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ORIGINAL ARTICLE

Comparison of Core Biopsy and Excision Materials in Terms of Histological Type, Histological Grade, Hormone Receptors, and Human Epidermal Growth Factor Receptor 2 Expression in Invasive Breast Carcinomas

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OBJECTIVE

Pre-operative diagnosis using core biopsy (CB) is one of the goals of the current approach for breast cancer, to learn the biological behavior of the tumor and reduce the costs by appropriate treatment planning.

METHODS

The histologic type, grade, estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) status of 201 patients were recorded for both the CB and excision specimens and compared with each other.

RESULTS

When we compared both materials, we found 89% concordance for histologic type and 75% for grade. There was a strong concordance for ER, PR, and HER2 status (96%, 89%, and 96%, respectively).

CONCLUSION

Higher rates of ER, PR, and HER2 positivity in core biopsies may be related to easier fixation, shorter unfixed time, or taking the CB from the tumor periphery. The CB is a reliable tool for pre-operative diagnosis and management of the breast cancer treatment. However, because of the CB may not represent the entire tumor, final decision for histologic type and grade should be made on excision specimens. Although it is low, the discordance rates in terms of hormone receptor (HR) and HER2 expression between two materials should be considered. If there is a discordance between histological type/grade and HR/HER2 status, especially in HR and HER2 negative cases, these studies should be repeated in the excision material. Internal and external controls should be used during immunohistochemical study, attention should be paid during fixation.

Keywords: Breast; core biopsy; grade; HER2; histological type; hormone receptor.

Introduction

The breast cancer constitutes 31% of cancers in women and 17-18% of cancer-related deaths.[1] In breast cancer, hormone receptors (HR) and human epidermal growth factor receptor 2 (HER2) expressions are the most important markers in patient-focused therapy. Due to the heterogeneous behavior of the disease, the current treatment guidelines are primarily based on HR status. The United States National Comprehensive

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Cancer Network guideline suggests determining the status of estrogen receptor (ER) and progesterone receptor (PR) in all primary invasive breast cancers, regardless of patient age, axillary lymph node status or adjuvant chemotherapy history.[2]

Pre-operative diagnosis using core biopsy (CB) is one of the goals of the current approach for breast cancer. Thus, it is aimed to learn the biological behavior of the tumor preoperatively and reduce the costs by appropriate treatment planning.[3] Tumor histology, grade, and expression of various prognostic markers can be determined by CB preoperatively. Thus, systemic chemotherapy and hormonal treatment decisions can be made and prognosis can be predicted.[4-6]

In the literature, the concordance rate of biomarker expressions of tumors between CB and excision materials (EM) has been reported to be 90% or more. Therefore, not to study the tumor markers in EM in patients whose tumor markers have been determined in CB is seen as a cost-saving practice. However, the number of studies about this subject and the number of patients studied are not sufficient.[4-8] It was reported that further studies with larger series are needed to optimize the approach to breast cancer patients.[9-14]

In this study, by comparing CB and EM of breast cancer patients in terms of histological type, grade, ER, PR, and HER2 expression status, we aimed to determine the concordance rates between two methods and to contribute to the determination of optimal treatment approach.

Materials and Methods

Patient Selection and Determination of Clinicopathological Parameters

The study was approved by a Local University Ethics Committee (No: A-36, Date: 02.09.2014).

The study included 201 patients who were diagnosed as invasive breast cancer using CB and then were performed mastectomy/partial mastectomy in a university hospital, between 2010 and 2013. The time between CB and excision ranged from 2 weeks to 1 month. Patients who received neoadjuvant therapy were excluded from the study.

In 78 cases, immunohistochemical ER, PR, HER2 study, and SISH method had already been applied in both CB and EM during routine pathological evaluation. In the remaining 123 patients, these studies had been performed to only one material. For the latter, immunohistochemistry (IHC) and SISH methods were performed during the study.

