Acta Cytologica

Fine Needle Aspiration

Acta Cytologica 2024;68:206–218
DOI: 10.1159/000539692

Received: February 3, 2024
Accepted: June 3, 2024
Published online: June 11, 2024

Overcoming Pitfalls in Breast Fine-Needle Aspiration Cytology: A Practical Review

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Keywords
Breast cytology · Fine-needle aspiration cytology · Diagnostic pitfalls · Yokohama system · Triple approach

Abstract
Background: Fine-needle aspiration cytology (FNAC) is a cornerstone technique for the initial assessment of breast lesions, offering a rapid and minimally invasive option for cytological evaluation. While FNACs can forego the need for core needle biopsies (CNBs), variations in technique, subjective interpretation, and intrinsic limitations present diagnostic challenges. The International Academy of Cytology (IAC) established the Yokohama system and is developing the WHO Reporting System for Breast Cytopathology jointly with IARC, to standardize diagnostic criteria, aiming to enhance diagnostic precision and consistency. Due to the preference for CNBs, expertise in breast FNAC is low in the developed world. ***Summary:*** This review assesses common pitfalls in breast cytopathology. These common and uncommon entities may easily lead to false-negative or false-positive diagnoses, due to morphological overlap or misleading clinical and radiological contexts. For instance, pauci-cellular lesions, such as lobular carcinomas, often lead to false-negative diagnoses, whereas complex sclerosing lesions, fibroadenomas, and papillary lesions may show concerning features, resulting in a false positive. The same is true for some benign inflammatory pathologies, such as steatonecrosis, and uncommon lesions, such as collagenous spherulosis. Ductal carcinoma in situ can lead to both false-negative and false-positive diagnoses, and high-grade lesions are impossible to tell apart from invasive carcinomas. These are discussed in detail. Procedural and preanalytical conditions, and the role of ancillary testing, are also briefly addressed. ***Key Messages:*** Breast FNAB is a powerful diagnostic technique, fast and minimally invasive. Even in contexts which lack expertise, this technique can be successfully adopted with a cautious approach and as long as pitfalls are kept in mind, benefiting patients and healthcare systems.

Introduction

Fine-needle aspiration cytology (FNAC) remains a mainstay for the initial assessment of breast lesions in many institutions all over the world, particularly in developing nations and in specialized practices in the West and in Asia. FNACs are quicker and less expensive to

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perform compared to core needle biopsies (CNBs). Furthermore, they are minimally invasive, resulting in less discomfort for the patient, particularly in difficult-to-reach locations such as axillary extensions and breast folds, enabling the collection of biological material for further molecular testing and immunocytochemistry (ICC), with few, if any, complications [1]. Despite their well-established utility, however, FNACs have fallen out of favor in most of the developed world, resulting in a lack of expertise among pathologists, outside of the aforementioned specialized practices [2].

Breast lesions are complex and require a diagnostic approach that is both comprehensive and nuanced, particularly in cytology. Variations in sampling technique, varying levels of experience, and the intrinsic limitations of FNAC contribute to a diagnostic spectrum which may at times be daunting to the novice and expert alike [1, 3].

Against this backdrop, the International Academy of Cytology (IAC) sought to establish the Yokohama system for reporting breast cytology, with the aim of standardizing diagnostic criteria, defining risks of malignancy, and aligning terminologies [1]. This system offers a framework for enhanced diagnostic precision, lowering interobserver variability, and providing clinicians with clearly actionable results [1]. The system is being improved with the development of the World Health Organization's (WHO) reporting system for breast cytopathology.

Diagnostic pitfalls still often arise in daily practice, however, requiring awareness to properly navigate [1]. In this review, we explore these pitfalls in detail, with an emphasis on practical aspects. We also briefly discuss the preanalytical challenges and nuances of ancillary testing.

Procedural and Preanalytical Considerations

Although not strictly pitfalls, artifacts resulting from poor procedural and preanalytical workflows may significantly alter samples, hindering or impeding a correct diagnosis. During the FNAC procedure itself, an adequate technique is necessary to both accurately locate the lesion and obtain representative material. Both an incorrect localization and a poorly done procedure may lead to nonrepresentative or suboptimal material, potentially leading to false-negative results, false reassurance for clinicians and the patient, and a delayed diagnosis. The former is particularly important in smaller lesions, which may be hard to immobilize. It can be improved with imaging methodologies, and both situations benefit from proper training in the procedure, which is essential for success [1].

Simple errors must also be kept in mind at all stages of specimen handling, namely, during labeling, coverslipping, and staining, possibly leading to diagnostic discrepancies and inaccuracies. These must be taken care of through standardized laboratory procedures and traceability, as they may be very difficult or impossible to resolve after they take place. Additionally, the choice of sample preparation can greatly impact the diagnostic process downstream and must be adequately considered [1, 4].

Smears are still the most common and accessible sample type. Preparing these slides – smearing – is a manual process which requires experience to adequately perform. Too much pressure may, for instance, result in significant crushing artifacts, and too little pressure often yields an inadequately spread slide, making it difficult to correctly appreciate cellular morphology. This has the potential to render a sample nondiagnostic or lead to false atypical or positive diagnoses [4].

