Review

The diagnosis and management of pre-invasive breast disease

The role of new diagnostic techniques

Ashutosh Nerurkar and Peter Osin

Department of Histopathology, The Royal Marsden Hospital, Fulham Road, London, SW3 6JJ, UK

Corresponding author: Ashutosh Nerurkar (e-mail: Ashutosh.Nerurkar@rmh.nthames.nhs.uk)

Published: 9 October 2003

Breast Cancer Res 2003, **5**:305-308 (DOI 10.1186/bcr721)

Abstract

In recent years we have seen significantly increased use of minimally invasive diagnostic techniques in the management of breast disease. There is wide recognition of fine needle aspiration and core biopsy as the principal diagnostic methods. However, concerns exist regarding their reliability. This article provides a brief overview of the major diagnostic issues related to use of fine needle aspiration, core biopsy and ductal lavage. It summarizes areas of use for each technique, outlines the main diagnostic pitfalls and their causes, and provides a perspective on future developments in the field.

Keywords: biopsy, breast carcinoma, cytology, diagnosis, ductal lavage

Introduction

The introduction of breast screening programmes led to wider employment of minimally invasive diagnostic methods. Fine needle aspiration (FNA) and core biopsy are now universally accepted as methods that virtually eliminate the need for open biopsy or frozen sections in diagnosis of breast cancer. However, rapid growth in the use of these methods presents the pathologist with certain challenges relating to the reduced size of material obtained, in combination with the wide variety of breast lesions that may be identified. This article summarizes the major diagnostic issues related to these techniques.

Fine needle aspiration cytology

Aspiration cytology has been practised for more than 50 years [1]. It was initially introduced to replace incisional biopsy, which is an invasive method. Over this period the technique has been used extensively for the diagnosis of breast lesions, and it forms an integral part of the triple approach to management of breast cancer. Although the technique is well suited to the superficial nature of breast lesions, and is highly sensitive and specific in their diagnosis, like any other techniques it has limitations that can lead to false-negative and false-positive results. Nevertheless, its role in the diagnosis of breast lesions cannot be underestimated.

Cystic lesions

Use of FNA in the evaluation of cystic lesions can be both diagnostic and therapeutic. Complete aspiration of cyst contents can result in collapse of the cyst and stripping of the lining epithelium. Cytological findings are variable. Some fluids are acellular whereas others contain foam cells, inflammatory cells, benign epithelium and apocrine cells. Sometimes cytological atypia within cyst lining epithelium may be detected, and this can be worrisome. The frequency of carcinoma among all cystic lesions is around 2% [2,3]. Complex cysts with thick walls or intracystic masses may require further investigation because their association with carcinoma is much stronger [4].

Solid lesions

In solid lesions the benefit of FNA is that it may offer a prompt diagnosis. It is of paramount importance that the results of FNA be interpreted as part of triple assessment and not in isolation. This can avoid delays and overtreatment.

In certain cases, such as fibroadenoma, FNA can provide a specific histological diagnosis on the basis of benign cytology [5]. Sometimes the subtype of carcinoma can be identified on cytology. Other malignancies such as

FNA = fine needle aspiration; DCIS = ductal carcinoma *in situ*; PCR = polymerase chain reaction.

lymphoma, melanoma or sarcoma have specific cytological appearances.

Limitations of fine needle aspiration technique

The limitations of FNA can either be technical or related to the nature of the lesion itself. Furthermore, there are limitations that are specific to FNA regardless of technique or lesion type (i.e. intrinsic limitations).

Technical limitations

False-negative diagnoses can result in diagnostic delay and provide the patient with false reassurance. They may result from incorrect localization, which can lead to non-representative material. This can be overcome by using imaging guidance. False-negative diagnoses may also result from improper technique, which can yield inadequate or suboptimal material. Contamination with blood can cause difficulties in interpretation. In addition, the preparation of a thin, uniform smear is equally important for accurate interpretation. It is very important that the person conducting the FNA is well trained in the technique.

Sometimes, poor technique can mislead the unwary pathologist into making a false-positive diagnosis. Excessive application of force while spreading the smear can lead to crushing and nuclear distortion and dissociation (i.e. crushing artefacts), which can result in the false impression of hyperchromasia. Also, delay in fixation of the smear for Papanicolaou staining can result in cellular enlargement; comparison with air-dried Giemsa stained smears can be helpful in avoiding such false-positive diagnoses. Finally, poor quality staining can cause artefactual changes in the nature of the chromatin pattern.

Limitations related to the lesion itself

Apart from technical problems, sometimes the nature of the lesion itself can cause diagnostic error. Some lesions share similar features on FNA and are difficult to differentiate from each other.

