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ORIGINAL ARTICLE

Benefits of using the cell block method to determine the discordance of the HR/HER2 expression in patients with metastatic breast cancer

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Abstract
Background The discordance of the hormone receptor (HR) and human epidermal growth factor receptor 2 (HER2) expressions between primary cancer and metastatic lesions is an important issue when selecting the optimal treatments for patients with metastatic breast cancer. A rebiopsy for the metastatic cancer is recommended before selecting the treatment; however, it is not easy to take a tissue sample for all metastatic lesions. Fine needle aspiration cytology (FNA) for regional lymph nodes and aspiration for pleural effusions or ascites are less invasive procedures to obtain the necessary samples to examine the HR/HER2 expression. These cytologic materials are able to be stained as a tissue sample using the cell block method.
Patients We examined the HR/HER2 expression of 20 patients with breast cancer (8 with synchronous metastases and 12 with metachronous metastases) using the cell block method. Among 8 patients with synchronous metastases, 7 patients with axillary lymph node (LN) metastasis were examined by fine needle aspiration (FNA), and one patient with pleural metastases was analyzed for the aspirated fluid. While in 12 patients with metachronous metastases, 7 patients were examined for their pleural effusion, 3 patients were examined for regional lymph node metastases, and 1 patient were examined for aspirated ascites. We compared the HR/HER expression between primary cancer and metastatic lesion in 17 patients (5 cases of 8 synchronous metastases, and all of 12 metachronous metastases).
Results Discordance of HR was seen in 4 of 17 patients (24 %). Three cases with axillary LN metastasis (2 cases with synchronous metastases and one with metachronous metastasis) showed negative change of ER. Negative change of HER2 expression was seen in one patient with ascites caused by peritoneal dissemination.
Conclusions Cytology materials are easily obtained by FNA for LN metastases and aspiration for malignant effusions and analyzed for HR/HER2 expression using cell block method. We should take advantage of cell block analysis to determine the discordance of the HR/HER2 expression to select the optimal treatment for metastatic breast cancer.

Keywords Metastatic breast cancer · Hormone receptor and HER2 expression · Cell block · Discordance

Introduction

Comprehensive gene expression analysis has been able to characterize various types of breast cancer resulting in the establishment of five intrinsic subtypes [1]. Based on the intrinsic subtype, recurrent site, time to recurrence, and survival rates may vary [2], accordingly, therapeutic options for the subtypes are also diverse. In the present practice, breast cancer is divided into 4 subtypes by immunohistochemical analysis (IHC) for estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor 2 (HER2), and the labeling index of Ki67 [3].

The HR/HER2 status of metastatic lesions is also an important factor in order to select adequate treatments. However, the discordance of HR/HER2 expression

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between primary cancer and metastatic lesions has been an important issue in selecting adequate treatments for patients with metastatic breast cancer. Meta-analysis of 48 articles indicated pooled discordance of proportions were 20 % for ER, 33 % for PR, and 8 % for HER2 [4].

A rebiopsy for the metastatic sites is recommended before treatment, but it is not an easy procedure to perform for all metastatic lesions [5]. In our experience, 24 % (63/265) of metastatic lesion was diagnosed on biopsy specimens, while almost same number; 22 % (58/265) of metastatic lesion was diagnosed on cytology by fine needle aspiration (FNA) and aspiration for malignant effusions.

These procedures of cytology are less invasive and convenient to examine the HR/HER2 status [6], and cytology materials are able to be stained as a tissue block using the cell block method [7].

We herein present the benefits of using the cell block method to analyze the discordance of HR/HER2 expression in metastatic lesions.

Patients and methods

Patients

We examined the HR/HER2 expression of 20 patients with breast cancer (8 cases with synchronous metastases and 12 cases with metachronous metastases) using the cell block method.