Air-dried smears must be stained with May-Grunwald-Giemsa (MGG), which highlights noncellular elements present in samples and enables a detailed view of the cytoplasm. Alcohol-fixed smears, on the other hand, are usually stained with Papanicolaou, which highlights nuclear details. These smears should be placed in a solution of ethanol or methanol shortly after smearing. Inappropriate air-drying, in this context, can lead to cell enlargement, possibly leading to false positives and erroneous diagnoses [4].

When smears are used, it is also advised to consider collecting additional material directly to formaldehyde for cellblock preparation. ICC can indeed be performed successfully in smears, but as discussed in the next section, this requires expertise to obtain consistent results, as well as tests created from other smears using cell transfer methods, both of which may not be available in all pathology laboratories [2].

Liquid-based cytology (LBC) preparations have also grown in popularity since they were first introduced in the early 1990s, enabling a more automated and reproducible slide preparation, albeit at an increased cost. Since these early days, these techniques have been refined and are currently applied to almost all types of cytology samples [5]. LBC preparations result in a homogeneous distribution of cells in the slide, with great preservation of nuclear detail, but show less matrix and noncellular elements, which may increase the difficulty of certain diagnoses. Given the major differences between LBC and smears, specific training and experience in the interpretation of this sample type are required [1].

LBC samples usually lead to good results in ancillary testing, namely, ICC and HER2 in situ hybridization (ISH), and may obliviate the need for cellblocks. Caution must be taken when preparing cellblocks from LBC

Table 1. Comparison of specimen types and preparation techniques

Specimen type	Advantages	Disadvantages
Smears	Rapid on-site evaluation (ROSE) (air-dried; Diff-Quick stained) Better stroma and noncellular element preservation	Crush artifacts may simulate atypia Inhomogeneous cellular distribution Overlapping and obscuring possible Less reliable for ICC than other sample types
LBC	Homogeneous cell distribution No air-drying or crushing artifacts Preserves nuclear detail Enables reliable ICC and molecular testing	More costly Less noncellular elements ROSE not possible Specific criteria and artifacts, requiring specific training
Cellblocks	Optimal for ICC and molecular testing Preserves cellular detail	May require additional passages of the needle Requires extra sample processing If prepared from alcohol-fixated samples (such as LBC), they may lead to false negatives in ICC

solutions, however, as certain studies have reported on a higher rate of false negatives with double alcohol and formaldehyde fixation [2].

LBC slides are usually stained with Papanicolaou. Proper staining is essential for good results in all sample types. Problems in this step may lead to artifactual changes in the nuclear chromatin pattern, raising the risk for false positives in a given sample [1, 5]. A brief summary of different cytological preparations can be found in Table 1.

Ancillary Techniques

As highlighted in the previous section, considerations about ancillary testing in breast cytological material begin in the preanalytical phase. Both ICC and ISH are heavily affected by preanalytical conditions. This is particularly important in the context of breast cytology, given the routine use of these techniques for prognostication and defining adequate therapy in breast carcinomas. Furthermore, ICC adds significantly to the diagnostic value of breast FNAC [6–12]. A recent review compiled the extensive body of the literature on the subject, showing that indeed ICC can be performed in cytological material with results comparable to histology and CNBs [2]. However, preanalytical conditions must be considered and vary significantly according to specimen type. Smears showed the worst performance across publications, with air-dried smears performing worse than alcohol-fixated ones. Of note, false equivocal and positive HER2 ICC results seem to be more common in these sample types, whether air-dried or alcohol fixated; therefore, HER2 ISH is recommended in smears instead of ICC. LBC samples, on the other hand, were shown to be adequate for most ICC and ISH, with good, reproducible results overall. Cellblocks showed better performance than other sample types, enabled multiple sections, and were uniquely shown to be adequate even for the evaluation of Ki67 [2, 13]. Interestingly, when cellblocks were prepared from LBC solutions, which are methanol based, there seemed to be a risk of false negatives on ICC, as a result of double fixation. Regardless of sample type, the authors highlight the importance of using a proper antigen retrieval and adequate testing. Cellblocks hold another advantage in this regard, since the same tests that are used for tissue can be employed, whereas for smears and LBC tests made from the same sample type are recommended [2].

The diagnostic utility of molecular techniques like ISH, polymerase chain reaction, and next-generation sequencing also hinges on accurate specimen handling and appropriate quality control measures. In the context of breast cancer, particularly in metastatic cases, FNACs can provide high-quality material for these studies, particularly if collected fresh to a cell culture medium such as RPMI. Molecular tests may also be performed on air-dried, alcohol- or formaldehyde-fixed samples, with adapted workflows. To extract maximum value from these techniques, robust standardization, attention to detail, and adequate controls are essential. Namely, when performing ISH tests, one must ensure that the cells evaluated hail from malignant clusters, and not those of ductal carcinoma in situ (DCIS); for polymerase chain reaction and next-generation sequencing, a minimum neoplastic cellularity must be present, according to

