Acta Med. Nagasaki 59: 41－45

Is it practical to determine the therapeutic strategy for breast cancer by evaluating pathological findings in core needle biopsy specimens?

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Background; Core needle biopsy (CNB) specimens have been widely used not only for the diagnosis of breast cancer, but also for assessing biomarkers, including lymphovascular invasion (ly and v), nuclear grading, the estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER-2) and Ki-67. We herein compared the pathological biomarkers of ER+/HER2- invasive breast cancers in CNB with those in the subsequent surgical specimens.

Methods; Patients with ER+/HER2- invasive breast cancer who presented to our department from August 2011 to July 2013 who had CNB and subsequent surgery were included. Lymphovascular invasion (ly, v) and nuclear grading were determined by hematoxylin and eosin staining, and the ER, PgR, HER-2, and Ki-67 status were evaluated by immunohistochemistry.

Results; The concordance rates between CNB and surgical specimens for the ly, v, nuclear grading, ER and PgR were 2.4%, 2.9%, 63.0%, 96.4% and 82.1%, respectively. Lymphovascular invasion and nuclear grading tended to be underestimated with CNB in discordant cases. The Ki-67 labeling index in CNB specimens was strongly correlated with that in surgical specimens (correlation coefficient 0.75, $p<0.0001$). Consequently, there was a reasonable level of agreement between CNB and surgical specimens for surrogate subtyping (82.1%).

Conclusions; CNB provided reliable information on the expression of hormone receptors, Ki-67 in ER+/HER2- invasive breast cancers. However, because of the substantial discordance between CNB and surgical specimens, the status of lymphovascular invasion and nuclear grading should not be concluded based on CNB specimens.

ACTA MEDICA NAGASAKIENSIA 59: 41－45, 2014

Key words: breast cancer, core needle biopsy, hormone receptor, Ki-67, lymphovascular invasion.

Introduction

The use of core needle biopsy (CNB) specimens collected under image guidance has been widely accepted for the diagnosis of breast cancer. Additionally, CNB specimens can be used to assess the characteristics of tumors, including their lymphovascular invasion, nuclear grading, immunohistochemical (IHC) findings (estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER-2), Ki-67), and so on[1, 2]. Histological observations of CNB are sometimes the only information available to determine the therapeutic strategies, i.e., in cases with preoperative systemic therapy. Especially in patients with ER+/HER2- tumors, the IHC findings are widely used as surrogate markers to classify luminal A and B tumors[3]. The problem is whether a small amount of sample obtained by CNB reflects the overall histological features of the cancers that might have intrinsic heterogeneity. In order to evaluate the pathological reliability of CNB specimens for determining the therapeutic strategy for patients with

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Received March 20, 2014; Accepted June 27, 2014

ER+/HER2- invasive breast cancer, we compared the tumor biomarker findings, including the lymphovascular invasion, nuclear grade, ER, PgR, and Ki67 status in CNB with those obtained in the subsequent surgical specimens.

Patients and methods

We reviewed the clinical and laboratory data of 29 patients with ER+/HER2- primary invasive breast cancer determined by findings of surgical specimens, who underwent CNB and subsequent lumpectomy/mastectomy between August 2011 and July 2013 in our department. Patients were excluded if they were treated with systemic or local therapy before surgery. CNBs were performed under ultrasound guidance and were taken with an automated biopsy device fitted with 14-gauge needles (C. R. Bard, Inc., Covington, Georgia, U.S.A). The median number of CNBs was three (range; 2-3), and the median interval between CNB and surgery was 34 days (range; 20-61). CNB and surgical specimens were fixed in neutral buffered formalin and embedded in paraffin before the histopathological analysis. Fixative durations were 4 to 24 hours for the CNB samples and 24 to 48 hours for the surgical specimens.

