

Squamous Epithelial Abnormalities

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Atypical Squamous Cells of Undetermined Significance

The concept of atypical squamous cells of undetermined significance (ASC-US) as a diagnostic category for cervical cytology reflects the reality of the limitations of light microscopy in classifying cytologic changes. Under ideal circumstances, all lesions would have classic appearances and fall quite nicely into their respective categories. In reality, the cellular changes seen in a cervical/vaginal sample represent a broad and continuous spectrum of change. Often there will be cells demonstrating overlapping criteria or small numbers of abnormal cells, which makes an exact diagnosis problematic. These equivocal findings lead to the necessity for an indeterminate diagnostic category such as ASC-US. Because of these limitations, the initial version of the Bethesda System invented the term “atypical squamous cells of undetermined significance” (ASCUS). This diagnostic category was to be used for cellular abnormalities that were more than those attributable to reactive changes but that quantitatively or qualitatively fell short of squamous intraepithelial lesion (SIL).¹ The term “of undetermined significance” was used because the cellular changes in the ASCUS category might reflect either an exuberant benign change or alternatively a potentially serious lesion.² It was also suggested that qualifying terms, such as “probably reactive” or “suggestive of LSIL” (low-grade SIL), be added to the diagnosis of ASCUS in order to more accurately define the risk of having true disease.

Over the ensuing decade, the term ASCUS became well accepted in the clinical community, and studies have shown that the use of ASCUS as a diagnostic category significantly improves the clinical usefulness of the Pap test. Pitman et al demonstrated that the elimination of ASCUS as a diagnostic category (a series of ASCUS cases were reclassified as either benign or SIL) resulted in a significant decrease in the sensitivity of the Pap test from 100% to 41% for high-grade squamous intraepithelial lesion or worse (HSIL+), with no associated increase in the specificity of the test.³ While the relative risk for HSIL+ is low for a patient with ASC-US (less than 10%),^{4,5} ASCUS is by far the most commonly diagnosed Pap abnormality leading to a true abnormality found on subsequent work up. Kinney et al⁴ clearly demonstrated the importance of minimally abnormal Pap tests in a prospective study of over 46,000 women. In that study, the authors showed that the most common initial cytologic diagnosis in women with histologically-proven HSIL+ was ASCUS, with 38.8% of these

HSIL+ cases having ASCUS as the initiating event. They also noted that while a cytologic diagnosis of high-grade intraepithelial lesion was very specific, only 31.4% of biopsy-proven HSIL+ was diagnosed as HSIL on the initial Pap test.

With the support of similar data, the 2001 Bethesda System consensus conference retained the general category of atypical squamous cells (ASC), with minor modification of the terminology and definition. The qualifiers of “probably reactive” and “suggestive of LSIL” were eliminated because they were found to have no real clinical significance. Instead, two more specific categories of ASC were adopted: (1) atypical squamous cells of undetermined significance (ASC-US) and (2) atypical squamous cells, cannot exclude HSIL (ASC-H).⁶ ASC-US is defined as “cytologic changes suggestive of an LSIL that are quantitatively or qualitatively insufficient for a definitive diagnosis.” ASC-H is defined as “cytologic changes suggestive of a HSIL that are quantitatively or qualitatively insufficient for a definitive diagnosis.” It is important to note that both the clinical significance and clinical management vary considerably between the two entities. For example, the recommended management for ASC-H is colposcopic examination. On the other hand, ASC-US may be initially managed with either reflex testing for high-risk human papillomavirus (HPV), follow-up with cervical cytology at 6 and 12 months, or immediate colposcopic examination. The 2006 American Society for Colposcopy and Cervical Pathology (ASCCP) guidelines recommend, as the preferred method of triage to colposcopic examination, offering reflex HPV testing (if using liquid-based cytology or obtaining co-collected HPV samples) for women older than age 20 years who have an initial diagnosis of ASC-US.⁷

It is important to remember that ASC is not a single diagnostic entity but encompasses a spectrum of cellular changes and reflects a variety of pathologic processes. Thus, ASC should always be considered a diagnosis of exclusion, and it should never be used if a more specific result is possible.