Table 2. Pitfalls in breast cytology

Breast lesion	Cytological features	Differential diagnoses
Complex sclerosing lesions	Moderately to highly cellular 3D or monolayered epithelial fragments with myoepithelial cells Small sclerotic of fibromyxoid stromal fragments Similar to hyperplastic fibrocystic change of the breast	Invasive low-grade carcinoma • May be indistinguishable • ICC for myoepithelial markers (p63, calponin, CK5/6) DCIS • May involve the lesion • CK5/6 will not stain DCIS but will stain usual duct hyperplasia
Lobular carcinoma	Low cellularity Low atypia Marked decohesion Tumor cells may mimic lymphocytes and be obscured by hyperplastic changes	Nondiagnostic or benign diagnoses • High false-negative rate • Dispersed atypical small cells favor malignancy • E-cadherin, p120 for detection of sparse neoplastic cells LCIS • May be indistinguishable • More cellular • Less atypia
Papilloma	Moderately to highly cellular 3D fragments with or without true fibrovascular cores, and monolayered sheets Cystic background Apocrine metaplasia May harbor hyperplastic and metaplastic changes May be involved by DCIS	Malignant papillary lesions • Higher architectural complexity • More dissociation • Cytologic atypia • No macrophages or apocrine metaplasia Fibroadenomas and nonpapillary DCIS • Lack fibrovascular cores • Lack cystic background • Myoepithelial markers help highlight lesion architecture • CK5/6 stains hyperplasia but not DCIS • ER stains DCIS in a diffuse fashion, and hyperplasia in a spotty fashion
Fibroadenomas	Highly cellular Cohesive, large fragments of ductal and myoepithelial cells Stromal elements Dissociated myoepithelial cells and bipolar nuclei	Phyllodes tumors • Lack abundant bipolar nuclei • Larger, more atypical nuclei • Large hypercellular stromal fragments in malignant lesions Well-differentiated carcinomas • Lack of bipolar nuclei • More atypical nuclei
DCIS	Variable cellularity Monotonous, cohesive clusters Solid, cribriform, micropapillary, and papillary architecture Cellular atypia and a necrotic background in high-grade lesions Microcalcifications	Benign hyperplasia • More cohesive fragments, showing streaming • No cribriform or micropapillary architecture • Dispersed myoepithelial cells and bare bipolar nuclei in the background Invasive carcinoma • Association of atypical cells with adipocytes of stromal fragments • Low cellularity, highly atypical samples with a necrotic background, and microcalcifications favor high-grade DCIS • Myoepithelial markers may be useful • Differential with high-grade DCIS is usually not possible

Table 2 (continued)

Breast lesion	Cytological features	Differential diagnoses
Steatonecrosis	Macrophages and adipocytes, some necrotic Necrotic background Microcalcifications	Invasive carcinoma/high-grade DCIS • Higher epithelial cellularity • Higher cytologic atypia • No necrotic adipocytes
Intramammary lymph nodes and low-grade lymphomas	Highly cellular slides Small cells Clumping may occur, mimicking epithelial cells	Invasive ductal/lobular carcinoma • Nuclear detail differs from lymphoid cells • Higher cytologic atypia • ICC for epithelial and lymphoid markers
Apocrine metaplasia	May be highly cellular Dispersed cells and sheets Mild cytologic atypia Vast cytoplasm May have a cystic background	Invasive carcinoma/high-grade DCIS • No apocrine morphology, unless apocrine carcinoma • Higher cytologic atypia • Higher N:C ratio • Necrotic background • Stromal invasion
Radiation effects	Usually low or moderate cellularity, but may be high Cohesive epithelial fragments Large, pleomorphic, hyperchromatic nuclei Smudged chromatin Vacuolated cytoplasm Fat necrosis and atypical fibroblasts in the background	Invasive carcinoma • More cellular decohesion • No nuclear smudging • Less cytoplasmatic vacuolization • No fat necrosis or atypical fibroblasts • There may be invasive carcinoma in a background of radiation effects
Granulomatous lesions (mastitis, silicone, soya oil, or paraffin granulomas)	Epithelioid histiocytes, which may be pleomorphic Granulomas with or without associated necrosis Foreign body material in silicone, soya oil, or paraffin granulomas	Invasive carcinoma • Lack granulomas in almost all cases • Atypical epithelial cells, dispersed and in clusters • Higher N:C ratio
Granular cell tumor	May be highly cellular Dispersed epithelioid cells Small, monotonous nuclei Granular cytoplasm Low N:C ratio, No myoepithelial cell layer	Invasive carcinoma • More cohesive epithelial clusters • Higher cytologic atypia • Higher N:C ratio • Less granular cytoplasm
Collagenous spherulosis (CS)	Collagenous spherules surrounded by epithelial or myoepithelial cells No atypia Bare bipolar nuclei dispersed in the background	Adenoid cystic carcinoma (ACC) • Larger, more prominent spherules • Overlapping ICC profiles in myoepithelial cells, except for CD10, which is negative in ACC and positive in CS • ACC luminal cells are CD117 positive, which is negative in CS • ACC luminal cells are negative for ER, PR, which are positive in CS
Mucinous carcinoma	Variable cellularity Epithelial fragments with tubular, cribriform, rounded, or papillary architecture Mild to moderate cytological atypia Background of abundant fibrillary mucin	Mucocele-like lesions • Always low epithelial cellularity • Reactive atypia only • Overlap with low cellularity, low atypia mucinous carcinoma – sign out as atypical

Table 2 (continued)

Breast lesion	Cytological features	Differential diagnoses
Lactational change	Highly cellular samples Cohesive cysts Larger nuclei Low N:C ratio No atypia Background of fat globules	Secretory carcinoma • Slightly more atypia • Possible significant overlap • Clinical and radiological correlations are essential

manufacturer guidelines. Interestingly, some authors have shown good results using these techniques on just supernatants, although at a cost of sensitivity [14, 15].

