ABSTRACT

Fine-needle aspiration cytology (FNAC) of the breast is a rapid, cost-effective, and sensitive procedure to diagnose breast lesions, and was widely employed to diagnose breast lesions in the past. However, in recent times, core needle biopsy of the breast is gaining popularity and acceptability, although FNAC still looms large. There are some intrinsic disadvantages to FNAC, of which the most important is probably difficulty in classification of a significant percentage of breast lesions. Such lesions are usually denoted by the rubric “grey zone lesions of the breast.” This article attempts to review these grey zone lesions and highlight the difficulties in diagnosing them.

Key words: Breast; cytology; fine-needle aspiration cytology (FNAC); grey zone.
some uncommon features. These uncommon features include nuclear pleomorphism and cellular discohesion or therapy-related nucleocytoplasmic changes as an individual change or in combination. In addition, an increase in cellularity can also be associated with any of these features. The lesions termed as “suspicious of malignancy” (C4) include those with some cells showing features of malignancy, in a material that is not diagnostic due to its scantiness, poor preservation, or poor preparation. The lesions with a higher degree of malignant features than C3 are also included in this group even though frankly malignant cells are not present. Moreover, a lesion with overall benign features but with scattered, distinctively malignant cells is also included in this category. In common parlance, a C3 lesion is a benign one with some atypical features, whereas a C4 lesion is probably a malignant one with insufficient material to be diagnosed as a frank malignancy or a lesion with a greater degree of atypia than that of a C3 lesion.

The importance of these two categories (C3 and C4) lies in the buffering they provide to both cytopathologists and the clinicians. It denotes the limitation and diagnostic difficulty on the part of cytopathologists in accurately classifying some of the breast lesions despite adequate sampling at times, whereas it guides a clinician to follow up such lesions with a repeat FNAC or core needle biopsy after a reasonable interval. However, the diagnosis in these categories should not exceed 20% of the lesions and it is preferred if the percentage remains below 15%, as per the NHSBSP guidelines,[9] to prevent overuse and abuse of such equivocal categories. Different studies have shown the percentage of “true grey zone lesions” (lesions which pose diagnostic difficulty due to the presence of atypical/suspicious cells and not because of technical limitation) to be close to 2% of all the breast fine-needle aspiration (FNA) cases examined.[9,10]

The “true grey zone lesions” comprise both benign and malignant lesions [Table 1], including fibroadenoma, fibrocystic disease of the breast, papilloma and other papillary lesions of the breast, proliferative breast disease with or without atypia including radial scar and sclerosing adenosis, fat necrosis, phyllodes tumor, lactating breast, lobular carcinoma, tubular carcinoma, mucinous carcinoma, low-grade in situ carcinoma, or ductal carcinoma.[4-7,9,11-15] Gynecomastia in male breast is also a lesion that causes a dilemma in some cases.

### Biphasic Lesions of the Breast

#### Fibroadenoma

Fibroadenoma is probably the commonest breast lesion for which FNAC is performed.[12] Different studies auditing C3 and C4 cases have documented fibroadenoma as being one of the most commonly misdiagnosed lesions that appear as the grey zone lesions.[7,8,10,16-19] In cytopathology, the smears are hypercellular with a relatively monomorphic population of ductal cells, some forming monolayered sheets and some forming angulated clusters along with numerous bipolar cells (myoepithelial cells) in the background.

Some degree of atypia, nuclear enlargement, and cellular discohesion is often associated with the aspirates of fibroadenoma, raising the suspicion of a low-grade adenocarcinoma [Figure 1], often compounded by the hypercellularity of the lesions. However, the maintenance of the polarity, the relative blandness of the nuclear chromatin, and the presence of bipolar cells in the background often help in making the correct diagnosis. Fibroadenomas do not show sudden change in tubular diameter or pointed tips, unlike tubular carcinomas. Of special concern are the cases termed as “fibroadenoma with atypia,”[18,19] as they have the potential to be diagnosed as low-grade malignancies. These lesions are mostly conventional fibroadenomas, though some are truly associated with benign proliferative lesions.

