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Diagnostic diversity and heterogeneity of tumors: a real-world study of metastasis re-biopsy in advanced breast cancer

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Abstract

Background: Re-biopsy of metastasis in advanced breast cancer (ABC) has become an international convention to assist the diagnosis and evaluation of tumor heterogeneity. This study aimed to detect diagnostic diversity and inconsistencies among estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression levels between primary and metastatic lesions.

Methods: We conducted a retrospective analysis of 1670 cases of ABC patients who had undergone at least one lesion re-biopsy from January 2010 to December 2018. The pathological diagnosis of biopsies, distribution of biopsy sites, and severe puncture complications at each site were collected. In addition, the inconsistency rates and related factors of ER, PR, and HER2 expression between primary and metastatic lesions were analyzed fully considering patients' demographic profiles and disease characteristics.

Results: In total, 1670 cases of breast cancer (BC) patients diagnosed by pathology underwent one to four biopsies of recurrences or metastases in different sites or at different stages during the rescue treatment, producing 2019 histopathological specimens which were analyzed in the study. Pathological diagnosis showed that eight patients had benign pathological diagnoses, 11 patients had second primary malignant tumors but without recurrences of breast cancer, and 17 patients had pathologically confirmed breast cancer recurrences combined with second primary cancer. In 1173 patients who presented ER, PR, and HER2 expressions in primary and metastatic lesions, the inconsistency rates of ER, PR, and HER2 were 17.5% (205/1173), 31.3% (367/1173), and 13.9% (163/1173), respectively. The multivariate analysis showed that the age at the onset of breast cancer or adjuvant endocrine therapy was an independent factor affecting changes in PR expression level. Except one liver puncture with local hemorrhage and two lung punctures with hemopneumothorax, no other severe puncture complications occurred in 1950 non-surgical rebiopsies.

Conclusions: The pathological diagnosis of metastasis re-biopsy of ABC was diverse, and the ER, PR, and HER2 expression levels were inconsistent between primary and metastatic lesions. Therefore, more attention should be paid to perform biopsies of relapsed and metastatic breast cancers routinely in clinical practice.

Keywords: Biopsy; Breast cancer; Estrogen receptor; Human epidermal growth factor receptor 2; Progesterone receptor

Introduction

Breast cancer is one of the most common malignant tumors in women.[1] With the continuous improvement of diagnosis and treatment technologies as well as the comprehensive application of systemic therapies, the recurrence and mortality rates of breast cancer have gradually decreased,[2] although recurrences and metastases still occur in some patients. Metastatic breast cancer is still an incurable disease, and its systemic treatment is relatively complicated. There is still a lack of effective treatment methods, especially for metastatic breast cancer after first-line failure.

Breast cancer is a heterogeneous tumor characterized by differences in the expression levels of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), which are considered key biomarkers for clinical decision-making and prognosis. Pathological confirmation of new lesions is still the gold standard for the diagnosis of metastatic breast cancer. Pathological evaluation of new lesions can be applied to several aspects including identification of the benign and malignant tumors, determination of the source of new malignant tumors, and re-evaluation of the ER, PR, and HER2 status of metastases. A number of studies[3-9] have shown that the expressions of ER, PR,

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and HER2 in metastases and primary tumors are inconsistent, and may be related to differences in prognosis. Re-biopsy of metastasis is now recommended internationally, although there is no consensus on the optimal puncture site. A study showed the heterogeneity in ER, PR and HER2 expressions for primary and metastatic breast cancer is more common in patients with distant metastases than in patients with local recurrences and metastases.[8] Visceral metastasis (eg, liver and lung) is common in advanced breast cancer (ABC) patients, but the safety and feasibility of biopsy of these sites and the success rates of biopsy of metastatic lesions are rarely reported.[9] In this study, we retrospectively analyzed the metastatic biopsy sites, pathological details, severe puncture complications, and ER, PR, HER2 information of ABC patients.

Methods

Ethical approval

The study was approved by the medical Ethics Committee of Henan Cancer Hospital (No. 2107407) and informed consent was obtained from all the patients.

