

## Immunocytochemical Study of Smooth Muscle Actin (SMA) in Fine Needle Aspiration Cytology (FNAC) of Benign and Malignant Breast Tumors

Methaq M Najem *MSc, PhD*

Dept. of Pathology, College of Medicine, AL-Mustansirya University

### Abstract

- Background** Myoepithelial cells play an important role in the interpretation of breast fine needle aspiration cytology, since these cells are believed to be a component of the benign process in the breast lesion. Myoepithelial cells distinction may be difficult occasionally, so their demonstration by immunocytochemistry method through myoepithelial cell marker like smooth muscle actin is a useful diagnostic tool to differentiate between benign and invasive malignant lesions.
- Objective** To study the role of smooth muscle actin as immunocytochemical marker in the demonstration of myoepithelial cell as an aid in the diagnosis of benign breast lesions in fine needle aspiration cytology of breast.
- Methods** Forty five cases of fine needle aspiration cytology of palpable female breast lesions were selected. 25 out of these 45 cases comprised 20 invasive breast carcinoma and 5 fibrocystic diseases with atypia stained with smooth muscle actin by immunocytochemistry method directly and 20 cases comprised of 10 fibroadenoma and 10 fibrocystic disease stained with smooth muscle actin after destaining from H&E stain. All of these cases had a confirmatory histological diagnosis.
- Results** Smooth muscle actin staining consistently highlighted the myoepithelial cells in smears of all histologically proven benign lesions such as fibroadenomas and fibrocystic changes. In contrast, invasive breast cancers demonstrated absence of staining with smooth muscle actin that seen in benign breast lesions.
- Conclusion** Application of smooth muscle actin immunostaining in breast fine-needle aspirates is feasible and practical. The use of destained H&E smears provides an effective means to directly examine any atypical cluster of cells for the presence of MEC differentiation. The demonstration of presence or absence of MEC differentiation in atypical cases can provide sufficient evidence to decrease error in diagnosis (false positive) of breast fine-needle aspirates.
- Keywords** Smooth muscle actin, fine needle aspiration cytology, breast lesions

### Introduction

The identification of myoepithelial cells (MECs) located between ductal epithelial cells and the basal lamina is useful in breast pathology for differentiating benign breast lesions from invasive breast carcinoma<sup>(1)</sup>.

MECs also play an important role in the interpretation of breast fine-needle aspiration cytology (FNAC)<sup>(2)</sup>.

These cells are believed to be a component of a benign process in breast lesions. Since MECs are not always easily identified in routinely stained

cytologic slides, their immunocytochemical demonstration would be a reliable ancillary study<sup>(3)</sup>.

The various antibodies that have been studied for this purpose<sup>(4)</sup> include S100 protein<sup>(5)</sup>, smooth muscle myosin heavy chain (SMM-HC)<sup>(6,7)</sup> and p63<sup>(8,9)</sup>, CD10<sup>(10)</sup> smooth muscle actin (SMA)<sup>(1,11)</sup>. Previous studies have reported that SMA appeared to be the most reliable marker for the recognition of MECs<sup>(2,3,11-16)</sup>.

In the present study we apply SMA immunocytochemistry for staining breast FNAC for different benign and malignant tumors. Our purpose was to study the role of SMA as immunocytochemical marker in the demonstration of myoepithelial cell as an aid in the diagnosis of benign breast lesions in FNAC of breast.

## Methods

Forty five histologically proven cases of FNAC of palpable breast lesions were selected, 20 infiltrative ductal carcinoma and 25 cases of benign lesions which were from female breast (10 fibroadenoma and 10 fibrocystic changes and 5 fibrocystic changes with atypia).

Twenty five out of these 45 cases were prospective collected from breast center in Al-Kadhimiya teaching hospital and 20 were retrospective and they were retrieved from cytology files from Al-Yarmouk hospital laboratories for the period between Jan. 2010 - August 2010 (10 fibroadenoma, 5 fibrocystic changes and 5 invasive ductal carcinoma) all of these cases had a confirmatory histological diagnosis with excisional biopsy results.

