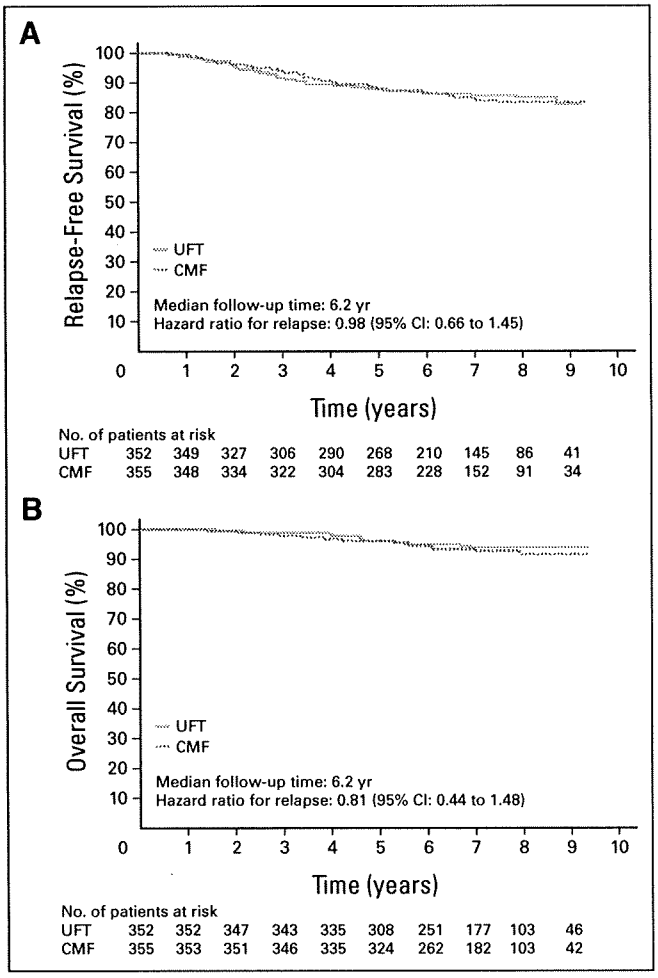

Fig 3. (A) Relapse-free survival, (B) overall survival of the total patients treated with uracil and tegafur (UFT) or cyclophosphamide, methotrexate, and fluorouracil. CPA, cyclophosphamide; MTX, methotrexate.

RFS subgroup analyses. The subgroups were not sufficiently large to allow definitive statistical conclusions to be established, but lower nuclear grade seemed to be beneficial in the UFT arm.

The 5-year survival rate was 96.0% in the CMF arm and 96.2% in the UFT arm, and the hazard ratio was 0.81 in the UFT arm (95% CI, 0.44 to 1.48) as compared with the CMF arm ($P = .49$, log-rank test; Fig 3B). The adjusted hazard ratio for OS was 0.82 (95% CI, 0.45 to 1.49; $P = .51$). Among the 19 deaths in the UFT arm, 14 patients died from breast cancer, two from a second cancer, and three from unknown causes. Among the 24 deaths in the CMF arm, 18 patients died from breast cancer, four from a second cancer, and two from other causes (one was an accident, the other was unknown).

Safety

The overall incidence of grade 2 or more severe adverse events did not differ statistically between the two arms (UFT *v* CMF, 85% *v* 80%). Grade 3 or 4 adverse events occurred in fewer than 10% of the patients in both arms (Table 2). The incidence of grade 3 or 4 leucopenia was significantly higher in the CMF arm, and the incidences of grade 3 or 4 diarrhea, anemia, elevated AST, and elevated serum total bilirubin were significantly higher in the UFT arm. The incidence of alopecia (any grade) was lower in the UFT group (9.7%) than in the CMF group (55.2%).

QOL

QOL assessments were conducted in 94% of patients at baseline, 90% after 1 month of treatment, 86% at 4 months, 79% at 12 months, and 61% at 27 months. The time courses of the QOL scores as assessed by EORTC QLQ-30/BR23 and the FACT-B questionnaire are shown in Figures 5A and 5B. QOL in the patients who received UFT was significantly better than that in the patients who received CMF with respect to variables such as social functioning ($P = .045$), nausea/vomiting ($P = .003$), constipation ($P = .011$), systemic adverse effects ($P = .02$), and upset by hair loss ($P = .02$) on QLQ-C30/BR23, and physical well-being and the breast cancer subscale on FACT-B. No significant differences were seen for other symptoms.

DISCUSSION

This randomized phase III trial evaluated postoperative chemotherapy regimens in patients with node-negative, high-risk breast cancer. One limitation of our trial is that the statistical power is not yet sufficient to undisputably establish the noninferiority of oral UFT as compared with classical CMF, at present, we cannot rule out the possibility that UFT may be inferior to CMF. However, there was no significant difference in efficacy between oral UFT and classical CMF in terms of RFS and OS. In addition, the Kaplan-Meier curves of these two regimens for RFS and OS appear to be superimposable (Figs 3A, 3B). Therefore our preliminary results strongly suggest that UFT is comparable to classical CMF in terms of inhibiting recurrence in patients with primary breast cancer.

The 5-year RFS was 88.0% in the CMF arm and 87.8% in the UFT arm, and the 5-year OS was 96.0% and 96.2%, respectively, indicating very good outcomes. The Early Breast Cancer Trialists' Collaborative Group reported that the 5-year survival rate was 86.6% in patients with node-negative breast cancer who received polychemotherapy and were younger than 50 years and 84.1% in those age 50 to 69 years.[11] The INT-0137 (S9313) study reported that the rate of disease-free survival at 5 years in patients with node-negative breast cancer was 83% after four courses of concurrent treatment with AC and 82% after four courses of sequential treatment with AC.[20] OS and RFS in both the CMF and UFT arms of our study compared favorably with the outcomes with polychemotherapy or AC in those studies.

Grade 3 or 4 adverse events were not common in either arm. Leucopenia and alopecia were significantly more frequent in the CMF arm, while liver dysfunction, anemia, and diarrhea were significantly more frequent in the UFT arm. Although oral fluoropyrimidines, such as UFT, are generally considered to have a relatively low frequency of adverse events, the incidence of liver dysfunction was unexpectedly high in our study. Capecitabine has been reported to often cause hyperbilirubinemia.[21] One potential cause of hyperbilirubinemia associated with oral fluoropyrimidines is hemolysis.[22] In addition to hyperbilirubinemia, 5% of the patients in the UFT arm had grade 3 or 4 elevations of ALT and AST. Patients who receive UFT or other oral fluoropyrimidines should therefore be closely monitored for liver toxicity and undergo liver function tests at regular intervals.

QOL assessments with the use of EORTC QLQ30/BR23 and FACT-B showed that QOL was significantly better in the UFT arm

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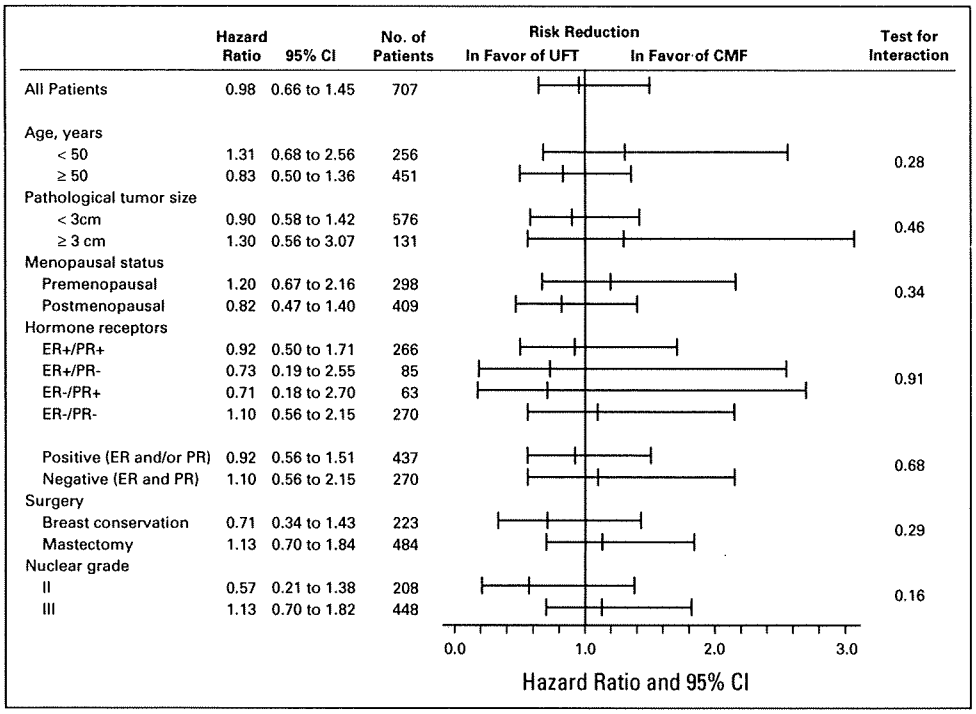

Fig 4. Results of subset analysis (uracil and tegafur [UFT] *v* cyclophosphamide, methotrexate, and fluorouracil): recurrence. CPA, cyclophosphamide; MTX, methotrexate.

than in the CMF arm. Duric et al[23] reported that patients perceive hair loss to be the worst type of toxicity. The difference in the incidence of hair loss was clearly reflected in the QOL scores for UFT and CMF. Patients with cancer generally prefer oral alternatives to intravenous chemotherapy, provided that efficacy is similar.[24] As the use of oral chemotherapeutic agents increases worldwide,[25] their safety and efficacy will receive further attention.

