Scientific Research

The Reliability of Assessment of Ki-67 Expression on Core Needle Biopsy and the Surgical Specimens of Invasive Breast Cancer: Comparison of Local Pathologists' Assessment and Central Review*#

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ABSTRACT

Purpose: The aim of this study was to assess the reliability of Ki-67 expression on core needle biopsy (CNB) and the surgical specimens of invasive breast cancer. We examined the concordance rate of Ki-67 expressions, hormone receptors, and human epidermal growth factor receptor 2 (HER2) status from a CNB with from a surgery in invasive breast cancer. **Methods:** A retrospective study was conducted on a clinical database of patients who underwent surgery for early breast cancer. Of these, 193 patients who underwent CNB before the surgery were enrolled. A cut-off value of 20% was used for Ki-67-positive criteria. Expression of estrogen receptor (ER), progesterone receptor (PgR), and HER2 were examined and compared with that of Ki-67. To evaluate discordance between the pathologists' earlier assessments, we re-examined Ki-67 expression among the Ki-67 discordant group in a central laboratory. **Results:** The concordance rate for Ki-67 expression between the two specimen types was 77.7%, which was significantly lower than that for ER, PgR, and HER2 expression (95.9%, 88.1%, and 91.6%, respectively). The concordance rate for re-examined Ki-67 expression among the Ki-67 discordant group improved to 93.8% and was not significantly different from that for the other receptors. **Conclusion:** The concordance rate for Ki-67 expression between biopsy and surgical specimens was significantly lower than that for ER, PgR, and HER2 expressions, but re-examination of Ki-67 expression in a central laboratory revealed no significant difference among the receptors, suggesting the need for standard pathological assessment of Ki-67 expression for clinical use as a predictive marker of breast cancer.

Keywords: Ki-67; CNB; Concordance Rate; Standard Assessment

1. Introduction

In recent years, focus has been placed on the usefulness of preoperative endocrine therapy for postmenopausal hormone-receptor-positive breast cancer, patients with the aim of improving breast conservation rates and selecting patients with high susceptibility to endocrine therapy [1-3].

Certain biomarkers provide clinically useful prognostic and predictive information in preoperative endocrine therapy. To identify alternative post-treatment factors that predict recurrence-free survival after preoperative endocrine therapy, Dowsett *et al*. examined changes in the Ki-67 index before and after 2 weeks of preoperative

endocrine therapy, and found that patients with higher Ki67 expression after 2 weeks of preoperative endocrine therapy had significantly lower recurrence-free survival [4-5]. In multivariable analysis, Ellis *et al*. recently demonstrated that among 228 postmenopausal women with ER-positive breast cancer who received preoperative endocrine therapy, 4 factors—pathological tumor size, node status, ER status, and the Ki-67 index—were determined to predict long-term outcomes after completion of preoperative endocrine therapy [6].

Based on this, considering pretreatment histopathological findings obtained using core needle biopsy (CNB) and biological markers are important for determining a therapeutic strategy. In addition, variations in the pre- and post-treatment using the Ki-67 index are reported to be related to preoperative hormone therapy as

a predictor of prognosis and effect [4-8]. Variations in the Ki-67 index are evaluated using CNB specimens before treatment and using surgical specimens after treatment. On the other hand, it is important to consider that there may be discrepancies in the Ki-67 index between the CNB and surgical specimens, even though preoperative treatment is not performed.

Consequently, to ascertain the necessity for standardizing Ki-67 index measurement methods in preoperative hormone therapy, we evaluated the Ki-67 index concordance rates between preoperative CNB and surgical specimens. Furthermore, we re-examined the discordance cases in the Ki-67 index through central review, in addition to comparing the concordance rates in the expression of other biological markers.

2. Patients and Materials

We retrospectively analyzed data from patients who underwent primary surgery for early breast cancer at Tokyo-West Tokushukai Hospital from August, 2008 to October, 2011. Of these, 193 consecutive patients who received CNB before surgery were enrolled. We retrospectively analyzed data from patients who underwent primary surgery for early breast cancer at Tokyo-West Tokushukai Hospital from August, 2008 to October, 2011. Of these, 193 consecutive patients who received core needle biopsies (CNB) before surgery were enrolled. The inclusion criteria included 1) diagnosis of breast cancer confirmed by CNB and 2) no evidence of metastases at initial assessment, and the exclusion criteria included 1) evidence of metastases at initial assessment; 2) inoperable tumors (stage T4, N2, or N3); 3) patients with neoadjuvant chemotherapy, and 4) patients with ductal carcinoma *in situ*. For CNB, a 16- or 18-gauge automated needle device with a 22-mm throw biopsy gun was used. Three or more CNB specimens were obtained per patient. CNB specimens were placed in 20% formalin for >6 hours to <48 hours. The original tumors had been fixed in buffered formalin and embedded in paraffin. One representative tissue block for each tumor was selected for routine evaluation of estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor 2 (HER2), and Ki-67 by immunohistochemical analysis. The clone MIB-1 (Dako, Denmark) was used for immunohistochemical analysis of Ki-67.

