exfoliative cytology

Department of Pathology - Cervical Cancer Screening Study Group
University of the Philippines

Women's Health & Safe Motherhood Project - Cancer Control Program
Department of Health
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Preface

This training manuscript is adopted and with gratitude from the 'Colposcopy for Beginners' training program of the UP-PGH Department of Obstetrics-Gynecology primarily for the standardization of procedures among gynecologist- and physician-participants of the Cervical Cancer Screening Project of the Women's Health & Safe Motherhood Project-Cancer Control Program of the Department of Health.

The Cervical Cancer Screening Research Project is conducted by the Cervical Cancer Screening Study Group, composed of the Clinical Epidemiology Unit, Department of Obstetrics-Gynecology, Department of Pathology, and Medical Oncology Section-Department of Medicine, all of the University of the Philippines-College of Medicine- Philippine General Hospital, and the International Clinical Epidemiology Network.

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**THE UP CERVICAL CANCER SCREENING STUDY GROUP**

Genara M. Limson, MD  
Corazon A. Ngelangel, MD, MS  
Mario R. Festin, MD, MS  
Laurie S. Ramiro, MD, PhD  
Agustina D. Abelardo, MD, MIAC  
Liberty Fajutrao, MD, MS  
Jose Maria V. Avila, MD  
Cynthia P. Cordero, MS  
Alexander Delgado, MD
The cervix is the lower part of the uterus and is divided from the corpus by the internal os. It projects through the anterior wall of the vagina at the vaginal vault. It is basically cylindrical, about 3 cm in length and 2.5 cm in diameter in nulligravids with an increase in size and eversion in multigravids. The endocervical canal is continuous with the uterine cavity above the level of the internal os and the vagina below the external os.

The cervix is held in position by the uterosacral and lateral ligaments. The former is thought to hold the cervix in its normal position, the latter being its principal means of support. There are cyclical changes in the cervix with a progressive increase in vascularity, congestion, edema, and secretion of cervical mucus during the proliferation phase of the menstrual cycle reaching a peak at ovulation. The cervical mucus is profuse, watery, alkaline, facilitating sperm penetration under estrogenic stimulation. It is scant, thick and acid, containing numerous leucocytes, and acting as a barrier for sperm penetration during post-ovulatory phase.

The cervix has a covering epithelium with an underlying stroma composed of an admixture of fibrous, muscular, and elastic tissue. It contains a well developed capillary network at the stromal epithelial junction. There are three basic types of cervical epithelium. These are the original squamous or columnar epithelium, mataplastic epithelium and atypical epithelium.

The original squamous epithelium is of the stratified squamous type similar to the vaginal epithelium. It contains variable amounts of glycogen and has five distinct layers or zones:

1. basal cell or stratum cylindricum
2. parabasal cell or prickle cell layer or stratum spinosum
3. intermediate, navicular, clear or stratum spinosum superficial cells
4. intraepithelial zone or condensation zone
5. stratum corneum.

It is separated from the fibrous stroma by a basement membrane. In young girls and elderly women, the epithelium is not usually stimulated and appears atrophic. During the reproductive period, the intermediate cell layer thickens and becomes rich in glycogen as a result of progesterone while the superficial layers develop with the influence of estrogen. The original columnar epithelium lines the endocervix and the underlying glandular structures, occasionally extends to the ectocervix and contains coarse secretory cells. Under the colposcope, the gross appearance of the epithelium exists in two forms:

1. rugae which are coarse subdivisions appearing as 2 or 3 mounds on the cervical lips, and
2. villi which appears as bunches of small "grapes".

Histologically the cells are tall and slender, one layer, closely packed in a cobblestone pattern. The nuclei are round or oval located in the lower third of the cell. There are two types of cell:

1. nonciliated secretory cells – have dome-shaped raised surface covered with many short microvilli
2. ciliated secretory cells – have fine chromatin distribution, numerous mitochondria, and free ribosomes.

Mitosis in the columnar epithelium is not seen under normal conditions.

The transformation zone is a physiologic process that occurs in late fetal life, at menarche, and during pregnancy with the latter producing the most dramatic alterations in cervical morphology. Exposure of the endocervical epithelium to the acetic pH of the vagina by eversion in primigravids and gaping of the lower part of the cervix in multigravids stimulate metaplasia. The metaplastic transformation in pubertal and adolescent cervix is most likely caused also by exposure of the original columnar
epithelium to the vaginal pH. The columnar epithelium is transformed to squamous epithelium in a matter of days and weeks. Once transformed, it does not revert to its original glandular state. Morphogenetically, there are two types of squamo-columnar junction:

1. the original squamo-columnar junction where the native squamous epithelium of the portio vaginalis joins the columnar epithelium, and
2. the physiologic or functional squamo-columnar junction, established between the newly formed transformation zone and the endocervical columnar cells

The visualization of the transformation zone is difficult with the naked eye and is enhanced with the application of 5% acetic acid and the use of the colposcope. The process under the colposcope can be divided into three stages:

Stage 1: Pallor in the villi
Stage 2: Downward growth of the new squamous epithelium to the sides of the villi (progressive fusion of the villi)
Stage 3: Completion of villi fusion with resultant smooth squamous surface

Histologically, the changes of metaplastic transformation can be divided into three stages:

Stage 1: Loss of mucus coat, decrease in height, and increase in the width of columnar cells
Stage 2: Mixture of both columnar and metaplastic epithelium
Stage 3: Smooth surface appearance of epithelium with occasional stromal papillae projecting into it and the original columnar epithelium seen underlying the new metaplastic epithelium

The colposcopic appearance of the atypical epithelium is that of a white structure as a result of the obstruction to the reflected light from the vascular epithelium and is caused by an increase in cellular and epithelial density. It may also have a honey-combed or stippled appearance which results from the shape of capillaries present within the atypical epithelium. The atypical epithelium includes two broad groups of lesions:

- atypical metaplastic epithelium
- dysplastic or CIS lesions

The former exhibits variations in nuclear size, shape, chromosome content, and epithelial differentiation. There are three distinct patterns:

- Abnormal epithelium – refers to a lesion where the prickle cell layer is increased in width in association with epithelial rete-like pegs; this term is not ideal, but confusing as all atypical epithelium is abnormal
- Basal cell hyperplasia – refers to marked proliferation, increased mitosis, and hyperchromatism of the cells in the basal layers. Its true potential is unknown and is not generally considered to have a malignant potential
- Dyplasia

REFERENCE:

THE PROCEDURE FOR TAKING A PAP SMEAR

Jose Maria V. Avila, M.D.

The smear should be taken before the bimanual examination and before other tests:

1. Instruct the patient not to douche or use any type of lubricant, and refrain from intercourse for 24 hours prior to obtaining the smear.

2. Use speculum lubricated only with warm water.

3. Cervix and adjacent vagina should be well visualized.

4. Sample endocervix and ectocervix separately:
   a) Cervix — rotate spatula with good pressure over entire endocervix
   b) Endocervix — recommended endocervical brush, rotate ¼ - ½ turn (can substitute cervical “brush” for (a) and (b)

5. Spread material thinly, but rapidly, on labeled glass slide(s) and fix immediately (less than 10 seconds) either by immersion (95% ethanol) or with a commercial spray fixative (held at least 10 inches from slide to prevent cellular distortion). Rapid fixation is crucial to providing an adequate specimen for proper evaluation of the Pap smear.

6. Particularly in older women, some method of detecting endometrial disease such as vaginal pool specimen or cervical canal aspiration, should be included.

Strengths and Limitations of the Pap Smear:

1. No other test ever invented has been as successful as the Pap smear in eradicating cancer. However, expectations of the Pap smear to detect pre-malignant lesions are now so high that any result short of perfection is likely to be considered malpractice (or even reckless homicide)

2. Cervical cancer, despite the Pap smear, has never been completely eradicated in any population even in the United States (which is a thoroughly screened community). Cervical cancer remains a leading cause of death in many countries.

3. Chain of events that cannot be broken in order for a screening process to work in cancer prevention:
   a) patient must come in for the test
   b) clinician must take adequate, representative, well prepared Pap smear
   c) cytologist must correctly identify any neoplastic lesion in the Pap smear
   d) result must go back to the clinician, who must act appropriately on the results
   e) patient must present herself either for therapy or her next Pap smear

4. Many, if not most diagnostic problems relate to sampling errors, i.e. few or no abnormal cells on the glass slide, rather than errors of interpretation.

5. Given a woman with a serious lesion, the Pap smear can detect the lesion in 90-95% of cases.

6. False-negative results occur at a low, but well-documented, and probably irreducible rate of 5-10% (at least 1 in 10-20 positive cases will be missed on routine screening), even in the finest laboratories.

7. False positive results also occur, but are of less concern because of abnormal Pap smears should always be confirmed histologically before therapy is undertaken.
**PITFALLS of DIAGNOSIS**

**Sampling and Processing Errors:**

1. The scraping has been taken directly from the bottom of an ulcerated area of the fungating tumor mass. This will produce only blood, inflammatory cells, and necrotic debris, but no intact, easy-to-recognize diagnostic tumor cells.

