grade of most tumors was grade 3 (11/12; 91.7%) (**Figure 1C**), and only one was grade 2. The ER and PR status in tumors was as follows: 7 cases were ER+/PR+, 3 cases were ER-/PR-, 1 case was ER+/PR-, and 1 case was unknown. Ki67 expression varied widely and ranged from 5% to 50%. In the HER2-positive group, a micropapillary pattern was observed in 1 case (8.3%, 1/12) (**Figure 1C**, **1D**), hypocellular PMBC (type A) was found in 5 cases (41.7%, 5/12) (**Figure 1A**), and hypercellular PMBC (type B) was found in 2 cases (16.7%, 2/12) (**Figure 1E**, **1F**).

Therapeutic methods also differed across patients. Nine patients received mastectomy, including axillary lymph node dissection, but surgical information in the other 3 patients was unknown. Among all patients, 3 (25%) received radiotherapy, 7 (58.3%) received chemotherapy, half (50%) received endocrine therapy and 3 (25%) received HER2-targeted therapy.

Prognostic factors in PMBCs

Kaplan-Meier analysis revealed that HER2-positive PMBCs (untreated with HER2-targeted therapy) had a significantly lower OS rate than HER2-negative PMBCs, while no significant dif-

Table 2. Summary of available clinicopathologic features in HER2-positive PMBCs (n=12)

Case no.	1	2	3	4	5	6	7	8	9	10	11	12
Age (y)/sex	41/F	32/F	38/F	75/F	44/F	49/F	49/F	57/F	39/F	78/F	54/F	51/F
Laterality	R	R	L	R	L	L	L	R	L	R	L	R
Menstrual status	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	U	No	U	U
Tumor size (cm)	3	2	2	3.5	U	4	9	U	1.5	2.5	U	2.5
TNM stage	IV (T2N1M1)	IIIA (T2N2M0)	IIA (T2N0M0)	IIA (T2N0M0)	IA (T1N0M0)	IIA (T2N0M0)	IV (T4N3M1)	U	U	IIB (T2N1M0)	IV (T2N0M1)	U
Cellularity	A	A	A+B	B	A	B	A	A+B	A	A+B	A+B	A+B
Nuclear Grade	3	2	3	3	3	3	3	3	3	3	3	3
ER	+	+	-	+	+	+	+	+	U	-	+	-
PR	+	+	-	-	+	+	+	+	U	-	+	-
HER2 IHC	1+	3+	3+	2+	3+	3+	2+	2+	3+	3+	2+	3+
HER2 FISH	Yes	ND	ND	Yes	ND	ND	Yes	Yes	Yes	ND	Yes	ND
Ki67	9%	16%	50%	50%	20%	15%	40%	30%	45%	10%	5%	25%
Follow-up (months)	BM (21), DOD (47)	NED (98)	NED (46)	NED (75)	NED (61)	NED (36)	BM (24), DOD (24)	U	U	NED (17)	CM (48), NED (48)	U
Surgical procedure	Mastectomy	Mastectomy	Mastectomy	Mastectomy	Mastectomy	Mastectomy	Mastectomy	U	U	Mastectomy	Mastectomy	U
Endocrine therapy	Yes	Yes	No	No	Yes	Yes	No	U	U	Yes	Yes	U
Chemotherapy	Yes	Yes	Yes	No	Yes	Yes	Yes	U	U	No	Yes	U
Radiotherapy	Yes	Yes	No	No	No	No	No	U	U	No	Yes	U
HER2-targeted therapy	No	Yes	No	No	No	Yes	Yes	No	No	No	No	U
Histologic Pattern	N	N	N	GP	N	SC	N	GP	N	Cribriform	MP	GP

L, left; R, right; F, female; M, male; ER, estrogen receptor; PR, progesterone hormone; HER2, human epidermal growth factor receptor 2; NED, no evidence of disease; A, hypocellular variant; B, hypercellular variant; BM, bone metastasis; CM, chest metastasis; DOD, died of disease; N, nests; SC, solid cribriform; MP, micropapillary pattern; GP, glandular pattern; IDC, invasive ductal carcinoma; U, unknown; ND, not done.

