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Reliability of Prognostic Factors in Breast Carcinoma Determined by Core Needle Biopsy

Shin Usami[1,2], Takuya Moriya[1], Masakazu Amari[2], Akihiko Suzuki[2], Takanori Ishida[2], Hironobu Sasano[1] and Noriaki Ohuchi[2]

[1]Department of Pathology and [2]Breast and Endocrine Surgery, Tohoku University Hospital, Sendai, Japan

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Objective: The aim of this study was to evaluate the reliability of information obtained by core needle biopsy (CNB).
Methods: We studied 111 women (112 lesions) with breast cancer who underwent CNB and subsequent surgical excision. Six factors (histological type, nuclear grade, histological grade, estrogen receptor (ER) status, progesterone receptor (PR) status, and human epidermal growth factor receptor-2 (HER2) status) were evaluated in a blinded fashion at CNB and at surgical excision.
Results: The histological type at CNB correlated exactly with that of the excisional specimen in 83% (87/105) of the cases. Of the 45 *in situ* lesions at CNB, 16 (36%) were found to have invasive carcinoma at surgical excision. The difference between the specimens from CNB and those from surgery in terms of the absolute concordance rate and κ statistic value were 61% with a fair κ value (0.26) in the nuclear grade, 75% with a moderate κ value (0.55) in the histological grade, 95% with an almost perfect κ value (0.84) in ER, 88% with a substantial κ value (0.70) in PR and 88% with a substantial κ value (0.65) in HER2. Regarding the evaluation of nuclear and histological grades, a trend toward greater accuracy was observed when thicker specimens were used.
Conclusions: CNB provided reliable information on the histological type of invasive carcinoma. It also evaluated ER, PR and HER2 (only in cases where the score was 3+) accurately in spite of the limited quantity of the specimen obtained with the thin (16-gauge) needle.

Key words: breast cancer – core needle biopsy – HER2 – hormone receptor – prognostic factor

BACKGROUND

Recently, core needle biopsy (CNB) has been widely used as an alternative to surgical open biopsy. There have been several studies on the diagnostic accuracy of CNB (1–6), which showed high diagnostic accuracy for both palpable and non-palpable breast lesions. One of the reasons for the preference of CNB over fine needle aspiration biopsy cytology (FNA) is that the incidence of ductal carcinoma *in situ* (DCIS) has increased among all breast cancers.

However, for therapeutic advancement in the treatment of breast cancer, many clinicians would like CNB to provide information not only on the histological diagnosis but also on various predictive factors because such information is very important when deciding the therapeutic strategy. Particularly in settings where neo-adjuvant therapy is used, such information would be unique to each patient because at the time of the operation, we cannot obtain the native tissue samples, i.e. those that have not been modified by the treatment. In addition, a recent study (7) on metastatic breast cancer has shown a high level of discordance for both the estrogen receptor (ER) and the progesterone receptor (PR) between primary and metastatic disease. This reinforces the importance of obtaining biopsy material at the first presentation of metastasis. Furthermore, survival after metastasis is related to the ER status of the metastatic tumor rather than that of the primary tumor. In clinical practice, material from metastatic lesions is often obtained by CNB.

There are few studies that detail the predictive factors from CNB, in particular, the impact of the needle size on the accuracy of several prognostic factors. Therefore, the aim of

For reprints and all correspondence: Takuya Moriya, Department of Pathology, Tohoku University Hospital. 1-1 Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan. E-mail: moriya@patholo2.med.tohoku.ac.jp

this study is to evaluate the reliability of the information obtained from CNB with respect to certain histopathological factors as well as those influenced by needle thickness.

PATIENTS AND METHODS

We studied 111 women (112 lesions) with breast cancer whose ages ranged from 29 to 80 years (mean: 55.3 years). Between January 2000 and May 2005, they underwent CNB and subsequent surgical excision at Tohoku University Hospital. Patients who received neo-adjuvant therapy or radiotherapy during the period between the CNB and the surgical excision were excluded. All the core biopsies were performed under image guidance. Ultrasound guidance using a 16-gauge true-cut needle with an automated biopsy device was employed in 91 cases (81%), while stereotactic guidance using an 11-gauge vacuum-assisted biopsy device was employed in 21 cases (19%), which demonstrated only calcification upon mammography. The number of cores ranged from 1 (88 cases) to 2 (3 cases) when the 16-gauge needle was used and ranged from 1 to 17 with a mean value of 7.7 when the 11-gauge needle was used.