Among 8 patients with synchronous metastases, 7 patients with axillary lymph node (LN) metastasis were examined by fine needle aspiration (FNA), and one patient with pleural metastases was analyzed for the aspirated fluid. While in 12 patients with metachronous metastases, 8 patients were examined for their pleural effusion, 3 patients were examined for regional lymph node metastases, and 1 patient was examined for aspirated ascites.

Cell block method

Smears of FNA specimens and malignant effusions were prepared using Carbowax coated slides. These were fixed immediately in 95 % ethanol, and Papanicolaou stained to confirm the existence of malignant cells.

Cytologic specimens containing malignant cells were prepared to make cell blocks, using Hold Gel 110 (Asia Kizai Co. Ltd.), according to the manufacture's instructions. The FNA specimens suspended in 10 % buffered formalin and aspirated fluid were centrifuged at 2500 rpm. Then the supernatant was discarded, and specimens were resuspended in 10 % buffered formalin. These were centrifuged again, supernatant was removed, and the samples were eosin stained. After dehydration with ethanol, hold gel 110 and methanol were added to the sediment to create the cell block. The cell block was subsequently embedded in paraffin. These cell blocks were utilized similarly to tissue samples, and immnohistochemical staining was performed using similar procedures.

Determination of HR/HER expression

The ER and PR expression levels were scored according to the Allred score [8] (a score of 3+ or greater indicated positivity). Some cases containing an insufficient number of malignant cells were judged to be either positive or negative instead of the Allred score. While the HER2 expression was determined by the ASCO/CAP guideline (a score of 3+ indicated positivity and 2+ was equivocal) [5]. For the equivocal cases, fluorescent in situ hybridization (FISH) for the HER2 amplification gene was applied.

The staining of the cell block was compared with the tissue sample in 5 cases.

Discordance between the metastatic site and primary cancer

We compared the HR/HER expression between primary cancer and metastatic lesion. in 17 patients (6 cases with synchronous axillary LN metastases, all with metachronous metastases). The remaining 3 cases were not analyzed for primary breast cancer; 2 cases suffered from occult breast cancer, and primary breast cancer of one case was distinguished by neoadjuvant chemotherapy.

Results

Validation of the cell block method to judge the HR/HER2 expression

Case 4 with synchronous metastases showed the concordant expression of HR/HER2 in both primary breast cancer and axillary LN metastases (Fig. 1). The strong expression of HR/HER2 was confirmed in the cell block sample for axillary metastasis similar to the tissue sample for primary cancer.

Next, case 7 with metachronous metastases indicated the concordant expression of both HR and HER2 (Fig. 2) in pleural metastases. Immunohistochemical staining for HR of the pleural effusion was satisfactory as well as in other cases. While HER2 expression of malignant effusion was detected in this case only. Therefore, we performed the FISH analysis to confirm the HER2 expression, resulting positive expression of HER2, (HER2/CEP17 ratio; 4.6). This case indicated the validity of analyzing the HER2

