ORIGINAL ARTICLE OPEN ACCESS

Scoring system of fine needle aspiration cytology samples for the detection of non-high-grade ductal breast carcinoma

Ayumi Ryu, Akemi Takenaka, Shigenori Nagata, Yasuhiko Tomita

ABSTRACT

Introduction: Although fine needle aspiration cytology (FNAC) is usually performed for breast lesions that are difficult to diagnose clinically and radiologically, in cases of carcinoma definitive diagnosis by FNAC is difficult in cases lacking obvious nuclear atypia, that are non-high-grade ductal carcinoma (NHGDC). Aims: We aimed to clarify cytopathological characteristics useful for differential diagnosis between NHGDC and benign breast lesions. Methods: We enrolled 62 patients who were difficult to diagnose or who were suspected of malignancy by routine FNAC examination. The definitive histopathological diagnosis was breast cancer in 42 patients and others in 21. The slides were re-examined to classify under the following 12 categories: dissociation of cells, cell size, cell uniformity, prominent nucleoli, nuclear margins, chromatin pattern, overlapping pattern of cells, presence of necrosis, presence of mucin, appearance of myoepithelial cells, appearance of bipolar bare nuclei, and appearance of foam cells. Results: Univariate analysis revealed that chromatin pattern, nuclear margins, irregular overlapping of cells, appearance of myoepithelial cells, and appearance of bipolar bare nuclei were statistically significant. Multivariate analysis revealed that irregular overlapping of cells and appearance of myoepithelial cells were independent factors that were useful for differential diagnosis. Combination of these factors proved to be a useful marker in the differential diagnosis of breast lesions. Conclusion: Application of the scoring system to future cases of breast lesions might clarify the usefulness of the system.

Keywords: Fine needle aspiration cytology, Non-high-grade ductal carcinoma, Multivariate analysis, Scoring system

Ayumi Ryu[1], Akemi Takenaka[2], Shigenori Nagata[3], Yasuhiko Tomita[4]

Affiliations: [1]Cytologist, Department of Cytology, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Osaka, Japan; [2]Chief Cytologist, Department of Cytology, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Osaka, Japan; [3]Staff, Department of Pathology, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Osaka, Japan; [4]Chief, Department of Pathology, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Osaka, Japan.

Corresponding Author: Tomita Yasuhiko, MD, PhD, Department of Pathology, Osaka Medical Center for Cancer and Cardiovascular Diseases, 1-3-3 Nakamichi, Higashinari, Osaka, Osaka 537-8511, Japan; Ph: 81-6-6972-1181; Fax: 81-6-6981-8336; Email: yasuhiko-tomita@umin.ac.jp

Received: 18 February 2012
Accepted: 25 February 2012
Published: 09 January 2013

****

Ryu A, Takenaka A, Nagata S, Tomita Y. Scoring system of fine needle aspiration cytology samples for the detection of non-high-grade ductal breast carcinoma. Oncocytology Journal 2013;3:1–7.

Article ID: 100004OCYAR2013

****

doi:10.5348/ocy-2013-4-OA-1

INTRODUCTION

The recent increase in use of mammography and ultrasonography has enabled the detection of breast

cancer at an early stage [1]. Cancers detectable only with these diagnostic imaging methods are usually ductal carcinoma in situ or small-sized invasive ductal carcinoma, with lower histological grades than those detected by conventional methods [1, 2]. Therefore, the chance of encountering cases that are difficult for differential diagnosis is increasing. At the same time, the chance of accidental detection of benign breast lesions such as mastopathy and papilloma by mammography and/or ultrasonography have rapidly increased [3].

Histological examination of needle biopsy specimens or fine needle aspiration cytology (FNAC) is usually performed for cases that are difficult to diagnose clinically and radiologically [4]. As it is less invasive to the patients compared with histological examination, FNAC is being increasingly performed [5]. Cytopathological features of malignant and benign lesions have been previously reported [6]; nevertheless, the differential diagnosis between benign and malignant lesions is difficult in some cases [7-9].

In the cytological examination of breast tumors, the following features are considered as general criteria for malignancy: presence of necrosis and dissociated cells, irregular overlapping of cells, absence of myoepithelial cells, and presence of mucin and intracytoplasmic lumina [6]. In addition, nuclear atypia such as enlarged nuclei, nuclear pleomorphism and irregular chromatin are the core factors for the diagnosis of malignancy. However, lack of nuclear atypia in cases of non-high-grade ductal carcinoma (NHGDC) might diminish the diagnostic utility of these criteria [7, 8].