#### Phyllodes tumor

Being the other important type of biphasic tumor, the phyllodes tumor also constitutes an important grey zone lesion. Distinguishing a benign phyllodes tumor from a (cellular) fibroadenoma is often difficult and depends on the clinicopathological correlation. A lesion of more than 4 cm size with abundant and hypercellular fibromyxoid stroma is usually diagnosed as a phyllodes tumor. Moreover, the epithelial component of phyllodes is broad and rounded as compared to the angulated or staghorn clusters of fibroadenoma. Cytology aspirates cannot also properly distinguish between benign, borderline, and malignant phyllodes tumors.[12,20,22] [Figure 2]. Nuclear pleomorphism and number of mitosis increase progressively from a borderline to a malignant phyllodes tumor. However, these features are subjective and difficult to assess on cytology smears. Though the presence of significant cellularity and/or the presence

### Table 1: Spectrum of grey zone lesions of breast

| C3 and C4: Benign | C3 and C4: Malignant |
|-------------------|---------------------|
| Fibroadenoma      | Lobular carcinoma   |
| Fibrocystic disease | Tubular carcinoma |
| Radial scar       | Mucinous carcinoma  |
| Papilloma         | Low-grade in situ carcinoma |
| Proliferative breast disease with or without atypia | Low-grade ductal carcinoma |
| Gynecomastia      |                     |
| Lactating breast, pregnancy |               |
| Fat necrosis, phyllodes tumor | |

C3: Atypia possibly benign, C4: Atypia possibly malignant
of stromal blood vessels is/are individual indicator/s of a diagnosis of phyllodes tumor, these features lack universality. For example, a sampling error can be the reason for less cellularity, and many fibroadenomas can also be associated with stromal blood vessels.

An adenomyoepithelioma is a rare cellular biphasic tumor and is difficult to diagnose without the help of ancillary techniques, as the myoepithelial cells can adopt a different morphology, namely, spindle cell, plasmacytoid, epithelioid, clear cell, or even oncocytic. A cellular smear with both epithelial and myoepithelial cells should raise a suspicion of this lesion. In a few cases, intranuclear inclusions are also noted. The atypia associated with some of these lesions often poses problems and portends a false positive diagnosis.[23-26]

**Fat necrosis**

Fat necrosis usually presents as a breast lump or a radiological density in the breast. The history of trauma is not always obtained. FNA smears show a dirty necrotic background, foamy histiocytes, and numerous other inflammatory cells. These inflammatory cells include neutrophils, lymphocytes, and plasma cells. The preponderance of the cell type depends on the timing of FNA. A neutrophilic infiltrate being predominant in an acute stage, whereas lymphoplasmacytoid infiltrate steals the show at a later phase. In addition, epithelioid histiocytes, occasional ill-formed granuloma, and multinucleate giant cells can also be seen. Sometimes there can be capillary proliferation when the lesion reaches an organizing phase.

The major difficulties in this lesion can be because of a clinicoradiological suspicion of a mass, lack of a history of trauma in some cases, reactive atypia in the ductal cells [Figure 3] that may look malignant, and a necrotic background.[9] However, the preponderance of inflammatory cells and foamy histiocytes as compared to the ductal cells, often scanty in a typical dirty necrotic background, alert an experienced cytopathologist toward the right diagnosis.