2. To avoid possible bleeding or other complications, the smear has been taken from an adjacent normal healthy mucosa, thus missing the margins of the ulceration.

3. A cotton-tipped swab has been used rather than a plastic or wooden spatula, scraper, or endocervical brush. The cotton fibers may retain most of the diagnostic cells.

4. The lesion has been “cleaned” before sampling or the patient had a vaginal douche, using intravaginal drugs (suppository), or had coitus shortly before the smear.

5. The smear has been allowed to air-dry before fixation. This may produce various artifacts (apparent nuclear and/or cytoplasmic size increase and orangeophilia of the cells) that may prevent the identification of the turnover cells.

6. The smear has been poorly smeared and flakes of tumor cells may slide off the slides into the fixative during their transportation or during the staining of the cells in the laboratory.

7. The reliability of the smears decreases when taken during menses.

**Interpretation of Errors:**

1. Because of fatigue, ignorance, or carelessness, the atypical cells present in the smear, especially if scanty, are missed by the overworked or negligent cytologist (true false negative)

2. An abundance of inflammatory cells, debris, contaminant foreign bodies, and tumor diathesis has hidden the diagnostic cells

3. An eroded, atrophic mucosa has generated an abundance of parabasal cells with repair changes that can mimic the cancer cells and confuse the screener.

4. The malignant cells, especially if not well preserved, are confused for repair, dysplastic, or radiated cells.
THE PAPANICOLAOU SMEAR

Jose Maria V. Avila, M.D.

- The Pap stain has 3 cytoplasmic dyes: Orange G, Eosin Y, and Light Green, plus Hematoxylin for nuclear detail
- The Pap stain can stain cells blue or blue-green (basophilia, cyanophilia), pink (acidophilia, eosinophilia), or indeterminate (grey-blue).
- Orange G and Eosin Y are acid dyes that stain basic proteins such as prekeratin. Cytoplasmic orangeophilia is associated with keratinization.

Pap Smear STAINING PROCEDURES

**Progressive Method**

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>95% ethanol (fixative)</td>
<td>15 minutes</td>
</tr>
<tr>
<td>80% ethanol</td>
<td>6 to 8 dips</td>
</tr>
<tr>
<td>70% ethanol</td>
<td>6 to 8 dips</td>
</tr>
<tr>
<td>50% ethanol</td>
<td>6 to 8 dips</td>
</tr>
<tr>
<td>Water, distilled</td>
<td>6 to 8 dips until glossy look disappears</td>
</tr>
<tr>
<td>Harris hematoxylin, undiluted</td>
<td>45 secs</td>
</tr>
<tr>
<td>Water, distilled</td>
<td>rinse</td>
</tr>
<tr>
<td>Water, distilled</td>
<td>rinse</td>
</tr>
<tr>
<td>Water, distilled</td>
<td>rinse</td>
</tr>
<tr>
<td>50% ethanol</td>
<td>6 to 8 dips</td>
</tr>
<tr>
<td>Ammonium hydroxide (1.5% in 70% ethanol)</td>
<td>70% ethanol</td>
</tr>
<tr>
<td>70% ethanol</td>
<td>6 to 8 dips</td>
</tr>
<tr>
<td>70% ethanol</td>
<td>6 to 8 dips</td>
</tr>
<tr>
<td>80% ethanol</td>
<td>6 to 8 dips</td>
</tr>
<tr>
<td>95% ethanol</td>
<td>6 to 8 dips</td>
</tr>
<tr>
<td>OG-6</td>
<td>1 ½ min</td>
</tr>
<tr>
<td>95% ethanol</td>
<td>rinse</td>
</tr>
<tr>
<td>95% ethanol</td>
<td>rinse (do not allow slides to remain in alcohol)</td>
</tr>
<tr>
<td>EA-50 or 65</td>
<td>3 min</td>
</tr>
<tr>
<td>95% ethanol</td>
<td>rinse gently</td>
</tr>
<tr>
<td>95% ethanol</td>
<td>rinse gently</td>
</tr>
<tr>
<td>95% ethanol</td>
<td>rinse gently</td>
</tr>
<tr>
<td>100% ethanol</td>
<td>6 to 8 dips</td>
</tr>
<tr>
<td>100% ethanol (if large volumes)</td>
<td>6 to 8 dips</td>
</tr>
<tr>
<td>100% ethanol: xylene (1:1)</td>
<td>6 to 8 dips</td>
</tr>
<tr>
<td>Xylene</td>
<td>6 to 8 dips</td>
</tr>
<tr>
<td>Xylene</td>
<td>6 to 8 dips</td>
</tr>
<tr>
<td>Xylene</td>
<td>until ready to mount</td>
</tr>
</tbody>
</table>

**Regressive Method**

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>95% ethanol (fixative)</td>
<td>15 to 30 minutes</td>
</tr>
<tr>
<td>80% ethanol</td>
<td>6 to 8 dips</td>
</tr>
<tr>
<td>70% ethanol</td>
<td>6 to 8 dips</td>
</tr>
<tr>
<td>50% ethanol</td>
<td>6 to 8 dips</td>
</tr>
<tr>
<td>Water, distilled</td>
<td>15 sec to 2 min until glossy look disappears</td>
</tr>
<tr>
<td>Harris hematoxylin (stock solution) distilled water (1:1)</td>
<td>6 min</td>
</tr>
<tr>
<td>Distilled water</td>
<td>rinse to remove excess stain</td>
</tr>
<tr>
<td>Distilled water</td>
<td>rinse</td>
</tr>
<tr>
<td>Aqueous HCl sol’n (0.25%)</td>
<td>6 dips</td>
</tr>
<tr>
<td>Gentle running tap water (lukewarm)</td>
<td>6 min</td>
</tr>
<tr>
<td>50% ethanol</td>
<td>6 to 8 dips</td>
</tr>
<tr>
<td>70% ethanol</td>
<td>6 to 8 dips</td>
</tr>
<tr>
<td>80% ethanol</td>
<td>6 to 8 dips</td>
</tr>
<tr>
<td>95% ethanol</td>
<td>6 to 8 dips</td>
</tr>
<tr>
<td>OG-6</td>
<td>1 ½ min</td>
</tr>
<tr>
<td>95% ethanol</td>
<td>rinse off excess stain</td>
</tr>
<tr>
<td>95% ethanol</td>
<td>rinse (do not allow slides to stand in alcohol)</td>
</tr>
<tr>
<td>EA-36, -50, or -65</td>
<td>1 ½ min</td>
</tr>
<tr>
<td>95% ethanol</td>
<td>rinse gently</td>
</tr>
<tr>
<td>95% ethanol</td>
<td>rinse gently</td>
</tr>
<tr>
<td>95% ethanol</td>
<td>rinse gently</td>
</tr>
<tr>
<td>100% ethanol</td>
<td>6 to 8 dips</td>
</tr>
<tr>
<td>100% ethanol (if large volume)</td>
<td>6 to 8 dips</td>
</tr>
<tr>
<td>100% ethanol xylene (1:1)</td>
<td>6 to 8 dips</td>
</tr>
<tr>
<td>Xylene</td>
<td>6 to 8 dips</td>
</tr>
<tr>
<td>Xylene</td>
<td>6 to 8 dips</td>
</tr>
<tr>
<td>Xylene</td>
<td>until mounted</td>
</tr>
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</table>
**ULTRAFAST Pap Smear**

<table>
<thead>
<tr>
<th>Step</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline -</td>
<td>30 sec</td>
</tr>
<tr>
<td>95% ethanol (optional) -</td>
<td>10 sec</td>
</tr>
<tr>
<td>Alcoholic formalin -</td>
<td>6 slow dips</td>
</tr>
<tr>
<td>Water -</td>
<td>2 slow dips</td>
</tr>
<tr>
<td>Richard-Allan Hematoxylin 2 -</td>
<td>2 slow dips</td>
</tr>
<tr>
<td>Water -</td>
<td>6 slow dips</td>
</tr>
<tr>
<td>95% alcohol -</td>
<td>6 slow dips</td>
</tr>
<tr>
<td>Richard-Allan Cytoestain –</td>
<td>4 slow dips</td>
</tr>
<tr>
<td>(alcoholic mixture of orange G</td>
<td></td>
</tr>
<tr>
<td>eosin Y, light green and</td>
<td></td>
</tr>
<tr>
<td>aniline blue) -</td>
<td></td>
</tr>
<tr>
<td>95% ethanol -</td>
<td>6 slow dips</td>
</tr>
<tr>
<td>100% ethanol -</td>
<td>6 slow dips</td>
</tr>
<tr>
<td>Xylene -</td>
<td>10 slow dips</td>
</tr>
<tr>
<td>Mount &amp; coverslip -</td>
<td></td>
</tr>
</tbody>
</table>

**TOTAL TIME** 90 sec
STAINING TECHNIQUE: CYTOCOLOR
Cytological Standard Stain According to Szczepanik

Szczepanik’s standard cytological stain with modified solutions is chiefly used for early recognition of female genital carcinoma. Within the space of about 3 minutes a stained microscopic specimen can be obtained giving reliable results on malignancy, hormone status and vaginal flora. The patient can be told the results of her test at first visit to the physician. The Szczepanik stain can also be used to stain smears prepared from tumors, punctuate and excised specimens, and sediment or centrifugate obtained from body fluids.