Cancer and Leukemia Group B (CALGB) has reported the results of a phase III study comparing six courses of capecitabine with six courses of CMF or four courses of AC in patients with breast cancer who were 65 years or older (CALGB 49907). Capecitabine was clearly shown to be inferior to CMF or AC in terms of RFS and OS.[26] In our study, UFT and CMF were associated with similar RFS and OS. One reason for the difference in response between our study and the CALGB 49907 study may be the longer treatment period for UFT (2 years) than for capecitabine (six courses, 18 weeks). Patients with breast cancer have a long-term risk of recurrence.[27] One study showed a trend toward better suppression of recurrence with 2 years of UFT treatment than with 1 year.[9] Experimental evidence suggests that the prevention of recurrence by UFT involves not only the cytotoxic activity of fluorouracil, but also the inhibition of angiogenesis.[28] Long-term treatment with UFT might maintain the dormant status of micrometastasis and thereby prevent recurrence.

Table 2. Most Common Adverse Events

	Grade (%)								
	UFT (n = 352)			CMF (n = 355)			*P*		
Toxicity	Any	2	3/4	Any	2	3/4	Any	2	3/4
Hematologic									
Anemia			1.4			0.0			.03
Neutropenia			3.5			5.5			.27
Leukopenia			0.3			3.1			< .01
Nonhematologic									
AST			5.7			1.4			< .01
ALT			8.9			5.1			.07
Total bilirubin			5.5			0.3			< .01
Nausea and vomiting			1.1			2.8			.18
Diarrhea			2.0			0.3			.04
Hair loss*	9.7	0		55.2	2.5		< .01	< .01	

Abbreviations: UFT, uracil and tegafur; CMF, cyclophosphamide, methotrexate, fluorouracil.
*No category for hair loss of grade 3 or higher.

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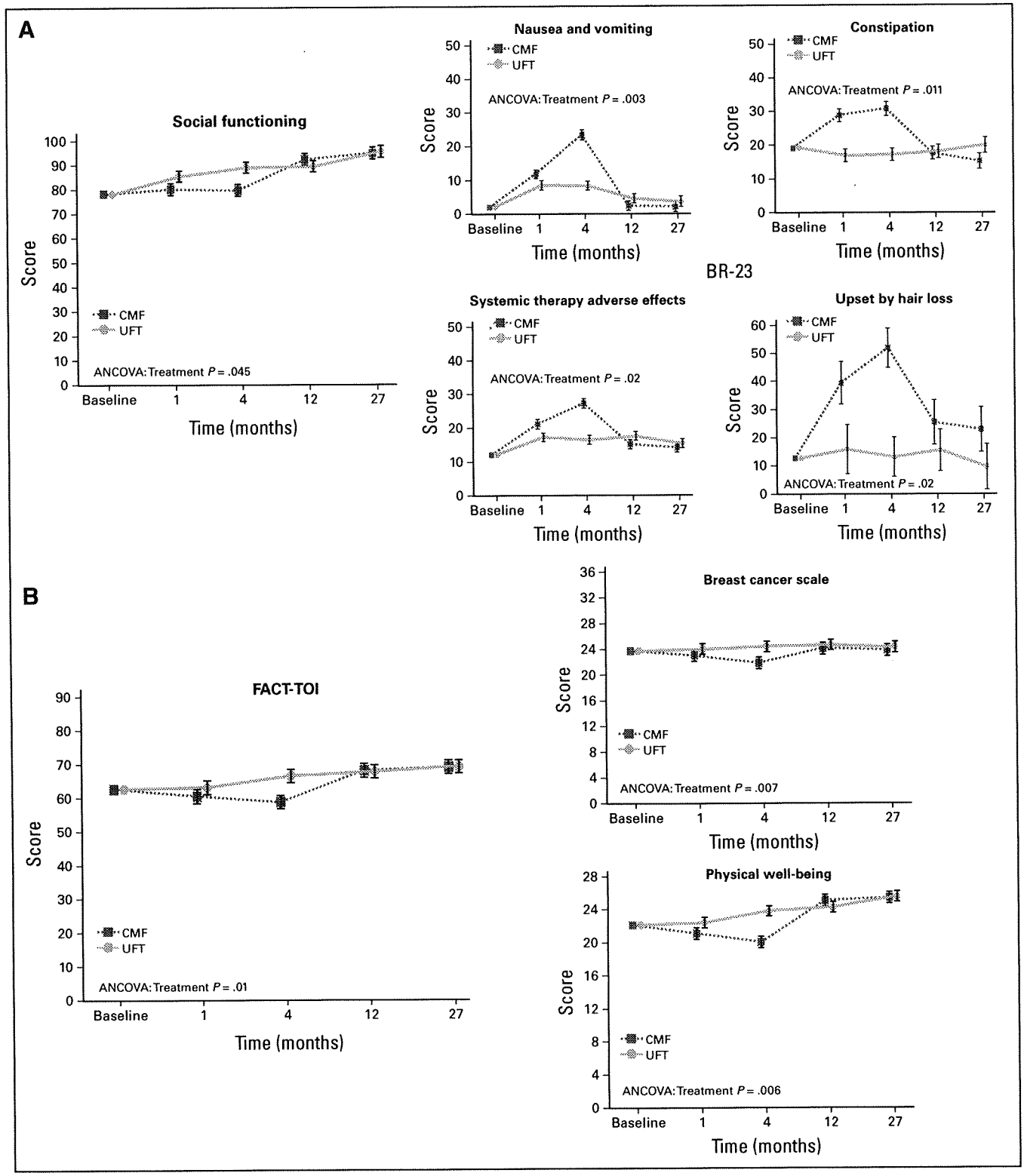

Fig 5. Impact of cyclophosphamide, methotrexate, and fluorouracil and uracil and tegafur (UFT) on quality of life (QOL). (A) European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 Breast 23. In the graph for social functioning, higher score indicates better QOL. In the symptom's items/domains, lower score indicates better QOL. (B) Functional Assessment of Cancer Therapy-Breast. Higher score indicates better QOL. TOI, total outcome index; ANCOVA, analysis of covariance.

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In conclusion, both UFT and CMF are associated with high rates of RFS in women with node-negative, high-risk breast cancer. However QOL is clearly better in patients given UFT than in those given CMF. Therefore, UFT is considered a promising treatment option that maintains QOL in women with node-negative, high-risk breast cancer.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Original Article

Histological differences between invasive ductal carcinoma with a large central acellular zone and matrix-producing carcinoma of the breast

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Carcinoma with a large central acellular zone (central acellular carcinoma, CAC) and matrix-producing carcinoma (MPC) have been recently noted as basal-like-type breast cancers, but the two entities are often confused. To clarify their histological differences, the histopathological sections of 15 CAC and seven MPC were examined and the following features were compared by reviewing slides: (i) mode of invasion; (ii) alteration of cancer cell adhesion in the transitional area between cellular and acellular zones; (iii) staining of the stromal matrix; (iv) lymphocyte infiltration; and (v) tumor grade. Complete agreement was required between two observers for the assessments of these features. All CAC had relatively sharp margins but showed infiltrative growth accompanied by eosinophilic intercellular matrix. In CAC there was abrupt transition between peripheral cellular and central acellular zones without alteration of cancer cell adhesion. In contrast, all MPC showed expansive growth with a well circumscribed margin, accompanied by basophilic and myxoid intercellular matrix. In MPC there was gradual transition from cellular to acellular areas with gradual loss of cancer cell adhesion. Histological grade 3 and peripheral lymphocyte infiltration were common features. It is suggested that CAC and MPC are histologically distinct entities, and that the aforementioned features are helpful for differential diagnosis.

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Key words: breast carcinoma, carcinoma with a large central acellular zone, matrix-producing carcinoma

Recently, using DNA microarray techniques, it has been shown that operable breast cancers can be classified into biologically distinct groups based on their gene expression profiles.[1,2] These groups consist of luminal A, luminal B, ERBB2 (c-erbB-2, HER2), basal-like, and normal breast types.