**Lactating breast**

Breast FNA from a pregnant or lactating woman often poses a diagnostic challenge. In cytology, a cellular smear with a dispersed/discohesive population of cells with round nuclei, coarse chromatin or hyperchromatic chromatin, and prominent nucleoli raises a suspicion of malignancy. However, numerous naked nuclei, cells containing multivacuolated cytoplasm, scattered inflammatory cells and foamy macrophages, and a bubbly proteinaceous background often help in navigating the cytopathologists toward the right diagnosis. However, it should be kept in mind that sometimes malignancy can be associated with lactation, and lactation in such a situation masks the evidence of malignancy.[27-29]
Granulomatous inflammation
Cytology smears in granulomatous inflammation show multiple epithelioid cell granulomas, multinucleate giant cells, inflammatory cells comprising of lymphocytes and plasma cells, and often a necrotic background. Granulomatous inflammation of the breast can be caused by infectious diseases such as tuberculosis or fungus; or it can have noninfectious causes such as sarcoidosis or idiopathic granulomatous mastitis; or sometimes as a part of foreign-body reaction against silicone implant.[30-36] Idiopathic granulomatous mastitis is difficult to diagnose in cytology smears as it is a lobulocentric lesion, which is a difficult feature to appreciate in cytology smears. Only the possibility can be suggested, based on history and cytomorphology.[27] The reactive atypia of the ductal epithelium associated with granulomatous inflammation can sometimes cause a problem in categorization of the lesion.

Radiation-induced changes
Radiation induced changes occur in the breast as a result of receiving radiotherapy for breast malignancy itself or some other malignancies such as Hodgkin’s lymphoma. The major differentials considered are a recurrence of the breast cancer or a radiation induced atypia or a radiation induced sarcoma in rare cases.[38] The first two differentials are often quite difficult to distinguish and require significant expertise. The major problem is faced because of radiation-induced atypia, which can be significant. The atypia is seen in the ductal epithelial cells showing loose clusters in a paucicellular FNA smear. A dirty necrotic background can also be seen to add to the problem. Usually the nucleocytoplasmic ratio in these cells is maintained due to an enlargement in both the nucleus and the cytoplasm. The degree of atypia, nuclear membrane irregularity, and nucleolar prominence are often less than what is expected in a case of frank carcinoma, although a cytopathologist is often forced to exercise a sufficient degree of caution to issue a straightforward report in such cases. The cellular cohesion is usually more than that of a case of carcinoma, and the smear has less cellularity.[39,40]

Papillary lesions of breast
The diagnostic challenge with papillary lesions of the breast is twofold: The distinction of the papillary lesions from the other nonpapillary lesions, namely, fibroadenoma, and the distinction of a benign from a malignant papillary lesion. The challenge is compounded by the fact that some of the papillary lesions are impossible to distinguish by cytology, such as intracystic or solid variants of papillary carcinoma.

Cytology smears of papillary lesions are highly cellular, with epithelial cell clusters, singly scattered epithelial cells, and papillary fronds, some of which are “true” in the sense that they contain central fibrovascular cores. Some complex and branching papillae are also noted [Figure 4a and b]. The singly scattered cells have a columnar look or sometimes they look plasmacytoid with eccentric nuclei and moderate to abundant amount of cytoplasm. The background of such lesions is equally important and is characterized by foamy macrophages, apocrine cells, and bipolar cells in a fluid backdrop. The presence of macrophages, apocrine cells, and bipolar cells is associated with a benign papillary lesion rather than a malignant one. In addition, a malignant papillary lesion is more often associated with a higher degree of cellularity, a greater number of singly scattered cells, and more complex papillae with fibrovascular cores. Atypia can be seen in both benign and malignant papillary lesions and is not a discriminating feature.[12,41-46] It is worth mentioning here that a proper classification of a papillary lesion is not always possible even by core needle biopsy and subsequent histopathology.[47,48]

Fibroadenomas and fibrocystic change lack in “true” papillae containing fibrovascular cores, and in addition fibroadenomas contain stromal fragments distinguishing them from papillary lesions.[41]

Proliferative breast diseases
After fibroadenoma, this is probably the second most common group of grey zone lesions.[9,12] Benign proliferative breast diseases, rhetorically called the nightmare of breast pathologists, are radial scar and complex sclerosing lesions that show adenosis and stromal fibrosis, often misunderstood as malignant glands causing infiltration and eliciting desmoplasia around them in histopathology. It should be remembered that despite best efforts, the accurate diagnosis of these two lesions is nearly impossible using cytology smears.

