

Essentials of

# **Pap Smear and Breast Cytology**

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2012

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**Pap Smear and Breast  
Cytology**

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# Preface

This monograph "Essentials of Pap Smear and Breast Cytology" is prepared at the request of a large number of students in cytology who wish to have a small and concise book with numerous illustrations for easy reference during their laboratory training. Most information and illustrations in this book are extracted from the authors' monograph entitled "Essentials of Gynecologic Cytology", and they are rearranged according to The Bethesda System-2001. This book should be used in conjunction with the above-mentioned book on gynecologic cytology.

For improving the future editions of this monograph, constructive comments from the reader will be highly appreciated.

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## Related material by the same author

Essentials of Needle Aspiration Cytology, Igaku-Shoin, New York, 1991  
Essentials of Exfoliative Cytology, Igaku-Shoin, New York, 1992  
Essentials of Cytology. An atlas. Igaku-Shoin, New York, 1993  
Critical issues in Cytopathology, Igaku-Shoin, New York, 1996  
Essentials of Lung Tumor Cytology, UBC pathology, Vancouver, 2008  
Essentials of Abdominal Fine Needle Aspiration Cytology, UBC pathology, 2008  
Essentials of Head and Neck Cytology, UBC pathology, 2009  
Essentials of Fluid Cytology, UBC pathology, 2010  
Essentials of Gynecologic Cytology, UBC pathology, 2011

## Key for abbreviations

CP smear: conventional Pap smear

DQ: Diff-Quik stain

FNA: fine-needle aspiration/aspirate

IHC: immunohistochemistry/immunohistochemical

LBP: liquid-based preparation

MGG: May-Grünwald-Giemsa stain

Pap: Papanicolaou stain

## Remarks

In this monograph:

- Histologic sections are stained with hematoxylin and eosin.
- Pap smears are stained with the standard Papanicolaou method.
- Fine needle aspirates are stained either with the Papanicolaou method or with another method, as indicated in the figure legends.
- Almost all cell samples from breast lesions are prepared by conventional technique. Breast FNAs prepared by LBP technique are so indicated in the figure legends.

## Chapter 1:

# Pap Smear: An overview

The Pap smear/test or cervicovaginal smear was initially introduced to medical practice by Papanicolaou and Traut in 1943. Since that time the test was gradually adopted worldwide and it proved to be the most successful screening test to detect premalignant and malignant lesions of the uterine cervix. It has been credited to the decreased incidence of invasive cervical cancers from 32.6 in 1940s to 8.3 per 100,000 women in 1983 and 1984 in the United States. However, cervical cancer is far from being eradicated as about 12,710 new cases are expected to be diagnosed in 2011. It is now established that Human papilloma virus is the most important agent in cervical carcinogenesis and HPV testing, therefore, plays an important role in the current cervical cancer screening.

## Cervical cancer screening

Prior to 2002, various national organizations generally recommended that screening should commence at the beginning of sexual activity. It should continue annually throughout life, and women with several prior negative Pap smears may have the test repeated at longer intervals. The revised cervical cancer screening guidelines of the American Cancer Society and those of the American College of Obstetricians and Gynecologists were published in 2002 and 2003 are summarized below:

- Both organizations recommended that screening should start 3 years after sexual debut or by the age of 21, whichever comes first.
- If a woman has had 3 consecutive negative tests in the preceding 10 years the screening may stop at age 70.
- For women aged 30 and older dual screening by Pap smear and HPV testing to triage equivocal cases is an option. If both tests are negative, screening should not be repeated for 3 years.
- Both organizations do not recommend cytology screening for hysterectomized women without a cervix, unless the surgery was performed for cervical premalignant or malignant lesions.

Most European countries also have established cervical cancer screening programs. Their recommended screening starts between the ages of 20 to 25 years and continues every 3 to 5 years until age 60 to 65. However, many developing countries do not currently have any cervical cancer screening programs. For those countries, it has been estimated that the lifetime risk of cervical cancer could be reduced by up to 30% if a screening program uses a combination of Pap smear and HPV testing in women aged 30 to 59 at least once per lifetime.

The prophylactic HPV vaccines for unexposed girls and women will likely to have an impact on the future of cervical cancer screening. Currently, Gardasil, a vaccine targeting HPV types 6, 11, 16 and 18; and Cervarix, a vaccine targeting HPV types 16 and 18, are the two FDA approved vaccines for females aged 9 to 26. The two HPV types 16 and 18 are responsible for about 70% of cervical cancers, but other HPV types that are not included in Gardasil and Cervarix are still responsible for the remaining 30% of cervical cancers. Therefore, women should continue to have cervical cancer screening by Pap smear regardless of their vaccination status.

## Sampling techniques

### Conventional Pap smear

Cervicovaginal cell samples should be collected prior to all digital pelvic examinations as lubricant may obscure cell morphology. If an excessive mucous secretion is present, it should be removed by a cotton ball moistened with normal saline solution. The most commonly used cell collecting devices for conventional Pap smears are the modified Ayre spatula and the cytobrush. Cell samples from the uterine cervix and the posterior vaginal fornix are usually collected by the pointed end and the blunt end of an Ayre spatula, respectively. They are either deposited onto 1 or 2 glass slides or mixed together on 1 glass slide. The use of a cytobrush in conjunction with a spatula can help ensure an adequate representation of the squamocolumnar junction or transformation zone (T zone) that is the origin of over 90% of cervical cancers and their precursor lesions. A major cause of a false negative test is the failure to sample the T-zone. During the reproductive years, the T-zone is generally located on the ectocervix, and it recedes within the endocervix in the post-menopausal years.

A cervicovaginal cell sample can be obtained with a spatula and/or a cytobrush:

A. If the T-zone is visible:

- Rotate the spatula 360 degrees once to obtain a single sample.
- Smear the sample onto a labeled slide and fix the sample immediately with a cytospray fixative to prevent air-drying.
- Hold the fixative 15-20 cm away from the slide and evenly spray the slide by depressing the plunger 2 or 3 times.

- B. If the T-zone is not visible:
- First use a spatula for the ectocervical specimen.
  - Then use a cytobrush, or the elongated end of the spatula, for the endocervical sample. Rotate the cytobrush 180 degrees only.
  - Place both specimens side-by-side lengthwise on a single slide and fix immediately.
- C. For detecting vaginal adenosis, a circumferential vaginal scraping of the upper vagina or a four-quadrant downward scraping of the vaginal mucosa should be performed. If a lesion is present, a direct scraping of the lesion should be made.

The slides are then air-dried and placed in a rigid container for mailing to a referral cytology laboratory. If the slide is broken during transportation, the cytologic material may be transferred to a new glass slide for examination by using a special technique.

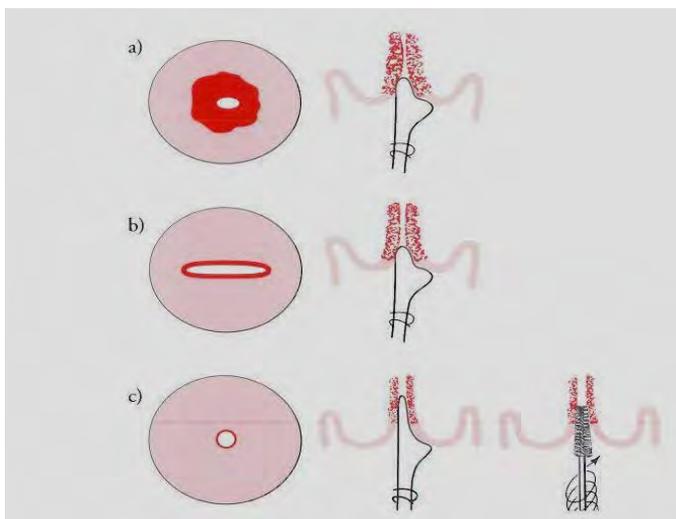


Fig.1.1. Location of the T-zone and optimal devices to take cervical cell samples:

- a. T-zone visible on ectocervix: sample with a spatula
- b. T-zone at or near external os: sample with a spatula
- c. T-zone not visible and small cervical os: sample with the elongated end of a spatula, or cytobrush.

Note: Figure 1.1 is reproduced from the BC Cancer Agency's Cervical Cancer Screening Program's Office Manual for Health Professionals with permission.

### Liquid-based preparation

Collecting devices for liquid-based preparations may be either a broom-like device or a plastic spatula in combination with a cytobrush. The collection device is rinsed into a

vial of a methanol-based fixative media that is processed semi-automatically or automatically to make a thin-layer cell film. The liquid-based preparation is then fixed in 95% ethanol or one of its substitutes and stained by the Papanicolaou method. Residual fixative media can be used for HPV-DNA testing in lieu of a new cell sample.

## The Papanicolaou stain

The Papanicolaou stain is the standard method for staining cervicovaginal cell samples worldwide. For optimal cytologic interpretation, an adequate well-prepared, well-stained and well-preserved cell sample is mandatory. For Papanicolaou staining, fixation of cell samples with alcohol is essential. The usual fixative is 95% ethanol or a spray cytofixative, however, substitutes such as 100% methanol, 80% isopropanol or denatured alcohol are also suitable. Papanicolaou staining consists of two main consecutive steps: nuclear staining with hematoxylin and cytoplasmic staining using Orange G and EA 36 or 50 polychrome.

### Trouble shooting in cytologic preparation

Many technical problems may be encountered in conventional Pap smear preparations, and those related to staining are summarized in Tables 1.1 and 1.2.

**Table 1.1 Nuclear staining problems\***

<b>Problem</b>	<b>Possible Reason(s)</b>
Nuclei too dark	<ol style="list-style-type: none"> <li>1. Over-staining with hematoxylin</li> <li>2. Excess stain not adequately removed by rinsing with tap water</li> <li>3. Inadequate rinsing in HCl solution</li> <li>4. HCl concentration too weak</li> <li>5. Ammonium chloride solution (or other bluing agent) too strong</li> </ol>
Nuclei too pale	<ol style="list-style-type: none"> <li>1. Diluted hematoxylin solution</li> <li>2. Inadequate time in hematoxylin</li> <li>3. Polyethylene glycol coating not adequately removed prior to staining with hematoxylin</li> <li>4. HCl not completely removed by tap water</li> <li>5. HCl solution too concentrated</li> <li>6. Slide dipped too long in HCl solution</li> <li>7. Ammonium hydroxide solution too weak</li> <li>8. Excessive time in chlorinated tap water</li> <li>9 pH of tap water after hematoxylin not alkaline enough</li> </ol>

**Table 1.2 Cytoplasmic staining problems\***

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<b>Problem</b>	<b>Possible Reason(s)</b>
Inconsistent cytoplasmic staining	<ol style="list-style-type: none"><li>1. Air drying prior to fixation</li><li>2. Polyethylene coating not adequately removed prior to staining</li><li>3. Slides left too long in ethanol rinses or clearing solution following OG/EA staining</li><li>4. Time in hematoxylin too long</li><li>5. Excess hematoxylin not removed prior to OG/EA staining</li><li>6. Inadequate rinsing between solutions</li><li>7. Inadequate rinsing following staining with dyes</li><li>8. Inadequate draining of slides between rinses</li><li>9. Inappropriate pH of tap water or EA solution</li><li>10. Change of cell pH by bacterial infection</li><li>11. Variable thickness of smear</li></ol>
Cytoplasm too green	Green dye too strong in EA solution
Lack of contrasting cytoplasmic stain	Exhausted hematoxylin and EA dye
Hazy grey appearance of cells	<ol style="list-style-type: none"><li>1. Dehydrating &amp; clearing solutions contaminated with water</li><li>2. Incomplete removal of polyethylene glycol coating prior staining</li></ol>
Opaque white color	Inadequate rinse after Scott tap water substitute on the back of slide
Pink, orange or yellow slides	Oven temperature too high
"Cornflake" artifact	Air bubbles entrapped on cell surfaces

\*\* These 2 Tables are adapted with modifications from: Holmquist MD, Keebler CM. Cytopreparative techniques. In: Manual of Cytotechnology, Keebler CM, Somrak TM, eds. 7<sup>th</sup> edition, 1993. Chicago, ASCP Press, p. 410-448.

## Cytologic evaluation

The entire slides should be meticulous screened with x10 and x40 objectives. This can be accomplished by means of a mechanical stage to move through the slide in a linear manner, or by manually moving the slide. With a x10 objective, the following information can be detected:

- the background smear pattern, including any microorganisms, blood or inflammatory exudate
- the presence or absence of endocervical cells
- the appropriateness of hormonal response

After this stage a search for abnormal cells should begin. Diagnostic criteria for exfoliative cytology are based mainly on examination of individual cells. There are major and minor criteria. Major criteria consist of:

- alteration in nuclear/cytoplasmic (N/C) ratio
- chromatin changes
- anisocytosis and anisonucleosis
- nuclear contour irregularity
- macronucleoli
- malignant diathesis

Minor criteria include cytoplasmic keratinization, "cannibalization", presence of cytoplasmic vacuoles or secretion and architectural disarray of minute tissue or epithelial fragments. For squamous cells, the cell shape, chromatin pattern and alteration in N/C ratio are of paramount importance. However, for glandular cells, the most important features include nuclear contour irregularity, anisonucleosis, macronucleoli, nuclear crowding and overlapping in cell clusters or minute epithelial fragments.

## Reporting cervicovaginal cytology

Since the introduction of cervical cancer screening programs in the 1940s, different classification systems have been used to report Pap smears. Prior to The Bethesda System (TBS), which was developed to standardize nomenclature and establish more consistent reports, the Papanicolaou numerical classification, the Reagan classification of "mild, moderate, severe dysplasia" and "carcinoma in situ", and Richart's concept of "cervical intraepithelial neoplasia" (CIN) have been used extensively to report cervicovaginal cytology worldwide. The TBS was introduced in 1988 and it has been revised twice in April 1991 and in April/May 2001. Shortly after its publication in 2001, most cytology laboratories in the United States had adopted TBS 2001 for reporting Pap smears (85.5% by 2003), and TBS is now used extensively worldwide to report cervicovaginal cytology.

## The Bethesda System - 2001

The Bethesda System-2001 consists of several components, as outlined below:

### SPECIMEN TYPE

Indicate conventional (Pap smear) vs. liquid-based preparation versus other

### SPECIMEN ADEQUACY

- Satisfactory for evaluation (describe presence or absence of endocervical or transformation zone component and other quality indicators, e.g., partially obscuring blood, inflammation, etc.)
- Unsatisfactory for evaluation (specify reason)
- Specimen rejected/not processed (specify reason)
- Specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormality because of (specify reason)

### GENERAL CATEGORIZATION (Optional)

- Negative for Intraepithelial Lesion or Malignancy
- Epithelial Cell Abnormality: See Interpretation/Result
- Other: see Interpretation/Result (e.g. endometrial cells in a woman  $\geq 40$  yrs)

### INTERPRETATION/RESULT

#### A. Negative for Intraepithelial Lesion or Malignancy

When there is no cellular evidence of neoplasia, state this in the General Categorization above and/or in the Interpretation/Result section of the report –whether or not there are organisms or other non-neoplastic findings

#### 1. Organisms:

- Trichomonas vaginalis.
- Fungal organisms morphologically consistent with *Candida* spp
- Shift in flora suggestive of bacterial vaginosis
- Bacteria morphologically consistent with *Actinomyces* spp
- Cellular changes consistent with herpes simplex virus

#### 2. Other Non-neoplastic Findings (Optional to report; list not inclusive):

- Reactive cellular changes associated with
  - inflammation (includes typical repair)
  - radiation
  - intrauterine device (IUD)
- Glandular cells status posthysterectomy
- Atrophy

### 3. Other

- Endometrial cells (in a woman  $\geq 40$  years of age) (specify if “negative for squamous intraepithelial lesion”)

## B. Epithelial Cell Abnormalities

### 1. Squamous cell:

- Atypical squamous cells
  - of undetermined significance (ASC-US)
  - cannot exclude HSIL (ASC-H)
- Low-grade squamous intraepithelial lesion (LSIL) (encompassing: HPV/mild dysplasia/CIN1)
- High-grade squamous intraepithelial lesion (HSIL)(encompassing: moderate and severe dysplasia, CIS, CIN 2 and CIN 3) with features suspicious for invasion (if invasion is suspected)
- Squamous cell carcinoma

### 3. Glandular Cell:

- Atypical
  - endocervical cells (NOS or specify in comments)
  - endometrial cells (NOS or specify in comments)
  - glandular cells (NOS or specify in comments)
- Atypical
  - endocervical cells, favor neoplastic
  - glandular cells, favor neoplastic
- Endocervical adenocarcinoma in situ
- Adenocarcinoma
  - endocervical
  - endometrial
  - extrauterine
  - not otherwise specified (NOS)

## C. Other Malignant Neoplasm: (specify)

## ANCILLARY TESTING

Provide a brief description of the test method(s) and report the result so that it is easily understood by the clinician.

## AUTOMATE REVIEW

If specimen was examined by automated device, specify the device and the result.

## EDUCATIONAL NOTES AND SUGGESTIONS (optional)

Suggestions should be concise and consistent with clinical follow-up guidelines published by professional organizations (references to relevant publications may be included).

## Specimen adequacy

Evaluation of specimen adequacy is important. There are 2 categories of specimen adequacy in The Bethesda System 2001:

- Satisfactory for evaluation
- Unsatisfactory for evaluation (lack of patient identification or unacceptable specimen due to slide broken beyond repair, for example)

Depending on the specimen type, the estimated minimum numbers of well-preserved squamous cells required for a specimen to be regarded as adequate or satisfactory for cytologic evaluation are different:

- 8,000 to 12,000 for a CP smear, and
- 5,000 cells for a LBP

The numbers of squamous cells constitute an additional criterion besides the presence of at least 10 well-preserved endocervical or metaplastic squamous cells. Any specimen with abnormal cells is, by definition, satisfactory for evaluation.

For obscuring factors, if a specimen has more than 75% of squamous cell nuclei obscured by white blood cells, blood, drying artifact, other, it should be termed unsatisfactory, assuming no abnormal cells are identified. (Fig.1.2). When 50% to 75% of the epithelial cells are obscured, a statement describing the specimen as partially obscured should follow the satisfactory term. Abundant cytolysis does not qualify the specimen as "unsatisfactory" unless nearly all nuclei are devoid of cytoplasm. (Fig.1.3).

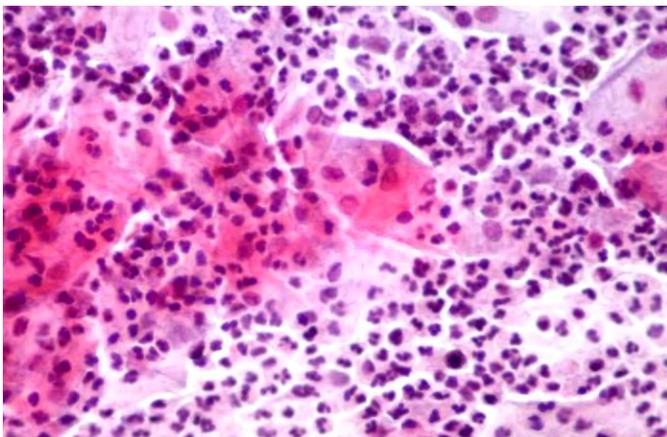


Fig.1.2. "Unsatisfactory" CP smear showing inflammatory exudate obscuring over 75% of the squamous cell nuclei.

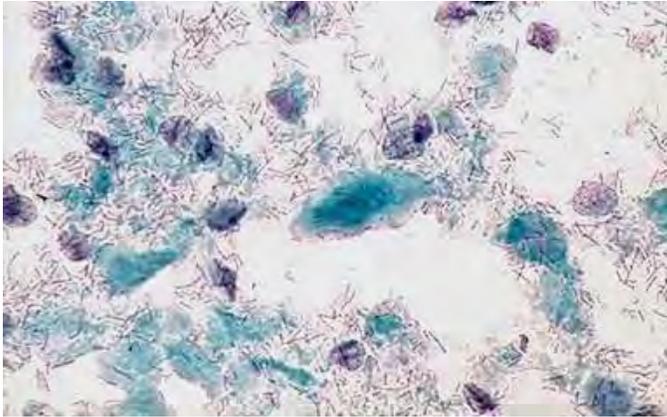


Fig.1.3. CP smear showing extensive cytolysis by Döderlein bacilli but still displaying fairly well-preserved nuclei, some of which are surrounded by a small amount of cytoplasm.

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## Chapter 2

# Pap smear: Normal uterus and vagina

## Histology of the uterus and vagina

**The uterine cervix** consists of an ectocervix and an endocervical canal. The ectocervix is covered by a nonkeratinizing, stratified squamous epithelium and the endocervix is lined by a single layer of columnar epithelium with complex folding. With advancing age, the distal part of the cervical canal is replaced by metaplastic squamous cells. The squamocolumnar junction is on the ectocervix and the transformation zone (T-zone), also known as ectropion, is located between the original squamocolumnar junction and the inner border of metaplastic squamous epithelium. (Fig.2.1). For cervical cancer screening the T-zone should be sampled, as it is the site of origin of over 90% of cervical cancers and its precursor lesions.

**The uterine corpus** consists of a thick smooth muscle wall and a triangular cavity lined by an endometrium that is comprised of a columnar glandular epithelium supported by endometrial stroma. The endometrium consists of 2 layers: the basalis and the functionalis. The functionalis layer above the lower uterine segment responds to ovarian hormones and displays cyclic changes. The length of the menstrual cycle varies considerably among female individuals, but it is 28 days long in most women. The proliferative phase is variable in length but the secretory phase is almost always 14 days long.

**The vagina** is covered by a layer of nonkeratinizing squamous epithelium that is similar to that of the ectocervix and it yields abundant squamous cells similar to those of the ectocervix.

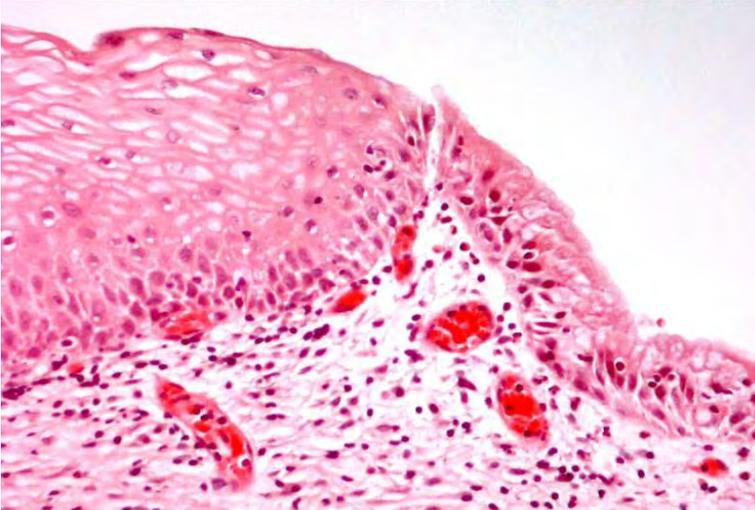


Fig.2.1. Histology of normal uterine cervix: Squamocolumnar junction showing an abrupt change between the nonkeratinizing squamous epithelium of the exocervix and the endocervical glandular epithelium.

## Cervicovaginal cytology

A representative cell sample from the T-zone commonly contains abundant squamous cells, some metaplastic squamous cells and many endocervical canal glandular cells. The cellular compositions of a normal Pap smear vary with the age of the patient.

### Reproductive age

A cervical smear from a **non-pregnant woman of reproductive age** is cellular and shows numerous epithelial cells. Normal squamous cells exfoliate predominantly singly and endocervical glandular cells usually exfoliate singly or in sheets of different sizes. Vaginal smears show only squamous cells that are similar to those of the ectocervix. Squamous cells of the cervix and vagina are classified as superficial, intermediate and parabasal, according to their characteristic features described below. A maturation index is expressed as a ratio of different types of squamous cells (parabasal: intermediate: superficial).

During the ***proliferative phase*** of the menstrual cycle, the number of superficial cells increases gradually under the influence of increased levels of serum estrogen. Prior to ovulation the serum estrogen reaches its peak and superficial cells predominate in the smear with the maturation index shifting to the right (for example, 0:20:80). After ***ovulation*** and under the influence of an increasing level of serum progesterone, the intermediate squamous cells predominate in the smear, shifting the maturation index to the middle (for example, 0:80:20). Normal endometrial cells may also be seen in Pap smears, depending on the phase of the menstrual cycle. Normal endometrial cells are described below.

Main cytologic features of normal cervical cells are summarized below:

- ***Superficial squamous cells*** are polygonal in shape with translucent, eosinophilic thin cytoplasm that may contain brownish keratohyalin granules. Their nuclei are pyknotic, centrally located and measure 16 to 20  $\mu\text{m}^2$  in area. These cells are seen singly and in loose clusters. (Fig. 2.2).

- ***Intermediate squamous cells*** are oval or polygonal in shape with translucent, eosinophilic or basophilic cytoplasm that commonly shows folding. Their nuclei are vesicular, with fine chromatin and measure about 35  $\mu\text{m}^2$  in area. Occasionally, superficial and intermediate squamous cells have a spindle-shape or display a long cytoplasmic extension or "tail" that is a rare collection of different types of intracytoplasmic filaments called Herxheimer's spirals. (Fig. 2.2).

- ***Parabasal squamous cells*** are rarely encountered in a smear from a premenopausal woman unless she is in the post partum period. They are commonly seen in post menopausal atrophy. They are seen singly and are oval in shape with opaque, basophilic cytoplasm. They have centrally located vesicular nuclei with fine chromatin which measure about 50  $\mu\text{m}^2$  in area. (Fig. 2.2).

- ***Endocervical glandular cells*** are columnar in shape with pale, abundant, mucinous cytoplasm and basally located vesicular nuclei displaying a granular chromatin and micronucleoli. A few are ciliated. In conventional Pap smears, endocervical cells are present singly or in monolayered sheets with characteristic honeycomb and picket-fence arrangements. Naked nuclei within mucus are a common finding, but they are not regarded as evidence of Pap smear adequacy. Endocervical glandular cells commonly present singly in liquid-based preparations. (Fig. 2.3).

- ***Metaplastic squamous cells*** arise from the reserve cells of endocervical columnar epithelium. They exfoliate singly or in pavement-like sheets and are polygonal or oval in shape. Their cytoplasm varies with the level of cell maturation and in immature cells it may be thin and vacuolated and with cytoplasmic extensions. It is waxy, basophilic or eosinophilic in mature cells. Their vesicular nuclei have granular chromatin and have an area of about 50  $\mu\text{m}^2$ . (Fig. 2.4).

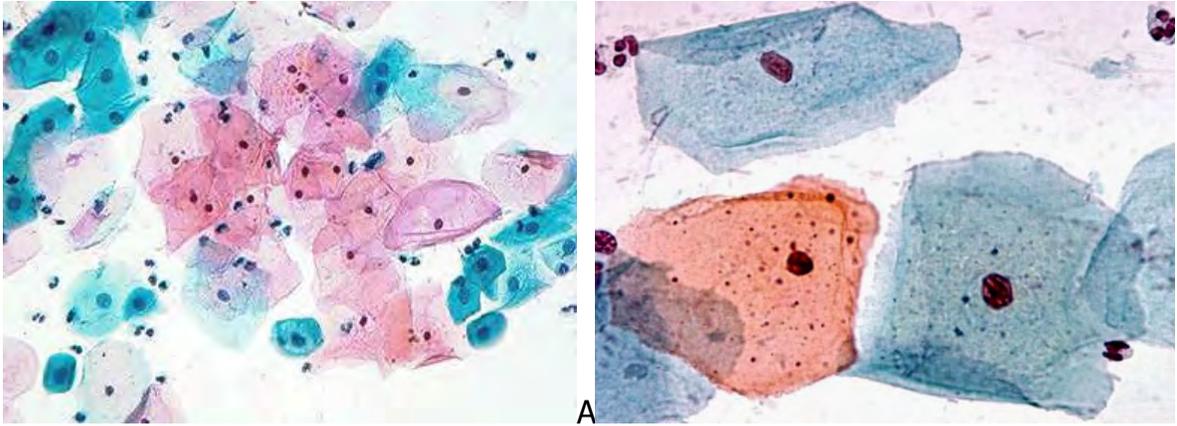


Fig.2.2. Normal cervical and vaginal squamous cells in a CP smear, (A): Superficial, intermediate and metaplastic cells seen singly and in aggregates. A few smaller, oval parabasal cells are also present. (B): One superficial cell with pyknotic nucleus and intracytoplasmic keratohyaline granules and 2 intermediate cells with vesicular nuclei.

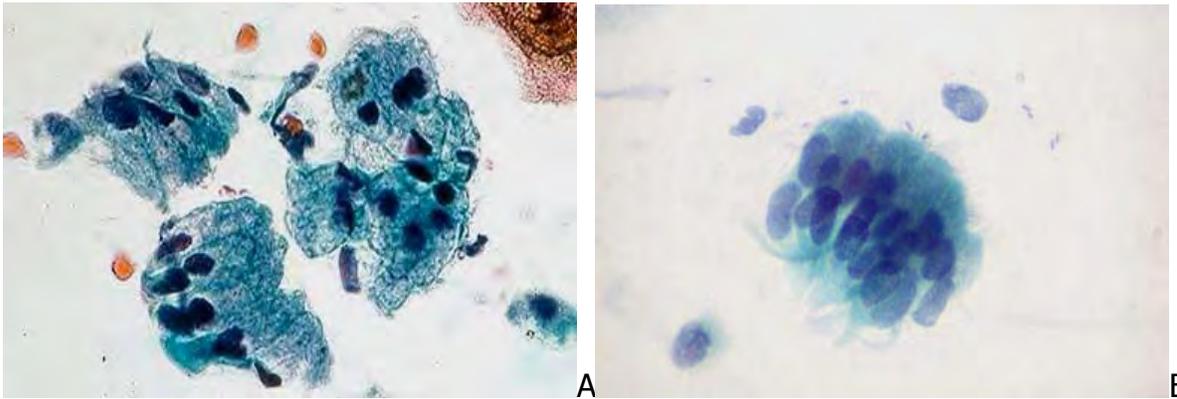


Fig.2.3. Normal endocervical cells in a CP smear: Mucus secreting columnar cells in small epithelial fragments (A) and ciliated glandular cells (B).

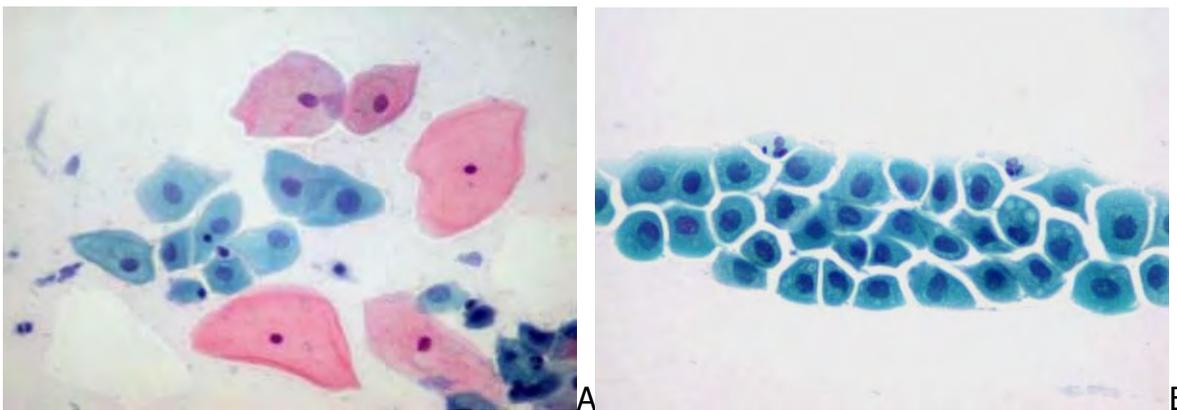


Fig.2.4. Metaplastic squamous cells in a CP smear, (A): A few smaller metaplastic cells admixed with superficial and intermediate cells. (B): Mature and immature metaplastic cells.

Exfoliated *endometrial cells* are more abundant during the first 10 to 12 days of the cycle, and they may be detectable in cervicovaginal smears. The normal endometrial cells are small, cuboidal cells with scant cytoplasm, round nuclei, chromatin clumping and small nucleoli, and they are commonly seen in cohesive clusters of different size. Superficial stromal cells resemble histiocytes and are present singly or in loosely cohesive sheets. Deep stromal cells appear as small loose clusters of spindle cells. Histiocytes and large masses of endometrial cells or wreaths (exodus) are more commonly observed on the above-mentioned days of the cycle. Histiocytes may occur in clusters or small groups and may be mistaken for endometrial cells. Histiocytes typically show bean-shaped nuclei with minimal indentation. (Figs.2.5 and 2.6).

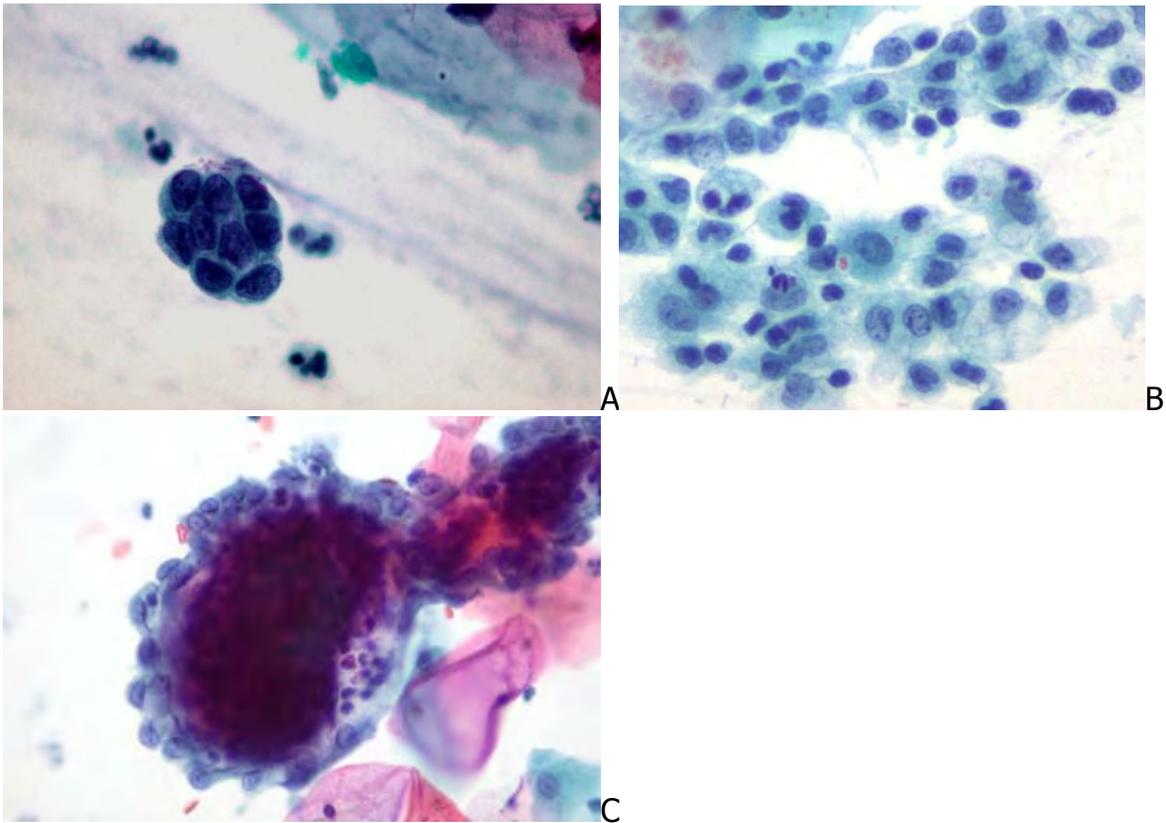


Fig.2.5. Normal, spontaneously exfoliated endometrial cells in CP smears, (A): A cluster of round endometrial epithelial cells showing scant cytoplasm. (B): Superficial stromal cells, resembling histiocytes, present singly and in loose clusters. (C): An endometrial wreath consisting of a large cluster of stromal cells (at the center) surrounded by a layer of endometrial epithelial cells.

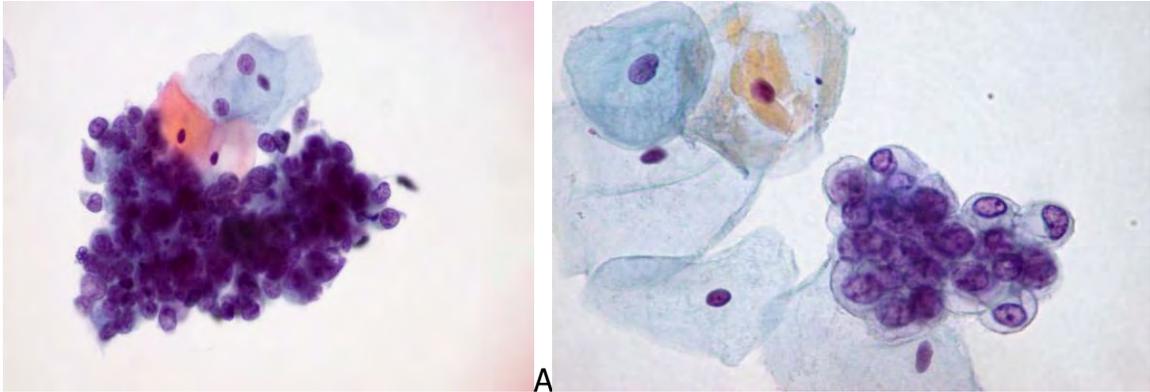


Fig.2.6. Benign endometrial cells in LBP, (A): A tridimensional cluster of endometrial glandular cells with scant cytoplasm. (B): Clustered histiocytes mimicking endometrial cells. Note the bean-shaped nuclei and more defined and more abundant cytoplasm.

An Ayre-type spatula with a longer tip or a cytobrush may inadvertently sample fragments of **endometrium from the lower uterine segment (LUS)**. These LUS endometrial tissue fragments are more commonly seen in women with prior cervical cone biopsy and appear as large, thick cell sheets with folding. The epithelial cells at the periphery display nuclei in picket-fence arrangement. (Fig.2.7). Smaller endometrial cell clusters and aggregates of stromal cells are commonly present.

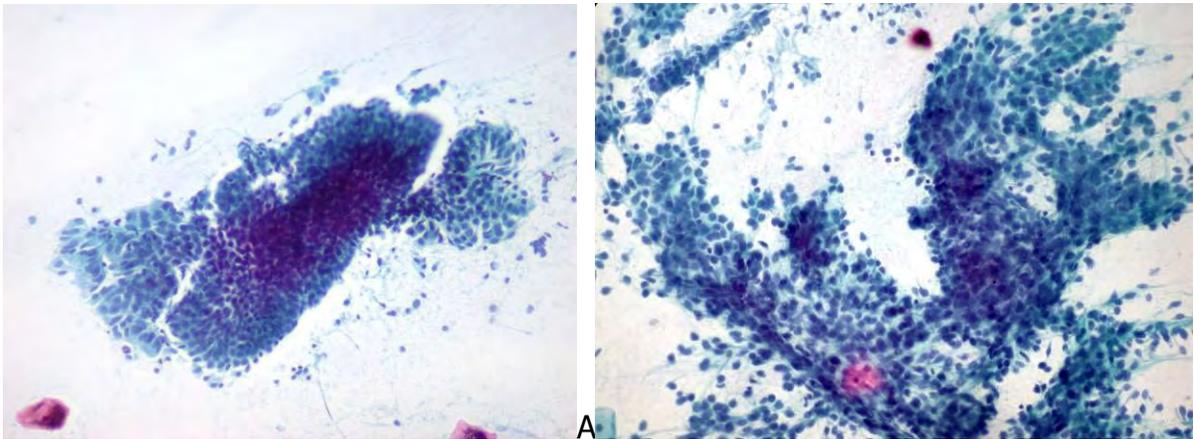


Fig.2.7. (A, B): CP smear showing thick fragments and cell clusters of lower uterine segment endometrium scrapped by a cervical cell sampler with a longer tip.

During **pregnancy** the placenta secretes large amounts of estrogen and progesterone, and as a result, intermediate squamous cells predominate in the smear, accounting for at least 80% of the total cell population. Intermediate cells are rich in glycogen and display an elongated, boat-shaped configuration (navicular cells). Usually the smear consists entirely of intermediate squamous cells by the 4<sup>th</sup> or 5<sup>th</sup> month of pregnancy. (Figs.2.8 and 2.9).

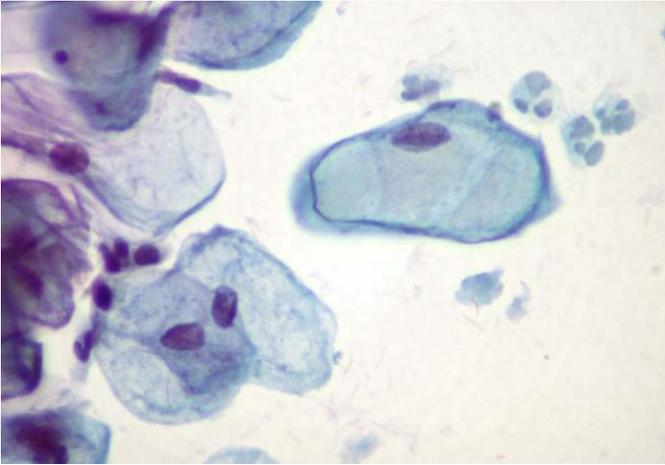


Fig.2.8. CP smear showing a few intermediate squamous cells and a navicular cell containing a large amount of intracytoplasmic glycogen that is yellowish in color.

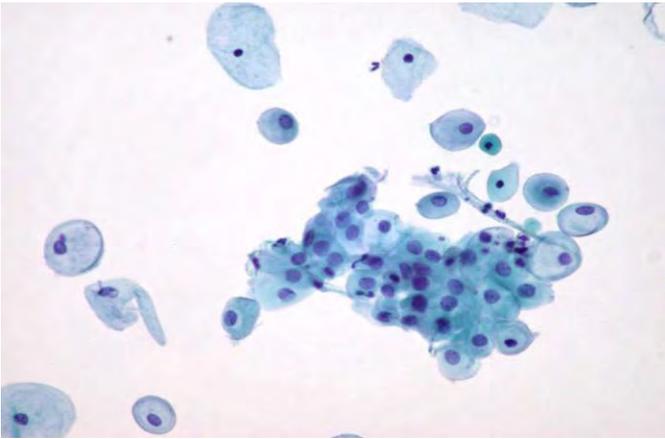


Fig.2.9. LBP from a woman in postpartum period showing parabasal cells singly and in a sheet.

During pregnancy the Pap smear may rarely show a few decidual cells. **Decidual cells** are of the same size as parabasal cells but occur in clusters and have thick, granular cytoplasm and larger oval or round nuclei without prominent nucleoli. (Fig.2.10). It is important to note that decidual nodules may occur in the cervix during pregnancy and in women taking progesterone-rich oral contraceptives. An Arias-Stella reaction affects endometrial cells in early pregnancy but it may also occur in the endocervical canal. **Arias-Stella cells** are large cells with vacuolated cytoplasm, hyperchromatic multiple nuclei and prominent nucleoli. They may mimic cells derived from a clear cell adenocarcinoma. **Cytotrophoblasts** and multinucleated **syncytiotrophoblasts** are rarely observed, except in patients with threatened abortion.

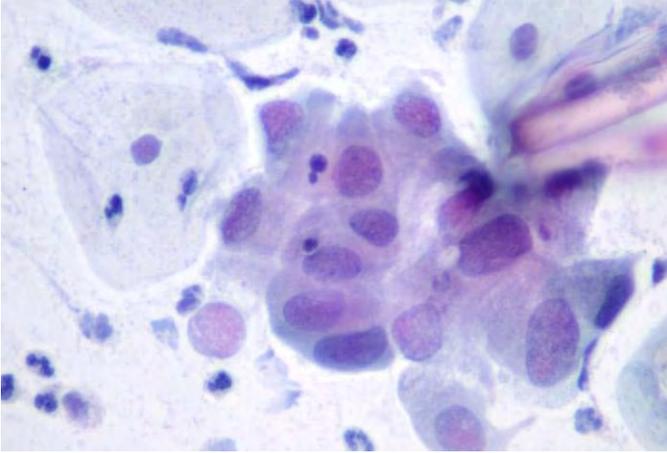


Fig.2.10. A group of decidual cells in a CP smear.

**Cockleburrs** are hematoidin crystal arrays that are often surrounded by histiocytes. They measure up to 100  $\mu\text{m}$  in greatest dimension. They are more commonly found in pregnant women and rarely in nonpregnant women. (Fig.2.11).

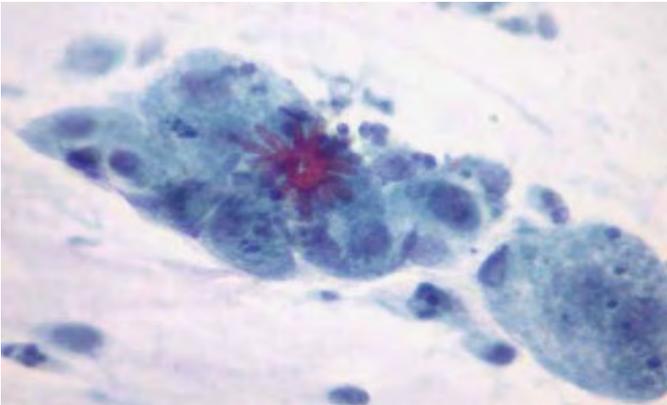


Fig.2.11. Cockleburrs surrounded by histiocytes in a CP smear.

In the **postpartum period** the Pap smear is predominated by parabasal cells. This is due to placental parturition and suppressed ovarian functions.

## Menopause

In early **menopause** the Pap smear is predominated by superficial squamous cells. This is caused by the development of nonovulated graafian follicles. As menopause progresses the smear is predominated by either intermediate cells or parabasal cells. (Fig.2.12).

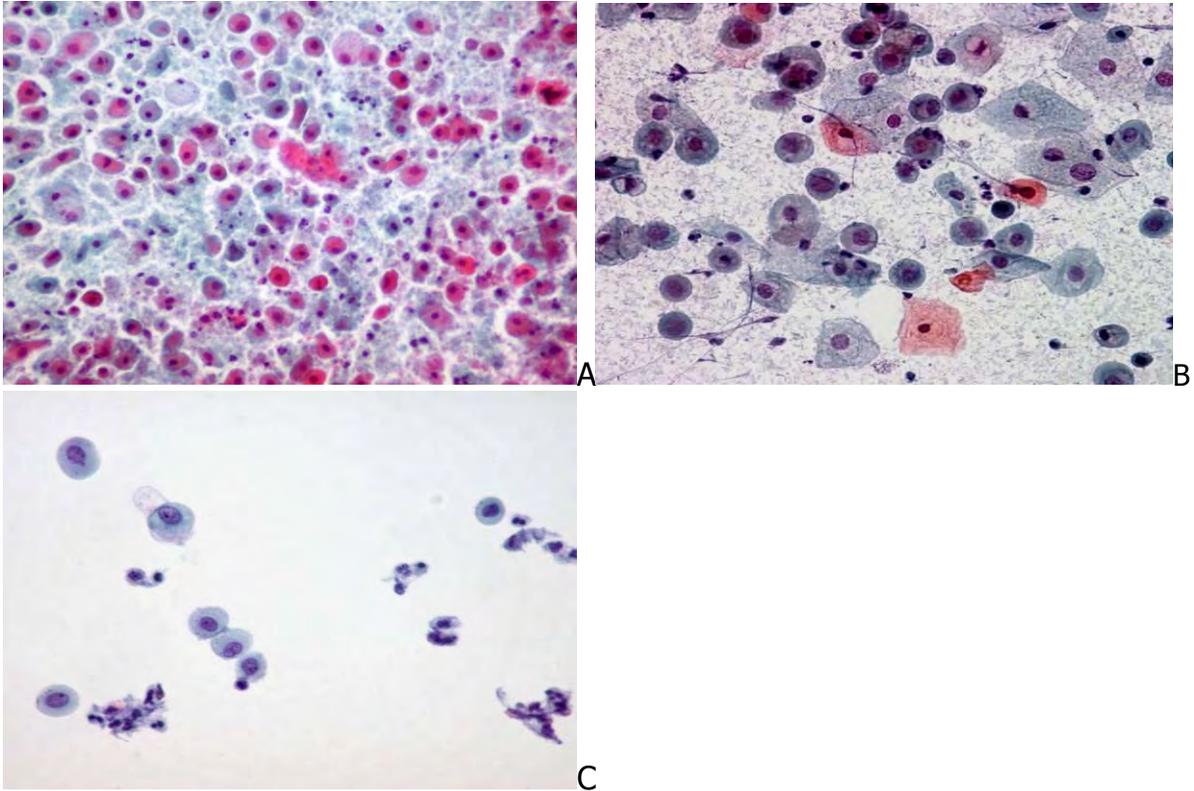


Fig.2.12. Atrophic cervix, (A, B): A CP smear showing a parabasal cell predominant pattern. Some cells display eosinophilic cytoplasm. Abundant necrotic debris is present in the smear background. (C): LBP showing parabasal cells in a clean background.

Parabasal cells from *atrophic vaginitis* may exhibit nuclear enlargement, mimicking dyskaryotic squamous cells. A repeat Pap smear taken immediately after a course of topical treatment with estrogen cream (to induce cell maturation) will be helpful to solve this diagnostic dilemma, as any dyskaryotic squamous cells will remain unchanged, and normal parabasal cells will have matured. (Fig.2.13)

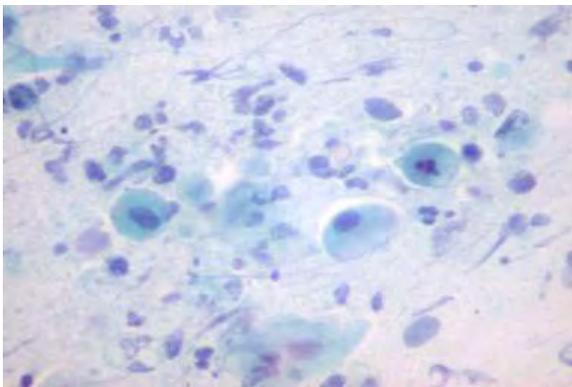


Fig.2.13. Atrophic vaginitis showing, in a CP smear, degenerated polymorphonuclear leukocytes and rare parabasal cells with slightly enlarged nuclei.

