The International Academy of Cytology Yokohama System for Reporting Breast Fine-Needle Aspiration Biopsy Cytopathology

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Abstract
The International Academy of Cytology (IAC) gathered together a group of cytopathologists expert in breast cytology who, working with clinicians expert in breast diagnostics and...
management, have developed the IAC Yokohama System for Reporting Breast Fine-Needle Aspiration Biopsy (FNAB) Cytology. The project was initiated with the first cytopathology group meeting in Yokohama at the 2016 International Congress of Cytology. This IAC Yokohama System defines five categories for reporting breast cytology, each with a clear descriptive term for the category, a definition, a risk of malignancy (ROM) and a suggested management algorithm. The key diagnostic cytopathology features of each of the lesions within each category will be presented more fully in a subsequent atlas. The System emphasizes that the crucial requirements for diagnostic breast FNAB cytology are a high standard for the performance of the FNAB and for the making of direct smears, and well-trained experienced cytopathologists to interpret the material. The performance indicators of breast FNAB, including specificity and sensitivity, negative predictive value, positive predictive value and ROM stated in this article have been derived from the recent literature. The current practice of breast FNAB has evolved with the increasing use of ultrasound guidance and rapid on-site evaluation. Two recent publications have shown a range of ROM for the insufficient/inadequate category of 2.6–4.8%, benign 1.4–2.3%, atypical 13–15.7%, suspicious of malignancy 84.6–97.1%, and malignant 99.0–100%. The management algorithm in the System provides options because there are variations in the management of breast lesions using FNAB and core-needle biopsy in those countries utilizing the “triple test” of clinical, imaging, and FNAB assessment, and also variations in the availability of CNB and imaging in low- and middle-income countries. The System will stimulate further discussion and research, particularly in the cytological diagnostic features of specific lesions within each category and in management recommendations. This will lead to continuing improvements in the care of patients with breast lesions and possible modifications to the IAC Yokohama System.

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Introduction

The International Academy of Cytology (IAC) System for Reporting Breast Fine-Needle Aspiration Biopsy (FNAB) Cytology has been developed following an initial meeting at the Yokohama International Congress of Cytology in 2016, by a group of cytopathologists, radiologists, surgeons and oncologists expert in the management of breast lesions [1]. The process included the writing of draft documents based on a review of the literature and the expertise of the members of the group, followed by discussion and review within the group. Minor modifications were based on peer responses to an international questionnaire carried out in mid-2018 (hosted at the University of Wisconsin State Laboratory of Hygiene and Department of Information Technology using Qualtrics software [Provo, Utah]). Further discussion and editing then took place. This article is a brief summary of the proposed system.

The overarching aims of the System are to standardize and improve the reporting of breast cytology, establish best practice guidelines, improve training in the performance and interpretation of breast cytology, clarify communication between cytopathologists and breast clinicians and to link this reporting system with patient management so as to facilitate optimal breast care.

Since the National Cancer Institute consensus document was published following a 1996 conference in Bethesda, Maryland, USA [2], the role of FNAB has changed in the developed world reflecting the increasing role of core-needle biopsy (CNB) particularly in the setting of mammographic screening for breast cancer. In the developing world breast FNAB is one of the most common FNAB procedures and is increasingly used as a key element in the campaign to reduce breast cancer mortality rates [3–9].

Breast FNAB is a rapid, accurate and highly cost-effective diagnostic procedure with a minimal complication rate for the broad spectrum of benign and malignant breast lesions. It has a high sensitivity in the range of 90–95% and a high positive predictive value (PPV) approaching 100% for the diagnosis of breast carcinoma [10–17]. Breast FNAB has a low false negative rate with any errors related to diagnostic difficulties with low-grade carcinomas and sampling error, and a very low false positive rate, which is usually due to misinterpretation of fibroadenomas, intraductal papillomas and papillary lesions [16–18]. Breast FNAB in the developed world with a high level of medical resources is a component of the “triple test” comprising clinical, imaging and FNAB cytology, and the triple test has a PPV close to 100% [19].

Ideally, except in certain circumstances such as pregnancy, the vast majority of breast lesions should be assessed initially clinically and then by bilateral mammography and ultrasound, and, where required, by FNAB, which may be directed by ultrasound. Rapid on-site evaluation (ROSE) of the FNAB direct smears provides a reduction in insufficient rates, as well as a reduction in atypical and suspicious rates, and a concomitant increase in benign and malignant diagnoses [17]. ROSE also allows immediate triage of the lesion and, dependent on the
FNAB findings, CNB may then be utilized in an appropriate and cost-effective manner. There is a long and successful tradition of using palpation to direct the FNAB and this is totally appropriate where medical infrastructure and resources are not readily available, and, specifically, where access to ultrasound equipment is limited or not available.

