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Multifocal and Multicentric Breast Carcinoma: A Significantly More Aggressive Tumor than Unifocal Breast Cancer

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Abstract. *Background/Aim: There are still many questions that surround multifocal or multicentric breast carcinoma (MMBC). The aim of this study was to analyze the clinico-pathological characteristics of MMBC and provide feasible suggestions for therapy. Patients and Methods: A total of 156 cases of MMBC in 3,597 invasive ductal breast carcinomas were collected and reviewed. Some factors related with prognosis such as tumor size, lymph node metastasis and others were assessed in each tumor focus, and mismatches among foci were recorded. Results: The majority of MMBC had aggregate dimensions over 2 cm (85.90%). The rate of axillary lymph node metastasis was 56.41% (88/156) compared to unifocal tumors of 33.01% (1,136/3,441). Most cases had higher Ki-67 proliferative indices (91/156). Mismatches in ER status were present in 6 cases, PR in 4 cases, proliferative index (Ki-67) in 9 cases and HER2-positive status in 2 cases. Conclusion: The larger aggregate dimension of tumor, the higher metastatic rate of axillary lymph node and the high Ki-67 proliferative index seen in most cases, suggest that MMBC is biologically more aggressive than unifocal breast cancer. In addition, every focus should be tested owing to the existence of different expressions of immunostaining between foci.*

Breast carcinoma is the most prevalent malignant disease in the world. With the gradually increasing incidence and the development of mammography, more and more foci are found to be ipsilateral, which were defined as multifocal and multicentric breast carcinoma (MMBC) (1-4). In developed countries the incidence of MMBC has been reported to be between 9-75% depending on the diagnostic criteria applied (5), while in China it is only around 2% according to a recent multicenter study (6). One of the most important reasons for this difference is the lack of uniform standardization. Some researchers defined MMBC as cancer with multiple invasive foci (of any size) separated by at least 4 mm of normal breast tissue, while more studies use the definition of "more than one focus of invasive carcinoma separated by benign tissue whether in the same or a different quadrant and regardless of the distance between the foci" (7). In addition, there are various clinical standards and mammography methods. Although great amount of work remains to be done to study the clinico-pathological features and related factors of prognosis, no agreement has been achieved. The aim of this study was to analyze the clinico-pathological features, observe the differences between different foci and provide feasible suggestions for clinical treatment.

Patients and Methods

Patients. Data from patients with the diagnosis of invasive ductal carcinoma from Yantai Yuhuangding Hospital between January 2005 and June 2016 were reviewed and 156 cases of MMBC were confirmed by two experienced pathologists. MMBC was defined as two or more invasive carcinomas separated by benign tissue whether in the same or a different quadrant and regardless of the distance between the foci. Only the cases with invasive ductal carcinoma were included and multiple lesions with different histological features in different breasts were excluded. Age, location, number of foci, size of the tumor and grade, lymph node metastasis status and expression of estrogen receptor (ER), progesterone receptor (PR), HER2, Ki67 proliferative index and E-cadherin were recorded. All breast cancer specimens were routinely analyzed by pathologists according to the World Health Organisation (WHO) (2012) guidelines.

Methods. Immunohistochemical methods were used to assess the status of ER, PR, HER2, Ki67 and E-cadherin. The ER and PR staining was further classified as 'positive' (staining of at least 1% of cells) or 'negative'. HER2 positive staining was defined according to the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines (2013) (8). Ki67 staining was labeled 'high' (in at least 20% of the cells)

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or 'low'. E-cadherin staining was classified as 'positive' (staining of at least 10% of cells) or 'negative'. In addition, all differences between different foci were recorded and compared. Moreover, the *HER2* gene was detected in each mass by fluorescence *in situ* hybridization (FISH) with HER2 immunohistochemical mismatches according to the new guidelines from the ASCO/CAP(8). All of the experiments were processed using licensed and validated reagents and methods in the same laboratory. The antibody used for HER2 immunohistochemistry was provide by Roche Group (4B5) and the experiments of FISH were accomplished using PathVysion™ HER2 DNA Probe Kit.

