False-positives in Fine-needle Aspiration Cytology of Breast Disease can be Reduced with p63 Immunostaining – A Preliminary Report

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Abstract. Myoepithelial cells of the mammary gland are considered to be a key to distinguishing benign from malignant disease in fine-needle aspiration (FNA) cytology. However, identification of these cells with Papanicolaou staining is not easy. The identification of myoepithelial cells was investigated using p63 antibodies to carry out immunostaining of FNA specimens that had been used at the time of Papanicolaou staining for 37 patients who yielded false-positives in FNA. Positively-stained cells were observed in overlying cell clusters or the background in 67.6% of the patients. There is a possibility that over-diagnosis could have been avoided by performing p63 staining for these patients. The controls consisted of stamp samples of fresh specimens obtained from 23 patients at the time of surgery for invasive carcinoma and the results of p63 immunostaining did not reveal any positive staining of tumor cells. Accordingly, these results indicate that there is a strong likelihood that there is no invasive carcinoma when many p63-positive cells are observed in the tumor cell population or the background and that p63 immunostaining has the potential to aid in reducing false-positives at the time of FNA diagnosis of breast disease.

Fine-needle aspiration (FNA) biopsy is widely used in the diagnosis of breast disease and shows excellent sensitivity and specificity. Moreover, when used in combination with estimation of the histopathological type on the basis of cytological diagnosis, immunostaining of cytological specimens, etc., FNA biopsy is capable of diagnosing the histological grade and, thus, has extremely high clinical significance (1, 2). However, since it is a cytological diagnosis, there is the possibility of false-negative and false-positive results; the accuracy of the technique is approximately of 90%. Therefore, in actual clinical practice, a global judgment is made on the basis of image diagnosis, etc. At present, there is a growing general trend to perform core needle biopsy in patients showing discordance with the findings of the image diagnosis. As a result, the burden on patients due to local anesthesia, is increased and it would, thus, be desirable to achieve a more definitive diagnosis on the basis of the cytological specimens.

With the ordinary Papanicolaou staining performed in FNA diagnosis, the identification of myoepithelial cells, i.e., their quantitative ratio, morphological characteristics and architectural distribution, is extremely important for making the diagnosis (3). A diagnosis of benign disease can be made in the case that the findings indicate proliferation of myoepithelial cells inside cell clumps, whereas malignancy is suspected in the case that clumps of atypical cells which do not include myoepithelial cells are observed. With only the usual staining methods, the identification of myoepithelial cells can be difficult, resulting in many cases to be diagnosed as class III breast disease. On the other hand, there are myoepithelial markers, such as α-SMA (smooth muscle actin), myosin and others. Immunostaining using such markers is useful for histological specimens, but in the case of cytological specimens, vascular walls and fibroblasts can also show positive reactions, thus, rendering the results of the diagnostic staining unreliable (4–6).

It was recently reported that the p63 antibody was useful for staining mammary myoepithelial cells since it is a nuclear
staining antibody and does not stain fibroblasts or the cells of the vascular walls (7-11). In particular, p63 staining gives a positive result in almost all patients with benign disease (12) and was also reported useful in distinguishing between DCIS and invasive carcinoma (13).

In consideration of this background, a retrospective study was performed to evaluate the utility of p63 immunostaining by applying this technique to specimens from patients diagnosed as class IV or V on the basis of ordinary Papanicolaou staining and then subsequently diagnosed as having benign breast disease on the basis of pathological studies.

Materials and Methods

Breast FNA was performed on 5,798 patients between June of 1996 and December of 2004. Diagnosis of these patients on the basis of Papanicolaou staining showed class 0 or I disease in 1,471 patients (25.4%), class II disease in 3,224 patients (55.6%), class III disease in 161 patients (2.8%), class IV disease in 45 patients (0.8%) and class V disease in 897 patients (15.1%). Probe lumpectomy under local anesthesia is regularly performed on patients who show discordance between the Papanicolaou staining results and the image findings. As a result, in 40 out of the 942 patients considered to have malignant disease (class IV or V) by FNA were judged to have benign breast disease on the basis of subsequent histopathological studies. These 40 patients (10 class IV and 30 class V patients) were the source of the cytological specimens used in the present study. The final pathological diagnosis of these patients included 14 patients with epithelial proliferative lesion, such as ductal hyperplasia or adenosis, 18 with papilloma, 8 with fibroadenoma, 5 with mastopathy, and 2 cases of mastitis.