Conceptually, ASC-US can be divided into two subcategories, both of which show changes suggestive but not diagnostic of LSIL. First there are cases where some, but not all, of the diagnostic features of HPV infection are present (Figure 5-1). For example, a hallmark of HPV infection is the perinuclear halo or koilocytosis. However, if this change is present in small numbers or with relatively normal nuclear morphology, a definitive diagnosis of LSIL may not be possible, and the correct interpretation would

be ASC-US. Cytoplasmic vacuolization or ill-defined perinuclear halos may be reactive in nature and, if possible, should be placed into a benign category (see Figures 7-6 and 7-16). Another atypical cellular change often associated with HPV infection is atypical or pleomorphic parakeratosis. These cells are small keratinizing squamous cells with atypical features, such as variable nuclear size and shape or elongated, sometimes snakelike, nuclei and dense eosinophilic cytoplasm. While these cells are not of themselves diagnostic of SIL, they are certainly suggestive of possible HPV cytopathic effect (see “Low-Grade Squamous Intraepithelial Lesions” below) (Figure 5-1, E through G). Again, these changes need to be differentiated from benign parakeratosis, often described as miniature superficial squamous cells with round, regular, pyknotic nuclei (see Figure 7-19).

Secondly, there are cases with nuclear abnormalities that approach those of LSIL (Figure 5-2). These nuclei are enlarged two to three times the size of a normal intermediate cell. There may be mild variation in size and shape, slight hyperchromasia, and occasional binucleation. These changes are seen in mature squamous cells with mature cytoplasm and low nuclear to cytoplasmic (N:C) ratios. These changes should not be confused with benign nuclear enlargement, which may be seen in the perimenopausal age group. This perimenopausal nuclear enlargement consists of mature squamous cells with large nuclei (two to three times normal), with a concomitant increase in cytoplasmic size and no nuclear hyperchromasia or membrane wrinkling, and with a finely granular chromatin structure. This change and other benign ASC-US mimics are illustrated in chapter 7 (see Figure 7-6).

Atypical Squamous Cells, Cannot Exclude HSIL

Most commonly, ASC-H is represented by changes in immature squamous metaplastic cells or reserve cells, which fall between benign cellular changes and HSIL. These cells have immature cytoplasm and larger nuclei than normal metaplastic cells. There is an increase in the N:C ratio along with some degree of anisonucleosis, nuclear membrane irregularity, and mild hyperchromasia. However, the changes seen fall short of the criteria needed for a diagnosis of HSIL (Figure 5-3). At other times, cells with the cytologic features of HSIL may be present on the slide, but there are not enough cells to confidently make the diagnosis. In many cases, these cells are seen as scattered single cells that are easily missed during the screening process and may be a cause of false-negative cases (Figure 5-4).^{8,9} Cells suggestive of HSIL may also be seen in hyperchromatic crowded groups, where the density of the cells makes evaluation of the nuclei difficult and therefore precludes a definitive diagnosis (Figure 5-5).

Another variant of ASC-H consists of cellular clusters with features of both repair (a benign cellular change) and an epithelial abnormality, often suggesting the possibility of invasive carcinoma (Figure 5-6). The cells in question are typically immature squamous or glandular cells with

prominent nucleoli. In a benign reparative process, the cells are present in flat, cohesive, syncytial sheets, with streaming of the cells (see Figure 4-8 and Figure 7-21). An atypical reparative process will contain groups of cells with piled up nuclei, more anisonucleosis, and uneven chromatin distribution, or loosely cohesive groups with occasional single cells. Numerous single cells or tumor diathesis is not present. Most often these changes represent an exuberant reactive or reparative process. Because the differential diagnosis includes invasive carcinoma, these cases should be included in the generic category of ASC-H, with a suggestion that the features are suggestive of carcinoma in order that appropriate clinical follow-up will be obtained.⁷ Pleomorphic parakeratosis may also fall into this category when the abnormal cells suggest the possibility of keratinizing dysplasia (HSIL) or even keratinizing squamous cell carcinoma (Figure 5-7).

The spectrum of change seen in atrophy is broad and may include cells that are difficult to distinguish from an epithelial cell abnormality. The differential diagnosis of the immature cells present in atrophy may include LSIL, HSIL, or even carcinoma. Thus, atypical cellular changes in atrophy may be included in either ASC-US or ASC-H (depending on the severity of the abnormality). Atypical findings in atrophy may include nuclear enlargement with concomitant hyperchromasia, marked irregularity in the nuclear membranes, irregular chromatin distribution, hyperchromatic crowded groups, or the presence of pleomorphic spindle or tadpole cells (Figure 5-8).

