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4.1 Epithelial Cell Abnormalities

Squamous Cell

- Atypical squamous cells (ASC)
 - Atypical squamous cells – undetermined significance (ASC-US)
 - Atypical squamous cells – cannot exclude a high-grade squamous intraepithelial lesion (ASC-H)

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4.2 Background

The forerunner of the category “atypical squamous cells” (ASC) was the more broadly defined interpretation of “atypical squamous cells of undetermined significance” (ASC US) [1]. In the second edition of the Atlas, the ASC classification was separated into two categories “atypical squamous cells – undetermined significance” (ASC-US) and “atypical squamous cells – a high grade squamous intraepithelial lesion cannot be excluded” (ASC-H) [2]. This separation reflected the fact that while most equivocal samples contained features suggestive of a low-grade squamous intraepithelial lesion, a small percentage of specimens were indeed equivocal, but their features were more suggestive of a high-grade squamous intraepithelial lesion. This dichotomous reporting terminology for atypia is in keeping with the 2-tiered reporting scheme for HPV-related squamous lesions which is based on our current understanding of the natural history of HPV-related infections – low-grade changes represent largely transient HPV infection, and high-grade morphology represents a precancerous lesion.

ASC does not represent a single biologic entity; it subsumes changes that are unrelated to oncogenic human papillomavirus (HPV) infection and neoplasia as well as findings that suggest the possible presence of an underlying squamous intraepithelial lesion (SIL) and rarely carcinoma. Numerous nonneoplastic conditions may produce cytologic changes that raise consideration for an ASC designation, including inflammation, air-drying, atrophy with degeneration, hormonal effects, and other artifacts. In many instances, the process that resulted in the ASC interpretation remains undefined, even following a diagnostic workup. In screening programs representative of the US population, approximately 40–50 % of women with ASC are infected with high-risk/oncogenic types of human papillomaviruses (HPV) [3–5].

The category of atypical squamous cells (ASC) is the most prevalent of all abnormal cervical cytology interpretations. In the 2014 Bethesda System, ASC continues to be included under squamous epithelial cell abnormality, with subcategorization as “atypical squamous cells – undetermined significance” (ASC-US) and “atypical squamous cells – cannot exclude a high-grade squamous intraepithelial lesion” (ASC-H). ASC-US refers to changes that are suggestive of LSIL but which are insufficient for a definitive interpretation as such. Although most ASC-US interpretations are suggestive of LSIL, the qualifier “undetermined significance” is preferred because approximately 10–20 % of women with ASC-US prove to have an underlying HSIL (CIN 2 or CIN 3) [3]. ASC-US is expected to comprise more than 90 % of ASC interpretations in most laboratories. The ASC-H category is a designation reserved for the minority of ASC cases (expected to represent less than 10 %) in which the cytologic changes are suggestive of HSIL but which are insufficient for a definitive interpretation. Only equivocal specimens specifically worrisome for HSIL should be distinguished from the bulk of ASC using the designation of ASC-H. Cases classified as ASC-H are associated with a higher positive predictive value for detecting an underlying HSIL (CIN 2 or CIN 3) than ASC-US but are less predictive of a high-grade lesion than definitive interpretations of HSIL [6, 7].

Because of its inherently equivocal nature, there have been arguments put forth suggesting entire elimination of this category, moving ASC into either NILM or SIL. However, after attempts to study how cervical cytology might perform in such a scenario, it has been shown that such elimination would diminish the sensitivity of

detection of precancer, the very lesions that this screening test was designed to identify [8]. ASC, by nature of being the most prevalent abnormal category, is also the interpretation that precedes the majority of identified HSIL (CIN3) cases [9].

4.3 Atypical Squamous Cells

4.3.1 Definition

ASC refers to cytologic changes *suggestive* of SIL, but which are qualitatively or quantitatively insufficient for a definitive interpretation as such [1, 2]. Cytologic findings that are most consistent with benign reactive changes should be carefully reviewed and judiciously classified as “negative for intraepithelial lesion or malignancy” whenever possible.

The interpretation of ASC requires that the cells in question demonstrate three essential features: (1) squamous differentiation, (2) increased nuclear to cytoplasmic ratio, and (3) minimal nuclear changes which may include hyperchromasia, chromatin clumping, irregularity, smudging, and/or multinucleation. Unequivocally normal-appearing cells on the same slide should be used for comparison in determining whether the interpretation of ASC is warranted [10]. Abnormal-appearing nuclei are a prerequisite for the interpretation of ASC. The finding of cytoplasmic and nuclear changes associated with HPV infection (perinuclear halos/koilocytes) warrant an interpretation of SIL. However, incomplete changes suggestive of koilocytosis (e.g., cytoplasmic halos closely resembling koilocytes but with no or minimal nuclear abnormalities) or poorly preserved cells with features suggestive of LSIL are generally designated as ASC-US [10].

It must be emphasized that the ASC category was developed to designate the interpretation of an entire specimen, not individual cells. The subtle and subjective findings in specimens with ASC have resulted in poor reproducibility, compounding the difficulty in developing and illustrating strict criteria [11, 12]. Furthermore, the almost infinite appearances that ASC may assume, including non-photogenic degenerative and artifactual changes, permit only a fractional representation of changes that experts might accept, if not agree upon, as ASC [12].

4.4 Atypical Squamous Cells – Undetermined Significance (ASC-US) (Figs. 4.1–4.19)

4.4.1 Definition

ASC-US refers to changes that are suggestive of LSIL.

4.4.2 Criteria

Nuclei are approximately two and one half to three times the area of the nucleus of a normal intermediate squamous cell (approximately 35 μm^2) or twice the size of a squamous metaplastic cell nucleus (approximately 50 μm^2) [12] (Fig. 4.1).

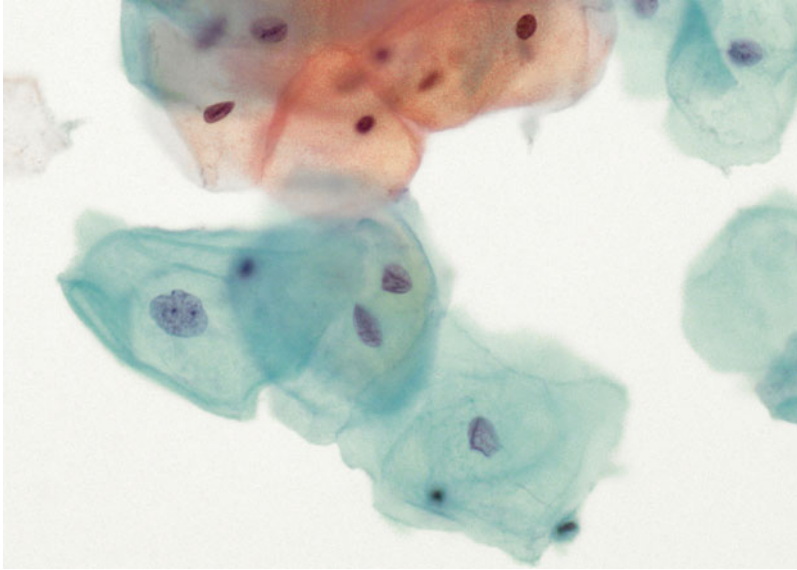


Fig. 4.1 ASC-US (*LBP, ThinPrep*). A 32-year-old woman. Atypical intermediate squamous cells with a nucleus 2–3× the area of a normal intermediate squamous cell nucleus and mild irregularity of nuclear contour. This isolated cell has some features suggestive of HPV infection, hrHPV was positive. Follow-up biopsy revealed LSIL (CIN1)

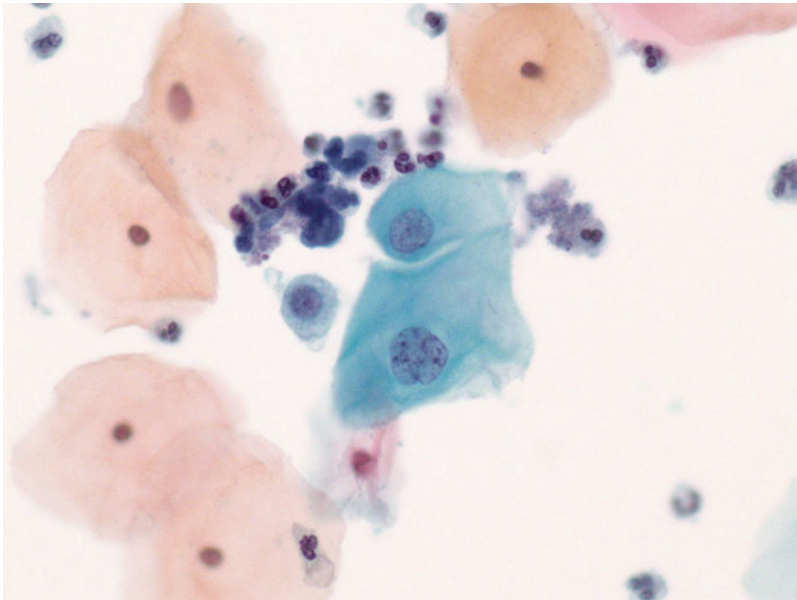


Fig. 4.2 ASC-US (*LBP, ThinPrep*). A 28-year-old woman. An intermediate squamous cell with an enlarged nucleus and slight nuclear membrane irregularity. The atypical features do not meet the criteria for LSIL, hrHPV was positive. Follow-up biopsy revealed LSIL (CIN1)

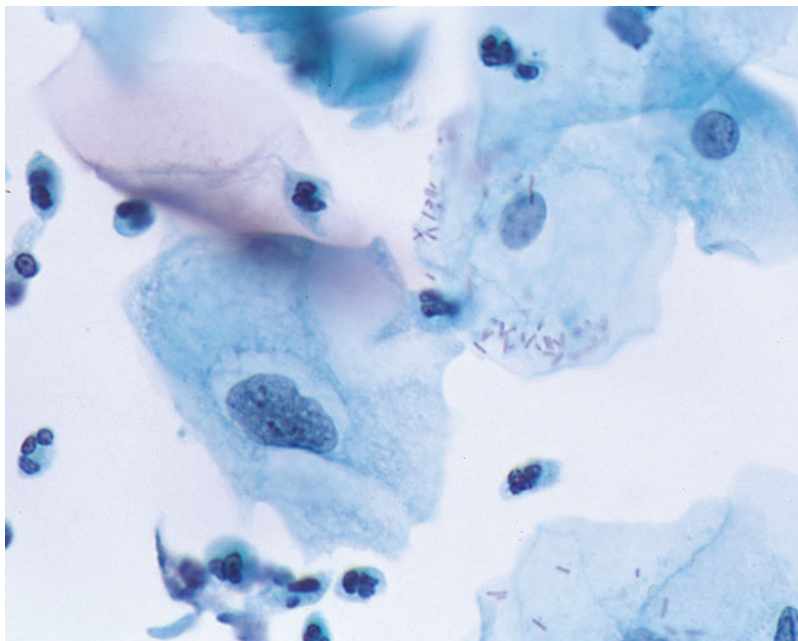


Fig. 4.3 ASC-US (*LBP, SurePath*). Routine screen from a 32-year-old woman. Single atypical squamous cell with ill-defined cytoplasmic halo in a background of inflammation. Adjacent squamous cell shows adherent lactobacilli. HPV testing was not performed on this sample

Slightly increased ratio of nuclear to cytoplasmic area (N/C) (Fig. 4.2).

