

# 1 CYTOLOGY OF THE UTERINE CERVIX AND CORPUS

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## INTRODUCTION

Cervical cytology is a screening tool that helps to categorize patients for their risk of cervical neoplasm depending on the degree of shift from normal cervical cytology and the amount of the abnormal change seen. Since the cervix is subject to hormonal effects, the clinical context (exogenous estrogens, pregnancy, etc.) should be considered when evaluating cervical cytology specimens.

The first cervical cytology screen is recommended for women starting at age 21, or 3 years after the onset of sexual intercourse, whichever comes first<sup>1,2</sup>. Women under the age of 30 should be screened annually with conventional cytology, or biennially using liquid-based methods. For women over the age of 30, human papillomavirus (HPV) testing can be used in conjunction with cytology, and if both tests are negative, screening can be reduced to every 3 years<sup>3</sup>. After the age of 70, women may choose to stop screening if no abnormal results were found in three or more consecutive cervical cytology specimens over the prior 10 years. Cervical cytology results are reported according to the 2001 Bethesda System (Table 1.1)<sup>4</sup>.

Cervical cytology screening allows for evaluation of exfoliated cells from the transformation zone, where most cervical cancers arise. Traditionally, cervical cytology samples have been smeared directly onto a slide, fixed with ethanol, and Papanicolaou stained. These conventional smears are rapidly being replaced by liquid-based methods such as ThinPrep and SurePath. For liquid-based preparations, the cervical sample is rinsed directly into a vial of proprietary fixative solution – methanol-based CytoLyt<sup>®</sup> for ThinPrep (Hologic, Bedford, MA) and ethanol-based CytoRich<sup>®</sup> for SurePath (BD Diagnostics, Burlington, NC). Both ThinPrep and SurePath utilize proprietary methods for minimizing obscuring material such as blood, mucus, and debris to produce a thin layer of evenly distributed cells on a slide. Although some studies have shown fewer unsatisfactory specimens with the liquid-based method,

**Table 1.1** 2001 Bethesda System

|   |   |
|---|---|
| <b>Specimen type</b>                                | Conventional<br>Liquid-based (specify type, e.g., ThinPrep, SurePath)<br>Other  |
| <b>Specimen adequacy</b>                            | Satisfactory for evaluation<br>Unsatisfactory for evaluation  |
| <b>General categorization (optional)</b>            | Negative for intraepithelial lesion or malignancy<br>Epithelial cell abnormality<br>Other: endometrial cells in a woman over 40 years of age  |
| <b>Interpretation/result</b>                        | Negative for intraepithelial lesion or malignancy (specify organisms, other non-neoplastic findings)  |
| <b>Squamous</b>                                     | Atypical squamous cells<br>– of undetermined significance (ASC-US)<br>– cannot exclude HSIL (ASC-H)<br>Low-grade squamous intraepithelial lesion<br>High-grade squamous intraepithelial lesion<br>Squamous cell carcinoma |
| <b>Glandular</b>                                    | Atypical endocervical, endometrial or glandular cells (NOS or favor neoplastic)<br>Endocervical adenocarcinoma in situ<br>Adenocarcinoma  |
| <b>Other</b>  | Endometrial cells in a woman over 40 years of age<br>Other malignant neoplasms  |
| <b>Ancillary testing</b>                            | Human papillomavirus (HPV), gonorrhea (GC), chlamydia: include description of test method(s) and results  |
| <b>Automated review</b>                             | Specify device and result, if slide is examined by an imaging system  |
| <b>Educational notes and suggestions (optional)</b> | Based on ASCCP Management Guidelines  |

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the performance of conventional and liquid-based methods in detecting high-grade lesions is similar<sup>5,6</sup>. Lower cost is an advantage for the conventional method, whereas the ability to use the same specimen for HPV and gonorrhea (GC)/chlamydia testing is an advantage for the liquid-based method. In addition, automated imaging systems such as the ThinPrep Imager or BD FocalPoint are optimized for liquid-based preparations. The American College of Obstetricians and Gynecologists (ACOG) accepts both conventional and liquid-based methods<sup>7</sup>.

Despite the effectiveness of cervical cytology as a screening tool for cervical dysplasia, the reported false-negative rate of cervical cytology varies greatly, ranging from 0 to 94% (average of 51.9%)<sup>8</sup>. Human papillomavirus testing is a more sensitive method compared to cytology, although it is less specific<sup>9</sup>.

### PROGNOSIS

#### Cervical squamous intraepithelial lesion

The risk of harboring at least cervical intraepithelial neoplasia (CIN) 2 based on an abnormal cervical cytology result is as follows: 27% for HPV-positive atypical squamous cells of undetermined significance (ASC-US) or low-grade squamous intraepithelial lesion (LSIL); 50% for atypical squamous cells – cannot exclude high-grade squamous intraepithelial lesion (ASC-H); and over 70% for high-grade squamous intraepithelial lesion (HSIL)<sup>10</sup>.

#### Atypical glandular cells

Atypical glandular cells (AGC) are diagnosed in about 0.2–0.9% of cervical cytology specimens, with significant disease present in 12–36% of the cases. The risk of finding a significant lesion on follow-up is 9–41% for atypical glandular cells NOS (not otherwise specified) and 27–96% for atypical glandular cells, favor neoplastic<sup>13,14</sup>. High-grade squamous intraepithelial lesion is the most common abnormality found on follow-up<sup>15,16</sup>. Other abnormalities detected on biopsy include endocervical adenocarcinoma in situ (AIS), invasive carcinomas (cervical, endometrial, and extrauterine), and other benign endometrial pathology. In patients whose cervical cytology test also detected a squamous abnormality, such as high-grade squamous intraepithelial lesion, ASC-H, or ASC-US, in addition to the atypical glandular cells, it is significantly more likely

that the glandular abnormality originated from the cervix and not from a non-cervical site (e.g., endometrium)<sup>17</sup>.