Results

From January 2005 to June 2016, 3,697 cases of invasive ductal breast carcinoma received surgical treatment, of which 156 cases (4.34%) were MMBC. Patients were aged from 36-80 years (average age 50.94 years, mean=53 years). One hundred and thirty six of 156 cases (87.18%) had two foci and most cases had aggregate dimensions over 2cm (85.90%, 134/156). The number and location of foci in the right and left breasts was almost equal. Invasive ductal carcinoma was present in all cases and the number of WHO grade I, II and III were 24, 78 and 54 respectively. Most cases were assessed at a higher rate of the Ki-67 proliferation index (91/156, 58.33%) and more than half of the cases (56.41%, 88/156) had axillary lymph node metastasis. In particular, 2 cases were metastatic in all lymph nodes, with 16 and 22 lymph nodes respectively (Table I). Mismatches in ER status were present in 6 cases, PR in 4 cases, proliferative index (Ki-67) in 9 cases and HER2 status in 2 cases. All cases were strongly positive for expression of E-cadherin. Mismatches in tumor grading were present in 6 cases. The majority of cases showed positive expression of ER (112/156, 71.80%) and PR (101/156, 64.74%) (Table II and Figure 1).

In addition, one case with discordant HER2 staining (larger focus 1+/smaller focus 3+) received testing for the *HER2* gene by FISH. The result demonstrated that the *HER2* gene amplification (HER2/CEP17=4.33) existed in the smaller focus (1.2*1.1cm) with negative amplification (HER2/CEP17=1.41) in the larger one (4*1.5cm) (Figure 2).

Table I. *Patient clinico-pathological characteristics.*

	n (%)
Age (mean=53; range=36-80), years	
<50	72 (46.15)
≥50	84 (53.85)
Number of foci	
2	136 (87.18)
3	20 (12.82)
Location	
Left mammary	79 (50.64)
Right mammary	77 (49.36)
Grade	
I	24 (15.38)
II	78 (50.00)
III	54 (34.62)
Axillary lymph node metastasis	
Positive	88 (56.41)
Negative	68 (43.59)
Ki-67 proliferation index	
≥20%	91 (58.33)
<20%	65 (41.67)

Table II. *Mismatches in biological features among foci of MMBC.*

	Number	Rate (%)
Mismatches in ER	6	3.85
Mismatches in PR	4	2.56
Mismatches in grading	6	3.85
Mismatches in Ki-67	9	5.77
Mismatches in HER2	2	1.28

Discussion

The presence of multiple foci of unilateral breast has long been recognized (9-11), and its significance debated (3). The reported incidence of MMBC varies widely in the literature, owing to the different methods used to detect it and the definition applied. Controversial features include the definition, the incidence, the biological characteristics, prognostic significance and the ensuing implications for therapy, particularly surgical therapy (12-16). MMBC has a reported incidence of 9-75% (5). This wide range of reported incidences also reflects the lack of a standard definition of MMBC. Many types of tumors could be seen in MMBC, including invasive ductal carcinoma, lobular neoplasia, ductal carcinoma *in situ* and others. Our study may present a few differences in some aspects compared to similar research in the same field due to the above reasons. Here, we focused on the features of invasive ductal carcinoma in MMBC due to the rarity of other types.

Tumor size is a well-recognized prognostic factor in breast cancer, but multifocality is not included as a prognostic factor in the earlier American Joint Committee on Cancer /Union for International Cancer Control (AJCC/UICC) system. The largest study to assess multifocality as a prognostic factor was reported by Joergensen *et al.* (2008) (17). A number of studies have consistently shown that multifocality is associated with a higher risk of nodal involvement than unifocal disease (12, 18, 19). The current staging system implies that each tumor which arises independently should be used to estimate the patient's prognosis, based on the size of the largest deposit (12, 18-