Minimal nuclear hyperchromasia and irregularity in chromatin distribution or nuclear shape.

Nuclear abnormalities associated with dense orangeophilic cytoplasm (“atypical parakeratosis”), cytoplasmic changes that suggest HPV cytopathic effect (incomplete koilocytosis) – including poorly defined cytoplasmic halos or cytoplasmic vacuoles resembling koilocytes but with absent or minimal concurrent nuclear changes (Figs. 4.3 and 4.4).

Preparation Specific Criteria

Conventional Preparations:

Cells may appear larger and flatter due to smearing and/or air-drying artifact (Figs. 4.5 and 4.6).

Liquid-Based Preparations:

Cells may appear smaller and have higher nuclear to cytoplasmic ratios in two-dimensional views due to fixation in liquid media (which leads to rounding up of cells) and lack of flattening on the slide (Fig. 4.7).

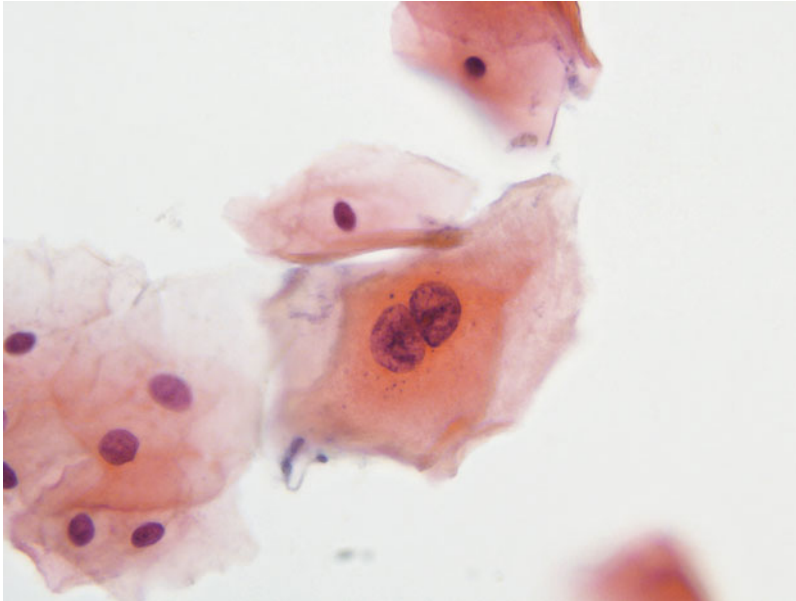


Fig. 4.4 ASC-US (*LBP, ThinPrep*). A 28-year-old female. An atypical binucleated intermediate cell with molded nuclei and orangeophilic cytoplasm suggestive but not diagnostic of LSIL. hrHPV was positive. Follow-up biopsy revealed LSIL (CIN1)

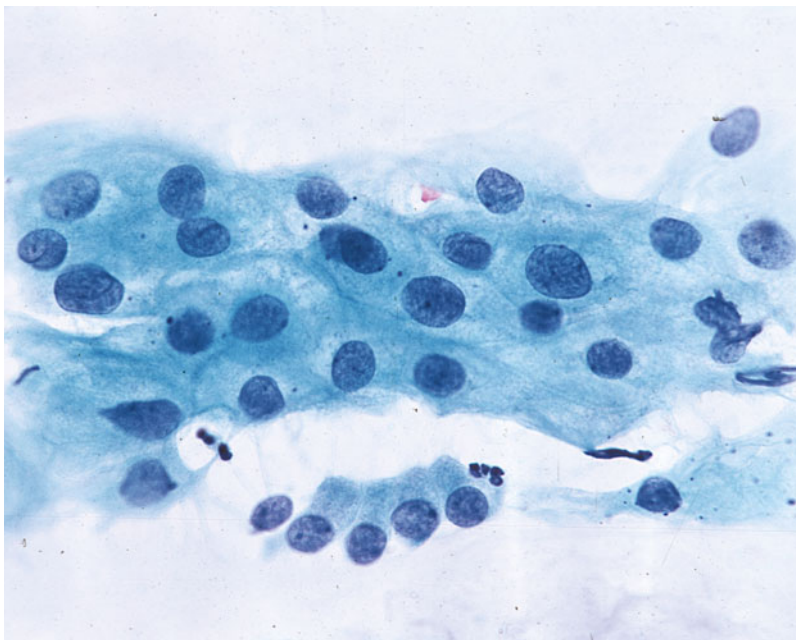


Fig. 4.5 Negative for intraepithelial lesion or malignancy (NILM) versus atypical squamous cells – undetermined significance (ASC-US) (*CP*). Perimenopausal woman. Mature squamous cells show mild nuclear enlargement, binucleation, and even chromatin distribution. Note benign endocervical cells at bottom of field

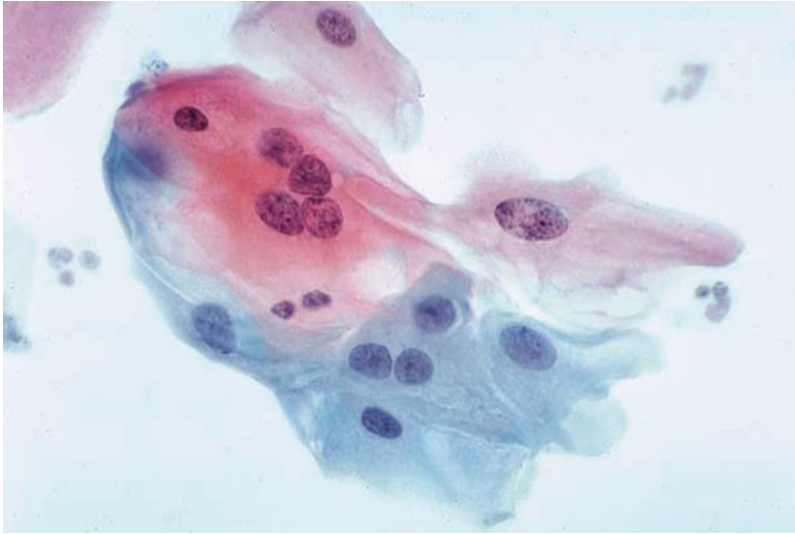


Fig. 4.6 ASC-US (CP). Cells with multinucleation, nuclear enlargement, and air-drying artifact, possibly representing LSIL (CIN1)

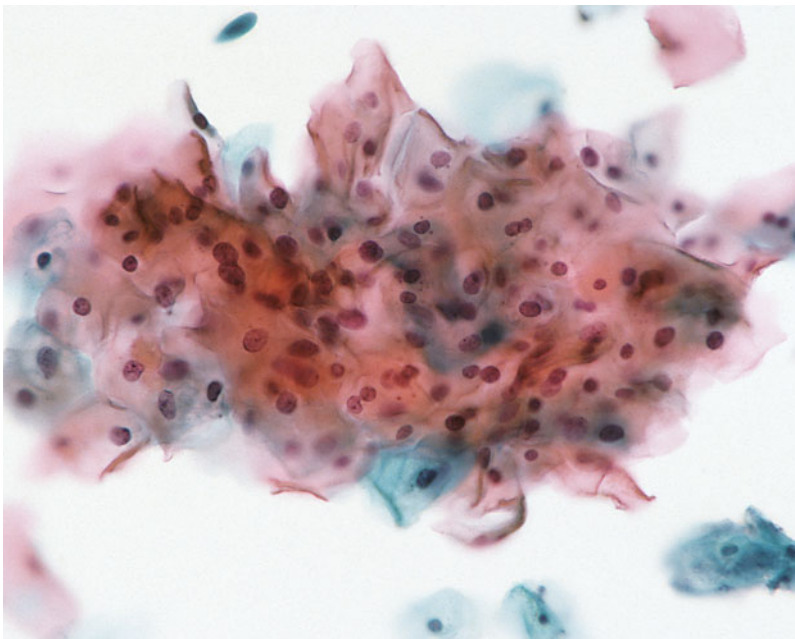


Fig. 4.7 ASC-US (LBP, SurePath). A 21-year-old woman. Thick cohesive sheet of cells with focal nuclear enlargement, orangeophilic cytoplasm, poorly formed cytoplasmic vacuoles, and binucleation. Follow-up biopsy was LSIL (CIN1)

4.4.3 Explanatory Notes

The normal-appearing intermediate cells that are present on a slide provide an appropriate source of comparison for assessing whether nuclear size and appearance meet criteria for ASC-US or SIL. Cells which might lead to an ASC-US designation for the slide typically have the overall size and shape of superficial or intermediate squamous cells. Round or ovoid cells that are approximately one-third the size of superficial cells and therefore resemble large metaplastic or small intermediate cells may also be classified as ASC-US. Criteria for ASC-US may differ subtly among laboratories, reflecting differences in stains and techniques for slide preparation (Figs. 4.8 and 4.9).

