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

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ISBN 978-3-030-26882-4 ISBN 978-3-030-26883-1 (eBook)
https://doi.org/10.1007/978-3-030-26883-1

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

I dedicate this atlas to my wife, Alison Field, who has supported me during my pathology training, my cytopathology career, and my research and writing.

Andrew S. Field

I dedicate this atlas to my husband, Grant, and daughters, Catie and Jacqui, who have always supported my academic endeavours despite the consequence of time taken away from the family.

Wendy A. Raymond

I dedicate this atlas to my partner, Sule Canberk, for being understanding and supportive during this period as well as to all people who have helped me during my career.

Fernando Schmitt

In addition, we dedicate this atlas to all those who have taught us the art of cytopathology, especially Torsten Lowhagen and Svante Orell, and to those who currently and in the future will perform and report breast fine needle aspiration biopsies.

Andrew S. Field
Wendy A. Raymond
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Preface

The development of this atlas began with a meeting sponsored by the International Academy of Cytology (IAC) of a group of cytopathologists interested in breast fine needle aspiration biopsy cytology at the International Congress of Cytology in Yokohama, in May 2016. This leads to the publication of a proposal in Acta Cytologica in 2017 and an expansion of the steering group to include a team of writers each with a specific role in performing a literature search and drafting material for a particular chapter in an atlas.

The proposal was presented at a number of national and international meetings, and the drafts were collated and edited into chapters, which were then distributed to the members of each chapter's writing team and the wider group of authors for comment. A series of questions based on the definitions, discussions, and management options were presented in a questionnaire posted on a website, and the broader community of pathologists and clinicians were invited to comment. The response to the questionnaire was very positive, and the various suggestions were assessed by the editorial team and incorporated where appropriate. The final drafts were recirculated and re-edited, photographic illustrations were collected, and this atlas was produced.

The IAC Yokohama System for Reporting Breast Fine Needle Aspiration Biopsy Cytopathology uses five clearly defined categories described by specific terms, and each has a specific risk of malignancy. The five categories are insufficient/inadequate, benign, atypical, suspicious of malignancy, and malignant. Each category and its risk of malignancy are linked to management recommendations, which include several options because it is recognized that diagnostic infrastructure, such as the use of core needle biopsy and ultrasound guidance, varies between developed and low- and middle-income countries. The system is intended for global use and is based on cytomorphology and includes key diagnostic cytological criteria for each of the many lesions and tumors found in the breast.

In addition, the atlas includes chapters on current and potential future ancillary tests, liquid-based cytology, nipple cytology, and management. There is also a chapter providing an overview of an approach to the diagnosis of direct smears of breast fine needle aspiration biopsies: breast cytology crucially relies on the expertise of those performing the biopsy and preparing the direct smears and on the cytopathologist interpreting the material on the slides.

The authors believe that the development of this system will provoke discussion and comment and enhance breast cytology reporting internationally. The authors sincerely hope that the system will encourage the use of breast

fine needle aspiration biopsy cytology and that it will lead to a greater recognition of the importance of the expertise required to perform the procedure, improve interpretation of the material, and standardize reporting. In addition, the system will facilitate communication with breast clinicians, further research into breast cytology and related molecular pathology, and improve patient care.

The editors thank all the authors involved in this project, the publication team at Springer, and the International Academy of Cytology community.

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A. S. Field et al. (eds.), *The International Academy of Cytology Yokohama System for Reporting Breast Fine Needle Aspiration Biopsy Cytopathology*, https://doi.org/10.1007/978-3-030-26883-1_1

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Introduction

The technique and diagnostic interpretation of fine needle aspiration biopsy (FNAB) cytology of the breast has developed over the past 60 years into an extremely useful, accurate, highly specific and sensitive, and cost-effective test for the diagnosis of benign and malignant breast lesions [1–7]. There has been a long-standing, highly successful and widespread practice of FNAB for palpable lesions and, more recently, for the assessment of mammographically and ultrasonographically detected lesions. FNAB has been readily accepted by patients and clinicians as a minimally invasive, cost-effective and valuable tool for diagnosis and management [8–15].

Breast FNAB can attain a sensitivity of 90–99%, a positive predictive value (PPV) of malignancy approaching 100% and a high degree of accuracy that is up to 96.2% [1–3, 11–15]. There is a very low false-positive rate usually related to FNAB of fibroadenomas, papillomas and papillary lesions, and a low false-negative rate usually related to low-grade ductal and lobular carcinomas [13–15]. In medically under-resourced developing countries, which represent more than 80% of the world's population, breast is one of the most common FNAB sites and FNAB is the most appropriate test for all palpable breast lesions when preoperative imaging, core needle biopsy (CNB) and histopathology are not readily available and expensive options [8, 9, 16–22].

The IAC Yokohama Breast FNAB Reporting System has been developed by a group of experts in cytopathology assisted by oncologists, radiologists and surgeons [23, 24]. The reporting system is based on a review of the literature and the expertise of the IAC breast group. The rationale for the development of this international reporting system is to have a standardized reporting system, which will improve the performance, interpretation and reporting of breast FNAB cytology and clarify communication between cytopathologists and clinicians by linking the reporting system with suggested management options. Ultimately, the system will benefit patient care and facilitate research and the ongoing utilization of FNAB breast cytology. The system and the suggested management algorithms have been designed to be applicable in all medical infrastructure settings.

The Role of Breast FNAB

FNAB offers significant benefits as a diagnostic test with its rapidity of diagnosis, low cost, high rate of acceptance by patients, low complication rates, virtually no contra-indications and high accuracy [10, 12–15].

This is optimized when the FNAB is performed by cytopathologists or experienced radiologists or clinicians in multidisciplinary clinics, utilizing ultrasound guidance as required with rapid on-site evaluation (ROSE) of Giemsa-stained slides to triage cases [12, 14, 15, 25–27]. The FNAB provisional results can be correlated immediately with the clinical and imaging findings in the 'triple test' [14, 28]. In these settings, the sensitivity and specificity rates for FNAB and CNB are comparable [12, 14, 15].

ROSE increases sensitivity and benign and malignant rates and reduces inadequate and recall rates, thus decreasing patient anxiety and waiting times and costs [12, 14, 28]. When the provisional report is insufficient/inadequate, atypical or suspicious of malignancy or the findings do not correlate in the triple test, the patient can be triaged for immediate repeat FNAB or CNB [12, 14, 28]. At the time of ROSE the cytopathologist or cytotechnologist can provide immediate feedback on the adequacy of the material, which often relates to the quality of the FNAB technique, and this continual feedback steadily improves the quality of the FNAB procedure and the quality of the smear making [14]. Ideally, the cytopathologist performs the FNAB with the assistance of ultrasound guidance, but if this is not possible the cytopathologist should work with the radiologist or clinician to develop their technique to optimize results for the patient and the system.

Breast FNAB does require specific training in techniques and slide interpretation, and continuing exposure to a significant caseload is essential to maximize reporting accuracy [29, 30]. FNAB cytology does have particular interpretative difficulties. It is generally accepted that FNAB cannot consistently distinguish in situ from invasive carcinoma, but there are specific cytological criteria that suggest low- and high-grade ductal carcinoma, and some authors have suggested criteria that enable a diagnosis of unequivocal invasive carcinoma [7, 31–35]. Correlation with imaging is required. It can also be difficult when material is limited to precisely diagnose and distinguish some proliferative lesions, which include epithelial hyperplasia with or without atypia, fibroadenomas [36], intraductal papillomas [37], radial scars and columnar cell change and its variants, from atypical ductal hyperplasia, low- and intermediate-grade DCIS, papillary carcinomas and low-grade invasive carcinomas. This is also the case on occasion with CNB [7, 29, 38–40].

It is crucial that cytopathologists are aware of the diagnostic criteria for each of these lesions, and that the diagnosis of malignancy is only made when it is unequivocal.

It is essential to avoid false-negative and particularly false-positive diagnoses with their potential risks of patient distress and inappropriate management.

FNAB can be used to diagnose the vast majority of palpable and impalpable lesions in a breast clinic where women present with a lesion or for routine imaging [1–7, 14, 15]. These lesions will most commonly be cysts, fibrocystic change, fibroadenomas, papillomas and a relatively small number of carcinomas. The same is true for mammographically detected mass lesions in a screening programme assessment clinic, while CNB is preferred for the workup of microcalcifications and less discrete or diffuse lesions. FNAB and CNB are regarded as complementary in many institutions [11, 12, 14, 30], while in other centres in parts of the developed world CNB has virtually replaced breast FNAB [41, 42]. This is particularly the case in mammographic screening programme assessment clinics where a large proportion of the cases involve workup for calcifications. However, the screening programme experience of the use of FNAB and CNB has been inappropriately extrapolated into the assessment of all breast lesions, whether palpable or impalpable, in clinical breast units managing women with symptomatic lesions [31, 43].

Even in a practice where CNB is available and generally preferred, FNAB still offers advantages and is preferred for specific clinical situations:

- Confirmation and drainage of simple and complex cysts

- Diagnosis of infections/abscesses and to procure material for microbiological studies
- Difficult to biopsy lesions such as those that are retroareolar or close to the chest wall or prosthetic implants
- Possible recurrences in reconstructed breasts
- Diagnosis of palpable lesions that lack an imaging abnormality
- Lesions where ROSE is required prior to possible CNB
- Patients who are pregnant or lactating where CNB risks creating a sinus tract
- Patients taking anti-coagulants or with a history of bleeding diatheses
- Patients considered at low risk on clinical and imaging findings, where the FNAB provides the final diagnosis within the triple test
- To provide a malignant diagnosis and material for ER, PR and HER2 testing in patients with advanced carcinoma or metastatic disease [10, 11].

FNAB can also be readily performed on axillary lymph nodes found on palpation or ultrasound examination, with or without CNB where required [44]. The FNAB can thus stage a patient with breast carcinoma providing a significant cost benefit over a sentinel lymph node biopsy, which can still be performed if the FNAB is negative [44, 45].

The Role of CNB

CNB is a more invasive biopsy procedure with a higher rate of complications, a less rapid turnaround time to diagnosis, and greater expense, both for the purchase of the CNB equipment and consumables and the requirement for a surgical pathology laboratory to process and interpret the samples [46, 47]. It precludes ROSE. CNB increases the risk of carcinoma seeding the needle track [48], and recently it has been suggested may adversely affect prognosis [49]. CNB is particularly inappropriate in a low resource setting for the diagnosis of the most common lesions [50–52]. CNB has limitations due to sampling error similar to FNAB, and often shows greater crush artefact than FNAB [46]. There are similar diagnostic problems in distinguishing papillomas from intraductal papillary cancer, cellular fibroadenomas from low-grade phyllodes tumours, and atypical ductal proliferations from low-grade DCIS [14, 46].

CNB does offer greater specificity than FNAB in the diagnosis of lesions associated with microcalcifications and the diagnosis of certain proliferative lesions and low-grade DCIS and for confirming invasive carcinoma [41, 42]. The interpretation of CNB is very similar to routine breast surgical pathology. IHC for prognostic and predictive markers can be performed on CNB, as it can on cell blocks of FNAB material [53, 54].

FNAB Techniques

A successful breast FNAB cytology service relies crucially on the performance of the FNAB and the subsequent making of direct smears. Poor technique is the major source of quality assurance problems and the ‘elephant in the room’ in any discussion of the role of breast FNAB [31]. Traditionally, cytopathologists performed the FNAB and had immediate feedback on the quality of their technique when viewing their direct smears [15, 27]. In the current setting in the developed world, the FNAB is frequently performed by a radiologist, who may have minimal contact with the reporting pathologists. The radiologist may not receive feedback and may remain unaware of the quality or shortcomings of their technique. The presence of a cytopathologist performing ROSE or at least a close working relationship between the pathologist and the radiologist or clinician performing the FNAB can assist with feedback and improvement in FNAB quality [14].

FNAB is a simple test that requires good training and ongoing experience with constant monitoring of the diagnostic yield and adequacy rates [4, 7, 27, 29, 30]. Currently in the developed world, the number of breast FNAB is decreasing and this provides fewer opportunities for radiologists to develop expertise, fewer opportunities for adequate training of radiology and pathology residents in performing FNAB, and fewer reporting opportunities for pathologists [31]. This contrasts with the majority of countries, that have limited medical resources including a lack of breast imaging and CNB, where FNAB of breast has a rapidly increasing role as the initial diagnostic test of any palpable breast lesion [8, 9, 16–22].

The key elements in performing breast FNAB are fixing the target lesion and a rapid technique where the fine needle (22 to 25 Gauge) is introduced into the lesion and ten to fifteen rapid passages of the needle are made into and across the lesion utilizing the cutting action of the needle bevel. The entire sampling process should take less than 10 seconds so as to prevent clotting in the needle. Aspiration can be applied during the procedure particularly if the lesion yields cyst fluid or if there is no material in the needle hub after the first few passages of the needle [55, 56].

Ultrasound is a very useful adjunct in the FNAB procedure and is essential if the lesion is impalpable. It can confirm that the needle has actually sampled the target lesion. However when ultrasound is used it can be more difficult for inexperienced operators to immobilize the lesion adequately, and there is a potential for the dwell time of the needle in the lesion to be increased resulting in increased blood contamination of the sample and clotting of the material in the needle.

The making of direct smears is the second crucial step in FNAB of the breast. Poor smearing technique can ruin good FNAB material. Liquid-based cytology has been suggested as a solution to poor smearing and fixation [57], but the cost is greater than that for direct smears, the cytological diagnostic criteria are not fully developed, and, most importantly, there is a loss of most of the key diagnostic elements in pattern recognition.

The alcohol-fixed Papanicolaou stain and the air-dried Giemsa stain are complementary and allow assessment of different cytological features. It is recommended that ideally each stain should routinely be used in every breast FNAB although this will vary with local preferences. Multiple smears may be produced by sample splitting methods [55, 56].

Routine cell block preparation from buffered saline washings of the needle and syringe and a single extra dedicated entire FNAB pass are recommended for any lesion, particularly those which are malignant [53, 58]. Cell blocks can assist the FNAB diagnosis in proliferative lesions, subtyping of carcinomas and the diagnosis of invasion [58]. The full range of immunohistochemistry for prognostic and predictive markers, including oestrogen and progesterone receptors and HER2, and for cytokeratin 5/6 or 14 for basal type carcinomas and E-cadherin to help confirm lobular carcinomas can be performed on cell blocks [59, 60]. HER2 ISH including dual colour ISH and other molecular testing may be also performed if required [53, 54, 59, 60]. Some authors have recommended that LBC preparations can be used in similar fashion for immunocytochemistry [57].

The Breast FNAB Report

A breast FNAB cytology report should be in an established format and provide one of the specific diagnostic categories as a heading using a standardized descriptive terminology [23, 24]. This should be followed by a clear cytological description including the degree of cellularity and the presence or absence of key cytological diagnostic features. There should be a concise comment or conclusion, which gives as specific a diagnosis as possible, or, if this is not possible, the most likely diagnosis with a differential diagnosis. The aim is to facilitate communication

between the cytopathologist and the clinician, and a code number should never be used in isolation as a replacement for the category and description. A code can be placed in the body of the report to facilitate quality assurance measures and research.

The categories utilized in the IAC Yokohama reporting system and detailed in this Atlas stratify the risk of malignancy and are:

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

An individual cytopathologist and a department with more than one cytopathologist should choose to use either the 'insufficient' or the 'inadequate' term. The 'insufficient/inadequate' category is not used for lesions where the cytopathology does not explain the expected imaging or clinical diagnosis [24]. In that situation, the FNAB cytology should be reported based on the findings on the slide, and then correlated in the triple test. This is further discussed in Chap. 2, Insufficient.

The 'atypical' category allows for a high negative predictive value for a 'benign' diagnosis, while the 'suspicious of malignancy' category will maintain a high positive predictive value for a malignant diagnosis. The categories allow for stratification of the risk of malignancy (ROM) and management recommendations. The 'atypical' category will include proliferative breast lesions that show some atypia related to individual cell dispersal, nuclear atypia or atypia of the architecture of tissue fragments. The 'suspicious of malignancy' category will in most cases represent low- or high-grade DCIS and low-grade invasive carcinomas and include some cases where there is scant or poorly smeared material.

Structured reporting will improve the quality, clarity and reproducibility of reports within individual pathologist departments and between countries, and will improve patient management and facilitate research and quality assurance measures [61–63]. Standard guidelines for cell block preparation, immunohistochemistry, ISH and other molecular tests of prognostic and predictive markers will improve accuracy, reduce cost and improve patient care, and LBC can potentially offer similar opportunities [53, 54, 57]. Structured reports are based on key diagnostic cytological findings, which act as a checklist for the reporting cytopathologist, who should use an analytical approach based on low power pattern recognition combined with high power assessment of nuclear and other cytological features. Low power pattern and high power assessment are then integrated into a final diagnosis [7, 24]. These criteria are presented in this Atlas.

Risk of Malignancy and Management Guidelines

Table 1.1 summarizes the categories stratified by ROM obtained from the most recent literature reflecting current practice [14, 15]. Suggested management guidelines are linked to each of the five diagnostic categories. The management options attempt to take into account the considerable differences in practice between well-resourced and less well-resourced countries with limited availability of imaging, CNB, surgical pathology and the various treatment and surgical options [50–52]. Further discussion of the ROM and management protocols is presented in the individual category and management chapters.

Table 1.1 Categories, risk of malignancy and summary of management recommendations

Category	ROM[a, b]	Management[c]	LMICMX[d]	Comment
Insufficient	2.6–4.8%	Review clinical & imaging findings: If imaging indeterminate or suspicious, repeat FNAB or proceed to CNB; if imaging benign consider repeat FNAB	Review clinical; if indeterminate or suspicious repeat FNAB	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
Benign	1.4–2.3%	Review clinical & imaging; if 'triple test' benign, no further biopsy required and review depends on the nature of the lesion; if clinical &/or imaging indeterminate or suspicious, repeat FNAB or proceed to CNB	Review clinical: if benign nil further; if suspicious repeat FNAB	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 centres review in line with screening programme policy
Atypical	13–15.7%	Review clinical & imaging: 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.[e]	Review clinical and repeat FNAB; manage based on FNAB category. If further FNAB atypical, consider excisional biopsy	At ROSE, if atypia is considered due to a technical issue, repeat FNAB; if cellular material adequate and atypical, proceed to CNB
Suspicious	84.6–97.1%	Review clinical & imaging: CNB is mandatory. [f]	If no CNB available, excision biopsy	At ROSE proceed to CNB
Malignant	99.0–100%	Review clinical & imaging: proceed to CNB if any discrepant findings. If 'triple test' is concordant and malignant, proceed to definitive management. [g, h]	If no CNB available, excision biopsy	At ROSE may proceed to CNB

ROM risk of malignancy, *FNAB* fine needle aspiration biopsy, *CNB* core needle biopsy, *ROSE* rapid on-site evaluation
[a]Montezuma et al. [15]
[b]Wong et al. [14]
[c]Best practice recommendation where imaging and CNB available
[d]Best practice recommendation where imaging and/or CNB not available in Low Middle Income Countries Management
[e]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
[f]If FNAB is 'suspicious' or 'malignant', then regardless of clinical and imaging findings, the FNAB dictates management
[g]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
[h]FNAB with or without CNB is recommended on palpable or suspicious on ultrasound axillary lymph nodes to assist in staging the lesion

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Insufficient/Inadequate

2

Wendy A. Raymond, Andrew S. Field,
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Introduction

There is no international consensus on the definition of an 'inadequate' or 'insufficient' or 'non-diagnostic' or 'unsatisfactory' breast fine needle aspiration biopsy (FNAB). A precise definition needs to consider what is an 'adequate' biopsy and should ideally encompass all clinical settings.

The FNAB specimen is determined as adequate or inadequate based on the assessment of the material on the slides, and if inadequate then it is categorized as 'insufficient/inadequate' for diagnostic purposes. However, it is recognized that the key to assessment of breast lesions is the 'triple test' approach utilizing clinical and imaging examination combined with pathological assessment and multidisciplinary discussion. An interpretation may be offered in the report that a specimen can be considered adequate if it provides a diagnosis for a particular breast lesion, for example, a proteinaceous background with no epithelium is consistent with cyst contents from a lesion that drained completely on imaging or left no residual palpable mass [1]. In this setting, the FNAB material is regarded as providing a reliable result when consistent with the other two components of the triple test.

This descriptive approach differs from publications promoting a specified minimum number of epithelial cell tissue fragments to determine adequacy. Suggested cut points for adequacy have included: a single fragment of epithelial cells; at least 6 epithelial tissue fragments of 5 or more cells; at least 10 bipolar cells in each of 10 medium power (×200) fields [2–4]; a minimum of 7 tissue fragments each consisting of more than 20 cells [1]; or any number of appropriately smeared and fixed epithelial cells [2, 5, 6].

The use of a minimum number of epithelial cell tissue fragments as a requirement for adequacy in the setting of a FNAB of a mass lesion offers the best approach to standardizing the definition of adequacy, and is reported to reduce the risk of missed carcinomas [5, 6]. However, it does not necessarily ensure adequate sampling and is not appropriate for non-epithelial lesions.

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A. S. Field et al. (eds.), *The International Academy of Cytology Yokohama System for Reporting Breast Fine Needle Aspiration Biopsy Cytopathology*, https://doi.org/10.1007/978-3-030-26883-1_2

This approach may promote further unnecessary FNAB or core needle biopsies (CNB) with no significant difference in false-negative rates [2].

Breast FNAB samples also may be inadequate due to poor smearing and fixation, crush artefact, thick smears, smears obscured by blood, air-drying artefact in alcohol-fixed Papanicolaou-stained smears and slow air-drying artefact in Giemsa-stained smears.

Reported inadequate rates range from 0.7% to 47% [7–12] reflecting:

1. Differences in definition. In some studies, if a minimum number of epithelial cell tissue fragments definition is used, up to 35–40% of the true negative FNAB would become 'inadequate/unsatisfactory' in certain clinical settings, requiring further potentially unnecessary workup [13–14].
2. Differences in workup protocols for different lesion types and differences in the type of clinical practice. In a community breast health clinic setting, the inadequate rate was reported as 25% due to non-pathologists performing a high proportion of the FNAB and to sampling of a proportionately greater number of lesions with a low suspicion of malignancy, including non-proliferative breast disease [14].
3. Differences in patient cohorts, such as mammographic screening program assessment clinics versus clinics assessing women with clinical lumps [12].
4. Use of rapid on-site evaluation (ROSE) which reduces insufficient rates [12].

There are very few studies analysing predictive values in relation to inadequate samples, and so it is not possible to establish an accurate risk of malignancy (ROM). Most studies have excluded unsatisfactory cases from the statistical analyses of PPV and NPV, because only patients proceeding to a surgical biopsy were included and the indication for surgery was often clinical or radiological suspicion of carcinoma [5, 11, 12]. Clinical follow-up is usually only 1 or 2 years, reflecting the fact that in the appropriate clinical and radiological setting, a low cellularity inadequate FNAB has not diagnosed a benign lesion, but does equate to a benign lesion with an extremely low false-negative risk (0.05–1.5%) [2, 15].

Overall the significance of an inadequate FNAB of breast is dependent on the clinical and radiological findings, which then determine the appropriate further management.

Definition

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

Discussion and Background

The role of the cytopathologist is to assess cytological material and the definition of the inadequate category is based on the cytological assessment. The material is categorized, and then triple assessment by the multidisciplinary team is required. If the smears do not explain or correlate with the clinical or imaging findings, further investigation, with or without biopsy, is required in almost all cases. If there is adequate diagnostic material, the cytology is not categorized as 'non-diagnostic/inadequate' when the cytological findings and imaging findings are discrepant. This is analogous to a core needle biopsy (CNB) of a clinically or radiologically malignant lesion, which shows fibrocystic change and is correctly reported as benign, but repeat biopsy is required. The terms 'insufficient' or 'inadequate' are recommended rather than 'non-diagnostic'.

The specific features of the cytological specimen that make it insufficient/inadequate should always be stated.

If any atypical features, such as dispersal of single epithelial cells, significant nuclear atypia or necrosis, are found in a smear, which is otherwise considered inadequate, then the smears should be regarded as 'atypical' and not 'inadequate'.

In cases where a palpable or impalpable solid mass lesion is seen on imaging, it is reasonable to require approximately 6–7 epithelial tissue

fragments, each consisting of at least 10–20 cells (so that the architecture of the tissue fragments and the presence or absence of myoepithelial cells can be assessed), as a guide to adequacy [1, 16]. However, it must be recognized that some tumours, such as invasive lobular carcinoma, may yield few or no tissue fragments and the smears may only show dispersed cells, in which case the material would be regarded as at least 'atypical'.

In a number of clinical settings, a smear may not be adequate using the definition above, but despite the paucity of epithelium, the cytological findings are consistent with the clinical and imaging findings [4, 16]:

- *Abscess* – acute inflammatory cells and debris (pus) are present.
- *Cyst contents or fluid* – there is a proteinaceous background with or without histiocytes. The report should state there is no apocrine or other epithelium. The palpable cyst disappears following the FNAB with no residual mass, or the cyst seen on ultrasound is completely drained by the FNAB with no residual lesion.
- *Lipoma/fatty nodule* – usually diagnosed by ultrasound, the FNAB yields a considerable number of fibrofatty tissue fragments. The report should state there is no epithelium.
- *Spindle cell lesions* – fibroblasts, other spindle cells or stromal tissue fragments are obtained by the FNAB, but no epithelial cells are seen.
- *Scar* – stromal cells or sclerotic tissue fragments are seen in the FNAB without epithelium and may be associated with fat necrosis.
- *Fat necrosis* – degenerate cellular material, histiocytes, multinucleated histiocytes and fragments of necrotic fat tissue are seen in a background of granular debris. The report should state that there is no epithelium.
- *Hyalinized/sclerotic fibroadenomas* – these may yield no material or minimal stromal fragments or only bare bipolar nuclei. If the imaging is characteristic, this may be regarded as adequate.

In all of these situations it would be reasonable to suggest a repeat FNAB or CNB if any clinical or imaging doubt exists as to the diagnosis.

If there are no clinical or imaging findings made available to the reporting cytopathologist, for example, 'cyst fluid completely drained', the cytopathologist should attempt to contact the clinician and obtain imaging information if it is available, before diagnosing the material as 'inadequate'. Alternatively, a report can be issued stating what the cytological findings are, and a caveat added, that the 'sample may not be representative and clinical and imaging correlation is required'.

Insufficient FNAB Rates

There are a number of factors affecting the potential inadequate rate.

1. *The inherent qualities of the lesion*:
   - Both benign and malignant smaller, scirrhous and difficult to stabilize lesions have higher inadequate rates [17]. Inadequate rates were 9.5% in pT1, 5% in pT2 and 0% in pT3 tumours aspirated in a study of 1472 cases with an overall inadequate rate of 16.2% [18].
     Lesions with a lesser degree of epithelial proliferation produce fewer cells on FNAB smears [15]: inadequate FNAB are more frequent from benign than from malignant lesions, both for masses and for microcalcifications [3]; FNAB of fibroadenomas are more likely to be diagnostic than FNAB from fibrocystic change [17, 19]; inadequate rates are higher in invasive lobular carcinoma than in invasive carcinoma of no special type, in the scirrhous carcinoma subtype than in other histological types in the Japanese classification, and in ductal carcinoma in situ than in invasive carcinoma [17, 19–21]. Any of these factors may result in hypocellular or acellular smears.
   - Impalpable lesions accessed by ultrasound have a higher inadequate rate than palpable lesions [7, 22] and lesions identified by

mammographic microcalcifications without a corresponding mass lesion have the highest inadequate rates [3, 19, 23].
- Necrotic or infarcted material or, rarely, suppurative material may obscure the epithelial component.

2. *The qualities of the FNAB operator*:
   - The experience of the FNAB operator directly correlates with the adequacy rate [24–27] and pathologists have half to a third of the inadequate rate of other clinical staff [7, 14, 27–30]. In a breast health clinic setting, the inadequate rate was 6% for pathologists, 14% for breast FNAB performed by other clinicians and 25% for other clinic health workers [14]. This may be related to adequate stabilization of the lesion, accurate placement of the needle, too gentle or too aggressive passaging of the needle through the lesion, application too early or overuse of aspiration, failure to release negative pressure before withdrawing the needle and the smearing technique. The cytopathologist is immediately aware of a poor quality FNAB on reading the slides and can adjust their technique.
   - Increasing the number of needle punctures increases the chance of a diagnostic sample, although the highest yield is usually from the first pass [31–33].
   - Utilizing ROSE decreases insufficient rates [12].
3. *The qualities of the actual smear*, usually related to the experience of the direct smear – maker. The following factors result in poor-quality and potentially inadequate smears:
   - Delay in smearing material deposited on the slide.
   - Failure to deliver the smears for alcohol fixation and Papanicolaou staining immediately into alcohol and failure to rapidly air-dry slides for Giemsa staining.
   - Smear technique too forceful, leading to crush artefact.
   - Thick smears or smears with an excessive amount of blood.

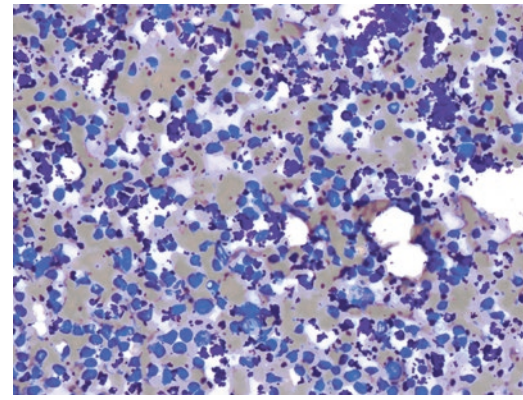

Fig. 2.1 Finely granular pink–purple obscuring ultrasound gel with erythrocytes. (Giemsa ×20)

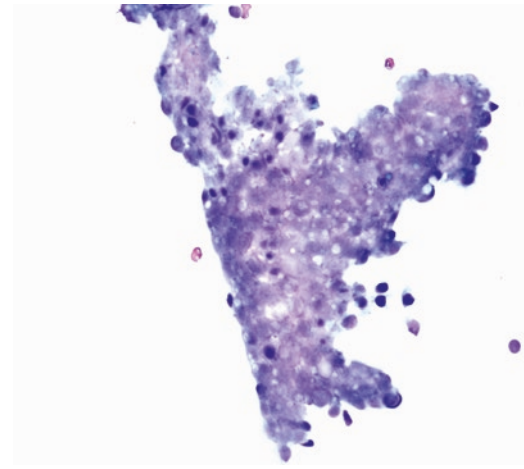

Fig. 2.2 Formalin vapour effect causes distortion of nuclei, prohibiting assessment. (Giemsa stain ×20)

   - Ultrasound gel, which has not been properly cleaned from the skin and the ultrasound probe, obscuring cellular details (Fig. 2.1).
   - Poor rapid Giemsa staining at rapid on-site evaluation (ROSE) or poor staining in the laboratory.
   - Formalin vapour artefact due to transport of the slides in a container with a core needle biopsy in formalin (Fig. 2.2).

It should be noted that the factors contributing to an inadequate FNAB also contribute to missing

the diagnosis of a carcinoma: small size [24, 34, 35], lobular type [12, 34] and intraductal carcinoma [36]. The most frequent explanation is that the FNAB has not adequately sampled the lesion [19, 34] either because the needle has not hit the target or there are too few epithelial cells within the tissue to be sampled to allow an atypical or suspicious diagnosis. Occasionally the smear is misinterpreted, generally because the pathologist misses scanty atypical cells [17, 19, 34].

The inadequate rate in breast FNAB can be reduced by:

- Adequate initial training and ongoing supervision and mentoring of FNAB operators, whether using a palpation or ultrasound-directed technique [25–28].
- Use of ultrasound guidance [31].
- Feedback about smear quality and results so that operators are aware of any inadequacies in their technique.
- ROSE, which gives immediate information on adequacy, highlights any need for an immediate repeat FNAB and enables triage of cases for immediate CNB when required [12]. This facilitates a considerable cost saving for the patient, FNAB operator, clinic and health system.
- Three passes if ROSE is not available [32].
- Rapid correlation with the imaging and clinical findings in the triple test in all cases to determine if the material is inadequate.

Cytopathologists and other experienced FNAB operators should aim for inadequate rates of less than 5% and, if providing ROSE, this rate should be even lower [10–12]. If the rate is 5–20%, the situation should be reviewed and may reflect the actual FNAB practice and the patient population it serves, or it may reflect a less experienced group of operators. An inadequate rate > 20% suggests a need to alter technique.

Management

If the FNAB is insufficient/inadequate there should be a review of the clinical and radiological findings to decide whether repeat FNAB or a CNB should be performed. If the smear is technically suboptimal a repeat smear with attention to the specific technical problem should be performed, if at all possible with ROSE. If the imaging is indeterminate or atypical then a further biopsy is regarded as mandatory. If there is a low clinical and imaging suspicion the patient may be followed up with clinical and/or imaging assessment with FNAB, usually at 3–6 months.

When ROSE is performed and the smears are insufficient/inadequate, the FNAB is repeated up to 2 or 3 times [12], ideally with ultrasound guidance. If still inadequate, immediate CNB can be performed, or in situations where CNB is not immediately available, the cytopathologist can wait and examine all air-dried and alcohol-fixed slides.

If CNB is not available at any time, and the clinical and imaging findings are indeterminate or atypical, then repeat FNAB at follow-up, or excision biopsy should be considered. If clinical and imaging suspicion is low, repeat FNAB at a reasonable follow-up is recommended although some centres may prefer to follow-up initially with clinical and imaging review.

Sample Reports

Specific scenarios where the diagnosis of 'insufficient' is appropriate:

Example 1

A solid nodule is palpable or present on imaging, but there are very few epithelial cells or tissue fragments on the smears.

Inadequate/insufficient

The smears are insufficient for diagnostic purposes due to insufficient cells being present. Clinical and radiological correlation is required, and repeat FNAB with ROSE or core needle biopsy is recommended if clinically indicated.

Example 2
A proteinaceous background without epithelium is present.

Inadequate/insufficient
The smears consist of a proteinaceous background and in the absence of clinical or imaging information are considered insufficient. No epithelial cells or histiocytes are present to confirm a cyst. Correlation with imaging is required.

Note:
(a) This is the appropriate report IF there is no clinical information about the target lesion.
(b) If the lesion completely drained under direct ultrasound imaging, or if the palpable lesion has been drained without any residual mass lesion, the specimen is NOT considered inadequate and the material is reported as 'consistent with cyst contents', and at the discretion of the operator may be managed without further workup at this time. If not, repeat FNAB is recommended of the residual lesion.

Example 3
Considerable epithelial material is present, but poor fixation and/or smearing prohibit interpretation of the material.

Inadequate/insufficient
The smears are insufficient for diagnostic purposes due to poor fixation and cell preservation. Repeat FNAB is recommended.

Example 4
There is high or adequate cellularity but ultrasound gel obscures the material.

Inadequate/insufficient
The smear is inadequate for diagnostic purposes due to background ultrasound gel obscuring cellular detail. Repeat FNAB is recommended.

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Benign

3

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Introduction

The principal roles of breast fine needle aspiration biopsy (FNAB) are to correctly diagnose benign lesions so as to avoid unnecessary invasive diagnostic techniques, and to diagnose malignancy, while recognizing atypical and suspicious categories that require further core needle biopsy (CNB) or excision biopsy. Benign diagnoses constitute 24–77.5% of breast FNAB, depending on the patient population and the skill and experience of those performing and reading the cases. The risk of malignancy (ROM) in lesions diagnosed as benign on FNAB ranges from less than 1% to approximately 3% [1–5]. Two recent reports based on cases with histopathological follow up had a ROM of 1.7% and 1.4%, and a negative predictive value (NPV) of 97.1% and 98.7% [6, 7].

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A. S. Field et al. (eds.), *The International Academy of Cytology Yokohama System for Reporting Breast Fine Needle Aspiration Biopsy Cytopathology*, https://doi.org/10.1007/978-3-030-26883-1_3

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.

Discussion and Background

In most follow-up studies of FNAB a confirmatory histological diagnosis was not required after a benign FNAB diagnosis to determine the NPV and PPV of the benign category. In practice, a negative clinical and/or imaging follow-up at 6–12 months is regarded as sufficient to confirm the original 'triple negative' diagnosis including a benign FNAB as correct. The 'overall' sensitivity of a benign diagnosis, which includes all the benign diagnoses with or without histopathological follow-up, can be calculated as 'the number of correctly identified benign lesions' expressed 'as a percentage of the total number of benign FNAB diagnoses' [8]. One recent report that utilized category definitions virtually the same as those in the IAC Yokohama System had an 'overall sensitivity' of 96.9% for the benign category [6].

Patients with a benign FNAB diagnosis of 'proliferative disease without atypia' have an increased Relative Risk (RR) of developing carcinoma of 1.88 anywhere in the breast, while 'non-proliferative disease' has a RR of 1.27, suggesting the need for an increased awareness and general follow-up of a 'proliferative' benign diagnosis, especially in women with a family history of breast cancer [9, 10]. However, lesions with a FNAB diagnosis of non-proliferative changes or proliferative changes without atypia do not require specific follow-up unless another component of the triple test is atypical or indeterminate. This compares to a RR of 4.24 in women with 'proliferative disease with atypia', who are included in the atypical category [10].

The cytological features associated with benign lesions include:

- A pattern of predominantly large, *cohesive monolayered sheets of uniform ductal epithelial cells or cohesive 3-dimensional epithelial tissue fragments* showing streaming of epithelial cells around irregular slit-like holes ('secondary lumina'); there may be a mix of smaller tissue fragments and sheets, but dispersal is usually not prominent [11] (Fig. 3.1a, b).

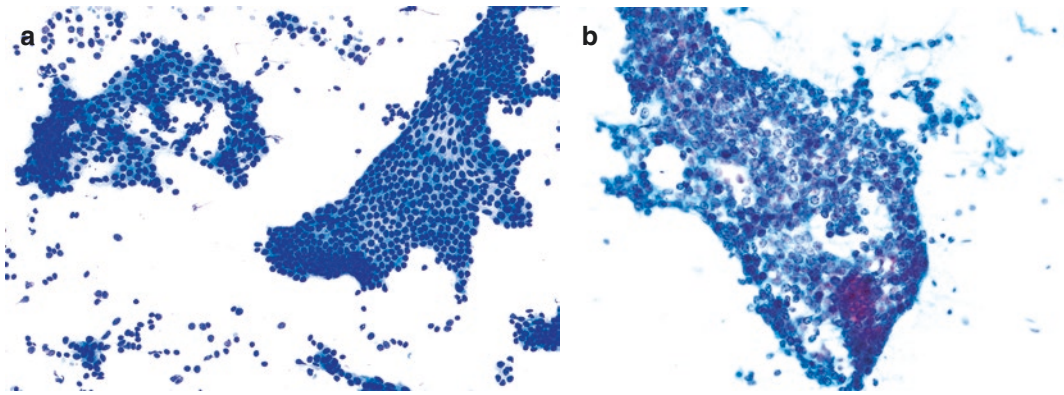


Fig. 3.1 (**a**) Typical low power pattern of a benign smear showing epithelial hyperplasia with predominantly large tissue fragments of ductal epithelial cells and bare bipolar nuclei in the background (Giemsa ×10); (**b**) Cohesive large ductal epithelial tissue fragment showing dark myoepithelial nuclei overlaying ductal cells with irregular slit-like holes, and bare bipolar nuclei in the background (Pap ×20)

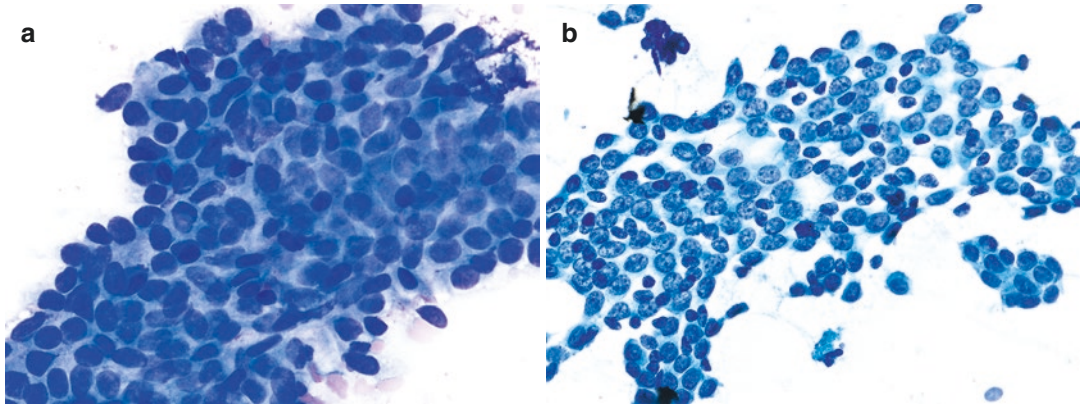


Fig. 3.2 (**a**) Ductal epithelial tissue fragment with dark oval myoepithelial cell nuclei overlying ductal epithelial cell nuclei (Giemsa ×40); (**b**) ductal epithelial tissue fragment with dark oval myoepithelial cell nuclei overlying ductal epithelial cell nuclei and a bare bipolar nucleus at the bottom right (Pap ×40)

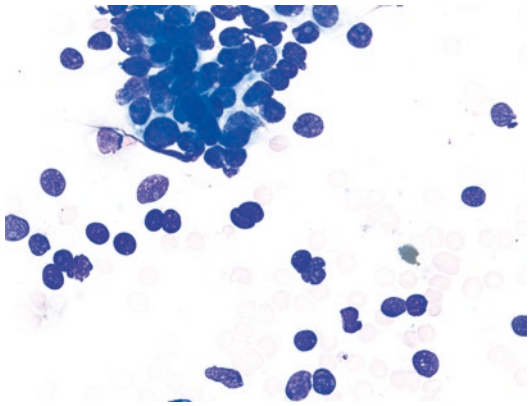


Fig. 3.3 Bare bipolar nuclei and small ductal epithelial cell tissue fragment

- *Myoepithelial cells* represented by perfectly ovoid nuclei with fine even chromatin and no nucleoli or definable cytoplasm are seen on the cohesive sheets and tissue fragments, in a slightly different focal plane, imparting a 'bimodal' pattern to these tissue fragments [12] (Fig. 3.2a, b).
- Stripped myoepithelial nuclei or '*bare bipolar nuclei*' in the background, which may occur as 'benign pairs' when the oval nuclei gently touch each other on one extremity; these nuclei are oval with fine chromatin and no nucleoli [13, 14] (Fig. 3.3).
- *Epithelial nuclei* from terminal ductules and smaller ducts are small, uniform and round, with fine to mildly clumped coarse chromatin, with or without small nucleoli. Nuclear size gradually increases to moderate with mildly coarse chromatin and small- to medium-sized round nucleoli in benign proliferative lesions [15, 16] (Fig. 3.4a, b).
- *Normal breast* may show a pattern of small terminal ductular tissue fragments with myoepithelial cell nuclei, intact or fragments of lobules and bare bipolar nuclei in the background (Fig. 3.5a–g).
- *Apocrine sheets, foamy histiocytes and a granular proteinaceous background* are commonly seen and are evidence of fibrocystic change (Fig. 3.6a–c).

Highly cellular smears showing the key cytological features of a specific benign breast lesion such as a fibroadenoma can be safely diagnosed as benign, even in the presence of minor degrees of dispersal or minimal nuclear atypia. The ability to recognize a specific lesion and accept minor degrees of atypia will vary with the reporting pathologist's experience. Correlation with imaging findings in the triple test is essential if imaging is available.

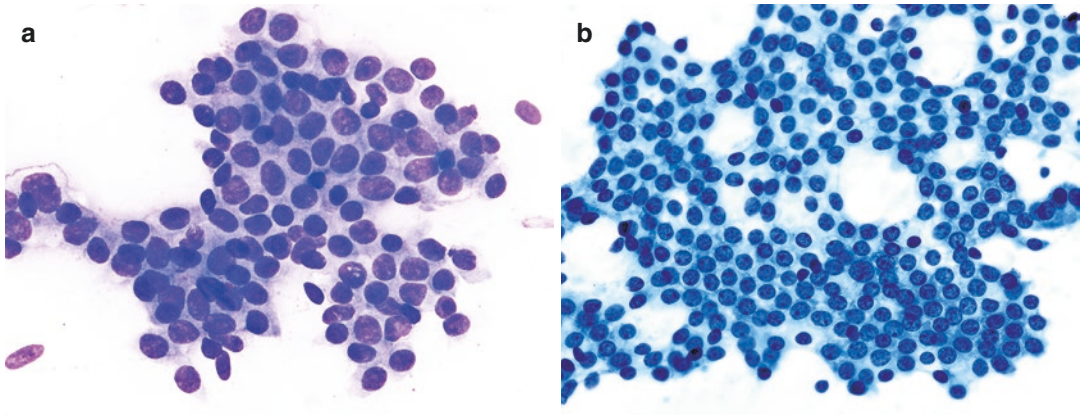


Fig. 3.4 (**a**) Small ductal epithelial cell tissue fragment showing evenly spaced ductal nuclei and small dark oval myoepithelial nuclei (Giemsa ×40); (**b**) small ductal epithelial cell tissue fragment showing evenly spaced ductal nuclei and small dark oval myoepithelial nuclei, with irregular secondary lumina (Pap ×40)

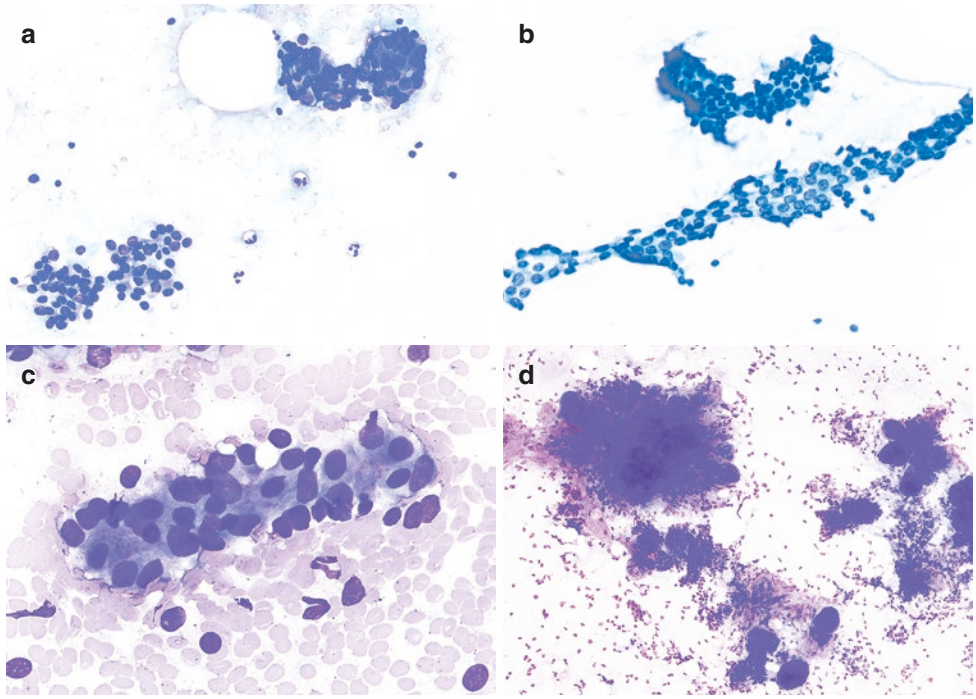


Fig. 3.5 (**a**) Normal breast showing small terminal ductular tissue fragments and bare bipolar nuclei (Giemsa ×20); (**b**) normal breast showing small terminal ductular tissue fragment and small ductal tissue fragment with myoepithelial nuclei and bare bipolar nuclei in the background (Pap ×20); (**c**) small terminal ductular tissue fragment with ductal cells and myoepithelial nuclei, and bare bipolar nuclei in the background (Giemsa ×40); (**d**) intact and fragmented lobules and several terminal ductular tissue fragments (Giemsa ×10); (**e**) intact lobule with terminal ductules in specialized lobular stroma and several terminal ductular fragments (Giemsa ×10); (**f**) intact lobule with terminal ductules and specialized lobular stroma (Pap ×10); (**g**) intact lobule with secretions in terminal ductules (Pap ×20)

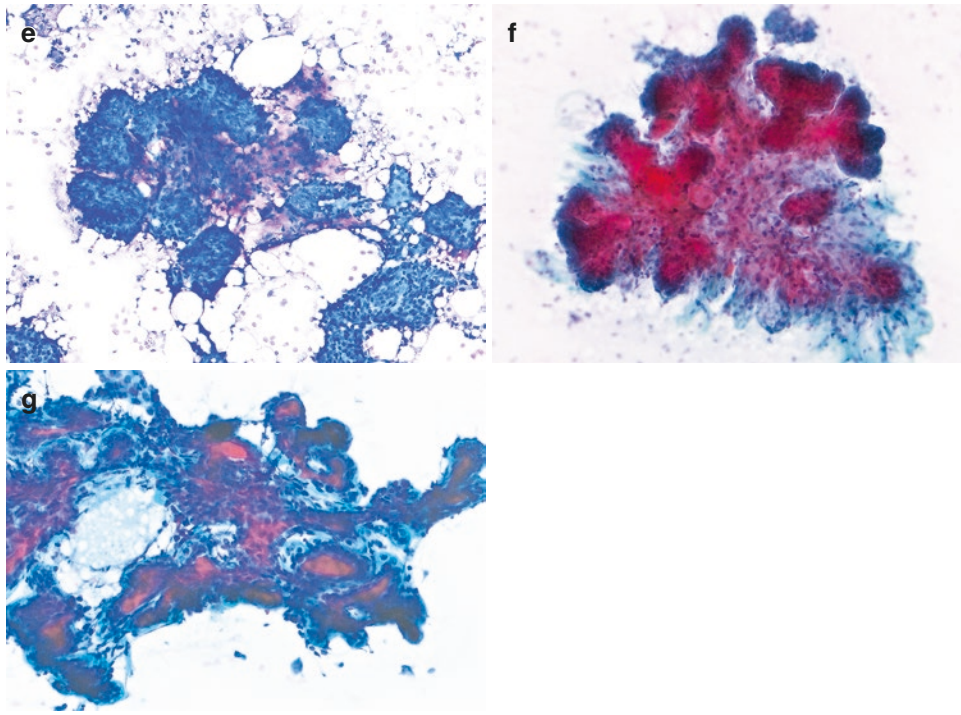


Fig. 3.5 (continued)

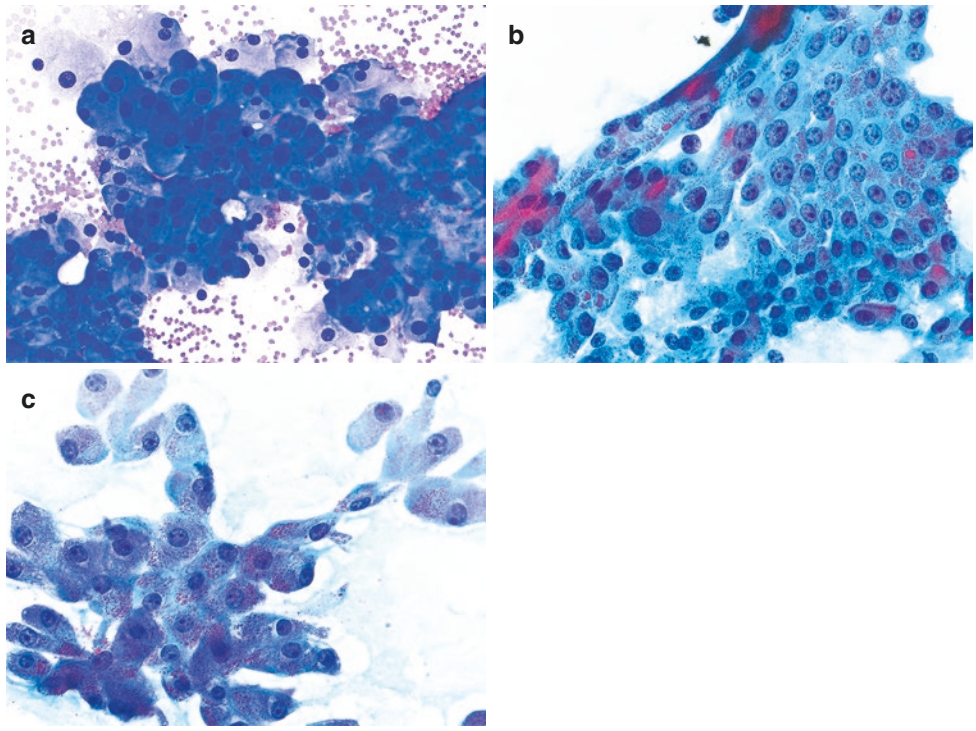


Fig. 3.6 (**a**) Apocrine cell sheet (Giemsa ×20); (**b**) apocrine sheet with mild anisonucleosis (Pap ×40); (**c**) apocrine cells showing columnar differentiation and some dispersal (Pap ×40)

Entities that Fall into the Benign Category

This is a short list of the entities that make up the bulk of benign lesions recognized on FNAB, accompanied by the main cytological features to suggest that diagnosis. These lesions will be further described in detail with photomicrographs later in this Chapter.

1. *Acute mastitis and breast abscess*: numerous neutrophils with scattered foamy histiocytes and absent or a small number of small tissue fragments of inflamed ductal or apocrine epithelial cells, in a necrotic suppurative background.
2. *Granulomatous mastitis*: with epithelioid granulomas, multinucleated giant cells and varying degrees of necrosis, which can be due to specific infections such as mycobacterial infection or be related to a foreign body reaction such as to silicone.
3. *Fat necrosis*: coarsely granular multicoloured necrotic debris, occasional infarcted, anucleate necrotic fat tissue fragments and a small number of macrophages and multinucleated histiocytes. Few or no epithelial cells are seen.
4. *Cyst*: aspirated fluid shows a variable number of metaplastic apocrine epithelial sheets, single apocrine cells and histiocytes in a proteinaceous background, that varies from finely granular to thick and dense with cholesterol crystals and debris.
5. *Fibrocystic change*: mix of large and small cohesive ductal epithelial tissue fragments and apocrine sheets and foamy histiocytes in a proteinaceous background.
6. *Normal breast tissue*: low cellularity smears consisting of small cohesive terminal ductular tissue fragments of relatively uniform epithelial cells with myoepithelial cells and occasional intact lobules in a clean background with a small number of bare bipolar nuclei.
7. *Epithelial hyperplasia*: moderately to highly cellular smears with plentiful large and some small cohesive epithelial tissue fragments with bland nuclei and both myoepithelial cells on the tissue fragments and bare bipolar nuclei in the background.
8. *Fibroadenoma*: moderately to highly cellular smears showing cohesive, sometimes branched 'staghorn' epithelial cell tissue fragments with myoepithelial nuclei, fibrillary to rounded or scalloped fibromyxoid stromal fragments and numerous bare bipolar nuclei in the background.
9. *Intraductal papilloma*: moderate to high cellularity with mainly large and some small cohesive ductal epithelial cell tissue fragments with myoepithelial cells together with papillary stellate or complex fragments with fibroelastotic stromal cores, in a proteinaceous background with apocrine epithelial cell sheets, siderophages and histiocytes.
10. *Lactational change*: variably cellular smears with small epithelial sheets comprising cells with generally micro-vacuolated cytoplasm and mildly enlarged rounded nuclei with single small nucleoli, in a milky background of fat globules and thin proteinaceous material, along with isolated intact acinar cells and round stripped nuclei with a single nucleolus.
11. *Adenosis and sclerosing adenosis*: moderately to highly cellular smears with small cohesive terminal ductular epithelial tissue fragments with myoepithelial nuclei, associated with small, dense stromal tissue fragments. Bare bipolar nuclei and scattered isolated epithelial cells are present in the background.
12. *Gynaecomastia*: often low cellularity smears with hyperplastic ductal epithelial tissue fragments with myoepithelial cells and bare bipolar nuclei and some fibrillary stromal fragments.
13. *Intramammary lymph nodes*: a mixed lymphoid population with small lymphocytes predominating and possible germinal centre material.

Management

There is a long and successful history of more than 50 years of utilizing FNAB to assess palpable breast lesions without necessarily performing imaging. This is exemplified by the situations in which a specific benign FNAB diagnosis correlates with the clinical findings, such as an abscess yielding pus, a cyst that drains without a residual palpable nodule, or a rounded firm mobile nodule with characteristic cytological features of a fibroadenoma. This is particularly the case in the situation of restricted local medical infrastructure resources without imaging, where FNAB of the breast is a highly accurate diagnostic technique, which advances the management of palpable breast lesions and breast care.

However, where imaging is readily available, best practice requires that the benign FNAB findings must be correlated with the clinical and imaging findings in the 'triple test', in part to ensure that the lesion of concern has been sampled. In most situations the imaging will and should precede the FNAB, and imaging particularly ultrasound will be used to direct the FNAB ensuring the lesion under study has been sampled. In this setting, when experienced personnel perform the FNAB and interpret the slides using key diagnostic criteria, a benign cytological diagnosis requires only routine clinical or imaging follow-up.

If the imaging is indeterminate or atypical and the FNAB shows a benign process such as fibrocystic change, with or without epithelial hyperplasia, and does not explain the imaging findings, then the FNAB should still be reported as 'Benign' and the imaging reviewed. A follow-up biopsy, most commonly a CNB, should be recommended. This can occur in lesions considered suspicious on imaging with radial scar in the DD, where the FNAB provides good material showing fibrocystic change with epithelial hyperplasia. If the FNAB is performed in a clinic with ROSE, immediate CNB should be recommended, and if not in a clinic with ROSE, CNB should be recommended at a later date, to establish a pre-operative diagnosis and the appropriate management of the sentinel node. If this CNB also shows benign changes, then simple excision biopsy or MRI is appropriate.

The ROM of a benign FNAB diagnosis does decrease when imaging is correlated [2, 3]. FNAB should be repeated or a CNB performed if a lesion changes its characteristics at follow-up.

There is considerable variation in clinical practice as to whether a patient with a benign FNAB diagnosis is recalled for any further investigation and the exact timing of any follow-up varies according to the different guidelines used in individual centres or programs, the specific lesion that has been diagnosed and the imaging features. A return to routine screening at 12–24 months is the most typical outcome.

Specific Benign Lesions

Reporting breast FNAB requires a cytopathologist to reach as specific a diagnosis as possible so that correlation with clinical history and examination and particularly with the imaging findings using mammography, ultrasound and magnetic resonance imaging (MRI) can be as accurate and effective as possible in the 'triple test'. Checklists of cytological criteria that are diagnostic of specific lesions assist in making specific diagnoses, foster uniformity and reproducibility of the diagnosis and also highlight discrepant findings that alert the cytopathologist to the possibility of an alternative diagnosis.

Inflammatory Changes

Clinical, Imaging and Histopathological Features

Acute abscesses typically present with red painful swelling of a region of the breast. Chronic recurrent subareolar abscess (Zuska's disease) is a distinct entity and related to squamous metaplasia of the lactiferous ducts associated with recurrent abscesses. Granulomatous inflammation due to specific infection such as mycobacterium can

present with painful and palpable swelling. Granulomatous reaction to silicone may produce hard palpable swelling around a prosthesis. Fat necrosis may or may not have a history of trauma or more commonly previous surgery to the breast. Imaging of chronic inflammatory processes and particularly fat necrosis can resemble carcinoma with stellate scarring.

Key Cytological Diagnostic Criteria

Abscesses yield neutrophils in large numbers showing varying degrees of degeneration in a fibrinous proteinaceous background or 'pus', with varying numbers of histiocytes and fragments of myxoid granulation tissue with branched, anastomosing capillaries as the lesion ages (Fig. 3.7a–c). Bacteria such as streptococcus or staphylococcus may be seen in the Giemsa-stained smears or Gram stain. Sheets of inflamed ductal epithelial or metaplastic apocrine cells show low nuclear to cytoplasmic (N:C) ratios, and within each sheet there is a uniform increase in nuclear size and uniform chromatin, despite the hyperchromasia and more prominent nucleoli (Fig. 3.7d). Cultures are required to facilitate antibiotic therapy. A neutrophilic infiltrate rarely is seen associated with carcinomas.

Large numbers of neutrophils and histiocytes and apocrine sheets infiltrated by neutrophils and showing inflammatory reactive atypia can also be seen in aseptic '*inflamed cysts*' in cases where cyst rupture has produced an inflammatory reaction that may or may not have caused pain or redness, and may clinically have suggested an abscess.

Recurrent subareolar abscess (*Zuska's disease*) shows smears with pus and considerable keratinous debris and superficial squamous cells, which may show inflammatory reactive changes (Fig. 3.8a, b). These lesions are differentiated from inflamed epithelial cysts of the skin of the

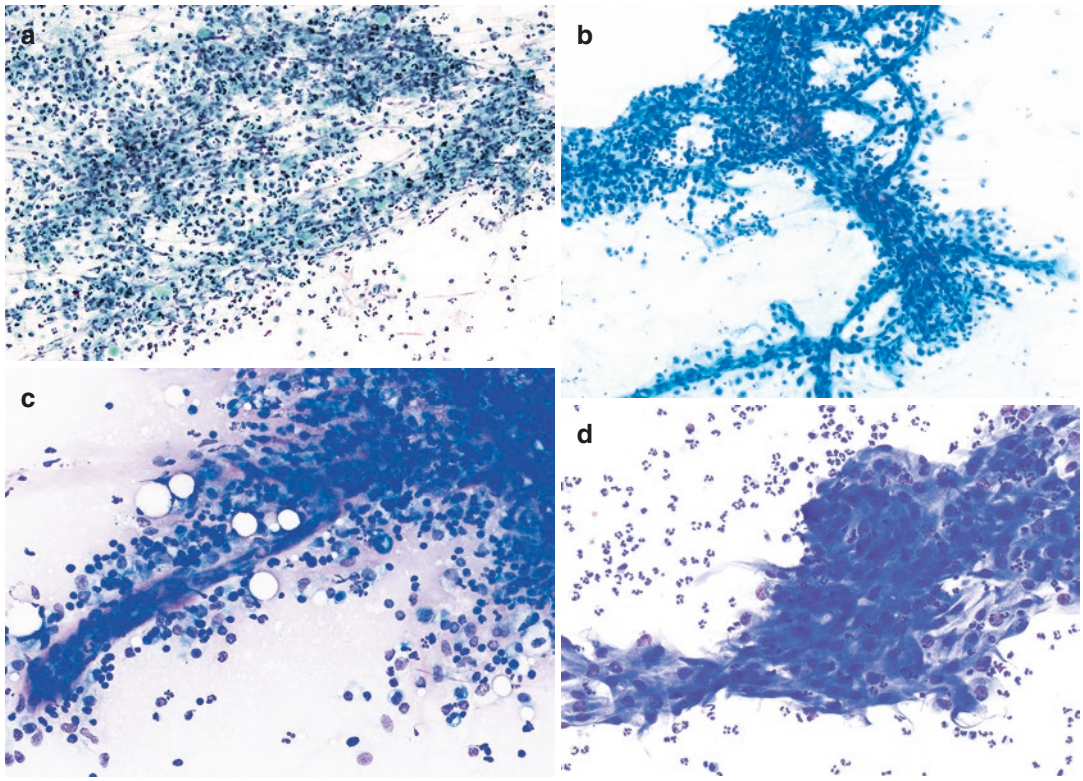


Fig. 3.7 (**a**) Pus from abscess (Pap ×10); (**b**) Granulation tissue fragment from abscess (Pap ×10); (**c**) Histiocytes and neutrophils with a capillary consistent with granulation tissue (Giemsa ×20); (**d**) Apocrine cells infiltrated by neutrophils and neutrophils in the background from an inflamed cyst or abscess (Giemsa ×20)

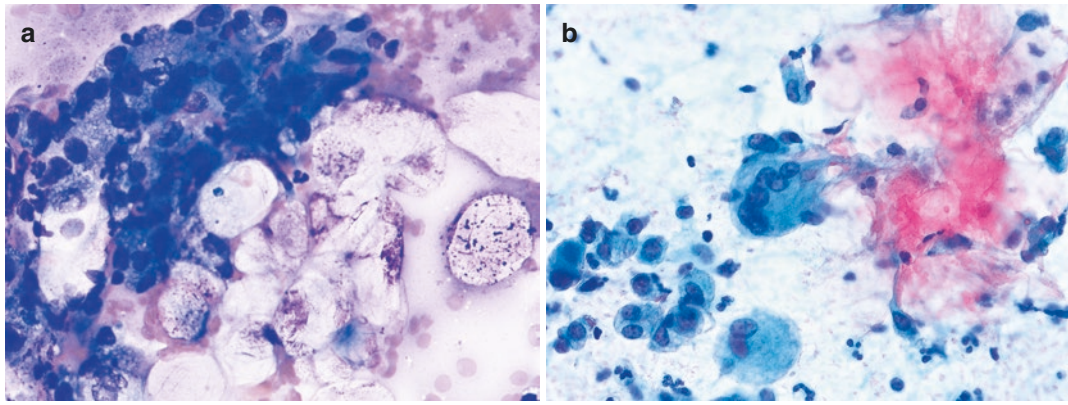


Fig. 3.8 (**a**) Recurrent sub-areolar abscess showing keratinous anuclear debris and adjacent histiocytes and neutrophils (Giemsa ×40); (**b**) Recurrent sub-areolar abscess showing keratinous anuclear debris, single histiocytes, multinucleated histiocytes and neutrophils (Pap ×40)

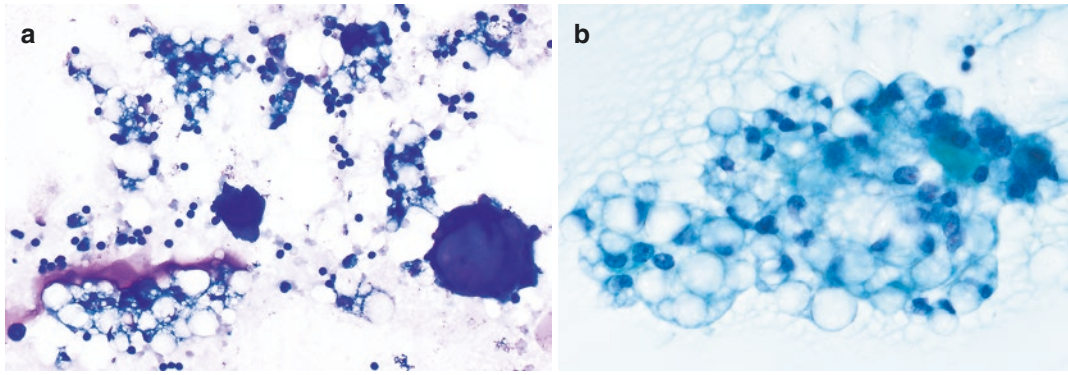


Fig. 3.9 (**a**) Epithelioid histiocytes and multinucleated histiocytes containing vacuoles and a calcification (Giemsa ×20); (**b**) Granulomatous aggregate of histiocytes in a case of leaking silicone from a breast prosthesis (Pap ×40)

breast by their location within and deep to the nipple and by their history.

Granulomatous mastitis can be seen in specific infections such as tuberculosis involving the breast but can also be a nonspecific inflammatory process, characterized by multinucleated giant cells, plentiful histiocytes, occasionally forming vague granulomas, and lymphocytes in a proteinaceous background with mildly atypical ductal tissue fragments [17, 18]. Culture or PCR is required to exclude mycobacterial infection. Similar findings can be seen in granulomas in the breast and axillary lymph nodes reacting to silicone derived from breast prostheses (Fig. 3.9a, b). The silicone is identified in histiocytes and multinucleated giant cells with foamy cytoplasm containing faintly refractile non-birefringent globules [19, 20].

Dilated ducts with periductal sclerosis and inflammation, known as *periductual mastitis* or *duct ectasia* when deep to the nipple, in FNAB produce a proteinaceous background, with histiocytes, lymphocytes, plasma cells, granular debris, multinucleated histiocytes and usually some apocrine or ductal epithelium. The distinction from a cyst relies on ultrasound findings.

Fat necrosis can be seen at a previous biopsy or operative site, or at sites of trauma, or can occur incidentally. It can be unsuspected on imaging or may mimic malignancy. Giemsa-stained smears show a range of yellow to orange to blue to black, punctate, and irregular granular debris with his-

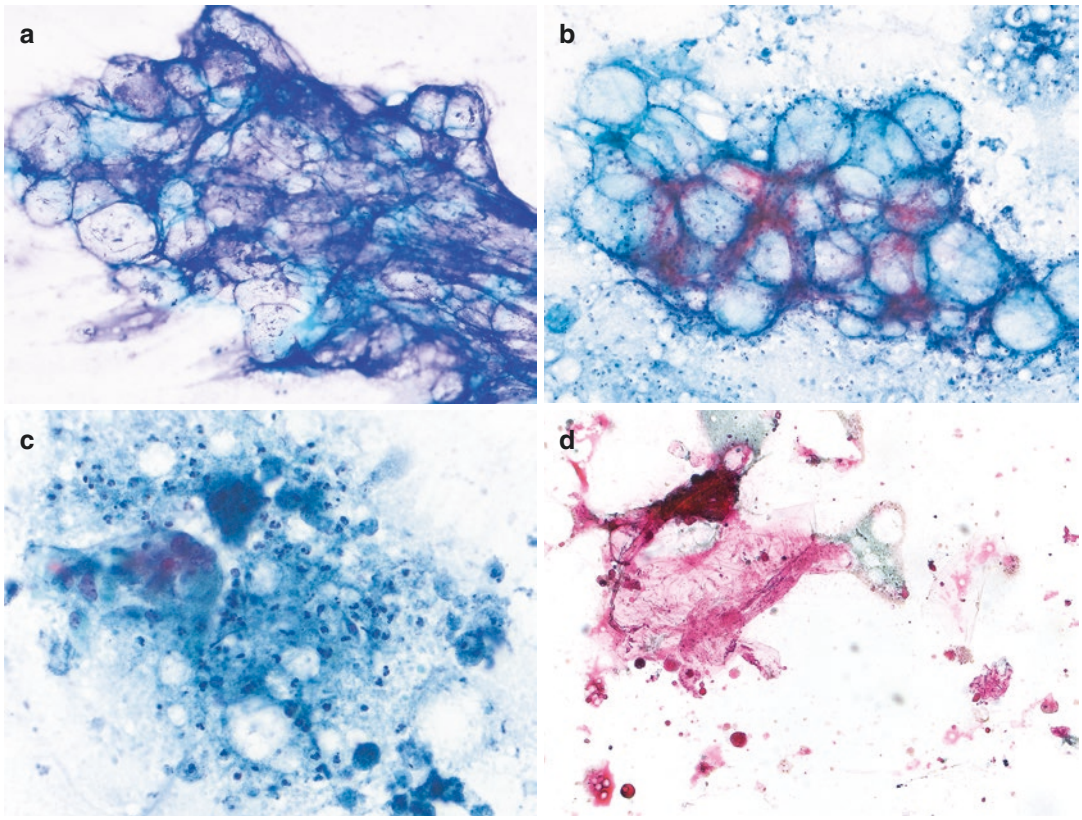


Fig. 3.10 (**a**) Fragment of infarcted fat tissue in fat necrosis (Giemsa ×10); (**b**) Fragment of infarcted fat tissue in a necrotic background in fat necrosis (Pap ×10); (**c**) Fat necrosis with multinucleated histiocytes, macrophages and neutrophils in a necrotic background (Pap ×20); (**d**) Fat necrosis with degenerate lipocytes in a granular multicoloured debris background (Pap ×20)

tiocytes, multinucleated histiocytes, hemosiderophages and fragments of fat, some of which may be necrotic with the reticular outline of anucleate adipocytes [11] (Fig. 3.10a–d). If fat necrosis is associated with large numbers of multinucleated histiocytes with bubbly vacuolated cytoplasm, the possibility of a reaction to silicone should be considered. 'Oil cysts', seen on imaging, typically fully aspirate and produce viscous yellow to white colloidal material on the unstained smears, but after fixation and staining only a little debris suggesting fat necrosis is seen.