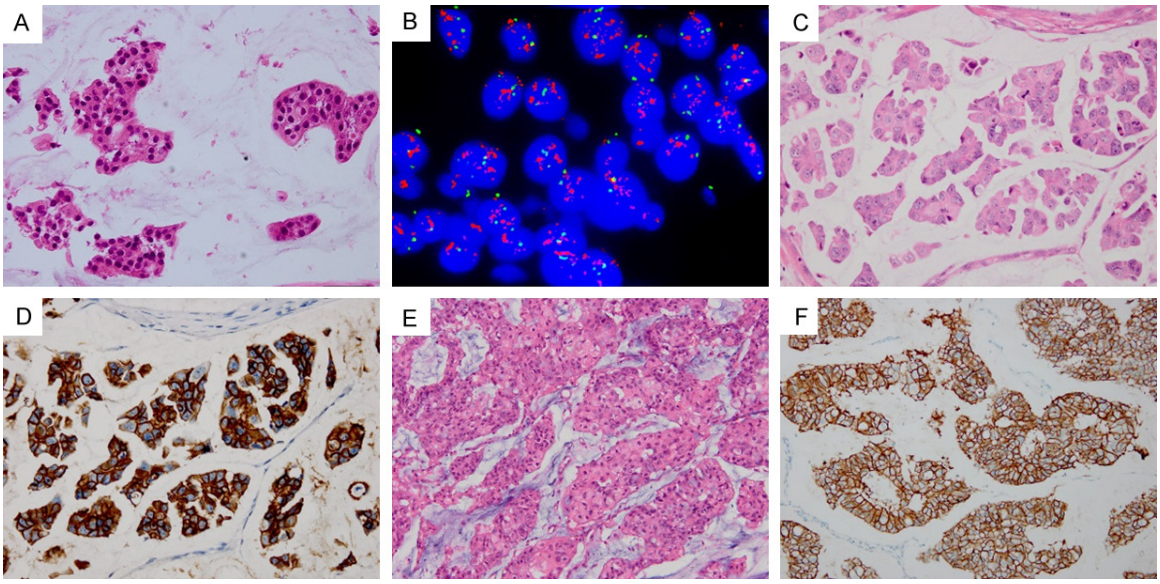

Figure 1. Histologic features and HER2 status of PMBCs. A. Photomicrograph of hypocellular PMBC (type A) with an intermediate nuclear grade (case 7). B. FISH analysis of HER2-positive PMBC; HER2/CEP17 ratio was 3.02 and average HER2 copy number was 9.50 signals per cell (case 8). C. Photomicrograph of micropapillary pattern in PMBC with a high nuclear grade (case 11). D. Immunohistochemistry of PMBC with micropapillary pattern (the HER2/CEP17 ratio was 4.79) (case 11). E. Photomicrograph of hypercellular PMBC (type B) (case 6). F. Immunohistochemistry of hypercellular PMBC showing positivity for HER2 (case 6).

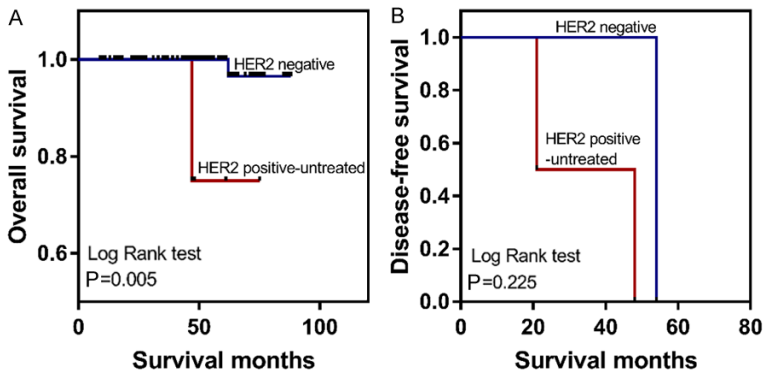

Figure 2. Kaplan-Meier analysis of HER2-positive and HER2-negative PMBCs. A. OS comparison between the HER2-positive (untreated with HER2-targeted therapy) and HER2-negative groups (P=0.005). B. DFS comparison between the HER2-positive (untreated with HER2-targeted therapy) and HER2-negative groups (P=0.225).

ference was found for DFS (**Figure 2A**, **2B**, P=0.005 and P=0.225, respectively). In addition, nodal metastasis, higher TNM stage and higher nuclear grade were associated with poorer OS in PMBC patients (**Figure 3A-C**, P=0.016, P<0.001, and P<0.001, respectively). Due to the limited adverse events, some of the DFS curves could not be presented.

Table 3 shows the univariate analyses of the clinicopathological predictors of OS in PMBCs. Larger tumor size (P=0.008), lymph node metastases (P=0.027), higher TNM stage (P=0.001), higher nuclear grade (P=0.012) and HER2 positivity (P=0.003) were identified as predictors of unfavorable prognosis. Other factors such as age, sex, menstrual status, tumor location, hormone receptors and Ki67 status, surgical procedure and adjunctive therapy, lacked prognostic significance for OS.