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## Chapter 3

# Pap smear: Negative for intraepithelial lesion or malignancy: infections and nonneoplastic findings

## Bacterial infections

**Gonorrhea** is caused by *Neisseria gonorrhoeae*, a Gram-negative diplococcus. It can be asymptomatic or manifested by purulent vaginal discharges associated with a burning sensation. The bacteria may be seen within the cytoplasm of neutrophilic polymorphonuclear leukocytes in Papanicolaou-stained smears, but the infection is confirmed by bacterial culture.

**Bacterial vaginosis** is also called a “shift in flora”. It is a common, nonspecific cervicovaginitis and is often asymptomatic. On Pap smears characteristic “clue cells” are present in a filmy background of small coccobacilli. These are superficial and intermediate squamous cells covered by a layer of bacteria (coccobacilli) that obscures the cell membrane (Fig.3.1.). These cells should be differentiated from “false-clue cells” that are also squamous cells covered with bacillary organisms.

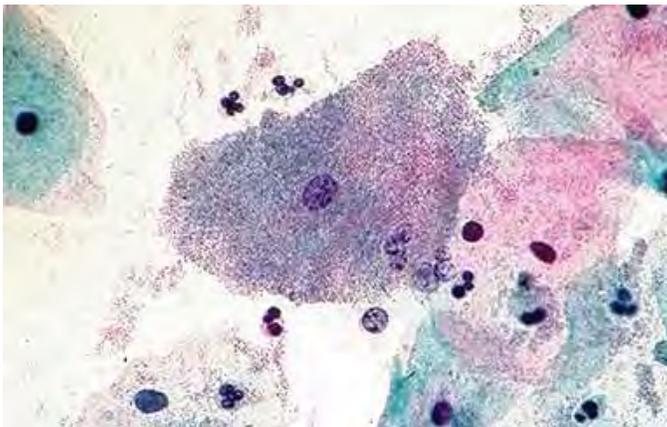


Fig.3.1. A “clue cell” covered with numerous coccobacilli in a CP smear.

**Actinomycosis.** Actinomyces normally resides in the female genital tract, so its presence is not an indicator of disease. Actinomycosis is characterized by a foul-smelling vaginal discharge containing sulfur granules. It is commonly caused by *Actinomyces israelii* in patients with IUDs or pessaries for contraception. These microorganisms are gram-positive and present as irregular, thick bundles or clusters of filaments (Gupta bodies). (Fig.3.2).

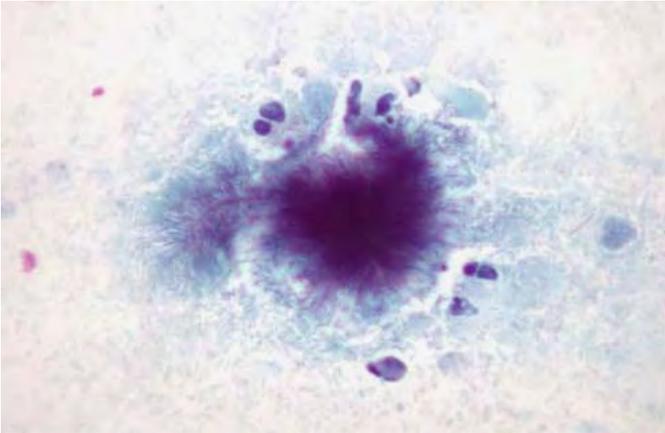


Fig.3.2. Actinomyces in a CP smear showing a thick cluster of filamentous elements.

**Granuloma inguinale** is an uncommon disease. Material scrapped from the ulcerated lesion reveal inflammatory exudates with vacuolated macrophages containing Donovan bodies (safety pin-shaped, gram-negative microorganisms). They are best demonstrated by Giemsa stain.

**Chlamydia trachomatis** is the 2<sup>nd</sup> most common STD in the Western world after HPV infection. The infection is usually asymptomatic in females and affects the cervix, uterus and its annexae, but not the vulva or vagina. The microorganism is an obligate intracellular parasite with 2 forms: the metabolically inactive form called the elementary body, and the metabolically active form called the reticulate body. The parasite mainly involves the endocervical columnar epithelium but may spread to the endometrium and fallopian tubes. On Pap smears the infection may be suspected by the presence of intracytoplasmic vacuoles containing aggregates of small coccoid bodies within columnar or metaplastic squamous cells. (Fig.3.3). The diagnosis is now made by molecular testing. Metaplastic squamous cells with mucous globules may be mistaken for Chlamydial infected cells.

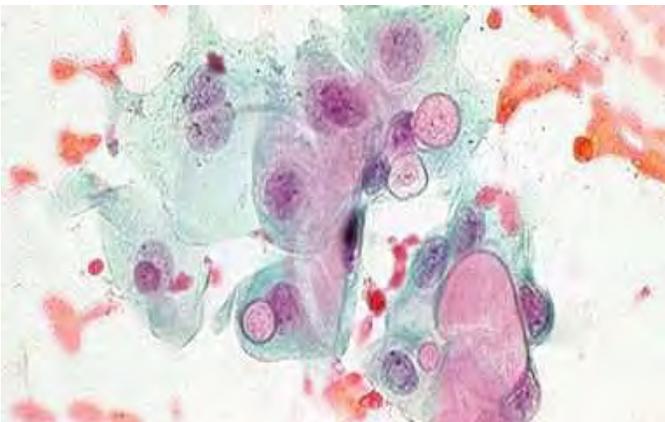


Fig.3.3. Chlamydial infection showing in a CP smear metaplastic squamous cells with vacuoles containing small coccoid bodies.

**Follicular cervicitis** is seen in about 50% of patients with Chlamydial infection, but the converse is not true. Numerous lymphoid cells at different stages of maturation and macrophages with tingible bodies are seen. (Fig.3.4).

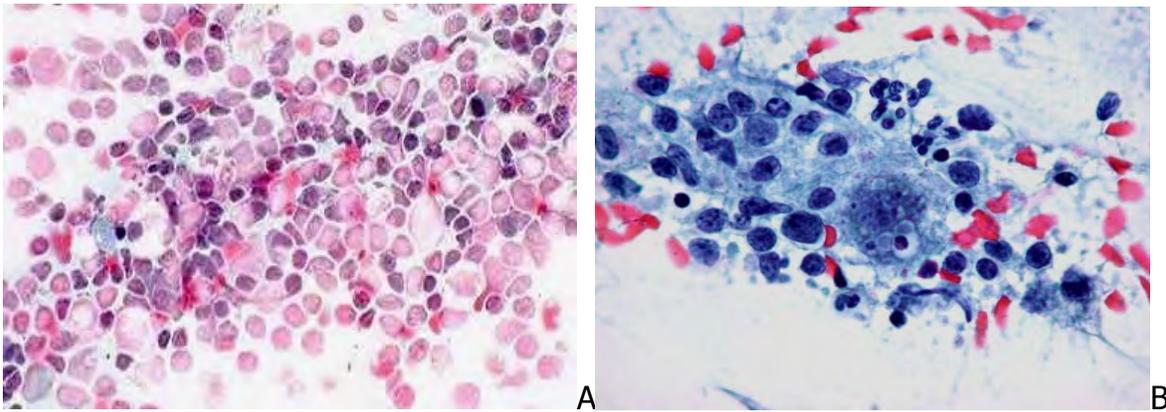


Fig.3.4. Benign lymphoid cells at different stages of maturation (A) and a large histiocyte with tingible bodies (B) in a CP smear from a follicular cervicitis.

## Viral infections

### HPV infection

Genital HPV infection is common, almost exclusively sexually transmitted and self-limiting in young women. HPVs are a member of papovirus family and are divided into low- and high-risk types. Low-risk HPVs are virtually never associated with cervical cancer and the most common types include 6,11,42 and 44. High-risk HPV types are found to be associated with cervical cancer and the most important types are 16,18,31,33,35,39,44,52,56,58,59 and 68. As a high percentage of women harbor these viruses but only a small number of them develop cervical intraepithelial lesions (CIN) and cancer, there is a suggestion of additional roles of risk factors. Early age of first intercourse is among the most significant of risk factors, and other important factors include: multiple sexual partners, a male partner with multiple previous sexual partners and persistent infection by high-risk HPVs.

The precancerous changes referred to as CIN may begin as CIN 1 (flat condyloma) and progresses to higher grades CIN 2 and 3, or they may begin at the outset as CIN 2 or 3 depending on the type of HPV infection (low- or high-risk virus) and other risk factors. According to some studies, CIN 1 regresses in 50% to 60%, persists in 30% and progresses to CIN 3 in 20% of cases. With progression, only 1% to 5% will eventually develop into an invasive cancer. With CIN 3, the rate of regression is about 33% and that of progression to invasive cancer is about 60% to 74%. The peak

incidence of CIN is about 30 years and that of invasive carcinoma is about 45 years. About 85% to 90% of CIN 1 lesions are caused by high-risk HPVs but they can also be caused by low-risk viral types. CIN 2 and CIN 3 are almost always caused by high-risk HPVs. CIN lesions usually have cytologic abnormalities that often reflect their severities. It is important to note that in women with normal cervical cytology, 10% to 15% harbor high-risk HPVs. HPV types 6 and 11 are the commonest low-risk types that are most commonly associated with condylomas or genital warts. HPV types 16 and 18 are responsible for 70% of all cervical cancers; and together with the other high-risk types (45,31,33,35,52 and 58) are responsible for 87% of all cervical cancers.

The usefulness of HPV testing as a screening test for cervical cancer is limited. Most sexually active women will contract a cervical HPV infection at some point in their lifetime, and cervical cytology will remain as the main test for this purpose. In future years, with the introduction of HPV vaccines [Gardasil (targeting HPV types 6,11,16 and 18) and Cervarix (targeting HPV types 16 and 18)] administered to females aged 9 to 26, the number of high-grade CIN and carcinoma of the cervix, vulva and vagina is expected to decrease by 70%. Genital warts (caused by HPV types 6 and 11) are also expected to decrease by 90% subsequent to Gardasil vaccination.

There are a few commercially available HPV-DNA tests and the most commonly used one is the Hybrid Capture 2 (hc2), "Digene<sup>®</sup> test" (Qiagen, Gaithersburg, Maryland). It is the only test approved by the US Food and Drug Administration for detecting HPV for patient care. The test is an in vitro nucleic acid hybridization assay that can differentiate between 2 HPV DNA groups: low-risk HPV types 6,11,42,43,44 and high-risk HPV types 16,18,31, 33,35,39,45, 51,52,56,58, 59 and 68. However, it cannot determine the specific HPV type present in the specimen. It can be performed on the residual cell samples collected for the ThinPrep<sup>®</sup> Pap Test or with the Standard Transport Medium<sup>™</sup> (Qiagen). It has a high sensitivity and low specificity, as not all patients with positive results have CIN or invasive cancer. For high-volume sample-throughput testing, the hc2 HPV DNA test can be performed using the Rapid Capture<sup>®</sup> system Instrument Application, but only the oncogenic high-risk HPV Probe was approved for high-volume testing. **As HPV infection is self-limiting in young women, HPV testing is not useful in women under 30 years of age.**

**Goal of HPV DNA testing:** The test is not intended for use as a screening tool in the general population. It is designed to augment existing methods for the detection of cervical disease and should be used in conjunction with clinical information. The test results should not be used as the sole basis for clinical assessment and treatment of patients. **Its main utility is to screen patients with ASC-US Pap smear results to determine the need for referral to colposcopy.** However, results of the test are not intended to prevent women from proceeding to colposcopy.

**Interpretation:** HPV DNA test results should be interpreted in conjunction with clinical findings and data derived from other diagnostic procedures:

- If the high-risk HPV probe is negative: there is a high probability that a high-grade CIN lesion will be not found at colposcopy.
- If the high-risk HPV probe is positive: there is a low but increased probability that a high-grade CIN lesion or a more severe lesion will be detected at colposcopy.

### Herpes simplex virus

HSV commonly infects cervix and vagina. Genital herpes virus infection is caused by HSV-1 and HSV-2. The infection causes inflammatory epithelial ulcers. Multinucleated giant squamous cells with nuclear molding and intranuclear inclusions or chromatinic liquefaction with "ground-glass" appearance are seen at the ulcer borders and are characteristic for the infection. When the cytomorphologic changes are equivocal, an immunocytochemical staining of the infected cells with a commercial Herpes simplex antibody will be helpful for confirmation. (Figs.3.5 and 3.6). This antibody is specific for HSV-2 but also cross-reacts with HSV-1. This immunocytochemical confirmatory test has 91% sensitivity and 95% specificity.

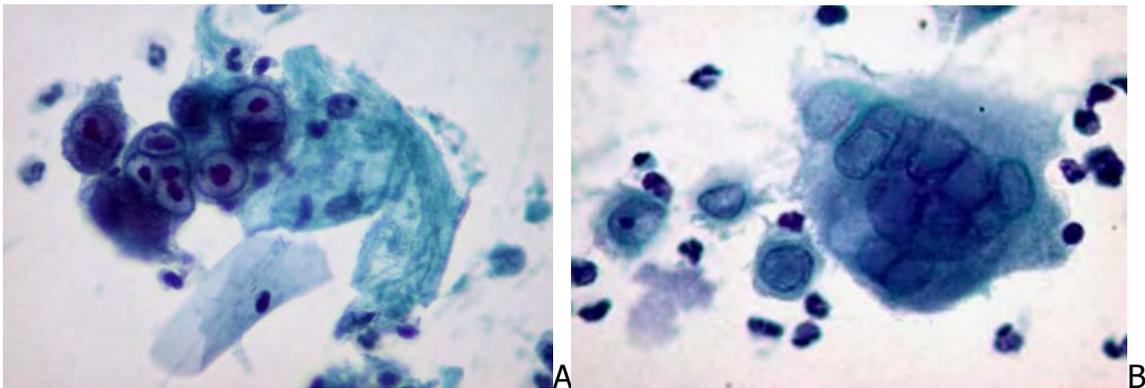


Fig. 3.5. Herpes simplex infection in a CP smear, (A): Infected epithelial cells with intranuclear inclusions. (B): Infected cells displaying multiple nuclei with "ground glass" change.

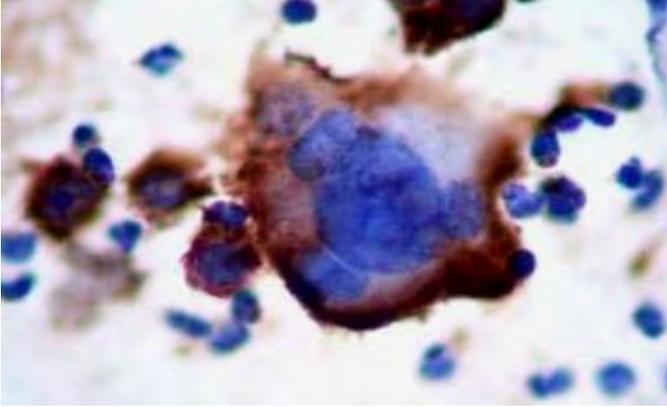


Fig.3.6. Infected cell demonstrating a positive cytoplasmic reaction to Herpes simplex antibody.

**Cytomegalovirus infection** is rarely detected by Pap smear. The infection affects endocervical and endometrial cells with production of characteristic large eosinophilic or amphophilic intranuclear inclusions.

## Fungal infection

### Candidiasis

Elements of *Candida species* are normally found in the cervix and vagina and are present in 3% of all Pap smears. Its presence is not indicative of a fungal infection requiring treatment. *Candida albicans* is the most common microorganism causing cervical vaginal candidiasis. Both yeasts and nonseptated pseudohyphae are seen. The budding yeasts are 3 to 7  $\mu\text{m}$  in greatest dimension and the pseudohyphae are eosinophilic to gray-brown. The pseudohyphae are formed by elongated budding that display constrictions along their length. (Fig.3.7).

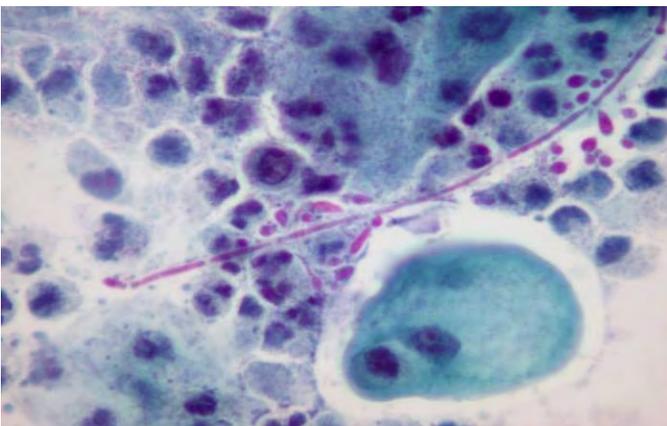


Fig.3.7. Vaginal candidiasis showing in a CP smear inflammatory exudate containing yeasts and nonseptated pseudohyphae of *Candida species*.

## Parasitic infection

### Trichomonas vaginalis

Trichomonas vaginalis infection is the most common STD of the lower female genital tract. *Trichomonas vaginalis* is a facultative anaerobic protozoan parasite without mitochondria or peroxisomes. The infection is characterized by a dense inflammatory exudate containing "pus balls" or large aggregates of polymorphonuclear leukocytes and *Trichomonas vaginalis* organisms. The organisms can be identified in routinely stained Pap smears. They are pear-shaped, oval or round cyanophilic organisms ranging in size from 15 to 30  $\mu\text{m}$ . The nucleus is pale, vesicular and eccentrically located. Intracytoplasmic eosinophilic granules are often present and flagella are usually not observed. Identification of *Trichomonas vaginalis* organisms in conventional Pap smears can be difficult, but its identification in LBP is highly accurate and does not require a confirmatory test. Leptothrix infection is a commonly associated infection with *Trichomonas vaginalis*. Elements of *Leptothrix* have a "spaghetti and meat balls" configuration. (Fig.3.8).

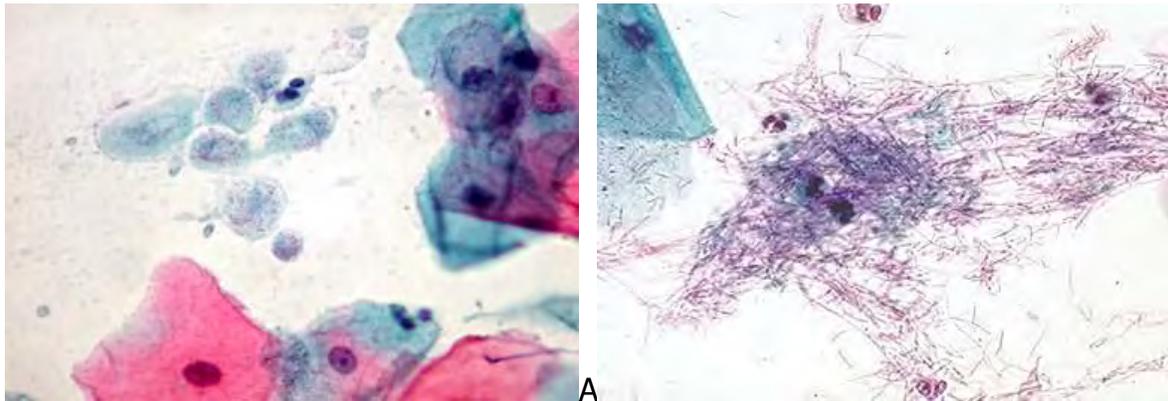


Fig. 3.8. A CP smear showing *Trichomonas vaginalis* organisms with intracellular eosinophilic granules (A) and elements of *Leptothrix* (B).

## Inflammation-associated cellular changes

Acute inflammation is often associated with changes in squamous cells. The affected cells show perinuclear halos or cytoplasmic vacuolization and enlarged, hyperchromatic nuclei with regular contours and clumped or fuzzy chromatin. (Fig.3.9). The smear background contains numerous polymorphonuclear leukocytes. Marked cytolysis may be seen in Pap smears containing abundant Döderlein bacilli.

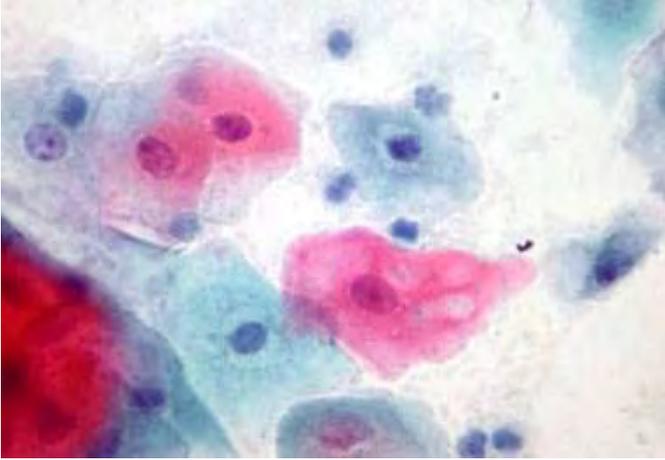


Fig.3.9. Perinuclear halos and slight nuclear enlargement in intermediate squamous cells caused by acute inflammation seen in a CP smear.

## Nonneoplastic epithelial changes

The cervical epithelium is under the effects of hormonal stimulation, inflammation or physical irritation. It may undergo hyperplasia, metaplasia and keratinization.

### Reserve cell hyperplasia

Reserve cells form a discontinuous layer between the endocervical columnar cells and the basement membrane. These cells are capable of differentiating into either squamous cells or endocervical glandular cells and proliferate as a response to physical or chemical irritation. They are usually seen in clusters and show oval, bland nuclei with scant, ill-defined, vacuolated cytoplasm. Rarely, they present singly. They may also be seen in tissue fragments. (Fig.3.10).

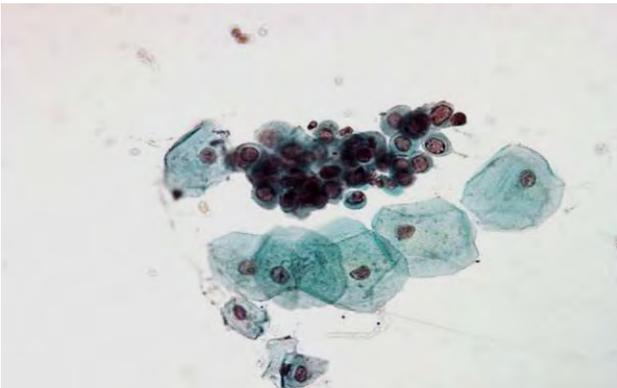


Fig.3.10. Clustered of hyperplastic reserve cells with oval, bland nuclei and scant cytoplasm.

## Squamous cell metaplasia

Hyperplastic reserve cells gradually transform into immature squamous cells that exfoliate in sheets or singly. The immature squamous cells have pale, vacuolated cytoplasm and may show cytoplasmic extensions or tails (spider cells). Their oval nuclei have fine chromatin and are slightly hyperchromatic. With time, immature metaplastic squamous cells change into mature squamous cells with more waxy, basophilic or eosinophilic cytoplasm. (Figs.3.11 and 3.12).

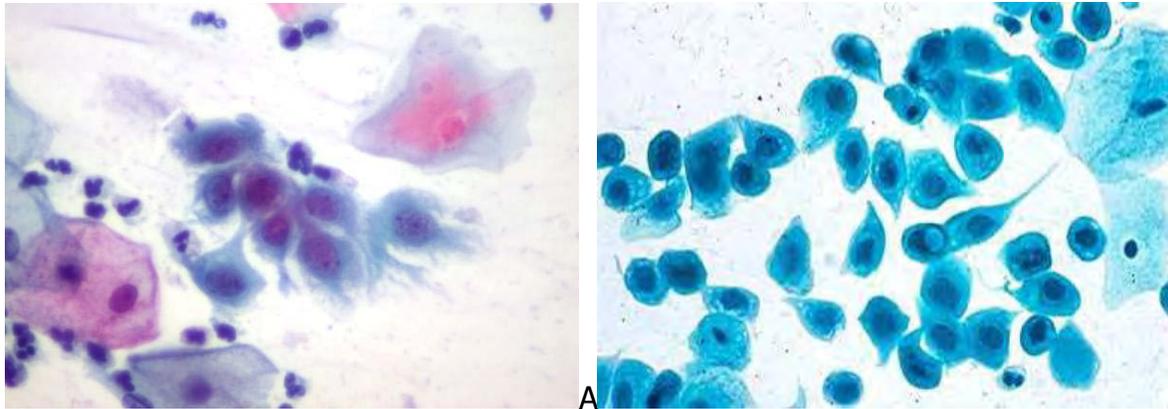


Fig.3.11. Endocervical epithelium with squamous metaplasia, (A): Metaplastic cells showing "spider leg" cytoplasmic extensions. (B) Metaplastic cells with some displaying intracytoplasmic mucinous vacuoles in CP smears.



Fig.3.12. Mature metaplastic cells with mild nuclear enlargement.

## Hyperkeratosis and parakeratosis

**Hyperkeratosis** is a protective process by which nonkeratinized squamous epithelium protects itself from injuries. It commonly occurs on the cervixes of prolapsed uteri. Thick orangeophilic layers of anucleated squamous cells with keratohyaline granules are observed. (Fig.3.13).

**Parakeratosis** is a form of hyperkeratosis in which small round or spindle-shaped keratinized squamous cells retain their pyknotic nuclei. (Fig.3.14). These cells exfoliate singly or in loose clusters. It is important to differentiate these cells from *pseudokeratotic cells* that are also small squamous cells with eosinophilic or basophilic cytoplasm and nonpyknotic oval nuclei with fine chromatin. Both *parakeratotic* and *pseudoparakeratotic cells* are commonly seen in condylomatous lesions of the cervix and vagina.

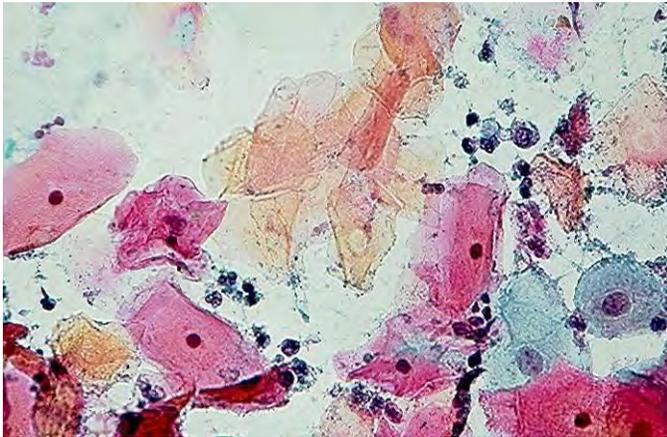


Fig.3.13. An irregular aggregate of anucleated, orangeophilic squamous cells in a CP smear.

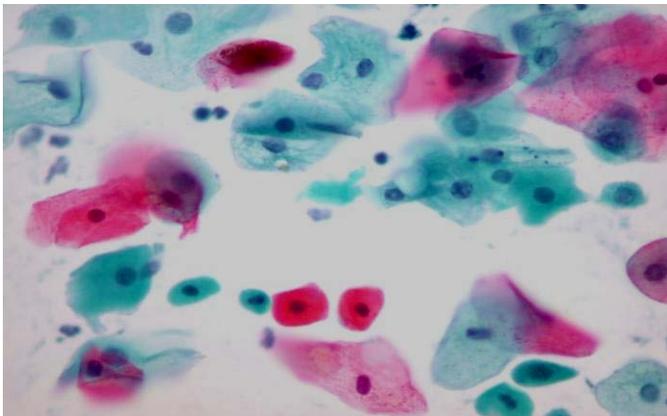


Fig.3.14. Small parakeratotic squamous cells with keratinized, eosinophilic cytoplasm and pyknotic nuclei in a CP smear.

### **Tubuloendometrial metaplasia**

This type of metaplasia is common, affecting about 30% of women. The lesion is located in the upper portion of endocervical canal, often in deep clefts. It may represent a response to injury. Cytologic material from this lesion may reveal ciliated cells with clear cytoplasm and abundant apical ciliae, secretory cells and intercalated cells with scant cytoplasm and thin, long nuclei (peg cells).

## Urothelial metaplasia

Urothelial metaplasia may be seen on exocervical atrophic squamous epithelium in elderly patients. The exfoliated metaplastic cells display oval, bland nuclei with longitudinal grooves. (Fig.3.15)

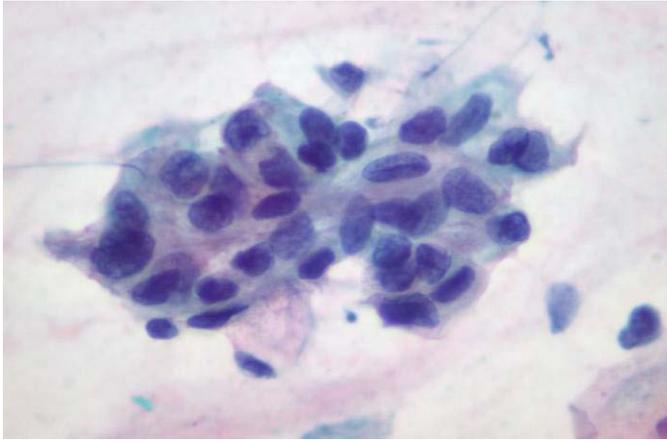


Fig.3.15. A cluster of metaplastic urothelial cells showing thin cytoplasm and oval nuclei with some displaying longitudinal grooves in a CP smear.

## Reactive cellular changes due to inflammation and repair cells

Reactive cellular changes are benign in nature. They are often associated with inflammation, radiation, an IUD and other nonspecific causes. Criteria for reactive changes are not always well defined, and as the result, the interpretation may lack reproducibility.

### Repair cells

Repair cells are seen in Pap smears of patients with inflammatory epithelial ulcers, and with previous biopsy, cautery and cryosurgery of their uterine cervixes and they are either squamous or glandular in type. Repair squamous cells are difficult to be differentiated from repair glandular cells. These cells commonly show nuclear enlargement (1, 1.5 or 2 times the area of the nucleus of a normal intermediate squamous cell). Endocervical cells may show a greater nuclear enlargement. Nuclei may be double or multiple with smooth contours, and mildly hyperchromatic with fine chromatin. Prominent single or multiple nucleoli are observed. The cell cytoplasm may display polychromasia, vacuolization and perinuclear halos without thick cytoplasmic rims. Squamous metaplastic cells display similar nuclear and cytoplasmic changes; and cytoplasmic processes (spider cells) may be observed. (Figs.3.16 and 3.17).

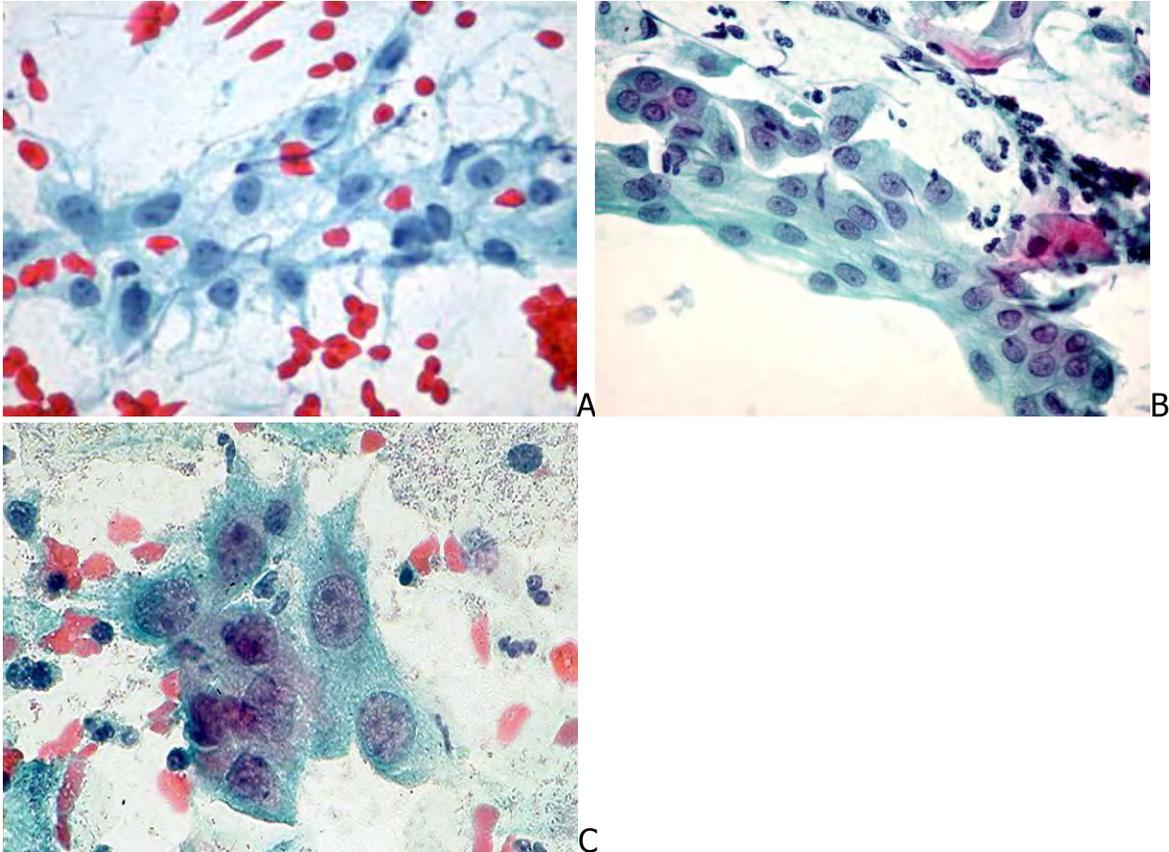


Fig.3.16. Epithelial repair cells in CP smears, (A): Repair cells with cytoplasmic processes. (B): Repair cells with granular or vacuolated cytoplasm in a monolayered sheet. (C): Repair cells with prominent nucleoli and cytoplasmic processes.

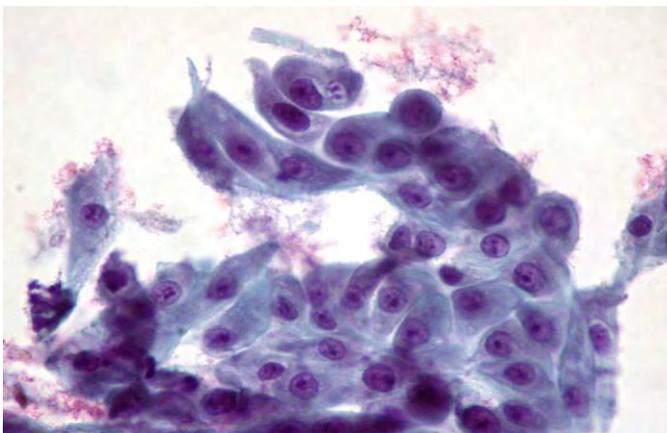


Fig.3.17. A monolayered sheet of squamous repair cells in a LBP.

### Vitamin B12 and Folic acid deficiency-induced cellular changes

These cellular changes are characterized by an enlargement of squamous cells and their nuclei. The nuclei are single or double and slightly hyperchromatic with fine

chromatin. The presence of hypersegmented nuclei within polymorphonuclear leukocytes on the smear is evidence supporting pernicious anemia.

## Radiation and Chemotherapy Effects

Radiation and chemotherapy are routinely used in the treatment of patients with advanced solid cancers, lymphomas and leukemias. Their mechanisms of action on protein metabolism and mitosis on human cells differ, however they produce similar cellular changes.

### Radiation effects

Injury is caused by radiation-induced ionization of intracytoplasmic molecules from inhibition of DNA synthesis and destruction of cellular proteins and enzymes. The extent of cellular injury varies with the type of radiation, the duration of exposure and the radiosensitivity of the cells. Hematopoietic cells, germ cells, gastrointestinal cells and anaplastic tumors are highly susceptible to radiation injury because they have a high mitotic rate. Radiation changes may be classified as acute and chronic.

- **Acute radiation changes** may appear a few days after the conclusion of treatment, persist for 6 to 8 weeks and then gradually subside. In the uterine cervix the changes affect mainly the squamous cells, however the endocervical glandular cells are also affected albeit to a lesser degree. Acute cellular radiation changes consist of (Figs.3.18 to 3.20):

- Inflammatory exudates with cellular debris, histiocytes and polymorphonuclear leukocytes forming "pus balls" in smear background
- Repair cells are markedly increased in size, with normal N/C ratio
- Enlarged nuclei may show vacuolization, pallor, wrinkling and smudging of chromatin
- Bi- or multinucleation and mild hyperchromasia
- Prominent single or multiple nucleoli
- Cytoplasmic vacuolization, hyalinization or polychromasia, intracytoplasmic aggregates of leukocytes

- **Chronic radiation changes** appear about 6 months after cessation of the initial radiotherapy and may persist for years. The Pap smear displays an atrophic pattern with parabasal cells and intermediate squamous cells with pleomorphic giant cells. Cellular changes as seen in acute post-radiation injuries may still be seen but they are less prominent.

- It is important to note that if during radiotherapy and after its conclusion

malignant cells are still present, a persistence of the original tumor should be suspected and a tissue biopsy should be obtained for histologic confirmation.

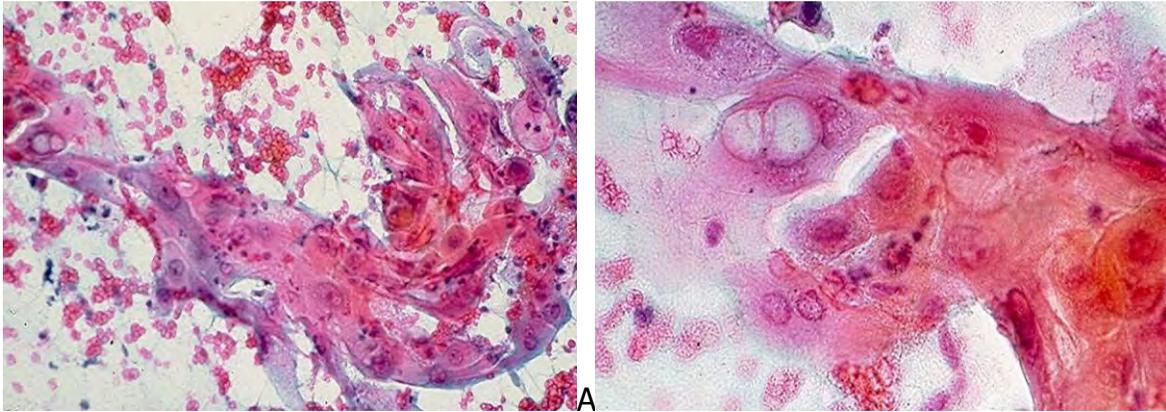


Fig.3.18. CP smear showing squamous cells with radiation changes, (A): An aggregate of squamous cells with slightly pleomorphic, hyperchromatic nuclei and polychromatic cytoplasm. (B): A fragment of squamous epithelium shows large epithelial cells with mild nuclear enlargement, conspicuous nucleoli. Intracytoplasmic vacuoles are noted in some cells.

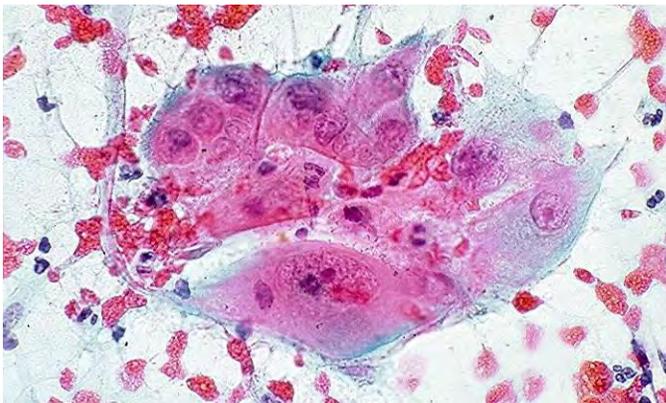


Fig.3.19. A cluster of large epithelial cells with enlarged nuclei, prominent nucleoli showing thick, polychromatic cytoplasm with cytoplasmic extensions suggesting repair cells with radiation changes in a CP smear.

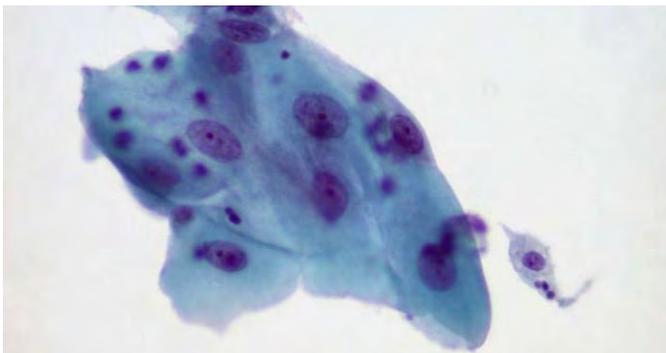


Fig.3.20. Squamous cells with radiation effects in a LBP.

## Chemotherapeutic effects

Many drugs used to treat malignant diseases are alkylating agents, which are derivatives of nitrogen mustard. They alter the cellular DNA, RNA and proteins by different mechanisms. The cellular changes caused by alkylating agents are similar to those caused by radiation but they are systemic. (Fig.3.21). In a Pap smear, the number of abnormal cells is much smaller than in the case of radiation therapy.

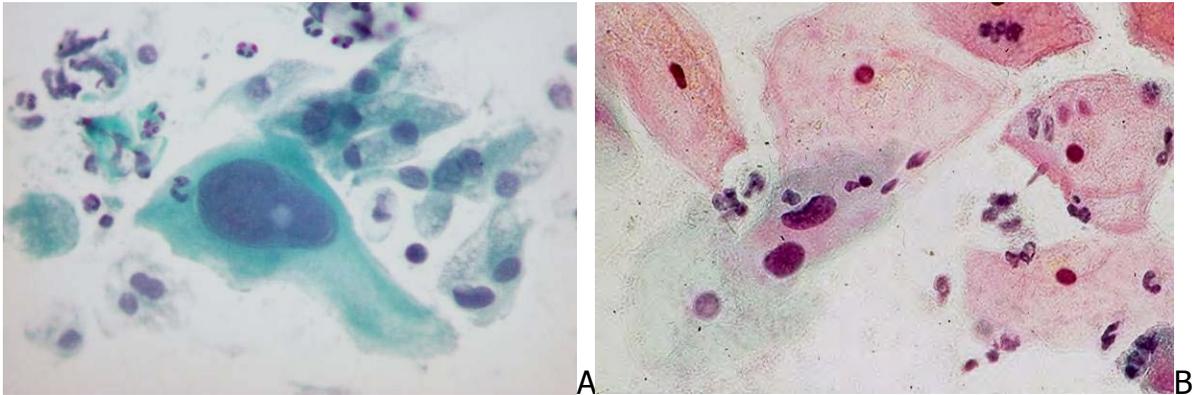


Fig.3.21. CP smear showing in (A) and (B) squamous cells with chemotherapeutic effects (Methotrexate) displaying enlarged, hyperchromatic nuclei.

## Benign-appearing endometrial cells in women over 40 years of age

The presence of **spontaneously exfoliated benign-appearing endometrial cells** in Pap smears of women over 40 years of age is not regarded as an epithelial abnormality in The Bethesda System-2001. These cells are seen in small tridimensional, round clusters and they are present in less than 1% of all Pap smears. In the majority of cases, these benign endometrial cells are from a normal and cycling endometrium, and in other cases their exfoliation is secondary to a benign endometrial polyp, an IUD and a hormonal replacement therapy. Only in about 1% of these women an endometrial hyperplasia or carcinoma is found. Therefore, the presence of spontaneously exfoliated benign-appearing endometrial cells in an asymptomatic woman does not constitute an indication for endometrial biopsy for histologic evaluation.

## Glandular cells in post hysterectomy Pap smears

Benign glandular cells similar to normal endocervical cells may be occasionally seen in Pap smears of patients with total hysterectomy. They are formed as the result of mucinous or glandular metaplasia of vaginal squamous epithelium, and they are more commonly seen in women with postoperative radiotherapy. (Fig.3.22).

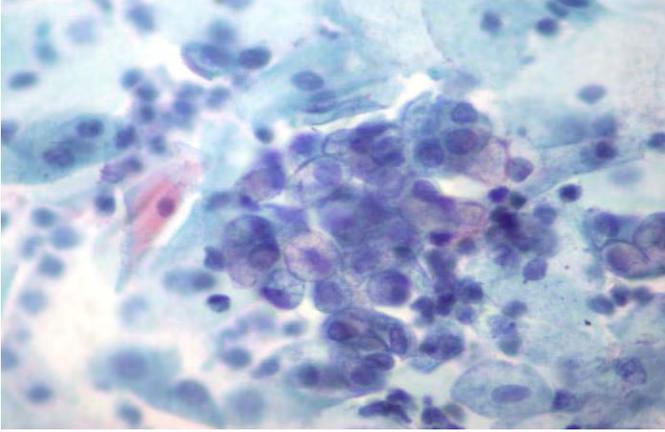


Fig.3.22. A cluster of benign glandular cells with mucin-filled clear cytoplasm in a CP smear of a woman with prior total radical hysterectomy and radiotherapy for endometrial carcinoma.

### Abnormal shedding of normal-appearing endometrial cells

Depending on a woman's menstrual status, abnormal shedding of normal-appearing endometrial cells may have different endometrial pathologies, according to several studies. Normal appearing endometrial epithelial cells have scant cytoplasm and a small bland, round or oval nucleus that is of the same size as the nucleus of a normal intermediate squamous cell. These cells usually occur in small groups or clusters and have no nuclear contour irregularity or conspicuous nucleoli. (Fig.3.23).

In a *premenopausal woman*, shedding of normal-appearing endometrial cells, epithelial and/or stromal types, beyond day 10 to 12 of the menstrual cycle is an abnormal finding that should be interpreted with caution in the light of clinical information. It can be secondary to an IUD, endometritis, anovulatory cycle, prior endometrial curettage or uterine endoscopy, endometrial polyp, hormonal therapy, submucosal myometrial leiomyoma, endometrial hyperplasia and rarely endometrial cancer.

In a *postmenopausal woman*, hormonal therapy, endometrial polyp, endometrial hyperplasia and endometrial cancer are the main causes of abnormal shedding of normal-appearing endometrial cells. Of these etiologies, hormonal replacement therapy is the most common one; a benign endometrial polyp is found in 23% of patients, endometrial hyperplasia and endometrial carcinoma are found in 5% and 5% of cases, respectively. Thus, the presence of unexplained normal-appearing endometrial cells in Pap smears in an asymptomatic and postmenopausal woman needs further investigation to rule out a significant endometrial pathology, especially when clinical risk factors for endometrial carcinoma are present (hypertension, obesity, nulliparity and hormonal replacement therapy). It should be born in mind that

a high percentage of patients with abnormal shedding of normal-appearing endometrial cells show no pathology in biopsied endometrial tissues.

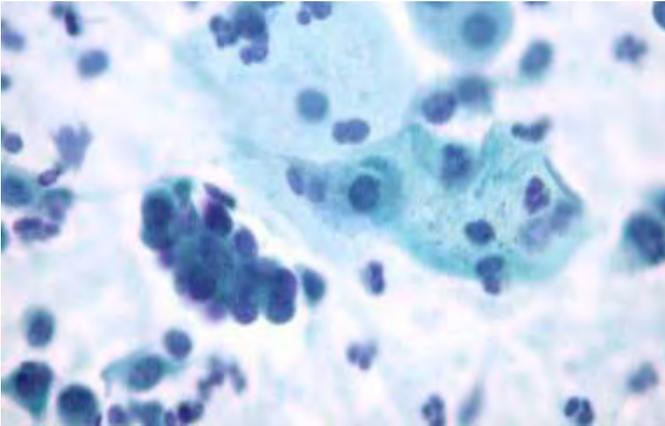


Fig.3.23. A group of normal-appearing endometrial cells in a CP smear. The endometrial cell nuclei are of the same size with those of intermediate squamous cells.

### Vaginal endometriosis

Vaginal endometriosis may be formed by implantation of viable endometrial cells discharged during the menstrual period. The lesion may appear as a bluish submucosal cyst with chocolate colored liquid contents. In typical cases of a scraping smear or fine needle aspiration, it yields fragments of endometrial epithelium, clusters of endometrial stromal cells, degenerated erythrocytes and a few hemosiderin laden macrophages. (Fig.3.24).

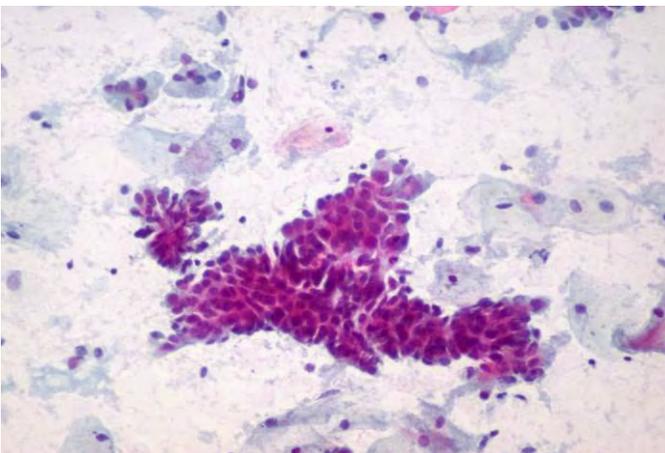


Fig.3.24. Endometriosis in a vaginal scraping CP smear showing a fragment of benign endometrial epithelium.

## Vaginal adenosis

Vaginal adenosis is defined by the presence of either endocervical glandular epithelium or tuboendometrial epithelium in the vagina. It most commonly affects the upper third of the anterior vaginal wall. Approximately 35% to 90% of female offspring with in utero exposure to diethylstilbestrol (DES) develop this lesion. However, the lesion is also found in women without in utero exposure to DES, and an incidence up to 41% has been reported. In a scraping smear, the lesion yields benign endocervical glandular cells singly, in loose clusters and in monolayered sheets. Metaplastic squamous cells may also be observed.