Determining whether to classify a specimen as NILM or ASC-US may be difficult in cases showing mild diffuse nuclear enlargement, the presence of reactive/reparative or degenerative changes, organisms, air-drying with artifactual nuclear enlargement, atrophic patterns, and in the presence of other artifacts (Figs. 4.10–4.13). In such specimens, the patient's age and history should be considered, and previous

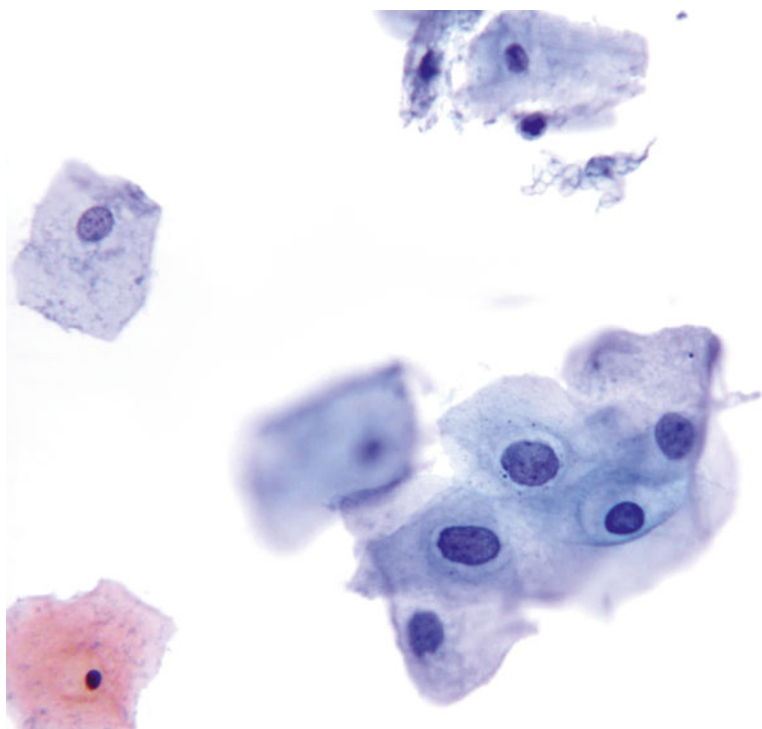


Fig. 4.8 ASC-US (*LBP, ThinPrep*). A 35-year-old woman. A group of cells featuring mild nuclear enlargement, slight nuclear membrane irregularity and mild hyperchromasia in a clean background. The cytologic features do not meet the criteria for LSIL. hrHPV was positive. Follow-up biopsy revealed LSIL (CIN1)

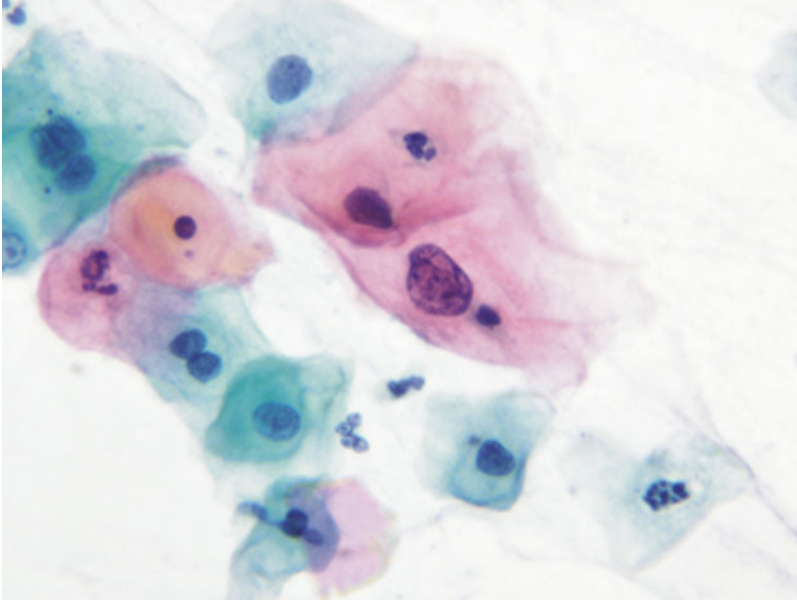


Fig. 4.9 ASC-US (*LBP, ThinPrep*). A 25-year-old woman. Intermediate cells with nuclear enlargement $\times 2$ – 3 that of normal intermediate squamous cell nucleus. There are rare binucleated cells. Slight nuclear irregularity and hyperchromasia are present that do not meet the diagnostic criteria for LSIL. A repeat cervical cytology showed similar findings. Follow-up biopsy revealed LSIL (CIN1)

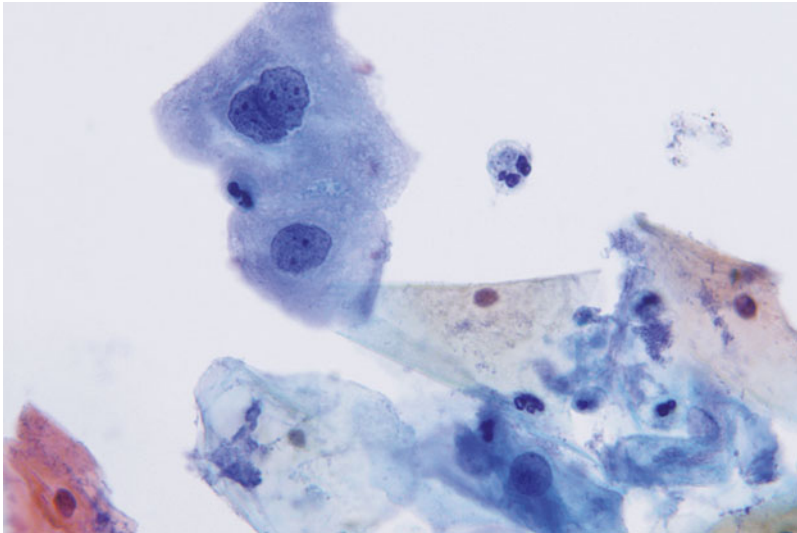


Fig. 4.10 ASC-US (*LBP, ThinPrep*). A 40-year-old woman. Binucleated atypical intermediate squamous cell with slightly enlarged irregular nuclei in an inflammatory background. hrHPV was positive. Follow-up biopsy showed LSIL (CIN1)

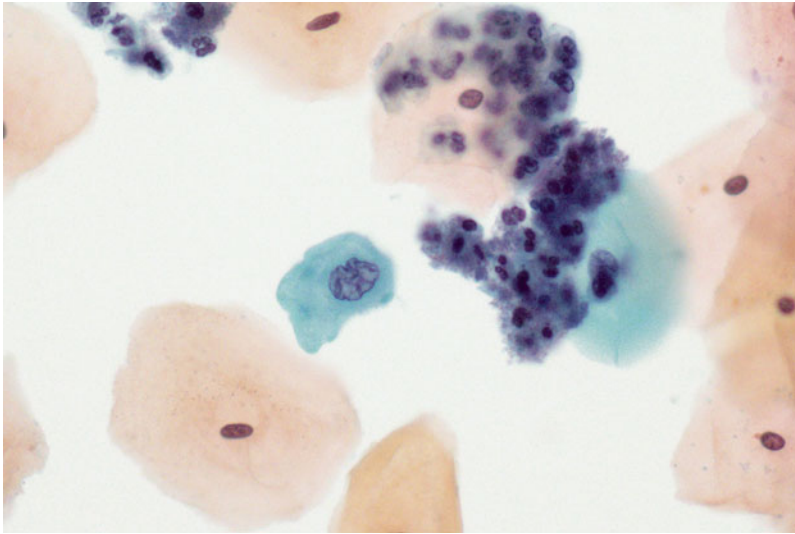


Fig. 4.11 ASC-US (*LBP, ThinPrep*). A 40-year-old woman. A single atypical intermediate squamous cell with a nucleus that is 2 to 3 times the area of a normal intermediate squamous nucleus and an irregular nuclear contour. The background shows acute inflammation. The cytologic features do not meet the criteria for LSIL

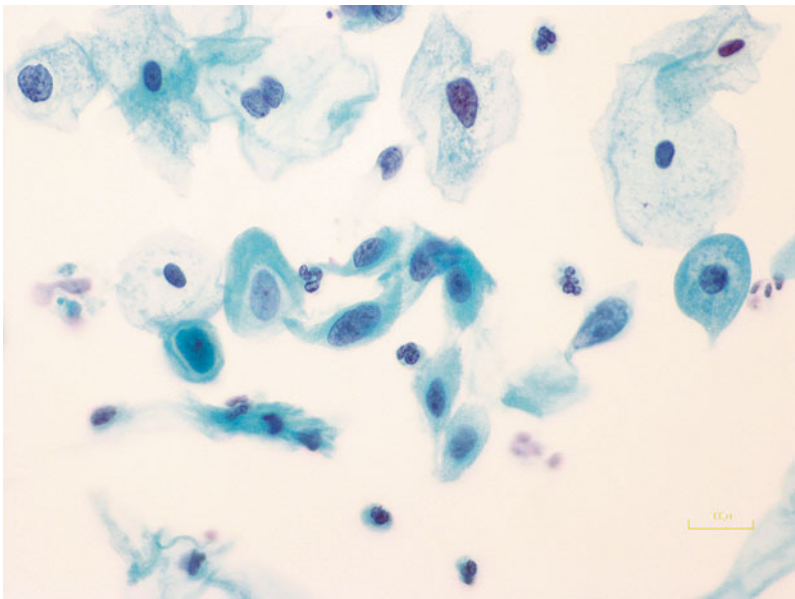


Fig. 4.12 ASC-US (*LBP, SurePath*). Routine screening in a perimenopausal woman. Several cells showing slightly increased nuclear hyperchromasia and nuclear to cytoplasmic ratios. Occasional bi-nucleation and cytoplasmic halos are seen. These features may be seen in a reactive/infectious process; however, given the absence of organisms and lack of history, an interpretation of ASC-US was rendered. Repeat cervical cytology was negative; hrHPV testing was also negative

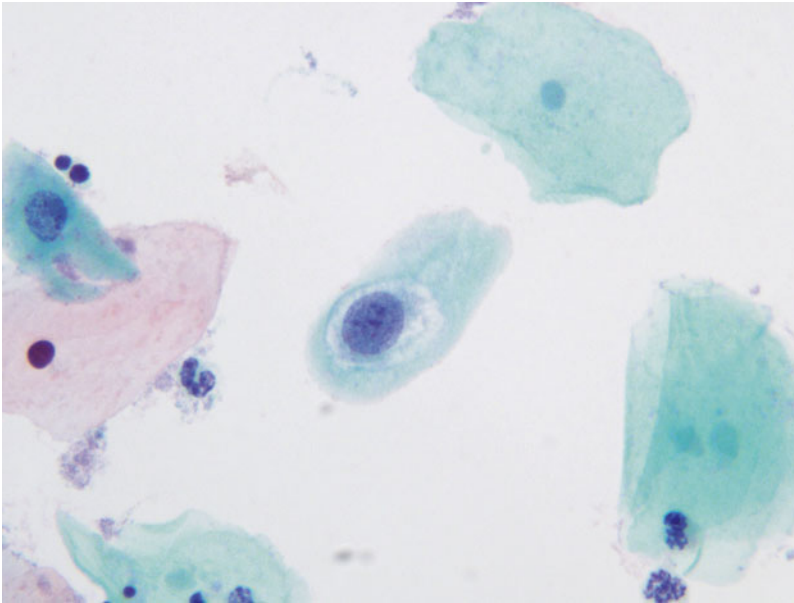


Fig. 4.13 ASC-US (*LBP, ThinPrep*). A 23-year-old woman. An atypical intermediate squamous cell with a mildly enlarged nucleus and a poorly-formed perinuclear halo. The atypical features are suggestive but not diagnostic of LSIL. hrHPV was positive. Follow-up biopsy revealed LSIL (CIN1)

specimens should be reviewed microscopically, if deemed relevant, to interpreting the current case. Generally, when the current cytologic findings favor a reactive process over an SIL and the patient has a history of multiple prior negative specimens—particularly if there is a recent negative hrHPV result—the interpretation of NILM should be favored. Most specimens classified as ASC demonstrate a numerically minor subpopulation of atypical cells that are either isolated or occur in small sheets or groupings (Fig. 4.14).