Due to the limited number of adverse events, multiple clinicopathologic variables could not be included simultaneously in the Cox proportional hazards model for the multivariate analysis of OS. **Table 4** shows the multivariate analysis for OS, which suggests that HER2 status was an independent prognostic factor for PMBCs (P=0.012).

Discussion

HER2 overexpression or amplification is rare in some subtypes with an indolent course. Both the HER2 gene and protein are prognostic fac-

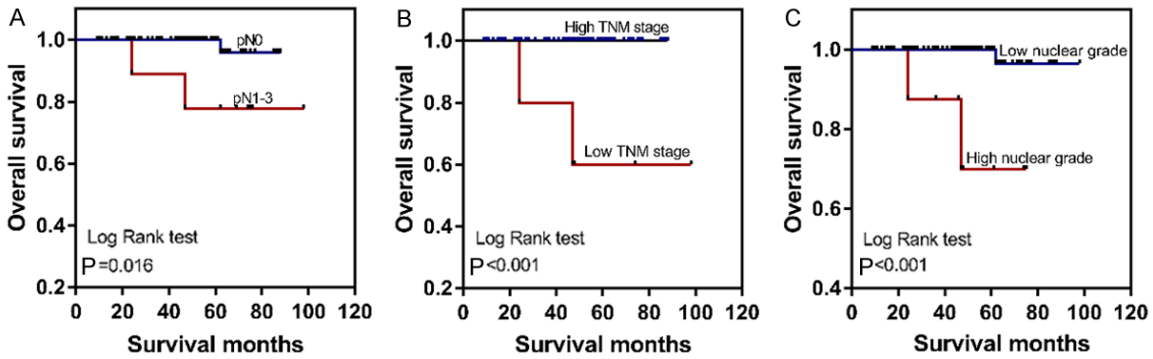

Figure 3. A-C. Kaplan-Meier analysis showed that frequent lymph node metastasis (P=0.016), higher TNM stage (III and IV vs. I and II) (P<0.001) and nuclear grade (3 vs. 1 and 2) (P<0.001) were associated with poor OS.

Table 3. Summary of univariate Cox model estimates for OS (n=119)

Variable	*P* Value	HR	95% CI
Age	0.881	1.01	0.93-1.09
Sex (female vs. male)	0.941	0.05	NA
Menstrual status (no vs. yes)	0.547	0.49	0.05-5.08
Laterality (left vs. right)	0.584	0.61	0.10-3.64
Tumor size	0.008	2.02	1.21-3.41
Lymph node status (pN0 vs. pN1-3)	0.027	7.80	1.27-47.96
TNM stage (I, II vs. III, IV)	0.001	47.82	5.28-433.26
Nuclear grade (3 vs. 1, 2)	0.012	22.34	2.01-248.86
HER2 status (negative vs. positive)	0.003	15.85	2.62-95.93
ER status (negative vs. positive)	0.767	27.70	NA
PR status (negative vs. positive)	0.653	23.22	NA
Ki67 status (negative vs. positive)	0.300	3.58	0.32-39.98
Chemotherapy (yes vs. no)	0.861	1.22	0.13-11.04
Radiotherapy (yes vs. no)	0.089	4.77	0.79-28.91
Endocrine therapy (yes vs. no)	0.193	0.23	0.03-2.10
Surgical procedure (yes vs. no)	0.755	21.53	NA

OS, overall survival; HR, hazard ratio; CI, confidence interval; NA, not applicable.

Table 4. Multivariate Cox model for OS (n=119)

Variable	*P* Value	HR	95% CI
Age	0.992	1.00	0.94-1.07
Lymph node status (pN0 vs. pN1-3)	0.364	2.89	0.29-28.59
Tumor size	0.089	1.80	0.92-3.53
HER2 status (positive vs. negative)	0.012	13.82	1.77-107.88

OS, overall survival; HR, hazard ratio; CI, confidence interval.

tors that indicate an unfavorable prognosis, and they are expressed in approximately 25-30% of invasive breast carcinomas [1, 27]. The overexpression or amplification rate of HER2 in PMBCs was markedly lower than that in IDCs. Some studies have revealed that the rate of HER2-positive PMBCs is 5.8-9.5% [8, 16, 17, 28]. In the present study, HER2-positive PMBCs accounted for approximately 10.1% of the PMBCs examined, which was consistent with previous studies.