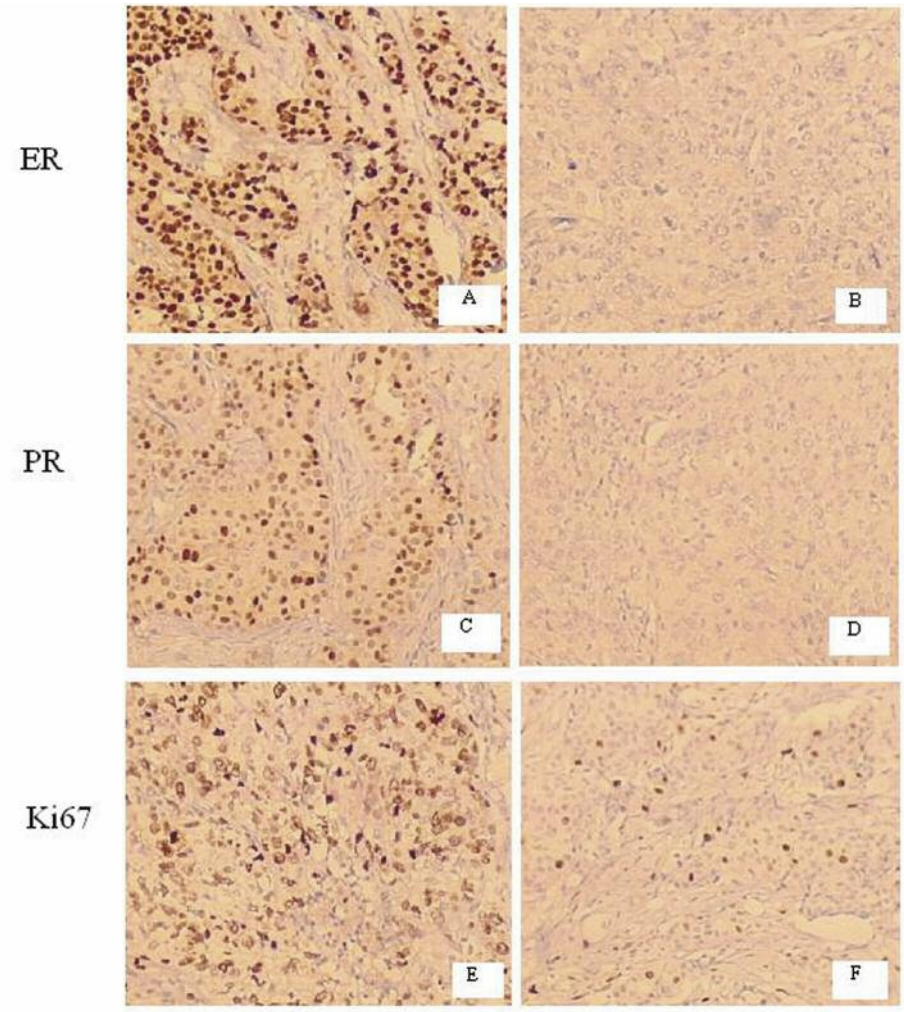

Figure 1. *Immunohistochemical demonstration of ER, PR and Ki67 expression in MMBC. The expression is different in different foci. ER, PR and Ki67 were all expressed in the cell nucleus. The expression of ER (A) is different from (B); the expression of PR (C) is different from (D); the Ki67 proliferation index (E) is different from (F).*

21). The current WHO guidelines (2012) agree with this opinion. This may be incorrect because of a higher risk of axillary nodal metastasis associated with multiple foci, which is in turn associated with a higher risk of local relapse (22-24), and a poorer prognosis (9, 21, 25). Our data showed that the size of most foci was smaller than 2 cm (the clinical stage was 58.97%, 38.46% and 2.56% respectively), which seems be inconsistent with the high metastatic lymph node rate. While the fact that more than half of the cases (85.90%, 134/156) had aggregate dimensions over 2cm (the clinical stage was 14.10%, 72.44%, 13.46% respectively) could support the aggressive behaviour of MMBC. Thus, we suggest that all foci of MMBC should be considered to estimate the risk of recurrence and metastasis. Our findings are in line with the results of Boyages *et al*. (26).