STAINING DIRECTIONS

Pour the reagents in the order given into closeable tubes or staining troughs. Slides must be repeatedly immersed in the solutions. Simply holding the slide in a solution gives poor results.

Immerse the smear following fixation in:
1. Distilled water – 10 x 1 sec
2. Hematoxylin solution – 1 x 1 min
3. Rinse under running water – 1 x 5 sec
4. 2-Propanol GR – 2 x 1 sec
5. Polychromic solution – 1 x 1 min
6. Distilled water – 5 x 1 sec
7. 2-Propanol GR – 5 x 1 sec
8. 2-Propanol GR – 5 x 1 sec
9. Xylene GR – 5 x 1 sec
10. Xylene GR – 5 x 1 sec

Cover the smear immediately (i.e., while still moist from immersion in xylene) with Merkcoglas spray or, if a cover glass is to be used, with Entellan new.

FINISHED STAIN

The staining effect is the same as that obtained with the Papanicolaua stain –
- Cyanophilic (basophilic) cytoplasm – blue green
- Eosinophilic (acidophilic) cytoplasm – pink
- Erythrocytes – red
- Keratinized cytoplasm – pink orange
- Cell nuclei – blue, dark violet, brown-black
- Microorganisms – blue-violet
- Trichomonas – grey-blue, grey-green

FILLING PREPARED SPECIMENS

Specimens covered with Merkcoglas or Entellan new remain color fast for over 5 years.

STORAGE CONDITIONS/ SHELF LIFE

The staining kit should be stored at a temperature of between 15 and 25 degrees Celsius. It is usable for at least 24 months when kept under proper conditions. Bottles remain usable for 4 weeks after opening. Staining solutions in use should be filtered daily and renewed if the color of the stain departs from the color normally obtained. The staining set is sufficient for about 1,000 prepared specimens.

CONTENTS OF THE STAINING KIT

The staining kit contains six 500 ml bottles:
- 1 x 500 ml modified hematoxylin solution
- 1 x 500 ml modified polychromic solution
- 3 x 500 ml 2-propanol GR
- 1 x 500 ml xylene GR
The Clinical Laboratories Improvement Act (CLIA 88)

In the U.S.A., this regulates in detail the practice of cytology by doing the following:

1. Requiring yearly proficiency testing of cytotechnologies and cytopathologists.
2. Mandating specimen adequacy.
3. Evaluating the physical layout and performance of cytology laboratories and their personnel with announced and unannounced inspections.
4. Establishing the new workload limits.
5. Comparing the cytology diagnosis with surgical biopsy diagnosis.
6. Mandating the review of prior negative smears seen in the last 5 years in the case of diagnosis of a new high-grade lesion.
7. Requiring increased record keeping for statistical analysis.
8. Issuing other directives and requirements whenever needed.

The aim of this law is to improve the quality of cytologic screening for female genital tract carcinoma and their precursors. Unfortunately, it may also have some negative effect by increasing the cost of the Pap smear and contributing to the shortage of personnel and cytology laboratories.

Quality Assurance

- Rescreening at least 10% of the initial negative smears.
- Review of previous negative smears when a serious atypia is discovered.
- Comparison of the cytologic and histologic findings and review of the smears in case of disagreement.
- Keeping records of the percentage of abnormal smears reported by each cytotechnologists.
- Participation in proficiency programs.
- Inserting new or old previously missed abnormal smears into the routine work.
- Participation in continuing and special remedial education.
NORMAL PAP SMEAR
Teresita V. Tuazon, M.D.

I. NORMAL CELLS IN THE PAP SMEAR:

The cells normally seen in a cervico-vaginal smear represent those originating from the normal squamous epithelium of the cervix and vagina.

A. Basal cells

These are typically small, undifferentiated cells that resemble small histiocytes. The nucleus is round or oval, vesicular and centrally located with a small amount of delicate cytoplasm. These cells are seldom recognized as isolated cells in Pap smears. They are however seen in severe atrophy, at the edge of an ulcer or when there is vigorous scraping. When present in a smear, these are usually associated with the next larger cells, the parabasal cells.

B. Parabasal cells

These are moderately large, with rounded cell borders. The cytoplasm is moderately dense which typically stains blue-green or gray. The nucleus is round or oval with finely granular, evenly distributed chromatin and occasional chromatoids and is approximately of the same size as the vesicular nucleus of the intermediate cell. Nucleoli are inconspicuous or absent, unless the cells are reactive or inflamed. Parabasal cells are more commonly seen in Pap smears than thebasals. However, in a fully mature epithelium, these are unlikely to be found.

C. Intermediate cells

These may range in size from that of a parabasal cell (low intermediate cell) to that of a superficial cell (high intermediate cell). The cytoplasm is usually basophilic, thin and abundant, with polygonal outlines. The nucleus is centrally placed, round or oval with a clearly defined nuclear membrane surrounding well-preserved nucleoplasm. The chromatin is finely divided and evenly distributed with occasional chromatoids. The term vesicular is applied to such a configuration. The size of the nucleus approximates that of a red blood cell and is somewhat smaller than the parabasal nucleus.

A variant of the intermediate cell is the navicular cell which is somewhat elongated and boat-shaped with a thicker cytoplasm. Navicular cells can be seen late in the menstrual cycle and during pregnancy.

D. Superficial cells

These are large cells of polygonal shape possessing a delicate, flat, transparent cytoplasm. The cell borders are well defined and have polygonal outlines. The nucleus of a superficial cell is normally smaller than that of a red blood cell. It is dense or pyknotic, like India ink-dot.

II. CELLS ORIGINATING FROM THE ENDOCERVICAL EPITHELIUM:

Endocervical cells frequently appear in tight cluster. These display a characteristic columnar appearance with finely vacuolated cytoplasm which is faintly basophilic and occasionally with clear mucus. When the endocervical cluster is flattened along the long axis and is seen “on end”, one may observe a tightly fitting network of cells resembling a honeycomb because of the clear cytoplasm surrounding the nuclei. The fragile cytoplasm of the endocervical cells may disintegrate, with resulting “stripped nuclei”. The nuclei of endocervical cells are finely granular and of approximately the same size as the nuclei of intermediate or parabasal squamous cells.

III. CELLS ORIGINATING FROM THE NORMAL ENDOMETRIUM:

Endocervical cells may be seen readily in vaginal smear from the time of the beginning of the menstrual flow until the 10th or even the 12th to the 14th day of the cycle. Normal endometrial cells are round to oval, and smaller than endocervical cells. The cytoplasm is generally scant and ill defined. It usually stains basophilic and may be finely vacuolated. The nuclei are round to oval and usually eccentrically located in the cell. These are usually degenerated, but when well preserved, these show evenly dispersed fine chromatins. The nucleus is about the size of an intermediate cell nucleus.
Spontaneously exfoliated endometrial cells usually form "double contour cell balls" with darkly staining stroma inside and lighter staining epithelium outside. These are common during "exodus", days 6 to 10 of the cycle, and can be seen also during menses as well as in patients with benign polyps and hyperplasia.

Endometrial Stromal Cells

There are two types of endometrial stromal cells: superficial and deep.

1. Superficial stromal cells tend to form loose aggregates ("sticky histiocytes") and are exclusively small. They have a moderate amount of cytoplasm that is poorly stained and ill defined, with fine vacuolation. The nuclei are round to oval or bean shaped and often eccentrically located in the cell.

2. Deep stromal cells are slightly smaller than superficial stromal cells and typically have spindle or stellate shapes, with scant cytoplasm. The nuclei are oval to spindle-shaped and frequently have a longitudinal groove in the nuclear membrane.

Endometrial stromal cells, like endometrial glandular cells are present only in the first half of the cycle, particularly during "exodus" (days 6 -10), together with histiocytes. During the secretory phase, only 2% of smears contain endometrial cells; the percentage again rises in the premenstrual phase (after day 25).