FNAB cytology has faced challenges including the introduction of CNB, which should be regarded as a complementary rather than replacement test. The extrapolation of biopsy protocols developed in mammographic screening programs where the lesions are often small and frequently identified as calcifications without a mass lesion, to the work up of all breast lesions is inappropriate [20]. CNB offers advantages in definitively diagnosing invasive carcinoma, assessing mammographically detected calcifications and assisting with proliferative lesions, but similarly to FNAB, it is a sampling device. CNB is more invasive, time consuming, has a higher rate of complications, and has a greater cost for both the biopsy equipment and the required histopathological laboratory processing and reporting [16, 21, 22]. There are reports of a risk of track seeding by carcinoma [23], and of a possible worse prognosis after CNB [24].

Performing breast FNAB, making direct smears and interpreting the material does require specific training and ongoing experience. In many institutions interventional cytopathologists performing the biopsy procedures have largely been replaced by radiologists due to practice patterns and in some cases disparities in reimbursement schedules. The number of breast FNAB procedures has significantly dropped in some institutions, reducing the experience and teaching potential for radiologists and pathologists [20]. The greatest quality assurance issue in providing a breast FNAB service is the quality of the technique of the FNAB proceduralist and the quality of the technique in the making of the direct smears. A cytopathologist performing and then interpreting the smears is at a great advantage to reach an accurate diagnosis [16, 25–27]. Liquid-based cytology has been utilized quite successfully but is more costly and many key diagnostic cytological findings are lost [28].

The IAC Yokohama Reporting System emphasizes the importance of skilled biopsy and smear making techniques to optimize quality and enhance FNAB diagnosis. The key elements to the breast FNAB procedure are the careful selection of a line of approach for the needle, fixation of the lesion by palpation or palpation around an ultrasound probe, and a rapidly performed puncture of the lesion using a rapid movement of the needle into and out from the immobilized lesion, with appropriate use of aspiration. Ultrasound can provide guidance and visualization of the needle tip throughout the procedure. Good training and continued monitoring are essential.

The System suggests that ideally both air-dried Giemsa-stained direct smears and alcohol-fixed Papanicolaou-stained slides should be prepared utilizing a method of splitting the material obtained on each needle pass. Routine rinsing of the needle or separate passes for cell block preparation to enable immunohistochemistry for prognostic indicators can be utilized [12, 29]. Liquid-based cytology preparations can also be considered [28].

The IAC Yokohama System has five categories that can be stratified by their risk of malignancy (ROM):

- Insufficient/inadequate
- Benign
- Atypical
- Suspicious of malignancy
- Malignant

The standardized structured report should state one of these five descriptive terms as a diagnostic heading [1]. A laboratory and its cytopathologists should select either “insufficient” or “inadequate” and use this term consistently. The term “non-diagnostic” is used in various reporting systems in different ways, and is not recommended. An FNAB report should always be correlated with the clinical and imaging findings in the triple test, and if there is a discrepancy, the FNAB should be categorized by the material on the slide and not as “non-diagnostic.” In this situation, the report should contain a statement that the material may not be representative of the lesion seen on imaging and that further biopsy by FNAB or usually CNB is required. If imaging is not available, but the clinical findings and FNAB are discrepant, then the management is similar, that is, further biopsy is required.

The decision was made to include an “atypical” category in order to maximize the negative predictive value (NPV) of a “benign” diagnosis, and a “suspicious of malignancy” category to maximize the PPV of a “malignant” diagnosis. The majority of “atypical” cases will be benign proliferative lesions and the majority of “suspicious of malignancy” lesions (see sections below) will be in situ or low-grade carcinomas, although in both categories scant or poorly smeared limited material may prevent a more definitive diagnosis.

The structured report headed by a category term should then include a brief cytological description noting where possible the presence or absence of key diagnostic features, which will be detailed in the forthcoming IAC Yokohama System for Reporting Breast FNAB Atlas [1].
This description is followed by a conclusion or summary in which the cytopathologist should give as specific a diagnosis of the lesion as possible (for example, “fibroadenoma”), or, if the diagnosis is uncertain, provide the most likely differential diagnoses. Ideally, both the imaging and cytology findings should be as precise as possible to maximize the specificity of the triple test, and to highlight any discrepancies. Finally, a category number, 1, 2, 3, 4, or 5 for insufficient, benign, atypical, suspicious of malignancy, and malignant, respectively, can be stated in the body of the report. This category number will assist with quality assurance activities and research. The category number is not to be used as a replacement for the actual diagnosis or the descriptive category terminology. The aim of utilizing consistent categories and a clear diagnosis is to enhance communication between the cytopathologist and clinician.

The ROM for each category has been extracted from the most recent literature [16, 17], so as to include current best practice and minimize confounding differences between studies, which include patient cohorts, experience of the FNAB operators, use of ultrasound guidance and statistical analyses [15, 17]. The IAC Reporting categories were then linked with management algorithms, which are dependent on the availability of local medical resources and local practices (Table 1). To meet this challenge, management options have been provided. However, many studies in the literature have used different methodologies, statistical calculations and categories which do not align with the categories in the IAC System, so the current ROM will be refined by future research, analogous to the modifications of the Bethesda System for Reporting Thyroid FNAB Cytology [30].