Postmenopausal women have a higher rate of significant abnormality, with up to 50% of the cases showing cervical or endometrial lesions, including 15–20% endometrial carcinoma and 5% invasive cervical carcinoma. In women under 40, the most common precancerous or malignant lesions detected are high-grade squamous intraepithelial lesion and adenocarcinoma in situ<sup>17</sup>.

### MANAGEMENT AND TREATMENT

The American Society for Colposcopy and Cervical Pathology (ASCCP) first published consensus guidelines for the management of women with abnormal cervical cancer screening tests in 2001, and revised the guidelines in 2006<sup>3</sup>. There are also similar consensus guidelines for the management of intraepithelial neoplasia and adenocarcinoma in situ diagnosed histologically<sup>3</sup>. The guidelines specify different management algorithms for the general population vs. special populations, as the risk of high-grade squamous intraepithelial lesion or cancer differs in these various groups. A significant change with the 2006 guidelines is the identification of adolescents (defined as women 20 years and younger) as a special population that should be treated less aggressively than the general population. Human papillomavirus testing as specified in the guidelines refers to testing for high-risk types only. Currently, there are two high-risk HPV assays approved by the US Food and Drug Administration (FDA) – Digene Hybrid Capture 2 (used in the ASCUS-LSIL Triage Study) and Cervista HPV HR. (See the *Ancillary diagnostic tests* section.)

#### Negative cervical cytology

For women aged 30 years and older, HPV testing can be used in conjunction with cervical cytology for screening. If the cervical cytology and HPV tests are both negative, routine screening can be decreased to every three years. If the HPV test is positive, the patient can be followed with repeat cervical cytology and HPV testing at 12 months or, if HPV genotyping is available, the sample can be further evaluated to determine if HPV types 16/18 are present<sup>3,18</sup>. If the cervical cytology sample is positive for HPV16/18, immediate colposcopy is indicated. If the HPV16/18 test is negative, then the patient can be followed with repeat cytology and HPV testing in 12 months. Genotyping can be

performed with Cervista HPV 16/18 (Hologic, Bedford, MA) or with HPV polymerase chain reaction (PCR). There are currently no FDA-approved HPV PCR systems available in the United States, but PCR can be internally validated by individual pathology laboratories.

For women under the age of 30, HPV testing is contraindicated given the high prevalence of HPV infections and spontaneous clearance rates of up to 90%<sup>19</sup>. These patients should be followed with cytology only.

### Atypical squamous cells of undetermined significance

Based on data from the ASCUS-LSIL Triage Study, there are three acceptable methods for managing ASC-US in the general population: (1) reflex testing for high-risk HPV; (2) immediate colposcopy; or (3) repeat cervical cytology at 6 and 12 months<sup>3,20</sup>. It should be noted that performing reflex HPV testing together with repeat cervical cytology at 6 months is not recommended; when the two tests are combined for follow-up, sensitivity remains the same while specificity drops. If the HPV test or colposcopy is negative, the patient can be followed with a repeat cervical cytology test at 12 months. However, if the HPV test is positive, the patient should be referred for immediate colposcopy with biopsy of visible lesions. If no visible lesions are identified or colposcopy is unsatisfactory, endocervical curettage is recommended. If cervical dysplasia is not identified, recommended follow-up is HPV testing at 12 months or cervical cytology at 6 and 12 months. Colposcopy is recommended if the subsequent HPV test is positive or at least ASC-US is identified on the cervical cytology. Human papillomavirus testing should not be performed more frequently than once every 12 months.

In adolescent women, HPV infection and minor cytologic abnormalities are common, but the risk of invasive cervical cancer is very low, and most HPV infections clear within two years without treatment. Consequently, it is recommended that adolescent women with ASC-US be managed less aggressively, with repeat cervical cytology at 12 and 24 months. Immediate colposcopy and reflex HPV testing are considered unacceptable. Only patients with high-grade squamous intraepithelial lesion at the 12-month cervical cytology test or at least ASC-US at the 24-month cervical cytology test should be referred for colposcopy.

Immunosuppressed (including HIV-positive), postmenopausal, and pregnant women should be managed the same as patients in the general population, except endocervical

curettage is contraindicated in pregnant patients. In addition, for pregnant women, colposcopy can be deferred until at least six weeks postpartum.

### Atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H)

Colposcopic examination with biopsy is recommended for ASC-H. If high-grade squamous intraepithelial lesion is not identified on biopsy, there are two options for follow-up: HPV testing at 12 months or repeat cervical cytology at 6 and 12 months. If the HPV test is positive or if cervical cytology shows ASC-US or above, colposcopy is recommended. If the HPV test is negative or both the follow-up cervical cytology tests are negative, the patient can resume routine yearly screening.

### Low-grade squamous intraepithelial lesion

Women with a cytologic diagnosis of low-grade squamous intraepithelial lesion have the same risk of harboring high-grade squamous intraepithelial lesion as high-risk HPV-positive ASC-US, and should be managed similarly, i.e., with immediate colposcopy and biopsy of visible lesions. If no visible lesions are identified or colposcopy is unsatisfactory, endocervical curettage is recommended. If squamous intraepithelial lesion (SIL) is not identified, recommended follow-up is HPV testing at 12 months or cervical cytology at 6 and 12 months. Human papillomavirus triage of low-grade squamous intraepithelial lesion is not indicated, except in postmenopausal women.

For adolescents, the recommended management is follow-up with annual cytology testing and referral to colposcopy only if at least high-grade squamous intraepithelial lesion is found at the 12-month cervical cytology or at least ASC-US at the 24-month cervical cytology.

Preferred management for pregnant women is immediate colposcopy, but it is also acceptable to defer colposcopy until at least six weeks postpartum. If the initial colposcopy does not show any colposcopic, histologic, or cytologic features to suggest high-grade squamous intraepithelial lesion or cancer, the patient can be further evaluated postpartum; additional exams are considered unacceptable. Endocervical curettage is also contraindicated in pregnant women.

