Abstract

Aim: The objective of this study was to apply a scoring method to fine needle aspiration cytology on breast duct dilatation and cystic lesions, to set an optimum cut-off value to differentiate between benign and malignant cases, and to identify features useful for cell judgment.

Materials and Methods: Samples were 23 preparations of specimens (12 benign and 11 malignant cases) suspected with intraductal lesions or cystic change by ultrasonography or mammography and cytology. The scoring system comprised the following 10 items, and each item was scored 1–3, with a total score of 10–30. Three items were concerning structural atypia: 1, scattered epithelial cells; 2, uneven irregular cluster edge; and 3, overlapping nuclei of epithelial cells, and seven items were concerning cellular atypia: 4, irregular nuclear size; 5, irregular nuclear morphology; 6, deep dyeing chromatin; 7, chromatin granularity; 8, chromatin distribution; 9, nucleolus; and 10, absence of myoepithelial cells.

Results: (1) Scoring cut-off value: malignancy is to be suspected when the score is 20.75 or higher (diagnostic accuracy: 95.7%). (2) Findings useful for cancer judgment: the sensitivity of the following four findings was high: uneven irregular cluster edge, irregular nuclear overlapping, chromatin granularity, and absence of myoepithelial cells. (3) Correlation among the findings: the findings correlated with malignancy were as follows: scattered epithelial cells versus uneven irregular cluster edge (r = 0.8).

Conclusion: Cytological evaluation by scoring lesions accompanied by intraductal dilatation and cystic change was a useful method capable of differentiating between benign and malignant cases at a high accuracy.

Keywords: Breast cytology, fine needle aspiration cytology, cytological scoring

Introduction

The prevalence and mortality of breast cancer have been increasing in Japanese women. According to the latest Cancer Statistics in Japan, one in 11 females develop breast cancer in lifetime, and one of 70 dies of breast cancer. Early cancer cases, which were previously impossible to discover, have recently increased with development of imaging diagnostic technology, such as ultrasonography and mammography, and penetration of breast cancer screening. Hence, cases difficult to differentiate by fine needle aspiration cytology (FNAC) have been increasingly encountered.

Moreover, chances extracting images without malignant findings and microcysts have increased with an increase in the accuracy of mammary ultrasonographic screening. However, core needle biopsy (CNB) is frequently inapplicable to small breast ductal dilatation and cystic lesions. Even if CNB can be performed, it is difficult to make a judgment due to an insufficient amount of the specimen, and a definite diagnosis cannot be made in many cases.

Therefore, FNAC is an essential examination for these lesions, and improvement of the judgment accuracy of FNAC, standardized techniques, and report of liquid-based cytology are necessary. The objective of this study was to set an optimum cut-off value to distinguish between benignity and malignancy.

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and malignancy and identify findings useful to judge breast cancer by applying a cytological scoring method, aiming at improvement of the cytological accuracy of these lesions.

**Materials and Methods**

**Cytological Materials**

This study was approved by the Hirosaki University School of Medicine and Hospital Ethics Committee (2015-033). This study provides retrospective analysis. The study involved 23 (2.1%) of 1076 cases examined by breast FNAC between August 2008 and October 2013 who were suspected with intraductal lesions or cystic change by imaging diagnosis and foamy cells in the background of cytology. These cases were definitely diagnosed histopathologically by surgery or needle biopsy. Twelve cases were benign on mammary gland FNAC, and the disease was mastopathy in nine and intraductal papilloma in three. Eleven cases were malignant on FNAC, and the disease was ductal carcinoma in situ in eight and invasive ductal carcinoma in three. After fine needle aspiration, all samples were sprayed on slide glasses and pressed, and the preparations were rapidly fixed in 95% ethanol and subjected to routine Papanicolaou staining.

**Cytological scoring method**

First, 4–27 typical cytology images were acquired in each case. The magnification of the objective lens was set at 4, 10, and 40 times, and images of each cell cluster were acquired at each magnification. All images of each case were scored by four raters with experience (2–30 years) in cytology. The following 10 items were scored 1–3, and a high grade was scored high. The total score of the items was within a range of 10–30. Cluster atypia was evaluated in three items: 1, scattered epithelial cells; 2, uneven irregular cluster edge; and 3, irregular nuclear overlapping. Cellular atypia was evaluated in seven items: 4, irregularity of the nuclear size; 5, nuclear morphological irregularity; 6, deep dyeing chromatin; 7, chromatin granularity; 8, chromatin distribution; 9, large nucleoli; and 10, absence of myoepithelial cells. Myoepithelial cells were scored as follows: The presence of bare bipolar or round nuclei in the background with the presence of myoepithelial cells in the cluster was scored 1, the presence in either background or cluster was scored 2, and the absence in both background and cluster was scored 3. The scores were input into an original scoring template prepared using Excel, and the mean of scores judged by the four members was calculated. To maintain the accuracy of scoring evaluation, all samples were subjected to routine Papanicolaou staining.