Patient age, gender, grade, histological type, ER, PR, HER2 status, and SISH results were recorded for each cases. The WHO-2019 classification was used for histopathological classification.[15] Histological grading was performed according to the modified Bloom-Richardson system.[16]

Immunohistochemical Staining

Three μm thick sections obtained from tissues fixed with 10% buffered formalin and embedded in paraffin blocks and transferred into positively charged slides. IHC was performed by an automated staining device (Ventana Benchmark XT, Ventana Medical Systems, Tucson, Arizona). A ready kit (ultraView Universal DAB Detection Kit, Ventana Medical Systems, Tucson, Arizona) containing biotin-free HPR multimer-based hydrogen peroxide substrate and 3,3'-diaminobenzidine tetrahydrochloride chromogen was used. 1/400 dilution for ER (Thermo; clone SP1); 1/100 dilution for PR (Biocare; clone SP2); and 1/100 dilution for HER2 (Thermo; clone SP3) were applied. It was completed using hematoxylin and bluing solution, and the process was terminated after dehydration and xylene stages.

Interpretation of IHC

ER, PR, and HER2 expressions were evaluated according to the CAP protocol. For ER and PR, 1% and above staining was considered positive.[17] The percentage of stained cells and staining intensity were recorded.

For HER2, no staining in invasive tumor cell membranes or ≤10% very weak, hardly visible, and incomplete membranous staining was scored as 0; >10% very weak, hardly visible, and incomplete membranous staining was scored as 1+; >10% incomplete and/or weak-moderate, complete membranous staining or ≤10% complete, strong membranous staining was scored as 2+; >10% complete, strong membranous staining was scored as 3+.[17] SISH method, which shows HER2 gene amplification on chromosome 17, was studied in cases with score 2+.

Silver *In Situ* Hybridization (SISH)

SISH procedure was performed by the automated device (Ventana Benchmark XT, Ventana Medical Systems, Tucson, Arizona). SISH detection kit, double probes showing HER2, and chromosome 17 (HER2 probe and Cr17 probe) were used.

Signals in the nuclei of a total of 40 cells were counted with ×100 immersion in areas that meet the

scoring criteria. The HER2/chromosome 17 ratio was recorded using ASCO/CAP protocol.[18]

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) 21.0 (SPSS, version 21.0, SPSS Inc., Chicago, Illinois) was used for statistical analysis. Kappa coefficient and concordance rate were calculated to evaluate the concordance between two methods. Kappa coefficient valued between 0 and 1 (0.93-1.00: Excellent; 0.81-0.92: Very well; 0.61-0.80: Well; 0.41-0.60: Moderate; 0.21-0.40: Below the middle, and 0.01-0.20: Poor concordance).[19] Pearson's Chi-square test and Fisher's exact test were used to compare qualitative data. Independent samples t-test was used to compare quantitative data. The results were evaluated at 95% confidence interval and $p<0.05$ significance level.

Results

Patients

The study included 201 cases (two males and 199 females). The mean age was 55 (31-90). These cases diagnosed for invasive breast carcinoma of no special type (IBC-NST), invasive lobular carcinoma (ILC), mucinous carcinoma (MC), invasive micropapillary carcinoma (IMPC), tubular carcinoma, metaplastic carcinoma, and mixed type carcinoma.

Comparison of Histological Type in CB and EM

Comparison of the histological types between two methods is summarized in Tables 1 and 2. The overall concordance rate was 89% (179 of 201 cases), moderate, and significant (kappa: 0.597, p=0.000).

Twelve cases reported as IBC-NST in CB were diagnosed as mixed type carcinoma with EM (Fig. 1). One case diagnosed as IMPC and two cases diagnosed as MC in CB were evaluated as mixed type carcinoma in EM. MC component was added in EM of one case which was diagnosed as mixed type carcinoma in CB. Three patients who were diagnosed as mixed type carcinoma in CB were diagnosed as IBC-NST in EM.

Comparison of Histological Grade in CB and EM

Comparison of the histological grades between two methods is summarized in Tables 1 and 3. The concordance rate was 75% (150 of 201 patients), the concordance was moderate and significant (kappa: 0.433, p=0.000).