### High-grade squamous intraepithelial lesion

In the general population, high-grade squamous intraepithelial lesion can be managed by colposcopy with

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endocervical sampling or immediate loop electrosurgical excision procedure (LEEP) of visible lesions. If colposcopy is satisfactory and high-grade squamous intraepithelial lesion is not identified histologically, the options for further evaluation are (1) diagnostic excision or (2) follow-up with colposcopy and cervical cytology at 6 and 12 months. Review of the cytology, histology, and colposcopy results should always be done in cases where there is a major discrepancy between the cytologic and histologic diagnosis, and management revised if any diagnoses are changed. If colposcopy is not satisfactory and high-grade squamous intraepithelial lesion is not identified on biopsy, diagnostic excision is recommended. Human papillomavirus triage or follow-up by repeat cervical cytology is unacceptable for the management of high-grade squamous intraepithelial lesion cytology.

Adolescent women with a cytologic diagnosis of high-grade squamous intraepithelial lesion should be evaluated with colposcopy and endocervical sampling. Immediate loop electrosurgical excision procedure is not acceptable. If colposcopy is satisfactory and high-grade squamous intraepithelial lesion is not identified histologically, preferred management is to follow the patient with colposcopy and cervical cytology at 6 month intervals for up to 24 months. Biopsy is recommended if a high-grade lesion is identified on colposcopy or if there is persistent high-grade squamous intraepithelial lesion by cytology for a year; diagnostic excision is recommended if high-grade squamous intraepithelial lesion persists by cytology for 24 months without identification of high-grade squamous intraepithelial lesion on biopsy. Patients can return to routine screening after two consecutive negative cervical cytology results, if no high-grade lesions are identified by colposcopy.

In pregnant women, endocervical sampling and loop electrosurgical excision procedure are unacceptable unless invasive cancer is suspected. They should be evaluated with colposcopy and biopsy of lesions suspicious for high-grade squamous intraepithelial lesion or cancer. If high-grade squamous intraepithelial lesion is not identified, repeat colposcopy and cervical cytology should be deferred until at least six weeks postpartum.

### Endometrial cells in a woman over 40

Endometrial cells are found in 0.5–1.8% of cervical cytology specimens in women over 40 years. Endometrial sampling is recommended in all postmenopausal women with endometrial cells in their cervical cytology sample. However, if they are premenopausal, asymptomatic, and

without risk factors, no further evaluation is needed; if they are symptomatic or have risk factors for endometrial neoplasms, such as unexplained vaginal bleeding or chronic anovulation, endometrial sampling is recommended.

### Atypical glandular cells

The diagnosis of “atypical glandular cells (AGC)” is often associated with benign, reactive conditions, but there is also a significant risk of underlying high-grade squamous intraepithelial lesion, endocervical adenocarcinoma in situ and invasive adenocarcinoma. Consequently, colposcopy with endocervical curettage is recommended for all categories of atypical glandular cells (i.e., atypical glandular cells NOS; atypical endocervical cells NOS; atypical glandular cells, favor neoplasia; atypical endocervical cells, favor neoplasia), except for “atypical endometrial cells.” Endometrial sampling is also indicated in women over the age of 35 years or in younger women with risk factors for endometrial pathology (e.g., vaginal bleeding or chronic anovulation).

For a diagnosis of “atypical endometrial cells,” endocervical and endometrial sampling are recommended; colposcopy can be performed at the initial evaluation or deferred. If no endometrial pathology is identified then colposcopy is indicated. For all categories of atypical glandular cells, HPV testing should be performed at the time of colposcopy, if not previously obtained. However, it is unacceptable to triage atypical glandular cells with HPV testing or to follow with repeat cytology.

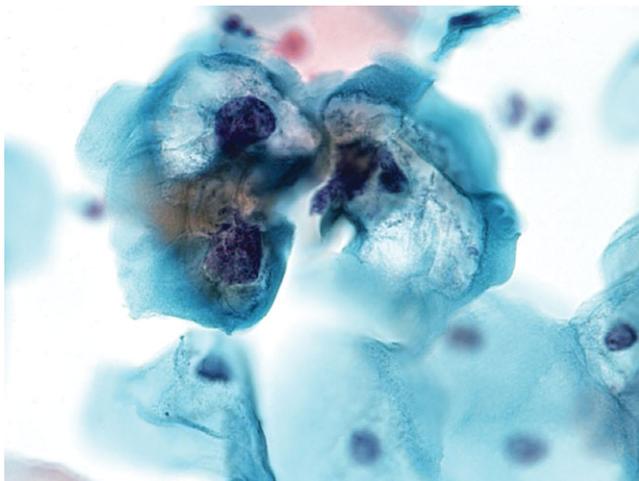
## MORPHOLOGY

### Low-grade squamous intraepithelial lesion

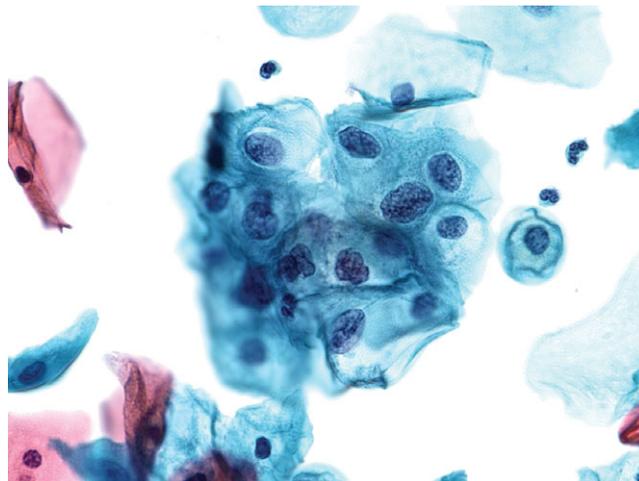
Low-grade squamous intraepithelial lesion (LSIL) is characterized by nuclear enlargement (three times the size of intermediate cell nuclei), hyperchromasia, and irregular nuclear membranes (Figure 1.1). Binucleation and multinucleation are common. Koilocytes, which have a sharply defined, irregularly shaped perinuclear halo with a peripheral rim of thickened cytoplasm, are characteristic of low-grade squamous intraepithelial lesion but are not required for the diagnosis. Of note, cells seen in low-grade squamous intraepithelial lesion have abundant cytoplasm with low nuclear-to-cytoplasmic (N:C) ratios.