### Immunocytochemical staining protocol:

#### Prospective cases

Preparation of cytological specimen to be examined immunocytochemically was the same procedure of conventional cytology, after smearing the slides; they are quickly placed in the fixative (95% ethanol) to decrease the air drying artifact for about 20-30 min. Then we

proceed in the immunocytochemistry staining protocol which is similar to immunohistochemistry (IHC) protocol except in:

1. Cytology smears are not contain wax so there was no need for overnight backing in oven and no need for putting the slides in xylol.
2. Cytology smears are not subjected to formalin so the epitope retrieval technique is unnecessary for immunocytochemical staining of these specimens.

**We begun with Rehydration:** through descending alcohol series (Fresh absolute ethanol, 95% ethanol, 70% ethanol, 30% ethanol and distilled water) for 5 minutes for each step then **Peroxidases block:** 50µL of peroxidase blocking reagent was placed onto the sections and incubated for 20 minutes in the humid chamber; then washing in phosphate buffer saline. Slides are drained and blotted.

**Primary antibody** 50µL of pre diluted primary antibody smooth muscle actin (Dako Clone HHF35 code M0635, Mouse- antihuman, monoclonal) was placed onto the sections (the dilution was 1:50) and incubated in the humid chamber at 37°C for 15 minutes then we leave the humid chamber for one hour. Then we place the slides in fresh buffer bath for 5 minutes. Drain and blot gently.

Enough drops of **secondary (biotinylated link) antibody** (Dako, Denmark) were applied to cover the specimen and slides were incubated for 1 hour at 37°C in humid chamber. The slides were rinsed with tris phosphate buffer solution and then drained and blotted gently.

Enough red drops of **streptavidin HRP complex** reagent were applied onto the section and then were incubated for 30 minutes at 37°C in humid chamber. Slides are rinsed with PBS then drawn and blotted gently.

**Substrate-chromogen solution:** we apply enough drops of diamino benzedine (DAB) substrate chromogen solution in dark field. Substrate chromogen solution was prepared freshly in each run by adding the substrate drops in graduated

tube until 1mL then we add one drop of chromogen. The slides then are put in the humid chamber for 10 minutes at 37°C. Rinse gently with distilled water. Slides are immersed in the Mayer's *haematoxylin* for about 10 seconds then slides are rinsed with slowly running tap water. Slides are then immersed in distilled water for 3 minutes. The slides are drained and blotted and left to dry.

One to two drops of aqueous mounting media (Dako, Denmark) are applied onto the sections and the sections are quickly covered with cover slips and left to dry overnight. Slides are examined under light microscope for the assessment of immunostaining .

#### Retrospective cases

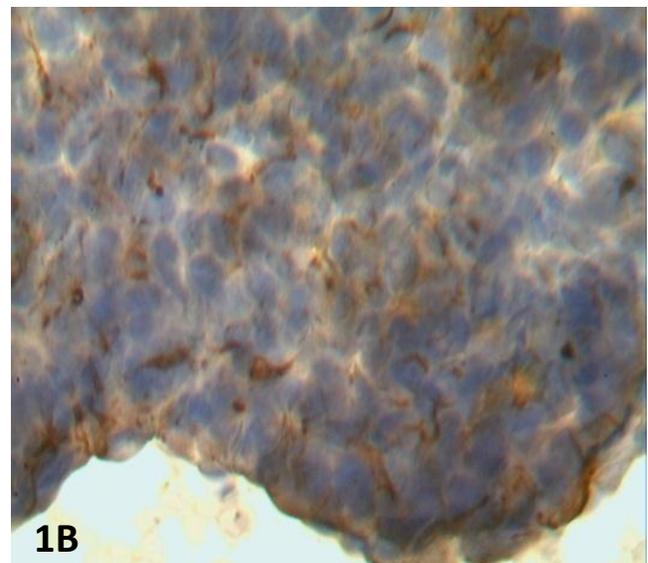
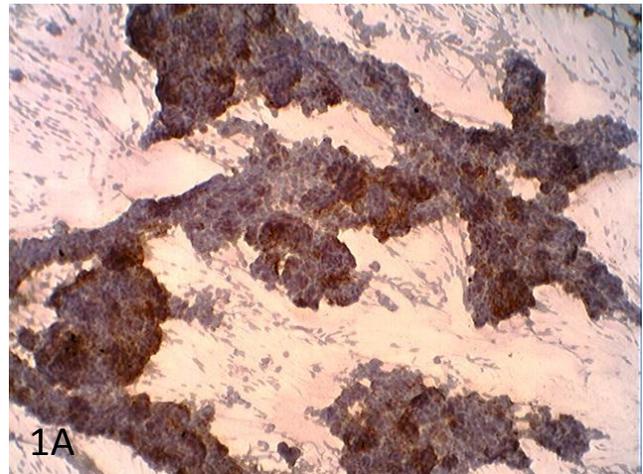
FNAC cases were selected and Immunocytochemical studies were performed following destaining of hematoxylin and eosin stained slides by immersion in 1% acid alcohol (prepared by adding 1 ml of HCL to every 100 ml of 70%of alcohol) for 10-30 min. then we proceed in the same above protocol (rehydration, peroxidase block. Slides were then incubated for 30 min in a humid chamber using a monoclonal mouse anti-human SMA. Slides were then incubated consecutively with a biotinylated anti-mouse secondary antibody, peroxidase labeled streptavidin detection, and 3-3 diaminobenzidine (**LSAB Kit code K0675**, Dako) and **DAB** substrate. Following hematoxylin counter staining, dehydration, and cover slipping were performed, slides were examined by light microscopy.

