BULLETIN OF TAIPEI MEDICAL COLLEGE, VOL. 14, MARCH 1985
P. 143-153

The Cytological Diagnosis in the Imprint Smear of Breast Biopsy

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In recent years, imprint cytology has been used increasingly in combination with frozen section histology for intraoperative biopsies. As a diagnostic tool, the imprint cytology has a very high rate of agreement (95%) with the histologic diagnosis and may be correlated with the frozen section to produce an accurate histologic diagnosis in the early stage of breast cancer, but it cannot replace a good frozen section. For example, when the frozen block consists of adipose tissue, attempts to cut thin sections will result in crumbling. However, imprint cytology smears can provide the information to support the diagnosis of fat necrosis or other metabolism diseases. The definitive diagnosis must be based on the permanent paraffin histologic section. The purpose of this study is to correlate cellular features in imprint cytology with frozen tissue and permanent sections for the possible use of imprint cytology as a diagnostic tool.

INTRODUCTION

Breast cancer is the most common malignant disease in women, and the death rate from it has been constant for over 45 years. Breast cancer is the leading cause of death for 20% of American women who die from malignant diseases as well as the leading cause of death in women over 40 years old[1,2]. The overall five-year survival rates for all stages of breast cancer are 62% and 47% for white and black American females respectively. Every fifteen minutes one woman dies of the disease. Nearly 33,000 deaths in the United States can be attributed to breast cancer annually[1,2].

While 9% of all women will develop breast cancer during their normal life expectancy, breast cancer is not a chance event that occurs randomly throughout the population. The exact etiology of breast cancer is not understood, but a group of patients exists with an increased risk of breast cancer. Factors for developing breast cancer are listed as follows[1,2,3]:

1. Over 99% of breast cancers occur in the female.
2. More than 80% of breast cancers are clinically detected in patients over 40 years of age.
3. Patients with a familial history of breast cancer are at increased risk.

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Received for Publication: October 27, 1984.

4. Nulliparous women or those with their first parity after the age of 34. Stress is placed on age of the first parity; protection seems to be against post-menopause breast cancer.
5. Patients with a previous cancer in one breast or patients with primary endometrial or ovarian cancer.
6. Those with a mastopathy type of benign breast disease.
7. Women with an adverse hormonal milieu such as prolonged menstrual activity or lowered estriol fractions.
8. Those who have had an excess exposure of the breast to ionizing radiation.
9. Those with other organ cancers, especially the endometrium and ovary cardinoma.

Based on these known statistical facts about benign and malignant breast lesions, combined with a careful history, a systematic and thorough breast examination, and the judicious and selective use of diagnostic aids; a tentative diagnosis of a given breast lesions can usually be made. The differential diagnosis of breast lesions should start with a careful patient history. First, the age of the patient is elicited, since different breast lesions have common age periods in which they occur.

Next, the patient's chief complaint with all pertinent and relative details is obtained. The chief complaints of patients with breast lesions include: lump or mass; pain, nipple symptoms (discharge, retraction, elevation, eczema); skin symptoms (dimpling, redness, edema, ulceration); other signs or symptoms such as suspicious axillary nodes, evidence of local advancement or distant metastasis[1].

In the clinic, the recognition of breast lesions may be accomplished by different types of examinations; clinical, radiologic (e.g., mammography and thermography), cytologic, and histological. For a cytological examination, cellular elements may be obtained in three ways: nipple discharge, needle aspiration, direct imprint of biopsied tissue or surgical specimens[4]. For this study of imprint cytology of breast lesions, a preliminary evaluation was conducted on 53 cases of nipple discharge or needle aspiration cytology at the University of South Alabama Medical Center during the years 1978 to 1981. The cytologic diagnoses were: 2 cases of Class 0 (invaluable specimen), 46 cases with Class I (negative for malignancy), 3 cases with Class II (atypical duct cell clusters present), none with malignant disease. These cytologic smears did not contain enough cellular representation to set up good cytological criteria for early diagnosis of breast lesion. This might be caused by the difficult desquamation of malignant cells in the breast or the inadequate technique of samplings from the lesions[5].