Certain types of lesions can lead to false-negative diagnoses. For example, it is difficult to fix the small mobile lesion by hand, and thus it may be missed. Also, it is difficult to aspirate fibrous lesions, and samples are often hypocellular and haemorrhagic. The smears may show only stromal fragments. Carcinomas can sometimes induce dense fibrotic stroma, and in such cases a careful search for malignant cells is necessary. In the case of complex sclerosing lesions, the smears show small uniform cells with mild or no atypia. The presence of bare nuclei may be helpful in identifying the benign nature of the lesion. However, the presence of concurrent *in situ* or invasive carcinoma can be difficult to diagnose. In a proportion of cases, further investigation with imaging modalities and core biopsies may be necessary [6]. In the case of necrotic and vascular lesions, the smears may not contain any viable cells or may be haemorrhagic. Finally, smears from lobular carcinoma can be hypocellular and cells may not show significant pleomorphism. Their resemblance to lymphocytes may result in false-negative diagnosis. Cytology of tubular carcinoma can resemble many benign conditions, including adenoma, microglandular adenosis and fibroadenoma [7].

There are also types of lesions that can lead to false-positive diagnoses. In epithelial hyperplasia it is sometimes difficult to differentiate between usual type hyperplasia, hyperplasia with atypia, and low-grade intraductal carcinoma. Three-dimensional clusters of cells with atypia can cause diagnostic problems. Also, with respect to fibroadenomas, hyperplastic foci can mimic low-grade carcinoma. Similarly, fibroadenomas with myxoid degeneration can be mistaken for mucinous carcinoma [8]. Cytologically, epithelial cells show mild nuclear pleomorphism with prominent nucleoli during lactational changes, which can be a cause of false-positive diagnosis. Finally, iatrogenic changes following previous FNA/biopsy can result in false-positive diagnoses. Stromal cells of granulation tissue, inflammatory cells and histiocytes can mimic carcinomas. Similarly, radiation-induced atypia in benign epithelium can be worrisome.

Intrinsic limitations

There are a number of limitations that are intrinsic to FNA cytology. First, identification of benign fibroadenoma or frankly malignant phyllodes tumour may not be difficult, but distinguishing between cellular fibroadenoma and a phyllodes tumour can cause problems. Stromal cellularity and the presence of a number of long spindle cells may be helpful in some cases [9]. Second, the cytological appearances of papillary lesions, which range from benign papilloma to invasive papillary carcinoma, can be similar. In addition, benign papillomas can harbour areas of ductal carcinoma *in situ*. All papillary lesions need complete excision, and in our opinion the cytopathologist should therefore not attempt to make a definitive diagnosis on the basis of FNA findings, and often on the basis of a core biopsy as well, unless frank carcinoma is present. Third, it can sometimes be difficult to distinguish between a mucocele-like lesion and mucinous carcinoma on cytology. The presence of high cellularity, single or small three-dimensional groups of tumour cells, and cytological atypia should raise suspicion of carcinoma [10]. Finally, in the absence of architectural information, the distinction between ductal carcinoma *in situ* (DCIS) and invasive carcinoma may be difficult cytologically [11].

Role of cytology in the evaluation of prognostic markers

Material obtained by aspiration techniques can be used to evaluate the expression of receptors such as oestrogen receptor and progesterone receptor, as well as the levels of expression of other markers such as E cadherin and p53. Cyto-centrifuged material is better with respect to

yield of tumour cells and in terms of antigen preservation [12]. Encouraging results in evaluating the expression of HER-2 by fluorescent *in situ* hybridization and immunocytochemistry using aspiration material were recently reported [13,14].

Needle core biopsy

The use of needle core biopsy has gained a wide acceptance, particularly with the advent of stereotactic guidance. Use of smaller gauge needles has avoided the complications of trauma, pain, use of anaesthetic agents and tumour implantation in a biopsy tract. With needle core samples, accurate subcategorization of carcinomas as well as study of hormone receptors and other prognostic markers is possible [15]. The false-positive rate with needle biopsy is very low (0.2–0.3%); it is slightly higher for nonpalpable lesions than for palpable ones [16]. However, some lesions can cause diagnostic problems, and these are described below.

Fibroepithelial lesions

The distinction between fibroadenoma and phyllodes tumour may be difficult on core biopsy. Stromal cellularity, vesicular nuclei of stromal cells, mitotic figures and epithelial hyperplasia should raise suspicion of the presence of phyllodes tumour. In difficult cases excision biopsy is recommended [17].

Papillary lesions

Needle core biopsies of papillary lesions frequently show the presence of loose papillary fragments. Occasionally, architectural distortion caused by the needle can simulate stromal invasion. Cytological atypia in a benign papilloma is not uncommon. Similarly, benign papilloma can harbour focal papillary carcinoma. In a recent study conducted by Irfan and coworkers [18], 14.3% of the papillary lesions diagnosed on stereo core biopsy demonstrated cancer on subsequent excision. All of these problems cause great difficulty in the diagnosis of papillary lesions, and therefore papillary lesions should be completely excised, regardless of cytological and architectural atypia.