## IUD-induced cellular changes

Women bearing IUDs usually show cellular atypias affecting endometrial glandular cells and cervical metaplastic squamous cells. By mechanical effects, reactive and regenerative endometrial cells are formed. These cells occur in groups or clusters and show cytoplasmic enlargement with intracytoplasmic vacuoles, conspicuous or prominent nucleoli thus mimicking malignant glandular cells. The cervical metaplastic squamous cells may show prominent nucleoli. Single endometrial cells with high N/C ratios and hyperchromatic nuclei with irregular nuclear membrane or contours mimicking HSIL/CIN 3 cells may be observed. (Fig.3.25). In difficult cases a colposcopy with cervical biopsy and endometrial curettage are necessary for histologic confirmation.

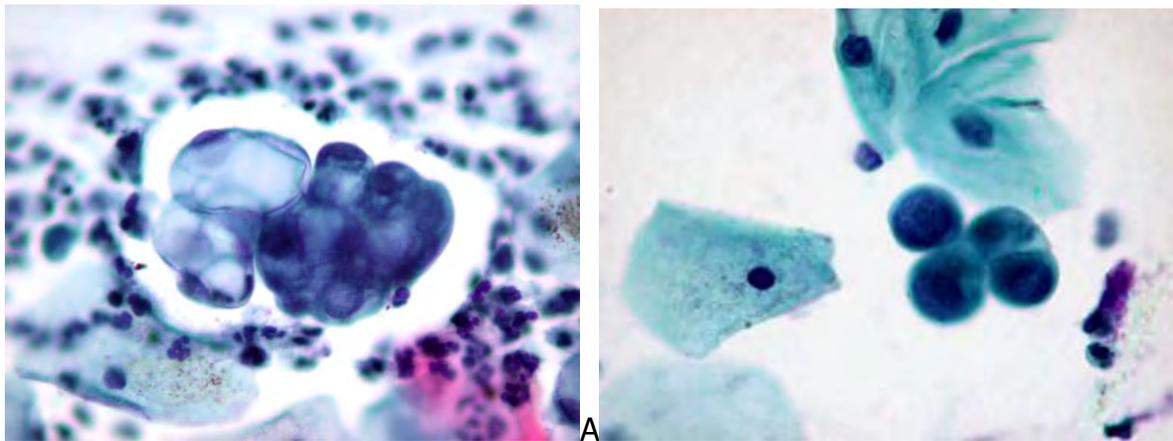


Fig.3.25. CP smear showing IUD-induced cellular changes, (A): A cluster of glandular cells with vacuolated cytoplasm. (B): Four small HSIL-like cells.

## Other cytologic findings

- "**Cornflakes**" or brown artifact "cornflaking" is due to evaporation of xylene before coverslipping with deposition of air on superficial squamous cells. Cornflaking is more commonly seen in conventional Pap smears than in LBP. (Fig.3.26).

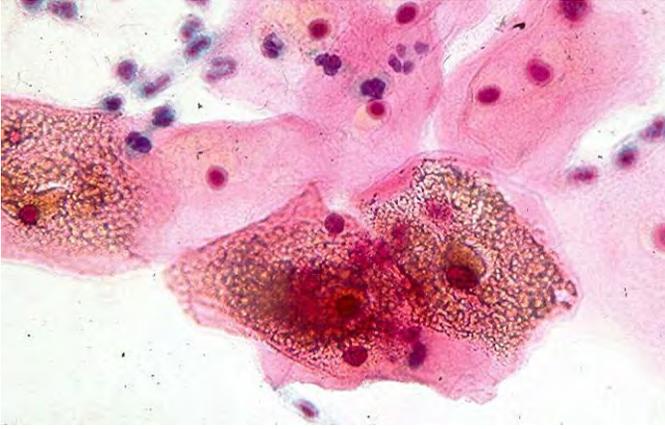


Fig.3.26. CP smear showing superficial squamous cells with "cornflakes".

- Blue blobs represent condensed mucus, degenerated bare nuclei and precipitating hematoxylin. In postmenopausal women, they may represent parabasal/intermediate squamous cells with various degree of degeneration. Blue blobs appear as dark blue, round, oval, amorphous masses in CP smears. (Fig.3.27).

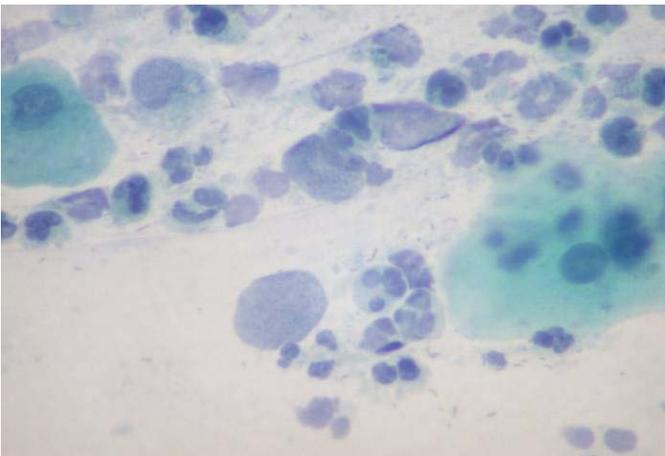


Fig.3.27. A few "blue blobs", 2 intermediate squamous cells and several polymorphonuclear leukocytes in a CP smear.

- Psammoma bodies are laminated calcified round bodies. (Fig.3.28). They are rarely seen in Pap smears. In about 50% of patients a benign condition or no lesion is found. In other cases, in particular postmenopausal women, an ovarian papillary serous carcinoma is present. In those cases, the psammoma bodies are usually seen

admixed with malignant epithelial cells that may wrap around some bodies. Therefore, further investigation is recommended in all patients showing psammoma bodies in their Pap smears to rule out the possibility of a clinically occult ovarian serous cancer.

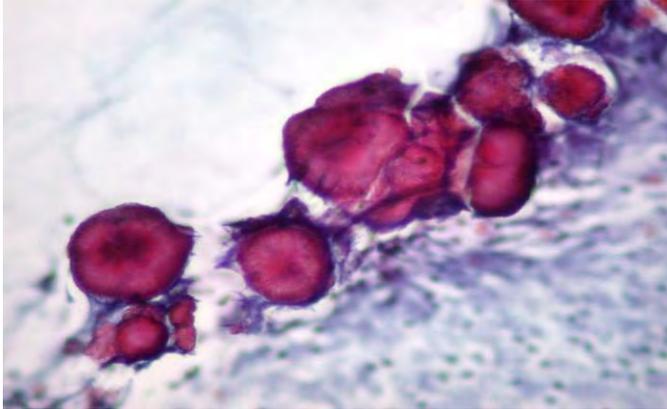


Fig.3.28. Laminated round psammoma bodies in a CP smear.

- Carpet beetle part is a contaminant from cotton applicator or tampon. It has a distinctive morphology permitting the correct identification when present. (Fig.3.29).



Fig.3.29. Carpet larva part with distinctive morphology in a CP smear.

- Curschmann spirals are rarely found in Pap smears. They are inspissate mucous threads within cervical glands or clefts and have no diagnostic significance. (Fig.3.30).

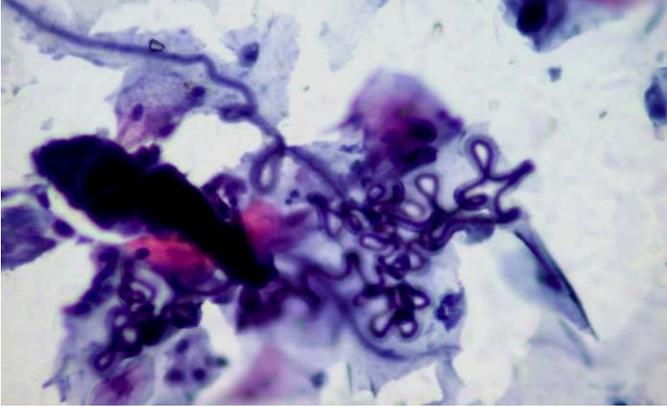


Fig.3.30. Curschmann spiral in a CP smear.

Common cellular features of abnormal and nonneoplastic squamous cells of the cervix and vagina are summarized in Table 3.1.

**Table 3.1** Common Cellular Features of Abnormal and Nonneoplastic Squamous Cells\*

Cellular features	Inflammation associated cellular changes	Vitamin B12 or Folic Acid deficiency	Radiation or Chemotherapy	Repair cells
Architecture	Singly Clusters, loose	Singly	Clusters, cohesive Singly	Sheets, monolayered Singly
Cytoplasm - Border - Perinuclear halo with thin cytoplasmic rim - Vacuolization - Enlargement - Extensions	Distinct +, small  +/- + -	Distinct +, small  +/- + -	Distinct +/-  + ++ +/-	Distinct -  - ++ ++
Nucleus - Irregular contours - Enlargement - Multinucleation - Chromatin	-  +/- - Clumped, fuzzy	-  + - Granular, fine	-  ++ + Granular, fine	-  + - Fine
Increased N/C ratio	-	+	-	-
Inflammatory background	+++	-	++	+
Other - Intracytoplasmic vacuoles - Macronucleolus	- -	- -	+ +/-	+/- +

\* Adapted with modifications from Nguyen GK, Kline TS: Essentials of Cytology. An Atlas. New York, Igaku-Shoin, 1993, p. 4 and 6.

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## Pap smear: Squamous cell abnormalities

Studies over the past 5 decades have demonstrated that cervical squamous cell carcinomas (SCC) develop through a multistep process involving preinvasive lesions. In the majority of cases, the tumor occurs as the end result of a series of epithelial changes, ranging from mild to severe atypia, at the T-zone of the cervix. These lesions have been given various names: dysplasia and carcinoma in situ, cervical intraepithelial neoplasia (CIN) and squamous intraepithelial lesion (SIL).

In recent years, studies of cervical SILs and SCCs using molecular biology techniques have documented that the sexually transmitted human papillomaviruses (HPV) play an important role in the pathogenesis of these lesions. Of over 80 different types of HPV which have been identified, only about 40 are found in female anogenital tract lesions. The HPV types 6 and 11 are mainly associated with cervical condylomas and CIN 1 but only rarely with HSILs (CIN 2 and 3) and almost never with cervical SCCs. Among the high-risk viruses HPV type 16 is most commonly detected in cervical SCCs. Of the SILs, LSILs are extremely heterogeneous with regard to their association with HPV types. In contrast to HSILs, LSILs may be caused by any single or combined HPV types of low- or high-risk HPV types, while HSILs, in about 88% of cases, are associated with HPV types 16, 18 or 31; and only 7% of them contain more than one HPV type.

### Squamous intraepithelial lesions

Cervical SILs have been generally regarded as precursors of cervical SCCs. SILs are predominantly a disease of women in their reproductive years. CIN lesions consist of 3 grades with grade 1 equivalent to mild dysplasia, grade 2 to moderate dysplasia, and grade 3 to severe dysplasia and carcinoma in situ (CIS). In TBS only 2 grades are recognized: LSIL that is equivalent to flat condyloma and CIN 1; and HSIL that is comprised of CIN 2 and CIN 3 or CIS.

LSIL is defined as an intraepithelial lesion showing a preservation of differentiation, maturation and organization of squamous epithelium with mitoses confined to basal and parabasal epithelial layers, koilocytosis, dyskeratosis, multinucleation, and enlarged hyperchromatic nuclei. HSIL, on the other hand, is characterized by a lack of squamous differentiation, epithelial disorganization, and severe cellular dyskaryosis with the presence of mitoses throughout the entire or lower 2/3 of the epithelium.

## Low-grade squamous intraepithelial lesion

LSIL includes flat condyloma, mild dysplasia and CIN 1. LSIL is caused by a large number of different HPVs of low-risk and high-risk types. LSIL cells are found in about 2% of all Pap smears. The majority of women with LSIL Pap results have LSIL (CIN 1), but 18% of them are found to have HSIL (CIN 2 and 3) on cervical biopsy.

**LSIL/Flat condyloma** is characterized by the presence of koilocytes with dyskaryotic nuclei presenting singly and in sheets. These koilocytes are superficial and intermediate squamous cells displaying enlarged, hyperchromatic, single or multiple nuclei with granular or smudged chromatin pattern and irregular nuclear contours or membranes. The nuclei are surrounded by a perinuclear clear halo with a well-defined, thick cytoplasmic rim. The perinuclear halo is caused by degeneration of perinuclear cytoplasmic microorganelles caused by the HPV. (Fig.4.1).

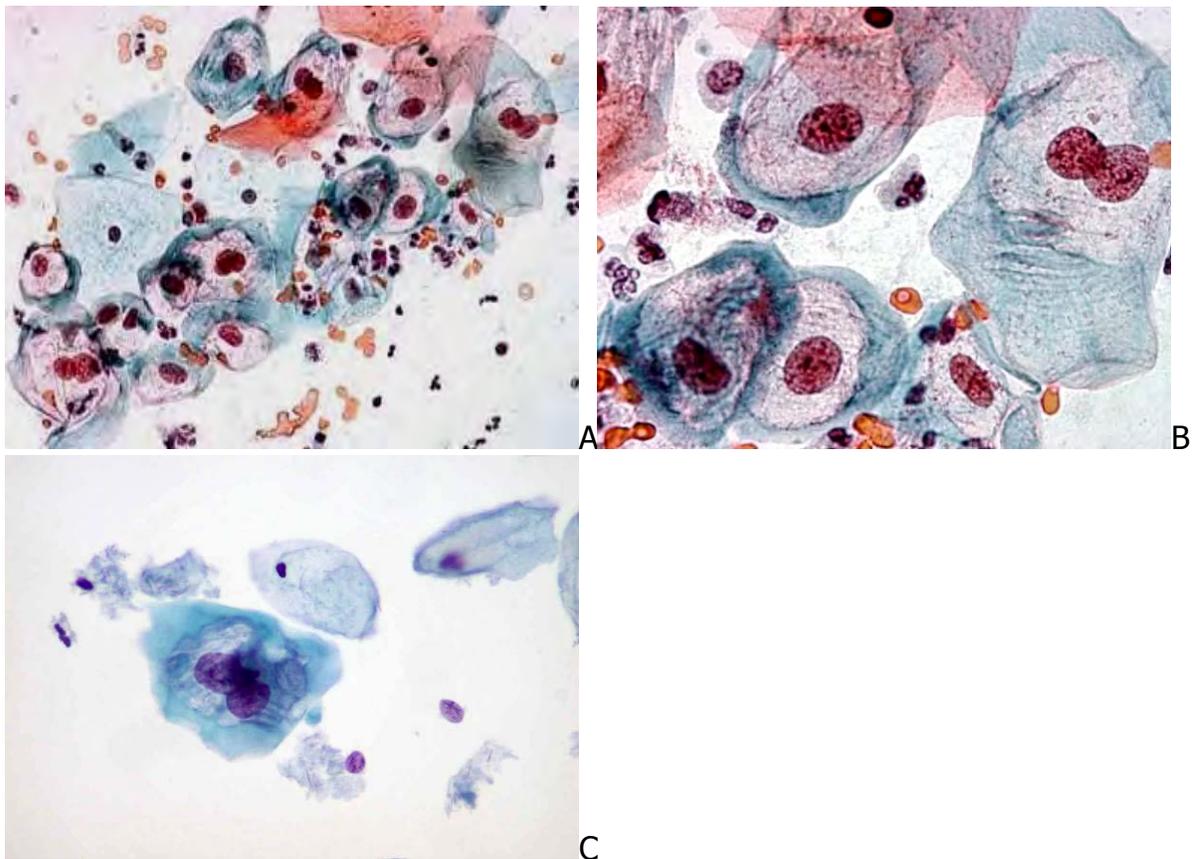


Fig.4.1. LSIL/Flat condyloma, (A, B): Classic koilocytes with dyskaryotic nuclei in a CP smear. (C): A koilocyte with two nuclei in a LBP.

**LSIL/Mild dysplasia/CIN 1** exfoliates superficial and intermediate squamous cells with enlarged, hyperchromatic and irregularly contoured nuclei. Nucleoli are absent and koilocytic changes are common. (Fig.4.2).

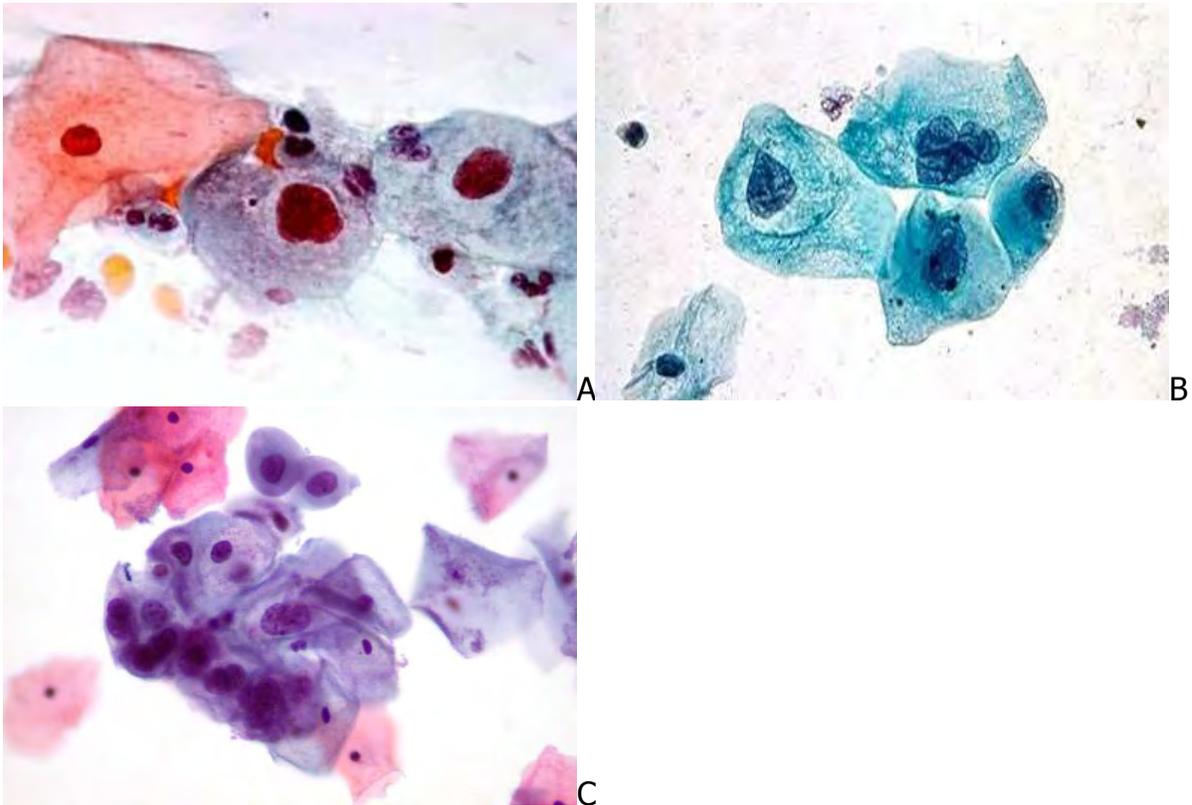


Fig.4.2. LSIL, (A, B): CP smears showing mildly dyskaryotic superficial and intermediate squamous cells. One of the cells in (B) shows koilocytic change with a perinuclear halo. (C): Mildly dyskaryotic squamous cells with some cells showing perinuclear halos in a LBP.

### Management of patients with LSIL result

The ASCUS/LSIL triage study has found that high-risk HPV types were detected in 85% of LSIL cases and that HPV DNA testing was not useful for triage strategy. Colposcopy is generally recommended for initial management of LSIL patients. For pregnant women, a colposcopically directed biopsy may be performed, but an endocervical tissue sampling is contraindicated, and the colposcopic evaluation may be deferred to 6 weeks postpartum.

### High-grade squamous intraepithelial lesion

HSILs include moderate and severe dysplasias, CIS, CIN 2 and CIN 3. HSIL accounts in about 0.5% of all Pap smears and 97% of women with HSIL Pap result are positive for high-risk HPV. If left untreated about 14% of them will develop cervical invasive squamous cell carcinoma.

**CIN 2 lesions** exfoliate parabasal-type cells singly or in sheets with thick, well-defined cytoplasm and enlarged hyperchromatic nuclei showing smooth or irregular nuclear contours. The chromatin is evenly distributed and may be finely or coarsely granular. Nucleoli are absent and koilocytic change may be present. (Fig.4.3).

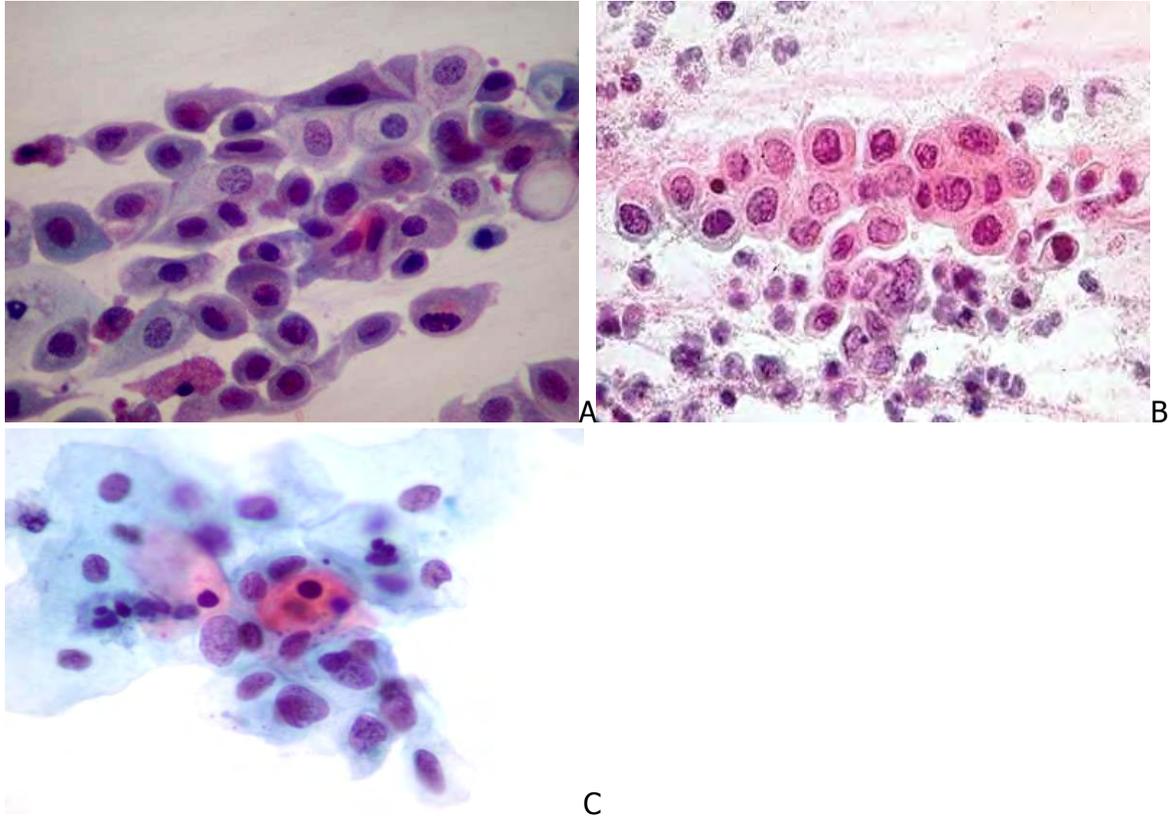


Fig.4.3. HSIL/CIN 2, (A, B): In a CP smear, moderately dyskeratotic squamous cells of low intermediate/parabasal cell type with enlarged, hyperchromatic nuclei, irregular nuclear contours and no nucleoli. (C): Similar moderately dyskaryotic squamous cells and normal intermediate cells in a LBP.

**CIN 3 lesions** have 3 main histologic patterns: large cell non-keratinizing, keratinizing and small cell patterns. A mixed cellular pattern is common.

Cells exfoliated from a **nonkeratinizing CIN 3** are large and pleomorphic. They show abundant, well- or ill-defined cytoplasm and enlarged, hyperchromatic nuclei displaying irregular nuclear contour. The nuclear chromatin is evenly distributed, and may be coarsely or finely granular. Cells in syncytial clusters are commonly encountered as well as epithelial fragments. (Figs.4.4 and 4.5). Nucleoli are absent, and the smear background is free of necrotic debris. Rarely, a nonkeratinizing CIN 3 is composed of spindle-shaped cells. (Fig.4.6).

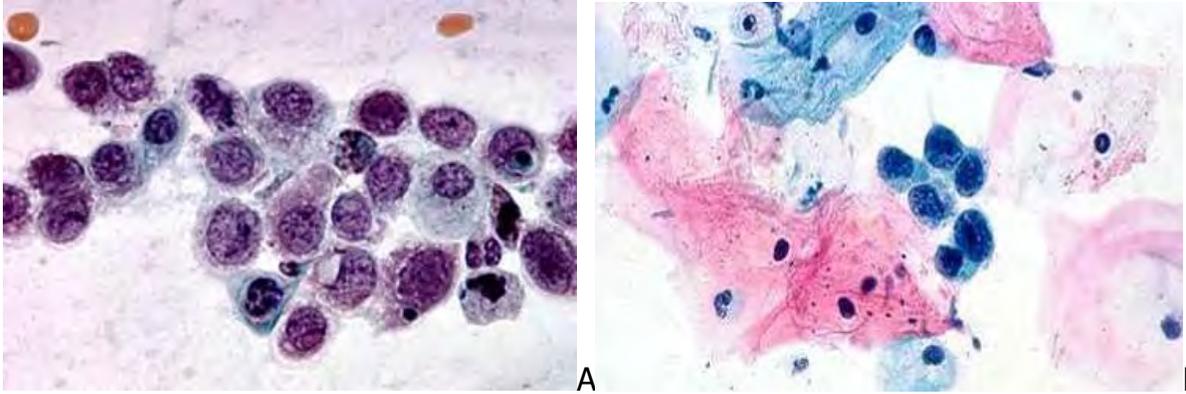


Fig.4.4. Cytology of HSIL/CIN 3, nonkeratinizing type in CP smears, (A): Markedly dyskaryotic cells with hyperchromatic nuclei and irregular contours. (B): A cohesive cluster of markedly dyskaryotic small cells showing nuclei with similar changes with those of the cells in A.

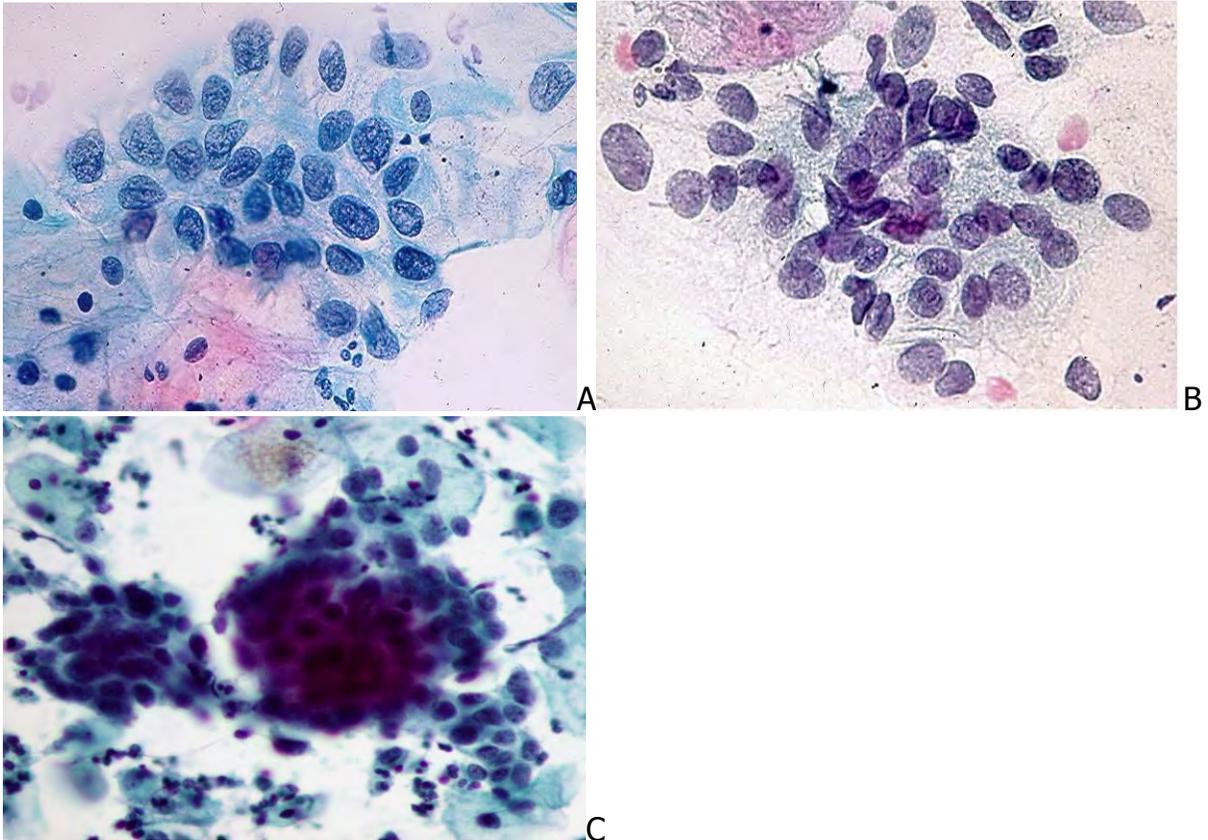


Fig.4.5. A-C. A,B. Cytology of HSIL/CIN3, nonkeratinizing type in CP smear showing syncytial-like clusters of markedly dyskaryotic cells with ill-defined cytoplasm and pleomorphic, hyperchromatic nuclei with irregular nuclear contours. (CP smears). C. Two large tridimensional clusters of markedly dyskaryotic squamous cells removed by cytobrush from another case of HSIL/nonkeratinizing CIN. (CP smear).

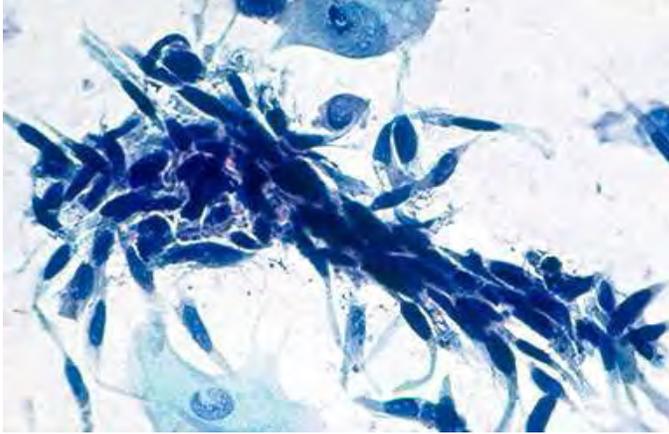


Fig.4.6. Nonkeratinizing HSIL/CIN 3 showing in a CP smear spindle-shaped cells with hyperchromatic, spindle nuclei.

**Keratinizing CIN 3** yields spindled-shaped cells with orangeophilic, well-defined, thick cytoplasm and enlarged hyperchromatic nuclei. They are chiefly seen as single cells and rarely in clusters. A **small-cell CIN 3** exfoliates cells with hyperchromatic nuclei and scant, ill-defined cytoplasm, singly or in loose aggregates, with or without nuclear molding. (Fig.4.7).

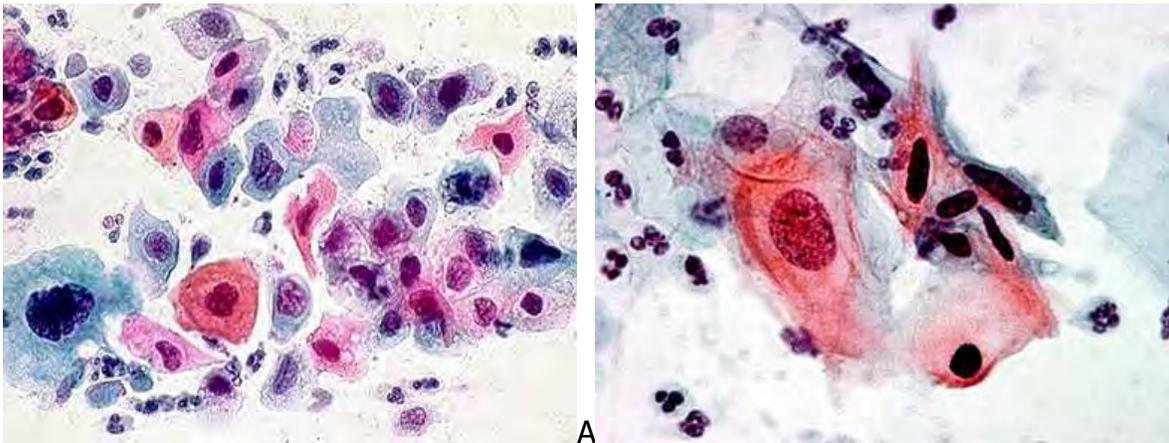


Fig.4.7. (A, B): Keratinizing markedly dyskaryotic pleomorphic squamous cells in a CP smear from a HSIL/keratinizing CIN 3.

### Management of patients with HSIL result

For patients with a HSIL Pap result, colposcopic evaluation is mandatory, as most patients will have confirmed biopsies of CIN 2 or 3. However, for pregnant women, the colposcopy may be deferred to 6 weeks postpartum. In pregnant women a colposcopically directed biopsy may be performed but an endocervical tissue sampling is contraindicated. If a CIN is not identified histologically at colposcopy, all cytologic and histologic materials of the patients should be reviewed. If the cytologic diagnosis

of HSIL is correct, a diagnostic excisional procedure (cone biopsy) should be performed.

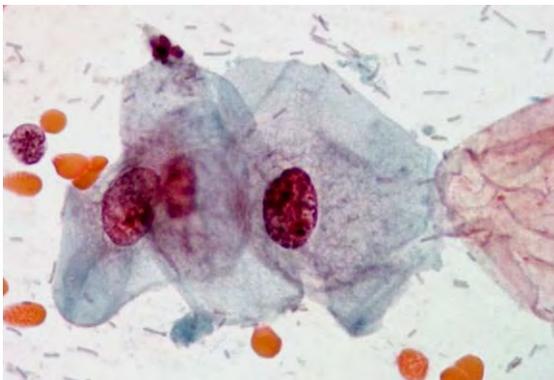
## Atypical squamous cells

Atypical squamous cells (ASC) are seen in less than 5% of all Pap smears and the ASC/SIL ratio is about 3:1. Patients with ASC diagnosis are found to have a CIN lesion on colposcopically directed cervical biopsy in 10% to 20% of cases. In TBS-2001, ASCs are divided in 2 categories: ASC of undetermined significance (ASC-US) and ASC, cannot exclude a high-grade squamous intraepithelial lesion (ASC-H). An ASC diagnosis is made when an SIL is suspected cytologically. Cytologic criteria for identification of ASC-US and ASC-H cells are somewhat subjective, and the diagnoses suffer high inter-observer and intra-observer variation rates. ASC-US represents about 90% of all ASC cases.

### ASC-US

ASC-US cells show cellular features that are more severe than those of squamous cells with reactive changes but less than those of a SIL. (Figs.4.8 and 4.9). Thus, the diagnosis of ASC-US is made by exclusion of cells with known cytologic features. Cytologic criteria of ASC-US cells include:

- ASC-US cells are of superficial or intermediate type with
- Enlarged mono-nucleus or bi-nuclei that are 2.5 to 3 times larger than the nucleus of a normal intermediate squamous cell ( $\sim 35 \mu\text{m}^2$ )
- Slightly increased N/C ratio
- Slightly hyperchromatic nuclei with irregular chromatin distribution.
- Regular nuclear contours, but it may show focal irregularity.
- Dense and eosinophilic or orangeophilic (keratinized) cytoplasm
- Perinuclear halo may be present
- Nucleoli with repair features may be seen in ASC-US repair cells



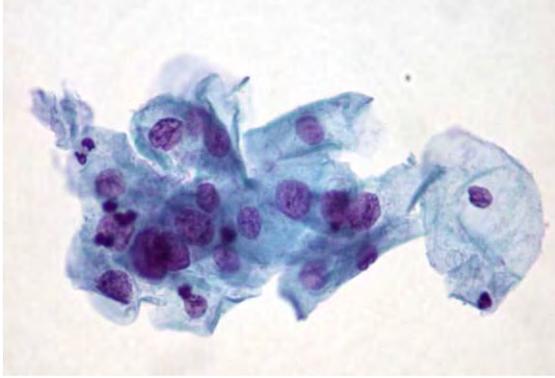


Fig. 4.8. ASC-US cells, (A, B): Single and loosely clustered ASC-US cells in a CP smear showing smooth nuclear contours with minimally hyperchromatic nuclei and no nucleoli are seen. (C): Similar ASC-US cells are seen in a LBP.

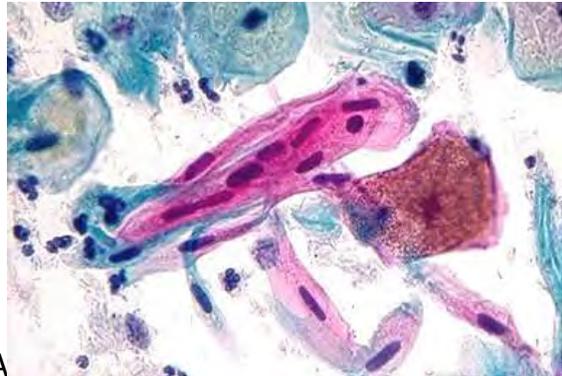
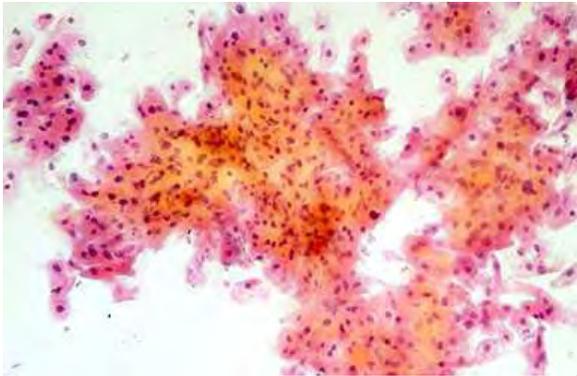
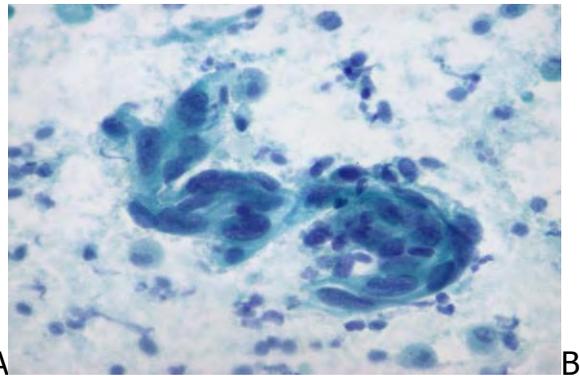
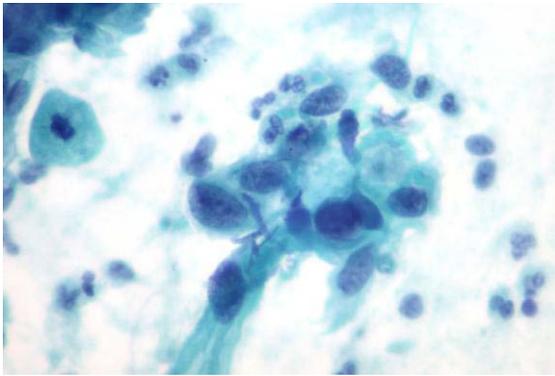


Fig. 4.9. (A, B): ASC-US cells with keratinized cytoplasm in CP smears.

**Atrophic vaginitis, atypical parakeratosis and atypical repair** may yield cells with features suggesting ASC-US changes. (Fig.4.10). In atrophic vaginitis, a short course of intravaginal treatment with estrogen cream for 4 to 7 days will be helpful to solve this diagnostic dilemma. This treatment will induce a maturation of squamous cells, but dyskaryotic cells will remain unchanged.



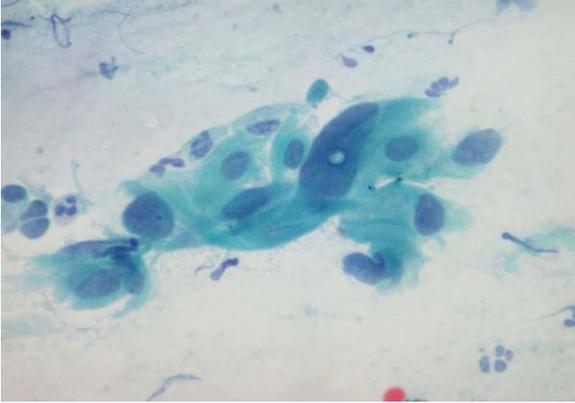


Fig.4.10. A-C: Atrophic vaginitis showing in CP smears ASC-US cells.

### Management of patients with ASC-US result

Oncogenic (high-risk) HPV DNA testing is the preferred management for patients with ASC- US Pap results, especially when it can be performed concurrently. The test should not be performed in women younger than 30 years of age because the HPV infection in these patients is often caused by a mixture of low- and high-risk virus types, making the interpretation of the test results difficult, if not impossible. Follow-up with repeat Pap tests at 6-month intervals or immediate colposcopy is also acceptable. In pregnant women, the colposcopy may be deferred to 6 weeks postpartum.

If the HPV DNA testing is positive for high-risk viruses, the patient should be referred to colposcopy. If the test is negative for high-risk viruses, she should be followed by a repeat Pap smear every 6 months for 2 years. If the cellular atypia is cleared within 2 years, she can return to routine annual screening.

If HPV DNA testing is unavailable and if the cellular atypia persists over 2 years, she should be referred to colposcopy for further evaluation.

### ASC-H cells

ASC-H cell represents 5% to 10% of all ASC cases. ASC-H cells are metaplastic squamous cells with nuclear atypia that fall short of a definitive diagnosis of HSIL. (Figs.4.11 and 4.12). Cytologic criteria of ASC-H cells include:

- ASC-H cells are usually small in number and,
- Occur singly, in small groups or in epithelial fragments with less than 10 cells.
- ASC-H cells are polygonal in shape, have dense cytoplasm and are the size of a squamous metaplastic cell.
- Their hyperchromatic nuclei are 1.5 to 2.5 times larger than that of a normal metaplastic squamous cell, have irregular chromatin and mildly irregular contours.

- No nucleoli.
- The N/C ratio is increased and is about that of an HSIL cell.

ASC-H cells in a crowded sheet or epithelial fragments may show a loss of nuclear polarity. On smears these thick tissue fragments may display peripheral cells in vague palisades mimicking those of a cervical adenocarcinoma in situ.

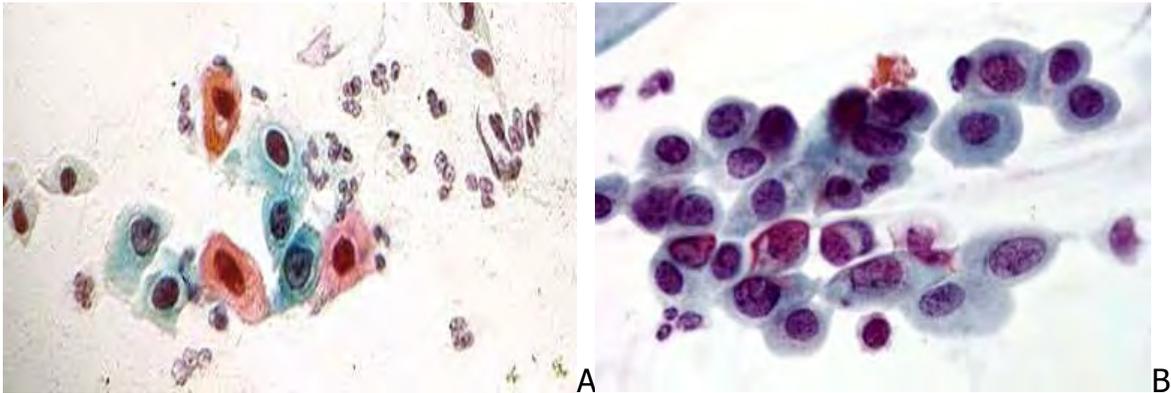


Fig.4.11. (A, B): ASC-H cells present singly and in loose aggregates in CP smears.

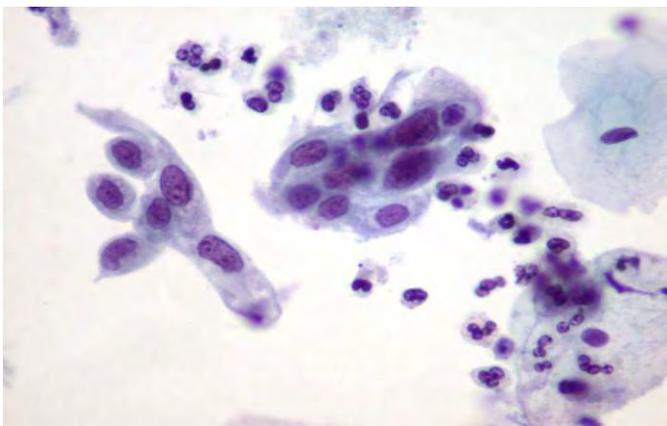


Fig.4.12. Loosely clustered ASC-H cells in a LBP.

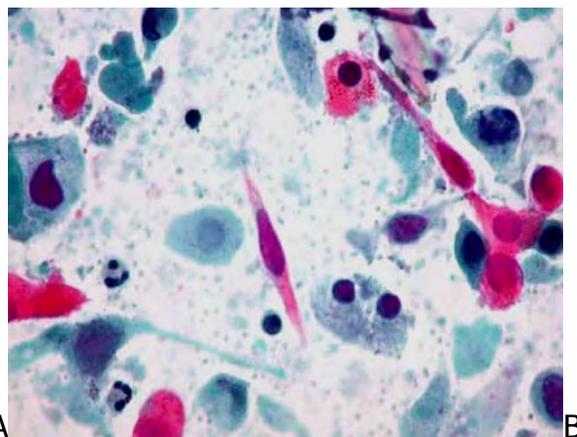
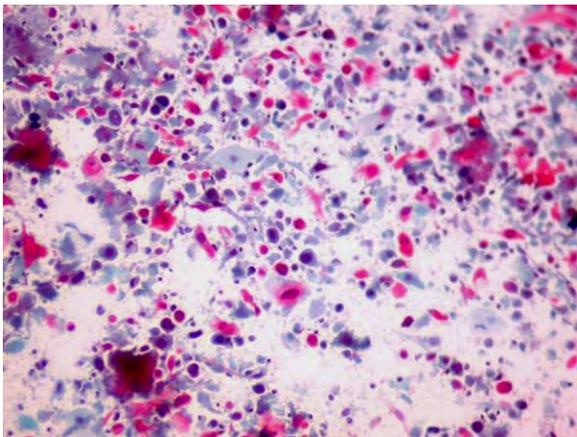
### Management of patients with ASC-H result

Patient with ASC-H diagnosis should be referred to colposcopy as ASC-H has a positive predictive value for histologic CIN 2 or 3 much higher than that of ASC-US (50% versus 17%). If a CIN 2 or 3 is not found she should have a repeat Pap test in 6 months or a HPV DNA test. If her repeat Pap test result is ASC-US or worse, or if her HPV DNA test is positive for high-risk viruses, she should be referred to a second colposcopy. If the patient is pregnant, the colposcopy may be deferred until 6 weeks postpartum.

## Cervical invasive squamous cell carcinoma

Invasive SCCs are the most common type of cervical cancers, accounting for 60 to 80% of all malignant tumors of the cervix. They occur mainly in adults with a peak incidence in the 5<sup>th</sup> and 6<sup>th</sup> decades of life. Their common clinical manifestation is abnormal vaginal bleeding that may occur spontaneously or following a sexual intercourse. Cervical SCCs are histologically classified as well- and poorly-differentiated (keratinizing and non-keratinizing SCCs). Cervical epithelium adjacent to SCC commonly shows foci of LSIL or HSIL. Cervical SCCs have distinctive cytologic manifestations. (Figs.4.13 to 4.16). Common cytologic features of cervical SCC include:

- Cancer cells with keratinized cytoplasm are seen predominantly singly.
- Nonkeratinized cancer cells predominantly seen in small aggregates and in clusters.
- Prominent nucleoli are present mainly in nonkeratinized tumor cells.
- Necrotic debris or tumor diathesis is almost always observed in conventional Pap smears, but it is subtle or minimal in liquid-based preparations in which the necrotic debris is collected at the periphery of tumor cell groups (“clinging diathesis”).
- Cells characteristic of SILs may be present, as SILs may coexist with SCC
- Cervical adenocarcinoma in situ (AIS) cells may be seen if the AIS coexists with an SCC.



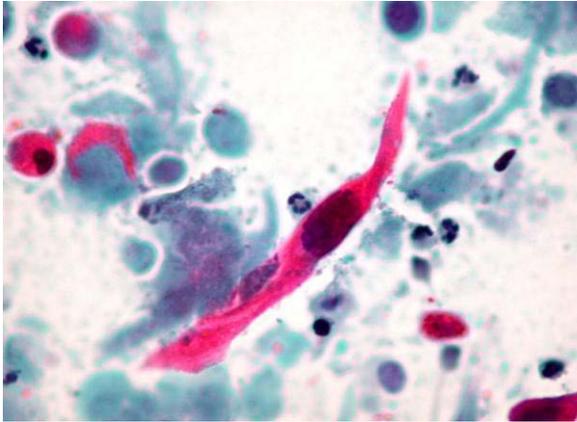


Fig. 4.13. (A, B and C): Keratinizing SCC in a CP smear showing keratinizing, pleomorphic malignant squamous cells in a necrotic background (tumor diathesis).