Atypical squamous cells of undetermined significance (ASC-US) are defined as squamous cells with nuclei that are 2.5–3 times the size of an intermediate cell nucleus, minimal

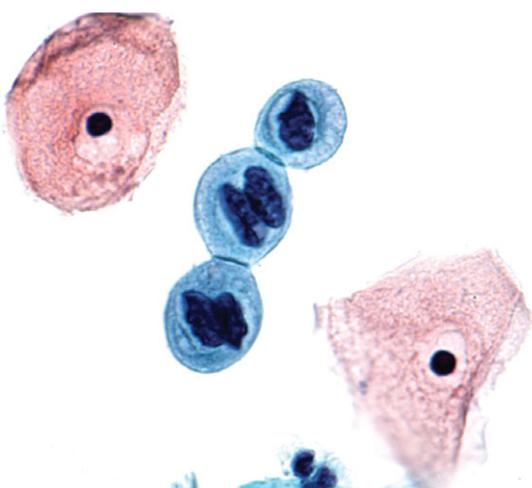
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**Figure 1.1** Low-grade squamous intraepithelial lesion (LSIL). Koilocytes with sharply punched-out, irregular halos surrounding enlarged, hyperchromatic, irregular nuclei.



**Figure 1.2** Atypical squamous cells, of undetermined significance (ASC-US). Atypical squamous cells with mild nuclear enlargement, minimal hyperchromasia, and slight nuclear membrane irregularities.



**Figure 1.3** High-grade squamous intraepithelial lesion (HSIL). Cluster of cells with hyperchromatic, irregular nuclei, and high nuclear-to-cytoplasmic ratios.

nuclear hyperchromasia, and slightly irregular nuclear membranes (Figure 1.2). Halos, if present, lack the characteristic punched-out appearance of koilocytes or appear regular in shape without a thickened rim of cytoplasm. Cells with dark, pyknotic nuclei and dense orangeophilic cytoplasm (atypical parakeratosis) may also be seen. The diagnosis of ASC-US is best applied to cases where the atypical cells have some but not all the features of low-grade squamous intraepithelial lesion.

### High-grade squamous intraepithelial lesion

High-grade squamous intraepithelial lesion is characterized by single cells, syncytial aggregates, and clusters of

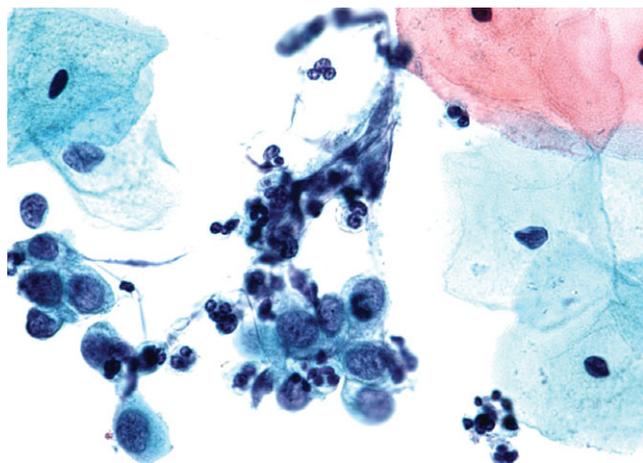
cells with irregular nuclear contours and high nuclear-to-cytoplasmic ratios of at least 1:1 (Figure 1.3). The nuclei are larger than an intermediate cell nucleus and, while they vary in size, they are not as large as those seen with low-grade squamous intraepithelial lesion. The cytoplasm varies from delicate to dense. The cells are typically hyperchromatic. However, with non-imaged ThinPrep slides, the cells may lack significant hyperchromasia. This is seen when the standard Richard-Allan hematoxylin or cyto-stain is used. However, it is not an issue with the proprietary ThinPrep Imager stain, which is a quantitative nuclear stain that allows computerized assessment of DNA content. With both Sure-Path and ThinPrep, dispersed abnormal single cells are more common and the cells may appear smaller and less abnormal. It is important to screen for single cells with high nuclear-to-cytoplasmic ratios and irregular nuclear membranes.

The diagnosis of “atypical squamous cells, cannot exclude HSIL” should be used for cases where the features raise concern for, but fall short of, a definitive diagnosis of high-grade squamous intraepithelial lesion (Figure 1.4). These are often cases where it is difficult to distinguish high-grade squamous intraepithelial lesion from one of its mimics, such as endometrial cells or squamous metaplastic cells.

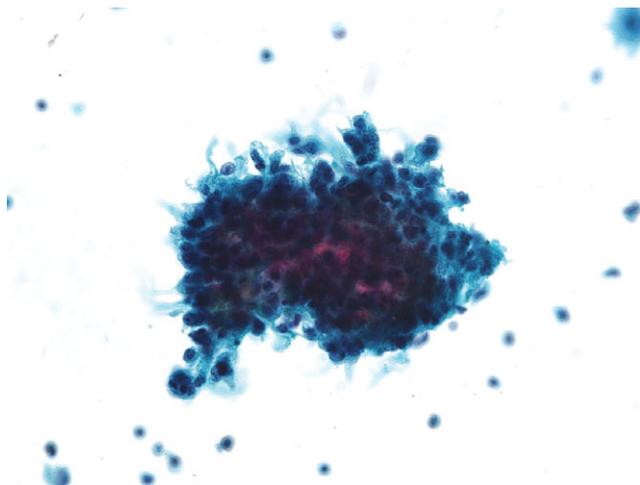
### Squamous cell carcinoma

Cervical squamous cell carcinoma (SCC) is commonly non-keratinizing, but keratinizing squamous cell carcinoma can also occur. Non-keratinizing squamous cell carcinoma can appear similar to high-grade squamous intraepithelial