Category: Insufficient/Inadequate

Definition: The smears are too sparsely cellular or too poorly smeared or fixed to allow a cytomorphological diagnosis.

FNAB smears are regarded as adequate or inadequate based on the assessment of the material on the slides. If there is sufficient material on the slides to reach a diagnosis the FNAB is categorized based on that material. However, if the triple assessment is discrepant and the FNAB material does not explain the imaging or clinical findings, then the FNAB report should contain a statement that “the material may not represent the lesion,” and further FNAB or usually CNB is required. The term “non-diagnostic” is not recommended.

The assessment of adequacy may not require epithelial material to be present if the cytological findings correlate with the clinical and imaging findings in the triple test and are sufficient to make a precise diagnosis [11, 13]. Such situations include: pus consistent with an abscess; a proteinaceous background with or without histiocytes consistent with cyst contents when the cyst has been drained under imaging or has no residual mass to palpation; fat tissue fragments consistent with a lipoma or fatty nodule; spindle cell lesions; fat necrosis; reactive lymphoid material consistent with an intramammary lymph node, or in some cases of a hyalinized fibroadenoma which correlates with imaging.

However, if there is a mass lesion on palpation or imaging, that does not decrease in size and drain on FNAB, it is recommended that seven tissue fragments each consisting of 20 or more epithelial cells to allow assessment of the architecture and the presence or absence of myoepithelial cells, should be a measure of adequacy [2, 13]. It is recognized that some epithelial lesions such as lobular carcinoma may on occasion yield only scant material and not provide tissue fragments of this size, but their pattern and cellular detail will usually allow them to be categorized as at least atypical [31–33]. If there are any atypical epithelial features or necrosis, even if there are fewer than seven tissue fragments, the case should be reported as atypical [13]. The reason for the categorization as insufficient/inadequate should always be stated in the report.

There are considerable variations in the literature in the definition of insufficient/inadequate and in the rates of insufficiency. These variations include the varying mix of patients, the experience of the FNAB operators, the type of practice (mammographic screening assessment clinic or clinic for patients with symptomatic lumps and other concerns) and types of lesions (palpable or impalpable), and it is not unexpected that insufficient rates also vary from 0.7 to 47% [10, 12, 14–17]. Similarly, an accurate ROM cannot be established because insufficient cases are usually excluded from PPV and NPV analyses, because only patients with surgical biopsy follow-up are included in these studies and the indication for surgery was often clinical or radiological suspicion of carcinoma [15, 17, 34].

When no clinical or imaging information is provided to the cytopathologist by those who have performed the FNAB, such as “completely drained cyst,” and there is no reasonable opportunity to review the case with the clinician, it is recommended that a report should be issued with a caveat that “correlation with clinical and imaging findings is required because the FNAB findings may not represent the lesion.”

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The insufficient rate in breast FNAB is influenced by:

- the skill of the practitioner who performs the FNAB with cytopathologists and practices utilizing ROSE having the lowest rates of insufficiency [16, 25, 26, 27, 35]
- the nature of the lesion with high insufficient rates in lesions of small size [26, 27, 36–39], lesions that are difficult to stabilize [40, 41], or scirrhous or sclerotic.

### Table 1. Management recommendations

<table>
<thead>
<tr>
<th>Category</th>
<th>ROM, %</th>
<th>Management</th>
<th>LMICMX</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insufficient</td>
<td>2.6–4.8</td>
<td>Review clinical and imaging findings; if imaging indeterminate or suspicious, repeat FNAB or proceed to CNB; if imaging benign consider repeat FNAB</td>
<td>Review clinical findings; if suspicious repeat FNAB</td>
<td>At ROSE, if inadequate due to a technical issue or the material does not explain the clinical or imaging findings, repeat FNAB up to a total of 3 times, ideally using ultrasound guidance; if FNAB still insufficient, proceed to CNB</td>
</tr>
<tr>
<td>Benign</td>
<td>1.4–2.3</td>
<td>Review clinical and imaging findings; if “triple test” benign, no further biopsy required, and review depends on the nature of the lesion; if clinical and/or imaging indeterminate or suspicious, repeat FNAB or proceed to CNB</td>
<td>Review clinical findings; if benign, nothing further; if suspicious, repeat FNAB</td>
<td>At ROSE, if the cellular material does not explain the clinical or imaging findings, repeat FNAB, up to a total of 3 times, using ultrasound guidance; follow-up depends on the nature of the lesion, e.g., abscess – 2 weeks after antibiotics, fibroadenoma – 12 months; some centers review in line with screening program policy</td>
</tr>
<tr>
<td>Atypical</td>
<td>13–15.7</td>
<td>Review clinical and imaging findings; repeat FNAB if atypia considered likely to be due to a technical issue; if good material available and atypical, repeat FNAB or preferably proceed to CNB</td>
<td>Review clinical findings and repeat FNAB; manage based on FNAB category; if further FNAB atypical, consider excisional biopsy</td>
<td>At ROSE, if atypia is considered due to a technical issue, repeat FNAB; if cellular material adequate and atypical, proceed to CNB</td>
</tr>
<tr>
<td>Suspicious</td>
<td>84.6–97.1</td>
<td>Review clinical and imaging findings; CNB is mandatory</td>
<td>IF no CNB available, excision biopsy</td>
<td>At ROSE proceed to CNB</td>
</tr>
<tr>
<td>Malignant</td>
<td>99.0–100</td>
<td>Review clinical and imaging findings; CNB if any discrepant findings. If “triple test” is concordant and malignant, proceed to definitive management</td>
<td>If no CNB available, excision biopsy</td>
<td>At ROSE may proceed to CNB</td>
</tr>
</tbody>
</table>