Molecular targeting therapies are now playing an important role in the control of operable breast cancers. Endocrine therapies are considered effective for luminal A and B types, whereas trastuzumab is considered effective for luminal B and ERBB2 types. In contrast, the basal-like type, which does not express both estrogen receptor (ER) or HER2, is not a candidate for endocrine or trastuzumab therapy. In this type, the incidence of visceral metastasis is higher, and patient outcome tends to be poorer in comparison with the other three types, although systemic chemotherapies are effective in 20–30% of cases.[2–4]

Several histological characteristics of basal-like-type breast carcinoma have been reported. In invasive ductal carcinoma not otherwise specified, two representative forms of the basal-like type have been reported: One is carcinoma with a large central acellular zone, or central acellular carcinoma (CAC), and the other is solid-type carcinoma, such as atypical medullary carcinoma.[5–8] CAC is invasive ductal carcinoma with a central acellular zone covering >30% of the tumor area. The central acellular zone is mostly composed of ghost cells and collagenous or hyalinous materials, suggesting extensive tissue infarction. The periphery of the tumor is composed of relatively small but high-grade cancer cells,

Table 1 Similar features between CAC and MPC

HE stain	Peripheral carcinoma cells surrounding central acellular zone
	Histological grade 3
	Concomitance as components in a tumor
Immunophenotype	Expression of basal-like type markers: CK5/6, CK14, CK17, vimentin, S-100 protein, and α-SMA
	Overexpression of KIT and EGFR
	Neither expression of ER, PgR, or HER-2
Clinical findings	Higher recurrence rate

α-SMA, α-smooth muscle actin; CAC, carcinoma with a large central acellular zone; CK, cytokeratin; EGFR, epidermal growth factor receptor; ER, estrogen receptor; MPC, matrix-producing carcinoma; PgR, progesterone receptor.

which are arranged in cords, and/or solid nest structures. CAC is known to be associated with poorer patient outcome and tends to metastasize to the brain and lung.[9]

Among the special types of breast carcinoma, metaplastic carcinoma and adenoid cystic carcinoma are known as basal-like-type breast carcinomas.[10,11] Metaplastic carcinoma is subclassified into carcinoma with squamous metaplasia, carcinoma with osseous and/or cartilaginous metaplasia, carcinoma with spindle cell metaplasia, and matrix-producing carcinoma (MPC). MPC is composed of overt invasive carcinoma and a cartilaginous/osseous matrix with absence of an intervening zone of spindle-cell or osteoclast component between the carcinoma and the matrix. Most of the MPC cells are high grade, and are arranged in cords, clusters, and/or tubular structures.[12]

CAC and MPC, known to be relatively frequent basal-like types, have common characteristic histological features, including the presence of a central acellular zone surrounded by peripheral carcinoma cells, a relatively clear cancer margin, and high histological grade (Table 1).[11,12] They commonly show a myoepithelial cell immunophenotype, for example expression of vimentin, S-100 protein, α-smooth muscle actin, cytokeratin 14 (CK14), and CK5/6. They frequently also show overexpression of KIT and EGFR,[11] but are negative for hormone receptors or HER2 overexpression and are known to have a poor clinical outcome. Sometimes, the CAC and MPC components coexist within a tumor.[13] From these similarities, CAC and MPC are often confused even by pathologists. In order to clarify whether CAC and MPC are histologically distinct entities, we compared several histopathological features of CAC and MPC by reviewing HE-stained sections.

MATERIALS AND METHODS

We examined representative HE-stained sections of 15 CAC and seven MPC. Fourteen of the CAC and three of the MPC were diagnosed at the National Cancer Center Hospital (Tokyo, Japan), three MPC were diagnosed at the Suzuka Central General Hospital (Suzuka, Japan), one MPC was diagnosed at the Yaizu City General Hospital (Yaizu, Japan), and one CAC was diagnosed at the National Defense Medical College Hospital (Tokorozawa, Japan).

CAC was defined as invasive ductal carcinoma with a central acellular zone consisting of infarcted tissue with ghost cells and deposition of hyaline materials and collagen. The central acellular zone occupied >30% of the area of the representative sections of the tumor.[5] MPC was diagnosed according to the criteria of Wargotz and Norris: overt carcinoma with direct transition to a cartilaginous and/or osseous stromal matrix without intervening spindle cells or osteoclastic cells.[12] All MPC examined in the present study produced cartilaginous matrices, and no case showed production of an osseous matrix.

For these 22 tumors, two observers (Y.S. and H.T.) independently assessed the following parameters: (i) tumor extension pattern, or the outer tumor margin; (ii) alterations of cancer cell adhesion in the transitional area between the peripheral cellular and central acellular zones; and (iii) staining of the intercellular stromal matrix. In addition, we examined lymphocyte infiltration in the peripheral zone and the histological grade of the carcinoma cells. Significance of differences was analyzed using the χ^2 test or Fisher's exact test.

RESULTS

The results are presented in Table 2. Complete agreement was acquired between two observers for the assessments of all parameters.

On microscopy the margins of the 15 tumors of CAC were relatively irregular, and the tumor cells with cord-like or single-cell patterns and a collagenous matrix showed invasion to the surrounding tissue in an infiltrative manner. In contrast, the margins of seven tumors of MPC on microscopy were well circumscribed, and the tumor cells with solid-nest patterns and a myxoid cartilaginous matrix showed invasion to the surrounding tissue in a pushing or expansive manner ($P < 0.0001$; Fig. 1).

The manner of tumor cell adhesion at the boundary between the peripheral cellular and central acellular zones differed between 15 tumors of CAC and seven tumors of MPC. In the 15 CAC, the boundary of these two zones was sharp, and the transitional zone was absent. Cell adhesion was maintained throughout the peripheral cellular zone in CAC. In contrast, a transitional zone was evident in the seven MPC ($P < 0.0001$; Fig. 2). MPC cells gradually became less cohesive and more scanty toward the central acellular zone.

In all CAC, the intercellular matrix was eosinophilic, while that in all MPC was basophilic ($P < 0.0001$; Fig. 3). In a CAC

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Table 2 Histopathological differences between CAC and MPC

	MPC ($n = 7$)	CAC ($n = 15$)	P
I. Tumor margin			
Circumscribed, expansive	7	0	<0.0001
Infiltrative	0	15	
II. Changing from cellular zone to acellular zone			
Gradual	7	0	<0.0001
Abrupt	0	15	
III. Intercellular matrix			
Basophilic	7	0	<0.0001†
Acidophilic	0	15	
IV. Lymphocyte/plasma cell infiltration in cancer margin			
Present	6	15	NS
Absent	1	0	
V. Histological grade			
Grade 1 or 2	0	0	NS
Grade 3	7	15	

†One case of CAC accompanied with a component of MPC had mostly eosinophilic matrix, but basophilic myxoid matrix was observed not only in the MPC component but also in part of the CAC component.

CAC, carcinoma with a large central acellular zone; MPC, matrix-producing carcinoma; NS, not significant.

tumor with a MPC component, there was a predominant eosinophilic matrix, but the basophilic mucinous matrix observed in the MPC component was also intermingled with the eosinophilic matrix in the CAC component.

All 15 CAC and six (86%) of the seven MPC showed lymphocyte and plasma cell infiltration in the peripheral zones. All 22 tumors examined were histological grade 3.

Eight of the 15 CAC and none of the seven MPC had a ductal carcinoma *in situ* (DCIS) component. All DCIS components in these eight CAC were high nuclear grade, and their area was ≤10% of the tumor.

DISCUSSION

We clarified histopathological differences between CAC and MPC by examination of HE-stained sections. The differences were remarkable in the tumor margin, the alteration of tumor cell adhesion in the transitional zone, and the staining of the intercellular matrix.

Although the margins of CAC and MPC have been described in imaging diagnosis as well circumscribed, the microscopic features of the margin differed between the two. In MPC, cancer cell nests and myxoid chondroid matrix proliferated concomitantly and extended to the surrounding tissue in a pushing manner. Because of its high viscosity, the myxoid matrix of MPC may form a circumscribed margin and lead to expansive tumor growth. In CAC, cancer cells infiltrated the surrounding structures with the induction of a cancer cell stroma rich in collagen fibers. Therefore, the margin of CAC was histologically microdenticulated.

The gradual loss of cell adhesion and basophilic matrix in MPC were explained by the effect of the cartilaginous matrix produced by the carcinoma cells. The major components of the cartilaginous matrix are chondroitin sulfate and acid mucopolysaccharides, which are basophilic upon HE staining. The matrix produced by MPC cells pervaded the intercellular spaces, making the cancer cells gradually less cohesive and more scanty toward the central acellular zone. In contrast, the central acellular zone of CAC was considered to be formed by tissue infarction and replacement, which may be caused by the relative paucity or oppression of blood vessels due to rapid proliferation of cancer cells and stroma. Loss of blood flow to the tumor center resulted in coagulation necrosis, with rapid disappearance of the cancer cells, and the necrotic area was replaced by collagen and hyaline materials. These materials were eosinophilic upon HE staining.

Lymphocyte and plasma cell infiltration was observed in the peripheral zones of almost all MPC and all CAC. These phenomena might reflect the fact that both tumor types induce a host immune response.

Both CAC and MPC were accompanied by a small amount of DCIS component, if any, and these DCIS components had high nuclear grade. Both of these cancer types appeared to occur as highly malignant DCIS and to begin stromal invasion from a very early stage in tumor progression.