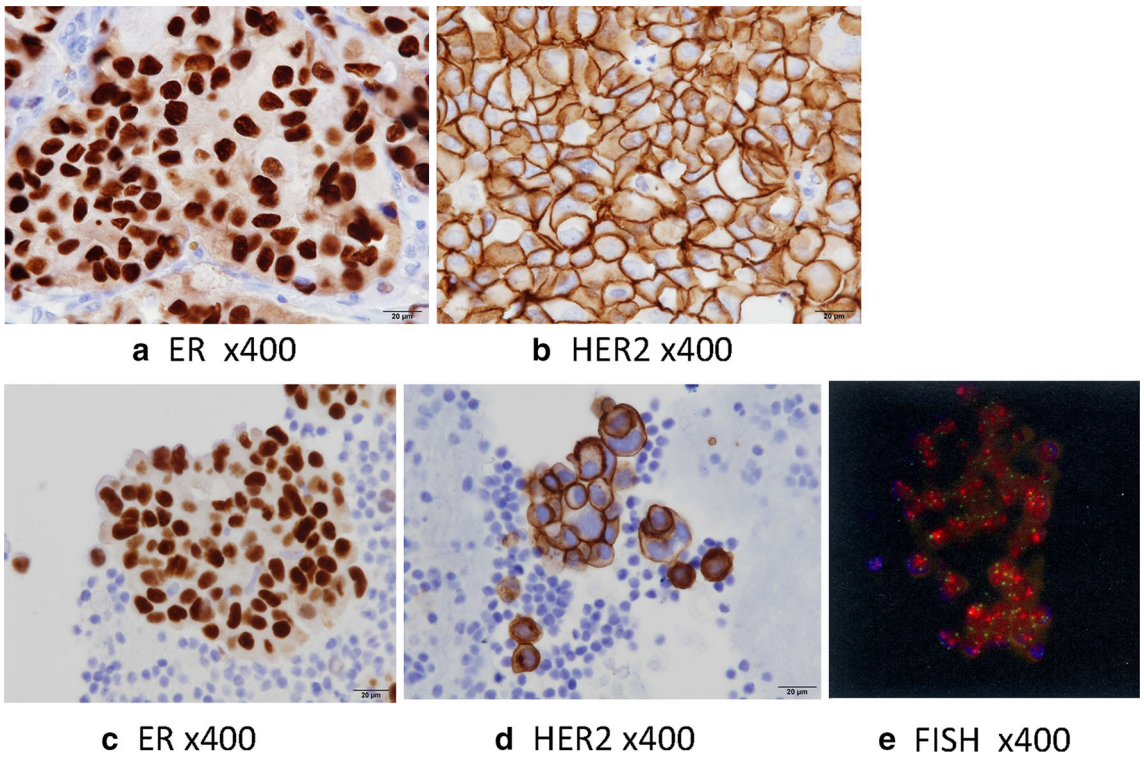

Fig. 1 Case 4; 65-year-old woman with synchronous metastasis of axillary LN. Both of biopsied tissue for primary cancer (**a**, **b**) and the cell block sample (**c**, **d**) showed the HR/HER2 expression. ER and HER2 expression was judged 8 of the Allred score, and 3+ of the ASCO/CAP criteria, respectively