The prevalence of ASC-US declines with increasing age in the screening population, as does the prevalence of hrHPV DNA (including genotypes 16 and 18) [13]. ASC-US cytology in younger women is more prevalent and more often reflective of an HPV-related lesion than in older women [13]. Regardless of age, the knowledge of a patient’s concurrent hrHPV result could potentially bias the perspective of the cytotechnologist or cytopathologist when making an interpretation of NILM vs. ASC-US, especially in specimens with minimal cytologic changes [14–16]. Hence, care should be taken when reviewing specimens with a priori knowledge of HPV status.

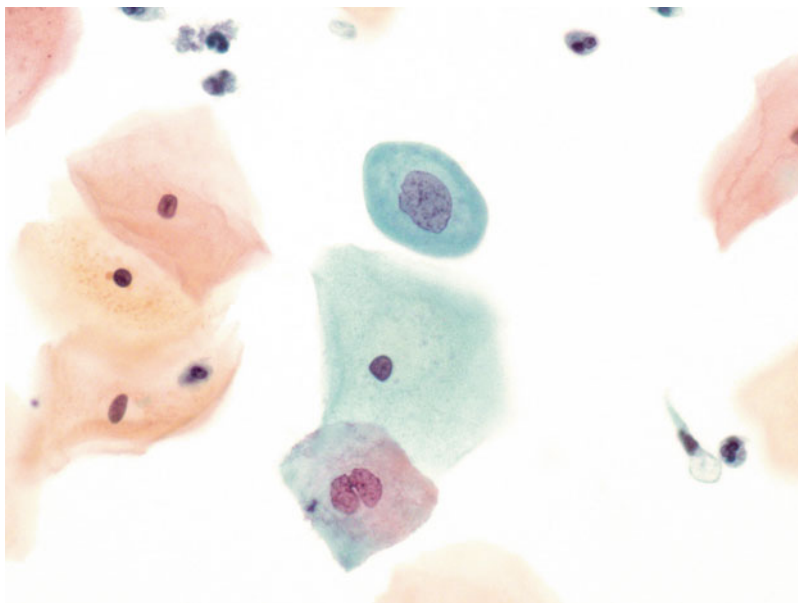


Fig. 4.14 ASC-US (*LBP, ThinPrep*). A 30-year-old woman. A metaplastic cell with dense cytoplasm, slightly enlarged nucleus and mild nuclear membrane irregularity is seen in the center. Below it is a binucleated intermediate squamous cell with irregular nuclear contour. The cytologic features are suggestive but do not meet the criteria for LSIL. hrHPV was positive. Follow-up biopsy revealed LSIL (CIN1)

4.5 Common Patterns Classified as ASC-US (Figs. 4.15–4.19)

4.5.1 Atypical Parakeratosis (APK) (Figs. 4.15 and 4.16)

Cells with dense orangeophilic or eosinophilic cytoplasm and small pyknotic nuclei (“parakeratosis”) should be classified as NILM if the nuclei appear normal (see Figs. 2.15 and 2.16). However, if the nuclei are enlarged, hyperchromatic, or irregular in contour or if the cells occur in three-dimensional clusters (referred to by some as “atypical parakeratosis”), an interpretation of ASC-US, ASC-H, or SIL should be considered depending on the degree of the abnormality [10, 17] (Figs. 4.15 and 4.16; see Figs. 5.8, 5.9, 5.26, 5.43, and 5.44).

4.5.2 Atypical Repair (Figs. 4.17 and 4.18)

Reparative changes that manifest some degrees of cellular overlap, dyscohesion, anisonucleosis, and/or loss of nuclear polarity may be designated as “atypical repair” which can be classified under the ASC-US category. The incidence of subsequent SIL among women with atypical repair has been reported to range from 25 to 43 % in high-risk population groups; however, the incidence of SIL in a more diverse population has been shown to be much lower (5.2 %) [18]. The differential diagnosis of atypical repair is wide. Changes that are at the lower end of the spectrum of atypia are generally designated as ASC-US (Figs. 4.17 and 4.18), while

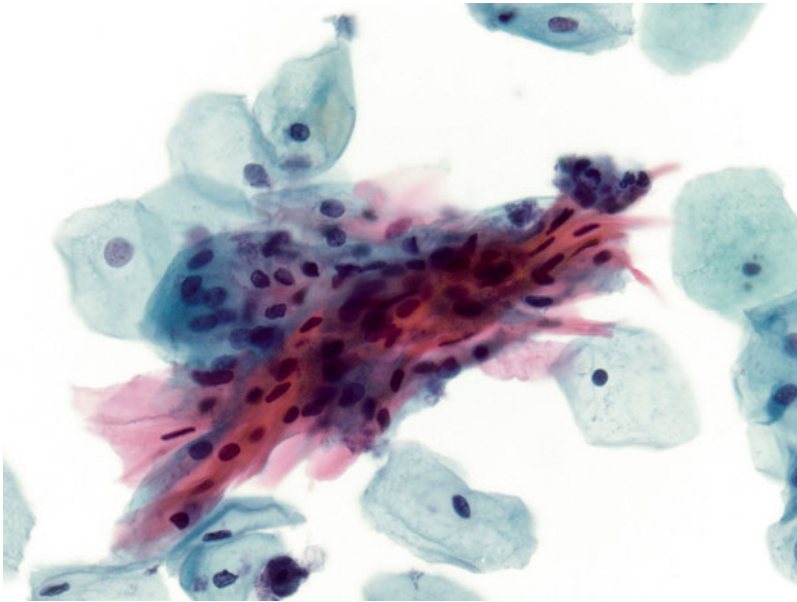


Fig. 4.15 ASC-US – atypical keratinized cells (*LBP, ThinPrep*). A 25-year-old woman. A cohesive sheet of spindled keratotic cells with nuclear enlargement, hyperchromasia and orangeophilic cytoplasm. hrHPV was positive. Follow-up biopsy revealed LSIL with prominent keratinization

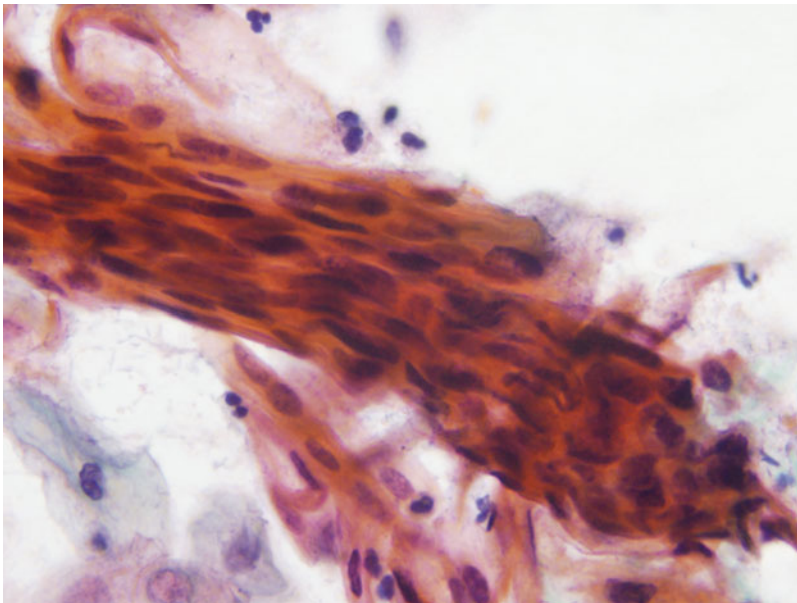


Fig. 4.16 ASC-US – atypical keratinized cells (*LBP, ThinPrep*). A 32-year-old woman. Cohesive sheet of atypical squamous cells with orangeophilic cytoplasm and elongated, hyperchromatic crowded nuclei. hrHPV was positive. Follow-up biopsy revealed HSIL (CIN 2) with prominent keratinization

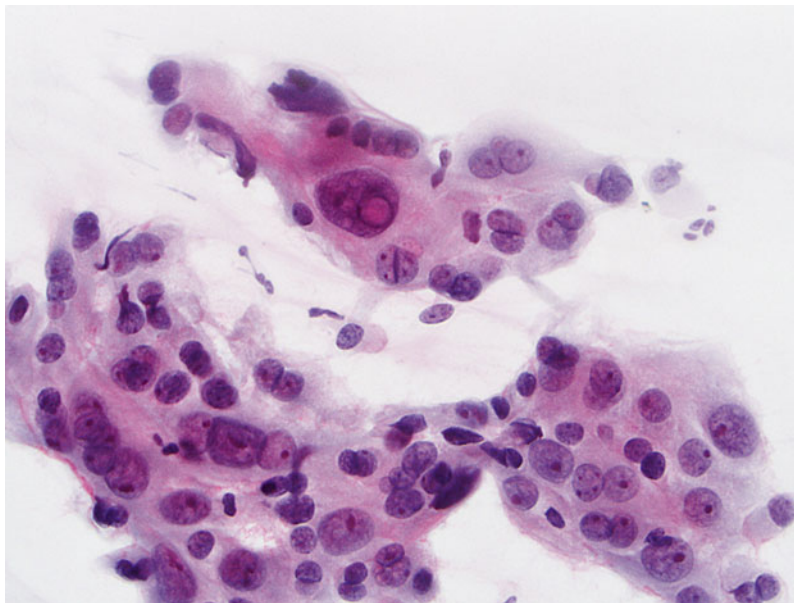


Fig. 4.17 ASC-US – atypical repair (*CP*). In this image, cells are arranged in two-dimensional sheet with abundant cytoplasm showing a “pulled-out” or streaming effect. Nuclei show pleomorphism of size and shape with some cells having multiple nuclei. Most nuclei show prominent nucleoli. These changes, while indicative of a reparative reaction, may be classified as ASC-US because of the nuclear pleomorphism noted. In favor of a reactive process is the generally fine granularity of the chromatin pattern