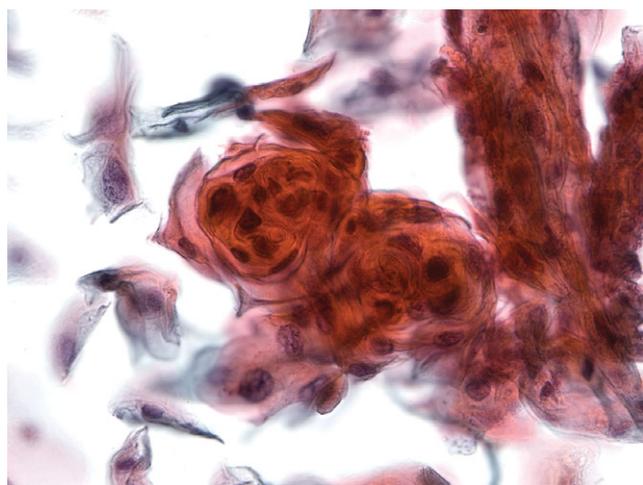
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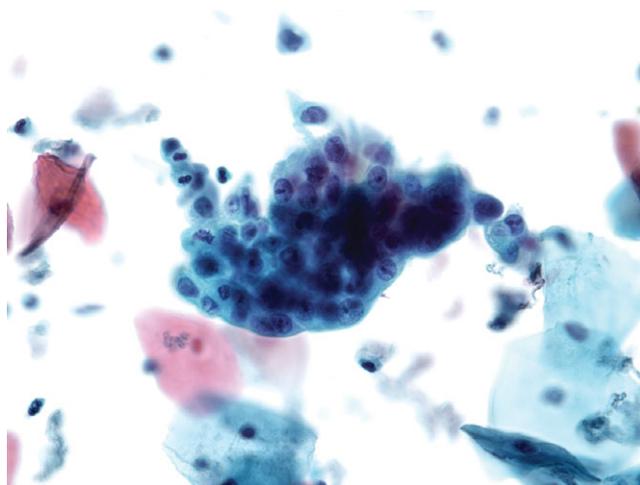
**Figure 1.4** Atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H). High nuclear-to-cytoplasmic-ratio cells with slight nuclear membrane irregularities and minimal hyperchromasia.



**Figure 1.5** Squamous cell carcinoma (SCC). Syncytial aggregates of hyperchromatic cells with dense cytoplasm. Necrosis appears as granular debris along the edges of cell clusters on liquid-based preparations.



**Figure 1.6** Keratinizing squamous cell carcinoma. Squamous pearls and dysplastic cells with high nuclear-to-cytoplasmic ratios help distinguish keratinizing squamous cell carcinoma from low-grade squamous intraepithelial lesion.



**Figure 1.7** Atypical endocervical cells NOS. Crowded group of endocervical cells with enlarged nuclei and mitotic activity.

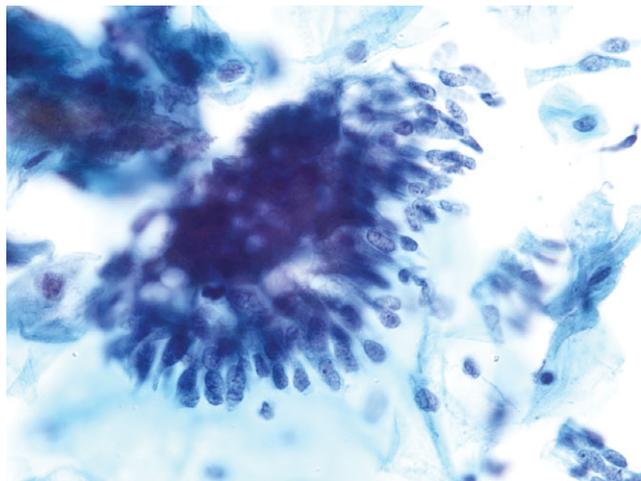
lesion, with syncytial aggregates of hyperchromatic cells, but with squamous cell carcinoma the cells will also exhibit macronucleoli and occur in a background of necrotic debris and degenerating blood (Figure 1.5). On liquid-based preparations, tumor diathesis appears as granular debris clinging to the edges of cell clusters.

Keratinizing squamous cell carcinoma can appear similar to low-grade squamous intraepithelial lesion, with heavily keratinized low N:C ratio cells. The presence of keratin pearls, tadpole or spindled cells, and isolated single cells with scant cytoplasm and marked nuclear pleomorphism will support a diagnosis of squamous cell carcinoma (Figure 1.6).

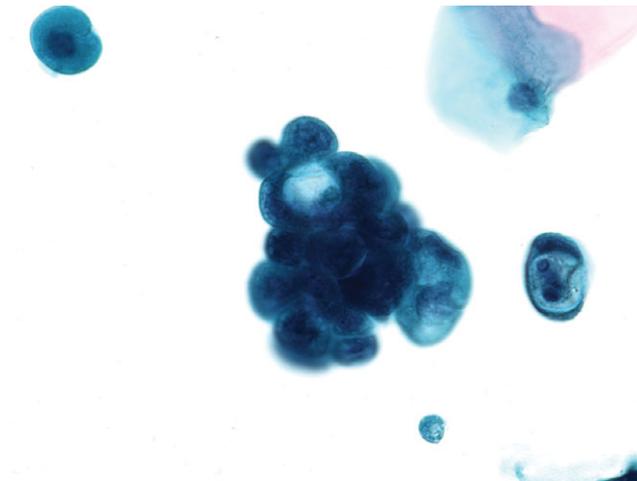
### Atypical endocervical cells

The diagnosis of “atypical endocervical cells NOS” applies to endocervical cell atypia which exceeds that of reactive or reparative changes, but does not meet criteria for a definitive diagnosis of endocervical adenocarcinoma in situ (AIS) or invasive adenocarcinoma. In general, the atypical features are mild but reflect the types of changes that are seen with glandular neoplasia; for example, nuclear crowding, oval-to-cigar-shaped nuclei, nuclear membrane irregularities, nuclear enlargement (3–5 times normal), hyperchromasia, and nucleoli (Figure 1.7). Mitotic figures are typically rare, but if mitotic activity is identified within a group of glandular cells, it is best to issue a diagnosis of