In the present study, cases that were difficult to diagnose definitively by FNAC were collected and their cytopathological characteristics were re-examined. The results were analyzed using multivariate analysis to determine sets of parameters on FNAC which will be useful in differential diagnosis and a scoring system using these factors was formulated.

MATERIALS AND METHODS

This study included 5682 patients who underwent FNAC for examination of breast lesions at our institute from August 2005 to October 2010. After aspiration of the breast lesion, samples were spread on glass slides, fixed in 95% ethanol and stained according to Papanicolaou's method. Out of 5682 patients, 174 (3.1%) and 205 (3.6%) patients were considered difficult to diagnose or were suspected of malignancy, respectively. The cytology slides from these patients were retrospectively reviewed. Forty-two cases were defined as being suitable for definitive diagnosis, and 246 specimens were considered inadequate for diagnosis. Twenty-eight patients were diagnosed with tumors with special histological features, such as invasive lobular carcinoma, mucinous carcinoma, and stromal sarcoma, and they were excluded from the analysis. Finally, 63 cases were enrolled for the present study. The definitive histopathological diagnosis was breast cancer in 42 cases and others in 21 cases (Table 1).

The slides were re-examined to classify the following 12 categories: dissociation of cells, cell size, cell uniformity, prominent nucleoli, nuclear margins, chromatin pattern, overlapping pattern of cells, presence of necrosis, presence of mucin, appearance of cells, appearance of bipolar bare nuclei and appearance of foam cells (Table 2). The former six categories were determined according to the criteria by Robinson et al. [10] (Figure 1). Overlapping pattern of cells was determined as follows: cellular structure was determined as regulated (Figure 2A), or deregulated (Figure 2B), then overlapping pattern was determined as score 1 (mostly regulated), score 2 (combined regulated and deregulated), or score 3 (mostly deregulated). Representative cases with myoepithelial cells, bipolar bare nuclei and foam cells are shown in figure 3.

For nuclear characteristics, slides were carefully examined using a 100× objective lens. The results were statistically examined for their diagnostic utility using the $\chi 2$ test and Fisher's exact probability test. Then, a logistic regression model was applied to clarify independent factors.

RESULTS

The relationship between cytological parameters is summarized in table 3 and the statistical significance of each parameter for the diagnosis in table 4. The

Table 1: Histological diagnosis of the 62 breast lesions.

	Initial FNAC Diagnosis		
	Difficult to Diagnose	**Suspect of Malignancy**	**Total**
Benign lesions			
Fibroadenoma	4	1	5
Papilloma	3	1	4
Mastitis	1	3	4
Sclerosing adenosis	2	1	3
Ductal adenoma	2		2
Ductal hyperplasia	1		1
Phyllodes tumor (benign)	1		1
Phyllodes tumor (borderline)	1		1
Malignant lesions			
Ductal carcinoma in situ	1	6	7
Invasive ductal carcinoma with a predominant intraductal components	2	5	7
Invasive ductal carcinoma	4	24	28

Table 2: Criteria for cytological classification of breast lesions.

Categories	**Score 1**	**Score 2**	**Score 3**
Dissociation of the cells	Cells mostly in clusters	Mixture of single cells and clusters	Mostly single cells
Size of the cells	1-2 ×RBC size	3-4 ×RBC size	≥5 ×RBC size
Cellular uniformity	Monomorphic	Mildly pleomorphic	Pleomorphic
Nucleoli	Indistinct	Noticeable	Prominent or multiple
Nuclear margin	Smooth	Folds	Buds or clefts
Pattern of chromatin	Vesicular	Granular	Clumped and cleared
Overlapping pattern of the cells	Mostly regulated	Combined regulated and deregulated	Mostly deregulated
Presence of necrosis	Absent	Few	Marked
Presence of mucin	Absent	Few	Marked
Appearance of myoepithelial cell	Absent	Mixture of cell clusters with and without myoepithelial cell	Most cell clusters observed with myoepithelial cell
Appearance of bipolar bare nuclei	Absent	Few	Many
Appearance of foam cell	Absent	Few	Many

Table 3: Univariate analysis of each cytological category for the differentiation between benign and malignant lesions.