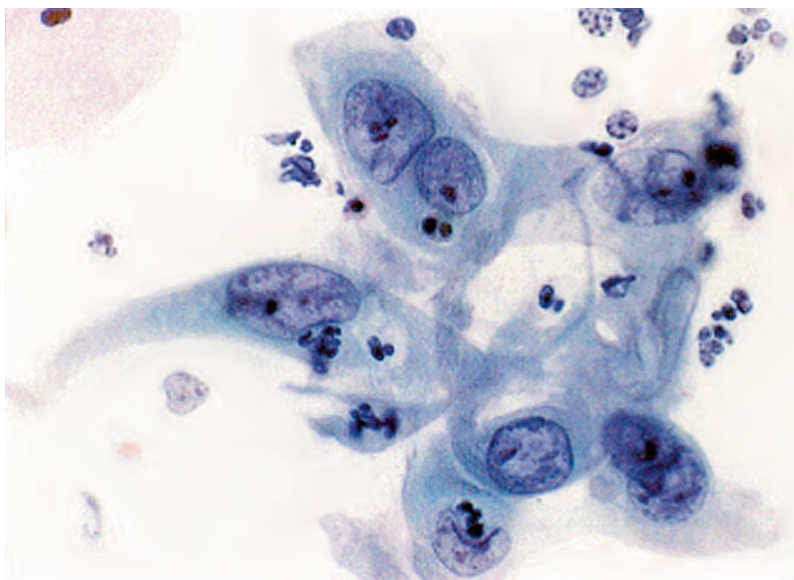


Fig. 4.18 ASC-US – atypical repair (*CP*). Group of cells with features of repair; however, the presence of irregular chromatin distribution and the increased nucleus to cytoplasmic ratio are not typical (see Figs. 2.38 and 2.39). Atypical reparative squamous cells may be classified as ASC-US, or sometimes as ASC-H if invasive carcinoma is a morphologic consideration

those that are concerning for the possibility of invasive carcinoma, especially in high-risk patients, should be placed in the ASC-H category.

4.5.3 Atypia in Postmenopausal Women and in Atrophy (Fig. 4.19)

Atrophic samples showing nuclear enlargement with hyperchromasia that fall short of a definitive interpretation of SIL may also be designated as ASC-US. Occasionally, and especially in the case of a high-risk patient, the atypia in atrophy may warrant an interpretation of ASC-H, if it raises concern for HSIL (see Fig. 4.29). The interpretation of HSIL may be difficult to make in an atrophic background because of the lack of maturity (and hence high nuclear to cytoplasmic ratio) of the parabasal cells. In low-risk scenarios, it may be prudent to categorize such atypias as ASC-US rather than ASC-H and allow adjunctive hrHPV testing to determine downstream management which may avoid overtreatment.

In peri- and postmenopausal women, mild bland nuclear enlargement is a common cause for ASC over utilization. Changes of mild nuclear enlargement without significant hyperchromasia or nuclear irregularity have sometimes been termed “postmenopausal atypia” and are not generally associated with HPV-related disease (Fig. 4.19). In the absence of definitive abnormalities, such cases are

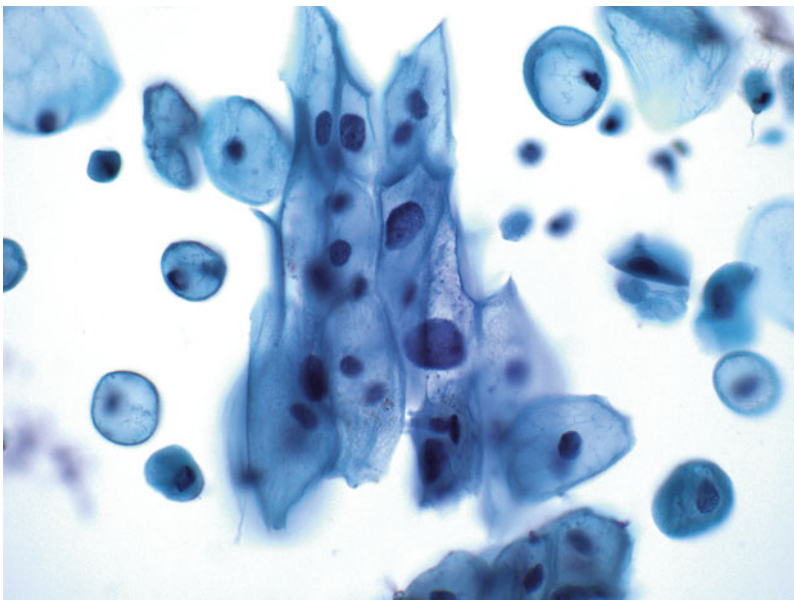


Fig. 4.19 Postmenopausal atypia (*LBP, SurePath*). Postmenopausal woman with an atrophic cell pattern, predominantly comprised of parabasal cells. The presence of occasional enlarged nuclei is a characteristic feature of postmenopausal atypia and is often overcalled as ASC-US. hrHPV testing is usually negative in such cases

preferably interpreted as NILM, especially in women who have no prior history of squamous cell abnormalities or do not have a prior positive hrHPV test [19, 20].

4.5.4 Other Patterns

Rarely, the difficult distinction between SIL and decidual and trophoblastic cells may also prompt an interpretation of ASC-US (see Figs. 2.28, 2.29, and 5.53).

ASC may also be an appropriate designation for some specimens that contain abnormal-appearing naked nuclei without associated cytoplasm, since isolated nuclei may be associated with SIL in some cases (see Fig. 5.39).

4.6 Atypical Squamous Cells – Cannot Exclude an HSIL (ASC-H) (Figs. 4.20–4.33)

4.6.1 Definition

ASC-H is a designation reserved for the minority of ASC cases (expected to represent less than 10 % of all ASC interpretations) in which the cytologic changes are suggestive of HSIL.

ASC-H cells are usually sparse. Several patterns may be present including atypical immature metaplastic cells, crowded sheets of cells, markedly atypical repair, severe atrophy, and postradiation changes that are concerning for recurrent or residual carcinoma.

4.7 Common ASC-H Patterns

4.7.1 Small Cells with High N/C Ratios (“Atypical Immature Metaplasia”) (Figs. 4.20–4.26)

4.7.1.1 Criteria

Cells usually occur singly or in small groups of less than ten cells; occasionally, in conventional preparations, cells may “stream” in strands of mucus (Figs. 4.24 and 4.25).

Cells are the size of metaplastic cells with nuclei that are about 1.5–2.5 times larger than normal (Fig. 4.20).

Nuclear to cytoplasmic ratio may approximate that of HSIL (Figs. 4.21 and 4.22).

In considering a possible interpretation of ASC-H or HSIL, nuclear abnormalities such as hyperchromasia, chromatin irregularity, and abnormal nuclear shapes with focal irregularity favor an interpretation of HSIL (Figs. 4.23 and 4.26).

Preparation Specific Criteria

Liquid-Based Preparations:

ASC-H cells may appear quite small with nuclei that are only two to three times the size of neutrophils. In some instances, differentiating two overlapping nuclei from a single irregular nucleus may pose difficulties, although this can usually be resolved by focusing up and down at high power.

Cells in the size range of metaplastic cells may also possess perfectly round pale nuclei, but which nonetheless appear to occupy the majority of the cytoplasm (Fig. 4.31).

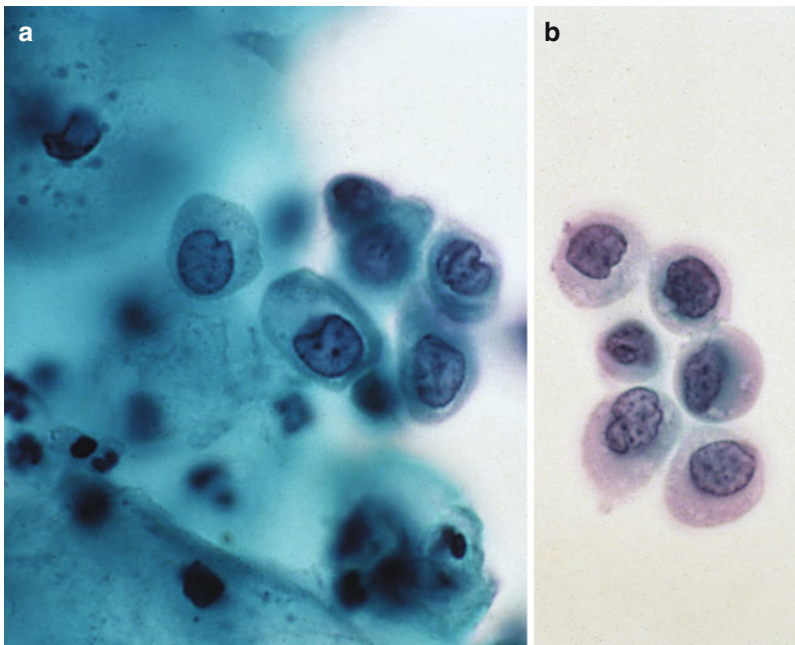


Fig. 4.20 ASC-H (*LBP, ThinPrep*). A 27-year-old woman. **(a)** On the *left* are isolated small cells with variable N/C ratios and some cells displaying prominent nuclear irregularity. **(b)** On the *right* is a high-magnification view of six small cells with enlarged and irregular, but degenerated, nuclei. Follow-up was HSIL (CIN 3)

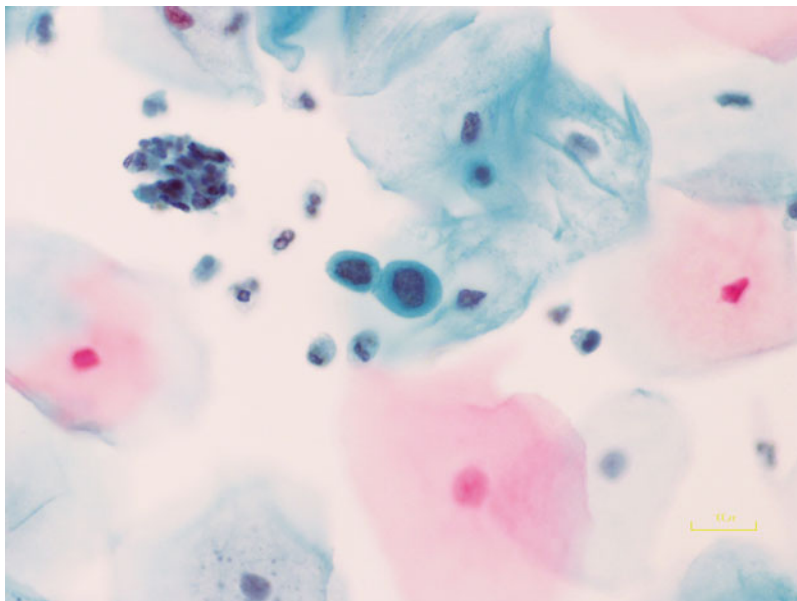


Fig. 4.21 ASC-H (*LBP, SurePath*). Routine cytology for a 30-year-old woman. Rare metaplastic cells with dense cytoplasm and nuclear enlargement with hyperchromasia are present in a background of scattered acute inflammation. An interpretation of ASC-H was rendered. Follow-up cervical biopsies revealed immature squamous metaplasia. Immature squamous metaplasia is one of the most common mimics of HSIL. An interpretation of ASC-H is appropriate, especially when only rare abnormal cells with “metaplastic” cytoplasm and high nuclear to cytoplasmic ratio are present

4.7.1.2 Explanatory Notes

Normal metaplastic squamous cells within a specimen may vary considerably in cell size and shape, nuclear size, and nuclear to cytoplasmic ratios. When cells with a metaplastic appearance demonstrate relatively mild nuclear enlargement, membrane irregularity, uneven chromatin distribution, or hyperchromasia, HSIL is a concern because the nuclear to cytoplasmic ratio may be similar to that found in definite HSIL. The range in size and nuclear appearance of normal metaplastic squamous cells provides a standard for judging whether cells of concern warrant an interpretation of ASC-H.