Eligibility

We screened all patients with ABC who underwent at least one metastasis biopsy at our department from January 2010 to December 2018. The following inclusion criteria were applied: (1) Breast cancer was confirmed by pathology in our hospital or other hospitals; (2) Both pathology and imaging information were available for those diagnosed as metastatic breast cancer; (3) Information on ER, PR, and HER2 expression levels in primary and metastatic lesions were available.

Data source

A total of 1670 cases were collected, including demographic characteristics, previous treatment information, etc. Pathological biopsy of metastasis was performed according to the requirements for diagnosis and treatment. The re-biopsy of the lesion was conducted according to the following principles: during or before the rescue treatment, the biopsy sites were selected according to factors such as the organs involved, the size and location of the lesion, and the response to treatment. The methods used to obtain tissue samples varied according to the organs in which the lesions were located. Samples of the liver, superficial lymph nodes, and other superficial lesions were obtained mainly by ultrasound-guided core needle biopsy; samples of lung and deep tissues were obtained mainly by computed tomography (CT)-guided cored needle biopsy; skin, chest wall, ovary, bone, or brain samples obtained mainly by surgical biopsy; samples of cavity organs were obtained by endoscopic biopsy. All patients provided written informed consents to the re-biopsy operation [Figure 1].

In the above data source collection, every one of the 1670 patients, whose median age is 46 (27–82) years, had experienced one to four biopsies of recurrences or
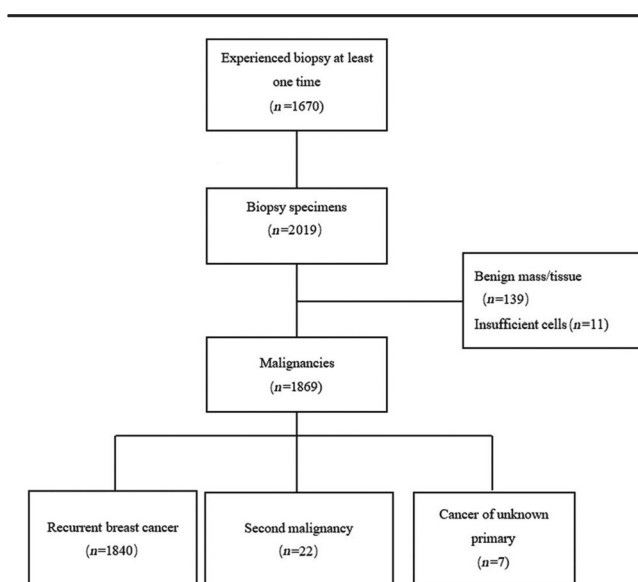

Figure 1: The flow chart of the cases enrolled in this study. There were 1670 cases of advanced breast cancer who underwent at least one metastasis biopsy at our department from January 2010 to December 2018.

metastases in different sites and at different stages during the rescue treatment stage [Table 1]. Among these patients, 1368 (81.9%) received one biopsy, 260 (15.6%) received two biopsies, 37 (2.2%) received three biopsies, and five (0.3%) received four biopsies. A total of 689 patients (41.3%) had previously received salvage treatment, while 981 patients (58.7%) did not receive any treatment for the metastatic breast cancer.

Observation index

We conducted a retrospective analysis of the pathological diagnosis of metastatic breast cancer in our department to observe the metastatic biopsy sites, pathological details, severe puncture complications, second primary malignant tumors, and discordance in ER, PR, or HER2 between the primary and the metastatic biopsy. Besides, we analyzed inconsistencies in ER, PR, and HER2 expression levels between primary and metastatic lesions by etiologic factors. ER/PR/HER2 were categorized as positive (+) or negative (−). The ER/PR/HER2 inconsistencies, defined as the proportion of + to − or − to + occurring between metastatic and primary lesions, were assessed.

Statistical analysis

We leveraged the software SPSS 19.0 (IBM Corp., Armonk, NY, USA) for the statistical analysis, specifically, descriptive statistical analysis. The chi-squared test was used for the inconsistency rate comparison among ER, PR, and HER2 expression levels. The binary logistic regression model was adopted for multivariate analysis with the level of significance (α) was 0.05 (bilateral).