	Benign lesions (n=21)			**Malignant lesions (n=42)**			
Categories	**Score 1**	**Score 2**	**Score 3**	**Score 1**	**Score 2**	**Score 3**	p **value**
Dissociation of the cells	17	4	0	34	8	0	N.S.
Size of the cells	18	3	0	37	5	0	N.S.
Cellular uniformity	17	4	0	37	5	0	N.S.
Nucleoli	17	4	0	31	11	0	N.S.
Nuclear margin	18	3	0	20	22	0	<0.01*
Pattern of chromatin	21	0	0	37	5	0	<0.05*
Overlapping pattern of the cells	6	8	7	4	8	30	<0.01**
Presence of necrosis	21	0	0	42	0	0	N.S.
Presence of mucin	21	0	0	42	0	0	N.S.
Appearance of myoepithelial cell	2	11	8	31	11	0	<0.0001***
Appearance of bipolar bare nuclei	3	14	4	29	10	3	<0.0001*
Appearance of foam cell	13	4	4	29	10	3	N.S.

Abbreviations: N.S. - Not Significant, * - Score 1 vs. 2+3, ** - Score 1+2 vs. 3

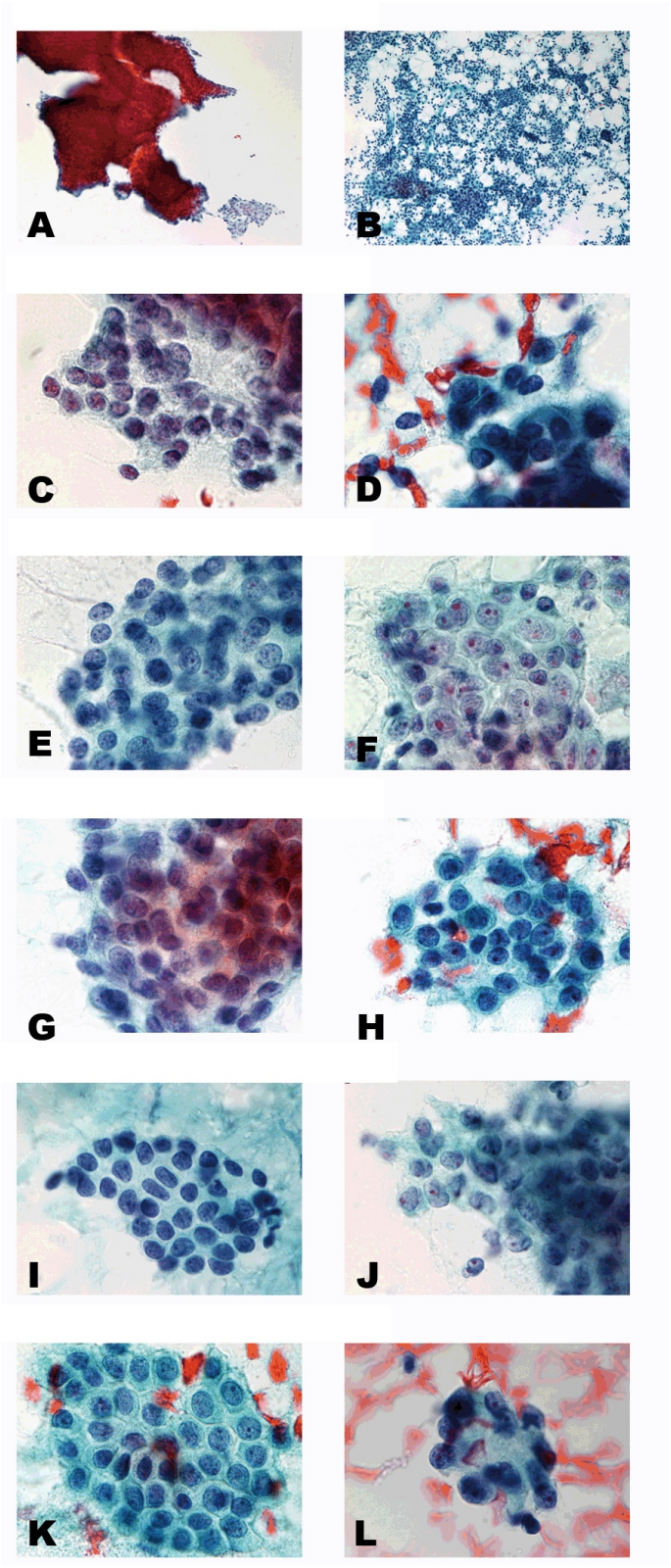

Figure 1: Representative pattern of cytological characteristics: A, B) Dissociation of the cells (A: Score 1, 10x, B: Score 2, 10x), C, D) Size of the cells (C: Score 1, 100x, D: Score 2, 100x), E, F) Cellular uniformity (E: Score 1, 100x, F: Score 2, 100x), G, H) Nucleoli (G: Score 1, 100x, H: Score 2, 100x), I J) Nuclear margin (I: Score 1, 100x, J: Score 2, 100x), K, L) Pattern of chromatin (K: Score 1, 100x, L: Score 2, 100x). (All slides were stained by Papanicolaou's method.)

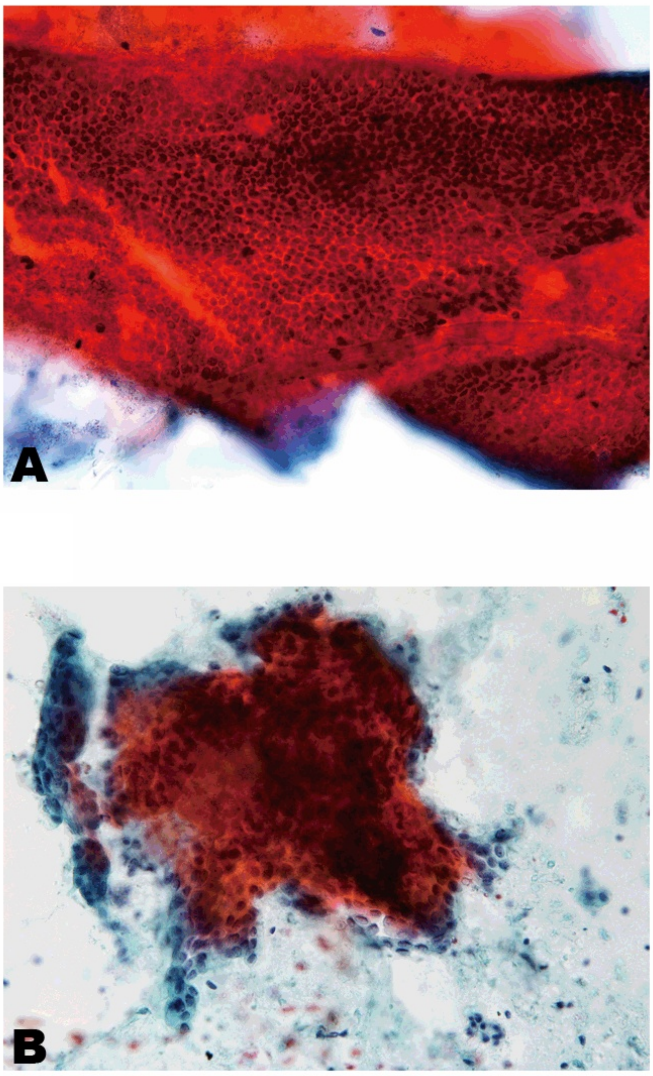

Figure 2: Overlapping pattern of the cells: A) Regulated, 20x, B) Deregulated, 20x (Stained by Papanicolaou's method).

following factors were shown to be statistically significant for detection of malignancy: chromatin pattern ($p < 0.05$: score 1 vs. score 2); nuclear margins ($p < 0.01$: score 1 vs. score 2); overlapping pattern of cells ($p < 0.01$: scores 1+2 vs. score 3); appearance of myoepithelial cells ($p < 0.0001$: score 1 vs. scores 2+3, scores 1+2 vs. score 3); and appearance of bipolar bare nuclei ($p < 0.0001$: score 1 vs. scores 2+3). Multivariate analysis revealed that three factors were independently significant: overlapping pattern of cells (scores 1+2 vs. score 3); and appearance of myoepithelial cells (score 1 vs. scores 2+3 and scores 1+2 vs. score 3) (table 4).