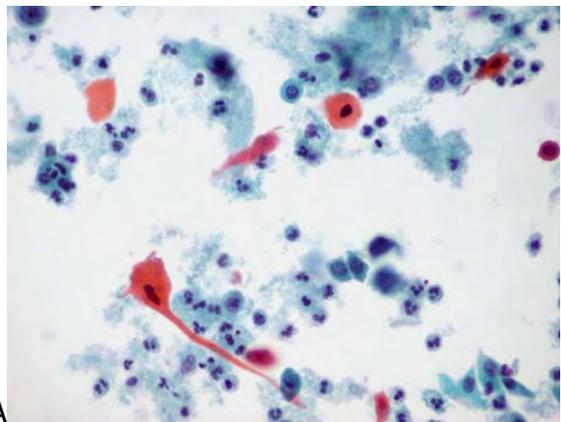
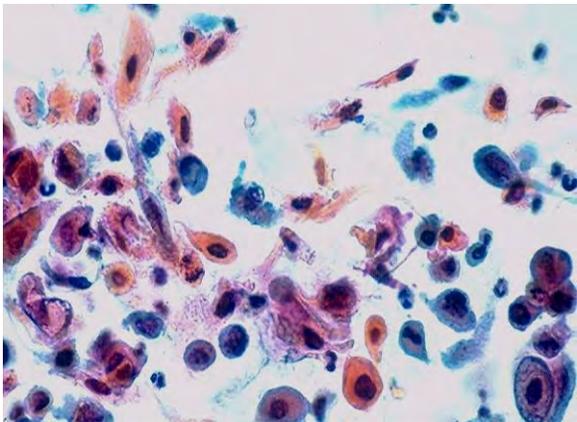


Fig. 4.14. Keratinizing SCC, (A): In a CP smear, pleomorphic malignant cells with keratinized cytoplasm and necrotic debris. A few tumor cells with tadpole configuration are present. (B): Similar cancer cells in a LBP.

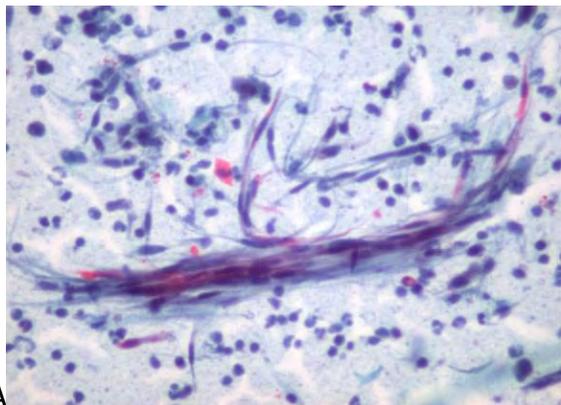
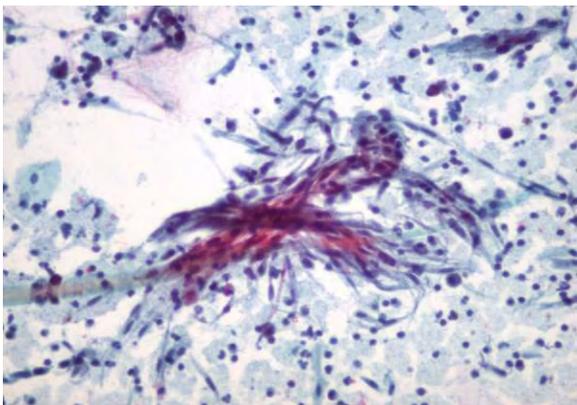


Fig.4.15. (A and B): Keratinizing SCC showing in a CP smear spindle-shaped, "fiber" tumor cells, singly and in bundles.

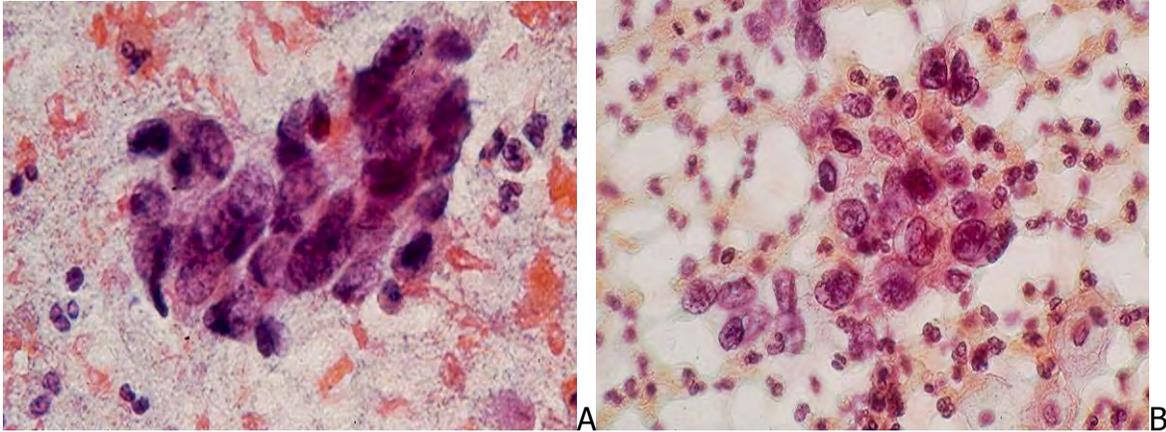


Fig.4.16. (A, B): Two poorly differentiated SCC showing in CP smears syncytial clusters of tumor cells with ill-defined, nonkeratinized cytoplasm, pleomorphic nuclei and tumor diathesis.

## Cervical microinvasive squamous cell carcinoma

Microinvasive squamous cell carcinoma (MICA) of the cervix is defined as an early invasive cancer up to 3 mm below the overlying basement membrane in which no vascular or lymphatic tumor invasion is identified. Cervical MICA, so-defined, has an incidence of pelvic lymph node metastasis lower than 1%. The tumor cannot be diagnosed by Pap smear but it can be suspected cytologically. The cytologic manifestations of cervical MICA in CP smears include:

- Pleomorphic, single HSIL cells with or without keratinization
- HSIL cells or malignant nonkeratinizing squamous cells in loose clusters, syncytia and hyperchromatic cell groups or tissue fragments (Fig.4.17)
- Nuclei with irregular chromatin clumping and distribution
- Micro- and macronucleoli
- Small amount/focal tumor diathesis

Of these criteria, the first three are the most important ones, however they are not present in every single case. MICA may be suspected cytologically, but its definitive diagnosis must be made by careful histologic examination of the excisional cervical cone biopsy.

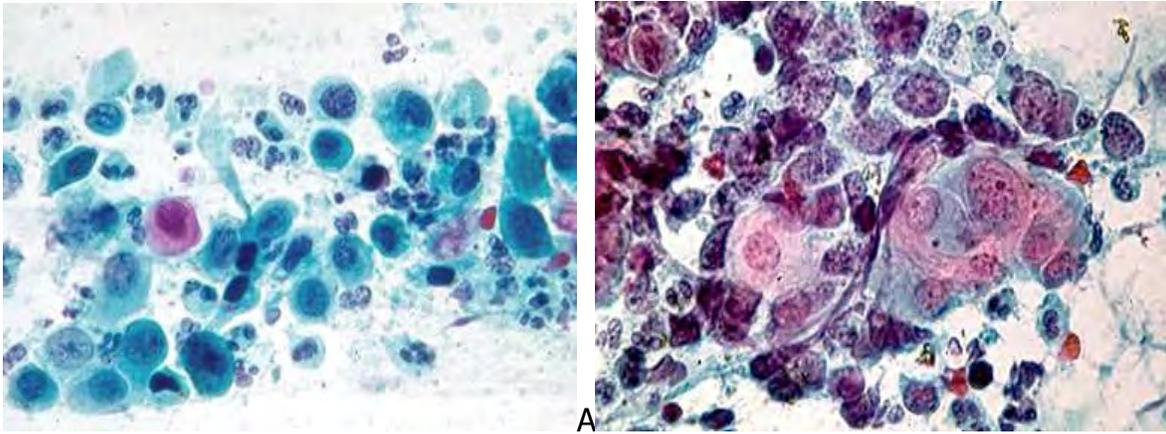


Fig. 4.17. Cervical microinvasive squamous cell carcinoma in CP smears, (A): Slightly pleomorphic HSIL cells and tumor diathesis. (B): Clustered malignant nonkeratinizing squamous cells with conspicuous nucleoli.

## Variants of cervical squamous cell carcinoma

**Verrucous carcinoma, papillary squamous (transitional) cell carcinoma, lymphoepithelioma-like carcinoma and sarcomatous squamous carcinoma** are rare and distinctive variants of squamous cell carcinoma of the cervix and vagina. Their cytologic manifestations on Pap smears have been reported. The reader is referred to selected references listed at the end of this chapter for consultation.

## Premalignant and malignant lesions of the vagina

**Squamous intraepithelial lesions** of the vagina or **Vaginal intraepithelial neoplasia (VIN)** are less common than those of the cervix. It is caused by HPV infection and is commonly associated with SIL or squamous cell carcinoma of the cervix or vulva. VIN lesions are histologically similar to those of the cervix and are also graded as VIN grade 1, 2 and 3.

**Post-radiation dysplasia** of vaginal mucosa more commonly develops following radiotherapy to the lower genital tract. The vaginal cells show radiation changes as described in Chapter 2. Post-radiation dysplasia may develop after a latency period ranging from months to years. It exfoliates dyskaryotic squamous cells as seen in a cervical SIL. Diagnosis should be confirmed by tissue biopsy. (Fig. 4.18).

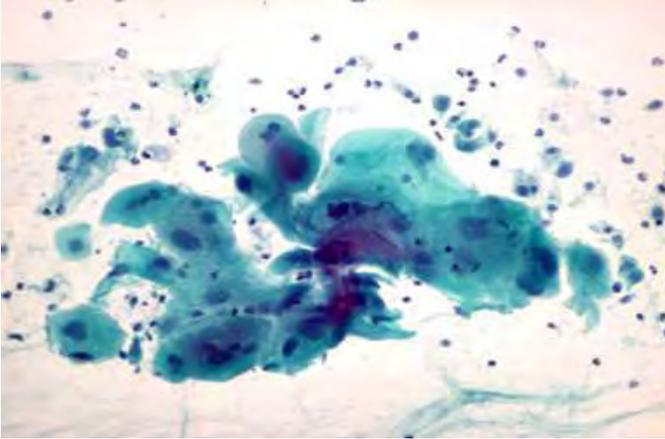


Fig.4.18. Mildly dyskaryotic squamous cells from a post-radiation dysplastic vaginal lesion.

**Primary carcinomas** of the vagina are rare and account for 1% to 2% of female genital tract cancers. Of these, squamous cell carcinoma is the most common neoplasm. However, most vaginal squamous cancers represent an invasion of either a cervical or vulvar squamous carcinoma. Vaginal squamous cell carcinoma is most commonly of nonkeratinizing type, and its exfoliated cells are indistinguishable from those of the cervix of the same histologic type.

## Diagnostic accuracy and errors

- In the screening of cervical cancer, about 85% to 90% of women in the general population have a normal Pap result, while about 10% of them show squamous cell atypia, and less than 5% of the cases are diagnosed as having a SIL. Of these cervical SILs, 75% to 90% are low-grade lesions, and the remainder are high-grade lesions.
- The cytodiagnosis of SIL is subjective and suffers remarkable interobserver variations. On rare occasions, a SIL cannot be graded as low- or high-grade, and a diagnosis of ungraded SIL has been made.
- Squamous cells with perinuclear haloes and normal nuclei are not specific for LSILs. (Fig.4.19). These changes are non-specific, and HPV-DNA is often not detected within the cell cytoplasm by in situ hybridization.

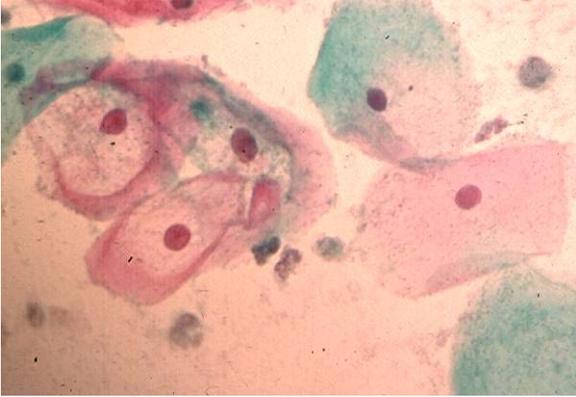


Fig.4.19. CP smear showing intermediate squamous cells with perinuclear haloes and normal nuclei.

- The diagnostic accuracy rates of LSILs varied tremendously in different reported series. The sensitivity rate of the Pap test in detecting cervical SILs varied widely, ranging from 30% to 80%, with a mean of 47%. Its specificity rate ranged from 86% to 100%, with a mean of 95%.

- For LSILs, a correct cyto–histologic correlation was obtained in about 38% to 56% of cases, and in one series, the cervical biopsy showed HSIL in 12% and was unremarkable in 50% of the cases. In another series, a poor correlation between cytologic and histologic diagnoses of various grades of CIN was observed: 50% of patients with a cytodiagnosis of CIN 1 showed a higher grade CIN in biopsied cervical tissues, and the overall false-negative rate of cervical smears for CIN 2 and 3 was 19%. In Koss' experience, about 20% of cases with a cytodiagnosis of LSIL show HSIL in biopsied tissues. For HSILs, a cytodagnostic accuracy rate of 85% to 100% has been reported.

- For invasive cervical cancers, the Pap test shows a more variable sensitivity rate, ranging from 16% to 82%; and many patients had one or more negative test result. SCCs with keratinized surfaces are often under diagnosed as scraping these tumors may yield only benign appearing keratinized squamous cells. It should be borne in mind that a false–negative cytodiagnosis is potentially dangerous as the cancer may be left untreated. A false–positive diagnosis is undesirable but it is less dangerous as the patient will be subsequently evaluated by colposcopy and biopsy.

- False-positive diagnoses of cervical cancer occurred in 10% to 15% of cases, and the 3 most common errors were: atrophic smear with benign atypia in a granular pseudonecrotic background, followed by reparative changes, and keratinizing HSIL.

**Table 4.1.** Cytology Features of Low- and High-grade SILs and Invasive Squamous Cell Carcinoma (SCC)\*.

<b>FEATURES</b>	<b>LSIL/CIN 1</b>	<b>HSIL/CIN 2</b>	<b>HSIL/CIN 3</b>	<b>INVASIVE SCC</b>
Abnormal cell number	+	++	+++	++/variable
Architecture	Singly Clusters, loose	Singly Clusters, loose	Singly Clusters, loose Syncytia	Singly Clusters, loose Syncytia
Defined cytoplasm	++	++	+/-	+/-
Perinuclear halo	++	+/-	-	-
Increased N/C ratio	+	++	+++	+ to ++
Irregular nuclear contours	+	+	+	++
Chromatin				
- Granular	Fine	Fine	Fine	Coarse/Dense
- Regular	+	+/-	-	-
Nucleoli	-	-	-	+
Tumor diathesis	-	-	-	+++
Others				
- Cervical AIS cells**	-	-	+/-	+/-

\* Adopted with modifications from Nguyen GK, Kline TS. Essentials of Cytology. An Atlas. New York, Igaku-Shoin, 1993, p 3 and 4. \*\* Adenocarcinoma in situ (AIS) cells.

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## Pap smear: Glandular cell abnormalities

Cervical adenocarcinomas are believed to originate from the multipotential subcolumnar reserve cells of the endocervical canal. These tumors display complex growth patterns consisting of different cell types. Of these, adenocarcinoma of endocervical cell type is the most common one, and it may occur in a pure form or coexist with a squamous cell carcinoma. The etiology of cervical adenocarcinoma has not been fully elucidated. Recently, HPV types 16 and/or 18 have been identified in cervical adenocarcinoma tissues suggesting a common etiology with squamous cell cancer. In contrast to cervical squamous cell carcinoma, the sequential changes of cervical glandular epithelium leading to the development of adenocarcinoma have not been well documented. Currently, adenocarcinoma in situ (AIS) is widely accepted as the immediate precursor to cervical adenocarcinoma.

### Cervical adenocarcinoma in situ

Cervical AIS tends to occur in women in their 3<sup>th</sup> and 4<sup>th</sup> decades of life. Histologically, it involves the transformation zone of the cervix in the majority of cases and consists of three main cell types: endocervical, endometrioid and intestinal; with endocervical-type lesions being the most common. Lesions containing more than one cell type are not uncommon. Cervical AIS has been found in association with squamous dysplasia and squamous carcinoma in about 50% of cases. On the other hand, AIS is found only in about 5% of cervical HSILs.

Depending on the degree of cellular differentiation, AIS may be classified as well and poorly differentiated. A well-differentiated AIS displays fairly distinctive cellular manifestations permitting its identification in a high percentage of cases, while a poorly differentiated tumor does not have any specific cytological pattern and may be readily mistaken for an invasive adenocarcinoma. (Fig.5.1).

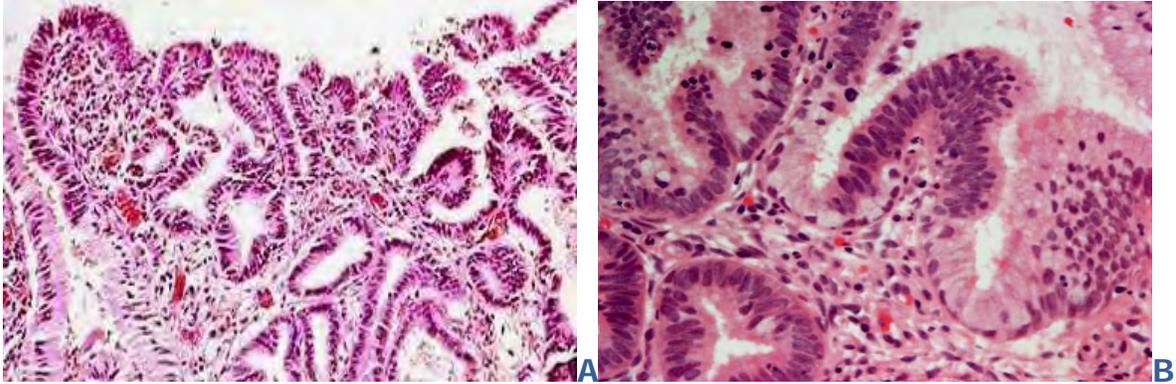


Fig. 5.1. (A, B): Histology of cervical adenocarcinoma in situ.

**Well-differentiated AIS, endocervical cell type** displays the following cytologic features in a CP smear. (Figs.5.2 and 5.3):

- Large sheets of malignant glandular epithelium with crowded columnar tumor cells showing nuclear stratification that is well visualized at the edges of the sheets.
- Short strips of tumor cells with cytoplasm extending off the edges of the tumor cell sheets (feathering).
- Short strips of tumor cells with palisading nuclei.
- Rosettes of tumor cells.
- Isolated tumor cells and cells in papillary clusters may be seen.
- The smear background is free of necrotic debris or tumor diathesis.
- The individual tumor cells are two to three times larger than normal endocervical glandular cells and have enlarged hyperchromatic nuclei with finely or coarsely granular chromatin pattern.
- Nucleoli are absent in about 50% of cases.

Atypical or malignant squamous cells may be seen if there is a coexisting squamous cell lesion that is usually present in about 50% of cases. Each of these features provides a key to an accurate cytodiagnosis which can be made in about 90% of cases.

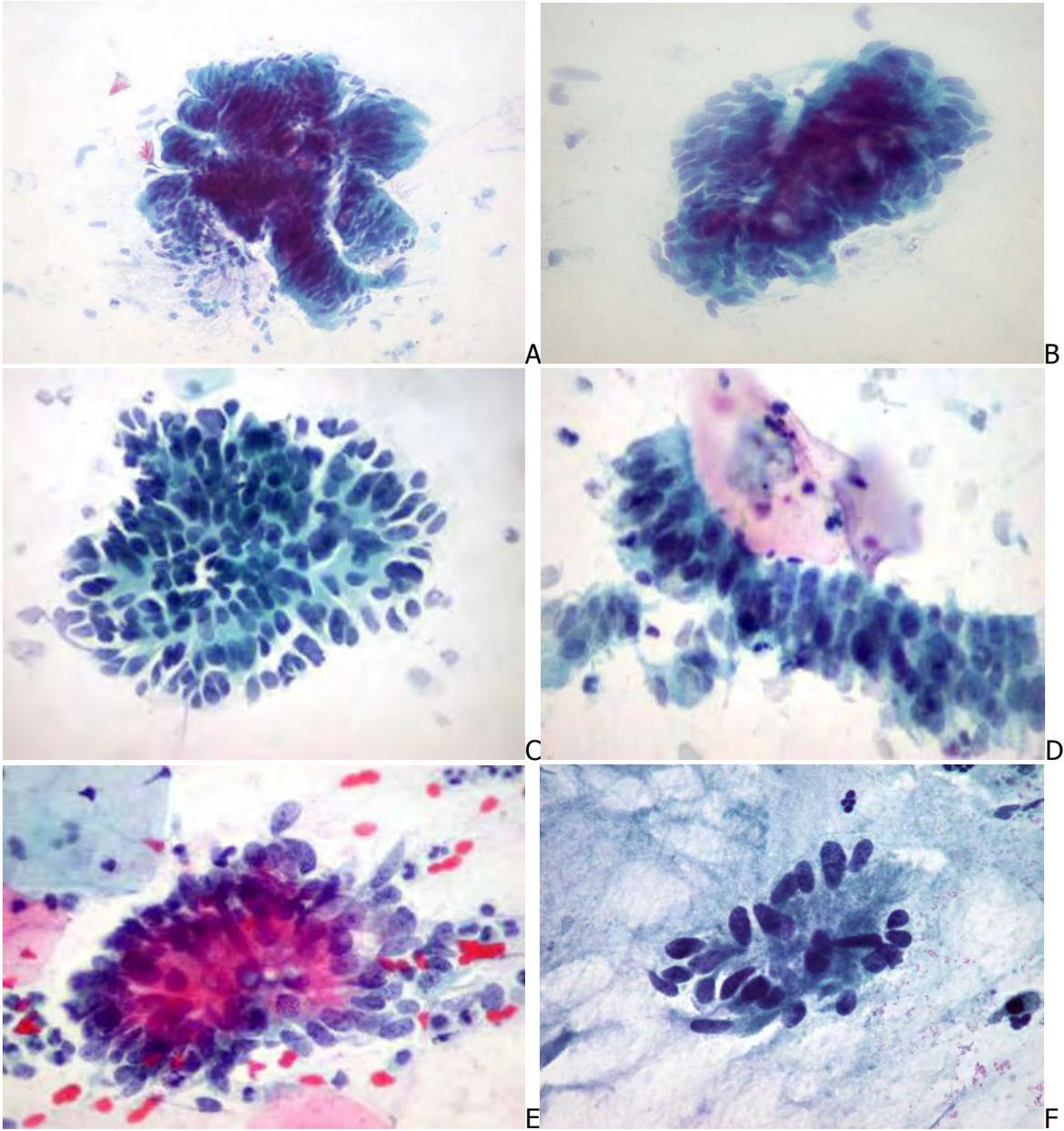


Fig. 5.2. Cytology of cervical AIS in a CP smear, (A-C): Irregular monolayered sheet of tumor cells with cytoplasmic extensions or feathering. (D): Strips of tumor cells with pseudostratified nuclei. (E, F): Tumor cells forming rosettes.

**AIS, intestinal variant** almost always coexists with AIS, endocervical type. Cytologically, the tumor cells are large and occur singly, in clusters and in large epithelial fragments. They show intracytoplasmic mucous vacuoles, resembling colonic epithelial sheets. (Fig.5.4).

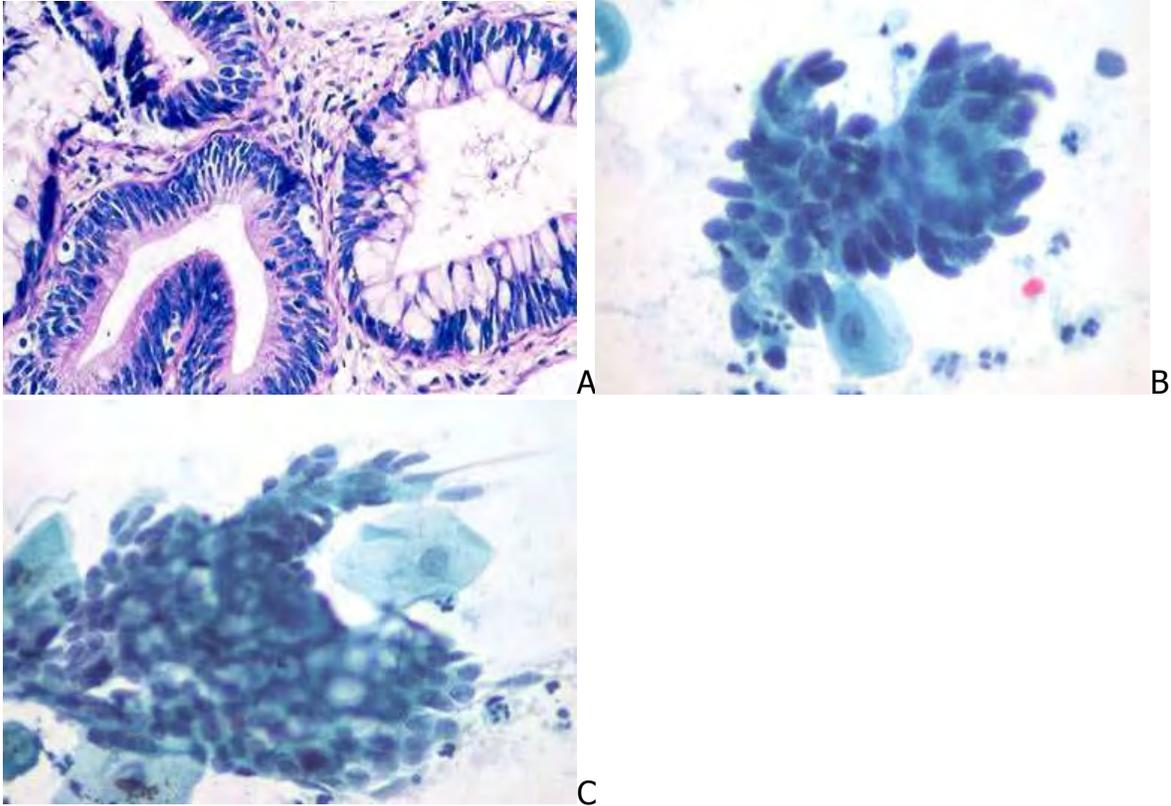


Fig.5.4. Cervical AIS, intestinal variant, (A): Histology of the tumor. (B): Epithelial fragment with elongated nuclei in vague palisade. (C): Epithelial fragment with multiple, round, clear spaces or vacuoles.

### Differential diagnosis

Cells exfoliated from cervical AIS should be distinguished from cells derived from a cervical endometriosis, tubal metaplasia, post cone biopsy smear, reactive endocervical cells and atypical glandular cells:

**Cervical endometriosis** exfoliates cells that may be mistaken for those of cervical AIS. Efforts should be made to look for endometrial glandular fragments and clusters of endometrial stromal cells to avoid a false-positive diagnosis.

**Tubal metaplasia**, a fairly common lesion of the cervix, may yield cell clusters that are readily mistaken for those of AIS. Ciliated cells can be recognized in well-preserved cellular strips. (Fig.5.5). However, loss of cilia due to degenerative changes is not uncommon. An awareness of tubal metaplasia and the potential for cytodiagnostic error is necessary to avoid an unnecessary cone biopsy.

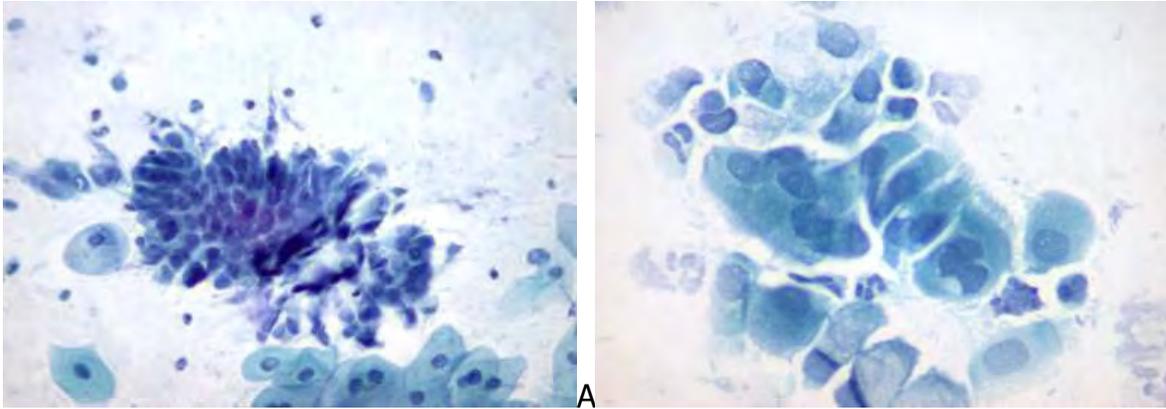
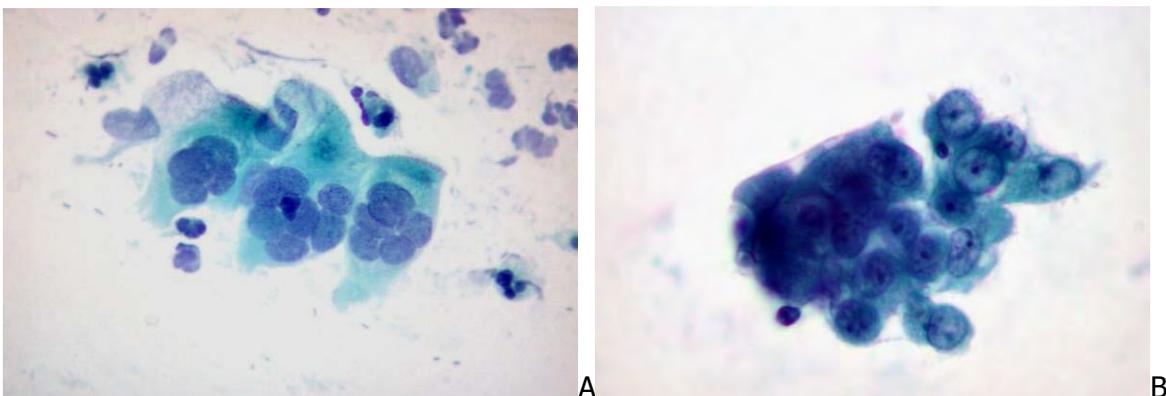


Fig.5.5. (A, B): Tubal metaplasia showing in a CP smear ciliated columnar cell singly and in row.

**Post-cone biopsy smears** may contain fragments of endometrium from lower uterine segment with crowded, hyperchromatic, and pseudostratified nuclei simulating those of cervical AIS. These endometrial epithelial fragments are usually mixed or surrounded by endometrial stromal cells. Clinical data will be helpful in avoiding a false-positive diagnosis in this setting. The reader is referred to Chapter 2 for illustrations of lower uterine segment endometrium.

**Reactive endocervical cells** display the following features:

- Occurring in flat sheets with minimal nuclear crowding
- Fairly abundant cytoplasm with well-defined cytoplasmic borders. (Fig.5.6)
- Slightly enlarged multiple nuclei, fine chromatin and prominent nucleoli
- N/C ratio is within normal limits or slightly increased



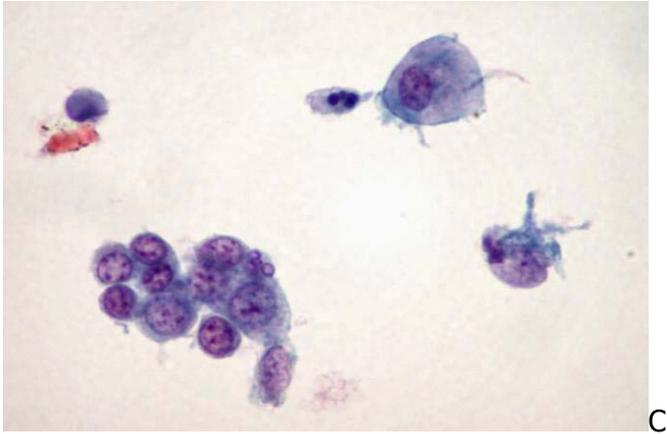


Fig. 5.6. Reactive endocervical glandular cells, (A, B): In CP smear these cells have well-defined cytoplasm and multiple, enlarged nuclei with prominent nucleoli. (C): Similar cells present singly and in small clusters are seen in a LBP.

**Atypical cervical glandular cells.** The reader is referred to the section on Atypical glandular cells below for discussion and illustrations.

## Atypical glandular cells

In TBS-2001 Atypical glandular cells (AGC) are defined as cells showing cellular changes that fall between those of definite benign reactive process and those of an unequivocal AIS or adenocarcinoma. AGCs are divided into 2 subtypes: AGC, NOS and AGC, favor neoplastic. AGCs are further divided into endocervical and endometrial types. AGC accounts for about 0.2% of all Pap tests, with about 30% of patients having a significant cervical lesion: 5% being AIS and adenocarcinoma, and 20% being CINs.

**Atypical endocervical cells, NOS.** The cytologic criteria of AGCs, NOS include:

- AGCs occur in sheets and strips with some cellular crowding (Fig. 5.7)
- Nuclei are enlarged, up to 3 to 5 times the area of normal endocervical nucleus
- Some variation in nuclear size and shape
- Fairly abundant, distinct cytoplasm
- Increased N/C ratio
- Mild nuclear hyperchromasia
- Nucleoli may be present

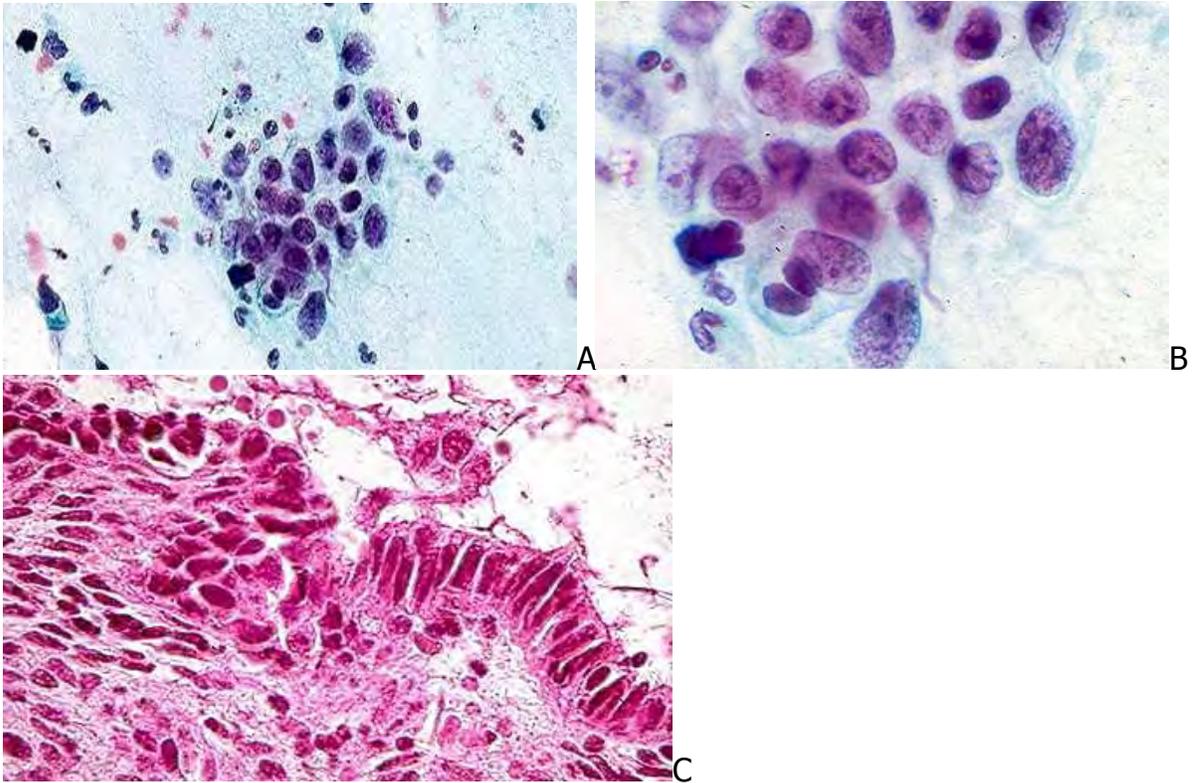


Fig. 5.7. Atypical endocervical glandular cells, NOS, (A, B): In a CP smear, a sheet of atypical endocervical glandular cells displaying enlarged, slightly hyperchromatic nuclei and conspicuous nucleoli. (C): Cervical biopsy in this case revealed atypical endocervical glandular epithelium with no definitive histologic features of an AIS.

**Atypical endocervical cells, favor neoplastic**, by definition, are AGCs with morphologic changes that qualitatively fall short of the cells derived from a cervical invasive or in situ adenocarcinoma. (Figs.5.8 and 5.9). Their cytologic criteria include:

- Cells exfoliate in sheets, clusters and strips with nuclear crowding and overlap
- Rare cell groups with rosette or acinar formation with feathering
- Hyperchromatic, enlarged nuclei
- Increased N/C ratios
- Relatively scant cytoplasm with ill-defined cell borders
- Nucleoli are rarely observed

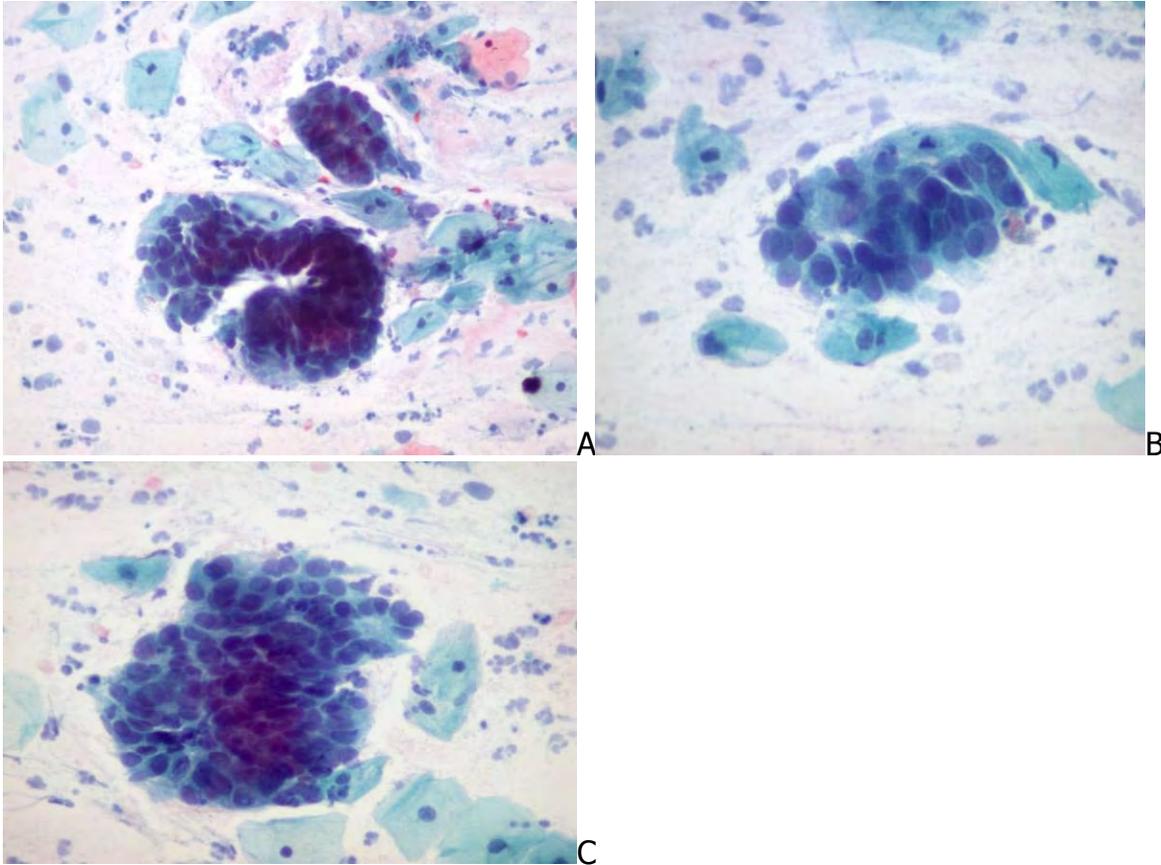


Fig.5.8. Clusters of atypical cervical glandular cells, favor neoplastic displaying nuclear Crowding and overlapping. Nuclei in palisade are seen in (A) and (B).

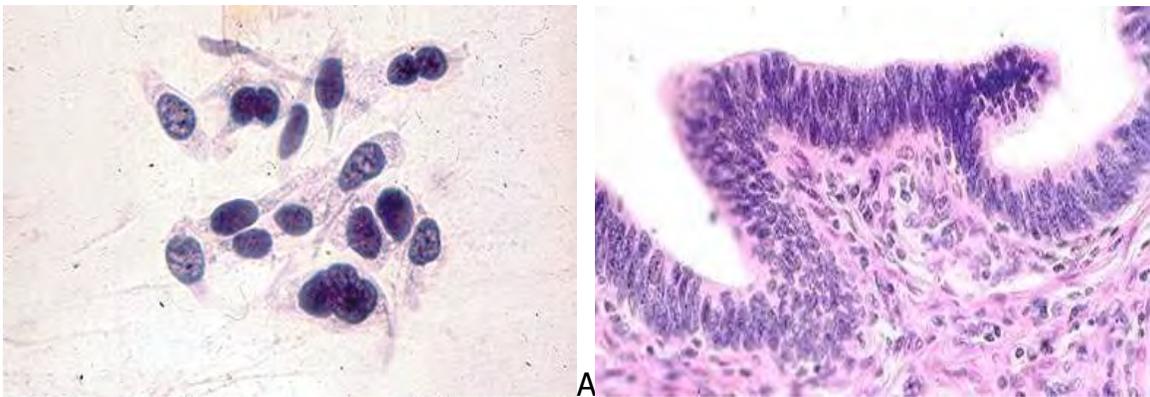


Fig.5.9. (A): CP smear showing atypical endocervical glandular cells, favor neoplastic in a dyshesive cluster. The atypical cells show enlarged, hyperchromatic nuclei and ill-defined cytoplasm. The patient was subsequently found to have AIS on cervical biopsy (B).

**Atypical endometrial glandular cells** have the following cytologic features:

- Small glandular cells present singly or in rounded clusters
- Scant or moderately abundant or vacuolated cytoplasm (Fig.5.10)
- Enlarged, hyperchromatic nuclei with 1 of the 2 additional nuclear changes below:
  - Irregular nuclear contours, or
  - Prominent nucleoli

These cellular changes may also be seen in association with endometrial polyp, chronic endometritis, endometrial hyperplasia and IUDs. The endometrial cell atypias can be difficult to identify because of cellular degeneration. On the other hand, normal endometrial cells exfoliated in menses may display reactive changes with slight nuclear enlargement and pleomorphism or degeneration that could be misinterpreted as abnormal. As about 50% of postmenopausal women shedding atypical endometrial cells have a significant endometrial pathology including hyperplasia with atypia and carcinoma, it is, therefore, more practical to lump all degrees of endometrial cell atypia into one category of "endometrial cell atypia" and rely on histologic examination of endometrial tissue samples for grading of endometrial cell atypias. The presence of a high maturation index of squamous cells or a tumor diathesis also represents a risk for malignancy.

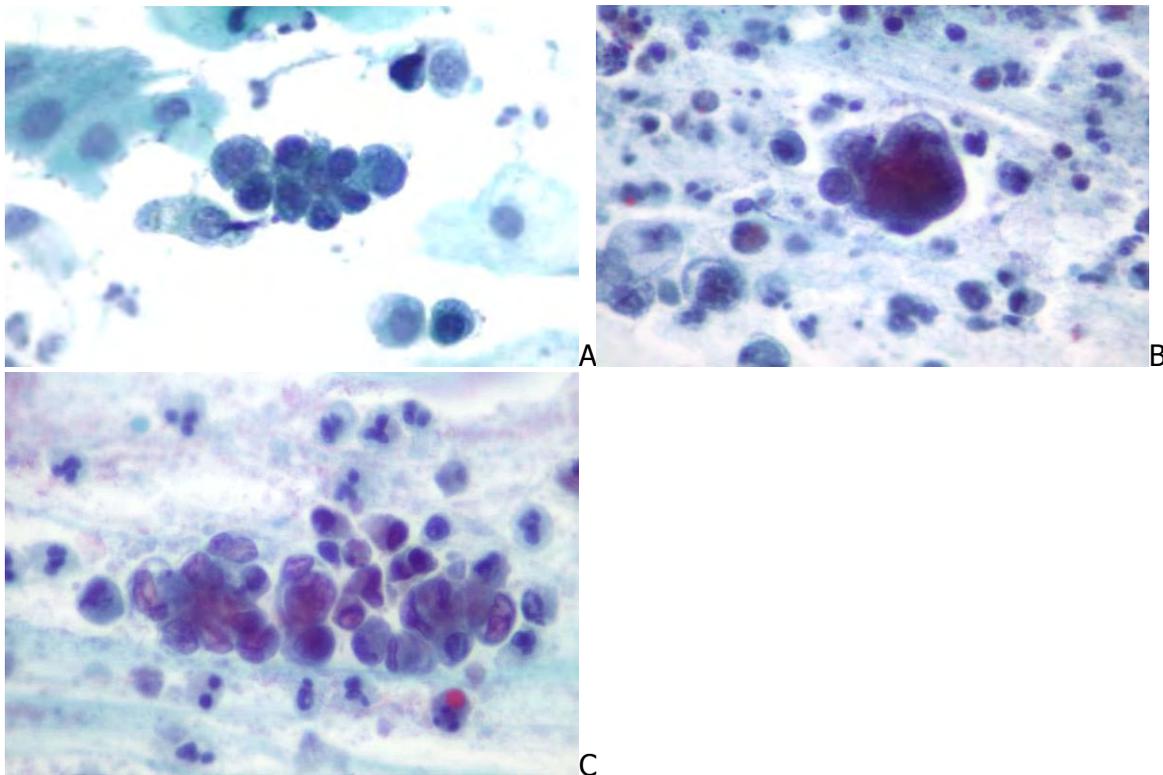


Fig.5.10. (A-C): CP smears showing 3-dimensional clusters of atypical endometrial cells.

## Management of patients with AGC result

Cervical AGCs, favor neoplastic are qualitatively slightly more severe than those of AGCs, NOS. On several occasions AGCs are difficult to distinguish from atypical squamous cells, and in many patients with AGCs on Pap smears, the cervical biopsy revealed a squamous cell lesion or no significant epithelial abnormality. However, according to DeMay, AGCs constitute a high-risk finding that predicts adenocarcinoma in 5% to 10% of cases. Therefore, patients with Pap smears showing persistent AGCs, NOS or AGCs, favor neoplastic should undergo colposcopic examination with biopsy and fractional uterine curettages to rule out a squamous cell lesion of the cervix and glandular neoplasm of the uterus. Testing for oncogenic HPV-DNA may be of diagnostic help, as cervical adenocarcinoma is strongly associated with HPV types 16 and 18.

## Invasive cervical adenocarcinoma

Invasive adenocarcinoma of the cervix occurs more frequently in the 5<sup>th</sup> decade of life and accounts for up to 25% of all cervical cancers. AIS is found at the edge of invasive cervical adenocarcinomas in 43% to 100% of patients. The tumor may be associated with an ovarian mucinous or endometrioid adenocarcinoma. It is p16, HPV16, HPV18, ER and PR positive in almost all cases. It may be well to poorly differentiated, and a mixed pattern consisting of areas of well and poor differentiation is not uncommon. Endocervical mucinous carcinomas are the most common type, accounting for 70% to 90% of cases followed by carcinoma of endometrioid type. Intestinal and signet-ring adenocarcinomas are rarely encountered.

**Well-differentiated cervical adenocarcinoma** has cytologic manifestations similar to those of AIS, endocervical type, previously described.

**Moderately differentiated cervical invasive adenocarcinoma** usually has cytologic manifestations different from those of a well-differentiated tumor. Its cytologic criteria include:

- Abundant malignant glandular cells commonly forming acini, balls, sheets, papillary clusters, strips, rosettes or syncytia. (Fig. 5.11).
- Dyshesive tumor cells are more commonly found (than AIS).
- Oval or pleomorphic nuclei with normo- or hyperchromasia.
- Finely or coarsely granular chromatin.
- Single or multiple micro- or macronucleoli.
- Cytoplasm is variable, ill defined, and rarely vacuolated.
- Tumor diathesis is present in about 30% of cases.
- Dyskaryotic or malignant squamous cells are found in about 20% of cases.

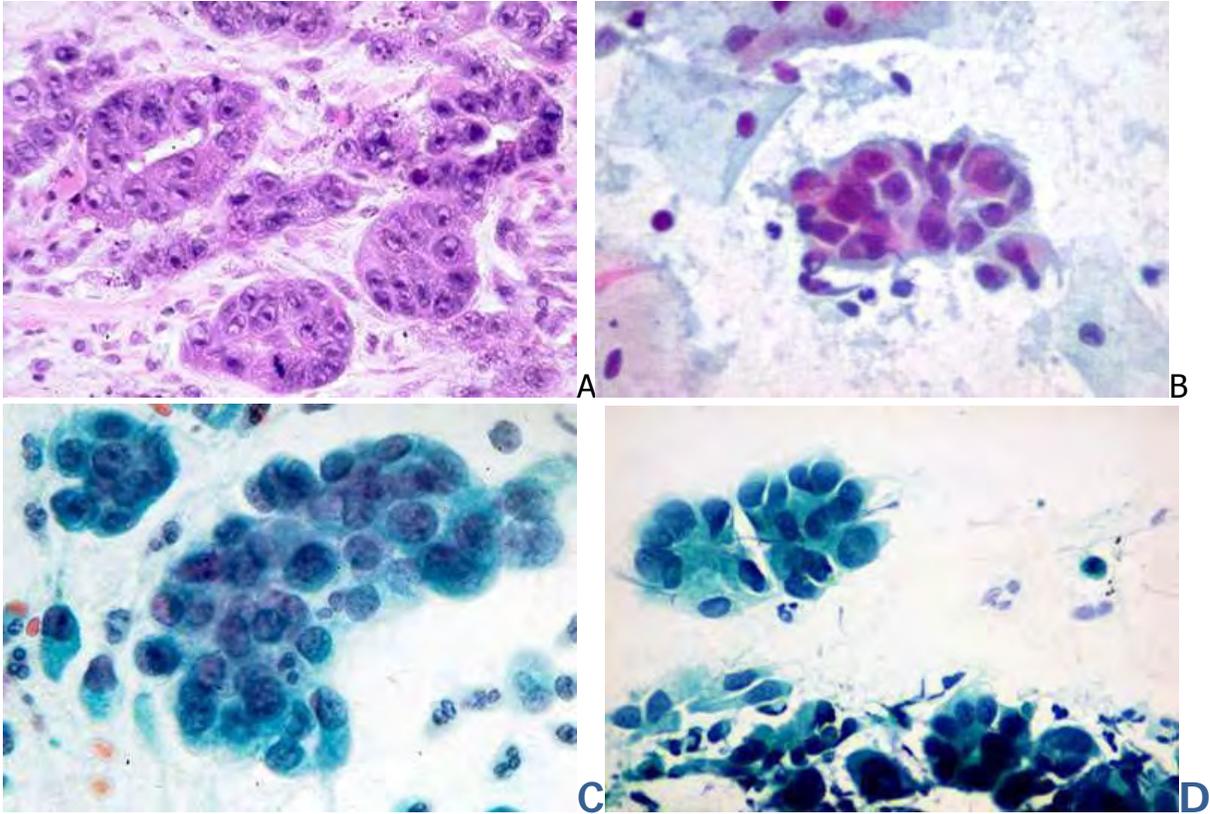


Fig.5.11. (A): Histology of a moderately differentiated cervical adenocarcinoma. (B-D): Clusters of fairly polygonal malignant glandular cells with ill-defined cytoplasm, hyperchromatic nuclei and small or inconspicuous nucleoli in CP smears from 3 cases of moderately differentiated endocervical adenocarcinoma.