FNA smears are variably cellular with epithelial cells in clusters or as a dispersed population in the background of bipolar (myoepithelial) cells as well as hyalinized stromal fragments. The cytological picture is complicated by hypercellularity, discohesive cell population, nuclear atypia, tubular structures, paucity of myoepithelial cells in some lesions, and a dirty background.[49-51]
Usual ductal hyperplasia (UDH), atypical ductal hyperplasia (ADH), and low-grade ductal carcinoma are extremely difficult to distinguish from each other on cytology smears. While the first two are essentially proliferative breast disease with or without atypia and are preneoplastic conditions, the last one is a low-grade malignancy. UDH without atypia shows epithelial cell clusters with bland chromatin of the epithelial cells. Cellular monomorphism is not a feature of UDH. Myoepithelial cells are seen admixed with the epithelial cell clusters, and cellular streaming can also be noted. The epithelial cell clusters can also adopt complex structures. In contrast, low-grade ductal adenocarcinoma shows cellular monomorphism, absence of myoepithelial cells, nuclear atypia, conspicuous nucleoli, and some features of invasion, which will be discussed later. ADH has features intermediate to UDH and low-grade ductal adenocarcinoma [Figure 5a and b]. The presence of the myoepithelial cells is an important point of discrimination from ductal carcinoma in situ (DCIS).\textsuperscript{[52-55]}

**Ductal Carcinoma in Situ (DCIS) and other Invasive Carcinomas of Breast**

**DCIS and ductal adenocarcinoma**

DCIS can be low-grade or high-grade. Low-grade DCIS is non-comedo DCIS (solid sheet, cribriform; cells around a central space and papillary type). Low-grade DCIS shows epithelial cell clusters, sometimes with papillary or cribriform pattern. They show a relatively monomorphic population of cells having round nuclei and small nucleoli. Myoepithelial cells are absent. Macrophages and calcification are seen and necrosis can also be noted. Although necrosis is almost invariably seen in comedo DCIS, the presence of necrosis is not pathognomonic of a high-grade DCIS. In contrast to low-grade DCIS, high-grade DCIS shows cohesive clusters as well as a dispersed population of tumor cells. These tumor cells show a greater degree of nuclear pleomorphism, coarse chromatin, prominent nucleoli, mitotic activity, calcification, and necrotic background. Myoepithelial cells are absent. Low-grade DCIS and ADH are difficult, if not impossible, to distinguish as the distinction is based on quantitative criteria in histopathology and is not just qualitative. In contrast, high-grade DCIS is difficult to differentiate from an invasive ductal carcinoma.\textsuperscript{[94-96]} The cytological features of invasion are 1) fat infiltration, 2) fibroblast proliferation, 3) elastoid stromal fragments, 4) stromal infiltration, 5) tubule formation by the tumor cells, 6) intracytoplasmic lumina formation, and 7) the presence of lymphocytes around the tumor cells. In addition, 1) macrophages, 2) myoepithelial cells in the epithelial cell clusters, and 3) calcification are more commonly associated with an in situ lesion than invasion.\textsuperscript{[52,57-61]}

**Tubular carcinoma**

FNA smears of tubular carcinoma are cellular and show monolayered or angulated epithelial cell clusters such as fibroadenomas. However, tubular carcinomas show sudden changes in the diameter of the tubules with pointed tips. The cells arrange perpendicularly to the edge of the clusters. Atypia is minimal. The discohesive population of tumor cells is lower and many myoepithelial cells can be seen. Sometimes nuclear grooves and intracytoplasmic vacuoles can also be noted. A stromal component can be seen in the background, which is usually acellular.