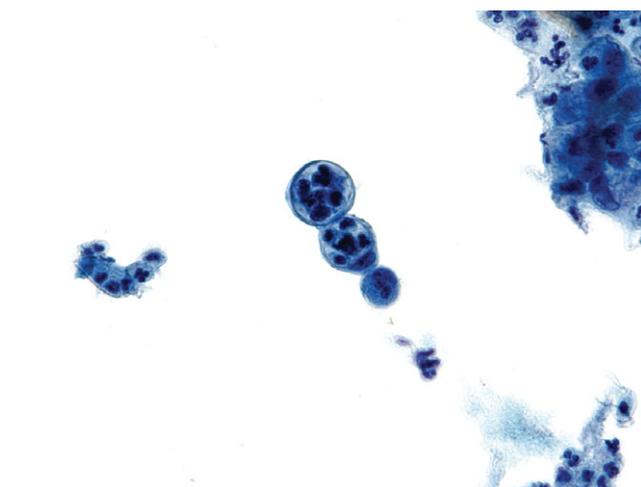
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**Figure 1.8** Atypical glandular cells, favor neoplastic. Atypical endocervical cells with feathering but only minimal nuclear membrane irregularities and minimal crowding.



**Figure 1.9** Atypical endometrial cells. Cluster of endometrial cells with nuclear enlargement and prominent nucleoli.



**Figure 1.10** Atypical endometrial cells. The presence of cytoplasmic vacuoles filled with neutrophils is an abnormal finding in endometrial cells.

atypical endocervical cells. The distinction between benign endocervical glands and a minimal deviation or well-differentiated endocervical adenocarcinoma can be difficult even on histologic sections, and mitotic activity can be a helpful feature in detecting these well-differentiated carcinomas.

The diagnosis of “atypical endocervical cells, favor neoplastic” applies to cases where the degree of endocervical atypia is more pronounced but continues to fall qualitatively or quantitatively short of a definitive diagnosis of endocervical adenocarcinoma. In addition to the features described above, the cell groups may also show rosetting or feathering, with splaying of the cytoplasm at the edges of the cell clusters (Figure 1.8).

### Atypical endometrial cells

Atypical endometrial cells will be recognizable as endometrial in origin but should exhibit atypia beyond what is expected for normal exfoliated endometrial cells. The cell clusters have slightly enlarged nuclei, mild hyperchromasia, and small nucleoli (Figure 1.9). The presence of numerous neutrophils within a cytoplasmic vacuole of an endometrial cell is an abnormal finding that should lead to a diagnosis of “atypical endometrial cells” (Figure 1.10).

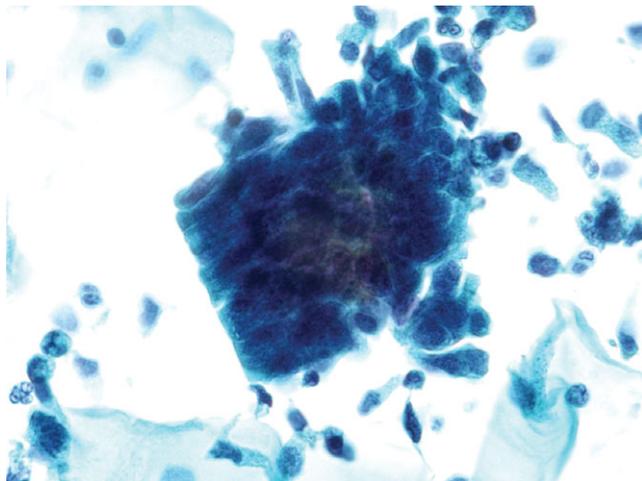
### Atypical glandular cells

If atypical glandular cells are present but it cannot be determined whether they are endocervical or endometrial in origin, a diagnosis of “atypical glandular cells NOS” or “atypical glandular cells, favor neoplastic” may be used.

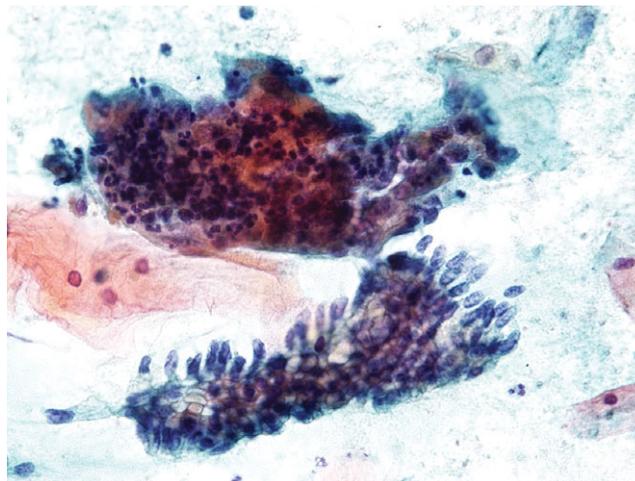
### Endocervical adenocarcinoma

The distinction between endocervical adenocarcinoma in situ and adenocarcinoma may not be clear cut on cervical cytology and, in many instances, clinical and radiographic correlation and a tissue specimen are required to differentiate the two. Adenocarcinoma in situ is characterized by strips of cells with crowded, palisading nuclei that are enlarged, elongate, and irregular (Figure 1.11). Nuclear-to-cytoplasmic ratios are increased and the chromatin is coarse and dark. On conventional smears, the apical cytoplasm frequently strips away, giving the impression of a row of feathers

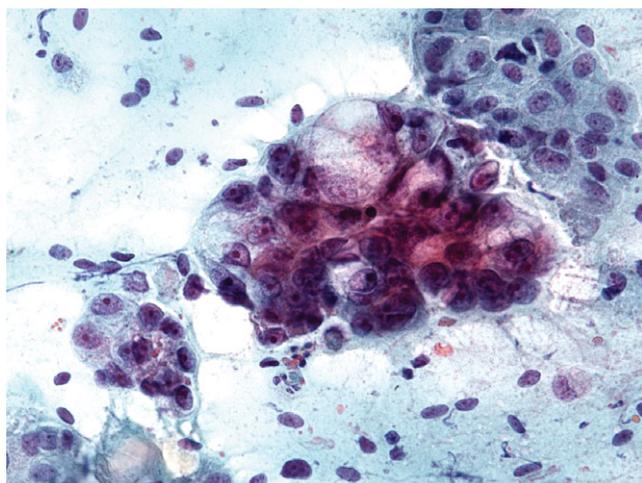
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**Figure 1.11** Endocervical adenocarcinoma in situ: Strips of abnormal endocervical cells with elongate, markedly irregular nuclei and nuclear crowding.