Results

Puncture site

A total of 1670 patients underwent at least one biopsy and obtained pathologic results. Thus, 2019 biopsy specimens

Table 1: Information about the 2019 biopsy specimens from 1670 advanced breast cancer patients underwent at least one biopsy.

Factors	N (%)
Biopsy method	
Ultrosound-guided	1333 (66.0)
Surgical biopsy	469 (23.2)
CT-guided	177 (8.8)
Endoscopic biopsy	19 (0.9)
Bone marrow biopsy	9 (0.4)
Unknown	12 (0.6)
Biopsy hospital	
Our hospital[*]	1926 (95.4)
Other hospitals	93 (5.6)
Biopsy site	
Liver	597 (29.6)
Lymph nodes	572 (28.3)
Chest wall	422 (20.9)
Lung	182 (9.0)
Breast	130 (6.4)
Skin and soft tissue	46 (2.3)
Bone and adjacent tissue	17 (0.8)
Pelvic masses	14 (0.7)
Bone marrow	9 (0.4)
Stomach	4 (0.2)
Colon	4 (0.2)
Thyroid	3 (0.1)
Pleura	2 (0.1)
Brain	2 (0.1)
Adrenal gland	2 (0.1)
Nasopharyngeal	2 (0.1)
Kidney	1 (<0.1)
Abdominal wall	1 (<0.1)
Parotid gland	1 (<0.1)
Pericardium	1 (<0.1)
Cervix	1 (<0.1)
Submandibular gland	1 (<0.1)
Vocal cord	1 (<0.1)
Vulva	1 (<0.1)
Endometrium	1 (<0.1)
Bladder	1 (<0.1)
Rectus femoris	1 (<0.1)

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were obtained. The sources of the 2019 biopsy tissue samples were: liver (597 cases, 29.6%), lymph nodes (572 cases, 28.3%), chest wall (422 cases, 20.9%), lung (182 cases, 9.0%), breast (130 cases, 6.4%), bone and adjacent tissues (17 cases, 0.8%), skin and soft tissues (46 cases, 2.3%), pelvic masses (14 cases, 0.7%), and other sites (39 cases, 2.0%), comprising kidney (one case), adrenal gland (two cases), parotid gland (one case), bone marrow (nine cases), brain (two cases), pleura (two cases), pericardium (onecase), nasopharynx (two cases), submandibular gland (one case), stomach (four cases), colon (four cases), thyroid (three cases), vocal cords (one case), bladder (one case), vulva (one case), abdominal wall (one case), cervix (one case), endometrium (one case), and rectus femoris (one case) [Table 1].

Pathological diagnosis

A total of 2019 tissue specimens were pathologically diagnosed, of which 1869 (91.1%) were diagnosed as malignant tumors, 139 (6.9%) were diagnosed as benign tumors or normal tissues, and 11 (0.5%) could not be diagnosed owing to insufficient tissue sampling. Among the malignant tissue samples, 1840 were diagnosed as breast cancer metastasis and 29 were diagnosed as non-breast cancer metastasis, specifically speaking, 18 lung malignant tumors (5 adenocarcinoma, 3 squamous cell carcinoma, 3 leiomyosarcoma, 1 phyllodes malignant tumor, 1 small cell carcinoma, and 5 carcinoma of unknown origin), 3 ovarian cancer specimens, 1 gastric adenocarcinoma, 1 gastrointestinal stromal tumor, 1 chest wall sarcoma, 1 liver sarcoma, 1 renal carcinoma, 1 melanoma, 1 vulva and 1 bone marrow carcinoma of unknown primary.