<table>
<thead>
<tr>
<th>Differential Diagnosis of Benign Glandular Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endocervix</strong></td>
</tr>
<tr>
<td>Larger</td>
</tr>
<tr>
<td>More variable</td>
</tr>
<tr>
<td>Loose two-dimensional arrangement</td>
</tr>
<tr>
<td>Honeycomb sheets</td>
</tr>
<tr>
<td>Multinucleation relatively common</td>
</tr>
<tr>
<td>Finer, paler chromatin</td>
</tr>
<tr>
<td>Abundant cytoplasm</td>
</tr>
<tr>
<td>Better preservation</td>
</tr>
<tr>
<td><strong>Endometrium</strong></td>
</tr>
<tr>
<td>Smaller</td>
</tr>
<tr>
<td>More uniform</td>
</tr>
<tr>
<td>Crowded three-dimensional clusters</td>
</tr>
<tr>
<td>Double contoured balls</td>
</tr>
<tr>
<td>Multinucleation rare</td>
</tr>
<tr>
<td>Coarser, darker chromatin</td>
</tr>
<tr>
<td>Scant cytoplasm</td>
</tr>
<tr>
<td>Degeneration</td>
</tr>
</tbody>
</table>
THE NORMAL PAP SMEAR

Squamous cells

a. Superficial cells - most mature squamous cells
   small pyknotic nucleus, 5 to 6 um in diameter
   polygonal with pink or green cytoplasm

b. Intermediate cells - less mature than superficial cells
   vesicular nuclei, 8um in diameter
   polygonal with pink or green cytoplasm

c. Parabasal cells - immature squamous cells
   vesicular nuclei
   round or oval, with green cytoplasm
Endocervical cells

- often arranged in strips & look like picket fence or in sheets rather than isolated cells
- when in sheets they resemble a honeycomb with well defined borders

Endometrial cells

- exfoliated cells are seen during the first 12 days of the menstrual cycle
- seen as small cells with dark nuclei, scanty cytoplasm, & often arranged in three dimensional clusters
- shedding of endometrial cells after day 12 has been associated with endometrial polyps, IUD and endometritis

Inflammatory cells

- neutrophils are seen in all cervical specimens & do not necessarily indicate infection
- their numbers increase after injury or infection

Lactobacilli

- non pathogenic gram positive, rod shaped bacteria
- organisms metabolize glycogen in the squamous cells resulting in the cell pattern called cytolysis, leaving behind only bare intermediate cell nuclei
HORMONAL CYTOLOGY

Teresita V. Tuazon, M.D.

The cervicovaginal epithelium is sensitive to a variety of stimuli, particularly hormones. The vaginal mucosa responds to changes in hormones produced by the body, particularly estrogen and progesterone. It may also be sensitive to androgens, corticoids, thyroxin, vitamins, antibiotics, mechanical stimulation, digitalis and inflammation. During the first half of the menstrual cycle, the follicular phase, the squamous mucosa is influenced primarily by estrogen, which promotes full maturation of the epithelium to the level of the superficial cell. During the second half, or luteal phase of the cycle, and also during pregnancy, the epithelium is primarily influenced by progesterone. Progesterone is produced by the corpus luteum of the ovary, which is formed after ovulation. During pregnancy, progesterone is first formed by the corpus luteum, and later by the placenta. Progesterone inhibits full squamous differentiation, allowing maturation only to the intermediate, rather than the superficial cell layer. Normal mucosal thickness is maintained by functional hyperplasia of the intermediate zone.

There are many ways of expressing the degree of maturation of the vaginal epithelium, but the most reproductive and commonly used method is the Maturation Index (MI).

Maturation Index

Parabasal % Intermediate % Superficial %

1. Complete history:
   a) Clinical
   b) Menstrual (including last menstrual period, postmenstrual, regularity)
   c) Pregnanacies
   d) Hormonal therapy
   e) Drugs
   f) Surgery
   g) Radiation
   h) Previous neoplasia

2. Gentle scrape; two thirds up lateral vaginal wall
3. No evidence of inflammation
4. No more than two cell types present

An MI smear should be free of inflammation, glandular cells, metaplastic cells, hyperkeratosis and parakeratosis, dysplastic cells; usually no more than two cell types should be present. An adequate clinical history is very important in the proper interpretation of the maturation index. The report should indicate whether the MI is, or is not, compatible with the patient’s history.

Parabasal Predominant Maturation Index (Atrophy)

1. Postmenopausal (classic)
2. Childhood (> 1 month)
3. Postradiation
4. Ovarian insufficiency
5. Postpartum
6. Lactation
7. Androgens (exogenous, tumors)
8. Androgenic atrophy
9. Intrauterine deficiency
10. Estrogen deficiency
11. Ulcer (cervicovaginal)
12. Turner’s syndrome (XO)
13. Hypopituitarism, including starvation
14. Hypothyroidism (severe)
15. Gonadal dysgenesis
16. Prolactin

Intermediate Predominant Maturation Index (Progesterone Effect)

1. Newborn (< 1 month)
2. Postovulatory
3. Pregnancy
4. Early menopause
5. Progesterone therapy
6. Cortisone therapy
7. Adrenocortical hormones
8. Tetracycline (perhaps others)
9. Late childhood (premenarchy)
10. Acromegaly
11. Digitalis (premenopausal)
12. Ovarian dysfunction
13. Low dose estrogen therapy
14. Androgens, including tumors
15. Luteinized follicle
16. Corpus luteum cyst
Superficial Cell Predominant Maturation Index (Estrogen Effect)

1. Preovulatory (peaks at ovulation)
2. Estrogen therapy (very variable)
3. Obesity
4. Cirrhosis
5. Excess androgens
6. Testicular feminization
7. Ovarian tumors
8. Granulosa cell
9. Thecoma
10. Metastasis
11. Primary endometriosis
12. Digitalis (postmenopause)

Representative Maturation Patterns

<table>
<thead>
<tr>
<th></th>
<th>Parabasal %</th>
<th>Intermediate %</th>
<th>Superficial %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn</td>
<td>0</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>Child</td>
<td>80</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Preovulatory</td>
<td>0</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>Postovulatory</td>
<td>0</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Postmenopause</td>
<td>80</td>
<td>20</td>
<td>0</td>
</tr>
</tbody>
</table>
1. Best site for obtaining a smear is the lateral vaginal wall.

2. Sample should be free from inflammatory changes, glandular cells, metaplastic cells, and anucleated squames.

3. Smear should have an adequate clinical history especially concerning hormonal treatment.

4. Sample should be rejected in the presence of three cell types. It is usually caused by inflammation and by evaluating the cervical component.

5. Randomized: count the first two squamous epithelial cells noted in each field. Use fields scattered widely throughout the smear for reproducibility.

6. Identify cells. Use 40x objective, counting only normal squamous cells.

7. Be consistent in the method of sampling or randomizing and in identifying cell types.

8. Maturation index is the relation of the parabasal cells to intermediate cells to superficial cells expressed in percentages and written as, e.g. 0/70/30.

9. Various cytologic patterns (Normal averages):

<table>
<thead>
<tr>
<th></th>
<th>Variability</th>
<th>Maturation Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>At birth (1st week)</td>
<td>Slight</td>
<td>0/95/5</td>
</tr>
<tr>
<td>Childhood (to 6 years)</td>
<td>Moderate</td>
<td>90/10/0</td>
</tr>
<tr>
<td>Perimenarche</td>
<td>Great</td>
<td></td>
</tr>
<tr>
<td>Reproductive Age (Superficial cells = 0%)</td>
<td>Moderate</td>
<td>0/40/60</td>
</tr>
<tr>
<td>Ovulation</td>
<td>Moderate</td>
<td>0/70/30</td>
</tr>
<tr>
<td>Menstruation</td>
<td>Moderate</td>
<td>0/95/5</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>Slight</td>
<td>90/10/0</td>
</tr>
<tr>
<td>Postpartum</td>
<td>Moderate</td>
<td></td>
</tr>
<tr>
<td>Perimenopausal (Superficial cells = 10%)</td>
<td>Moderate</td>
<td>0/100/0</td>
</tr>
<tr>
<td>Estratrophy</td>
<td>Moderate</td>
<td>100/0/0</td>
</tr>
<tr>
<td>Teleatrophy</td>
<td>Moderate</td>
<td></td>
</tr>
</tbody>
</table>

10. A few agents affecting cytohormonal pattern are:
   a. estrogen: clean background, flat single cells, M.I. shift to the right
   b. progesterone: dirty background, curled clumped cells, M.I. shift to midzone
   c. cortisone: clean background, flat cells, M.I. shift to midzone
REACTIVE CELLULAR CHANGES

1. Inflammation

- Cytoplastic changes: vacuolization, decrease staining intensity, polychromasia, perinuclear halos, and irregularity of shape
- Nuclear changes: bi or multinucleation, vacuolization, chromatin clumping, karyorrhexis and karyopyknosis, nuclear enlargement (1.5-2x the area of intermediate squamous cell nucleus)

2. Typical Repair

- initially characterized by a predominantly inflammatory reaction with fibrin, debris, PMN’s and hemorrhage
- subsequently, the cells have enlarged nuclei with increased activity
- immature cells arranged in large cohesive sheets
- cytoplasmic borders are indistinct resulting in syncytia
- cytoplasm stains variably while the nuclei are ovoid to round, enlarged to 1 – 2.5x the size of the nucleus of an intermediate cell and without nuclear irregularities
- there is regular nuclear polarity
- nucleoli are prominent and normal mitotic figures are not unusual

3. Cellular Changes induced by IUD

- IUDs may elicit an inflammatory reaction in the endometrial cavity
- String in IUD may produce cervical glandular atypia
- Endocervicals may contain vacuoles, engulfing PMNs, producing mild to moderate variation in nuclear size and shape and hyperchromasia
- Metaplastic squamous cell can also have vacuoles in the cytoplasm, with slight increase in nuclear size but with bland chromatin pattern
- Endometrials occur singly or in clusters, in a clean background, being shed throughout the menstrual cycle