In recent years, imprint cytology has been used increasingly in combination with frozen section histology for intraoperative biopsies. As a diagnostic tool, the imprint cytology has a very high rate of agreement (95%) with the histologic diagnosis and may be correlated with the frozen section to produce an accurate histologic diagnosis in the early stage of breast cancer, but it cannot replace a good frozen section[6]. For example, when the frozen block consists of adipose tissue, attempts to cut thin sections will result in crumbling. However,

imprint cytology smears can provide the information to support the diagnosis of fat necrosis or other metabolism diseases. The definitive diagnosis must be based on the permanent paraffin histologic section. The purpose of this study is to correlate cellular features in imprint cytology with frozen tissue and permanent sections for the possible use of imprint cytology as a diagnostic tool.

MATERIALS AND METHODS

From January 20 to April 26, 1982, 22 surgical breast specimens from 21 women were examined simultaneously by histology and imprint cytology. These patients had the clinical diagnosis of breast masses or lumps; their ages ranged from 16 years to 74 years with the average age being 49 years. The imprint preprations were made from suspect areas of breast surgical tissue for frozen sections whose freshly cut surface was gently pressed against the albuminized glass slides. The imprint smears were immediately fixed by alcohol aerosal spray fixative and were stained by Papanicolaou's stain for cytological study. The remaining tissue was then fixed in formalin and embedded in paraffin for conventional histopathologic diagnosis. The permanent paraffin sections were used to make the final diagnoses.

RESULT

Final interpretation of the 22 surgical specimens are as follows: four with fat necrosis, ten with benign mammary dysplasia (including fibrocystic disease, fibrous mastopathy, chronic mastitis, and intraductal pappillomatosis[7], three with fibroadenoma, and five with malignant diseases (including three infiltrating ductal carcinoma, one intraductal carcinoma, one metastasis carcinoma). The agreement between frozen section and permanent histologic section is shown in Table 1; one frozen section, one fat necrosis and one malignancy were classified as benign fibrous tissue and fibrocystic disease (Benign mammary dysplasia). The agreement between imprint cytology and

Table 1. The Agreement Between Frozen Histologic Section and Permanent Histologic Section. On Frozen Histologic Section, One Fat Necorsis and One Malignancy were Classified as Benign Mammary Dysplasia

Diagnoses	Permanent histologic section	Frozen histologic section	No. of disagreement
Fat necrosis	4	3	1
Benign mammary dysplasia	10	12	2
Fibroadenoma	3	3	0
Malignancy	5	4	1
Total	22	22	4

Table 2. The Agreement between Permanent Histologic Section and Imprint Cytology. In Imprint Cytology, Two Benign Mammary Dysplasia were Classified as Suspicious for Malignancy

Number of Patients

Methods	Permanent histologic section	Imprint cytology	No. of disagreement
Diagnoses			
Fat Necrosis	4	4	0
Benign Mammary Dysplasia	10	8	2
Fibroadenoma	3	3	0
Malignancy	5	5	0
Suspicious for Malignancy	0	2	0
Total	22	22	2

Table 3. The Relationship between Ages and Breast Lesions of Patients. Fibroadenoma Appeared in Patients Aged under 30 Years Old and Malignant Carcinoma Occurred in the Ages Over 40

Number of Patients

Ages	10-19	20-29	30-39	40-49	50-59	60-69	70-79	Total
Histologic Diagnoses								
Fat Necrosis		1		1		2		4 (18%)
Benign Mammary Dysplasia			1	4	1	2	2	10 (45%)
Fibroadenoma	1	2						3 (14%)
Malignancy				2		2	1	5 (23%)
Total	1 (4.7%)	3 (14%)	1 (4.7%)	7 (31%)	1 (4.7%)	6 (27%)	3 (14%)	22 (100%)

permanent histologic section is shown in Table 2; with imprint cytology, two benign mammary dysplasias were classified as suspicious for malignancy. The relationship between ages and breast lesions of patients is shown in Table 3; fat necrosis occurred at any age from 20 years to 74 years; benign mammary dysplasia occurred in ages from 30 years to 74 years, Fibroadenoma appeared in patients ages 16 years to 29 years; breast carcinoma occurred in the ages of 40 years to 74 years. This data shows that fibroadenoma of the breast was often found in young women under the age of 30 and the