Processing of samples

Five sections were prepared from each paraffin block. One slide was stained for hematoxylin and eosin (HE), and the remaining slides were kept for IHC staining. In the IHC analysis, sections were incubated with antibodies against the ER (clone SP1, Ventana Medical Systems, Inc., AZ, USA.), PgR (clone 1E2, Ventana Medical Systems), and Ki-67 (clone MIB1, DAKO, Tokyo, Japan). Blots were developed by the labeled streptavidin biotinylated antibody method using automated staining system (BenchMark XT, Ventana Medical Systems).

Pathological evaluation

The histological findings of the surgical specimen were taken as the gold standard. Although the existence of lymphovascular invasion (ly and v) in cancers was basically evaluated by HE staining, Elastica van Gieson staining or IHC analysis for anti-D2-40 was performed in the obscure cases with HE staining. The nuclear grading was based on the sum of the nuclear atypia score and the mitotic count score as follows [4]: Grade 1: 2 or 3 points, Grade 2: 4 points, Grade 3: 5 or 6 points. The nuclear atypia score was graded from 1 to 3 points according to the variation in the size and shape of nuclei and unevenness of chromatin. The mitotic counts score was graded from 1 to 3 points, increasing with the mitoses in the area in which mitoses were most abundant. The ER- and PgR-positive cell counts were determined by the J-score as follows [5]: J-Score 0: not stained; J-Score 1: stained cells <1%; J-Score 2: stained cells ≥ 1% but <10%; J-Score 3a: stained cells ≥10% but <50% and ;J-Score 3b: stained cells ≥50%. The Ki-67 labeling index (LI) was measured in approximately 1,000 malignant invasive cells counted in hot spots in a high-power field (400×) [6]. The IHC-based surrogate subtyping was determined using the following definitions adopted by the 2013 St. Gallen Consensus Panel [3]: Luminal A-like; ER positive / PgR positive (≥ 20%) / HER2 negative / Ki-67<14%, Luminal B-like; ER positive / HER2 negative, and at least one of: Ki-67 ≥14%, PgR negative or low.

Statistical analysis

The agreement of the ly, v, nuclear grading and hormonal receptors was tested using the kappa test. The agreement of the Ki-67 LI between the results from CNB and those from surgical samples were statistically analyzed using Pearson's correlation coefficient test. The results obtained were considered to be significant at $p < 0.05$.

Results

Lymphovascular invasion

Pathological ly and v was identified in the CNB specimen in 21 of 29 cases, while the remaining eight CNB samples were too little to evaluate these parameters. The concordance rates for ly and v between CNB and surgical samples were only 23.8%, with a kappa value of 0.07 (Table 1) and 28.6%, with a kappa value of -0.18 (Table 2), respectively. In 71.4% of cases, ly was underrated on CNB samples, and the v was underdiagnosed in 57.1%. Notably, ly and v were estimated to be absent in 90.5% and 76.2% of cases in CNB samples, although the final rates of absence in surgical samples were only 28.6% and 38.1% respectively.

Nuclear grading

The nuclear grading was evaluated in 27 cases in both CNB and subsequent surgical samples, and there was 63.0% (17 out of 27 cases) agreement, with a kappa of 0.41 (Table 3). The grading was underrated in 33.3% of the cases, while it was overrated in only 3.7% of the cases.

Table 1. Lymphatic invasion

CNB	Surgical sample				
	0	1	2	3	Total
0	5 (83.3%)	8	4	2	19
1	1	0 (0%)	0	0	1
2	0	0	0 (0%)	1	1
3	0	0	0	0 (0%)	0
Total	6	8	4	3	21

Percentages in parentheses indicate the degree of concordance.

Table 2. Vessel invasion

CNB	Surgical sample				
	0	1	2	3	Total
0	5 (62.5%)	8	3	0	16
1	3	1 (11.1%)	1	0	5
2	0	0	0 (0%)	0	0
3	0	0	0	0 (0%)	0
Total	8	9	4	0	21

Percentages in parentheses indicate the degree of concordance.

Table 3. Nuclear grading

CNB	Surgical sample			
	1	2	3	Total
1	12 (92.3%)	0	5	17
2	1	2 (100%)	4	7
3	0	0	3 (25.0%)	3
Total	13	2	12	27

Percentages in parentheses indicate the degree of concordance.