See text for further discussion. ROM, risk of malignancy; FNAB, fine-needle aspiration biopsy; CNB, core-needle biopsy; ROSE, rapid on-site evaluation.

* References: Montezuma et al. [16]; Wong et al. [17].
  
* Best practice recommendation where imaging and CNB available.
  
* Low- and middle-income countries management: best practice recommendations where imaging and/or CNB not available.
  
* Atypical cases with good material and atypical features should have clinical and imaging review: there is considerable variation in management protocols at this point, including immediate CNB if the imaging is atypical or indeterminate and review with imaging at 3 or 6 months if imaging is benign.
  
* If FNAB is “suspicious” or “malignant,” then regardless of clinical and imaging findings, the FNAB dictates management.
  
* Concordant “triple test” is mandatory before surgery and prognostic markers can be performed on the cell block, but it is recognized that in some institutions CNB is required prior to neoadjuvant chemotherapy or definitive surgery, while in other institutions the patient will proceed to definitive surgery and prognostic markers will be performed on the excised specimen.
  
* FNAB with or without CNB is recommended on palpable or suspicious on ultrasound axillary lymph nodes to assist in staging the lesion.
lowly proliferative or lowly cellular, such as lobular carcinomas, or lesions that are microcalcifications without a corresponding mass lesion [33, 42]

- the number of FNAB passes: two or three is recommended if ROSE is not utilized [43].

The insufficient rate can be reduced by better initial training, a greater case load to build experience, use of ultrasound guidance, immediate feedback on the adequacy and quality of the specimen through ROSE or a cytopathologist performing the FNAB, and rapid correlation with imaging and clinical findings [16, 17, 25, 35, 37]. It is recommended that experienced personnel performing the FNAB should aim for less than a 5% insufficient rate, particularly if utilizing ROSE, while a rate of 10–20% requires review of the procedure. If the rate is greater than 20% urgent review of the techniques of those performing the FNAB is required.

**Management**

If the smear is insufficient due to technical issues the FNAB should be repeated to a maximum of three passes [43]. If the insufficiency is due to a lack of sufficient cellularity to explain the clinical or imaging expected diagnosis, it should be repeated. Correlation with the clinical and imaging findings in the triple test determines further management. This is facilitated by the use of ultrasound guidance and ROSE. If the imaging is indeterminate or suspicious, repeat FNAB or CNB is mandatory. If the imaging is regarded as benign or at low risk, in some practices follow-up with clinical or imaging review may be regarded as appropriate and this usually occurs at 3–6 months. In a setting where no imaging is available, the clinical and FNAB findings should be closely correlated, and repeat FNAB usually recommended.

**Category: Benign**

**Definition:** A benign breast FNAB diagnosis is made in cases that have unequivocally benign cytological features, which may or may not be diagnostic of a specific benign lesion.

In most follow-up studies of breast FNAB a histological diagnosis was not required after a benign FNAB diagnosis to determine the NPV and PPV of the benign category. A benign FNAB diagnosis with negative clinical and imaging findings is regarded as sufficient diagnostic work up, and if still negative at 6–12 months the benign FNAB is regarded as correct. The ROM is reported as in the range of 1–3% [14, 15, 44–46]. Two recent studies reported an NPV of a benign FNAB with histopathological follow-up as ranging from 97.1 to 98.97% [16, 17].

Because the vast majority of benign FNAB do not proceed to histopathological biopsy, an “overall” sensitivity for benign cytology can be calculated from the total number of FNAB in a series as “the number of correctly identified benign lesions, “expressed as a percentage of the total number of benign FNAB diagnoses” [47]. This was calculated in a recent study as 96.9% and reflects the sensitivity of benign breast FNAB in practice [17]. Rates of benign diagnoses vary between different practices, for instance between mammographic screening program assessment clinics and a breast clinic for patients presenting with breast lumps or pain [17].

The key cytological features of benign lesions include a pattern of predominantly large cohesive three-dimensional tissue fragments and flat mono-layered sheets consisting of evenly spaced, ductal epithelial cells with myoepithelial cells creating a “bimodal” pattern, as well as, “bare bipolar nuclei” representing stripped myoepithelial nuclei, in the background [11, 13]. Although rarely sampled, normal breast tissue yields intact lobules and small terminal ductular tissue fragments showing ductal nuclei and myoepithelial nuclei. (Fig. 1) The nuclei of terminal ductules and ductal epithelium vary in size and chromatin pattern from small and fine, in normal epithelial components, to moderately large and mildly coarse in proliferative lesions, while nucleoli vary from small and inconspicuous to single larger round nucleoli [11, 13, 48].