Pitfalls in Breast FNAC

Interpretation of certain breast lesions in cytology samples can be challenging, regardless of procedural or preanalytical conditions. Due to significant morphological overlap between lesions, difficulties in differential diagnoses may lead either to false-negative or false-positive diagnoses [16]. These lesions and the interpretative pitfalls they pose are discussed in detail below. A summary can be found in Table 2.

Complex Sclerosing Lesions

Complex sclerosing lesions, also known as radial scars, are often found incidentally and usually have a high level of radiological suspicion, due to spiculated contours. This is exemplified in Figure 1. Histologically, they show a central sclerotic scar surrounded by sclerotic ducts. These ducts may show metaplastic and proliferative changes, such as apocrine metaplasia, duct hyperplasia, with or without atypia, and sclerosing adenosis. DCIS may also involve these structures. Cytologically, they result in moderately to highly cellular slides, composed of large fragments of ductal epithelium, disposed in monolayered sheets and three-dimensional fragments, both featuring myoepithelial cells. Mild nuclear atypia can sometimes be present. In the background, bare bipolar nuclei are often present amid foamy macrophages, proteinaceous material, and small sclerotic or fibromyxoid stromal fragments [1, 17]. These findings are not specific and are similar to those observed in hyperplastic fibrocystic change of the breast. Concurrent DCIS is often difficult to identify, and, particularly in a hyperplastic background, tubular carcinomas or low-grade ductal carcinomas may be indistinguishable. The latter are particularly concerning given the possible overlap in radiological findings. ICC can be helpful in these differential diagnoses. Myoepithelial markers, such as p63, calponin, and CK5/6 stain myoepithelial cells around acini and ducts, are absent from invasive neoplasms. CK5/6 stains hyperplastic lesions and is absent from DCIS. Conversely, estrogen receptors stain DCIS diffusely, while staining hyperplastic lesions in a patchy fashion [18–20]. These markers are often difficult to interpret, however, and false-negative or false-positive diagnoses remain common in the context of both complex sclerosing lesions and low-grade carcinomas. Therefore, if complete certainty cannot be achieved in the context of the triple approach, it is recommended to excise these lesions for a proper histopathological evaluation, and the exclusion of malignancy [16, 17, 21, 22].

Lobular Carcinomas

Pauci-cellular lesions with a dense fibrotic stroma often lead to hypocellular and hemorrhagic slides. If such a lesion is malignant, for instance, a lobular carcinoma, this can easily lead to a false-negative diagnosis, particularly if clinical and radiological suspicion is low, which is often the case for these tumors [1, 23]. When aspirated, they lead to samples composed of small, somewhat uniform cells, sometimes plasmacytoid, dispersed singly in small decohesive aggregates or linear strands, mimicking histological findings. Nuclei are eccentric, round to polyhedric, and show only mild to moderate pleomorphism with bland chromatin and inconspicuous nucleoli. Cytoplasm may contain mucin droplets or intracytoplasmatic lumina and at times indent the nucleus resulting in signet ring cell morphology [1, 22, 24]. This combination of a low, bland cellularity and a low clinical suspicion makes lobular carcinomas particularly challenging, especially if the background is fibrocystic and/or hyperplastic. For instance, tumor cells may be confused with lymphocytes. One should always keep this pitfall in mind, paying close attention to dispersed cells in the background, looking for the typical features of lobular carcinoma outlined above.

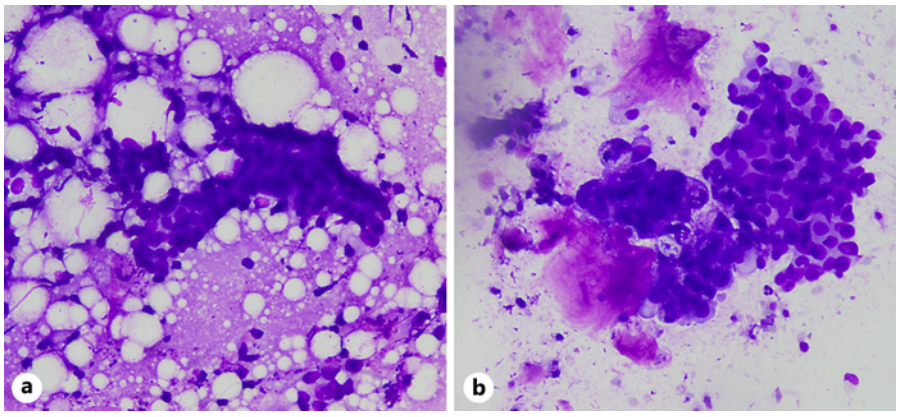

Fig. 1. Highly suspicious lesions on imaging. In (**a**), we can see an isolated epithelial fragment, without atypia, amid adipocytes of varying sizes admixed with debris and histiocytes – these findings enabled a benign diagnosis of steatonecrosis. In (**b**), an irregular fragment can be seen, with macrophages and stromal elements admixed – although this image could be mistaken for a malignancy; it is in fact a sample of a radial scar. Notice the lack of significant atypia and the myoepithelial cells in the background.