In total, 1840 specimens were pathologically diagnosed as breast cancer metastasis. The common metastatic sites included liver, lung, lymph nodes, chest wall, skin and other soft tissues, pelvic masses, bone and parabone tissue, with composition rates of 95.6%, 78.0%, 93.5%, 91.7%, 82.2%, 78.6%, and 100%, respectively. Among them, the second primary malignant tumors in the lung and the pelvic mass were diagnosed as primary ovarian cancer, with composition rates of 9.9% and 21.4%, respectively. The rare metastatic sites included the adrenal gland, pleura, pericardium, thyroid, parotid gland, and abdominal wall. Breast cancer metastases were also found in the bone marrow and colon biopsies. The second primary malignant tumors occurred in lung, ovary, kidney, stomach, bone marrow, and vulvar, while no malignant cells were found in nasopharynx, submandibular gland, vocal cords, bladder, cervix, endometrium, or rectus femoris [Figures 2 and 3].

The treatment of second primary malignant tumor

Twenty-nine tissue samples with pathological diagnosis of second primary malignant tumor were obtained from 28 patients, one of whom had two kinds of second primary cancers of vulva and lung simultaneously. Among these 28 patients, 11 patients had no progress in breast cancer, so the adjuvant treatment of breast cancer was not changed. While other 17 patients were diagnosed as metastatic breast cancer, which were treated through multidisciplinary discussion to avoid delaying the treatment of the second primary malignant tumor.

Inconsistency rate of ER, PR, and HER2 expression levels

The data of ER, PR, and HER2 expression levels in primary and metastatic lesions of 1173 patients with confirmed ABC were collected. Information on ER, PR, or HER2 expression levels in primary and metastatic lesions were collected from 1173, 1170, and 1074 patients, respectively. Compared with the primary lesion status, the changes in ER, PR, and HER2 expression levels in the metastatic lesion were observed in 205, 366, and 149 patients, respectively. The inconsistency rates were 17.5% (205/1173), 31.3% (366/1170), and 13.9% (149/1074),

respectively ($P < 0.0001$); 10.6% (124/1173) and 6.9% (81/1173) of the patients showed a loss and gain of ER expression, respectively ($P = 0.002$); 22.1% (259/ 1170) and 9.1% (107/1170) of the patients showed a loss and gain of PR expression, respectively ($P < 0.0001$); 3.8% (41/1074) and 10.1% (108/1047) of the patients showed a loss and gain of HER2 expression, respectively [Table 2].

Univariate analysis showed that the changes in PR expression correlated with the duration of disease-free survival (DFS) as well as the use of adjuvant chemotherapy or adjuvant endocrine therapy ($P < 0.05$). The inconsistency rate of PR expression was higher in patients who had received chemotherapy or radiotherapy, or had shorter DFS duration. The changes in the HER2 expression level correlated with the use of adjuvant chemotherapy, adjuvant endocrine therapy, and the puncture site in the metastatic lesion ($P < 0.05$). No factors were identified to correlate with changes in the ER expression level [Table 3]. Binary logistic regression multivariate analysis showed that the age at breast cancer onset and the use of adjuvant endocrine therapy were the factors affecting PR expression ($P < 0.05$), while no factors were found to correlate with the changes in ER and HER2 expression levels ($P > 0.05$) [Table 4].

Safety

In total, 1950 non-surgical specimens were obtained in our hospital, with a few serious adverse events reported as follows: ultrasound-guided needle biopsy of the liver mass resulted in one case of local liver hemorrhage; CT-guided biopsy of the lung mass caused two cases of mild pneumothorax. Symptoms improved after symptomatic
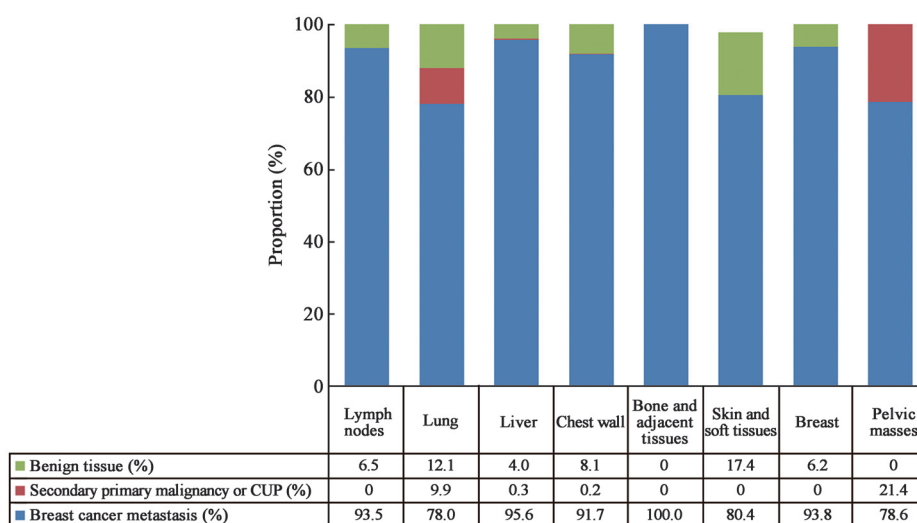