Therapeutic excision was performed in each case. Partial mastectomy was performed in 92 cases (82%) while total mastectomy was carried out in 20 cases (18%). The partial mastectomy specimen was serially sectioned into 5-mm thick slices and all the slices were processed for histological diagnosis (8). In the total mastectomy cases, an adequate number of slices were retained for diagnosis. All the specimens were fixed in 10% formalin, embedded in paraffin and sectioned into slices 2–3 μm thick; staining with hematoxylin-eosin (HE) and immunohistochemicals was then performed. For determining the hormone receptor status, we employed the avidin-streptavidin immunoperoxidase method using the clone 6F11 antibody (Ventana) for ER and the clone 6 antibody (Ventana) for PR in an automated immunostainer (Benchmark System: Ventana Medical Systems, Inc.). To evaluate the human epidermal growth factor receptor-2 (HER2) status, we used a standardized immunohistochemistry kit (HercepTest for Immunoenzymatic Staining: DAKO) (9).

Slides from both CNB and the surgery were independently reviewed in a blinded fashion by two pathologists. The following six factors were considered: histological type, nuclear grade (pleomorphism), histological grade, ER status, PR status and HER2 status. In cases of inter-observer disagreement, a conclusion was reached after sufficient discussion.

Assignment of the histological type was based on the WHO classification from 2003 (10). When we could only observe a malignant (ductal) cell cluster without a border in the stroma in the CNB slides, we diagnosed only 'ductal carcinoma' (in which the invasive component of the tumor could not be determined). The histological grade was assigned using the Nottingham grading system (11). The nuclear grade was evaluated based on three tiers, namely, grades 1 (mild), 2 (intermediate) and 3 (severe), according to the same WHO classification system. The ER and PR results were assessed semi-quantitatively using Allred's scoring system (12). The results were categorized as positive when the total score (TS), expressed as the sum of the proportion score (PS) and the intensity score (IS), was more than two. With regard to HER2, membranous staining was graded as negative (score 0 or 1+), weakly positive (score 2+) and strongly positive (score 3+).

In order to determine the impact of the needle size on diagnostic accuracy, we also analyzed the effect of the relationship between the needle size and the absolute concordance rate on the evaluation of each factor.

Agreement between the results from CNB and those from surgical excision was statistically analyzed using the absolute concordance rate and κ statistic values. The χ^2 test or Fisher's exact test was used to examine the association of the needle size and the absolute concordance rate. The Cochran–Cox method was used for evaluating the relationship between the size of invasion and the accuracy of the estimation of the invasion. In this study, P values less than 0.05 were considered significant.

RESULTS

Histological Type

All the cases that were diagnosed to be malignant on CNB proved to be breast cancer upon subsequent surgical excision (positive predictive value = 100%). In the final (excisional) diagnosis, there were 70 (62%) cases of invasive ductal carcinoma not otherwise specified (IDC), 30 (27%) cases of non-invasive ductal carcinoma (DCIS), seven (6%) cases of invasive lobular carcinoma (ILC), three (3%) cases of mucinous carcinoma, and one case each of apocrine carcinoma and tubular carcinoma (Table 1). The histological type on CNB correlated exactly (including the distinction between *in situ* and invasive carcinomas, and ductal and specific type carcinomas, i.e. lobular, mucinous and apocrine carcinomas) with that of the excisional specimen in 87 of 105 (83%) cases, except in seven cases in which the invasive component of the tumor could not be determined.

All 60 cases diagnosed as invasive carcinoma at CNB were also invasive carcinoma at excision. Of the 45 cases of *in situ* lesions diagnosed with CNB, 29 (64%) showed non-invasive ductal carcinoma at surgical excision. At CNB, 16 of the 45 (36%) *in situ* lesions were found to have invasive carcinoma at surgical excision.