Estrogen and progesterone receptor status

Based on the surgical samples, the ER was expressed in 100% of the cases (Table 4), while PgR was expressed in 85.7% (24/28) (Table 5). The concordance rate of the J-score was 96.4% (27/28), with a kappa of 0.74, for the ER and was 82.1% (23/28) for the PgR, with a kappa of 0.68.

Table 4. Degree of estrogen receptor expression by J-Score

CNB	Surgical sample					
	0	1	2	3a	3b	Total
0	0	0	0	0	0	0
1	0	0	0	0	0	0
2	0	0	1 (50.0%)	0	0	1
3a	0	0	1	0	0	1
3b	0	0	0	0	26 (100%)	26
Total	0	0	2	0	26	28

Percentages in parentheses indicate the degree of concordance.

Table 5. Degree of progesteron receptor expression by J-Score

CNB	Surgical sample					
	0	1	2	3a	3b	Total
0	4 (100%)	0	0	0	0	4
1	0	0	1	0	0	1
2	0	0	0 (0%)	0	0	0
3a	0	0	1	3 (100%)	3	7
3b	0	0	0	0	16 (84.2%)	16
Total	4	0	2	3	19	28

Percentages in parentheses indicate the degree of concordance.

Ki-67 labeling index

The correlation coefficient of the Ki-67 LI between the CNB and surgical samples was 0.75 ($p<0.001$, 95% CI 0.61 – 1.28) (Figure 1).

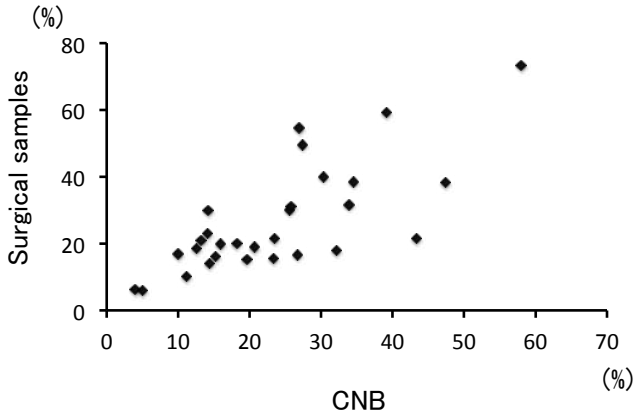

Figure 1. Comparison of the Ki-67 labeling index between CNB and surgical specimens (Pearson's correlation coefficient test, correlation coefficient 0.75, $p<0.0001$).

Surrogate subtyping

The concordance rate of the surrogate subtyping based on the IHC findings was 82.1% (23 out of 28 cases), with a kappa of 0.38 (Table 6). All five of the remaining cases were evaluated as Luminal A-like based on the CNB and Luminal B-like based on the corresponding surgical samples, four of which showed discordance in the Ki-67 LI and one in the PgR expression.

Table 6. Surrogated subtyping

CNB	Surgical sample		
	Luminal-A like	Luminal-B like	Total
Luminal-A like	2 (100%)	5	7
Luminal-B like	0	21 (80.8%)	21
Total	2	26	28

Percentages in parentheses indicate the degree of concordance.

Discussion

Breast cancer is regarded as a systemic disease, and most patients require individualized systemic therapy before or after surgery, based on the biology of the tumor [3]. Additionally, the most favorable treatment varies depending on the progression of the disease. CNB specimens have been widely used for the diagnosis of breast lesions that can be seen under image guidance. It is sometimes necessary to predict the prognosis or determine the therapeutic strategies of the patients with primary invasive breast cancer by evaluating only CNB samples, for instance, in cases where preoperative systemic therapy has been given, or in cases with accompanying metastatic disease.