4. Changes associated with Atrophy with or without inflammation

- generalized nuclear enlargement in atrophic squamous cells or parabasal type cells
- naked nuclei due to autolysis
- degenerated eosinophilic parabasal-like cells with nuclear pyknosis resembling parakeratotic cells
- basophilic amorphous material in the background (“blue blobs”)
- numerous PMNs
- air drying can cause artifactual nuclear enlargement
5. Radiation-induced cell changes

- marked cellular and nuclear enlargement without an increase in nucleocytoplasmic ratio
- nuclear changes: polychromasia, wrinkling, and smudging of chromatin, vacuolization, bi and multinucleation
- cytoplasmic changes: vacuolization and/or polychromasia
MICROBIOLOGICAL CLASSIFICATION in the FEMALE GENITAL TRACT

Doderlein’s bacilli
- 69% are lactobacillus acidophilus
- responsible for cytolysis of intermediate cells
- conditions seen: in pregnancy, luteal phase of menstrual cycle, postmenopause, premenarche, and during or after steroid therapy

Mixed bacteria
- encompasses admixtures of rods and coccoids
- can be properly identified only by culture

Cocci or coccoid bacteria
- often seen as complete overgrowth of organisms
- pseudoeosinophilia and pseudopyknosis
- usually observed in the presence of Trichomonads and Leptothrix

Gardnerella vaginalis (Hemophilus vaginalis)
- “clue cells”: epithelial cells covered with small bacilli giving them a grainy appearance
- materials from patients with Gardnerella vaginitis contain a few pus cells and lack lactobacilli
Leptothrix

- very thin, hairless structures
- morphologically indistinguishable from Doderlein bacilli
- identified only on cultures and biochemical characteristics

Chlamydia trachomatis

- infected cells show intracytoplasmic changes, often classified as "atypical" with enlarged hyperchromatic nuclei having uniformly distributed chromatin
- initially seen as fine eosinophilic, coccoid bodies followed by transformation of these particles into fine vacuoles and larger inclusions

Trichomonas

- trichomonads are small pear-shaped organisms with faint gray or pink staining reaction, with small oval eccentric nucleus
- smears with Trichomonads without inflammation exhibit a "clean smear"
- with Trichomoniasis, cell changes are seen such as perinuclear halos, nuclear enlargement, multinucleation, and chromatin degeneration
- Leptothrix may be seen in association with Trichomonas vaginalis
- False positive diagnosis of Trichomonads may be due to degenerated leucocytes, cellular fragments, parabasals with karyolysis, and granulated blobs or mucus in atrophic smears
Fungi
- yeast cells, budding yeast cells, pseudohyphae and true hyphae which are eosinophilic to gray-brown
- pseudohyphae formed by elongated budding, with constrictions along their length

Viruses (HPV, herpes simplex)

Herpes simplex virus type II
- infected cells initially have coarse chromatin pattern, nuclear vacuoles, and later on, the nucleus has a ground-glass appearance
- formation of syncytia and multinucleation
- partial to complete karyorrhexis of nuclei followed by karyolysis

Human papilloma virus
- cell samples contain anucleate squames, parakeratotic cells, koiocytes
- infected cells have enlarged nuclei with coarse chromatin and karyorrhexis

Actinomyces
- tangled clumps of filamentous organisms with acute angle branching, seen as “cotton ball” on low power
- “sulfur granules” formed by masses of leucocytes adherent to microcolonies of organisms with swollen filaments at the periphery
- numerous pus cells are usually evident

Other microorganisms
- parasites such as *Trichuris trichiura*, *Enterobius*, and *Schistosoma*
THE BETHESDA SYSTEM
for Reporting Cervical/Vaginal Diagnoses
Arnold Fernandez, M.D.

DEFINITIONS and CRITERIA for SPECIMEN ADEQUACY

“Satisfactory for evaluation” indicates that the specimen has all of the following:

- Appropriate labeling and identifying information
- Relevant clinical information
- Adequate numbers of well-preserved and well-visualized squamous epithelial cells
- An adequate endocervical/ transformation zone component (from a patient with a cervix)

Well-preserved and well-visualized squamous epithelial cells should cover more than 10% of the slide surface. An adequate endocervical transformation zone component should consist of at least two clusters of well-preserved endocervical glandular and/or squamous metaplastic cells, with each cluster composed of at least five cells. The definition applies to specimens from both premenopausal women with a cervix, except in the situation of marked atrophy in which metaplastic and endocervical cells often cannot be distinguished from parabasal-like cells. (In case of marked atrophic changes, the absence of an identifiable endocervical/transformation zone component does not affect the specimen adequacy categorization of a specimen otherwise determined to be “Satisfactory for evaluation”.)

A specimen is “Satisfactory for evaluation but limited by...” if any of the following apply:

- Lack of pertinent clinical patient information (age, date of last menstrual period as a minimum; additional information as appropriate)
- Partially obscuring blood, inflammation, thick areas, poor fixation, air-drying artifact, contaminant, etc. that precludes interpretation of approximately 50% to 70% of the epithelial cells
- Absence of an endocervical/transformation zone component as defined above.

“Satisfactory but limited by...” indicates that the specimen provides useful information; however, interpretation may be compromised. A report of “Satisfactory for evaluation but limited by absence of endocervical/transformation zone component” does not necessarily require a repeat smear. Patient factors such as location of the transformation zone, age, pregnancy, and previous therapy may limit the clinician’s ability to obtain an endocervical sample. The ultimate determination of specimen adequacy rests with the clinician, who must correlate the findings described in the cytopathology report with clinical knowledge of the individual patient.

A specimen is “Unsatisfactory for evaluation...” if any of the following apply:

- Lack of patient identification on the specimen and/or requisition form
- A slide that is broken and cannot be repaired
- Scant squamous epithelial component (Well-preserved and well-visualized squamous epithelial cells covering less than 10% of the slide surface)
- Obscuring blood, inflammation, thick area, poor fixation, air-drying, contaminant, etc. that precludes interpretation of approximately 75% or more of the epithelial cells

The “Unsatisfactory...” designates that the specimen is unreliable for the detection of cervical epithelial abnormalities.

Specimen adequacy is evaluated in all cases. However, any epithelial abnormality is of paramount importance and must be reported regardless of compromised adequacy. If abnormal cells are detected, the specimen is never categorized as “Unsatisfactory”. Such cases may be considered “Satisfactory but limited by...” based on the above criteria.
DESCRIPTIVE DIAGNOSIS

BENIGN CELLULAR CHANGES

Infection
- *Trichomonas vaginalis*
- Fungal organism morphologically consistent with *Candida* spp.
- Predominance of cocobacilli consistent with shift in vaginal flora
- Bacteria morphologically consistent with *Actinomyces* spp.
- Cellular changes associated with *Herpes simplex* virus

Reactive Changes

Reactive cellular changes associated with:
- Inflammation (includes typical repair)
- Atrophy with inflammation (“atrophic vaginitis”)
- Radiation
- Intrauterine contraception device (IUD)
- Other

EPITHELIAL CELL ABNORMALITIES

Squamous Cell

Atypical squamous cells of undetermined significance (ASCUS): Qualify**

Low grade squamous intraepithelial lesion (LSIL)
Encompassing: Human papilloma virus (HPV)*/Mild dysplasia/cervical intraepithelial neoplasm (CIN I)

High grade squamous intraepithelial lesion (HSIL)
Encompassing: Moderate dysplasia, severe dysplasia and carcinoma in situ /CIN 2 and CIN 3

*cellular changes of HPV cytopathic effect, previously termed "koilocytosis", "koilocytosis atypia" or condylomatous atypia, are included in the category of LSIL.
**Atypical squamous or glandular cells of undetermined significance should be qualified further, if possible, as to whether a reactive or a neoplastic process is favored.

Glandular Cell

Endometrial cells, cytologically benign, in a postmenopausal woman

Atypical granular cells of undetermined significance (AGUS): Qualify @
- Endocervical adenocarcinoma
- Endometrial adenocarcinoma
- Extraterine adenocarcinoma
- Adenocarcinoma, NOS
- Other Malignant Neoplasm, specify

Selection of criteria for diagnosing cervical lesion

<table>
<thead>
<tr>
<th>Findings</th>
<th>Diagnosis</th>
<th>Implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Koilocytosis, maturation,</td>
<td>Low-grade squamous intraepithelial</td>
<td>Low risk of progressing may</td>
</tr>
<tr>
<td>minimal basal atypia</td>
<td>lesion (flat or exophytic condyloma; CIN I)</td>
<td>persist</td>
</tr>
<tr>
<td>Koilocytosis, maturation</td>
<td>High-grade squamous intraepithelial</td>
<td>High risk of persisting/</td>
</tr>
<tr>
<td>diffuse atypia</td>
<td>lesion (CIN II)</td>
<td>progressing</td>
</tr>
<tr>
<td>Minimal koilocytosis or</td>
<td>High-grade squamous intraepithelial</td>
<td>High risk of persisting/</td>
</tr>
<tr>
<td>maturation, diffuse atypia</td>
<td>lesion (CIN II)</td>
<td>progressing</td>
</tr>
</tbody>
</table>

Source: Christopher P. Crum. M.D. and Gerard J. Nuovo, M.D. *Genital Papillomaviruses and Related Neoplasms*

Reference:
Cytology and Histology of Cervical Intraepithelial Neoplasm (SIL)

Arnold Fernandez, M.D.