According to our institutional ethics committee's policies, general consent is taken from all the patients who undergo medical care.

3. Methods

All cases were evaluated by registered local pathologists. Although many different systems for grading of patho-

logical responses by local pathologists have been proposed, no standard method has been adopted. The concordance rates for assessment of ER, PgR, HER2, and Ki-67 by local pathologists were reviewed, and in cases of non-matching Ki-67, the tumor diameters (approximately indicative of tumor heterogeneity) and operative method (approximately indicative of formalin fixation condition) were studied. Next, the non-matching cases from August, 2008 to October, 2011 were reassessed by central review. The central review was performed by scanning magnification to count at least 1000 cells in the most densely labeled areas. For all non-matching cases, the percentage of tumor cells with any nuclear staining was recorded.

4. Statistical Analysis

Statistical significance for concordance rates between the 2 types of specimens was evaluated by Wilcoxon t-test. To evaluate the consequence of formalin and genetic heterogeneity, parameters, such as the operative method and tumor size, were analyzed by χ^2 analysis.

5. Results

The mean patient age was 56.3 years (median, 55.5 years; range, 30 - 91 years).

Seventy-three patients ultimately underwent mastectomy and the remainder underwent breast-conserving surgery.

For ER, 152 cases showed positive and 33 cases showed negative findings for both CNB and surgical specimens; 3 cases showed positive findings for CNB specimens and negative findings for surgical specimens; and 5 cases showed negative findings for CNB specimens and positive findings for surgical specimens. For PgR, 111 cases showed positive findings for CNB and surgical specimens, 59 cases showed negative findings for CNB and surgical specimens, 12 cases showed positive findings for CNB specimens and negative findings for surgical specimens, and 11 cases showed negative findings for CNB specimens and positive findings for surgical specimens. For HER2 expression, 27 cases showed positive findings for CNB and surgical specimens; 137 cases showed negative findings for CNB and surgical specimens; 11 cases showed positive findings for CNB specimens and negative findings for surgical specimens; and 4 cases showed negative findings for CNB specimens and positive findings for surgical specimens. For Ki-67, 85 cases showed positive findings for CNB and surgical specimens; 65 cases showed negative findings for CNB and surgical specimens; 28 cases showed positive findings for CNB specimens and negative findings for surgical specimens; and 15 cases

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showed negative findings for CNB specimens and positive findings for surgical specimens. Concordance between CNB and surgical specimens for marker expression is shown in **Tables 1-4**. In our series, the concordance rate for Ki-67 expression between the two specimen types was 77.7%, significantly lower than that for ER, PgR, and HER2 expression (95.9%, 88.1%, and 91.6%, respectively) (**Table 5**).

Table 1. Concordance rate between CNB and surgical specimens for ER status.

	Positive surgical specimen	Negative surgical specimen
Positive (CNB)	152	3
Negative (CNB)	5	33

Concordance rate for ER: 95.9%.

Table 2. Concordance rate between CNB and surgical specimens for PgR status.

	Positive surgical specimen	Negative surgical specimen
Positive (CNB)	111	12
Negative (CNB)	11	59

Concordance rate for PgR: 88.1%.

Table 3 Concordance rate between CNB and surgical specimen for HER2 status.

	Positive surgical specimen	Negative surgical specimen
Positive (CNB)	27	11
Negative (CNB)	4	137

Concordance rate for HER2: 91.6%.

Table 4. Concordance rate between CNB and surgical specimen for Ki-67 expression.

	Positive (surgical specimen)	Negative (surgical specimen)
Positive (CNB)	85	28
Negative (CNB)	15	65

Concordance rate for Ki-67: 77.7%.

Table 5. Comparison of concordance rates between ER, PgR, and HER2 with that of Ki-67.