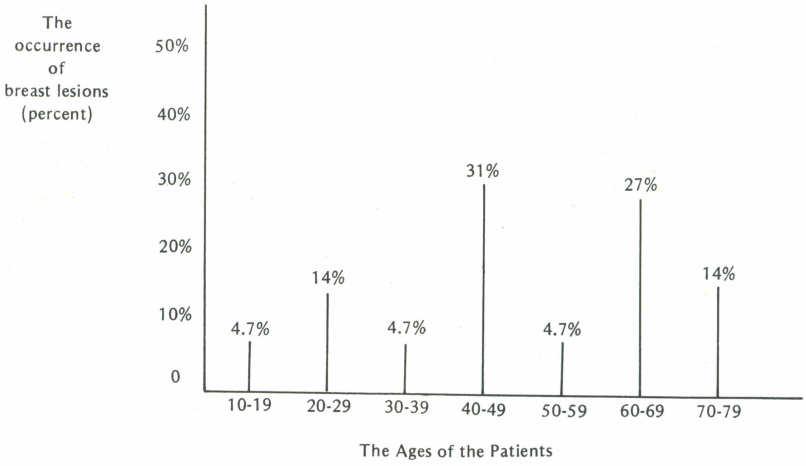

Fig. 1. The relationship between the occurrence of the breast lesions and the ages in the patients.

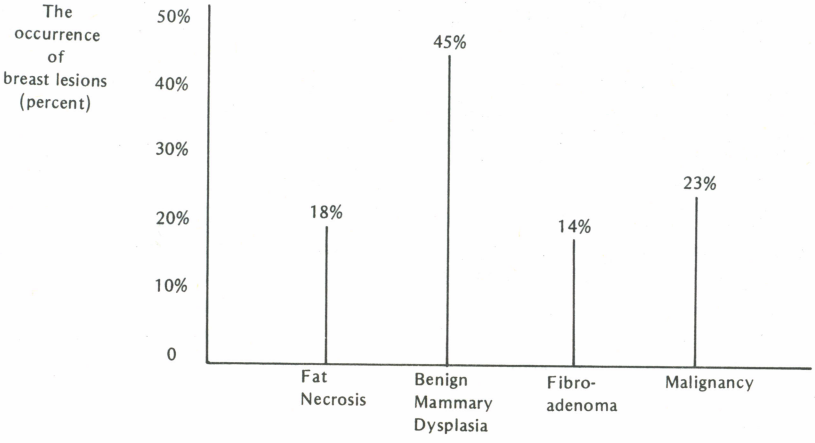

Fig. 2. The relationship between the occurrence of breast lesions and the diagnoses of breast lesions in the patients.

malignant lesions of the breast were usually found in women over 40 years of age[4,7].

The breast lesions in both benign and malignant conditions were commonly found in the ages of 40 to 49 and 60 to 69. (as Fig. 1). The histologic diagnoses of breast lesions were: 4 cases (18%) with fat necrosis, 10 cases (45%) with benign mammary dysplasia, 3 cases (14%) with fibroadenoma, 5 cases (23%) with malignancy. (Fig. 2)

BENIGN FEATURES IN CYTOLOGY IMPRINT SMEARS

On the imprint cytology of benign lesions, there were several types of epithelial and inflammatory cells. These cells included: [4,5,7,8,9,10,11]

1. Duct cels and acinar cells — cubodial cells often found in tight clusters, showing more cellular molding and forming

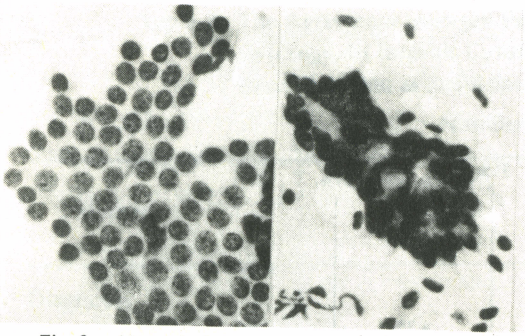

Fig. 3. Normal duct cells. Small cuboidal cells in sheet (left) and in cluster (right), both are shown in good adherence. The oval nuclei have evenly distributed chromatin and many have small chromocenters. (papanicolaou stain, x400)

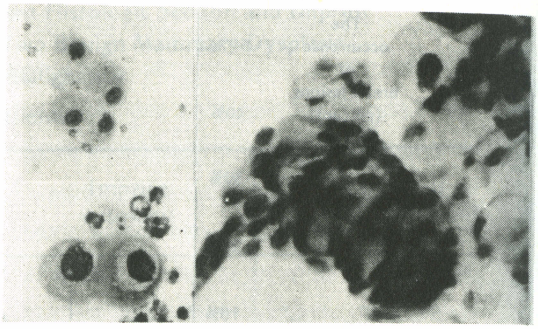

Fig. 4. Foam cells (left upper and lower) are usually found in single or in loose clusters with eccentric round or oval nuclei, abundant foamy cytoplasm. (panapnicolaou stain, Left upper x200, Left lower x400) Apocrine metaplastic cells (Right). Note the abundant cytoplasm and round nuclei. (papanicolaou stain, x400)