	Lymph nodes	Lung	Liver	Chest wall	Bone and adjacent tissues	Skin and soft tissues	Breast	Pelvic masses
Benign tissue (%)	6.5	12.1	4.0	8.1	0	17.4	6.2	0
Secondary primary malignancy or CUP (%)	0	9.9	0.3	0.2	0	0	0	21.4
Breast cancer metastasis (%)	93.5	78.0	95.6	91.7	100.0	80.4	93.8	78.6

Figure 2: The proportion of benign tissue, secondary primary malignancy or cancer of unknown primary and breast cancer metastasis in common biopsy sites.
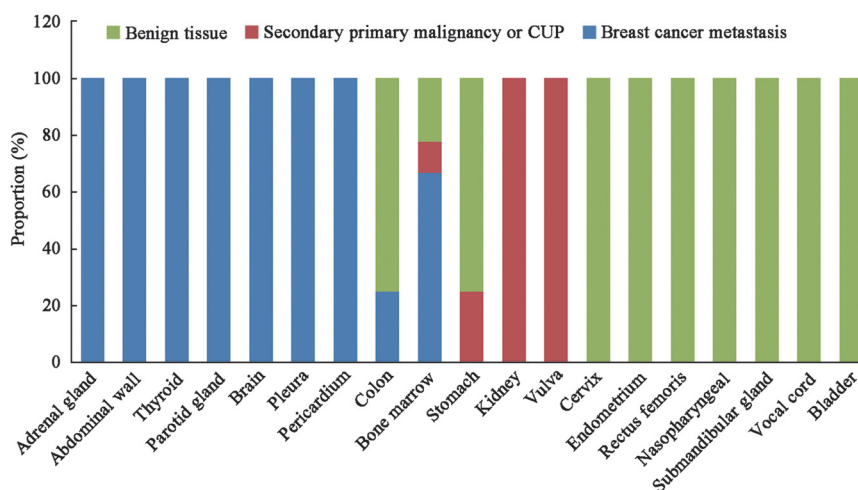

Figure 3: The proportion of benign tissue, secondary primary malignancy or cancer of unknown primary and breast cancer metastasis.

Table 2: Changes in ER, PR, and HER2 between the original pathology report of the primary tumor and metastasis.

| | Metastasis | | | | | |
| | ER | | PR | | HER2 | |
Primary tumor	+	−	+	−	+	−
+	550 (51.3)	124 (10.6)	358 (30.6)	259 (22.1)	310 (28.9)	41 (3.8)
−	81 (6.9)	432 (35.6)	107 (9.1)	446 (38.1)	108 (10.1)	615 (57.3)
Inconsistency	205 (17.5)		366 (31.3)		149 (13.9)	

Data are shown as n (%). ER: Estrogen receptor; HER2: Human epidermal growth factor receptor 2; PR: Progesterone receptor.

Table 3: Univariate analysis of ER/PR/HER2 inconsistency between primary and metastatic lesions.