Lymphovascular invasion is a prognostic factor [7] or a predictive factor for loco-regional recurrence in node-negative breast cancer [8] and its initial precise evaluation is desirable. In this study, ly and v were accurately diagnosed in only 23.8% and 28.6% of CNB samples, respectively. Lymphatic vessel invasion in invasive breast cancer is seen only in the peripheral areas, not in the central areas of tumors in resected specimens [9], and this heterogeneous distribution might be one of the reasons for the low accuracy by CNB. In early breast cancer patients, a high nuclear grade is associated with occult metastases and isolated tumor cells in non-sentinel lymph nodes [10]. Our concordance rate for nuclear grading was 63.0%, and this value was comparable to that reported by Badoual et al. and Harris et al [11, 12]. Lymphovascular invasion and nuclear grading tended to be underestimated with CNB, as reported previously [13, 14]. In terms of the number of CNB, the accuracy of the histological grading or hormone receptor status increased with the number of CNB passes [15, 16]. This implies that the portions sampled and number of passes of CNB are relevant for the precise evaluation of the cancer characteristics.

One of the purposes of preoperative systemic therapy is to permit breast-conserving surgery for patients who would otherwise require total mastectomy. Additionally, preoperative systemic therapy can provide information on the drug sensitivity, which could be beneficial during the subsequent treatment. Rapidly proliferating tumor subtypes, such as the nonluminal HER2 type and triple negative diseases, could subsequently turn into a pCR with systemic cytotoxic chemotherapy [3]. Preoperative hormone therapy is, in general, carried out for the patients with hormone receptor-positive breast cancer, for whom chemotherapy is unsuitable [17]. ER+/HER2- invasive breast cancer consists of heterogeneous entities, and the tumor subtypes vary according to the pathological biomarkers, including the degree of ER and PgR expression, nuclear grading and markers of proliferation such as Ki-67 LI. In randomized trials of preoperative hormone therapy in postmenopausal patients with ER+ breast cancer, higher ER levels significantly correlated with a higher response [18]. On the other hand, the degree of expression of the ER and PgR was inversely correlated with the response to neoadjuvant chemotherapy [19]. These reports suggest that the proportion of hormone receptor expression might be more useful to predict the therapeutic efficacy and to determine the therapeutic strategy than a dichotomous evaluation of their expression.

In our study, the proportion of ER and PgR expression showed good concordance rates (96.4 and 82.1%, respectively) between CNB and surgical samples, comparable to the findings of previous reports. With respect to proliferation, hormone therapy and chemotherapy showed similar clinical response rates in the patients with a low Ki-67 LI, but patients with a high Ki-67 LI had a better response with chemotherapy [20]. The 2013 St. Gallen Consensus Panel strongly endorsed preoperative hormone therapy for postmenopausal patients with highly positive expression of hormone receptors and disease with a low proliferation rate [3]. We demonstrated that the Ki-67 LI in CNB was positively correlated with that in surgical samples (correlation coefficient 0.75, $p<0.0001$).

In addition to the ER and Ki-67 LI, the PgR expression has been considered as the factor that distinguishes Luminal A-like and Luminal B-like/HER2-negative disease, based on the 2013 St. Gallen Consensus Panel. The concordance rate of the surrogate subtyping based on the IHC findings was 82.1% (23 out of 28 cases) in our study. The remaining five cases showed discordance due to an underestimation of the Ki-67 LI or PgR. The problem with the Ki-67 LI and PgR measurement is that it is not standardized among laboratories, and the former is lacking a consensus regarding the threshold for the use of adjuvant cytotoxic chemotherapy for patients with ER+/HER2- disease [3]. That is why the ready availability of multigene molecular assays is expected to help provide accurate and reproducible prognostic information and prediction of the response to chemotherapy.

In conclusion, since the ER, PgR and Ki-67 LI, all of which are factors defining surrogate subtyping, were well correlated between CNB and surgical samples, the appropriate systemic therapy could be determined based on the findings of CNB specimens. On the other hand, since the lymphovascular invasion and nuclear grading were frequently discordant, their status should be determined based on the surgical samples whenever possible.

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