Although CAC and MPC have been considered different entities, one of the 22 tumors showed coexistence of both components.[13] Coexistence and/or histological transition in a tumor has been reported among the components of metaplastic carcinomas, that is, squamous cell carcinoma, spindle cell carcinoma, MPC, and carcinoma with cartilaginous/osseous metaplasia. Furthermore, metaplastic carcinoma components can coexist with the component of atypical medullary carcinoma and/or of CAC.[13,14] Therefore, distinct

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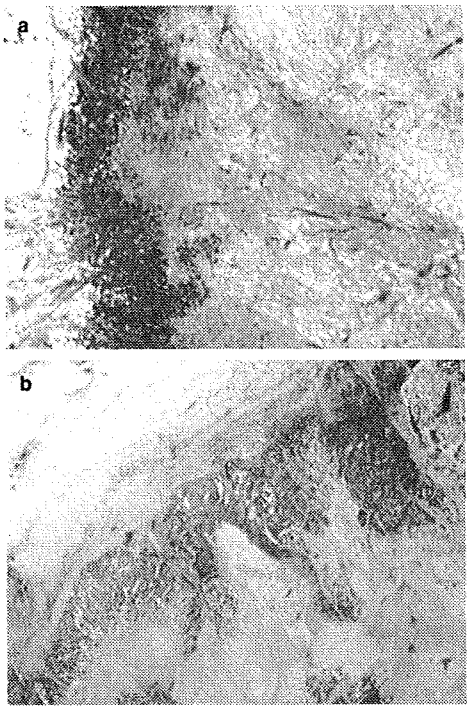

Figure 1 Characteristics of the microscopic tumor margin. (a) Carcinoma with a large central acellular zone (central acellular carcinoma). The margin of the tumor is relatively irregular, and the tumor cells show invasion to the fat tissue in an infiltrative manner. (b) Matrix-producing carcinoma. The margin of the tumor is well circumscribed, and the tumor cells show invasion to the surrounding tissue in a pushing or expansive manner (HE, original magnification ×40).

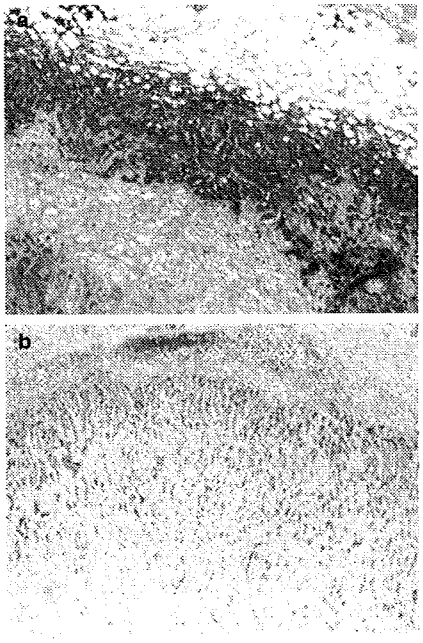

Figure 2 Mode of tumor cell adhesion in the boundary between the peripheral cellular and central acellular zones. (a) Carcinoma with a large central acellular zone (central acellular carcinoma). Intercellular adhesion is constant throughout the peripheral zone. The boundary between the peripheral cellular and central acellular zones is sharp, and the transitional zone is absent. (b) Matrix-producing carcinoma. Carcinoma cells gradually become scanty and less cohesive toward the central acellular zone (HE, original magnification ×100).

histological types of basal-like-type breast carcinoma would often coexist as components in a tumor. Basal-like-type breast carcinomas are speculated to arise from mammary gland stem cells or early transit cells that have potential for pluripotent differentiation. Therefore, multidirectional differentiation, as represented by histological types and immunophenotype, would be a unique feature of basal-like-type breast carcinomas.

In summary, we have shown that CAC and MPC have distinct histological features by examination of HE-stained sections. In order to identify differences in DNA structure, mRNA and protein expression, and clinical behavior, additional molecular biological and clinical studies are required.

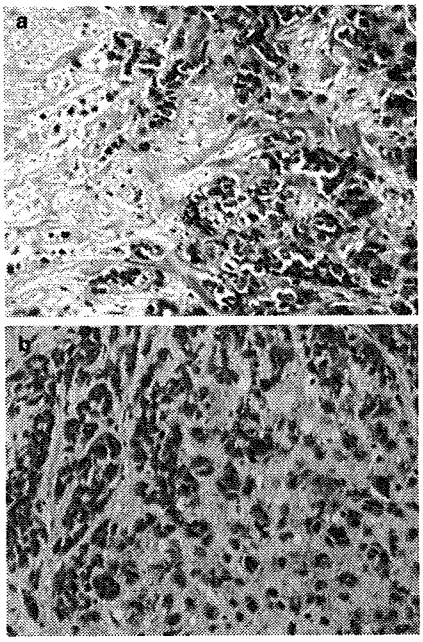

Figure 3 Features of the intercellular matrix in the peripheral cellular zones. (a) Acidophilic matrix in a carcinoma with a large central acellular zone (central acellular carcinoma). (b) Basophilic matrix in a matrix-producing carcinoma (HE, original magnification ×200).

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SPECIAL FEATURE

Current issues and perspectives on breast cancer diagnosis

Morphological characteristics of basal-like subtype of breast carcinoma with special reference to cytopathological features

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Abstract Expression profiling of invasive breast carcinomas by DNA microarray techniques has identified five distinct subtypes of tumors (luminal A, luminal B, normal breast-like, HER2 overexpression, and basal-like) that are associated with different clinical outcomes and with different chemotherapy. Basal-like carcinoma is associated with younger patient age, high histological grade, aggressive clinical course, development of distant metastasis, poor prognosis, and relatively high mortality rate. Basal-like carcinomas do not express estrogen receptor, progesterone receptor, or HER2 (triple-negative phenotype). Therefore, patients with basal-like carcinomas are not likely to benefit from endocrine therapies or trastuzumab, but are likely to benefit from systemic chemotherapy. Although genetic, morphological, and immunohistochemical features of basal-like carcinomas have been reported, there is no universal definition for those tumors. Furthermore, there are no specific morphological and immunohistochemical features that can identify those tumors in routine diagnostic materials. In the present paper, we present data of histological and cytological features of basal-like breast carcinoma, and discuss about its morphological spectrum.

Keywords Breast carcinoma · Cytopathology · Histopathology · Triple-negative · Basal-like subtype

Abbreviations

CK	Cytokeratin
ER	Estrogen receptor
PR	Progesterone receptor
HER2	Human epidermal growth factor receptor 2
EGFR	Epidermal growth factor receptor
IHC	Immunohistochemistry
IDC	Invasive ductal carcinoma

Introduction

Recent DNA microarray profiling of breast carcinoma has identified distinct subtypes of breast carcinoma with clinical, biological, and therapeutic implications. Sorlie et al. [1, 2] analyzed the expression profiles and categolized breast tumors into five distinct molecular subtypes as follows; luminal A and B, HER2, normal breast-like, and basal-like subtypes. These breast carcinoma subtypes are associated with different clinical outcomes, from the relatively good prognosis of patients with luminal A tumors to the worst prognosis of those with basal-like and HER2 tumors [2, 3].

Basal-like carcinoma does not express hormone receptors (ER and PR) or HER2 (triple-negative phenotype), but express genes characteristic of basal epithelial cells. Basal-like carcinoma is characterized by younger patient age, an

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aggressive clinical course, development of distant metastasis (particularly in the first 5 years), poor prognosis, and relatively high mortality rate [4]. In addition, basal-like tumors show a specific pattern of distant visceral metastases to brain and lung, and, less likely, metastases to bone and liver [5, 6]. Those tumors that expressed the basal cytokeratin (CK5/6) and EGFR were significantly more likely to demonstrate CNS and lung recurrence [5].

These molecular subtypes are potentially associated with response to specific therapeutic agents. Luminal A and luminal B tumors are expected to be sensitive to endocrine therapy, HER2-overexpression tumors, and luminal B tumors can be candidates for molecule targeted therapies with antibodies against HER2. In contrast, patients with basal-like carcinomas are not candidates for hormonal therapies or trastuzumab therapy, but are likely to benefit only from targeted systemic chemotherapy [4].

It has recently been demonstrated that patients with the basal-like subtype of breast carcinoma have higher sensitivity to neoadjuvant anthracycline-based chemotherapy than the luminal subtype, and higher rates of pathologic complete response [7, 8]. Patients with pathologic complete response to chemotherapy had a good prognosis regardless of subtype. However, a part of basal-like breast carcinomas are resistant to the systemic chemotherapy. The poor prognosis of basal-like carcinomas could be explained by a higher likelihood of relapse in those patients in whom pathologic complete response was not achieved [7, 9].

Currently, there is no universal definition for basal-like carcinomas. The majority of basal-like tumors have common histological features, but there are no specific detectable morphologic features that identify those tumors in routine diagnostic materials.

In the present report, we examined the percentage of the basal-like carcinoma in a consecutive series of 308 breast carcinomas that were surgically resected from patients in the Matsusaka Chuo General Hospital. In addition, we examined histopathological and cytopathological characteristics of basal-like breast carcinomas.

Immunohistochemical identification of basal-like breast carcinoma

Ducts and lobules of normal human breast are lined by two cell layers consisted of inner/luminar epithelial cells and outer/myoepithelial cells. These cells can be distinguished by their immunophenotype, and several markers have been used to define them. The luminar epithelial cells are characterized by epithelial membrane antigen (EMA), ER, PR, BCL2, GATA3, low molecular weight CKs such as CK7, CK8, and CK19, and epithelial cell adhesion molecules. The myoepithelial cells usually express high molecular weight basal CKs including CK5/6, CK14, CK17, smooth muscle actin (SMA), calponin, p63, beta4 integrin, laminin, p-cadherin, nerve growth factor receptor (NGFR), CD10, caveolin 1, and S-100 protein [1, 4]. Myoepithelial cells are also called basal cells which are negative for luminal CKs, EMA, ER, and PR.