Low-Grade Squamous Intraepithelial Lesion

At the first Bethesda consensus conference, the terms low-grade squamous intraepithelial lesion (LSIL) and high-grade squamous intraepithelial lesion (HSIL) were devised to cover the preinvasive squamous lesions seen in cytologic samples. The term squamous intraepithelial lesion (SIL) is a cytologically specific concept; cervical intraepithelial neoplasia (CIN) is a histological term. While the cytologic features of SIL correlate with histological features, SIL should be reserved for cytologic samples. The 2001 Bethesda System confirmed the use of these terms as clinically relevant. This support is based on the strong biological justification for a two-tiered diagnostic terminology in which the dividing line is placed between the histologic entities of mild dysplasia (CIN I) and moderate dysplasia (CIN II).¹⁰ Both LSIL and HSIL encompass the spectrum of squamous precursors leading to carcinoma of the cervix.

LSIL on Pap tests is characterized by cells with the diagnostic features of mild dysplasia or the definitive changes associated with HPV infection—so-called HPV cytopathic effect. The category is inclusive of the older diagnostic classes of mild dysplasia as well as the previously utilized terms of HPV infection, such as “koilocytic atypia.” The cells of LSIL may be seen as single cells or as sheets of dysplastic cells, with or without HPV cytopathic effect. The hallmark of this HPV-associated change is the perinuclear cytoplasmic halo known as koilocytosis. A diagnostic

koilocyte has a well-defined, optically clear, perinuclear halo, with a dense peripheral rim of cytoplasm and nuclear abnormalities (Figure 5-9). In preparations with the features of “classic” nonkeratinizing (or mild) dysplasia, LSIL is characterized by mature squamous cells with large nuclei, ranging from four to six times the size of a normal intermediate nucleus. The nuclei also demonstrate hyperchromasia, irregular nuclear membranes, and frequent binucleation. The chromatin is typically finely granular and evenly distributed. Nucleoli are infrequent (Figure 5-10). The nuclear changes of LSIL may consist of enlargement, nuclear membrane wrinkling, hyperchromasia, binucleation and multinucleation, and often some degenerative changes, such as chromatin smudging or pyknosis, resulting in changes described as “raisinoid” (Figure 5-11). Most often, slides with LSIL will demonstrate a combination of the above features, with both “classic” mild dysplasia and HPV cytopathic change.

In liquid-based cervical samples, the cytologic criteria for LSIL are essentially the same as for conventional smears, although in some cases there is a tendency towards nuclear normo- or even hypochromasia.

High-Grade Squamous Intraepithelial Lesion

The category of HSIL encompasses the histologic categories of moderate and severe dysplasias and carcinoma in situ (CIN II-III). The cytologic features of HSIL are characterized by cells with immature cytoplasm, abnormal nuclear features, and an increased N:C ratio.

The nuclei of HSIL are smaller than those of LSIL, especially in the more severe lesions. The nuclear size typically ranges from two to five times the size of an intermediate cell nucleus. Of importance, the cytoplasmic area is always decreased, yielding a marked increase in the N:C ratio. The nuclei are hyperchromatic with a fine to coarsely granular, evenly distributed chromatin pattern. The nuclear membranes are often wrinkled, and there is typically a significant degree of anisonucleosis. Nucleoli are generally absent (Figure 5-12).

The two most important differences between LSIL and HSIL are the immaturity of the cytoplasm and the high N:C ratio in cases of HSIL. The cells of HSIL are present singly, in sheets, and, at the high end of the spectrum, may be seen in syncytial-like aggregates (Figures 5-13 and 5-14). Some sampling devices will often pull aggregates of HSIL off from the surface or from endocervical glands, yielding three-dimensional clusters or hyperchromatic crowded groups of abnormal immature cells (Figure 5-15).

Keratinizing dysplasia is a variant of HSIL. Unlike the immature cells of classic HSIL, this variant consists of cells with a glassy keratinizing (often pleomorphic) cytoplasm. These cells may take on a caudate or tadpole shape. The N:C ratio is very high, and the nuclear membranes are irregular. The chromatin is typically very dense and may be opaque (Figure 5-16). These cells are placed in the HSIL category because the biopsy is most often CIN II+ and these features are similar to those of invasive keratinizing

carcinoma. Indeed, the only difference between keratinizing dysplasia and keratinizing invasive carcinoma is the presence of a tumor diathesis and/or nucleoli and subtle chromatin irregularity (clearing), which may be present in invasive carcinoma (see Figure 7-19).