The most common benign lesions diagnosed by FNAB include: acute mastitis and breast abscess; granulomatous mastitis due to specific infections, including tuberculosis [49] and nonspecific inflammatory processes that require microbiological studies, including culture or molecular testing to exclude tuberculosis; foreign body reactions such as that to silicone [50]; fat necrosis; cysts with apocrine sheets in a proteinaceous background (Fig. 2); material “consistent with cyst contents” when there is a granular proteinaceous background with no epithelium and there is correlation with imaging and clinical findings and the cyst completely drained; fibrocystic change with apocrine sheets and small cohesive ductal epithelial tissue fragments in a proteinaceous background (Fig. 3); lactational change with small acinar sheets of vacuolated cells and stripped acinar nuclei in a milky proteinaceous background including fine fat globules; normal breast with lobules and small terminal ductular tissue fragments in a clean background with bare bipolar nuclei (Fig. 1); usual epithelial hyperplasia with cohesive large ductal epithelial tissue fragments with myoepithelial cells and with bare...
bipolar nuclei in a clean background (Fig. 4); fibroadenoma with similar large ductal epithelial tissue fragments and plentiful bare bipolar nuclei and fibrillary or rounded stromal fragments (Fig. 5, 6); and intraductal papilloma with similar large ductal epithelial tissue fragments, along with papillary stellate and more complex meshwork fragments, apocrine sheets and siderophages in a proteinaceous background [51]. In male patients, gynaecomastia resembles epithelial hyperplasia with or without scanty stroma. Intra mammary lymph nodes show a heterogeneous lymphoid population with predominantly small lymphocytes.

Cellularity is increased in the proliferative lesions such as usual epithelial hyperplasia and fibroadenomas, and is
often associated with increased dispersed single cells and admixed smaller tissue fragments with usually bland nuclei, while myoepithelial nuclei and bipolar nuclei are still present. If the overall pattern is diagnostic of a specific lesion such as a fibroadenoma, the high cellularity and dispersal and a degree of nuclear enlargement are still acceptable for a benign diagnosis [13]. The experience of the reporting cytopathologist will determine the threshold between a confident benign diagnosis and an atypical diagnosis in reporting these proliferative cases.

Management

For more than 50 years, FNAB of breast directed by palpation has provided an accurate diagnostic work up of breast lesions without necessarily any imaging, and this is particularly the case where clinical findings clearly correlate with the FNAB, for example, a cyst that completely drains with no palpable residual lesion, or a clinical abscess that yields purulent material, or a rounded mobile mass that has the characteristic cytological findings of a fibroadenoma, or a fixed gritty mass that has the findings of a carcinoma.

However, where imaging is available, best practice is to perform pre-FNAB imaging, utilize ultrasound or other imaging to direct the FNAB, and correlate the clinical, imaging and cytological findings in the triple test. The use of ultrasound guidance assists in ensuring the target lesion has been sampled. Correlation is essential and a benign FNAB diagnosis that correlates with the clinical and imaging findings does not require any further biopsy and no specific recommendation is needed in the report. However, if the clinical or imaging assessment is indeterminate and not explained by the cytology, or if it is suspicious, the cytology should be reported as benign, and follow-up biopsy usually by CNB should be recommended. Conversely, indeterminate cases on imaging, such as an apparent fibroadenoma that may have some irregularities, when reported as benign on FNAB do not require further work up.

Follow-up for a benign FNAB diagnosis varies with the nature of the benign lesion, for example, follow-up for an abscess may be after 2 weeks of antibiotics. Follow-up also varies between institutions and countries, but it is usually at 12–24 months, with the patient returned to routine screening in mammographic screening programs.

Category: Atypical

Definition: The term atypical in breast FNAB cytology is defined as the presence of cytological features seen predominantly in benign processes or lesions, but with the addition of some features that are uncommon in benign lesions and which may be seen in malignant lesions.

Fig. 5. Fibroadenoma: hyperplastic ductal epithelial tissue fragment with myoepithelial nuclei, branched dumb-bell ended epithelial tissue fragments, smaller epithelial tissue fragments, a folded ductal epithelial tissue fragment and a ragged fragment of stroma with some bare bipolar nuclei in the background (Pap, ×100).

Fig. 6. Fibroadenoma: hyperplastic ductal epithelial tissue fragments, an irregular fragment of myxoid stroma and bare bipolar nuclei with some dispersed cells in the background; a small aggregate of histiocytes is also present (Giemsa, ×100).
The ROM of an atypical diagnosis varies in the literature from 22 to 39% [10, 14, 52–57], reflecting the variability of the definition and usage of the term “atypical” in the literature. Two more recent studies that looked at applying the IAC Yokohama System had ROM of 13 and 15.7% [16, 17]. As the body of literature regarding the System grows, the ROM of an atypical cytologic diagnosis will be refined.