Basal-like carcinomas have been characterized for gene expression analysis as having a gene expression pattern similar to basal/myoepithelial cells [1, 2]. The basal-like carcinomas are characterized by an IHC expression signature similar to that of the myoepithelial cells of the breast [10]. Moreover, tumors arising in BRCA1 germline mutation carriers are remarkably similar to basal-like carcinoma at the morphologic features and are often found to express basal markers [11]. It was recently reported that basal-like carcinomas showed an incomplete basal/myoepithelial cell phenotype [12]. Basal-like carcinomas showed expression of CK5/6 and EGFR; however, the majority of basal-like carcinomas were negative for myoepithelial markers, including SMA, CD10, and p63. Therefore, SMA, CD10, and p63 appear to be of limited value in the immunohistochemical detection of basal-like carcinomas. Further studies will be needed to discover whether basal-like carcinomas have the characteristics of myoepithelial cells.

The basal CKs expression is one of the main characteristic features of basal-like carcinomas. In most IHC studies, CK5/6, CK14, combined CK5/6 and CK14, CK5/6, and CK17, or a combination of the three basal CKs have been used to defined basal-like carcinomas.

Nielsen et al. [13] suggested that the use of a panel of four markers (ER, HER2, CK5/6 and EGFR) could accurately identify basal-like tumors with widely available standard pathologic tools. They classified HER2 positive tumors as HER2 subtype, ER-positive tumors as luminal subtype, tumors of negative ER, negative HER2, and positive CK5/6 or positive EGFR as basal-like subtype, and tumors that were negative for all these four markers as undetermined subtype. This panel showed a high sensitivity and specificity to identify breast carcinomas with a basal-like phenotype. Other authors also adopted these criteria for definition of basal-like carcinomas. Livasy et al. [12] reported that the most consistent immunophenotype of basal-like tumors was ER−, HER2−, and positive for vimentin, EGFR, CK5/6, and CK8/18. According to Carey et al. [3], IHC intrinsic subtypes were defined as follows: luminal A (ER+ and/or PR+, HER2−), luminal B (ER+ and/or PR+, HER2+), basal-like (ER−, PR−, HER2−, CK5/6+ and/or EGFR+), HER2+/ER− (ER−,PR−, and HER2+), and unclassified (negative for all five markers). The expanded surrogate immunopanel of ER, PR, HER2, EGFR and CK5/6 provides a more specific definition of basal-like carcinoma that better predicts breast carcinoma

Table 1 Incidence of intrinsic subtypes of invasive breast carcinoma (Matsusaka Chuo General Hospital 2003–August 2008)

Intrinsic subtype	No. of cases	%
Luminal A	224	73
Luminal B	14	5
HER2+/ER−	22	7
Basal-like	32	10
Unclassified	16	5
Total	308	100

Table 2 Distribution of basal-like carcinoma among different histological tumor types (Matsusaka Chuo General Hospital 2003–August 2008)

Histology	No. of cases	%
Invasive ductal carcinoma	26	81.2
Papillotubular	2	6.2
Solid-tubular	21	65.6
Scirrhous	3	9.4
Medullary carcinoma	2	6.2
Invasive micropapillary carcinoma	1	3.1
Squamous cell carcinoma	1	3.1
Spindle-cell carcinoma	1	3.1
Matrix-producing carcinoma	1	3.1
Total	32	100

survival [14]. Other biomarkers that have been included in the panel of basal markers are vimentin, laminin, c-KIT, p63, CD10 caveolin-1, nestin, osteonectin, and NGFR [4].

Histological features of basal-like breast carcinoma

Basal-like breast carcinoma is a distinct group of tumors with heterogenous morphologic features. They comprise 8–20% of all breast carcinomas [3, 10, 11, 15, 16]. A very low prevalence (8%) of basal-like breast tumors in Japanese patients was reported [16]. We found that 10% were of the basal-like subtype from a series of 308 invasive breast carcinomas at the Matsusaka Chuo General Hospital from 2003 to August 2008 (Table 1).

Of these 308 cases, the histological type of basal-like tumors was most commonly high histological grade (grade 3) [17] IDC (particularly of solid-tubular subtype), but was occasionally medullary, metaplastic, or invasive micropapillary carcinomas (Table 2). The common histologic features of solid-tubular subtype grade 3 IDC comprised solid pattern in association with syncytial architecture and indistinct cell borders and a well-defined border of invasion with a pushing manner. Central acellular zones (central scar and infarcted necrosis) [18], geographic necrosis or

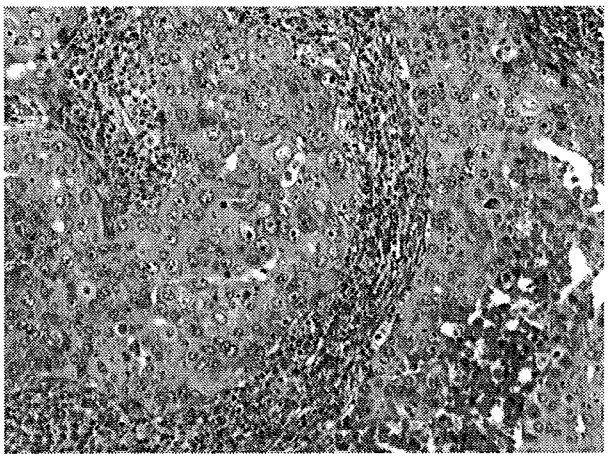

Fig. 1 A basal-like carcinoma shows foci of tumor necrosis and peritumoral lymphocytes infiltration (HE ×100)

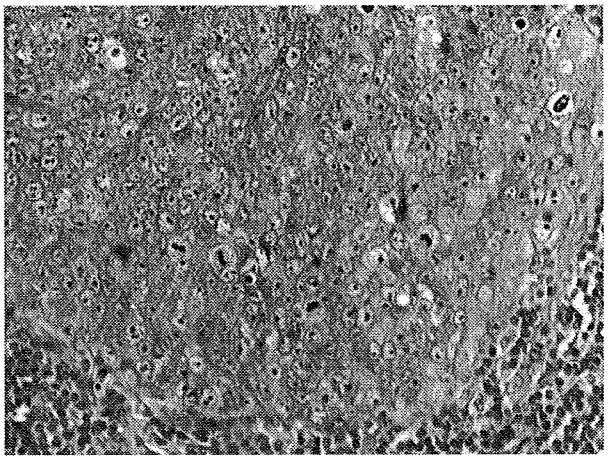

Fig. 2 A basal-like carcinoma shows syncytial arrangement with indistinct cell borders, cellular pleomorphism, prominent nucleoli, and high mitotic activity. Apoptotic tumor cells were demonstrated (HE ×200)

comedo type necrosis and stromal lymphocytic infiltration were often also present (Fig. 1). These basal-like tumors were usually lacking in tubular formation, and showed marked cellular pleomorphism, high nuclear-to-cytoplasm ratio, and high mitotic index (Figs. 2, 3) [4, 12, 19]. Numerous apoptotic tumor cells were identified in the cases with necrotic foci. They sometimes showed metaplastic elements such as spindle shaped cells, clear cells and squamous metaplasia (Fig. 4) [12, 19].

Almost all metaplastic and medullary carcinomas are basal-like phenotype [4, 20, 21]. Typical immunoprofile of basal-like tumors was observed in 91% of metaplastic carcinomas [22], regardless of the type of metaplastic elements (e.g., sindle cell carcinoma, squamous cell carcinoma, and matrix-producing carcinoma). Moreover, these neoplasms frequently (57%) overexpress EGFR [22].

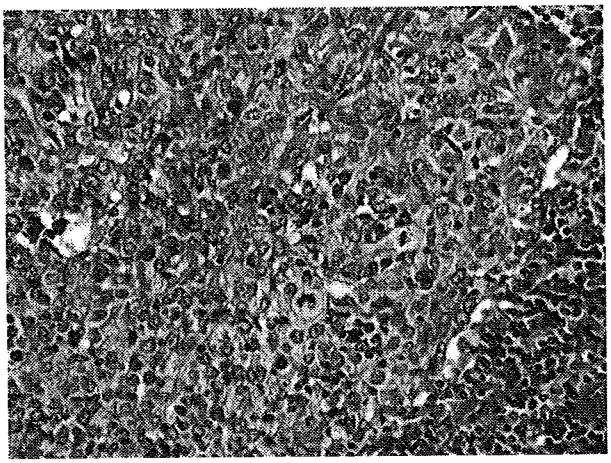

Fig. 3 Proliferation of oval to spindle shaped cells with tapering cytoplasm. Cell size and nuclear appearance can be variable (HE ×200)

Fig. 5 Most tumor cells of basal-like carcinoma show strong CK5/6 expression (IHC ×200)