**Poorly differentiated cervical adenocarcinoma** exfoliates pleomorphic malignant glandular cells singly and in clusters. The tumor cells display pleomorphic nuclei, ill-defined cytoplasm and prominent nucleoli. (Fig. 5.12).

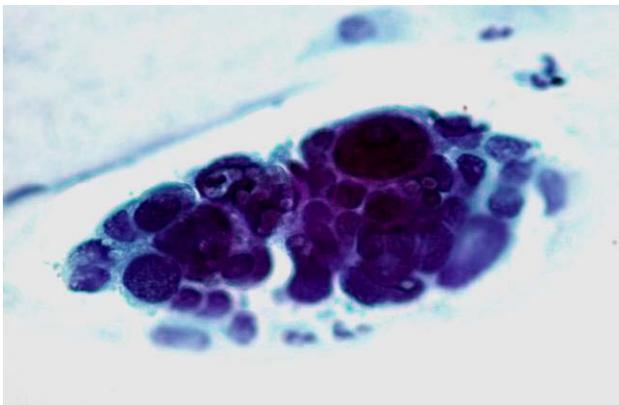


Fig.5.12. Clustered pleomorphic malignant glandular cells in a CP smear from a poorly differentiated cervical adenocarcinoma.

Cells from a cervical adenocarcinoma should be differentiated from cells derived from a reparative epithelium, endometrial adenocarcinoma and extrauterine cancer. The reader is referred to Chapter 2 in this book for discussion and illustration of repair cells.

## Variants of cervical adenocarcinoma

Relatively common variants of cervical adenocarcinoma are mixed adenosquamous carcinoma, serous papillary carcinoma, and clear cell carcinoma. Adenoma malignum and adenoid cystic carcinoma rarely occur in this location.

**Glassy cell carcinoma** is a poorly differentiated adenosquamous cell carcinoma and accounts for 1% to 2% of all cervical cancers. It more commonly occurs in relatively young patients, with a mean age of 41 and is HPV types 16 and 18 positive. It most often appears as a bulky exophytic mass consisting of nests and masses of polygonal cells with granular "glassy" cytoplasm and oval nuclei with prominent nucleoli. Patients with this type of tumor may show blood eosinophilia. In typical cases, the tumor presents in Pap smears as single and clustered large malignant epithelial cells with oval nuclei and prominent nucleoli, similar to those of a nonkeratinizing squamous cell carcinoma. (Fig.5.13). Tumor cells with ground-glass cytoplasm may be found. In other cases the exfoliated tumor cells do not display a ground glass cytoplasm, and a diagnosis of glassy cell carcinoma can be only made by tissue biopsy.

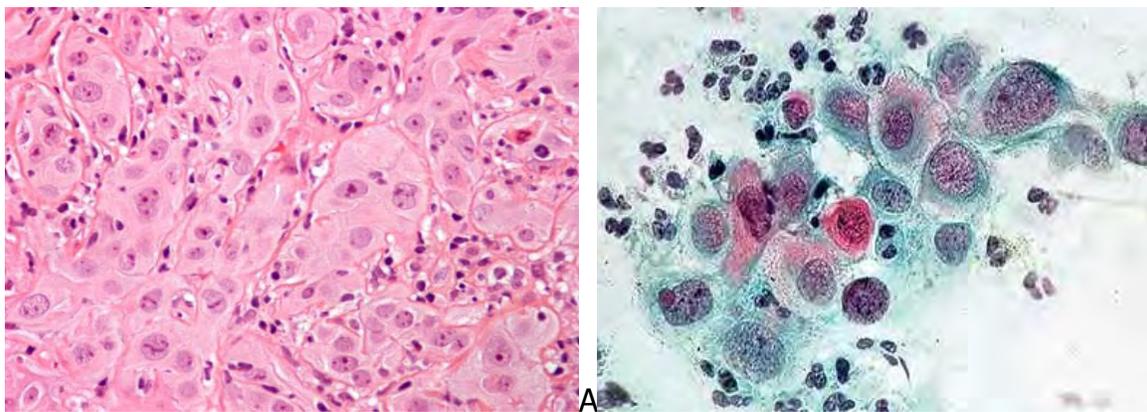


Fig.5.13. Glassy cell carcinoma, (A): Tumor histology showing cells with abundant, homogenous, eosinophilic, "glassy" cytoplasm, oval nuclei and prominent nucleoli. (B): CP smear showing clusters of malignant epithelial cells with abundant, "glassy" or granular cytoplasm and large and oval nuclei. Small nucleoli are noted in some cells.

**Adenoid cystic carcinoma** accounts for about 1% of all cervical adenocarcinomas. The tumor occurs mainly in elderly patients, but it may occur in patients under 50 years of age. The neoplasm commonly forms a polypoid friable mass and consists of small cancer cells forming clusters, cords and trabeculae with lumens containing hyaline eosinophilic material. The neoplasm may be associated with a cervical squamous cell carcinoma. The tumor is HPV type 16 positive and has a poor prognosis. It shows in Pap smears clusters of small cells with scant cytoplasm and small, oval, hyperchromatic nuclei. Globules of basophilic material may be observed. (Fig.5.14).

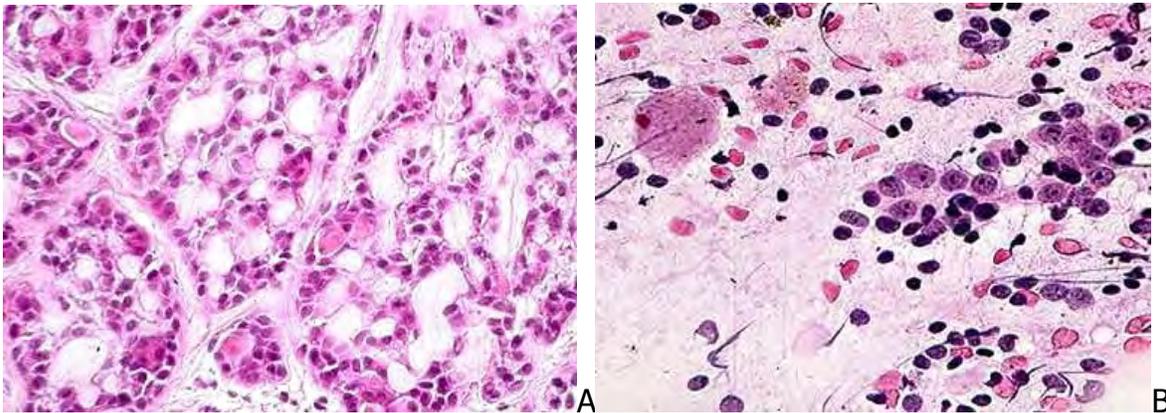


Fig. 5.14. Adenoid cystic carcinoma, (A): Tumor histology showing small tumor cells forming round spaces containing dense, hyaline eosinophilic material. (B): A round hyaline body and smaller clusters or sheets of tumor cells with scant, ill-defined cytoplasm and round nuclei with conspicuous nucleoli seen in a CP smear.

**Minimal deviation adenocarcinoma** is a rare tumor accounting for about 1% of all primary cervical adenocarcinomas. Histologically, it may be divided into 3 types: cervical, endometrioid and non-specific.

- **Cervical minimal deviation adenocarcinoma (MDA), mucinous type** is the most common type, occurring in young women between 32 to 42 years of age. It is usually sporadic but it may rarely occur synchronously or precede an ovarian tumor that is commonly mucinous in nature. The tumor is usually HPV negative and often missed by small cervical biopsy. Due to diagnostic delay, this neoplasm may be diagnosed at a high stage and therefore will have a poor prognosis. In about 50% of cases, foci of moderately or poorly differentiated adenocarcinoma are present. Cervical MDA, mucinous type, yields in Pap smear sheets and clusters of glandular cells with monomorphic nuclei, small nucleoli and clear cytoplasm that may show wispy cytoplasmic extensions or tails. (Fig.5.15).

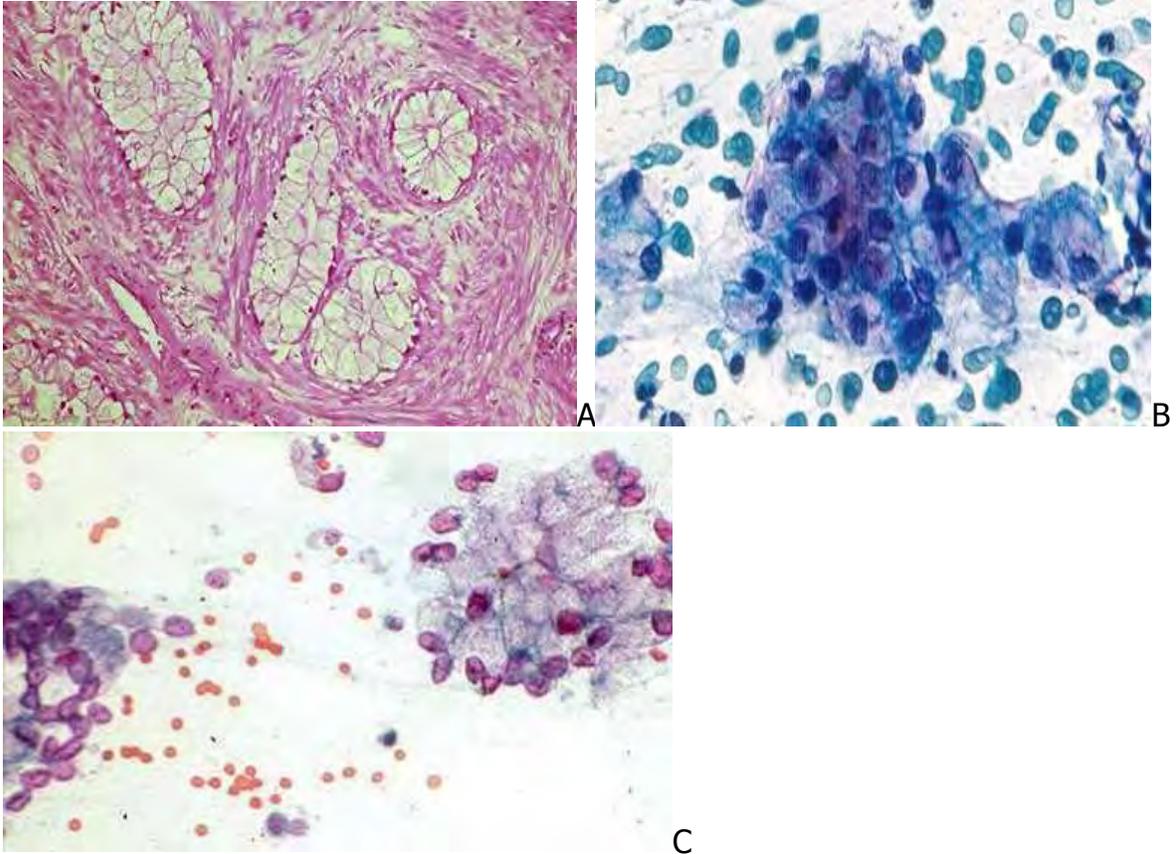


Fig.5.15. Cervical MDA, mucinous type, (A): Histology of the tumor showing invasive mucous glands with small, bland nuclei. (B, C): Cervical MDA showing in a CP smear irregular sheets and clusters of benign-appearing mucus secreting cells.

- **MDA, endometrioid type.** The cytologic manifestations of this neoplasm have recently been reported. This type of tumor exfoliates sheets of columnar glandular cells with low-grade oval nuclei in palisade at free borders. Similar tumor cells forming cell stripes with vague pseudostratified nuclei and rosettes, as seen in cervical AIS, are present. Tumor cells with higher nuclear grade may also be found. (Fig.5.16). Cytologic manifestations of a cervical **MDA, non-specific type** have not been reported so far.

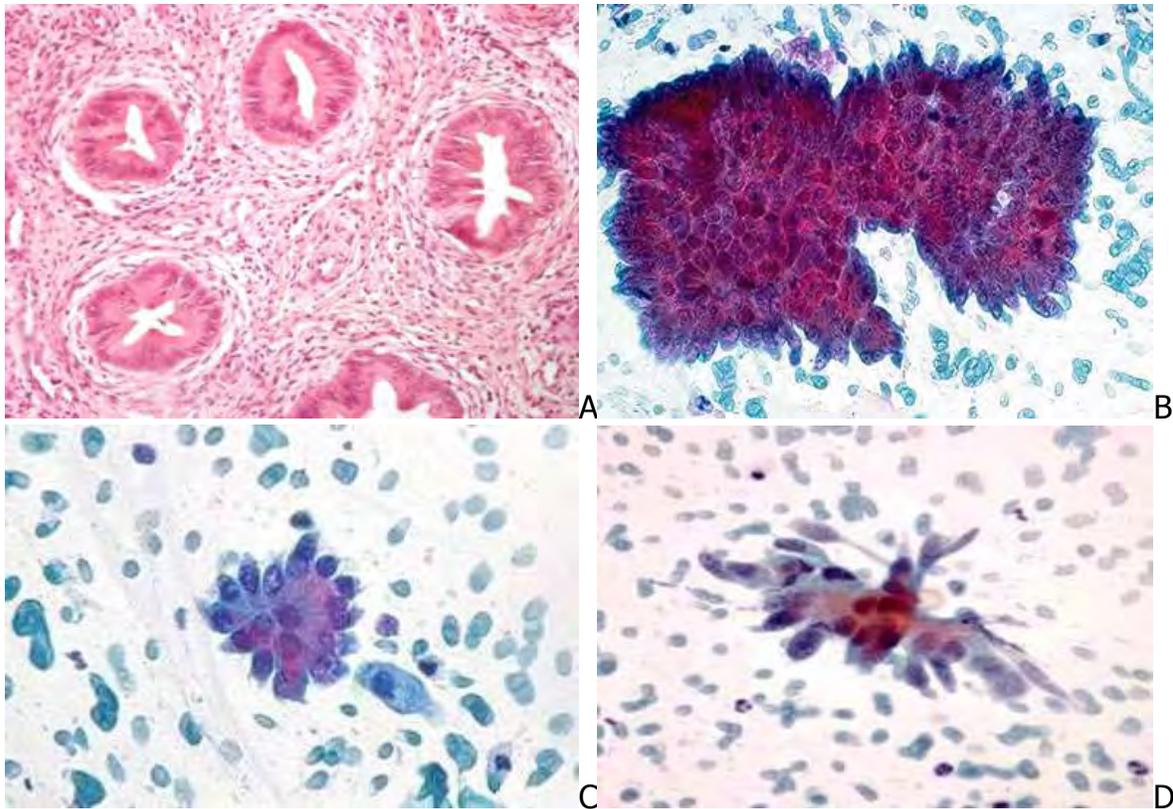


Fig. 5.16. Minimal deviation adenocarcinoma, endometrioid type, (A): Histology of the tumor. B-E. CP smear showing tumor cells in a large sheet with nuclei feathering and in palisade formation at the periphery (B), tumor cells with low-grade nuclei and cytoplasmic "tails" in a rosette and cell strip (C,D).

**Clear cell carcinoma** accounts for about 4% of all cervical adenocarcinomas. About 2/3 of cases occur in young women who had an in utero exposure to diethylstilbestrol (DES). It may occur in older women without DES exposure. In about 50% of cervical clear cell carcinoma related to DES exposure a vaginal adenosis is present. Grossly, the tumor appears as a nodular or an ulcerated lesion. Histologically, it is characterized by a papillary, microcystic, tubular or solid pattern. The tumor cells show a clear or eosinophilic, granular cytoplasm and often have a "hobnail" configuration. The nuclei are oval and show prominent nucleoli. Cervical clear cell carcinoma shows in Pap smears irregular sheets and clusters of epithelial cells with clear, granular or vacuolated cytoplasm and oval nuclei with prominent nucleoli. (Figs. 5.17 and 5.18). The 5- and 10-year survival rates of patients with clear cell carcinoma are 55% and 40%, respectively.

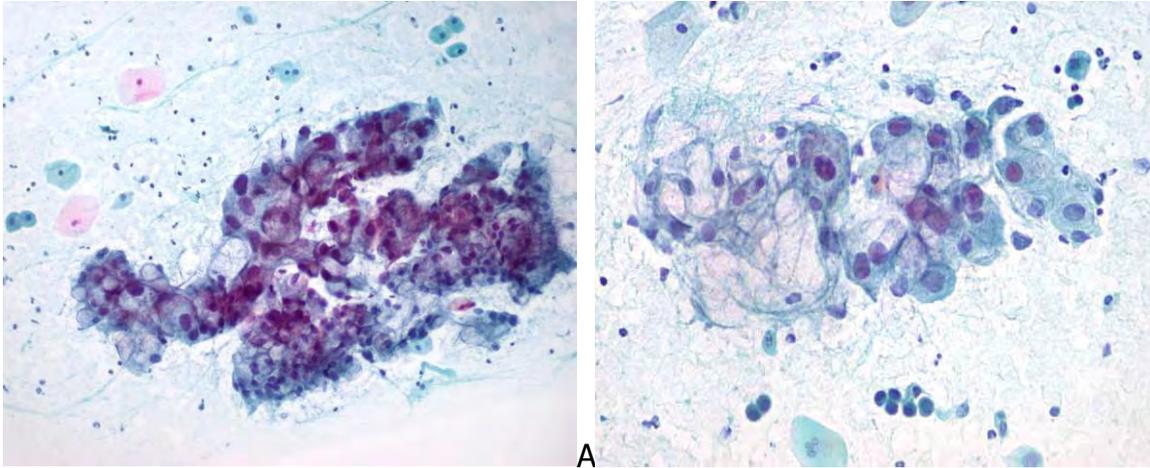


Fig.5.17. (A, B): A CP smear of a case of clear cell carcinoma showing sheets of malignant glandular cells with hyperchromatic, slightly pleomorphic nuclei and clear, vacuolated or granular cytoplasm.

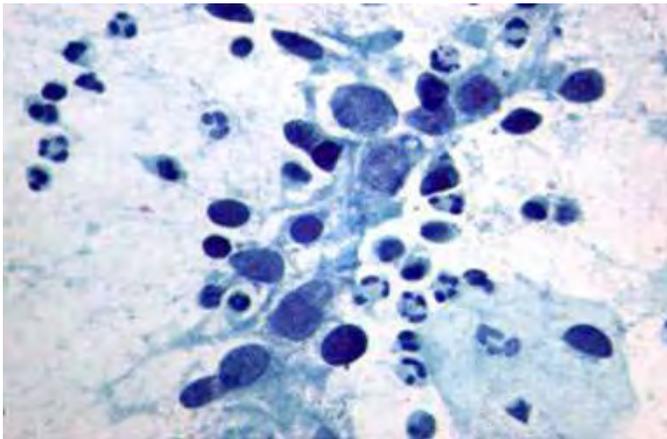


Fig.5.18. Clear cell carcinoma with hobnail pattern showing in CP smear aggregates of tumor cells with prominent, large, hyperchromatic nuclei and ill-defined, granular cytoplasm displaying a vague "bulging" pattern.

**Villoglandular carcinoma** is a rare cervical cancer with low-grade nuclei and an excellent prognosis. It is composed of epithelial papillae with thick fibrovascular cores. It presents in Pap smears as monolayered sheets of malignant epithelial cells with folding and nuclear crowding. The nuclei are oval, hyperchromatic and show inconspicuous nucleoli. (Fig.5.19).

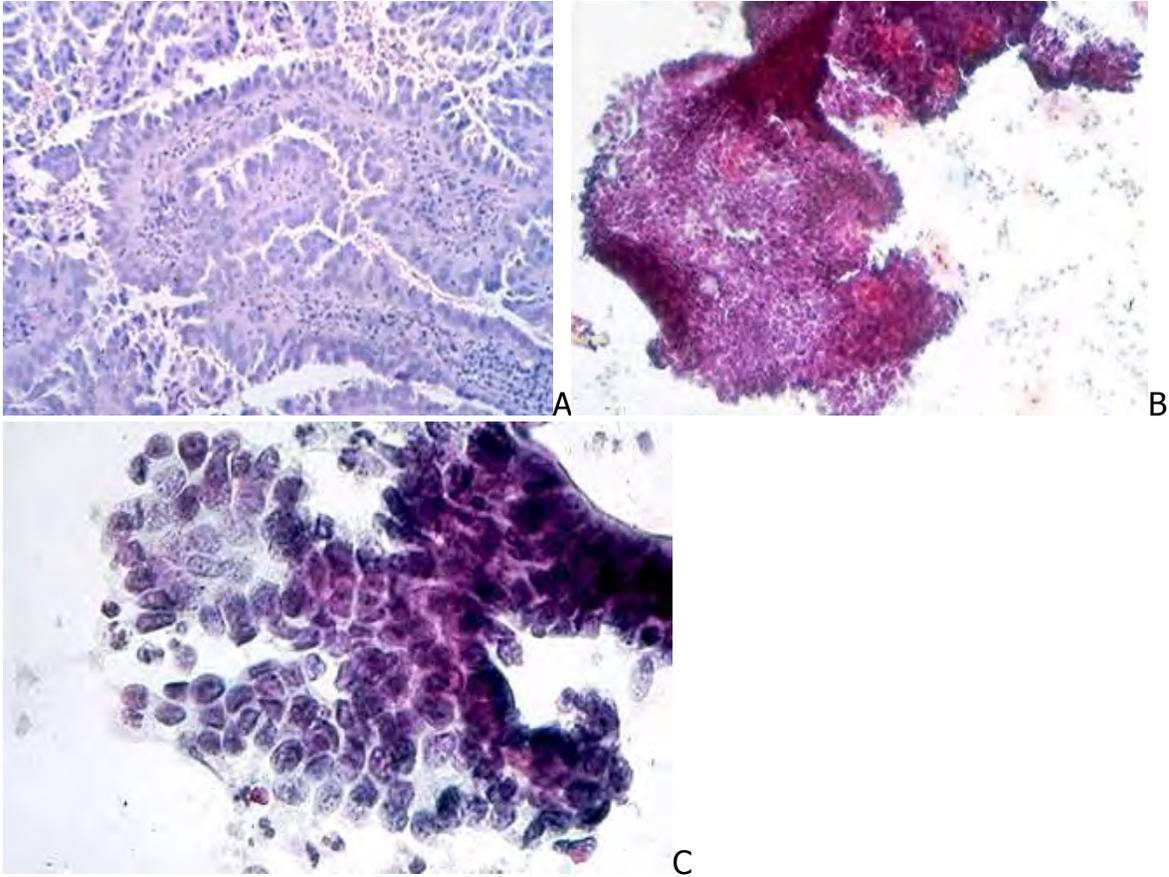


Fig.5.19. Villoglandular carcinoma, (A): Histology of the tumor. (B, C): CP smear showing a large monolayered sheet of tumor epithelium with folding and thick tumor cell clusters showing round, hyperchromatic monomorphic nuclei and inconspicuous nucleoli.

**Papillary serous carcinoma** accounts for about 1% of all cervical carcinomas. The tumor is aggressive and has early pelvic and periaortic lymph node metastases. In some studies, tumors in young patients are HPV positive and those in older patients are HPV negative. Histologically the tumor is characterized by the presence of thin fibrovascular cores and covered by pleomorphic malignant glandular cells with prominent nucleoli. In a Pap smear, it displays tri-dimensional clusters of malignant glandular cells or irregular, monolayered sheets of similar cells. (Fig.5.20).

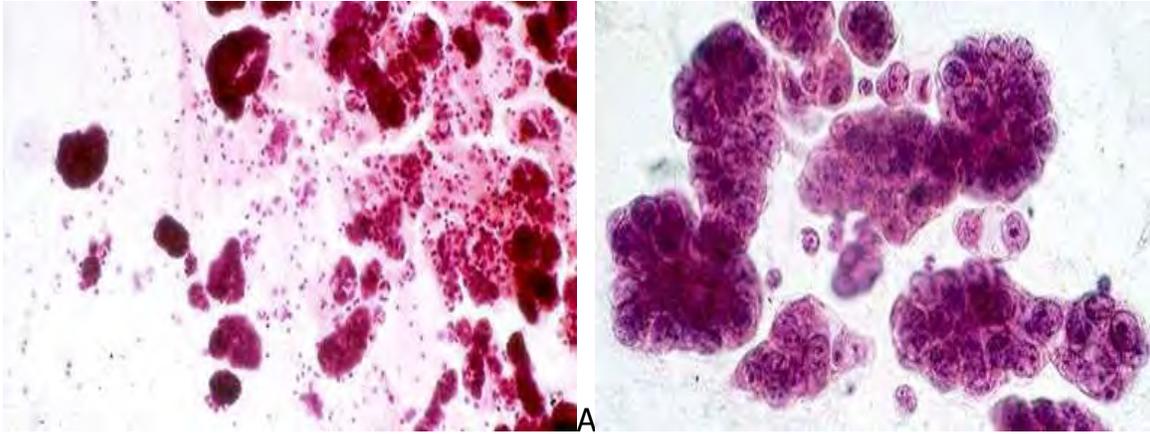


Fig.5.20. (A, B): Papillary serous carcinoma: Tridimensional papillary clusters of tumor cells with prominent nucleoli seen in a CP smear.

## Diagnostic accuracy of cervical adenocarcinoma

Cytodiagnosis of cervical AIS is challenging. In one series consisting of 94 patients with cervical AIS, 65 (69%) cases showed a glandular lesion and 29 (31%) displayed a squamous or unspecified lesion. For pure cervical AISs a diagnostic sensitivity of 40% to 69% has been reported. When a combined lesion consists of AIS and HSIL, the reported rate of detection of glandular cell abnormality was 16% to 23% only, depending on the type of cell preparation (CP smear or LBP). However, if the cell samples were diagnosed as having either an AIS plus HSIL or a HSIL, the sensitivity rates were about 63% and 74%, respectively. The diagnostic accuracy rate of invasive cervical adenocarcinomas in different reported series ranged from 86% to 97.4%. The cytologic detection of cervical adenosquamous carcinomas appears to be more difficult leading to a false-negative rate up to 55%. It is important to note that cervical biopsy is satisfactory for confirming an invasive adenocarcinoma but it is not adequate for diagnosing cervical AIS which requires a deep cone biopsy for histologic confirmation.

## Endometrial adenocarcinoma

Endometrial adenocarcinoma is the most common malignancy of the female genital tract in North America. It occurs mainly in postmenopausal women in their 6<sup>th</sup> and 7<sup>th</sup> decades of life, with an average age of 60 years at the time of diagnosis. However, patients younger than 40 years constitute about 5% of all cases. The tumor may arise from a hyperplastic endometrium or from a normal or atrophic endometrium. The most common clinical manifestation of endometrial carcinoma is an abnormal uterine bleeding. However, about 10% of patients with early disease present with leukorrhea only.

From the cancer screening point of view, **routine Pap smears are not efficient in detecting endometrial carcinomas as the tests fail to detect cancer cells in about 50% of cases.** Cytologic features of endometrial adenocarcinomas in Pap smears include:

- Tumor cells present singly and in small, tight clusters. (Figs.5.21 to 5.24)
- Scant, basophilic and often vacuolated cytoplasm
- Variation in nuclear size and loss of nuclear polarity
- Nuclei with moderate hyperchromasia and irregular chromatin distribution
- Prominent nucleoli with parachromatin clearing
- Increased nuclear and nucleolar sizes are observed with higher tumor grade
- Tumor diathesis variably present

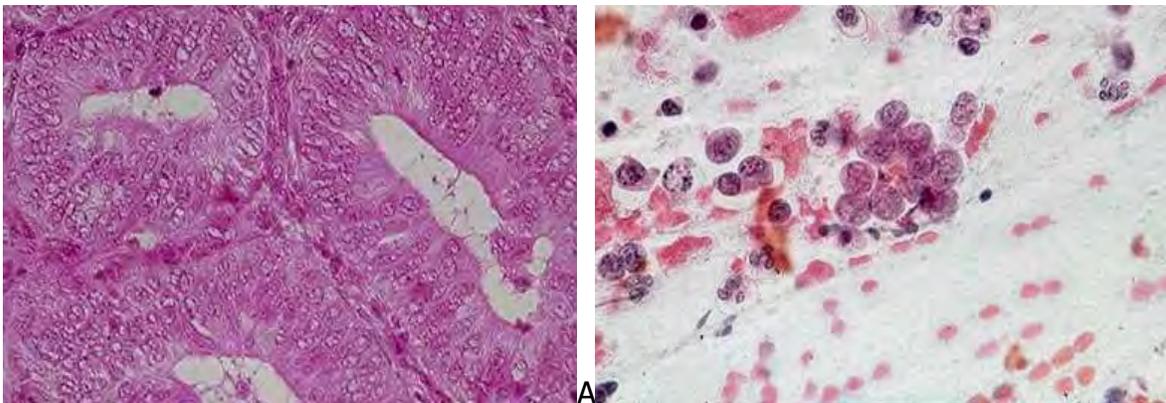


Fig.5.21. Low-grade endometrial adenocarcinoma, (A): Histology of the tumor. (B): CP smear showing clustered monomorphic tumor cells with enlarged, hyperchromatic nuclei and small nucleoli.

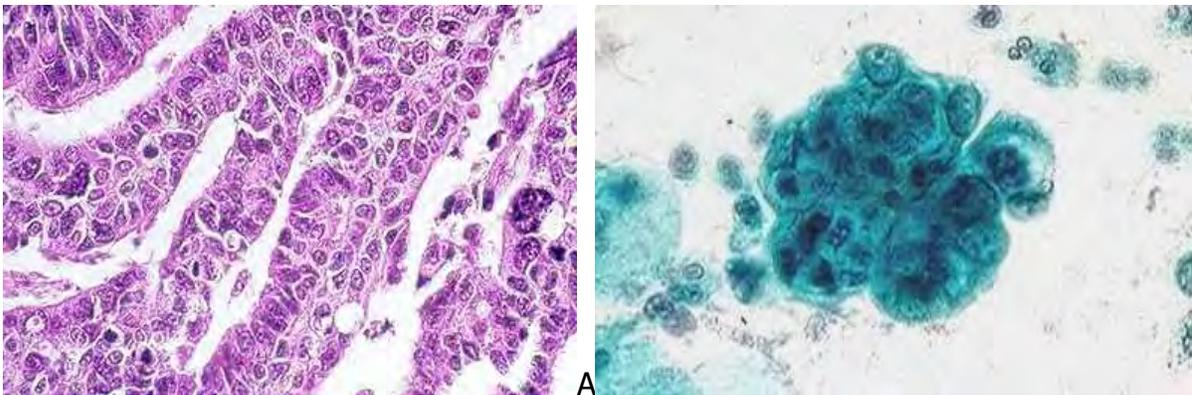


Fig.5.22. Poorly differentiated endometrial adenocarcinoma, (A): Histology of the tumor. (B): CP smear showing a large, cohesive cluster of tumor cells with pleomorphic nuclei, irregular chromatin clumping, parachromatin clearing and prominent nucleoli.

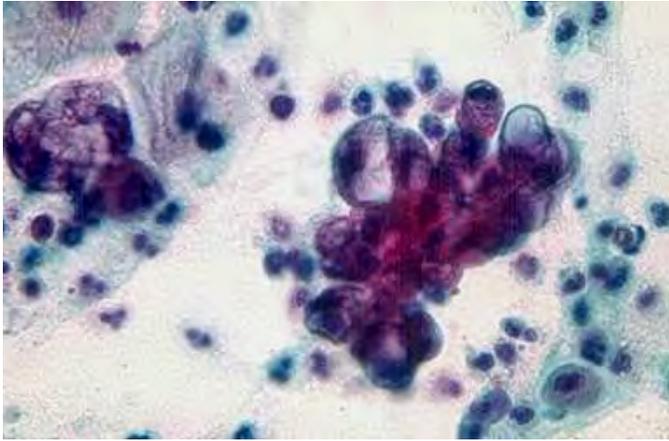


Fig.5.23. Pleomorphic tumor cells with vacuolated cytoplasm in the CP smear of a patient with a poorly differentiated endometrial adenocarcinoma.

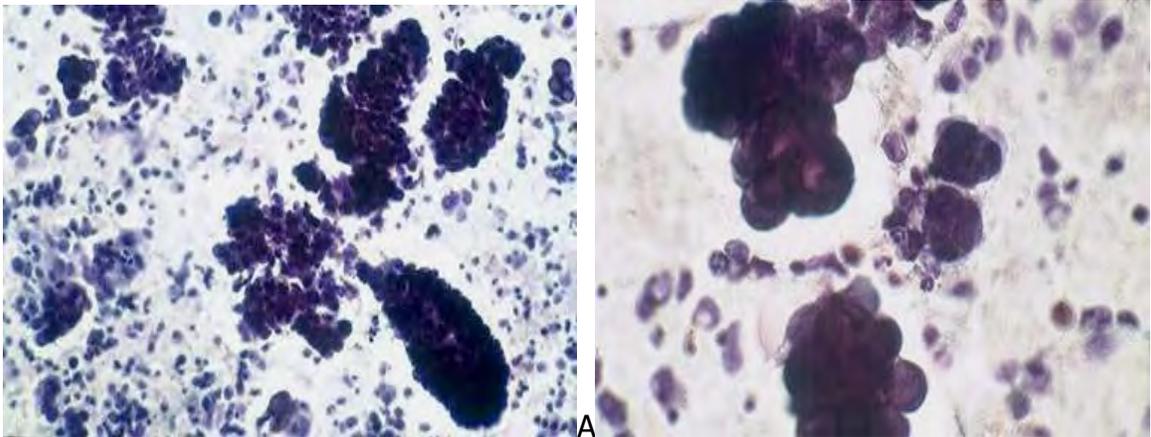


Fig.5.24. (A, B): Papillary serous endometrial carcinoma showing in CP smears abundant 3-dimensional papillary clusters of malignant glandular cells.

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## Pap smear: Other malignant tumors

### Neuroendocrine carcinoma of the cervix

Neuroendocrine carcinoma of the cervix is a rare tumor that may occur alone or in association with a cervical adenocarcinoma of usual type. Histologically, these tumors are classified into 4 subtypes: typical and atypical carcinoid tumors, small cell carcinoma and large cell carcinoma with neuroendocrine differentiation. Carcinoid tumors are rare and highly aggressive with a 3-year survival rate of 12% to 33%. Large neuroendocrine carcinoma is also a very rare tumor.

**Typical carcinoid tumor** yields, in a CP smear, single and loosely clustered oval cells with plasmacytoid configuration and oval nuclei with chromatin clumping. (Fig. 6.1).

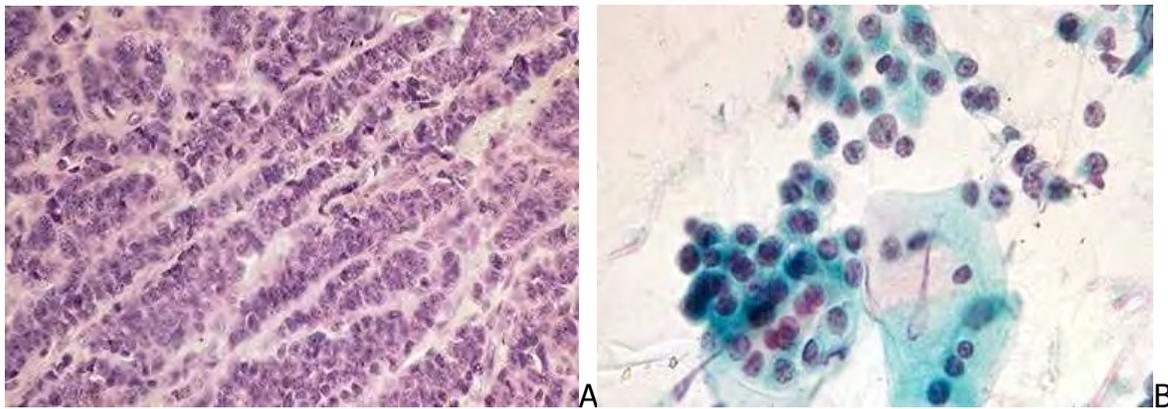


Fig.6.1. Typical carcinoid tumor, (A): Histology of the tumor. (B): CP smear showing single and loosely clustered oval or polygonal tumor cells with eccentrically located nuclei.

**Small-cell carcinoma** (oat cell carcinoma) of the cervix is an aggressive neoplasm, accounts for 2% to 5% of all cervical cancers and is strongly associated with HPV type 18. It is occasionally associated with Cushing syndrome or symptoms of other peptide hormones. It rarely coexists with SIL and its 5-year survival rate is 30% to 40%. It is histologically similar to small cell lung cancer and it yields in Pap smears small cancer cells with hyperchromatic nuclei with "salt and pepper" chromatin, nuclear molding and linear basophilic nuclear debris. (Fig.6.2).

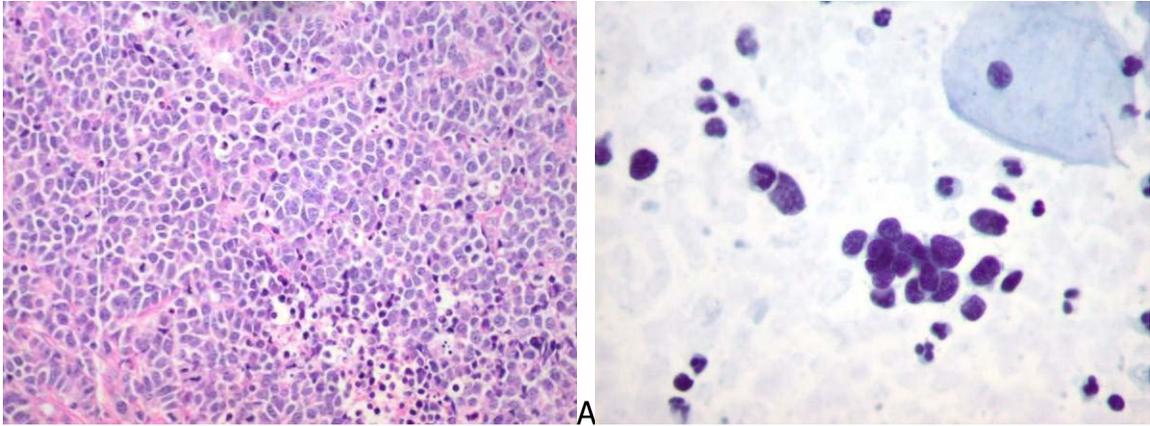


Fig.6.2. Small cell carcinoma, (A): Histology of the tumor. (B): CP smear showing single and clustered tumor cells showing scant cytoplasm, round hyperchromatic nuclei with nuclear molding.

### Endometrial stromal sarcoma

Cervical and endometrial stromal sarcomas rare neoplasms. These tumors occur mainly in postmenopausal women. It may be classified as low- or high-grade, depending on the degree of cellular atypia and the number of mitotic figures. The cytologic manifestations of the tumor in vaginal pool smears and direct endometrial samples are similar and consist of numerous single or loosely clustered malignant round cells with oval or pleomorphic, hyperchromatic nuclei and scant, ill-defined cytoplasm. (Fig.6.3).

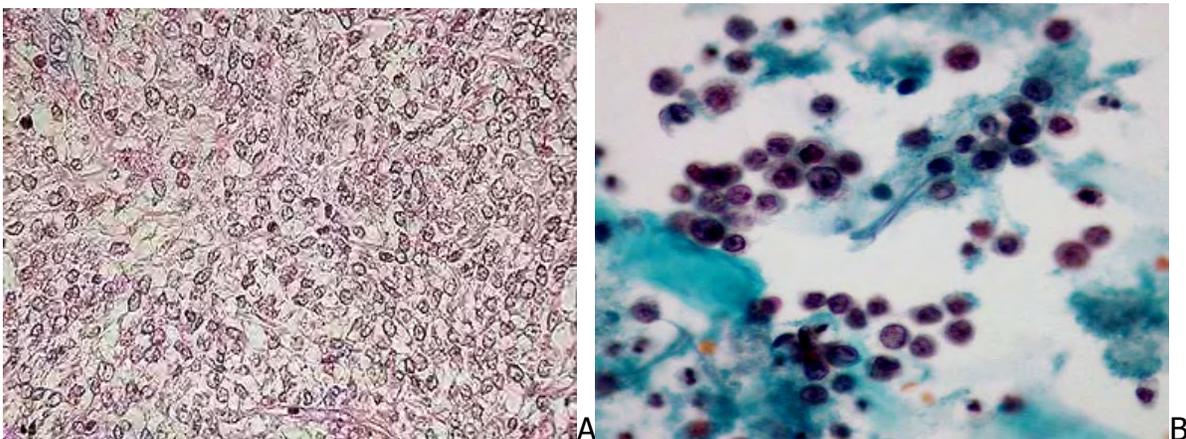


Fig.6.3. Low-grade endometrial stromal sarcoma, (A): Tumor histology. (B): Single and loosely clustered round tumor cells in a direct endometrial sample.

## Malignant mixed müllerian tumor

Malignant mixed müllerian tumor (MMMT) is a rare tumor arising more commonly in the endometrium than in the cervix. It occurs predominantly in postmenopausal women in the 6<sup>th</sup> or 7<sup>th</sup> decade of life, with up to 30% having a history of exposure to radiation. Histologically, the tumor is classified as homologous or heterologous. In most patients with endometrial MMMT only malignant glandular cells are seen in Pap smears and neoplastic stromal cells, and/or mesenchymal cells are rarely observed. (Fig.6.4).

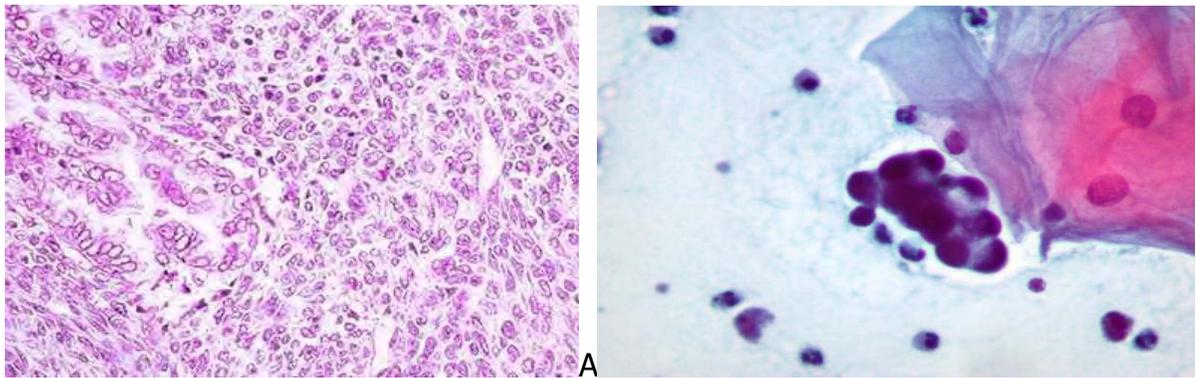


Fig.6.4. Homologous MMMT, (A): Histology of the tumor. (B): CP smear showing a cohesive cluster of monomorphic malignant glandular cells.

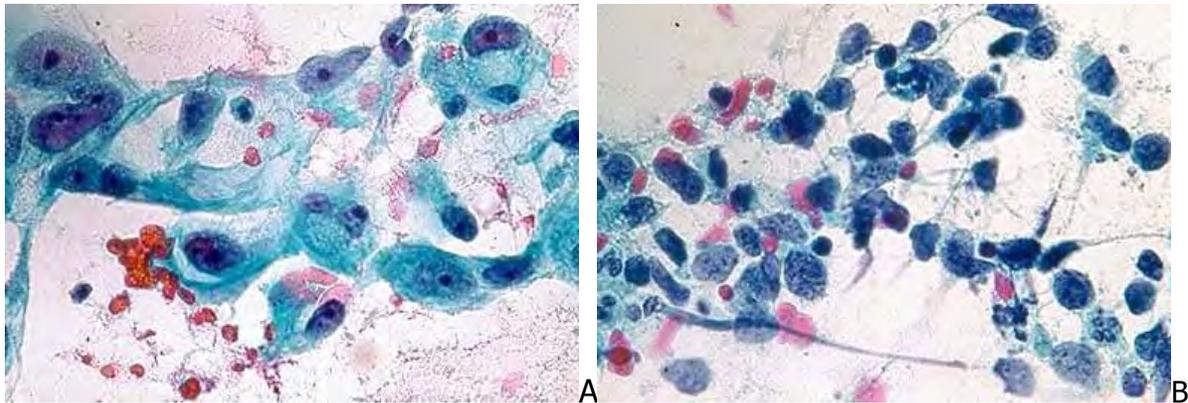


Fig.6.5. Homologous MMMT, (A, B): Malignant glandular/epithelial cells with prominent nuclei (A), malignant stromal cells present singly and in syncytial cluster (B).

## Nonepithelial malignant tumors of the vagina

Primary nonepithelial cancers of the vagina are rare neoplasms. Of these **melanoma** is the most common one in adult patients. In Pap smears it presents as single and loosely clustered pleomorphic malignant cells with variable cytoplasm that may

contain intracytoplasmic melanin pigment granules. (Fig.6.6). When the tumor is amelanotic, a positive cytoplasmic reaction to S-100 protein, HMB-45 and MART-1 antibodies will confirm the diagnosis of melanoma.

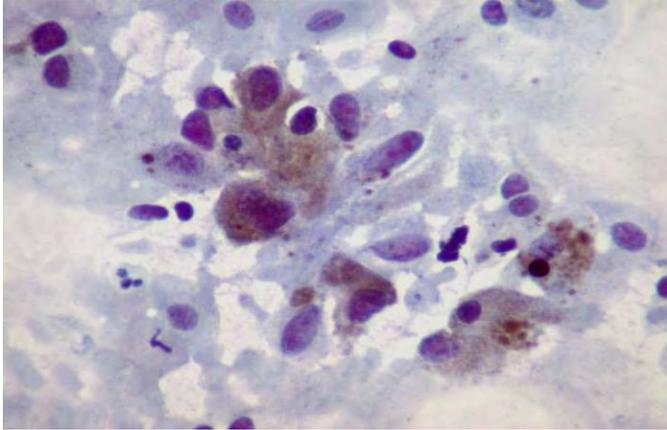


Fig.6.6. Vaginal melanotic melanoma showing in CP smear loosely clustered malignant cells with intracytoplasmic brownish melanin pigment granules.

## Metastatic cancer

Almost all **adenocarcinomas** arising from extragenital organs can metastasize to the uterine cervix. Of these tumors, carcinomas of the breast, stomach and colon are the most common primaries while those arising from other anatomic sites such as the lung, pancreas, bladder, liver, kidney and gallbladder are sporadic. Regardless of the primary site, nearly 90% of women with metastasis to the cervix have evidence of a disseminated cancer, and the most common symptom is vaginal bleeding, occurring in 75% of patients.

The cytologic manifestations of metastatic adenocarcinomas in Pap smears are rather distinctive and different from those of a usual primary endocervical adenocarcinoma. A metastatic moderately differentiated colonic adenocarcinoma yields abundant necrotic debris and irregular sheets of glandular cells with elongated nuclei in palisade at free borders and syncytial tumor cell clusters. (Fig.6.7). Metastatic mammary duct carcinoma to the cervix yields malignant glandular cells in clusters and in Indian file arrangement. (Fig.6.8).

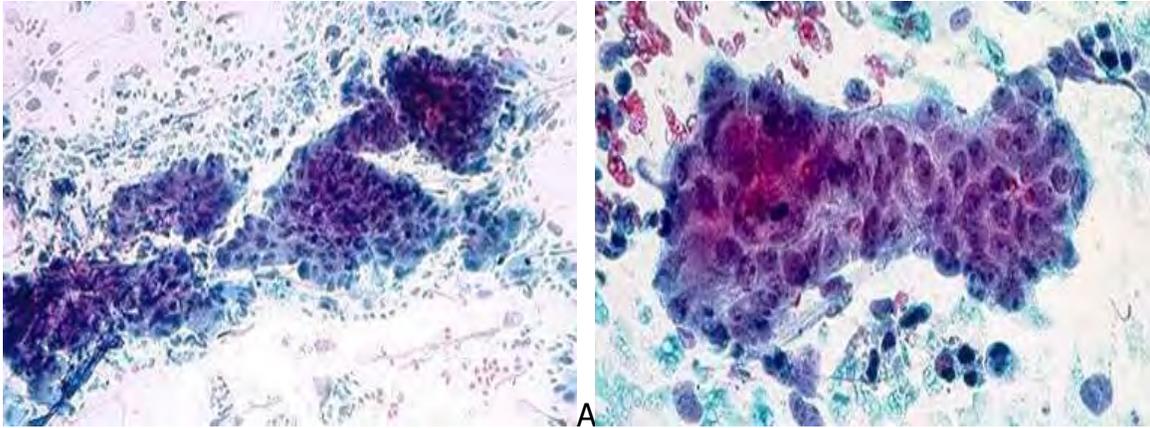


Fig. 6.7. Metastatic well-differentiated colonic adenocarcinoma to the cervix showing in CP smear irregular large sheets of tumor cells with cells at the periphery arranged in picket-fence (A, B). A large amount of necrotic debris is present.