ASC-H may present as “atypical immature metaplasia” in both conventional and liquid-based preparations, although this finding is more common in the latter. Note that degenerated nuclei, in the absence of a bona fide SIL, are often irregular or hyperchromatic, but the irregularities tend to involve the *entire* nuclear outline, imparting a wrinkled appearance, and the chromatin is smudgy (Fig. 4.26). ASC-H cells are usually sparse. When numerous small atypical cells are identified, the interpretation of HSIL is more likely.

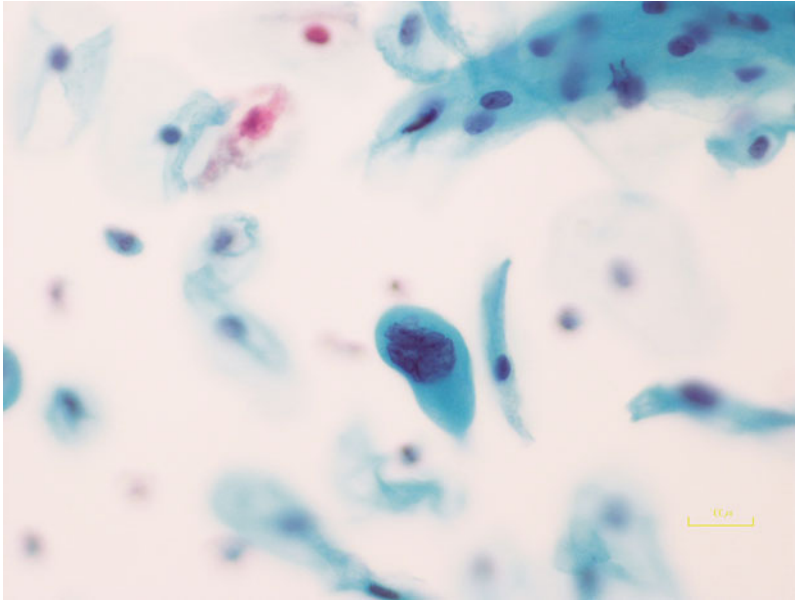


Fig. 4.22 ASC-H (*LBP, SurePath*). Perimenopausal woman with history of LSIL. Unremarkable slide with only a single large atypical cell in a clean background. The nuclear irregularity and hyperchromasia were worrisome but not definitive for SIL. Cervical biopsies were performed and showed tubal metaplasia but no intraepithelial neoplasia. A solitary cell of this nature is difficult to classify. Cyto-histologic correlation favored this to be a reactive endocervical cell, although a terminal bar and cilia were not conclusively identified

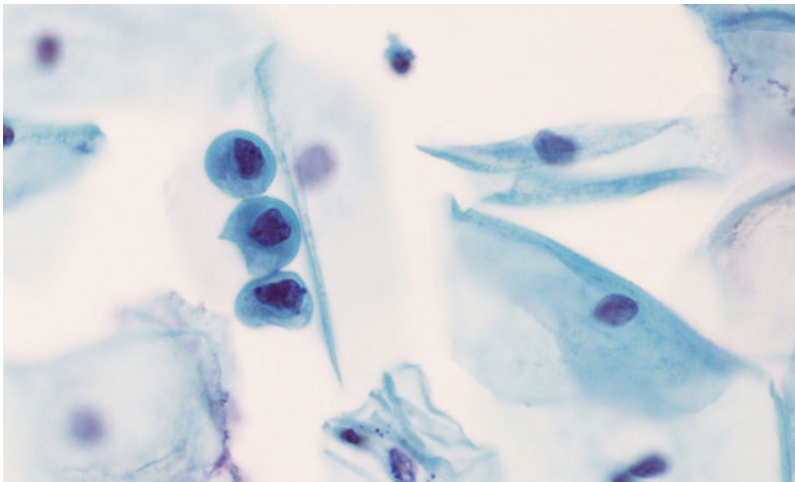


Fig. 4.23 ASC-H (*LBP, SurePath*). Perimenopausal woman with history of atypical cytology (ASC-US). Three small atypical metaplastic cells with hyperchromatic nuclei and irregular nuclear membranes are identified. The interpretive considerations included immature metaplasia; however, a high-grade lesion could not be excluded, thus an interpretation of ASC-H was rendered. Loop electrical excision procedure (LEEP) revealed focal areas of HSIL as well as immature metaplasia. Concomitant review of the cytology favored these cells to represent HSIL

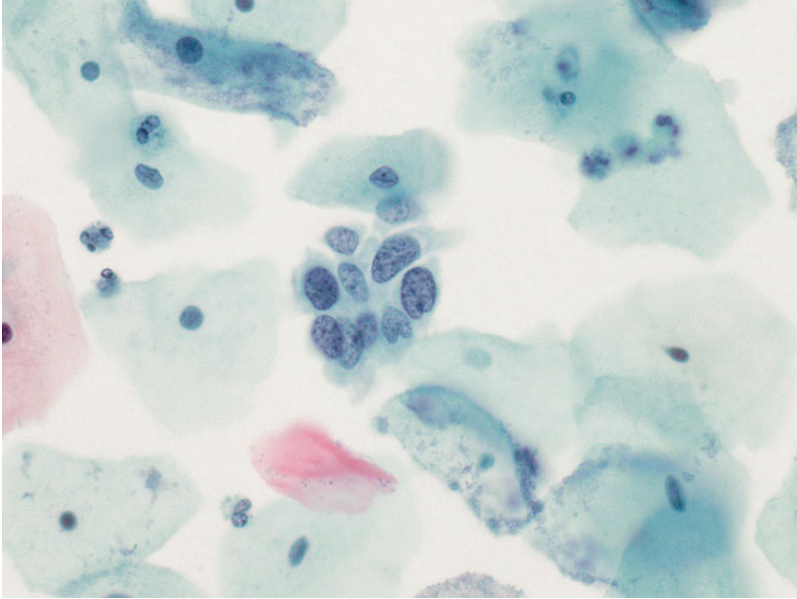


Fig. 4.24 ASC-H (*LBP, SurePath*). A group of atypical immature metaplastic cells with enlarged nuclei, high nuclear to cytoplasmic ratio, coarse chromatin and irregular nuclear contour. The cytologic features are worrisome but insufficient for an interpretation of HSIL. Follow-up biopsy revealed HSIL (CIN3)

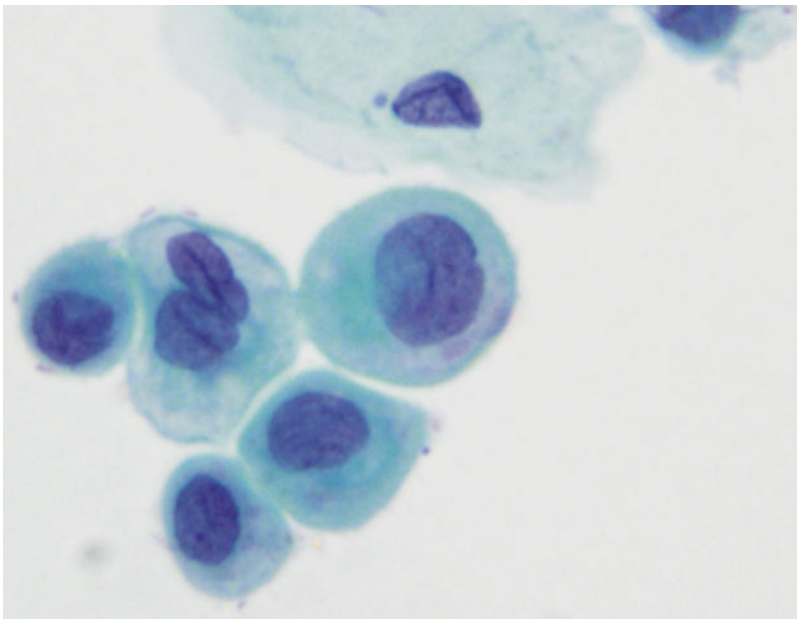


Fig. 4.25 ASC-H (*LBP, ThinPrep*). A 35-year-old woman. An isolated group of atypical immature metaplastic cells with dense cytoplasm, high nuclear to cytoplasmic ratio, enlarged nuclei, irregular nuclear contour and nuclear grooves. Follow-up biopsy revealed HSIL (CIN2)

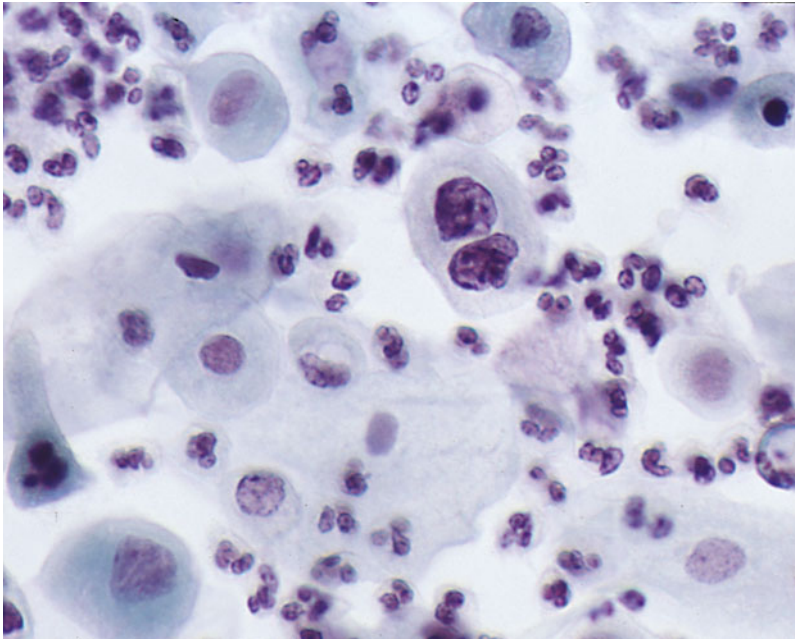


Fig. 4.26 ASC-H (*LBP, ThinPrep*). Vaginal specimen obtained from patient with prior history of vaginal HSIL (VAIN 3) and endometrial carcinoma. Cells present show degenerated, markedly hyperchromatic nuclei, worrisome for HSIL. Follow-up histology was HSIL (VAIN 3)

4.7.2 “Crowded Sheet Pattern” (Fig. 4.27)

4.7.2.1 Criteria

A microbiopsy of crowded squamous cells containing nuclei that may show atypical features as noted above, loss of polarity, or are difficult to visualize. Dense cytoplasm, polygonal cell shape, and fragments with sharp linear edges generally favor squamous over glandular (endocervical) differentiation.