Adjuvant *radiation therapy* following surgery can produce scattered cohesive epithelial tissue fragments showing radiation changes which are characterized by nucleomegaly with pleomorphic, large hyperchromatic nuclei with intranuclear vacuoles and smudged dark chromatin, and cytomegaly with a low to moderate N:C ratio and considerable vacuolated cytoplasm (Fig. 3.11). Debris and fat necrosis can be seen in the background with plump atypical

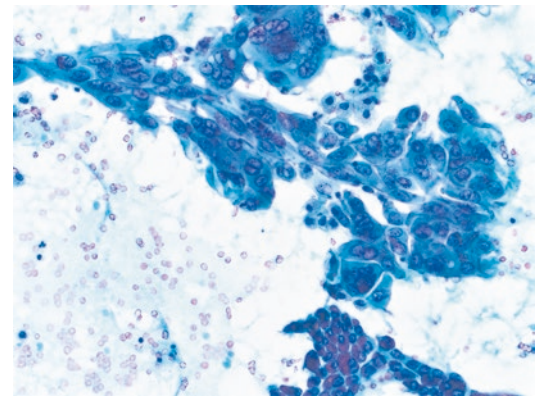


Fig. 3.11 Radiation therapy changes with a discohesive sheet of reactive apocrine cells, histiocytes and a sheet of apocrine cells (inferior) (Pap ×20)

fibroblastic cells. Residual carcinoma should be suspected if the epithelial cellularity is moderate to marked or there is dispersal of atypical cells with preserved chromatin, and CNB should be recommended [21].

Cysts and Fibrocystic Changes

Clinical, Imaging and Histopathological Features

Cysts may present as palpable, sometimes painful breast masses or they may be asymptomatic and present as rounded and typically well-defined masses on mammography and ultrasound. The imaging appearances may be diagnostic of a simple cyst or they can resemble fibroadenomas or papillomas. When inflamed they may appear similar to high grade carcinoma of no specific type, carcinoma with medullary features or mucinous carcinomas. Most cysts disappear on ultrasound and mammography after aspiration, but thick cyst fluid may be difficult to aspirate and associated fibrocystic change may constitute a residual mass requiring a further FNAB pass. Aseptic inflammation, scarring, a focal epithelial proliferative component or multiple juxtaposed cysts may create a 'complex' or 'multilocular cyst'. Fibrocystic change can produce irregular palpable masses with nonspecific findings on mammography and ultrasound.

Cyst fluid is usually thin, watery and lightly stained or it may contain fresh blood from the FNAB or be brown or black due to old haemorrhage, and very variable in viscosity and colour. Such cases cannot be distinguished by macroscopic examination from necrotic or cystic carcinomas, including the uncommon encysted papillary carcinoma. If a cyst appears typical on clinical and imaging and is fully aspirated with no residual palpable or ultrasound lesion, cytology is still recommended even though the risk of malignancy is low. Cytology is required if there are any clinical or imaging concerns or the fluid is thick or blood stained or the cyst does not fully aspirate.

Key Cytological Diagnostic Criteria

(Fig. 3.12a–c)

- Cellularity is low.
- A proteinaceous background varying from thin to thick granular material is present, with or without cholesterol crystals and debris.
- Foamy histiocytes, multinucleated histiocytes and siderophages are seen in variable numbers, singly or in aggregates.
- Apocrine cells are present in sheets and dispersed singly.
- Small cohesive tissue fragments of ductal epithelial cells with myoepithelial cells, and a variable number of bare bipolar nuclei are present in the diluting proteinaceous background.
- Granular calcific debris or irregular calcific fragments can be seen in the proteinaceous background.

Histiocytes have small, round, kidney-shaped or irregular, indented nuclei and copious, finely vacuolated or granular cytoplasm, which may contain blue-black (Giemsa) or yellow-brown (Papanicolaou) hemosiderin granules ('haemosiderophage') representing previous haemorrhage (Fig. 3.13). Multinucleated histiocytes may be present. Histiocytes with dense epithelioid cytoplasm may also be seen and should not be mistaken for atypical epithelial cells.

Metaplastic apocrine cells occur in flat sheets of evenly spaced polygonal cells with clearly defined cytoplasmic margins and abundant finely granular cytoplasm, which is greyish blue with fine red granules in the Giemsa smears, and green with varying dark green to reddish brown granules in the Pap stain (Fig. 3.6a–c). They have single or binucleated round central nuclei and often quite large single nucleoli, and can show columnar differentiation or spindling in small sheets, consistent with the attenuated lining of cysts. Degenerating apocrine cells can have intracytoplasmic lumina, containing eosinophilic material in the Pap stained smear or bluish purple material in the Giemsa stained smear, and there may be nuclear enlargement, anisonucleosis, and hyperchromasia with blurring of the chromatin

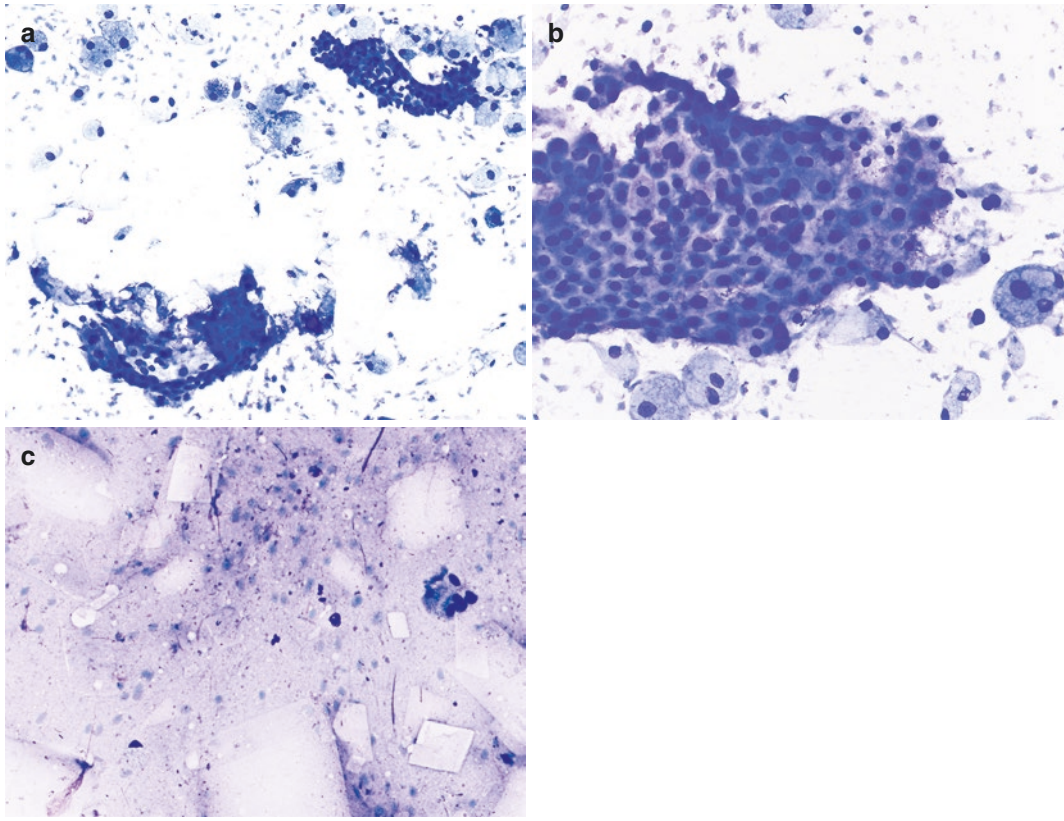


Fig. 3.12 (**a**) Fibrocystic change showing a small ductal epithelial tissue fragment, a small apocrine sheet and histiocytes in a proteinaceous background (Giemsa ×10); (**b**) Apocrine sheet and histiocytes in a proteinaceous background (Giemsa ×20); (**c**) Cyst contents with a minute apocrine sheets and cholesterol crystals in a proteinaceous background (Giemsa ×20)

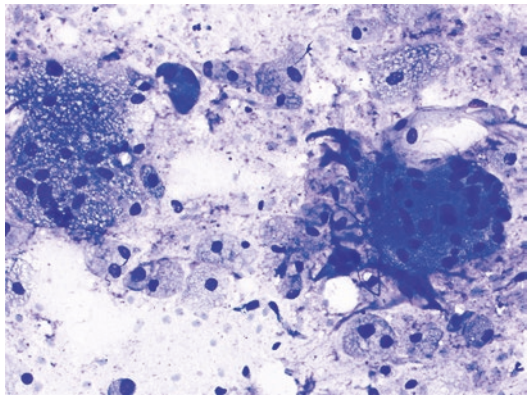
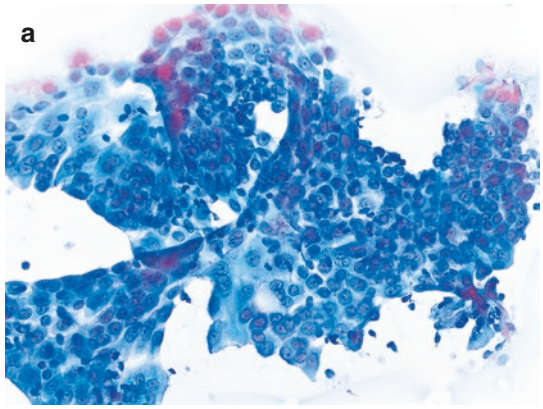


Fig. 3.13 Multinucleated histiocytes and histiocytes in a proteinaceous background with some containing blue hemosiderin granules (Giemsa ×20)

(Fig. 3.14a, b). Apocrine sheets usually have scant to no visible myoepithelial cells and can be hyperplastic and micropapillary (Fig. 3.15a, b). In some cases, dispersal of apocrine cells may be marked and when associated with nuclear degeneration may cause some concern. However, in distinction to apocrine carcinoma, the overall cellularity is low, the N:C ratio is low, and marked anisonucleosis, increase in nuclear size, coarse chromatin with perinucleolar clearing, large spiculated irregular nucleoli and 3-dimensional (3-D) or cribriform tissue fragments are not seen.

The diagnosis of '*cyst contents*' is made when there is a proteinaceous background with histiocytes and no apocrine epithelium and there is correlation with imaging findings, specifically complete drainage under real-time ultrasound imaging, or no palpable residual mass (Fig. 3.12c). A diagnosis of '*cyst with apocrine cells*' is made when apocrine sheets are present in a proteinaceous background (Figs. 3.12b and 3.16a, b). When ductal epithe-



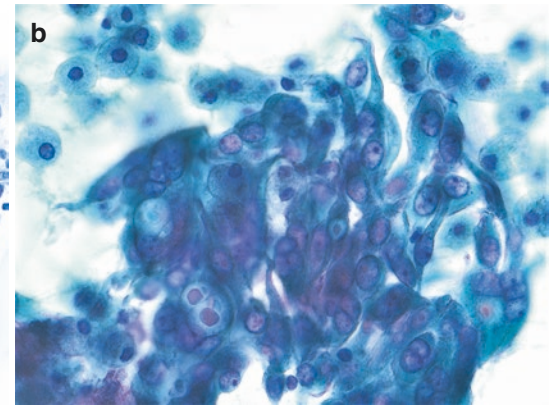
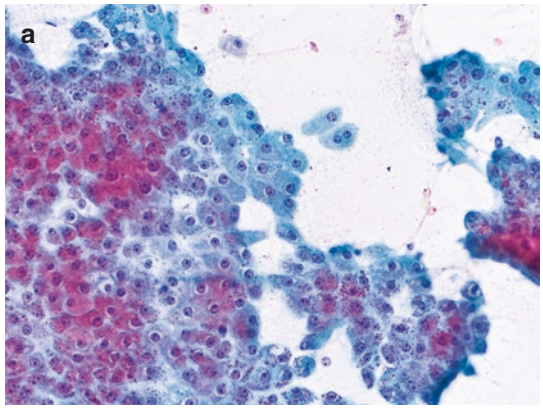


Fig. 3.14 (**a**) Apocrine sheet including myoepithelial nuclei, with reactive nuclear enlargement, mild pleomorphism and multinucleation of the apocrine cells (Pap ×20); (**b**) Apocrine sheet infiltrated by histiocytes from a cyst, with vacuolation of cytoplasm and reactive nuclear enlargement with fine chromatin (Pap ×40)



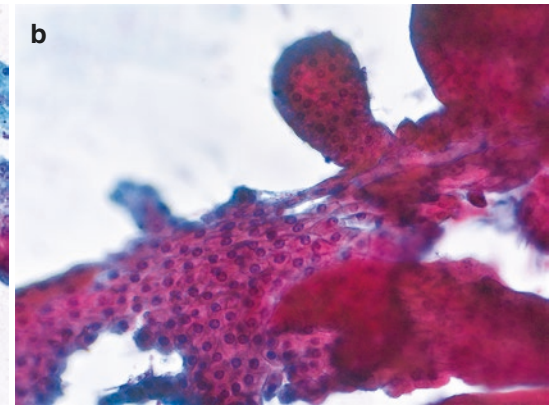
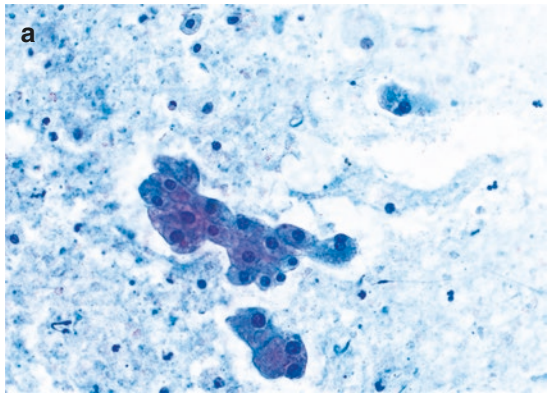


Fig. 3.15 (**a**) Hyperplastic apocrine sheet (Pap ×20); (**b**) Micropapillary apocrine hyperplasia (Pap ×20)



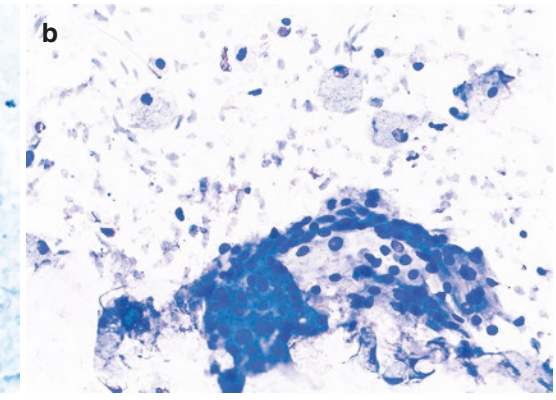


Fig. 3.16 (**a**) Cyst with small sheet of apocrine cells and histiocytes in a proteinaceous background (Pap ×20); (**b**) Cyst with small apocrine sheet and histiocytes in a proteinaceous background (Giemsa ×20)

lial tissue fragments with myoepithelial cells are also present with apocrine cells and histiocytes in a proteinaceous background, the diagnosis of '*fibrocystic change*' is made to facilitate correlation with ultrasound findings (Figs. 3.12a and 3.17). A small number of bare bipolar nuclei are usually present but the proteinaceous background may dilute their number. Bare bipolar nuclei can be distinguished from stripped apocrine cell nuclei, which are round with a single nucleolus. Ductal epithelial cells can show a transition to apocrine metaplasia or columnar cells, which can be associated with nuclear enlargement and mild degrees of atypia and dispersal [11] (Fig. 3.18a, b).

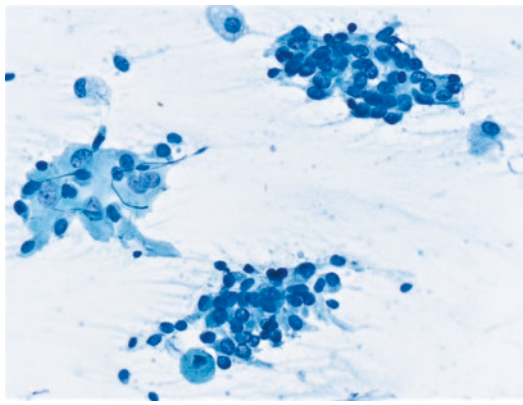


Fig. 3.17 Fibrocystic change with ductal epithelial tissue fragments and a small apocrine sheet and histiocytes in a proteinaceous background (Pap ×20)

Galactoceles occur in pregnant or lactating women and resemble cysts on imaging. They have a proteinaceous background that variably resembles cyst fluid or milk, with fat globules in a thin proteinaceous material and scattered histiocytes (Fig. 3.19).

Lactational change and lactational nodules (sometimes referred to inappropriately as 'lactating adenomas') also show a milky background of micro and macrovesicular fat globules in a thin casein proteinaceous background. A small number of acinar cells in sheets and as single cells, with pale vacuolated fragile cytoplasm and round nuclei containing a single central nucleolus are present (Fig. 3.20). Stripped, round acinar cell nuclei with a single nucleolus, and large lobules in which the terminal ductules are expanded, may be seen [11]. Acinar cells can show marked dispersal, but the milky background and lack of nuclear atypia help in the distinction from lobular and the rare secretory carcinoma.

Epithelial Hyperplasia

Clinical, Imaging and Histopathological Features

'Usual' epithelial hyperplasia is commonly associated with fibrocystic change, and may present with palpable lumps, mammographic asymmetry or nonspecific irregular densities or

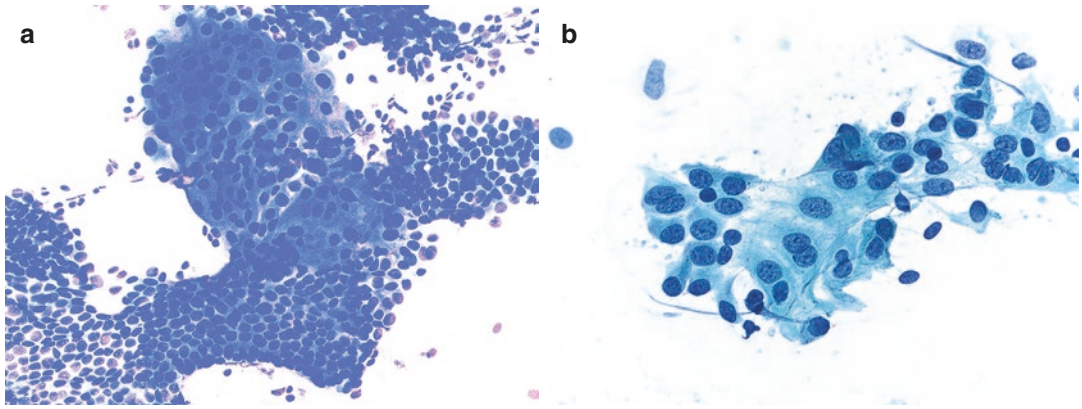


Fig. 3.18 (**a**) Ductal epithelial tissue fragment showing transition to apocrine metaplasia associated with nuclear enlargement (Giemsa ×20); (**b**) Ductal epithelial cells with myoepithelial cells showing transition to partial apocrine metaplasia (Pap ×40)

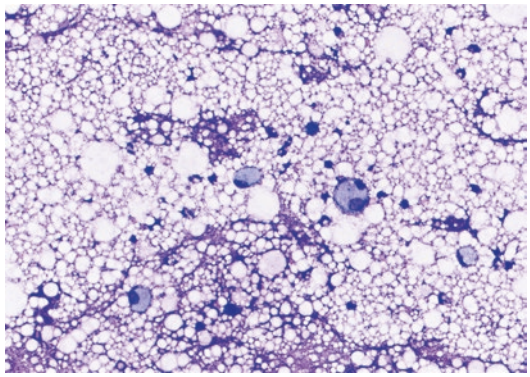


Fig. 3.19 Milky background with histiocytes from a galactocele (Giemsa ×20)

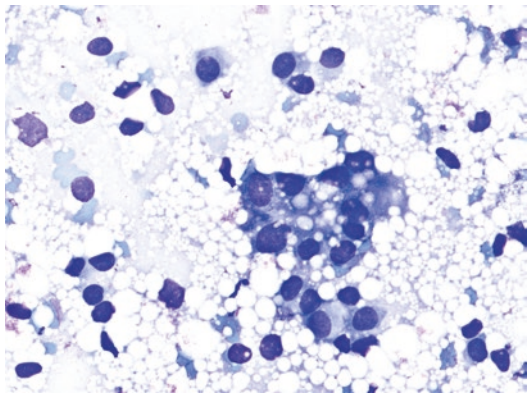


Fig. 3.20 Lactational change showing small tissue fragment of vacuolated acinar cells and single acinar cells and stripped round acinar nuclei in a milky background (Giemsa ×40)

architectural disturbances and benign-appearing microcalcifications. Ultrasound usually shows similar non-specific features regarded as 'fibrocystic change'. Epithelial hyperplasia can also be seen in intraductal papillomas, radial scars, fibroadenomas and phyllodes tumours, and it is the presence of other features such as stromal fragments that allow a more specific diagnosis to be made. In surgical pathology, epithelial hyperplasia is characterized by expansion of ducts by a proliferation of irregularly arranged epithelial cells showing small mildly pleomorphic nuclei with occasional notches, folds, pseudoinclusions, small round nucleoli and infrequent mitoses [22]. There is no necrosis. The epithelial cells stream around small slit-like irregular secondary lumina. There may be residual columnar cells lining the duct lumen and focal apocrine change can occur.

Key Cytological Diagnostic Criteria

(Fig. 3.21a–e)

- Cellularity is moderate to high.
- Predominantly large ductal epithelial tissue fragments with myoepithelial cells are present with a variable number of cohesive smaller epithelial tissue fragments and bare bipolar nuclei.
- The large hyperplastic ductal cell sheets have regularly arranged ductal epithelial cells and nuclei, with myoepithelial cells.
- Larger 3-D tissue fragments have irregularly arranged cells and nuclei that lack orientation, stream around irregular slit-like secondary lumina, and show variable mild nuclear enlargement and pleomorphism, with numerous myoepithelial cells.
- A variable but usually small number of dispersed single epithelial cells may be present, especially at the tail of the smears.

The '*bare bipolar nuclei*' (*stripped myoepithelial nuclei or possibly intralobular stromal nuclei*) in the background must be perfectly oval in shape with fine, even chromatin and no nucleoli, to distinguish them from stripped malignant nuclei [11, 12] (Fig. 3.2a, b). Myoepithelial cells are present at a focal plane above the larger ductal cells in the 'bimodal' tissue fragments, and have small, perfectly oval nuclei with uniform fine chromatin and no nucleoli, and must be distinguished from irregular apoptotic debris in carcinomas.

The *ductal cells* show round to oval to mildly variable and indented nuclei with fine chromatin, single nucleoli and occasional nuclear pseudoinclusions and grooves (Fig. 3.22a, b). The cells are arranged in a relatively uniform pattern when seen in a flat sheet, but are more haphazardly arranged in a multilayered overlapping pattern in the thicker 3-D tissue fragments reflecting their origin in ducts, or show streaming around irregular secondary lumina or holes in the hyperplastic epithelial fragments

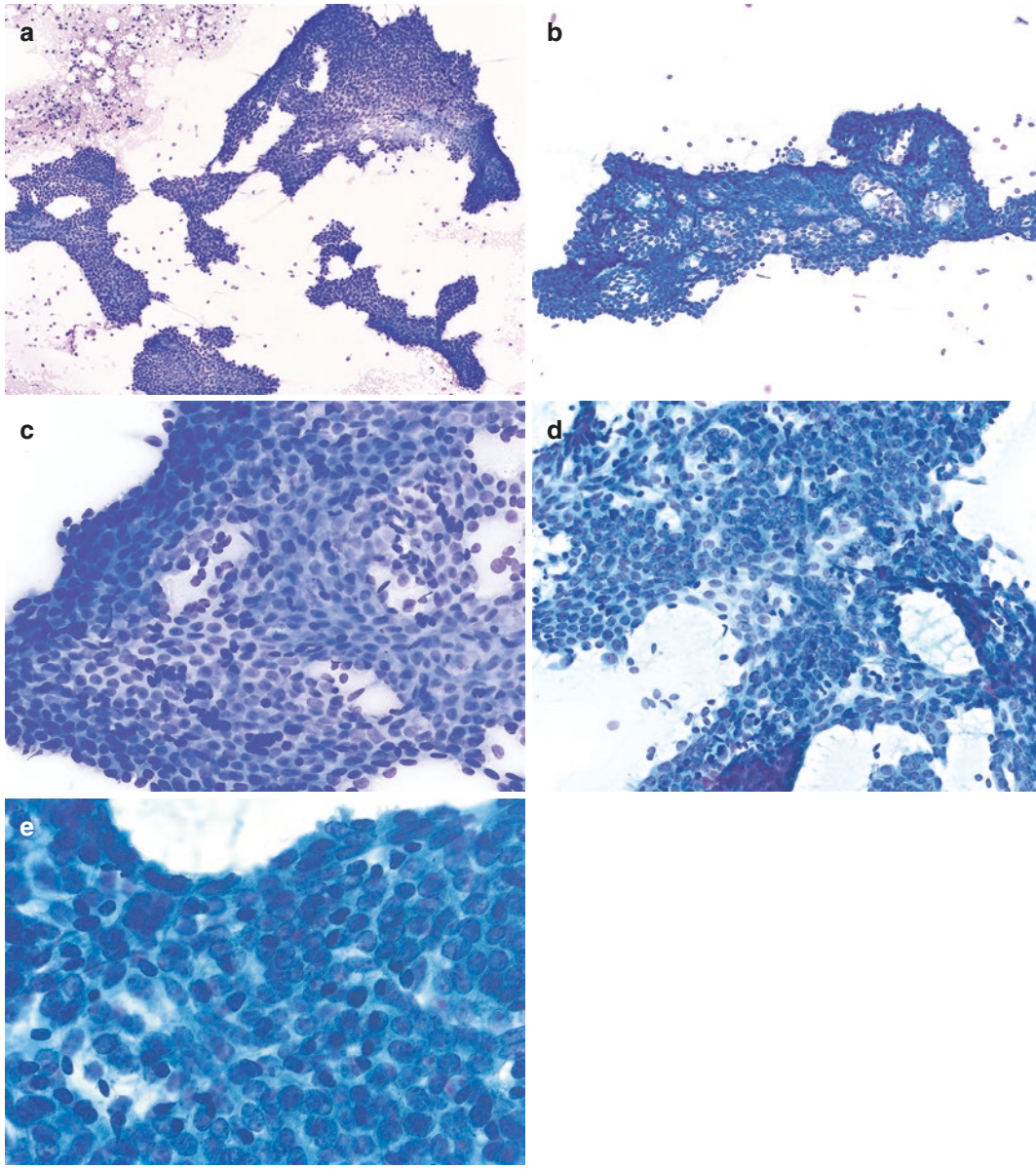


Fig. 3.21 (**a**) Epithelial hyperplasia showing large tissue fragment pattern (Giemsa ×5); (**b**) Hyperplastic ductal epithelial tissue fragment with irregular secondary lumina (holes) and bare bipolar nuclei in the background (Giemsa ×10); (**c**) Hyperplastic ductal epithelial tissue fragment with myoepithelial cells and irregular holes (Giemsa ×20); (**d**) Hyperplastic ductal epithelial cell tissue fragment with irregular holes and myoepithelial cells (Pap ×20); (**e**) Irregularly arranged ductal epithelial nuclei and myoepithelial cells (Pap ×40)

(Fig. 3.23a, b). The nuclei may be larger, with prominent single nucleoli, but the N:C ratio remains low and the nuclear features are usually uniform or predictable throughout a particular tissue fragment or sheet. Nuclear hyperchromasia or irregular chromatin clearing such as peri-nucleolar clearing and prominent nuclear envelope abnormalities are not usually seen. The nucleoli can be quite large and prominent, particularly in younger women, possibly related to the proliferative phase of the menstrual cycle or to the oral contraceptive pill.

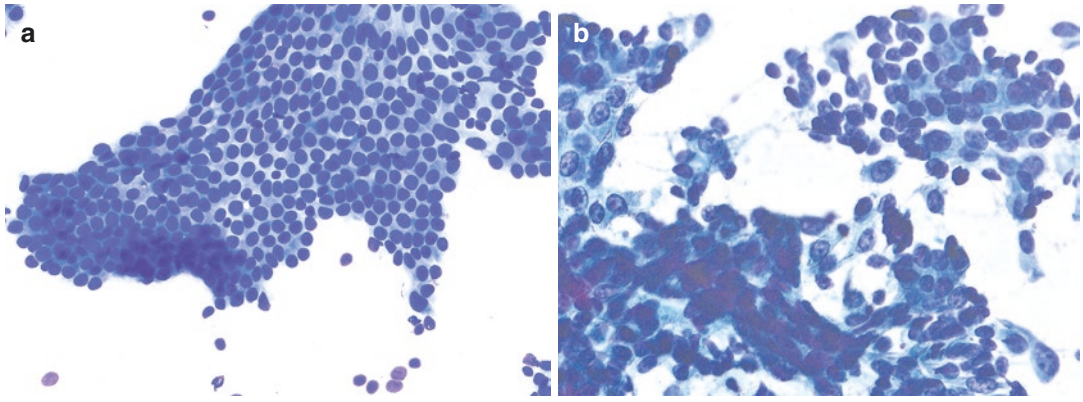


Fig. 3.22 (**a**) Flat sheet of hyperplastic ductal epithelial cells with myoepithelial cell nuclei and bare bipolar nuclei (Giemsa ×20); (**b**) Three-dimensional ductal epithelial tissue fragments with plentiful myoepithelial cell nuclei (Pap ×40)

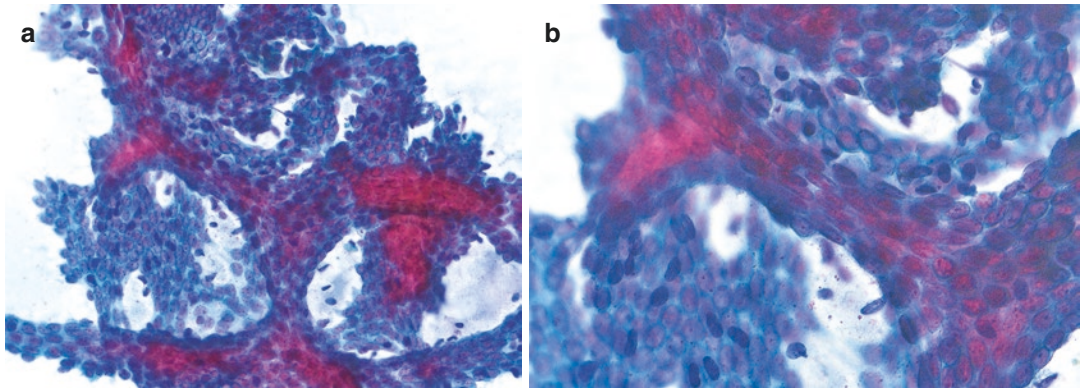


Fig. 3.23 (**a**) Cohesive hyperplastic relatively complex ductal epithelial tissue fragment with large irregular holes and prominent myoepithelial nuclei (Pap ×20); (**b**) High power showing streaming of ductal epithelial cells and myoepithelial cells (Pap ×40)

Hyperplastic ductal epithelial tissue fragments and plentiful bare bipolar nuclei are seen in:

- 'Fibrocystic change with epithelial hyperplasia' with a proteinaceous background, histiocytes and apocrine sheets.
- Radial scars with high cellularity, histiocytes, apocrine sheets and a proteinaceous background [23].
- Fibroadenomas with large, scalloped, irregular, myxoid or fibrillary stromal fragments.
- Gynaecomastia in male patients with fragments of fibrillary stroma.
- Benign and borderline phyllodes tumours with hypercellular and variably atypical stroma.
- Intraductal papilloma with stellate papillary or complex meshwork fragments and apocrine sheets.
- Columnar cell change.

Columnar cell change may be seen in FNAB of mammographic calcifications, or as an incidential finding. In surgical pathology, the calcifications are present in the dilated terminal ductules of lobules, which are lined by columnar cells featuring a luminal apical cytoplasmic bleb

[24]. There is a spectrum of change through columnar cell hyperplasia where the columnar cells are multilayered but still orientated, flat epithelial atypia, where the nuclei of the thickened epithelium lack orientation to the lumen and show nuclear atypia, to atypical ductal hyperplasia and low grade clinging, cribriform and micropapillary Ductal carcinoma in situ (DCIS). The interpretation of these changes, which are frequently intermingled with fibrocystic change and epithelial hyperplasia in the same or serially sectioned slides, shows considerable inter-observer variability in surgical pathology. Columnar cell change in FNAB cytology shows bulbous, hyperplastic epithelial tissue fragments, which are three-dimensional and ballooned with a central lumen that can be demonstrated by focusing up and down on the fragment. Myoepithelial nuclei are seen on the outer surface, and there is columnar cell orientation at the margins of the fragments and columnar cells are present in the background [11] (Fig. 3.24a–d). Calcifications are often present in a proteinaceous background.

In FNAB the specific diagnosis of *proliferative changes* requires careful assessment utilizing the key cytological diagnostic criteria, and an attempt should be made to maximize correlation with imaging and to avoid a false-positive diagnoses of carcinoma [11, 25]. The features should be clearly described and a differential diagnosis provided that emphasizes the most likely diagnosis. The cytopathologist should assess if the features are consistent with epithelial hyperplasia or if they are 'atypical' in which case a diagnosis of 'epithelial hyperplasia with atypia' is appropriate [11, 25]. The specific diagnosis of 'atypical ductal hyperplasia' should not be attempted [25].

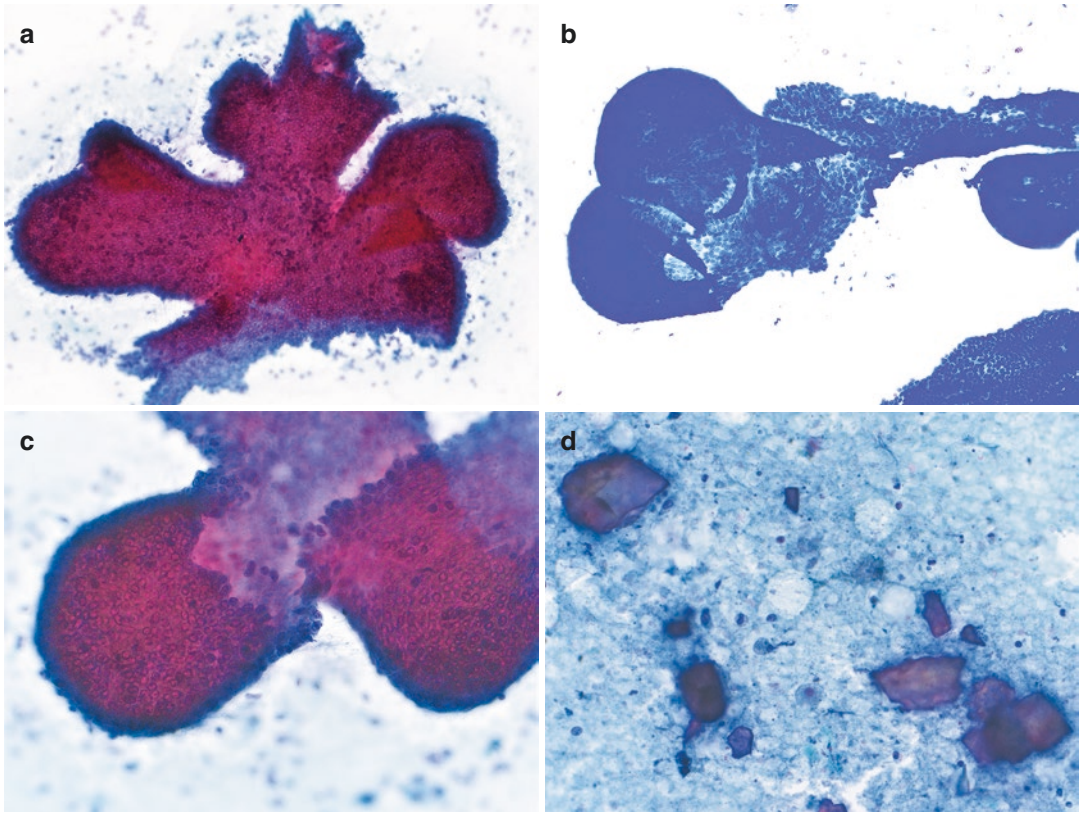


Fig. 3.24 (**a**) Columnar cell change with ballooned terminal ductules (Pap ×10); (**b**) Columnar cell change (Giemsa ×10); (**c**) Columnar cell change with the open lumen of the dilated terminal ductule and myoepithelial nuclei on the outer aspect of the epithelium (Pap ×20); (**d**) Calcifications in a proteinaceous background (Pap ×20)

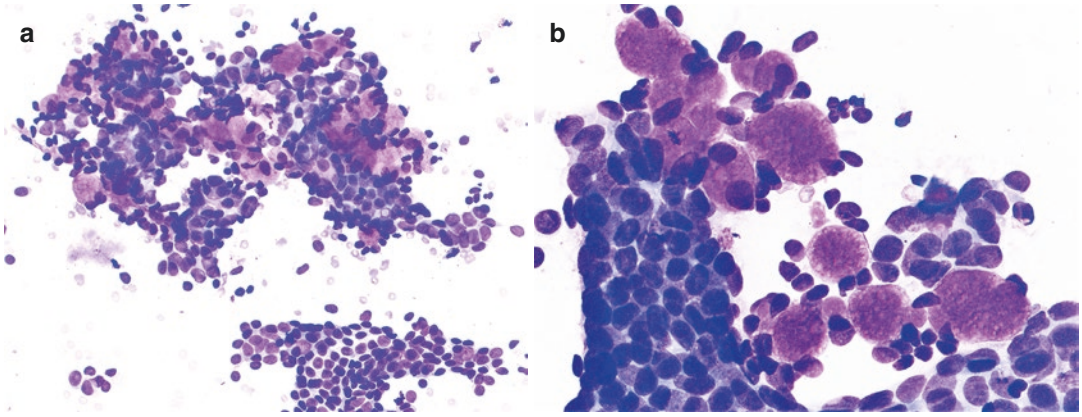


Fig. 3.25 (**a**) Collagenous spherulosis with rounded globular bodies with ductal epithelial cells and myoepithelial cell nuclei (Giemsa ×20); (**b**) Collagenous spherulosis with myoepithelial cells and ductal cells (Giemsa ×40)

Correlation with imaging is required and further biopsy suggested.

Hyperplastic ductal epithelial tissue fragments may include magenta (Giemsa) or pale green (Pap) globules derived from basement membrane material and known as *collagenous spherulosis* (Fig. 3.25a, b). These tissue fragments are usually seen in a background of fibrocystic change [11, 12]. The collagen balls need to be distinguished from the larger and more numerous hyaline globules seen in adenoid cystic carcinoma in which the background is usually clean and there is no evidence of fibrocystic change. If a definite distinction cannot be made the smears should be labelled 'atypical' and CNB recommended.

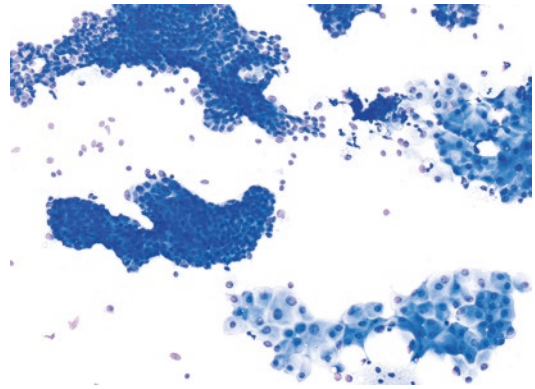


Fig. 3.26 Fibrocystic change with hyperplastic ductal epithelial tissue fragments and apocrine sheets in a proteinaceous background consistent with radial scar (Giemsa ×10)

Radial Scars/Complex Sclerosing Lesions

Clinical, Imaging and Histopathological Features

Radial scars are usually found incidentally on mammography and mimic stellate carcinomas. They may be presented to the cytopathologist with a diagnosis of 'carcinoma'. Histologically, they are characterized by central and radiating sclerosis, which contains small distorted tubules, and ductal epithelial hyperplasia with or without atypia, apocrine change, adenosis, sclerosing adenosis, and in some cases, atypical ductal hyperplasia. In a small number of cases, these proliferative changes may be associated with low grade DCIS or tubular carcinomas [22].

Key Cytological Diagnostic Criteria (Fig. 3.26)

- Cellularity is moderate to high.
- The pattern is florid fibrocystic change with epithelial hyperplasia.
- Plentiful large ductal epithelial tissue fragments, including monolayered sheets of apocrine cells and 3-D ductal epithelial cell tissue fragments with myoepithelial cells are present (Figs. 3.15a, b, 3.21a–e).
- Variable numbers of smaller epithelial tissue fragments, some of which may be tubular

with myoepithelial cells, and dispersed epithelial cells.
- Bare bipolar nuclei are present but may be diluted by a proteinaceous background.
- Mild nuclear atypia is seen in some cases.
- Foamy macrophages and a proteinaceous background are usually present.
- Small sclerotic or elastotic tufts as well as myxoid stromal fragments may be present [23].

The features of radial scar in FNAB cytology resemble fibrocystic change with epithelial hyperplasia and are not diagnostic. If the imaging features are suspicious and the cytological findings are benign, CNB is recommended.

Fibroadenoma

Clinical, Imaging and Histopathological Features

Fibroadenomas are one of the commonest breast tumours and can present in all age groups, usually as a palpable, often mobile mass, or on mammogram and ultrasound as an ovoid, well-defined mass, which may be lobulated and may contain calcifications. The imaging features can be diagnostic but the differential diagnosis (DD) includes complex cysts, phyllodes tumours, fibrocystic change, intraductal papillomas, high grade carcinomas of no special type, mucinous carcinomas and carcinoma with medullary features.

Fibroadenomas are biphasic, fibroepithelial lesions with varying components of epithelial slits and large tubules, which are lined by ductal cells with myoepithelial cells. The epithelium may show columnar cell change and varying degrees of epithelial hyperplasia and apocrine metaplasia. The stroma varies from myxoid to sclerotic and is variably cellular [22]. Calcifications may be present.

Key Cytological Diagnostic Criteria

(Fig. 3.27a–h)
- Cellularity is moderate to high.
- A pattern of large and often some smaller epithelial tissue fragments with stromal fragments and plentiful bare bipolar nuclei is seen.
- Large, cohesive, 3-D branching ductal epithelial tissue fragments, which may show 'chicken drumstick' or 'antler-shaped' architecture, and folded monolayered sheets of regularly ordered ductal epithelial cells with an overlay of myoepithelial cells imparting a bimodal pattern are present.
- Irregular or rounded, scalloped, myxoid, sclerotic or fibrillary stromal fragments with variable cellularity, typically containing occasional branching blood vessels are present. Residual myoepithelial cells may be seen clinging to the edges of scalloped stromal fragments.
- Plentiful bare bipolar nuclei are often a clue to the diagnosis.
- Variable single epithelial cell dispersal is seen, and may be most marked and associated with smearing artefact towards the tail end of the smear.
- Often mild nuclear enlargement, anisonucleosis and crowding may be seen with prominent nucleoli, particularly in younger patients [11, 12].

Sclerotic fibroadenomas or poor quality FNAB technique may yield a low cellularity sample consisting of dispersed and often partially crushed epithelial cells, small epithelial tissue fragments, few bare bipolar nuclei, and scant stroma, resulting in an atypical or even false-positive diagnosis of carcinoma [26, 27] (Fig. 3.28).

On occasion, a fibroadenoma can yield high cellularity associated with prominent single cell dispersal, mild nuclear pleomorphism and hyperchromasia and prominent nucleoli leading to an atypical or false-positive diagnosis of carcinoma [26–30]. Precedence should be given to the overall pattern to avoid a false malignant diagnosis [11]. If significant nuclear atypia is present, a diagnosis of '*fibroadenoma with atypia*' should be made and CNB recommended to exclude the alternative diagnosis of low grade DCIS or invasive carcinoma, or the rare occurrence of lobular neoplasia, DCIS or invasive carcinoma within or adjacent to a fibroadenoma [31].

Myxoid fibroadenomas can have a granular magenta proteinaceous background mimicking

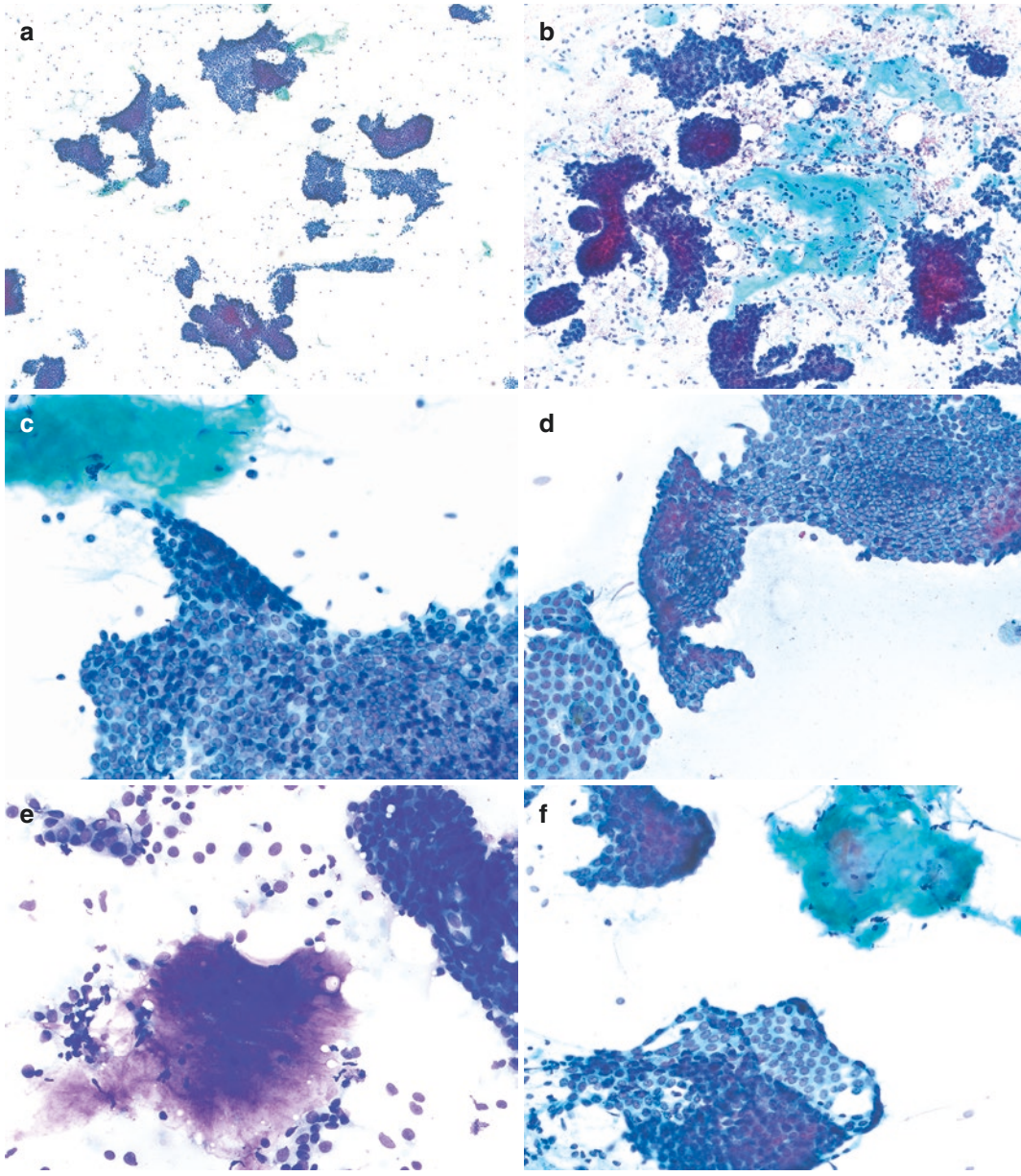
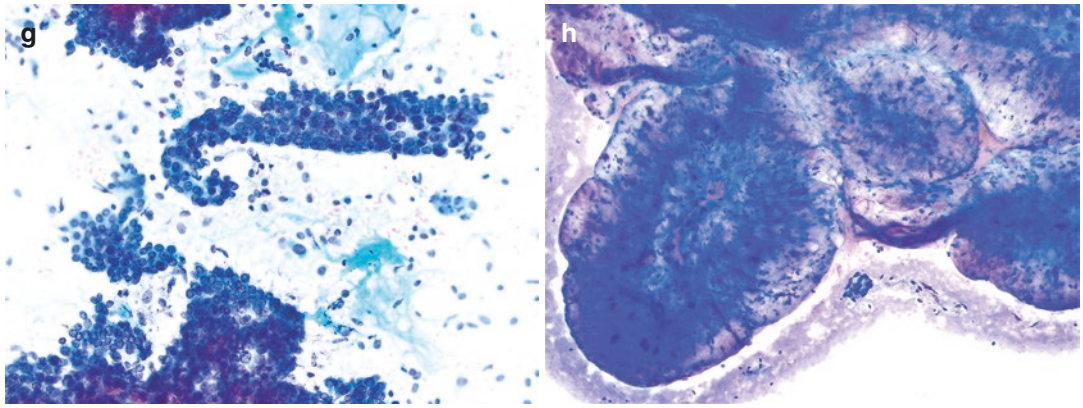


Fig. 3.27 (**a**) Fibroadenoma pattern of large tissue fragments and stroma (Pap ×5); (**b**) Fibroadenoma with typical pattern of large tissue fragments and stroma (Pap ×10); (**c**) Fibroadenoma with stromal and a hyperplastic ductal epithelial tissue fragment with myoepithelial nuclei, and bare bipolar nuclei in the background (Pap ×20); (**d**) Fibroadenoma with two ductal epithelial tissue fragments, one with prominent myoepithelial nuclei and one showing partial apocrine metaplasia and associated nuclear enlargement (Pap ×20); (**e**) Stromal fragment, ductal epithelial tissue fragments and bare bipolar nuclei in a clean background (Pap ×20); (**f**) Stromal tuft and ductal epithelial tissue fragments (Pap ×20); (**g**) Tubular and irregular ductal tissue fragments with myoepithelial nuclei and stroma and bare bipolar nuclei (Pap ×20); (**h**) Myxoid rounded stroma (Giemsa ×10)



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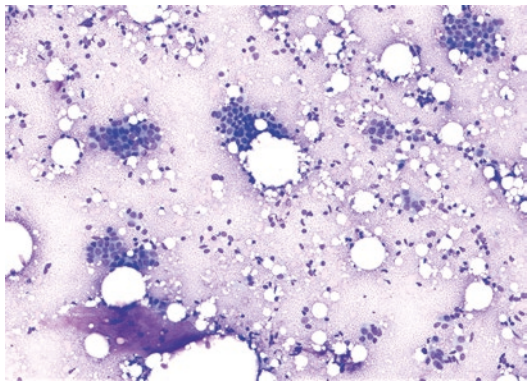

Fig. 3.28 Fibroadenoma showing a small ductal epithelial tissue fragment pattern with stroma and bare bipolar nuclei (Giemsa ×10)

mucinous carcinoma, but myxoid stromal fragments and the lack of nuclear atypia prevent a false-positive diagnosis. Mucinous carcinoma also often includes branching capillary vessel fragments in the characteristically fibrillary mucin [11, 32] (Fig. 3.29).

Differential Diagnosis

The DD of fibroadenomas includes all lesions that can produce large epithelial tissue fragments with myoepithelial cells and bare bipolar nuclei in the background:

- Epithelial hyperplasia lacks stromal fragments.
- Intraductal papillomas have large epithelial hyperplastic tissue fragments, apocrine sheets, a proteinaceous background and siderophages, in addition to stellate papillary and complex meshwork tissue fragments featuring a fibroelastotic branching stroma [33].
- Low grade DCIS can present with large tissue fragments, usually showing a rigid nuclear array or micropapillary or cribriform architecture with mild nuclear atypia, more marked single cell dispersal and no rounded stromal fragments [34].
- Invasive carcinomas may present with large epithelial tissue fragments but there is usually more marked nuclear atypia, greater dispersal of single cells and a lack of myoepithelial cells and bare bipolar nuclei.
- Benign/borderline phyllodes tumours in surgical biopsies can have heterogeneous regions varying from low cellularity and sclerosis to high stromal cellularity. The distinction from fibroadenomas with a cellular stroma is difficult, although the stroma of fibroadenomas lacks nuclear enlargement and atypia typical of phyllodes tumours [35–40] (Fig. 3.30). When stromal hypercellularity and nuclear atypia are present in the FNAB and when dispersed spindle stromal cells with elongated nuclei are numerous, the diagnosis of phyllodes tumour can be suggested [36, 40] (See further discussion in Chap. 4, Atypical). Excision biopsy to assess the whole tumour and its margins is recommended rather than a CNB which is subject to sampling error.
- *Tubular adenomas* are rounded lesions on imaging and regarded by many as a fibroade-

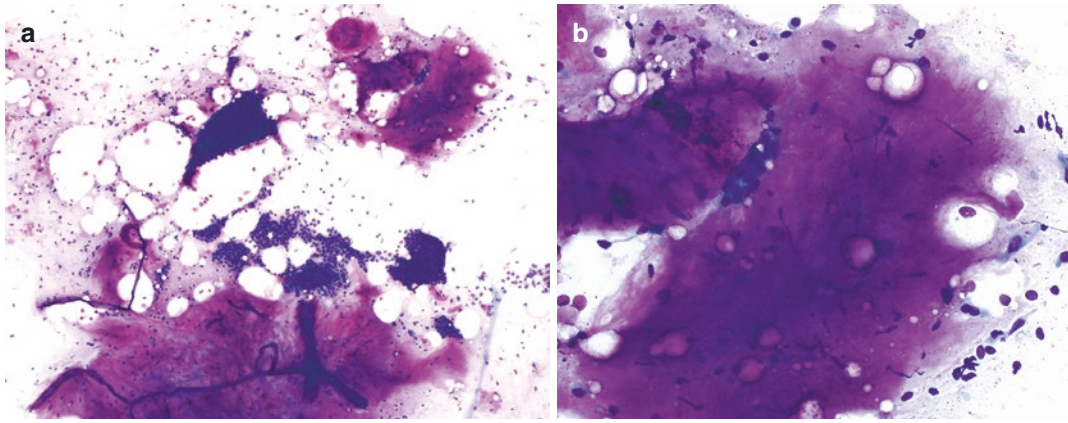

Fig. 3.29 (**a**) Fibroadenoma with myxoid rounded stroma containing a branching capillary (Giemsa ×10); (**b**) Myxoid stroma which is granular at its margin (Giemsa ×20)

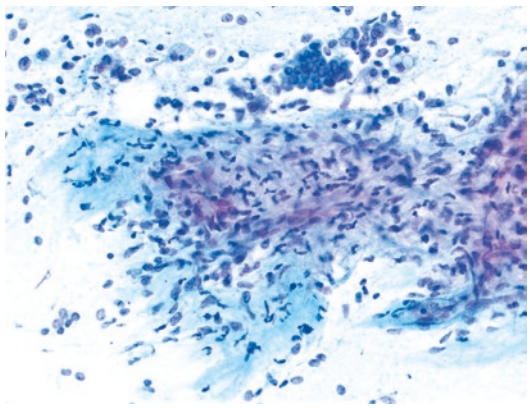

Fig. 3.30 Fibroepithelial lesion with mildly hypercellular stroma showing mild nuclear enlargement and pleomorphism raising a differential diagnosis of cellular fibroadenoma and low grade phyllodes tumour (Pap ×20)

noma variant with a prominent tubular component and scant stroma. FNAB reflects the histology and shows moderate to high cellularity with small acinar and tubular tissue fragments, some of which resemble lobular units, associated with myoepithelial cells, bare bipolar nuclei and scanty stromal fragments [41].

Intraductal Papilloma

Clinical, Imaging and Histopathological Features

Intraductal papillomas occurring in the subareolar region may present with spontaneous, haemoserous nipple discharge usually from a single duct, or on occasion a mass. On imaging, larger papillomas may produce a rounded mass, sometimes with an associated dilated duct on ultrasound and Doppler blood flow in the stalk. In surgical pathology intraductal papillomas include:

- Large papillomas most commonly retroareolar with a single or branching thick fibrovascular sclerotic core covered in varying degrees of epithelial hyperplasia.
- Small, sometimes multiple papillomas with thin fibro-elastotic cores found incidentally anywhere in the breast and usually associated with fibrocystic change with epithelial proliferations.
- Complex proliferations resembling adenosis or sclerosing adenosis forming a papilloma within a dilated duct [22].

In all of these variants, the epithelium ranges from a low cuboidal or columnar single-layer to proliferative epithelial hyperplasia. In histopathology, intraductal papillomas are usually easily distinguished from low grade micropapillary DCIS, papillary DCIS, encysted papillary carcinoma and the rare invasive papillary carcinoma. Similarly, the range of cytological features for the varying types of intraductal papilloma can be distinguished from papillary DCIS in FNAB cytology, and benign intraductal papillomas can be diagnosed in up to 75% cases [33, 42].

As in other benign proliferative lesions, the FNAB diagnosis requires careful assessment for epithelial atypia and correlation with imaging, to avoid a false-positive diagnosis [42].

Key Cytological Diagnostic Criteria
(Figs. 3.31a–d and 3.32a–f)

- Cellularity is moderate to usually high.
- The pattern is predominantly large 3-D epithelial tissue fragments with a lesser number of small cohesive ductal epithelial cell tissue fragments with myoepithelial cells, scattered papillary stellate and complex tissue fragments, and a variable number of dispersed columnar cells, siderophages, histiocytes and bare bipolar nuclei in a proteinaceous background.
- The large 3-D epithelial tissue fragments often have irregular slit-like secondary lumina and the flat monolayered sometimes folded sheets show well-ordered ductal cells with myoepithelial cells.
- The stellate papillary tissue fragments consist of stellate fibro-elastotic or sclerotic strands of stroma radiating from a central point with small sheets of ductal epithelial cells with myoepithelial cells attached.
- The complex meshwork tissue fragments consist of a 'chicken wire' mesh of criss-crossing, fine fibro-elastotic stromal strands surrounding tubules of bland epithelium with myoepithelial cells.
- Sheets of apocrine cells or focal apocrine change are usually present.
- A proteinaceous background often with debris suggestive of haemorrhage with haemosiderophages and histiocytes is present.

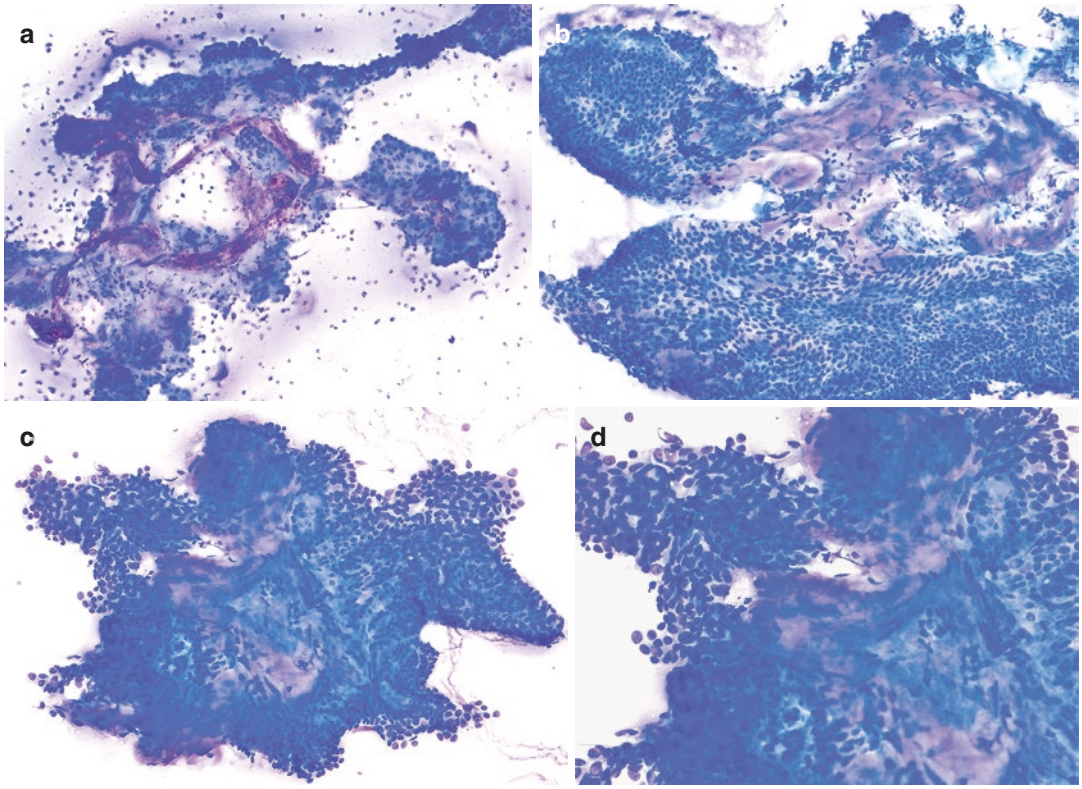

Fig. 3.31 (**a**) Papilloma with complex branching magenta coloured stroma and attached epithelial sheets in a proteinaceous background (Giemsa ×10); (**b**) Papilloma showing sheets of ductal epithelial cells with myoepithelial cells attached to and partially enclosed by magenta stroma in a complex meshwork tissue fragment (Giemsa ×10); (**c**) Complex tissue fragment with magenta stroma covered in ductal epithelium with myoepithelial cells (Giemsa ×10); (**d**) Same fragment at high power showing ductal epithelial cells and myoepithelial cells and stromal core (Giemsa ×20)

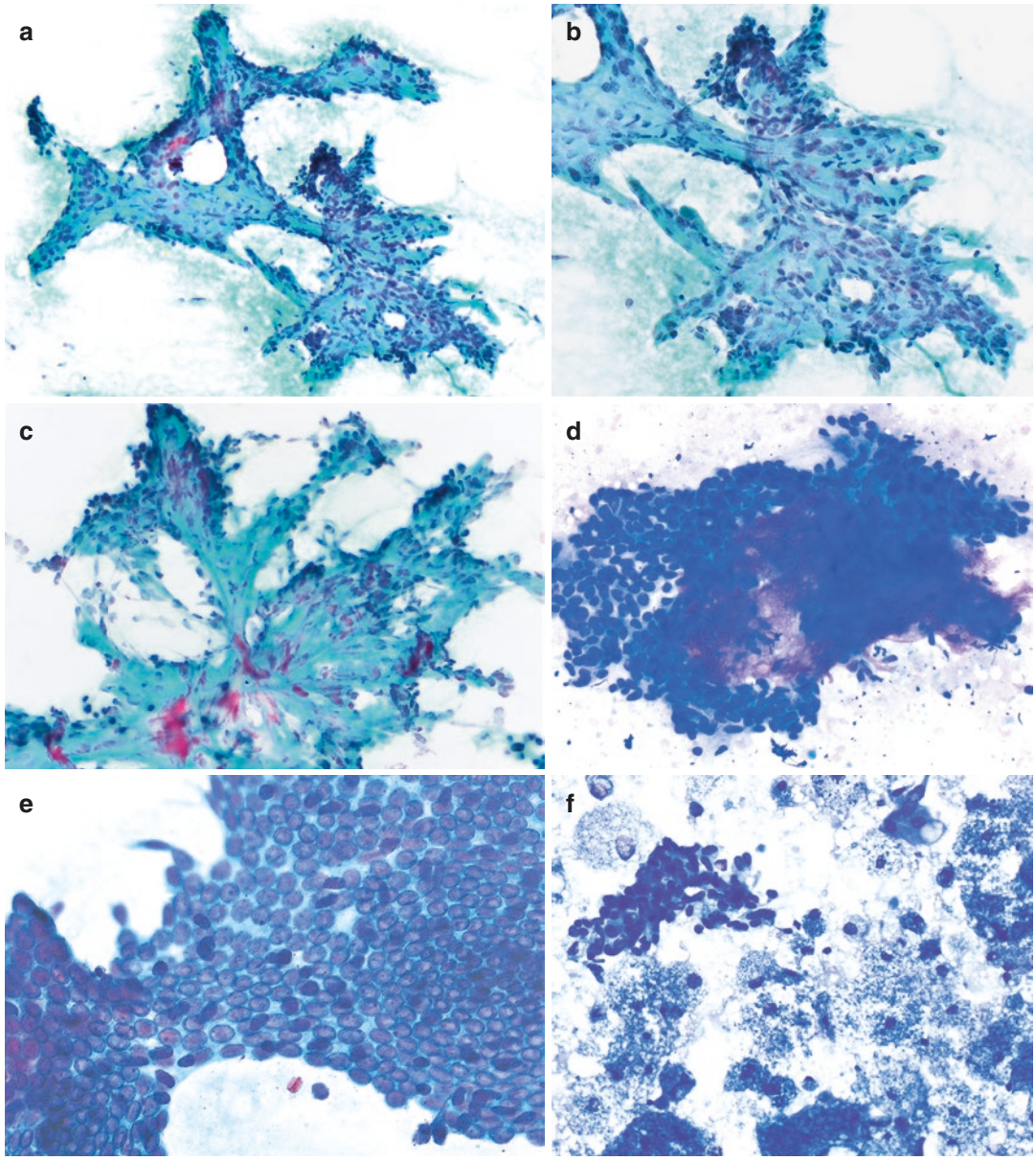

Fig. 3.32 (**a**) Stellate fragment of a papilloma (Pap ×10); (**b**) Same stellate fragment at high power showing branching stromal core (Pap×20); (**c**) Stellate fragment of papilloma (Pap ×20); (**d**) Stellate fragment with magenta core and ductal epithelial cells with myoepithelial cells (Giemsa ×20); (**e**) Ductal epithelium with myoepithelial nuclei in papilloma with epithelial hyperplasia (Pap ×40); (**f**) Single ductal epithelial tissue fragment in a proteinaceous background with histiocytes and siderophages (Giemsa ×20)

- Usually small but occasionally a large number of dispersed often columnar epithelial cells, with bland round to oval nuclei are seen.
- A variable number of bare bipolar nuclei are present [11, 42].

The complex meshwork and stellate papillary tissue fragments are highly specific in the diagnosis of papillomas [42]. Apocrine sheets, columnar epithelial cells, siderophages, histiocytes and a proteinaceous background are usually found, but

are not specific for the diagnosis of intraductal papilloma [42]. The meshwork tissue fragments represent the intraductal proliferations of small tubules separated by stroma seen in surgical pathology and variously called 'intraduct adenosis', 'sclerosed papilloma' or 'complex intraductal papillary lesions', and resemble adenosis seen in fibrocystic change or as a lobulated mass in 'nodular adenosis'. Partially crushed lobules of adenosis in FNAB smears can mimic meshwork fragments but lack the branching fibro-elastotic stroma, size and complexity of meshwork fragments [42]. The stroma of the stellate papillary and meshwork tissue fragments varies from fibroblastic to fibroelastotic, in which non-staining negative-image elastic fibrils can be seen, to densely hypocellular and sclerotic collagen (Fig. 3.33a, b).

In some cases which present with a bloody nipple discharge, and usually a tender subareolar mass, FNAB may only yield scattered, rounded-up, papillary-type tissue fragments of partially degenerate, often mildly atypical ductal, apocrine or squamous epithelial cells in a bloody background with siderophages. Such findings on FNAB should suggest intraductal papilloma, which may have undergone focal or complete infarction [43].

It is essential in every case to assess for the presence of epithelial nuclear atypia in the meshwork and stellate papillary tissue fragments, as well as in the dispersed cells and 3-D tissue fragments. Nuclear enlargement, anisonucleosis, pleomorphism and hyperchromasia can suggest atypia, in which case CNB or excision biopsy is recommended.

Differential Diagnosis

The DD of intraductal papilloma includes any lesion that produces a pattern of large hyperplastic ductal epithelial tissue fragments with apocrine sheets, histiocytes and a proteinaceous background:

- Fibrocystic change with epithelial hyperplasia and radial scars lack complex meshwork and stellate papillary fragments.
- *Apocrine hyperplasia* can include micropapillary tissue fragments with bulbous heads and narrow necks representing 'papilliform apocrine hyperplasia', which can line ducts or cysts (Fig. 3.15b). The low N:C ratio and typical apocrine features of the epithelium should avoid any misdiagnosis of intraductal carcinoma [11].
- Fibroadenomas are distinguished by their typical rounded, myxoid or irregular fibrillary stromal fragments, which are different from meshwork and stellate papillary tissue fragments in architecture and composition, and by their large numbers of bare bipolar nuclei and branched staghorn epithelial fragments (Fig. 3.27a–h).
- Low- to intermediate-grade DCIS has a similar low-power pattern, but typically shows

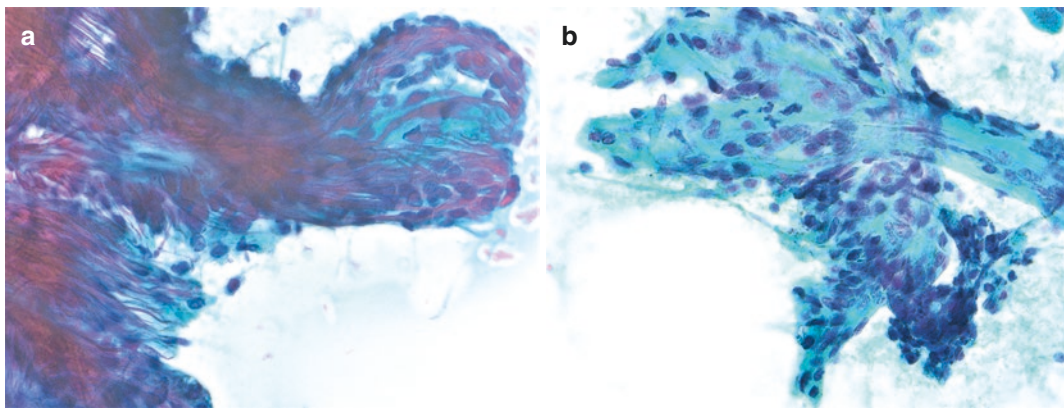

Fig. 3.33 (**a**, **b**) Papilloma showing fibroelastotic stroma with elastic fibrils; note the residual myoepithelial cells adherent to the stroma (Pap ×40)

rigid, micropapillary and cribriform tissue fragment architecture and increased dispersal with scanty or no myoepithelial cells and scant bare bipolar nuclei. Micropapillary tissue fragments have bulbous tips, narrow necks, nuclear crowding and overlapping and variable, often mild nuclear atypia. They lack prominent myoepithelial cells. Cribriform tissue fragments have punched-out holes in the Pap-stained smears and nuclear orientation to these holes, and they have a rigid architectural pattern of nuclei, rather than the streaming, disorderly pattern of crowded small cells of epithelial hyperplasia (see further discussion in Chap. 5, Suspicious of Malignancy).