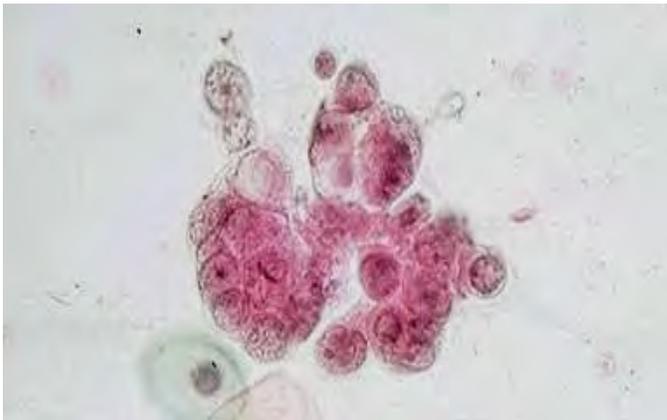


Fig. 6.8. Metastatic breast carcinoma showing in CP smear an irregular cluster of malignant glandular cells with conspicuous nucleoli.

**Metastatic cancers to the uterine corpus** are rare, with breast, gastrointestinal and kidney being the most common primary carcinomas followed by cutaneous melanoma. They may involve the myometrium but it may first appear in endometrial curettings, particularly in the case of breast lobular carcinoma. Their neoplastic cells may be seen in cell samples collected from the posterior vaginal fornix.

**Secondary tumors of the vagina** may occur by direct extension or via hematogenous or lymphatic spread. The most common primary cancer sites are cervix, endometrium, ovary, colon and urinary bladder. These neoplasms are usually submucosal and may be diagnosed by transvaginal fine needle aspiration cytology.

## Extrauterine cancer

**Extrauterine cancers involving the abdominal peritoneum** may traverse through the fallopian tubes and the uterine cavity to accumulate in the posterior vaginal fornix. A vaginal smear in this case may reveal tumor cells in irregular, tight tridimensional clusters. The smear background is more commonly free of tumor diathesis. The presence of psammoma bodies should alert the observer to the possibility of a papillary serous ovarian carcinoma, and effort should be made to identify psammoma bodies surrounded by malignant epithelial cells to confirm the diagnosis. However it should be born in mind that psammoma bodies may be seen in cervicovaginal cell sample in patients without intra-abdominal cancer and in women with IUD. (Figs. 6.9 and 6.10).

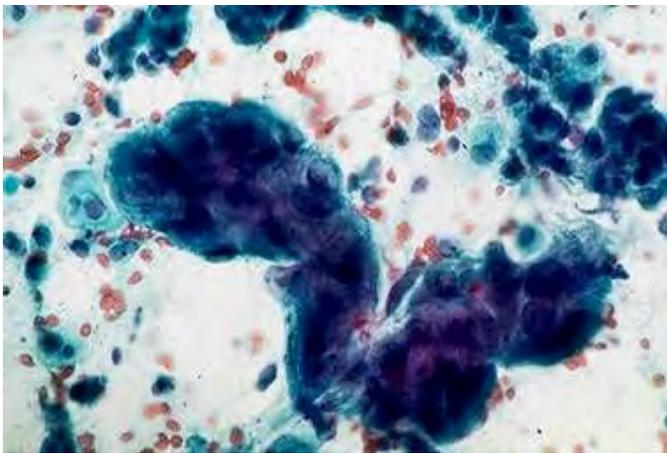


Fig.6.9. Papillary serous ovarian carcinoma showing in CP smear thick tridimensional clusters of malignant glandular cells in a clean smear background.

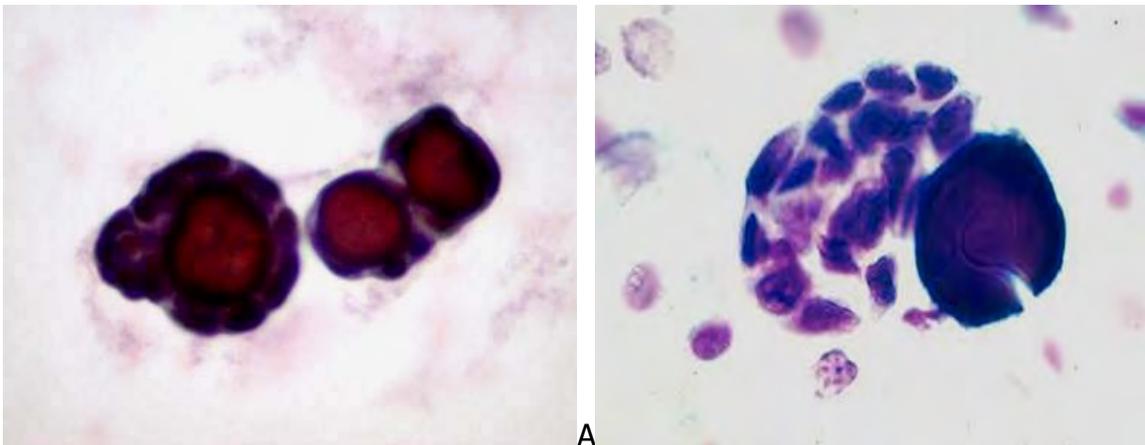


Fig. 6.10. Ovarian carcinoma, (A): Borderline ovarian carcinoma showing in CP smear 3 psammoma bodies surrounded by low-grade epithelial tumor cells in a clean background. (B): High-grade ovarian serous carcinoma showing in CP smear fairly pleomorphic malignant cells partially surrounding a psammoma body.

**Primary fallopian tube carcinoma** is a very rare neoplasm. Most patients are postmenopausal and nulliparous. Abnormal vaginal discharge or bleeding is a common clinical manifestation. Histologically, the cancer is of papillary serous type. It may shed cancer cells into the uterine cavity and these cells may be accumulated in the posterior vaginal fornix. The tumor cells commonly display features of a serous carcinoma and often have a vacuolated cytoplasm. (Fig.6.11). Psammoma bodies may be observed. A fallopian tube cancer should be suspected in an adult woman who shows malignant glandular cells in her Pap test and who also has a negative cone biopsy and endometrial curettage and who has no known primary cancer. Fallopian tube carcinoma may also be diagnosed cytologically by laparoscopic FNA.

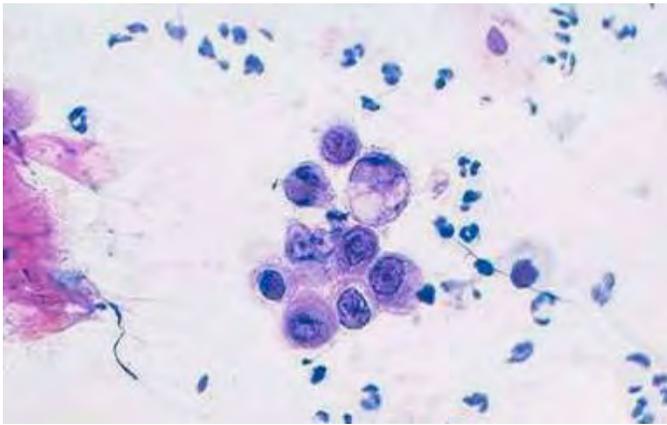


Fig.6.11. A fallopian adenocarcinoma showing in posterior vaginal fornix CP a loose cluster of malignant epithelial cells with vacuolated or granular cytoplasm and prominent nucleoli. (Pap).

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## Anal Pap smear: Anal-rectal cytology

### General considerations

Anal squamous cell carcinoma (ASCA) is a rare disease, has an annual incidence of about 1 in 100,000 population and is more common in women than in men. The tumor incidence has increased in recent years, in particular in homosexual men. Among the individuals in this group, the incidence of ASCA in HIV positive patients is estimated to be twice higher than in HIV negative patients. Anal intraepithelial neoplasia (AIN) is present in 30% to 40% of homosexual men, and progression from AIN 1 and 2 to AIN 3 is uncommon, as is progression from AIN 3 to invasive cancer. ASCA and AIN are strongly associated with HPV infection. The presence of the HPV genome, as demonstrated by polymerase chain reaction, was identified in 80-85% of cases.

Screening with anal-rectal cytology or anal Pap test proved to be cost-effective in detecting AIN in HIV-infected individuals. Anal cytology has a low sensitivity and specificity for AIN lesions with poor detection of high-grade lesions, and the grades of lesions on anal cytology do not always correspond to their histologic grades. The presence of any abnormal anal cytologic finding indicates a potential for HSIL on biopsied tissue. A diagnosis of ASC-H or HSIL accurately predict the presence of AIN 2 and 3 in 90% of cases while a diagnosis of ASC-US and LSIL has a 46% to 56% chance to be associated with a high-grade AIN on biopsy. Therefore, it is recommended that all HIV patients with abnormal anal Pap tests (ASC-US and above) should be evaluated with high-resolution anoscopy with biopsy to detect high-grade AIN.

### Sampling method

The target of sampling is the entire anal canal including the keratinized and nonkeratinized portions and the transformation zone that is an analogue to the T-zone in the uterine cervix in women and that is thought to be highly susceptible to HPV infection. The cell samples are usually collected without direct visualization of the anal canal, using a Dacron fiber swab, cotton swab and cytobrush. Both CP smears and LBPs are used for cytologic evaluation of anal lesions. The LBP has fewer factors that can interfere with the cytologic evaluation such as air-drying and mechanical artifacts and fecal materials.

## Adequacy

Experts involved in anal cytology recommended a minimal cellularity adequacy of 2000 to 3000 nucleated squamous cells for a CP smear. This is translated to an equivalence of 1 or 2 nucleated squamous cells per high-power field for ThinPrep slides.

## Cytologic findings/Results

Normal cellular elements include anucleated squamous cells, nucleated squamous cells, squamous metaplastic cells and rectal columnar cells. (Fig.7.1). Cell samples showing poor preservation and marked contamination with fecal material are regarded as unsatisfactory for evaluation. The anal cell samples reflect anal lesions that are predominantly keratinizing in type.

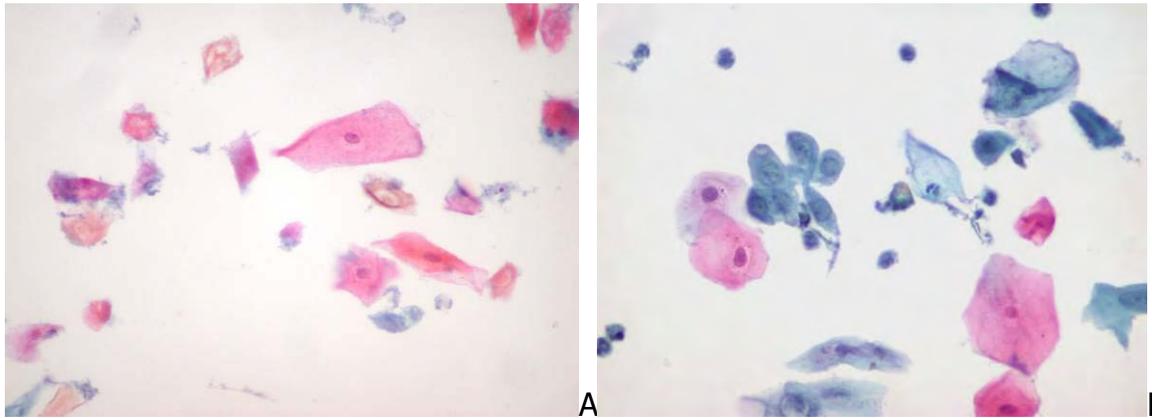


Fig.7.1. Anal Pap smear: Negative for intraepithelial lesions or malignancy: note the clean background and the presence of many keratinized squamous cells, anucleated squames in (A) and a group of metaplastic squamous cells in (B). (LBP).

The Bethesda System terminologies are generally used to report anal lesions. And the cytologic criteria for HPV-related anal lesions are essentially similar to those of the uterine cervix. Some examples of LSIL, HSIL, ASC-US and ASC-H are illustrated below. It is important to note that typical koilocytes are infrequently observed in anal Pap smears. (Figs. 7.2 to 7.4).

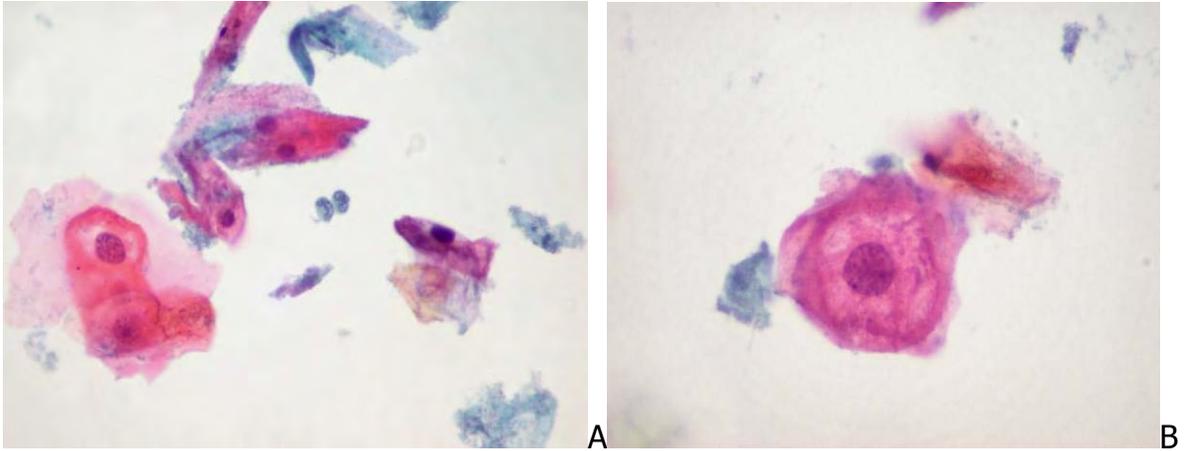


Fig.7.2. Anal Pap smear: (A, B) LSIL cells. (LBP).

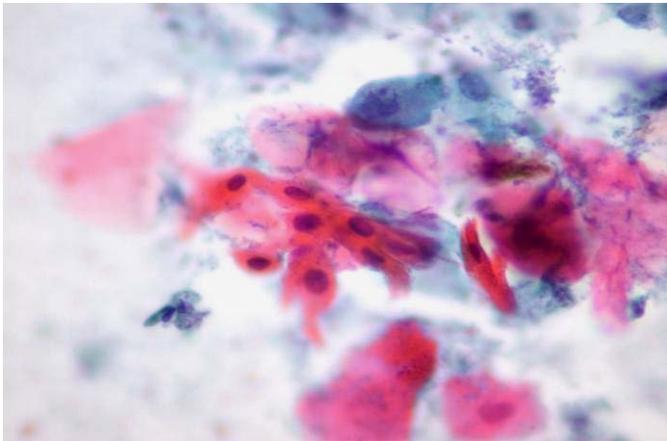


Fig.7.3. Anal Pap smear: ASC-H cells. (LBP).

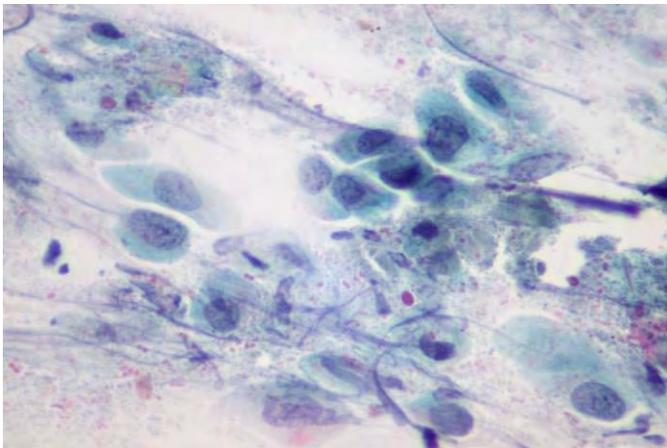


Fig.7.4. Anal Pap smear: HSIL cells. (CP).

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## Breast cytology: An overview

Cytologic evaluation of breast lesions by fine-needle aspiration (FNA) and examination of nipple discharge (ND) is now practiced worldwide. Ductal lavage, a special technique for investigation of small intraductal epithelial lesions, is available only in a small number of tertiary healthcare centers. In this chapter, essential information on FNA and ND cytology of the breast are discussed.

### Fine needle aspiration

The technique of FNA of the breast depends upon whether the abnormality is palpable or non-palpable.

**1. Palpable lesions** are best investigated by manual FNA:

- Fix the mass with one hand and the needle is manipulated with the other hand.
- At least 3 passages through the mass lesion in different directions using a cutting motion back-and-forth or drill-like are made.
- A needle with an attached a syringe and or syringe holder may be used.
- As an alternative, a bare needle held by the hub provides excellent results.
- The cell sample is expressed onto glass slides and spread.
- The needle can be rinsed for cell block or cytospin preparation.
- Air-dried smears are suitable for staining with MGG or Diff-Quik method.
- Smears obtained can be fixed immediately in 95% ethanol and stained with hematoxylin and eosin or by the Papanicolaou technique.
- Excess of aspirated material is used for cell block preparation for histologic evaluation and IHC studies, if indicated.

**2. Non-palpable cystic or solid mass lesion:**

- Can be localized under imaging guidance (ultrasound, mammography or MRI).
- Is aspirated and handled in a similar manner as with a palpable lesion.
- Lesions that are suspicious for malignancy by imaging findings can also be sampled by core needle biopsy in the same session.

## Specimen adequacy

- An adequate FNA has been generally defined by the presence of more than 6 well-preserved cell clusters containing at least 5 epithelial cells each
- Exceptions to this rule include:
  - Cyst contents which are sometimes acellular
  - Lipoma or other stromal lesion

## Diagnostic accuracy

- An FNA cannot distinguish an in situ carcinoma from an invasive carcinoma, or suggest a vascular or lymphatic invasion.
- The diagnostic accuracy of breast cytology is highly operator dependant.
- The sensitivity of FNA for palpable and non-palpable malignant lesions performed under imaging guidance is comparable.
- A sensitivity rate for malignancy ranging from 65% to 98% and a specificity rate ranging from 34% to 100% have been reported.
- False-positive results in 0% to 2% and false-suspicious results in 1 to 13% of cases have been reported.
- False-negative results may be due to error in sampling, interpretation or both.
- The majority of breast cyst fluids are benign but about 2% are malignant.

FNA cytologic findings of any breast lesion should be interpreted in the light of its clinical and imaging characteristic findings. These findings form the basis of the "Triple Test" for breast cytodagnosis and the clinicopathologic assessment of a breast lesion is best achieved by communication with the clinician and radiologist involved in the case.

## Reporting results

The report should include the following information:

- The exact site of the biopsy
- The type of sample – FNA, nipple discharge
- Result: One of the 5 following conclusions/results should be given
  - Insufficient for cytological diagnosis
  - Benign
  - Atypical (Atypical cells present favor benign)
  - Suspicious for malignancy (Atypical cells present favor malignant)
  - Malignant
- Comments:
  - Further investigation (e.g. Histological assessment by open or core needle tissue biopsy for an atypical or suspicious result) or
  - Follow-up with repeat FNA, and
  - Histologic confirmation is required in all malignant cases.

## Nipple discharge

Nipple discharges (ND) can be physiologic or pathologic:

- Physiologic discharges are typically related to pregnancy or lactation.
- Pathologic conditions such as a prolactinoma that can result in ND and this can be seen in association with some medications (e.g. antidepressants).
- Inflammatory conditions of the breast such as acute mastitis and abscess can cause a purulent discharge.
- Most bilateral discharges occur in association with fibrocystic changes. The discharge in fibrocystic changes can be clear, greenish or brownish in color.
- Frankly blood-stained discharges occur with intraductal papilloma or intraductal carcinoma.
- A ND that contains erythrocytes or hemosiderin-laden macrophages requires further investigation of the underlying breast tissue to exclude a neoplasm.
- According to one study, cancer is most prevalent when the ND is bloody (4%), less prevalent when it is purulent (0.8%), serous (0.2%) and milky (0.1%).
- ND is useful in identifying a minimal ductal carcinoma or intraductal papilloma. A sensitivity up to 85%, a sensitivity of 85%, a specificity of 97%, a positive predictive value of 92%, a negative predictive value of 94% of ND cytology have been reported.

ND can be expressed and put directly onto glass slides and spread to make a smear in the similar manner as making a blood film. The smears are air-dried or fixed in 95% ethanol for staining by the MGG or Papanicolaou method respectively. Cytologic examination of a ND may be challenging, as duct cells from benign lesions shed into the discharge undergo degenerative changes, resulting in nuclear enlargement and irregularity. Therefore, a conservative approach to diagnosis is, advisable.

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## Nonneoplastic breast lesions

### Normal breast tissue

Normal breast consists of 15 to 25 lactiferous branching ducts that originate from the nipple. The distal branches of the duct are the terminal ducts that end in a grape-like cluster of blind sac-like dilatations or acini. The ducts and lobular acini are lined by a dual layer of cells: an inner luminal cell layer and an outer myoepithelial cell layer which are separated from the periductal stroma by a basement membrane. The stroma of the breast consists of the specialized periductal or lobular stroma. It is composed of delicate collagen fibers, fibroblasts, adipose tissue and dense collagenous fibrous tissue that is hypocellular and contains coarse closely packed collagen bundles. Other mesenchymal elements include lymphatics, blood vessels, nerves and myofibroblasts.

Normal breast cells and minute breast tissue fragments may be seen in an FNA. Ductal epithelial cells are columnar in shape and have benign, oval nuclei and granular, defined cytoplasm. They are commonly seen in small sheets with honeycomb pattern or in small clusters. Myoepithelial cells appear singly and show hyperchromatic, bipolar nuclei and no visible cytoplasm. Acinar cells are polygonal in shape and display oval nuclei, inconspicuous nucleoli and clear or granular cytoplasm. The acinar cells appear singly, in small clusters or groups. Lobules of acinar cells partially surrounded by a thin basement membrane are observed occasionally. (Figs.9.1 and 9.2).

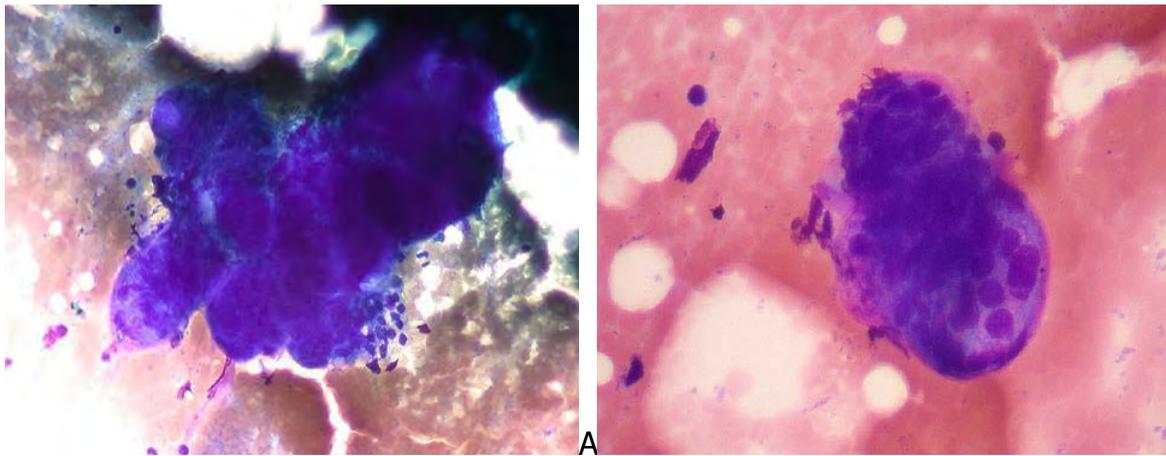


Fig.9.1. Normal breast tissue FNA showing a cluster or group of acini (A) and an acinus partially surrounded by basement membrane (B). (MGG).

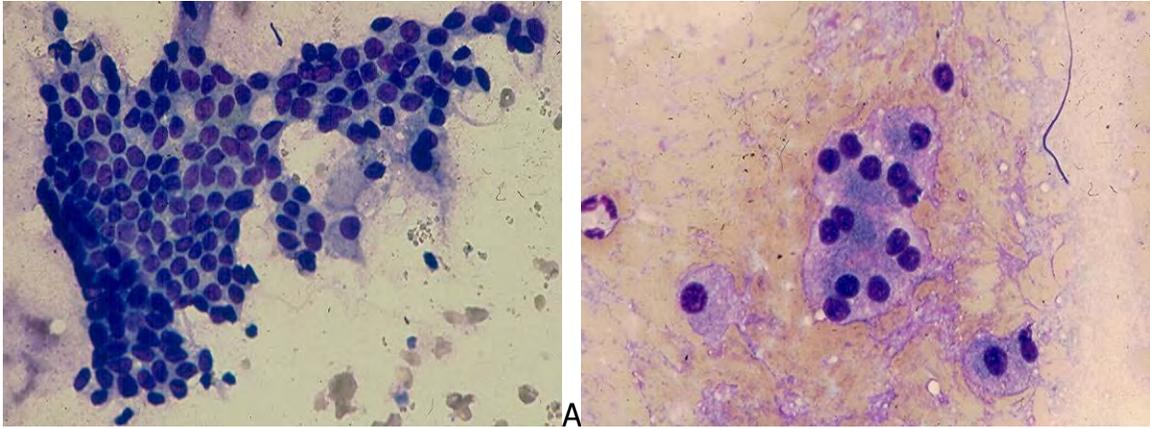


Fig. 9.2. Normal breast tissue FNA showing a monolayered sheet of ductal epithelium with regularly spaced, oval nuclei and scant, granular cytoplasm (A) and single and clustered polygonal acinar cells with eccentrically located oval nuclei (B). (DQ).

## Nonneoplastic lesions

### Fibrocystic changes and Ductal epithelial hyperplasia

Fibrocystic changes (FC) are commonly found in women in their reproductive years but can occur at any age. FCs may manifest clinically as a palpable mass lesion and are rarely associated with a clear ND. Histologically, breast tissue with FCs is characterized by any combination of small and large cysts, apocrine metaplasia, fibrosis, adenosis and ductal epithelial hyperplasia.

**FNA cytology varies with the lesions sampled.** (Figs.9.3 to 9.5):

#### 1. Cysts:

- Foamy macrophages
- Cell debris
- Apocrine cells arranged in cohesive sheets or singly
- Nuclear atypia may be present
- Liesegang rings and calcifications may be seen

#### 2. Ductal hyperplasia:

- Hypercellular cell samples
- Cohesive groups/sheets of uniform ductal epithelial cells with
- Myoepithelial cells present within the ductal epithelial cell groups/sheets
- Some small hyaline stromal globules may be present
- Some stripped nuclei – less numerous than seen in fibroadenoma
- Abundant bipolar, hyperchromatic naked nuclei of myoepithelial cells
- Mild pleomorphism of ductal cell nuclei may be observed

### 3. Stromal hyperplasia:

- Irregular mildly cellular stromal fragments without the club- or "clover-leaf" shape of those seen in fibroadenoma
- Small hyaline stromal globules in collagenous spherulosis

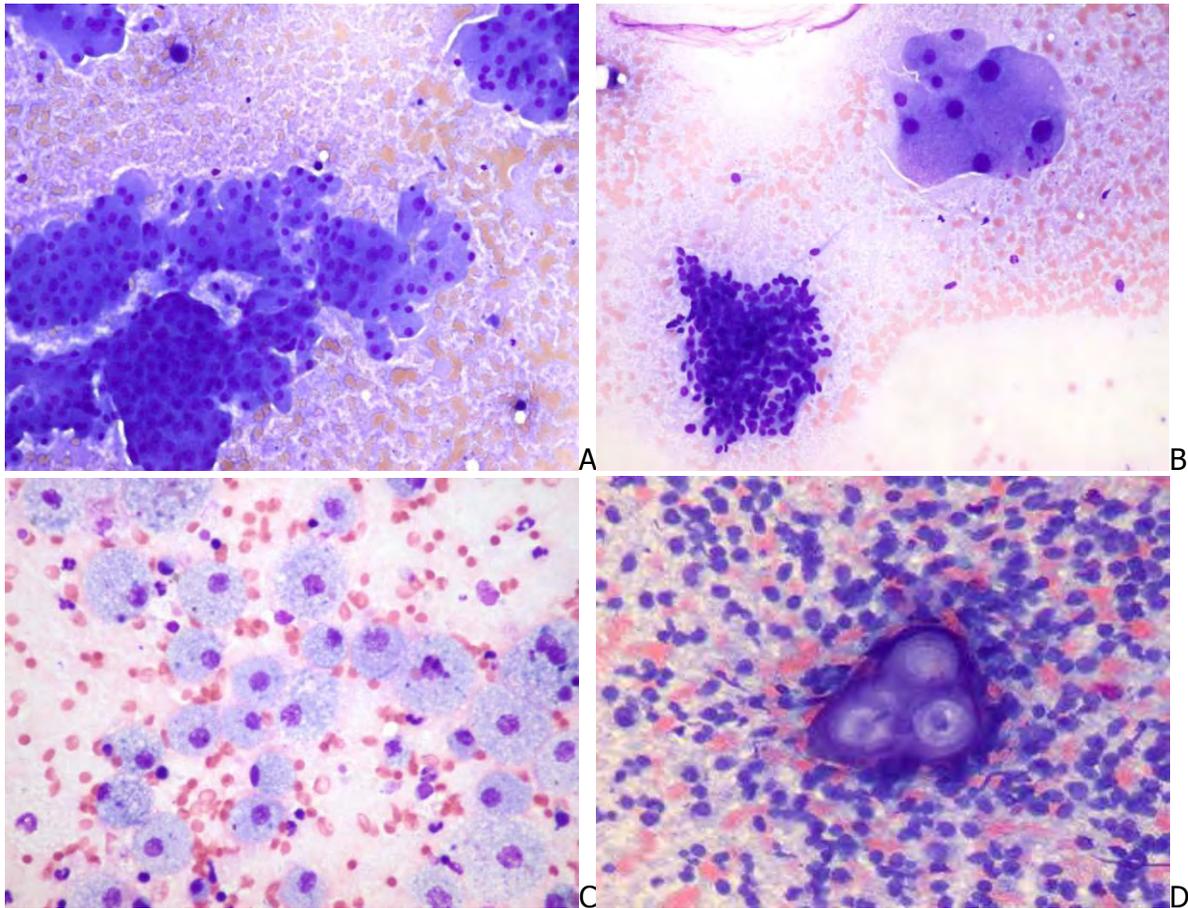


Fig.9.3. Breast FCs showing in FNA: irregular, monolayered sheets of apocrine cells (A), a group of apocrine cells and a fragment of ductal epithelium with crowded nuclei (B) and numerous foamy histiocytes (C), concentric Leisgang rings and abundant foamy histiocytes (D). (MGG)

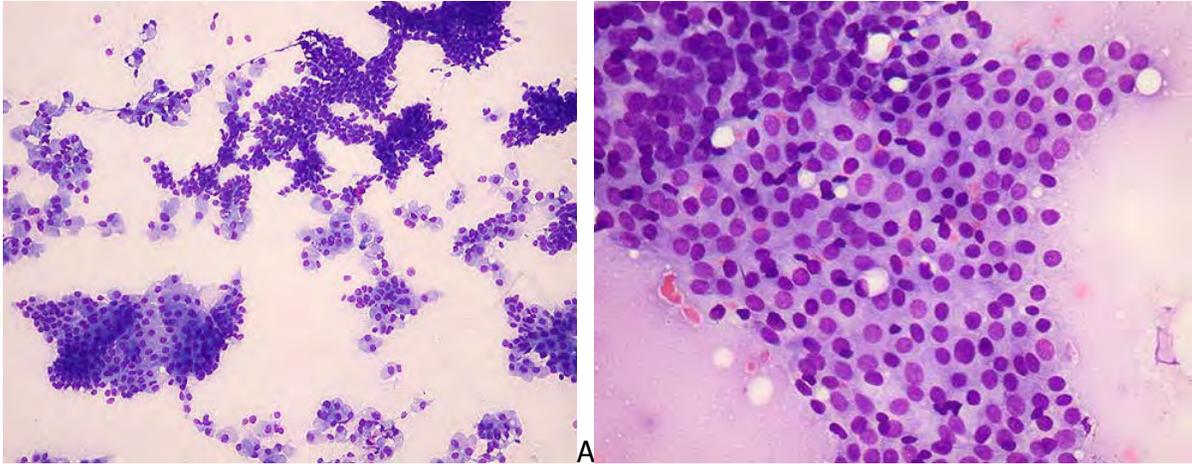


Fig.9.4. (A): Breast FCs showing in FNA irregular sheets of ductal epithelial cells with evenly spaced oval nuclei. (B): A monolayered sheet of benign ductal epithelial cells with evenly spaced oval nuclei and interspersed myoepithelial cells with dark, bipolar nuclei. (MGG).

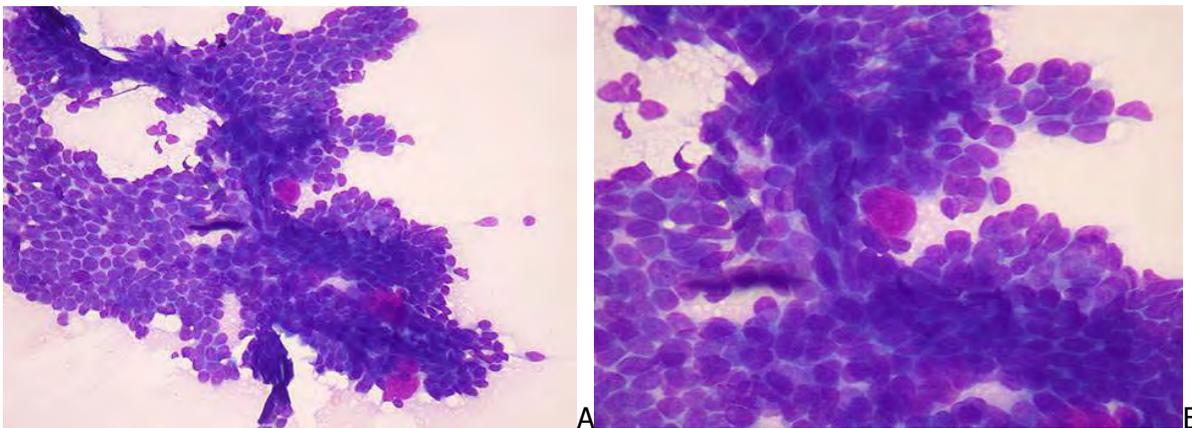


Fig.9.5. Irregular sheets of hyperplastic ductal epithelial cells showing focal nuclear crowding and collagenous stromal globules. (MGG).

Diagnosis of FCs of the breast by FNAC is usually straightforward. However, diagnostic difficulties may be encountered on the following occasions:

- When abundant markedly atypical apocrine cells are present an apocrine carcinoma of the breast should be ruled out. **Apocrine carcinoma** usually yields numerous dyshesive large malignant epithelial cells with abundant, finely vacuolated or granular cytoplasm and prominent nucleoli. A "Triple test" should be exercised to avoid this diagnostic dilemma.
- In the presence of proliferated ductal epithelial cells, a fibroadenoma, phyllodes tumor, intraductal papillary neoplasm and ductal carcinoma should be ruled out:

1. **Breast fibroadenoma or phyllodes tumors** are characterized by large sheets of ductal epithelium, abundant naked oval nuclei and bipolar naked nuclei of myoepithelial cells, and fragments of stromal tissue. Abundant naked oval nuclei, bipolar spindle nuclei and cellular stromal tissue fragments are practically not seen in FNA of a breast with FCs.

2. An **Intraductal papillary neoplasm** is characterized by a bloody nipple discharge, a discrete subareolar mass lesion (in about 50% of cases) and papillary epithelial fragments with fibrovascular cores. Excision of the tumor mass lesion is necessary for a firm diagnosis of an intraductal papillary neoplasm.

3. A mammary **ductal epithelial hyperplasia or adenosis with atypia** yields cells indistinguishable from those of a ductal carcinoma in situ or a low-grade ductal carcinoma. Cells from these three lesions are present in clusters or sheets with variable degrees of nuclear atypia. A variable number of naked oval nuclei and spindle, bipolar, dark bare nuclei of myoepithelial cells may be present. (Fig.9.6). A core needle biopsy of the lesion is needed for further histologic confirmation.

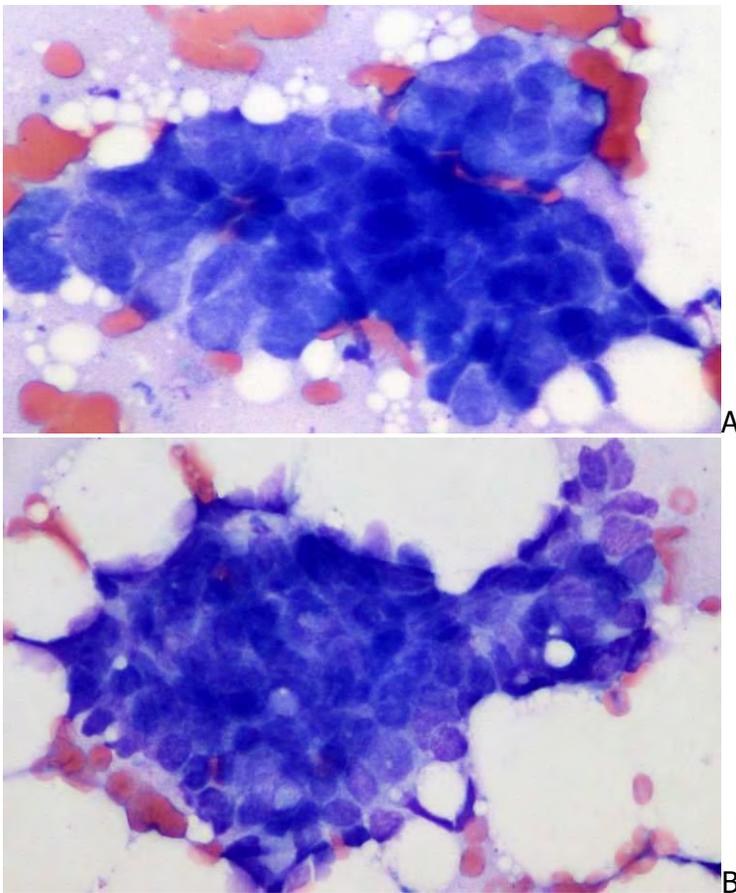


Fig. 9.6. (A, B): Two large fragments of ductal epithelium showing nuclear crowding and pleomorphism from a case of atypical ductal epithelial hyperplasia confirmed by core needle biopsy. (MGG)

## Mastitis and abscess

Mastitis is a bacterial infection of the breast caused by nipple erosion by breast feeding in lactating women. In some patients it is unassociated with lactation and presents as a tender mass. Occasionally, the clinical diagnosis is not obvious and the lesion is evaluated by FNA. The cytologic findings of breast abscess vary with the phase of inflammation (acute versus chronic).

### FNA cytology:

- Highly cellular sample (Fig.9.7)
- Abundant neutrophils, histiocytes and cell debris in acute phase
- Ductal epithelial cells with reactive changes
- Granulation tissue fragments with capillary blood vessels and inflammatory cells
- High contents of lymphocytes and histiocytes in chronic phase

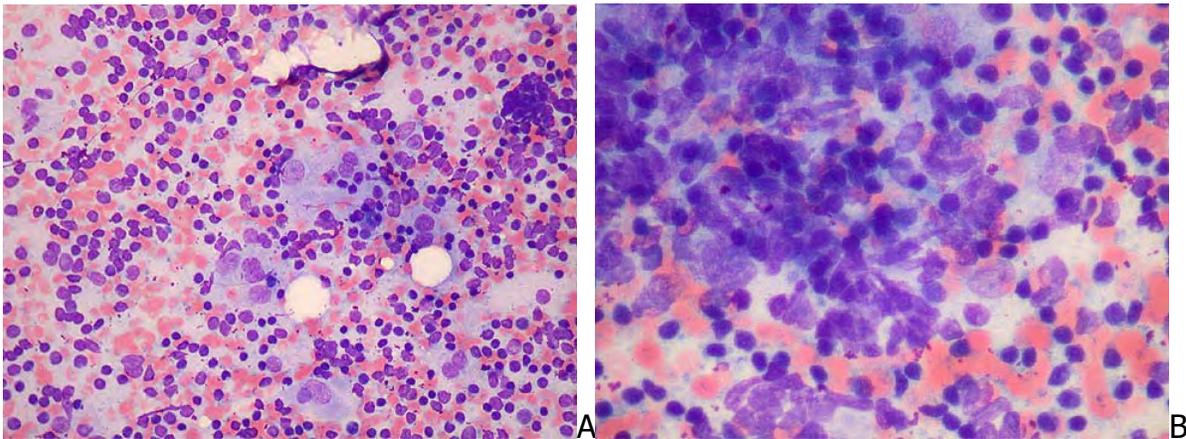


Fig.9.7. Chronic mastitis showing in FNA numerous lymphocytes admixed with a few dyshesive and single histiocytes are seen in (A). A large aggregate of histiocytes mimicking atypical epithelial cells is seen in (B). (MGG).

In **granulomatous mastitis** caused by tuberculosis or fungal infection, proliferative epithelioid histiocytes with abundant vacuolated cytoplasm, enlarged nuclei and prominent nucleoli may be observed, as well as a few multinucleated giant cells, lymphocytes and eosinophils.

## Periareolar abscess and fistula

Plugging of lactiferous duct by ductal squamous metaplasia causes dilation, inflammation and rupture of the duct with formation of a sinus. FNA of the lesion may be performed to rule out a neoplastic lesion.

### **FNA cytology:**

Highly cellular FNA with abundance of neutrophils in acute phase. (Fig.9.8)

Abundant lymphocytes, plasma cells and histiocytes in chronic lesions.

Squamous cells from squamous metaplasia of the duct lining cells.

Anucleated squamous cells and multinucleated giant cells may be present.

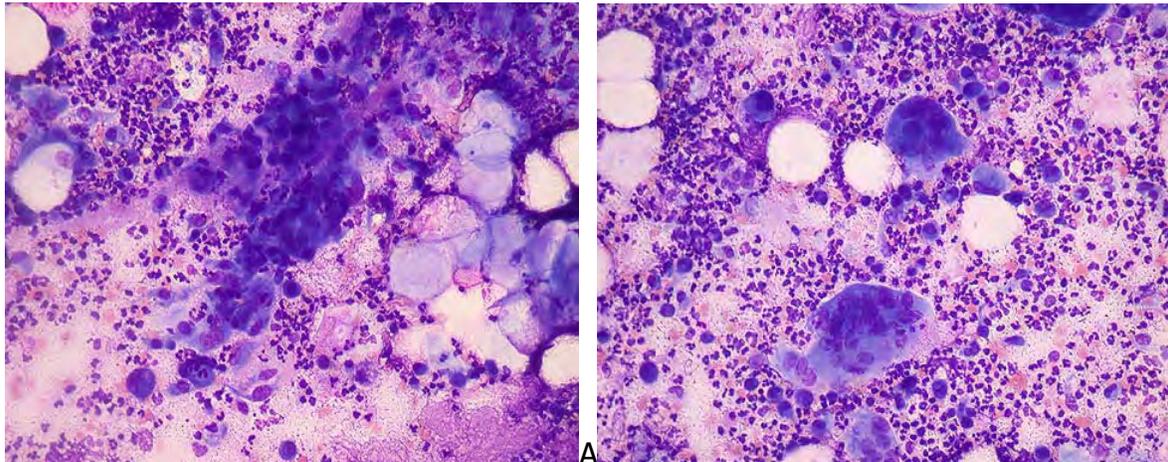


Fig.9.8. Periareolar abscess showing in FNA, (A, B): squamous epithelial cells, anucleated squamous cells, neutrophils, histiocytes and multinucleated histiocytic giant cells. (MGG).

### **Fat necrosis**

Fat necrosis is commonly seen in patients with prior trauma or surgery to the breast. It may mimic a breast carcinoma clinically and may be investigated by FNA.

### **FNA cytology:**

- Lipid debris and fat vacuoles. (Fig.9.9)
- Haemosiderin laden macrophages may be present
- Predominantly histiocytic infiltrate with few or no ductal cells
- Histiocytes with foamy cytoplasm, enlarged nuclei and prominent nucleoli
- Loose aggregates of histiocytes may mimic groups of atypical ductal cells
- Metachromatic reactive stromal fragments may be present

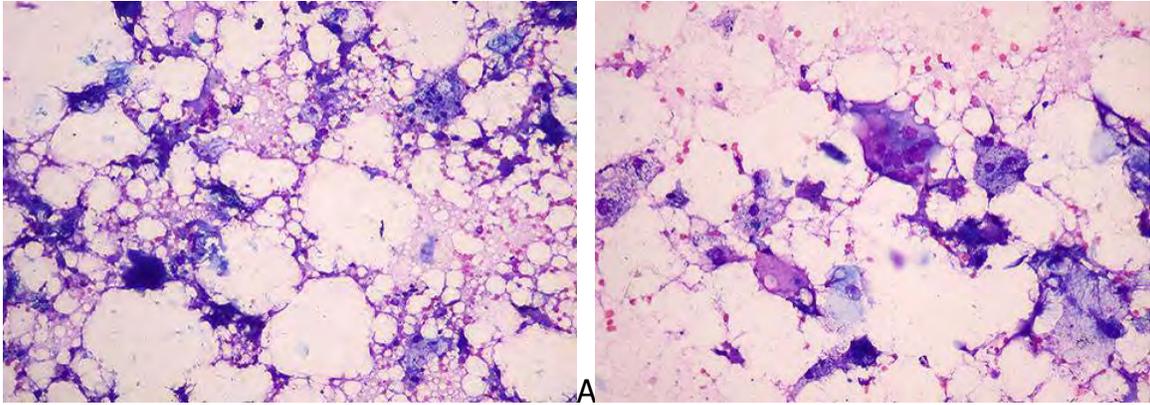


Fig. 9.9. Fat necrosis showing in FNA fat vacuoles, minute necrotic fatty tissue fragments, foamy histiocytes (A) and a few multinucleated giant cells in (B). (MGG)

### Lactational changes and adenoma

During lactation ductules of the terminal ductal lobular units become hyperplastic with vacuolated cytoplasm and secretion. Rarely, a palpable mass lesion is formed and the term "lactating adenoma" is used clinically to indicate this lesion. Occasionally, secretory changes can occur outside of pregnancy in patients who have a prolactinoma, hypothyroidism or who are on exogenous hormones or antidepressant drugs.

### FNA cytology:

- Cellular aspirate showing sheets of epithelial cells with vacuolated/foamy cytoplasm, uniform medium-sized round nuclei and central nucleoli. (Fig.8.10).
- Stripped round nuclei, lipid droplets and cell debris in background.

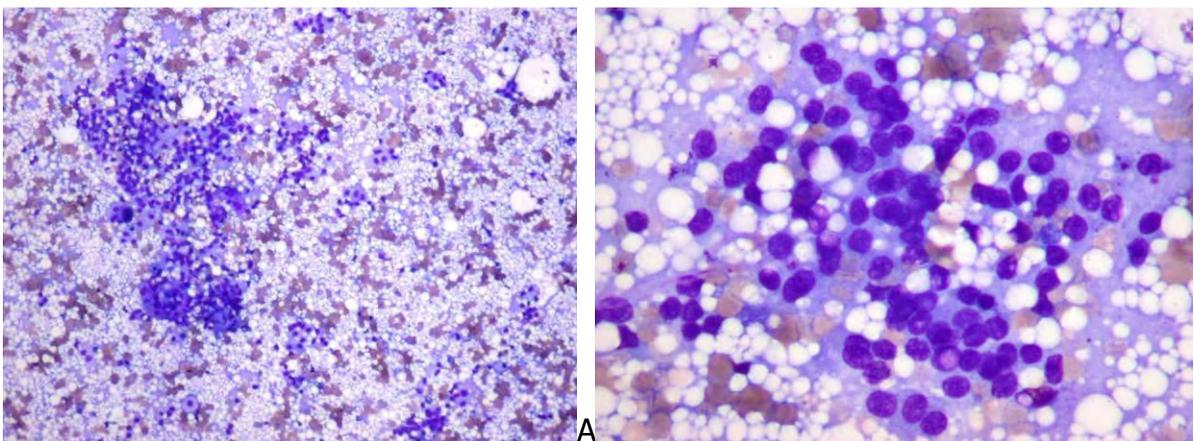


Fig.9.10. Lactating breast showing in FNA sheets of epithelial cells with vacuolated cytoplasm and numerous fat droplets in smear background (A, B). (MGG).

## Gynecomastia

Gynecomastia is the most common abnormality in male breasts. It can be diffuse or nodular and it is frequently bilateral. It may occur as a physiologic condition in teenage boys but can develop in adult patients and mimic a breast carcinoma, especially in elderly men. Gynecomastia may occur as a side effect of some drugs, liver disease, as a paraneoplastic phenomenon and in cannabis smokers.