Papillary Lesions

In contrast, papillary lesions often lead to false-positive diagnoses. These include a wide gamut of entities, from benign papillomas to invasive papillary carcinomas, which may show significant morphological overlap in FNAC. Generally, papillomas yield moderately to highly cellular samples composed of three-dimensional tissue fragments and monolayered sheets, with minimal or mild cytologic atypia. True papillary fragments, which are characterized by columnar cells polarized against a well-developed fibrovascular core, may or may not be present, which, although more common in malignant papillary lesions, are not reliable indicators of malignancy. Typical of papilloma samples are also stellate papillary tissue fragments, which consist of fibrotic strands of tissue originating in a single central point with small ductal and myoepithelial cells attached and are thought to be highly specific to this type of lesion. In the background, small bland columnar cells can be seen, as well as numerous histiocytes, typical of cyst contents. Apocrine metaplasia is usually present, at least focally [25–30]. An example of these features can be seen in Figure 2. Of note, papillomas may harbor hyperplastic and columnar cell changes, which can raise suspicion for malignancy, and may also be involved by DCIS, further muddying the diagnostic waters [1]. Malignant papillary lesions, such as encapsulated papillary carcinomas, solid papillary carcinomas, invasive papillary carcinomas, and papillary DCIS, often present with more architectural complexity, including branched and complex epithelial fragments, higher degrees of cell dissociation, plasmacytoid cells, cytologic atypia, mitotic figures, and an absence of histiocytes or apocrine metaplasia. Solid papillary carcinomas can display distinctive glomeruloid vascular structures [26, 27].

Nonpapillary lesions, such as fibroadenomas or nonpapillary DCIS, may also show a papillary-like pattern on FNAC samples, entering the differential. They usually lack true fibrovascular cores and the typical features described above, however. Since DCIS may involve papillomas, telling the two entities apart might not be feasible. When presented with a few atypical features in an otherwise typical papilloma sample, it is reasonable to render a diagnosis of "atypical papillary lesion" and recommend excision [1]. ICC can be useful in this context; myoepithelial cells around epithelial nests and in fibrovascular cores can be highlighted with myoepithelial markers, as described above. CK5/6 and estrogen receptors can be used similarly in this context [1, 31].

Fibroadenomas

Fibroadenomas may also lead to false-positive diagnoses, due to difficulties in the differential with phyllodes tumors and, sometimes, well-differentiated carcinomas. Typical fibroadenomas produce samples which are moderately to highly cellular, composed of cohesive ductal and myoepithelial cells organized into large fragments, varying from monolayered to three dimensional. Stromal elements are also present, dotted with myoepithelial cells at their periphery. Dissociated myoepithelial cells and naked bipolar nuclei are usually seen in the background. These features can be appreciated in Figure 3. Cells may show mild atypia, particularly in younger women. As above, ICC can be helpful in this setting [1, 3, 29, 32].

Naked bipolar and stromal fragments are the hallmark features of fibroadenomas. Phyllodes tumors lack these bipolar nuclei but may show abundant dispersed spindle-shaped cells, particularly in benign lesions. Nuclei are larger than in fibroadenomas, and there is usually some