- Papillary DCIS shows marked hypercellularity and thin fibrovascular papillary fronds covered in mildly to moderately atypical epithelium.
- Solid papillary DCIS has plentiful dispersed atypical cells and small discohesive tissue fragments, as well as distinctive capillary 'glomeruloid' structures that distinguish this lesion from invasive carcinoma (see further discussion in Chap. 5, Suspicious of Malignancy).
- *Juvenile papillomatosis* yields high cellularity, with a large epithelial tissue fragment pattern with large apocrine sheets, a variable number of hyperplastic ductal epithelial tissue fragments, and tissue fragments resembling stellate papillary tissue fragments but lacking the elastotic fibrils and thickness of the stromal core of intraductal papilloma [11]. Myoepithelial cells are often prominent on the epithelial fragments, while histiocytes are scant in the proteinaceous background.
- *Nipple adenoma* (florid papillomatosis of the lactiferous ducts, subareolar duct papillomatosis or papillary adenoma) is a rare benign tumour-like lesion presenting beneath the nipple and subareolar region, which clinically can mimic Paget disease because it may ulcerate and cause crusting of the nipple or present with nipple discharge. Histologically, tubules showing columnar cell change or hyperplastic tufts or papillomatous protrusions form a circumscribed mass around lactiferous ducts, which may dilate, in a background of limited stroma. The FNAB yields moderately cellular smears showing a pattern of large, cohesive, 3-D epithelial tissue fragments and sheets of ductal cells with myoepithelial cells [44]. There is a background of siderophages, histiocytes, bare bipolar nuclei, focal necrotic debris and a variable number of dispersed cells showing bland chromatin of their round nuclei. The cytological diagnosis relies on the clinical site of the lesion but often raises a DD due to its pronounced epithelial hyperplasia.

The emphasis is to avoid false-positive diagnoses in benign intraductal papillomas with epithelial hyperplasia, hypercellularity and dispersal because the imaging findings of some papillomas, especially the smaller incidental papillomas, are not specific. A cytological diagnosis of 'intraductal papilloma' requires close correlation with imaging and clinical findings, and in most circumstances, excision biopsy is preferred to CNB, which has the same sampling problems in regard to distinguishing atypical ductal hyperplasia and low grade DCIS. Lesions that have characteristic features of papillomas, including meshwork or stellate papillary tissue fragments, but show high cellularity (particularly in postmenopausal women not on hormone replacement therapy), nuclear atypia or marked dispersal should be reported as a 'papilloma with atypical epithelial features' or 'atypical papillary lesion' and biopsy recommended. These lesions may represent papillomas with focal atypical ductal hyperplasia or low grade DCIS.

Immunohistochemistry for P63 and calponin on cell block material can demonstrate myoepithelial cells in intraductal papillomas, recognizing that low grade DCIS has a very attenuated, layer of myoepithelial cells at least at its periphery on similar testing in excision biopsies. Lack of any myoepithelial layer infers intracystic papillary or invasive carcinoma. Hyperplastic ductal epithelial cells show patchy CK5/6 and oestrogen receptor positivity while low grade DCIS shows uniform oestrogen receptor positivity and is negative for CK5/6. CNB is often required to confirm the FNAB diagnosis [45].

Sample Reports

Specific scenarios where the diagnosis of 'benign' is appropriate:

Example 1

A pattern of scattered small apocrine sheets in a proteinaceous background with occasional histiocytes.

Benign

Occasional apocrine sheets are seen in a proteinaceous background with histiocytes.

Comment: the features are those of a cyst.

Example 2

Highly cellular smears showing a pattern of frequent cohesive large and some smaller epithelial tissue fragments and folded sheets, with regularly arranged rounded nuclei showing minimal nuclear enlargement or pleomorphism and with associated myoepithelial nuclei and bare bipolar nuclei in a clean background. There are few small epithelial tissue fragments or dispersed single cells.

Benign

These highly cellular smears show a pattern of predominantly large epithelial tissue fragments with plentiful myoepithelial nuclei, and bare bipolar nuclei in the clean background.

Comment: the features are those of epithelial hyperplasia.

Example 3

A mildly cellular pattern of cohesive large and small epithelial tissue fragments with myoepithelial nuclei and a small number of dispersed columnar cells and bare bipolar nuclei in the background.

Benign

These mildly cellular smears show scattered large and small epithelial tissue fragments with well- ordered round epithelial nuclei and myoepithelial nuclei, and a small number of dispersed intact columnar cells with bland nuclei and bare bipolar nuclei in the background.

Comment: the features are those of epithelial hyperplasia.

Example 4

Moderately cellular smears showing a pattern of large epithelial tissue fragments with well-ordered nuclei and myoepithelial nuclei, scattered fibrillary stromal fragments, a small number of dispersed intact epithelial cells with bland nuclei, and plentiful bare bipolar nuclei.

Benign

These moderately cellular smears show large epithelial tissue fragments of ductal epithelial cells and myoepithelial cells with scattered fibrillary stromal fragments, plentiful bare bipolar nuclei and a few bland dispersed cells in the background.

Comment: The features are those of a fibroadenoma.

Example 5
Moderately cellular smears showing a pattern of mainly large epithelial tissue fragments with myoepithelial nuclei, plus stellate fibroelastotic papillary tissue fragments and/or complex meshwork fragments, apocrine sheets and siderophages in a proteinaceous background.

Benign
These moderately cellular smears show large hyperplastic epithelial cell tissue fragments with myoepithelial cells, stellate papillary tissue fragments, apocrine sheets and siderophages and histiocytes in a proteinaceous background.

Comment: The features are those of an intraductal papilloma with epithelial hyperplasia.

Example 6
Mildly cellular smears showing a pattern of cohesive small epithelial tissue fragments consisting of small ductal cells with bland nuclei and myoepithelial cells, very occasional intact lobules and bare bipolar nuclei in the background.

Benign
These hypocellular smears show scattered small epithelial tissue fragments with myoepithelial nuclei, bare bipolar nuclei and occasional lobules.

Comment: the features are those of benign breast tissue showing no specific lesion.

Example 7
A background of coarse granular material with scattered histiocytes and fragments of anucleate necrotic fat tissue is present with considerable debris and very occasional small cohesive epithelial tissue fragments that include myoepithelial nuclei, with or without a history of surgery and radiation.

Benign
Considerable granular necrotic material is present with occasional histiocytes and siderophages and fragments of infarcted acellular fat tissue, consistent with fat necrosis. Several epithelial tissue fragments are present and show mild anisonucleosis and myoepithelial nuclei.

Comment: the features are those of fat necrosis.

Example 8
Moderately cellular smears showing plentiful apocrine sheets with scattered small cohesive tissue fragments of ductal epithelial cells with myoepithelial cells, in a proteinaceous background with histiocytes and plentiful neutrophils.

Benign
These moderately cellular smears show apocrine sheets, scattered small ductal epithelial tissue fragments with myoepithelial nuclei, and histiocytes in a proteinaceous background with plentiful neutrophils.

Comment: the features are those of fibrocystic change with evidence of inflammation.

Example 9

In a male patient, mildly cellular smears showing scattered large cohesive epithelial tissue fragments with myoepithelial cells, occasional bare bipolar nuclei and very occasional small fibrillary stromal fragments.

Benign

These mildly cellular smears show large epithelial tissue fragments of ductal epithelial cells and myoepithelial cells with scattered fibrillary stromal fragments and bare bipolar nuclei in the background.

Comment: The features are those of gynaecomastia.

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Atypical

4

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Introduction

In breast fine needle aspiration biopsy (FNAB) cytology the terms 'atypical' and 'suspicious of malignancy' show the most variation in definition and application between cytopathologists, including those working within a single department or a single city in addition to national and international variations [1, 2]. The interpretation of these terms and reports and subsequent patient management by clinicians also vary. In the literature, the risk of malignancy (ROM) in follow-up core needle biopsies (CNB) or excision biopsies ranges from 22% to 39% in 'atypical' and 81% to 88% in 'suspicious of malignancy' cytological diagnoses respectively, such that two distinct categories are required in a standardized reporting system with five diagnostic categories [1, 3–9]. Two very recent publications utilizing the IAC Yokohama System definitions and categories had a ROM of 13 and 15.7% for the atypical category [10, 11].

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.

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A. S. Field et al. (eds.), *The International Academy of Cytology Yokohama System for Reporting Breast Fine Needle Aspiration Biopsy Cytopathology*, https://doi.org/10.1007/978-3-030-26883-1_4

These features include prominent single intact cell dispersal, nuclear enlargement and pleomorphism, high cellularity, necrosis, mucin and complex micropapillary and cribriform architectural features in tissue fragments.

Discussion and Background

When reporting a breast FNAB as 'atypical' the cytopathologist should always describe the material present on the slides, including the degree of cellularity, and then state which specific cytological features are atypical. If possible, the differential diagnoses (DD) and the most likely specific diagnosis should be provided [12, 13].

The causes of an 'atypical' cytological diagnosis include technical problems, interpretative problems related to the inherent characteristics of the lesion, or a combination of these factors influenced by the expertise of the cytopathologist.

The training and experience of the cytopathologist impacts on the rate of atypical diagnoses in reporting breast FNAB cytology, but interpretive expertise has been shown to play a smaller role than the quantity and quality of the material [14, 15]. However, an experienced cytopathologist will be able to recognize the smear pattern in the vast majority of benign lesions, such as fibroadenoma or intraductal papilloma, and then evaluate any high power atypical features, such as high cellularity or marked single intact cell dispersal, to determine whether the features lie within the range acceptable for that specific lesion. An inexperienced cytopathologist may give an inappropriate weighting in their interpretation to an usual atypical feature, while not recognizing the overall diagnostic pattern and features, leading to a higher 'atypical' rate.

Limitations in specimen technical quality play a significant role in a proportion of cases falling into both the atypical and the suspicious of malignancy categories and are due to a variety of factors:

- Cases with low cellularity and only scanty interpretable material or cases where ample material is present but smearing and fixation artefacts limit its interpretation, can both lead to atypical and, in some cases, suspicious of malignancy or false-positive diagnoses.
- In smears intended for Papanicolaou staining, air-drying artefact occurs when smeared slides are not immediately immersed in alcohol, resulting in apparent nuclear enlargement and lack of chromatin structure. This compromises the ability to assess nuclear characteristics important for distinguishing benign from malignant cells.
- In smears intended for Giemsa staining, slow air-drying of directly smeared material containing considerable watery fluid can also lead to severe artefact due to rupture of cells and nuclear distortion.
- Smearing which is too forceful leads to either crush artefact or dispersal of otherwise benign cohesive material, particularly towards the tail of the smear, mimicking the loss of cell adhesion seen in carcinoma.
- Blood or ultrasound gel and clotting of material in the needle can obscure cells.

The *inherent nature of some breast lesions or processes* produces interpretative problems for even experienced cytopathologists.

In surgical pathology, the diagnostic features of certain benign and atypical proliferative lesions and of low-grade ductal carcinoma in situ (DCIS) show a degree of overlap and there is frequent inter-observer variability. The lesions may also vary from block to block or level to level. The spectrum of intraductal and intralobular proliferative changes ranges from benign, including usual epithelial hyperplasia, columnar cell change and columnar cell change with hyperplasia, through to atypical, including flat epithelial atypia, atypical ductal hyperplasia and atypical lobular hyperplasia [16]. The atypical spectrum merges with low-grade cribriform, papillary and solid DCIS and lobular carcinoma in situ.

Reflecting this surgical pathology spectrum, in breast cytology it is recognized that distinguishing benign proliferative lesions from atypical intraductal and intralobular lesions and from low-grade in situ and invasive carcinoma is difficult [17]. When good material is available the atypical cases will raise a DD of a benign

proliferative lesion such as an intraductal or intralobular hyperplastic proliferation or fibroadenoma versus a low-grade DCIS or low-grade invasive carcinoma. The DD of a suspicious of malignancy diagnosis usually will include low- and high-grade DCIS and low- to intermediate-grade invasive carcinoma, including lobular carcinoma and grade 1 carcinoma of no special type, rather than high-grade invasive carcinomas (for further discussion see Chap. 5, Suspicious of Malignancy).

A diagnosis of low/intermediate-grade DCIS can be suggested on FNAB and correlated with imaging, where calcifications without a mass lesion would support the diagnosis, although a specific diagnosis is not possible because criteria overlap with proliferative lesions. Almost all these cases will be regarded as atypical or, less commonly, suspicious of malignancy. The aim is to avoid a false-negative diagnosis of a benign proliferative lesion and a false-positive diagnosis of an invasive carcinoma in cases that are purely in situ.

The following breast lesions cause most of the interpretative difficulties:

- *Fibroadenoma* is a common cause of atypical FNAB diagnoses and the most common source of suspicious or false-positive diagnoses [18, 19]. Well-sampled and smeared fibroadenomas with characteristic features of a pattern of large ductal epithelial tissue fragments with myoepithelial cells, stromal fragments and plentiful bare bipolar nuclei in the background are diagnostic. However, fibroadenomas can show high cellularity, dispersal of intact single cells, varying degrees of nuclear enlargement and pleomorphism and the presence of nucleoli, and all of these individual features can be seen in carcinomas (Fig. 4.1a–d). In rare cases lacking the typical fibroadenoma features and with these atypical features, fibroadenomas can be misdiagnosed as carcinoma. Application of the triple test before definitive treatment and further biopsy of discordant cases will minimize or eliminate inappropriate management. Coexistence of in situ or invasive carcinoma within or adjacent to a fibroadenoma is extremely rare.
- *Intraductal papillomas and fibrocystic change with epithelial hyperplasia, including radial scars,* can also produce high cellularity, which may raise a suspicion of malignancy [20, 21]. The epithelial proliferation in papillomas can produce marked dispersal of intact, sometimes columnar, cells and there can be complexity of the tissue fragments and partial degeneration resulting in nuclear atypia (Fig. 4.2). Papillomas can be associated with various stromal fragments, some of which are diagnostic of papilloma [22], but in some cases the features raise the DD of papillary DCIS with its characteristic finer branching epithelial strands, nuclear atypia and marked dispersal. Conversely, low-grade DCIS lacking a micropapillary or cribriform architecture, but showing dispersal and lack of myoepithelial cells and bare bipolar nuclei, may produce an atypical diagnosis.
- *'Usual' epithelial (ductal) hyperplasia and sclerosing adenosis* can produce highly cellular smears with a degree of dispersal or significant nuclear enlargement and atypia in tissue fragments and sheets, leading to concern regarding a low-grade DCIS or invasive carcinoma [17, 23] (Fig. 4.3). However, epithelial hyperplasia is usually associated with myoepithelial nuclei and streaming of epithelial cells around irregular slit-like spaces ('secondary lumina') in the epithelial fragments as well as bare bipolar nuclei in the background.
- *Atypical apocrine cells* showing varying degrees of degeneration and proliferation can be seen in cysts (Fig. 4.4a–c).
- *Lobular neoplasia* is in the differential diagnosis in cases of low cellularity with scattered single intact epithelial cells, particularly if the cells show eccentric cytoplasm with or without intracytoplasmic vacuoles containing cytoplasmic mucin. These features are regarded as atypical [13] (Fig. 4.5a, b). Normal breast tissue or an undersampled proliferative lesion or fibroadenoma typically show small cohesive ductal epithelial fragments with bland nuclei and myoepithelial nuclei, accompanied by some bare bipolar nuclei in the background, and lack the subtle nuclear

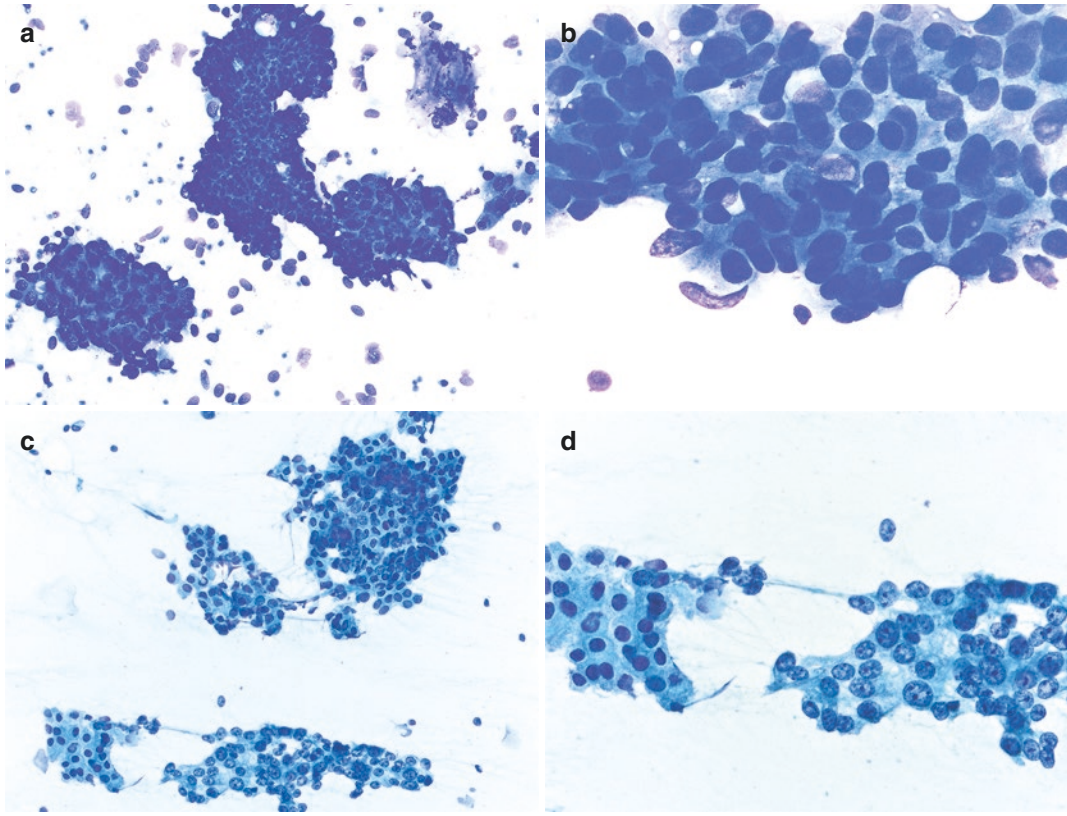

Fig. 4.1 (**a**) Fibroadenoma with typical features elsewhere on the slides but showing two crowded tissue fragments with no definite myoepithelial cells adjacent a larger more typical ductal epithelial tissue fragment with myoepithelial cells and in a background of bare bipolar nuclei (Giemsa ×20); (**b**) Epithelial tissue fragment from a fibroadenoma showing mild nuclear enlargement and atypia although probable myoepithelial cells and a bare bipolar nucleus are present (Giemsa ×40); (**c**) Fibroadenoma showing two typical benign epithelial tissue fragments adjacent to a crowded tissue fragment (Pap ×20); (**d**) High power of **c**, showing atypia with nuclear enlargement, pleomorphism and nucleoli and an apparent lack of myoepithelial cells, adjacent to a ductal epithelial tissue fragment with prominent myoepithelial cells (Pap ×40)

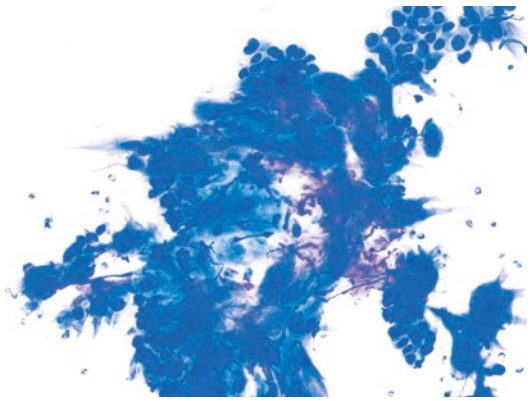

Fig. 4.2 Intraductal papilloma showing atypia due to a complex architecture and crowded epithelium showing focal apocrine change (Giemsa ×20)

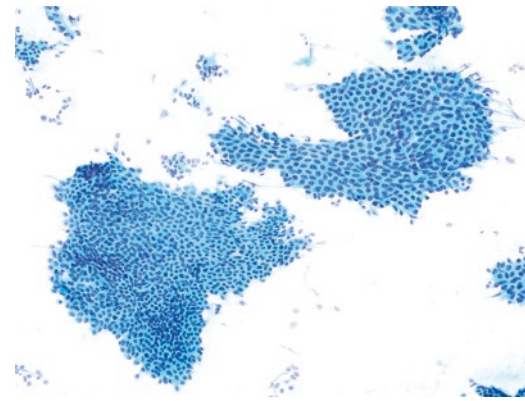

Fig. 4.3 Epithelial hyperplasia showing mild atypia in one (top right) large sheet with nuclear enlargement and nucleoli and few myoepithelial cells adjacent to a tissue fragment showing myoepithelial nuclei and no ductal nuclear enlargement (Pap ×10)

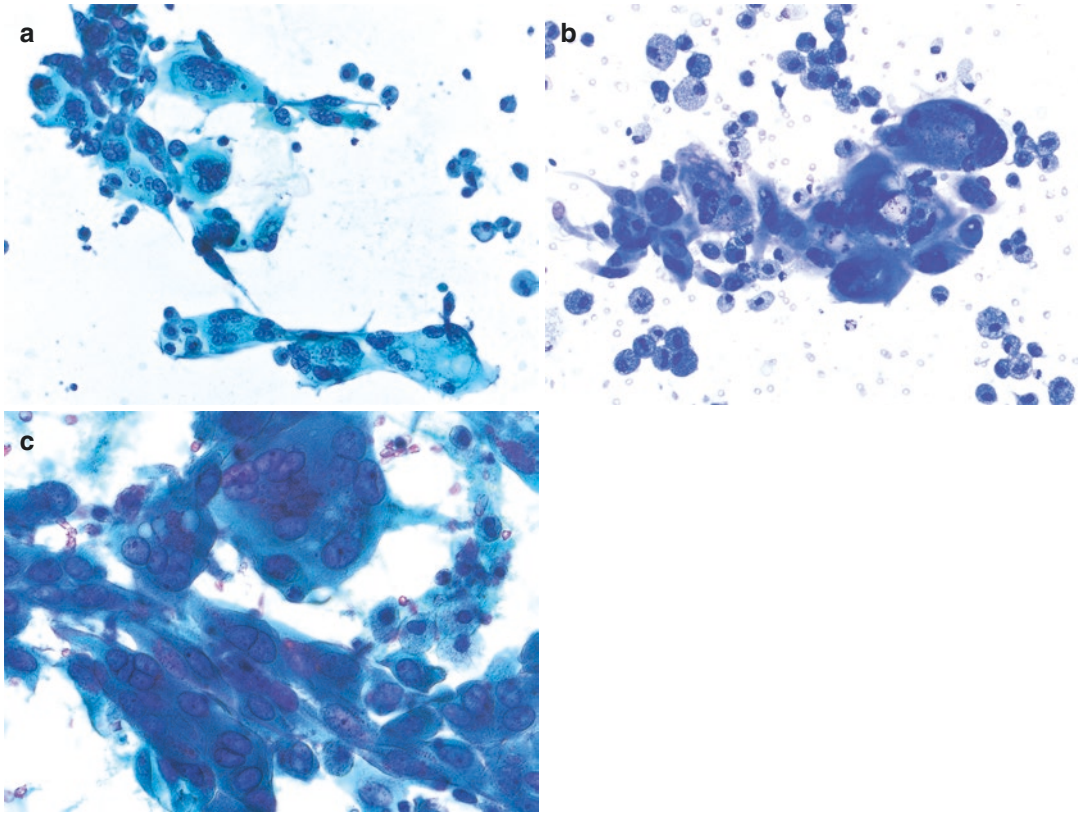

Fig. 4.4 (**a**) Atypical apocrine cells showing nuclear pleomorphism, hyperchromasia and multinucleation from a cyst (Pap ×20); (**b**) Atypical apocrine cells showing degenerative nuclear atypia in a proteinaceous background with histiocytes (Giemsa ×20); (**c**) Atypical apocrine cells showing multinucleation and nuclear enlargement with few eosinophilic granules in their cytoplasm, in a proteinaceous background with histiocytes from a cyst (Pap ×40)

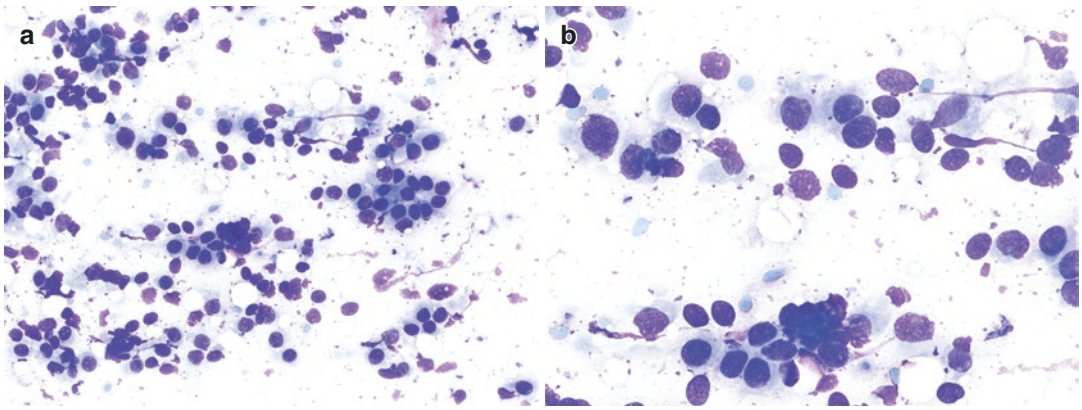

Fig. 4.5 (**a**) Dispersed epithelial cells showing mild nuclear enlargement and pleomorphism along with bare bipolar nuclei and evidence of smearing artefact, which if taken in isolation from the findings on the rest of the smear could be regarded as atypical (Giemsa ×20); (**b**) High power of (**a**) to confirm the bare bipolar nuclei and dispersed single cells (Giemsa ×40)

enlargement and variation in nuclear shape of lobular carcinoma in situ or lobular carcinoma.
- *Low-grade ductal carcinoma in situ* usually presents a large tissue fragment pattern with plentiful dispersed single cells and may have cribriform, micropapillary or papillary architecture leading to an atypical or suspicious of malignancy report [23–25]. (See Chap. 5, Suspicious of Malignancy for further discussion)
- *Low-grade invasive carcinomas of no special type* can yield a large tissue fragment pattern with limited single cell dispersal and low-grade nuclear atypia, producing an atypical rather than a suspicious of malignancy or malignant diagnosis [23–25]. (See Chap. 6, Malignant for further discussion)
- *Fibroepithelial lesions* such as cellular fibroadenomas and low-grade phyllodes tumours with cellular and minimally atypical stroma are indistinguishable on FNAB. The stromal hypercellularity may be regarded as atypical. (See below)
- *Adenomyoepithelioma* frequently produces highly cellular smears with crowded tissue fragments consisting of a dual population of epithelial cells and prominent spindle myoepithelial cells. (See below)
- *Spindle cell lesions* most commonly a fibromatosis may mimic carcinoma on imaging, and frequently produce smears of low cellularity and variable stromal components (See below).

Management

The management of a FNAB atypical case is correlation with the clinical and imaging findings, constituting the 'triple test'. If the imaging or clinical findings are indeterminate or suspicious, CNB should be carried out. If no CNB is available, a repeat FNAB or simple excision biopsy is recommended.

If the imaging and clinical findings are not atypical or indeterminate and if rapid on-site evaluation (ROSE) has been employed, repeat FNAB or CNB can be carried out immediately. If the repeat FNAB or the CNB is negative the patient can be reviewed at 3 to 6 months, at which time if the lesion has altered repeat FNAB or CNB can be performed.

If imaging and CNB are not available, repeat FNAB or excision biopsy is recommended, depending on the clinical findings.

The difference in the management of a suspicious of malignancy FNAB, which also involves correlation with clinical and imaging findings, is the mandatory requirement for that category of immediate repeat biopsy, preferably utilizing CNB and/or excision biopsy. The communication with the patient will also differ for the two categories, with the aim to avoid unnecessary high levels of anxiety in the setting of an atypical FNAB report.

Specific Breast Lesions That May Be Associated with Atypical Reports

Low-Grade and Borderline Phyllodes Tumours

Clinical, Imaging and Histopathological Features

Low-grade and borderline phyllodes tumours may be found incidentally on imaging or may present as ovoid or rounded masses that are rapidly increasing in size in women, who tend to be over 40 years of age and older than those presenting with fibroadenomas. They make up less than 1% of breast tumours and between 2% and 3% of fibroepithelial lesions [16]. Imaging usually shows a relatively defined ovoid, rounded or lobulated mass. They are fibroepithelial lesions characterized in histopathology by increased stromal cellularity, mild to moderate stromal nuclear atypia and enlargement, and a prominent leaf-like pattern of growth lined by epithelium, and a low mitotic rate [16, 26–28]. Borderline tumours may have an infiltrating margin and moderate mitotic rate.

Key Cytological Diagnostic Criteria

[29–32] (Fig. 4.6a–d)
- Cellularity is high
- The pattern is of large epithelial tissue fragments and usually prominent large hypercellular stromal fragments.

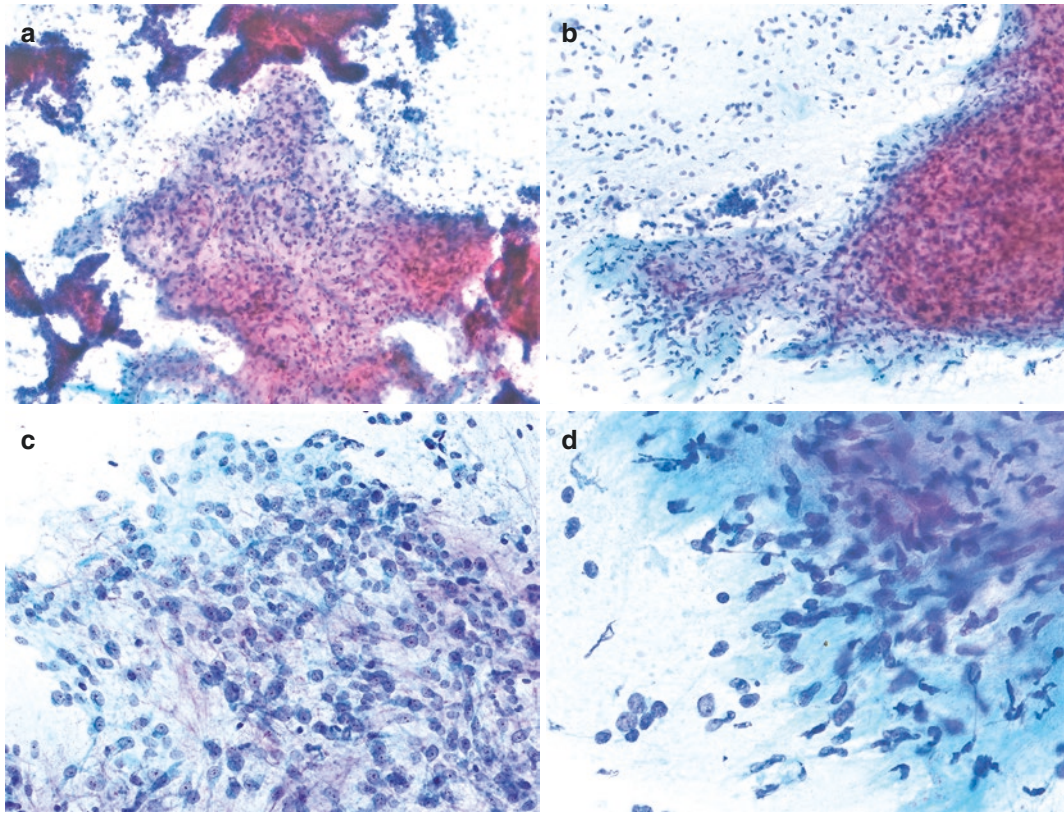

Fig. 4.6 (**a**) Large stromal tissue fragment showing uniform mild hypercellularity and epithelial tissue fragments from a confirmed low-grade phyllodes tumour (Pap ×10); (**b**) Same low-grade phyllodes tumour showing a large hypercellular stromal fragment with the increase in stromal nuclei extending to the fragment margin (Pap ×10); (**c**) High power to show the increased cellularity and mild nuclear enlargement and atypia of the stromal nuclei in this low-grade phyllodes tumour (Pap ×20); (**d**) Mildly hypercellular stroma showing mild nuclear enlargement and atypia of a low-grade phyllodes tumour (Pap ×40)

- The epithelial tissue fragments consist of ductal epithelial cells with myoepithelial cells and may show apocrine or squamous metaplasia.
- The stromal fragments show varying degrees of nuclear enlargement, irregular nuclear outlines including bent nuclei, irregular mildly hyperchromatic chromatin, more prominent small nucleoli and occasional mitoses.
- There are bare bipolar nuclei, degenerate cells, stripped nuclei and an increase in spindle stromal cells in the background.
- There is no necrosis and no heterologous elements are seen.

Low-grade and borderline phyllodes tumours often show variable regions of hypercellular and more sclerotic stroma, and this makes distinguishing fibroadenomas with cellular stroma and low-grade phyllodes tumours problematic on FNAB and CNB. Phyllodes tumours will show enlarged and atypical stromal nuclei particularly the borderline tumours, but stromal nuclei in fibroadenomas do vary in size and shape and may show bent and elongated nuclei and the presence of nucleoli. If there are plentiful stromal fragments showing hypercellularity throughout the fragments and to their margins and there is nuclear enlargement, then it is appropriate to regard the smears as atypical, suggest the DD of a cellular fibroadenoma and low-grade phyllodes tumour, and recommend simple excision biopsy. This is supported by a history of a rapidly growing tumour or a tumour that is greater than 3 cm [27, 29, 32].

High-grade phyllodes tumours usually have a history of rapid growth and on FNAB often appear sarcomatous with large plump spindle cells seen more frequently as single cells along with crowded tissue fragments [13]. These spindle cells show a high N:C ratio, with nuclear enlargement, marked hyperchomasia and variable chromatin clearing, and large prominent nucleoli are seen. Mitoses are frequent and may be atypical. Magenta stroma may be seen between the malignant stromal cells with their pale blue cytoplasm (Giemsa stain). Necrosis with some foamy histiocytes is often present, and the epithelial component is often sparse.

Adenomyoepithelioma

Clinical, Imaging and Histopathological Features

Adenomyoepithelioma is a rare lesion presenting clinically and on imaging as a rounded or lobulated, relatively circumscribed, solid lesion. It may recur if not fully excised but does not metastasize, although there is a rare malignant counterpart.

Histopathologically, adenomyoepitheliomas are multilobulated and biphasic, composed of tubules with an inner cuboidal lining, which can show squamous or sebaceous metaplasia, and an outer hyperplastic myoepithelial cell layer [16]. The myoepithelial cells usually have clear cytoplasm but may be spindled or, less commonly, epithelioid or myoid, and merge into the surrounding sclerotic stroma. If the tubular component becomes malignant, the carcinoma resembles infiltrating carcinoma of no special type or metaplastic carcinoma, and if both components are malignant, the tumour is an epithelial-myoepithelial carcinoma. Myoepitheliomas are monophasic tumours without the tubular component.

Key Cytological Diagnostic Criteria

[33–37] (Fig. 4.7a–f)

- The cellularity usually is moderate to high.
- The pattern is a mix of small and larger tissue fragments, sometimes accompanied by small fragments of fibrillary myxoid material and bare bipolar nuclei.
- The larger tissue fragments are a mix of crowded, cuboidal tubular epithelium with uniform round nuclei, surrounded by prominent, usually spindled, myoepithelial cells with clear to more commonly pale cytoplasm and round to oval nuclei, which "spin off" the epithelial tissue fragments and merge with less cellular stroma.
- Smaller tissue fragments consisting of spindle cells can be relatively hypercellular and myxoid or sclerotic and tufted.
- Metachromatic rounded collagen globules may be seen.
- Bare bipolar nuclei, foamy histiocytes and occasional apocrine cells are seen in the background.

The epithelial tissue component can show squamous differentiation and dispersed similar cells with atypical hyperchromatic nuclei and intranuclear pseudo inclusions can be seen. The myoepithelial cells can show epithelioid or myoid differentiation with considerable pale eosinophilic cytoplasm [13]. A specific diagnosis can be difficult, but is suggested by the biphasic nature of the tumour, with a variable number of bare bipolar nuclei, and the transition from ductal to myoepithelial cells with clear or pale cytoplasm at the periphery of the tissue fragments. The aim is to recognize the unusual features and avoid a false-positive diagnosis.

Differential Diagnosis

- Fibrocystic change with epithelial hyperplasia.
- Fibroadenomas with staghorn epithelial tissue fragments, usually rounded stromal fibromyxoid fragments and plentiful bare bipolar nuclei.
- Low-grade phyllodes tumours, which have prominent hypercellular stroma with atypia of the stromal nuclei and increased spindle cells.
- Rare pleomorphic adenoma in the breast, which has a myxofibrillary stroma.
- Adenoid cystic carcinoma with basaloid cells in tissue fragments and similar, but larger and more plentiful, metachromatic globules.

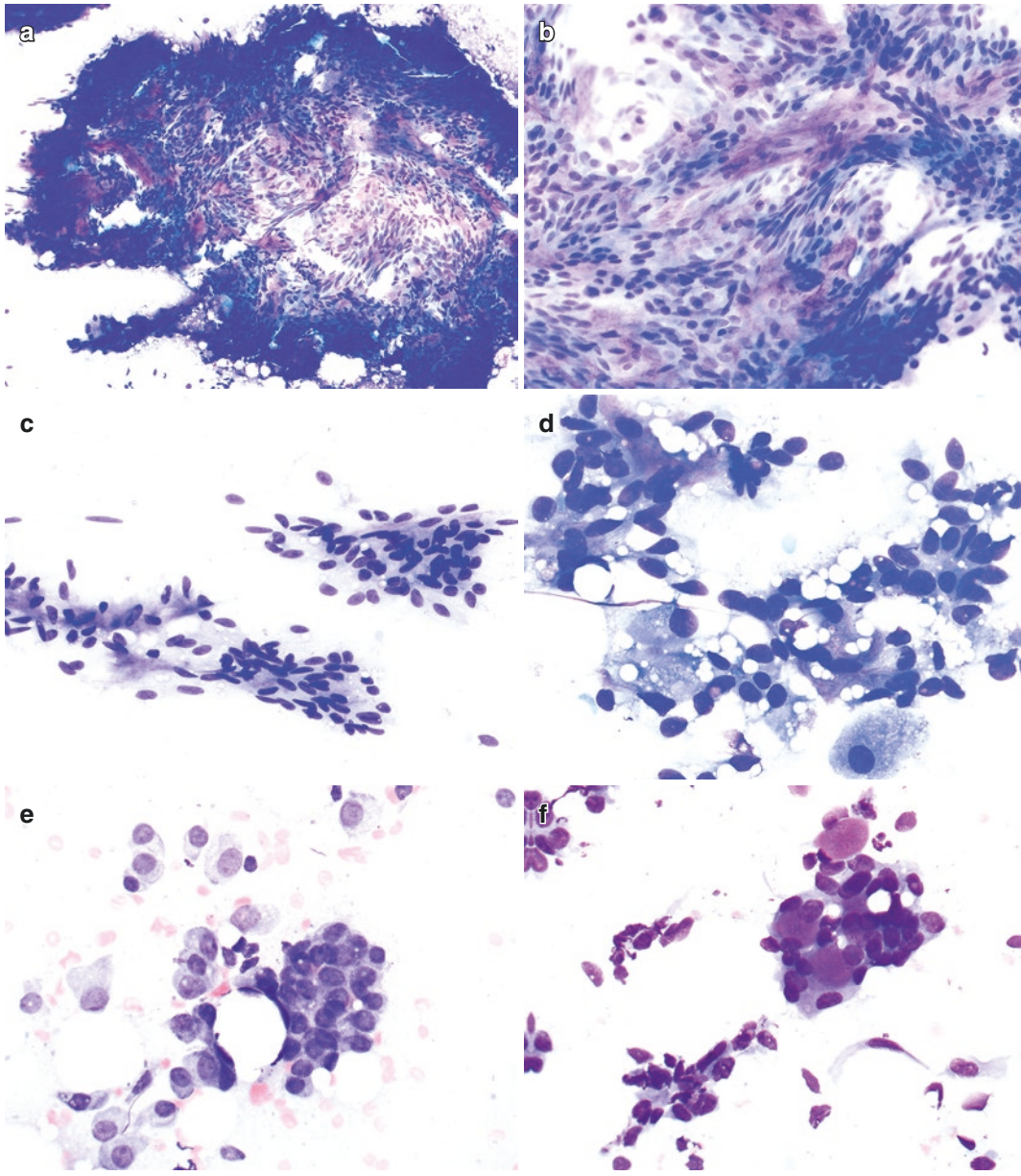

Fig. 4.7 (**a**) Adenomyoepithelioma showing a large tissue fragment with a biphasic architecture (Giemsa ×10); (**b**) High power showing the biphasic architecture of islands and strands of tubular epithelium merging into spindle myoepithelial cells and magenta stroma (Giemsa ×20); (**c**) Small tissue fragments consisting mainly of spindle cells, with spindle cells in the background (Giemsa ×20); (**d**) Small tissue fragments showing a biphasic architecture with central more cuboidal epithelial cells with uniform nuclei and outer spindle cells, a stromal strand and rounded large single epithelial cells (Giemsa ×40); (**e**) High power showing the dual population of polygonal epithelial cells and plump spindle myoepithelial cells (Giemsa ×40); (**f**) Rounded collagenous magenta globules (Giemsa ×40)

- Low-grade adenosquamous carcinomas, a variant of low-grade metaplastic carcinoma.
- Malignant change in the setting of adenomyoepithelioma with increased pleomorphism of the epithelial and myoepithelial cells.

Adenomyoepithelial carcinoma is rare, associated with necrosis and nuclear atypia and usually diagnosed as carcinoma [38]. Malignant myoepithelioma is an invasive, storiform spindle cell tumour, which is immunohistochemically smooth muscle actin and high molecular weight cytokeratin positive, and is regarded as a spindle cell variant of malignant adenomyoepithelioma or a spindle cell metaplastic carcinoma. It produces moderately to highly cellular smears consisting of spindle or pleomorphic polygonal cells seen singly or in small tissue fragments with pleomorphic nuclei. The DD is of a spindle cell neoplasm [39, 40].

Ancillary Diagnostic Studies

In cell blocks, the ductal cells stain with cytokeratin 7, while the myoepithelial cells stain variably with p63, calponin, smooth muscle actin and S100. In addition, the myoepithelial component may stain with CK5/6 and CK14, which may also be positive in low-grade adenosquamous and occasional metaplastic carcinomas.

Fibromatosis (Extra-abdominal Desmoid Tumour)

Clinical, Imaging and Histopathological Features

Fibromatoses may be found incidentally on imaging or may present sporadically as a firm mass that may cause skin retraction, and may follow breast injury or surgery including prosthetic implants or be associated with familial adenomatous polyposis [16, 28]. Mammography may show minimal changes or a non-specific density that is irregular and may be spiculated, while ultrasound demonstrates an ill-defined, irregular, hypoechoic, indeterminate to suspicious mass. Histopathologically, irregularly arranged and poorly formed fascicles of spindle cells within collagenous stroma, are interwoven. The tumour is solid and does not include breast lobules or ducts, while the margin is typically infiltrating [28, 39]. Management is wide excision. Recurrences can occur.

Key Cytological Diagnostic Criteria [40, 41] (Fig 4.8a–f)

- Cellularity varies but is often low.
- Small stromal fragments consist of spindle cells orientated in parallel or irregularly arranged, with some single slender spindle cells in a clean background.
- Spindle stromal cells show oval nuclei with uniform fine chromatin, mild pleomorphism of shape and small or inconspicuous nucleoli, low N:C ratio, and thinly tapered pale cytoplasm, that in tissue fragments is light blue and separated by magenta collagen stroma (Giemsa stain).
- A small number of lymphocytes, stromal tufts and occasional tissue fragments of ductal epithelial cells may be seen.
- No necrosis and scanty mitoses are seen.

The DD includes scarring related to previous surgery, which is usually associated with fat necrosis and hemosiderin-laden macrophages, low-grade phyllodes tumour that has fibroepithelial components and greater degrees of nuclear enlargement and atypia, myofibroblastoma, pseudoangiomatous stromal hyperplasia and metaplastic spindle cell carcinoma, which usually shows more marked nuclear atypia [40–43].

Myofibroblastoma usually occurs in older men and postmenopausal women, and on FNAB shows single spindle cells or spindle cells separated by collagen in tissue fragments, admixed with a background of fat, tufted collagen and myxoid material [44]. Nuclear grooves and pseudoinclusions may be seen in the uniform oval nuclei, which lack conspicuous nucleoli. Distinction from fibromatosis and other spindle cell lesions requires correlation with the age of the patient, CNB and immunohistochemistry.

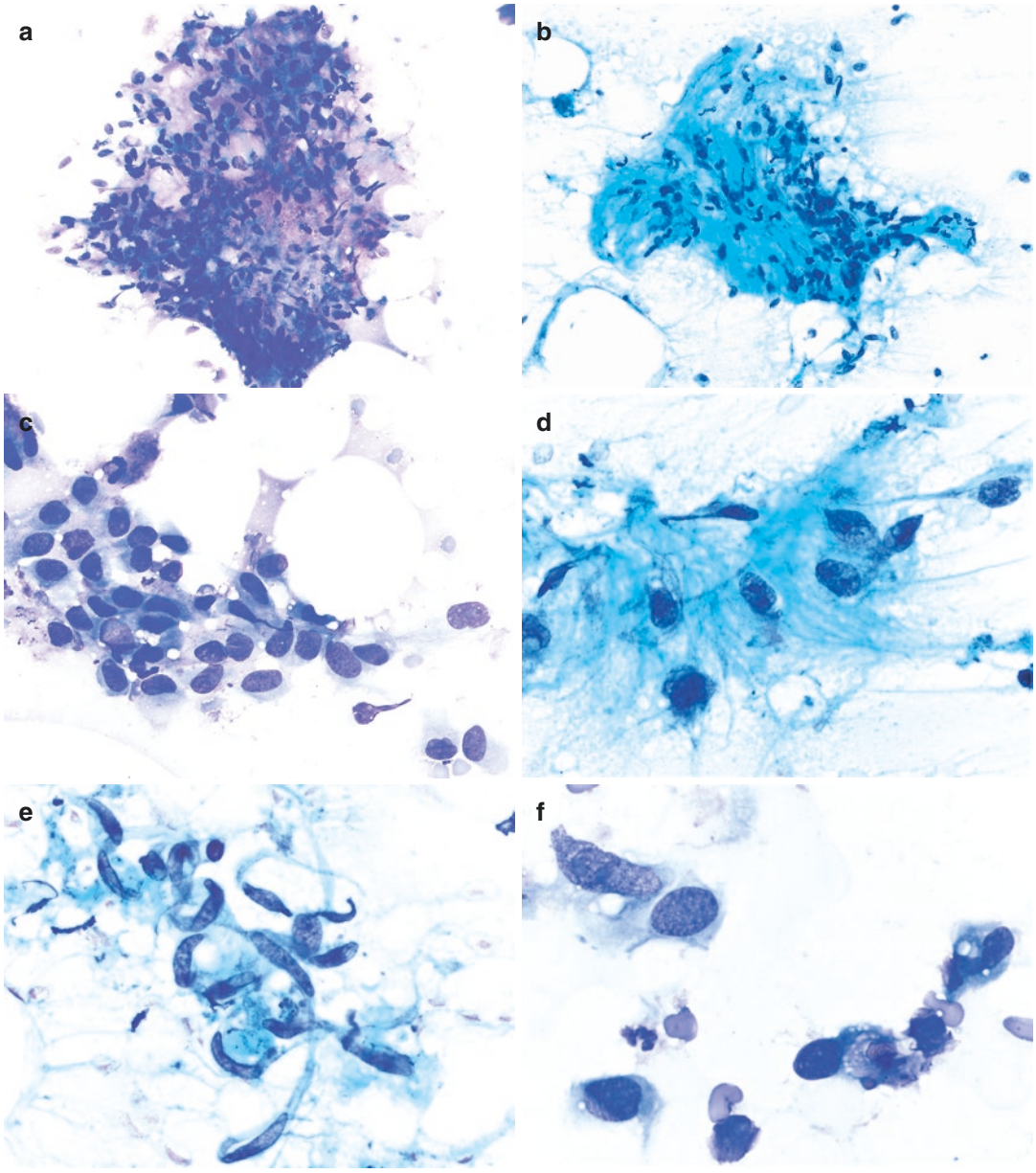

Fig. 4.8 (**a**) Fibromatosis showing a small stromal fragment in which spindle cells are present in magenta collagenous material (Giemsa ×20); (**b**) Thin spindle cells are seen within a collagenous dense fragment, and several spindle cells are seen in the background (Pap ×20); (**c**) Single plump small spindle cells are present with oval nuclei with bland even chromatin and no nucleoli (Giemsa ×40); (**d**) Spindle cells with oval nuclei, fine chromatin and tiny nucleoli are present within wispy fibrillary stroma; note the spindle cell (top right) with tapering pale cytoplasm (Pap ×60); (**e**) Spindle cells with thin tapering cytoplasm and elongated nuclei with fine chromatin (Pap ×60); (**f**) Several plump spindle cells showing mild nuclear pleomorphism and, in one enlarged nucleus, two nucleoli (Giemsa ×60)

Pseudoangiomatous stromal hyperplasia (PASH) presents as a painless palpable mass or an irregular density lacking calcifications on imaging, in younger women or postmenopausal women on hormone replacement therapy. On FNAB, cellularity is low with single plump spindle cells, small tissue fragments of hypocellular stroma and bland, oval nuclei, and small terminal

ductular tissue fragments or lobules with few bare bipolar nuclei [45]. The epithelial fragments are smaller than those seen in fibroadenomas.

Cell block material is very useful in distinguishing these lesions using immunohistochemistry: fibromatosis is B-catenin and smooth muscle actin positive, variably weakly positive for oestrogen receptor and desmin, and negative in the CD34, pankeratin and p63; myofibroblastoma is positive for CD34 and desmin; PASH is positive for Bcl-2 and progesterone receptor and negative for CD31; metaplastic spindle cell carcinoma is positive for pancytokeratins and negative for B-catenin and CD34; and phyllodes tumour is CD34 positive and B-catenin negative [46].

Fibromatosis may show CTNNB1 gene mutations or APC gene mutations in patients with familial adenomatous hyperplasia.

Nodular fasciitis occurs in the subcutis with a history of a rapidly growing sometimes painful lesion. The lesion usually involutes after some 3 months, but should be considered in the DD of spindle cell lesions of the breast, as the history may not always be provided on the FNAB request form. FNAB often yields hypercellular material consisting of tissue fragments that can be myxoid and contain spindle and more rounded epithelioid cells, with occasional 'ganglion-like' cells exhibiting large rounded hyperchromatic nuclei and a moderate amount of dense cytoplasm in a mix of similar spindle, polygonal and occasional

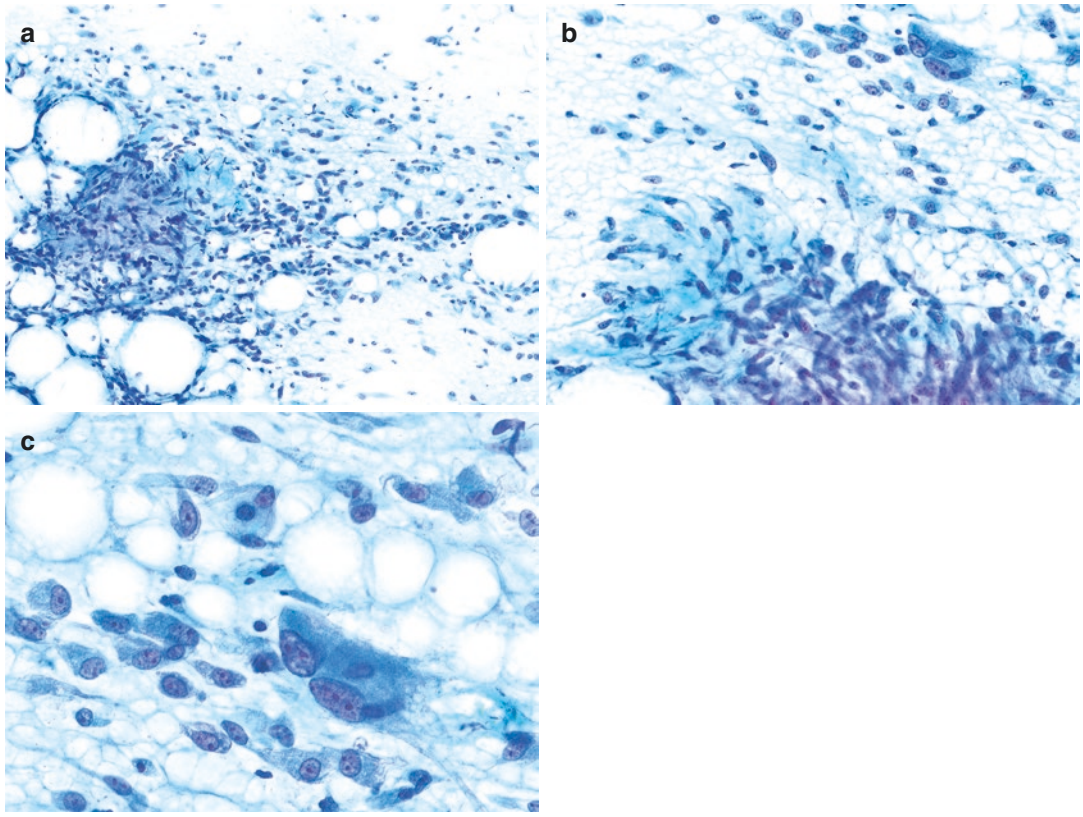

Fig. 4.9 (**a**) Nodular fasciitis showing moderate cellularity with spindle cells and a fragment of myxoid stroma and adjacent adipocytes (Pap ×10); (**b**) Fragment of myxoid stroma containing spindle cells and occasional polygonal cells, along with single spindle cells showing some nuclear pleomorphism and several much larger cells with large nuclei and prominent nucleoli in a granular background (Pap ×20); (**c**) High power of (**b**) showing the larger 'ganglion-like' cells with larger nuclei and several nucleoli in a background of plump spindle cells and an occasional lymphocyte; note the granular background with some fat globules (Pap ×40)

multinucleated cells in the typically granular background [41, 47, 48] (Fig. 4.9a–c). A variable number of lymphocytes, neutrophils, eosinophils and macrophages are present [48]. The plump spindle cell nuclei show bland chromatin and mitoses may be seen.

Sample Reports

Specific scenarios where the diagnosis of 'atypical' is appropriate:

This is not an all-inclusive list and what is atypical to one cytopathologist may be regarded as within the normal limits of a specific lesion by another cytopathologist, often reflecting the pathologists' experience in breast FNAB cytology.

Example 1

A pattern of frequent large epithelial tissue fragments showing increased crowding of cells with a mild degree of nuclear enlargement or pleomorphism and/or a more complex pattern suggesting a cribriform or micropapillary architecture.

Atypical.

These highly cellular smears show a pattern of large epithelial tissue fragments with a suggestion of a cribriform architecture, as well as crowding, a mild degree of nuclear enlargement and atypia, few myoepithelial cells and only a small number of dispersed cells and bare bipolar nuclei.

Comment: the features raise a differential diagnosis of epithelial hyperplasia and possible low-grade ductal carcinoma in situ. Core biopsy is recommended.

Example 2

A large epithelial tissue fragment pattern with a few fibrillary stromal fragments suggesting a fibroadenoma, but with increased small epithelial tissue fragments and dispersal, and focal or more diffuse epithelial nuclear enlargement, pleomorphism, granular hyperchromatic chromatin and larger nucleoli.

Atypical.

These moderately cellular smears show large and small epithelial tissue fragments of ductal epithelial cells and myoepithelial cells with scattered fibrillary stromal fragments, but there are few bare bipolar nuclei and plentiful bland dispersed cells.

Comment: The features suggest a fibroadenoma but low-grade intraduct carcinoma should be considered in the differential diagnosis. Core biopsy is recommended.

Example 3

A large epithelial tissue fragment pattern with stellate fibroelastotic papillary tissue fragments and/or complex meshwork fragments of an intraductal papilloma, but with focal more diffuse epithelial cell dispersal and nuclear enlargement, pleomorphism, granular hyperchromatic chromatin and large nucleoli.

Atypical.

Large hyperplastic epithelial cell tissue fragments are present with myoepithelial cells, along with stellate papillary tissue fragments and numerous dispersed single cells showing mild nuclear atypia.

Comment: The features suggest an intraductal papilloma but there is prominent dispersal and mild nuclear atypia. Core or excision biopsy is recommended.

Example 4

A large epithelial tissue fragment pattern with rounded, scalloped and fibrillary stromal fragments suggesting a fibroadenoma, but with increased stromal cellularity, nuclear pleomorphism, enlargement and hyperchromasia

Atypical
These highly cellular smears include hyperplastic epithelial tissue fragments and stromal fragments showing a degree of stromal nuclear atypia in a background of bare bipolar nuclei and scattered spindle cells.

Comment: the features raise a differential diagnosis of a cellular fibroadenoma and a low-grade phyllodes tumour. Excision biopsy is recommended.

Example 5
Scanty material consisting of a small tissue fragment pattern more commonly indicative of a malignant process, with increased dispersal and few myoepithelial cells and bare bipolar nuclei, but with minimal nuclear enlargement or pleomorphism.

DD: under-sampled proliferative lesion, including a fibroadenoma versus a low-grade invasive carcinoma.

Atypical.
These hypocellular smears show scattered small epithelial tissue fragments with scant or no myoepithelial cells, rare bare bipolar nuclei and scattered dispersed intact cells with minimal nuclear atypia.

Comment: the pattern raises a differential diagnosis of an undersampled proliferative lesion or possibly a low-grade carcinoma. Core biopsy is recommended.

Example 6
Scanty material showing a dispersed cell pattern with few minute tissue fragments, minimal nuclear atypia, limited eccentric cytoplasm and few bare bipolar nuclei.

Atypical
There is scanty material to assess consisting of a small number of dispersed cells and few bare bipolar nuclei. The dispersed cells are not enlarged and show minimal nuclear atypia.

Comment: the features favour paucicellular benign breast tissue, but lobular neoplasia cannot be excluded. Core biopsy is recommended.

Example 7
A mucinous background with low cellularity consisting of occasional single cells or small tissue fragments showing minimal nuclear enlargement or pleomorphism.

Atypical
There is abundant mucin in the background with only scanty dispersed small tissue fragments and single epithelial cells showing minimal pleomorphism.

Comment: the features favour a mucocele-like lesion but mucinous carcinoma cannot be excluded. Core biopsy is recommended.

Example 8
Scanty material as small epithelial tissue fragments showing minimal nuclear enlargement or pleomorphism in a background of fat necrosis, with or without a history of surgery and radiation.

Atypical
Considerable granular necrotic material is present with occasional histiocytes and siderophages and fragments of infarcted acellular fat tissue, consistent with fat necrosis, in addition to occasional small epithelial tissue fragments showing nuclear hyperchromasia and pleomorphism and scanty myoepithelial cells.

Comment: the features are consistent with fat necrosis related to previous treatment but the epithelial atypia raises the possibility of recurrent carcinoma. Core biopsy is recommended.

Example 9

Plentiful apocrine cells showing nuclear enlargement, pleomorphism and hyperchromasia with an increased nuclear to cytoplasmic ratio and some dispersal in either a cystic or suppurative background.

Atypical

These moderately cellular smears show apocrine cells in small sheets and singly dispersed, with mild nuclear enlargement and atypia in a suppurative background.

Comment: the features favour apocrine reactive atypia but raise the possibility of apocrine neoplasia. If the lesion does not resolve with or without antibiotic treatment, further biopsy should be considered at follow-up.

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Suspicious of Malignancy

5

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Introduction

As discussed in Chap. 4, Atypical, the definitions and applications of the terms 'atypical' and 'suspicious of malignancy' in breast FNAB show the most variability within and between departments. The interpretation of these terms and the management of patients with these diagnoses also vary greatly amongst clinicians. The published risk of malignancy (ROM), based on the subsequent CNB or excision biopsy, ranges from 22% to 39% in 'atypical' and 60–95% in 'suspicious of malignancy' [1–14]. Two more recent publications utilizing the category definitions of the IAC Yokohama system had a ROM for the 'suspicious of malignancy' category of 97.1% and 84.6% [15, 16]. Two distinct diagnostic categories of 'atypical' and 'suspicious of malignancy' maintain the high positive predictive value (PPV) of a 'malignant' diagnosis, while retaining a high degree of sensitivity for FNAB.

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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.

Discussion and Background

The suspicious of malignancy diagnosis will potentially show a wide inter-observer variability and be dependent on the cytopathologist's

A. S. Field et al. (eds.), *The International Academy of Cytology Yokohama System for Reporting Breast Fine Needle Aspiration Biopsy Cytopathology*, https://doi.org/10.1007/978-3-030-26883-1_5

experience, but will also be affected markedly by the quality of material provided by the FNAB operator, the quality of the direct smear preparation and the availability and quality of imaging guidance and reporting.

Limitations in specimen quality include:

- Low cellularity is one of the commonest causes and is related to the skill of the FNAB operator, suboptimal smear preparation and staining, as well as, the nature of the lesion.
- Thick or heavily blood-obscured direct smears.
- Crush and smearing artefact, which cause dispersal of single cells.
- Air-drying artefact due to delay in alcohol fixation for Papanicolaou staining.
- Slow air-drying of thickly smeared or watery slides for Giemsa staining.

The expertise of the cytopathologist plays a smaller, but still significant, role in determining whether a case is reported as suspicious of malignancy rather than atypical or malignant [17, 18]. An experienced cytopathologist assesses the whole slide for a pattern, critically analyses the components at high magnification for benign or malignant features, and does not rely on a high power assessment of a limited area of the slide.

The inherent nature of some breast lesions highlights the need for a suspicious of malignancy category:

- *High cellularity* in breast FNAB is a feature of carcinoma, but can also be associated with proliferative changes such as usual type epithelial hyperplasia [5, 9, 19], and is seen in fibroadenomas [20], papillomas [21], and radial scars [22], and so is not necessarily an indicator of malignancy.
- There is *overlapping of the cytological criteria* for benign and malignant tumours [23, 24]. Fibroadenomas can produce a high degree of cellularity, associated in some cases with marked dispersal, nuclear enlargement and prominent nucleoli, which can also be found in malignant smears [20]. Conversely, lobular carcinomas tend to result in low cellularity, a dispersed cell population with only minute tissue fragments, and only mild nuclear enlargement and atypia, features that may be seen in undersampled benign lesions [25, 26] (Fig. 5.1a–c).
- The pattern of large epithelial tissue fragments, with some showing a *cribriform or micropapillary architecture*, in association with smaller tissue fragments and plentiful dispersed cells showing low- to intermediate-grade nuclear atypia, can suggest low-grade ductal carcinoma in situ (DCIS) although an unequivocal diagnosis of low-grade DCIS cannot be made [19, 27, 28]. It is preferable in these cases to use the category suspicious of malignancy so as to avoid a potential overcall of malignant. These features may also overlap with those of a low-grade invasive carcinoma. (See further discussion below).
- *Necrosis* can be seen in some high-grade invasive carcinomas of no special type, metaplastic or basal-like carcinomas and in high-grade DCIS. High-grade DCIS is usually associated with small numbers of dispersed markedly atypical cells and epithelial tissue fragments and calcifications admixed with the granular necrotic debris [29, 30]. (See further discussion below and in Chap. 6, Malignant).
- *Distinction between lymphoma and carcinoma*, particularly in the setting of a basal – like carcinoma or carcinoma with medullary like features in which there is also a prominent lymphoid background, may be difficult as both may present as dispersed large atypical cells associated with stripped atypical nuclei. In this scenario it may be prudent to render a suspicious of malignancy overall diagnosis to prevent unnecessary surgery and a CNB should be suggested.

Management

Follow up is mandatory in cases diagnosed as suspicious of malignancy on cytology, irrespective of the ‘triple test’ imaging and clinical find-

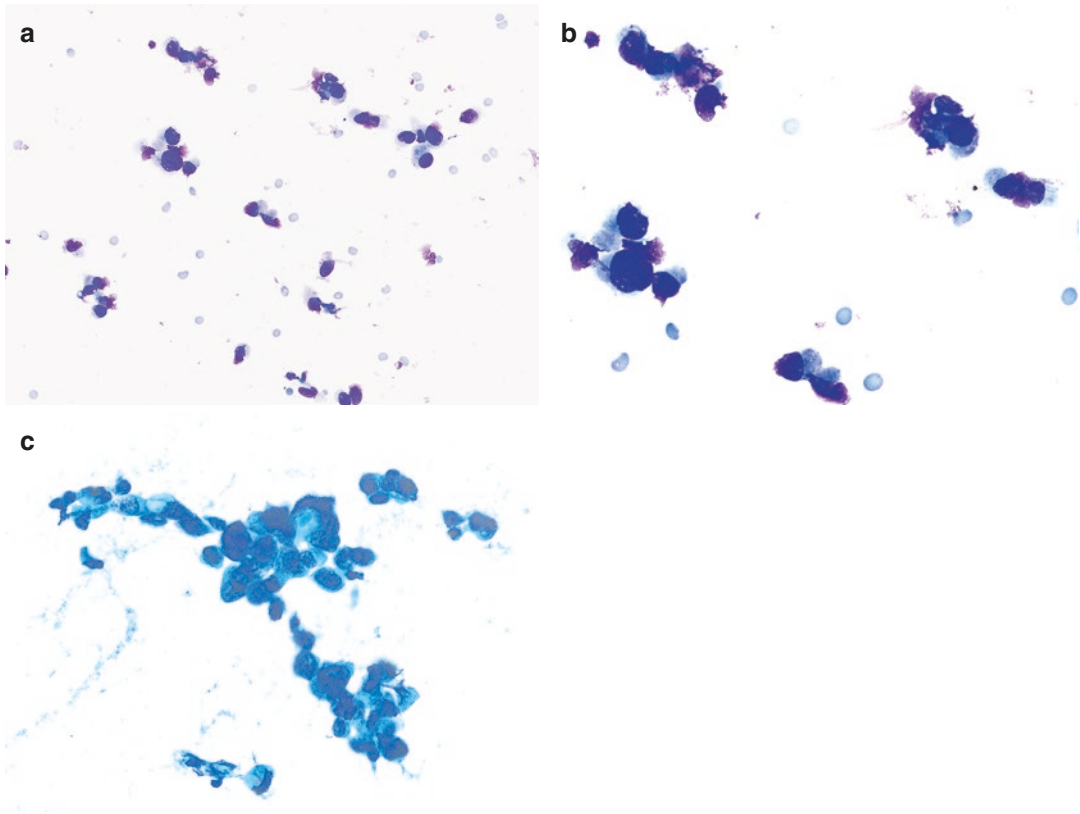

Fig. 5.1 (**a**) A very small amount of material is present consisting of dispersed single cells with eccentric cytoplasm in the presence of some crush artefact, should be reported as suspicious of malignancy. Lobular carcinoma on histopathology (Giemsa ×20); (**b**) High power of (**a**) showing features suspicious of lobular carcinoma with a small number of cells with eccentric cytoplasm and mildly to moderately atypical nuclei (Giemsa ×40); (**c**) A small amount of material showing a discohesive tissue fragment made up of cells with a moderate to high nuclear to cytoplasmic ratio, eccentric cytoplasm and occasional intracytoplasmic vacuoles containing mucin is regarded as suspicious of lobular carcinoma (Pap ×40)

ings. This may include a repeat FNAB in an environment where CNB is not available, but usually will entail CNB or excision biopsy. If a cytological provisional diagnosis of suspicious of malignancy is made at rapid on-site evaluation (ROSE) by a cytopathologist, the clinician can discuss the 'triple test' findings with the patient and immediately proceed to CNB. Counselling and discussion with the patient about the continuing management can commence at this time. When a suspicious of malignancy cytology report is issued some time after the FNAB, the cytology and imaging findings form the basis of discussions with the patient and CNB or excision biopsy is the usual recommended follow up.

Specific Breast Lesions that May Be Associated with Suspicious of Malignancy Reports

Ductal Carcinoma In Situ

The FNAB diagnosis and categorization of low-grade DCIS and high-grade DCIS are controversial, with differences in the degree of confidence of diagnosis amongst experienced cytopathologists. Low-grade DCIS and high-grade DCIS can be suggested as the diagnosis but cannot be precisely diagnosed by FNAB cytology, and in these cases invasive carcinoma cannot be absolutely

excluded [23, 27, 28]. Low-grade DCIS and high-grade DCIS have different molecular pathways and different histopathological features [31]. Similarly, in FNAB cytology low- and high-grade DCIS have different key cytological features, which raise different differential diagnoses (DD), although an intermediate-grade may show features seen in both. The discussion and presentation of the cytological features and the DD of low-grade DCIS are presented in this chapter, while high-grade DCIS is presented more extensively in Chap. 6, Malignant.

Clinical, Imaging and Histopathological Features

About 80–85% of DCIS cases are detected in the absence of clinical findings in opportunistic ad hoc or organized mammography screening programs as microcalcifications with or without a mass lesion. The microcalcifications vary from granular, where the DD is fibrocystic or proliferative changes, including columnar cell change or sclerosing adenosis, to casting calcifications, characteristic of high-grade DCIS. In clinical work-up with FNAB of palpable lesions, only 2% of carcinomas will be pure DCIS on histopathology and the vast majority of these will be high-grade DCIS [32]. A few cases present as nipple discharge (with or without a mass) or as Paget's disease of the nipple.

There is no universal agreement on a histopathological classification system for DCIS, but most systems place more weight on the nuclear grading than on the architecture and the presence of necrosis [33]. Most grading systems utilize three grades, low, intermediate and high, although a two-tiered system such as the Van Nuys [34] is preferred for FNAB. A two-tier system can attain a concordance between preoperative cytological and final histopathological grading of up to 94% in low-grade DCIS and 97% in high-grade DCIS [35].

Biomarkers are of limited value in the clinical handling of in situ lesions. Direct alcohol-fixed smears, LBC preparations and cell blocks can be stained to demonstrate the diffuse 'clonal' oestrogen receptor positivity and CK5/6 negativity of DCIS, but residual P63 positive myoepithelial cells can still be present and apocrine lesions are CK5/6 negative [36].

Low-Grade Ductal Carcinoma In Situ

The WHO defines low-grade DCIS histopathologically as small monomorphic epithelial cells with evenly spaced, mildly enlarged and pleomorphic nuclei proliferating in ducts and forming rigid arcades, 'Roman' bridges, micropapillae and cribriform patterns with sharply defined punched out holes [33]. These features distinguish it from usual epithelial hyperplasia with its chaotically arranged variable small nuclei, linear streaming of cells and irregularly sized and shaped holes or 'secondary lumina'. Intermediate-grade DCIS has larger, more pleomorphic, atypical nuclei, forming cribriform, solid or micropapillary architectural patterns and can occasionally show luminal necrosis. Microcalcifications tend to be smaller and more rounded rather than the coarse granular calcifications of high-grade DCIS. In FNAB these two grades are grouped as low-grade DCIS.