Comparison of ER Status in CB and EM

Comparison of ER status and staining intensities between two methods is summarized in Tables 1 and 4. The concordance rate was 96% (193 of 201 cases), the concordance was very well and significant (kappa: 0.880, p=0.000).

The mean ER staining percentage of ER positive cases was 81.4% (±18) in CBs and was 74.7% (±23.2) in

Table 1 Comparison of the tumor characteristics in core biopsy and excision materials

Parameter	Core biopsy		Excision materials	
	n	%	n	%
Histological type				
IBC-NST	171	85.1	162	80.6
ILC	13	6.4	9	4.5
MC	4	2.0	2	1
Tubular carcinoma	1	0.5	1	0.5
IMPC	1	0.5	-	-
Metaplastic carcinoma	1	0.5	1	0.5
Mixed type carcinoma	10	5.0	26	12.9
IBC-NST+ILC	6		14	
IBC-NST+MC	2		5	
IBC-NST+IMPC	2		3	
MC+IMPC	-		1	
IBC-NST+MC+IMPC	-		2	
IBC-NST+ILC+IMPC	-		1	
Histological grade				
I	9	4.5	5	2.5
II	158	78.5	126	62.5
III	34	17	70	35
Estrogen receptor				
Positive	162	80.6	156	77.6
Weak	4	2.5	16	10.3
Moderate	44	27.1	50	32.1
Strong	114	70.4	90	57.7
Negative	39	19.4	45	22.4
Progesterone receptor				
Positive	139	69.2	135	67.2
Weak	18	12.9	11	8.1
Moderate	31	22.3	31	23
Strong	90	64.8	93	68.9
Negative	62	30.8	66	32.8
HER2 score (IHC)				
Score 0	77	38.3	50	24.9
Score 1+	60	29.9	63	31.3
Score 2+	24	11.9	58	28.9
Score 3+	40	19.9	30	14.9
HER2 score (IHC+SISH)				
Positive	44	21.9	37	18.4
Negative	157	78.1	164	81.6
Total	201	100	201	100

IBC-NST: Invasive breast carcinoma of no special type; ILC: Invasive lobular carcinoma; MC: Mucinous carcinoma; IMPC: Invasive micropapillary carcinoma; IHC: Immunohistochemistry; SISH: Silver *in situ* hybridization

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Table 2 Comparison of the core biopsy and excision materials in terms of the histological type of the tumor

		Excision material							
	Carcinoma type	**IBC-NST**	**ILC**	**MC**	**IMPC**	**Tubular carcinoma**	**Metaplastic carcinoma**	**Mixed type**	**Total**
Core biopsy	IBC-NST	**159 (98%)**	0	0	0	0	0	12	171
	ILC	0	**9 (100%)**	0	0	0	0	4	13
	MC	0	0	**2 (100%)**	0	0	0	2	4
	IMPC	0	0	0	**0**	0	0	1	1
	Tubular carcinoma	0	0	0	0	**1 (100%)**	0	0	1
	Metaplastic carcinoma	0	0	0	0	0	**1 (100%)**	0	1
	Mixed type	3	0	0	0	0	0	**7 (27%)**	10
	Total	162	9	2	0	1	1	26	201

In the table, bold fonts show the concordant results and the accordance rates are given in parentheses. IBC-NST: Invasive breast carcinoma of no special type; ILC: Invasive lobular carcinoma; MC: Mucinous carcinoma; IMPC: Invasive micropapillary carcinoma