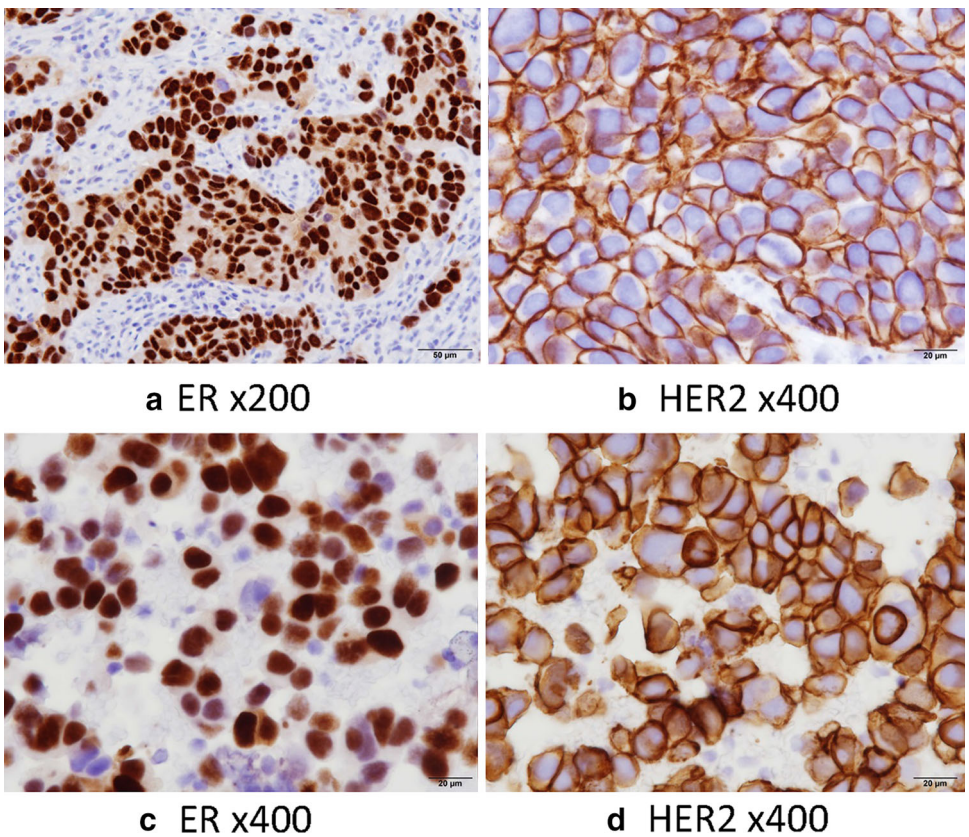

Fig. 2 Case 7; 87 year-old woman with metachronous metastasis of pleural effusion. Both of primary breast cancer (**a**, **b**) and malignant cell (**c**, **d**) exhibit the positive staining of ER and Her2. The HER2 expression of malignant cell of pleural effusion was confirmed by FISH (HER2/CEP17 ratio; 4.6) as shown at (**e**)

expression for malignant effusion using the cell block method.

Furthermore, we compared the immunohistochemical staining between the cell blocks and tissue specimens on same metastatic site in 5 cases. (cases 1, 3, 7 with synchronous metastases and cases 9, 11 with metachronous metastases) (Fig. 3). The judgment of the HR/HER2 status of both samples was the same. However, if the number of cells was insufficient to determine the Allred score for HR, we judged the sample to be positive or negative for HR expression.

Furthermore, we also experienced cases that demonstrated negative staining for HR/HER2, in which it was sometimes difficult to diagnose the existence of malignant cells.

Breast cancer patients with synchronous metastasis

The comparison of the HR/HER2 expression between primary breast cancer and metastatic lesions was performed in 5 of 7 patients with axillary lymph node (Ax LN) metastasis (Table 1). Two patients with Ax LN metastasis and one patient with pleural effusion were not evaluated for the primary sites. In case 2, primary cancer was distinguished after neoadjuvant chemotherapy. Cases 6 and 8 were patients with occult breast cancer who were treated according to the cell block analysis.

The discordance of the HR expression (negative change) of axillary LN was seen in two cases (cases 1 and 5) (Table 2).

Breast cancer patients with synchronous metastases

The ER/HER2 status for recurrent sites was examined in 12 cases (8 with pleural effusion, one with ascites, and 3 with regional LN metastasis), and all cases were compared to the ER/HER2 status of primary breast cancer.

We could not judge the Allred score of HR status in three cases due to the insufficient number of cancer cells (cases 2, 6, and 7).

Although a negative change of the PR expression (3–0 according to the Allred score) was seen in case1, the HR/HER2 status was judged to be concordant in all cases with pleural effusion.

We experienced two cases that indicated discordance with primary breast cancer as follows. Case 8 with ascites indicated discordance of both of the HR and HER2 expression between abdominal metastatic tumor (HR: positive; HER2: negative) and primary breast cancer (HR: negative; HER2: 3+). The number of cells was insufficient