In histopathology solid papillary ductal carcinoma in situ shows ductal structures expanded by a solid proliferation of usually intermediate sized cells with moderate nuclear enlargement, among which fine fibrovascular papillary strands are present. Papillary ductal carcinoma in situ is usually of low or intermediate nuclear grade and consists of branching, arborizing fine fibrovascular fronds covered in a single or multilayered crowded epithelial proliferation with moderate nuclear enlargement and atypia. The features of encapsulated papillary carcinoma are the same, although the proliferation occurs in a rounded, sclerotic walled cystic structure. Invasive carcinoma arising from a papillary DCIS is usually of no special type, and invasive carcinoma retaining a papillary architecture (true invasive papillary carcinoma) is rare in the breast [33].

In histopathology, proliferative breast lesions range from usual epithelial hyperplasia to columnar cell change, columnar cell change with hyperplasia, flat epithelial atypia and atypical ductal hyperplasia, and then overlap with low-grade

DCIS. Inter-observer variability in distinguishing proliferative disease and low/intermediate-grade DCIS is well recognized in the literature [33].

The cytological distinction between proliferative breast disease and low/intermediate-grade DCIS is equally challenging, with overlapping diagnostic criteria. Some of these cases may yield the histopathological diagnosis of 'atypical ductal hyperplasia', but this is a histopathologically defined term and not a meaningful FNAB diagnosis [23]. Low-grade DCIS typically does not present as a clinical mass and its specific cytological diagnosis is problematic, but the aim is to avoid an over-diagnosis of malignancy (i.e. invasive malignancy) or the under-calling of low-grade DCIS as proliferative breast disease.

Key Cytological Diagnostic Criteria [19, 23, 27, 28, 35, 37] (Figs. 5.2a–e, 5.3a–c, and 5.4a–h)

- Cellularity varies but can be high in papillary DCIS.
- The pattern is mainly of large 3-D epithelial tissue fragments with a variable number of

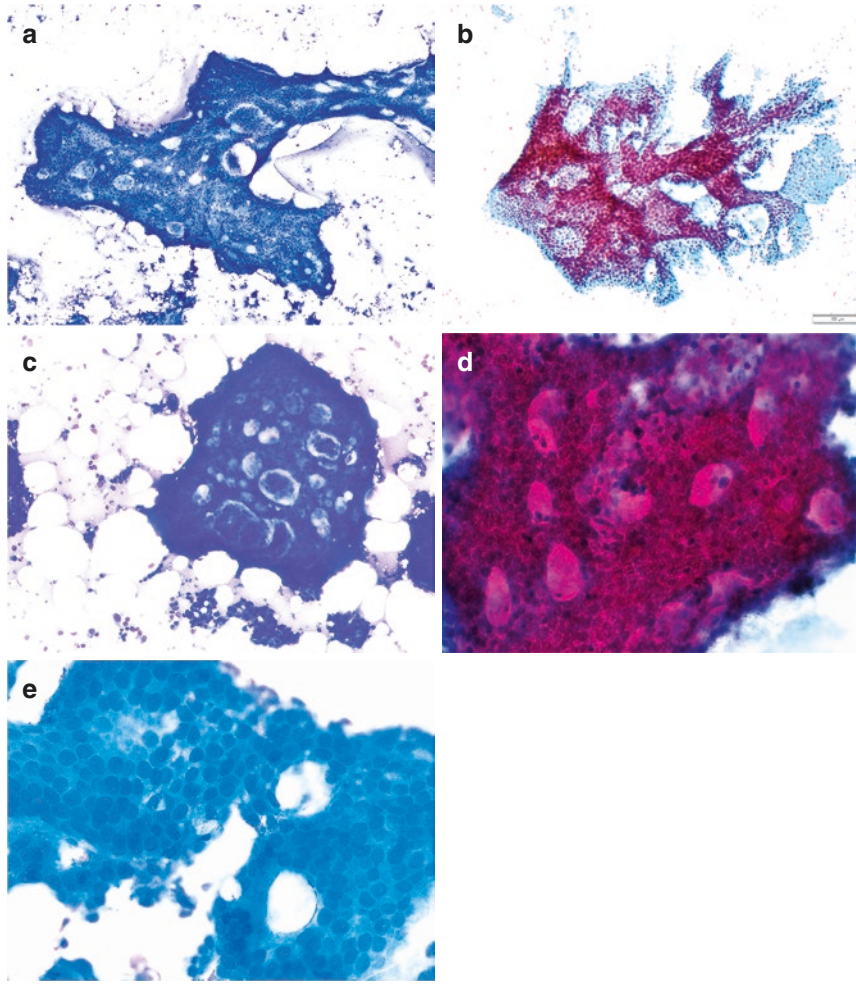

Fig. 5.2 (**a**) Low-grade cribriform ductal carcinoma in situ (DCIS) showing a low power pattern of large three-dimensional complex tissue fragments with punched-out holes in a background of a small number of dispersed cells (Giemsa ×5); (**b**) Large complex tissue fragment showing a cribriform pattern (Pap ×10); (**c**) Cribriform DCIS tissue fragment with punched-out holes in a background of smaller crowded epithelial tissue fragments and a small number of dispersed single cells (Giemsa ×10); (**d**) Cribriform DCIS showing punched-out holes with nuclear orientation to the lumen, and apoptotic debris simulating myoepithelial nuclei (Pap ×20); (**e**) Cribriform DCIS showing several punched-out holes with nuclear orientation to the lumen (Giemsa ×40)

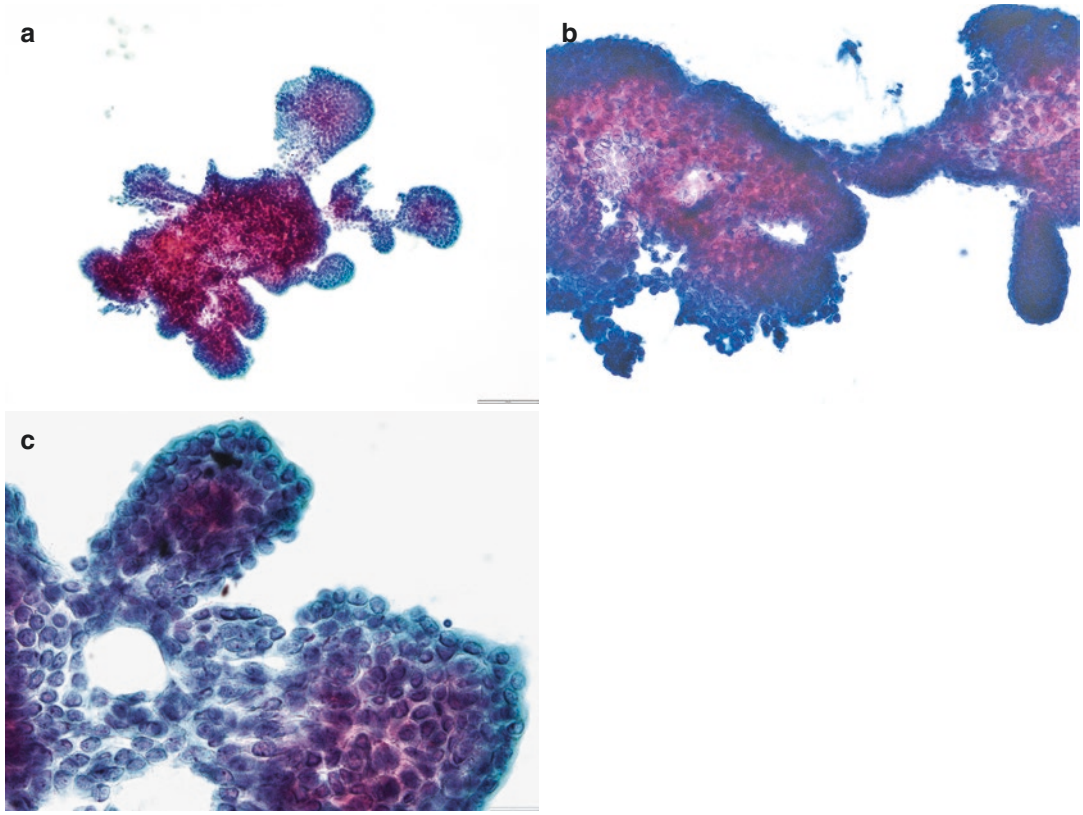

Fig. 5.3 (**a**) Low-grade ductal carcinoma in situ (DCIS) showing micropapillary architecture (Pap ×10); (**b**) Low-grade DCIS showing micropapillary (right) and cribriform (left) architecture (Pap ×20); (**c**) Low-grade micropapillary DCIS with crowding and a lack of myoepithelial nuclei (Pap ×40)

discohesive smaller fragments and often plentiful dispersed single cells.

- Epithelial tissue fragments show a solid or complex cribriform, micropapillary or true papillary architecture, and these patterns are frequently mixed.
- Cribriform tissue fragments in Papanicolaou stained smears have relatively uniformly sized punched out holes with nuclear orientation to the hole rather than linear streaming of the nuclei around an irregular space. This feature is more difficult to recognize in Giemsa-stained air-dried slides.
- Micropapillae are narrow necked, bulbous tipped, papillary extensions from an epithelial tissue fragment lacking a fibrovascular core or a lumen and without myoepithelial cells.
- True papillary tissue fragments have branching, arborizing, thin fibrovascular cores covered in a multilayered, orientated epithelial proliferation; these are seen in a small proportion of DCIS [35].
- Some complex tissue fragments with a mix of cribriform and micropapillary fragments may have anatomical borders along which there is strict columnar orientation of the nuclei to the edge.
- Low-grade DCIS shows epithelial tissue fragments with a regular, orientated uniform honeycomb arrangement of minimally atypical or enlarged nuclei, ranging to more enlarged and pleomorphic nuclei in intermediate-grade DCIS. There are usually no, or only a few, myoepithelial nuclei.

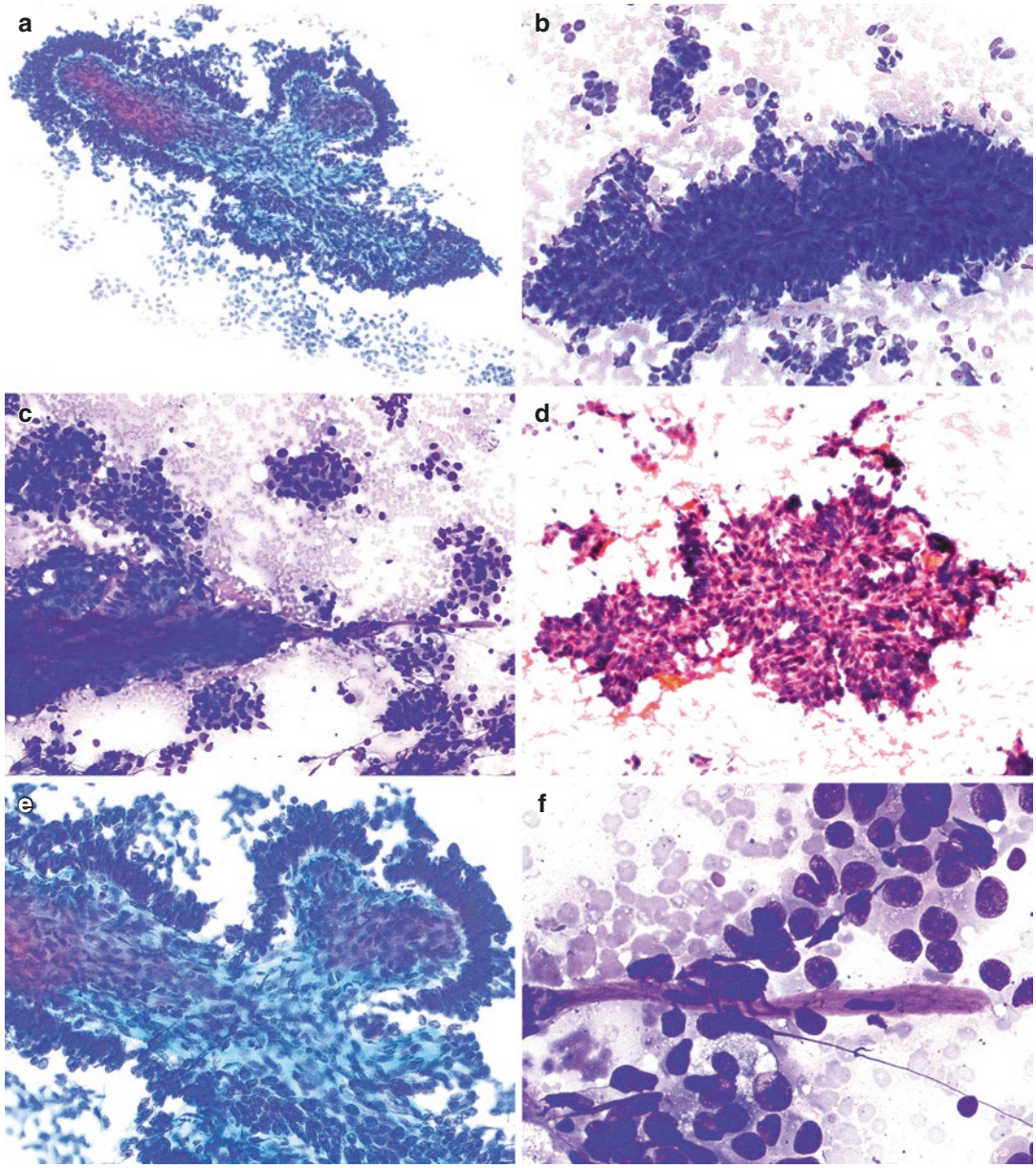

Fig. 5.4 (**a**) Papillary ductal carcinoma in situ (DCIS) showing a cellular fibrovascular core covered in a multilayered, crowded epithelial proliferation with dispersed single often columnar cells in the background (Pap ×10); (**b**) Papillary DCIS showing a thin fibrovascular core covered in a multilayered crowded columnar cell proliferation, with small crowded tissue fragments and some single cells in the bloody background (Giemsa ×10); (**c**) Papillary DCIS showing a fibrovascular core, small discohesive tissue fragments of crowded cells and some single cells in the background (Giemsa ×10); (**d**) Papillary DCIS showing a small branching fibrovascular core covered in a multilayered crowded epithelium with some dispersed single cells in the background (Pap ×10); (**e**) High power of (**a**) showing the fibrovascular core covered in a multilayered crowded columnar epithelial proliferation (Pap ×20); (**f**) High power of (**c**) showing a thin dense fibrovascular core with small discohesive tissue fragments of cells with mildly to moderately atypical nuclei and some dispersed cells in the background (Giemsa ×20); (**g**) Papillary DCIS showing a columnar cell array and columnar cell differentiation in single cells with mild to moderate nuclear atypia (Giemsa ×40); (**h**) Dispersed columnar cells from papillary DCIS showing mid nuclear atypia; note the absence of bare bipolar nuclei Giemsa ×40)

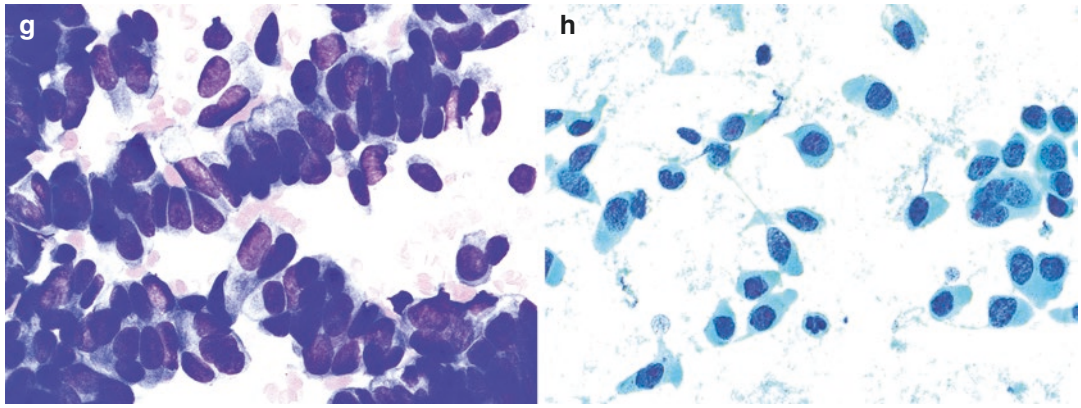

Fig. 5.4 (continued)

- Bare bipolar nuclei are scanty or not present in the background.
- Dispersed cells vary in number but can be numerous and show varying degrees of nuclear enlargement (up to less than the combined diameters of two red cells) and pleomorphism.
- Calcifications, when present, are usually granular or fragmented rather than psammomatous.
- Histiocytes and hemosiderophages can be present in a proteinaceous background.

In solid papillary DCIS the branching fibrovascular cores have rounded bulbous tips of capillary loops resembling glomeruli ('glomeruloid bodies') and reflect the surgical pathology of fine fibrovascular papillae ramifying through the solid intraductal epithelial proliferation [37] (Figs. 5.5a–f and 5.6a–f). The nuclear grade is intermediate with often plentiful dispersed cells (Fig. 5.6e). Solid papillary DCIS may be mixed with papillary and cribriform DCIS, and in many cases is positive for neuroendocrine markers such as chromogranin and synaptophysin (Fig. 5.7c). Distinguishing solid papillary DCIS and invasive solid papillary carcinoma, which is usually a circumscribed lesion and has a relatively better prognosis than most invasive carcinomas of the breast, is not possible on FNAB, and is problematic on CNB and requires excision biopsy.

True papillary tissue fragments are more commonly seen in papillary DCIS and in encapsulated papillary carcinoma with intermediate-grade nuclei, while micropapillary architecture suggests low-grade DCIS where there is a rigid architecture and a monotony of cell type.

Differential Diagnosis

- *Usual epithelial hyperplasia* has a low power pattern similar to low-grade DCIS in smears with large cohesive 3-D epithelial tissue fragments, but these are cohesive and show streaming of nuclei around slit-like secondary lumina in the Pap stain, have plentiful myoepithelial cells and lack the cribriform or micropapillary architecture that suggest low-grade DCIS. There are usually plentiful bare bipolar nuclei in the background with a small number of dispersed epithelial cells. Particular fragments of stroma in this situation may help identify a *fibroadenoma* or an *intraductal papilloma*. With the addition of hyperplastic apocrine sheets, histiocytes and a proteinaceous background, '*fibrocystic change with epithelial hyperplasia*' can be diagnosed and correlation with imaging may show a radial scar.
- When assessing complex or 3-D epithelial tissue fragments, apoptotic debris and small

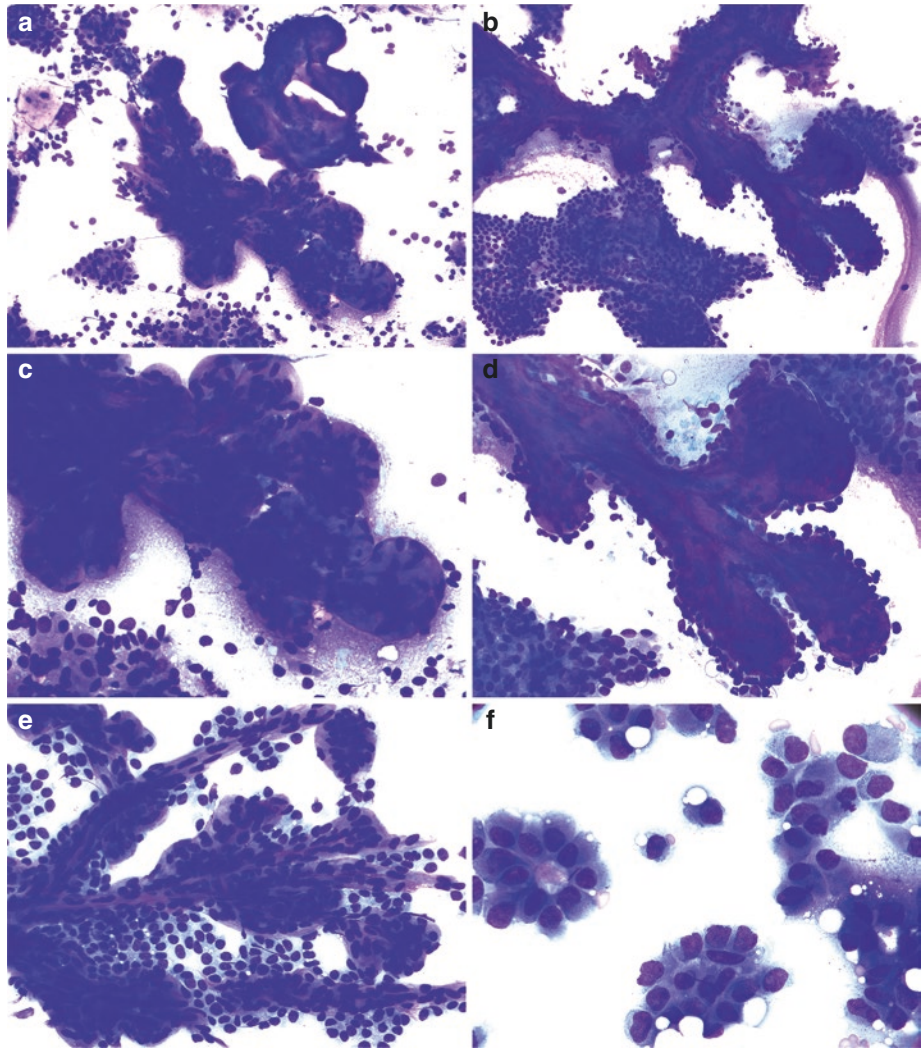

Fig. 5.5 (**a**) Solid papillary ductal carcinoma in situ (DCIS) showing a complex branching fibrovascular "glomeruloid" body, a sclerotic fibrovascular core, small discohesive tissue fragments and dispersed single cells in the background (Giemsa ×10); (**b**) Solid papillary DCIS showing a branching fibrovascular 'glomeruloid' body with rounded capillary loop ends, and a large crowded tissue fragment (Giemsa ×10); (**c**) High power of (**a**) showing a 'glomeruloid' body with distinct rounded capillary loops in which endothelial cells can be seen; note the stripped irregular nuclei in the background which at this power mimic bare bipolar nuclei (Giemsa ×20); (**d**) High power of (**b**) showing the 'glomeruloid' body with the rounded looped capillaries and the adherent myoepithelial nuclei (Giemsa ×20); (**e**) Fine branching strands of the fibrovascular cores with rounded ends, and attached sheets of mildly atypical epithelium (Giemsa ×20); (**f**) Small papillary tissue fragments and dispersed single cells showing moderate nuclear atypia and columnar cell differentiation in a solid papillary DCIS (Giemsa ×63)

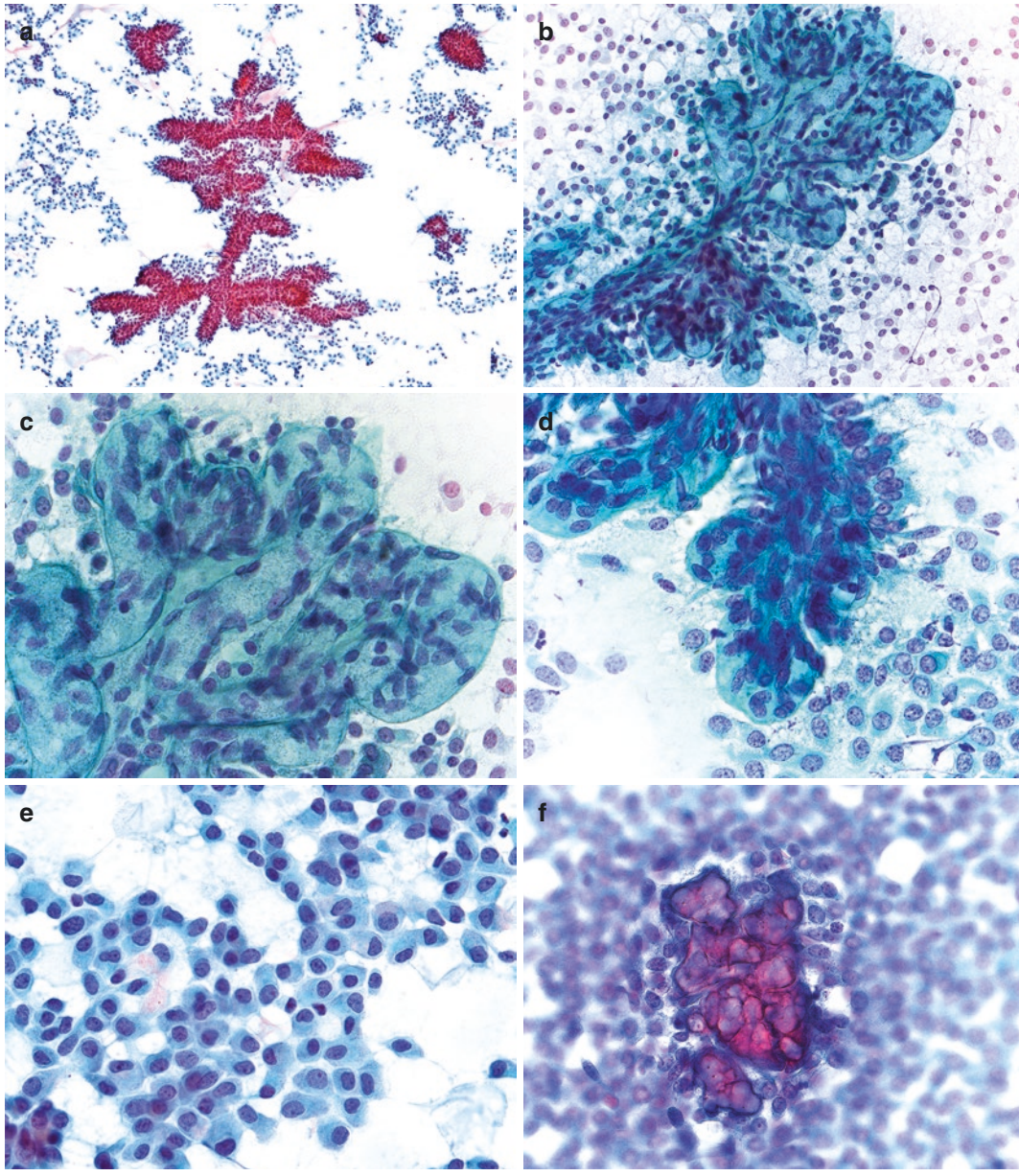

Fig. 5.6 (**a**) Solid papillary ductal carcinoma in situ (DCIS) showing papillary branching tissue fragments and dispersed cells in the background (Pap ×10); (**b**) Solid papillary DCIS complex capillary tissue fragment or 'glomeruloid' body showing the capillary loops, and plentiful dispersed single cells in the background (Pap ×20); (**c**) High power of (**b**) showing the capillary loops lined by endothelium and the dispersed epithelial cells in the background (Pap ×40); (**d**) Solid papillary DCIS showing the capillary loops covered (top right) by columnar epithelial cells with plentiful dispersed single cells in the background (Pap ×40); (**e**) Dispersed epithelial cells of solid papillary DCIS showing eccentric cytoplasm and mild nuclear atypia, which may lead to a 'malignant' report if the 'glomeruloid' bodies are not recognized as suggesting DCIS (Pap ×63); (**f**) Solid papillary DCIS calcifications in a background of a large number of dispersed single cells (out of focus) (Pap ×40)

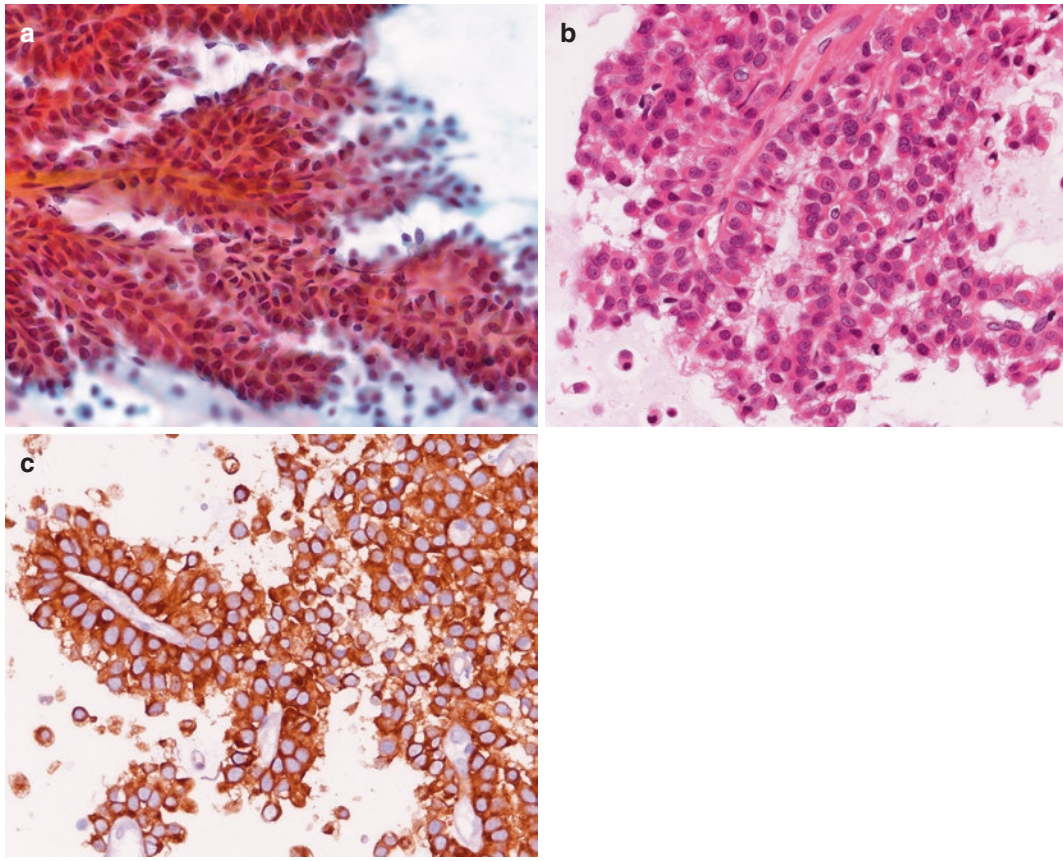

Fig. 5.7 (**a**) Fine branching fibrovascular cores of a solid papillary ductal carcinoma in situ (DCIS) showing the crowded arrays of epithelial cells with mild nuclear atypia (Pap ×40); (**b**) Cell block preparation showing the fine branching fibrovascular strands in a solid papillary DCIS (H&E ×40); (**c**) Same cell block as (**b**), showing solid papillary DCIS staining for the neuroendocrine marker synaptophysin (Synaptophysin immunohistochemistry ×40)

partially degenerate, rounded dark epithelial nuclei in low-grade DCIS can mimic *myoepithelial nuclei* in epithelial hyperplasia (Fig. 5.2d). Stripped epithelial nuclei in the background can mimic *bare bipolar nuclei* (Fig. 5.8). Criteria for both the myoepithelial nuclei seen in tissue fragments and the bare bipolar myoepithelial nuclei in the background must be strictly applied (Fig. 3.2a, b).

- *Intraductal papilloma* also has a low power pattern of large, hyperplastic epithelial tissue fragments, often with some smaller fragments and, in some cases, plentiful dispersed cells. The dispersed cells may be columnar with bland nuclei and are admixed with bare bipolar nuclei, apocrine sheets and siderophages in

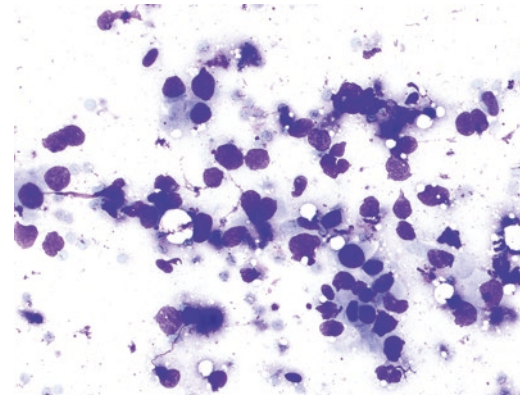

Fig. 5.8 Dispersed intact cells and plentiful stripped nuclei mimicking bare bipolar nuclei, from a low-grade infiltrating carcinoma, no specific type (Giemsa ×20)

a proteinaceous background. Stellate papillary tissue fragments with branched fibro-elastotic cores and bland attached epithelium with myoepithelial cells, and tissue fragments composed of a complex meshwork of fibrotic stroma surrounding tubules with myoepithelial and ductal cells, help distinguish papillomas from papillary DCIS [21, 37] (Figs. 3.31a–d, 3.32a–f). Small cohesive tissue fragments of rounded cells with squamoid denser cytoplasm, round hyperchromatic nuclei and intracytoplasmic vacuoles, can also be present and may show a hobnail outer aspect.

- Cytologically distinguishing *low-grade invasive carcinoma* from the spectrum of proliferative disease (usual epithelial hyperplasia, intraductal papillomas and a small subset of fibroadenomas) and from low-grade DCIS can be difficult, because all can present with a smear pattern of large epithelial tissue fragments [23, 27, 37]. Low-grade carcinomas may have high cellularity, marked dispersal and mild to moderate nuclear enlargement and pleomorphism, while tubules may be prominent in tubular carcinoma (Fig. 6.2a–h). The diagnostic features that suggest intraductal papillomas, fibroadenomas and radial scars, as well as those that suggest low-grade DCIS, should be rigorously sought, and if not present, these cases should be reported as suspicious of malignancy. Correlation with the imaging and CNB or excision biopsy is required.

In summary, it is recommended that in cases suspicious of low-grade DCIS on cytological criteria, it is appropriate to give a diagnosis of 'atypical' or 'suspicious of malignancy'. Correlation with imaging may suggest a pure DCIS when no mass lesion is present, or a mass lesion may point to an invasive carcinoma. CNB or excision biopsy should be recommended. The key is to recognize the features that suggest low-grade DCIS to avoid false-negative diagnoses of proliferative breast changes, and to avoid false-positive diagnoses of malignancy, which may lead to more aggressive surgery.

Distinguishing High-Grade DCIS from Invasive Carcinoma

The lack of consensus as to whether FNAB can distinguish high-grade DCIS and invasive breast carcinoma was a major reason for the replacement of breast FNAB with CNB. Many cytopathologists in their current practice do not attempt to suggest that a high-grade DCIS component is present, and simply call these cases malignant. If there is a clinical mass or a mass on imaging, pure high-grade DCIS is highly unlikely and it is appropriate to call these cases malignant. The triple test is used to determine management and if the FNAB cytology and imaging are discrepant CNB should be performed.

Necrosis can be seen in high-grade invasive carcinomas. However, if extensive necrosis, calcifications and only small numbers of single, highly atypical epithelial cells and tissue fragments of crowded similar cells are seen, consideration should be given to reporting the case as suspicious of malignancy and raising the possibility of a high-grade DCIS component (29, 30, 35, 37). CNB and correlation with clinical and imaging findings are required.

The aim of this approach is not to definitively diagnose high-grade DCIS to the exclusion of invasive carcinoma, but rather to encourage correlation with imaging and avoid a possible overcall of malignant in cases that may be purely high-grade DCIS. As FNAB and CNB are both sampling procedures and cannot exclude invasive carcinoma, the triple test is required before commencing any treatment. Any axillary surgery, including sentinel node biopsy, will be determined by the constellation of clinical, radiological and, where appropriate, lymph node FNAB findings [38]. (See further discussion in Chap. 6, Malignant)

Sample Reports

Specific scenarios where the diagnosis of 'suspicious of malignancy' is appropriate

This is not an all-inclusive list and what is 'suspicious of malignancy' to one cytopathologist may be regarded by another as a specific proliferative lesion showing some atypical features or, at the other end of the spectrum, 'malignant', reflecting both the pathologists' experience in breast FNAB and the quality of the material.

Example 1

Highly cellular smears with a pattern of small epithelial tissue fragments with mild to moderate nuclear enlargement and pleomorphism, few, if any, myoepithelial cells or bare bipolar nuclei, and an increase in dispersed cells.

Suspicious of malignancy

These highly cellular smears show plentiful dispersed cells and small epithelial tissue fragments consisting of similar cells, with scanty myoepithelial cells or bare bipolar nuclei.

Comment: the features are suspicious of a low-grade carcinoma. Core needle biopsy is recommended.

Example 2

Scanty small- to intermediate-sized epithelial cells dispersed singly or in minute tissue fragments, some with eccentric cytoplasm, with or without intracytoplasmic vacuoles.

Suspicious of malignancy

There is scanty material to assess consisting of small epithelial cells with small to intermediate nuclei showing mild nuclear enlargement and atypia, eccentric cytoplasm and occasional vacuoles.

Comment: suspicious of lobular neoplasia, either lobular carcinoma in situ or invasive lobular carcinoma. Core needle biopsy is recommended.

Example 3

Prominent necrosis is present in the background with scanty epithelium consisting of occasional pleomorphic cells with large markedly atypical nuclei, with or without calcifications.

Suspicious of malignancy

There is a large amount of necrosis with occasional calcifications and scattered large atypical cells.

Comment: suspicious of high-grade ductal carcinoma in situ, with or without an invasive component. Core biopsy is recommended.

Example 4

Scattered occasional pleomorphic large cells with large, markedly atypical nuclei are seen singly or in small tissue fragments, in a background of cohesive ductal epithelial tissue fragments with myoepithelial cells and scattered bare bipolar nuclei.

Suspicious of malignancy

These mildly cellular smears show occasional crowded small tissue fragments consisting of cells with a high N:C ratio and large atypical nuclei, and occasional single dispersed atypical cells, in a background of hyperplastic ductal epithelial tissue fragments with myoepithelial cells and bare bipolar nuclei.

Comment: the features are suspicious of carcinoma admixed with benign elements. Core needle biopsy is recommended.

Example 5

Highly cellular smears of dispersed cells, with or without large tissue fragments, with probable nuclear enlargement, but showing air-drying artefact in the alcohol-fixed Papanicolaou stained smears

Suspicious of malignancy
These moderately cellular smears show dispersed single cells and occasional large tissue fragments but there is marked air-drying artefact.

Comment: the features are suspicious of malignancy but a definitive diagnosis is precluded by poor fixation. Repeat FNAB or core biopsy is recommended.

Example 6
Highly cellular smears consisting of large epithelial tissue fragments with increased crowding of cells showing a mild to moderate degree of nuclear enlargement and atypia and a complex pattern suggesting a cribriform or micropapillary architecture.

Suspicious of malignancy
These highly cellular smears show large epithelial tissue fragments with a probable cribriform and micropapillary architecture and mild nuclear atypia, plentiful dispersed similar cells and no definite myoepithelial cells in the background. Bare bipolar nuclei and stroma are not seen.

Comment: suspicious of low-grade intraduct carcinoma. Core biopsy is recommended.

Example 7
Large epithelial tissue fragments consisting of scattered hyperplastic epithelial cells with myoepithelial cells, and irregular hypercellular fibrillary stromal fragments with nuclear pleomorphism, enlargement and hyperchromasia.

Suspicious of malignancy
These highly cellular smears show hyperplastic ductal epithelial tissue fragments with myoepithelial cells along with large, hypercellular stromal fragments showing moderate nuclear atypia of the spindled mesenchymal cells. There are a small number of spindle cells and bare bipolar nuclei in the background. There is no necrosis.

Comment: the features are suspicious of a low grade to borderline phyllodes tumour. Excision biopsy is recommended.

Example 8
Mucinous background with low cellularity consisting of occasional single cells or small tissue fragments showing mild to moderate nuclear enlargement or pleomorphism.

Suspicious of malignancy
There are scattered moderately atypical epithelial cells in a background of abundant fibrillary mucin.

Comment: suspicious of mucinous carcinoma. Core needle biopsy or simple excision biopsy is recommended.

Example 9
Mildly cellular smears showing singly dispersed intact cells or sheets of cells with apocrine type cytoplasm, nuclear enlargement, pleomorphism and hyperchromasia, an increased nuclear to cytoplasmic ratio and a proteinaceous background with some evidence of necrosis and occasional calcific fragments.

Suspicious of malignancy
There are a small number of sheets of atypical apocrine type cells and occasional single similar dispersed cells in a background that suggests necrosis with an occasional minute calcification.

Comment: suspicious of apocrine ductal carcinoma in situ, with or without an invasive component. Core needle biopsy is recommended

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Malignant

6

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Introduction

The positive predictive value (PPV) of a malignant breast fine needle aspiration biopsy (FNAB) diagnosis should approach 100%, based on adherence to specific key cytological criteria, that diagnose carcinoma and distinguish it from proliferative lesions, metastases and other primary breast malignancies. In the recent literature, the PPV averages 98.5% with a range of 92–100% [1–18]. Two recent publications, which used the category definitions of the IAC Yokohama System, reported a risk of malignancy (ROM) of 99.0% and 100% [19, 20]. False-positive breast cytology is very rare [5, 14, 19, 21, 22] and is usually caused by errors in the interpretation of proliferative breast lesions, in particular, intraductal papillomas and fibroadenomas [10, 23, 24]. On review, false-negative results most frequently occurred in cases where there was minimal material or only benign elements, suggesting that inadequate sampling was the major underlying cause, but interpretation remains a factor [11, 14, 19, 25].

The cytological features that are associated with malignancy in a breast FNAB include

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A. S. Field et al. (eds.), *The International Academy of Cytology Yokohama System for Reporting Breast Fine Needle Aspiration Biopsy Cytopathology*, https://doi.org/10.1007/978-3-030-26883-1_6

marked cellularity, crowded small discohesive tissue fragments with nuclear overlapping, prominent dispersed single intact cells and, crucially, atypical nuclei showing enlargement, pleomorphism of shape, nuclear margin and chromatin and large nucleoli [26, 27]. But none of these features is individually diagnostic of malignancy, and all may be seen at times in proliferative lesions, so that a 'malignant' diagnosis should only be made when a constellation of features are identified in one lesion. The fundamental aim is to avoid false-positive malignant diagnoses. Several other features have been suggested as being pathognomonic for invasive carcinoma, including stromal fragments infiltrated by carcinoma cells resembling the smaller fragments seen in CNB [27, 28], tissue fragments with a tubular architecture, intracytoplasmic vacuoles in cells with atypical nuclei, and elastoid fragments, but these remain controversial [29–31].

The diagnosis 'suspicious of malignancy' should be used if there is any question regarding the adequacy of material, if the features raise a differential diagnosis (DD) that includes a proliferative lesion or in situ carcinoma, if there are unusual specific features or a mix of benign and malignant features or if there are some features of malignancy but insufficient for an unequivocal diagnosis. It should be noted that suspicious of malignancy cases will include some proliferative lesions, but in some published studies, this category is sometimes included with malignant diagnoses in determining the PPV, whereas in other studies, it is separated out, allowing the PPV malignant rate to be close to 100% [10, 14, 19]. A suspicious of malignancy diagnosis should be managed by core needle biopsy (CNB) rather than definitive surgery.

In practical terms, in the developed world where there is good availability of medical resources, malignant FNAB diagnoses along with clinical and imaging findings make up the triple test, applied to both palpable and non-palpable breast lesions. The PPV of the triple test exceeds 99% [32]. Patients with triple test components that are discordant in any way, such as a malignant FNAB in a case with benign imaging and clinical examination, require further investigation, most commonly a CNB, prior to definitive management. A false-negative diagnosis may lead to delay and a false-positive diagnosis, potentially may result in inappropriate surgery, but in the setting where the triple test is applied, both false-positive and false-negative cytological diagnoses are usually superseded by correlation with imaging and clinical findings [11, 25, 32, 33].

Definition

A malignant cytopathological diagnosis is an unequivocal statement that the material is malignant, and the type of malignancy identified should be stated whenever possible.

The majority of cytopathologists reporting breast FNAB carcinomas attempt to categorise carcinoma into carcinoma of no special type (NST) (previously called 'ductal'), lobular carcinoma and mucinous carcinoma and, to a lesser extent, micropapillary, tubular, medullary, metaplastic, metastatic, neuroendocrine, apocrine and adenoid cystic carcinomas. Very small numbers of secretory, histiocytoid, glycogen-rich and clear cell carcinoma have also been reported. Malignant lymphomas and sarcomas are also specifically recognised. Some carcinomas, such as pleomorphic lobular carcinoma, however, are indistinguishable from high grade carcinoma NST. The degree of nuclear atypia may vary within the NST category from low to high grade [27].

Management

If the malignant FNAB correlates with the clinical and imaging findings in the triple test, and cell block material is available for any required prognostic markers to facilitate treatment planning (see Chap. 9, Ancillary Techniques), then definitive management, including neoadjuvant chemotherapy and surgery, can proceed as appropriate. Clinical practice varies, but some oncology

services will proceed to surgical excision on the basis of a positive triple test, with prognostic marker studies carried out on the excision specimen, whereas other centres and clinical trials require CNB prior to neo-adjuvant chemotherapy or definitive surgery.

If the FNAB diagnosis does not correlate with the imaging findings, CNB or excision biopsy if CNB is not available, is mandatory.

If the axillary lymph nodes are clinically enlarged or are abnormal on ultrasound examination, then FNAB ideally with rapid on-site evaluation (ROSE) is recommended to assess these lymph nodes, followed by CNB if required. Some centres utilise CNB of axillary nodes without a preliminary FNAB, which may be in part due to a lack of availability of ROSE to immediately assess and triage the FNAB smears. If the lymph node shows metastatic carcinoma consistent with the carcinoma in the breast, this stages the patient [34, 35]. If the lymph node FNAB is benign or suspicious, then sentinel lymph node biopsy is recommended.

Specific Breast Lesions Producing Malignant Reports

Invasive Carcinoma of No Special Type

Clinical and Histopathological Features

Invasive carcinoma NST constitutes approximately 75% of breast carcinomas [36, 37] and usually presents as palpable masses and as solid, stellate, rounded or irregular lesions on imaging.

Histologically, carcinoma NST includes low grade or well-differentiated carcinoma with plentiful tubules, mild nuclear enlargement, mild atypia and a low mitotic count; intermediate grade or moderately differentiated carcinoma; and, most commonly, high grade or poorly differentiated carcinoma with minimal tubule formation and nuclear atypia, which varies from moderate to marked with a moderate to high mitotic count. Carcinoma NST may have a sclerotic, elastotic, hyaline or more myxoid desmoplastic stroma, which can limit FNAB material. 'Inflammatory carcinoma of the breast' is a clinical term describing a tensely swollen and erythematous breast with dermal oedema ('peau d'orange') and often no palpable mass or discrete mammographic or ultrasonographic lesion and can be caused by a number of different carcinoma subtypes, most commonly carcinoma NST [37].

Imaging

Invasive carcinoma may present mammographically: as a focal asymmetry or an asymmetric density; as an irregular, circumscribed (suggests the pushing margin of high grade carcinoma without fibrosis), or spiculated mass (suggests a low grade carcinoma with peritumoural desmoplastic fibrosis); as an architectural distortion; or as microcalcifications alone or in combination with any of the above features.

The ultrasound features of invasive carcinoma include a solid mass with irregular shape, typically taller than wide with margins that are spiculated, ill-defined, microlobulated or angulated, and with variable but typically hypoechogenic internal echogenicity and variable through transmission but typically with posterior shadowing. The carcinoma may be seen as an architectural disturbance without a mass, and the presence of microcalcifications or duct extension indicates association with in situ disease.

Inflammatory breast cancer on imaging may show only subtle inflammatory changes of the skin and trabecular thickening and a subtle diffusely increased breast density with enlarged lymph nodes.

Key Cytological Diagnostic Criteria

(Figs. 6.1a–i and 6.2a–h)

The features vary between low and high grade carcinoma NST.

- Cellularity is high in most cases.
- The pattern in low grade carcinomas is predominantly of large irregular 3D epithelial tissue fragments, with some smaller tissue fragments and dispersed single cells, but in most high grade carcinomas, the pattern is

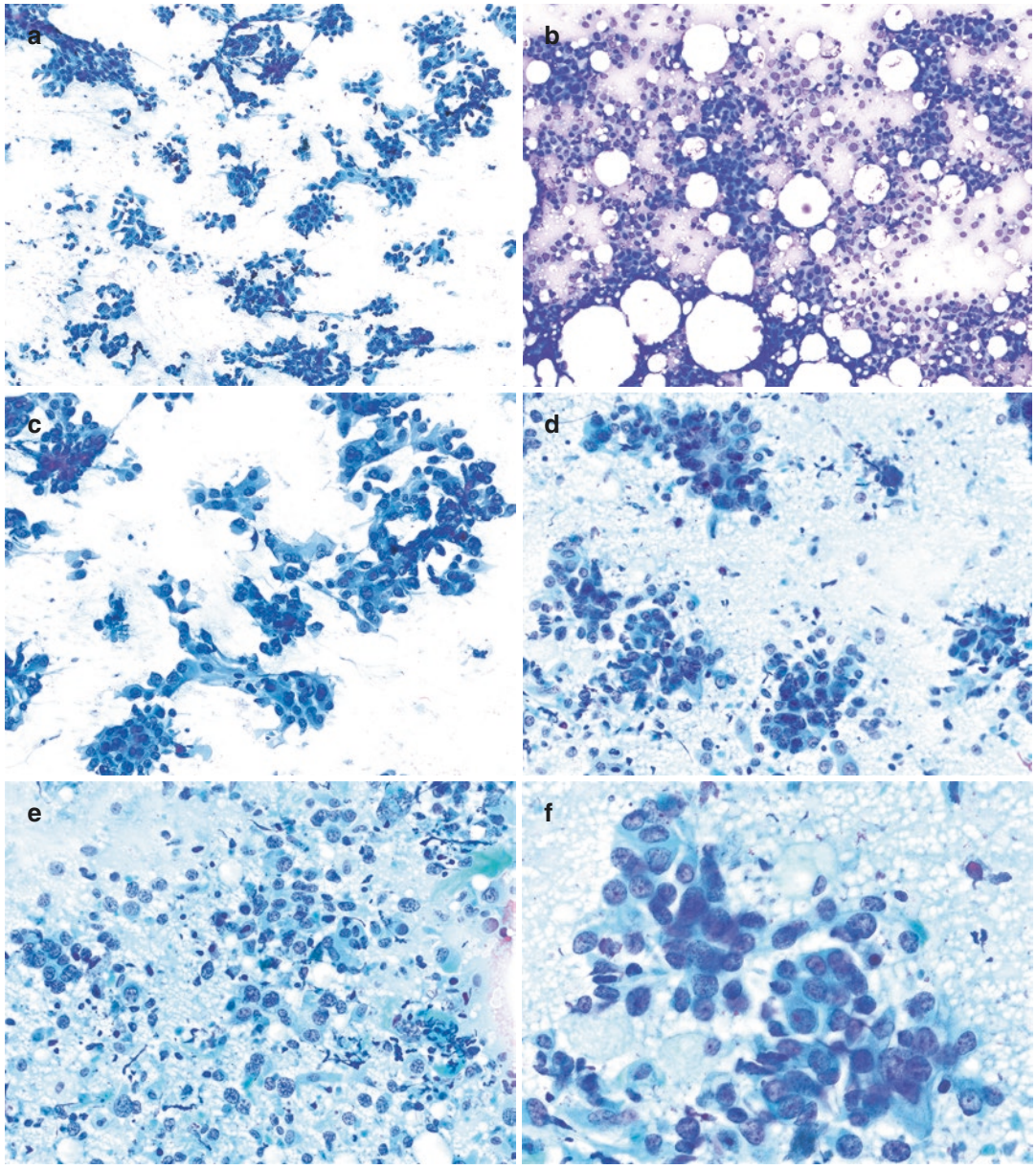


Fig. 6.1 (**a**) High grade invasive carcinoma of no special type (NST) showing a predominantly small tissue fragment pattern with dispersed cells (Pap ×5); (**b**) High grade invasive carcinoma NST showing a pattern of predominantly small tissue fragments with large numbers of dispersed single cells with interspersed fat globules (Giemsa ×10); (**c**) High grade invasive carcinoma NST showing small tissue fragments which are discohesive with some dispersed single cells (Pap ×10); (**d**) High grade invasive carcinoma NST showing small tissue fragments and dispersed single cells (Pap ×20); (**e**) High grade invasive carcinoma NST showing predominantly dispersed single intact cells along with several small tissue fragments which are fraying and two sclerotic stromal fragments (Pap ×20); (**f**). High grade invasive carcinoma NST showing small tissue fragments consisting of cells with high N:C ratio and highly pleomorphic nuclei and some single intact cells (Pap ×40); (**g**) High grade invasive carcinoma showing a moderate to high N:C ratio and marked nuclear pleomorphism with prominent nucleoli (Giemsa ×60); (**h**) High grade invasive carcinoma showing a moderate to high N:C ratio, marked nuclear pleomorphism and hyperchromatic irregular chromatin and prominent nucleoli (Pap ×60); (**i**) High grade invasive carcinoma showing a moderate to high N:C ratio, nuclear pleomorphism and two atypical mitoses (Giemsa ×40)

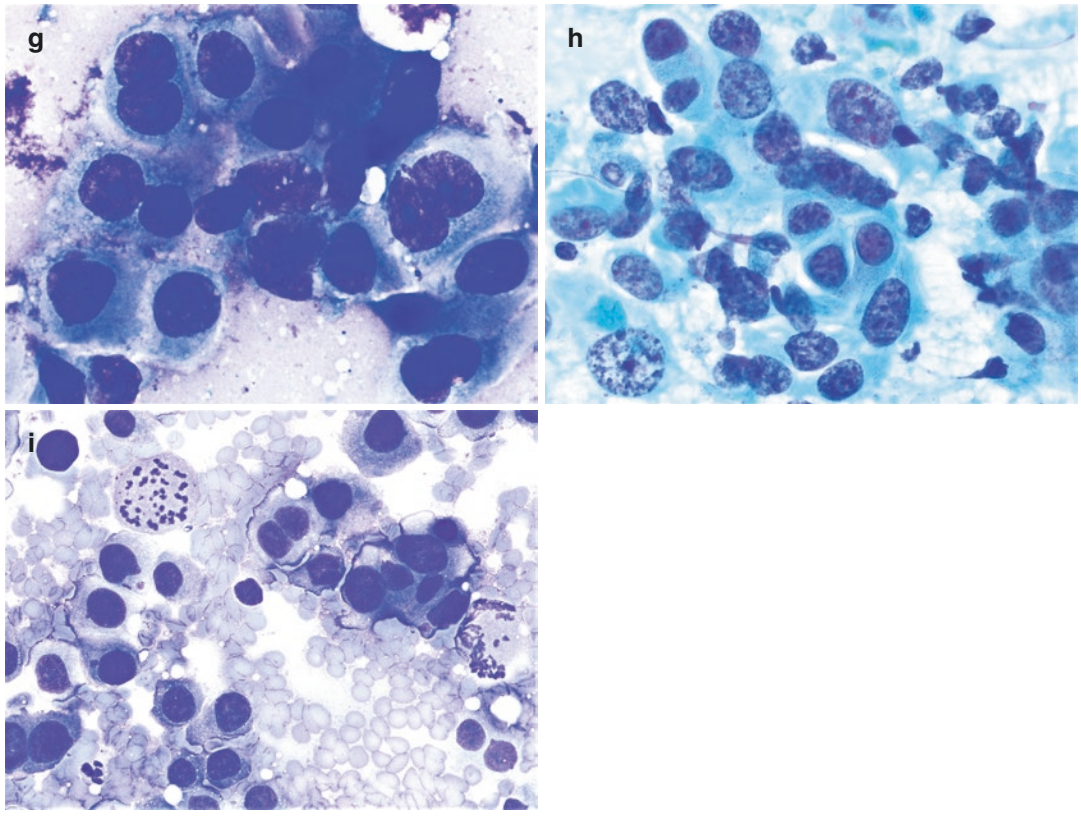


Fig. 6.1 (continued)

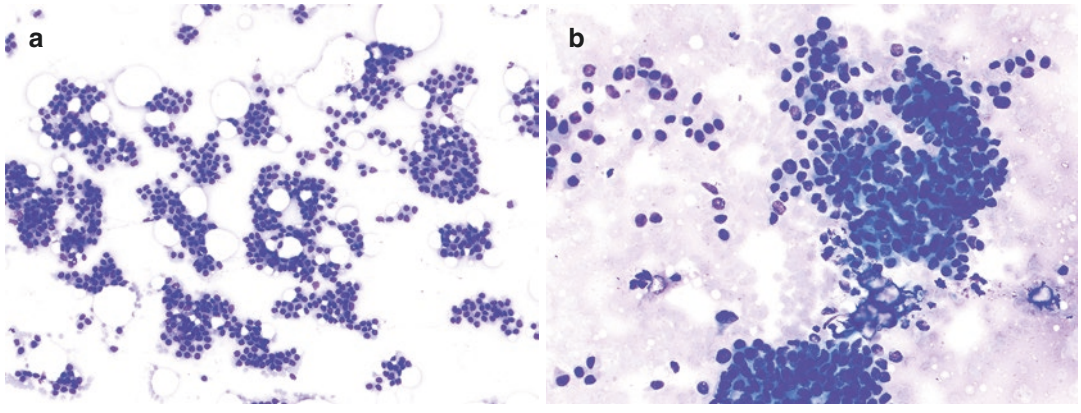


Fig. 6.2 (**a**) Low grade invasive carcinoma NST showing predominantly small tissue fragments and dispersed cells (Giemsa ×10); (**b**) Low grade invasive carcinoma showing a large tissue fragment pattern with some dispersed single intact cells in the background (Giemsa ×20); (**c**) Low grade carcinoma showing small tissue fragments with mildly to moderately pleomorphic small- to intermediate-sized nuclei and some dispersed cells in the background (Giemsa ×20); (**d**) Low grade carcinoma showing cohesive tubular tissue fragments (Giemsa ×40); (**e**) Low grade invasive carcinoma NST showing a cohesive tubular tissue fragment consisting of cells with mildly to moderately pleomorphic nuclei (Pap ×40); (**f**) Low grade invasive carcinoma showing a more dispersed pattern, a moderate to high N:C ratio and mildly to moderately pleomorphic nuclei (Giemsa ×40); (**g**) Low grade invasive carcinoma showing small relatively cohesive tissue fragments consisting of small- to moderate-sized cells (Pap ×40); (**h**) Low grade invasive carcinoma showing a discohesive tissue fragment consisting of cells with mildly pleomorphic rounded nuclei with small or absent nucleoli

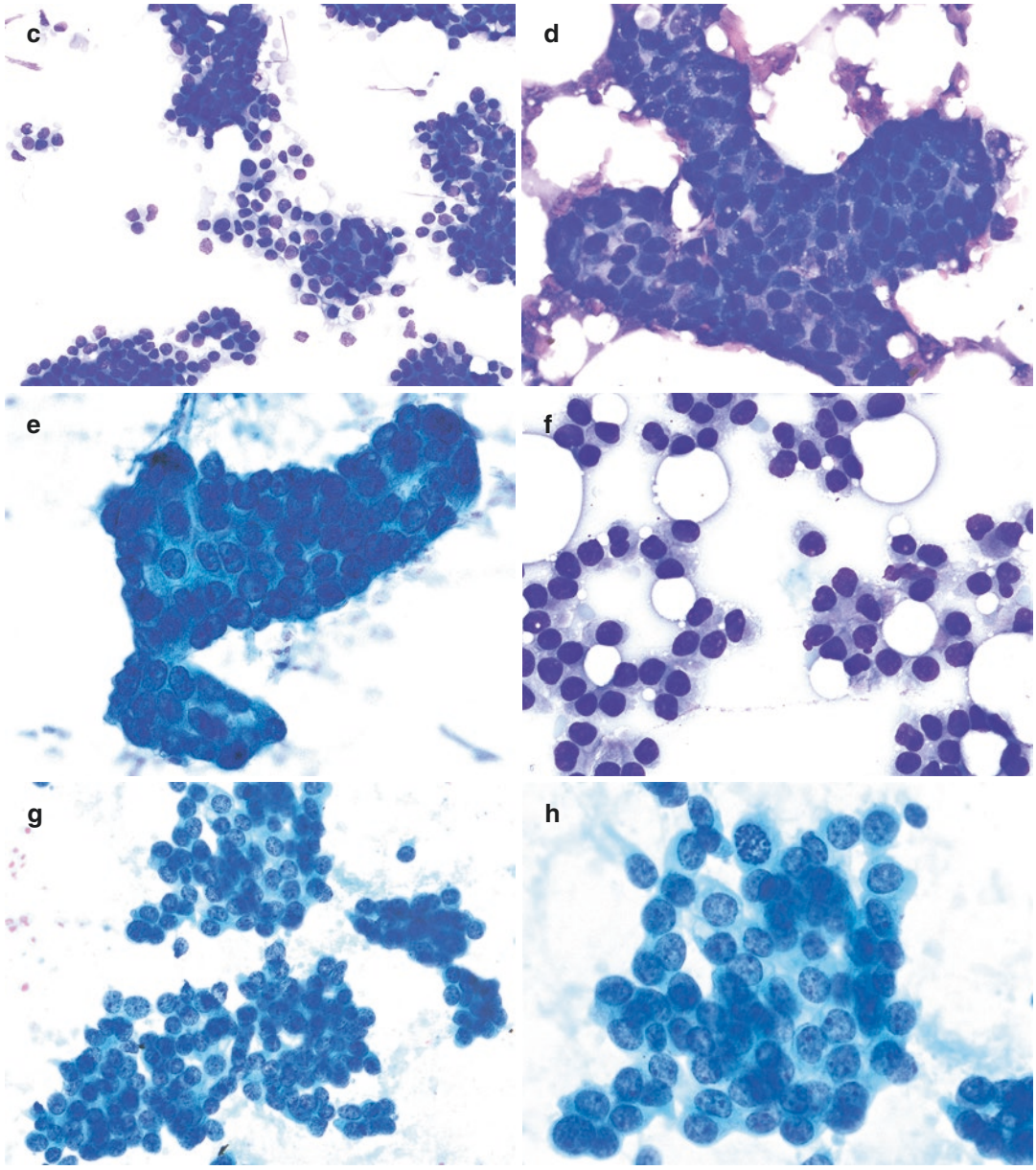


Fig. 6.2 (continued)

predominantly smaller tissue fragments with plentiful dispersed cells.

- Tissue fragments in low grade carcinoma are relatively cohesive and crowded with some nuclear overlapping. The fragments can be tubular or large and 3D with a possible cribriform architecture, whereas in high grade carcinomas, they are less cohesive and fray at the edges, with more marked crowding and overlapping and loss of nuclear orientation.
- Dispersed cells in low grade carcinoma are relatively monotonous and moderately enlarged, and in high grade carcinoma, they are larger and often markedly pleomorphic,

with denser cytoplasm and a high N:C ratio. There may be eccentric intracytoplasmic vacuoles in both.

- Nuclei in low grade carcinoma tend to be monotonous but can have mildly to moderately increased size, round to pleomorphic shape, mild to moderate hyperchromasia and small nucleoli, whereas in high grade carcinoma, the nuclei show marked enlargement, pleomorphism of shape and denser coarse chromatin with prominent large, irregular or spiculated nucleoli and perinucleolar clearing.
- Nuclear debris can be seen within some tissue fragments, especially in high grade carcinomas.
- Sclerotic stromal tufts and tufts associated with or infiltrated by carcinoma cells may be seen in both low and high grade carcinoma.
- No myoepithelial cells or bare bipolar nuclei, which are features of benign breast, are seen.

A malignant diagnosis should be made only when a constellation of these diagnostic criteria is identified, with no discrepant findings. None of the criteria of high cellularity, marked dispersal or nuclear atypia is individually diagnostic of invasive carcinoma, and these individual features can be seen in some fibroadenomas, intraductal papillomas and complex sclerosing lesions and in some intraductal carcinomas [26, 27]. Similarly, although the presence of tubules (also seen in fibroadenomas), elastoid stromal fragments, intracytoplasmic vacuoles and reactive or multinucleated carcinoma giant cells has been suggested as favouring carcinoma [27–31], none are specifically diagnostic of malignancy (Fig. 6.3a, b). The finding of stromal fragments infiltrated by carcinoma cells in 'micro-biopsies', resembling small CNB fragments, has been described as diagnostic of invasive carcinoma but reportedly is not seen in a large number of malignant FNAB [27, 28] (Fig. 6.4a, b). Fat fragments infiltrated by carcinoma are rarely seen [27] (Fig. 6.10i, j).

It is important to assess the low power pattern of the whole smear and combine this with high power confirmation of cell type and nuclear features rather than focus purely on nuclear atypia.

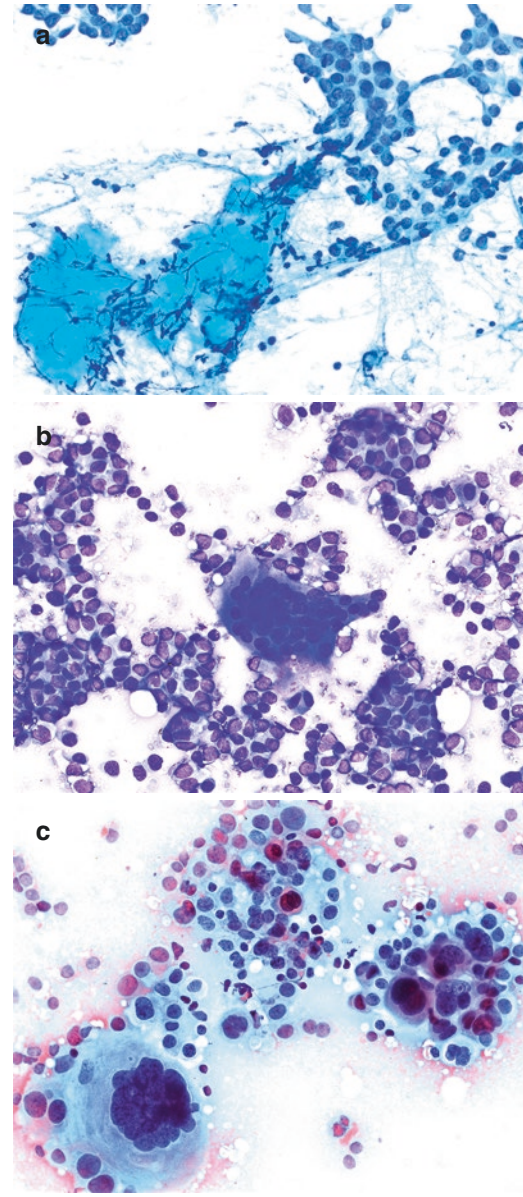


Fig. 6.3 (**a**) Sclerotic stromal tuft in a low grade invasive carcinoma NST (Pap ×20); (**b**) Multinucleated benign reactive giant cell in a background of a low grade invasive carcinoma NST (Giemsa ×20); (**c**) Multinucleated tumour giant cell in a high grade invasive carcinoma (Pap ×40)

In some FNAB there may be mixed benign and malignant features due to the biopsy sampling of a benign component adjacent to a carcinoma or the carcinoma infiltrating benign

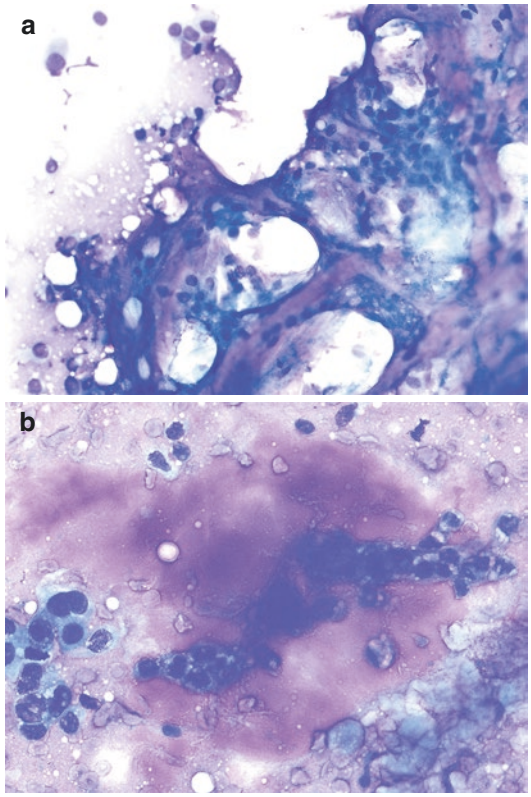


Fig. 6.4 (**a**) Magenta coloured stromal fragment infiltrated by strands of carcinoma (Giemsa ×20); (**b**) Strand of carcinoma infiltrating dense sclerotic stroma (Giemsa ×40)

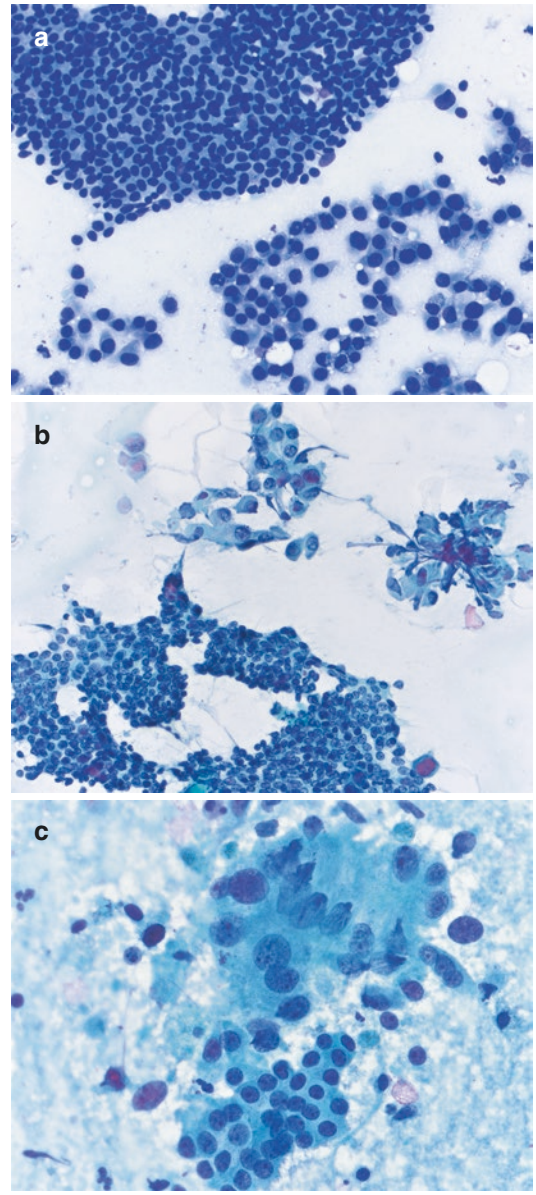


Fig. 6.5 (**a**) Hyperplastic ductal epithelial cell tissue fragment with myoepithelial cells next to carcinoma NST (Giemsa ×20); (**b**) Large hyperplastic ductal epithelial cell tissue fragment with myoepithelial cells adjacent to carcinoma NST (Pap ×20); (**c**). Small ductal epithelial cell tissue fragment adjacent to a high grade invasive carcinoma (Pap ×40)

breast tissue (Fig. 6.5a–c). In this setting a 'suspicious of malignancy' report should be issued unless there is considerable material with marked nuclear atypia in plentiful single cells and discohesive tissue fragments, as well as a distinct second population of benign tissue fragments with myoepithelial cells. This approach is prudent to avoid a false-positive diagnosis.