### FNA cytology:

- FNAs are usually scanty in cellularity but can be cellular
- Benign columnar epithelial cells with oval nuclei present in cohesive groups, large clusters or in papillary tissue fragments showing myoepithelial component (Figs.9.12 and 9.13)
- Nuclear atypia can be observed
- Isolated oval nuclei and naked, spindle nuclei of myoepithelial cells may be present in smear background

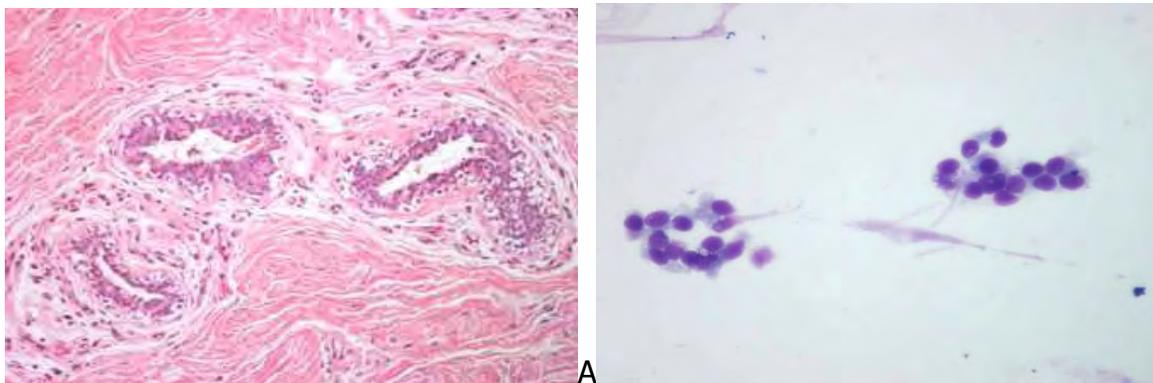


Fig.9.12. Gynecomastia, (A): Histology of gynecomastia showing mammary ducts lined by slightly hyperplastic epithelium with scattered myoepithelial cells and surrounded by loose, edematous stroma. (B): It yields in FNA clusters of benign ductal epithelial cells with oval nuclei and ill-defined cytoplasm. Bipolar, spindle nuclei of myoepithelial cells may be observed. (DQ).

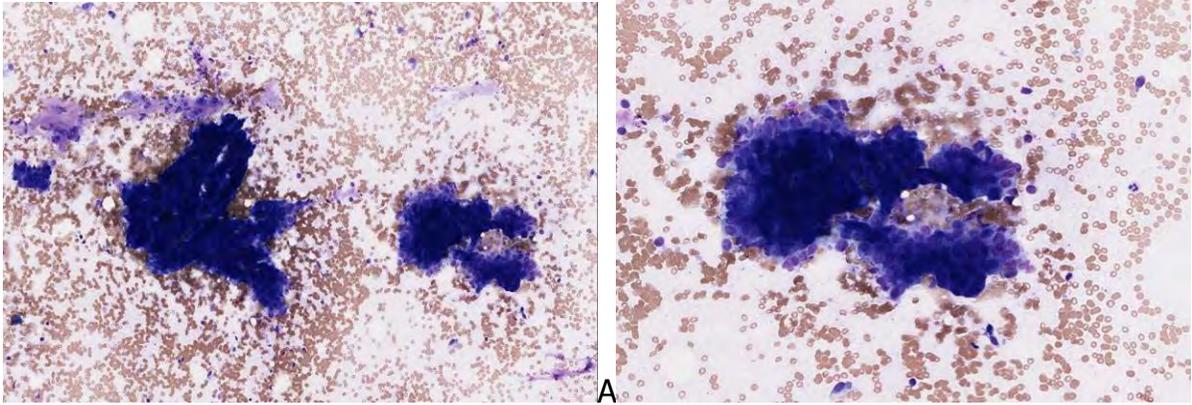


Fig.9.13. (A, B): Gynecomastia showing in FNA large irregular, papillary fragments of ductal epithelium surrounded by a few naked oval nuclei and spindle, bipolar nuclei of myoepithelial cells. (MGG)

### Radiation changes

Commonly present in breast cancer treated by lumpectomy and radiation. It is characterized in FNA by a hypocellular aspirate showing enlarged cells, nuclear and cytoplasmic enlargement with a low nuclear cytoplasmic ratio, hyperchromatic, bi- or multinucleation, prominent nuclei and nucleoli.

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## Chapter 10

# Breast neoplasms

## Benign neoplasms

### Fibroadenoma

Breast fibroadenoma (FA) occurs in a wide age range but it is uncommon in children. It is characterized by a smooth hypoechoic mass in diagnostic imaging, and it is the most common solid mass in the breast that is sampled by FNA. Histologically, a FA is composed of ducts surrounded and compressed by proliferating stromal tissue that forms nodules with club-shaped formations. The ducts within a FA are always lined by a dual layer of luminal epithelial and myoepithelial cells and can display epithelial hyperplasia that is occasionally florid.

#### **FNA cytology:**

- Highly cellular aspirates. (Fig.10.1).
- Cohesive groups of ductal cells with demarcated borders with branching glove-like or antler horn-like shapes.
- Flat or folded sheets of ductal cells with evenly spaced nuclei.
- Crowded poorly cohesive groups of ductal cells often present.
- Myoepithelial nuclei within and at the periphery of epithelial fragments.
- Naked nuclei (ductal, myoepithelial and fibroblastic in origin) present.
- Stromal fragments with a club-shaped or clover-leaf appearance.
- Apocrine cells, histiocytes, multinucleated giant cells are rarely observed.
- Mildly atypical ductal cells often present.
- Absence of free-laying atypical stromal cells in the smear background.

Epithelial cell atypia may be observed in cell samples from a breast FA and the differential diagnosis between a FA and a low-grade ductal carcinoma is usually not problematic. In some FAs isolate atypical epithelial cells are present and mimic those of a low-grade ductal carcinoma, and a core needle biopsy of the lesion is necessary to solve this diagnostic dilemma.

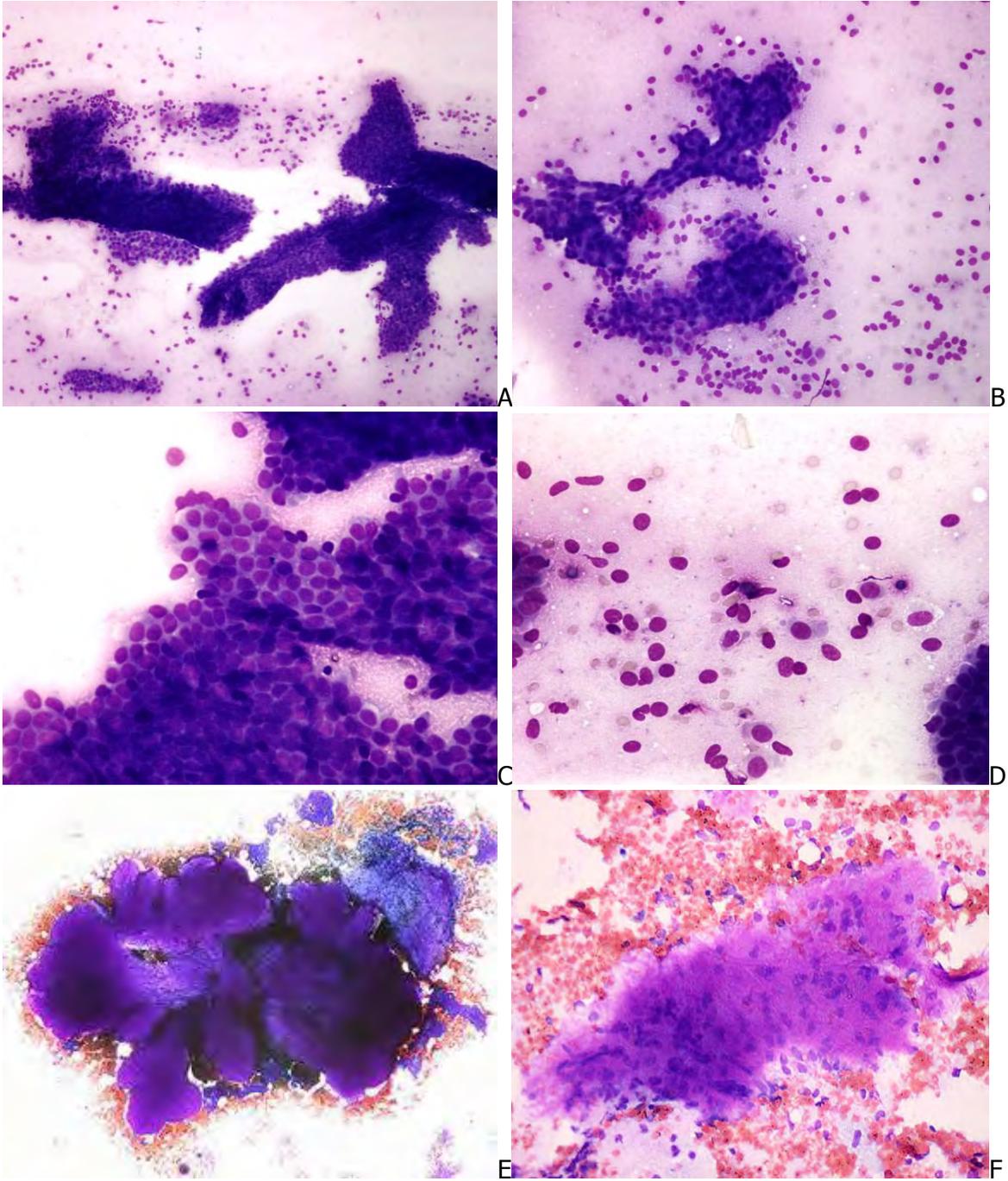


Fig.10.1. Breast fibroadenoma showing in FNA, (A, B): Irregular, branching fragments of ductal epithelium with antler-like horns admixed with naked nuclei and bipolar, naked nuclei of myoepithelial cells. (C): Epithelial fragment with interspersed myoepithelial cells. (D): Naked oval nuclei and bipolar dark nuclei of myoepithelial cells. (E): Stromal tissue fragment with clover-leaf configuration. (F): Smaller stromal fragment containing fibroblastic cells. (MGG).

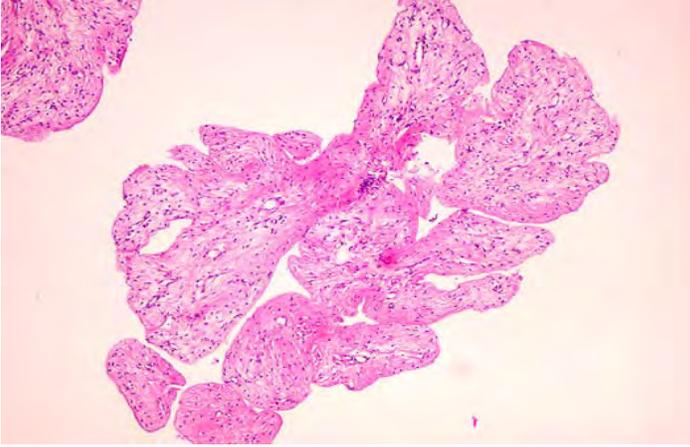


Fig.10.2. Fibroadenoma: Cell block section from an FNA showing a stromal tissue fragment with clover-leaf configuration. (HE)

From the cytologic point of view, diagnosis of a breast fibroadenoma by FNA is usually straightforward. However, the distinction between a florid ductal proliferation in breast tissue with FCs, a phyllodes tumor and a ductal carcinoma can be challenging:

- The differential diagnosis between a fibroadenoma and a phyllodes tumor is extremely difficult, as fragments of hypercellular stroma are present in FNAs of both tumors. A core needle biopsy or excision of the breast lesion is necessary for histologic confirmation.
- The distinction between a fibroadenoma and a ductal carcinoma is usually straightforward. But epithelial fragments with atypia from a fibroadenoma may be mistaken for epithelial fragments/sheets derived from a low-grade ductal carcinoma. Abundant naked oval nuclei are one of the main cytologic features of fibroadenoma, but a small number of naked nuclei are also present in FNA from a low-grade ductal carcinoma. When the distinction is not clear a core needle or excisional biopsy of the breast is necessary for further histologic confirmation.

### **Myofibroblastoma**

This is a rare and benign mesenchymal tumor occurring predominantly in adult males and females in their 7<sup>th</sup> decade of life. It is subdivided into 6 histologic subtypes such as classic, fibrous, epithelioid, cellular, myxomatous and infiltrative variants. The fibrous variant is characterized by bundles of slender, bipolar and uniform tumor cells separated by a scanty amount of hyalinized collagen. A myofibroblastoma, fibrous type yields in FNA abundant randomly arranged monomorphic benign spindle cells with oval or spindle nuclei with finely granular chromatin and no nucleoli. (Fig.10.3). An epithelioid type of myofibroblastoma displays in FNA single and loosely cohesive clustered oval tumor cells with granular cytoplasm and oval, bland nuclei.

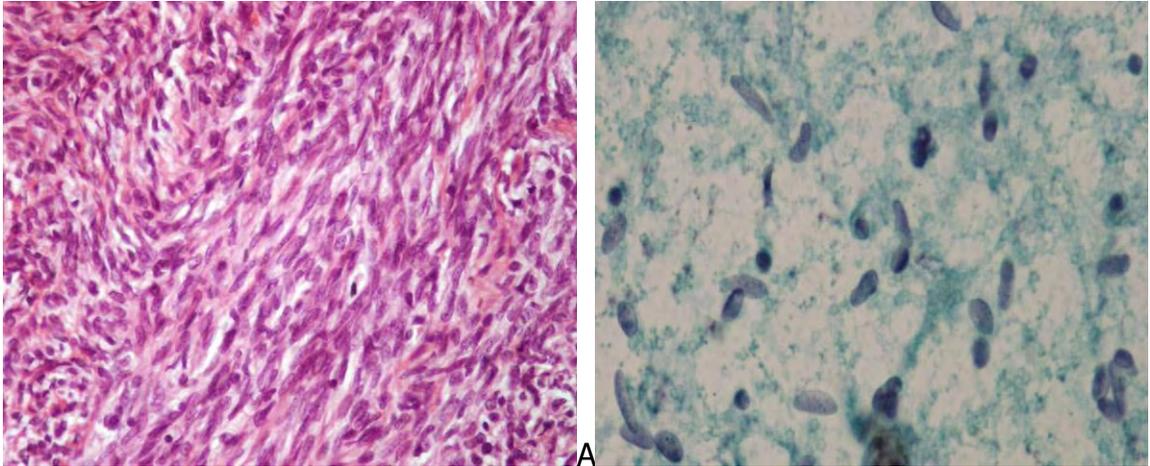
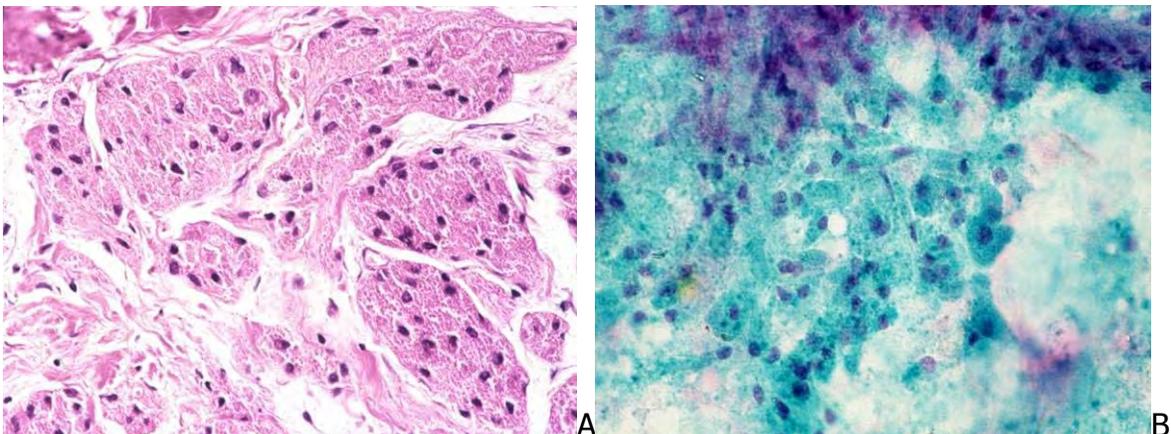


Fig.10.3. Breast myofibroblastoma, (A): Histology of the tumor showing irregular bundles of spindle fibroblast-like cells. (B): FNA of the tumor showing single elongated tumor cells with scant cytoplasm. (Pap)

### Granular Cell Tumor

This tumor is of Schwann cell origin and rarely arises in the breast. It occurs more commonly in females than in men with a wide age range from 17 to 75 years. The neoplasm may mimic a breast cancer clinically, radiologically and grossly. Histologically, it is composed of cells with ill-defined, granular cytoplasm and oval, bland nuclei. The granular cytoplasm, due to the presence of numerous intracytoplasmic lysosomes, reacts positively with periodic-acid Schiff with prior diastase digestion and S-100 protein antibody. The tumor shows in FNA single and clustered spindle cells with granular, ill-defined cytoplasm and bland, oval nuclei. (Fig.10.4). These cells are indistinguishable from histiocytes with granular cell metaplasia. As both cell types express S-100 protein and CD68 (KP1), a lysosomal marker, biopsy of the lesion is necessary for histologic confirmation.



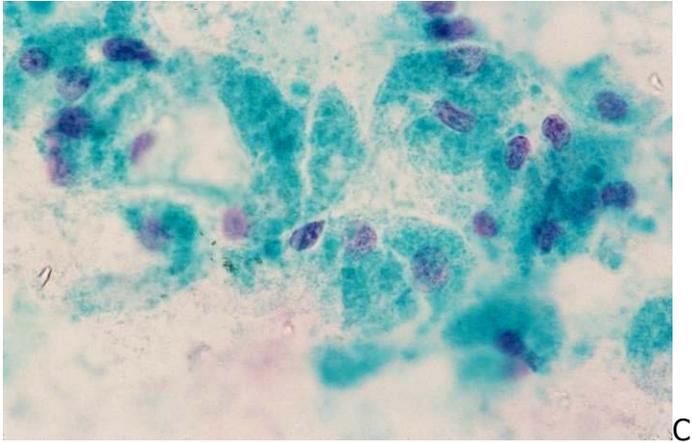


Fig.10.4. Breast granular cell tumor, (A): Histology of the tumor showing irregular nests of polygonal cells with bland nuclei and abundant, granular cytoplasm. (B, C): Single and loosely aggregated polygonal cells with bland, oval nuclei and abundant, granular cytoplasm. (Pap). (Courtesy of Dr. Nour Sneige, Houston, Texas, USA).

### Adenomyoepithelioma

This is a very rare benign tumor occurring most commonly in adult women. It consists of nests of proliferated myoepithelial cells around glandular spaces. An adenomyoepithelioma with inconspicuous glandular spaces or tubular structures and abundant matrix yields in FNA irregular and tight bundles of spindle myoepithelial cells arranged in 3-dimensional, lobulated clusters with strong cellular cohesiveness. Scattered bipolar, naked nuclei of myoepithelial cells and myoepithelial cells with ill-defined, granular cytoplasm are present in the smear background. (Figs.10.5 and 10.6).

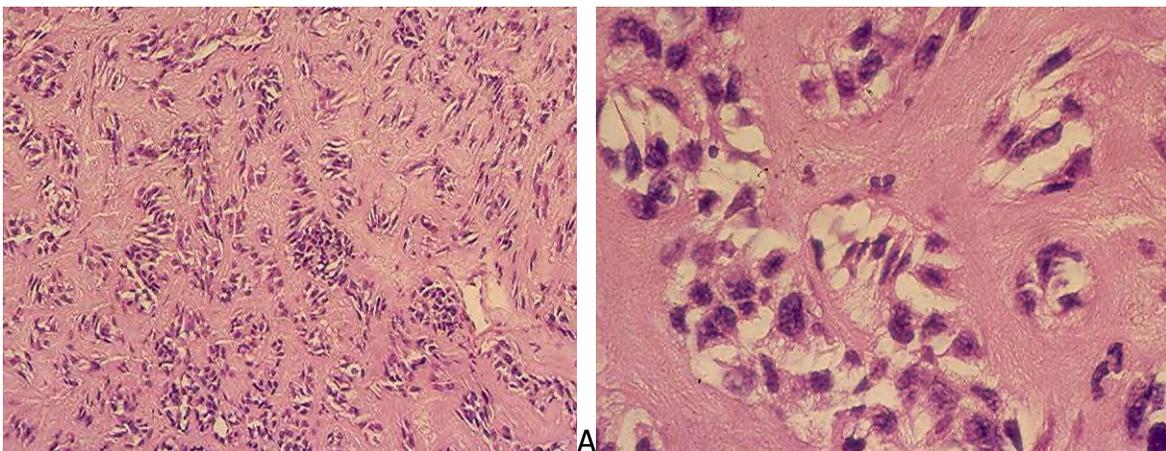


Fig.10.5. (A, B): Histology of breast adenomyoepithelioma showing nests of tumor cells with clear or granular cytoplasm and round or spindle, hyperchromatic nuclei surrounded by a large amount of amorphous basement membrane-like material.

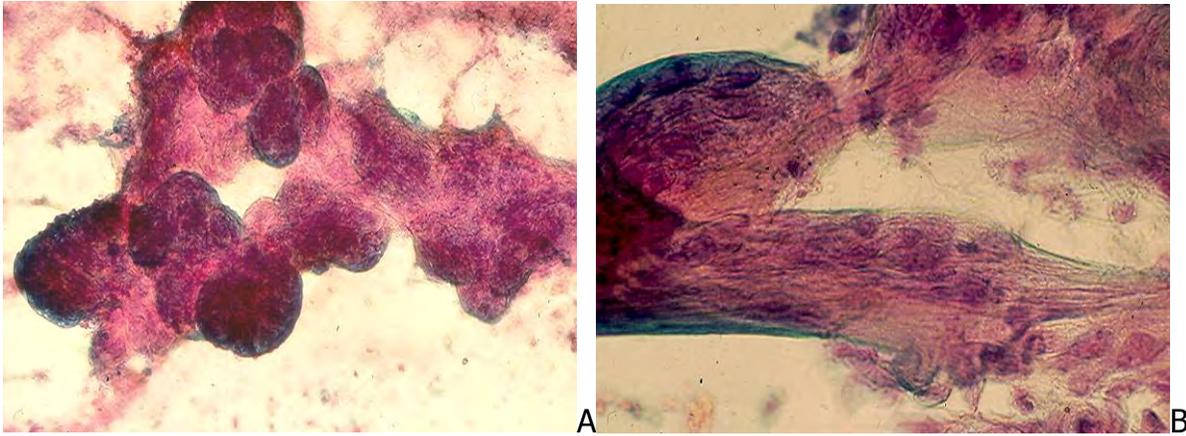


Fig.10.6. Breast adenomyoepithelioma showing in FNA, (A): Irregular, thick, 3-dimensional, lobulated, cohesive clusters of myoepithelial cells. (B): Cluster of spindle tumor cells with granular or fibrillary cytoplasm and round or oval nuclei (Pap). Fig. 10.6 B is from "Nguyen G.K et al. Aspiration biopsy cytology of mammary myoepithelioma. *Diagn Cytopathol.*1987;3:335-8" with permission.

## Intraductal papilloma and carcinoma

Intraductal papilloma is usually solitary and subareolar (in about 50% of patients). It is seen most commonly in young women < 40 years of age, but it may occur in postmenopausal women. The patients usually present with a unilateral bloody ND that shows clustered benign or atypical glandular cells.

Papillary carcinoma (PCA) represents 1% to 2% of all breast cancers and is defined histologically by the presence of a papillary growth pattern accounting for at least 90% of the tumor. PCA is seen more commonly in women > 40 and manifests clinically by a bloody ND and a subareolar mass. Depending on cellular atypia it can be graded as a low- or high-grade tumor. A low-grade PCA is composed of columnar epithelial cells with oval nuclei showing mild atypia and a high-grade PCA is composed of pleomorphic malignant cells. The ND in low-grade PCA may show small groups and pseudopapillary clusters of benign-appearing or atypical glandular cells, as seen in intraductal papilloma. A high-grade PCA may display in ND papillary clusters of malignant glandular cells.

FNA from a mammary papilloma may show many cellular features in common with a PCA. The cell samples are usually hypercellular and show a loss of cellular cohesion and myoepithelial cells. Epithelial cell atypia secondary to degenerative changes may be observed. Awareness of the central sub-areolar location of the mass can be helpful in minimizing interpretive errors. Myoepithelial cells are usually present in papillary cell fragments derived from a papilloma and absent in tissue fragments aspirated from a PCA. Unfortunately, this finding is not reliable to distinguish a papilloma from a PCA of

the breast. Therefore, it is wise to report the lesion as a papillary neoplasm and recommend an excision for histologic evaluation.

#### **ND cytology of papillary neoplasm:**

- Hypocellular cell sample
- Several erythrocytes, hemosiderin-laden macrophages and pseudopapillary clusters of benign-appearing ductal epithelial cells that may show nuclear atypia. (Fig.10.7)

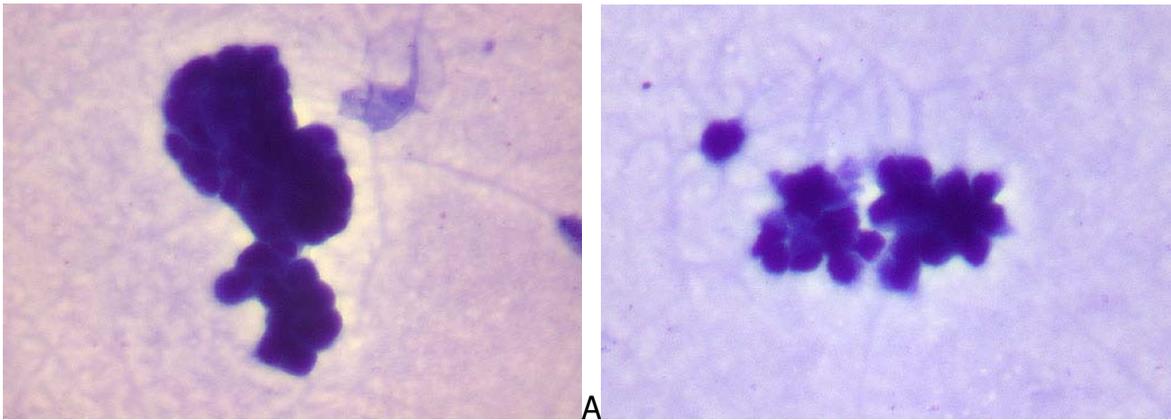


Fig.10.7. Intraductal papilloma showing in a ND a tridimensional, papillary cluster (A) and small groups (B) of benign ductal epithelial cells. (MGG)

#### **FNA cytology of papillary neoplasm. (Figs.10.8 to 10.10):**

- Cellular cell sample
- 3-dimensional papillary tissue fragments and/or
- Flat sheets of epithelial cells
- Myoepithelial cells are more commonly seen in papilloma and rare in PCA
- Epithelial cells can be small, cuboidal or columnar in configuration with oval nuclei with finely granular chromatin and conspicuous nucleoli
- Dissociated cells are commonly columnar in shape and may form rows
- Epithelial nuclear atypia may be present in papilloma and low-grade PCA
- Malignant epithelial cells are present in high-grade PCA
- Naked oval nuclei may be present
- Histiocytes and hemosiderin laden macrophages may be observed

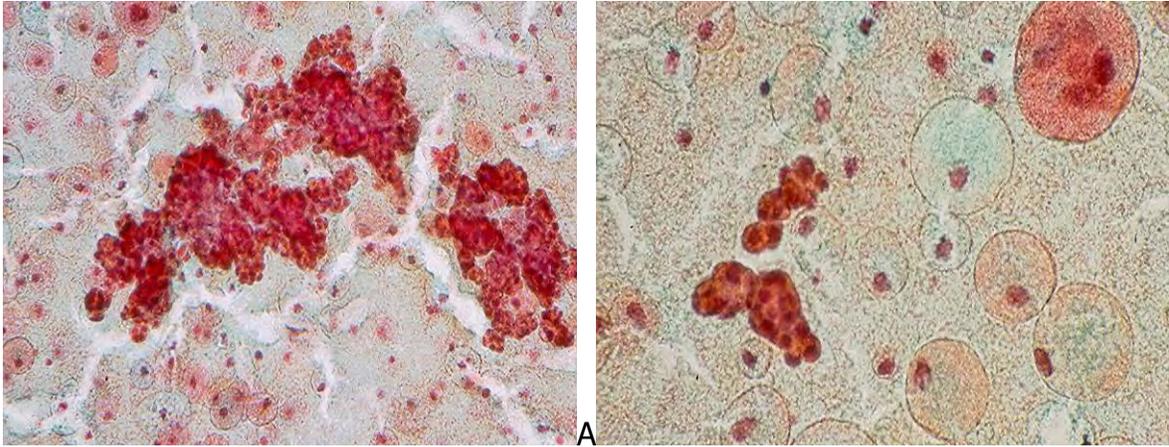


Fig.10.8. (A, B): Breast intraductal papilloma showing in FNA 3 dimensional, thick, branching, papillary clusters and smaller clusters of epithelial cells with oval, bland nuclei surrounded by numerous foamy histiocytes. (Pap).

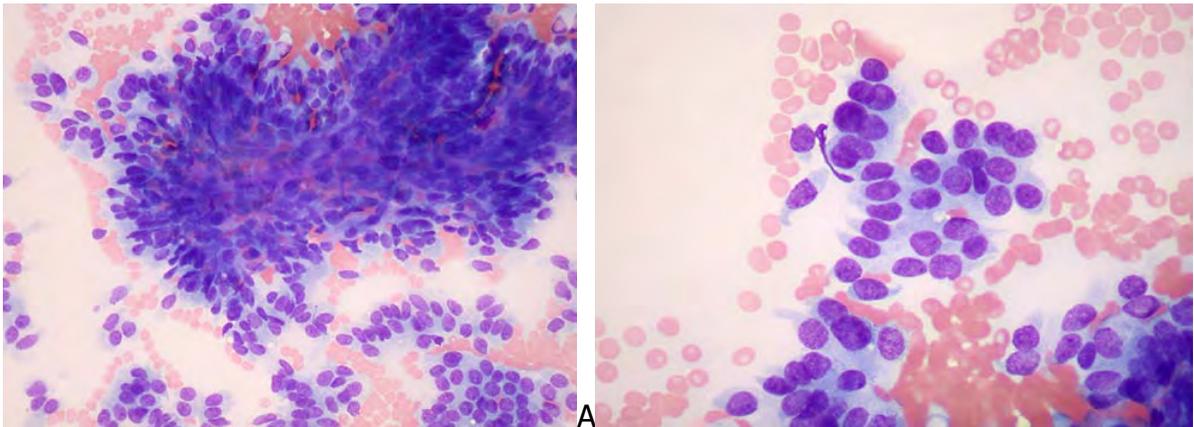


Fig.10.9. Low-grade breast PCA showing in FNA hypercellular clusters/sheets of tumor cells (A). Columnar tumor cells arranged in rows are seen in (B). (MGG).

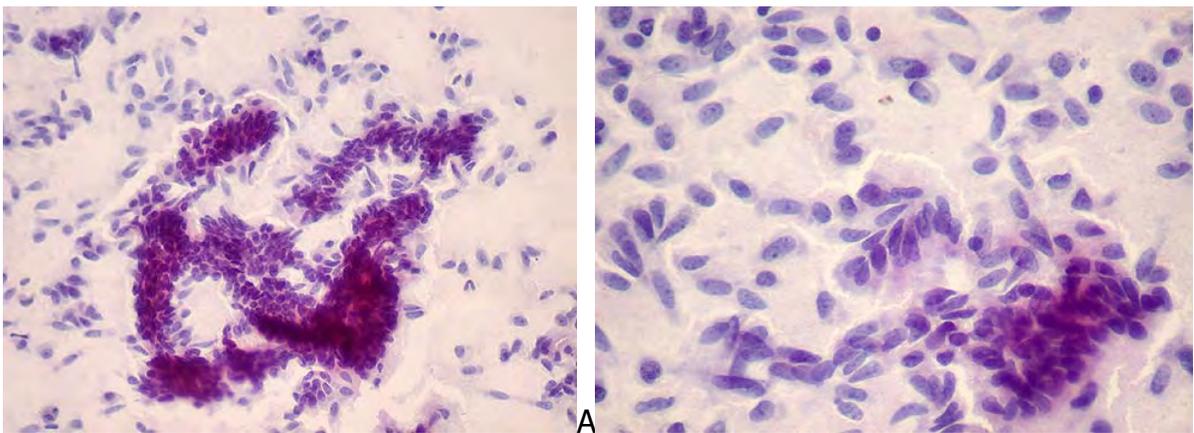
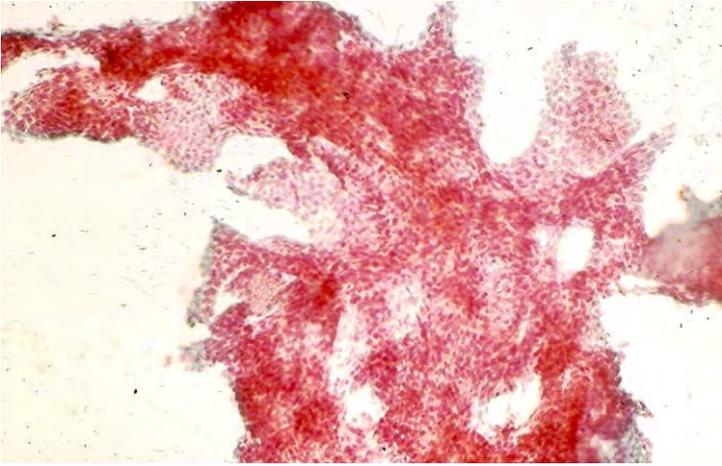
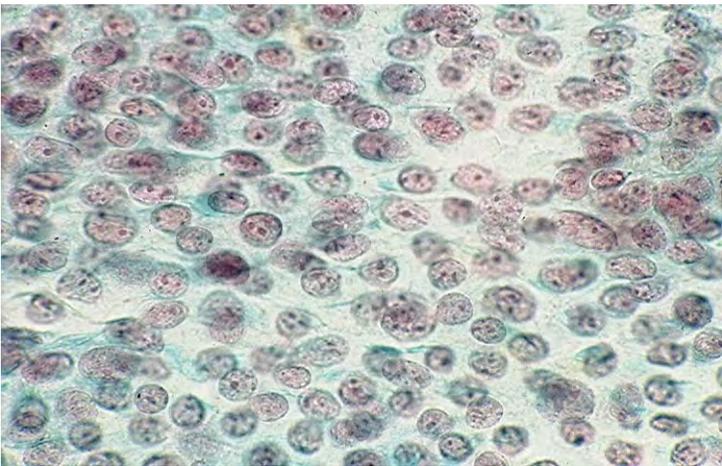


Fig.10.10. Low-grade breast PCA showing in FNA abundant columnar cells singly and in crowded epithelial fragments (A). Tumor cells present singly and in short rows (B). (Pap).

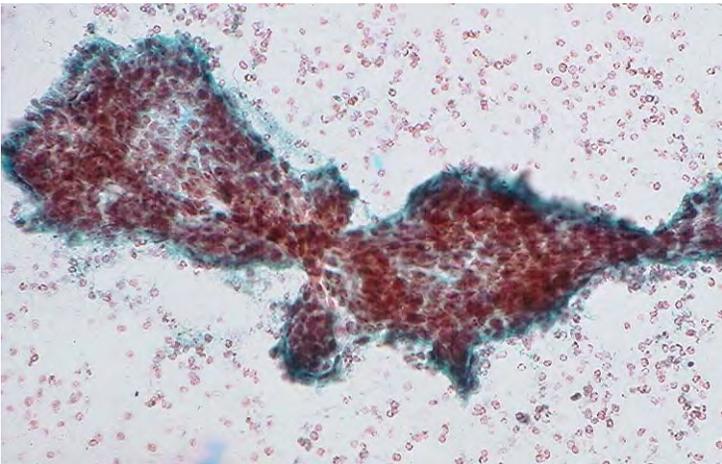


A

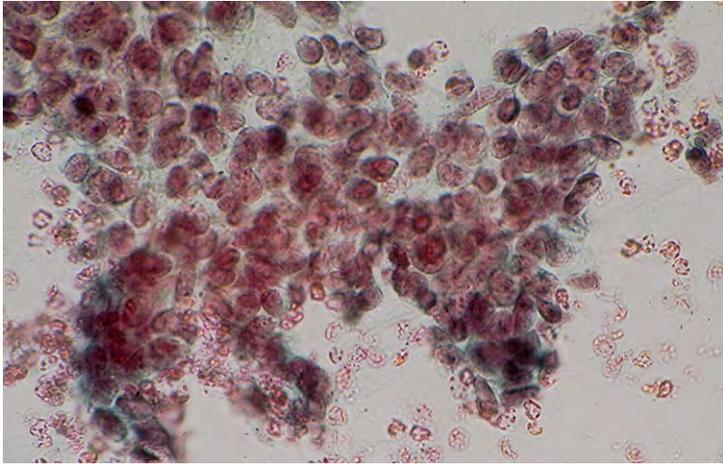


B

Fig.10.11. FNA of a low-grade breast papillary carcinoma showing in (A) complex large sheets of ductal epithelial cells displaying focal nuclear crowding, slightly pleomorphic nuclei, mildly irregular nuclear contours and conspicuous nucleoli (B). No bipolar, spindle nuclei of myoepithelial cells are clearly seen. (CP, Pap).

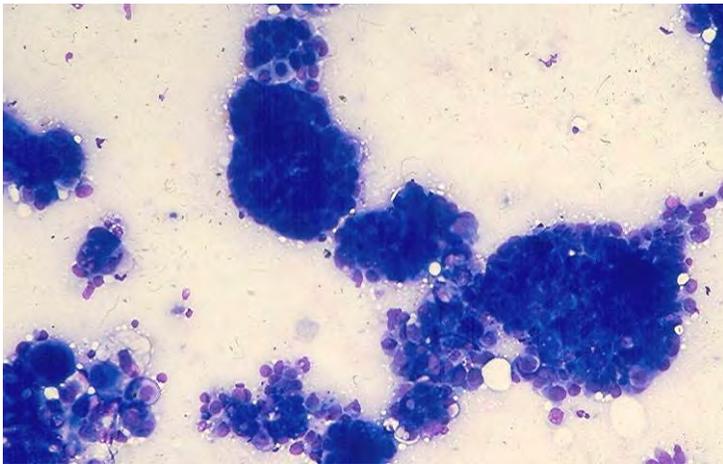


A

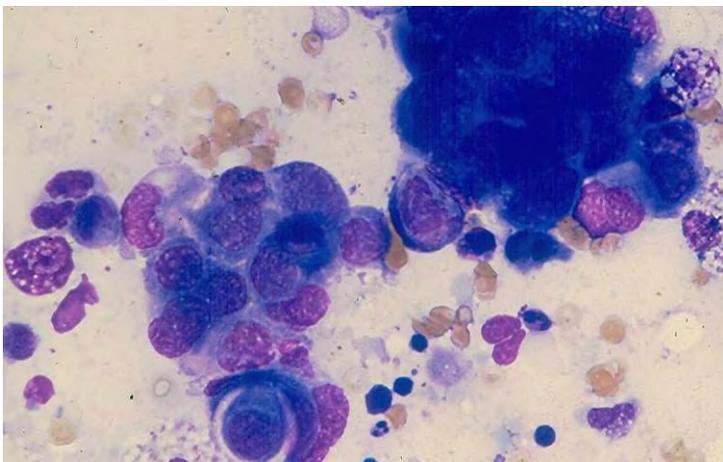


B

Fig.10.12. A,B. Low-grade PCA showing in FNA thick papillary tissue fragments showing malignant cells with nuclear crowding. (Pap)



A



B

Fig.10.13. A,B. High-grade PCA showing in FNA thick, papillary tissue fragments and clusters of pleomorphic malignant glandular cells with nuclear molding. (DQ)

## Phyllodes tumor

Phyllodes tumor is less common than fibroadenoma and accounts for less than 1% of all breast neoplasms. It occurs in women between 30 and 70 years of age and can be large, 5 cm in greatest dimension on average, and it can mimic a breast cancer clinically.

The tumor is formed as the result of neoplastic proliferation of stromal elements and nonneoplastic proliferation of ductal epithelial cells, and it can be benign or malignant. The stroma indents the ductal epithelium to show a "leaflet pattern". (Fig.10.11). The stroma is hypercellular and displays spindle stromal cells that may show mitoses and express smooth muscle actin. A malignant phyllodes tumor is characterized by a sarcomatous transformation of its stroma with local invasion. Benign phyllodes tumors are treated by local excision and malignant tumors by wide local excision without lymph node dissection. Malignant tumors often recur after wide local excision and may develop late metastases (15%).

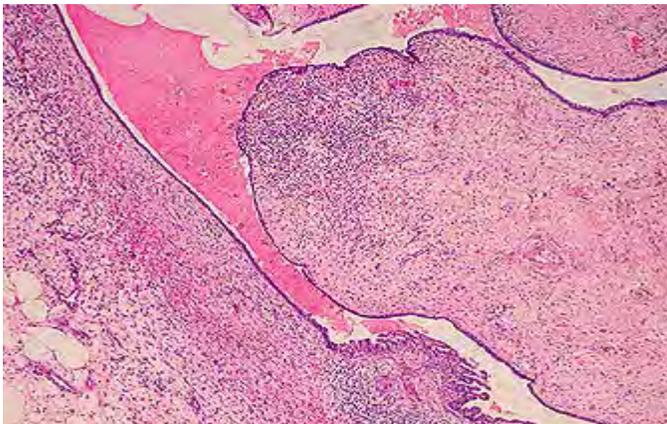


Fig.10.11. Histology of a mammary phyllodes tumor.

### FNA cytology:

- Abundant hypercellular stromal fragments. (Figs.10.14 and 10.15)
- Abundant groups of ductal epithelial cells and myoepithelial cells
- Free-laying atypical spindle stromal cells
- Stripped nuclei

The differential diagnosis between a fibroadenoma and a phyllodes tumor is difficult by FNA. The presence of hypercellular stromal fragments favors a phyllodes tumor, but similar stromal cell fragments can be seen in FNA of a fibroadenoma. As a phyllodes tumor can be benign or malignant and the distinction cannot be made with confidence by FNA, biopsy for histologic confirmation is necessary. Stromal

hypercellularity, stromal cell atypia and free-laying atypical stromal cells with mitoses favor malignancy.

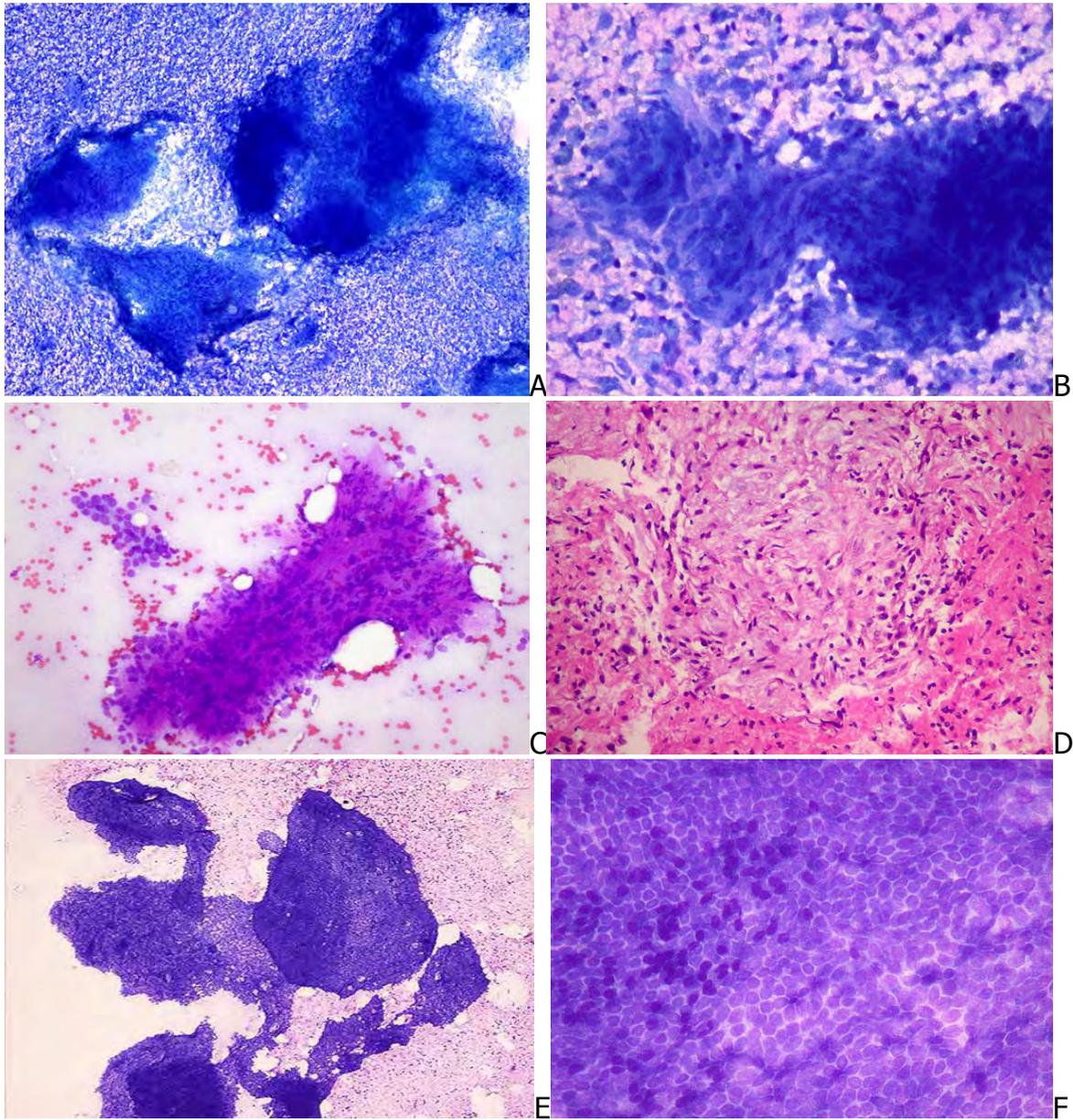


Fig.10.14. Breast phyllodes tumor showing in FNA (MGG), (A): Hypercellular tissue fragments surrounded by abundant spindle stromal cells. (B): Large fragment of stromal tissue surrounded by numerous stromal cells. (C): Smaller fragment of stromal tissue consisting of spindle cells. (D): Fragment of stromal tumor tissue in a cell block section. (HE). (E, F): Sheets of benign epithelium with regularly spaced oval nuclei.

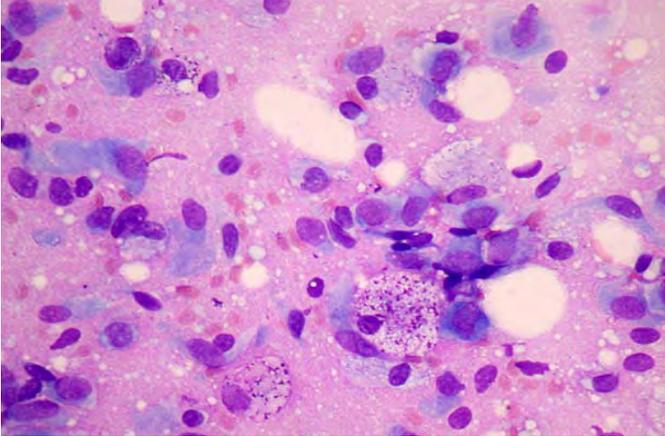


Fig.10.15. High-power view of the smear background reveals abundant spindle stromal cells without nuclear atypia. (MGG).

## Primary carcinomas

Breast carcinoma is the most common cancer in women in the United States. About 1 in 9 women will develop breast cancer at some point in their lives. Today, most breast cancers are asymptomatic and are detected by routine mammographic screening. Both invasive and in-situ carcinoma can present as a blood-stained nipple discharge. The cytologic manifestations of breast carcinomas vary depending upon the type and grade of the carcinoma.

### Invasive ductal carcinoma

Invasive ductal carcinoma is the most common breast malignancy and accounts for over 80% of all breast cancers. It can occur in any age groups and often manifests clinically as a palpable mass lesion. It may be associated with a bloody ND.

#### ND cytology:

- Malignant epithelial cells singly, in groups and in pseudopapillary clusters. (Figs.10.16 and 10.17)
- Nuclear molding in some tumor cell clusters
- Erythrocytes, haemosiderin-laden macrophages and necrotic debris.

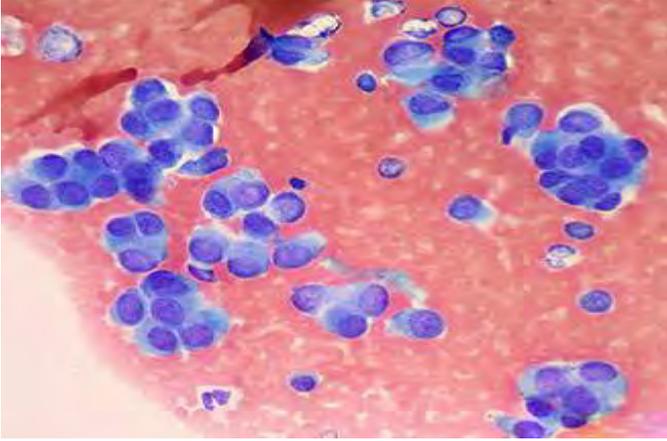


Fig.10.16. Invasive ductal carcinoma of the breast showing, in a bloody ND, single and clustered malignant epithelial cells with eccentrically located oval nuclei. (MGG).

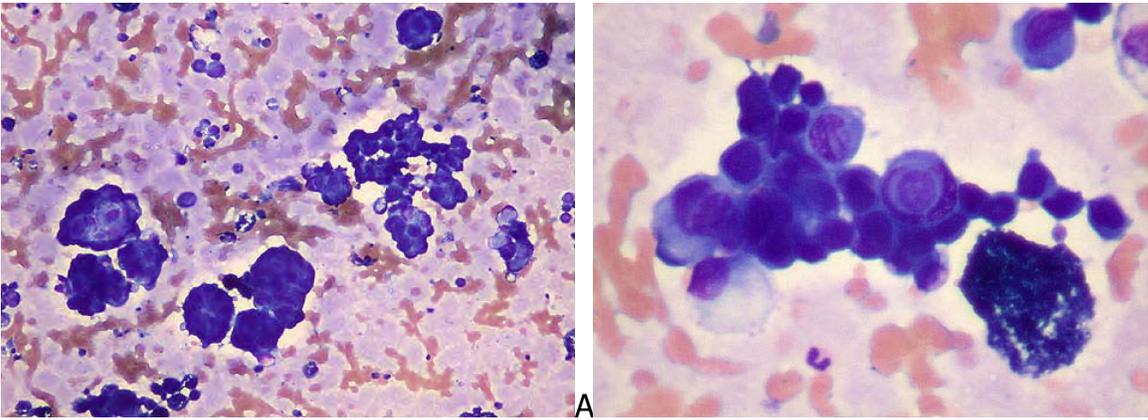


Fig.10.17. ND from an intraductal carcinoma showing tridimensional, pseudopapillary clusters of tumor cells and hemosiderin-laden macrophages (A) with marked cellular atypia (B). (MGG).