	Concordance rate	Comparison of concordance rates with Ki-67
ER	95.9%	$P < 0.01$
PgR	88.1%	$P < 0.01$
HER2	91.6%	$P < 0.01$
Ki-67	77.7%	-

Analysis of the operative methods used among the 151 concordant cases showed that breast-conserving surgery was performed in 94 cases (62.3%) and mastectomy in 57 cases (37.7%). No significant difference in parameters, such as operative method (mastectomy vs. breast-conserving surgery), were observed between the two patient groups (**Table 6**).

Analysis of tumor size among the 151 concordant cases showed that pT1 tumor occurred in 92 cases and pT2 tumor in 59 cases. No significant difference in parameters, such as tumor size (pT1 vs. ≥ pT2) was observed between the two patient groups (**Table 7**).

The central laboratory re-examination of Ki-67 expression among the Ki-67 discordant group cases showed that 30 of the 43 non-matching cases should have been placed in the concordant group. The concordance rate for Ki-67 after re-examination by the central review improved to 93.8% (**Table 8**).

6. Discussion

The Ki-67 index is a crucial factor as a predictor of therapeutic effect and prognosis in preoperative hormone therapy [9-12]. There are, however, certain identified problems with regard to the Ki-67 index including standardization of measuring method, reproducibility of

Table 6. No significant difference between operative methods.

	Breast-conserving Surgery	Mastectomy	N
Concordance	94 cases (62.3%)	57 (37.7%)	151
Discordance	26 (61.9%)	16 (38.1%)	42

Table 7. No significant difference between tumor size <pT1 and ≥pT2.

	<pT1	≥pT2	n
Concordance	92 cases (60.9%)	59 (39.1%)	151
Discordance	29 (69.0%)	13 (31.0%)	42

Table 8. Comparison of concordance rates between ER, PgR, and HER2 with that of Ki-67 (central review).

	Concordance rate	Comparison of concordance rates with Ki-67 (central review)
ER	95.9%	$P = 0.42$
PgR	88.1%	$P = 0.02$
HER2	91.6%	$P = 0.08$
Ki-67	77.7%	-

measurement results, and establishment of cutoff values [13-16].

Matthew *et al.* evaluated 209 breast cancer patients using concordance rates between biological markers in needle biopsy and surgical specimens. Their report indicates that concordance rates of Ki-67, ER, PgR, HER2, tumor grade, mitotic rate were 59%, 88%, 78%, 81%, 59%, and 61%, respectively; and the concordance rates of Ki-67, PgR, HER2, tumor grade, and mitotic rate were lower than that of ER [17].

The current investigation indicated that the concordance rate of Ki-67 expression in CNB and surgical specimens was 77.7%, significantly lower than those of ER (95.9%), PgR (88.1%), and HER2 (91.6%). The following factors were thought to cause discordance: 1) tumor heterogeneity, 2) differences in formalin fixation conditions, and 3) a lack of standardization of the measuring method by pathologists. Although the concordance rates between tumor diameter and its equivalence to tumor heterogeneity, as well as Ki-67 expression were compared to determine the effect on tumor heterogeneity, no significant correlation was observed. Further, although the concordance rates between the operative method and its equivalence to formalin fixation conditions as well as Ki-67 expression were compared to consider the effect on formalin fixation, no significant correlation was observed. In order to consider the lack of standardization of the measurement method by pathologists, we reexamined the discordant cases in the Ki-67 index through central review.

In reexamination through central review, the concordance rate of Ki-67 expression was 93.8%, which was almost equivalent to that of ER, PgR, and HER2. Ki-67 measurements were examined microscopically by the pathologists; the percentage of positive cells to total tumor cells was calculated and this was reported as the Ki-67 labeling index. Because the pathologists used visual judgment, there was some discordance reported between the pathologists in the percentage of positive cells. Finally, because the concordance rate of Ki-67 expression was almost equivalent to that of ER, PgR, and HER2 in our reexamination through central review, it was suggested that standardization of the measurement method by pathologists is important.

In order to ascertain the necessity of whether the methods to measure the Ki-67 index should be standardized in preoperative hormone therapy, we evaluated the index concordance rates between preoperative CNB and surgical specimens, and reexamined the discordance cases in the Ki-67 index through central review. In CNB and surgical specimens, the concordance rate of Ki-67 was lower than that of other biological markers. However, in the reexamination through central review, the concor-

dance rate of Ki-67 was almost the same as that of other biological markers. Therefore, for the clinical application of Ki-67, it is necessary to standardize the methods to measure Ki-67 index.

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