Factors	ER inconsistency rate (%)	χ^2	P	PR inconsistency rate (%)	χ^2	P	HER2 inconsistency rate (%)	χ^2	P
Age (years)		0.696	0.404		4.677	0.031		1.962	0.161
≤ 35	19.7			38.2			17.4		
> 35	17.1			30.0			13.3		
Adjuvant chemotherapy		1.954	0.162		4.295	0.038		0.062	0.803
Yes	18.0			30.6			14.0		
No	12.8			21.4			13.1		
Chemotherapeutic drugs		0.413	0.521		0.110	0.740		9.687	0.002
Anthracyclines only	18.7			33.1			24.2		
Taxanes and anthracyclines	17.0			32.0			11.6		
Adjuvant endocrine therapy		3.561	0.059		38.800	<0.001		4.680	0.031
Yes	19.5			39.5			16.1		
No	15.3			22.6			11.6		
Endocrine drugs		1.819	0.177		0.244	0.636		0.103	0.748
Tamoxifen	18.6			40.9			15.9		
AI	24.7			38.2			17.2		
Adjuvant radiotherapy		0.093	0.760		4.001	0.045		0.073	0.788
Yes	17.9			34.2			13.6		
No	17.2			28.7			14.2		
Source of metastatic foci		0.542	0.461		3.334	0.068		7.713	0.005
Viscera	18.4			34.1			12.6		
Non visceral	16.8			29.1			18.9		
DFS (years)		0.172	0.678		5.207	0.027		3.187	0.075
≤2	17.9			28.3			12.1		
>2	17.0			34.5			15.9		

AI: Aromatase inhibitors; DFS: Disease free survival; ER: Estrogen receptor; HER2: Human epidermal growth factor receptor 2; PR: Progesterone receptor.

treatment. No serious puncture or surgical complications occurred for any other patients.

Discussion

Breast cancer is a heterogeneous disease, and stratification of tumors is paramount to achieve better clinical outcomes.[10] Tumor heterogeneity refers to the existence of subpopulations of cells within a primary tumor and its metastases that have distinct genotypes and phenotypes, and leads to different biological behaviors.[11] Previous small-sample studies have confirmed that re-biopsy of breast cancer recurrence and metastasis can confirm its pathological diagnosis, exclude the presence of a second primary tumor, clarify changes in the hormone receptor

and HER2 status of the tumor recurrence and metastasis, and serve as a reference for guiding the treatment plan.[12]

In this retrospective study, we conducted the re-biopsy of metastases and detected 19 cases where recurrences in breast cancer were ruled out, and 28 cases where the second primary tumors, including 18 cases lung cancer and three cases ovarian cancer. Therefore, solitary nodules should undergo pathological examination whenever possible to confirm their diagnosis, especially for lung or pelvic masses, to avoid misdiagnosis and inappropriate treatment. A meta-analysis showed that breast cancer patients have a 17% higher risk of other malignancies compared with the other population.[13] As the survival time of breast cancer patients increases, the risk of recurrence of a second primary

Table 4: Binary logistic regression analysis of inconsistency between PR and HER2.

	PR			HER2		
Factor	OR	95% CI	*P* value	OR	95% CI	*P* value
Age ≤ 35 years	0.704	0.502–0.988	0.042	1.394	0.886–2.194	0.151
Adjuvant radiotherapy	0.859	0.296–2.493	0.780	2.068	0.262–16.330	0.491
DFS	0.950	0.726–1.243	0.708	0.814	0.560–1.183	0.280
Adjuvant chemotherapy	0.803	0.487–1.322	0.388	0.875	0.461–1.658	0.681
Accessory endocrine	0.485	0.367–0.640	<0.001	0.681	0.463–1.002	0.051
Puncture site	0.861	0.666–1.113	0.763	1.277	0.889–1.833	0.185

CI: Confidence interval; DFS: Disease-free survival; HER2: Human epidermal growth factor receptor 2; OR: Odds ratio; PR: Progesterone receptor.

malignant tumor also increases.[14,15] A study showed that thyroid cancer was the most common second primary cancer in breast cancer patients.[16] However, in our study, we underestimated the proportion of primary thyroid cancer because it was reported be not related to breast cancer prognosis.[16] Therefore, we did not perform biopsies on all patients with thyroid masses.