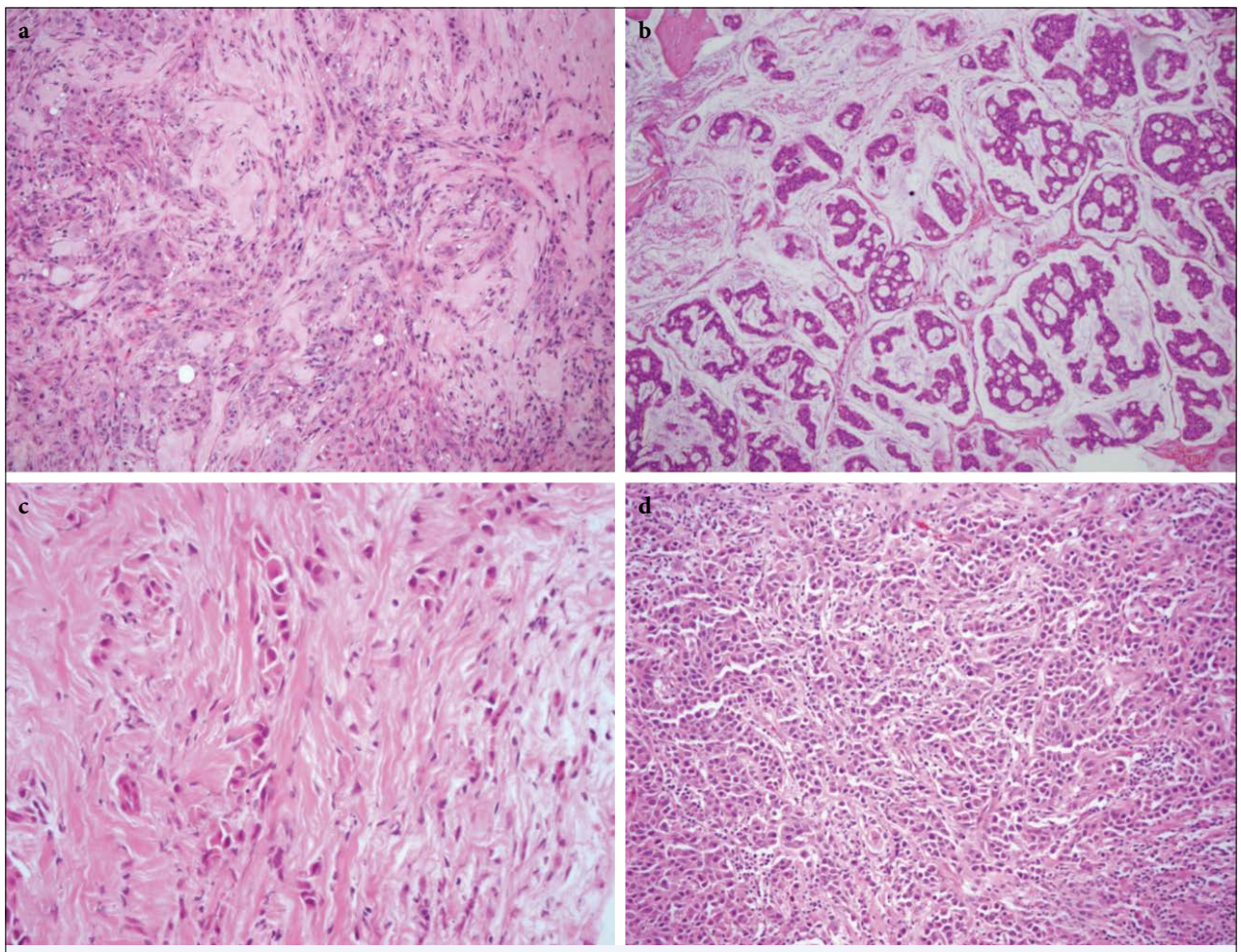

Fig. 1. (a, b) A case of mixed type carcinoma, (a) invasive breast carcinoma of no special type (IBC-NST) component sampled by core biopsy ×200, (b) mucinous carcinoma component of mixed type carcinoma in excision material ×100). (c, d) A case of IBC-NST, (c) in core biopsy, carcinoma cells were trapped in the stroma, formed single cell rows and mimicked invasive lobuler carcinoma ×400, (d) IBC-NST in the excision material, ×200).