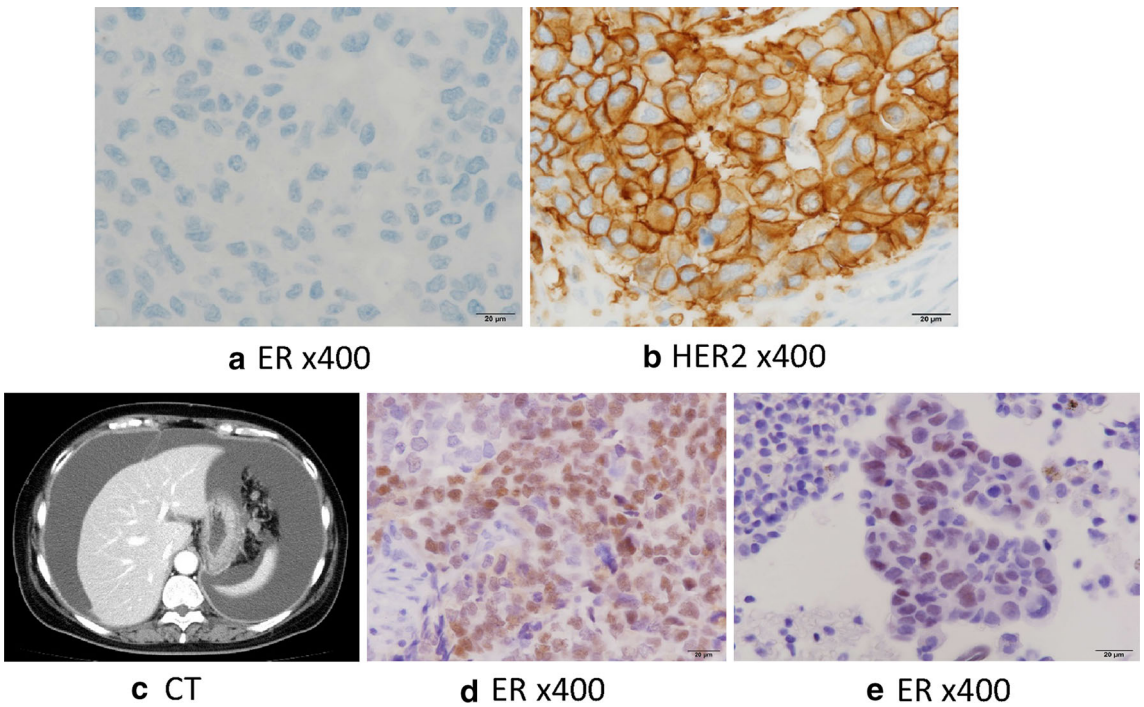

Fig. 3 Case 9; 56-year-old woman with ascites. Primary breast cancer exhibited HR (−), HER2 (3+) as shown at **a**, **b**. She had adjuvant chemotherapy containing trastuzumab. 3 years later, contrast CT indicated massive ascites (**c**). Abdominal metastasis was diagnosed by open biopsy for the omental tumor, indicating HR (positive) as shown in (**d**). The positive expression of ER was detected in the cell block analysis for ascites (**e**)

Table 1 Breast cancer patients with synchronous metastases

Case	Age	Metastatic sites	Metastatic site (cell block)			Primary site (tissue specimen)			Concordance
			ER	PR	HER2	ER	PR	HER2	
1	48	Axillary LN	0	0	0	7	8	1	Discordance
2	60	Axillary LN	0	3	0	–	–	–	Unknown
3	71	Axillary LN	0	0	2	3	2	2	Concordance
4	65	Axillary LN	8	6	3	8	6	3	Concordance
5	72	Axillary LN	0	0	3	5	2	3	Discordance
6	42	Axillary LN	7	7	3	–	–	–	Unknown
7	56	Axillary LN	8	7	1	7	6	2	Concordance
8	43	Pleural effsion	8	6	0	–	–	–	Unknown