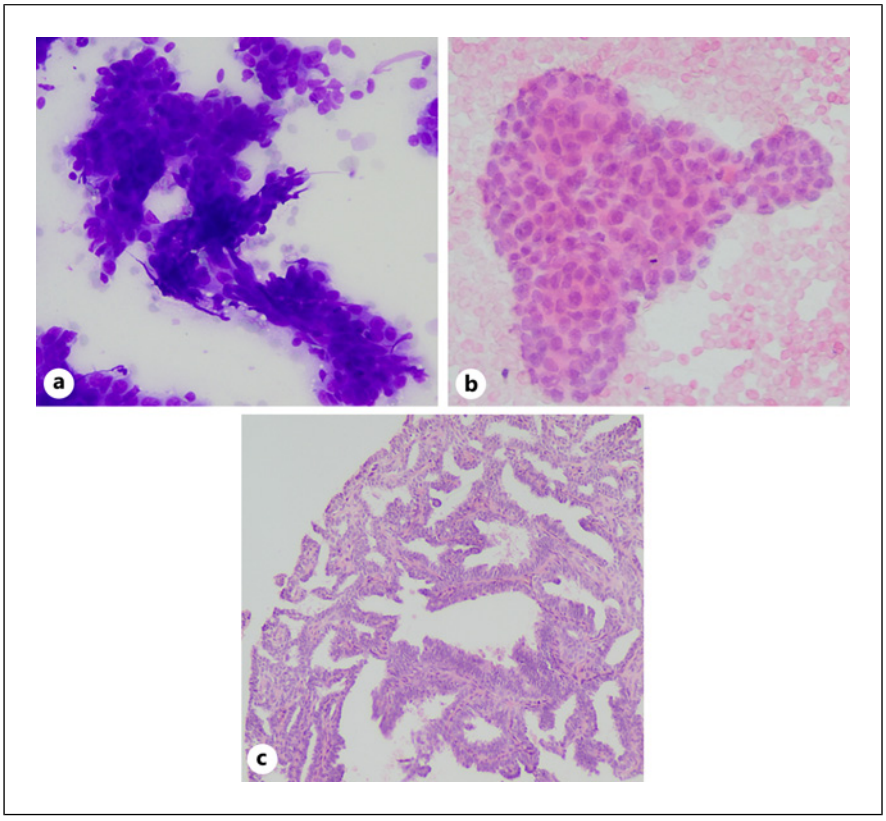

Fig. 2. Papilloma of the breast. In (**a**), a branched papillary fragment can be seen, composed of epithelial cells without atypia. Apocrine metaplasia can be focally seen at the periphery of the smear. In (**b**), from the same case, a smaller fragment can be seen, composed of cells showing monotony and lacking apocrine metaplasia and presence of columnar cells. In (**c**), we can see the lesion in histology, a benign papilloma.

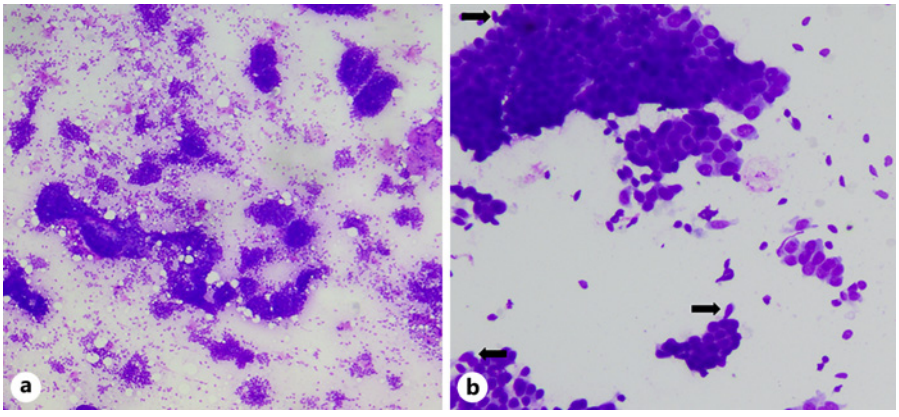

Fig. 3. Typical morphological features of a fibroadenoma in breast FNAC cytology. In (**a**), a highly cellular smear, composed of cohesive fragments with dispersed elements in the background, can be seen. In high magnification (**b**), cytological detail can be appreciated – note the lack of atypia in the epithelial cells, myoepithelial cells in the periphery of clusters (arrows), and naked bipolar nuclei in the background.

degree of atypia, both in the dispersed background cells and those in the stromal fragments. The latter are usually more abundant than in fibroadenomas and more cellular as well. The typical phyllodes pattern, characterized by large, hypercellular stromal fragments with cytological atypia interspersed with epithelial cells, is usually only conspicuous in malignant lesions and shouldn't be relied upon for this diagnosis [33–36]. When atypical features are present, rendering an atypical diagnosis is adequate, with a recommendation for excision [1, 16, 37]. Low-grade carcinoma samples may also mimic fibroadenomas, particularly on low magnification. On closer inspection, however, one should notice a lack of bipolar nuclei in malignant samples and a higher degree of cytologic atypia. ICC for myoepithelial markers can be useful in this context [38, 39].