Some studies have demonstrated that PMBCs tend to be characterized by an indolent course with infrequent lymphatic or hematogenic dissemination and a favorable prognosis [1, 3, 4, 29]. The superior prognosis of PMBC was also highlighted in a retrospective analysis of more than 11,400 cases, which examined survival rate, epidemiology, clinicopathologic features, and therapy [1]. The indolent behavior of PMBCs can be linked to the relatively low level of genomic instability in these tumors, rare recurrent amplifications, uniform and strong positivity for hormone receptors, and infrequent expression of HER2 [1, 30, 31].

Unlike the indolent behavior of traditional PMBCs, HER2-positive PMBCs were outliers. Studies on the comparison between HER2-positive and HER2-negative PMBCs are limited. In one study, Flynn et al [17] suggested that HER2-positive PMBC patients were younger than HER2-negative PMBC patients, while the patient age in the two groups was not shown to

be significantly different in our study. Furthermore, the average tumor size measured 3.3 cm in the HER2-positive group and 2.6 cm in the HER2-negative group; however, in our study, tumor size in the HER2-positive PMBC group was larger than that in the HER2-negative PMBC group, but the difference was not significant. On the one hand, the size of HER2-positive and HER2-negative tumors may be related to their biologic characteristics, and HER2-positive tumors may grow faster. On the other hand, these differences could be related to the tumor detection methods: screen-detected or spontaneous presentation. A previous study has indicated that screen-detected cancers were more likely to be small, node negative, and well differentiated compared with clinically detected cancers [32]. Although studies have confirmed that tumor size is closely related to the tumor detection methods and the tumors found by screen detected tend to be smaller, there were no significant differences in the two groups as determined by Fisher's exact test (P=0.568). Therefore, it is currently difficult to answer whether the tumor detection methods explain the difference in tumor size in the HER2-positive and HER2-negative groups. Factors such as race and medical conditions available to Chinese patients may affect the size of the tumor at discovery.

In our study, most HER2-positive patients (58.3%) exhibited triple-positive expression (ER, PR and HER2), which was consistent with a previous study in which over two-thirds of HER2-positive patients had triple-positive tumors [17]. Although the HER2-positive group had a high percentage of triple positive tumors, the expression of ER and PR was lower than that in HER2-negative PMBCs. In addition, HER2-positive tumors showed a much higher frequency of Ki67 expression (P=0.001). As a marker of cell proliferation, Ki67 is universally expressed among proliferating cells and is absent in quiescent cells. An analysis of 46 studies including 12,155 patients showed that high Ki67 labeling is correlated with increased relapse and decreased survival [33]. However, in our HER2-positive group, there were 2 cases with Ki67 indexes lower than 10%. A literature search found that the Ki67 index of HER2-positive invasive breast cancer (no special type) can also be lower. HER2 amplification occurred in 7.5-16.2% low Ki67 cases (the cut-off value is 14%), and the lowest Ki67 index is 4% [34]. At the same time, other subtypes of mucinous carcinoma, such as mucinous micropapillary carcinoma, which is often considered to be more aggressive than classic type, have 46% of cases with Ki-67 index <10% [8]. Therefore, a Ki67 index of <20% may also be an intrinsic characteristic of this group of mucinous carcinomas, which is slightly different from non-specific cancers.

HER2-positive PMBCs had a higher frequency of lymph node metastasis (P=0.038), TNM stage (P<0.001), and nuclear grade (P<0.001) than the HER2-negative PMBCs. Lymph node metastasis was an important predictor of disease-specific survival, and most patients had axillary metastases that developed into distant metastases [1]. A study on HER2-positive PMBCs also showed that patients with HER2-positive tumors had a higher rate of axillary lymph node metastases (30%) than patients with HER2-negative tumors (21%) [17]. Axillary staging in surgical intervention is not suggested in MBCs due to their indolent behavior, but in the present study, HER2-positive PMBCs had a higher frequency of lymph node metastases, which was associated with unfavorable OS. Clinically, axillary stage should be assessed in patients with PMBCs. In addition, a previous study has indicated that most HER2-positive tumors were moderately or poorly differentiated, whereas over 57% of the HER2-negative tumors were well-differentiated [17]. In our cohort, the nuclear grades of HER2-positive PMBCs were predominantly high and were associated with poorer OS. Therefore, nuclear grade should also be evaluated regularly in patients with PMBCs.