In consideration of this background, a retrospective study was performed to evaluate the utility of p63 immunostaining by applying this technique to specimens from patients diagnosed as class IV or V on the basis of ordinary Papanicolaou staining and then subsequently diagnosed as having benign breast disease on the basis of pathological studies.

**Table I. Pathological diagnosis of 40 patients with benign lesions.**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of cases</th>
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<tbody>
<tr>
<td>Mastopathy</td>
<td>14 cases</td>
</tr>
<tr>
<td>Fibroadenoma</td>
<td>16 cases</td>
</tr>
<tr>
<td>Papilloma</td>
<td>8 cases</td>
</tr>
<tr>
<td>Mastitis</td>
<td>2 cases</td>
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Results

It was possible to evaluate the immunohistochemical staining for p63 in 37 out of the 40 cytological specimens. In 23 (62.2%) of the evaluated specimens, p63-positive cells were observed in the overlying cell clusters and background cells (Figure 1), while positive staining of cells in the background but not in the cell population was observed in one specimen (Figure 2). In addition, the specimen from one patient showed positive staining for cells in the overlying cell clusters, but the background cells were negative. In total, 25 (67.6%) out of 37 cases over-diagnosed with malignancy by FNA were positive staining for p63. Double negative staining was observed in 12 cases. Thus, concordance of the staining in the overlying cells and the background cells was found in 35 out of the 37 (94.6%) specimens showing positive or negative p63 staining.

The rate of positive staining was evaluated as a function of the age of the patients. For 24 patients under 50 years of age, positive staining was observed in 18 specimens, while 6 specimens were negative. For 11 patients aged 50 years or more, the staining was positive in 4 specimens and negative in 7. These results, thus, indicate that the rate of positive p63 staining was clearly higher in the patients under 50 years of age. As a function of the histological type, of the 20 specimens from patients with epithelial proliferative lesion, such as ductal hyperplasia or adenosis, 18 were positively stained and two were negatively stained. Five out of the eight specimens from patients with papilloma were positively stained and 3 negatively, while for the 16 specimens from fibroadenoma patients 12 were positive and 3 were negative stained (Table II).

In contrast, p63 staining was negative in 19 out of the 23 stamp samples of fresh specimens obtained at the time of surgery from patients with invasive carcinoma. For all 4 of the positively stained specimens, staining was observed in the cells of the cell population but not in the background cells.

**Table II. p63 Staining for 40 patients with benign lesions according to age and histological type.**

<table>
<thead>
<tr>
<th>Age</th>
<th>p63 positive</th>
<th>p63 negative</th>
</tr>
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<tbody>
<tr>
<td>&lt;49 years</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td>≥50 years</td>
<td>4</td>
<td>7</td>
</tr>
</tbody>
</table>
| With epithelial proliferative lesion*** | 18 | 2 
| papilloma   | 5            | 3            |
| fibroadenoma| 13           | 3            |

*p<0.05; **NS; ***includes mastopathy, fibroadenoma, papilloma, mastitis.
Figure 1. Aspiration cytology of benign lesion. P63-positive cells were observed in the nuclei of overlying epithelial clusters and background cells (x200).

Figure 2. Aspiration cytology of benign lesion. P63-positive staining only in background cells. Note the absence of p63 immunoreactivity in the nuclei of cell clusters (arrows) (x200).
These positive cells seemed to be benign epithelial cells. Concordance of the staining was seen in 19 out of the 23 specimens and all were negatively stained.

Discussion

Breast disease is globally diagnosed on the basis of the findings from palpation, mammography, ultrasonography and FNA. In particular, cytological diagnosis is performed by estimating the histopathological type of the disease and also by performing immunohistochemical staining of the cytological specimen and it has become possible to diagnose the grade of malignancy. The clinical significance of this approach is very high (1, 2). In recent years, there has been increasing application of core needle biopsy as a diagnostic tool, but this increases the burden on patients due to local anesthesia and other complications. Thus, it would be desirable to achieve a more definitive diagnosis on the basis of the cytological specimens.