**Cut-off value for differentiation between benign and malignant cells by cytological scoring method**

The total score of each disease, the median, and quartile ranges [value of the first quartile, Q1; value of the third quartile, Q3; interquartile range (IQR)] of the total 12 benign cases (190 images analyzed) and 11 malignant cases (237 images analyzed) were determined. To determine the optimum cut-off score to differentiate between the benign and malignant cases, the receiver operating characteristic (ROC) curves were drawn by ROC analysis, and the value with the highest "sensitivity-(1-specificity)" and the area under the ROC curve (AUC) were determined. AUC is an index to evaluate the usefulness of the ROC curve numerically, and the value ranges were from 0.5 to 1.0. The usefulness of the ROC curve increases as the AUC value comes close to 1, and the accuracy of AUC is evaluated as follows: 0.9–1.0 high accuracy, 0.7–0.9 moderate accuracy, and 0.5–0.7 low accuracy. In addition, the sensitivity, specificity, and diagnostic accuracy of the optimum cut-off value to differentiate between the benign and malignant cases were analyzed.

**Findings of breast cancer cytology from the viewpoint of each scoring item**

The score was compared between the benign and malignant cases by the scoring items. In statistical analysis, since the distribution showed non-normality on a normality test in both diseases (Shapiro–Wilks test), Mann–Whitney U-test was performed, and all items showing a significant difference were subjected to ROC analysis as described in the section "Materials and Methods" 3 and the optimum cut-off score for differentiation between the benign and malignant cases was determined, and the sensitivity, specificity, and accuracy were investigated.

**Findings of breast cancer cytology from the viewpoint of correlation between scoring items**

The breast cancer cytology findings were investigated with regard to the correlation among the scoring items, aiming at identifying the combination of the items likely to develop simultaneously and clarifying whether these items are positively or negatively correlated in the benign and malignant cases. The correlations among the scoring items were investigated using Spearman’s rank correlation separately in the benign and malignant cases. The correlation coefficient was compared and the scoring items with a characteristically high correlation coefficient (rs > 0.8) were extracted.

**Statistical analysis**

The software used for ROC analysis in Methods 3 and 4 was Excel 2012 for Windows. SPSS 16.0 Japanese for Windows was used for the normality test (Shapiro–Wilks test), test of significant differences (Mann–Whitney U-test), and Spearman’s rank correlation performed in Methods 3, 4, and 5. A P value of less than 0.05 was considered as statistically significant. All P values used were two-tailed.

**Results**

The scores [median (Q1, Q3), IQR] of the diseases are shown in Table 1. The scores [median (Q1, Q3), IQR] of the benign cases (190 images of 12 cases) and malignant cases (237 images of 11 cases) were 18.0 [(17.1, 18.9), 1.8]
and 23.0 [(21.8, 24.1), 2.3], respectively. The results of ROC analysis are shown in Figure 2. The optimum cut-off score to distinguish the benign and malignant cases was 20.75 ($P < 0.01$). AUC of this cut-off score was 0.93, showing a high accuracy, and the sensitivity, specificity, and diagnostic accuracy were 90.9, 100%, and 95.7%, respectively, clarifying that the lesion can be judged as malignant at a high accuracy when the score is 20.75 or higher.