Stripped malignant nuclei can mimic bare bipolar nuclei but are usually larger and irregular in shape and resemble those in the dispersed cells or the tissue fragments (Fig. 6.6a, b). Apoptotic debris can mimic myoepithelial cells on tissue fragments, although typically the apoptotic debris is seen in the focal plane of the carcinoma nuclei rather than in a plane superficial to the epithelial cells, where myoepithelial cell nuclei are seen.

Cytological grading of carcinoma as low or high grade can be based purely on nuclear grade and correlates well with subsequent histopathology, but most cytopathologists do not formally

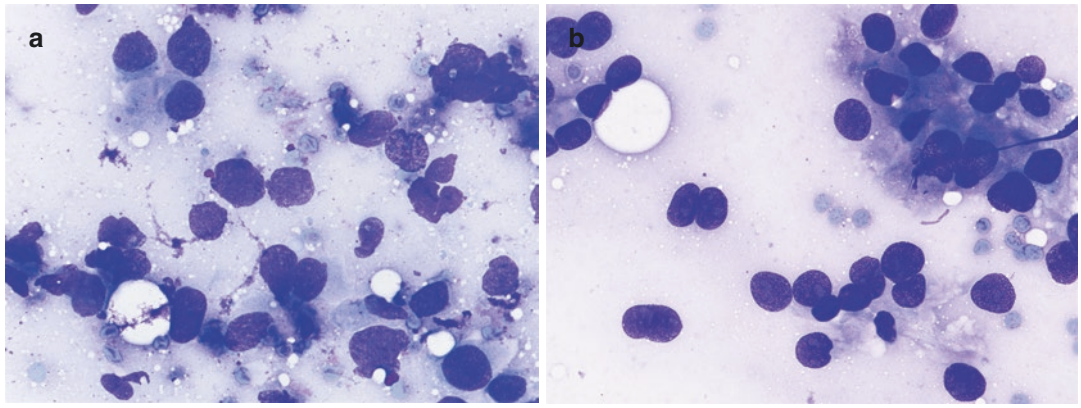


Fig. 6.6 (**a**) Stripped pleomorphic nuclei of an invasive carcinoma NST (Giemsa ×40); (**b**) Stripped large atypical nuclei with prominent nucleoli from a high grade invasive carcinoma NST (Giemsa ×40)

grade breast carcinomas [38] (compare Figs. 6.1g, h and 6.2h). The Robinson System is based on the degree of dissociation (mostly tissue fragments, mixed or mainly dispersed), cell size (twice a red blood cell (RBC) in size, up to five times a RBC), cell uniformity (monomorphic through to pleomorphic), nucleoli (indistinct through to prominent and pleomorphic), nuclear margin (smooth to clefted) and chromatin (bland through to clumped with clearing) [39].

Differential Diagnosis

- High grade ductal carcinoma in situ (DCIS) and high grade invasive carcinoma (Grade 3), NST, share high grade nuclei and cannot be distinguished consistently on FNAB. Necrosis can occur in high grade carcinomas, including HER2-positive, basal-like and metaplastic carcinomas [40]. However, the combination of prominent necrosis and calcifications and overall low cellularity consisting of highly atypical epithelial cells suggests high grade DCIS, with or without an invasive component [41]. Micro-biopsies of stromal fragments infiltrated by cells which show the same atypia as those seen in the background have been reported as being diagnostic of invasive carcinoma [27, 28] (Fig. 6.4a, b).
- Low grade carcinoma NST can share features with some benign proliferative breast processes, including dispersal of single cells, large tissue fragments and mild nuclear atypia and pleomorphism. The diagnostic features of fibroadenoma, intraductal papilloma and radial scar, and also of low- to intermediate-grade DCIS, should be sought, and if present, an atypical or suspicious of malignancy report should be issued. The differential diagnosis (DD) of each of these lesions is discussed more fully in their chapters, but in general terms, high cellularity, prominent dispersal, increasing degrees of nuclear atypia and lack of benign features, such as bare bipolar nuclei and myoepithelial cells, favour carcinoma NST.
- Metaplastic apocrine cytoplasmic change is a common benign feature, but can occur in NST, lobular and micropapillary carcinomas and in DCIS and lobular carcinoma in situ. Apocrine carcinomas show moderate to high cellularity, usually with a malignant pattern of dispersed large cells with moderate to sometimes high N:C ratio and some small crowded discohesive tissue fragments. The cytoplasm is densely granular with well-defined cell margins, and the nuclei are usually large, round to oval or irregular, hyperchromatic with coarse chromatin, often with single spiculated macronucleoli, and occasional mitoses may be seen [42] (Fig. 6.7a–d). Necrosis can occur in apocrine intraductal carcinoma, with associated high grade nuclei.
- Granular cell tumour has even and coarsely granular, paler cytoplasm, with less well-defined cell margins, a lower N:C ratio and

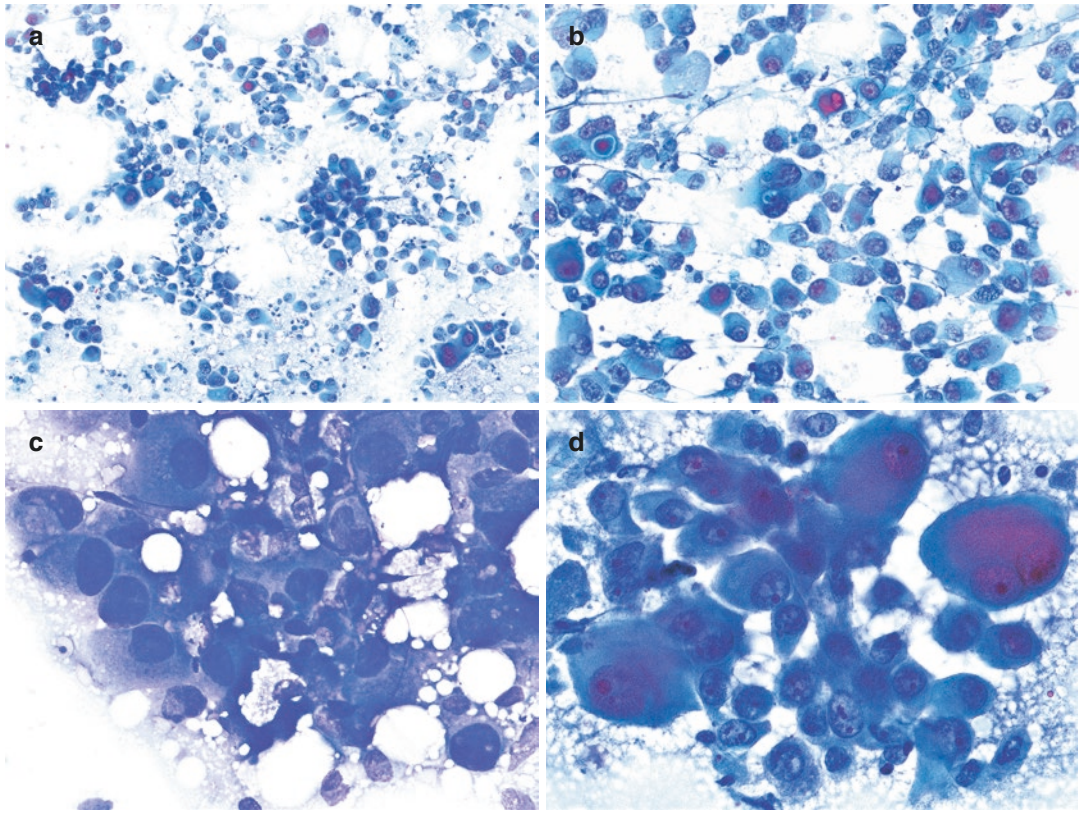


Fig. 6.7 (**a**) Apocrine carcinoma showing a pattern of predominantly dispersed cells with some discohesive tissue fragments (Pap ×10); (**b**). Apocrine carcinoma showing a dispersed pattern of large cells with considerable cytoplasm (Pap ×20); (**c**) Apocrine carcinoma showing marked nuclear enlargement and pleomorphism and apocrine-type cytoplasm (Giemsa ×40); (**d**) Apocrine carcinoma showing marked nuclear pleomorphism, prominent nucleoli and abundant apocrine-type cytoplasm (Pap ×40)

small, bland nuclei. There may be stromal tissue fragments associated with the granular cells [43] (Fig. 6.8a, b, c).

- Malignant lymphomas, whether primary or, more commonly, secondary, show a largely dispersed pattern of generally monotonous and large- or intermediate-sized lymphoid cells. The marked dispersal, moderate nuclear size, distinctive lymphoid cell type and a background of lymphoid cell cytoplasmic fragments ('lymphoglandular bodies') assist in the DD from carcinoma NST.
- Malignant melanoma and other metastatic carcinomas with their distinctive features can usually be differentiated from carcinoma NST, but if a lesion which is malignant on imaging does not have the typical features of a breast carcinoma variant, metastatic carcinoma should be considered, particularly in young women, in whom primary breast carcinoma is less common.
- Classic lobular carcinoma produces highly dispersed intermediate-sized single cells and small, loosely cohesive tissue fragments, in which there may be 'windows' between the cells demonstrating discohesion [27]. The nuclei are small to intermediate in size and round to angulated with subtle nuclear envelope indentations and have single small nucleoli. Characteristically there is eccentric, relatively dense cytoplasm containing, in a variable but often frequent number of cells, single intracytoplasmic vacuoles (see below for further discussion and photo-images).

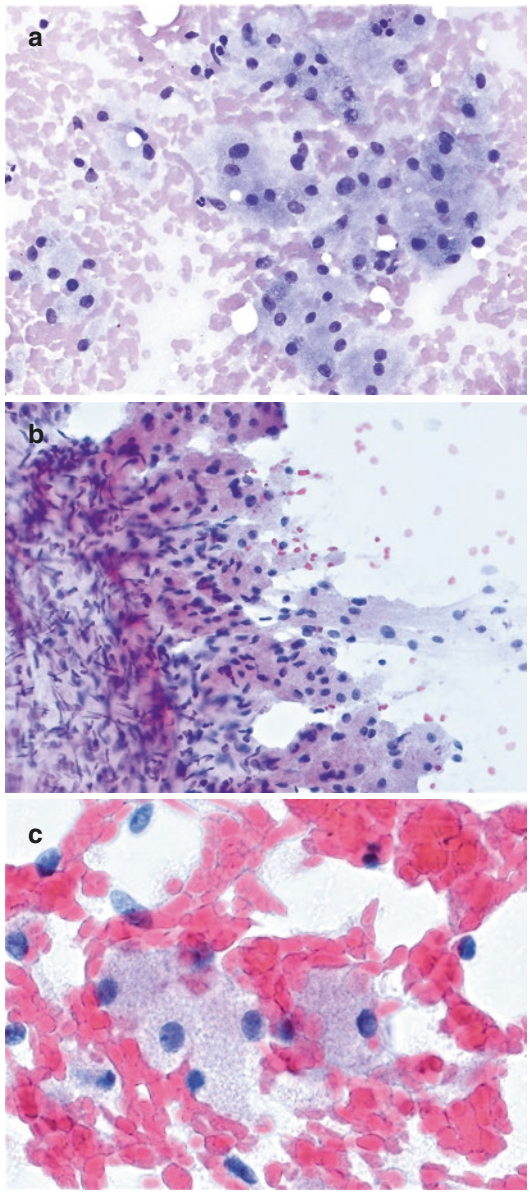


Fig. 6.8 (**a**) Small sheets of granular cell tumour consisting of plump cells with low N:C ratio, abundant pale granular cytoplasm and rounded nuclei (Giemsa ×20); (**b**) Granular cell tumour with sheets of large plump cells with copious granular cytoplasm and adjacent fibroblastic stroma (H + E ×20); (**c**) Granular cell tumour showing abundant granular eosinophilic cytoplasm and minimally pleomorphic rounded nuclei with occasional small nucleoli (H + E ×60). (Courtesy of Professor Pamela Michelow, Johannesburg)

Pleomorphic lobular carcinoma shows cells of high nuclear grade with marked dispersal, mimicking high grade carcinoma NST [44].

- Variable degrees of nuclear atypia can be seen in epithelial tissue fragments in the setting of scar tissue related to previous surgical excisions, or following radiation therapy. The latter is associated with nucleomegaly, cytomegaly, nuclear and cytoplasmic vacuolation, hyperchromasia and blurring of chromatin, a variable but often low N:C ratio and myoepithelial cells [45]. Macrophages can also show nuclear enlargement and atypia. Fat necrosis, with its multi-tinctorial granular background material, siderophages, foamy histiocytes and multinucleated histiocytes, is often present in the background, and the epithelioid histiocytes can mimic carcinoma cells. If there is high epithelial cellularity with nuclear atypia, CNB is recommended to exclude recurrent carcinoma.

High Grade Ductal Carcinoma In Situ

Clinical and Histopathological Features

High grade DCIS rarely presents as a palpable mass in the absence of an invasive carcinoma and is usually found in the workup of calcifications found on mammography. Histologically it is characterised by pleomorphic enlarged cells showing marked nuclear hyperchromasia, pleomorphism and prominent nucleoli, which expand and proliferate in ducts and extend into the terminal ductules of lobules, with a solid, 'comedo' or central necrosis, cribriform or micropapillary architecture, although frequently the patterns are mixed [37]. Microcalcifications are often present, especially in the central necrosis pattern. Mitoses are common.

(Note: Low grade DCIS, which includes low and intermediate nuclear grades, is discussed in Chap. 5, Suspicious of Malignancy)

Imaging

The mammographic findings are usually those of pleomorphic or casting or linear microcalcifications of varying sizes, sometimes with a surrounding soft tissue density. The microcalcifications are present within intraluminal necrosis, and the soft tissue density is indicative of the surrounding inflammatory/immune response.

The microcalcifications typically follow a disordered ductal pattern and extend towards the nipple. Rarely in situ malignancy may present as a non-calcified mass. However an irregular or spiculated mass associated with microcalcifications strongly suggests an associated invasive carcinoma. As the region of calcification and HGDCIS increases, so does the probability of finding an invasive carcinoma that may be occult on mammography.

Ultrasound may show no findings to correspond to the microcalcifications. The fibroglandular tissue may show focal hypoechogenicity changes due to the immune response, and microcalcifications may then be recognised within this tissue. If an associated mass lesion is seen on ultrasound, then it raises the possibility of an invasive carcinoma.

Key Cytological Diagnostic Criteria:

(Fig. 6.9a, b)

- Variable cellularity, but can be abundantly cellular.
- Pattern of prominent granular necrosis in most cases.
- Small varying to large 3D discohesive epithelial tissue fragments showing a solid or cribriform architecture.
- Variable number of dispersed single pleomorphic epithelial cells and some stripped nuclei.
- Large, highly atypical hyperchromatic nuclei, with coarse chromatin and large irregular nucleoli, often with perinucleolar chromatin clearing.
- Calcifications of variable shapes and sizes, reminiscent of 'broken glass' fragments; psammoma bodies are uncommon and suggest a metastasis.
- Scanty or usually no myoepithelial cell nuclei on the epithelial tissue fragments and no bare bipolar nuclei in the background.
- Apocrine cytoplasmic differentiation can occur.

Other variants, including signet ring, neuroendocrine, squamous, spindle or clear cell in situ carcinoma, are rare.

Differential Diagnosis

- Unequivocally distinguishing high grade DCIS from high grade invasive carcinoma NST is not possible on FNAB, but the presence of epithelial tissue fragments and dispersed intact cells with high grade nuclear features in a background of necrotic debris and irregular microcalcifications has a high specificity for high grade DCIS [40, 41]. An invasive carcinoma may be associated with high grade DCIS, or an invasive high grade carcinoma can exhibit necrosis, most frequently in basal-like and metaplastic carcinomas, although these carcinomas are usually not associated with casting calcifications on

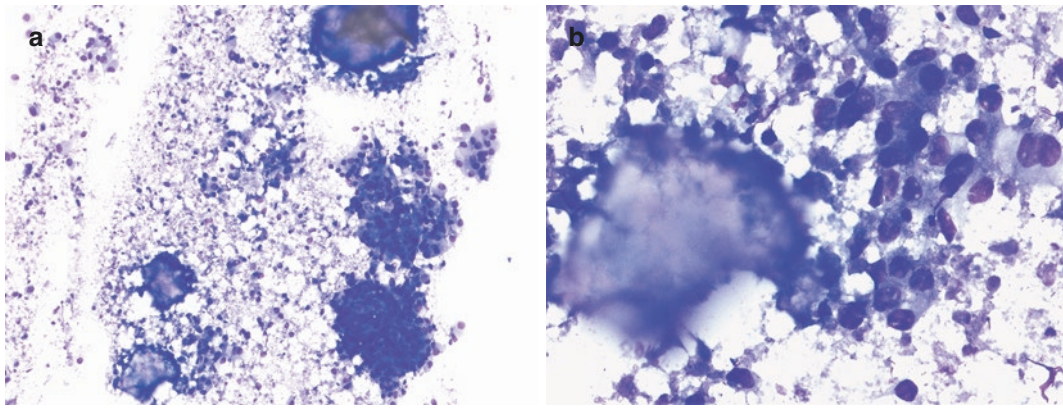


Fig. 6.9 (**a**) High grade ductal carcinoma in situ with three calcifications and a necrotic background (Giemsa ×10); (**b**) High power of (**a**) showing high grade ductal carcinoma in situ showing a calcification (out of focus) and adjacent carcinoma showing marked nuclear pleomorphism in a necrotic background (Giemsa ×40)

mammography [46]. An appropriate report in these cases could be the following: 'the features suggest high grade DCIS, with or without an invasive component, and correlation with imaging findings is required' (see also Chap. 5, Suspicious of Malignancy).

- Granulomatous mastitis can be due to bacterial, mycobacterial or fungal infections, can be idiopathic, can be associated with necrosis and smears and can include pleomorphic epithelioid histiocytes or reactive epithelial cells which can be misinterpreted as dispersed carcinoma cells. It is rare for breast carcinomas to be associated with granulomas, although some carcinomas can have associated reactive multinucleated giant cells.
- Silicone leaked from breast implants can produce a foreign body granulomatous response. Characteristically, histiocytes have variably sized vacuoles containing non-birefringent, weakly refractile globular material, which is also dispersed in the background, particularly well seen at ROSE and in Giemsa-stained smears [47].

  Fat necrosis shows a granular necrotic background, which in Giemsa stained smears is multi-tinctorial, yellow and red with dense blue small globules, and scattered histiocytes, siderophages and fragments of necrotic fat lacking nuclei [27]. Epithelial cells are usually scanty or absent.

Invasive Lobular Carcinoma

Clinical and Histopathological Features

Lobular carcinomas constitute 10–15% of breast carcinomas [36]. Lobular carcinoma may present with a palpable mass but is frequently clinically occult until advanced. It infiltrates diffusely as single cells and strands of cells, often around surviving breast lobules and ducts, and is associated with a sclerotic desmoplastic stroma [37]. This carcinoma often yields low cellularity or insufficient FNAB specimens. Variants include solid or alveolar subtypes, the tubulo-lobular variant with scant small tubules [48], and the pleomorphic lobular variant with larger more pleomorphic mitotically active cells demonstrating a single-file infiltrating pattern.

Imaging

Mammography can show a typically malignant irregularly shaped mass with spiculated margins, sometimes corresponding to a palpable tumour, but frequently the clinical findings are indeterminate, and imaging will show only nonspecific architectural distortion and diffuse changes. The diffusely infiltrating margins and the size of a lobular carcinoma are frequently not apparent. Occasionally lobular carcinoma may be mammographically occult or seen in only one view or identified due to a contracted, 'shrunken' breast secondary to fibrosis. Calcification is not a feature except in the pleomorphic lobular variant when the calcifications have a typically malignant appearance of varying shapes and sizes.

As with mammography, ultrasound examination may show an irregular discrete mass but often only shows a nonspecific architectural disturbance with localised shadowing. Again the extent of the disease is difficult to estimate due to the poor definition of tumour margins.

Key Cytological Diagnostic Criteria

(Fig. 6.10a–j)

- Cellularity is variable but can be low.
- The pattern is of highly dispersed single cells with short linear strands and, in some cases, small discohesive tissue fragments.
- The dispersed cells are relatively uniform, small to intermediate in size, with scanty to a moderate amount of eccentric cytoplasm and a variable N:C ratio.
- Mild to moderate nuclear pleomorphism is present with round or irregularly polyhedral nuclei, relatively bland chromatin and inconspicuous nucleoli.
- The cytoplasm is eccentric in most cells and may be plasmacytoid or contain intracytoplasmic lumina (vacuoles) with mucin droplets ('targetoid' or 'magenta bodies') and which may indent the nucleus ('signet ring cells'). Single cells or strands of cells identical to those in the background may infiltrate sclerotic stromal fragments.

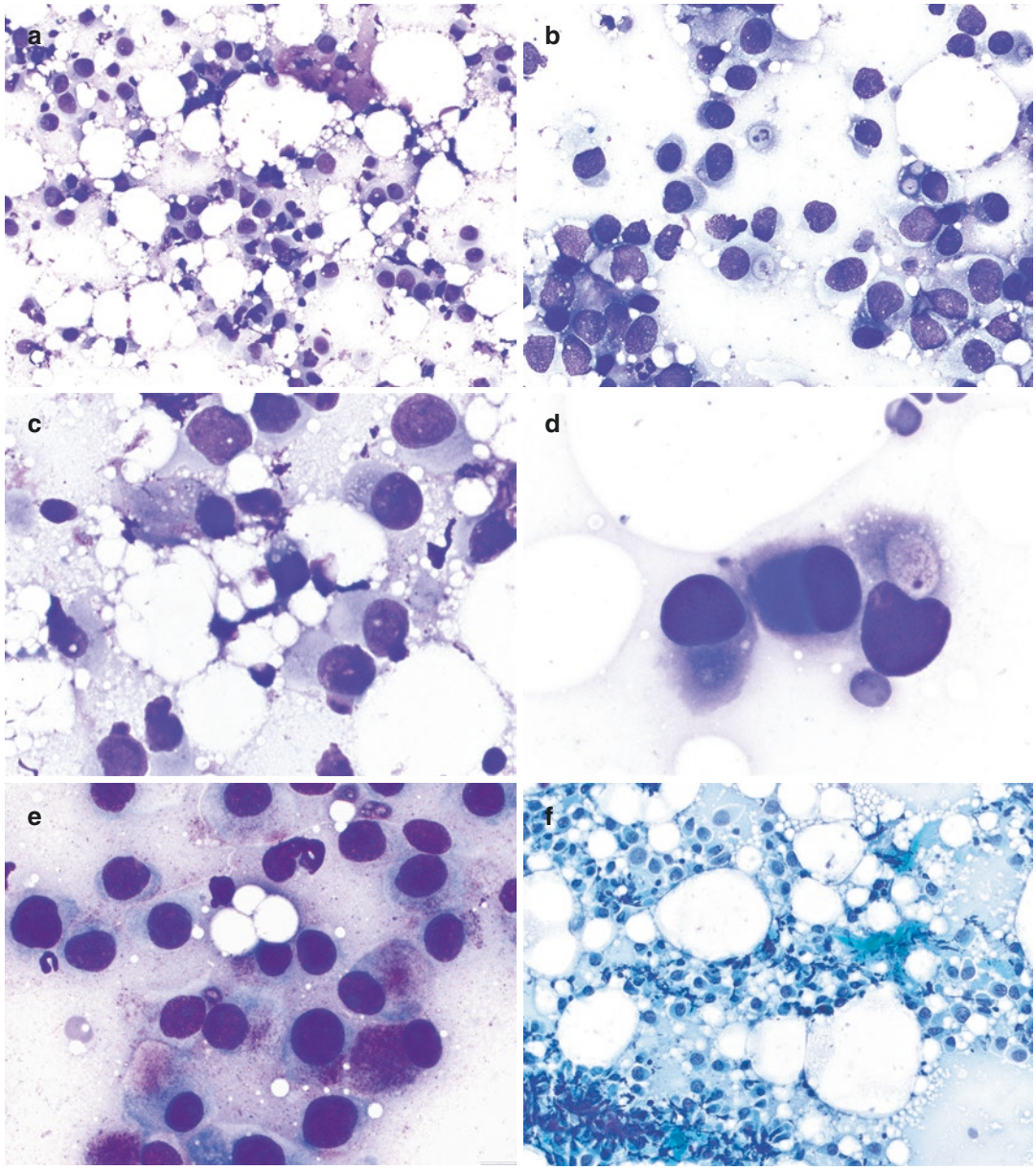


Fig. 6.10 (**a**) Lobular carcinoma with dispersed cells and strands of cells and a stromal tuft (Giemsa ×20); (**b**) Lobular carcinoma showing a dispersed cell pattern with small discohesive tissue fragments and several cells containing intracytoplasmic vacuoles (Giemsa ×40); (**c**) Lobular carcinoma showing cells with eccentric cytoplasm and mildly to moderately atypical nuclei (Giemsa ×60); (**d**) Dispersed single cells of lobular carcinoma showing eccentric cytoplasm and in one cell a vacuole (Giemsa ×60); (**e**). Lobular carcinoma showing eccentric cytoplasm with granular mucin (Giemsa ×60); (**f**) Lobular carcinoma showing a dispersed cell pattern and two stromal tufts (Pap ×20); (**g**) Lobular carcinoma showing a dispersed cell pattern and a moderate degree of nuclear pleomorphism amid fat globules (Pap ×40); (**h**) Lobular carcinoma showing dispersed single cells with eccentric cytoplasm and mild to moderate nuclear atypia (Pap ×60); (**i**) Lobular carcinoma infiltrating fibrofatty tissue (Giemsa ×10); (**j**) High power of (**i**) showing lobular carcinoma infiltrating fat with carcinoma strands surrounding adipocytes (Giemsa ×40)

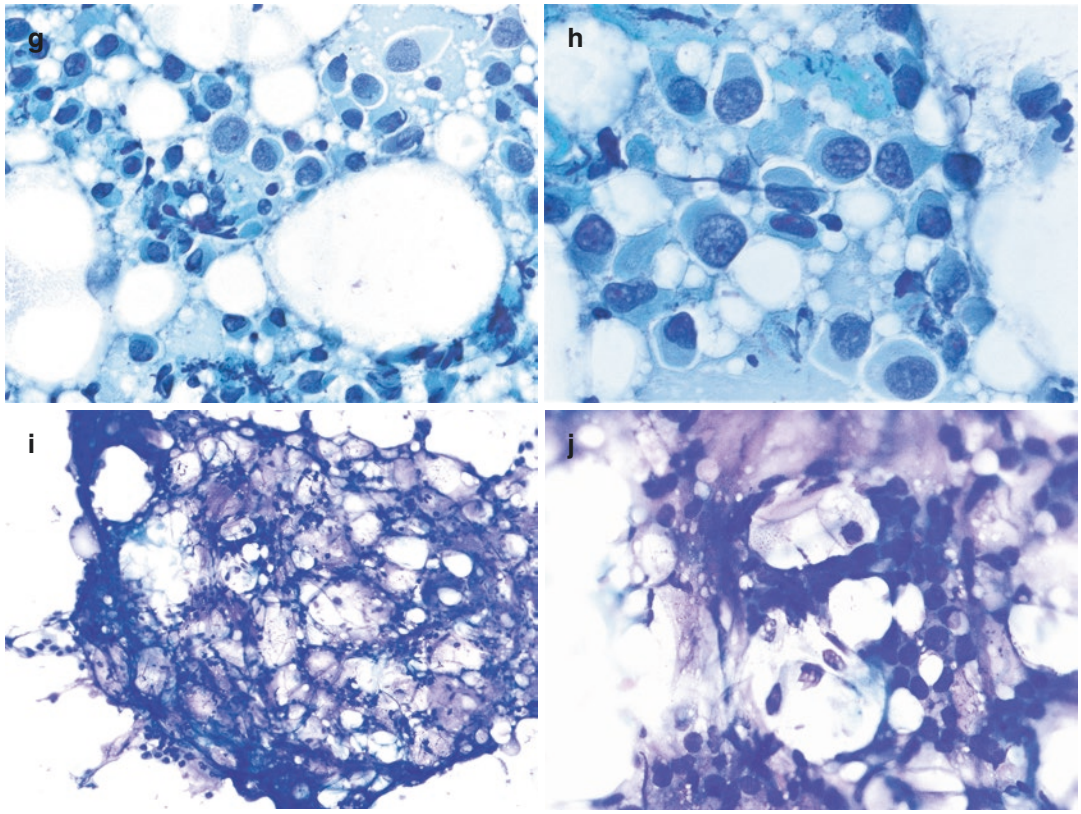


Fig. 6.10 (continued)

Smearing artefact with stripped nuclei and chromatin smearing can be present. The characteristic single cells and short strands of lobular carcinoma can be overlooked when benign components, such as 'fibrocystic change', with large epithelial hyperplastic tissue fragments and apocrine sheets, are also present [49, 50]. Correlation with imaging and clinical findings, and in many cases, the patient's concern about a palpable 'new' breast lesion, should lead to FNAB or CNB to complete the triple test.

Intracytoplasmic vacuoles raise a suspicion of invasive lobular carcinoma but are seen regularly in lobular carcinoma in situ, invasive carcinoma NST and mucinous carcinomas and occasionally in benign conditions, such as epithelial hyperplasia, columnar cell change and apocrine metaplasia [49, 50]. On some occasions, lobular carcinoma in situ and pleomorphic lobular carcinoma in situ involving expanded ducts may undergo necrosis and calcification and may present as an imaging abnormality, primarily calcifications. The FNAB of lobular carcinoma in situ is characterised by a similar pattern and cells to those of invasive lobular carcinoma [27, 51].

Paucicellular FNAB smears with scattered single, mildly atypical cells in patients with minimal imaging findings are an indication for further workup, including CNB or repeat FNAB and MRI.

Differential Diagnosis

- Undersampled benign lesions, most typically fibroadenomas, may produce smear regions where dispersed cells are prominent, and rarely some of these may contain intracytoplasmic lumina. Recognition of benign features, such as bare bipolar nuclei, will prevent a false-positive diagnosis [52].
- Carcinoma NST can produce a markedly dispersed pattern, particularly when it is high grade, but the high grade nuclear features and

usually at least some crowded tissue fragments will exclude classic lobular carcinoma.
- The rare alveolar lobular carcinoma variant produces moderately cellular smears with discohesive sheets of mildly atypical cells with pale eccentric cytoplasm, resembling lactating epithelium, but lacks a milky background and sheets of vacuolated acinar cells.

Pleomorphic lobular carcinoma cannot be distinguished from high grade infiltrating carcinoma NST because both may show high cellularity with predominantly single dispersed cells with large malignant nuclei, occasional intracytoplasmic lumina and a small number of discohesive tissue fragments [44, 53] (Fig. 6.11a–d). Both pleomorphic lobular carcinoma and pleomorphic lobular carcinoma in situ can show apocrine differentiation [53–55]. Pleomorphic lobular carcinoma may also exhibit necrosis and calcifications resembling high grade DCIS [53].

Tubular Carcinoma

Clinical and Histopathological Features

Tubular carcinomas constitute up to 2% of breast carcinomas and are typically detected as an impalpable, small (less than 1 cm), screen-detected tumour [36, 37]. A diagnosis of pure tubular carcinoma requires more than 90% of the tumour to be composed of open tubules lined by a single layer of mildly to moderately enlarged low columnar cells showing apical apocrine

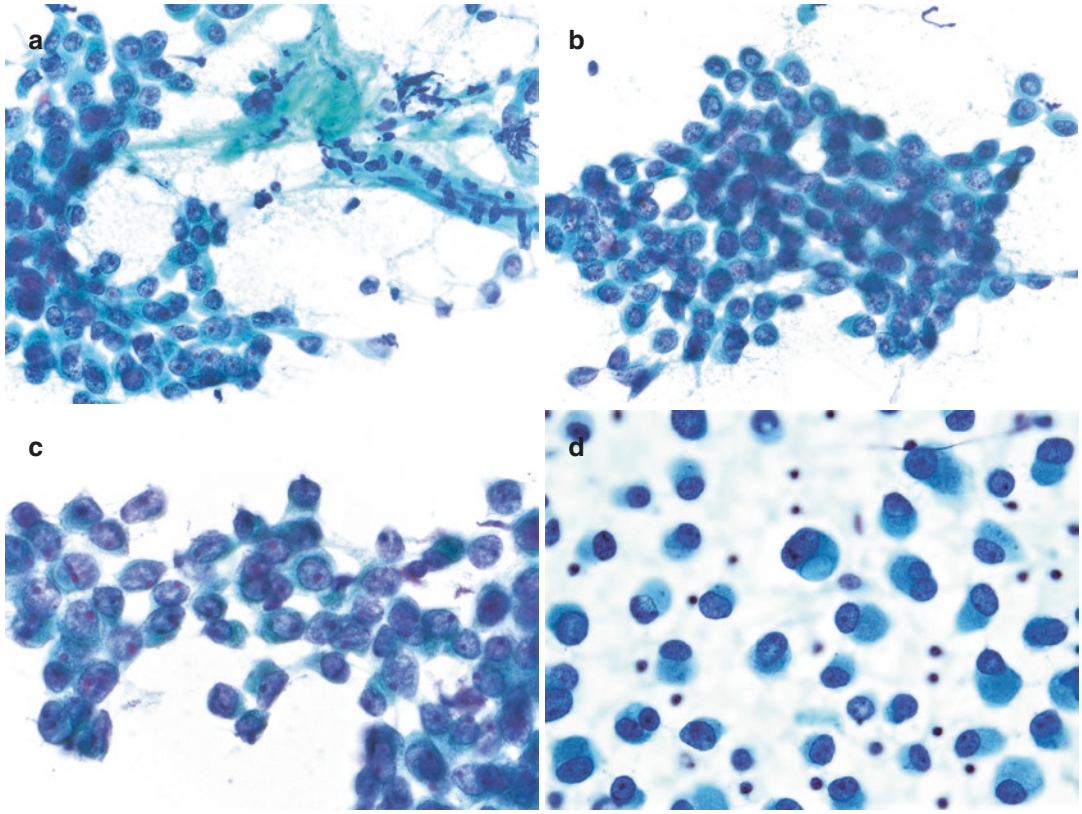


Fig. 6.11 (**a**) Pleomorphic lobular carcinoma with a stromal tuft that includes a capillary (Pap ×40); (**b**) Pleomorphic lobular carcinoma in a small sheet showing discohesion throughout the sheet (Pap ×40); (**c**) Pleomorphic lobular carcinoma showing a discohesive pattern and prominent nucleoli (Pap ×60; (**d**) Pleomorphic lobular carcinoma showing dispersal, eccentric cytoplasm and marked nuclei atypia (Pap ×63)

snouts and mildly to moderately pleomorphic nuclei. The invasive component may be associated with cribriform low grade DCIS and invasive cribriform, lobular or low grade carcinoma NST. There is frequently a background of benign proliferative breast disease.

Imaging

Tubular carcinomas present mammographically as a small, highly spiculated mass, sometimes with fine scattered microcalcifications, indicative of associated ductal carcinoma in situ, within and/or around the tumour mass.

On ultrasound examination, they have classic malignant features of a low-echogenicity tumour centre with surrounding spiculations and tissue disruption.

Key Cytological Diagnostic Criteria

(Fig. 6.12a–e)

- Cellularity is variable, but can be moderate to high.
- The pattern is of small, rigid, angulated or comma-shaped narrow cohesive tubules with occasional monolayered or 3D cribriform tissue fragments lacking myoepithelial cells.
- A variable number of dispersed, atypical epithelial cells with mildly irregular atypical nuclei showing subtle indentations and grooves, mild hyperchromasia and single small nucleoli is precent.
- Cribriform and micropapillary 3D tissue fragments may suggest an intraduct cribriform component or invasive cribriform carcinoma. Sclerotic stromal tufts may be present.
- Bare bipolar nuclei are absent or present in very small numbers [56, 57].

In the alcohol-fixed Pap-stained smears, the focus can be adjusted to demonstrate the 3D tubule, focussing from the most superficial epithelial cells down through the lumen to the deep layer. Apoptotic debris can mimic myoepithelial cell nuclei on the tissue fragments.

Differential Diagnosis

- Tubules can also be seen in fibroadenomas, but in this setting, they lack the rigid architecture, monomorphic cell type, mild nuclear enlargement and atypia of tubular carcinoma and have associated myoepithelial cells with bare bipolar nuclei in the background.
- Low grade intraductal cribriform carcinoma or invasive cribriform carcinoma can be found admixed with tubular and low grade invasive carcinoma NST.
- Sclerosing adenosis can present as a mammographic mass, calcification or architectural

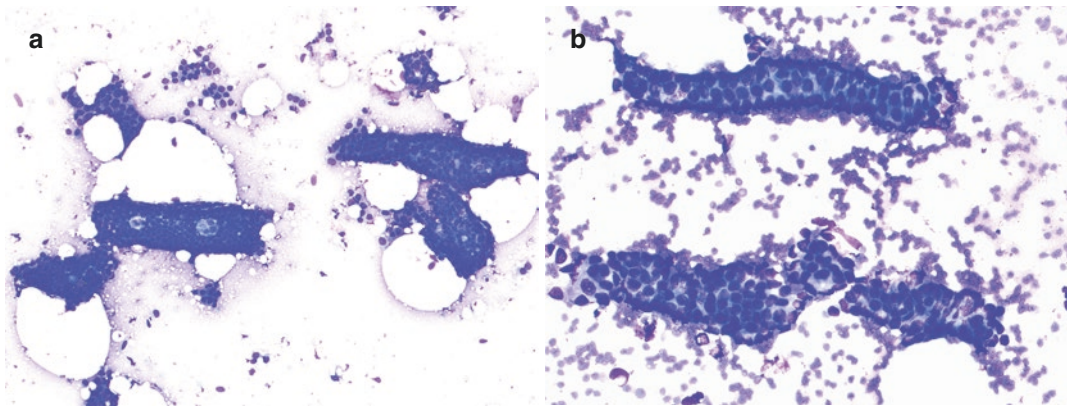


Fig. 6.12 (**a**) Tubular carcinoma showing rigid tubules and occasional small tissue fragments and dispersed cells (Giemsa ×10); (**b**) Tubular carcinoma showing rigid tubules with a tubular architecture (Giemsa ×20; (**c**) Tubular carcinoma showing rigid tubules and small tissue fragments (Pap ×20); (**d**) High power of (**b**) showing tubular carcinoma showing mild nuclear pleomorphism and an absence of myoepithelial cells (Giemsa ×40); (**e**) Tubular carcinoma showing a tubule with mild nuclear atypia and absence of myoepithelial cells and adjacent small tissue fragment (Pap ×40)

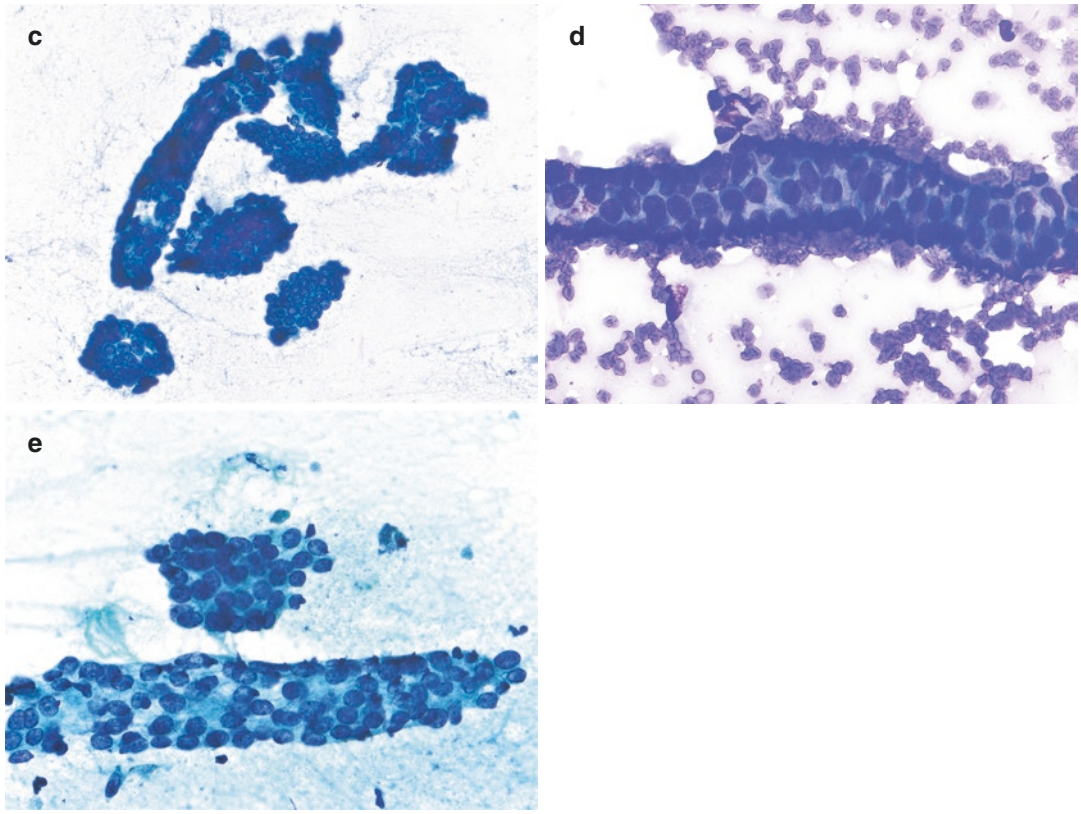


Fig. 6.12 (continued)

distortion and, rarely, as a palpable nodular mass. In FNAB sclerosing adenosis has very small cohesive terminal ductular tissue fragments which have bland small nuclei and usually myoepithelial cells in a clean background with some bare bipolar nuclei, and a specific diagnosis is not usually possible [58]. There may also be partially crushed lobules or portions of lobules.

Carcinoma with Medullary Features

Clinical and Histopathological Features

Carcinomas with medullary features typically present as rapidly growing masses and are formed of syncytial sheets of crowded large cells with highly atypical, sometimes bizarre, large pleomorphic vesicular nuclei and a heavy lymphoplasmacytic infiltrate at the circumscribed peripheral margins [36, 37]. The rare lymphoepithelioma-like carcinoma shows more variable atypia and a prominent lymphoid infiltrate throughout the epithelial component.

This carcinoma is often triple negative and basal-like and may be associated with *BRCA1* germline mutations [59, 60]. Although many *BRCA1*- related tumours show medullary features, only approximately 13% of carcinomas with medullary features are associated with a *BRCA1* mutation [60].

Imaging

Typically carcinomas with medullary features on both mammography and ultrasound examination are relatively circumscribed, rounded masses with pushing margins, rather than spiculations. The margins reflect their rapid growth. The DD

includes benign lesions, such as fibroadenomas and cysts, and malignant lesions, such as mucinous carcinomas, high grade invasive carcinoma NST, lymphoma and metastases.

Key Cytological Diagnostic Criteria

(Fig. 6.13a–c)

- Cellularity is high.
- The pattern consists of small, syncytial sheets of large epithelial cells often infiltrated by small numbers of lymphocytes, and a variable number of dispersed epithelial cells.
- Large tumour cells show a high N:C ratio, considerable pale cytoplasm and large pleomorphic nuclei with coarse chromatin, perinucleolar clearing and macronucleoli.
- Mitoses can be numerous.
- Stripped bizarre nuclei are often plentiful.
- Plentiful lymphocytes, occasional plasma cells, cellular debris and lymphoid cytoplasmic fragments ('lymphoglandular bodies') are present in the background [61, 62].

Differential Diagnosis

- High grade lymphomas are characterised by an almost completely dispersed population of large lymphoid cells, usually resembling immunoblasts or centroblasts, with lymphoid cell cytoplasmic fragments in the background. The cells of a 'large cell lymphoma' are smaller than the large pleomorphic cells of carcinoma with medullary features, which have vesicular nuclei and spiculated large nucleoli.
- High grade ductal carcinoma metastatic to intramammary or axillary tail lymph nodes cannot be readily distinguished from this cancer subtype, and clinical-radiological correlation is required.
- The rare 'lymphoepithelioma-like carcinoma' shows similar epithelial syncytial sheets with high grade, large pleomorphic nuclei, poorly defined cell cytoplasmic margins and plentiful infiltrating lymphocytes in a rich lymphoid background [63].

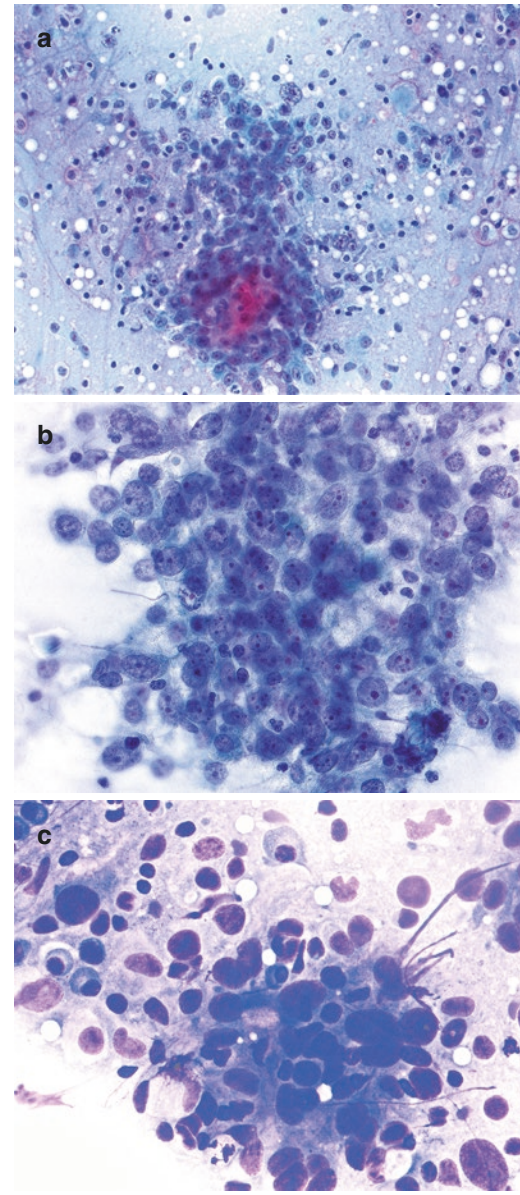


Fig. 6.13 (**a**) Medullary carcinoma showing a small syncytial sheet of high nuclear grade tumour cells infiltrated by occasional lymphocytes with a background of lymphocytes and stripped nuclei (Pap ×20); (**b**) Medullary carcinoma showing tumour cells with markedly enlarged and pleomorphic nuclei and prominent nucleoli with scattered lymphocytes infiltrating the epithelium (Pap ×40); (**c**) Medullary carcinoma showing a small number of lymphocytes infiltrating a syncytial tissue fragment of carcinoma with lymphocytes and plasma cells in the background (Giemsa ×40)

Mucinous Carcinoma

Clinical and Histopathological Features

Pure mucinous carcinoma is rare, constituting approximately 2% of breast carcinomas, and more commonly occurs in patients over 55 years of age who present with a mass or incidentally on screening mammography [36, 37].

In surgical pathology mucinous carcinoma is characterised by small epithelial cell tissue fragments and single cells of small to intermediate size that are dispersed through large amounts of extracellular mucin, which has a pushing margin and dissects around capillaries. Mucinous differentiation can occur focally in association with invasive carcinoma NST and micropapillary, solid papillary and other carcinoma subtypes.

Imaging

Mucinous carcinoma frequently presents as a rounded mass on mammography. It may have multiple small, rounded protrusions or adjacent lesions of similar appearance to the main tumour. Microcalcifications and coarse calcifications may develop within the mucin.

On ultrasound the mucinous content may appear homogeneous with posterior enhancement, suggesting a cystic lesion, but the margins are generally indistinct. In other cases mucinous carcinoma may show mixed solid and cystic components, microlobulation or irregular margins suspicious of malignancy.

The DD includes benign lesions, such as fibroadenomas and cysts, and malignant lesions, such as medullary-like carcinomas and high grade invasive carcinoma NST.

Key Cytological Diagnostic Features

(Fig. 6.14a–e)

- Cellularity is variable but may be high.
- The pattern is of tissue fragments of variable size in a background of copious fibrillary mucin, which is blue to purple in the Giemsa stain and pale green to orange in the Pap stain.
- Epithelial tissue fragments may be balled up, rounded, cribriform, papillary or tubular.
- Mild to moderate nuclear atypia and enlargement are typically present with occasional intracytoplasmic vacuoles or signet ring cells. Occasionally the nuclei may show high grade features.
- Myoepithelial cells and bare bipolar nuclei are not seen.
- Branching anastomosing capillary tissue fragments can be seen.
- Occasionally calcifications may be present [64–66].

Differential Diagnosis

- Mucocele-like lesions with considerable fibrillary mucin can be associated with fibrocystic change and may include ductal epithelial tissue fragments with bland nuclei and myoepithelial cells. They result from extravasation of mucin from ruptured ducts and cysts and show low cellularity.
- Infiltrating carcinoma NST may have focal mucinous differentiation and generally shows high grade nuclei.
- Invasive micropapillary carcinoma has papillary tissue fragments which lack a fibrovascular core and may have an outer mucinous blush and is usually of high nuclear grade.
- Fibroadenomas can have a granular myxoid or ‘mucin-like’ finely granular proteinaceous background.
- Metastatic adenocarcinomas of the colon, rectum and lung can have associated mucin production and necrosis.
- Ultrasound gel is coarsely granular and pinkish purple on the Giemsa stain and should not be mistaken for mucin.

Invasive Micropapillary Carcinoma

Clinical and Histopathological Features

Invasive micropapillary cancers comprise 1–2% of all invasive breast cancers, although a focal micropapillary component can be found in up to 7.4% of breast cancers [37]. This carcinoma has a distinctive architecture of small micropapillary and rounded tissue fragments, without fibrovascular cores, which are found in clear spaces delineated by thin stromal strands. The tissue fragments show reverse polarity with the luminal

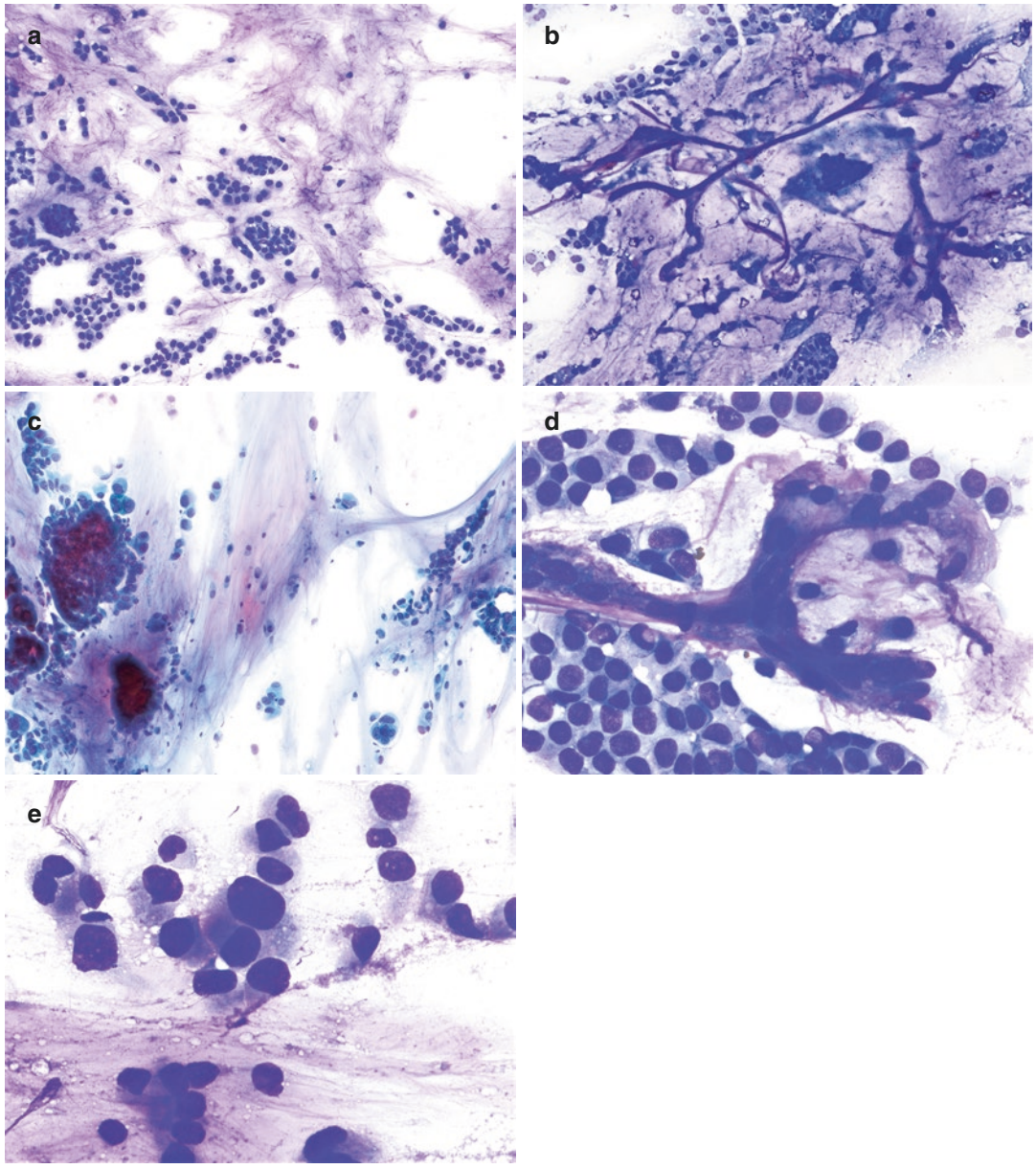


Fig. 6.14 (**a**) Mucinous carcinoma showing fibrillary mucin with sheets and single cells (Giemsa ×10); (**b**) Mucinous carcinoma showing a dissected branching capillary in a background of fibrillary mucin (Giemsa ×10); (**c**) Mucinous carcinoma showing small tissue fragments and dispersed single cells in a fibrillary mucinous background (Pap ×10); (**d**) Mucinous carcinoma showing a dissected capillary and small tissue fragments of carcinoma and a few dispersed single cells in a fibrillary mucinous background; (**e**) Mucinous carcinoma showing dispersed cells in a fibrillary mucinous background (Giemsa ×60)

surface orientated peripherally. Invasive micropapillary carcinoma shows frequent lymphovascular invasion and lymph node metastases.

Imaging

The imaging features are the same as for carcinoma NST.

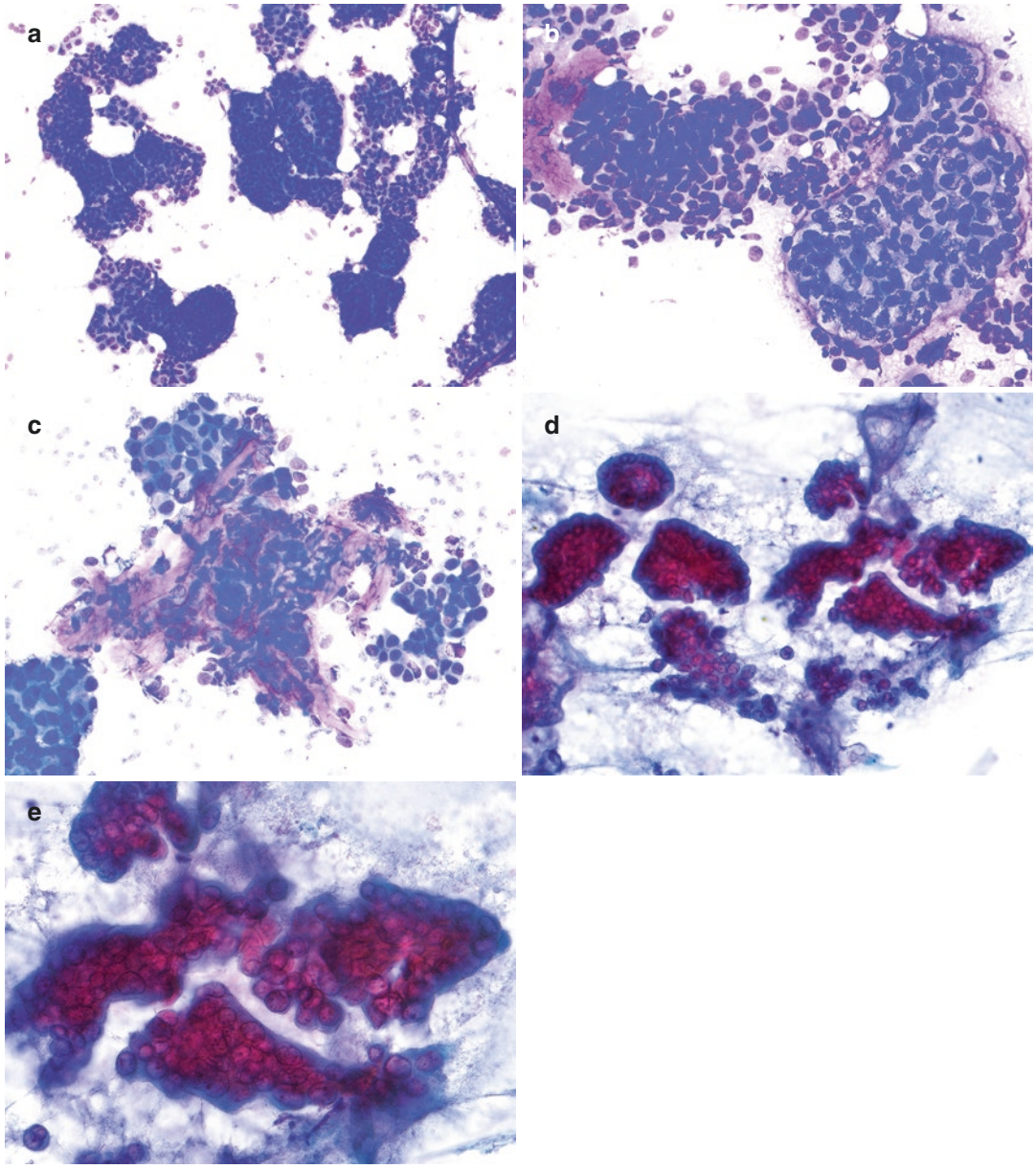


Fig. 6.15 (**a**) Micropapillary carcinoma showing a typical jigsaw pattern of apposed small tissue fragments (Giemsa ×10); (**b**) Micropapillary carcinoma showing two small tissue fragments with a mucinous blush (Giemsa ×20); (**c**) Micropapillary carcinoma showing invasion of a stromal fragment (Giemsa ×20); (**d**) Micropapillary carcinoma showing a typical jigsaw pattern (Pap ×20); (**e**) Micropapillary carcinoma showing jigsaw-like small epithelial tissue fragments of crowded cells with enlarged nuclei and a high N:C ratio (Pap ×40)

Key Cytological Diagnostic Features

(Fig. 6.15a–e)

- Cellularity is moderate to high.
- The pattern is predominantly small tissue fragments and plentiful dispersed cells.
- Micropapillary and rounded or irregularly shaped epithelial tissue fragments are typically closely apposed and may show moulding in a 'jigsaw' pattern.

- The tumour cells have large, atypical, hyperchromatic nuclei with prominent nucleoli and a moderate to high N:C ratio. Some cells may have dense, apocrine-type cytoplasm or eccentric columnar cytoplasm.
- A mucinous blush may be present on the outside of the epithelial tissue fragments, and there may be mucin in the background.
- Necrosis can be present.
- Calcifications may be seen [67, 68].

Differential Diagnosis

- High grade infiltrating carcinoma NST lacks the micropapillary architecture.
- Stellate papillary structures with thick fibroelastotic-vascular cores can be seen in intraductal papillomas, in which the often hyperplastic epithelium is bland.
- Papillary DCIS shows a more delicate branching fibrovascular architecture with low grade nuclear atypia.

Metaplastic Carcinomas

Clinical and Histopathological Features

These are a rare, heterogeneous group of tumours that include squamous cell carcinoma, spindle cell carcinoma and tumours in which a squamous or poorly differentiated carcinoma component is admixed with a sarcomatous element, which can be chondroid, spindled or, rarely, osteogenic or rhabdoid [37]. Low grade adenosquamous carcinoma with tubules, small glands, focally keratinised epithelium and rounded squamous cell nests in a spindle cell background is also included in this category [69].

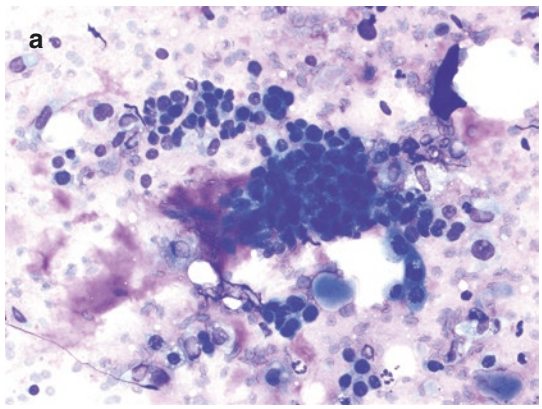
Imaging

Imaging is usually that of a typical invasive cancer, although their appearance is variable, and they may resemble complex cysts when necrosis has occurred.

Key Cytological Diagnostic Criteria

(Figs. 6.16a–d and 6.17a–e)

- Cellularity is moderate to high.
- Variable patterns occur:
  1. Poorly differentiated carcinoma with small discohesive tissue fragments and marked dispersal.
  2. Squamous cell carcinoma in sheets and tissue fragments and a background of keratinous debris.
  3. Low grade adenosquamous carcinoma with small glands and tubules showing focal keratinisation and a prominent spindle cell component.
  4. Sarcomatous plump spindle cells, dispersed or in small tissue fragments.



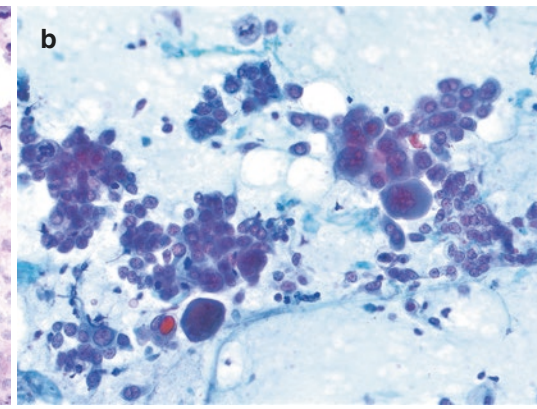


Fig. 6.16 (**a**) Metaplastic squamous cell carcinoma with a stromal tuft (Giemsa ×40); (**b**) Metaplastic squamous cell carcinoma in small tissue fragments with dispersed cells; (**c**) High power of figure (**a**) showing squamous cell carcinoma infiltrating stroma (Giemsa ×40); (**d**) Metaplastic squamous cell carcinoma showing giant cells with markedly atypical nuclei and an atypical mitosis (Pap ×40)

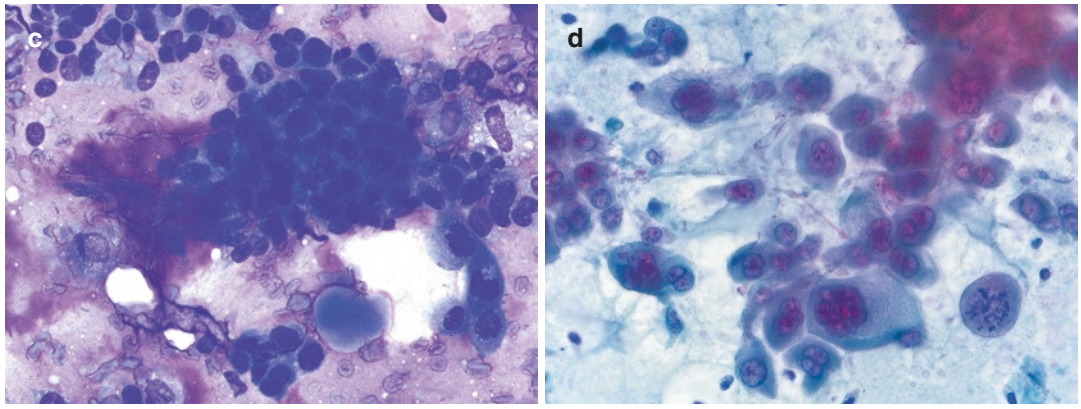
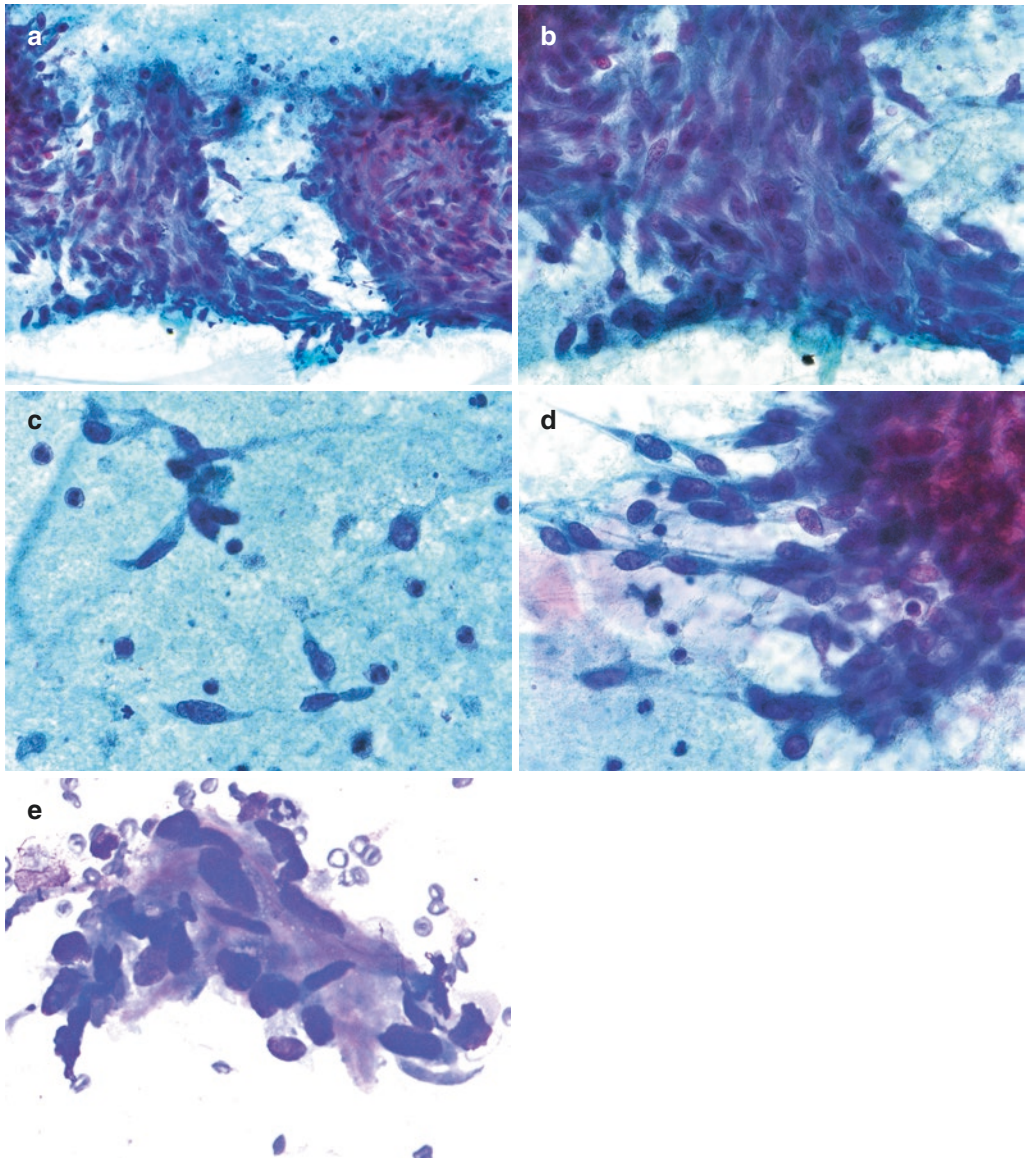


Fig. 6.16 (continued)



5. Fibroblastic, fibromatosis-like spindle cells or chondroid sarcomatous stroma.

- Necrosis is often present.
- Neutrophils, histiocytes, siderophages and debris are frequently seen [70–72].

Differential Diagnosis

- Subareolar chronic abscess with squamous metaplasia of the lactiferous ducts frequently has a history of recurrent subareolar abscesses and shows considerable anucleate keratinous debris, minimal nuclear atypia in the squamous cells and plentiful neutrophils.
- Squamous metaplasia can be seen in infarcted intraductal papillomas or in residual ducts at the necrotic margin of previous excision biopsies, with or without radiation therapy.
- Metastatic squamous cell carcinoma has similar features, and clinical history should always be correlated.
- Skin lesions, including epidermal inclusion cysts [73].
- Malignant phyllodes tumours usually have a biphasic pattern with epithelial and spindle cell tissue fragments, but the epithelial component may be scanty, and this diagnosis should always be considered along with metaplastic spindle cell carcinomas and rare sarcomas in a malignant spindle cell neoplasm in the breast.

Secretory Carcinoma

Clinical and Histopathological Features

Secretory carcinoma is a rare carcinoma, constituting less than 0.15% of all breast cancers and presenting at a wide variety of ages with a median age of 40 [37]. Patients present with a slow-growing, painless, circumscribed, mobile palpable mass, with rare nodal metastases. The carcinoma is characterised histopathologically by intra- and extracellular secretory material and low-nuclear-grade tumour cells with vacuolated or amphophilic cytoplasm. Most secretory carcinomas are oestrogen, progesterone and HER2-negative and cytokeratin 5/6 and cytokeratin 14-positive, falling within the spectrum of triple negative, basal-like carcinoma [74]. They also show the same ETV6-NTRK3 gene fusion that is characteristic of mammary analogue secretory carcinoma of the salivary gland [75].

Imaging

There is usually a solid, well-circumscribed mass, with smooth or mildly irregular margins, mimicking both fibroadenomas and well-circumscribed carcinomas, such as medullary, solid papillary and mucinous carcinomas.

Key Diagnostic Cytological Criteria

(Fig. 6.18a–c)

- Cellularity is moderate to high.
- The pattern consists of cohesive or loosely cohesive sheets and small tissue fragments.
- Epithelial cells are round to polygonal with abundant cytoplasm containing prominent intracytoplasmic vacuoles. On occasion, there may be abundant granular cytoplasm [74, 76].
- Abundant intensely staining ‘bubbly’ secretory material is present in the background.
- Nuclei are round with fine to moderately coarse chromatin and show mild atypia with small nucleoli.
- Occasional signet ring cells or plasmacytoid cells are present.
- Stripped nuclei with mild atypia may be present [76, 77].

Differential Diagnosis

- *Lactational change* has a milky proteinaceous background with fine fat globules which mimic the background seen in secretory carcinoma, but there are plentiful monotonous bland, round stripped acinar nuclei with single small nucleoli, some single dissociated small acinar cells and small acinar cell sheets with individual cells showing vacuolation.