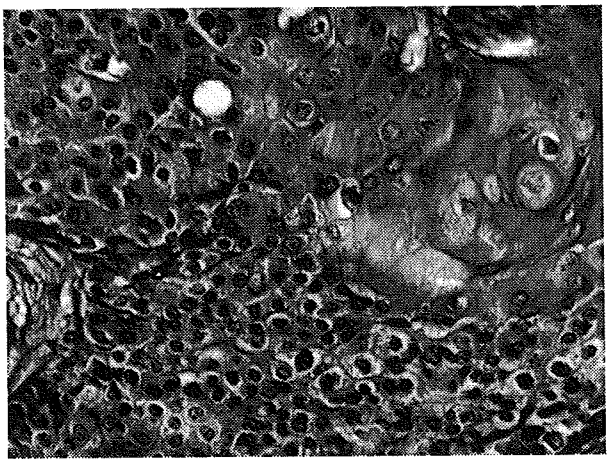

Fig. 4 Basal-like carcinoma shows foci of squamous metaplasia (HE ×200)

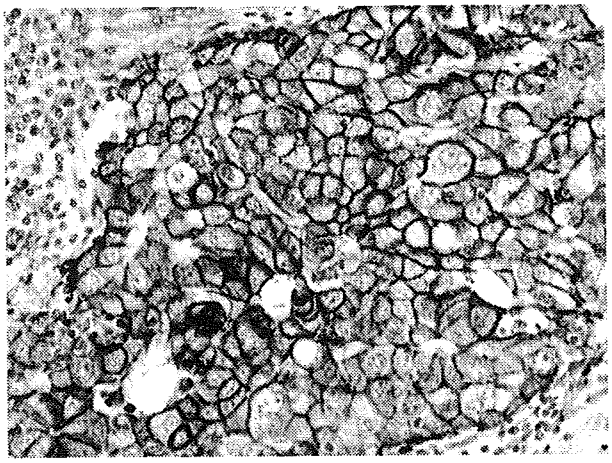

Fig. 6 Basal-like carcinoma shows positive EGFR immunoreaction on cell membrane (IHC ×200)

Medullary carcinoma of the breast is defined as a well-circumscribed carcinoma composed of a poorly differentiated cells growing in a syncytial pattern, with no glandular structures, sparse tumor necrosis, scant stroma, and a prominent stromal lymphocytic infiltration which, despite its high grade tumor, has a favorable prognosis compared with high grade IDC. Basal-like tumors were correlated with IDC of solid-tubular subtype grade 3. The solid-tubular subtype grade 3 may contain atypical medullary carcinoma [23], IDC with a large central acellular zone [18], and matrix-producing carcinoma. Atypical medullary carcinoma has been described as having an intermediate prognosis. Medullary features have been found in 35–60% of breast carcinomas arising in BRCA1-mutation carriers.

Although basal-like tumors are associated with aggressive behavior and relatively high mortality rate, absence of lymphatic permeation, or lymph node metastasis has frequently been demonstrated [4, 11, 12, 19].

Cytopathological features of basal-like carcinoma

Fine-needle aspiration cytology (FNA) of the breast has become a widely accepted procedure for the diagnosis of breast masses. Although histological and immunohistochemical findings of basal-like breast carcinoma have been reported [12, 19], a detailed cytological analysis of known basal-like tumors has not been performed. This prompted us to examine if basal-like tumors can be recognized on cytological examination.

We performed a detailed cytological review of FNA specimens of 28 basal-like carcinomas determined by IHC

Table 3 Cytological findings of basal-like breast carcinoma

Characteristic	No. of patients	%
Cellularity		
Scant	5	17.9
Moderate	10	35.7
Marked	13	46.7
Syncytial status		
Mild	2	7.1
Moderate	10	35.7
Marked	16	57.2
Isolated cells		
Mild	3	10.7
Moderate	11	39.3
Marked	14	50
Naked nuclei		
No	2	7.1
Mild	14	50
Marked	12	42.9
Nucleoli		
No	1	3.5
Small	12	42.9
Large	15	53.6
Nuclear grade		
Grade 1	1	3.5
Grade 2	3	10.7
Grade 3	24	85.8
Mitosis[a]		
0–1	5	17.9
2–4	10	35.7
5≦	13	46.4
Necrosis		
No	13	46.5
Yes	15	53.6
Lymphocytes		
No	8	28.6
Yes	20	71.4
Squamous metaplasia		
No	23	82.1
Yes	5	17.9
Spindle cells		
No	23	82.1
Yes	5	17.9

[a] 10 high-power fields (×400)

analysis. They were identified from the patient database of the Matsusaka Chuo General Hospital from 2003 to August 2008. We defined the basal-like phenotype according to the criteria of Carey et al. [3] which were ER−, PR−, HER2−, and CK5/6+ (Fig. 5) and/or EGFR+ (Fig. 6). ER and PR status were determined by IHC. The cutoff value for receptor positivity was 10%. The HER2 status was also

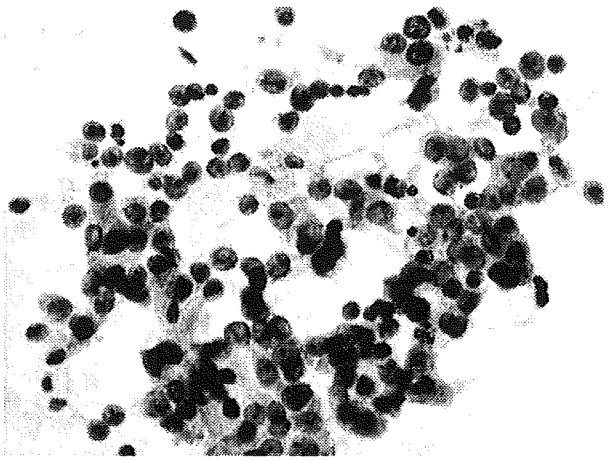

Fig. 7 FNA cytology showing loose syncytial sheets with indistinct cell borders and many isolated naked nuclei (Pap ×200)

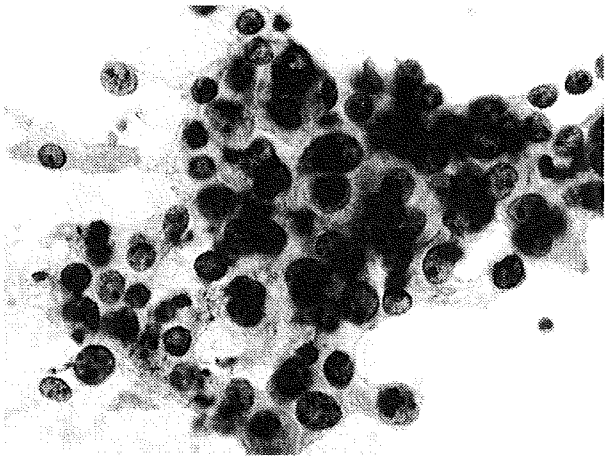

Fig. 8 High-power view of tumor cells with large round nuclei, prominent nucleoli and mitotic figures (Pap ×400)

determined by IHC. According to the criteria of the Hercep test, scores 0 and 1 were defined HER2-negative and scores 2 and 3 were defined HER2-positive. Triple-negative (ER−, PR−, and HER2−) breast carcinoma samples were examined by IHC for CK5/6 and EGFR. CK5/6 and EGFR were considered positive when more than 10% of the tumor cells were labeled.

The cytomorphological findings of basal-like breast carcinoma are presented in Table 3. The FNA specimens were cellular, syncytial sheets of tumor cells with indistinct cell borders (Fig. 7). Isolated tumor cells were also frequent. The neoplastic cells consisted of high-grade atypical cells with increased nuclear-to-cytoplasmic ratio, nuclear pleomorphism, prominent nucleoli, and high mitotic index (Figs. 8, 9). Naked nuclei and apoptosis were a prominent feature. The diathesis of necrotic debris and lymphocytes

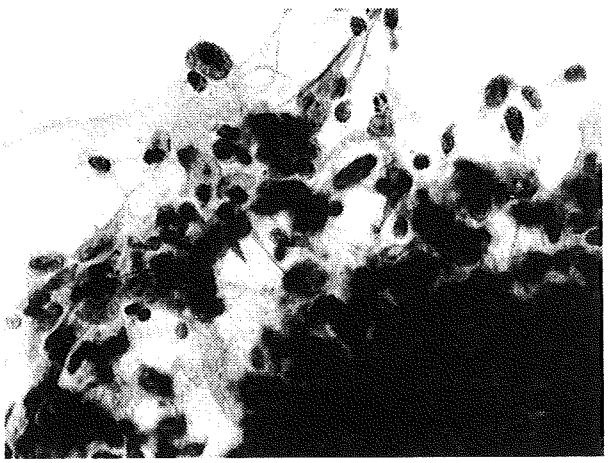

Fig. 9 Aggregation of oval to spindle shaped cells with nuclear pleomorphism and bizarre naked nuclei (same case as Fig. 3) (Pap ×200)

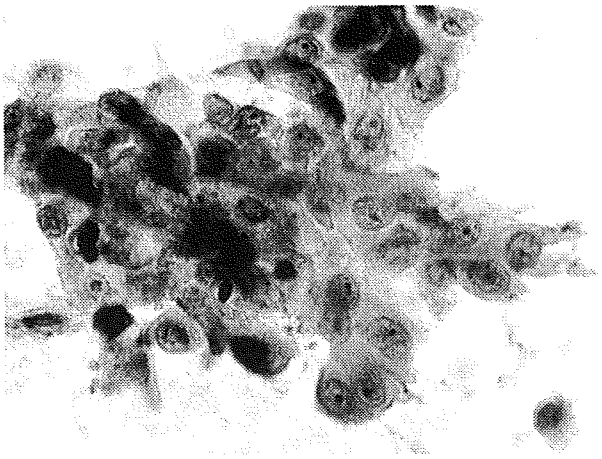

Fig. 11 Basal-like tumor cells with focal squamous differentiation (Pap ×400)