Table 3 Distribution of the cases according to the tumor grade in the core biopsy and excision materials

Excision material grade	Core biopsy grade				Kappa value
	I	II	III	Total	
I	**4 (80%)**	1	0	5	0.433
II	4	**117 (92%)**	5	126	
III	1	40	**29 (41%)**	70	
Total	9	158	34	201	

In the table, bold fonts show the concordant results and the accordance rates are given in parentheses

EMs. The mean ER staining percentage in CBs was significantly higher than EMs (p=0.000). In terms of ER staining intensity, the concordance rate was 68% (106 of 155 cases), concordance was below the middle and significant (kappa: 0.371, p=0.000).

Comparison of PR Status in CB and EM

Comparison of PR status and staining intensities between two methods is summarized in Tables 1 and 4. The concordance rate was 89% (179 of 201 cases), the concordance was well and significant (kappa: 0.748, p=0.000).

The mean PR staining percentage of PR positive cases was 60%, 7 (±32.5) in CBs and was 59.9% (±30.1) in EMs. There was no significant difference between two methods in terms of mean PR staining percentage (p=0.824). In terms of PR staining intensity, the concordance rate was 65% (82 of 126 cases), concordance was below the middle and significant (kappa: 0.262, p=0.000).

Comparison of HER2 Status in CB and EM

HER2 expression status between two methods is summarized in Tables 1 and 4.

When HER2 expressions were classified as score 0, 1+, 2+, and 3+, the concordance rate was 57% (114 of 201 cases), the concordance was moderate and significant (kappa:0.421, p=0.000). SISH was studied for EMs of the cases with score 3+ in CB but 2+ in EM; HER2 amplification was detected in six of 11 cases. In one case whose HER2 score was 2+ in CB but 3+ in EM, SISH result of the CB was positive. There was no amplification in the other cases with suspicious HER2 positivity.

When HER2 results were reported as positive, suspicious, and negative, the concordance rate was 73% (147 of 201 cases), the concordance was moderate and significant (kappa: 0.514, p=0.000).

SISH was applied to all suspected cases and results were as follow: In CB, positive in 1 (4%) and negative in 23 (96%) of 24 cases; in the EM, positive in 6 (10%) and negative in 52 (90%) of 58 cases.

When immunohistochemical HER2 study and SISH results were evaluated together, the concordance rate was 96% (192 of 201 patients), the concordance was very well (85.8%) and significant (kappa: 0.861, p=0.000).

Discussion

At present, the CB is an important method for pre-operative diagnosis and has high sensitivity and specificity rates reaching up to 97-99% in detecting breast cancer.[20,21] Since the tumor tissue obtained by CB is usually sufficient, many centers apply routine IHC to this tissue. The results are used to determine the treatment plan.[22] Endocrine treatment is preferred in patients with positive ER and PR, because it is safe and effective.[23] Transtuzumab is used as standard treatment in HER2-positive patients.[24] Chemotherapy can also be an effective treatment option in HR-negative or HER2-positive cases.[25]

IHC applied to CB is usually accepted accurate and it is not studied again in EM to reduce the cost.[14] The question is, considering the tumor heterogeneity and the small amount of tissue obtained by CB, whether the tumor grade and immunohistochemical profile evaluated in CB reflect the entire tumor. The ASCO/CAP guideline supports to study of tumor markers to CB with sufficient number and size, but if the results are inconsistent with the histopathological characteristics or the tissue is not prepared as recommended in the guideline, IHC is recommended to be repeated in EM.[18,26]

In our series of 201 cases, in terms of histological type, the concordance between two methods was 89%. The concordance rate was 100% in ILC, MC, tubular carcinoma, and metaplastic carcinoma, 98% in IBC-NST, and 27% in mixed type carcinoma. In similar studies, it was reported that this concordance was highest in IBC-NST and metaplastic carcinomas; however, the rates are decreased to 14% in mixed type carcinomas.[10,12] In a study, the overall concordance was 73.6%, and the concordance rates were 82% in IBC-NST, 30% in ILC, 50% in MC, and 25% in mixed-type carcinoma.[10] Greer et al.[12] found the overall concordance as 81%, and the concordance rates were 96% in IBC-NST, 77% in ILC, 100% in metaplastic carcinoma, and 14% in mixed type carcinoma.