Using these three parameters that were significant in multivariate analysis, a grading system for FNAC samples was established. Each case was scored by appearance of myoepithelial cells (0–2) and overlapping pattern of cells (0 or –1), then all the scores were added together. There were 21 cases with a score of –1 (all malignant), 24 with 0 points (19 malignant and five

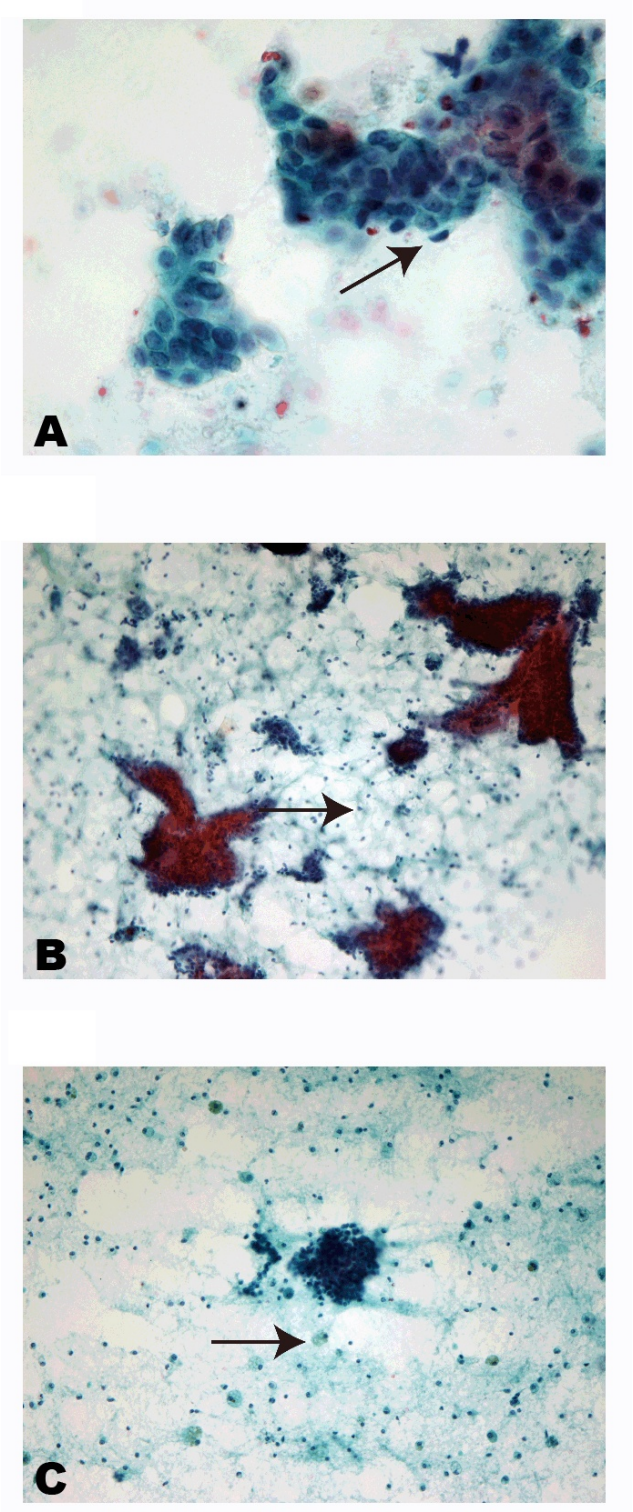

Figure 3: Arrows shows, A) Appearance of myoepithelial cells 40x), B) Bare bipolar nuclei (10x), and C) Foam cells (10x). All slides stained by Papanicolaou's method.

benign), 14 cases with 1 point (two malignant and 12 benign), and four cases with 2 points (all benign) (figure 4).

Table 4: Multivariate analysis of categories for the differentiation between benign and malignant lesions.

Categories	Classification	Chi-square value	p value
Overlapping pattern of the cells	1: Score 1+2 0: Score 3	10.71	0.0011
Appearance of myoepithelial cells	1: Score 2+3 0: Score 1	15.54	<0.0001
Appearance of myoepithelial cells	1: Score 3 0: Score 1+2	10.17	0.0014

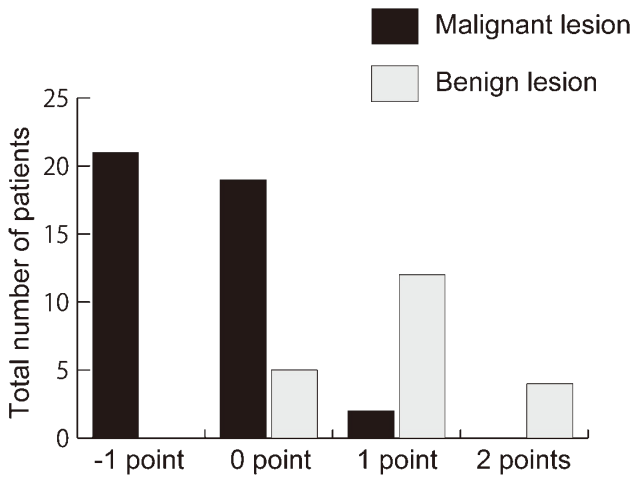

Figure 4: Stratification of breast lesion with the combination of appearance of myoepithelial cells (0, 1, 2) and overlapping pattern of the cells (0, -1).