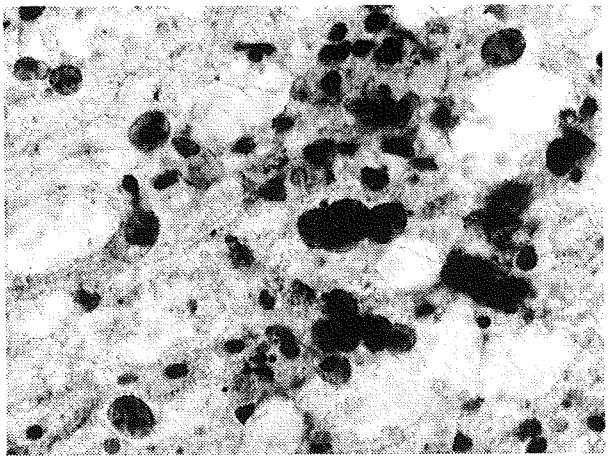

Fig. 10 Isolated naked nuclei in necrotic diathesis associated with macrophages and small lymphocytes (Pap ×400)

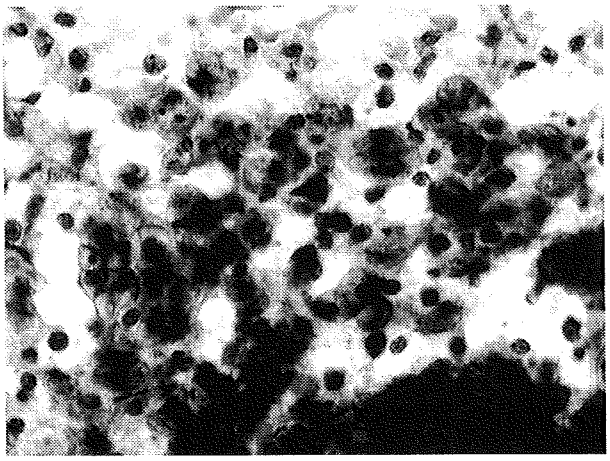

Fig. 12 Medullary carcinoma consisted of large tumor cells, which are isolated or in loose syncytia, with indistinct cell borders. The features of cellular pleomorphism, bizarre nuclei, mitotic figure, and lymphocytes response are characteristic (Pap ×200)

infiltration was demonstrated (Fig. 10). They were also characterized by the presence of metaplastic elements such as spindle-shaped cells and squamous metaplasia in several cases (Fig. 11).

Cytological specimens of medullary carcinoma of the breast usually consisted of large tumor cells with a small nuclear-to-cytoplasm ratio, which were isolated or in loose syncitia. Especially characteristics were the features of pleomorphism, large nuclei with anisonucleosis, prominent nucleoli, and bizarre naked nuclei. Abundant lymphocytes were almost always demonstrated (Fig. 12).

From the study of the 28 basal-like breast carcinomas, we learned that specific cytological features of basal-like carcinoma were associated with the presence of syncytial aggregation of tumor cells, indistinct cell border, prominent nucleoli, high mitotic index, naked nuclei, squamous metaplasia, and lymphocytes infiltration. These findings should help to identify basal-like carcinoma in routine diagnostic practice. It seems difficult to distinguish between HER2+/ER− subtype of breast carcinoma and basal-like carcinoma cytologically, since cytological features of HER2+/ER− subtype of breast carcinoma and those of basal-like carcinoma are very similar.

E-cadherin inactivation in breast carcinoma has been shown to be strongly associated with lobular carcinoma. A recent report demonstrated that reduction/lack of E-cadherin expression is preferentially found in basal-like breast carcinomas [24]. It may be suggested that reduction/lack of E-cadherin expression is associated with an isolated cell pattern in FNA cytology of basal-like carcinoma.

Conclusion

These histopathological and cytopathological findings should further assist in the identification of basal-like carcinomas in routine diagnostic practice.

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A new reporting form for breast cytology

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Abstract The Cytology and Core Needle Biopsy Subcommittee, organized under the Rules Committee of the Japanese Breast Cancer Society, has prepared a new form for breast cytology reports. This reporting form consists of "diagnostic categories" and "recommendations." The "diagnostic category" is either "specimen inadequacy" or "specimen adequacy." The judgment on "specimen adequacy" is subdivided into four categories: "normal or benign," "indeterminate," "suspicious for malignancy," and "malignant." The "recommendation" indicates descriptions of cytological features and estimated histological type of tumor (these descriptions should be as detailed as possible). On the basis of an analysis of cytological data from 3,439 cases performed before preparing this form, the subcommittee has attached the following recommended goals to this form: (1) the percentage of "specimen inadequacy" should be 10% or less of all samples, (2) the percentage of "indeterminate" samples should be 10% or less of all "specimen adequacy" cases, and (3) 90% or more of "suspicious for malignancy" cases should be diagnosed as "malignant" in a subsequent histological examination. We hope that modification of this form, if it requires revision in the future, will be evidence-based, as was the process for compiling this set of rules.

Keywords Breast · Fine-needle aspiration cytology · New reporting form

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Introduction

In Japan, the Papanicolaou classification (Pap classification) has long been used in breast cytology reporting forms. However, the *Journal of the International Academy of Cytology* has for some time now refused papers using the Pap classification. Several new forms of breast cytology reports were prepared in the 1990s, primarily by Western investigators [1–4]. It has been suggested that a new form for this kind of report in Japan should be prepared to meet these demands.

The Japanese Breast Cancer Society recently organized the Cytology and Core Needle Biopsy Subcommittee within the Rules Committee and began to prepare a new breast cytology reporting form to be included in the 15th edition of the General Rules for Clinical and Pathological Recording of Breast Cancer [5]. Cytological data from about 3,400 cases, supplied by its, were analyzed by the 10 subcommittee members and used as evidence for preparing a new reporting form. A draft reporting form was sent to several councilors of the Society (surgeons and pathologists) to ask their opinions and thus enable problems with the draft form to be identified and corrected.

This paper presents the new form, accompanied by a summarized presentation of the analysis of the data from the approximately 3,400 cases that served as the basis for its development.

Problems with the current breast cytology reporting form and countermeasures

Breast cytology is mostly based on specimens collected by fine-needle aspiration (FNA). The role of FNA differs from that of cervical exfoliative cytology in that the former is performed on individuals known to have a mass, while the latter is performed to check for presence/absence of lesions. The Pap classification, used for the current reporting form, is not suitable for FNA, which is capable of directly identifying the disease, i.e., FNA can categorize the tumor's histological type. This is probably the major reason that the *Journal of the International Academy of Cytology* now ignores the Pap classification. Therefore, before preparing a new reporting form, the subcommittee listed problems with the Pap classification as follows: (1) it does not include any conclusions on sample appropriateness, (2) it provides no clear criteria that delineate cytological categories, and (3) it does not include the concept of histological type characterization. The subcommittee discussed these three problems as well as the need to dispatch patient information from clinicians to pathologists.

The first problem, deciding on the appropriateness of samples, is crucial for precision control of cytology. The Bethesda system for uterine cancer includes clear instructions on how to arrive at a decision [6]. The Pap classification, however, does not include any. Medical facilities that use this classification sometimes add this concept at their own discretion. For example, numerous medical facilities place inappropriate samples in class I. Another problem that needs to be resolved is to demonstrate, with evidence, the maximum tolerable percentage of inappropriate samples among all cases undergoing cytological examination.

Regarding the second problem (lack of clear criteria for judgment), when the Pap classification I–V is reviewed in detail, the term "malignancy" is used for classes II to V, but no evidence is given for the finding of "malignancy." The new reporting form, therefore, should list lesions that fall under each class. Class III in the existing classification includes several concepts such as "dysplasia" (which often leads to the detection of cancer on follow up), "borderline lesions" (in the sense used in histology and cytology), and "difficult to distinguish" (inability to distinguish between benign and malignant lesions under the current diagnostic capacity). The meaning of these concepts differs among examiners and medical facilities. The new reporting form replaces class III with the category of "indeterminate."

Regarding the third problem (lack of a concept of histological type estimation), we must point out that "histological type" yields useful medical information only when its result is entered in a cytological report. Diagnostic imaging for breast diseases uses mammography, ultrasonography, CT, MRI, etc. Reports of the results of diagnostic imaging include identification of the histological types of lesions. Cytodiagnosis, which distinguishes between benign and malignant lesions, without referring to estimated histological types, is not sufficiently useful for surgeons when making a general diagnosis on the basis of various preoperative examinations. To what extent does cytology allow estimation of histological types? When the data collected by the subcommittee were analyzed, estimation of the histological type of benign lesions was possible in 93% of cases with fibroadenoma, 81% of fibrocystic change cases, and 92% of intraductal papillomas. Estimation of the histological type of cancers was possible in 85% of invasive ductal carcinomas, NOS, 91% of mucinous carcinomas, 54% of lobular carcinomas, and 23% of ductal carcinomas in situ. These results indicate that estimation of histological types on the basis of cytological data is possible at an accuracy comparable to or higher than that of diagnostic imaging.