The characteristics of these 16 cases were as follows. The mean size of invasive carcinoma was 8.8 mm (1–19 mm). The size (8.8 mm) was significantly smaller ($P < 0.05$) than that of the invasive carcinoma (2–80 mm, mean: 17.6 mm) diagnosed both at CNB and at surgical excision (histological type concordant cases). In 11 (69%) of these cases, the size was equal to or less than 4 mm. The mean number of invasive sites was 6.8 (1–16). Eleven (69%) cases had multiple

Table 1. Comparison of histological type in CNB and surgical excision

CNB	Surgical excision					
	IDC	DCIS	ILC	Muc	Apo	Tub
IDC	**50**	0	1	0	0	1
DCIS	14	**29**	0	0	1	0
DC	6	1	0	0	0	0
ILC	0	0	**5**	0	0	0
Muc	0	0	0	**3**	0	0
LCIS	0	0	1	0	0	0
	70	30	7	3	1	1
	62%	27%	6%	3%	1%	1%

IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; DCIS, ductal carcinoma *in situ*; DC, ductal carcinoma in which invasion could not be determined; LCIS, lobular carcinoma *in situ*; Muc, mucinous carcinoma; Tub, tubular carcinoma.

invasion sites. All 16 cases presented comedonecrosis in *in situ* carcinoma and the nuclear grade was intermediate or high in surgical excision specimens. Further, it should be noted that four cases had metastasis to the axillary lymph node.

Nuclear Grade (Pleomorphism)

Among 112 patients, with respect to the nuclear grade, the concordance between the results of CNB and those of surgical excision in 68 cases was 61% with a κ statistic value of 0.26 (Table 2). The remaining 44 cases (39%) were discordant: 33 cases were underestimation at CNB (1-grade discordant: 31 cases, 2-grade discordant: 2 cases), while 11 cases were overestimation at CNB (all cases were 1-grade discordant).

Histological Grade

The histological grade was evaluated in 60 cases that were identified as invasive carcinoma by both CNB and subsequent surgical excision. There was 75% (45 of 60 cases) agreement (Table 2) with a κ statistic value of 0.55. Based on the results of nuclear grade concordance, in 11 of the 15 discordant cases, the grade at CNB was lower, while in four cases, the grade at CNB was higher than that in subsequent surgical excision. However, every disagreement case was 1-grade discordant.

Estrogen Receptor and Progesterone Receptor

At surgical excision, ER was expressed in 80% (90/112) of the cases, while PR was expressed in 76% (85/112) of the cases (Table 3). The absolute concordance rate between the CNB and the surgical specimen was 95% (106/112) in ER and 88% (99/112) in PR. When the ER/PR status of the excisional specimen was regarded as the golden standard, the sensitivity, specificity and positive predictive values of CNB were 96, 91 and 98% in ER and 89, 85 and 95% in PR, respectively.

If cases in which at least one of the hormone receptors (ER or PR) was positive were defined as hormone receptor-positive cases, the number of discordant cases was seven (6%). Among these, four cases were negative at CNB but positive at excision. Three cases were positive at CNB but negative at excision. There was 94% concordance with a κ statistic value of 0.79. The sensitivity, specificity and positive predictive values at CNB were 96, 85 and 97%, respectively.

HER2

HER2 was also evaluated in 60 cases. In 13 (22%) of the 60 cases, HER2 was expressed in the excisional specimen. There was 88% (53 of 60 cases) agreement with a κ statistic value of 0.65. The sensitivity, specificity and positive predictive values of CNB were 69, 94 and 75%, respectively (Table 4). In cases where the score was 3+ (strongly positive) at either CNB or surgical excision, all six cases showed concordance between the results from CNB and those from the surgical specimen with respect to discrimination between positive and negative cases.