The scores of the scoring items [median (IQR)] of the benign and malignant cases and the cut-off score of each item to differentiate between the benign and malignant cases and their sensitivity, specificity, and accuracy are shown in Table 2. Of the cluster atypia items, the following items were useful to judge breast cancer: 1, scattered epithelial cells; 2, uneven irregular cluster edge; and 3, irregular nuclear overlapping. The cellular atypia items useful to judge breast cancer were as follows: 8, chromatin distribution and 10, absence of myoepithelial cells. Of these characteristics, the sensitivity of the following items was high (80% or higher): 2, uneven irregular cluster edge; 3, irregular nuclear overlapping; 7,
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Figure 2: ROC curve: best cut-off value for differentiation between benign and malignant cells; score = 20.75; AUD: 0.93 ($P < 0.01$), representing a sensitivity of 90.9%, a specificity of 100%, and an accuracy of 95.7%

Table 1: Score value of breast FNAC for each histological diagnosis

| Histological diagnosis       | Score value of cytology | Number of analysis images | Score value Median [Q1, Q3], IQR |
|------------------------------|-------------------------|---------------------------|---------------------------------|
| Benign Mastopathy            | 38                      | 19.0 [18.8, 19.3], 0.5    | 18.0 [17.1, 18.9], 1.8          |
| Intraductal papilloma        | 152                     | 17.8 [17.0, 18.3], 1.3    |                                 |
| Malignant Ductal carcinoma in situ | 168                | 21.8 [21.3, 22.4], 1.1    | 23.0 [21.8, 24.1], 2.3          |
| Invasive ductal carcinoma    | 69                      | 24.1 [24.1, 24.2], 0.1    |                                 |
| Total                        | 427                     | *P=0.003                  |                                 |

FNAC: fine needle aspiration cytology; Q1: the value of the first quartile; Q3: the value of the third quartile; IQR: interquartile range *Mann-Whitney U-test

Table 2: Score value of cytology for benign and malignant lesions

| Cluster atypia                | Benign, median [IQR] (range: 1-3) | Malignant, median [IQR] (range: 1-3) | $P^*$ | Cut-off value** | Sensitivity | Specificity | Accuracy |
|------------------------------|-----------------------------------|--------------------------------------|-------|-----------------|-------------|------------|----------|
| Scattered epithelial cells   | 1.8 [0.7]                         | 2.8 [1.0]                           | 0.002 | 2.25            | 72.7        | 92.0       | 82.6     |
| Uneven irregular cluster edge | 2.0 [0.6]                         | 2.5 [0.5]                           | 0.003 | 2.25            | 90.9        | 75.0       | 82.6     |
| Irregular nuclear overlapping | 1.8 [0.5]                         | 2.5 [0.5]                           | 0.001 | 2.25            | 90.9        | 83.0       | 87.0     |
| Irregularity of the nuclear size | 1.5 [0.4]                       | 1.8 [1.0]                           | 0.104 | 1.75            | 63.6        | 58.3       | 42.4     |
| Nuclear morphological irregularity | 1.5 [0.4]                   | 1.8 [0.5]                           | 0.122 | 1.75            | 72.7        | 58.3       | 65.2     |
| Deep dyeing chromatin        | 2.5 [0.5]                         | 2.5 [0.5]                           | 1.00  | 2.25            | 72.7        | 33.3       | 52.2     |
| Chromatin granularity        | 1.6 [0.7]                         | 1.8 [0.5]                           | 0.124 | 1.75            | 81.8        | 50.0       | 65.2     |
| Chromatin distribution       | 1.8 [0.5]                         | 2.3 [0.8]                           | 0.019 | 2.25            | 54.5        | 92.0       | 73.9     |
| Large nucleoli               | 1.5 [0.4]                         | 2.5 [1.3]                           | 0.057 | 2.5             | 63.6        | 83.0       | 73.9     |
| Absence of myoepithelial cells | 1.5 [0.4]                       | 3.0 [0.0]                           | 0.002 | 3.0             | 90.9        | 75.0       | 87.6     |
| Score total points (range: 10-30) | 18.1 [1.9]                    | 23.0 [2.5]                          | <0.001 | 20.75            | 90.9        | 100        | 95.7     |

IQR: interquartile range *Mann–Whitney U-test **Receiver operating characteristic (ROC) curve for differentiation between benign and malignant cells