The specific cytological features that are considered atypical, such as high cellularity, increased dispersal of single intact cells, enlargement and pleomorphism of nuclei, presence of necrosis or mucin, and complex micro-papillary or cribriform architecture of epithelial tissue fragments, should always be stated [11, 13]. The diagnosis, if possible, should include the differential diagnosis and the diagnosis considered most likely.

There are three major causes for “atypical” diagnoses on breast FNAB cytology, and in many cases these may all be contributing factors.

Firstly, the skill of the operator is most important [25, 35, 36]. Poor FNAB technique may result in low cellularity and obscuring blood or ultrasound gel, overly forceful smearing causing crush artefact and dispersal (Fig. 7), or poor handling of material, which results in air-drying artefact when there is a delay in immersing slides in alcohol for Papanicolaou-stained slides and slow drying artefact in wet slides intended for Giemsa-stained slides. All of these result in suboptimal smears making interpretation difficult.

Secondly, interpretative problems do occur, and are due to overlapping cytological features between proliferative breast lesions, such as usual epithelial hyperplasia, intraductal papillomas [58] and fibroadenomas [59], and low-grade in situ lesions and low-grade invasive carcinomas (Fig. 8) [11, 13, 60]. The specific diagnosis of low-grade ductal carcinoma in situ (LGDCIS) and lobular carcinoma in situ (LCIS) and the exclusion of invasive carcinoma in these cases is not possible in FNAB cytology, but recognition of their features can suggest the diagnosis and help to distinguish in situ lesions from proliferative lesions to prevent a false positive diagnosis as carcinoma and a false negative diagnosis as a proliferative lesion [13]. CNB can be recommended.

Thirdly, the degree of training, experience, on-going case load and expertise of the cytopathologist in interpreting the material impact on atypical rates. Less experienced pathologists may focus on dispersal or nuclear atypia and ignore the overall diagnostic features of the lesion, most typically, a fibroadenoma [59–61].

Lesions commonly associated with an atypical diagnosis include usual epithelial hyperplasia by itself or associated with fibrocystic change, fibroadenomas [17, 59–61] (Fig. 9), radial scars [62] and intraductal papillomas [17, 59], and the much less common adenomyoepithelioma [63, 64] and spindle cell lesions [65]. The distinction between cellular fibroadenomas and low-grade phyllodes tumors based on stromal hypercellularity and stromal...
atypia is problematic, as it is in CNB, due to the varying cellularity, atypia and mitotic counts in each phyllodes tumors (Fig. 10) [66–69]. LCIS and invasive lobular carcinoma may both produce low cellularity and a dispersed pattern of small cells with mildly atypical nuclei, which can be difficult to distinguish from poorly sampled proliferative lesions such as fibroadenomas [11, 13, 16, 70]. The key to the correct diagnosis of proliferative and low-grade malignancies is the strict application of key cytological features diagnostic for specific lesions, including the assessment of smearing patterns, the architecture of tissue fragments and the degree of nuclear atypia [13].

Management

Repeat FNAB is recommended if the atypical diagnosis is considered primarily due to a technical problem. When there is sufficient quality material for interpretation, the triple test should be applied. If either or both the clinical and imaging findings are indeterminate or suspicious a repeat FNAB or, more commonly if available, CNB is mandatory. If neither clinical nor imaging findings are of concern, there is considerable variation in management dependent on the lesion and between breast care centers. The management options include to repeat the FNAB or to perform a CNB, or to review the patient with imaging at 3–6 months, with subsequent repeat FNAB or CNB if the lesion has changed. If imaging and CNB are not available, repeat FNAB and close follow-up are recommended.

### Category: Suspicious of Malignancy

**Definition:** The term “suspicious of malignancy” in breast FNAB is defined as the presence of some cytomorphological features, which are usually found in malignant lesions, but with insufficient malignant features, either in number or quality, to make a definitive diagnosis of malignancy. The type of malignancy suspected should be stated whenever possible.

The definition and use of the term “suspicious of malignancy” varies in similar fashion to the term “atypical” and this is reflected in the published range of PPV of a suspicious diagnosis from 60 to 95% [3–6, 8, 12, 14, 15, 70–78]. Two recent studies utilizing the IAC Yokohama System had ROM for a “suspicious of malignancy” diagnosis of 97.1 and 84.6% [16, 17]. The inclusion of a suspicious category helps maintain the high PPV of a malignant diagnosis while maintaining the sensitivity of FNAB cytology. The causes of a “suspicious of malignancy” diagnosis are similar to those of the atypical category and include technical problems related to the skill of the operator performing the FNAB, making smears and handling the material, the experience of the interpreting cytopathologist, and the nature of the breast lesion (Fig. 11, 12).

The cytological features of proliferative lesions and low-grade or in situ carcinomas overlap and great care has to be taken in assessing smear patterns and nuclear atypia [13, 79]. This mirrors the difficulties in surgical
pathology in distinguishing atypical proliferative lesions from LGDCIS [80].