DISCUSSION

It is clear that the presence or absence of nuclear atypia is the core diagnostic factor in the differential diagnosis of breast FNAC samples [6, 11, 12]. However, the presence of breast cancer without obvious nuclear atypia, namely NHGDC, is a confusing event in the diagnostic process [11]. The present study was designed to detect factors useful in distinguishing NHGDC from its benign counterparts. We showed that combination of two factors was useful in the differentiation between malignant and benign lesions.

Lim et al. have found that no morphological criteria, such as cellularity, nuclear enlargement, and discohesion, were useful for differentiation between benign and malignant lesions in FNAC of breast tissue [12]. Kumarasinghe et al. have concluded that cell discohesion, bare atypical nuclei, bipolar bare nuclei, and necrosis are discriminating features between NHGDC in situ and benign breast lesions [7]. The

present study showed that chromatin pattern, nuclear margins, overlapping pattern of cells, appearance of myoepithelial cells and appearance of bipolar bare nuclei were significant in distinguishing between malignant and benign lesions, although the discriminating utility of each factor was limited, which is consistent with previous reports [7, 11, 12].

Nuclear atypia is a generic term for several characteristics that indicate malignancy, such as nuclear size, nucleus to cytoplasm size ratio, nuclear pleomorphism, nuclear membrane morphology, presence of nucleoli and pattern of chromatin [13, 14]. Nucleus to cytoplasm size ratio is not commonly used for diagnosis of breast lesions stained with Papanicolaou's method as it is usually increased in non-cancerous ducts because their cytoplasm is fragile and destroyed. All the specimens in the present study had small monomorphic nuclei and no difference was observed between the specimens. Some specimens had prominent nucleoli, irregular nuclear margins and granular chromatin. Among these, most specimens with irregular nuclear margins and granular chromatin were breast cancer and these two factors proved to be significant in differential diagnosis.

Structure of the cell cluster was examined in the following two categories: dissociation of the cells and overlapping pattern of the cells. We showed that overlapping pattern of the cells was useful in differential diagnosis. Lack of cell-to-cell cohesion is considered to be characteristic of malignant lesions [6], but the present study demonstrated that a considerable percentage of benign lesions contain dissociated cells or small clusters and therefore the utility of cell cluster size in differential diagnosis is limited. However, deregulated overlapping was observed in most cases of breast cancer, thus it could be a useful marker.

Presence of necrosis and mucin are important indicators of malignancy [6]; however, these findings were not observed in the present series.

Coexistence of myoepithelial cells and bipolar bare nuclei are useful markers that are suggestive of benign breast lesions [6, 12]. However, two of 21 benign lesions did not have myoepithelial cells. Even in benign breast lesions, lack of biphasic (epithelial and myoepithelial) cell pattern was observed in the specimen when excess proliferation of epithelial cells compared with myoepithelial cells developed. Bipolar bare nuclei are round or oval cells with faint cytoplasm, are often found in fibrous adenoma and originate from myoepithelial cells [15]. It has been demonstrated that fibroblasts also appear as spindle cells with bare nuclei [16]. Fibroblasts are observed in benign and malignant breast lesions, especially breast cancer with invasion [16]. The former (benign lesions) show myoepithelial characteristics, confirmed by immunoreactivity to p63 but the latter (malignant lesions) do not [15]. Nevertheless, morphological differentiation of the two on slides stained with Papanicolaou's method is difficult [16]. In the present study, two cases of breast cancers had bipolar bare nuclei, both of which proved to be invasive ductal carcinoma. Foam cells are found both in benign and malignant lesions; however, their appearance is not so common in malignant lesions. In the present study, marked foam cell appearance was observed in four cases, three of which were benign. Because of the small number of cases, this factor was not a significant factor in univariate analysis.