**Lobular carcinoma**

Numerous studies had concluded lobular carcinoma to be the most commonly misdiagnosed (false negative) variety of breast malignancy.\textsuperscript{[62-66]} Cytology smears show variable cellularity ranging from low to high with a dispersed population of relatively monomorphic cells with eccentrically placed nuclei, mild nuclear atypia, deceptively bland chromatin, and moderate amount of cytoplasm [Figure 6]. The presence of intracytoplasmic lumina is often considered a telltale feature, though it is not very specific. It has been documented that the cellularity of an invasive lobular carcinoma in cytology smears correlates with the histopathological architectural pattern rather than with the original histopathological cellularity.\textsuperscript{[12,67]} The help of ancillary tests such as immunocytochemistry can be taken in doubtful cases combining a “pancytokeratin” and E-cadherin.

**Mucinous (colloid) carcinoma**

Mucinous carcinoma is important to diagnose due to two reasons. First, it should be distinguished from the other benign mucinous lesions of the breast, and second, it has a better prognosis than the other invasive carcinomas of the breast. Cytology smears of mucinous carcinoma show cellular aspirate with the tumor cells in clusters, sheet, or as a discrete population dispersed in an abundant amount of extracellular pool of mucin [Figure 7]. The individual cells are relatively monomorphic with eccentrically placed vesicular nuclei, inconspicuous to small nucleoli, and mucin-containing
vacuolated cytoplasm. Capillary fragments are often noted in the pool of mucin.

The major differentials considered in such a lesion are myxoid fibroadenoma, mucocele-like lesion, and fibrocystic change. Mucocele-like lesions are acellular to paucicellular, and the cellularity is contributed by occasional epithelial or myoepithelial cells with minimal atypia. In contrast, a fibrocystic lesion is variably cellular, containing macrophages and apocrine cells in addition to the epithelial and the myoepithelial cells. A myxoid fibroadenoma contains all the classical cytomorphological features of fibroadenoma, such as staghorn epithelial cell clusters, stromal fragments, and bare nuclei in a myxoid background.[12,45,68-71]

Other rare lesions
Adenoid cystic carcinoma is a sufficiently rare tumor of the breast showing acellular hyaline, metachromatic globules (Giemsa) surrounded by basaloid tumor cells in clusters. The basaloid cells are relatively monomorphic and have a high nucleocytoplasmic ratio with round to oval hyperchromatic nuclei, inconspicuous nucleoli, and scant amount of cytoplasm. These cells are arranged around the globules in clusters, sheet, cribriform pattern, and sometimes as single cells. The major differential is collagenous spherulosis, showing similar kind of globules/spherules though the cells lack in the basaloid appearance and numerous myoepithelial cells are present in the background. Pleomorphic adenoma has a metachromatic, feathery, and fibrillary character to the stroma, distinguishing them from those of adenoid cystic carcinoma.[72-74]

Cribriform carcinoma shows a low nuclear grade and tumor cells in a sheet with cribriform spaces in between. The major difficulty lies in its deceptively benign cytoarchitectural appearance. Cribriform DCIS naturally appears in the differential. The presence of osteoclast-like giant cells is considered to be a rare and nonspecific feature of invasion.[75-77]

Conclusion
The categorization of reporting of breast cytology has provided a buffer for the pathologists while providing necessary information to the clinicians and the patients. Whether the presence of both C3 and C4 is needed is a matter of debate, but the presence of such borderline categories definitely provides scope for the cytopathologists to make mistakes and also speaks volumes of the limitations and difficulties in interpretation of the breast cytology smears. Nevertheless, most of the cytology smears are correctly reported and there are only a few lesions that fall in the true grey zone region, being almost unclassifiable, unidentifiable, or undiagnosable by cytology. In such situations, the use of uncertain categories guides the clinician for further management. We have discussed the major grey zone lesions and the reasons for their difficulty in interpretation. Although not an exhaustive one, this discussion should help cytopathologists make the right call.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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