Positive control (smooth muscle of uterine leiomyoma) and technical negative control by omitting primary antibody and adding diluents only were included in each run.

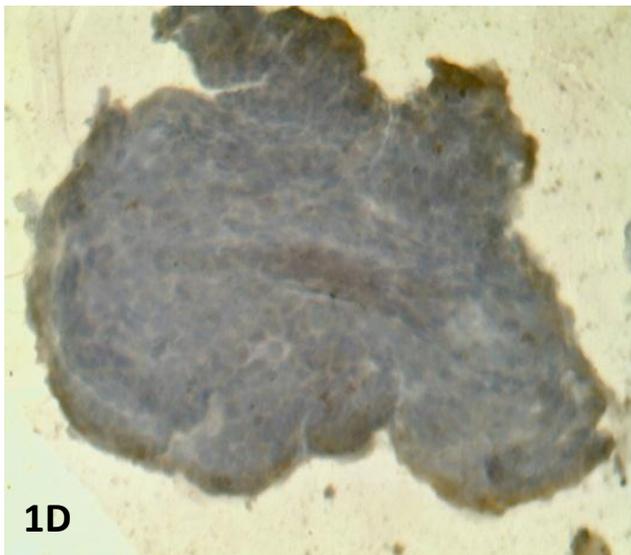
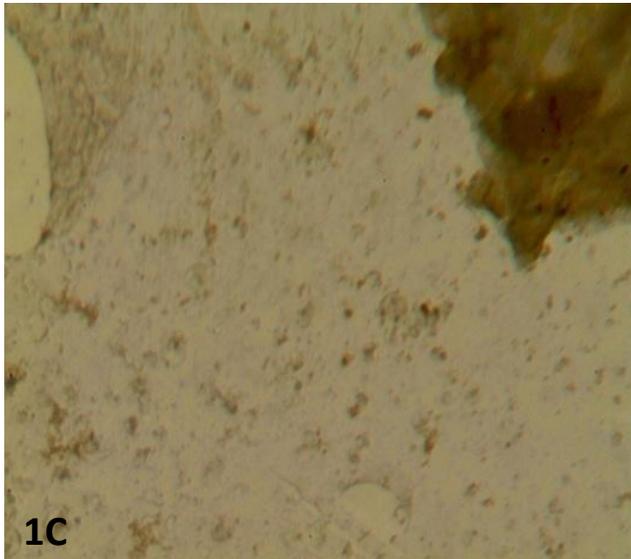
All the slides were examined under light microscope. A positive immunocytochemical reaction will appear as a brownish discoloration of the cytoplasm of myoepithelial cells.

#### Results

SMA staining consistently highlighted the myoepithelial cells in both smears as well as all histologically diagnosed benign lesions such as fibroadenomas and fibrocystic disease. The SMA positivity was demonstrated predominantly in the cytoplasm of individual spindle-shaped myoepithelial cells intertwined within the clusters of epithelial cells which characteristically showed no immunostaining (Figure 1 A,B ).