With the ordinary Papanicolaou staining performed in the FNA diagnosis, the diagnosis of benign or malignant disease is made on the basis of the identification of myoepithelial cells (3). However, the use of Papanicolaou staining alone generates false-negative and false-positive results and the diagnostic accuracy is approximately 90%. Thus, immunohistochemical staining for myoepithelial markers is being recommended as an auxiliary diagnosis. Myoepithelial markers include α-SMA, myosin, etc., but while these are useful for histological specimens, there is a lack of reliability regarding their staining for cytological specimens (4-6).

It was recently reported that the p63 antibody is useful for staining mammary myoepithelial cells due to its selective properties as a nuclear staining antibody which does not stain fibroblasts, the cells of the vascular walls (7-11). In particular, it was reported that p63 staining yielded a positive result in almost all patients with benign disease (12). It is also considered useful for distinguishing between DCIS and invasive carcinoma (13).

With this background, we performed p63 immunostaining on breast cytological specimens obtained from patients who had been diagnosed as class IV or V on the basis of ordinary Papanicolaou staining and were subsequently diagnosed with benign disease on the basis of histopathological studies. The utility of p63 immunostaining was, thus, retrospectively evaluated. For 23 out of the 37 (62.2%) patient specimens for which the results were evaluable, the cell nuclei of the overlying cell clusters and background cells stained positively for p63. One specimen showed positive staining of the background cells but no staining in the cell population, while another specimen positively stained for cells in the cell population while the background cells were negative. In total, specimens from 25 patients (67.6%) stained positively for p63. There is a possibility that we could have prevented false-positive by performing p63 staining for these 25 patients. Stratification of the p63 staining results as a function of patient age revealing a positive rate of 75% in patients who were under 50 years of age and a positive rate of 36% in those patients 50 years or older. These data, thus, show a clearly higher rate of positive p63 staining in the younger patient stratum (Table II).
Our observation of positive p63 staining in only 67.6% of the patient specimens differs from the earlier report that p63 staining yields a positive result in almost all patients with benign disease. However, the positive staining rate was 75% in the under-50 population and it was, thus, surmised that there was a change in antigenicity, as a result of the aging process. In addition, the positive staining rate was 90% for specimens from patients with marked hyperplasia of the ductal epithelium, 63% for papilloma specimens and 81% for fibroadenoma specimens. These results indicate that, regardless of the histological type of patients with benign disease, there is at least a possibility of false-negative test results. One possible explanation for these false-negative test results is that they were caused by the reactivation of antigenicity that was performed after removal of the cover glasses from the Papanicolaou-stained specimens used in this study. The fixation conditions used for the cytological specimens are another possible explanation. Future studies should be carried out using fresh FNA cytological specimens.

P63 staining was negative in 19 out of the 23 fresh stamp specimens obtained at the time of surgery on patients with invasive carcinoma. All 4 of the positively-stained specimens showed staining in the cells of the cell population but not in the background cells. This cell population was a portion that formed a clump of cells with little atypia and was benign. Therefore, there is a possibility that the invasive carcinoma cells that stained positively for p63 in this study were due to contamination by benign cells, and a strong possibility that negative p63 staining in invasive carcinoma has a high diagnostic value.

The reports of various researchers indicate that even invasive carcinomas showed some degree of p63-stain positivity. Explanations for this include such possibilities as that a small number of myoepithelial cells from a noninvasive focus in the invasive carcinoma were harvested together with tumor cells and that breast cancer cells also underwent myoepithelial-like or basal-like differentiation (8, 14, 15). However, in the present study, the invasive carcinoma cells did not stain positively for p63. It is also noted that we did not investigate DCIS in this study. It was reported that DCIS positively stained for p63 and that breast cancer cells also underwent myoepithelial-like or basal-like differentiation. In conclusion, the results of this preliminary study indicate the possibly high level of diagnostic value of p63 staining for patients with breast disease that is difficult to judge as benign or malignant on the basis of Papanicolaou staining of FNA specimens. In particular, it is surmised that there is a high likelihood that positive staining for p63 negates the possibility of invasive carcinoma. Future investigations should include a prospective study of p63 staining using fresh FNA breast disease specimens.

**References**


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