Unlike LSIL, there are sometimes significant variations in the diagnostic criteria for HSIL in liquid-based samples.¹¹ While the morphologic changes are generally similar for both preparations, HSIL cells from the liquid-based samples may appear to be somewhat smaller than their counterparts in conventional smears. This is especially true in the cells derived from the highest grade lesions, such as carcinoma in situ, and is especially marked in ThinPrep specimens. Cells from HSIL can be found singly and in groups. Syncytial aggregates noted in association with carcinoma in situ are clearly identified on liquid-based cytology preparations but may mimic glandular groups, due to smoother contoured borders and smaller nuclei.¹² Challenges occur when only a few high-grade cells are present in the background of the slide. These cells are often small and may approximate the size of a small histiocyte. In the clean background encountered on liquid-based preparations, these small cells are easily missed on routine screening (Figure 5-17). The increased N:C ratios seen in moderate and severe dysplasias are evident on liquid-based preparations. Irregular nuclear contours are clearly displayed and may even be accentuated in liquid-based preparations. Fine to coarse nuclear granularity of the chromatin pattern is preserved. Hyperchromasia may be somewhat decreased in liquid-based preparations and should be considered to be a lesser criterion (Figure 5-18).

Squamous Cell Carcinoma

Squamous cell carcinoma (SCC) comes in two common morphologic variants: keratinizing and nonkeratinizing. While the Bethesda System does not subdivide SCC into these categories, the cytologic features are somewhat different. Nonkeratinizing SCC features cells with immature cytoplasm, high N:C ratios, and nuclei with prominent nucleoli, irregular chromatin distribution, and irregular nuclear membranes (Figure 5-19). These cells may be seen in loose or syncytial groups or as isolated cells (Figure 5-20). Associated features may include a tumor diathesis composed of necrotic debris, old blood, and inflammation.

Keratinizing SCC displays all of the cellular characteristics of keratinizing HSIL, with the addition of variable numbers of cells demonstrating nucleoli or the addition of a tumor diathesis. Features include marked cellular variation with tadpole, spindle, and caudate shapes; dense eosinophilic cytoplasm; and markedly hyperchromatic, often opaque, nuclei with high N:C ratios. Cells may be present singly or in loose or even thick groups (Figure 5-21).

A definitive interpretation of SCC is often difficult to make on cervical/vaginal preparations, especially in women without a clinical history of cervical cancer or other suggestive physical findings. In these cases, the Bethesda System recommends the use of “HSIL with features sug-

gestive of invasive carcinoma." As mentioned above, often the only difference between keratinizing HSIL and keratinizing SCC is the presence of a tumor diathesis and nucleoli. To complicate matters, because of the frequent exophytic growth pattern, a diathesis may not be present in some cases of invasive carcinoma, and the dense nuclear features make nucleoli difficult to identify. Thus, a careful search should be made for more immature cells with less nuclear opacity to identify the presence of nucleoli. A mixture of keratinizing cells and the markedly atypical cells associated with nonkeratinizing SCC is another clue to the presence of invasive carcinoma.

The presence of a tumor diathesis suggests an invasive carcinoma. This finding is still noted on liquid samples but may be somewhat patchier. It has been described as "clinging" diathesis because it seems to cling to abnormal cells and often coagulates into aggregates of debris, in distinction to the diffuse diathesis in smeared specimens (Figure 5-22). The presence of background blood and inflammation in women with SCC often makes the smears difficult to evaluate. This material may plug the filters used for ThinPrep preparations, yielding scanty cellular smears.¹³ At times the diagnostic cells are hidden in this obscuring material and may be missed during screening. Look for numerous pleomorphic, pyknotic cells that are caught up in necrotic debris. It is always important to thoroughly examine bloody and inflamed smears for the presence of these hidden malignant cells, even—or especially—when they are otherwise technically unsatisfactory.

Figure 5-1. ASC-US with changes suggestive of LSIL (HPV cytopathic effect).

The changes seen in these images suggest the possibility of HPV cytopathic effect and LSIL. The atypical cells present with varying degrees of dyskeratotic and koilocytic change. In some cases, while the individual cells (as in C) strongly suggest an HPV infection, the limited number of atypical cells present on the entire slide may preclude a diagnosis of LSIL. The sheets of cells in A and B show variably sized nuclei with focal binucleation. In A there is a suggestion of halo formation, but definite koilocytosis is not seen. The two atypical cells in C show halo formation, but the nuclei are small and do not demonstrate the quantitative nuclear changes sufficient for LSIL. The nuclei in D are larger, irregular, and hyperchromatic but fall short of the features of LSIL. Atypical keratin pearls are seen in E and F, with irregular nuclei rather than the round nuclei that would be associated with a benign pearl. Two types of atypical parakeratosis are seen in G and H. The cells in G are probably derived from surface cells. The more atypical cells in H have increased N:C ratios, variably-sized large nuclei, and irregular membranes. These cells are on the borderline between ASC-US and cells suggesting keratinizing dysplasia (ASC-H).