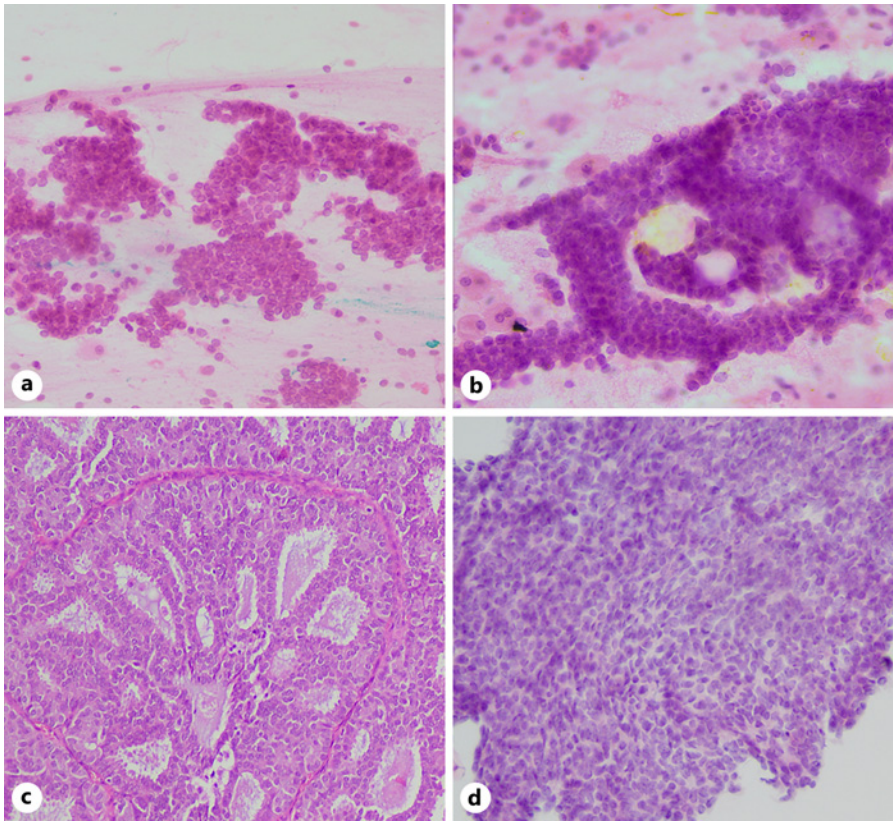

Fig. 4. Low-grade DCIS in an FNAC cytology sample (**a–c**) versus usual ductal hyperplasia (**d**). Small epithelial clusters can be seen, which are solid in (**a**) and show well-defined cribriform spaces in (**b**). Epithelial cells lack atypia, but nuclei are round and monotonous. Polarization can be seen, as well as a lack of streaming. In (**c**), the corresponding lesion in histology shows a well-defined fibrovascular core, which was not sampled in the FNAC. In (**d**), one can see a solid cluster of oval to round nuclei without atypia, showing streaming. Myoepithelial cells can be seen easily at the periphery. These features favor the benign nature of the lesion.

Ductal Carcinoma in situ

DCIS poses significant diagnostic challenges and can lead to both false-negative and false-positive diagnoses [1]. Low-grade DCIS typically features a monotonous cell population assembled in three-dimensional cohesive clusters, with accompanying small fragments or sheets and dispersed epithelial cells in the background. Tissue fragments may show a solid, micropapillary, papillary, or cribriform architecture. In the latter case, cells are usually polarized around a central lumen. Cellularity varies but may be high. Atypia is minimal. These features overlap significantly with atypical ductal hyperplasia, rendering this differential practically impossible. Differentiating low-grade DCIS from usual ductal hyperplasia, on the other hand, may be done by paying close attention to lesion architecture and background. Tissue fragments in usual ductal hyperplasia appear more cohesive, feature streaming, and lack the cribriform or micropapillary architecture of DCIS. Furthermore, in these benign lesions abundant myoepithelial cells and bare bipolar nuclei are often present in the background [22, 40–44]. This differential is illustrated in Figure 4.

High-grade DCIS displays more pronounced cellular atypia and, often, an accompanying necrotic background. Unlike low-grade lesions, abundant atypical, isolated cells are often present in the background, raising the differential with high-grade invasive carcinomas. Calcifications are also often seen here, and bare bipolar nuclei are absent [1]. Low cellularity, highly atypical samples with a necrotic background, and microcalcifications are highly suggestive of high-grade DCIS, in the context of a compatible triple test. Additionally, unlike high-grade DCIS, invasive carcinomas lack myoepithelial cells around glandular structures and may sometimes feature neoplastic cells within stromal or adipose tissue fragments, suggesting invasion. These findings are not easily observed in FNAC samples, however, and literature suggests they are not entirely specific and may also be seen in DCIS. Other cytologic features have been put forward as possible predictors of invasion, such as the presence of tubular structures within malignant clusters, cast macrocalcifications, or intracytoplasmic lumina in dissociated tumor cells, but none have proved specific to either entity [42, 45–48]. A few authors have reported on good performance using myoepithelial markers for this differential diagnosis, but this is controversial [2]. The current consensus is that one cannot reliably tell apart high-grade DCIS from invasive carcinomas in FNAC

samples. These should always be considered in the context of the triple approach, and a definite diagnosis made either on CNBs or in the final surgical sample [1].

Inflammatory, Metaplastic, and Degenerative Lesions

The practicing cytopathologist should also beware of mistaking inflammatory, physiological, metaplastic, or degenerative conditions, such as steatonecrosis, intramammary lymph nodes, apocrine metaplasia, or radiotherapy changes, with malignancy. In steatonecrosis, a necrotic background with microcalcifications may be seen, but epithelial fragments are sparse and necrotic fatty tissue should be appreciable, as observed in Figure 1.

Intramammary lymph nodes, as well as malignant lymphomas, result in highly cellular slides. Lymphocytes may clump together, mimicking epithelial cells. This diagnosis can usually be made through close attention to nuclear detail, and ICC for epithelial and lymphoid markers when doubt arises. When apocrine metaplasia is extensive, samples can be highly cellular; if the typical vast cytoplasm is not recognized, this may raise a suspicion for malignancy, particularly given that apocrine metaplasia can show mild cytologic atypia.

Radiation exposure can lead to samples containing cohesive epithelial fragments with large, pleomorphic, and hyperchromatic nuclei. These cells will show a low N:C ratio, smudged dark chromatin, and a vacuolated cytoplasm. Furthermore, in the background, debris and fat necrosis are often present, alongside atypical fibroblastic cells, characteristic of radiation. If cellularity is moderate to high, and there are a few or more atypical cells with preserved chromatin present, one should consider the possibility of residual carcinoma being present and refer the patient to CNB [1].