Table 2 Breast cancer patients with metachroous metastases

Case	Age	Period	Reccurence sites	Metastatic site (cell block)			Primary site (tissue specimen)			Concordance
				ER	PgR	Her2	ER	PgR	Her2	
1	53	54	Pleural effsion	8	0	0	8	3	2	Concordance
2	49	66	Pleural effsion	Positive	0	0	8	2	1	Concordance
3	59	156	Pleural effsion	7	5	0	5	7	0	Concordance
4	76	78	Pleural effsion	7	6	0	Positive	Positive	Unknown	Concordance
5	56	132	Pleural effsion	0	0	0	0	0	0	Concordance
6	60	204	Pleural effsion	Positive	Positive	0	7	7	0	Concordance
7	87	280	Pleural effsion	Positive	Positive	3	8	7	3	Concordance
8	76	149	Pleural effsion	Positive	Negative	0	8	5	0	Concordance
9	56	74	Ascites	Positive	Positive	0	0	0	3	Discordance
10	43	240	Supraclavicular LN	8	4	0	8	5	0	Concordance
11	48	108	Axillary LN	0	0	0	6	3	1	Discordance
12	43	108	Axillary LN	6	3	0	3	5	0	Concordance

Period months between the operation for primary breast cancer and the cell block analysis for metastases

to judge the score of HR/HER2 expression; however, we were able to determine positive or negative expression. This result was confirmed comparing with biopsy sample as described above. These findings allowed us to determine the subsequent treatment.

Case 11 with a recurrent Ax tumor was diagnosed with a spindle-shaped tumor with no expression of ER/HER2 using the cell block method, which was discordant with the primary tumor (Fig. 4). We performed a wide resection of the tumor and the pathologic finding indicated a metaplastic carcinoma.

Discussion

Discordance of the HR/HER2 expression between the primary cancer and metastatic lesion is an important issue to select the optimal treatments for patients with metastatic breast cancer. However, it is difficult to determine the HR/HER2 expression of the metastatic sites in all cases. A large amount of data of discordance rate have been reported [9–11]. Meta-analysis of 48 articles indicated pooled discordance of proportions was 20 % for ER, 33 % for PR, and 8 % for HER2 [4]. Nishimura et al. [12] reported changed biomarkers (HR, HER2, Ki67, and P53) of recurrent breast cancer, and the decreased expression of the HR rerated to poor prognosis.

The tumor heterogenesity is indicated as a reason for the occurrence of discordance in analysis of primary cases. Yao et al. [13] reported that the discordance between primary breast cancer and synchronous axillary lymph node metastasis was approximately 20 % for HR and 10 % for HER2 expression, and Krishnamurthy et al. [14] reported that the HER2 discordance of circulating tumor cell (CTC) and disseminated tumor cell (DTC) was approximately 20 %.