Squamous intraepithelial lesion (SIL) encompasses the entire morphological spectrum of precursor to invasive squamous cell carcinoma, previously called dysplasia, carcinoma in situ (CIS), and cervical intraepithelial neoplasia (CIN). It is characterized by abnormal cell proliferation, maturation and cytologic atypia. The cytologic abnormalities include hyperchromatic nuclei, abnormal chromatin distribution, nuclear pleomorphism, and increase nucleo-cytoplasmic ratio.

The histologic features which are taken into account when assessing CIN are:

1. **Differentiation (maturation, stratification)**
   a) presence or absence
   b) proportion of epithelium showing differentiation

2. **Nuclear abnormalities**
   a) nucleo-cytoplasmic ratio
   b) hyperchromasia
   c) nuclear pleomorphism and
   d) anisokaryosis

3. **Mitotic activity**
   a) number of mitotic figures
   b) height in epithelium
   c) abnormal configuration

Based on the above features, the grades of CIN can be categorized as CIN Grade I, CIN Grade II and CIN Grade.

<table>
<thead>
<tr>
<th>Maturation</th>
<th>CIN Grade 1</th>
<th>CIN Grade 2</th>
<th>CIN Grade 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear abnormalities</td>
<td>Upper 2/3</td>
<td>Upper half</td>
<td>Absent or confined</td>
</tr>
<tr>
<td>Mitotic figures</td>
<td>Slightly marked on basal third</td>
<td>More marked than CIN 1</td>
<td>Marked throughout most or full thickness</td>
</tr>
<tr>
<td></td>
<td>Confined to basal third; Rare abnormal forms</td>
<td>Confined to basal 2/3; Presence of abnormal forms</td>
<td>Numerous; found at all levels; frequent abnormal forms</td>
</tr>
</tbody>
</table>

Cytologic criteria of low grade SIL (LSIL) and high grade (HSIL)

<table>
<thead>
<tr>
<th></th>
<th>LSIL</th>
<th>HSIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Architecture</td>
<td>isolated cells or sheets</td>
<td>isolated cells or pseudosyncytia</td>
</tr>
<tr>
<td>Cell size</td>
<td>mature</td>
<td>immature or mature keratinized cells</td>
</tr>
<tr>
<td>Nuclear size</td>
<td>more than 3X the area of normal intermediate cell nucleus</td>
<td>more than 3X the area of normal intermediate cell nucleus</td>
</tr>
<tr>
<td>Nucleocytoplasmic ratio</td>
<td>↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>Hyperchromasia</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Nuclear outlines</td>
<td>slightly irregular</td>
<td>markedly irregular</td>
</tr>
</tbody>
</table>

References:

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THE CYTOLOGY AND HISTOPATHOLOGY OF MICROINVASIVE SQUAMOUS CELL CARCINOMA OF THE UTERINE CERVIX

Arnold Fernandez, M.D.

Definition

Microinvasive carcinoma of the cervix (MICA) is a preclinical carcinoma diagnosed only by microscopy and correspond to clinical Stage Ia of the 1985 FIGO Staging of Carcinoma of the Cervix.

Over the past decades, the definition of MICA has been controversial. The depth of stromal invasion, the significance of vascular permeation, and the degree of tumor confluence in relation to the frequency of pelvic node metastases, tumor recurrence and death are the main subjects of discussion. Several classification have been developed but have not been generally satisfactory. The depth of stromal invasion reported in literature varies from 1-5 mm. Some investigators regard microinvasion by the absence of vascular permeation whereas others include lymphatic infiltration under MICA.

Studies suggest that lesions with 1-3 mm stromal penetration and without vascular invasion have virtually no potential for metastases or recurrence. On the other hand, lesions with vascular invasion may potentially metastasize although the risk is low. Pooled data has indicated that a maximum depth of invasion of 3 mm or less is associated with a risk of lymph node metastasis of only 0.3% and a risk of invasive recurrence of 0.2%. On the other hand, invasion of 3.1 - 5.0 mm is associated with an overall risk of lymph node metastasis of 7.4% and a recurrence rate of 5.4-6%. With such contention, measurements in the definition of MICA have been included.

Microinvasive carcinoma (MICA) using the 1995 Modification of FIGO staging of cervical carcinoma divides Stage IA carcinoma into two categories. Stage Ia1 and Stage Ia2, both of which are defined by precise measurement.

Stage Ia1 includes all lesions with measured invasion of stroma up to 3mm. in depth and no wider than 7 mm. This category embraces all the minimally invasive and unmeasurable lesions included in stage Ia1 in the previous (1985) classification as well as the smaller lesions that were previously included in the Stage Ia2.

Stage Ia2 includes all lesions with measured invasion of stroma 3-5mm. in depth and no wider than 7mm. If there is more than one focus of invasion, the lateral spread of each individual invasive lesion is measured and the figures are added together to give a total horizontal spread.

Clinical Aspects

Of the women with microinvasive cancer, majority are asymptomatic, seen on routine pelvic examination. Affected females are in their 40s with cervixes that either appear normal or eroded.

The colposcopic diagnosis of MICA is based on abnormal vascular patterns of the cervical epithelium. There is a characteristic naked degree of whiteness in the epithelium consistent with cervical epithelial neoplasia in which one or more foci contain bizarre surface branching vessels. The recommended approach in the diagnosis is through a colposcope-oriented punch biopsy of the suspicious area followed by adequate conization as punch biopsies without colposcopic assistance and endocervical curettage specimens are inadequate to provide pertinent information required of cases with microinvasive cancer. For optimal evaluation, the cone should be properly fixed, totally and serially sampled vertically for microscopic study. Cervical cone reduces errors due to misinterpretation as a result of tangential cuts, glandular involvement and surface erosion. Multifocal invasion, extension of tumor in the deep and peripheral margins of the specimen and accurate recognition of vascular involvement cannot be possibly missed.
Cytopathology of MICA:

It is essential to recognize this lesion in cellular samples as initial detection or suspicion of microinvasion is dependent on cellular studies. Samples are better obtained using both cervical scrape and aspirate.

<table>
<thead>
<tr>
<th>Cellular Features</th>
<th>In-Situ Cancer</th>
<th>Micro-Cancer</th>
<th>Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distribution:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>single</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>syncytiul</td>
<td>+</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Pleomorphism</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Chromatin Pattern:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uniformly coarse</td>
<td>++++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Irregular finely granular</td>
<td></td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Irregularly coarsely granular</td>
<td></td>
<td>+/-</td>
<td>+++</td>
</tr>
<tr>
<td>Nucleoli:</td>
<td></td>
<td></td>
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<tr>
<td>Nuclear</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Micronucleoli</td>
<td>-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Macronucleoli</td>
<td>-</td>
<td>+</td>
<td>++++</td>
</tr>
<tr>
<td>Tumor diathesis</td>
<td>-</td>
<td>+/-</td>
<td>++++</td>
</tr>
</tbody>
</table>

Legend: +++++ increasing frequency

Histopathology of MICA

Microinvasive cancer shows distinct microscopic features related to the changes in the tissue of origin, growth pattern, cellular characteristics and stromal reactions at the site of infiltration.

Microinvasion usually originates from carcinoma in-situ and less frequently from dysplasia, the abnormal surface epithelium being more commonly involved than an altered epithelium within the gland. Typically, the malignant cells that penetrate through the basement membrane are pleomorphic with inconstant polarity, abundant cytoplasm, prominent nucleoli and occasional foci of keratinization.

In early microinvasion, the infiltrating cord of malignant cells tend to be unifocal, in continuity with the parent tissue of origin. The surface epithelium at the site of infiltration is intact. The cellular features of the infiltrating cells are similar to those observed in the tissue of origin. The advancing margins appear either blunt or sharp. Lesions developing into keratinizing cancer tend to have sharp irregular margins while those that become large cell non-keratinizing cancer have blunt margins. Lymphocytic response is noted in the cervical stroma.

As the extent of microinvasion increases up to 3mm., the infiltrating epithelial cells become multifocal, still continuous with the parent tissue of origin. The surface epithelium at the site of infiltration remains intact. Infiltrating cells have prominent nucleoli, loss of polarity, mitotic figures, and individual cell keratinization.

There are more epithelial infiltrations and confluence in microinvasions up to 5 mm. Lesions tend to be discontinuous from the parent tissue of origin. Ulceration of surface epithelium at the site is encountered although less frequently. Lymphocytic and desmoplastic reactions are conspicuous.
The most accurate method of measuring depth of stromal penetration is through a calibrated slide of ocular microscale. A practical but less accurate method is through the use of a microscopic field that corresponds to a diameter of 1 mm, determined by direct visualization with a transparent metric ruler.