Park et al.[5] investigated the accuracy of CB in breast carcinomas and found that the average number of cores required to ensure 100% concordance in tu-

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Table 4 Comparison of the core biopsy and excision materials ER status and staining intensities, PR status and staining intensities, immunohistochemical HER2 results and HER2 status according to both IHC and SISH methods

Excision material ER	**Core biopsy ER**			**Kappa value**
	Negative	**Positive**	**Total**	
Negative	**38 (84%)**	7	45	0.880
Positive	1	**155 (99%)**	156	
Total	39	162	201	
Excision material ER intensity	**Core biopsy ER intensity**			
	Weak	**Moderate**	**Strong**	**Total**
Weak	2	9	5	16	0.371
Moderate	2	**23**	25	50	
Strong	0	8	**81**	89	
Total	4	40	111	155	
Excision material PR	**Core biopsy PR**			
	Negative	**Positive**	**Total**	
Negative	**53 (80%)**	13	66	0.748
Positive	9	**126 (93%)**	135	
Total	62	139	201	
Excision material PR intensity	**Core biopsy PR intensity**			
	Weak	**Moderate**	**Strong**	**Total**
Weak	**4**	1	4	9	0.262
Moderate	2	**12**	14	28	
Strong	9	14	**66**	89	
Total	15	27	84	126	
Excision material HER2 (IHC)	**Core biopsy HER2 (IHC)**				
	Score 0	**Score 1+**	**Score 2+**	**Score 3+**	**Total**
Score 0	**36 (72%)**	8	6	0	50	0.421
Score 1+	25	**35 (56%)**	3	0	63	
Score 2+	16	17	**14 (24%)**	11	58	
Score 3+	0	0	1	**29 (97%)**	30	
Total	77	60	24	40	201	
Excision material HER2 (IHC)	**Core biopsy HER2 (IHC)**			
	Negative	**Suspected**	**Positive**	**Total**
Negative	**104 (92%)**	9	0	113	0.514
Suspected	33	**14 (24%)**	11	58	
Positive	0	1	**29 (97%)**	30	
Total	137	24	40	201	
Excision material HER2 (IHC+SISH)	**Core biopsy HER2 (IHC+SISH)**			
	Negative	**Positive**	**Total**	
Negative	**156 (95%)**	8	164	0.861
Positive	1	**36 (97%)**	37	
Total	157	44	201	

In the table, bold fonts show the accordant results and the accordance rates are given in parentheses. ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor 2; IHC: Immunohistochemistry; SISH: Silver *in situ* hybridization

mor type was 5.1. The number of cores taken in our center varies between 1 and 6.

When we evaluated the cases with discordance of histological type, we found that 12 IBC-NST, 1 IMPC, and 2 MC cases according to the CB were diagnosed as mixed type carcinoma in EM. In one case which was diagnosed as mixed type carcinoma (IBC-NST+IMPC) by CB, MC component was also added in EM. Since

it is not always possible to sample each component of heterogeneous tumors, in mixed type carcinomas, the diagnosis given by CB may be incomplete.[10,12]

Three cases diagnosed as mixed IBC-NST +ILC in CB were diagnosed as IBC-NST in EM. In revision, possibly depending on the artifact during taking biopsy, it was observed that the carcinoma cells were trapped in the stroma, formed single cell rows, and mimicked ILC in CB (Fig. 1). Similar diagnostic differences for mixed type carcinomas have been reported in the literature.[11,12]

In our study, the concordance rate in terms of grade between two methods was 75% and the concordance was moderate. The concordance rates were 80% for Grade I, 92% for Grade II, and 41% for Grade III tumors.