Fig. 6.17 (**a**) Spindle squamous cell carcinoma in a necrotic background (Pap ×20); (**b**) High power of spindle squamous cell carcinoma showing crowded spindle cells with atypical nuclei (Pap ×40); (**c**) Spindle squamous cell carcinoma showing discrete spindle cells in a necrotic background (Pap ×40); (**d**) Spindle cell squamous cell carcinoma showing spindle cells at the margin of a larger tissue fragment (Pap ×40); (**e**) Spindle cell squamous cell carcinoma (Giemsa ×40)

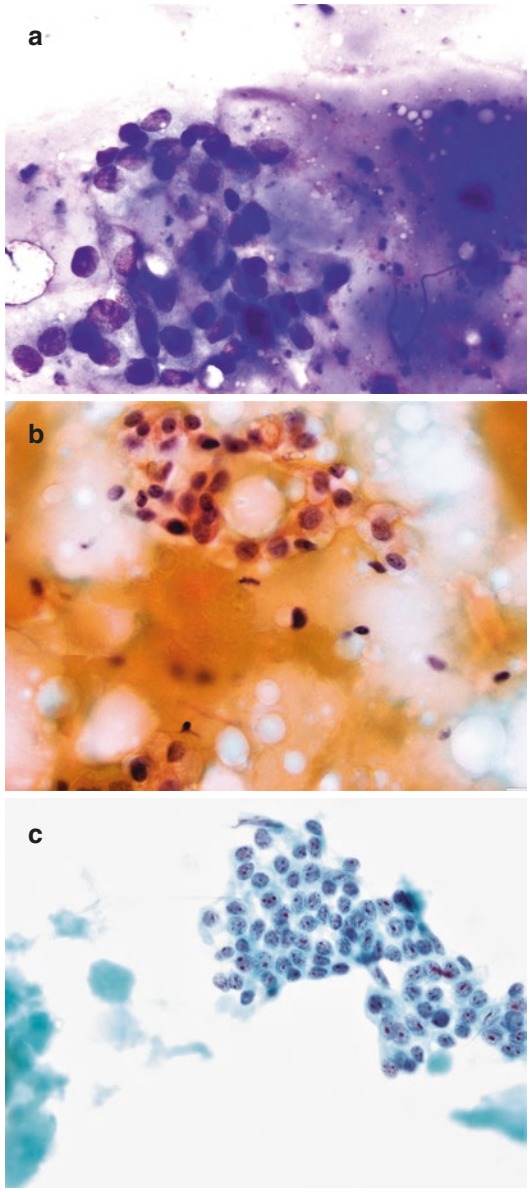


Fig. 6.18 (**a**) Secretory carcinoma showing a secretory background with a small tissue fragment of carcinoma (Giemsa ×40); (**b**) Secretory carcinoma showing a bubbly background and small carcinoma cells with low grade nuclei and often several secretory vacuoles (Pap ×40); (**c**) Secretory carcinoma showing a small sheet of small- to intermediate-sized cells with rounded nuclei and nucleoli (Pap ×40)

Occasional lactational lobules may be seen. Distinction may be difficult, and clinical correlation is essential.

- Cystic hypersecretory hyperplasia shows background extracellular secretions, which appear colloid-like and dense, and lack the bubbly features of secretory carcinoma. The ductal epithelial cells present have only occasional cytoplasmic vacuoles.
- Mucinous carcinoma has fibrillary mucin rather than bubbly extracellular material, and the epithelial component shows mild to moderate nuclear atypia.

Glycogen-Rich Clear Cell Carcinoma

Clinical and Histopathological Features

This is a rare subtype of breast cancer constituting 1–3% of all carcinomas, depending on the definition [37]. The current WHO Classification requires more than 90% of the tumour to be composed of clear cells showing a variable architecture which can be papillary, solid, nested or sheetlike and may also show focal apocrine change.

Imaging

These tumours present as a mass with an irregular border and may show fine to coarse calcifications.

Key Cytological Diagnostic Criteria

- Cellularity is moderate to high.
- The pattern shows small loosely cohesive syncytial sheets and single cells in a finely granular 'tigroid' background.
- Tumour cells show finely granular, variably 'cleared', often eccentric cytoplasm with well-defined cell membranes and a possible plasmacytoid appearance.
- Moderate to marked nuclear pleomorphism is seen.
- Koilocyte-like cells can occur [78].

Differential Diagnosis

- Lipid-rich carcinoma shows similar cells, although cytoplasmic vacuoles contain lipid, which is oil red O-positive and PAS glycogen-negative [79].
- Infiltrating carcinoma NST can show focal clear cell components.
- Apocrine carcinomas can show considerable eosinophilic granular cytoplasm.

- Metastatic carcinoma, such as renal cell carcinoma and ovarian carcinoma, can show clear cell features.
- Histiocytoid carcinoma has large cells with considerable granular to finely vacuolated, often eccentric cytoplasm resembling histiocytes, in addition to large, hyperchromatic, pleomorphic nuclei and prominent nucleoli [80].

Adenoid Cystic Carcinoma

Clinical and Histopathological Features

These rare breast carcinomas generally present in older women and have a good prognosis. Histological features are similar to those seen in the salivary gland, including cribriform, tubular, solid or basaloid growth patterns and varying degrees of nuclear atypia [37].

Imaging

Radiological presentation is variable, from a rounded mass to a stellate lesion.

Key Cytological Diagnostic Criteria

- Cellularity is moderate.
- The pattern consists of variably small to large epithelial sheets and 3D tissue fragments, which often contain rounded stromal globules, and plentiful dispersed epithelial cells and stripped epithelial nuclei.
- The basaloid epithelial cells have poorly defined, scanty cytoplasm and mildly enlarged, rounded, mildly pleomorphic nuclei with coarse chromatin and small single nucleoli.
- Hyaline globules are magenta in the Giemsa stain and pale greyish green in the Pap stain.
- Stripped oval epithelial nuclei resembling bare bipolar nuclei can be prominent in the background [81, 82].

The cytological features are the same as adenoid cystic carcinoma arising in the salivary glands. Scattered large epithelial tissue fragments with cribriform spaces containing hyaline or granular material may be present. If a cell block is available, the epithelium of the tubules is CD117 and cytokeratin 5/ 6-positive, with associated diastase-resistant PAS-positive mucin, whereas the basal myoepithelial cells are S100, P63 and calponin positive. Oestrogen and progesterone receptors and HER2 are negative, and EGFR may be positive. This carcinoma subtype is also within the spectrum of triple negative, basal-like carcinomas with a good prognosis and low incidence of axillary metastases.

Differential Diagnosis

- Collagenous spherulosis is characterised by tissue fragments of ductal epithelial cells containing small collagenous spherules surrounded by bland epithelium with myoepithelial cells rimming the spherules and bare bipolar nuclei in the background (Fig. 3.25a, b). If fibrocystic change is present with epithelial hyperplasia, and the collagenous spherules are small and only seen focally, then collagenous spherulosis can be diagnosed. However, if the collagenous spherules are large and plentiful, the possibility of adenoid cystic carcinoma should be suggested and CNB requested.
- Pleomorphic adenoma is very rare in the breast but usually includes prominent myxofibrillary stroma and a bland epithelial component [83].

Carcinomas with Apocrine Differentiation

Clinical and Histopathological Features

Extensive apocrine differentiation is seen in approximately 4% of invasive breast carcinomas [37], whereas focal apocrine differentiation is a common feature in invasive carcinoma NST and does not alter the clinical outcome. It may also occur focally in most other special-type carcinomas, including lobular and medullary carcinoma, and in both lobular and ductal carcinoma in situ. The abundant eosinophilic cytoplasm is granular, and the enlarged pleomorphic nuclei contain large nucleoli with occasional inclusions. The typical immunophenotype of carcinoma with apocrine differentiation is the expression of GCDFP15, androgen receptor and HER2 and the absence of ER and PR, although these may be positive in some cases [37]. An androgen signature is also identified by gene expression array analysis, show-

ing increased androgen signalling and significant overlap with HER2-positive carcinomas [84].

Key Cytological Diagnostic Features

(Fig. 6.19a–c)

- Moderate to high cellularity.
- A pattern of dispersed single cells and plentiful crowded, small, discohesive tissue fragments.
- Large pleomorphic cells with considerable granular, well-defined cytoplasm resembling apocrine metaplasia and large pleomorphic nuclei with hyperchromatic, coarse chromatin, perinucleolar clearing and often single and spiculated large nucleoli. Necrosis can be present and, with calcifications, can suggest an intraductal component [42].

Differential Diagnosis

- Metaplastic apocrine epithelium may show some nuclear atypia in the setting of fibrocystic change or inflammation but retains an ordered arrangement of nuclei and generally a low N:C ratio, without the coarse hyperchromasia and pleomorphic nucleoli of apocrine carcinoma (Fig. 3.14a, b; 3.15a, b). Benign apocrine cells frequently disperse, and myoepithelial cells can be scanty in apocrine sheets, whereas a proteinaceous background may dilute bare bipolar nuclei (Fig. 3.6a–c).
- Granular cell tumour cells have finely granular, abundant cytoplasm with a low N:C ratio and central, rounded, minimally pleomorphic nuclei with small nucleoli (Fig. 6.8a–c). The cells are dispersed in a finely granular background with some fibrovascular tissue fragments. They are S100-positive and oestrogen receptor-negative.

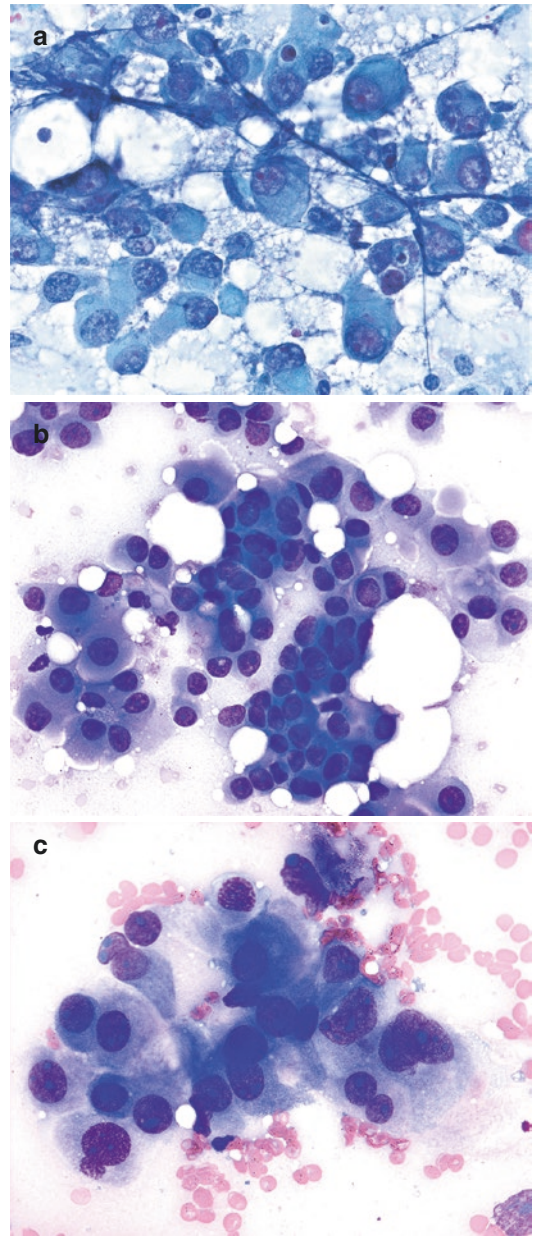


Fig. 6.19 (**a**) Apocrine carcinoma showing large cells with markedly pleomorphic nuclei, prominent nucleoli and abundant granular cytoplasm (Pap ×40); (**b**) Apocrine carcinoma showing abundant granular cytoplasm. (Giemsa ×40); (**c**) Apocrine carcinoma showing high grade nuclei and abundant granular cytoplasm. (Giemsa ×63)

Carcinoma with Neuroendocrine Features

Clinical and Histopathological Features

The presentation of carcinoma with neuroendocrine features is not distinguishable from other

breast carcinomas. They are rare, constituting less than 1% of breast carcinomas, and exhibit cytological and immunohistochemical similarities to neuroendocrine tumours of the gastrointestinal tract and lung [37].

There is a range of differentiation from well differentiated neuroendocrine tumours resembling carcinoid tumours, to carcinoma of the breast with focal neuroendocrine features to poorly differentiated small cell carcinoma, resembling its counterpart in the lung [85]. Mucinous and solid papillary intraductal carcinomas both frequently show neuroendocrine differentiation [85–87].

Key Cytological Diagnostic Features
(Fig. 6.20)

Well Differentiated Neuroendocrine Tumors

- Cellularity can be high.
- Dispersed cells, small sheets and larger fragments consisting of branching thin fibrovascular strands, and tumour cells are present.
- Tumour cells are monotonous with bland, small, rounded nuclei with coarse chromatin and small nucleoli and often appear plasmacytoid with eccentric pale granular cytoplasm.

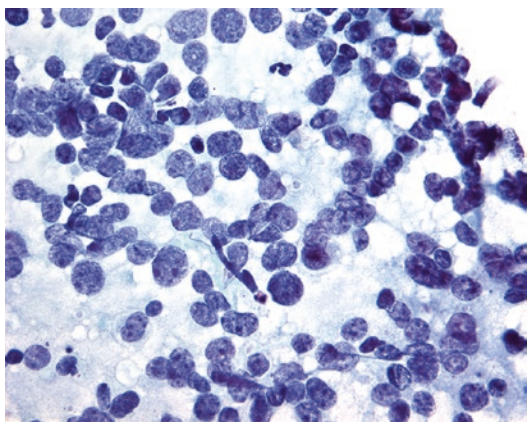


Fig. 6.20 High grade neuroendocrine carcinoma of the breast showing a dispersed pattern and neuroendocrine granular chromatin. (Pap ×63)

Poorly Differentiated (Small Cell Carcinoma)

- Resembles small cell neuroendocrine carcinoma of the lung [88].

Differential Diagnosis

The DD is primarily metastatic carcinoid tumour or small cell carcinoma of the lung and GIT, and clinical and imaging correlation, combined with immunohistochemistry on cell block material or CNB, is required [85].

Non-Hodgkin Lymphoma

Primary lymphomas of the breast comprise less than 0.5% of primary breast tumours [37] and are defined as dominant single or multi-nodular masses in the breast in patients without a prior history of lymphoma elsewhere [89]. The diagnosis of a primary lymphoma relies on the clinical and imaging exclusion of lymphoma at other sites.

FNAB of high grade lymphomas can be diagnostic of malignancy, whereas FNAB supported by flow cytometry, cytogenetics and/or gene rearrangement studies allows a specific diagnosis of lymphoma in almost all cases [89, 90]. CNB may be required for definitive diagnosis and subtyping.

Diffuse large B-cell lymphoma is the most common type, accounting for up to two thirds of all breast lymphomas [89–91]. The key cytological features are the marked dispersal of atypical large lymphoid cells resembling centroblasts or immunoblasts with eccentric basophilic cytoplasm, in a background of fragments of lymphoid cell cytoplasm. Marginal zone lymphoma involving mucosa-associated lymphoid proliferations shows a heterogeneous lymphoid population of plasmacytoid cells, lymphocytes, centrocytes, plasma cells and scattered larger lymphoid cells and is difficult to distinguish from a reactive process, requiring ancillary studies for confirmation of monoclonality and usually a CNB.

Burkitt lymphoma, follicular lymphoma, acute leukaemia [92] and T-cell lymphoma can all occur in the breast and show typical cytomorphology and lymphoma markers [89–93].

Anaplastic large cell lymphoma related to textured prosthetic breast implants is also now well recognised [94]. This tumour is most frequently recognised in the setting of a new peri-implant fluid accumulation and may be diagnosed on the fluid submitted for cytology. The tumour consists of large lymphoid cells with highly atypical hyperchromatic large nuclei with prominent one or two nucleoli and a rim of cytoplasm. Nuclear pleomorphism is marked, and horseshoe-shaped nuclei consistent with 'hallmark' cells may be present. The tumour is positive for CD30 and some T-cell markers on cell block material (Fig. 6.21a–d).

Angiosarcoma

Clinical and Histopathological Features

Angiosarcoma is a rare breast tumour but is the commonest primary sarcoma of the breast [37]. It can present de novo in young women as a mass or bluish discoloration of the skin and can also occur at a median of 6 years post radiation therapy in older women. It occurs primarily in the skin but sometimes involves the chest wall or breast parenchyma.

Histologically, it ranges from well-differentiated anastomosing tiny vascular channels dissecting fat tissue to poorly differentiated solid, spindled or epithelioid tumour sheets showing a range of nuclear atypia and proliferation.

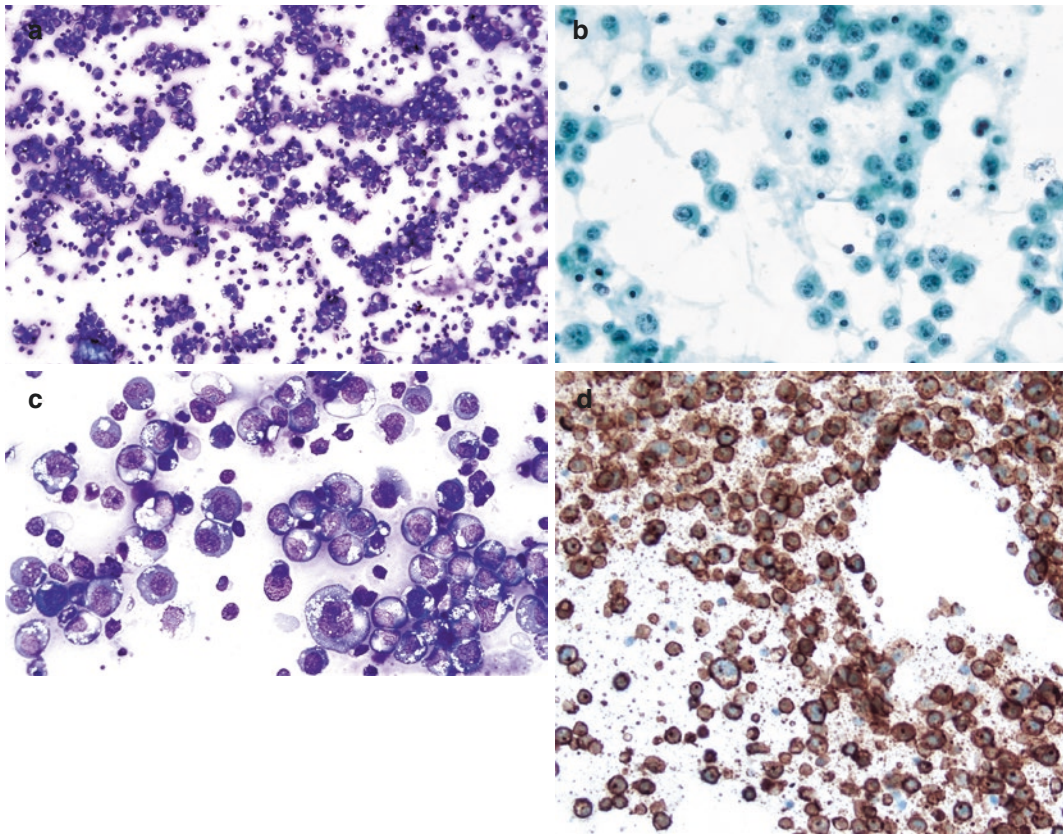


Fig. 6.21 (**a**) Anaplastic large cell lymphoma showing a dispersed lymphoid pattern (Giemsa ×10); (**b**) Anaplastic large cell lymphoma showing a dispersed pattern (Pap ×10); (**c**) Anaplastic large cell lymphoma showing large lymphoid cells with large pleomorphic nuclei and prominent nucleoli, some with fine cytoplasmic vacuoles (Giemsa ×40); (**d**) Anaplastic large cell lymphoma in a cell block showing positive staining for CD30 (IHC ×20)

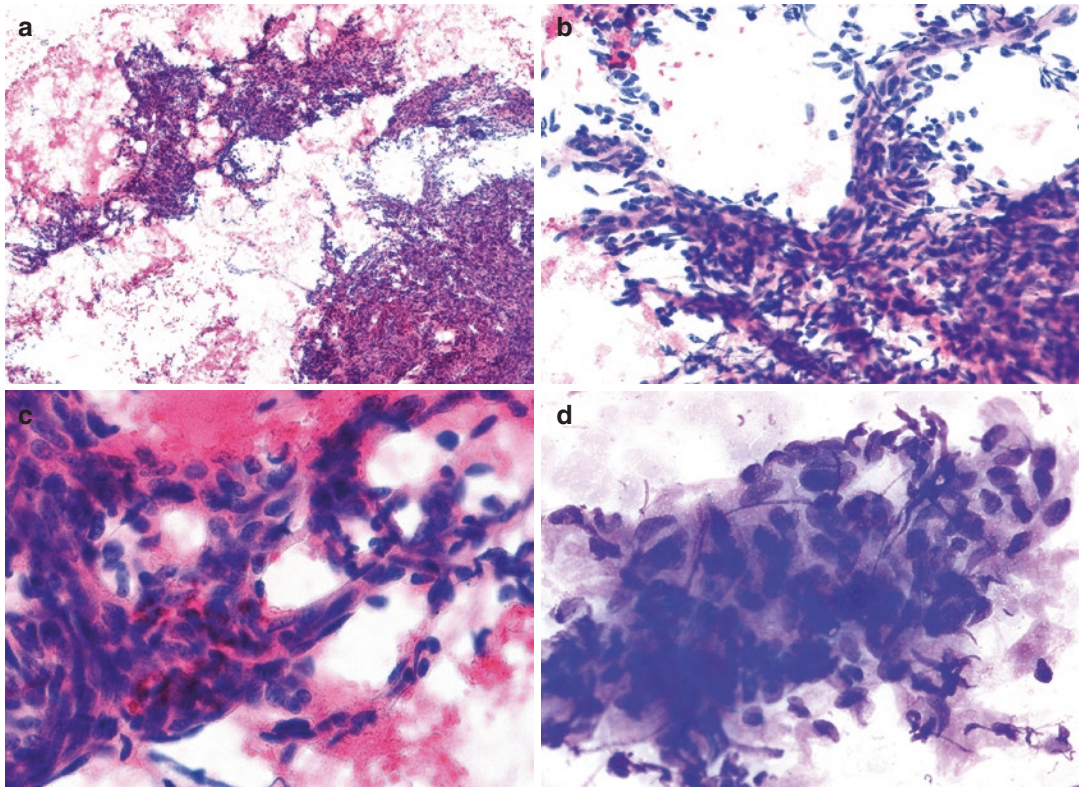


Fig. 6.22 (**a**) Angiosarcoma showing large tissue fragments of crowded spindle cells (H&E ×5; Fig. 22a–c Courtesy of Professor Pamela Michelow, Johannesburg); (**b**) High power showing branching small vessels and spindle cells (H&E ×20); (**c**) Angiosarcoma showing branching atypical vessels and spindle cells (H&E ×40); (**d**) Angiosarcoma showing small tissue fragment consisting of pleomorphic spindle cells and magenta stroma (Giemsa ×40). (Fig. 22d Sourced from A. Field)

Key Cytological Diagnostic Criteria

(Fig. 6.22a–d)

- Cellularity varies from usually low to high.
- Small, loosely cohesive spindle cell tissue fragments and larger tissue fragments containing capillary-like structures are seen in a haemorrhagic background.
- Single spindle, sarcomatous or rounded, epithelioid cells show variable but often marked, nuclear atypia with hyperchromasia, nuclear indentations and folds and one to two nucleoli.
- Intracytoplasmic haemosiderin and vacuoles may be present [95].

In cell block material, angiosarcomas are positive for CD31, CD34, ERG and D2–40 and negative for S100 and, in most cases, cytokeratins.

Once suggested on FNAB, CNB should be recommended to confirm the diagnosis.

Differential Diagnosis

- Granulation tissue shows tissue fragments consisting of branching, anastomosing, well-formed capillaries with surrounding fibroblasts, histiocytes and inflammatory cells with minimal, if any, nuclear atypia (Fig. 3.7b–c). There is usually a history of previous surgery or infection.
- Spindle cell lesions of breast include spindle cell carcinoma and a range of soft tissue lesions, including nodular fasciitis [96, 97]. Malignant fibrous histiocytoma and high grade phyllodes tumor must also be considered [96, 97], and Kaposi sarcoma, which usually occurs in immune-suppressed patients, is rare.

Metastases to the Breast

Metastases to the breast are relatively uncommon and range from 0.46% to 5% of malignant breast FNAB. There is usually a history of malignancy at another site, although the breast FNAB may be the first diagnosis of a metastasis from a known primary tumour or may be the first presentation of malignancy at another site. In children, the commonest metastases to the breast are rhabdomyosarcoma and lymphoma, and in young adults, the diagnosis of malignancy on a breast or chest wall FNAB demands exclusion of another primary malignancy with full IHC workup of a cell block or CNB [98]. The keys to the diagnosis are the clinical history and features that are more typical of a lesion from another body site and not of one of the variants of carcinoma of the breast. Imaging may show a single or multiple, rounded, circumscribed lesions.

Breast carcinoma can metastasise to the contralateral breast, and to determine whether bilateral breast carcinomas represent two primaries or a metastasis from one breast to the other, the cytologic features and the prognostic indicators by IHC studies on the cell block should be assessed. However, the distinction may not be possible.

An IHC panel for breast carcinoma should be used, including ER, PR, GATA3, CK7 and HER2, as well as specific IHC for any suspected non-breast primary, such as TTF1 for lung adenocarcinoma, MART1 and S100 for melanoma, CDX2 and CK20 for colorectal carcinoma and WTI and PAX8 for ovarian carcinoma [98].

Sample Reports

Specific scenarios when the diagnosis of 'malignant' is appropriate

Example 1

Highly cellular smears with a pattern of small epithelial tissue fragments consisting of large cells and similar dispersed single cells in the background, showing large, pleomorphic nuclei with coarse chromatin and large irregular nuclei.

Malignant

These highly cellular smears show plentiful dispersed cells and small epithelial tissue fragments consisting of similar cells, with large pleomorphic hyperchromatic nuclei.

Comment: The features are those of carcinoma of the breast.

Example 2

A moderate number of small- to intermediate-sized epithelial cells dispersed singly or in minute strands or tissue fragments are present and show mild nuclear atypia and eccentric cytoplasm, with or without intracytoplasmic vacuoles.

Malignant

These mildly cellular smears show dispersed small epithelial cells with small- to intermediate-sized atypical nuclei, eccentric cytoplasm and occasional vacuoles, some of which contain mucin droplets.

Comment: The features are those of carcinoma of the breast and suggest lobular carcinoma.

Example 3

Prominent necrosis is present in the background with a small amount of epithelium consisting of pleomorphic cells with large markedly atypical nuclei and occasional small sclerotic stromal fragments infiltrated by the same atypical cells, with or without associated calcifications.

Malignant

Scattered large atypical cells are seen singly and in small tissue fragments with occasional sclerotic stromal fragments

infiltrated by similar atypical cells, in a background of a large amount of necrosis with occasional calcifications.

Comment: Carcinoma of the breast is present with features suggestive of a high grade intraductal component.

Example 4

Plentiful single large pleomorphic cells with large markedly atypical nuclei, and tissue fragments of similar cells are seen with a background separate population of occasional cohesive ductal epithelial tissue fragments with myoepithelial cells.

Malignant

These moderately cellular smears show large pleomorphic cells with large atypical nuclei and single dispersed atypical cells. Benign epithelial material is also present.

Comment: The features are those of carcinoma of the breast.

Example 5

Numerous large irregular hypercellular fibrillary stromal fragments and dispersed single large plump spindle cells with marked nuclear pleomorphism, enlargement and hyperchromasia are present, with hyperplastic ductal epithelial tissue fragments with myoepithelial cells and necrosis.

Malignant

These highly cellular smears show large, hypercellular stromal fragments and marked nuclear atypia of dispersed plump spindle cells with focal necrosis.

Comment: The features suggest a high grade phyllodes tumor, but a high grade spindle sarcoma or metaplastic spindle cell carcinoma should also be considered. Core needle biopsy is recommended.

Example 6

There is a fibrillary mucinous background with moderate epithelial cellularity consisting of single epithelial cells and small tissue fragments showing mild to moderate nuclear enlargement or pleomorphism.

Malignant

These moderately cellular smears show moderately atypical epithelial cells in a background of abundant fibrillary mucin.

Comment: The features are those of mucinous carcinoma.

Example 7

Moderately cellular smears with dispersed large cells, which are also seen in small discohesive tissue fragments, in a background of focal necrosis, apoptotic debris, histiocytes and occasional calcifications.

Malignant

These moderately cellular smears show small tissue fragments and dispersed large atypical cells in a background of focal necrosis. Calcifications are seen.

Comment: The features are those of carcinoma with features suggestive of a high grade intraductal component. Core needle biopsy is recommended to confirm that invasive carcinoma is present.

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7 An Approach to the Interpretation of Breast Fine Needle Aspiration Biopsy Cytopathology Direct Smears

Andrew S. Field, Wendy A. Raymond, and Fernando Schmitt

Introduction

In interpreting breast fine needle aspiration biopsy (FNAB) direct smears, it is important to recognise that common benign lesions of the breast can produce high cellularity, similar to that seen in high-grade invasive carcinomas. In addition, some benign lesions can show varying degrees of single cell dispersal, mild nuclear enlargement and atypia and prominent nucleoli, which can also be seen in carcinoma. It is therefore recommended that when assessing breast FNAB smears, the cellularity and pattern of the material on the slides should be assessed at low power, such as the 5, 10 or 20× objective, before proceeding to high power on the 40× objective to assess individual tissue fragments, cells and nuclei [1]. The low power impression is then confirmed or altered by the high power assessment, with further switching from low to high power until the features can be integrated into a diagnosis or a differential diagnosis (DD) can be established.

When assessing the *cellularity*, each cytopathologist and each department will have its own degrees of cellularity, based on the material that they regularly see. The cellularity can be labelled as scanty, low, moderate or marked. Cellularity will vary with the technique used to perform the FNAB and with the skill and expertise of the operator. Cellularity is a summation of the material present on all the slides and only relates to epithelial cells, without any inflammatory component, although some lesions do not require epithelium for a diagnosis to be made.

When assessing the *pattern at low power* (a 2×, 4×, 5×, 10× or 20× objective), the degree of smearing artefact should be assessed because this will influence the pattern of the spread of material on the slide. Usually the central part of a smear will show the diagnostic pattern. Overly firm smearing will cause single cell dispersal admixed with crushed cells and chromatinic smearing, while insufficient pressure will lead to poor distribution of tissue fragments and overlapping material.

The *pattern at low power* can be diagnostic or it may raise a DD that can be refined by assessment of the various components of the smears at *high power*. Initially this will involve looking at

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A. S. Field et al. (eds.), *The International Academy of Cytology Yokohama System for Reporting Breast Fine Needle Aspiration Biopsy Cytopathology*, https://doi.org/10.1007/978-3-030-26883-1_7

the shape and size and architecture of the epithelial and stromal tissue fragments, and assessing the degree of crowding or nuclear enlargement of the epithelial and stromal cells and the presence or absence of myoepithelial cells. The dispersed cells if any in the background should then be assessed for their nuclear to cytoplasmic (N:C) ratio and nuclear atypia, and the presence or absence of stripped myoepithelial 'bare bipolar nuclei' should be established. The features of the tissue fragments and the dispersed cells are correlated with the low power pattern impression. If the high power assessment does not correlate and is discrepant from the low power appearance, then the diagnostic process is recommenced until the low and high power features can be integrated in a report.

Specific Patterns

A normal breast produces smears of low cellularity with a pattern of *small epithelial tissue fragments containing both ductal and myoepithelial nuclei and a small number of stripped myoepithelial nuclei or bare bipolar nuclei in the background* (Fig. 7.1). The small tissue fragments represent terminal ductules and small ducts, and fragmented or whole lobules and some fat fragments may also be present (Fig. 3.5a–g). Other smears with a similar pattern of small epithelial cell tissue fragments

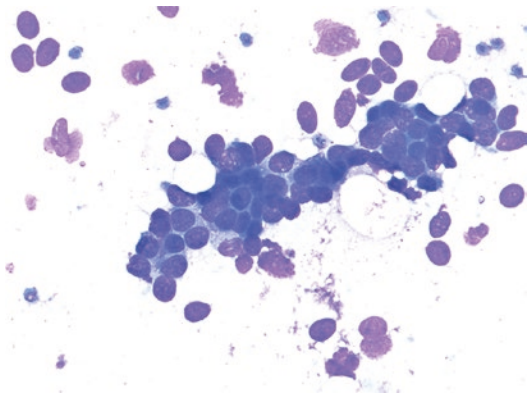


Fig. 7.1 Normal breast tissue showing a small tissue fragment of a terminal ductule with myoepithelial cells and bare bipolar nuclei in the background (Giemsa ×40)

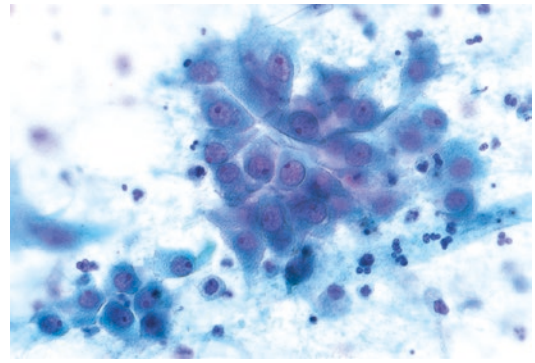


Fig. 7.2 Small sheet of inflamed apocrine cells with adjacent single apocrine cells in a background of suppuration and neutrophils; note the low nuclear to cytoplasmic ratio of the apocrine cells with uniform oval nuclei with fine chromatin and single nucleoli associated with the inflammation (Pap ×40)

can be seen due to undersampling of sclerotic lesions, such as sclerosed fibroadenomas or poor FNAB technique (see Chap. 3, Benign).

The pattern may be one of *predominantly inflammatory cells*, either suppurative, such as in a breast abscess or inflamed cyst (Fig. 7.2; Fig. 3.7a–d), or granulomatous as seen in mycobacterial infection or a reaction to foreign material, such as silicone (Fig. 3.9a, b). If the inflammatory cells are lymphoid, the DD includes an intramammary lymph node or possibly lymphoma, medullary carcinoma or the uncommon lymphocytic lobulitis (see Chap. 3, Benign).

A very common finding in breast FNAB cytology is that of a *cystic or finely granular proteinaceous background*, which can be associated with histiocytes, with multinucleated histiocytes or with debris. When this is found in a patient with a rounded lesion on mammography and ultrasound, which drains completely with the FNAB leaving no residual mass, then the lesion can be categorically regarded as a cyst and the cytology material as 'cyst contents' (Fig. 7.3a). If there are apocrine cells in sheets or as single cells admixed within the proteinaceous background along with histiocytes, then the lesion can be safely regarded as a 'cyst' (Fig. 7.3b, c). In some cases the apocrine cells may be hyperplastic or even show a micropapillary architecture, consistent with a cyst with apocrine hyperplasia (see Chap. 3, Benign).

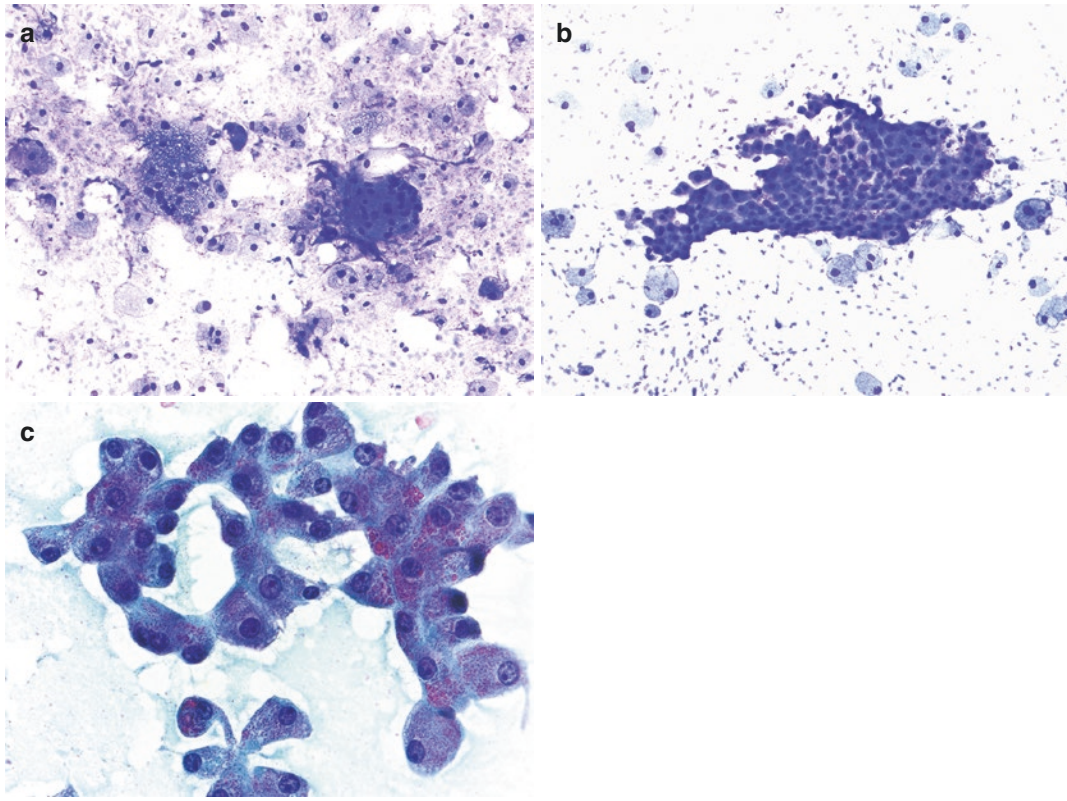


Fig. 7.3 (**a**) Cyst contents with plentiful histiocytes and multinucleated histiocytes and occasional cholesterol crystals in a proteinaceous background (Giemsa ×10); (**b**) A cyst showing a sheet of apocrine cells in a proteinaceous background with plentiful histiocytes (Giemsa ×10); (**c**) Sheet of apocrine cells with round nuclei, single nucleoli and reddish granules in their abundant cytoplasm in a proteinaceous background from a cyst (Pap ×40)

If there are *small tissue fragments of ductal epithelial cells with myoepithelial cells in a proteinaceous background with apocrine sheets*, then this is characteristic of fibrocystic change (Fig. 7.4a–c). A proteinaceous background may also be seen with any intraductal lesion (see Chap. 3, Benign).

Most common benign lesions of the breast, such as usual epithelial hyperplasia and fibroadenomas, produce moderate to high cellularity associated with a pattern of *predominantly large epithelial tissue fragments showing minimal nuclear pleomorphism and plentiful myoepithelial cells, bare bipolar nuclei in the background and a variable number of smaller cohesive epithelial tissue fragments and dispersed cells* (Fig. 7.5a–c) (see Chap. 3, Benign).

Epithelial hyperplasia is commonly associated with fibrocystic change in a pattern of *large epithelial tissue fragments with myoepithelial cells in a proteinaceous background with histiocytes and apocrine sheets* (Fig. 7.6a, b). Radial scars have similar features, but usually with more marked cellularity and occasional tubules, and require correlation with imaging to make the diagnosis (see Chap. 3, Benign) [2].

Fibroadenomas usually show the same pattern as epithelial hyperplasia of hyperplastic epithelial tissue fragments and add plentiful bare bipolar nuclei in the background plus large or small stromal fragments, which can be myxoid, fibrillary or fibrotic (Fig. 7.7a, b). Similarly, intraductal papillomas show the epithelial hyperplasia pattern, and add stellate papillary and more complex meshwork fragments of stroma and tubules, and apocrine sheets and siderophages in a proteinaceous background (Fig. 7.8a, b) (see Chap. 3, Benign).

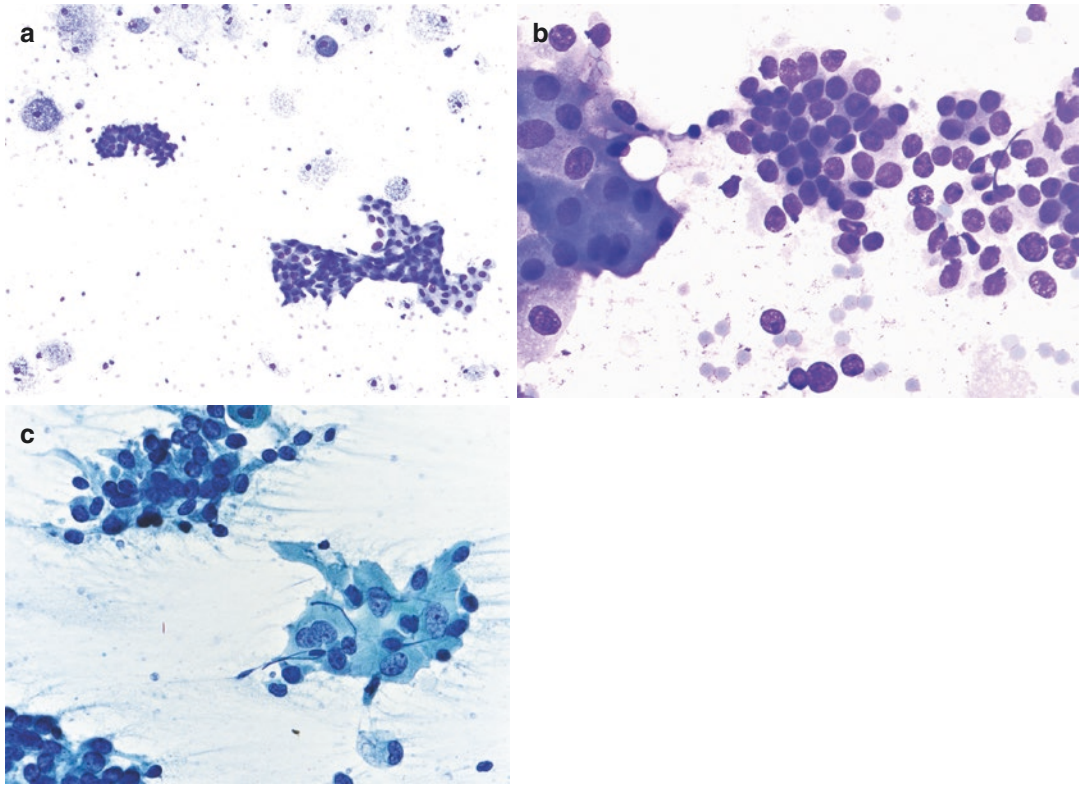


Fig. 7.4 (**a**) Fibrocystic change showing a small terminal ductular tissue fragment with occasional bare bipolar nuclei and a sheet of apocrine cells in a proteinaceous background with plentiful histiocytes (Giemsa ×10); (**b**) Fibrocystic change showing the edge of an apocrine sheet and adjacent ductal epithelial cell tissue fragment with myoepithelial cells; note the stripped round apocrine nuclei with nucleoli (Giemsa ×40); (**c**) Fibrocystic change showing a small apocrine sheet with mild nuclear pleomorphism and abundant cytoplasm and two small ductal epithelial cell tissue fragments with myoepithelial cells in a proteinaceous background (Pap ×40)

Low-grade and borderline phyllodes tumours will show a similar pattern to that seen in fibroadenomas, but the stromal fragments will show increased cellularity and atypia, and there may be more dispersed spindled stromal cells in the background (see Chap. 4, Atypical).

Both ductal carcinoma in situ (DCIS) and invasive carcinomas may show a *predominantly large tissue fragment pattern but usually exhibit marked dispersal of intact single cells and frequent small, crowded epithelial tissue fragments* with peripheral fraying (Fig. 7.9a–e). Intraductal and invasive carcinomas will also show varying degrees of nuclear atypia, with enlargement and pleomorphism and prominent nucleoli, and there is crowding within the tissue fragments. The tissue fragments may have a cribriform or micropapillary architecture, suggesting DCIS, and may include calcifications (see Chap. 5, Suspicious of Malignancy).

Invasive carcinomas most commonly are associated with a pattern of *mainly small tissue fragments of crowded cells with plentiful dispersed intact single cells in the background* (Fig. 7.10a–d). This is typical of invasive carcinoma of no special type (NST), which shows varying degrees of nuclear atypia reflecting the range of low to high grade carcinoma NST. Triple negative and basal-type carcinomas also typically have this pattern along with high-grade nuclei, as does carcinoma with medullary features where lymphocytes are prominent in the background and may be seen infiltrating small syncytial tissue fragments of carcinoma. Micropapillary carcinoma typically has a pattern of small tissue

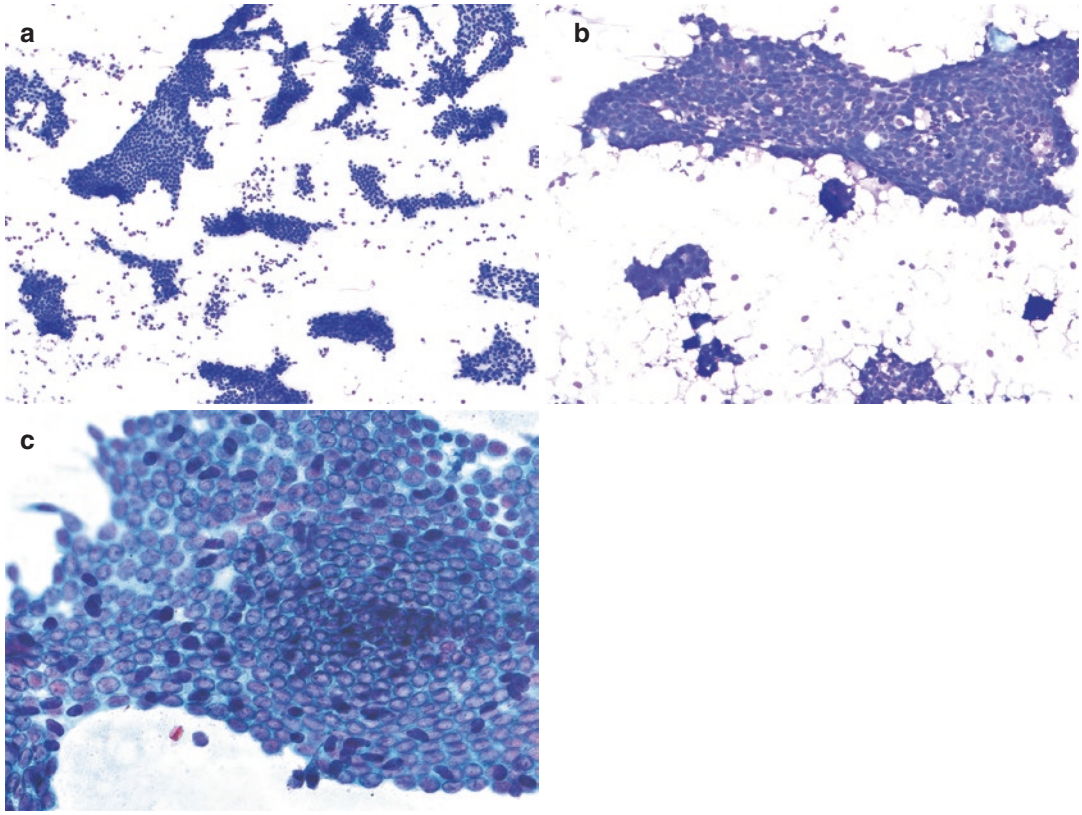


Fig. 7.5 (**a**) Epithelial hyperplasia showing a pattern of predominantly large epithelial tissue fragments with some smaller tissue fragments and some bare bipolar nuclei in the background (Giemsa ×5); (**b**) Epithelial hyperplasia showing a cohesive large ductal epithelial cell fragment with irregular holes ('secondary lumina'), and small epithelial tissue fragments and bare bipolar nuclei in the background (Giemsa ×10); (**c**). Cohesive ductal epithelial cell tissue fragment with uniform nuclei and myoepithelial nuclei (Pap ×40)

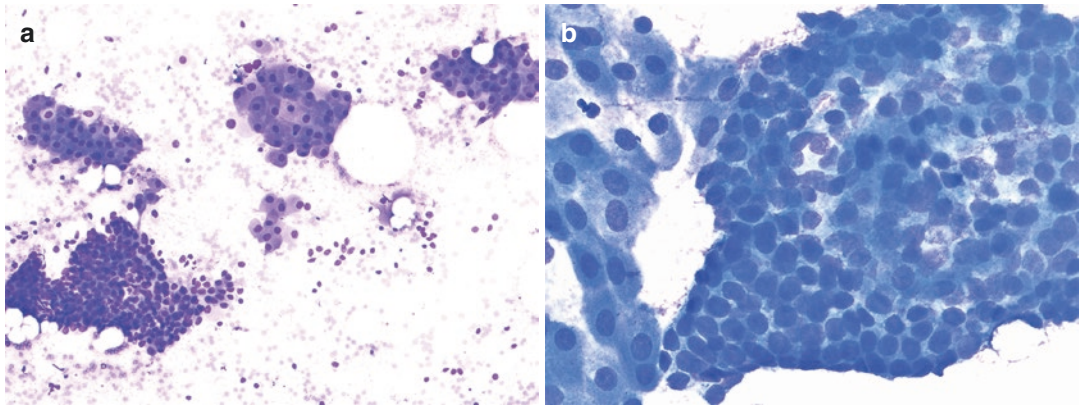
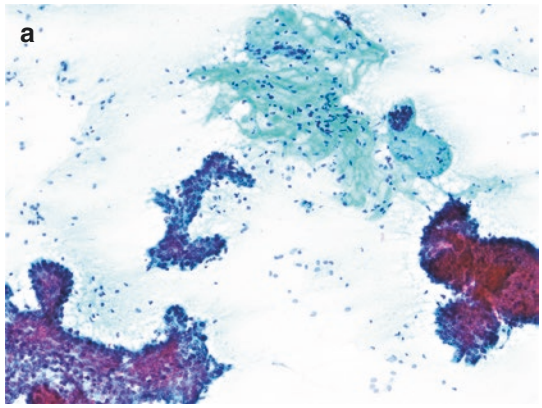


Fig. 7.6 (**a**) Fibrocystic change with epithelial hyperplasia showing a pattern of hyperplastic tissue fragments of ductal epithelial cells with myoepithelial cells and adjacent smaller sheets of apocrine cells in a proteinaceous background with bare bipolar nuclei (Giemsa ×10); (**b**) Fibrocystic change with a hyperplastic ductal epithelial cell tissue fragment with myoepithelial nuclei and an adjacent apocrine sheet in a proteinaceous background (Giemsa ×40)



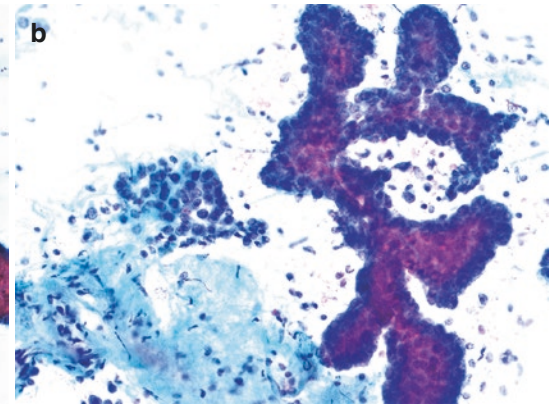
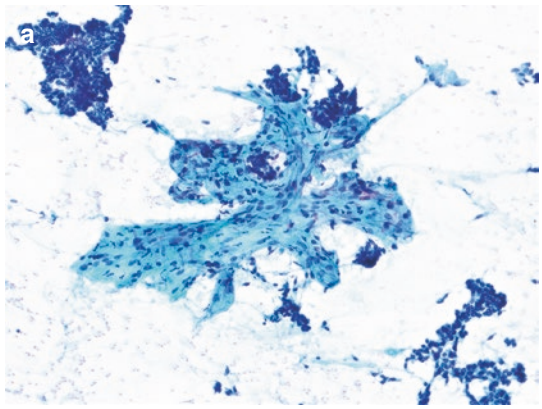


Fig. 7.7 (**a**) Fibroadenoma showing a pattern of large cohesive, three-dimensional ductal epithelial cell tissue fragments with myoepithelial nuclei and a large irregular stromal fragment in a background of bare bipolar nuclei (Pap ×10); (**b**). Typical fibroadenoma pattern with a large branching cohesive epithelial cell tissue fragment, a smaller epithelial tissue fragment with myoepithelial cells and a fragment of rounded stroma in a background of bare bipolar nuclei (Pap ×20)



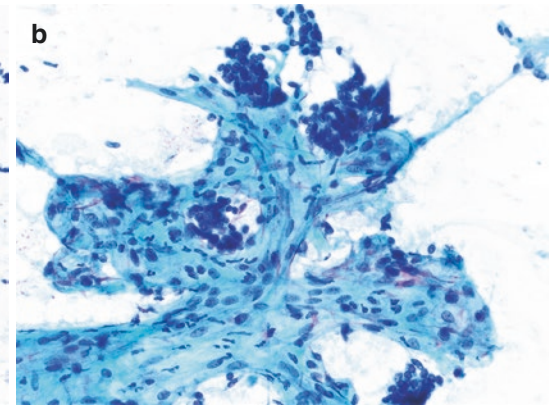


Fig. 7.8 (**a**) Intraductal papilloma showing a pattern of a stellate, papillary, fibro-elastotic tissue fragment with small ductal epithelial cell tissue fragments with myoepithelial cells in a proteinaceous background (Pap ×10); (**b**). High power of (**a**) showing the papillary, fibro-elastotic branching stellate stroma and small epithelial tissue fragments of small ductal epithelial cells with myoepithelial cells in a proteinaceous background (Pap ×20)

fragments, which can show a jigsaw-like architecture, along with usually plentiful dispersed cells and high nuclear grade. Tubular carcinoma has fewer dispersed single cells, and the small tissue fragments are often angulated, bent and rigid small tubules with a mild degree of nuclear atypia. Metaplastic carcinomas are most typically squamous cell carcinomas showing varying degrees of keratinisation and nuclear dysplasia, and some can show stromal and heterologous components. Many carcinomas can have mixed features (see Chap. 6, Malignant).

Classic invasive lobular carcinoma shows a distinctive pattern of *plentiful dispersed small- to intermediate-sized cells and minute, non-cohesive tissue fragments,* including small strands of cells (Fig. 7.11a–c). Nuclear grade is low to moderate, and the cells have eccentric cytoplasm that may contain intracytoplasmic vacuoles. Pleomorphic lobular carcinoma has a similar pattern and cells with eccentric cytoplasm and vacuoles, but the nuclear grade is high and it is difficult to distinguish from high-grade invasive carcinoma NST (Fig. 7.11d) (see Chap. 6, Malignant).

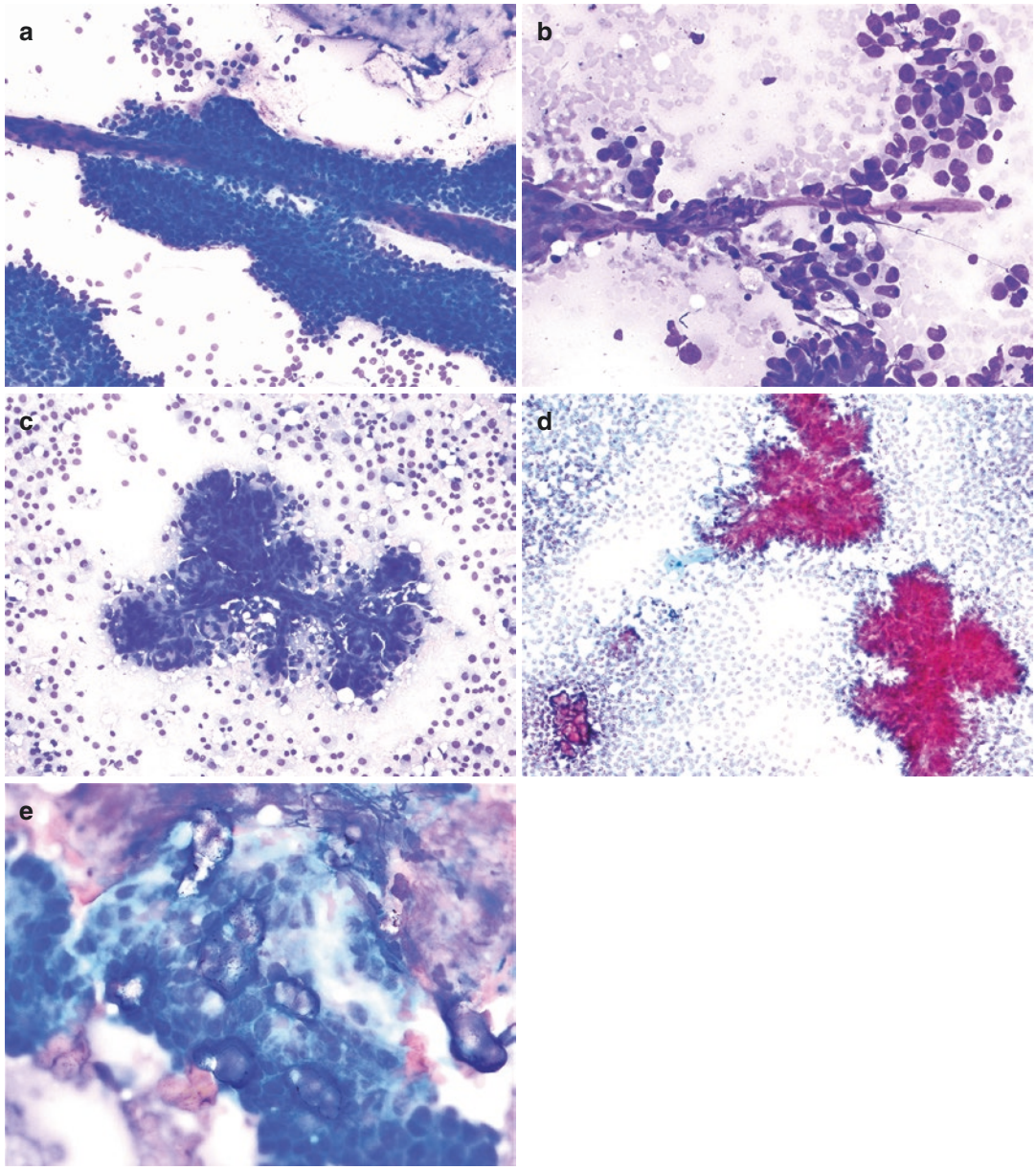


Fig. 7.9 (**a**) Low-grade ductal carcinoma in situ showing a pattern of a large tissue fragment consisting of a thin fibrovascular strand covered in an epithelial proliferation with small discohesive epithelial tissue fragments, single cells and some stripped nuclei in the background (Giemsa ×10); (**b**) Papillary ductal carcinoma in situ showing a thin fibrovascular strand with small discohesive tissue fragments and single cells showing moderate nuclear atypia (Giemsa ×20); (**c**) Solid papillary ductal carcinoma in situ showing a pattern of a branching complex capillary tissue fragment ('glomeruloid body') in a background of a large number of dispersed single cells (Giemsa ×10); (**d**) Solid papillary ductal carcinoma in situ showing 'glomeruloid bodies' in a background of a large number of dispersed single cells with a large calcification (bottom left) (Pap ×20); (**e**) Solid papillary ductal carcinoma in situ showing calcifications (in focus) on an epithelial tissue fragment (out of focus) with a stromal fragment (Giemsa ×40)

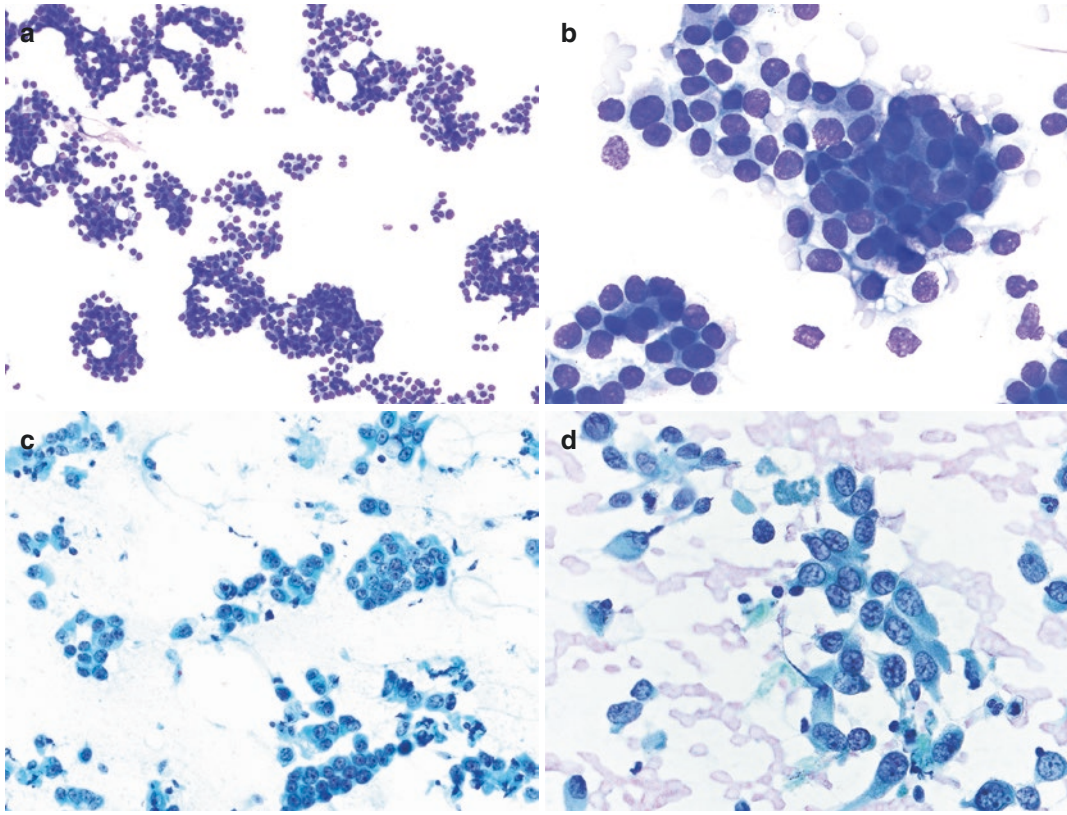


Fig. 7.10 (**a**) Low grade carcinoma no special type (NST) showing a pattern of small tissue fragments with some dispersed cells; note the occasional intracytoplasmic vacuoles (Giemsa ×10); (**b**) Low-grade carcinoma NST showing a small tissue fragment with occasional dispersed cells in the background and a lack of bare bipolar nuclei; note the irregular dark nuclei mimicking myoepithelial cells on the epithelial tissue fragment (Giemsa ×40); (**c**) High grade carcinoma NST showing a pattern of small tissue fragments and some dispersed single cells (Pap ×20); (**d**) High grade carcinoma NST showing dispersed single cells and a very small discohesive epithelial tissue fragment and high nuclear grade (Pap ×40)

Mucinous carcinomas usually show mild to moderate nuclear atypia in a pattern of *small non-cohesive tissue fragments and dispersed intact cells and distinctive 'naked' branching capillaries in a fibrillary mucinous background* (Fig. 7.12a, b). The DD includes benign lesions, such as cysts containing mucinous material and mucocele-like lesions (see Chap. 6, Malignant).

High grade carcinomas, including carcinoma NST and metaplastic carcinomas, can be seen in a pattern of *prominent granular necrosis*. This is also seen in high grade DCIS showing central or comedo-type necrosis, commonly associated with calcifications (Fig. 7.13a, b). Distinction between DCIS and invasive carcinoma is not possible on FNAB, unless small stromal fragments infiltrated by carcinoma are present confirming invasion (see Chap. 6, Malignant). Correlation with clinical and imaging findings is required. Fat necrosis can have calcifications and enters the DD but usually is not associated with epithelial tissue fragments and often has a history of previous surgery or trauma (Fig. 3.10a–d) (see Chap. 3, Benign).

Intramammary lymph nodes and lymphomas give a dispersed single cell pattern, which may be associated with pseudo-aggregation, and metaplastic apocrine epithelium may also show marked dispersal and varying degrees of nuclear atypia (see Chap. 3, Benign).

Finally, prominent *spindle cells* can be seen in relatively rare lesions in the breast. In nodular fasciitis, the spindle cells have relatively bland nuclei and are associated with multinucleated histiocytes and inflammatory cells (Fig. 4.9a–d) [3].

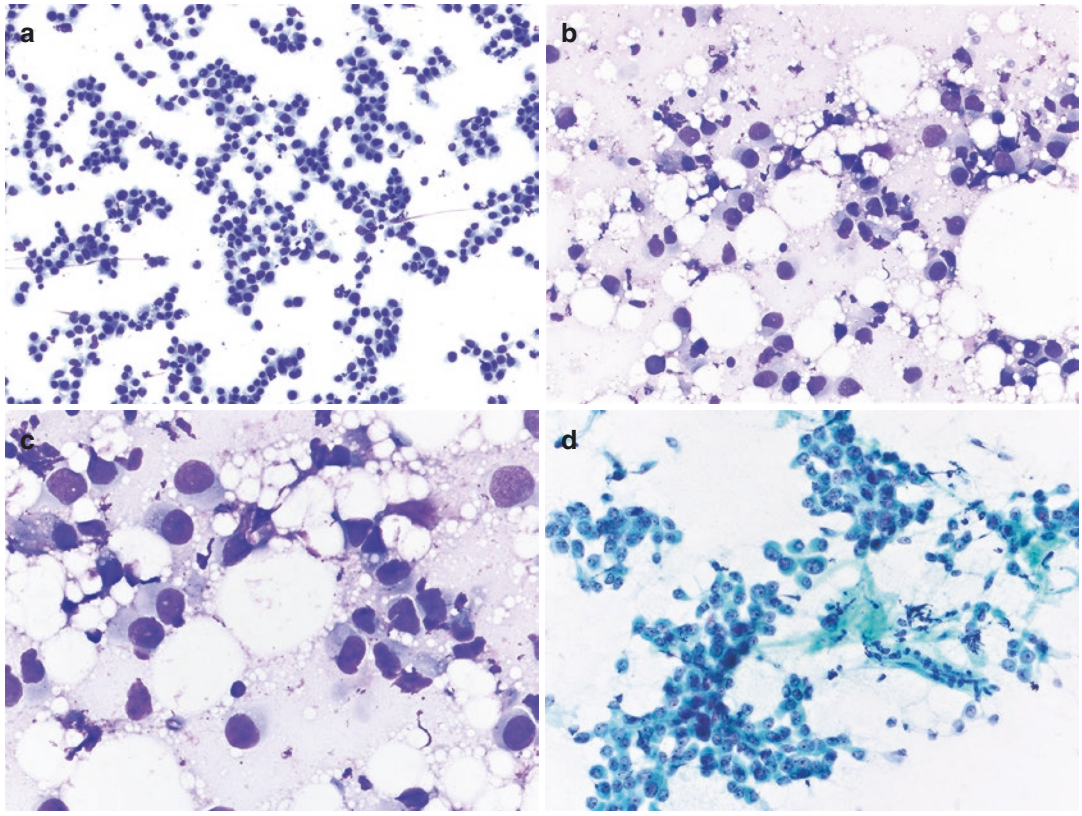


Fig. 7.11 (**a**) Lobular carcinoma showing a dispersed single cell pattern with tiny, very discohesive strands (Giemsa ×10); (**b**) Lobular carcinoma showing a dispersed cell pattern in a background of fat globules (Giemsa ×20); (**c**) Lobular carcinoma showing dispersed single cells with eccentric cytoplasm, occasional vacuoles (centre) and mildly to moderately enlarged and atypical nuclei (Giemsa ×60); (**d**) Pleomorphic lobular carcinoma showing discohesive tissue fragments of crowded cells with some single cells and a stromal fragment including a capillary (Pap ×20)

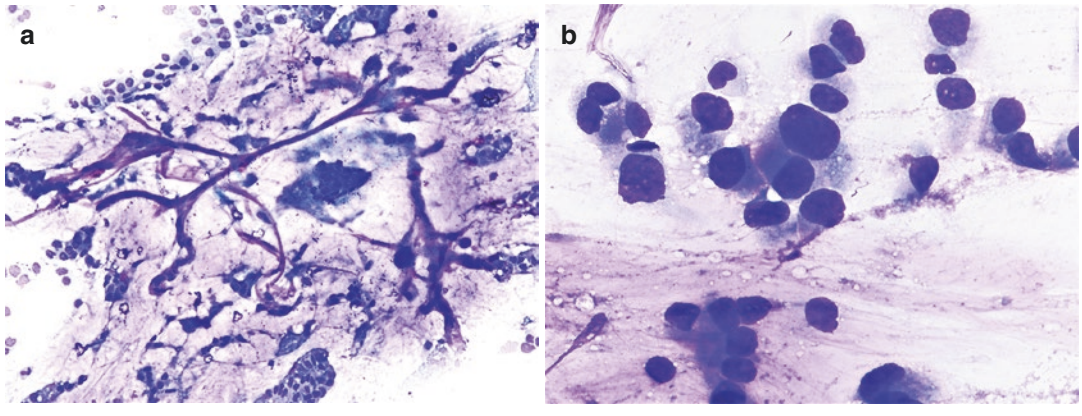


Fig. 7.12 (**a**) Mucinous carcinoma showing a dissected branching capillary with tissue fragments of carcinoma and some single cells in a mucinous background (Giemsa ×10); (**b**) Mucinous carcinoma showing fibrillary mucin with dispersed single cells showing moderately enlarged and atypical nuclei (Giemsa ×40)

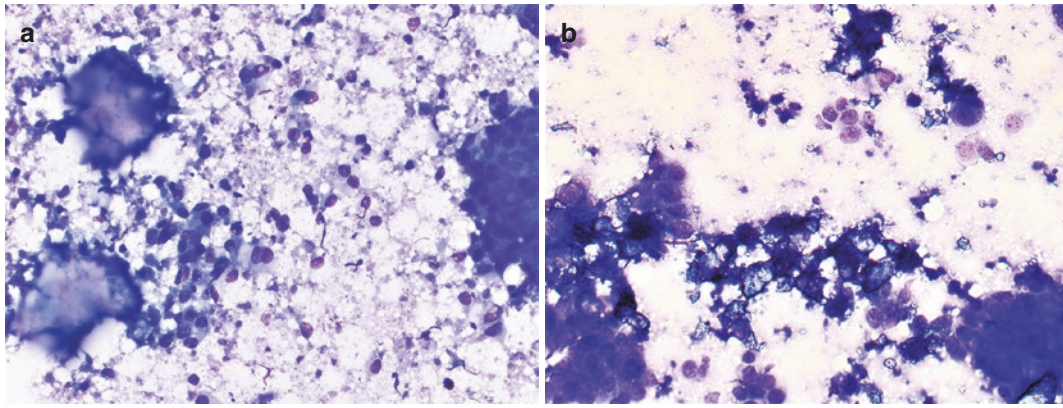


Fig. 7.13 (**a**) High grade ductal carcinoma in situ (proven on surgical biopsy) showing two calcifications, some dispersed single cells and a small tissue fragment in a background of necrosis (Giemsa ×20); (**b**) High grade ductal carcinoma in situ showing calcifications and some minute tissue fragments and dispersed cells with high nuclear grade in a necrotic background (Giemsa ×40)

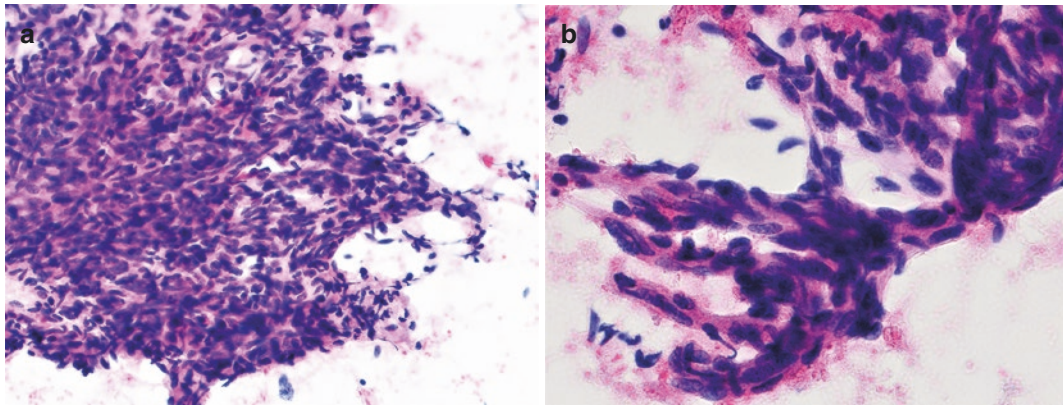


Fig. 7.14 (**a**) Angiosarcoma showing a crowded tissue fragment of spindle cells (H&E ×20); (**b**) Angiosarcoma showing poorly formed vessels with highly atypical spindle cells in a bloody background (H&E ×40) (Case for Fig. 7.14 (**a**) and (**b**) courtesy of Professor Pamela Michelow, Johannesburg)

Extra-abdominal desmoid ('fibromatosis') tumours may involve the breast and have paucicellular smears of small tissue fragments and single spindle cells (Fig. 4.8a–f). High-grade sarcomas, ranging from angiosarcomas to high-grade phyllodes tumours, with or without minimal epithelium, can be diagnosed on FNAB (Fig. 7.14a, b) (see Chap. 6, Malignant).