CONCLUSION

In the present study, several factors proved useful in the differential diagnosis of breast lesions, but none of these were found to be necessary or sufficient for differential diagnosis. Breast lesions without obvious nuclear atypia, necrosis and mucin are difficult to diagnose; therefore, diagnosis should be made by observation of several independent characteristics. The present scoring system showed that appearance of myoepithelial cells and overlapping of cells were useful for diagnosis of breast lesions. Application of the scoring system to future breast lesions might clarify the usefulness of the system.

****

Author Contributions

Ayumi Ryu – Conception and design, Acquisition of data, Analysis and interpretation of data, Drafting the article, Critical revision of the article
Akemi Takenaka – Drafting the article, Critical revision of the article
Shigenori Nagata – Drafting the article, Critical revision of the article
Yasuhiko Tomita – Drafting the article, Critical revision of the article, Final approval of the version to be published

Guarantor

The corresponding author is the guarantor of submission.

Conflict of Interest

Authors declare no conflict of interest.

REFERENCES

1. Allgood PC, Duffy SW, Kearins O, et al. Explaining the difference in prognosis between screen-detected and symptomatic breast cancers. Br J Cancer 2011;104(11):1680–5.

2. Dawson SJ, Duffy SW, Blows FM, et al. Molecular characteristics of screen-detected vs symptomatic breast cancers and their impact on survival. Br J Cancer 2009;101(8):1338–44.
3. Kneeshaw PJ, Lowry M, Manton D, Hubbard A, Drew PJ, Turnbull LW. Differentiation of benign from malignant breast disease associated with screening detected microcalcifications using dynamic contrast enhanced magnetic resonance imaging. Breast 2006;15(1):29–38.
4. Kocjan G, Bourgain C, Fassina A, et al. The role of breast FNAC in diagnosis and clinical management: a survey of current practice. Cytopathology 2008;19(5):271–8.
5. Feoli F, Paesmans M, Van Eeckhout P. Fine needle aspiration cytology of the breast: impact of experience on accuracy, using standardized cytologic criteria. Acta Cytol 2008;52(2):145–1.
6. Pathology Non-operative Diagnosis Subgroup of the National Coordinating Group for Breast Screening. Guidelines for non-operative diagnostic procedures and reporting in breast cancer screening. NHS Cancer Screening Programmes 2001;50.
7. Kumarasinghe MP, Poh WT. Differentiating nonhigh-grade duct carcinoma in situ from benign breast lesions. Diagn Cytopathol 2004;30(2):98–102.
8. Cangiarella J, Waisman J, Simsir A. Cytologic findings with histologic correlation in 43 cases of mammary intraductal adenocarcinoma diagnosed by aspiration biopsy. Acta Cytol 2003;47(6):965–72.
9. Ozkara SK, Ustun MO, Paksoy N. The gray zone in breast fine needle aspiration cytology. How to report on it? Acta Cytol 2002;46(3):513–8.
10. Robinson IA, McKee G, Nicholson A, et al. Prognostic value of cytological grading of fine-needle aspirates from breast carcinomas. Lancet 1994;343(8903):947–9.
11. Karimzadeh M, Sauer T. Diagnostic accuracy of fine-needle aspiration cytology in histological grade 1 breast carcinomas: are we good enough? Cytopathology 2008;19(5):279–86.
12. Lim JC, Al-Masri H, Salhadar A, Xie HB, Gabram S, Wojcik EM. The significance of the diagnosis of atypia in breast fine-needle aspiration. Diagn Cytopathol 2004;31(5):285–8.
13. Bjelkenkrantz K, Stål O, Gröntoft O, Olofsson J, Herder A, Hellquist H. HISTOSCAN: computer program for cytophotometry in tissue sections and its application in the evaluation of nuclear atypia. Histochemistry 1981;73(3):353–62.
14. Peterse JL, Koolman-Schellekens MA, van de Peppel-van de Ham T, van Heerde P. Atypia in fine-needle aspiration cytology of the breast: a histologic follow-up study of 301 cases. Semin Diagn Pathol 1989;6(2):126–34.
15. Reis-Filho JS, Albergaria A, Milanezi F, Amendoeira I, Schmitt FC. Naked nuclei revisited: p63 Immunoexpression. Diagn Cytopathol 2002;27(3):135–8.
16. Tsuchiya S, Maruyama Y, Koike Y, Yamada K, Kobayashi Y, Kagaya A. Cytologic characteristics and origin of naked nuclei in breast aspirate smears. Acta Cytol 1987;31(3):285–90.