In total, 41 of 201 cases had higher tumor grade in EM. In these cases except one, the tumor grade increased from II to III. In one case, tumor grade changed to Grade III from Grade I. When it is revised, it was seen that the tumor was mixed type carcinoma consisting of MC and IBC-NST. Probably, due to the small tumor area sampling, only MC component was sampled by CB. Badoual et al.[10] found 73.1% concordance rate between CB and EM in terms of grade, in a series of 110 cases. They observed that the concordance rates were 78.5% and 79.6% in terms of tubular formation and pleomorphism, respectively, and this rate decreased to 60.2% in terms of mitotic index. They suggested that the discordance in terms of tubular formation and pleomorphism was dependent on tumor heterogeneity and the tumor area in CB was too small to give mitotic index. Park et al.[5] found that lower grade in CBs than EMs was associated with the lower calculation of mitotic index in CBs. In this study, when the average number of CBs was 5.1, the concordance rate between two methods was 80.8% for grade and 59.6% for mitotic index. As a result, they emphasized that five CBs were insufficient to accurately determine the grade. In other studies, the concordance rates between two methods in terms of grade varied between 63% and 77%.[9,11-13] Based on these results, it is necessary to evaluate the grade in EM for optimal treatment and prognosis.[2]

In our study, the concordance rate between two methods was 96% for ER and 89% for PR, similar to the literature. The reported rates ranged from 81% to 99%.[5,9,11,13,14,27] Compared to EM, ER staining percentage and intensity were higher in CB. One of the important factors explaining these findings is tissue fixation (cold ischemia time, type of fixative, and fixation time). The ASCO/CAP guideline recommends keeping cold ischemia time under 1 h for breast excisions.[26]

Qiu et al.[28] fixed breast cancer samples using different fixation times and performed IHC by using the same clones, they showed that the staining scores of ER started to decrease in 2-8 h and PR in 1-8 h. As shown in this and similar studies, it is important to standardize the cold ischemia time to prevent the changes in the level of target protein expressions to which ER, PR, and HER2 antibodies can bind.[17,26,29] Formaldehyde permeates into the tissue at a rate of 1 mm/h, but tissue fixation is slower. Complete crosslink formation takes 24 h at room temperature and 18 h at 37oC. Tissue fixation time is the same, although the fixative permeates faster in smaller tissues.[14,30] ASCO/CAP recommends tissue fixation with 10% buffered formalin for at least 6 h.[26]

Alcohol, alcohol-based solutions (e.g. Carnoy's), acetone, and acidic fixatives (eg., Bouin's, and B5) are also used for tissue fixation. Since ER is degraded in acidic fixatives, they are not recommended by CAP.[17] Although alcohol-based fixatives and acetone are superior to formaldehyde in terms of preserving antigenicity, they lead to coagulation of tissue and are not recommended for routine usage.[30]

Another factor affecting the IHC is tissue processing. Prolonged using of alcohol solutions may cause insufficient dehydration of the tissue. Insufficient dehydration may cause poor or no staining. Some authors recommend changing the solutions at least once a week.[30] In our laboratory, automatic tissue processing device is used and solutions are renewed every week.

In our center, CBs are placed into formalin immediately, so fixation begins at the 0th min. Tissue processing is also performed the same day. EMs sent for frozen process are taken into formalin as soon as the process is over. For the others that kept in the operating room or refrigerated the time without fixation can sometimes extend up to 6 h. In case of delay, EMs are taken into formalin without sectioning and reaches to our laboratory the next day. In this case, formaldehyde penetration into the tissue center is insufficient and these areas are not fixed. In our department, EMs are divided into slices of 0.5-1 cm thickness to ensure formaldehyde penetration to the whole tissue, kept in formalin for 24 h, and sampled the next day and taken to the tissue processing device. Yıldız-Aktaş et al.[29] showed that ER and PR staining percentage and intensity of EMs significantly decreased when EMs kept more than 2 h at room temperature and 4 h refrigerated.

Another reason of higher ER expression in CBs is peripheral sampling. Douglas-Jones et al.[31] reported that ER expression is higher in the tumor periphery than in the center in breast tumors. They found that ER staining

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intensity decreased by approximately 2% per millimeter from the periphery to the center. It was emphasized that may be due increased biological activity and mitosis, as well as due to better fixation at periphery. Greer et al.[12] also compared the HR status between CB and EM and found stronger and higher positivity in CBs.