Preparation Specific Criteria

Conventional Preparations:

Cells may appear larger and flatter due to smearing and air-drying artifact (Fig. 4.28).

4.7.2.2 Explanatory Notes

The “crowded sheet pattern” may reflect HSIL (particularly involving endocervical glands), reactive or neoplastic endocervical cells, or atrophy with crush artifact [21, 22] (see Figs. 5.15, 5.16, and 5.34). These cases are sometimes classified as “atypical glandular cells” (AGC), leading to an unexpectedly strong association between the latter category and detection of HSIL on subsequent biopsy [23]. Dense cytoplasm, polygonal cell shape, and fragments with flattening of cells at the edge of the cluster generally favor squamous over glandular differentiation [24]. Excessively

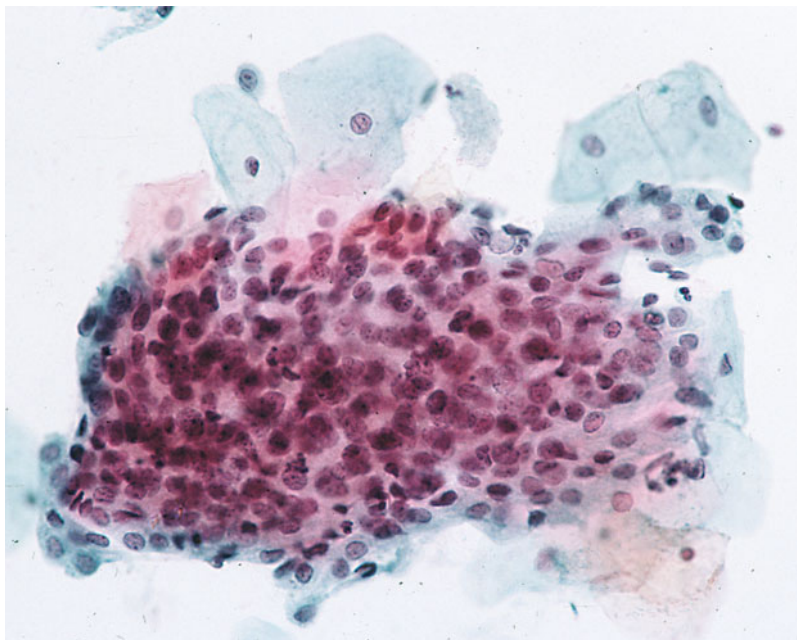


Fig. 4.27 ASC-H (CP). Thick aggregate of cohesive, air-dried, overlapping cells containing nuclei with even chromatin and regular borders. The thickness of the cluster makes it difficult to determine if the cells are squamous or glandular. The disorganization of the cells within the group is suggestive of a high-grade lesion; however, the individual nuclear features are insufficient for a definitive interpretation

vigorous scraping with sampling devices may represent an avoidable cause of thick cell fragments.

Identification of prominent nucleoli is more typical of repair than HSIL; however, nucleoli may be found in cases of HSIL, especially when associated with incipient or established invasion or when HSIL involves the necks of endocervical glands (see Fig. 5.32). Cohesive sheets of cells containing uniform-appearing nuclei with smooth contours and nucleoli favor a reparative process, but nuclear pleomorphism or loss of cohesion may require an interpretation of ASC-H in order to rule out a neoplastic lesion.

In atrophic specimens, the small size and high nuclear to cytoplasmic ratio typical of parabasal cells may raise concern about HSIL, especially when nuclear hyperchromasia and smudging associated with degeneration are present (Figs. 4.28 and 4.29). Hyperchromatic cellular groups of benign atrophy, when viewed at high magnification in a single focal plane, will generally show no nuclear overlapping in that focal plane, while dysplastic lesions, which are syncytial, will show nuclear overlapping in a single focal plane (see Figs. 5.45 and 5.46). This is a useful

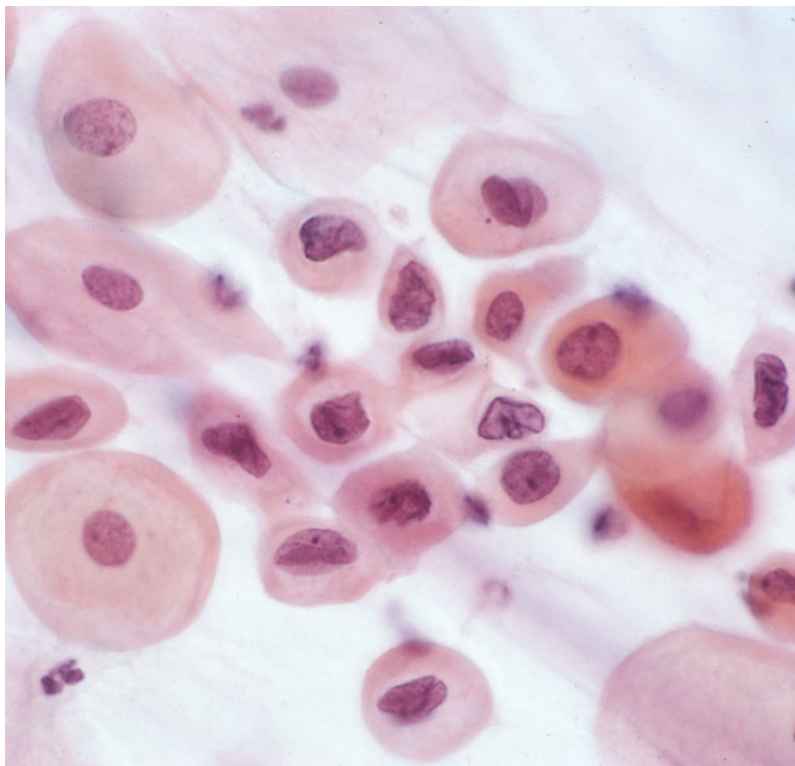


Fig. 4.28 ASC-H (CP). Smear from postmenopausal patient containing ovoid cells with irregular poorly preserved nuclei. Possible interpretations include NILM (atrophy), ASC-H and HSIL

differential diagnostic maneuver in equivocal cases. In addition, atrophy will generally not show evidence of cell proliferation, whereas proliferative cells may be noted in cases of SIL. Adjunctive hrHPV testing may also be helpful to clarify such cases. Application of topical estrogen may produce sufficient maturation to allow definitive classification of a repeat sample [25]; however, in the 2012 ASCCP management guidelines, it is recommended that colposcopy be performed for ASC-H. Blood and inflammation may be present in both atrophic vaginitis and carcinoma; however, the presence of a background containing frank cellular necrosis (diathesis) would favor a neoplasm.

Similar findings may prompt an interpretation of ASC-H following radiation therapy for carcinoma. Typical benign radiated cells show proportionate nuclear and cytoplasmic enlargement associated with cytoplasmic and nuclear degeneration (see Figs. 2.43 and 2.44), but an interpretation of ASC-H is appropriate when markedly atypical cells are present for which a clear distinction from HSIL or carcinoma is not possible. Comparison with the morphology of the original tumor, if available, may help.

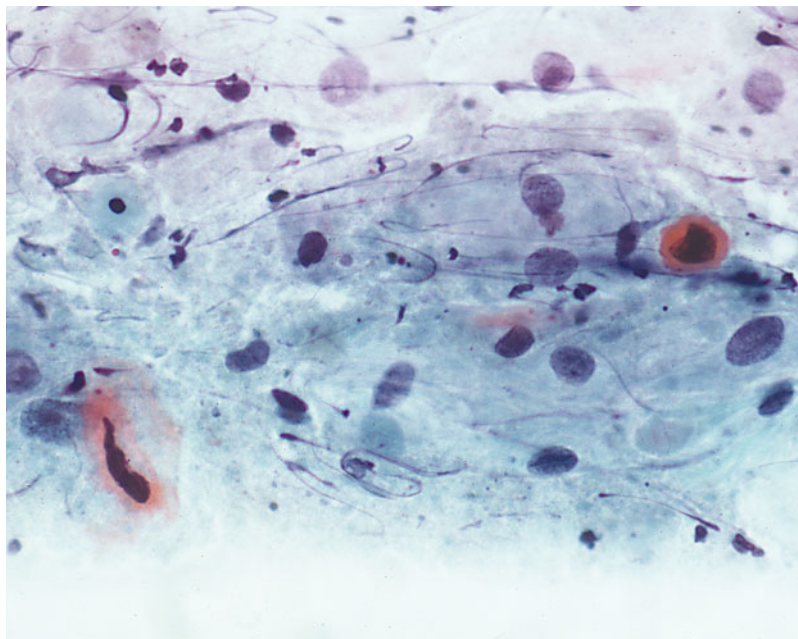


Fig. 4.29 ASC-H (CP). A 50-year-old postmenopausal woman with prior abnormal cytology. Two cells with extremely hyperchromatic, degenerated nuclei, and orangeophilic cytoplasm, in a background of atrophy with lysed cells and debris. Follow-up demonstrated HSIL (CIN 2)

4.8 ASC-H Mimics

4.8.1 Non-squamous Cells (Figs. 4.30–4.33)

Isolated endocervical cells (Figs. 4.30, 4.31 and 4.34), degenerated endometrial cells (Fig. 4.32), and macrophages (Fig. 4.33) may also possess nuclei that can closely mimic those of HSIL, leading to over interpretations as HSIL/ASC-H (see Figs. 2.4 and 2.5, 5.41 and 5.51). Similarly, some patients having an intrauterine device may shed rare cells with an extremely high nuclear to cytoplasmic ratio that resemble HSIL (see Fig. 2.47), and pregnant/postpartum patients may show atypical appearing decidualized stromal cells (see Figs. 2.28 and 5.53). These cells have a characteristic wrinkled nuclear contour and a distinct nucleolus. An interpretation of ASC-H or AGC may be appropriate if the etiology of the changes is not certain or the presence of an IUD is unknown (see Fig. 6.5).