LGDCIS includes a range of solid, cribriform, micropapillary, papillary and solid papillary subtypes, and although rarely producing a clinical or radiological mass it may be associated with microcalcifications. In surgical pathology, LGDCIS is often an incidental finding in association with both benign and malignant lesions, or a lesion that is found in the work up of mammographic calcifications [80]. FNAB cytology cannot specifically diagnose LGDCIS and at the same time exclude invasive carcinoma [79, 81, 82].

In FNAB cytology, LGDCIS most typically produces highly cellular smears, a pattern of large tissue fragments showing cribriform, micropapillary or papillary architecture, a variable but often marked increase in dispersed single cells showing mild to moderate nuclear atypia, a greatly reduced number or total lack of myoepithelial cells associated with the epithelial tissue fragments, and scant or absent bare bipolar nuclei in the background (Fig. 13, 14) [11, 13, 81–83].

Recognition of these features prevents under-calling LGDCIS as a proliferative lesion, and over-calling LGDCIS as invasive carcinoma [60, 79]. Although controversial in relation to the specific diagnosis of LGDCIS, the categorization of these lesions as “suspicious of malignancy” is recommended. Correlation with imaging is required, utilizing the triple test approach, and a more specific diagnosis in both cytology and imaging refines the triple test approach.

The diagnosis of high-grade ductal carcinoma in situ (HGDCIS) by FNAB is also controversial [83–86]. FNAB cannot diagnose HGDCIS to the exclusion of invasive carcinoma. HGDCIS is often associated with casting pleomorphic calcifications on mammography, and occasionally may produce a palpable or imaging mass lesion in the absence of invasive carcinoma [80].

In FNAB cytology smears, HGDCIS has been reported to be associated with extensive necrosis, calcifications and high-grade nuclear atypia in dispersed single epithelial cells and both small and larger crowded epithelial tissue fragments [87]. The smears are often low in cellularity reflecting the small volume of cancer cells in ducts relative to breast tissue [87]. These findings cannot exclude high-grade invasive carcinomas, particularly those of no special type and metaplastic carcinomas, which can also show necrosis. It is highly debated as to whether “suggesting that there is an HGDCIS component” assists management.

Few institutions and cytopathologists have attempted to diagnose the presence of HGDCIS “with or without an invasive component” or to use the “suspicious of malig-
nancy” category for these cases. In the past an outright malignant breast FNAB could lead to a full axillary clearance only to find just HGDCIS in the mastectomy. Currently, axillary dissection at the time of resection of the breast primary is guided by sentinel lymph node biopsy or a prior positive FNAB or CNB of axillary lymph nodes [88]. Furthermore, the use of sentinel lymph node biopsy and surgical management of HGDCIS and invasive carcinoma are similar in many institutions.

A highly dispersed pattern of large atypical cells may also raise the differential diagnosis of lymphoma, and a “suspicious of malignancy” categorization may be prudent to avoid unnecessary surgery. If lymphoma is suspected based on ROSE, material may be triaged for flow cytometry if available and/or cell block for immunohistochemistry.

**Management**

A “suspicious of malignancy” FNAB cytology diagnosis should lead to review of the imaging findings, but further biopsy is an absolute requirement and most commonly this will be a CNB. If CNB is not available, then surgical excision biopsy is required before specific treatment in almost all cases. When the “suspicious of malignancy” diagnosis is made at ROSE, immediate CNB is ideal.

The PPV of a malignant breast FNAB ideally should be 100%, but there is a reported range from 92 to 100% [3–6, 8, 12, 14, 15, 70–78]. Two recent studies utilizing the IAC Yokohama Reporting System had ROM of 100 and 99.0% for the malignant category [16, 17].

The malignant diagnosis should only be used when there is a full constellation of cytological findings and no discrepant features. These key cytological findings include high cellularity, prominent dispersal of single cells, crowded tissue fragments with overlapping nuclei, and most importantly, nuclear enlargement, anisonucleosis, pleomorphism of the nuclear margin, size and chromatin, hyperchromasia and prominent nucleoli (Fig. 15) [11, 13]. The nuclear features of malignancy will vary from low- to high-grade carcinomas, and none of these features taken separately is pathognomonic of carcinoma.

Various other cytological features have been suggested as evidence of invasive carcinoma, including tubular architecture of epithelial fragments, intracytoplasmic lumen in atypical epithelial cells and elastoid fragments [86, 89, 90]. The presence of stromal fragments infiltrated by...
carcinoma in breast smears resembling smaller fragments seen in CNB is regarded by some authors as being categorical evidence of invasive carcinoma [13, 91].

It is recommended that the type of malignancy should be mentioned or at least suggested in the report. It is recognized that this is not always possible, and lesions such as pleomorphic lobular carcinoma are not distinguishable reliably from high-grade invasive carcinoma, no special type [13].