CAP recommends to use internal control (normal breast tissue) during ER evaluation to avoid false negative results. In CBs without normal cells, if ER is negative, the procedure must be repeated with another block or EM. Staining of the external control as expected indicates that IHC procedure is correct.

The histological type and grade are also important in the evaluation of ER; if ER is negative in low-grade tumors such as mucinous and tubular carcinoma, where it is expected to be positive, the study must be repeated.[17]

In our study, the concordance rate between two methods for PR was 89%, and it was lower than ER as in the literature. It is thought that this was related to the tumor heterogeneity and PR had more heterogeneous distribution than ER within the tumor.[10,13,32] Although there was no significant difference in terms of mean PR staining percentage, intensity of staining was higher in EMs. Greer et al.[12] showed that the concordance between CB and EM in terms of ER, PR, and HER2 decreased in heterogeneous tumors, and the concordance increased with the increased number of CB samples. In the study, PR positivity was detected in more cases in CBs compared to EMs. In many studies, a higher rate of PR positivity in CBs was found associated with rapid fixation and better formaldehyde permeation into the tissue.[28,29,32]

Immunohistochemical HER2 results may vary between laboratories and this discordance rate can increase up to 18-26%.[33,34] In our center, for CBs, we can successfully provide fixation conditions recommended in ASCO/CAP guideline. However, cold ischemia time for EMs may sometimes exceed 1 h.[18]

In our study, the concordance rate of immunohistochemical HER2 expression between CB and EM was 73%, moderately concordant and significant. The concordance rates were 97%, 24%, and 92% in positive, suspicious, and negative cases, respectively. HER2 was positive in 20% of CBs and 15% of EMs. We detected a higher rate of immunohistochemical HER2 positivity in CBs compared to EMs. Park et al.,[5] in a series of 104 cases, showed positive HER 2 staining in 22 CBs and 20 EMs but HER2 amplification was not searched. In our study, SISH method was applied to all patients with score 2+. When IHC and SISH results were evaluated together, concordance rate between two methods reached to 96%. In the previous studies, high concordance rates ranging from 86.5% to 100% in terms of HER2 positivity have been reported.[5,11-13]

The general opinion is that CB represents a very small part of the tumor. Especially, at multiple tumors and at the tumors larger than 4.5 cm, heterogeneity is higher, and CB does not reflect the entire tumor.[12,35-37] Greer et al.[12] showed that the concordance between CB and EM in terms of HER2 expression was worse than ER and PR in heterogeneous tumors, so they emphasized that HER2 study should be repeated in EM.

In our study, since the SISH method was applied only to cases with suspicious HER2 positive, the concordance between two methods could not be examined in terms of SISH. Shousha et al.[38] found 89% concordance between two methods in terms of SISH results, and suggested that 11% discordance was associated with tumor heterogeneity.

Limitations of the Study

In the current approach, Ki67 is routinely studied together with HR and HER2 in patients with breast cancer. However, since our study was retrospective and there were no Ki67 results for most cases in the pathology reports, core biopsies and EM could not be compared in terms of Ki67.

Conclusion

The factors such as fixation time, fixative type, decalcification, and solutions used in tissue processing affect the correct evaluation of the histological type and grade, IHC, and SISH methods. True antigen titrations are also important for IHC. Higher rates of ER, PR, and HER2 positivity in CBs may be related to easier fixation, shorter cold ischemia time, or peripheral sampling. The results show that CB can be used safely in the pre-operative diagnosis of breast cancer and appropriate treatment planning. However, since CB may not represent the entire tumor, final decision for histologic type and grade should be made on EM. Internal or external controls should be used during IHC. If there is a discordance between histological type, grade, HRs, and HER2 status, especially in triple negative cases, these studies should also be repeated in EM.

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Conflict of Interest: All authors declared no conflict of interest.

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