Cervical cones with previous biopsy usually contain individual nests of malignant epithelium within the stroma at the site of punch biopsy. Such nests are clusters of intraepithelial carcinoma that may have been disrupted and incorporated in the stroma by the procedure, masquerading as MICA. The diagnosis of MICA should be carefully examined whenever this pattern is encountered at or near the site of biopsy.

Three features which can be helpful to the histopathologist in deciding whether early stromal invasion is present or not in ambiguous cases are: better differentiation of the invasive elements, clearing of the stroma and lymphocytic infiltrate. As the tumor becomes larger and progresses to a more complex pattern, other morphologic features must be taken into account. In the reporting of MICA, the following should be considered:

1. **Size of invasive tumor in two dimensions in the section which shows the greatest area of the lesion.**
2. **Presence or absence of lymphocytic channel involvement.**
3. **Whether growth pattern is confluent or finger-like.**
4. **Whether excision of CIN and invasive elements is complete or not.**

**Treatment**

Methods of treatment vary ranging from conization of the cervix to radical hysterectomy with pelvic lymph node dissection and from radium insertion to total pelvic irradiation. The present modality is toward a more conservative management with an individual approach to treatment based on the depth of penetration, lateral extent, and involvement of the cone margins.

**References:**

THE CYTOLOGY AND HISTOPATHOLOGY OF INVASIVE SQUAMOUS CELL CARCINOMA OF THE UTERINE CERVIX
Arnold Fernandez, M.D.

Definition
Preclinical invasive carcinoma of the cervix with dimensions greater than those accepted as stage Ia carcinoma have been previously referred to as "occult" invasive carcinomas. This word has not been included in the current FIGO classification; these tumors should be designated by the histopathologist simply as Stage Ib carcinomas. Stage II is frankly invasive carcinoma that extends beyond the cervix to involve the upper 2/3 of the vagina.

Cytology of Cervical Squamous Cell Carcinoma
Malignant squamous cell are relatively small with mean area of 229 +/- 83 square microns when compared to a superficial squamous cell. They may occur as singly syncytiat masses. Individual cells are round to oval in 75%, the remaining cells being caudate, elongate or irregularly formed. Cytoplasm is variable ranging from cyano philic, indeterminant, to eosinophilic. The nucleus measures 77 +/- 27 square microns, more than twice the size of the nucleus of a normal intermediate cell. Nuclearcytoplasmic ratio varies from 35-50%. All nuclei have some degree of hyperchromasia, round to oval, with irregularly distributed chromatin. Nucleoli are encountered only in 10% of the cells. Evidence of adverse host reaction or tumor diathesis is often present with a background of inflammation, hemorrhage, degenerative changes, and tissue destruction with cell debris.

Reagan & Ng proposed classifying cervical squamous carcinoma into large cell keratinizing, large cell non-keratinizing, and small cell non-keratinizing carcinoma.

In keratinizing carcinoma, tumor diathesis is usually not conspicuous. The cells are relatively large with mean area of 275 +/- 107 square microns, mostly isolated with 15% as caudate and elongated forms. Cytoplasmic eosinophilia is distinct and nuclear chromatin is usually coarsely granular.

An adverse host reaction is often seen in large cell non-keratinizing carcinoma. The cells are large with a mean area of 267 +/- 69 square microns. Cells are isolated or in syncytial sheets, with a cyanophilic cytoplasm coarsely granular hyperchromatic chromatin and macronucleoli.

Small cell carcinoma is usually isolated with tumor diathesis. The cells are small and uniform in size with a mean area of 169 +/- 37 square microns, isolated or in syncytia, with cyanophilic cytoplasm. Nuclei are usually oval and hyperchromatic with coarsely granular chromatin in majority of the cells. Nucleoli may be obscure.

The accuracy of cytologic evaluation of squamous cell carcinoma in adequate samples and competent cytopathologists can be highly sensitive and specific. The sensitivity for the detection of squamous cell cancer is 86.2% in stage Ia cancers, 89.8% in stage Ib, 95.7% in stage II, 94.6% in stage III and 100% in stage IV cancers. The specificity is 97% for keratinizing carcinoma, 96% for small cell cancers and 94% for large cell non-keratinizing cancers.

Pathology of the Three Cell Types of Squamous Cancer
Grossly, keratinizing cancers are exophytic, usually located at the ectocervical portion of squamocolumnar junction. Large cell keratinizing and small cell cancers are usually endophytic ulcerative lesions found at the endocervical portion of the squamocolumnar junction.

The most common and widely used histologic classification is based on the degree of differentiation. The lesions are divided into well differentiated (grade 1), moderate differentiated (grade 2), and poorly differentiated (grade 3) carcinomas. The most common squamous carcinomas are the moderately differentiated followed by the poorly differentiated and well differentiated tumors.

Wenz & Reagan classified the morphologic characteristics of the three different types of squamous cancer based on growth pattern, cellular characteristics and stromal reactions at the site of infiltration.
Microscopically, keratinizing squamous cell carcinoma of the uterine cervix is composed of irregular masses with sharp or irregular infiltrating margins. Desmoplastic reaction maybe marked and mononuclear infiltration is moderate. Keratin pearl formation and individual cell keratinizing are quite prominent. Tumor cells are comparable in size to large cell non-keratinizing carcinoma. Cytoplasm is moderately abundant, usually eosinophilic with well developed intercellular bridges. Nucleus is enlarged, hyperchromatic with coarse chromatin. Nucleoli are not appreciated. Mitotic activity is relatively low.

Large cell non-keratinizing carcinoma consists of nests of tumor cells with blunt or rounded borders, less frequently sharp infiltrating margins. A desmoplastic reaction with dense bands of connective tissue separate large or small tumor nests. Mild to moderate mononuclear inflammatory reaction maybe evident and focal necrosis is seen among large tumor sheets. Cellular pleomorphism is absent. Keratin pearls are not encountered although local individual cell keratinization maybe seen. Cells are round to polygonal with moderate homogenous cyanophilic cytoplasm. The nucleus is centrally located with irregular clumped chromatin. Mitotic figures are increased in number.

The neoplasm of small cell carcinoma tends to grow in diffuse pattern as syncytial nests with poorly defined boundaries between the tumor and cervical stroma. There are thin bands of supporting connective tissue. Tumor cells are small with high nucleocytoplasmic ratio, round to oval with scanty cytoplasm, large hyperchromatic nuclei with clumped chromatin and nucleoli. Mitotic figures and areas of necrosis are frequent. Occasionally, the neoplastic cells assume a spindle shaped configuration, resembling a sarcoma.

The five year survival rate of patients with large cell non-keratinizing carcinoma is 68.3%, 41.7% in large cell keratinizing type and 20% in small cell carcinomas.

It is not unusual to find more than one histologic type of tumor in the same patient. The most common combination is a keratinizing and a large cell non-keratinizing carcinoma. In this instances, the tumor with the poorer prognosis should be given the leading diagnosis.

Non-keratinizing small cell carcinomas have a complex variety of keratins with a different pattern and number from those of normal tissue. Keratinizing cervical carcinomas contain a less complex pattern of keratin intermediate filaments and polypeptides. Involuturin, another marker distinct from keratin, can be identified also in 93% of patients with keratinizing squamous cell carcinomas. It is likely that the intermediate filaments and differentiation marker maybe useful in better understanding the pathogenesis of cervical carcinoma and as tools in differential diagnosis.

The most common modes of spread of squamous cell carcinoma of the cervix is by direct local invasion to the adjacent tissue via the lymphatic with a preferential involvement of the paracervical, hypogastric and external iliac lymph nodes. Metastasis to the lateral sacral, common iliac, paraaortic and inguinal lymph nodes are occasionally encountered. Distant lymph node metastasis are uncommon and are usually part of a widespread disease.

Hematogenous route is least common, generally seen in stage IV lesions. Metastatic lesions are found in the lungs, liver, heart, bone and brain.

Comparison of the ureter and perireteral lymphatic obstruction leads to hydronephrosis, hydrothorax, and bronchitis with subsequent loss of renal function. Bilateral ureteral obstruction results in uremia, the leading cause of death as seen in 40-50% of patients. Other causes of deaths are cardiac and respiratory failure secondary to massive edema and pulmonary metastasis, peritonitis secondary to obstruction and perforation of the intestines, hemorrhage, and complication of radiation therapy.