The inadequacy of medical information supplied by clinicians to pathologists has been pointed out for many years. This problem is closely related to the view held by clinicians that cytology is merely an "adjuvant means" of making a definite diagnosis. However, as stated above, FNA, which is often used for breast cytology, is highly likely to provide data that can greatly contribute to making a definite diagnosis of a lesion, since it directly punctures the mass. When clinicians request a histological examination, they usually provide background information on individual patients to the pathologists, since histological examination is viewed as a means of making a definite diagnosis. Because FNA can also be viewed as a means of making a "definite diagnosis," pathologists should demand related medical information (including information from diagnostic imaging) from clinicians requesting cytological examination. This will contribute to improved accuracy of cytodiagnosis. The Revised General Rules for Clinical and Pathological Recording on Breast Cancer include this point with the aim of facilitating better cytodiagnosis.

Details of the new reporting form

The new reporting form has the following characteristics: (1) it is composed of "judgments" and "findings" (Table 1); (2) the "judgment" is first divided into two categories, "inadequate" and "adequate"; and (3) "adequate" is subdivided into four categories: "normal or benign," "indeterminate," "suspicious for malignancy," and "malignant." We avoided allocating numbers (such as class I, II, III, IV and V adopted in the Pap classification) to each finding to emphasize the fact that this new form has been designed to supersede the conventional Pap

Table 1 New reporting form for breast cytology

1. Judgments
Inadequate
Adequate
Normal or benign
Indeterminate
Suspicious for malignancy
Malignant
2. Findings: descriptions of cytological features and estimated histological type of lesion

classification. In the questionnaire survey, about half of the subcommittee members and 60% of the council members of the Japanese Breast Cancer Society affirmed the need for numbering. Individual medical facilities may attach numbers to the findings if they regard it as necessary. The "findings" are histological types that can be estimated from cellular characteristics using the histological classification included in the General Rules for Clinical and Pathological Recording of Breast Cancer.

Before preparing this new form, the subcommittee analyzed cytology data from 3,439 cases. Histological diagnosis had been performed on all of these cases. Table 2 classifies them according to the system adopted in the new reporting form.

Of the council members, 98% affirmed the need to adopt this new reporting form.

Diagnostic category

Inadequate

This category refers to cases where diagnosis is difficult due to inappropriately prepared specimens or an inadequate number of cells present. In reporting forms used in Western countries, numerical criteria (e.g., the minimum number of cell clusters acceptable as a valid sample) are sometimes used [7]. However, even when a sample contains only a few cells, the lesion can be suspected of being invasive ductal or lobular carcinoma if the cells assume a linear pattern (abnormal architecture). Therefore, our criterion does not refer to any particular number of cells or cell clusters.

Of the 3,439 cases analyzed by the subcommittee, 371 were deemed inadequate (10.8%; Table 2). We therefore attached a recommended goal to the criterion, stating that the percentage of inappropriate cases among all cytological cases should be 10% or less. This is because the significance of submitting samples to laboratories will be reduced if the percentage of inadequate cases is too high. Inadequate samples may be associated with puncture techniques, methods used for preparing samples, and other factors that may involve clinicians and pathologists. If this percentage exceeds 10%, the cause needs to be checked without delay by both clinical and pathological staff. Since 88 (23.7%) of these inadequate cases were histologically rated as malignant, efforts are needed to reduce inadequate cases as soon as possible (Table 2).

In the questionnaire survey, 98% of respondents supported the validity of the "inadequate" and "adequate" categories, and 90% affirmed the adoption of the four subcategories of "adequate."

Normal or benign

Of the 3,068 cases analyzed, excluding "inadequate" cases, 1,264 (41%) were rated as "normal or benign." Although there is still room for argument about what range of cytological features can be deemed as normal, we added the rating "normal" to this category, considering that normal mammary glands around the tumor may also be punctured by the needle. In addition to normal mammary gland cells, the following histological types are included in this category: most fibroadenomas, some fibrocystic changes, intraductal papilloma, and inflammatory lesions.

Of all cases rated as normal or benign, 85 (6.7%) were eventually found to have cancer (Table 2). In these 85 cases, cancer cells were not detected by repeated microscopy, and the inability to detect cancer appeared to be associated with methods of puncture or other skills, rather than careless microscopy. Ideally, clinicians should make efforts to acquire high-level puncturing skills, and to this end, adequate opportunities should be given to pre-graduate medical students and post-graduate doctors to enhance their puncture skills. Higher-level clinicians should also improve their teaching methods.

Table 2 Analytical results according to the new reporting form

	Normal or benign	Indeterminate	Possibly malignant	Malignant	Inadequate	Total
Benign	1,179	133	7	5	283	1,607
Malignant	85	103	87	1,469	88	1,832
Total (cases)	1,264	236	94	1,474	371	3439

Indeterminate

This category refers to cases where it is difficult to determine cytologically whether a lesion is benign or malignant. This category includes papillary lesions, epithelial hyperplasia, complex fibroadenoma, etc. Histological distinction of benign lesions from malignant lesions is often difficult with these lesions. The same can be said for cytological diagnosis.

A recommended goal has been attached to this criterion, stating that the percentage of cases rated as "indeterminate" should be 10% or less among all adequate cases (3,068 cases). This percentage was determined on the basis of the data that 236 samples (7.7%) of the 3,068 adequate cases were placed in this category when analyzed by the subcommittee (Table 2). In the questionnaire survey, 61% of respondents agreed with this percentage (10%), although some respondents said that this criterion was too severe. However, since the usefulness of cytodiagnosis will be weakened if this percentage is 20–30% or more, we adopted 10%. Every laboratory should ideally make efforts to reduce this percentage to below 10%. Of the 236 cases rated by the subcommittee as "indeterminate," 133 (56.4%) were later rated as benign and 103 (43.6%) as malignant. Thus, benign lesions predominated over malignant ones at a ratio of about 6:4. Of the 133 benign lesions, 51 were fibroadenomas, 42 were fibrocystic changes, and 13 were intraductal papillomas. These three types accounted for 80% of benign lesions. Of the 103 malignant lesions, about half were invasive ductal carcinoma (48 cases), followed by ductal carcinoma in situ (18 cases), papillary carcinoma (13 cases), and invasive lobular carcinoma (9 cases).

Suspicious for malignancy

This category was defined as primarily including ductal carcinoma in situ, invasive lobular carcinoma, and others with a few atypical features. A recommended goal was attached to this criterion, stating that the percentage of cases rated as "malignant" in subsequent histological examinations should be 90% or more of cases initially rated as "suspicious for malignancy." This percentage is based on the evidence that 87 (92.6%) of the 94 "suspicious for malignancy" cases were later found to have malignant lesions (Table 2). In the questionnaire survey, 64% of the respondents affirmed this percentage (90%) for use with this criteria. Benign lesions that were initially rated as "suspicious for malignancy" include three cases of fibrocystic change, two cases of fibroadenoma, one case of nipple adenoma, and one case of ductal adenoma. Nipple adenoma and ductal adenoma tend to be overestimated as malignant by clinical observation, diagnostic imaging, and histological examination.

Past reports on FNA of the breast using the conventional Pap classification have sometimes combined class IV with class V into a single category for statistical analysis. In recent years, some lawsuits have been brought against surgeons who performed mastectomy on class IV cases. This appears to be an outcome of regarding class IV as a synonym of class V. This new reporting form clearly states that 10% of "suspicious for malignancy" cases may actually be benign cases and that clinicians need to be aware of this fact when determining surgical treatment.

Malignant

This category refers to malignant tumors, including breast carcinoma (primary and metastatic), nonepithelial malignant tumor, etc. We cannot deny that histological diagnosis inevitably involves misdiagnosis, although its frequency is very low. Of the 1,474 cases cytologically rated as "malignant," 5 cases (0.3%; 2 cases of invasive ductal carcinoma, 2 cases of noninvasive ductal carcinoma, and 1 case of invasive lobular carcinoma) were later found to be benign. Although none of these cases suffered unfortunate outcomes, it is the duty of our pathologists to minimize this percentage.

Cytological findings

Needless to say, the most basic purpose of cytology reports is to report "estimated histological types" and accompanying "findings." If a good sample is available, it is possible to estimate the histological types of both benign and malignant lesions with relatively high accuracy, as described above in the section "Problems with the current breast cytology reporting form and countermeasures." When making a cytodiagnosis, emphasis tends to be placed on determining whether or not a given tumor is benign or malignant. However, when a histological type is estimated from cytological features, it is always accompanied by a judgment as to its benign or malignant nature. Therefore, this new reporting form emphasizes that the estimated histological type needs to be included whenever possible.

Conclusion

A new reporting form for breast cytology has been presented in this paper. The new form is designed to be independent of the conventional Pap classification. It is composed of the following factors: (1) appropriateness of sample (adequate or inadequate), (2) evident diagnostic criteria, (3) estimated histological type, and (4) recommended goals for the percentage of inadequate cases, the percentage of "indeterminate" cases, and the percentage of