Conclusion

In summary, the key steps in an approach to reporting breast FNAB direct smears are to initially assess at low power the cellularity and pattern of the material on the slides, and then to use high power to assess the presence or absence of myoepithelial cells and the architecture of the epithelial tissue fragments, assess the degree of nuclear enlargement and atypia in the tissue fragments and single dispersed cells, determine the presence or absence of bare bipolar myoepithelial nuclei in the background and look for distinctive stromal or combined stroma and epithelial tissue fragments. The crucial factors of breast FNAB reporting are to avoid rushing to high power and ignoring the overall pattern.

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Nipple Cytopathology

8

Andrew H. S. Lee and Andrew S. Field

Nipple Discharge

Introduction

Nipple discharge is the presenting complaint of 5–10% of patients in a breast clinic. The discharge can be milky, serous, bloody, suppurative or brown, the latter being suggestive of old hemorrhage. Nipple discharge can be divided into two types: physiological discharge, which is typically bilateral, comes from multiple ducts, and may be related to lactation, and pathological discharge, which is usually spontaneous, persistent, unilateral and comes from a single duct. The majority of causes are benign, particularly papilloma and duct ectasia, but 3–20% are associated with carcinoma [1–5].

In patients presenting with nipple discharge, a large proportion of carcinomas are detected by clinical examination and radiology, usually mammography and retroareolar ultrasound. Nevertheless, a small percentage of patients may have a carcinoma undetected by clinical examination or radiology. Ductography and ductoscopy may readily localize a lesion but are poor at differentiating between benign and malignant lesions. Fine needle aspiration biopsy (FNAB) or core needle biopsy (CNB) is ideal for the assessment of these lesions.

The reported sensitivity of nipple discharge cytology for the detection of carcinoma following a "suspicious of malignancy" or "malignant" cytological result is between 17% and 70%; specificity, 66–100%; positive predictive value (PPV) 63–100%; and negative predictive value (NPV), 80–95% [1–4, 6–9]. The PPV of a malignant result was 97% in one large series [7]. Comparison of studies is difficult because the definitions of positive cytology vary, and there are different criteria for surgical follow-up, with some series considering all patients, whereas others only including those having a surgical excision. A major bias is the lack of follow-up details for patients who did not undergo surgery. A recent meta-analysis found rates of malignancy of 25% and 12% in bloody and non-bloody discharge [10].

Preparation of Discharge Material

If the nipple discharge is spontaneous, a sample can be easily gained by touching the nipple to the slide. Alternatively, the breast can be gently massaged towards the nipple by the patient and any discharge touched to the slide. Smearing the

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A. S. Field et al. (eds.), *The International Academy of Cytology Yokohama System for Reporting Breast Fine Needle Aspiration Biopsy Cytopathology*, https://doi.org/10.1007/978-3-030-26883-1_8

nipple onto the slide is not recommended because this increases contamination by squamous cells and keratinous debris. Once material is applied to the slide, this can be smeared with a second spreader slide and immediately immersed into alcohol for Papanicolaou staining or rapidly air-dried for Giemsa staining. Using both stains is recommended. Cytological samples can also be obtained from ductoscopy fluid [11].

Cytopathology of Nipple Discharge

In cases of nipple discharge related to lactational change, a small number of histiocytes and degenerate cells are seen in a proteinaceous background that may appear milky.

Benign ductal epithelial cells are usually seen as rounded, papillary-like tissue fragments that lack fibrovascular cores. There may be molding of the cells with a scalloped edge to the tissue fragment. Single well-preserved epithelial cells are uncommon. The nuclear to cytoplasmic (N:C) ratio of the ductal cells is low to moderate, the cytoplasm variably dense and may show squamous differentiation, and the nuclei are round to elongated and often have dense, even chromatin, showing varying degrees of degeneration. There are variable numbers of foamy histiocytes in the background. In intraductal papillomas, the number of similar epithelial tissue fragments may be greater with a mild degree of nuclear atypia, and siderophages in a thin colloidal background consistent with old hemorrhage are usually seen. Plentiful siderophages in a bloody background with large ghost-like epithelial sheets and papillary tissue fragments are suggestive of infarction of a papilloma [12] (see Chap. 3, Benign for further discussion on intraductal papillomas).

Exclusion of low-grade ductal carcinoma in situ (DCIS) in the setting of small, rounded, papillary-like tissue fragments of epithelial cells with mild to moderate nuclear atypia may not be possible, although tissue fragments with features similar to those described in a FNAB may be seen (Fig. 8.1) (see Chap. 5, Suspicious of Malignancy for further discussion on ductal carcinoma in situ.)

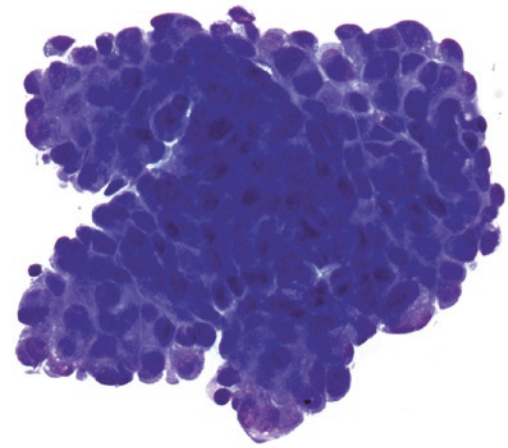

Fig. 8.1 Nipple discharge with a papillary tissue fragment of epithelial cells with increased nuclear-cytoplasmic ratio and nuclear hyperchromasia classified as atypical. The patient was found to have carcinoma (Giemsa ×40)

A definitive diagnosis of malignancy on nipple discharge cytology is rare, but atypical cells may be the only clue to underlying malignancy when clinical and radiological examination is normal [8]. The cytological features in discharge specimens are usually regarded as suspicious of malignancy and are similar to those in FNAB of breast carcinomas: a highly cellular sample with small tissue fragments and dispersed single cells showing moderate to marked nuclear atypia, including a high N:C ratio, nuclear enlargement and hyperchromasia (Figs. 8.2 and 8.3). Siderophages and necrotic debris may be present, and very occasionally a papillary architecture with thin branching fibrovascular cores may be seen.

Management

The management of nipple discharge requires a balance between avoiding unnecessary surgery for benign disease and not missing malignancy.

If the nipple discharge is associated with a significant clinical or radiological abnormality, further investigation is required. This can include nipple discharge cytology, FNAB or CNB. Assessment of subareolar lesions or lesions in the papilla can be easily done using FNAB.

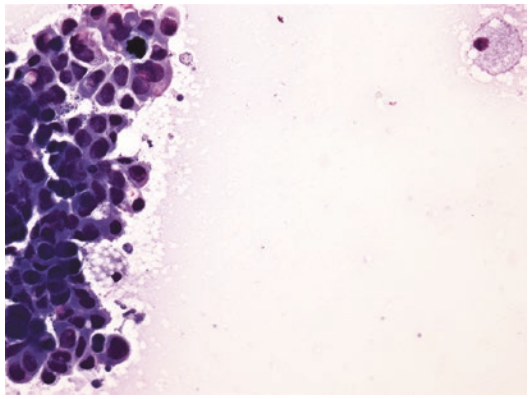

Fig. 8.2 Nipple discharge with a tissue fragment of epithelial cells with increased nuclear-cytoplasmic ratio and large nuclei (compared with the foamy macrophage on the upper right) classified as suspicious (Giemsa × 40)

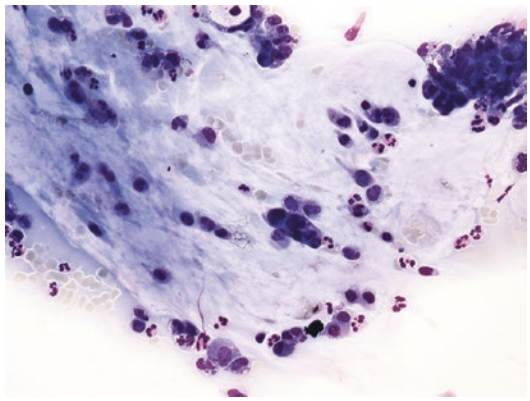

Fig. 8.3 Malignant nipple discharge with small groups and single atypical cells in a mucinous background (Giemsa × 40)

Nipple discharge cytology has a role in patients with normal clinical and radiological findings because a suspicious of malignancy or malignant result is an indication for surgery.

Management of patients with small, rounded, scalloped papillary-like tissue fragments with mild atypia on nipple discharge cytology is difficult as the atypia may represent degenerative change. In this situation, if there is no clinical mass or suspicious imaging findings, the patient can be followed up. If the nipple discharge continues or becomes bloody, duct excision should be considered. A negative cytology result does not exclude intraductal or invasive cancer.

Microdochectomy or duct excision is often performed to exclude malignancy or to treat persistent discharge. If the risk of malignancy is low, follow-up until resolution of the discharge may be considered. Age greater than 50 years is associated with a higher rate of malignancy in some studies. In modern series the risk of missing malignancy if clinical examination, mammography and ultrasound are normal is low.

Paget's Disease of the Nipple

Introduction

Paget's disease typically presents with an eczematous or erythematous appearance of the nipple. Nipple discharge may be present, and late ulceration and destruction of the nipple may occur. There is almost always underlying ductal carcinoma in situ, usually of high nuclear grade, and associated invasive carcinoma is identified in about half of the patients. The carcinoma may be palpable or show calcifications or a mass on imaging.

Paget's disease of the nipple is characterized histopathologically by carcinoma cells (Paget cells) within the epidermis of the nipple, and these may extend into the adjacent skin. The neoplastic cells have large nuclei with prominent nucleoli and abundant cytoplasm. Eczematous changes of the nipple show features of a spongiotic dermatitis that is easily diagnosed histopathologically. Melanoma is the main differential diagnosis but is very rare in the nipple. Direct invasion of the nipple and epidermis by underlying invasive breast carcinoma can give similar features, especially if of high nuclear grade.

Ideally, the nipple should be gently scraped with the edge of a glass slide or a scalpel and the cells immediately smeared on a slide with very rapid immersion in alcohol for Papanicolaou staining and a second scraping performed for air-drying and Giemsa staining.

If there is an underlying palpable lesion or a lesion on imaging in the subareolar region or papilla, then FNAB can be utilized ideally under ultrasound direction to supplement the nipple scraping.

Cytological Features

Cytology shows loosely cohesive minute tissue fragments and single Paget cells with large pleomorphic nuclei, coarse chromatin, prominent nucleoli and abundant cytoplasm that varies in density [13–16]. Inflammatory cells and necrotic debris may be present among squamous cells and keratinous debris. In some cases there are only scanty atypical cells [13]. A pitfall is that reactive squamous atypia may lead to an incorrect diagnosis of squamous carcinoma [16]. One small study found no false-positive and no false-negative results [14].

Ancillary Testing

The Paget cells can be scanty, resulting in diagnostic difficulty in some cases. A cell block for immunohistochemistry is helpful. Paget's disease is usually positive for cytokeratin 7, GATA3 and CAM5.2. HER2 in immunohistochemistry is positive in approximately 90% of cases [13], and estrogen receptor, GCDFP-15 and S100 are positive in about 30–50% of cases. MART1 and HMB45 are negative in Paget cells. Any underlying carcinoma usually has the same immunophenotype. The pathological differential diagnosis includes melanoma (S100, HMB45 and MART1 positive) and Bowen's disease (positive for basal cytokeratins CK5/CK6 and CK14). Toker cells are CK7 and CAM5.2 positive but are cytologically bland.

Management

A punch biopsy requires local anesthetic but ideally should be performed at the same time as the direct smears to allow exclusion of dermatitis and nipple adenoma and to facilitate the diagnosis of Paget's disease when Paget cells are scanty and require immunohistochemistry. Further management, in particular the extent of surgery, depends on the extent of any associated underlying carcinoma.

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9 Role of Ancillary Tests in Breast Fine Needle Aspiration Biopsy Cytopathology

Francisco Beca and Fernando Schmitt

Role of Immunocytochemistry in Breast FNAB Cytopathology

General Overview

Currently, fine needle aspiration biopsy (FNAB) cytology is used as the first line of pathological investigation for breast lesions in some developed countries and in many developing countries [1]. Although ancillary techniques, such as immunocytochemistry (ICC) and molecular studies, have been preferentially used on histological material, including core needle biopsies (CNB), they can be applied easily to FNAB material. FNAB and ancillary techniques can also be applied in situations where cytology specimens are the only material available, as occurs in locally advanced breast cancer and metastatic breast cancer, or in cases where the histopathological biopsy shows technical artifacts. Both ICC and molecular studies can be utilized for diagnosis and classification of breast cancers and for prognostication and prediction of a response to treatment [1, 2, 3].

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The diagnostic evaluation of breast carcinoma and other benign processes remains grounded in the careful evaluation of morphology in high-quality cytological or histological material. However, with advancements in our understanding of tumor biology and the development of new treatment options, cytopathologists have become increasingly prominent members of the multidisciplinary patient care team. Pathologists not only provide a pathological diagnosis but also deliver prognostic and predictive information about the tumor. ICC analysis has assumed a critical role in clarifying the diagnosis in challenging cases and resolving differential diagnoses. Moreover, ICC testing of estrogen receptor (ER), progesterone receptor (PR), HER2, and other markers can provide critical information for planning targeted therapies and can be used as a surrogate for the molecular classification of breast cancer into molecular subtypes, it can also help in combination with molecular techniques, to identify new prognostic and therapeutic targets. While ICC and molecular studies have proven to be useful in the diagnosis and treatment of breast carcinoma, it is important to emphasize that the results of these tests should always be interpreted within the clinical and cytomorphological context.

Technical Aspects

The main challenges in the application of ICC and molecular techniques to breast cytology are mostly due to technical aspects. These include

A. S. Field et al. (eds.), *The International Academy of Cytology Yokohama System for Reporting Breast Fine Needle Aspiration Biopsy Cytopathology*, https://doi.org/10.1007/978-3-030-26883-1_9

the major challenges of the selection of the most appropriate test for the limited sample quantity, the avoidance of blindly applying histopathology protocols which have been untested on cytology, and the recognition of the need to use appropriate controls for the cytological material [4]. Assay validation is also an essential step for any ancillary test applied to cytopathology. The lack of standardization in many applications of ICC to cytological material is perhaps the single most common factor leading to assay failure [5]. Lack of standardization includes the use of unsuitable controls, non-customized reagent concentrations, and different methodologies of fixation and preparation of the material, and all these problems are still frequent and can lead to errors, ultimately undermining the perceived usefulness of cytopathology and particularly breast FNAB [6, 7].

Cytological Specimens

Proper cytology specimen processing is of utmost importance for any ancillary techniques. Whether the sample is a direct smear, a cytospin, a cell block, or a liquid-based cytology (LBC) preparation, specimen processing is fundamental to the success of the assay. Direct smears prepared from FNAB material, brushings, or centrifuged sediment of effusions are all different sources and types of specimens each with their own advantages and disadvantages, but they frequently require slightly different processing for the success of an ancillary assay. Table 9.1 summarizes the advantages and disadvantages of the different cytological preparations.

Generally, with air-dried direct smears, the cytomorphology is excellent, and after fixation in formalin and alcohol, they perform well in ICC for nuclear antigens, such as Ki-67, TTF-1, and ER. However, they are less suitable for cell membrane markers due to frequent high background staining owing to cell crush damage. Additionally, since considerable antibody quantity is needed to cover the entire slide, direct smears are usually less cost-effective when ICC ancillary studies are needed.

On the other hand, cytospins that are prepared from cell suspensions from FNAB needle rinsings or effusions in non-fixative solutions, such as PBS and RPMI, offer an excellent source of staining for most antibodies. While this technique is less suitable for specimens with a rich admixture of blood or mucus, it has the advantage that in most cases a large number of cytospin slides can be prepared, allowing the use of more extensive antibody panels from a single specimen. If air-dried, cytospin slides can even be stored for many years at −70 °C without loss of antigenicity and with excellent DNA preservation.

Liquid-based cytology (LBC) systems, which are now available in most cytology laboratories in developed countries, can be used to prepare almost all types of cytological specimens and for staining with various antibodies. Whether the antibody is directed at nuclear, cytoplasmic, or membrane-bound antigens, slides from LBC systems usually stain reproducibly. Similar to cytospins, several slides can be produced from a single LBC sample allowing an extensive immunological workup if needed and can be stored for months at −70 °C without a change in immunoreactivity. However, as any experienced cytopathologist is well aware, the cytomorphology of LBC material is considerably different from that

Table 9.1 Different cytological preparations: advantages and disadvantages

Preparation type	Advantages	Disadvantages
Direct smears	ICC slide with the same cytomorphology as the initial preparation Good for nuclear markers	Limited by the availability of slides Increased amounts of antibodies needed
Cytospin	Large number of slides can be prepared	Frozen bank of air-dried cytospin slides should be prepared for controls Increased technical workload
LBC	Large number of slides can be prepared	A bank of control slides should be prepared
Cell blocks	Numerous slides can be prepared Controls similar to the ones used for histopathology	

of direct smears [8]. For the evaluation of standard prognostic and predictive markers in breast cancer, LBC preparations seem to perform better than direct smears in immunocytochemistry. In a multinational study, cytospins and LBC preparations were superior to direct smears for the evaluation of ER and PR on FNAB of breast carcinoma [9]. LBC preparations are suitable for preserving cell samples and DNA with sufficient quality to be used in several molecular analyses, such as PCR, restriction fragment length polymorphism (RFLP), and even sequencing [10].

Cell blocks can be considered a bridge to traditional histopathology and have several distinct advantages over other cytological preparations. Cell blocks can be prepared using a wide array of techniques, such as cytocentrifugation, either with direct formalin fixation or fixation after the addition of plasma thromboplastin. There are also commercially available systems that offer a standardized technique with a high reproducibility but with the trade-off of a greater cost [11]. No matter what the specific preparation technique, cell blocks usually stain in a highly reproducible way and can be used to prepare enough slides for extensive ICC panels, except in specimens with extremely low cellularity, such as cerebrospinal fluids. Additionally, since most ICC and molecular techniques are now standardized for paraffin-embedded tissues, they can be applied directly to the preparation of cell block with excellent results. Perhaps the biggest advantages of the use of cell blocks are that the morphology and staining characteristics are similar to traditional histological specimens. Cell blocks are frequently preferred in settings where both the laboratory infrastructure and the pathologists are more experienced handling histopathologic specimens. Therefore, cell blocks can bridge the gap between traditional cytopathology and histopathology for specimen evaluation and ancillary ICC assay technical performance and assessment.

Fixation

There are a number of fixation procedures used for FNAB and effusion specimens. Fixation procedures can be based on various fixatives, such as ethanol, methanol, acetone, and formalin. Air-drying is often used, and for LBC preparations, proprietary fixatives are available. The choice of fixative is of utmost importance for the optimal performance of ancillary techniques, and the variability in fixatives is one the major factors preventing standardization of ICC [6, 7]. Nuclear antigens, such as ER, PR, androgen receptor (AR), p63, and Ki67, perform best after fixation of air-dried specimens in buffered 4–10% formalin and then methanol-acetone [12] or microwave antigen retrieval. Formalin is a reliable fixative for the detection of HER2 by ICC and ISH. Commercially available LBC fixatives have also been used with good results for the detection of nuclear epitopes. On the other hand, the use of alcohol-fixed or air-dried or even the combination of these methods has been reported as less reproducible regarding antibodies to nuclear epitopes. Methods of fixation and antigen retrieval were the key points in obtaining good results in a comparative multinational study of ER and PR detection in breast cancer FNAB [9]. Membrane and cytoplasmic antigens seem to have less stringent requirements regarding the type of fixative use. Air-dried smears fixed in formalin and then ethanol generally give optimal staining results. For cell blocks, buffered formalin is suggested as the optimal fixative by most authors [12].

Controls

The basis of an optimal ancillary assay is the correct choice of positive and negative controls and knowing the expected and optimal sensitivity and specificity of the assay. A meta-analysis study demonstrated that in more than 50% of published papers on ICC applied to cytological material, controls were not even mentioned [13]. The preservation of the specimen integrity and quality must be a priority.

Procedures

ICC methods applied to cytology specimens have been refined, and currently there is no need to use in-house developed protocols and methods for standard clinical care in cytopathology. High-quality reagents and automation are more

Table 9.2 Main ancillary (ICC and molecular) assays in FNAB of the breast, most typical usage scenarios, and recommendations

Assay type	Assay/antibody combination	Typical usage scenario	Recommendation summary
ICC	Myoepithelial cell markers (inc. P63, 34BE12, SMA, calponin)	Differential diagnosis of epithelial proliferative lesions	Used to highlight the presence of myoepithelial cells in benign proliferative lesions and papillary lesions; ideally perform a nuclear (P63) and at least one cytoplasmic marker (i.e., 34BE12).
ICC	E-cadherin P120	Lobular neoplasia/ invasive lobular carcinomas	Consider using at least E-cadherin when the diagnosis of a lobular carcinoma/lobular neoplasia is in the differential diagnosis
ICC	HMW- CK P63	Spindle cell lesions of the breast	Priority to these markers should be given when a metaplastic carcinoma is in the differential diagnosis
ICC	Bcl-2 CD34 B-catenin	Benign spindle cell lesions of the breast	
ICC	Gata-3 GCDFP-15 Mammaglobin	Confirmation of breast origin for metastatic lesions	Use in combination and not as a single marker; consider combining with CK7/CK20/TTF-1/ Napsin A and CDX2 in case of an axillary/head and neck metastasis that is particularly challenging
ICC	Pax-8 WT-1	Differential diagnosis with ovarian and breast primary carcinomas	Especially useful in cases of ER-positive metastatic lesions and suspected ovarian carcinomas
ICC	Prognostic/predictive markers (ER/PR/ HER2)	New metastatic lesions/ progression of disease/ therapeutic failure	Should be performed in FFPE cell blocks and with the protocol and evaluation compliant with the most recent ASCO/CAP guidelines for ER and HER2
Molecular	Prognostic/predictive markers (HER2 by ISH)	Evaluation of HER2 amplification status by ISH (FISH/CISH/SISH)	ASCO/CAP guidelines for ER and HER2 should be followed
Molecular	ETV6-NTRK3	Diagnosis of secretory carcinoma of the breast	Confirmation of the diagnosis of secretory carcinoma of the breast
Molecular	MYB-NFIB fusions/ MYBL1 rearrangements/MYB amplification	Diagnosis of adenoid cystic carcinoma of the breast	MYB-NFIB fusions are the most frequent with the other rearrangements and amplifications having been recently described

widely available and assist standardization while simultaneously offering quality, reproducibility, and consistency while optimizing labor and reagent costs.

Applications

Diagnostic Applications

The main diagnostic applications for the use of ICC in breast cytology are (Table 9.2 and Fig. 9.1):

- Differentiating benign and malignant epithelial proliferative lesions
- Subtyping malignant lesions
- The particular challenge of spindle cell lesions
- Identifying the breast as the origin of metastatic carcinomas

Differential Diagnosis Between Benign and Malignant Epithelial Proliferative Lesions, Including Papillary Lesions

The fundamental guiding principle in differentiating benign from malignant breast aspirates is the presence of myoepithelial cells in benign lesions and their absence in malignant lesions. While this distinction sounds straightforward, the differential diagnosis of proliferative epithelial lesions is challenging in breast pathology, even when using standard histopathological preparations [14, 15]. In breast FNAB, proliferative epithelial lesions are especially difficult because frequently, myoepithelial cells can be difficult to recognize and may be confused with apoptotic cells, stromal cells, and even epithelioid histiocytes. Therefore, a robust myoepithelial cell

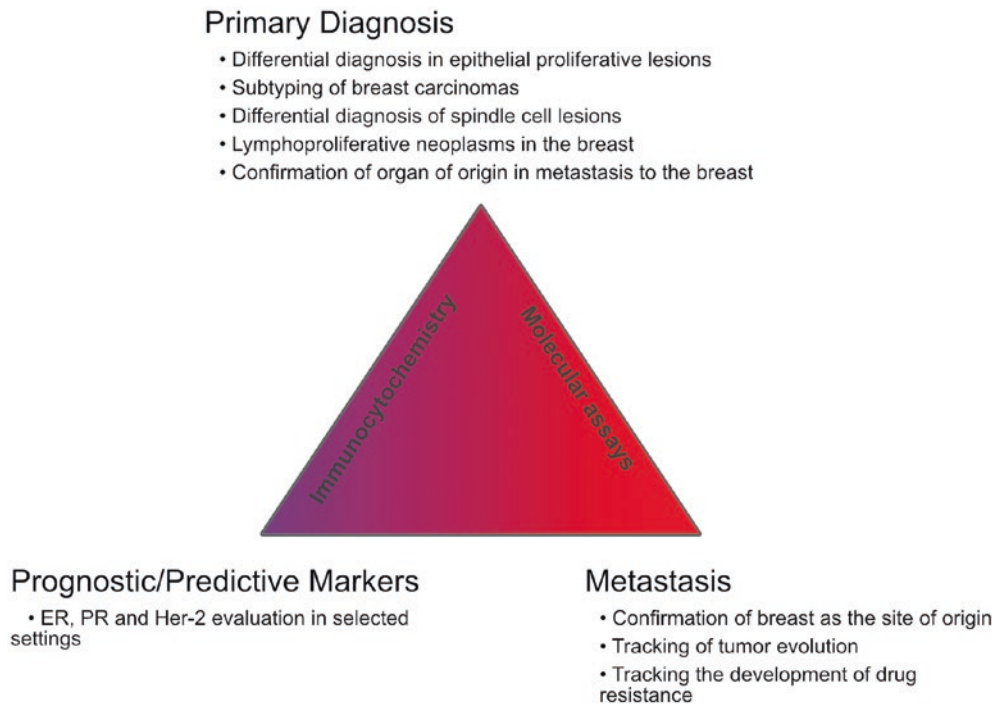

Fig. 9.1 Summary of the main application of immunocytochemistry and molecular assays in breast cytopathology. (Reproduced with permission from Beca and Schmitt [93])

marker, or more frequently a panel, should be in the arsenal of the cytopathologist confronted with the differential diagnosis of proliferative epithelial lesions of the breast, especially when these display atypia and raise the possibility of an invasive carcinoma. As in histopathology, myoepithelial cell markers, including smooth muscle actin (SMA), calponin, p63, or high molecular weight keratins, can be used to demonstrate the presence of myoepithelial cells and help differentiate an epithelial proliferation with atypia from an invasive carcinoma. Due to its higher specificity and easy interpretation, p63 has been the most widely investigated myoepithelial marker. In general, there is a high percentage of staining of p63 in benign aspirates, ranging from 75% to 86% [16] (Fig. 9.2). However, in malignant smears, the positivity rate ranged from 11% to 60% due to the staining patterns and the presence of an in situ component [16, 17]. Additional sources of errors are the positive staining of some of the epithelial cells, observed in up to 20% of invasive carcinomas and 37% of in situ carcinomas [17].

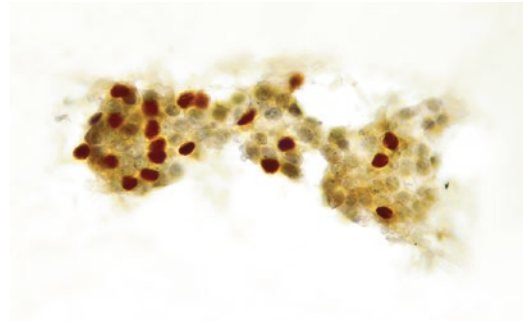

Fig. 9.2 P63 decorating myoepithelial cells overlapping a group of epithelial cells in a case of a benign epithelial proliferative breast lesion (ICC, P63)

Therefore, despite frequently being the easiest to interpret, p63 should only be used as a "soft sign," and the staining results have to be corroborated with other diagnostic considerations [2]. Other myoepithelial markers, such as SMA, calponin, and high molecular weight cytokeratins (HMW-CK), also have interpretation challenges because they show cross-reactivity with other cell types, including myofibroblasts, luminal cells,

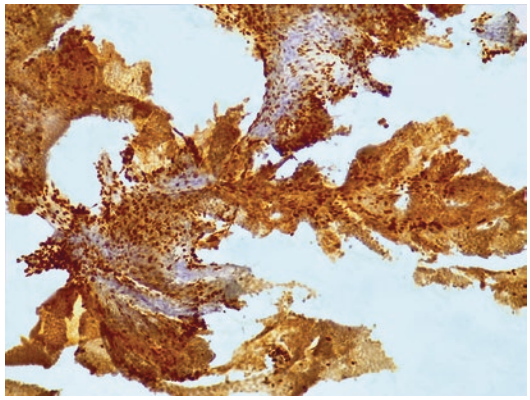

Fig. 9.3 Benign papillary lesion showing myoepithelial cells stained by P63 (ICC, P63)

stromal cells, and pericytes. Additionally, cytoplasmic markers expressed in the fragile cytoplasm of myoepithelial cells can easily be lost in direct smears. Possibly the best approach is the combined use of a nuclear p63 and a cytoplasmic marker, such as 34BE12, which recently was demonstrated to probably increase the detection sensitivity of myoepithelial cells on breast FNAB [18].

Based on our personal experience, p63 is also useful for the demonstration of myoepithelial cells in lesions with papillary architecture [19]. Their presence favors the diagnosis of a benign papillary lesion in contrast with papillary carcinoma (Fig. 9.3).

Subtype of Malignant Lesions

In many centers, the breast cancer preoperative therapeutic plan is frequently based on the "triple test" approach, integrating the clinical, pathologic and imaging findings. For tissue sampling in this setting, CNB is the procedure that is most commonly used. However, situations where the only material available is from FNAB are not uncommon, presenting particular challenges in the preoperative planning.

In the preoperative setting, distinguishing lobular and carcinoma of no special type (NST) is clinically crucial due to two main reasons. The first is that it influences the choice of the radiologic imaging system to be used, namely, MRI versus mammography. The second is that the potential patterns of recurrence are different: carcinoma NST tends to be unifocal in the breast with distant metastasis to liver, lung, and brain, whereas lobular carcinoma tends to be multifocal and bilateral in the breasts with distant metastases to serosal surfaces and the gastrointestinal and gynecologic tracts. The FNAB false-negative rate for lobular carcinoma ranges from 4% to 60% in different studies [20]. Most of the errors are due to inadequate sample quality, low cellularity, and difficulties in the interpretation of the cytological features of lobular neoplasia.

Both E-cadherin and P120 can be used to help distinguish lobular and other invasive carcinomas of the breast. E-cadherin is a calcium-dependent transmembrane, cell-cell adhesion protein. It plays a functional role in intracellular adhesion and cell polarity by binding the actin cytoskeleton through interactions with the catenin complex, including p120 alpha, beta, and gamma-catenin. Loss of E-cadherin affects cellular adhesion and tumor cohesion, motility, and possibly cellular proliferation and is frequently detected in lobular carcinomas; therefore it is a very useful adjunct in the diagnosis of lobular carcinomas [2]. However, the correct interpretation of E-cadherin loss is occasionally challenging, particularly in cases of invasive lesions with sparse single cells. P120, which is part of the E-cadherin/catenin membrane complex, demonstrates membrane staining for "ductal" and lobular carcinomas. Loss of E-cadherin leads to the release of P120 from the membrane complex, resulting in diffuse cytoplasmic staining in lobular carcinomas. Occasionally, E-cadherin can show aberrant expression in lobular lesions [21], whereas many of these cases carry an E-cadherin gene mutation and protein dysfunction. Therefore, E-cadherin aberrant expression should not automatically classify a carcinoma as a "ductal" lesion, and correlation with the cytomorphology is essential while the P120 staining may be helpful. The combination of loss of E-cadherin membrane expression and diffuse cytoplasmic staining for P120 can aid in the diagnosis of lobular carcinoma.

Spindle Cell Lesions

Spindle cell lesions on FNAB can be particularly challenging, and it is often difficult to make a definitive diagnosis of breast spindle cell lesions on cytology alone usually due to limited cellularity. In most cases, core needle biopsy (CNB) is required for the definitive diagnosis. However, FNAB can be used to exclude other benign or malignant lesions suggested by the imaging and to demonstrate whether a lesion is a pure or biphasic spindle cell lesion.

Fibromatosis, which on ICC shows nuclear positivity for beta-catenin and negativity for hormonal receptors, Bcl2 and CD34, can be distinguished from myofibroblastoma, which is positive for CD34, Bcl2, and sometimes hormonal receptors, when the cytomorphology and ICC are combined. Caution must be taken when using beta-catenin as a single marker in the assessment of mammary spindle cell lesions, because aberrant nuclear expression of this marker has been reported in the stroma of phyllodes tumor (PT), as well as in metaplastic carcinoma [22, 23, 24].

Among the malignant lesions, spindle cell metaplastic carcinoma and high-grade PT are seen most frequently. Metaplastic carcinoma can be predominantly composed of spindle cells, which can be deceptively bland, or overtly high grade and pleomorphic. Metaplastic carcinoma can also be composed of a mixture of epithelial and heterologous elements, including extracellular matrix.

ICC analysis with a panel of keratin markers is essential, including HMW-CK markers, such as 34BE12, CK5/6, CK14, and AE1/3, which often show variable or focal staining, whereas low molecular weight (LMW)-CK markers, such as CK7, are usually negative [25]. P63 is positive in more than 90% of metaplastic carcinoma, and it can be a very useful marker to distinguish metaplastic spindle cell carcinoma from other spindle cell lesions of the breast when combined with the HMW-CK [26].

PT are biphasic fibroepithelial lesions characterized by hypercellular stroma and elaborate leaf-like architecture, and depending on the degree of stromal cellularity, the number of mitoses, the degree of nuclear atypia, the presence of stromal overgrowth, and the nature of the tumor margins, they are divided into benign, which resemble fibroadenomas, borderline, and malignant categories [22]. A PT with extensive stromal overgrowth may be difficult to distinguish from a spindle cell metaplastic carcinoma in a limited FNAB or CNB specimen. Although HMW-CK or p63 expression supports the diagnosis of metaplastic carcinoma, focal expression of these markers has been described in stromal cells of PT [22, 27]. Another marker sometimes present in the stromal cells of PT is CD34. CD34 expression has been reported to be inversely related to adverse histopathological features, and therefore it is not expected to be expressed in malignant PT, which could be useful in differentiating high-grade spindle cell lesions of the breast [22, 28]. Other markers to consider when dealing with spindle cell lesions include Bcl2, which is more frequently expressed in PT, and CD117, which shows increased expression in higher-grade phyllodes tumors [22, 28]. Sarcoma-specific molecular cytogenetic alterations can also be critical diagnostic adjuncts in the setting of overtly malignant spindle cell lesions of the breast.

Identification of a Primary Breast Carcinoma in the Setting of Metastatic Carcinomas

The identification of the primary origin of a carcinoma is a frequent problem, especially in FNAB of the head and neck region. Breast carcinoma metastasizes to regional and distant lymph nodes and organs, such as liver, lung, brain, bone, gastrointestinal tract, and the gynecological systems, where FNAB is frequently diagnostic, as well as to the pleura, pericardial, and abdominal serosal surfaces leading to effusions [3]. The main challenge with the identification of the primary origin of a metastatic carcinoma is that the primary lesion may not be available for review or the metastasis may be sampled before the primary lesion has been diagnosed or even identified.

The primary site of origin of a metastatic carcinoma can be especially challenging in the setting of a patient with a history of multiple previous carcinomas and a new mass or enlarged lymph node. In these particularly challenging situations, ICC with mammaglobin, gross cystic disease fluid protein-15 (GCDFP15), GATA3, ER, and CK7 can help in the diagnosis of a breast carcinoma metastasis. Some 75–80% of breast cancers are positive for ER, and a strong ER expression is indicative of a breast primary, but lack of expression does not exclude a breast origin. Additionally, carcinomas with an ovarian origin can also be weakly positive for ER. The expression of ER is inversely correlated with nuclear grade. Therefore a low-grade metastatic lesion that is ER negative has probably another origin rather than a primary breast carcinoma [3]. GCDFP-15 is positive especially in lobular and apocrine carcinomas showing 98% of specificity but only 58% of sensitivity to identify breast as a primary site of a metastatic carcinoma [29, 30]. Mammaglobin is a secretory protein expressed in more than 50% of breast cancers [29], it is more sensitive but less specific than GCDFP-15 for breast lesions [30], and its expression is not correlated with tumor grade, tumor stage, or hormone receptor status, limiting its overall usefulness.

GATA3 is a more recent marker with several advantages. It is an excellent overall marker for breast cancer origin. It works very well in all types of cytological specimens, and almost 100% of ER-positive breast cancers express GATA3, as well as 60% of the triple-negative cases [31] (Fig. 9.4). Despite this diagnostic performance, it is not specific, as other tumors are positive for GATA3, including urothelial carcinoma, germ cell tumors, cutaneous basal cell carcinoma, and benign skin adnexal tumors [31]. GATA3 may be especially useful in the identification of breast as the primary site if the carcinoma is also CK7+/ER+/CK20- [32]. GATA3, PAX8, and WT1 are good markers for separating breast cancer from ovarian carcinoma, which is usually positive for PAX8 and WT1 and negative for GATA3 [33].

In the specific case of effusions, the sensitivity of GATA3 for detecting metastatic breast carcinomas is around 95% with a specificity of 89%, including for triple-negative carcinomas [34, 35]. While GATA3 positivity may be supportive of an effusion due to breast cancer, especially when urothelial carcinoma has been excluded, a small proportion of other tumors can exhibit GATA3 positivity. These include carcinomas with mullerian, pancreatobiliary, lung, and gastrointestinal tract origins [34]. Therefore, while GATA3 is a powerful and robust marker strongly suggestive of breast cancer origin, it should always be used as part of a panel aimed at ruling out other primary sites. Further, GATA3 can stain lymphocytes. In cases with rare tumor cells against an inflammatory background, the interpretation of the staining can be challenging [35].

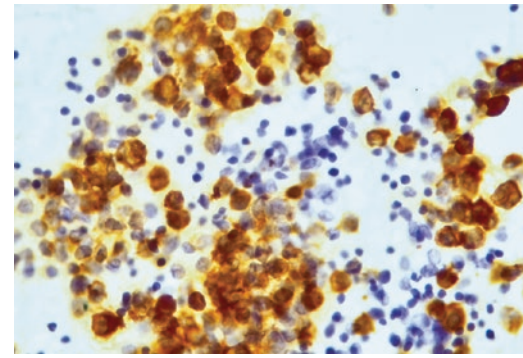

Fig. 9.4 FNAB of metastatic breast carcinoma in a lymph node showing intense nuclear positivity for GATA3 (ICC,GATA3)

Classification of Breast Cancer

Gene expression profiling has enabled the establishment of a molecular classification of breast cancers, with prognostic and predictive significance. ICC markers for ER, PR, HER2, and a proliferation marker (such as Ki67/MIB1) can act as surrogates for the molecular classification of breast cancer. Using these markers, breast carcinomas can be divided into luminal A (ER/PR positive, low proliferative index, and HER2 negative), luminal B (ER positive, PR positive or negative, high proliferative index, and HER2 negative or positive), HER2 overexpressing (ER/PR negative and HER2 positive), and triple negative (ER/PR and HER2 negative) or basal (ER/PR and HER2 negative and EGFR/CK5/6 positive) categories. All these markers can be used in

cytological specimens (see the section below), but presently there is no clinical utility to use this classification in cytological samples.

Prognostic/Predictive Markers

ER, PR, and HER2 status not only provide prognostic information but are also critical predictive markers for currently available anti-hormonal and anti-HER2 therapies. Thus, accurate, reliable, and reproducible evaluation of hormonal receptors and HER2 in breast cancer is critically important to help ensure appropriate treatment planning. These biomarkers are usually tested in surgically resected or CNB specimens of newly diagnosed primary breast carcinoma and require standardized fixation conditions, that is, fixation in 10% neutral buffered formalin for 6–48 h for ER, PR, and HER2 [36, 37]. They are also frequently tested in cytological specimens to determine their status in primary and especially metastatic breast carcinoma (MBC). The recommendations of some organizations, such as the European Society of Medical Oncology (ESMO), are that these markers should be retested in metastatic tumors even though the receptor status of the patient's primary tumor may be known. Tumor heterogeneity and possible clonal evolution during the biologic progression of the tumor may result in metastatic cells that lose or gain expression of these receptors, thus demonstrating a receptor status different from the primary tumor [38]. The receptor activity in metastatic breast cancer may be altered after systemic chemotherapy or targeted therapy due to clonal selection. Discrepancies between the primary tumor and the metastatic lesions have been reported to be as high as 30–40% for MBC that are hormone receptor (ER and PR) positive and up to 10% for HER2 overexpressing carcinomas [39, 40, 41, 42]. More importantly, the discrepancies between primary tumor and metastatic biopsies have been shown to be responsible for a therapy regimen change in 14% to 20% of patients [39, 40]. FNAB of a primary breast carcinoma can be used for testing these markers when patients are not able to undergo surgery owing to comorbidity, when the disease is already disseminated at presentation and when chemotherapy is the first choice of treatment.

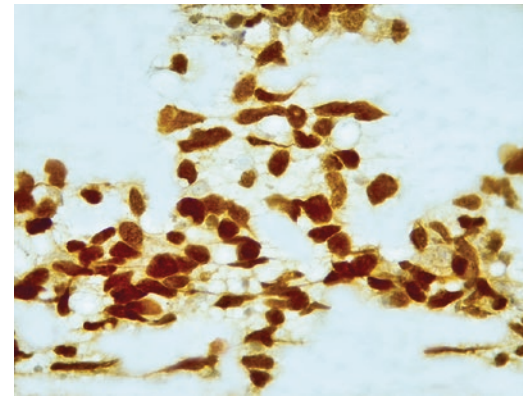

Fig. 9.5 Breast carcinoma cells showing strong nuclear positivity for ER in a smear (ICC, ER)

Hormonal Receptors: Estrogen (ER) and Progesterone (PR) Receptors

ER is a nuclear transcription factor with one DNA-binding domain and two AF (activation function) domains, and it is positive in 70–80% of breast cancer cases (Fig. 9.5). Expression of ER plays a significant role in tumor development in ER-positive tumors, drives disease progression in these tumors, and makes these carcinomas eligible for antiestrogen therapy [36, 43]. Clinically, ER-positive invasive breast cancers are usually better differentiated and have a more indolent course and favorable prognosis. There is a direct correlation between the likelihood of response to hormonal therapies and the levels of expression, but even tumors expressing very low levels of ER show benefit from hormonal therapy when compared with ER-negative tumors [36]. The American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines presently recommend that ER and PR be considered positive if $\geq$1% of tumor cells show nuclear staining of any intensity [36].

PR is also a transcription factor regulated mainly by ER [43] and, to some degree, by growth factors. PR is expressed in 55–65% of invasive carcinomas of the breast. The consensus opinion is that while the predictive role of PR may not be as clinically useful as ER [43], the assessment of this receptor provides some information. The loss of PR expression in ER-positive

tumors is associated with a worse prognosis and a decreased response to tamoxifen therapy [43], and some studies indicate a more significant benefit from endocrine therapy in ER-positive, PR-positive tumors [44]. In the rare situation of ER-negative, PR-positive disease (0.1–3.2% of breast cancers), there is not enough evidence of a clear benefit from endocrine therapy, but this may be related to the small number of such cases limiting the power of predictive testing, rather than real lack of benefit [43, 45]. Given the rarity of this phenotype and clinical uncertainty regarding the benefit from endocrine therapy, it would be prudent to retest both ER and PR before reporting these results, as several studies have demonstrated a significant proportion of cases yielding differing results on repeat testing [43, 46]. As with ER, PR is currently assessed by IHC, with a threshold of ≥1% tumor cells staining defining PR positivity.

All laboratories performing ICC assays for breast cancer biomarkers should closely follow quality control and quality assurance measures outlined in published guidelines [36, 37]. Although specific references for cytology are not currently included in CAP/ASCO guidelines, the following is recommended, when testing ER/PR in cytology specimens [1, 2, 9]:

- Formalin fixation is recommended at some stage for cytological sample preparation.
- If the material is fixed in alcohol or methanol, the controls used in the assay must have the same fixation and be validated.
- Cell blocks, liquid-based cytology, cytospins, and previously wet stained slides (previously air-dried slides are not recommended) can be used with the respective controls (Table 9.1).
- Antigen retrieval (heat-based) should be used.
- Positive and negative controls should be included in every run.
- It is highly desirable to maintain laboratory metrics for each prognostic/predictive test to monitor for potential analytical drift.
- All laboratories should participate in the external proficiency testing.

HER2 Testing

HER2 is a member of a family of transmembrane tyrosine kinase receptors and plays a vital role in the regulation of cellular signaling that affects cell growth, differentiation, and survival [43]. Gene amplification and overexpression of HER2 in 10–20% of invasive breast cancers are essential for prognosis, as HER2-positive breast cancer is associated with an aggressive clinical course and poor outcome [37, 43]. Because it is located on the cell surface, HER2 is an ideal therapeutic target for the drug trastuzumab, a humanized monoclonal antibody that directly targets the HER2 receptor by binding with high affinity to an extracellular epitope of the molecule [37, 43]. At the same time that the development of trastuzumab occurred, an IHC test was developed to evaluate the expression levels of the HER2 protein in formalin-fixed paraffin-embedded breast cancer tissue. This assay has been used to classify tumor cells as being negative (scored as 0 or 1+), equivocal (2+), or positive (3+) for HER2 expression, based on the degree of membranous staining, and so to select patients that will likely benefit from HER2-targeted therapies. Other HER2-targeted drugs, including lapatinib, pertuzumab, and the antibody-drug conjugate adotrastuzumab emtansine (T-DM1), have been developed and approved for the treatment of HER2-positive breast cancer. Given the continued expansion of options for targeting the HER2 pathway in breast cancer, accurate and reliable HER2 testing to help ensure that the patients receive the proper treatment is now more critical than ever [43].

For cytology, immunostaining of HER2 on direct smear or liquid-based preparations is not standardized and is insufficiently reliable for clinical use. It is associated with high variability in sample preparation, fixation, staining, and interpretation [1, 2, 3, 7]. A significant problem for air-dried smears and LBC was short-term cell conservation, but storage of LBC samples at −20 °C or −74 °C for 6 months did not appear to compromise immunoreactivity of the cells [47]. Compared with direct smears and LBC, cell

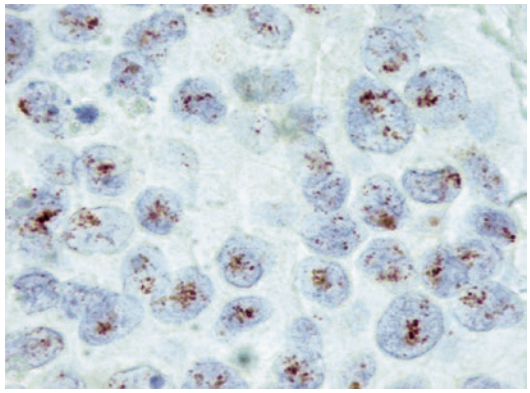

Fig. 9.6 Breast carcinoma showing amplification of HER2 in a cell block (SISH)

blocks make long-term cell conservation at room temperature feasible and allow the use of molecular testing protocols standardized for FFPE tissue [1, 36, 37]. HER2 ISH has been successfully performed on cell block material with concordance rates between 91% and 100% (Fig. 9.6) [1, 2, 3, 42, 48, 49]. Compared with paraffin sections for in situ hybridization (ISH) testing, the use of cytological smears or touch imprints has the advantage of assessing monolayered whole tumor cells and enumerating all the HER2 signals within an entire nucleus without a truncating artifact. FNAB specimens are acceptable for HER2 testing in metastatic sites, providing they are formalin fixed and safe to obtain. In situ hybridization should be used since cytological samples may have compromised cell membranes making ICC unsuitable [49, 50].

In summary, all the quality assurance principles mentioned for the determination of ER/PR should be used in the HER2 assessment. FISH on alcohol-fixed material should be avoided since it can cause autofluorescence that may hinder detection of HER2 amplification [49, 51].

Ki 67, Proliferation Marker

Ki67 remains one of the most controversial biomarkers in breast cancer. Ki67 antigen is expressed in all cycling cells, and it is the most commonly used ICC marker of cell proliferation. Clinical utility of Ki67 has been reported in the adjuvant setting as both a prognostic and predictive marker and an endpoint for neoadjuvant systemic therapy [43]. Neoadjuvant window-of-opportunity studies have become popular as a means of assessing short-term response to various treatments, utilizing Ki67 as a surrogate marker of response [52]. Despite recommendations in international guidelines, consensus on the method of evaluation of Ki67 in histological sections of breast cancer has not been achieved with a recommended cutoff of 14% based on gene expression profiling results still lacking clinical validation [43]. Much of the controversy surrounding Ki67 stems from issues surrounding reproducibility. Scoring methodology, fixation, antigen retrieval, choice of clone, and staining technique all influence Ki67 scores and contribute to poor reproducibility [43, 53]. Moreover, there is no clinical value in assessing Ki67 in metastatic breast lesions at present with unreliable data being generated by the direct application of guidelines outside individual laboratory references. In our opinion, presently there is no place to use Ki67 in breast cancer cytology for clinical purposes [7].

Conclusion

Despite its proven accuracy, time efficiency, and unequivocal cost-effectiveness, FNAB is currently underutilized in many developed countries. Globally, however, breast FNAB is increasingly used as a diagnostic modality because its equipment requirements are low and rapid on-site evaluation allows for immediate provisional diagnosis and patient triage for clinical management [1, 54]. Successful breast FNAB requires skilled aspirators, high-quality preparations of the material, and experienced pathologists in breast cytology interpretation [1]. Ljung et al. [55] demonstrated that physicians with formal training in FNAB sampling technique achieved more cellular samples and lower nondiagnostic rates. Establishing an effective FNAB training infrastructure and keeping an active real-time communication with clinicians and radiologists can avoid delays in reporting and can make this simple and efficient method for the diagnosis

of breast cancer available to a large number of patients around the world [1]. The combination of breast FNAB and ICC contributes to a more accurate diagnosis and allows the evaluation of breast cancer biomarkers in cytological material. FNAB is also ideally placed to monitor biological changes in metastases that may affect treatment and response, since it can be repeated with relatively little trauma even at different sites simultaneously, and it can be coupled with ancillary techniques even when the primary nature of a tumor is unknown [42, 49].

The Role of Molecular Testing, Including Fluorescent In Situ Hybridization and Multiple Parallel Sequencing in Breast FNAB Cytopathology

General Overview

Molecular techniques in routine pathologic examination are changing practice paradigms, as did the introduction of immunohistochemistry, and are preferentially used on histopathological material. However, they can be easily applied to cytological material. Presently, cytological samples present numerous advantages over histopathological material. These include the ability to check the quality of the tissue immediately after harvesting, better preservation of DNA and RNA [56, 57], and the possibility of conducting genomic studies on small amounts of cytological material obtained by FNAB or from effusions. In turn, this minimizes the need for invasive procedures and allows for more frequent re-biopsy enabling longitudinal monitoring of tumors and metastases [42, 58–60].

Molecular techniques in cytological samples have a wide array of applications. Depending on the method, they can be applied for diagnosis, subtype classification, and prognostic and predictive purposes (Table 9.2 and Fig. 9.1). Other more "exotic" applications where cytology is coupled with molecular techniques are, for example, the establishment of 3D organoid cell cultures and the establishment and monitoring of patient-derived tumor (PDX) models [61]. While these techniques are still mostly investigational, the transition to the clinical setting involving cytology techniques is taking place.

Technical Aspects

Cytological Specimens and Fixation Techniques

Like in any other diagnostic method, proper specimen processing in molecular cytopathology is fundamental to the success of the intended assay. While most cytological samples can be used for molecular techniques with minor differences depending on the specific method and material source, these technical aspects are even more critical than when using FFPE tissue samples due to the variation in the specimen type and fixation techniques.

Molecular testing in cytology specimens can be performed on a variety of specimens that range from fresh or frozen tissue to archival smears. Current molecular diagnostic procedures have been adapted to the workflow of the molecular diagnostic pathology laboratory, and, except for samples fixed in Bouin solution, most molecular methods can be performed on routine cytological specimens [62].

In specific molecular methods in breast cytopathology, in situ hybridization (ISH) techniques are ideally applied to monolayer direct breast FNAB smears that can be either ethanol-fixed or air-dried. ISH methods can also be used on cell blocks, following a more conventional protocol for FFPE preparations. On the other hand, polymerase chain reaction (PCR)-based techniques do not require monolayer smears and can be easily performed on FNAB material, either liquid-based or cells microscopically selected and scraped from slides. In this case, 50–100 cells are usually adequate to obtain satisfactory results given the conventionally used and clinically acceptable limits of detection. While low DNA or RNA concentrations are the most common limiting factor in the use of sequencing techniques, this is an area where the use of cytological techniques frequently outperforms the use of FFPE tissue samples. Cytological samples are a better

source of nucleic acids for sequencing techniques than the counterpart FFPE tissues, especially when dealing with challenging tissues, such as bone metastases, which are frequent in ER-positive breast cancer [63]. New nucleic acid extraction protocols and new sequencing platforms that require minimal nucleic acid quantities have contributed tremendously to the success of employing NGS on cytological samples and allow both targeted and whole exome sequencing in cytological specimens, including effusions. Liquid-based preparations once concentrated are usually considered ideal for NGS studies. Ethanol-fixed and Papanicolaou-stained or methanol-fixed and Giemsa-stained direct smears are equally useful. Cytological material collected from archival smears processed for routine diagnosis is a reliable source of nucleic acids for targeted and whole exome sequencing (WES) and single nucleotide polymorphism (SNP) array-based analysis [64]. Archival cytological specimens, previously processed and used for routine diagnostics, offer new opportunities for cytopathologists and oncologists and retrospective clinical and translational research in this field.

Like any other assay, the success depends not only on the interpretation of the results but also on the guarantee of valid results to start with. The correct choice of controls, positive and negative to verify the sensitivity and specificity of the technique against its reference is of the utmost importance. Additionally, laboratories should follow the accreditation requirements of relevant authorities, including the College of American Pathologists (CAP) in the USA, the Clinical Pathology Accreditation in the UK, or their national equivalents. Ideally, laboratories should also participate in external quality control assessments in clinical and molecular genetics as offered by the UK NEQAS or the NordicQC.

The Molecular Techniques

The molecular techniques classically used in cytology are based on PCR and ISH. Sequencing techniques utilizing massive parallel sequencing or next-generation sequencing (NGS) using target panels are also already routinely performed on cytology samples in many centers around the world, mostly for lung cancer specimens.

In breast cytopathology, like in other organ systems, the primary determinants in selecting the appropriate molecular technique are the target(s) to be identified, the type of alteration, and if there is a need to perform an in situ analysis rather than bulk sample/tumor analysis.

PCR-based methods have been used extensively in cytology and are ideal to identify specific targets, such as the detection of gross chromosomal alteration, including translocations or deletions and point mutations in particular genes. Reverse transcription polymerase chain reaction (RT-PCR) uses complementary DNA (cDNA) as a template for primer exon sequences to flank rupture points of the sequence/gene of interest. Then the target sequence of interest is amplified using traditional PCR, and a quantitative evaluation integration technique, such as qPCR measuring the amplification of DNA using fluorescent dyes, can be utilized.

ISH techniques, either with fluorescent or chromogenic markers, have also been applied extensively in breast cytology and are based on the hybridization reaction between the sequence of interest (usually DNA, but can be RNA) and a complementary sequence that is later detected using a fluorescent probe, a chromogenic reaction, or a silver precipitate. With ISH techniques it is possible to detect deletions, insertions, or translocations or, most frequently and routinely, amplifications, such as HER2 in breast carcinomas.

In contrast to these more specific approaches, NGS techniques can investigate more genes or sequences of interest simultaneously. NGS is highly scalable and allows the level of resolution to be tuned to meet specific experimental needs, making it possible to obtain a clinically significant resolution with minimal amounts of cytology material. Depending on the assay design, it makes possible an array of interrogations that includes mutation analysis, copy-number alterations, or even genome-wide methylation or DNA-protein interaction profiling in a single sample. Additionally, if the assay also includes RNA,

then translocations/fusion genes become technically easy to detect and report, as well as information based on gene expression, which can be especially important in the case of breast carcinoma. However, comprehensive profiling of tumors by NGS is still challenging in terms of clinical workflow integration due to the amount of data generated and the complex clinical interpretation. While the study of the multiple transcripts by NGS has the potential to replace a variety of assays using several techniques, thus making the use of an integrated NGS assay querying both somatic DNA and RNA more cost effective, the current trend for most departments is to offer only a targeted somatic mutation panel occasionally with information on copy-number alterations based on off-target reads. Despite some of these more comprehensive analyses remaining mostly investigational, the power of these techniques for detection of alterations is well established and can be easily translated to the clinical sphere once the clinical importance of detected alterations is clinically well established in breast cancer.

The Application of Molecular Techniques

Personalized diagnosis of carcinomas requires robust, validated diagnostic tests, which should be simple enough and reliable so that they can be applied in routine diagnostic pathology laboratories [65]. In the section on technical aspects, we have established that cytological material and, in particular, FNAB material is a reliable source of nucleic acids for molecular techniques, ranging from "simpler" FISH or qPCR to targeted sequencing, and WES. Presently, high-throughput molecular analysis can not only be performed on FNAB material but can also be used on routinely processed "residual" cytology FNAB material, which is particularly important in patients with either a paucicellular specimen or lack of a cell block [66]. Therefore, the precise molecular technique or the type of cytological sample should not be considered limitations to the application of molecular techniques in the clinical practice of molecular cytopathology. Like the use of their histologic "counterparts," as frozen or FFPE samples, current limitations in the application of molecular techniques in cytology and, in particular, in neoplastic diseases of the breast are not technical but due to the limited knowledge of the mechanisms of progression in breast cancer and the defined molecular hallmarks specific to each disease stage.

The frequent genetic alterations in breast cancer are well established. Due to the efforts of large consortiums, such as The Cancer Genome Atlas Network (TCGA), the mutational and copy-number alteration landscape of breast cancer has been established in cohorts with considerable numbers of patients. We have evolved from differentiating the type of breast cancers into intrinsic subtypes using expression studies, to understanding the mutational landscape of breast cancer. Besides confirming the occurrence of recurrent somatic mutations, including AKT1, CDH1, GATA3, PIK3CA, TP53, and PTEN, these studies have identified potential driver mutations and copy-number alterations in genes, such as AKT2, CASP8, or PPP2R2A [67, 68]. Despite this increased knowledge, specifically in breast cancer, the clinical significance of many of these alterations remains obscure. This has challenged the clinical translation of this knowledge [69] and led to reduced interest in widespread routine use of somatic cancer mutation panels in breast cancer, clearly contrasting to current clinical practice regarding lung carcinomas and hematologic malignancies. Additionally, the reduced interest in routinely performing somatic mutation and CNA testing in breast cancer has also led to the lack of clinical opportunities to use the integrative cluster classification of breast cancers [68, 70]. While of significant magnitude, these challenges highlight the current needs and the many opportunities for improvement of patient care in molecular cytopathology of the breast and how this field could be at the brink of a surge in newer clinical applications and demand.

Predictive Markers Including ISH for HER2

As mentioned above, the evaluation of the predictive and prognostic markers ER, PR, and HER2 has been successfully performed on FNAB material over a long period. More targeted

approaches using molecular techniques, such as qRT-PCR, to assess ER, PR, and HER2 status, qPCR for HER2 copy-number alterations, and comprehensive transcriptional profiling using microarrays have been successfully tested for breast cancer using FNAB material [71–73]. Despite this success, the implementation of many of these techniques in the clinical setting has been difficult because they are often erroneously perceived as more complicated, time-consuming, or not cost-effective because they use FNAB samples. Additionally, many of today's commercially available breast cancer multigene prognostic tests are not available and/or have not been validated in FNAB material, making FNAB a less popular source of samples for molecular studies. However, this is rapidly changing, and the popularity of FNAB samples for molecular diagnosis is presently on the rise.

The use of HER2-targeted agents requires confirmation of HER2 overexpression or amplification. FISH is currently regarded as the gold-standard method for detecting HER2 amplification. The main difficulty for adopting FISH in a clinical setting is the need for additional equipment for the analysis, such as fluorescence microscopes and multiband fluorescence filters. Silver in situ hybridization (SISH), which was developed to overcome these disadvantages, has been used with excellent concordance with FISH. A study showed that an overall concordance rate between CISH and FISH was higher than 95% [74]. HER2 assessment using FISH or SISH on FNAB material shows excellent correlation with the histopathological specimens and is also an excellent method for assessing HER2 status in the metastatic setting, including effusions (Fig. 9.6).

RT-PCR-based methodologies have been available to assess ER, PR, and HER2 status, as well as qPCR for HER2 CNA in cytology specimens. While feasible and usually showing good correlation with gold-standard testing, they are not widely used in most countries due to regulatory environments and where reimbursement issues might be a concern. However, there has been a recent resurgence in the interest in the use and validation of multiplexed qRT-PCR methods for accessing predictive markers in breast carcinomas [75, 76]. These assays can be delivered in a closed, packable system (cartridge-based), with insignificant standardization issues, and offered at locations with minimal human resources or infrastructure. As such, these types of assays are the ideal companion for a cytopathologist working in remote areas with limited access to a modern pathology/cytopathology laboratory. These assays can a provide both a diagnosis and an appropriate treatment plan based on ER, PR, and HER2 testing in a single trip to a cytopathologist working in a remote or resource-poor location and therefore could potentially transform breast cancer care in low- to medium-income countries.

FISH for Primary Diagnosis of Special Histopathological Subtypes of Breast Cancer, Secretory and Adenoid Cystic Carcinomas

Few breast cancer special histopathological types and variants are defined by recurrent genetic alterations as specific translocations. Presently, only secretory carcinoma of the breast and adenoid cystic carcinomas are known to harbor particular translocations that are subtype defining.

Secretory carcinomas of the breast are exceedingly rare entities that tend to occur in young individuals and, despite being triple negative, are indolent [77]. Cytologically these are characterized by the presence of globular structures, consisting of small centrally located, mucoid material with covering epithelium, usually composed of two or three, and occasionally more, cells [78]. However, some of these cytomorphologic characteristics are shared with the also rare acinic carcinoma of the breast. The distinctive feature of secretory carcinoma is the presence of a recurrent t(12;15)(p13;q25) translocation resulting in ETV6-NTRK3 gene fusion [79]. As such, while not traditionally performed on cytological material, ETV6 split signal probes are available, making the diagnosis of secretory carcinoma of the breast possible on ISH.

Another subtype that is rare and triple negative and has a favorable prognosis is adenoid cystic carcinoma of the breast. Adenoid cystic carcinomas of the breast are defined by a recurrent translocation, in this case t(6;9)(q22e23;p23e24),

which generates fusion transcripts involving MYB and NFIB [80]. An FNAB of this tumor most commonly shows tissue fragments of cohesive small uniform cells arranged around magenta-stained hyaline globules associated with tubular structures covered with uniform epithelial cells. The individual cells are small and have round or ovoid nuclei which are often naked in smears, but a narrow rim of cytoplasm may be present [81]. Due to this distinctive pattern, the use of ISH for the diagnosis of this type of tumor is rarely needed. However, break-apart FISH probes are commercially available and can be used as an ancillary study to establish the diagnosis of adenoid cystic carcinoma of the breast in a cytological specimen.

Tracking of Tumor Evolution, Including ESR1 Status and HER2 Amplification Status

With the increased recognition of breast cancer tumor heterogeneity and tumor evolution during treatment, frequent re-biopsying of the tumor and the metastases in the same patient is often needed and is part of the standard of care during treatment. For this purpose, FNAB has been shown to be an ideal method for tumor and metastasis sampling [42, 59]. While truly "liquid biopsies" based on circulating tumor cells or circulating tumor cells DNA (ctDNA) are not a clinical reality, FNAB is the ideal method for repetitive sampling of tumors and metastasis for tumor evolution tracking. FNAB is a minimally invasive method, and it is safe, cost-effectiveness, and an excellent source of genetic material for any modern molecular technique. Additionally, when coupled with advanced imaging techniques, most metastatic sites are accessible for FNAB sampling. These characteristics make FNAB the ideal tumor sampling technique to track tumor evolution. The application of molecular pathology techniques in FNAB samples is the perfect combination to deliver state-of-the-art care, thus making cytology a central discipline in modern precision medicine efforts.

Presently, one of the best-known consequences of tumor evolution with critical clinical implications is the change in ESR1 mutation status [82], although there is considerable debate regarding the prevalence and clinical significance of ESR1 gene amplification [83–85]. Amplification of ESR1 has been reported as an acquired aromatase inhibitor resistance mechanism, namely, in patient-derived xenograft (PDX) models and in the corresponding human ER-positive cancers that progressed on aromatase inhibitor therapy [86], but its clinical significance is yet to be established. On the other hand, ESR1 mutations are well established as clinically significant [87]. ESR1 point mutations are exceedingly rare in treatment-naïve primary breast cancer patients [67] but are frequently identified (11–55%) in patients who have progressed while on endocrine therapy [88, 89] and signal resistance to hormonal therapy in the metastatic setting. It should lead to consideration for the use of more potent ER antagonists. Whether ESR1 mutations can be identified in naïve-treatment tumors as the coverage of sequencing increases and could serve as predictors of endocrine therapy response, is yet to be demonstrated. Routine testing of ESR1 mutation status in patients with ER-positive breast cancer and metastatic disease resistant to endocrine therapy should be considered for clinical recommendations and perhaps even the tracking of the mutation development with sequential biopsies.

Recently, in addition to ESR1 mutations, other genomic alterations have been identified in breast carcinomas under hormonal treatment. An increased number of alterations in genes involved in the mitogen-activated protein kinase (MAPK) pathway and ER transcriptional machinery has been found in patients previously exposed to hormonal therapy [90]. Activating mutations of ERBB2 and loss-of-function mutations were also more frequent in endocrine-resistant tumors. Interestingly, MAPK pathway and ER transcriptional factor alterations seemed to be mutually exclusive with ESR1 mutations and were associated with shorter response to endocrine therapies. The identification of these different groups with clinical significance may have just opened the door to a new molecular classification of metastatic breast carcinoma based not only on the "primary" molecular subtype but also on the pathway of tumor evolution. Independently of

the success of a newer molecular classification for a metastasis, we foresee an increased demand for clinical massive parallel sequencing of breast carcinoma metastasis samples. FNAB is the ideal method for sampling breast carcinoma metastases in various locations and at different time points for sequencing studies.

While ESR1 mutation and possibly other gene testing are in their early clinical application stages, sequential testing of HER2 during the course of the disease in HER2-amplified carcinomas is already well established in clinical practice. Discrepancies between the pathology report for the primary tumor and that for the metastatic lesions are frequent, with Wilking and colleagues showing that HER2 status was similar in the primary carcinoma and the metastatic site in only 76% of the patients [91]. Additionally, the same authors showed a significantly worse outcome in the patient group, in which HER2 status changed between the primary and metastatic tumors, when compared with patients in whom positive HER2 status remained concordant. Thus, an unstable status for HER2 in breast cancer is clinically significant and should motivate retesting of tumor recurrences. In our experience, with use of FISH on FNAB material, around 15% of patients show disagreement between the HER2 assessment in primary and metastatic breast cancer. Thus, we recommend retesting of HER2 amplification status in any new metastasis or when there is therapeutic failure.

Conclusion

In summary, massive parallel sequencing can be performed on routine cytological samples, and FNAB biopsies are the ideal method for tumor sampling and re-sampling for most molecular studies. Specifically, regarding breast cancer, while the knowledge about breast carcinoma origin and evolution has been increasing exponentially, drivers of clinical behavior and new clinical targets have been difficult to identify and clinically translate. The first publications on studies tracking breast carcinoma evolution during treatment are beginning to emerge, and we hope these will shed light on new predictive genomic alterations [92].

In the meantime, and in addition to HER2-amplified carcinomas, ESR1 mutation status tracking is perhaps the only example of a defined mutation with clinical implications whose evolutionary dynamics are worth tracking clinically. Continued efforts are needed in the clinical and translational research setting to gather clinical and genomic data through routine whole exome sequencing or using target sequencing panels to discover new, actionable clinical alterations in breast cancer and to understand the complete genomic landscape of breast carcinomas even as further alterations emerge during different therapeutic cycles.

Precision oncology and diagnostics efforts which transition from research to clinical trials and to the clinical setting demand that the cytopathologist be prepared to request and correctly interpret molecular tests, to meet the patient's needs, and to provide meaningful diagnostic, prognostic, and predictive information.

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10 Fine Needle Aspiration Biopsy Cytopathology of the Breast Utilizing Liquid-Based Preparations

Fernando Schmitt and Rana S. Hoda

Introduction

Conventional direct smears and cell blocks have traditionally been used as the preferred preparations for breast fine needle aspiration biopsy (FNAB). However, liquid-based cytology (LBC) preparations, including ThinPrep [TP, Hologic, Boxborough, MA] and SurePath [SP, BD Diagnostics, Burlington, NC], are increasingly being used as either the sole preparation or in conjunction with the traditional smears for diagnosing breast lesions. Although the LBC produces minor changes in cytomorphology and background features, diagnostic sensitivity and specificity are similar to conventional preparatory techniques [1–3].

The LBC technique consists of an automated method for preparing thin-layer cytological samples onto glass slides from cell suspensions collected in alcohol-based preservative and stained with the Papanicolaou (Pap) stain. These methods are designed to improve the conventional cytological preparations by avoiding limiting factors, such as obscuring material, air-drying artifacts and irregular thickness of the smears [1–3].