The most common types of carcinoma which can be suggested in breast FNAB cytology based on their cytological characteristics are no special type (ductal) (Fig. 15), lobular (Fig. 16), mucinous (Fig. 17) [92], tubular and metaplastic. Ancillary studies such as E-cadherin for lobular carcinoma can be applied to cell blocks [11, 13, 29, 91] or liquid-based cytology [28, 93]. Less common carcinomas that might be suggested or diagnosable based on their key cytological features with or without ancillary studies are carcinoma with medullary features, adenoid cystic carcinoma, carcinoma with apocrine differentiation, carcinoma with neuroendocrine features, and carcinoma with osteoclastic giant cells [94]. Also, malignant lymphomas, angiosarcomas and some metastatic carcinomas and metastatic melanoma to the breast can be diagnosed. Rare cases of secretory, histiocytoid, glycogen-rich and clear cell carcinoma have been reported in the cytological literature.

**Management**

A malignant FNAB cytology diagnosis should be correlated with the clinical and imaging findings, and if the triple test is concordant and material is available in the cell block for prognostic and treatment markers [29], definitive therapy including neoadjuvant chemotherapy and surgery can be commenced. Some centers will proceed on the basis of a positive triple test to surgical excision with the markers being performed on the excised lesion. However, many centers and treatment and trial protocols in the developed world require CNB before neo-adjuvant chemotherapy or definitive surgery.

If the FNAB cytology diagnosis does not correlate with the other components of the triple test, CNB or, if this is not available, simple excision biopsy, is mandatory prior to commencing definitive treatment.

If the axillary lymph nodes are palpable or enlarged or suspicious on ultrasound of the axilla, FNAB is recommended to stage the patient who has presented with a breast lesion. Some centers will then proceed to CNB of the lymph node to confirm the FNAB findings. Other centres may use only CNB to assess the lymph node, possibly because ROSE at the time of a FNAB is not available [88]. If the lymph node cytology shows metastatic carcinoma the patient is staged. If the lymph node cytology is negative or suspicious of metastatic carcinoma then sentinel lymph node biopsy is required.
Ancillary Techniques

Independent of the preparation used, FNAB of breast cytology provides good and reliable material for ancillary studies [93]. This material can be found in direct smears fixed in alcohol with Papanicolaou or HE staining or air dried and stained with a Giemsa or similar stain, as well as, in liquid-based cytology preparations and formalin-fixed paraffin-embedded cell blocks. The ancillary studies include immunocytochemistry, immunohistochemistry and molecular techniques. These ancillary techniques are of value in specific situations that include: diagnostic difficulties, for example, utilizing myoepithelial cell markers on cell block material in atypical and suspicious lesions; immunohistochemistry on cell blocks for the prognostic and predictive markers for estrogen, progesterone and HER2 receptors; in situ hybridization for HER2 on cell blocks; immunohistochemistry for the determination of a primary site in metastatic lesions; and the study of prognostic and predictive markers in the metastatic breast cancer setting [12, 25, 93].

In some centers around the world, patients are treated preoperatively with neo-adjuvant chemotherapy on the basis of the FNAB in the context of the triple test, without a CNB or surgical biopsy. The FNAB smears and cell block can provide material for predictive markers [25]. Tracking breast cancer evolution by using high-throughput technology such as next-generation sequencing to detect ER or HER2 mutations using FNAB material from breast cancer metastases is one of the next developments in the changing world of the application of molecular testing in FNAB of the breast.

Conclusion

The IAC Yokohama System for Reporting Breast FNAB cytology defines five categories for reporting breast cytology and this article provides discussion on the use of these categories. The categories effectively stratify breast lesions by their ROM and provide guidance within a management algorithm for each category [1]. Each category has a specific recommended term and in the case of inadequate/insufficient a choice of two terms. The System emphasizes the crucial importance of high-quality performance of the FNAB procedure and of the making of direct smears. The interpretation of the smears also requires good training and on-going experience, and the authors believe will be greatly helped by the forthcoming Atlas.

The authors recognize that the performance indicators of breast FNAB, including specificity and sensitivity, NPV, PPV and ROM stated in this article are those derived from reviews of the literature and recent articles. It is hoped that the Reporting System will stimulate research into these indicators to test the current System and its management recommendations. The authors also recognize that reviews of past studies, which vary in patient population, diagnostic approach and statistical analysis, may not represent current best practice, which now includes sophisticated imaging and many patients the use of ultrasound to assist with guidance of the FNAB and the use of ROSE. ROSE provides the opportunity to immediately assess the FNAB material for adequacy and triage the case for the most cost-effective further diagnostic procedures including immediate CNB [17, 95]. When a cytopathologist has made the provisional diagnosis at ROSE this can guide discussion with the patient, greatly reducing patient anxiety [96].

The management recommendations provide options because the authors recognize that there are variations internationally and within countries in the application and the use of FNAB and CNB. The availability of imaging and CNB with its inherent need for readily available histopathology laboratories vary between those countries with well-developed medical infrastructure and those low- and middle-income countries with more limited resources [97]. The use of FNAB and CNB will also
vary with the availability of expertise and the clinical practice.

Finally, the IAC Yokohama System will invoke a response from the cytology community and also from all those who provide a component in the “triple” diagnostic approach, which is the best practice for women with breast lesions. The authors sincerely hope that this will lead to better patient care.

Disclosure Statement

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