In addition, compared to unifocal tumors the higher rate of axillary nodal metastasis (56.41%:33.01%) and Ki67 proliferation index (58.33%:42.96%) (Table III) also indicated a poorer outcome of MMBC, although not enough follow-up data were obtained. Many earlier studies have suggested that MMBC has a negative impact on prognosis (27, 28), while the latest study of Neri *et al*., (2015) demonstrated that MMBC was an independent prognostic factor for breast cancer-specific survival together with higher numbers of metastatic axillary nodes, absence of estrogen receptors and high proliferative activity (29). Therefore, no matter how much the impact on prognosis was, MMBC should behave more aggressively.

Hormone receptor and HER2 status is used to identify patients suitable for adjuvant therapy and has prognostic

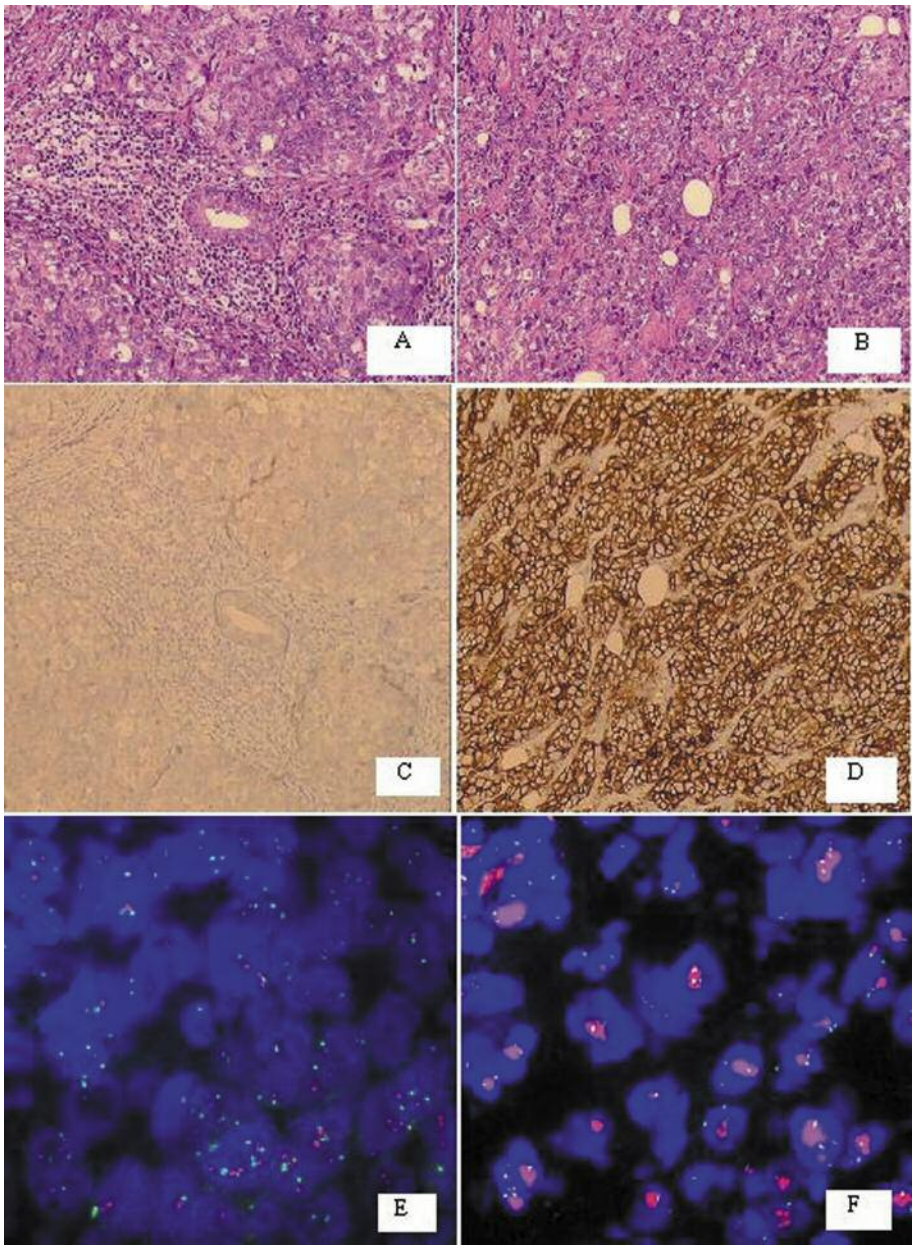

Figure 2. *Both the two foci in one case were grade III (A: the larger focus; B: the smaller focus). The lager focus showed HER2 (1+) (C) and the smaller one (3+) (D) by immunohistochemistry. HER2 testing showed negativity of the larger focus (E) and positivity of the smaller one (F) by FISH.*

implications (30). However, the variability of ER, PR and HER2 status between individual foci in MMBC has not been widely studied. According to current guidelines, ER/PR status should be assessed on the sample obtained at initial core biopsy or the main tumor foci in multifocal breast cancer (more than one distinct tumor foci in a quadrant). In our study we assessed receptor status of all individual foci in MMBC, and confirmed the variability

Table III. *Comparison between MMBC and unifocal IDC.*

	MMBC (%)	Unifocal IDC (%)
Axillary lymph node metastasis	56.41	33.01
Ki67 proliferation index over 20%	58.33	42.96
HER2(3+)	36.54	18.02
ER(–)	28.20	19.99
Tumor size over 2 cm	85.90	46.99

similarly to another study (31). To better identify how many blocks are enough for HER2 testing in cases of MMBC, Bethune *et al*., (2013), examined 246 consecutive cases by immunohistochemistry and FISH (32). Their results suggest evaluating HER2 on the largest focus is enough unless the smaller focus was either a higher grade or histologically different. However, we found an exception in our cases. There were two lesions in the upper outer quadrant of the left breast of a 52-year-old female. Both lesions were invasive ductal carcinoma grade III