Table 2. Comparison of the nuclear grade (pleomorphism) and the histological grade in CNB and surgical excision

CNB	Nuclear grade: surgical excision			
	1	2	3	
1	**3**	15	2	20 (18%)
2	5	**50**	16	71 (63%)
3	0	6	**15**	21 (19%)
	8 (7%)	71 (63%)	33 (30%)	**112**

Absolute concordance rate, **61**% (68/112); kappa statistic value, **0.26.**

CNB	Histological grade: surgical excision			
	I	II	III	
I	**11**	4	0	15 (25%)
II	2	**29**	7	38 (63%)
III	0	2	**5**	7 (12%)
	13 (22%)	35 (58%)	12 (20%)	**60**

Absolute concordance rate, **75**% (45/60); kappa statistic value, **0.55.**

Table 3. Comparison of ER and PR and surgical excision

CNB	ER: surgical excision		
	Positive	Negative	
Positive	**86**	2	88 (79%)
Negative	4	**20**	24 (21%)
	90 (80%)	22 (20%)	**112**

Absolute concordance rate, **95**% (106/112); kappa statistic value, **0.84.**

CNB	PR: surgical excision		
	Positive	Negative	
Positive	**76**	4	80 (71%)
Negative	9	**23**	32 (29%)
	85 (76%)	27 (24%)	**112**

Absolute concordance rate, **88**% (99/112); kappa statistic value, **0.70.**

Impact of the Needle Size

Table 5 shows the impact of the needle size on several prognostic factors. With respect to the evaluation of the nuclear and histological grades, in comparison with the 16-gauge core, the 11-gauge core showed a more marked trend toward greater accuracy; however, all the factors analyzed in this study did not reach statistical significance ($P = 0.17–0.96$).

DISCUSSION

CNB may be a good diagnostic procedure for distinguishing between *in situ* and invasive carcinoma, and this is a great advantage that CNB has over FNA. In addition, more information can be obtained by immunohistochemical analysis of the CNB specimen. However, underestimation of the invasion is an unavoidable problem in CNB because the quantity of the specimen is limited. Several reports have documented this (13–15). The incidence of underestimation has been reported to be 19–35% using the 14-gauge core, approximately 10% using the 11-gauge vacuum-assisted core and 4% using the 8-gauge vacuum-assisted core.

In comparison with the results of earlier studies, our results show a higher rate (11-gauge vacuum-assisted core: 25%, 16-gauge core: 41%) of underestimation of invasion. Unlike previously published studies, in this study, we performed minute pathological examination (the entire specimen was serially sectioned into 5-mm thick slices). This would explain the higher underestimation of the invasion rate. The diagnosis of *in situ* carcinoma is influenced to a great extent by the degree of pathological examination. The rate may become higher if even thinner slices are examined.

Therefore, when dealing with a case of *in situ* carcinoma diagnosed at CNB, we should consider the nuclear grade and comedonecrosis, and the diagnosis should be comprehensive, involving the use of various types of imaging techniques (mammography, ultrasonography (US), computed tomography (CT) and magnetic resonance imaging (MRI). Additionally, in this present series, there were four cases with axillary lymph node involvement. This shows that it is necessary to perform sentinel lymph node biopsy or lymph node dissection in some cases, even if the diagnosis at CNB is DCIS.

Information on the histological type is important in cases of invasive breast carcinomas. In 60 cases of invasive carcinoma that were diagnosed by CNB, our results showed excellent agreement (97%, 58 of 60 cases) with regard to the histological type even when the special types of carcinoma were included. In the case of invasive carcinomas, a 16-gauge core was sufficient to estimate the histological type. A recent study indicated (16) that the discordance was due to the inclusion of special types and variants of invasive carcinomas. In this series, since there were few incidences of special types of carcinomas and all of these showed typical histological findings, our results may not have been influenced by minor variations. In this study, we evaluated the nuclear and histological grades of specimens taken at CNB and compared these with those of specimens taken at

Table 4. Comparison of HER2 in CNB and surgical excision

CNB	HER2: surgical excision		
	Positive	Negative	
Positive	**9**	3	12 (20%)
Negative	4	**44**	48 (80%)
	13 (22%)	47 (78%)	**60**

Absolute concordance rate, **88**% (53/60); kappa statistic value, **0.65.**
HER2 positive: score 3+, 2+; negative: score 1+, 0 in the Hercep Test.