Multiple studies have demonstrated the utility of LBC for breast FNAB. The collection technique is uniform, and the sample collection vial containing preservative solution, is easy to transport and store. Other advantages include a single standardized and uniform preparation with no obscuring elements making it easier to screen and interpret, better cellular preservation and less cell overlapping in comparison to smears. Ancillary tests, such as immunocytochemistry, can be formed on additional LBC slides. The main disadvantages of LBC are that on-site adequacy assessment cannot be performed and that alterations in pattern, tissue fragment architecture and cellular morphology are present, and there is loss of or at least a less informative background [1–4].

The architecture and cytological alterations include the following: cell tissue fragments may become fragmented and more single cells may be seen; cells may appear smaller; due to immediate liquid fixation, nucleoli may become apparent even in benign lesions; myoepithelial cells may retain their cytoplasm and mimic cells of invasive ductal carcinoma; and in the background, stromal cells are reduced or absent and extracellular material, such as mucin or necrosis, may be missing, and the background material may clump instead of being diffuse as in smears [1–4]. Because of these differences, some authors have

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A. S. Field et al. (eds.), *The International Academy of Cytology Yokohama System for Reporting Breast Fine Needle Aspiration Biopsy Cytopathology*, https://doi.org/10.1007/978-3-030-26883-1_10

advocated that prior training in LBC and the use of LBC in conjunction with the traditional preparations for diagnosing breast lesions are necessary to avoid potential pitfalls and diagnostic errors [1–3].

In this chapter, we describe the architecture, cytological and background alterations, and the diagnostic performance of LBC in breast aspirates. These features will be illustrated with specific breast lesions.

General Cytomorphological Characteristics in Liquid-Based Slides

Cellularity

LBC shows similar epithelial cellularity to direct smears for both benign and malignant breast lesions. The quality and cellularity of the samples, however, largely depend on the number of FNAB passes and the skill of the practitioner performing the procedure.

Architectural Features

Epithelial tissue fragments are fragmented, shortened, and less distinct in LBC, resulting in smaller tissue fragments and increased cellular dissociation. Three-dimensional (3D) arrangements may be more pronounced in both benign and malignant breast lesions. The presence of small tissue fragments, loss of cohesion and three-dimensional (3D) fragments may lead to an erroneous diagnosis of malignancy.

Cell Changes

Because the cells are immediately fixed in a liquid medium, they tend to be rounded and smaller than the flattened cells of a smear. Cells are usually better preserved with enhanced nuclear detail, including well-defined, usually more pronounced nucleoli, even in benign lesions. Darker stained nuclei (hyperchromasia) can be seen in both benign and malignant breast cases. Nuclear changes can be very subtle in cases of tubular carcinoma, papillary carcinoma and low-grade invasive ductal carcinomas, resulting in a high rate of false-negative diagnoses before LBC training. The cytoplasm can be dense or overstained, but the cells located in the periphery of cell tissue fragments may show frayed cytoplasm. Myoepithelial cells as bare bipolar nuclei are usually less apparent or decreased in number in LBC preparations. Moreover, some myoepithelial cells may have an intact cytoplasm resembling fibroblasts or invasive ductal carcinoma.

Background Elements

The background is usually clean in LBC with less obscuring elements, such as blood, excessive inflammation and cellular debris. However, the background elements are usually retained sufficiently in LBC for diagnostic purposes, although reduced and altered. Mucus or necrosis may clump with the latter clinging to tumor cells, hence designated as “clinging” diathesis.

Selected Breast Lesions on LBC

Some benign and malignant breast lesions prepared by the LBC technique have different cytomorphological features compared to those on smears, including fibroepithelial lesions such as fibroadenoma and papillary lesions, and certain types of breast carcinomas. Recognizing these differences and potential pitfalls will help in making a correct diagnosis.

Fibroadenoma [1–5]

In conventional smears (CS), fibroadenomas show cellular preparations consisting of large and cohesive branching sheets of ductal epithelial cells which may have staghorn configurations, numerous bare bipolar nuclei and myoepithelial nuclei, and fragments of fibrous or fibromyxoid stroma. However, fibroadenoma is a well-recognized

source of false-positive diagnoses because it is misdiagnosed as low-grade carcinoma of no special type, due to shared cytomorphological features.

Similar features and pitfalls are seen in fibroadenomas processed as LBC. Cytologically in TP, ductal cells appear in staghorn tissue fragments with isolated bare bipolar nuclei and stromal fragments. But the diagnosis is more problematic on LBC, with low diagnostic rates compared to direct smears and more false-positive diagnoses of fibroadenomas as atypical or suspicious of malignancy. LBC shows small epithelial tissue fragments in contrast to large branching sheets, a decrease in the number of myoepithelial cells, and paucity or loss of stromal fragments (Fig. 10.1a–i). Most importantly, enhanced cellular discohesion and prominent nucleoli may be potential diagnostic pitfalls.

Lactational Change [1–3, 6]

In pregnancy, the terminal ducts develop acini and the lobules enlarge, with the latter showing different shapes, irregular distention and secretory changes. Histologically, the cells within the lobules enlarge and proliferate and display variably vacuolated cytoplasm, hyperchromatic nuclei and prominent nucleoli. Cytologically, the architectural and cellular features are similar to histology. Both direct smears and LBC show lipid droplets and proteinaceous material in the background containing "stripped" round nuclei with prominent nucleoli. FNAB of breast masses in pregnant or lactating women is an uncommon procedure, and cytological interpretation may be problematic due to atypia inherent to secretory change in glandular epithelium (Fig. 10.2a–d).

Papillary Lesions [1–3]

Papillary neoplasms of the breast include a wide spectrum of benign and malignant lesions, and the differential diagnosis can be difficult not only in FNAB but also in CNB. Fibroadenomas and papillary neoplasms can be morphologically distinguished on TP specimens. While staghorn tissue fragments, fibromyxoid stroma and bare bipolar nuclei are characteristic features of a fibroadenoma, the presence of papillary tissue fragments and single columnar cells is highly suggestive of a papillary neoplasm.

Intraductal Papilloma

Intraductal papillomas in direct smears usually reveal broad branching fragments with rounded contours, columnar cells, scarce-to-moderate numbers of myoepithelial cells, and apocrine cells. On LBC, pseudopapillary or papillary tissue fragments and cell balls, comprising columnar to round ductal cells with uniform distribution of myoepithelial cells, similar to direct smears can be seen. The background is either proteinaceous or bloody and contains macrophages (Fig. 10.3a–d). Immunostains on extra LBC slides for smooth muscle actin, calponin, or p63 may be useful in identifying myoepithelial cells which may favor papilloma.

Papillary Carcinoma

In direct smears, papillary carcinomas tend to show slender and complex papillae with thin fibrovascular cores, an increased number of dissociated tall columnar cells and variable nuclear atypia. LBC shows features similar to direct smears with branching frond-like papillae with less evenly distributed and more complex fibrovascular cores compared with papilloma. The epithelial cells show less orderly, hyperchromatic nuclei with uneven chromatin distribution and high N:C ratio. The myoepithelial cells are absent and more frequent mitoses are seen. The LBC differs from direct smears in that the fibrovascular cores appear as eosinophilic central cores, and there are fewer dissociated tall columnar cells (Fig. 10.4a–d).

Carcinomas

Invasive Carcinoma of No Specific Type (NST) [1–4, 7–9]

LBC and direct smears have comparable performance for the detection of breast carcinomas. It is

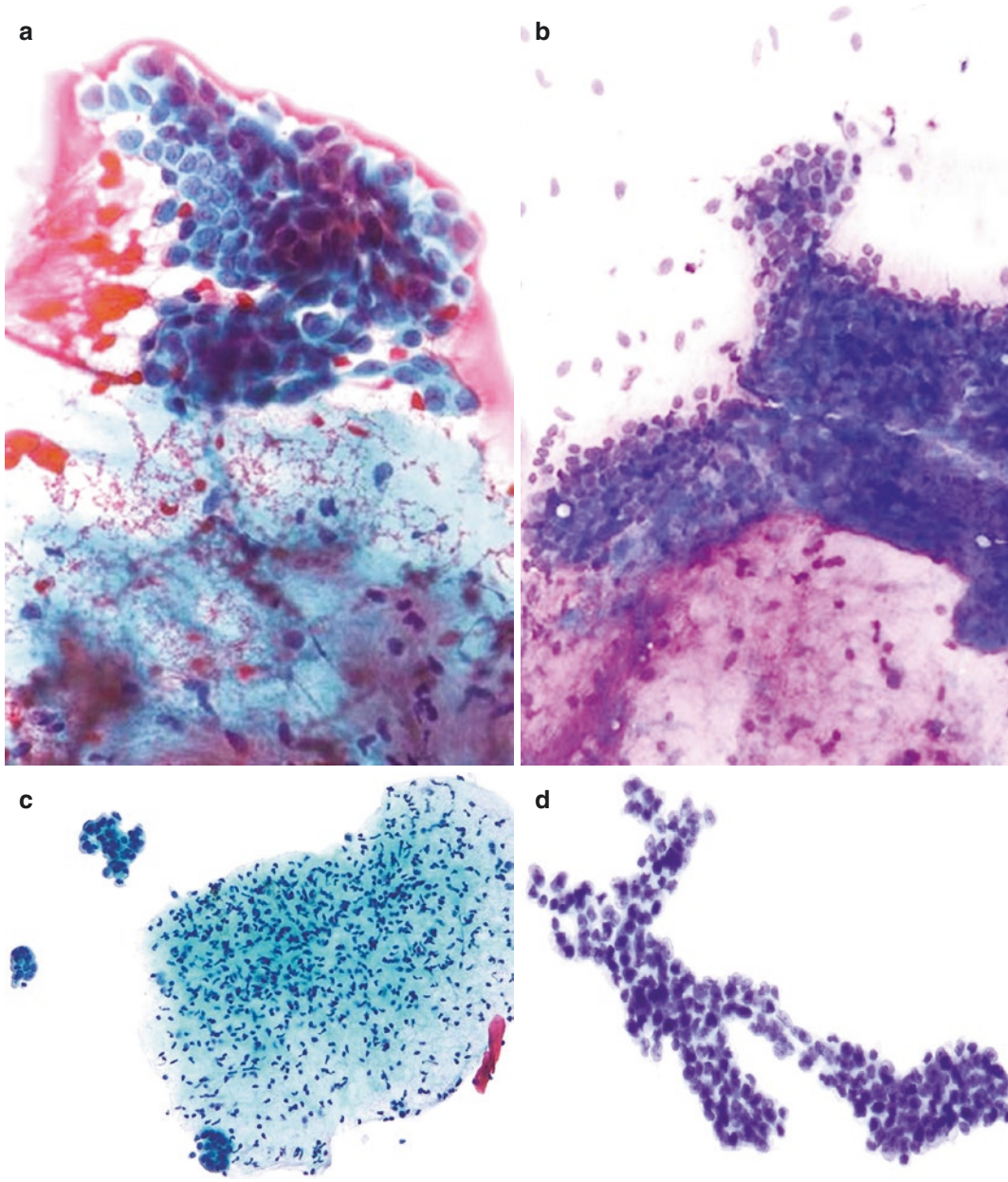

Fig. 10.1 Fibroadenoma (FA): (**a**, **b**) FA shows stroma, a staghorn arrangement of cohesive ductal epithelial cells intermixed with spindled myoepithelial cells (Pap and Giemsa direct smears); (**c**) Similar features are seen in LBC. Note the large stromal fragment with tissue fragments of epithelial cells, both close to it and dissociated from it. The background is clean with rare bare bipolar nuclei (Pap TP); (**d**) Another case of FA showing a staghorn tissue fragment of cohesive ductal epithelial cells in a clean background (Pap TP); (**e**) FA showing a small epithelial tissue fragment with spindle myoepithelial cells which have retained their cytoplasm (Pap, TP); (**f**, **g**) FA processed as TP and SP, respectively. Both show similar cellular and background features. The ductal epithelial cells in SP appear more 3D (Pap TP, Pap SP); (**h**) Atypia of nuclei and dispersal at high magnification seen in low-grade carcinoma of no special type (Pap, TP); (**i**) Reactive atypia in FA may mimic a low-grade ductal carcinoma. Myoepithelial cells are prominent in FA (Pap, TP)

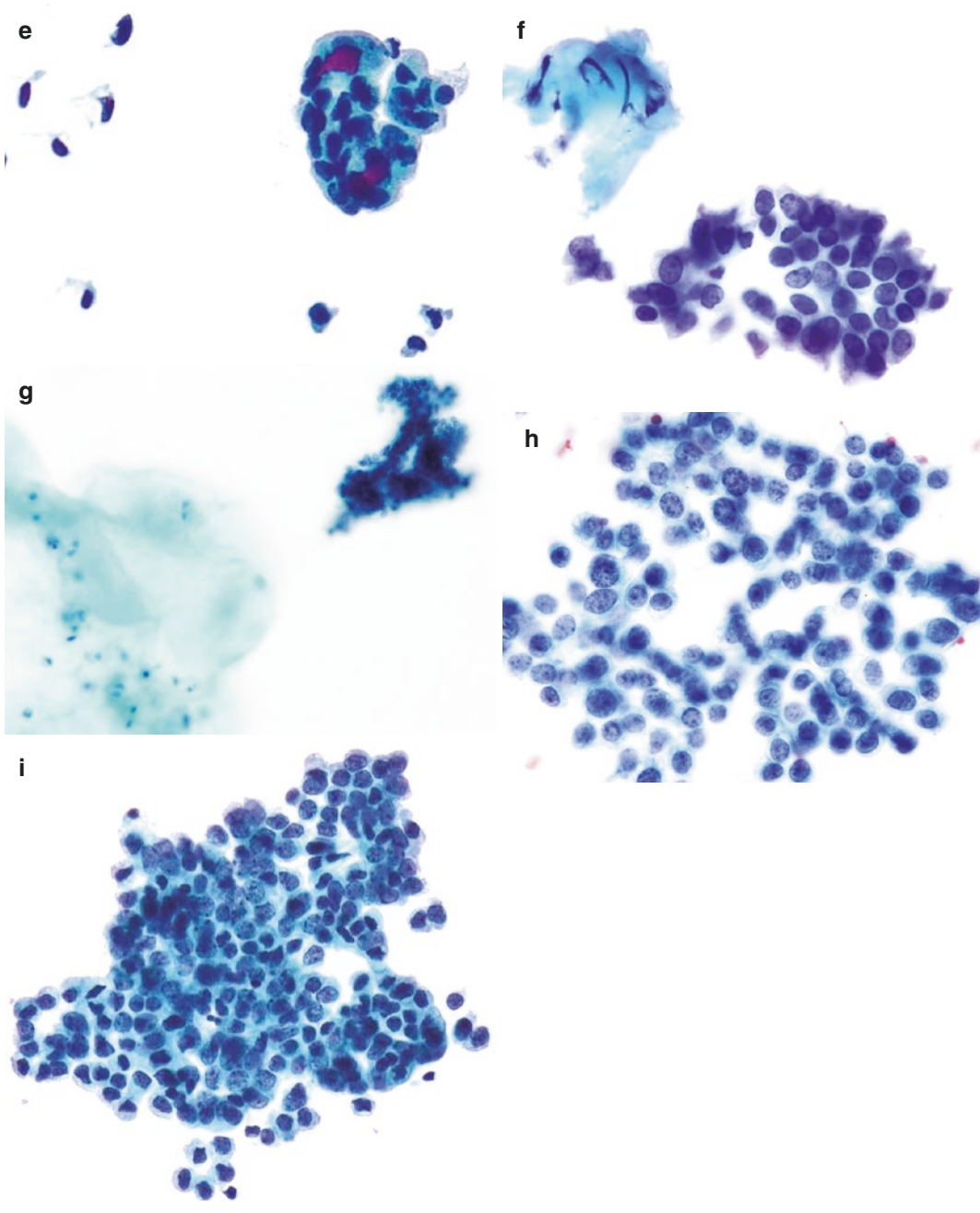

Fig. 10.1 (continued)

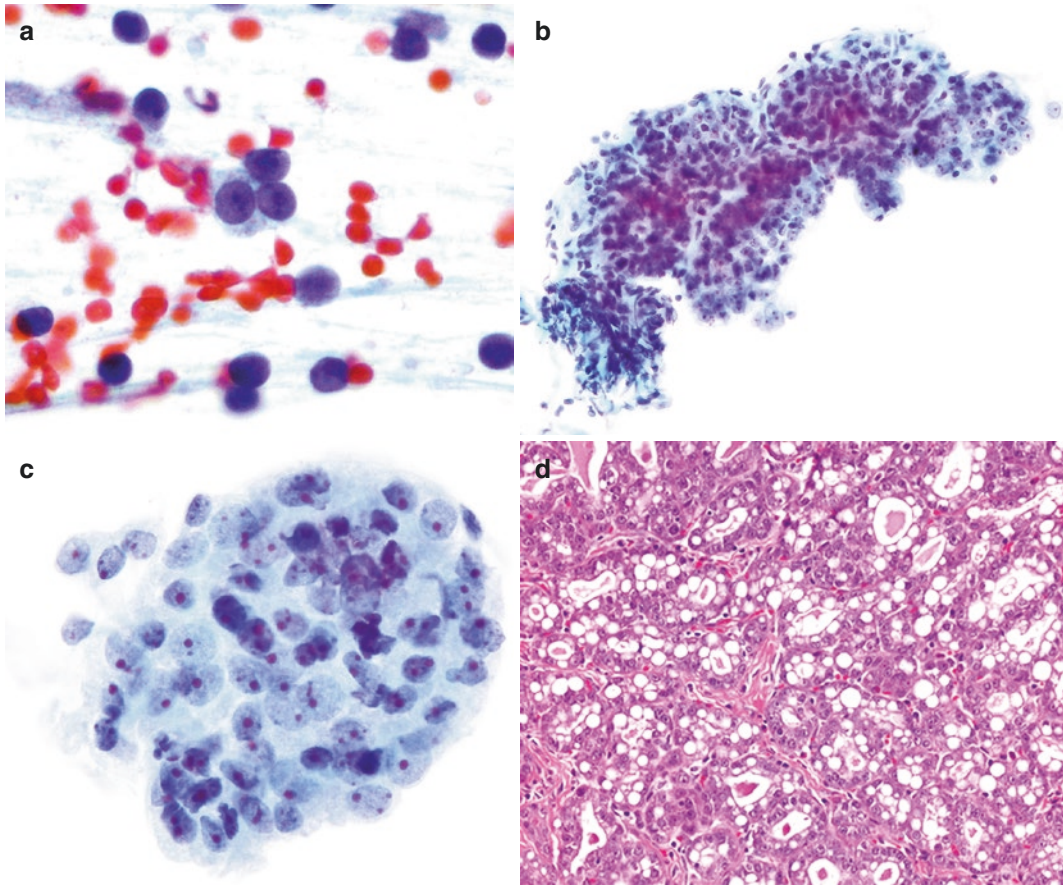

Fig. 10.2 Lactational nodule: (**a**) Proteinaceous background with a minute tissue fragment of acinar cells with finely granular cytoplasm and uniform, round, hyperchromatic nuclei with prominent nucleoli and scattered stripped nuclei (Pap, conventional smear); (**b**) Distended lobules comprising cells with foamy and vacuolated cytoplasm, uniform nuclei, and prominent nucleoli. Background is clean in this LBC (Pap, TP); (**c**) Small acinar tissue fragment of cells showing monotonous nuclei, prominent nucleoli, and foamy and delicate cytoplasm (Pap, TP); (**d**) The corresponding resection specimen contains cells with very similar features (H&E)

easier to diagnose carcinoma on LBC because of the clear background and the detailed nuclear features of the neoplastic cells. The background may show necrotic material clinging to tumor cells. The architecture in the LBC shows large tissue fragments of carcinoma cells reduced to smaller tissue fragments. Three-dimensional tissue fragments are more frequently found in SP preparations in contrast to the more common flattened epithelial tissue fragments in TP slides (Fig. 10.5, 10.6, and 10.7).

Tubular Carcinoma

LBC of tubular carcinoma shows small numbers of single abnormal cells and cohesive 3D tissue fragments with rigid borders, minimal cell stratification and absence of myoepithelial cells. The nuclei show mild atypia and are small and monomorphic with fine chromatin and inconspicuous nucleoli. Potentially, low-grade invasive ductal carcinomas are often diagnosed as negative or "atypical" (Fig. 10.5a, b).

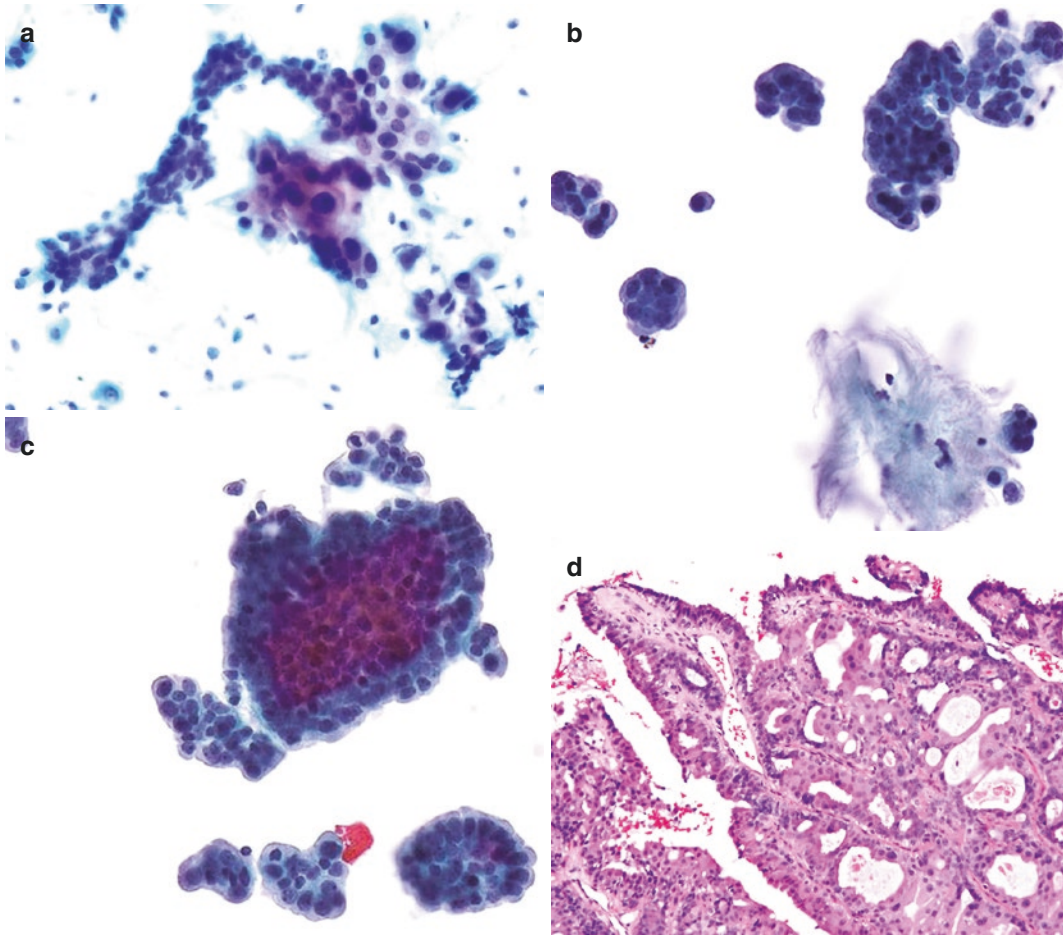

Fig. 10.3 Intraductal papilloma: (**a**) Cuboidal to columnar epithelial cells in minute tissue fragments with scalloped borders. Nuclei are oval to round, regular with fine chromatin and small nucleoli. The tissue fragment at 1 o'clock position shows small broad branching fragments. Compare this to the slender complex papillae of papillary carcinoma in Fig. 10.4 (Pap TP); (**b**) Papilloma displaying more pronounced broad papilliform fragments. Background is clean in both images (Pap TP); (**c**) Papilloma with apocrine metaplasia (Pap, SP); (**d**) Excisional specimen showing multiple papillae with well-developed vascularized connective tissue; apocrine metaplasia can be seen (H&E)

Mucinous Carcinoma

Mucinous carcinoma is better diagnosed on Giemsa-stained direct smears compared with Papanicolaou-stained smears and TP slides. In LBC the diagnosis of carcinoma with mucinous features may be challenging as the mucin in the background can be reduced or lost (Fig. 10.8a–c). The mucin stains magenta on air-dried Giemsa-stained slides and bluish-green on alcohol-fixed Papanicolaou-stained slides. The differential diagnosis includes mucocele of breast and a metastasis of mucinous carcinoma from other sites, such as the colon, the lung and the gynecological tract.

Invasive Lobular Carcinoma

In direct smears and LBC, the cells are discohesive and seen singly or in single-cell thick strands with well-preserved nuclear detail, intracytoplasmic lumina and high nuclear-cytoplasmic ratio. "Signet ring" cells with intracytoplasmic mucinous condensation or a "targetoid

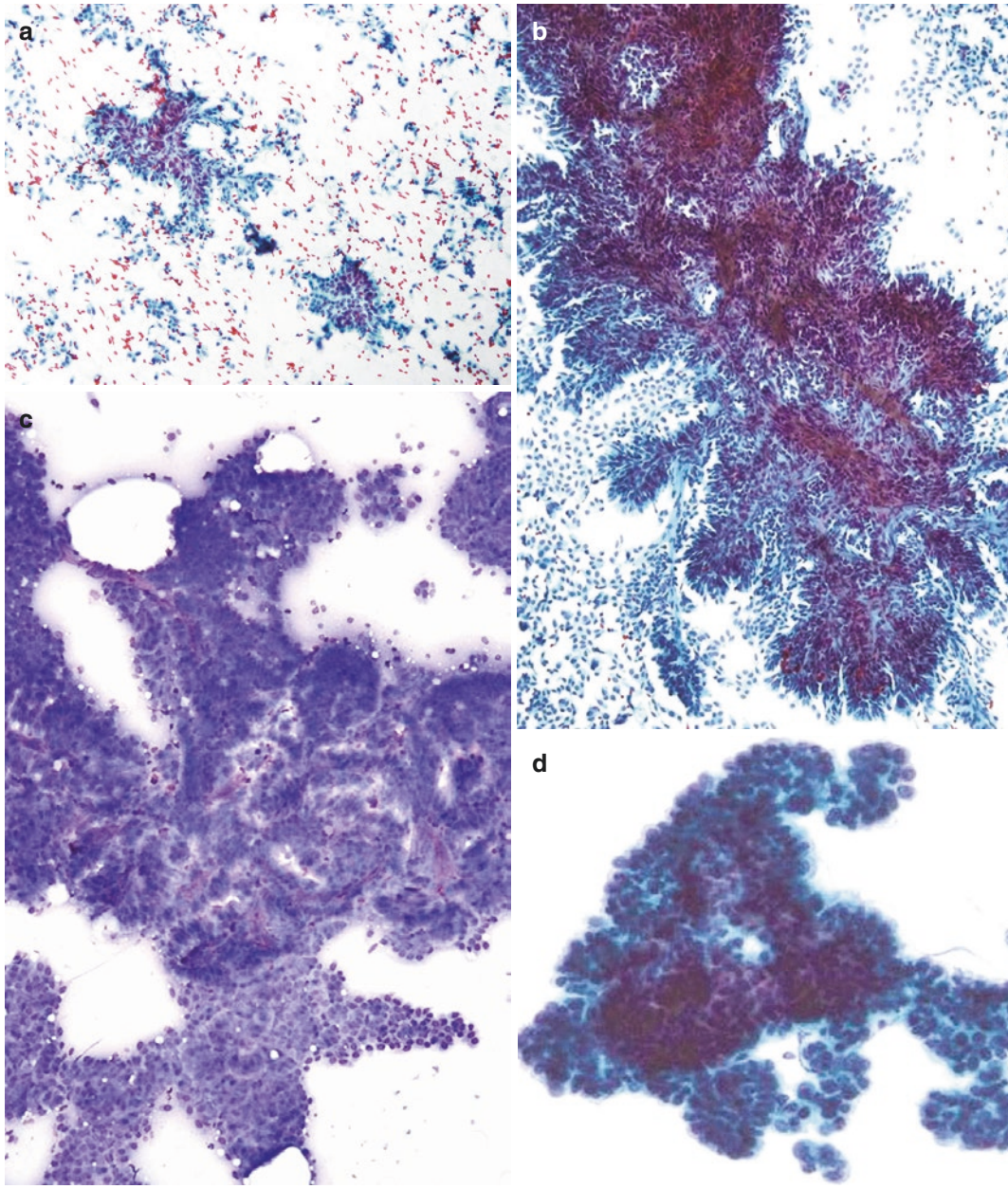

Fig. 10.4 Papillary carcinoma: (**a**) Multiple cellular fragments, some with thin branching and complex arborizing architecture, as well as small tissue fragments. The cells around the edges are columnar. Fibrovascular cores appear as central eosinophilic thin bands. The nuclei are hyperchromatic and overlapping. Background is clean (Pap, low power, TP); (**b**) Papillary carcinoma showing complex branching with the eosinophilic band of the fibrovascular cores and background with many single columnar cells (Pap, conventional smear); (**c**) Papillary carcinoma showing fine branching fibrovascular cores with a crowded columnar arrangement and plentiful dispersed cells in the background (Giemsa, low power, conventional smear); (**d**, **e**) LBP from the same case as Figures **b** and **c**, showing multiple cellular fragments, some with thin and complex arborizing architecture, as well as central eosinophilic thin bands (Pap stain, TP); (**f**) Resection demonstrating complex papillae with columnar epithelium (H&E)

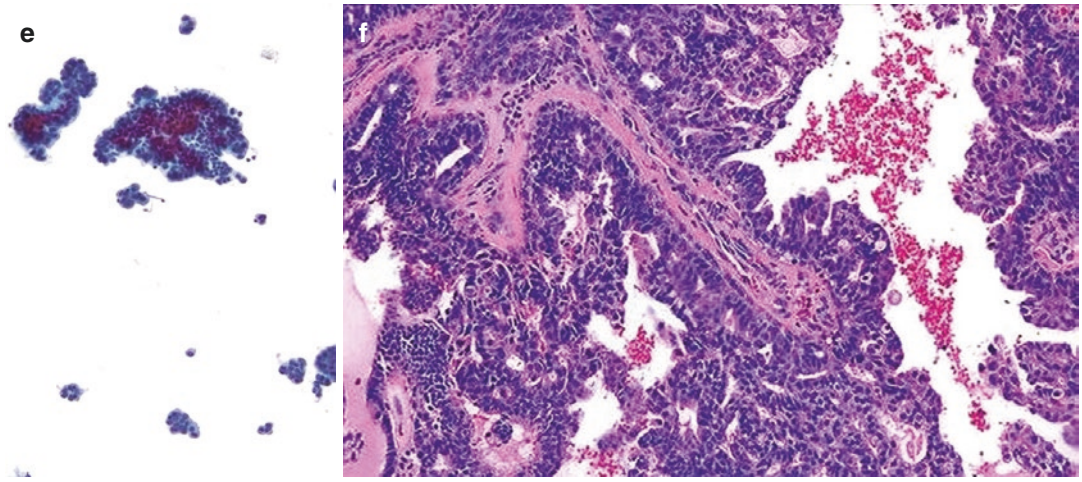

Fig. 10.4 (continued)

body" can be seen. The background is clean in LBC. Invasive lobular carcinoma is one of the most common causes of false-negative FNAB due to scant cellularity of tumor cells and small tumor cell size which may mimic lymphocytes [10] (Fig. 10.9a–c).

Pleomorphic lobular carcinoma is a well-defined variant of lobular carcinoma showing high nuclear grade and high N:C ratio in large cells with marked single-cell dispersal and small tissue fragments. The aggressive biology of this type may relate to overexpression of HER2 (Fig. 10.10a–c).

Metastatic Tumors to the Breast [1–3, 11]

Metastases to the breast account for 1.3–3% of malignant breast tumors. Hematopoietic neoplasms, malignant melanoma, and small cell carcinoma of the lung are the most commonly reported primary tumors to metastasize to the breast. Clinical history, awareness of the features suggesting a non-breast primary carcinoma, and immunohistochemical assessment on FNAB, using conventional preparations or LBC, are important in distinguishing between metastatic and breast primary carcinoma (Fig. 10.11).

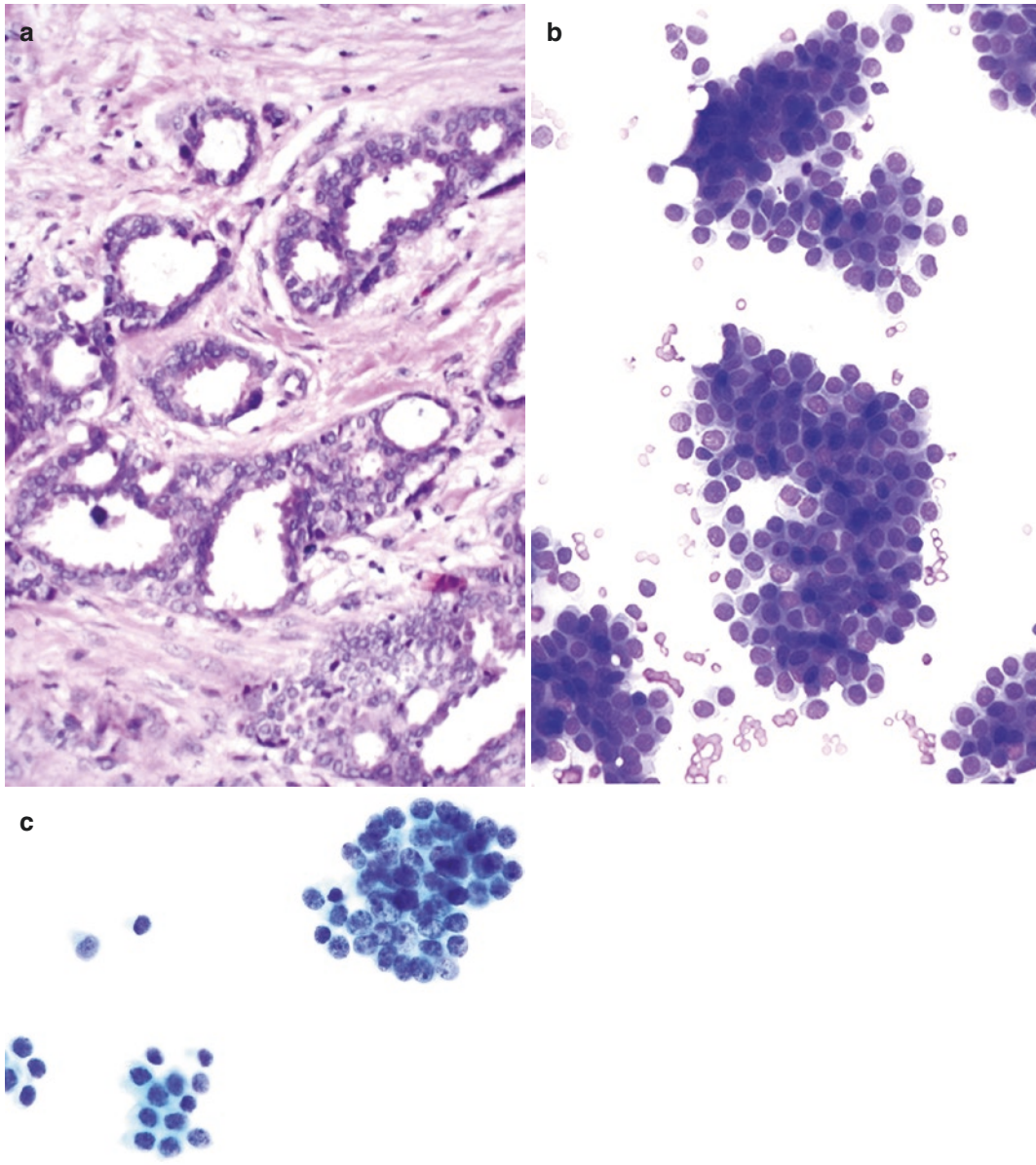

Fig. 10.5 Tubular carcinoma: (**a**) Tubular carcinoma showing well-defined, angulated and open tubules that haphazardly infiltrate the desmoplastic stroma in the resection (H&E); (**b**) Tubular carcinoma showing loosely cohesive 3D tissue fragments with rigid borders, minimal cell stratification and absence of myoepithelial cells. The nuclei show mild atypia and are small and monomorphic with fine chromatin and inconspicuous nucleoli (Giemsa, conventional smear); (**c**) LBC shows similar features (Pap, TP)

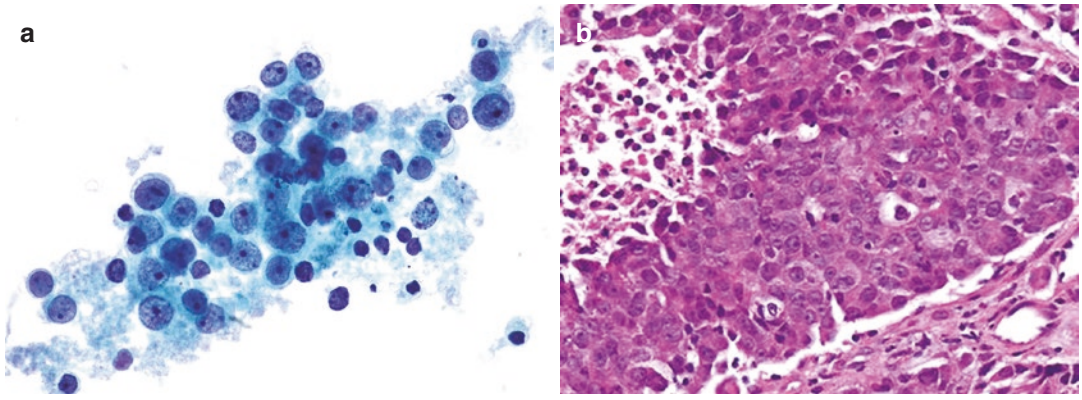

Fig. 10.6 Carcinoma of no special type (NST): (**a**) Loosely cohesive small tissue fragment and single malignant cells. The nuclei are enlarged, round to oval with coarse chromatin and single prominent nucleoli. Cytoplasm is scant to indistinct. Apoptosis is prominent. Note the "clinging" diathesis (Pap, TP); (**b**) Invasive carcinoma, NST in the resection shows similar high-grade features (H&E)

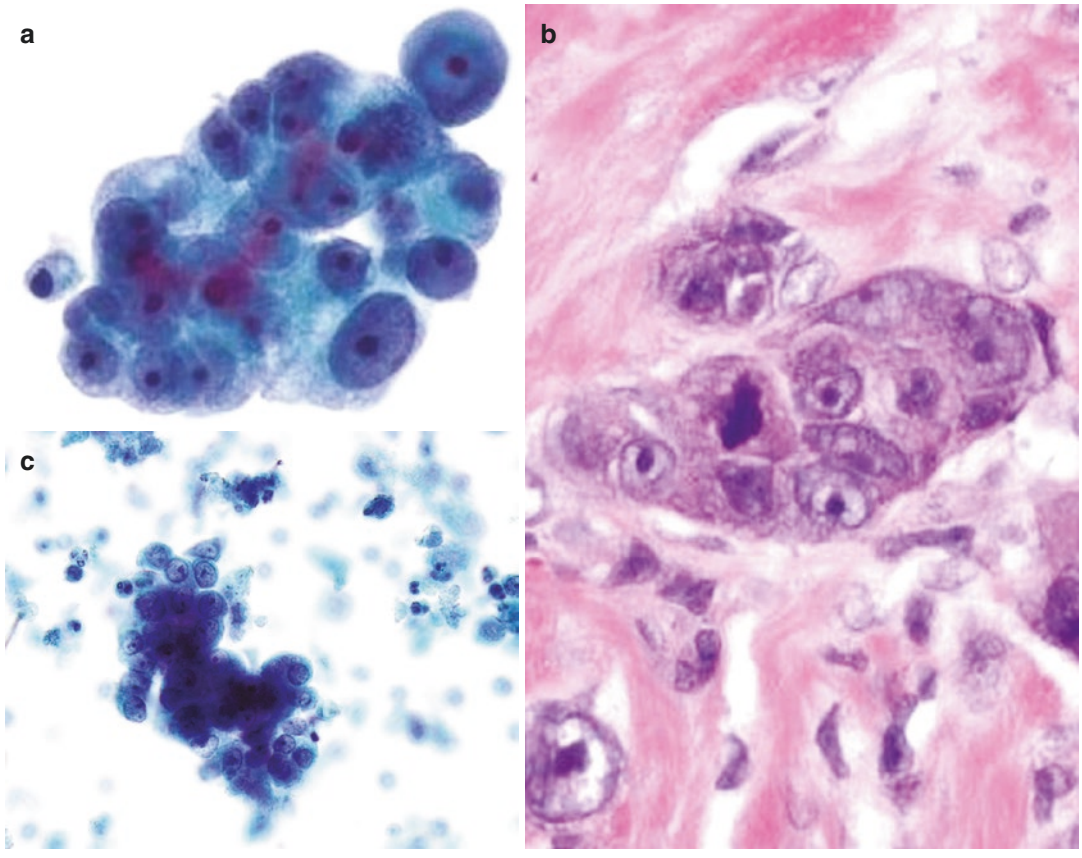

Fig. 10.7 Carcinoma of no special type: (**a**) 3D tissue fragment of cells with nuclear enlargement, pleomorphism, and macronucleoli. Cytoplasm is scant and finely vacuolated. A mitosis is noted. Morphology is similar to histopathology (Pap TP); (**b**) Histopathology of same tumor as seen in Figure a (H&E); (**c**) Note similar nuclear features in a SurePath preparation. Background tumor diathesis is more diffuse unlike the "clinging" diathesis seen in TP slides (Pap, SP)

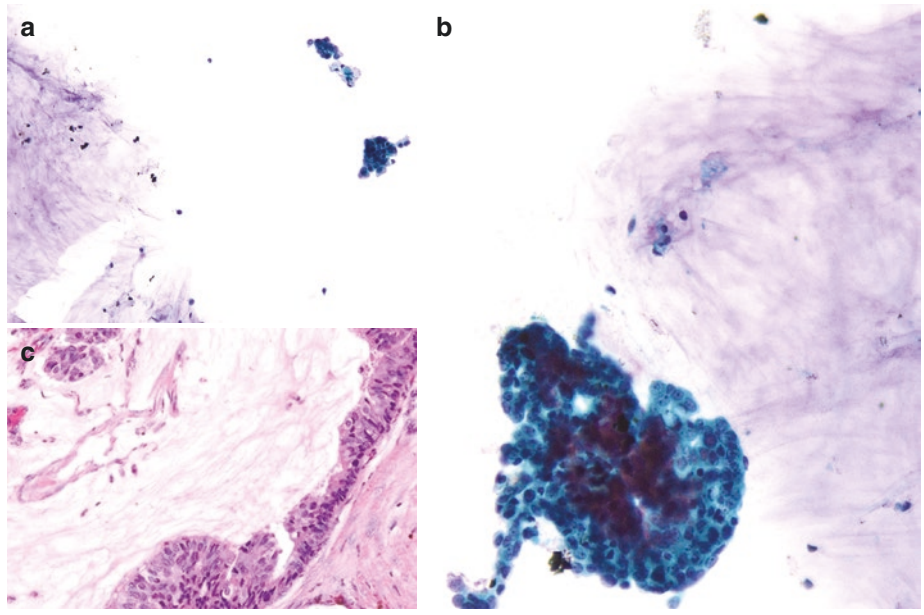

Fig. 10.8 Mucinous carcinoma: (**a**) Small tissue fragments and acinar architecture of tumor cells with smooth borders, close to abundant thick cellular mucin (Pap, TP); (**b**) A tissue fragment of tumor cells with smooth borders, close to abundant thick cellular mucin; moderate nuclear pleomorphism; intracytoplasmic mucin can be seen occasionally; prominent nucleoli. Thick pink mucin attached to malignant cells and thus remains visible in LBC (Pap, TP); (**c**) Resected mucinous carcinoma (H&E)

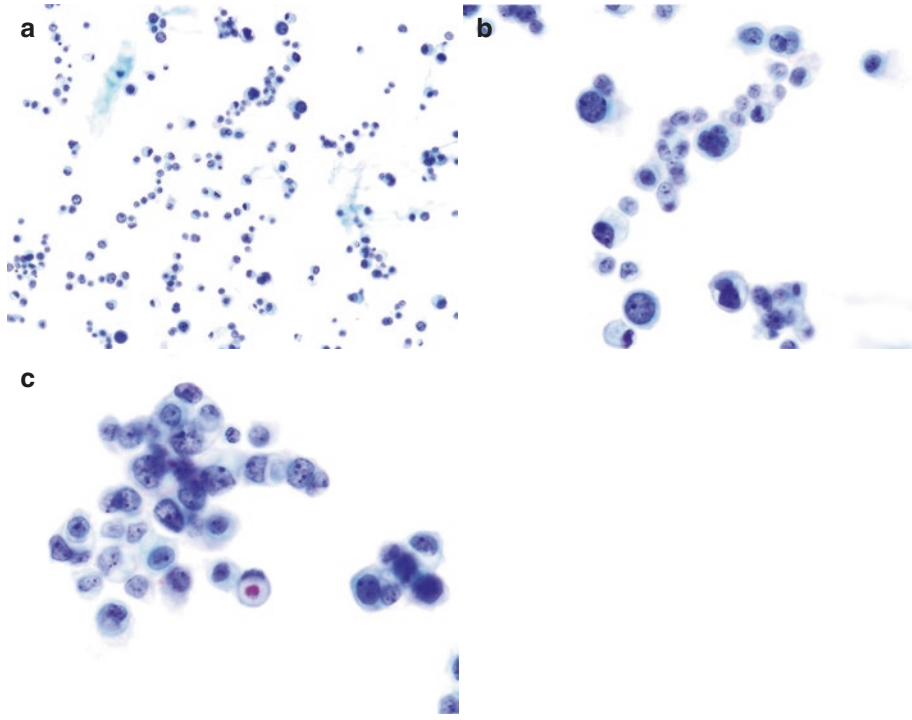

Fig. 10.9 Lobular carcinoma: (**a**) Small cells in a linear pattern and singly, in a clean background, show nuclear molding (Pap, low power, TP); (**b**) Lobular carcinoma showing the nuclear borders are irregular, with moderate variation in shapes and sizes (Pap, high power, TP); (**c**) Cytoplasm is foamy, with some cells showing mucin vacuoles, which can be large as "signet ring" cells (Pap, high power, TP)

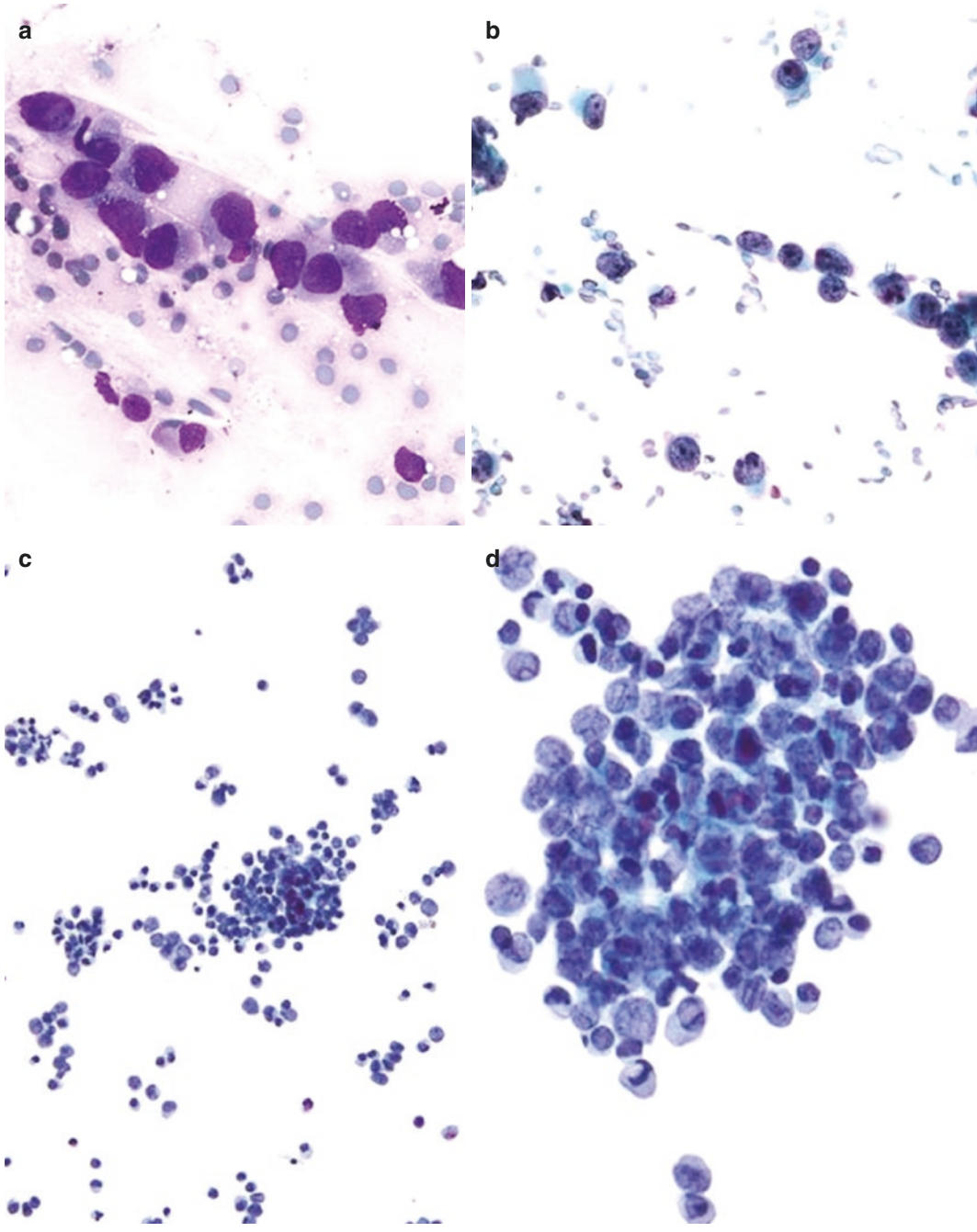

Fig. 10.10 Pleomorphic lobular carcinoma: (**a**, **b**) Discohesive malignant cells and minute tissue fragments showing a linear architectural pattern. The tumor cells are pleomorphic with enlarged, irregular nuclei and nucleoli and eccentric cytoplasm (Giemsa & Pap, conventional smear); (**c**) Discohesive cells occasionally forming small and larger tissue fragments with a linear pattern of growth with marked nuclear pleomorphism, prominent nucleoli, and abnormal chromatin distribution. Cytoplasm is scant, granular, or vacuolated (Pap, low power, TP); (**d**) Note the nuclear pleomorphism (Pap, high power, TP); (**e**, **f**) Note the architectural and cellular similarity in the histologic section and TP (H&E, Pap stain, high power, TP)

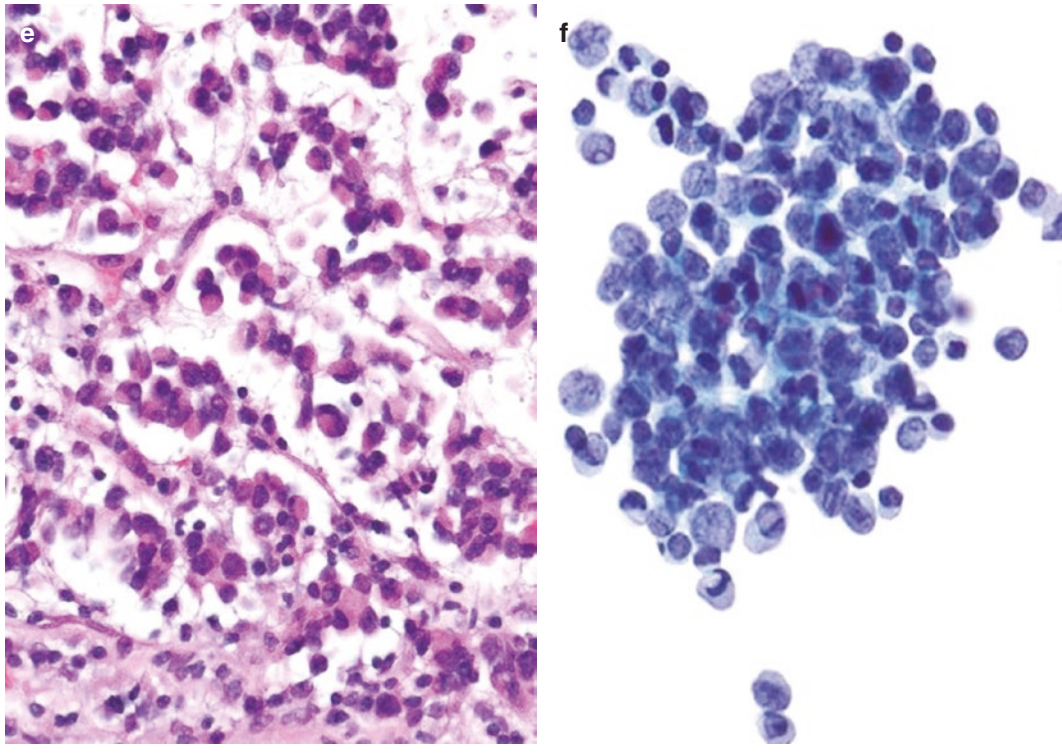

Fig. 10.10 (continued)

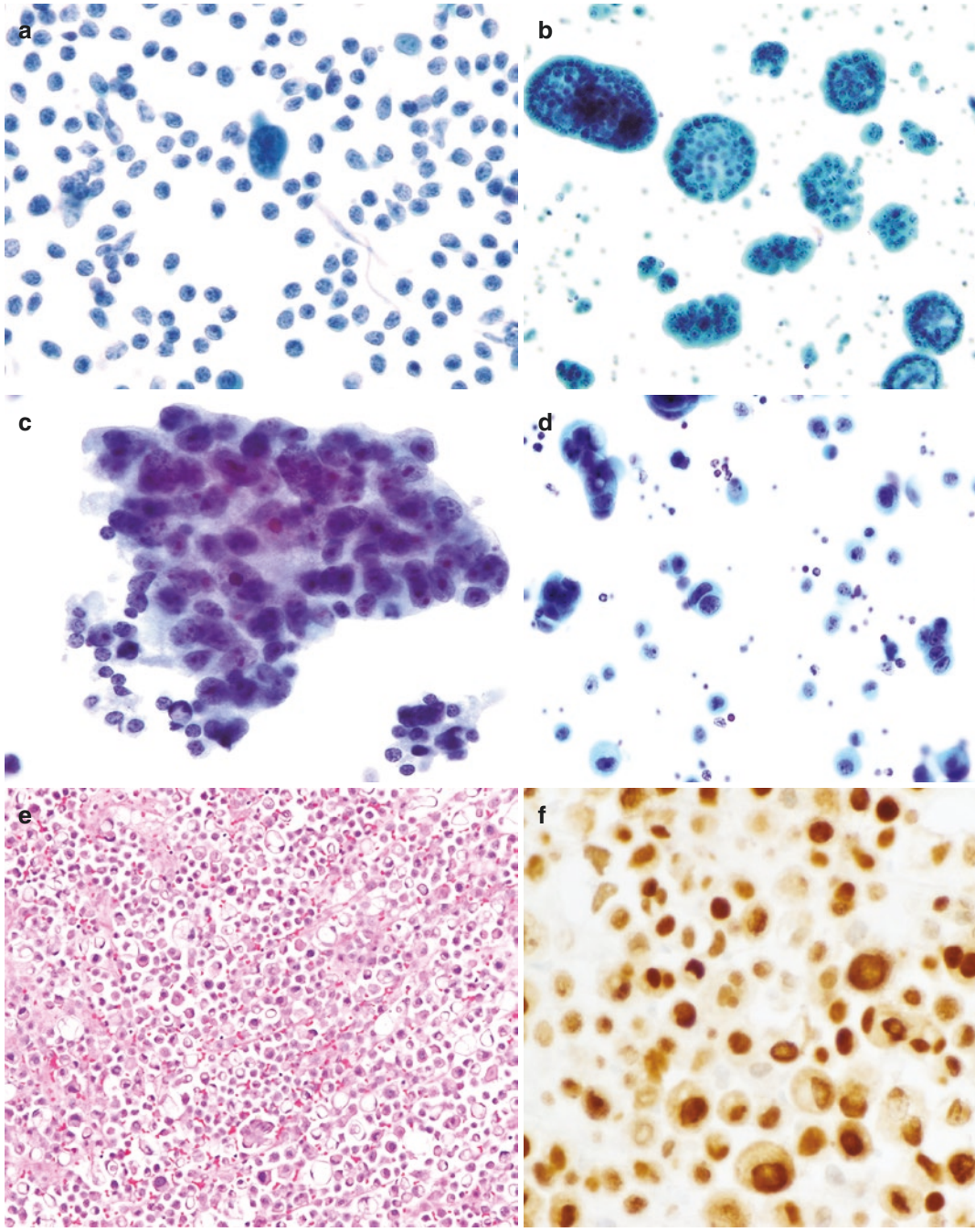

Fig. 10.11 Metastatic tumors to the breast: (**a**) Hodgkin lymphoma metastatic to the breast. A Reed-Sternberg cell with "mirror-image" nuclei is noted in a background of lymphocytes and occasional eosinophils. Note crisp nuclear morphology. Positivity for CD15 and CD30 immunostains, negativity for flow cytometry, and review of the previous primary confirmed the diagnosis (Pap, high power, SP); (**b**) Metastatic carcinoma, no special type, in ascitic fluid forming morulas with smooth community borders (Pap, low power, SP); (**c**) Metastatic lung adenocarcinoma to the breast. Note cellular crowding with enlarged round to oval nuclei and macronucleoli (Pap, TP); (**d**) Metastatic lobular carcinoma to ascitic fluid. Note "single-cell file" pattern or linear configuration of cells. Nuclei are enlarged, eccentrically located with prominent nucleoli. Some lymphoid cells appear in a different plane of focus (Pap, high power, SP); (**e**, **f**) Cell block section from the same case in the H&E and showing a positive GATA3 immunostain. Patient had a history of lobular carcinoma (H&E; GATA3 immunohistochemistry)

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Clinical Management

11

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Introduction

A breast cancer diagnosis is established using the triple assessment approach ('triple test'). This comprises the clinical, imaging and pathological assessment of a breast lesion. Not all components of the triple test are necessarily required to establish a benign diagnosis.

The required clinical history focuses on risk factors including early menarche, late menopause and delayed childbearing, positive family history of breast cancer raising the possibility of genetic mutations (including *BRCA1* and *BRCA2* and others), and a personal history of invasive breast cancer, ductal carcinoma in situ (DCIS) or some types of proliferative conditions. Other risks relate to endogenous and exogenous hormone exposure and lifestyle factors. Signs and symptoms vary, but the classic characteristics of a cancerous lesion include a hard, fixed, solitary dominant lesion with irregular borders and possible skin tethering. However, even these advanced carcinoma features cannot confidently distinguish a benign from a malignant lesion. With current breast screening programs, many cancers are detected early and are impalpable and diagnosed in women at average risk.

Mammography and ultrasonography are the most frequently used methods for assessing palpable breast lesions, and mammography is widely used for breast cancer screening before a palpable mass develops [1, 2]. Radiologists summarize

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A. S. Field et al. (eds.), *The International Academy of Cytology Yokohama System for Reporting Breast Fine Needle Aspiration Biopsy Cytopathology*, https://doi.org/10.1007/978-3-030-26883-1_11

Table 11.1 Breast Imaging Reporting and Data System classification of mammographic lesions

BI-RADS Category	Description	Probability of malignancy (%)	Follow-up
0	Needs additional evaluation		Diagnostic mammogram, ultrasonographic image
1	Normal mammogram	0	Routine screening
2	Benign lesion	0	Routine screening
3	Probably benign lesion	<2	Short interval follow-up
4	Suspicious for malignancy	20	Biopsy
5	Highly suspicious for malignancy	90	Biopsy
6	Biopsy-proven malignancy	100	Treatment

mammographic findings and breast mammographic density using the American College of Radiology Breast Imaging Reporting and Data System (BI-RADS) diagnostic assessment categories [2]. The BI-RADS classification categorizes mammographic findings and determines further recommendations including return to routine screening, short-interval follow-up or biopsy. Mammograms assigned as BI-RADS 3 require additional evaluation that may include additional mammographic views, ultrasound, magnetic resonance imaging (MRI) or tissue sampling. BI-RADS 4 or 5 strongly correlate with malignancy, may require additional imaging and routinely require biopsy to confirm the diagnosis (Table 11.1).

Significant findings that can be identified on mammography include a mass lesion (regular, irregular or spiculated), a non-specific or asymmetric density, an architectural disturbance and microcalcifications. The most common mammographic features of malignancy are a spiculated mass and suspicious microcalcifications [2, 3]. Ultrasonography provides important adjunctive information by determining whether a lesion is cystic or solid and by further characterizing solid masses on the basis of benign and malignant features. Ultrasound is also useful in assessing the axillary lymph nodes for evidence of metastatic disease.

MRI is used for screening women with dense breasts or those who have a significant family history of breast cancer or proven genetic mutations known to have an increased risk of breast cancer. It is also used to further evaluate findings that have not been fully resolved by mammography and ultrasound. MRI has a high sensitivity of over 90%; however, it has limited specificity, and biopsy confirmation with radiological-pathological concordance is still required [4, 5].

The final component within the 'triple assessment approach' is cytopathological or histopathological correlation. The goal of biopsy is to obtain sufficient material using the least invasive approach to obtain a diagnosis. In patients with an indeterminate or suspicious clinical or imaging finding, the obligatory initial diagnostic technique is percutaneous biopsy. However, surgical biopsy should be used if percutaneous biopsy is not feasible or available or where a diagnosis cannot be reached with FNAB or CNB. In all cases, correlation of the FNAB and/or CNB results with the clinical and imaging findings is essential for accurate diagnosis. If available, ultrasound examination of the axilla is recommended, and if there is a suspicious axillary lymph node on clinical or ultrasound findings, FNAB, followed if necessary by CNB, should be performed [6].

With the appropriate use of the triple test, it should be possible to obtain a definitive diagnosis in almost all cases, and the use of short-term clinical and radiological follow-up in patients with indeterminate clinical or imaging findings should be avoided whenever possible.

It is usually best to obtain a definitive pathological diagnosis and management by observation should be uncommon. But in certain scenarios, asymptomatic lesions with a low risk of malignancy on imaging and benign or atypical biopsy findings may be managed by clinical and imaging follow-up. Repeat clinical examination

and ultrasound in 3–6 months may be considered to ensure that no significant growth or change in imaging features has occurred. Repeat FNAB or CNB is required if changes have occurred.

Optimal management of breast cancer patients requires a multidisciplinary approach including surgeons, radiologists, radiation and medical oncologists, pathologists, geneticists and specialist nurses [7, 8]. Multidisciplinary teams help ensure that women with breast cancer receive appropriate assessment and consideration of all treatment modalities particularly in difficult clinical scenarios. Evidence suggests that a multidisciplinary breast clinic provides patients with the most accurate diagnosis and the best possible coordination and sequencing of management [8]. Once a benign diagnosis has been made, further assessment in a multidisciplinary meeting is typically not required.

Management Options for Each Diagnostic Category

The IAC Yokohama System suggests that management protocols are linked to the five categories and their risk of malignancy, and these are listed below. The system recognizes that there are current differences in the use of fine needle aspiration biopsy (FNAB) and core needle biopsy (CNB) in the diagnostic workup of breast lesions and that there are differences in the management of breast carcinomas between oncology services on a local, national and international basis. Some of these differences relate to established protocols and some to the availability of medical resources [7–10].

The management options are summarized in Table 1.1 in Chap. 1.

Category 1: Insufficient/Inadequate

Definition

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

Management

- Review the clinical and imaging findings to decide whether to repeat the FNAB or perform a CNB.
- If the smear is technically suboptimal, then repeat the FNAB, ideally under ultrasound guidance and with rapid on-site evaluation (ROSE).
- If the imaging is indeterminate or atypical, further biopsy is mandatory.
- If repeat FNAB is inadequate, then CNB, ideally under ultrasound guidance, is performed.
- If CNB is not available and the clinical and/or imaging features are indeterminate or atypical, then repeat the FNAB at follow-up or consider an excision biopsy.
- If there is low clinical and imaging suspicion, follow-up with clinical and imaging assessment, with or without FNAB, usually at 3–6 months is suggested.
- If the sample is 'cyst contents' or a 'cyst', completely aspirate the cyst ideally under ultrasound guidance. If there is a residual mass or if a solid component is present on ultrasound, immediately repeat the FNAB to sample the mass.

Category 2: 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.

Possible Specific Cytological Diagnoses

- Normal breast tissue
- Acute mastitis and breast abscess
- Granulomatous mastitis
- Cyst contents
- Cyst
- Fibrocystic change
- Usual epithelial hyperplasia
- Fibrocystic change with usual epithelial hyperplasia
- Fibroadenoma

- Intraductal papilloma
- Lactational change
- Adenosis and sclerosing adenosis
- Fat necrosis
- Intramammary lymph node

Management

- Lesions that are benign on clinical, imaging and FNAB investigation may be followed up with serial physical examination and imaging to ensure that no change occurs in the lesion, and if such a lesion remains stable, then the patient may be returned to routine screening and management. The timing of the follow-up varies with the different guidelines in mammographic screening programs and breast clinics but most commonly occurs at 3 or 6 months. It should be noted that in many clinical situations, the patient returns to routine screening without short-term follow-up after a benign triple test. Any change at follow-up in either the clinical or imaging examination requires a repeat FNAB.
- If the FNAB does not explain the clinical and/or the imaging findings, raising a question of sampling error, or if the FNAB is discordant with the clinical and imaging findings which may be indeterminate, atypical or suspicious, then CNB is required. If CNB is not available, immediate repeat FNAB or simple excision biopsy should be considered.

Category 3: 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.

Lesions that Cause most Interpretative Difficulty

- Fibroadenoma
- Intraductal papilloma
- Fibrocystic change with usual epithelial hyperplasia, including radial scars
- Usual epithelial hyperplasia and sclerosing adenosis
- Spectrum of atypical ductal hyperplasia and low-grade ductal carcinoma in situ
- Lobular neoplasia (non-pleomorphic)
- Fibroepithelial tumour, with features suggesting low-grade phyllodes tumour
- Columnar cell change with hyperplasia
- Lactational change with atypia
- Mucocele-like lesion
- Adenomyoepithelioma
- Spindle cell stromal proliferations

Management

- An atypical FNAB result requires correlation with clinical and imaging findings, which can be immediate if ROSE has been employed or at a later time if ROSE was not utilized.
- If the clinical or imaging findings are indeterminate or suspicious, CNB should be carried out preferably with ultrasound guidance. If no CNB is available, a repeat FNAB is recommended.
- If the clinical and imaging findings are benign, repeat FNAB should be considered. If the FNAB is benign, then the patient can be reviewed with clinical and imaging follow-up at 3–6 months. Some centres may prefer to use CNB rather than repeat FNAB.
- If the CNB shows lesions such as atypical ductal hyperplasia, intraductal papilloma, complex sclerosing lesion or radial scar, simple surgical excision should be considered so that the whole lesion can be completely assessed and intraductal carcinoma or concurrent underlying carcinoma can be excluded.
- A definitive preoperative FNAB or histopathological diagnosis is preferred, but in situations where imaging is not available, open biopsy or surgical excision may be considered for lesions with atypical FNAB cytology and a clinical presentation suspicious of malignancy, such as a hard, fixed, solitary dominant lesion or diffuse erythema or ‘peau d’orange’.
- Punch biopsy of the nipple is recommended if Paget’s disease is suspected.

- If the FNAB is atypical and CNB or excision biopsy is not performed due to the patient's choice or unsuitability for surgery, then 3–6-month follow-up with repeat clinical and imaging assessment is mandated to ensure stability of the lesion. If stable, repeat clinical and imaging assessment at 12 and 24 months is recommended. If the lesion has changed, then repeat FNAB or CNB or excision biopsy is recommended.
- Cross-sectional imaging with contrast-enhanced MRI may be beneficial.

Category 4: 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.

Lesions that Cause Most Interpretative Difficulty

- Proliferative breast lesions with high cellularity, with or without marked dispersal of single cells, including columnar cell change with hyperplasia, flat epithelial atypia, atypical ductal hyperplasia and intraductal papillomas with epithelial hyperplasia
- Lobular carcinoma in situ and invasive lobular carcinoma
- Prominent necrosis, with or without calcifications, and a small amount of highly atypical epithelium: possible high grade ductal carcinoma in situ
- High cellularity with complex epithelial tissue fragments with or without a cribriform and/or papillary architecture and associated with marked dispersal of intact cells: possible low grade ductal carcinoma in situ
- Low grade invasive carcinomas including low grade NST and tubular carcinoma
- Distinguishing high grade lymphoma from carcinoma

Management

- Follow-up is mandatory after a FNAB diagnosis which is suspicious of malignancy, regardless of clinical and imaging findings, and will require repeat tissue sampling ideally by CNB with ultrasound guidance, or, if CNB is not available, by repeat FNAB or excision biopsy.
- CNB may have sampling errors similar to FNAB but is the preferred modality to confirm diagnosis as it allows for a surgical pathology diagnosis and confirmation of invasive carcinoma or ductal carcinoma in situ.
- If CNB confirms unequivocal malignancy, then proceed to appropriate surgery and axillary management with or without neoadjuvant treatment.
- If a definite diagnosis is not possible with repeat FNAB or CNB, then a surgical diagnostic biopsy should be considered.
- Magnetic resonance-guided biopsy (MRB) may be indicated if the lesion is an impalpable MRI-identified lesion that is mammographically anod ultrasonographically occult or if CNB results are discordant.

Category 5: Malignant

Definition

A malignant cytological diagnosis is an unequivocal statement that the material is malignant, and the type of malignancy identified should be stated whenever possible.

Management

- Correlation with clinical and imaging findings is required, and if concordant with the malignant FNAB diagnosis in the triple test, prognostic and predictive markers can be performed on cell block material so that definitive treatment including surgery and neoadjuvant chemotherapy can commence. It should be noted that some centres and management protocols require CNB confirmation and other centres perform the markers only on the CNB or on the excised specimen.
- If the FNAB is not concordant with the clinical and imaging findings, then perform CNB,

or if CNB is not available proceed to surgical biopsy.

- Perform CNB to confirm diagnosis if appropriate, and perform immunohistochemistry for oestrogen and progesterone receptors and immunohistochemistry and/or in situ hybridization (ISH) for HER2 and a proliferation marker such as Ki67 if this is the local protocol.
- Clinical examination and, if available, ultrasound examination of the axillary lymph nodes are required. If the lymph nodes are suspicious of metastatic carcinoma, FNAB initially and, where necessary, CNB preferably under US guidance is recommended.
- Discuss each case at a multidisciplinary meeting and proceed with treatment as appropriate.

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A. S. Field et al. (eds.), *The International Academy of Cytology Yokohama System for Reporting Breast Fine Needle Aspiration Biopsy Cytopathology*, https://doi.org/10.1007/978-3-030-26883-1