Uncommon Lesions

Uncommon lesions may lead to false-positive diagnoses. Granulomatous mastitis and silicone, soya oil, or paraffin granulomas often show reactive, pleomorphic epithelioid histiocytes and epithelial cells, which can raise the possibility of malignancy. Clinical and imaging findings are usually not typical of carcinoma, however, and granulomas are often numerous, which is rare in invasive neoplasms. Granular cell tumors can be highly cellular and lack a myoepithelial cell layer but should not trigger a diagnosis of malignancy due to their bland nuclei, granular cytoplasm, and low N:C ratio.

Collagenous spherulosis is characterized by collagenous spherules surrounded by epithelial and myoepithelial cells, with bare bipolar nuclei dispersed in the background. When these features are not apparent, and/or spherules are large and conspicuous, one may suspect adenoid cystic carcinoma; ICC may be useful in this differential, but if not, or if it cannot be performed, CNBs should be recommended.

Mucinous carcinomas result in cytological samples of varying cellularity, composed of epithelial tissue fragments showing a tubular, cribriform, rounded, or papillary architecture, with mild to moderate cytological atypia and dispersed in a background of abundant fibrillary mucin. When cellularity is low and atypia is mild, these findings may overlap with those of mucocele-like lesions, a rare type of lesion which may occur from the rupture of ducts or cysts in the context of fibrocystic change. Given that mucinous carcinomas are much more frequent than mucocele-like lesions and may show radiological similarities, an aspirate from a mucocele-like lesion can easily result in a misdiagnosis of malignancy. Such samples should be classified as atypical and referred to CNB.

Lactational changes of the breast may be nodular clinically and result in samples with a background filled with small globules of fat. This can raise the suspicion for secretory carcinoma. This entity usually shows slightly more atypical cells, but there can be significant overlap. Attention to clinical history and radiological features is essential to resolve this differential.

Finally, it is important to mention cutaneous neoplasms and metastases. Skin tumors, namely from adnexa, can produce complex cytological samples and leave the unwary cytopathologist perplexed and on the way to an erroneous or noncontributing diagnosis. Metastasis from epithelial or mesenchymal neoplasms and primary or secondary involvement by hematogenous malignancies can happen in the breast, even without clinical suspicion, and cytopathologists should consider this possibility when faced with atypical or hard-to-fit findings. ICC studies may help in this situation. Breast primaries are usually positive for CK7 and negative for CK20. Other markers of breast lineage, such as TRPS1 and GATA 3, may also be used. TRPS1 is more specific, particularly in triple negatives, whereas GATA3 has been shown to stain a number of other neoplasms, namely, urothelial carcinomas [1, 49].

Conclusion

Although breast FNACs have fallen out of favor in the Western world, progress in this area of cytopathology has not stopped. The introduction of the Yokohama system provided a structured framework for breast cytopathologists, enabling the alignment of cytological criteria, fostering

consistency between pathologists and across laboratories and countries, and allowing for a reproducible communication with clinicians. However, this alone did not solve all problems with breast FNAC. Including this valuable technique in our daily practice requires knowing how and when to use it. In the right hands, it can be extremely powerful, saving costs and time and reducing complications for patients. But one must understand its limitations and where errors are more likely.

In this review, we identified and briefly covered most aspects a breast cytopathologist needs to pay more attention to procedural and preanalytical conditions, ancillary techniques, and interpretative pitfalls. Although not an extensive description, we have hopefully managed to provide an outline of what is most important, enabling further study.

The pitfalls described are the ones most likely to appear in daily practice. They are daunting, but not impossible to deal with. Additionally, the fact that specialized practices in the West still use breast FNAC to this day should alert us to the potential hidden in FNAC: the ease of performing the technique, the possibility of ROSE, the simple materials used, and minimal complications can be valuable in lowering costs for healthcare systems and managing increasing patient waiting lists.

One should not be afraid to include breast FNACs in their daily practice, even if they do not feel wholly comfortable with the diagnostic process: this comes with experience, and experience can only be gained by doing. In the meantime, a strong correlation with clinical and radiological data, and a cautious approach, deferring to biopsy or excision when in doubt, can enable a successful and productive practice, benefiting patients most of all.

Statement of Ethics

This review paper entails an analysis of existing literature, guided by expert opinion, and does not involve any new experiments or the collection of primary data from human or animal subjects. As such, it did not require ethical approval from an Institutional Review Board (IRB) or Ethics Committee. The research and analysis presented in this paper have been conducted following ethical guidelines for academic integrity and scholarly conduct. All sources and data referenced in this review have been appropriately cited to ensure proper credit is given to original authors and researchers.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This study was not supported by any sponsor or funder.

Author Contributions

Dr. Daniel Gomes Pinto wrote the paper. Prof. Dr. Fernando Schmitt reviewed the paper.

Data Availability Statement

This review paper is based on the analysis of previously published studies, it does not contain any original data that require sharing. The data supporting the findings of this study are derived from the cited sources. For any additional information or clarifications, the corresponding author can be contacted.

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