Ductal carcinoma *in situ* and atypical ductal hyperplasia

Identification of high grade, commedo-type DCIS is not difficult. However, bearing in mind the limited amount of material obtained with needle core biopsy, the distinction between low grade DCIS and atypical ductal hyperplasia can be troublesome. Bonnett and coworkers [19] showed that identification of severe atypical hyperplasia on core biopsy was associated with a high probability of finding DCIS on follow-up excision. Complete excision of these lesions is recommended.

Invasive carcinoma

Use of immunohistochemical markers of myoepithelial cells and basement membrane can be helpful in identifying areas of tumour invasion. In our practice we found use of immunostaining for S100 protein, smooth muscle actin, calponin, cytokeratin 5/6 and cytokeratin 14 to be simple and reliable. Identification of microinvasion is possible on core biopsy but this does not provide assurance that the material is representative of the entire tumour.

Ductal lavage

Alongside FNA and core biopsy, a number of noninvasive methods of sampling breast epithelium have recently attracted increased interest both from researchers and clinicians [20,21]. These methods include ductal lavage, ductoscopy and examination of spontaneous nipple discharge. The approach has some advantages; the noninvasive nature of the procedure makes it attractive to patients, medical practitioners and health service providers. The technique of ductal lavage, with or without ductoscopy, is less invasive than FNA and does not involve needles; it is therefore better tolerated by nervous patients. Technically, the method is not complicated and the necessary experience could be acquired in a shorter period of time than is required for other techniques. The cost of the method is comparable to that for FNA, and results are available quickly. In a recent study [22] it was demonstrated that a large number of breast epithelial cells can be collected by ductal lavage.

There are factors that limit the reliability of ductal lavage, seriously restricting its use. The main obstacle is varied cellularity of the sample and the degenerate nature of the cells. Interestingly, even the origin of the cells in the nipple lavage (histiocytic versus epithelial) was subject to controversy. Thus far it has been demonstrated that foam cells in the ductal lavage are undoubtedly of histiocytic origin, and a significant proportion of the cellular population in the lavage consists of cells from the ductal system [23].

The specificity of the ductal lavage method may vary depending on the degree of cell degeneration because degenerate cells can sometimes be mistaken for malignant ones. Another important issue relates to the sensitivity of the method (false-negative results may occur due to low cell output). It has been suggested that lavage is potentially a more sensitive method than nipple aspiration in detecting cellular atypia [24]. The biotechnological revolution that has occurred over recent years has given rise to attempts to overcome the limitations of the noninvasive approach by use of molecular biology methods. The potential utility of DNA amplification, protein gel electrophoresis and mutagenesis assays was recently demonstrated. Amplification techniques such as methylation-specific PCR may help to increase the sensitivity of the method [25]. The use of noninvasive methods is still very limited, but their role is likely to increase in the future.

This article is the sixth in a review series on *The diagnosis and management of pre-invasive breast disease – current challenges, future hopes*, edited by Sunil R Lakhani.

Other articles in the series can be found at http://breast-cancer-research.com/articles/review-series.asp?series=bcr_Thediagnosis

Conclusion

In summary, the use of minimally invasive and noninvasive methods in cytological diagnosis of breast cancer represents an integral component of the triple approach and is essential to the quality of the diagnostic process. An understanding of the limitations of the methods, and of their specificity and sensitivity is very important in optimizing their use in a multidisciplinary environment.

Competing interests

None declared.