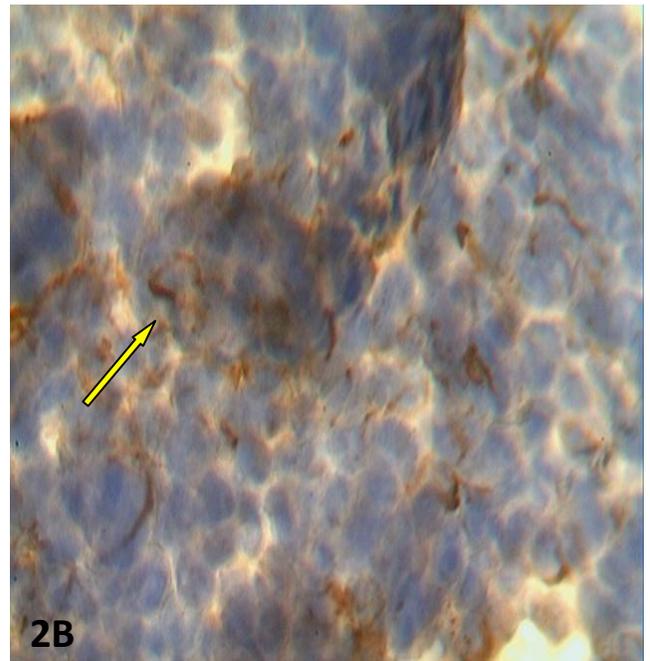
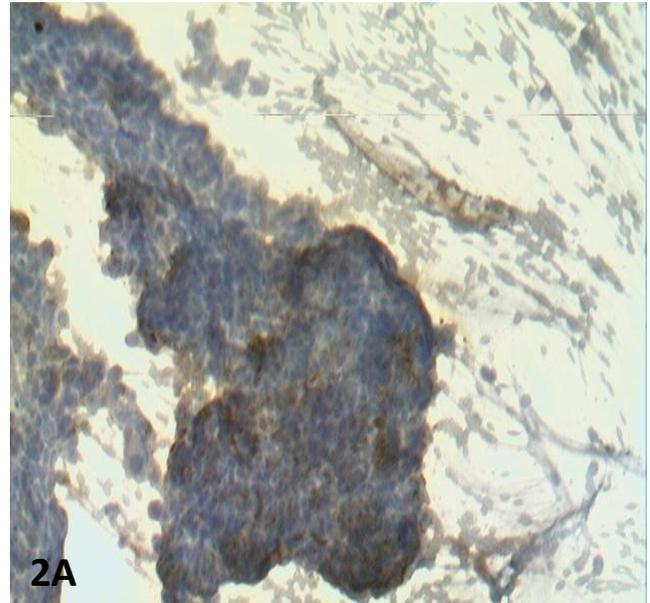
Some myoepithelial cells were contained along the perimeter of these fragments, and these also were decorated (Figure 1D).



**Figure 1. Expression of SMA by immunocytochemistry [A&B]: fibroadenoma showing positive staining for SMA as a brownish color [C]: fibroadenoma with positive MECs in the background of the specimen (400X)[D]: a case of fibrocystic changes showing positive staining of the peripheral cells surround the epithelial cells (400X)**