References (partial):
Atypical squamous cells of undetermined significance (ASCUS)

- Definition: cell abnormalities that are more marked than those attributable to reactive changes but quantitatively and qualitatively fall short of the definite diagnosis of squamous intraepithelial lesion.
- borderline cytologic changes
- not a single diagnostic entity
- a diagnosis of exclusion which should be used sparingly; general guide in the diagnosis-should not exceed 2-3x the rate of squamous intraepithelial lesion (SIL)
- nuclear changes: enlargement 2-3x that of normal intermediate squamous cell nucleus with slight increase in nucleocytoplasmic ratio, mild hyperchromasia with finely granular evenly distributed chromatin, smooth and regular nuclear outlines
- nuclear enlargement is usually seen with mature (superficial/intermediate) squamous cells

The following are included in the category of ASCUS

1. Cells with mature intermediate-type cytoplasm containing some but not all features of HPV changes (e.g. nucleus may be wrinkled with smudged chromatin but may not be enlarged)
2. Atypical squamous cells in the setting of atrophy: nuclear enlargement with concomitant hyperchromasia, marked irregularity in nuclear outline or chromatin distribution, marked pleomorphism in the form of tadpole or spindle cells

Note: A short course of estrogen therapy followed by repeat smear may be useful to establish the diagnosis. Benign changes associated with atrophy will resolve after treatment; atypical changes from a precancerous lesion will persist.

3. Atypical squamous metaplasia: nuclear enlargement 1-1.5x the size of nucleus of metaplastic squamous cells (differential diagnosis is high grade SIL)
4. Atypical parakeratosis/dyskeratosis: miniature small polygonal squamous cells with densely eosinophilic cytoplasm having cellular pleomorphism in the form of caudate or elongate shapes and/or increased nuclear size or chromasia, shed singly or in three dimensional clusters
5. Atypical repair: involves tissue fragments or sheets of immature squamous cells, nuclear piling, significant anisonucleosis, and irregular chromatin pattern; differential diagnosis is an exuberant repair vs. carcinoma
- Reporting of ASCUS needs to be qualified, whether a reactive process or a neoplastic process is favored, since management may differ if reported as ASCUS probably reactive or ASCUS probably SIL, or ASCUS in the setting of atrophy.

Squamous Intraepithelial Lesion (SIL)

- Definition: encompasses a spectrum of noninvasive cervical epithelial abnormalities traditionally classified as flat condyloma, dysplasia, and CIN (cervical intraepithelial neoplasia)
- The Bethesda System (TBS) divides SIL into low grade (LSIL) and high grade (HSIL) lesions

LSIL (Low Grade Squamous Intraepithelial Lesion)

- encompasses cellular changes associated with HPV, previously called koilocytosis, as well as CIN-1 (mild dysplasia)

- Criteria of LSIL
  - Cells occur singly or in sheets
  - Nuclear abnormalities are generally confined to cells with mature superficial or intermediate type cytoplasm
  - Nuclear enlargement is at least 3x the area of normal intermediate nuclei, resulting in an increase in the nucleocyttoplasmic ratio
  - Moderate variation in nuclear size and shape
  - Binucleation and multinucleation
  - Hyperchromasia with uniformly distributed chromatin, or chromatin may appear degenerated or smudged if associated with viral cytopathic effects (HPV)
  - Nucleoli are not identified
  - Cell borders are distinct
  - Cells that display a well defined, optically clear perinuclear cavity and a peripheral dense rim of cytoplasm must show above nuclear abnormalities to be diagnostic of LSIL
Three cell types compose the cellular pattern of LSIL: koiocyte, dyskeratocyte, and atypical parabasal cell

1. Koilocyte – an intermediate mature squamous cell with an almost empty appearing perinuclear cavity with irregular and sharply defined borders. It contains one, two, or more hyperchromatic, slightly irregular nuclei with dense or coarsely granular chromatin
2. Dyskeratocyte – looks like miniature keratinized cell which is spindly or oval in shape; nuclei are usually condensed, pyknotic, or hyperchromatic
3. Atypical parabasal cell – a small cell, with large, irregular, hyperchromatic nuclei; cytoplasm is amphophilic or cyanophilic, often showing a clear area in the vicinity of the nucleus

**HSIL (High Grade Squamous Intraepithelial Lesion)**

- encompasses moderate dysplasia, severe dysplasia, and carcinoma in situ/CIN 2,3

- Criteria of HSIL
  - Cells occur singly, in sheets or syncytial-like aggregates
  - Nuclear abnormalities involve cells with immature, lacy and delicate or metaplastic cytoplasm; occasionally, the cytoplasm is densely keratinized
  - Nuclear enlargement may actually be less than LSIL, but the cytoplasmic area is decreased, leading to a marked increase in nucleocytoplasmic ratio
  - Hyperchromasia is evident; chromatin may be finely or coarsely granular with even distribution
  - Nucleoli are generally absent
  - Nuclear outlines are regular
  - Overall, cell size is smaller than LSIL
Squamous Cell Carcinoma

- general criteria of malignancy in cells are present in the nucleus, its relationship to the cytoplasm, and the relationship of one cell to another
- Nuclear changes: increase in size, variation in size and shape, infolding of nuclear membrane, chromatin changes in the form of hyperchromasia, increased size of clumps, irregular distribution of clumps, and prominence of interchromatin areas
- Nucleolar changes: irregularity in shape with sharp pointed projections and indentations; increase in size and number

Three types:

1. Keratinizing
   - Relatively large cells, many appearing as isolated cells
   - Cytoplasm is eosinophilic, the ecto- and endoplasmic borders appearing sharp
   - Elongated and caudate forms are common
   - Chromatin patterns often coarsely granular, less frequently finely granular
   - Tumor diathesis is not conspicuous
2. Large cell, non-keratinizing
   - Usually large cells, many appearing as isolated cells
   - Chromatin pattern is often coarsely granular and hyperchromatic
   - Macronucleoli are seen
   - Tumor diathesis is inconspicuous

3. Small cell
   - Usually uniformly small cells, either appearing as isolated cells or in syncytia
   - Cytoplasm is cyanophilic
   - Chromatin pattern is coarsely granular
   - Nucleoli are relatively common
   - Tumor diathesis commonly encountered
Atypical Glandular Cells of Undetermined Significance (AGUS)

- Applies to glandular cells which demonstrate changes beyond those encountered in reactive processes, yet insufficient for a diagnosis of adenocarcinoma
- The diagnosis should be qualified to indicate whether the cells are thought to be endocervical or endometrial in origin

Atypical Endocervicals of Undetermined Significance

- Includes a wide spectrum of morphologic changes from atypical-appearing reactive processes to adenocarcinoma in situ (AIS)
- Lesions in this category should be further qualified, if possible, whether a reactive or neoplastic lesion is favored
- Atypical endocervical cells, probably reactive, have enlarged nuclei 3-5x the normal and slightly hyperchromatic; the honeycombed pattern with distinct cell borders is often maintained
- Atypical endocervical cells, probably AIS are characterized by sheets and tightly packed cells with marked pseudostratification, high nucleocytoplasmic ratio and hyperchromasia

Atypical Endometrial Cells of Undetermined Significance

- Usually seen in small clusters and exhibit nuclear enlargement and hyperchromasia
- Small nucleoli may be present
- Cytoplasm is scant and cell borders are ill-defined
- Criteria for separating atypical endometrial cells into possibly reactive vs. probably premalignant or malignant are not well-defined, and therefore, this diagnosis is not subdivided further
Adenocarcinoma

- Cells are characterized by marked nuclear membrane and chromatin irregularity and/or a background of tumor diathesis
- The type of adenocarcinoma, whether endocervical, endometrial, or extrauterine should be indicated if possible

Cytologic differences between endometrial and endocervical adenocarcinoma

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Endometrial</th>
<th>Endocervical</th>
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<tr>
<td>Cell number</td>
<td>low</td>
<td>High</td>
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<tr>
<td>Cell arrangement</td>
<td>Single or in groups</td>
<td>Also rosettes and palisades</td>
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<td>Cell size</td>
<td>smaller</td>
<td>Larger</td>
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<tr>
<td>Cell shape</td>
<td>Round or oval</td>
<td>Cylindrical or columnar</td>
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<tr>
<td>Cytoplasm</td>
<td>Finely or coarsely vacuolated</td>
<td>Often granular</td>
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<tr>
<td>Nuclear size</td>
<td>small</td>
<td>Large</td>
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<tr>
<td>Chromatin</td>
<td>Less hyperchromatic</td>
<td>More hyperchromatic</td>
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<tr>
<td></td>
<td>Evenly distributed</td>
<td>Irregularly distributed</td>
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<tr>
<td>Nucleoli</td>
<td>Single micronucleoli</td>
<td>Multiple micronucleoli</td>
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<td></td>
<td>Grade 3 and 4 macronucleoli</td>
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Salient practical differences between CIS, MICA, CA

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<th>CA</th>
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<td>Distribution</td>
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<tr>
<td>Single</td>
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<tr>
<td>Pleomorphism</td>
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<td>+</td>
<td>+++</td>
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<td>Chromatin pattern</td>
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<td>+</td>
</tr>
<tr>
<td></td>
<td>Irregularly finely granular</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Irregularly coarsely granular</td>
<td>-</td>
<td>+/</td>
</tr>
<tr>
<td>Nucleoli</td>
<td>-</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Micronucleoli</td>
<td>-</td>
<td>+++</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Tumor diathesis</td>
<td>-</td>
<td>+/</td>
<td>+++</td>
</tr>
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Tumor diathesis in a case of squamous cell carcinoma