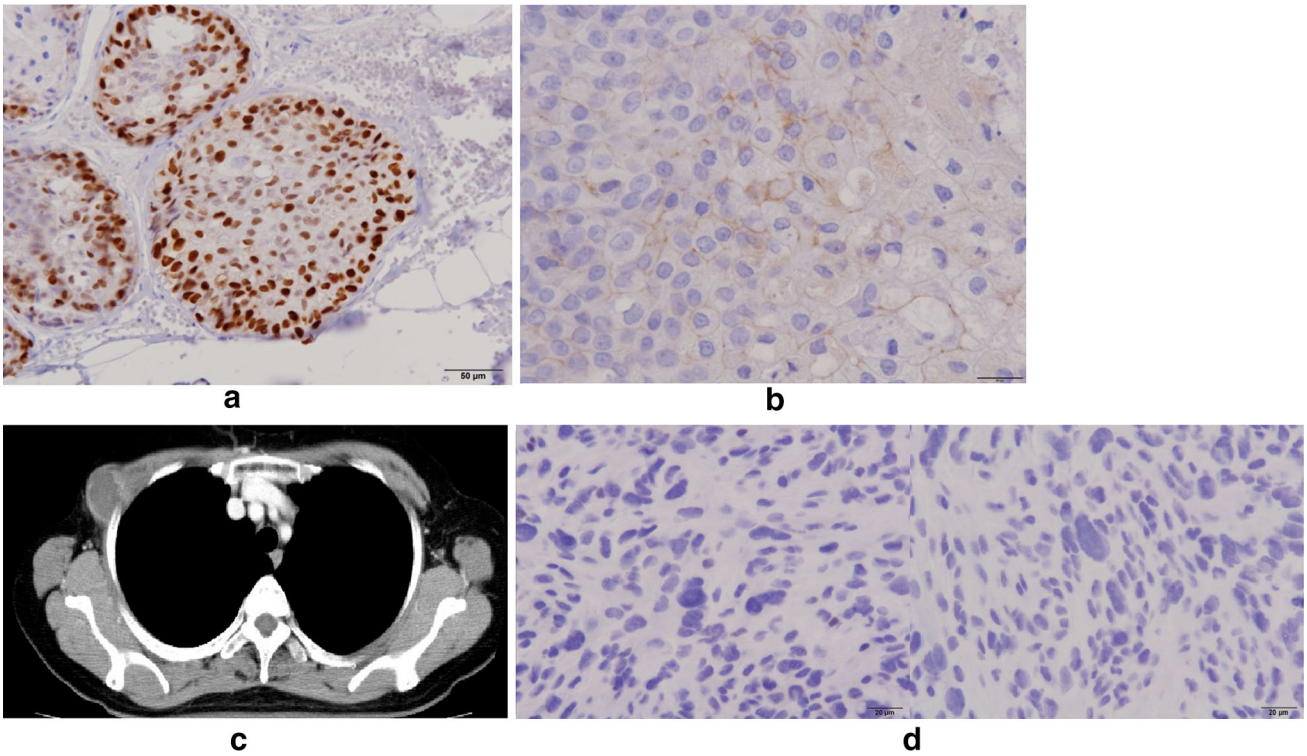

Fig. 4 Case 11; A 48 year-old woman with metachronous metastasis. Primary breast cancer was DCIS with 1 cm diameter and IHC indicated ER (Allred score; 6) HER2 (1+) expression (**a**, **b**). A chest wall tumor measuring 1 cm in diameter was developed 11 years later (**c**). FNA and cell block analysis indicated poorly differentiated adenocarcinoma with ER (negative), HER2 (0) as shown at (**d**). We performed a wide resection of the tumor and the pathologic finding indicated a metaplastic carcinoma (**e**)

A rebiopsy for the metastatic sites is recommended before treatment, but it is not easy to perform for all metastatic lesions [5]. In our experience, 24 % (63/265) of metastatic lesion was diagnosed on biopsy specimens, while 22 % (58/265) of metastatic lesion was diagnosed on cytology by fine needle aspiration (FNA) and aspiration for malignant effusions (data are not shown).

In the cell block method, specimens are embedded in paraffin and available for the immunohistochemical analysis or cancer genome testing. Furthermore, it is possible to utilize the block repeatedly for several analyses.

Furthermore, the cell block analysis for the HR/HER2 expression is sometimes useful for the patients with unknown primary site of breast cancers [15]. We also experienced two cases of occult breast cancer in this study.

It is important to note that the HR/HER2 expressions from the cell block method and tissue sample have been reported to be the same [16, 17]. In our experience, the immunohistochemical staining for HR and HER2 was validated to determine the HR/HER2 expression. However, in cases with cytology material containing small number of cancer cells, it is difficult to judge the Allred score as well as HER2 expression according to the ASCO/CAP criteria. We could make only positive/negative judgment for both of HR and HER2. Especially for HER2 expression, we could not distinguish positive case (3+) from equivocal cases. Therefore, we need to adopt the FISH to judge the HER2 expression.

The advantage of the cell block method is that it is a less invasive procedure to get sample and only takes a few days to obtain the results. We can begin therapy rather quickly without spending much time waiting for the diagnosis based on the biopsy.

We should take advantage of the cell block analysis to determine the discordance of the HR/HER2 expression.

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Conflict of interest The authors declare no conflicts of interest (COI).

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