Discussion

Several studies on cytological scoring as a breast FNAC evaluation method have been reported,[12-20] and Robinson grading[12,13] has been widely used in studies on a grading system comprising six items of nuclear findings. Robinson et al.[12,13] set the following six items as the criteria for nuclear atypia classification: “Cell dissociation,” “Nuclear size,” “Cell uniformity,” “Nucleoli,” “Nuclear margin,” and “Chromatin pattern.” The results of cell scoring using the Robinson grading were compared with those of the histological-diagnostic grading method (Bloom–Richardson histopathological grading[14-18]) and Mouriquand’s grading method[19] in recent studies. These studies reported that Robinson grading is a useful
method reflecting the prognosis and evaluating malignancy. In addition, a study on scoring a total of 12 items has been reported in which six items, “Overlapping pattern of the cells,” “Presence of necrosis,” “Presence of mucin,” “Appearance of myoepithelial cells,” “Appearance of bipolar bare nuclei,” and “Appearance of foam cell,” were added to the six items of Robinson grading.[20] Our scoring system was different in that it comprised the following 10 scoring items: 3 items of cluster atypia, “1: Scattered epithelial cells,” “2: Uneven irregular cluster edge,” and “3: Irregular nuclear overlapping,” and 7 scoring items of cellular atypia, “4: Irregularity of the nuclear size,” “5: Nuclear morphological irregularity,” “6: Deep dyeing chromatin,” “7: Chromatin granularity,” “8: Chromatin distribution,” “9: large nucleoli,” and “10: Absence of myoepithelial cells.” The characteristic of this study was the addition of the three findings related to cluster structure: “1: Scattered epithelial cells,” “2: Uneven irregular cluster edge,” and “3: Irregular nuclear overlapping,” and 7 scoring items of cellular atypia, “4: Irregularity of the nuclear size,” “5: Nuclear morphological irregularity,” “6: Deep dyeing chromatin,” “7: Chromatin granularity,” “8: Chromatin distribution,” “9: large nucleoli,” and “10: Absence of myoepithelial cells.” The characteristic of this study was the addition of the three findings related to cluster structure: “1: Scattered epithelial cells,” “2: Uneven irregular cluster edge,” and “3: Irregular nuclear overlapping.” No previous scoring method has closely evaluated atypia of the cluster structure. In addition, no previous study has investigated the chromatin by scoring three items: “6: Deep dyeing Chromatin,” “7: Chromatin granularity,” and “8: Chromatin distribution,” through which the nuclear findings could be closely evaluated. It was clarified that in the present scoring system, the lesion can be judged as malignant at a high accuracy when the cut-off score is 20.75 or higher (accuracy: 95.7%, sensitivity: 90.9%, specificity: 100%).

Regarding the characteristics of scoring in breast cancer, the scores of the following five items were significantly higher than those in the noncancer cases “1, Scattered epithelial cells,” “2, Uneven irregular cluster edge,” and “3, Irregular nuclear overlapping,” “6, Chromatin distribution” and “10, Absence of myoepithelial cells” of the cellular items. The sensitivity was especially high (80% or higher) in the following items: “2, Uneven irregular cluster edge,” “3, Irregular nuclear overlapping,” “7, Chromatin granularity,” and “10, Absence of myoepithelial cells.” The specificity was high (80% or higher) in the following items: “1, Scattered epithelial cells,” “3, Irregular nuclear overlapping,” “6, Chromatin distribution,” and “9, Large nucleoli.” Ryu et al.[20] reported that the scores of the breast cancer items: “Nuclear margin,” “Pattern of Chromatin,” “Overlapping pattern of the cells,” and “Appearance of myoepithelial cell” were significantly high, being consistent with the findings of this study. Furthermore, it has been reported that evaluation by simultaneously scoring the grades of nuclear atypia and structural atypia of clusters representing histological atypia is important because the grade of histological atypia is II or III.
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No

[12-20]

[14-16]

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The characteristics of the breast cancer cytology findings were investigated with regard to the correlation among the scoring items. The items likely to simultaneously develop in the same preparation were identified based on the correlation among the scoring items, suggesting that evaluation of differences in the correlation among the scoring items between benign and malignant cases serves as an important index of their differentiation. No previous study investigated scoring evaluation of breast FNAC from this viewpoint.[12-20] No correlation was noted in the benign cases in the following findings, whereas they appeared with significant correlation in the malignant cases: (1) 1, scattered epithelial cells became marked as 2; uneven irregular cluster edge became marked (rs = 0.8), (2) 4, irregularity of the nuclear size became marked as 5; nuclear morphological irregularity became marked (rs = 0.6), (3) 8, chromatin distribution became marked as 9; large nucleoli marked (rs = 0.6), and (4) 6, deep dyeing chromatin became marked as 7; chromatin granularity marked (rs = 0.5). These were novel findings for judging cancer by scoring evaluation of the breast.

The breast FNAC scoring method is a useful technique to differentiate benign from malignancy of breast lesions accompanied by breast ductal dilation and cystic lesions and to identify the useful findings for judging cells.

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Conflicts of interest

There are no conflicts of interest.

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