Morphologically, PMBC shares features with invasive micropapillary breast carcinoma. A similar micropapillary epithelial growth pattern has been described in PMBCs and was termed invasive micropapillary mucinous carcinoma (IMMC). IMMC is an uncommon special type of MBC that accounts for 4.5%-51.6% of HER2-positive PMBCs [8, 10, 16]. In our study, we found that the micropapillary growth pattern occurred with chest wall metastasis in 1 patient (8.3%, 1/12). The biologic behavior of PMBCs with a micropapillary growth pattern is controversial, but most studies have indicated that PMBCs with a micropapillary component had a higher frequency of lymph node metastasis and more aggressive behavior than conventional

PMBCs [8-10, 28, 35]. However, some have reported that PMBCs were not significantly influenced by the presence of micropapillary features [36]. Due to the limited number of micropapillary cases in our study, a statistical analysis could not be conducted. A micropapillary pattern in mucinous carcinoma may represent a possible histogenetic association with invasive micropapillary carcinoma [21, 37]. Therefore, further analysis of a larger series of patients is required to clarify the prognostic significance of micropapillary patterns in mucinous carcinoma of the breast. It is important to recognize growth patterns and to manage them appropriately.

Overexpression of HER2 was found in 20-25% of IDCs and is associated with a poor prognosis and resistance to some chemotherapeutic agents [1, 27]. A study enrolling 7458 breast cancer patients revealed that for hormone receptor-positive breast cancers, HER2-negative patients had a higher 5-year OS rate than HER2-positive patients (90.9% vs. 84.7%) [38]. In the present study, both univariate and multivariate Cox analyses indicated that HER2 status was an independent prognostic indicator of PMBCs for OS but not DFS. However, our study is limited by the small number of cases retrieved due to the relative rarity of HER2-positive PMBCs, and thus, a comparative prognostic analysis on HER2-positive PMBCs could not be performed. Long-term follow-up data on larger cohorts are needed to investigate the outcomes of patients with HER2-positive PMBCs.

At present, studies on the targeted therapy of HER2-positive PMBCs are circumscribed. A case report by Baretta et al emphasized that the presence of a mucinous component could act as a barrier against trastuzumab in HER2-positive mucinous IDCs. The authors defined the mucinous component as >50% of the lesion [20]. However, in another study, 3 HER2-positive PMBCs showed an excellent response to trastuzumab [39]. These authors required at least 90% of the invasive component of the carcinoma to be admixed with stromal mucin for a diagnosis of PMBC, which is consistent with our criteria [40]. The contradictory responses to trastuzumab may be caused by the different criteria for PMBC. In our study, 3 HER2-positive PMBC patients received HER2-targeted therapy, and distant metastasis occurred in 1 patient. Because of the limited number of HER2-positive cases, only 3 patients received HER2-targeted therapy, and it is difficult for this study to determine whether the prognosis of HER2-positive pure mucinous carcinoma is affected by HER2-targeted therapy (treated vs. untreated). Clinically, the determination of appropriate therapeutic options for these patients requires early identification of HER2-positive status and a deeper understanding of drug resistance mechanisms to recognize their existence, grasp their characteristics and fundamentally solve the problem.

Additionally, large amounts of extracellular mucin may slow the spread of PMBC, as it may serve as a physical barrier between neoplastic cells and the surrounding stroma [4, 31]. Many studies have demonstrated that abundant extracellular mucin in PMBC contributes to the slower spread of PMBC by functioning as a physical barrier between neoplastic cells and the surrounding stroma [41, 42]. In the present study, 5 of these HER2-positive cases were type A, 5 were mixed type A and B and 2 were type B. Chest wall metastasis was found in 1 patient with mixed type A and B, bone metastasis was found in 2 patients with type A, but none was found in patients with type B. This finding indicates that PMBC type B with abundant extracellular mucin may be associated with a more desirable prognosis than other types.

To the best of our knowledge, at many other centers, HER2-positive MBCs with high nuclear grade are normally diagnosed as HER2-positive mucinous IDCs. The name of this special tumor type remains controversial. Therefore, these diagnoses describe the same tumor with different names. Currently, few related systematic studies have been performed, and the clinicopathologic features are largely unknown.

In conclusion, this study may represent the first systematic investigation of the clinicopathologic features of patients with HER2-positive PMBCs. HER2-positive PMBC is a rare subtype of breast carcinoma. HER2-positive PMBCs showed more frequent lymph node metastasis, a significantly higher clinical TNM stage and nuclear grade, lower ER and PR expression, and higher Ki67 expression than HER2-negative PMBCs. Overexpression or amplification of HER2, node metastasis, higher TNM stage and

nuclear grade were associated with poorer OS in patients with PMBCs. HER2 status was an independent prognostic indicator of OS in PMBC. Given the unfavorable prognosis and aggressive clinical features of HER2-positive PMBCs, the determination of HER2 status in PMBC should warrant special attention. Recognition and a better understanding of the growth patterns, biologic behaviors and prognosis of HER2-positive PMBC can promote a more accurate pathologic diagnosis and better patient stratification for treatment.

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Disclosure of conflict of interest

None.

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