### FNA cytology:

- Hypercellular needle aspirates showing single and clustered tumor cells.
- Monomorphic or pleomorphic malignant epithelial cells. (Figs.10.18 to 10.20)
- Eccentrically located, enlarged, hyperchromatic, nuclei showing nuclear crowding, irregular nuclear contours and conspicuous or inconspicuous nucleoli.
- Absence of myoepithelial cells.
- Clean or necrotic background with inflammatory exudate.

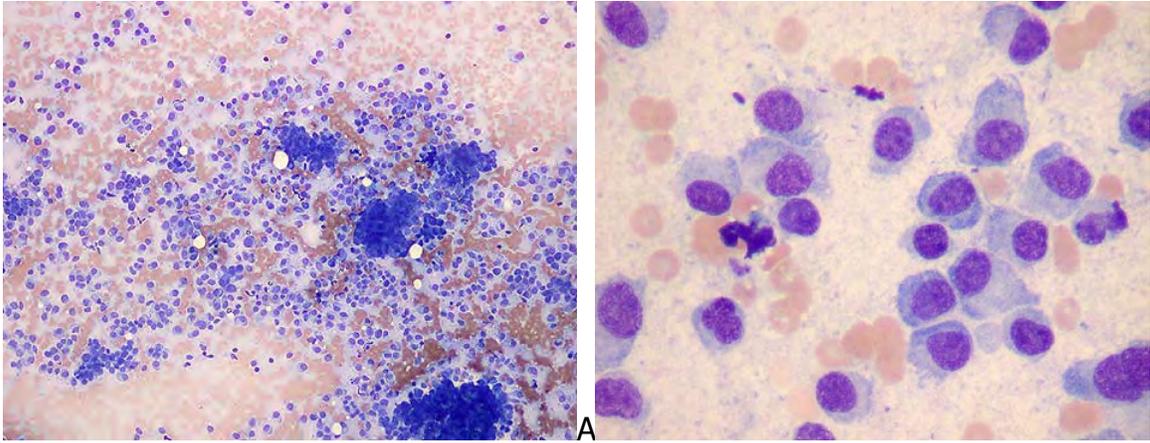


Fig.10.18. Single and clustered monomorphic malignant cells from a low-grade mammary ductal carcinoma. (MGG).

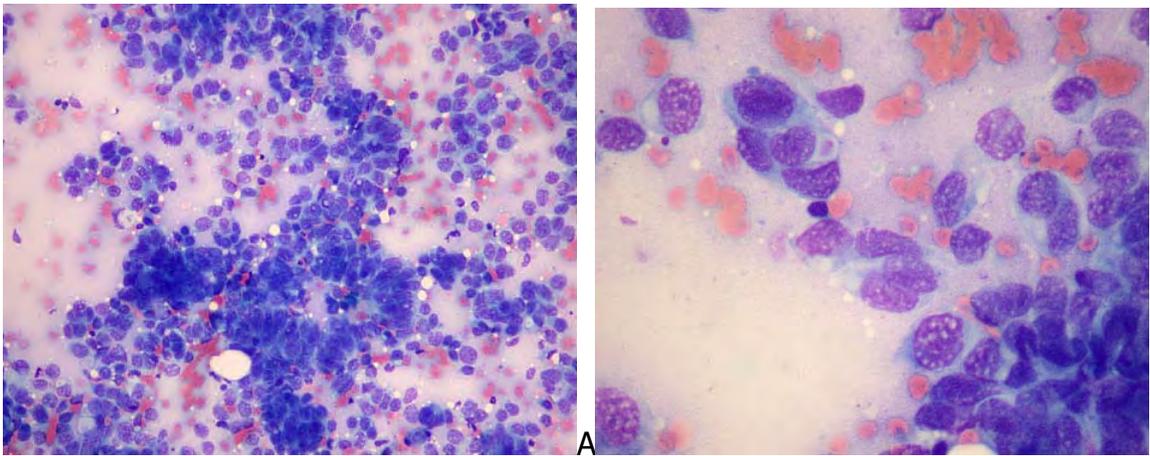


Fig.10.19. Single and clustered pleomorphic malignant cells from a high-grade mammary ductal carcinoma of the breast. (MGG).

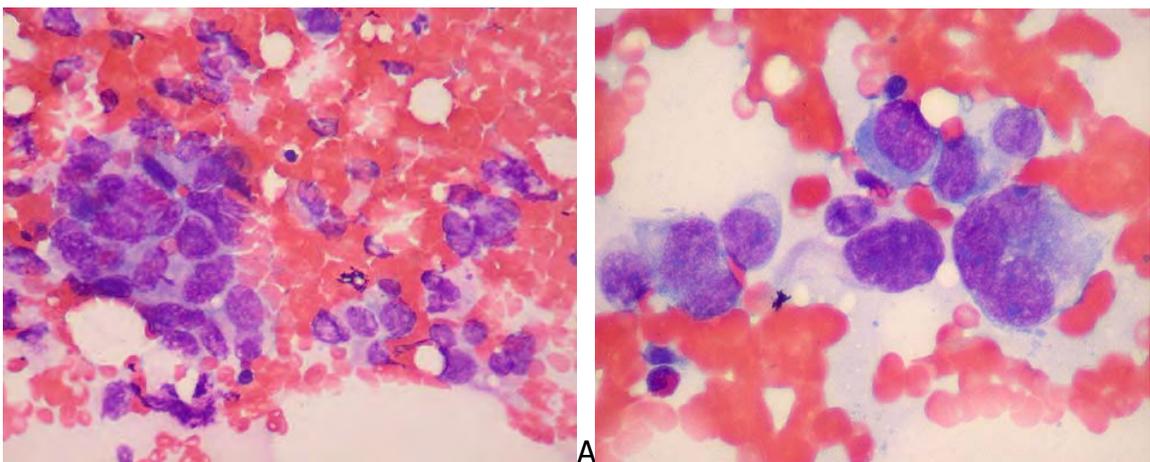


Fig.10.20. Single and clustered pleomorphic malignant cells from a high-grade ductal carcinoma of the breast. (MGG)

## Invasive lobular carcinoma

This tumor accounts for about 20% of all breast cancers. Histologically an invasive lobular carcinoma with classic pattern is characterized by infiltrating in cords of one cell thickness without evident of duct formation and desmoplasia. The malignant cells are small and have uniform nuclei lacking overt malignant nuclear features. The cords of cells are often arranged concentrically around pre-existing benign breast ducts. The tumor cells frequently contain a single vacuole of mucin in their cytoplasm and are e-cadherin negative. Other histologic variants are rare and include alveolar, signet ring, solid, tubulolobular, histiocytoid and pleomorphic lobular carcinomas. Cytodiagnosis of mammary lobular carcinoma by FNA is challenging as the needle aspirate is usually hypocellular and shows subtle malignant cellular changes:

### FNA cytology:

- Hypocellular needle aspirate (Figs.10.21 to 10.23)
- Small tumor cells predominantly present singly and in small groups
- Cytoplasmic vacuoles containing mucin with targetoid configuration
- Hyperchromatic and mildly atypical nuclei with small inconspicuous nucleoli

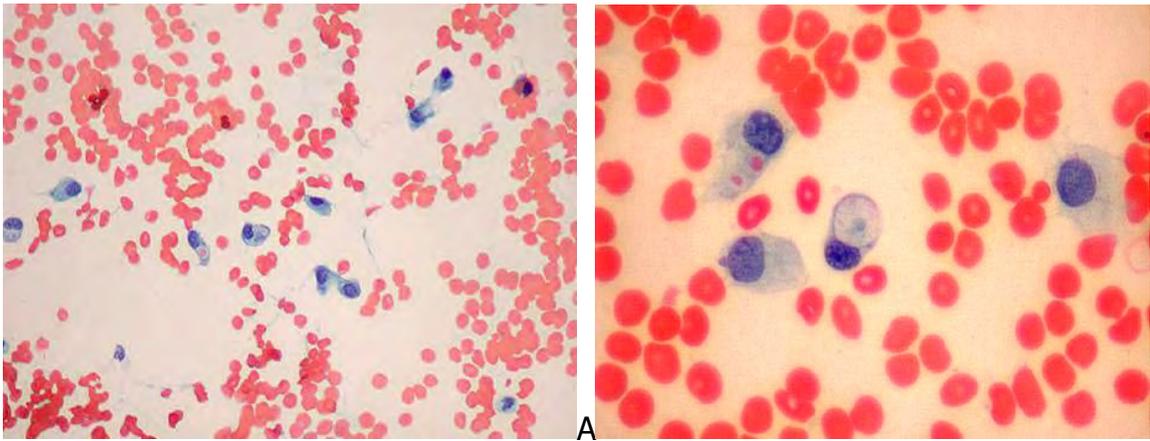


Fig.10.21. (A, B): Lobular breast carcinoma showing in FNA isolated, monomorphic malignant cells with eccentrically located oval nuclei. Some tumor cells display intracytoplasmic vacuoles with targetoid configuration (Pap).

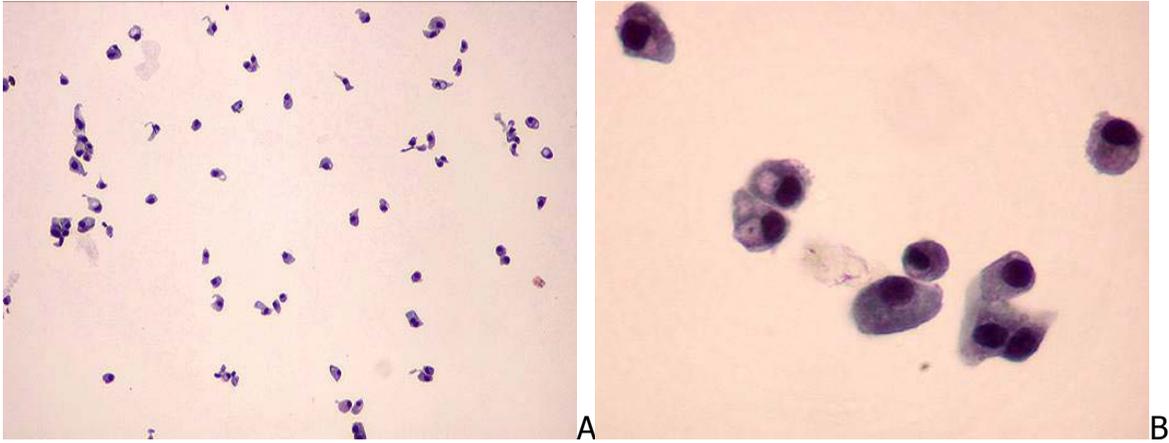


Fig.10.22. (A, B): Lobular breast carcinoma showing single malignant cells with intracytoplasmic vacuoles. (LBP, Pap).

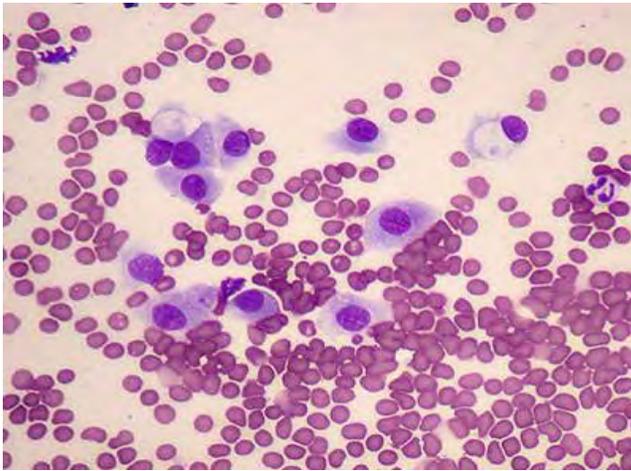


Fig.10.23. Lobular breast carcinoma showing in FNA small, single and polygonal epithelial cells with some cells displaying a large intracytoplasmic vacuole. (MGG).

### Adenoid cystic carcinoma

This is a low-grade epithelial-myoepithelial cancer of the breast and accounts for <1% of all breast cancers. It is a slow-growing neoplasm with a very low incidence of axillary lymph node metastases. Histologically, the tumor consists of 3 histologic subtypes: cribriform, tubular and solid. The cribriform pattern is the most common one and it is characterized by basaloid cells with little cytoplasm forming solid sheets and sieve-like aggregates of cells with tubular differentiation. Basophilic and slightly eosinophilic globular material within cell masses is present.

### FNA cytology:

- Cellular smear showing small uniform tumor cells with scant cytoplasm (Fig.10.24)
- Groups and nests of cohesive small cells wrapping around round globules
- Hyperchromatic nuclei, small nucleoli

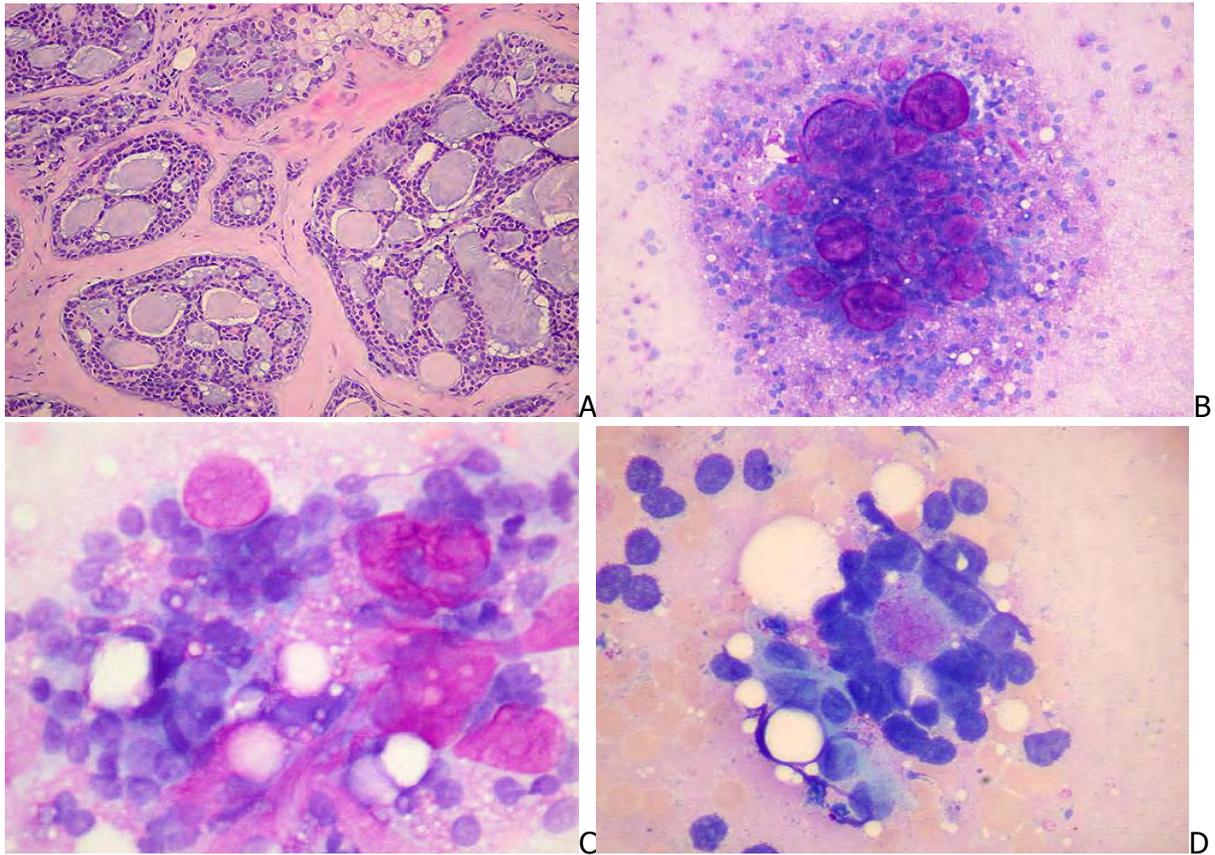


Fig.10.24. Adenoid cystic carcinoma of the breast, (A): Histology of the tumor. (B-D): Uniform small tumor cells with scant cytoplasm and hyperchromatic nuclei wrapping around bright red, round globules. (MGG).

### Tubular carcinoma

This cancer accounts for about 1% of all breast cancers and has a stellate configuration on mammogram. Histologically, the tumor has an infiltrative pattern on scanning power and consists of crowded tubules with open lumens and pointed ends making >90% tubular structures. The tubules are lined by columnar cells with low-grade nuclei with maintained polarity and rare mitoses. No myoepithelial cell layer is present.

**FNA cytology:**

- Cellular smear. (Fig.10.25)
- Predominantly tubular, angular clusters of tumor cells
- Uniform, medium-sized tumor cells with granular cytoplasm
- Round, uniform, low-grade nuclei
- Finely granular chromatin and inconspicuous, small nucleoli
- Rare stripped "naked" nuclei
- Myoepithelial cells and club-shaped stromal fragments are absent

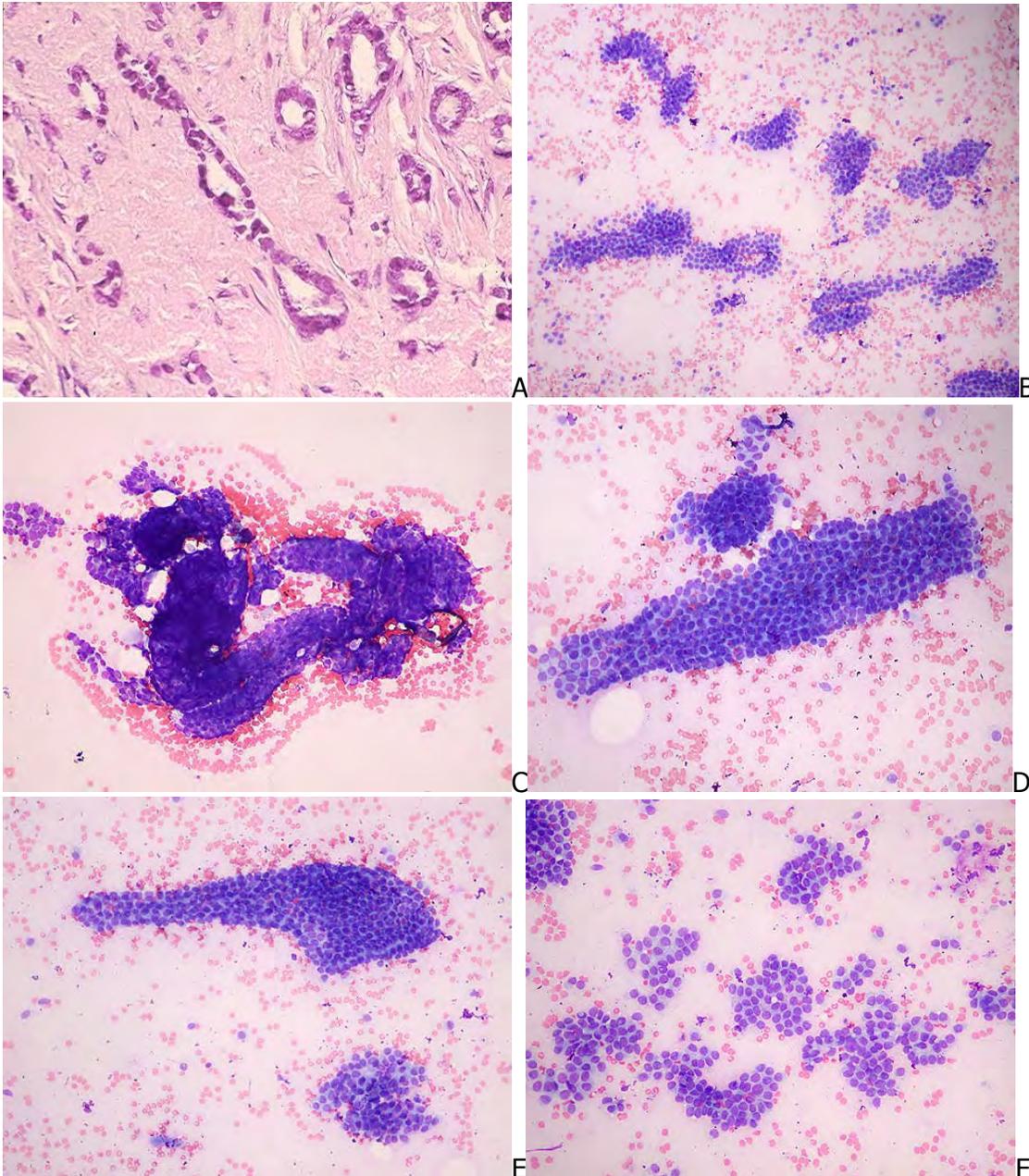


Fig.10.25. Tubular carcinoma of the breast, (A): Histology of the tumor. (B-F): Tubular, angular clusters and irregular sheets of uniform tumor cells. (MGG).

## Low-grade endocrine carcinoma

This is a rare tumor occurring mainly in elderly patients, >60 years of age. It often presents with a blood-stained nipple discharge. The tumour shows a solid growth pattern with tumor cell nests with pushing borders. The tumor cell nuclei are of uniformly oval and show small nucleoli. The cytoplasm is granular and strongly eosinophilic and reacts positively with neuroendocrine antibodies.

### FNA cytology:

- Abundant loosely clustered malignant cuboidal epithelial cells
- Eccentrically located low-grade nuclei (Fig.10.26)
- Intracytoplasmic metachromatic granules may be prominent
- Tumor cell clusters have a true papillary architecture with stromal core
- Myoepithelial cells difficult to identify and mucin may be present

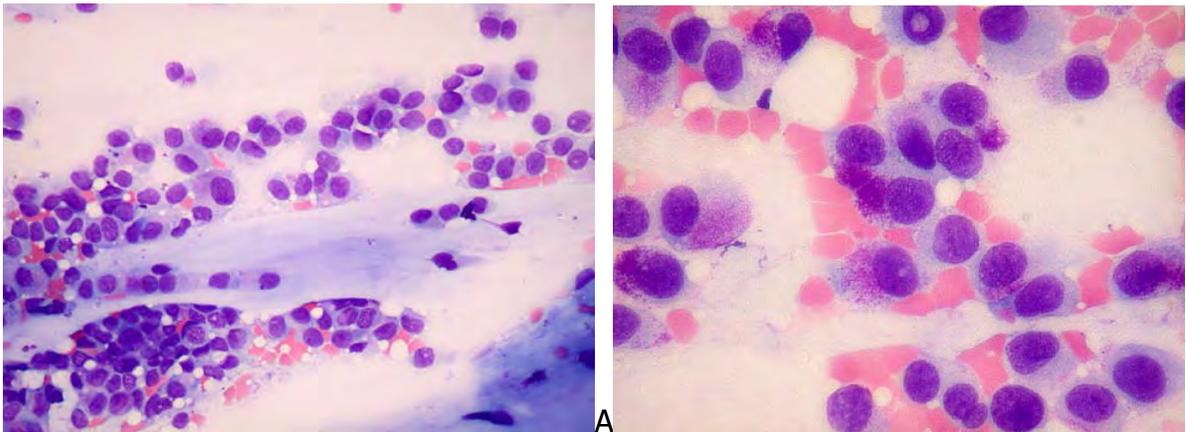


Fig.10.26. (A, B): Low-grade neuroendocrine carcinoma of the breast showing in FNA single and clustered malignant polygonal cells with low-grade, oval nuclei and intracytoplasmic metachromatic granules. (MGG).

## Mucinous (colloid) carcinoma

This tumor accounts for about 2% of all breast cancers. It usually presents as a circumscribed solid mass, mimicking a fibroadenoma and has an abundant extracellular mucinous stroma (must be >90% to be qualified). It usually has low-grade nuclei and a good prognosis.

### FNA cytology:

- Large pools of mucinous material (Figs.10.27 and 10.28)
- Cohesive groups of tumor cells with low-grade nuclei– often arranged in tight, ball-like clusters
- Proliferated capillary blood vessels
- Occasionally calcifications

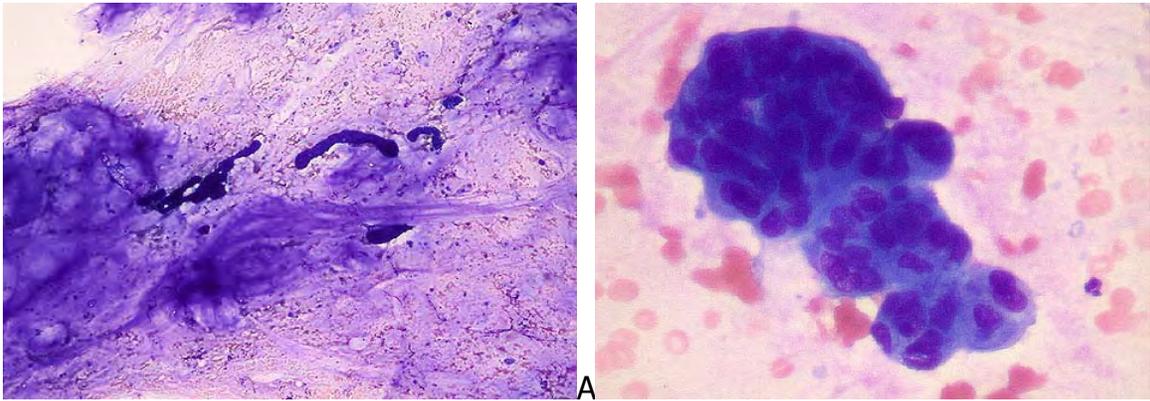


Fig.10.27. Mucinous carcinoma showing in FNA thick mucous material and rare tri-dimensional clusters of tumor cells. (MGG).

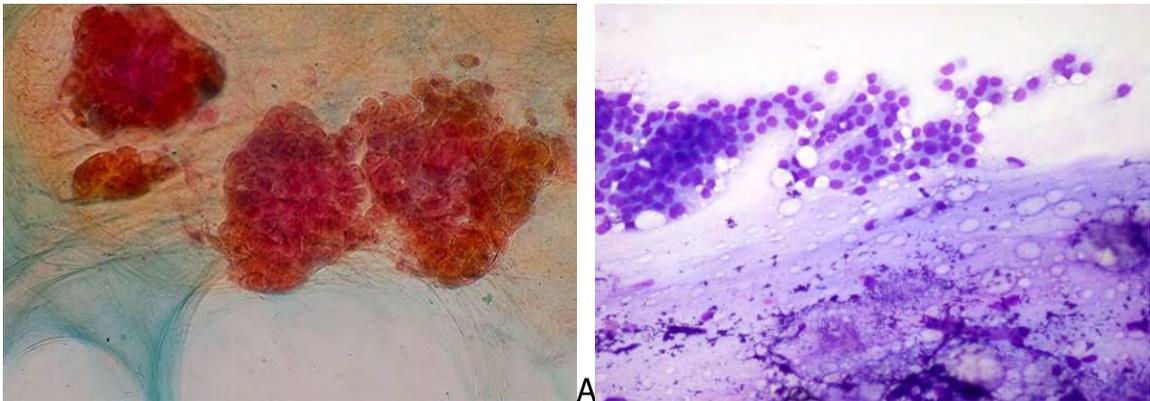


Fig.10.28. (A): Mucinous carcinoma of the breast showing in FNA thick mucous material and tri-dimensional, tight clusters of small tumor cells (Pap). (B): Another case of breast mucinous carcinoma showing in FNA thick mucous material, sheets of polygonal tumor cells and proliferated, branching capillary blood vessels (in the lower part of the figure). (MGG).

### Carcinoma with osteoclastic giant cells

This is a rare morphologic variant of ductal carcinoma of the breast. It usually presents clinically as a circumscribed brown or haemorrhagic mass lesion. Histologically, it displays features of a low-grade ductal carcinoma with cribriform pattern. The stroma is cellular and shows extravasated erythrocytes, abundant lymphocytes, histiocytes, plasma cells, haemosiderin pigment granules and many osteoclast-like giant cells – often hugging the clusters of carcinoma cells.

### **FNA cytology:**

- Monomorphic cuboidal malignant cells with oval nuclei (Fig.10.29)
- Many osteoclast-like giant cells
- Scattered lymphocytes and haemosiderin-laden macrophages

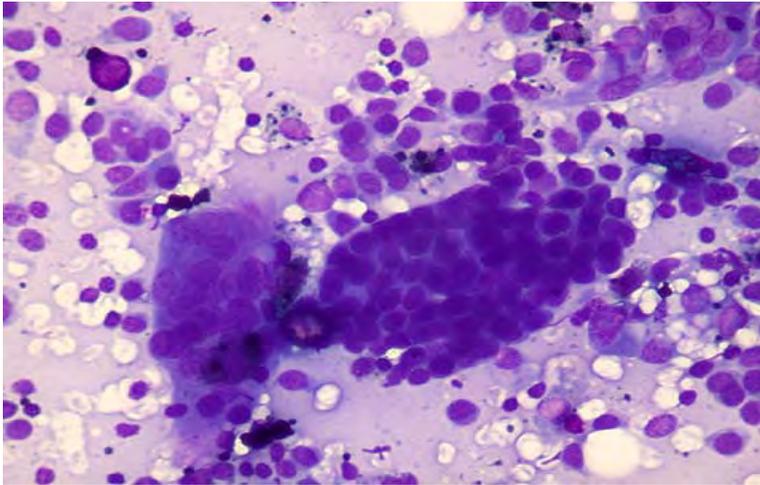


Fig.10.29. Carcinoma with osteoclastic giant cells showing in FNA abundant single and clustered cuboidal malignant epithelial cells admixed with a few benign appearing multinucleated giant cells. (MGG).

### **Medullary carcinoma**

This is a relatively uncommon variant of ductal carcinoma with a favorable prognosis, accounting for 5% to 7% of all breast cancers. Histologically it has a well circumscribed “pushing” margin with dense continuous cuff of lymphocytes and plasma cells. The neoplasm has a syncytial sheet-like growth pattern (>75%) with grade 3 nuclei, ill-defined cytoplasm and minimal fibrosis within the lesion. Cystic changes may be present.

### **FNA cytology:**

- Hypercellular smear (Figs.10.30 and 10.31)
- Abundant isolated tumor cells and loosely cohesive clusters
- Granular, variably abundant cytoplasm
- Enlarged, hyperchromatic nuclei and prominent nucleoli
- Background is usually rich in lymphoid cells

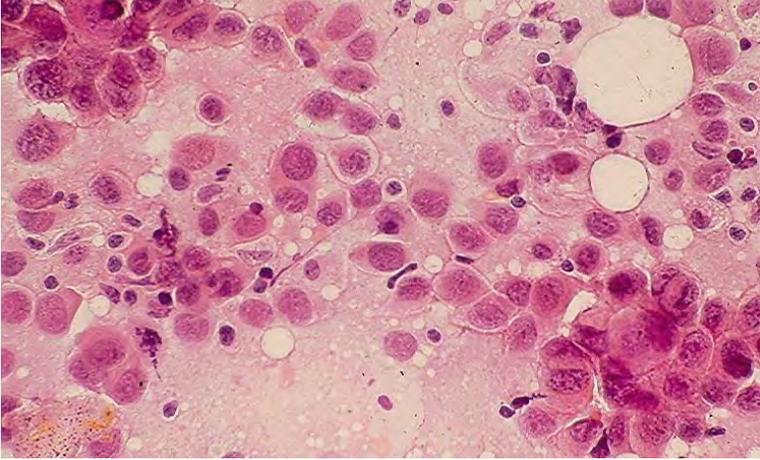


Fig.10.30. Mammary medullary carcinoma showing in FNA single and clustered polygonal malignant epithelial cells with granular cytoplasm and oval nuclei with small nucleoli, admixed with a few lymphocytes. (Pap).

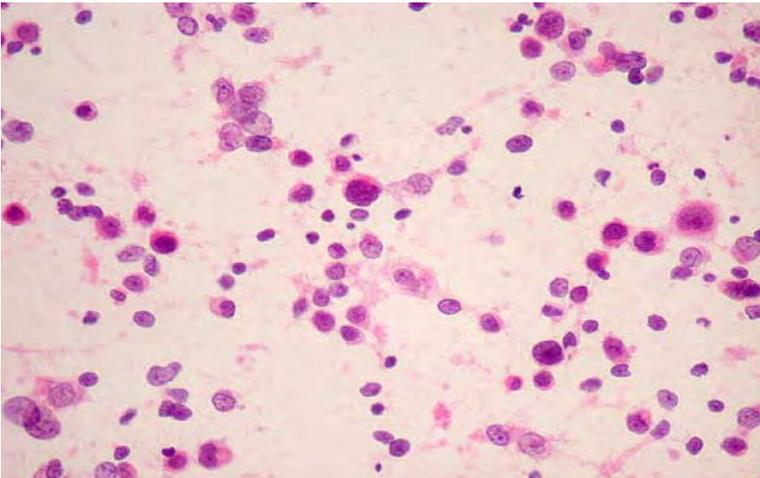


Fig.10.31. Numerous benign lymphoid cells and malignant cells with granular, ill-defined cytoplasm and inconspicuous nucleoli present in FNA of a mammary medullary carcinoma. (Pap).

### Secretory carcinoma

This tumor occurs most commonly in prepubescent children but it may occur in adult females and in males. Clinically, it often presents as a slow growing - occasionally large circumscribed ball-like or multilobulated mass. The tumor is composed of cells with typically low-grade nuclei, dense, vacuolated cytoplasm. Necrosis is unusual and mitoses are rarely observed. It has a good prognosis but may recur later.

### FNA cytology:

- Hypercellular aspirate (Fig.10.32)
- Single and loosely cohesive clustered malignant cells with foamy cytoplasm.
- Slightly pleomorphic nuclei with conspicuous nucleoli.

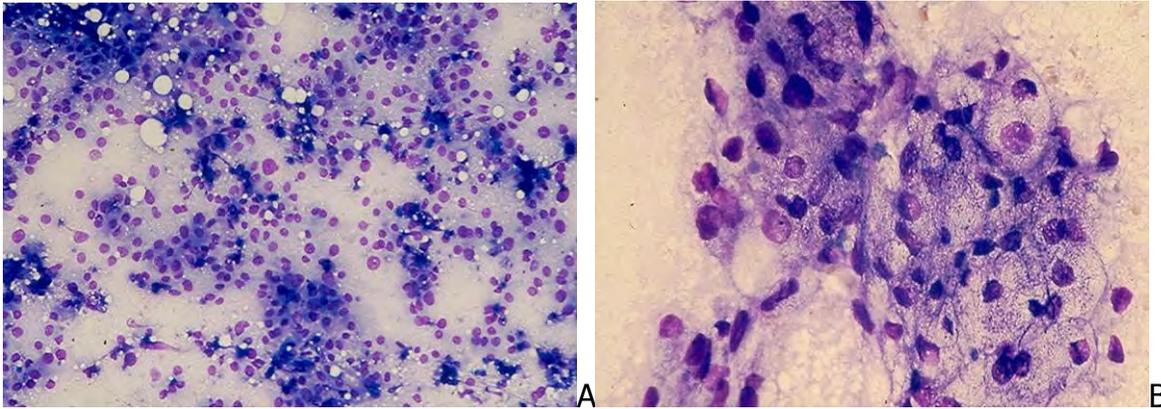


Fig.10.32. Secretory carcinoma of the breast showing in FNA, (A): Abundant polygonal epithelial cells singly and in clusters. (B): A cluster of tumor cells showing vacuolated cytoplasm and monomorphic oval nuclei with inconspicuous nucleoli. (Diff Quik stain)

### Apocrine carcinoma

This is a rare variant of ductal carcinoma accounting for < 1% of all breast cancers.

### FNA cytology:

- Cellular aspirate (Figs.10.33 and 10.34)
- Large polygonal tumor cells present singly and in loose clusters
- Granular cytoplasm
- Eccentrically located low-grade nuclei with conspicuous or inconspicuous nucleoli.

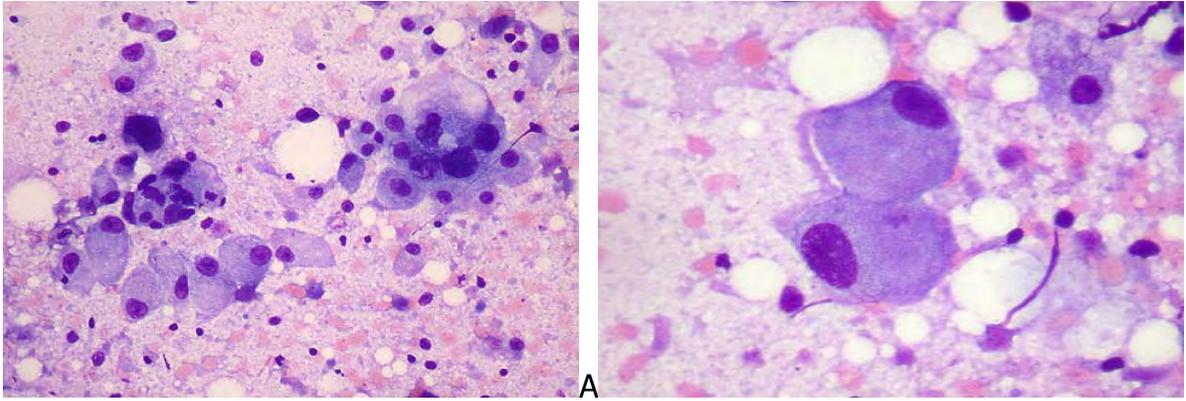


Fig.10.33. (A, B): Single and clustered polygonal, large tumor cells with abundant, granular or vacuolated cytoplasm and eccentrically located oval nuclei in FNA of an apocrine carcinoma of the breast. (MGG).

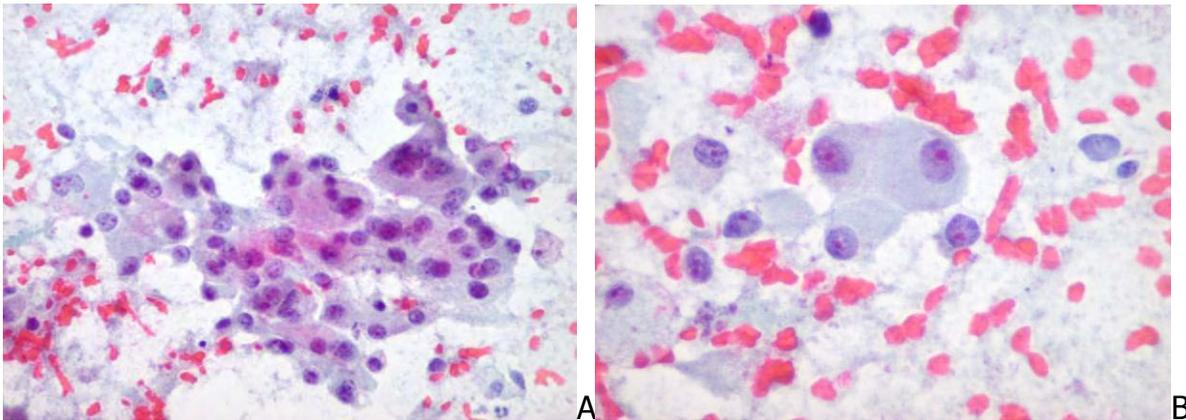


Fig.10.34. (A, B): Apocrine carcinoma of the breast displaying in FNA single and clustered tumor cells with well-defined, granular cytoplasm. (Pap).

## Other malignant tumors

- **Pleomorphic carcinoma** is a rare variant of high-grade ductal carcinoma. It is characterized by a proliferation of pleomorphic and bizarre giant cells comprising > 50% of the tumor cells in a background of adenocarcinoma with spindle or squamous differentiation. Patients with this tumor have a median age of 51 years, and in 12% of cases metastasis is present at initial clinical presentations. It shows in FNA single and clustered malignant pleomorphic giant cells with many displaying multiple nuclei.

- **Metaplastic carcinoma** accounts for less than 5% of all breast cancers. It is a ductal carcinoma with extensive mesenchymal or squamous differentiation. The tumor shows in FNA malignant glandular cells admixed with malignant squamous cells or mesenchymal cells and/or stromal tissue fragments.

- **Metastatic cancers** to the breast account for 0.5% to 6% of all breast cancers and women are affected about 5 times more than men, according to some studies. Cancers arising in any other body sites can metastasize to the breast and on rare occasions the primary tumor is clinically occult and the metastatic tumor is the initial clinical manifestation. The FNA cytology of a metastatic carcinoma to the breast usually displays cytologic manifestations that are usually different from those of a primary ductal or lobular carcinoma of the breast. (Fig.10.35)

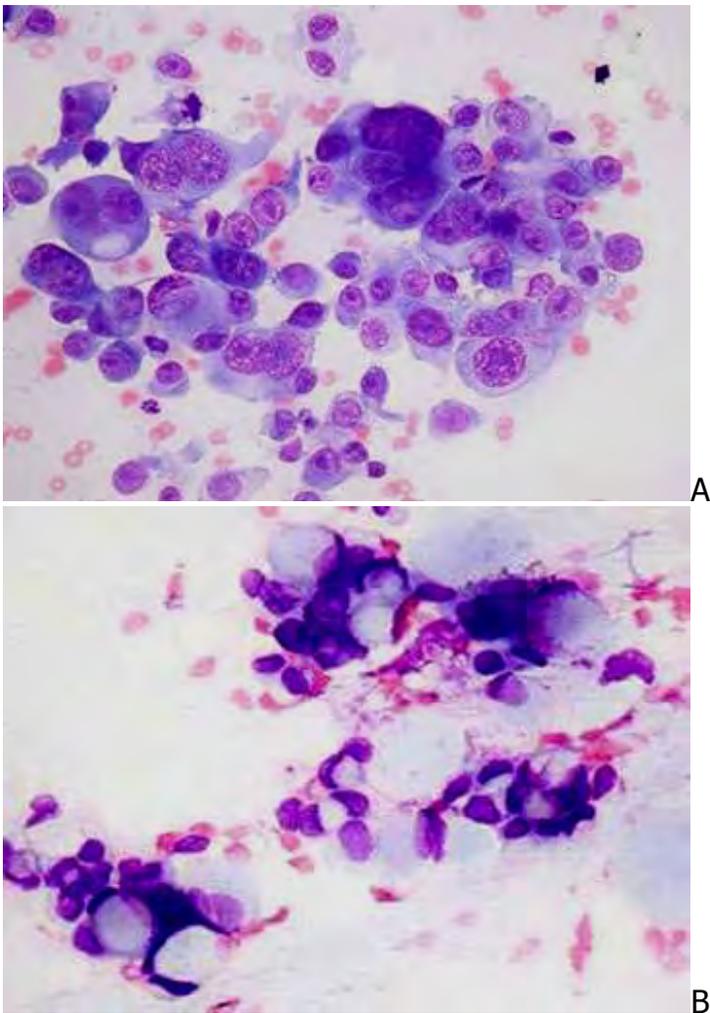


Fig.10.35 (A): Metastatic melanoma to the breast showing in FNA single and loosely clustered pleomorphic malignant cells with prominent nucleoli (MGG). (B): Metastatic mucus secreting colonic adenocarcinoma to the breast showing in FNA clustered malignant glandular cells with signet-ring configuration and intracytoplasmic mucus filled large vacuoles. (MGG).

- **Lymphoma.** Any types of lymphoma may occur in the breast involving either the breast parenchyma directly or indirectly through spread from an intramammary lymph node. Non-Hodgkin's lymphomas are more common than Hodgkin's lymphoma, and Burkitt's lymphoma is seen especially in endemic areas. In our practice B-cell lymphomas outnumber T-cell lymphomas by far. Most breast lymphomas are diffuse large B-cell tumors that yield in FNA abundant monotonous atypical lymphoid cells showing no cellular cohesion. Nuclear indentation and protrusion may be present. (Fig.10.36). Flow cytometry is helpful to show monoclonality and provides lymphocyte subtyping. Tissue biopsy is usually required for confirmation of the tumor and its subtype.

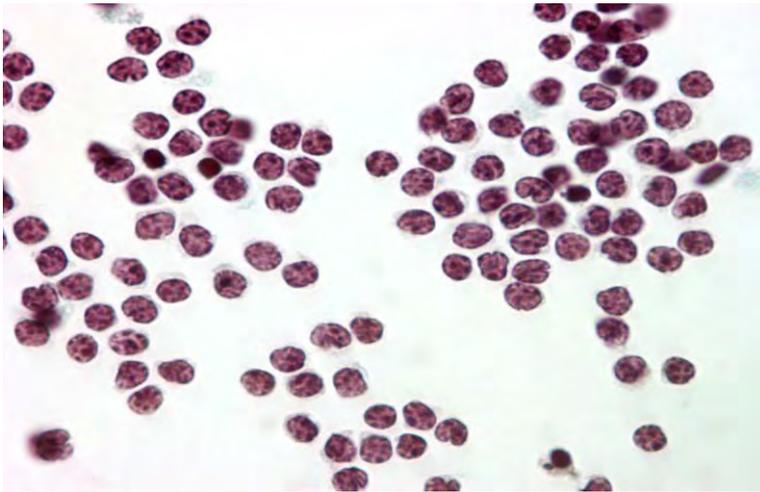


Fig.10.36. Breast Non-Hodgkin's lymphoma of showing in FNA abundant monomorphic hyperchromatic lymphoid cells. Nuclear indentation is noted in some cells. (Pap).

**Sarcomas.** All types of soft tissue sarcoma may arise in the breast and the most common one is angiosarcoma. Other types of soft tissue sarcoma are extremely rare.

*Angiosarcoma* of the breast accounts for less than 1% of all breast cancers. The tumor occurs most commonly in adult women in 6 and 7 decades of life and some patients had a prior history of radiotherapy to their breast cancers. The tumor is characterized by a poorly defined, reddish mass lesion. Histologically, it consists of irregular vascular spaces lined by neoplastic endothelial cells. Depending on the degree of nuclear atypia it can be classified as low-grade or high-grade tumor. Most post-irradiation angiosarcomas are high-grade epithelioid tumors. The tumor cells express Factor VIII-related, CD31 and CD34 antigens. A low-grade angiosarcoma yields in FNA a large amount of blood containing a few spindle cells with relatively bland elongated nuclei. A high-grade angiosarcoma, in contrast, shows a bloody cellular needle aspirate containing single and clustered pleomorphic malignant cells with variable cellular cohesiveness and focal acinar or vasoformative arrangement. The tumor cell cytoplasm expresses Factor VIII-related antigens. (Fig.10.37).

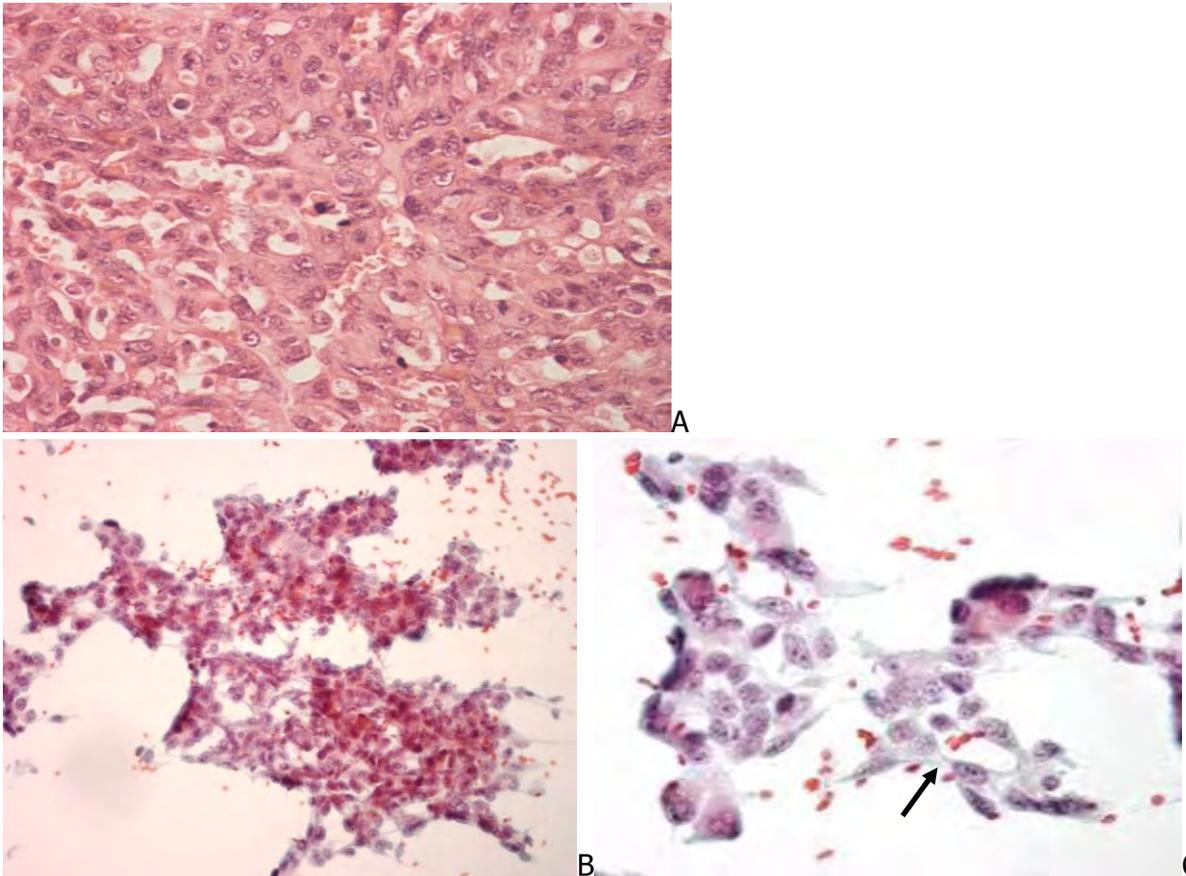


Fig.10.37. High-grade angiosarcoma of the breast, (A): Histology of the tumor. (B, C): Tumor FNA showing sheets and clusters of pleomorphic malignant cells with oval nuclei and prominent nucleoli. Cells forming vascular spaces (arrow) can be found in a few tumor cell clusters or sheets. (Pap).

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