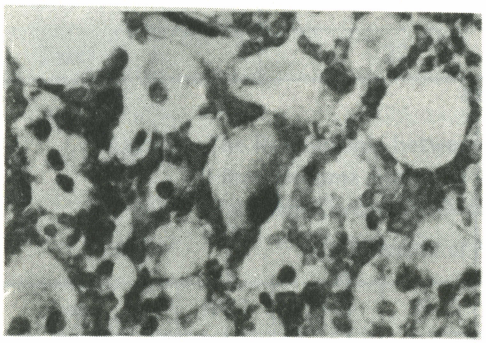

Fig. 5. Apocrine cells. Note Single apocrine cell with abundant eosinophilic cytoplasm and centric round nucleous and many foam cells in hemorrhagic smear. (pananicolaou stain x400)

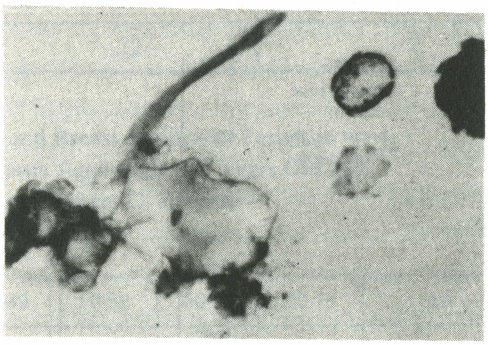

Fig. 6. Adipose tissue. Note the clustered fat cells (lower, left) and the single fat cell (middle) which has empty space cytoplasm with a pyknotic nucleous. (panpanicolaou stin, x400)

trabeculae of various sizes.

2. Naked bipolar nuclei — considered to derive from myoepithelial cells and often increased the numbers of fibroadenoma cases. Compared with duct cells, their nuclei had a much more uniform appearance and showed less variation in size and shape.
3. Foam cells — believed to derive either from epithelial cells or foam histiocytes. They were usually found singly or in loose clusters with round or oval shape, abundant foamy cytoplasm, and fine lipid - containing vacuoles of variable size.
4. Fat cells — found in either normal condition or fat necrosis. Sometimes, it might be difficult to distinguish free fat cells from large foamy phagocytes.
5. Fibrocytes — elements of the connective tissue have spindly appearance and centrally located nuclei that are round, oval or spindly.
6. Gint cells — found in pregnancy, early

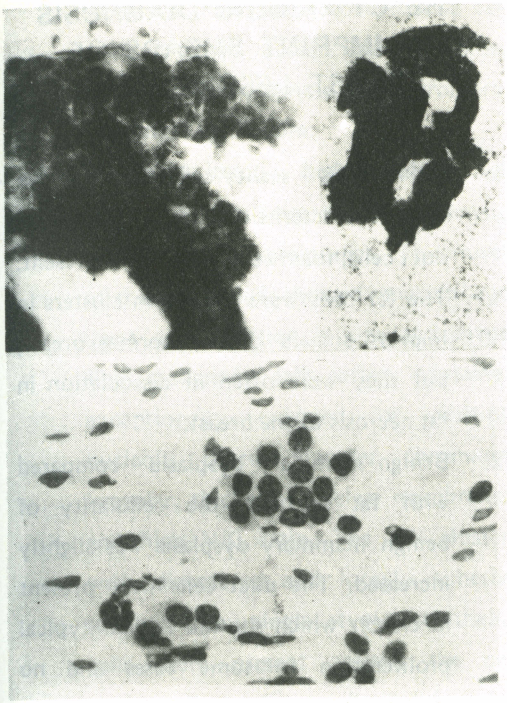

Fig. 7. Fibroadenoma. In imprint cytology, fibroadenoma has high cellularity, prominent of duct cells and biopolar nuclei. (upper right, x200). The cluster of duct cells are shown molding and prominent nuclei. (upper left, x400). Some mild atypical duct cells in sheet and many scattered biopolar nuclei are found in background. (lower, x400) (pananicolaou stain)