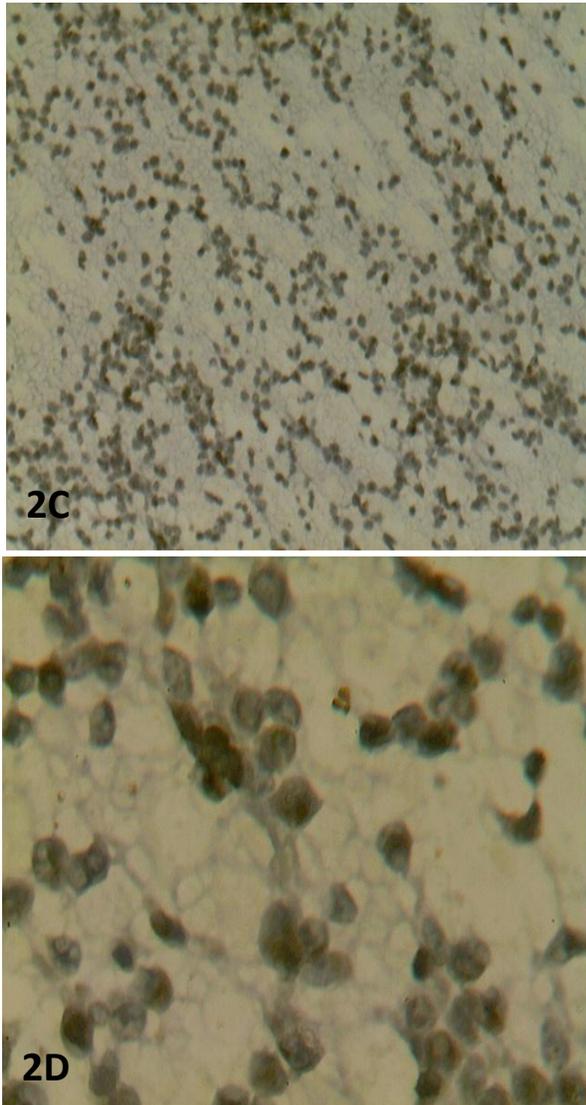
Expression of SMA was also clearly demonstrated in spindle cells scattered in the background. However, some spindle bipolar stromal cells, in the aspirates of proliferative fibrocystic breast disease or fibroadenomas,

showed no immunostaining while SMA immunostaining could be seen in the adjacent cells.



In 3 out of 15 fibrocystic disease cases showed cluster of atypical cells but with abundance of MECs in the background and within the clusters these cases proved to be on histopathological examination as proliferative fibrocystic changes with mild –moderate atypia.

In contrast, invasive breast cancers demonstrated absence of staining seen in benign breast lesions in 18 cases (Figure 2 C,D), while 2 of malignant cases showed positive staining of some scattered spindle cells in the background.



**Figure 2. Expression of SMA by immunocytochemistry: [A,B]: case of fibrocystic disease with atypia showing positive staining for SMA as a brownish color in different magnification (200x, 400x respectively) [C]: invasive ductal carcinoma showing negative staining for SMA(200X).[D]. Same previous case in (Figure C) in high magnification (400X)**

## Discussion

As the identification of MECs is useful in breast pathology for the differentiation between benign breast lesions and invasive breast carcinoma it gains the same importance in breast cytology<sup>(1)</sup>. The presence of myoepithelial cell (ME) has long been recognized as a prominent feature of benign breast diseases<sup>(11)</sup>.

In histology MECs identification based on both cytomorphologic features and their structural location on the other hand in cytology since the exfoliative cells do not demonstrate a lot of structural informations. MECs are almost always identified only by cytomorphological features. Therefore immunocytochemical staining is thus considered to be a reliable ancillary study to identify MECs<sup>(1)</sup>.

The MEC markers were not unique to MECs. In contrast, immunostaining for SMA has been found to be the most reliable marker for the recognition of MECs<sup>(1-3,11-15)</sup>.

The observed staining of MECs in benign and proliferative fibrocystic breast disease can be used as a strong differentiating feature in interpretation of atypical breast fine-needle aspirates.

This will potentially maximize the diagnostic accuracy of fine-needle aspiration biopsies and help to reduce the number of inconclusive cytologic diagnoses.

In the present study, spindle cells in benign cases occasionally showed no expression of SMA, while characteristic cytoplasmic staining as evidenced by SMA expression was clearly seen in the adjacent cells. This may be explained that these cells are connective tissue cells and not MECs<sup>(6)</sup>, or these cells may be the indeterminate cells may elongate and be mistaken for the MECs by light microscopy<sup>(2)</sup>.

The explanation to the presence of scattered positively staining cells with SMA in malignant cases is that these cells is possibly the SMA positive myofibroblast that exfoliate on cytologic smears and not MECs<sup>1</sup> and the tissue sections of

these cases proved to be positive for these cells, another possible explanation is that the cells had been entrapped during passage of the needle through the benign component of a malignant breast lesion<sup>(2)</sup>.