**Figure 1.12** Endocervical adenocarcinoma. Cluster of malignant glandular cells (upper) would not be classifiable as endocervical in origin without the strip of adenocarcinoma in situ cells (lower).



**Figure 1.13** Endometrial adenocarcinoma. Three-dimensional clusters of malignant cells with pleomorphic nuclei, prominent nucleoli, and large cytoplasmic vacuoles are characteristic of serous carcinoma.

(“feathering”). This is less commonly seen with liquid-based preparations. Apoptotic bodies and mitotic figures may also be identified but are not specific or required. Finding single atypical glandular cells is uncommon.

With invasive endocervical adenocarcinoma, the cells are clearly malignant with enlarged, pleomorphic nuclei and finely vacuolated cytoplasm; macronucleoli may also be seen. The tumor cells occur as syncytial aggregates and three-dimensional clusters (Figure 1.12). Tumor diathesis is a useful indicator of invasion but is less prominent on liquid-based preparations, where it will appear as granular debris along the edges of the tumor cell clusters.

While these features are diagnostic of adenocarcinoma, they are non-specific as to site of origin. The only way that a diagnosis of invasive endocervical adenocarcinoma can be made on a cervical cytology specimen is if these findings are present along with features of endocervical adenocarcinoma in situ.

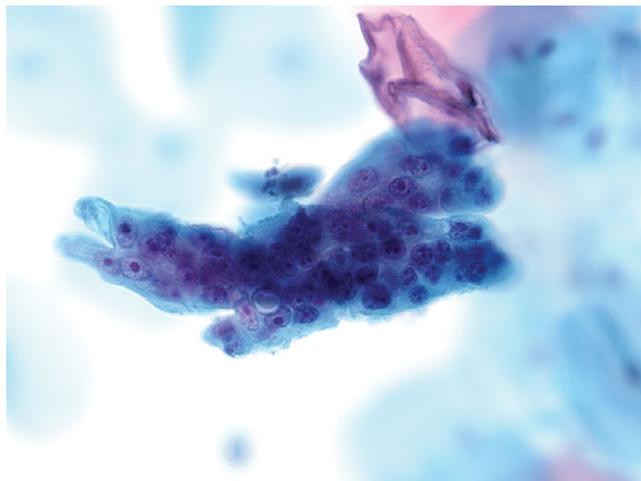
### Endometrial adenocarcinoma

Endometrial adenocarcinoma is frequently indistinguishable from endocervical adenocarcinoma based on cytology. However, there are certain features that are characteristic of endometrial origin, such as malignant cells with numerous intracytoplasmic neutrophils, or papillary clusters of cells with large cytoplasmic vacuoles and markedly pleomorphic nuclei (typically seen with serous carcinomas) (Figure 1.13). Cases that lack features characteristic of endometrial origin should be classified as adenocarcinoma NOS, with determination of primary site deferred to the resection specimen, as well as clinical and radiographic findings.

### Metastatic carcinoma

Metastatic adenocarcinoma should be suspected when the morphology is unusual for a uterine primary or the background lacks a tumor diathesis. Common sites that metastasize to the cervix include the urinary bladder, breast, ovary and fallopian tube, kidney, and gastrointestinal tract (e.g., colon, stomach) (Figure 1.14).

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**Figure 1.14** Metastatic breast carcinoma. Clearly malignant cells with enlarged nuclei and a cytoplasmic vacuole compressing the nucleus that do not resemble usual Mullerian tract primaries; the background also lacks tumor diathesis.

### ANCILLARY DIAGNOSTIC TESTS

#### Digene Hybrid Capture 2

Digene Hybrid Capture 2 (HC2) (Qiagen, Valencia, CA) is FDA approved for triaging patients with a diagnosis of ASC-US and for co-testing in women aged 30 years and older. It is the assay that was used in the ASCUS-LSIL Triage Study that led to the current recommendation for HPV triage of ASC-US<sup>20</sup>. Two probes are available – low risk and high risk – but only the high-risk probe has clinical utility. It targets 13 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68).

Digene HC2 has a detection limit of 5000 copies of HPV DNA (1 pg/ml) and has good interlaboratory reproducibility. However, the HC2 is unable to specifically type HPV, and lacks an internal control to assure specimen adequacy. It also has poor reproducibility near the 1 pg/ml cutoff. Results near the cutoff are best reported as equivocal; a significant number of these borderline cases are negative by HPV polymerase chain reaction.

The overall false-positive rate of HC2 is 6.2%. This is due to both cross-hybridization and signal leak. Cross-hybridization with low-risk HPV types has been reported to occur at a rate of 1.9%. Although this can lead to false-positive results, it also increases the clinical sensitivity of the assay and allows detection of high-risk HPV types that are not directly targeted by the test probe (e.g., HPV66)<sup>21</sup>. Signal leak in contiguous samples has been observed in 4.3% of cases.

#### Cervista

Cervista HPV HR and Cervista HPV 16/18, formerly known as Invader, (Third Wave Technologies, Hologic, Bedford, MA) are two signal-amplification HPV assays that were FDA approved in 2009. Cervista requires a smaller sample volume (2 ml vs. 4 ml) than HC2 and has an internal control which detects human histone 2 gene (*H2be*) that allows for evaluation of specimen adequacy. Cervista HPV HR targets 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68), which are the same as those targeted by Digene HC2 with the addition of HPV66. It is approved for ASC-US triage and for co-testing in women aged 30 years and older. Cervista HPV 16/18 targets HPV types 16 and 18, which are more oncogenic and responsible for the majority of cervical cancers. It is approved for use in conjunction with Cervista HPV HR, specifically for further evaluation of patients who are HPV positive.