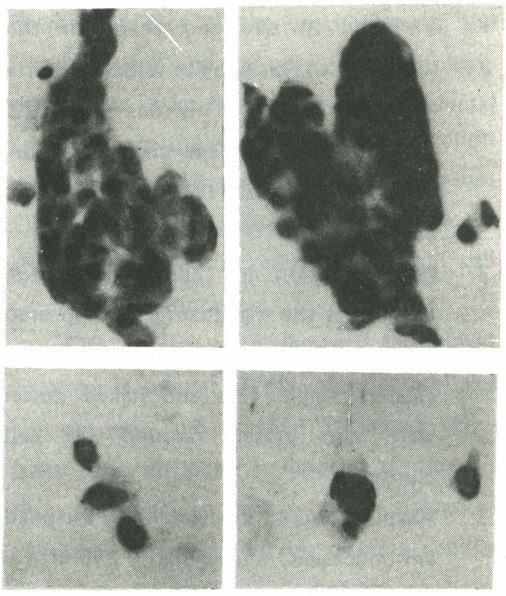

Fig. 8. Duct cell cluster (upper left) is shown nuclear molding and somewhat hyperchromasia in benign mammary dysplasia. The cluster of duct cells (upper right) is shown marked anisocytosis and poikilocytosis in disordered, loosely cohesive in carcinoma. Note the cancer cells (lower) have high N/C ratio, irregular & thickened nuclear membranes; hyperchromasia & macronucleolei (lower, right) (pananicolaou stain, x400)

postpartum period, inflammatery reactions, granulation tissue as typical foreign body type giant cell, and occasionally in cyst fluid. In carcinoma of the breast, numerous multinucleated giant cells may be found representing atypical tumor cells. Giant cells with extremely bizarre nuclei may be observed after radiation therapy.

7. Inflammatory cells - included histiocytes, lymphocytes, polymorphonuclear leukocytes present in varying numbers. An inflammatory smear should be screened for tumor cells with even more care than usual because of the frequent coexistence of inflammation and breast cancer.

ABNORMAL FINDING IN CYTOLOGY IMPRINT SMEARS

In imprint cytology of greast malignant lesions, there were some easily recognized features in the smears such as rich cellularity, accumulation and overlapping of features in thick aggregates, disordered arrangement

in cohesive clusters[4,5,7]. Criterial of malignant lesions of the breast are summarized as below[4,7,10,11,12]:

1. Variation in size and shape of the tumor cells. This was not reliable criteria when used alone. This was associated with abnormal nuclear chromatin pattern and abnormal nucleoli in breast cancer.
2. Poor cohesion in cellular aggregates. In general the malignant cells appeared less cohesive at the periphery of each cluster. Single cells and naked nuclei were also present around the cell clusters.
3. Atypical nuclear changes. They included anisonucleosis, irregular hyperchromatism, nuclear molding, increased N/C ratio, nucleolar hypertrophy, and irregular thick nuclear membrane.
4. Atypical cells consisting of only one cell type. No admixture of epithelial or myoepithelial cells was noticed within the tumor cell population which was a feature seen on almost all benign breast lesions.
5. Linear pattern arrangement in the small cell clusters. They were frequently seen in infiltrating ductal carcinoma.
6. Mitoses - the least reliable criterion in the malignancy of the breast. However, they were markedly increased in the malignant lesions.
7. Inflammatory diathesis. This also could be found in benign cystic lesions.

In general, the small clusters of epithelial cells, larger nuclei, and the presence of nucleoli are associated with poor prognosis and aggressive cancer.

THE CYTOLOGIC DIAGNOSIS IN IMPRINT SMEARS OF BREAST LESIONS:[5,6,10]

1. Fat necrosis - scanty cellularity was the main characteristics. There were few duct cells, foam cells, or fat cells present. The fat cells were usually in clusters in imprint smears of the normal breast, but they were often in dissociation in fat necrosis of the breast.
2. Benign mammary dysplasia - compared with fat necrosis, the cellularity of benign mammary dysplasia was slightly increased. The duct cells were present in cluster which showed regular typical proliferation in some cases and no obvious cellular atypia in imprint smears. Many epithelial cells were degenerated and some cells showed trends transitioning towards foam cells. Foam cells and clusters of apocrine cells were found. Single dissociated cells were few, indicating a high degree of intracellular cohesiveness. Free, naked nuclei of oval shape were commonly seen. Inflammatory cells were often present in clear background.
3. Fibroadenoma - The histologic characteristics of fibroadenoma are proliferation of connective tissue and, to a lesser extent, also of ductal epithelium. In imprint cytology, fibroadenoma had high cellularity in which the ductal epithelium was the dominant cell type - including both aggregated cells and naked bioplar nuclei. Foam cells and apocrine cells were present but in less numbers than that of benign mammary

dysplasia. Many atypical epithelial cells with enlarged nuclei and hyperchromatism might originate in the proliferating ductal epithelium.