The present study once again confirmed the inconsistency in ER, PR, and HER2 expression levels between primary and metastatic lesions, with the inconsistency rates of 17.5%, 31.3%, and 13.9%, respectively, which were in good agreement with the aforementioned results.[3-9] The differences in ER, PR, and HER2 expression levels between primary and metastatic lesions might correlate with the heterogeneity of tumor tissues, clonal selection of tumor cells, tissue fixation, antigen repair, differences in staining methods, subjective judgment of technicians in the staining results, tumor microenvironment, previous treatments, and so forth. In this study, 48.0% of the results pertaining to the primary lesion were reported by the department of pathology in other hospitals, while the majority of results pertaining to metastatic lesions were reported by the department of pathology of the study hospital. Thus, the fact that the results of ER, PR, and HER2 expression in primary and metastatic lesions were reported by different laboratories might pose certain biases on the conclusions of this study. It is to be reminded that the results of the primary tumor and metastasis detection interval in this study are long, the result of the inconsistency of ER, PR, and HER2 is caused by long-term clonal selection of tumor cells, or because of different periods of detection errors, still unsure as of now.

Previous studies on the inconsistencies in ER, PR, and HER2 expression levels and the contributing factors are not conclusive. One study suggested that previous anthracycline-based chemotherapy rendered ER expression more susceptible to change.[6] Another study in Turkey showed that endocrine therapy after chemotherapy led to a higher rate of change in the PR expression level.[7] However, a related published study found that changes in ER, PR, and HER2 expression levels showed no correlation with previous chemotherapy, endocrine therapy, or radiotherapy.[8] This study revealed that young patients (aged ≤35 years) or patients who received adjuvant endocrine therapies tended to have higher change rates of PR expression level. This study found that no factors correlated with the changes in ER or HER2

expression levels, consistent with previous studies. Nonetheless, given that these studies were retrospective in nature, the aforementioned results need further validation.

Meanwhile, some studies suggested[4,8,17] that temporal and spatial heterogeneity between the primary tumor and the metastasis resulted in different inconsistency rates of ER, PR, and HER2 in different metastatic sites, especially in the patients with distant metastasis. In this study, we obtained about 38.6% of the tissue samples from the liver or lung, but we did not find significant differences in the inconsistency rates of ER, PR, and HER2 in different metastatic sites. So, further prospective studies are required to clarify differences in the ER, PR, and HER2 status in different metastatic sites. Fortunately, no significant complications were found in the puncture of visceral metastases, so visceral puncture is safe and feasible and can be widely performed in clinical practice.

The study has some limitations. It is retrospective in design and there is no standard operating procedure for metastasis biopsy. There were selective biases in patient compliance, biopsy site determination, and biopsy method. The proportion of adverse events about biopsy is underestimated since not all adverse events of the biopsy were recorded. In addition, there is a lack of follow-up information for all patients diagnosed with second primary cancer because they were transferred to other departments for treatment.

Although a non-invasive liquid biopsy can characterize massive sequencing of circulating cell-free tumor DNA released from cancer cells into plasma, genomic alterations in solid cancers,[18-21] a study suggests that in the setting of previously treated, advanced pancreatic ductal adenocar cinoma, liquid biopsies are not yet an adequate substitute for tissue biopsies.[22] In addition, tissue biopsy will help us to establish biobanks of biopsy material taken from metastatic sites, which is important for exploring mechanisms of metastases.[23]

In summary, this study confirmed the pathologically diagnostic diversity and heterogeneity in the expression of ER, PR, and HER2 in primary and metastatic lesions by re-biopsy of the metastases, especially in patients who are young or have previously received endocrine therapy. The existence of the second primary cancer in breast cancer patients reminds clinicians to pay attention to the differential diagnosis of ABC. At the same time, our

findings reconfirmed the feasibility and safety of metastatic re-biopsy in clinical practice, including in visceral metastases. Therefore, the suspicious metastatic lesion required a biopsy to guide doctors in treatment planning and prognostic evaluation for metastatic breast cancer.

Acknowledgments

Conflicts of interest

None.

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