In the present study, it was found that SMA positive myoepithelial cells are commonly seen in the background of non-neoplastic conditions. This study suggests that the application of SMA immunostaining in breast fine-needle aspirates is feasible and practical. The use of destained H&E smears provides an effective means to directly examine any atypical cluster of cells for the presence of myoepithelial cell differentiation. The demonstration of presence or absence of myoepithelial cell differentiation in atypical cases can provide sufficient evidence to decrease the error in diagnosis (false positive) in breast fine-needle aspirates.

## References

1. Nambu M, Iwashita A, Iwasaki H, et al. The pattern of pitfalls in the application of smooth muscle actin immunostaining for breast cytological analyses. *Med Bull Fukuoka Univ* 2007; 34(1): 15-21.
2. Masood S, Lu L, Assaf-Munasifi M, et al. Application of immunostaining for muscle specific actin in detection of myoepithelial cells in breast fine needle aspirate. *Diag Cytopathol* 1995; 13(1): 71-74.
3. Nayar R, Breland C, Bedrossian U, et al. Immunoreactivity of ductal cells with putative myoepithelial markers: a potential pitfall in breast carcinoma. *Ann Diag Pathol* 1999; 3(3): 165-173.
4. Rudland PS, Leinster SJ, Winstanley J, et al. Immunocytochemical identification of cell types in benign and malignant breast diseases; variations in cell markers accompanying the malignant state. *J Histochem Cytochem* 1993; 41: 543-553.
5. Emberley ED, Murphy LC, Watson PH. S100A7 and the progression of breast cancer. *Breast Can Res* 2004; 6: 153-159.
6. Dabbs DJ, Gown AM. Distribution of calponin and smooth muscle myosin heavy chain in fine needle aspiration biopsies of the breast. *Diag Cytopathol* 1999; 20(4): 203-207.
7. Kalof AN, Tam D, Beatty B, et al. Immunostaining patterns of myoepithelial cells in breast lesions : a comparison of CD10 and smooth muscle myosin heavy chain. *J Clin Pathol* 2004; 57(6): 625-629.
8. Sato S, Kijima H, Suto A, et al. p63, a p53 homologue, is a selective nuclear marker of myoepithelial cells of the human breast. *Am J Surg Pathol* 2001; 25: 1054-1060.
9. Stefanou BD, Arkoumani E, Agnantis NJ. The usefulness of p63 as a marker of breast myoepithelial cells. *In Vivo* 2003; 12: 573-576.
10. Moritani S, Kushima R, Sugihara H, et al. Availability of CD10 immunohistochemistry as a marker of breast myoepithelial cells on paraffin sections. *Mod Pathol* 2002; 15: 397-405.
11. Pattari SK, Dey P, Gupata SK, et al. Myoepithelial cells: any role in aspiration cytology smears of breast tumors? *Cyto J* 2008; 5: 9.
12. Collins LC, Carlo VP, Hwang H, et al. Intracystic papillary carcinoma of the breast: A reevaluation using a panel of myoepithelial cell markers. *Am J Surg Pathol* 2006; 30(8): 1002-1007.
13. Fischler DF, Sneige N, Nelson G, et al. Tubular carcinoma of the breast: cytologic features in fine-needle aspirations and application of monoclonal anti-smooth muscle actin in diagnosis. *Diag Cytopathol* 1994; 10: 120-125.
14. Masood S, Assaf N, Hardy NM. The value of muscle specific actin immunostaining in differentiation between atypical hyperplasia and carcinoma in breast fine needle aspirates. *Acta Cytol* 1994; 38: 5, 860(A126).
15. Staerckel G, Sneige N, Ordoñez N. Fibroadenoma like (FA-like) carcinoma: cytologic features and utility of smooth muscle actin (SMA). *Acta Cytol* 1994; 38: 5, 863(A131).
16. Sato S, Kijima H, Suto A, et al. Fine-needle aspiration cytology of breast lesions: a review of cytological analysis using smooth muscle actin (SMA) immunostaining. *Anticancer Res* 2003; 23: 4175-4180.

---

Correspondence to: Dr. Methaq M Najem

E-mail: Meethaqmoen76@yahoo.com

Mobile + 964 7901147255

Received 24<sup>th</sup> Jul. 2011: Accepted 30<sup>th</sup> Oct. 2011