Table 5. Impact of the needle size on prognostic factors

	11G		16G		*P* value
	κ	ACR (%)	κ	ACR (%)	
Nuclear grade	0.57	76	0.18	57	0.17
Histological grade	1.00	100	0.45	70	0.47
ER	0.69	90	0.87	96	0.69
PR	0.86	95	0.67	87	0.48
HER2	–	100	0.58	86	0.96

ACR, absolute concordance rate; κ, kappa statistic value.

surgical excision. In particular, the histological grade is a powerful prognostic factor of invasive breast carcinoma. There are 61% cases with a fair κ value (0.26) and 75% cases with a moderate κ value (0.55). These results are similar to those reported previously (59–75%) (16–20).

In the discordant cases, the grade at CNB tended to be lower than that at surgical excision. Previous studies noted that this tendency of the histological grade was due to underestimation of the mitotic count at CNB. In this study, underestimation of the nuclear grade (pleomorphism) is not negligible. Similar results were reported in previous studies (17–19), but the reasons were not described in detail. In the present study, we also analyzed the impact of the needle size on the nuclear grade. The κ statistic value was calculated to be 0.57 using an 11-gauge core and 0.18 using a 16-gauge core. This result indicates that the nuclear grade predicted using the 11-gauge core tended to be more accurate than that obtained with the 16-gauge core, although a significant difference was not found between the two needle sizes. One plausible explanation for this observation is that a stronger experimental artifact, resulting from the crushing of specimens, occurs when the samples are obtained by the 16-gauge core than when they are removed by the 11-gauge vacuum-assisted biopsy device.

The hormone receptor status is also a very important and independent prognostic factor in breast cancer. In our study, hormone receptors were accurately evaluated at CNB. An almost perfect κ value (0.84) in ER and a substantial κ value (0.70) in PR were observed. The concordance of PR was lower than that of ER. This can be associated with the lower incidence of PR-expressing cells in the whole tumor because the Allred total score of PR (mean: 4.5) was lower than that of ER (mean: 5.6). In general, if the result of ER 'and/or' PR is positive, endocrine therapy is indicated. Based on this observation, an extremely high concordance (94%) was observed in this study, while the needle size had no influence. If we use a 16-gauge needle, the core could be reliably used for the assessment of the hormone receptor status.

Although the immunohistochemical staining results for ER/PR at CNB may be reliable for determining therapeutic indications, seven discordant cases (6%) were observed. There were four cases that were CNB-negative and excision-positive and three cases that were of the opposite type. The characteristics of the former cases are mainly due to intratumoral heterogeneity. In the latter cases, only the area of the CNB specimen was positive and some investigators (21–22) have noted that such situations could exist because of more rapid and constant fixation. Douglas-Jones et al. (23) suggested that ER expression was higher in the CNB than in the excised tumors. ER expression was higher at the periphery of tumors than at the center. The higher ER expression in CNB might reflect the greater possibility of the peripheral part of a tumor being sampled when CNB is performed. As long as these cases exist, routine immunostaining of specimens at CNB will have great significance in determining the indications of endocrine therapy. Especially when we determine post-operative systemic therapy in the cases given neo-adjuvant therapy, the results determined by the specimens from core needle biopsy will be more important than those from surgery, which are denatured by pre-operative therapy. With regard to the HER2 status, the diagnostic accuracy (particularly the sensitivity) of CNB was relatively low when compared to that of the hormone receptor status. Conversely, CNB demonstrated excellent sensitivity in cases where the score was 3+. This result is clinically useful in metastatic or inoperable patients. In contrast, cases with a score of 2+ or 1+ should be regarded as indeterminate.

In summary, the underestimation rate for detecting invasion was higher in this study than in previous reports; this is probably due to more precise examination of the excised specimen. Even if a thicker core is used, the problems cannot be completely resolved. If invasive components are detected among the CNB specimens, they can provide reliable information on the histological type, in spite of the use of a thin (16-gauge) needle. The accuracy with which the nuclear/histological grade is estimated is modest and it may be improved by optimizing the needle size and/or noting the nuclear/histological characteristics of the CNB specimen. Hormone receptor evaluation is accurate, even when the single 16-gauge core is used. Determination of the HER2 status in CNB was reliable only in cases where the score was 3+. In order to obtain information on the characteristics of the target lesions, it is necessary to choose a needle of appropriate size. Although CNB has performance limitations, it can provide reliable prognostic factors. It would be worthwhile using the information obtained from CNB in clinical practice.

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Conflict of interest statement
None declared.

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