The clinical validation study for Cervista HPV HR showed 93% clinical sensitivity for the detection of >CIN2 and 100% for >CIN3; negative predictive value was 99.1% for >CIN2 and 100% for >CIN3<sup>22</sup>. Analytic sensitivity ranges from 1250–7500 copies, and analytic specificity is high with no cross-reactivity reported when tested with seven non-oncogenic HPV types<sup>23</sup>. A separate population-based study in China showed similar sensitivity and specificity of Cervista HPV HR and HC2 for the detection of >CIN3: sensitivity 95.1% Cervista vs. 97.9% HC2; specificity 90.3% Cervista vs. 87.8% HC2<sup>24</sup>. However, in a study where Cervista HPV HR was used to evaluate 65 samples with borderline Digene HC2 results, there was a 6.1% false-positive rate<sup>25</sup> (line blot genotyping assay as gold standard).

The clinical validation study for Cervista HPV 16/18 showed a detection rate of 68.8% for >CIN2 and 77.3% for >CIN3, which correlates with the estimated prevalence of HPV16 and HPV18<sup>22</sup>. When stratified by age, Cervista HPV 16/18 showed similar sensitivity and negative predictive value (NPV) for women under 30 and over 30, but lower specificity (61.9 vs. 79.9%) and lower positive predictive value (15.2 vs. 21.9%) for women under 30<sup>26</sup>. Cervista HPV 16/18 has high analytic specificity with no reported cross-reactivity with low-risk types. Cross-reactivity with high-risk HPV type 31 has been reported when using high concentrations of HPV31 cloned DNA samples; however, when HPV31-positive clinical samples were tested, no cross-reactivity was observed<sup>27</sup>.

Kinney *et al.* evaluated the test performance data in the package inserts for Cervista HPV HR and Cervista HPV

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16/18, and raised concerns about the assays being overly sensitive, with 2–4 times more positive results than were reported in the premarketing approval trial<sup>28</sup>. Increased analytic sensitivity is not always desirable since the goal is detecting clinically relevant infections. Overly sensitive assays can lead to overtreatment of patients and increased cost of care. While Kinney *et al.* raised this concern, they also note that post-marketing studies evaluating Cervista will be important in determining the clinical sensitivity of these assays in actual practice.

### Slide-based assays

Human papillomavirus in situ hybridization (ISH) and immunohistochemistry (IHC) stains for p16 and ProEx C, surrogate markers for high-risk HPV, can be performed on cervical cytology slides. However, these assays are limited by the need for manual screening of the stained slide and the difficulty in interpreting the stains. While specificity is usually better with these assays than with HC2, the limited amount of material evaluated negatively impacts sensitivity. Various studies comparing Ventana Inform in situ hybridization with HC2 have shown insufficient sensitivity for Ventana in situ hybridization to be used for ASC-US triage (43–61% in situ hybridization vs. 97–100% HC2)<sup>29–31</sup>.

In addition to staining cells with integrated high-risk HPV, p16 and ProEx C can show focal staining of benign squamous metaplastic cells and endocervical cells<sup>32</sup>. This makes it difficult to interpret the stain, since only positive cells that are cytologically atypical cells should be scored as positive. Consequently, sensitivity and specificity will vary depending on the skill of the pathologist reading the slide.

### Human papillomavirus polymerase chain reaction

Polymerase chain reaction (PCR) is the traditional gold standard for detecting HPV, and can identify the specific type of HPV. Human papillomavirus PCR for types 16 and 18 can be used for triaging women over 30 with negative cervical cytology but positive HPV test. Currently, there are no commercially available platforms in the United States, but individual clinical laboratories have internally validated “home-brewed” HPV PCR assays.

Roche has developed two HPV detection systems – Amplicor (consensus) and LinearArray (type specific) – that are available in Europe but have not yet received

FDA approval for use in the United States. The Amplicor consensus assay detects the same 13 high-risk HPV types as Digene HC2 (i.e., types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68), while the LinearArray assay allows for type-specific detection of 37 high-risk (same as consensus assay) and low-risk (6, 11, 26, 40, 42, 53, 54, 55, 56, 61, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, 83, 84, CP6108, IS39) HPV types. Using LinearArray genotyping as the gold standard, Sandri *et al.* compared Roche Amplicor with Digene HC2 and showed increased sensitivity (100% vs. 85% for HC2) and similar specificity (95% vs. 93% for HC2)<sup>33</sup>. Overall, there was good concordance (83%) between the two assays, but Amplicor was positive in significantly more cases that were cytologically normal. This indicates that the increased sensitivity of Amplicor reflects a higher analytic sensitivity and corresponding lower clinical specificity. Improved sensitivity is not necessarily desirable since not all HPV infections lead to the development of a high-grade squamous intraepithelial lesion.

### Imaging

Imaging systems can be used for primary screening or quality control (QC) review. The goals are to improve quality by decreasing screening and interpretative error and to increase productivity. These systems are most useful in labs with poor sensitivity and are less useful in labs with a high degree of accuracy. The US Clinical Laboratory Improvement Amendment (CLIA) limits the number of slides screened by a cytotechnologist to 100 per 8-hour minimum workday. With location-guided screening, the Centers for Medicare and Medicaid Services (CMS) allows up to 200 slides. Actual productivity is less than the allowable maximum. The actual time savings of the imaging system depends on the experience of the cytotechnologist and whether the laboratory requires rapid QC rescreening<sup>5,34,35</sup>. For experienced cytotechnologists, the imaging system may decrease productivity.

There are currently three FDA-approved imaging systems: ThinPrep Imaging System (Hologic), FocalPoint GS (BD), and FocalPoint Slide Profiler (BD). The ThinPrep and FocalPoint GS systems allow for location-guided screening of liquid-based preparations. In contrast, the FocalPoint Slide Profiler performs primary screening of SurePath or conventional cervical cytology specimens and allows for archiving of up to 25% of successfully processed slides from non-high-risk patients without manual review.