4. Malignant lesion - high cellularity and cellular atypia were the characteristics of the breast carcinomas. The nuceli of the cancer cells showed much enlargement and variation in size and shape. The malignant cells often contained exceptionally enlarged or multiple nucleoli; coarse granular chromatin; irregular, thickened nuclear membranes; and high N/C ratio. The cell clusters were disordered and loosely cohesive so that many single atypical epithelial cells were present around the cell clusters. Some foam cells and apocrine cells were seen in tumor diathesis background.

DISCUSSION

The diagnosis of imprint cytology had good correlation with the histologic diagnosis in 20 of 22 surgical specimens. The two cases in disagreement were histologically diagnosed as fibrocystic disease. However, one imprint smear showed atypical epithelial cells with enlarged nuclei and multiple large irregular nucleoli against a clear backgrund. In fact, this patient had had a previous breast biopsy with poorly differentiated infiltrating ductal carcinoma four days prior to the second frozen section. Another imprint cytology showed high cellularity with mild atypical changes in the epithelial cells; this patient had had a previous breast biopsy and imprint cytologic smear with intraductal carcinoma one week prior to the second frozen section. This suspicious case also had a disagreement at the first intraoperative biopsy of the breast between the frozen section and the permanent histologic section. It was interpreted as intraductal carcinoma on the paraffin histologic section but fibrocystic disease on the frozen section. The imprint smear showed the malignant characteristics which suggested small cell type ductal carcinoma.

The criteria of malignancy in imprint cytology is cellular atypia, poor intracellular cohesiveness, and marked increased cellularity. The difficulty in imprint cytology is that some benign lesions show a marked cellularity accompanied by discrete cellular alteration. Greater intensity of these cellular atypias distinguish malignant tumors from intraductal papillomas, fibroadenoma, and epithelial hyperplasia of fibrocystic disease. The danger of false-positve diagnosis is eliminated if all radial therapy is sanctioned by biopsy. False-negative cytologic results, particularly in cases of suspicious clinical or radiologic reports should be considered with reservation and not be accepted as a definitive answer[4].

There is no doubt that imprint cytology can aid the frozen section examination for accurate intraoperative diagnosis. Imprint smears of surgical specimens can be rapidly processed for examination of suspicious lesions and can serve as an adjuvant to frozen histologic sections[4].

ACKNOWLEDGEMENTS

The author wishes to express her grati-

tude to Professor T.Y. Chen in the department of Pathology, Taipei Medical College for his kindness to review the manuscript.

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乳房活體組織印捺片的細胞學診斷

蘇 美 淑

摘 要

近年來，活體組織印捺片細胞學與其冷凍切片檢查同時應用在臨床上做爲急速病理組織診斷，有日漸增加的趨勢。印捺片細胞學檢查被應用爲一種診斷工具，主要是其診斷結果與最後的病理組織切片結果，有很高的符合率（約95％）；這顯示印捺片細胞學有相當高的臨床使用價值，如同時配合冷凍組織切片法檢查病人的乳房活體組織標本，可得到更精確的病理組織診斷，而能早期發現乳癌。但這並不表示印捺片細胞學可以完全取代良好的冷凍組織切片法。例如活體組織標本含有脂肪組織時，很難取得完整美好的冷凍病理切片，常常是破碎而妨礙結果的判讀。此時印捺片細胞學的輔助檢查常常可提供有效資料來支持脂肪壞死或其它新陳代謝疾病的臨時病理診斷。當然最後的正確病理組織診斷，則應倚靠永久性蠟包埋病理組織切片檢查。此研究的目的是在尋找細胞群的病變特性及其出現在印捺片細胞學、冷凍組織切片及蠟包埋組織切片三者之間的組互關係，次求證活體組織印捺片細胞學的臨床使用價值及對於病理組織診斷的可靠性。

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