References

1. Martin HE, Ellis EB: **Biopsy by needle puncture and aspiration.** *Ann Surg* 1930, **92:**169-198.
2. Kline TS, Joshi LP, Neal HS: **Fine needle aspiration of the breast. Diagnosis and pitfalls. A review of 3545 cases.** *Cancer* 1979, **44:**1458-1464.
3. Strwabridge HTG, Bassett AA, Foldes I: **Role of cytology in management of lesions of the breast.** *Surg Gynaecol Obstet* 1972, **152:**1-7.
4. Berg WA, Campassi CI, Ioffe OB: **Cystic lesions of the breast: sonographic–pathologic correlation.** *Radiology* 2003, **227:**183-191.
5. Maygarden SJ, Novtny DB, Johnson DE, Frable WJ: **Subclassification of benign breast disease by fine needle aspiration cytology. Comparison of cytologic and histologic findings in 265 palpable breast masses.** *Acta Cytol* 1994, **38:**115-129.
6. Orell SR: **Radial scar/complex sclerosing lesion: a problem in the diagnostic work-up of screen-detected breast lesions.** *Cytopathology* 1999, **10:**250-258.
7. Evans AT, Hussein KA: **A microglandular adenosis-like lesion simulating tubular carcinoma of the breast. A case report with cytological and histological appearances.** *Cytopathology* 1990, **1:**311-316.
8. Matsuda M, Wada A, Nagumo S, Ichida K: **Pitfalls in fine needle aspiration cytology of breast tumours. A report of two cases.** *Acta Cytol* 1993, **37:**247-251.
9. Bhatari S, Kapila K, Verma K: **Phyllodes tumour of the breast. A cytopathologic study of 80 cases.** *Acta Cytol* 2000, **44:**790-796.
10. Wong NL, Wan SK: **Comparative cytology of mucocelelike lesion and mucinous carcinoma of the breast in fine needle aspiration.** *Acta Cytol* 2000, **44:**765-770.
11. Shin H J C,Sniege N: **Is a diagnosis of infiltrating versus in situ ductal carcinoma of the breast possible in fine needle aspiration specimens?** *Cancer Cytopathol* 1998, **84:**186-191.
12. Kuenen-Boumeester V, Timmermans AM, De Bruijn EM, Henzen-Logmans SC: **Immunocytochemical detection of prognostic markers in breast cancer; technical considerations.** *Cytopathology* 1999, **10:**308-316.
13. Solomides CC, Zimmerman R, Bibbo M: **Semiquantitative assessment of c-erbB-2 (HER-2) status in cytology specimens and tissue sections from breast carcinoma.** *Anal Quant Cytol Histol* 1999, **21:**121-125.
14. Mezzelani A, Alasio L, Bartoli C, Bonora MG, Pierotti MA, Rilke F, Pilotti S: **c-erbB2/neu gene and chromosome 17 analysis in breast cancer by FISH on archival cytological fine-needle aspirates.** *Br J Cancer* 1999, **80:**519-525.
15. Denley H, Pinder SE, Leston CW, Lee AH, Ellis IO: **Preoperative assessment of prognostic factors in breast cancer.** *J Clin Pathol* 2001, **54:**20-24.
16. Orell SR, Farshid G: **False-positive reports in fine needle biopsy of breast lesions.** *Pathology* 2001, **33:**428-436.
17. Hoda SA, Rosen PR: **Practical consideration in the pathologic diagnosis of needle core biopsies of breast.** *Am J Clin Pathol* 2002, **118:**101-108.
18. Irfan K, Brem RF: **Surgical and mammographic follow up of papillary lesions and atypical lobular hyperplasia diagnosed with stereotactic vacuum assisted biopsy.** *Breast J* 2002, **8:** 230-233.
19. Bonnett M, Wallis T, Rossmann M, Pernick NL, Bouwman D, Caro KA, Visscher D: **Histopathologic analysis of atypical lesions in image guided breast biopsies.** *Mod Pathol* 2003, **1:**154-160.
20. Love SM, Barsky SH: **Breast-duct endoscopy to study stages of cancerous breast disease.** *Lancet* 1996, **348:**997-999.
21. Khan SA, Baird C, Staradub VL, Morrow M: **Ductal lavage and ductoscopy: the opportunities and the limitations.** *Clin Breast Cancer* 2002, **3:**185-191.
22. Domchek SM: **The utility of ductal lavage in breast cancer detection and risk assessment.** *Breast Cancer Res* 2002, **4:**51-53.
23. King BL, Crisi GM, Tsai SC, Haffty BG, Phillips RF, Rimm DL: **Immunocytochemical analysis of breast cells obtained by ductal lavage.** *Cancer* 2002, **96:**244-249.
24. Dooley WC, Ljung BM, Veronesi U, Cazzaniga M, Elledge RM, O'Shaughnessy JA, Kuerer HM, Hung DT, Khan SA, Phillips RF, Ganz PA, Euhus DM, Esserman LJ, Haffty BG, King BL, Kelley MC, Anderson MM, Schmit PJ, Clark RR, Kass FC, Anderson BO, Troyan SL, Arias RD, Quiring JN, Love SM, Page DL, King EB: **Ductal lavage for detection of cellular atypia in women at high risk for breast cancer.** *J Nat Cancer Inst* 2001, **93:**1624-1632.
25. Evron E, Dooley WC, Umbricht CB, Rosenthal D, Sacchi N, Gabrielson E, Soito AB, Hung DT, Ljung B, Davidson NE, Sukumar S: **Detection of breast cancer cells in ductal lavage fluid by methylation-specific PCR.** *Lancet* 2001, **357:**1335-1336.

Correspondence

Dr Ashutosh Nerurkar, Department of Histopathology, The Royal Marsden Hospital, Fulham Road, London, SW3 6JJ, UK. Tel: +44 (0)20 7352 8171; e-mail: Ashutosh.Nerurkar@rmh.nthames.nhs.uk