according to WHO guidelines (2012) and ER/PR were all negative. However, HER2 was 1+ on the lager focus (4x1.5cm) and 3+ on the smaller one (1.2 x 1.1cm). Further study by FISH showed the compatible results with immunohistochemistry. The HER2/CEP17 ratio was 1.41 on the larger and 4.33 on the smaller focus. It is clear that the larger should be considered as negative, while the smaller was positive according to the current ASCO/CAP guidelines (2013) (6). Therefore, some positive cases may be missed if detecting the largest focus only. We suggest that all individual foci should be tested in MMBC cases for better therapy and prognosis. The reasons most possible to explain the mismatch between foci were heterogeneity (31) or different clonality among tumor foci (33). However, testing every focus of MMBC to assess the gene abnormality and the difference of clonality does not seem to be an efficient approach, so before a new guideline concerning MMBC is published, immunohistochemistry is still the best way to identify HER2 status for further treatment, considering the experimental and economic level, especially in developing countries.

E-cadherin is an important cell adhesion molecule. Much research has demonstrated that down-regulation of E-cadherin is associated with breast cancer progression, poorer outcome and resistance to therapy (34, 35). Weissenbacher *et al*., (2013), stated that E-cadherin expression was significantly lower in MMBC compared to the unifocal group and suggested the down-regulation of E-cadherin in MMBC was causally connected with a worse prognosis (36). While our results showed that E-cadherin showed strong positive staining in all foci of MMBC, no down-regulation was found. Therefore, more research is necessary to elucidate the molecular mechanisms of MMBC.

Although we failed to collect the follow-up data and the tumor size was usually smaller than 2 cm, the larger aggregate dimension, higher rate of lymph node metastasis and Ki67 proliferative index and higher ER-negative rate than unifocal breast cancer suggests a more aggressive behaviour of MMBC. The variability of ER, PR and HER2 status support the idea that all individual foci should be tested in MMBC cases in order to provide the best therapy and prognosis.

Conflicts of Interest

The Authors have no conflicting or competing interests.

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