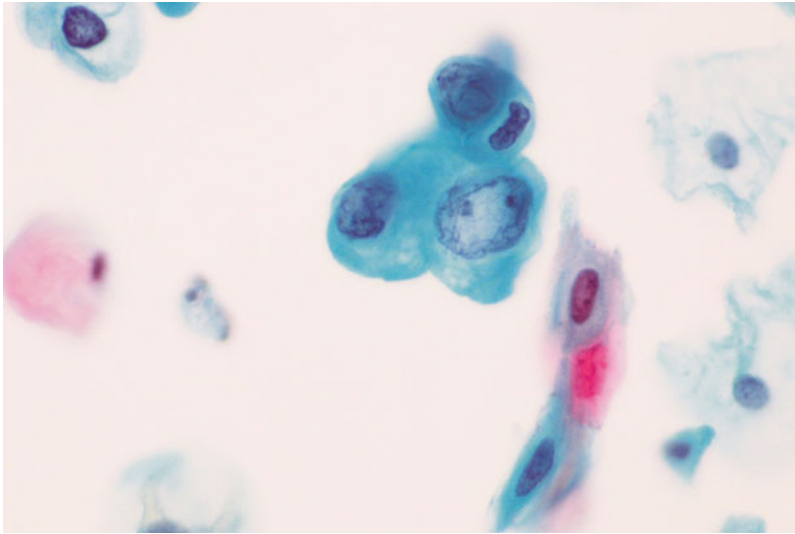


Fig. 4.30 ASC-H (*LBP, SurePath*). Routine cervical cytology from a perimenopausal woman. A group of metaplastic cells with increased nuclear to cytoplasmic ratios is identified in a relatively clean background. In addition to slightly increased nuclear size, the cells also show some nuclear clearing. In the absence of a history of prior abnormalities, an interpretation of ASC-H was made. Follow-up cervical biopsy and endocervical curettage were negative. The atypical cells were identified as degenerating endocervical cells on cyto-histologic correlation

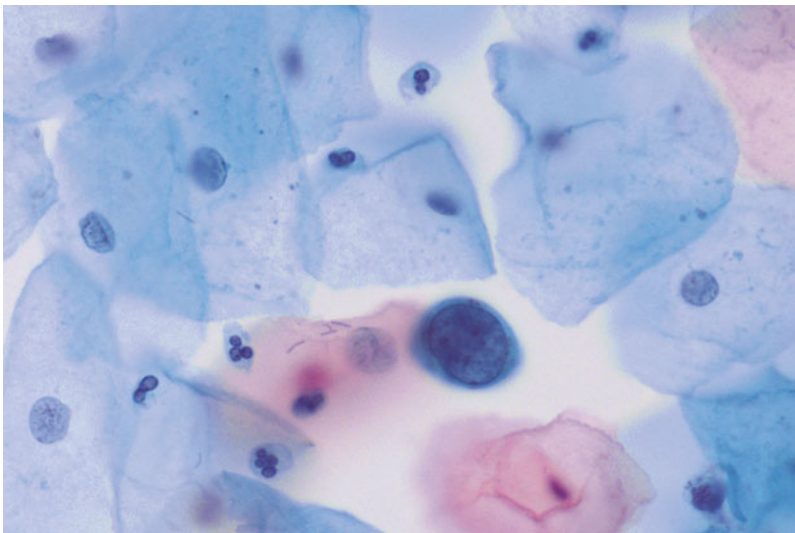


Fig. 4.31 ASC-H (*LBP, SurePath*). Perimenopausal woman with no significant medical history. Cervical cytology was unremarkable with the exception of a single enlarged cell with scant cytoplasm, a distinct, regular nuclear membrane and evenly distributed chromatin. An interpretation of ASC-H was made. Cervical biopsy and endocervical curettage were negative. Cyto-histologic correlation favored this atypical cell to be a degenerated endocervical cell seen *en face*. Review of other fields with comparison of other endocervical cells showed similar nuclear features

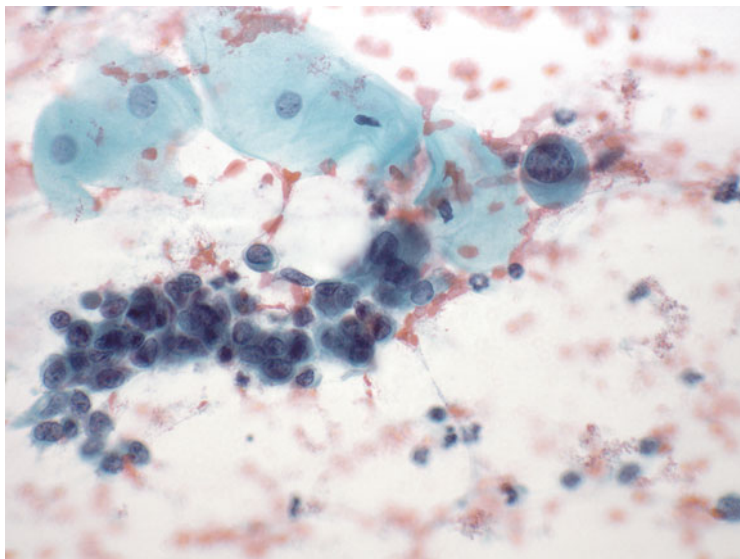


Fig. 4.32 Endometrial cells mimicking HSIL (CP). A crowded group of poorly preserved endometrial cells featuring small cells with hyperchromatic nuclei and high nuclear to cytoplasmic ratios

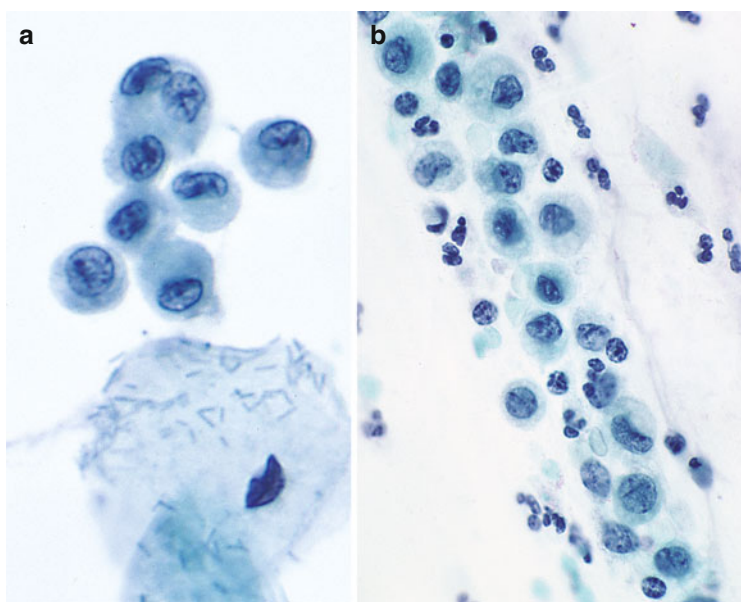


Fig. 4.33 Histiocytes: appearance on liquid based and conventional preparations. (a) *Left panel.* NILM, histiocytes (LBP, ThinPrep). Routine screen from a 32-year-old woman. Cells possess eccentric oval and round nuclei and foamy cytoplasm. The rounder shape of most cells in LBP as compared to CP may lead to uncertainty about the cell type; however, definitive assessment is usually possible under high magnification. (b) *Right panel.* NILM, histiocytes (CP). Streaming pattern of single cells with round, ovoid, and bean-shaped nuclei. Cells possess fine cytoplasmic vacuoles that may resemble degenerative vacuoles sometimes found in normal metaplasia, ASC-H, and HSIL. By contrast, cells of squamous lineage typically are polygonal in shape and possess dense cytoplasm. Follow-up was NILM in both cases

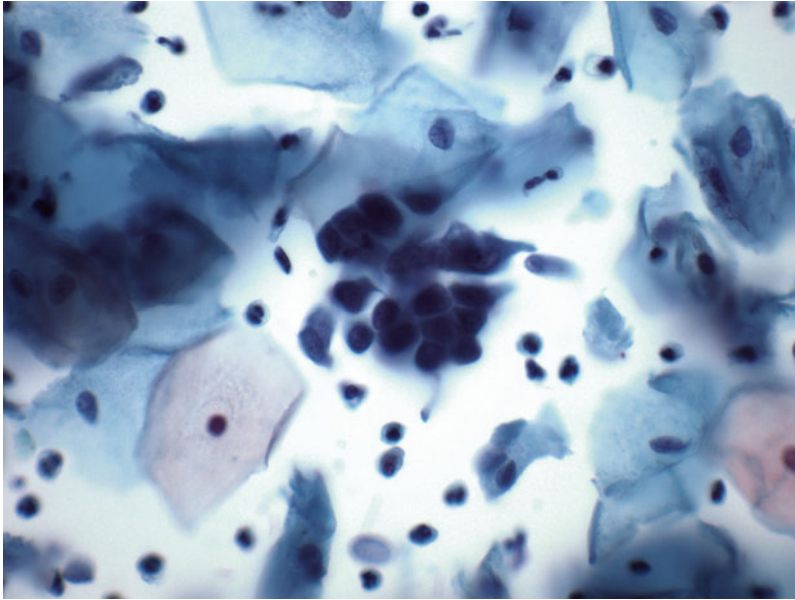


Fig. 4.34 NILM, Endocervical cell grouping (*LBP, SurePath*). Endocervical cells, when viewed on end, may mimic ASC-H, showing high nuclear to cytoplasmic ratios, and a configuration reminiscent of metaplastic cells. Maintenance of a “honey-comb” structure, and a mucus cap when focusing above the nuclear plane is helpful in distinguishing this mimic

4.8.2 Artifacts (Fig. 4.34)

In some instances, the perception of a high nuclear to cytoplasmic ratio represents an artifact resulting from layering of the cell (squamous metaplastic or endocervical) onto the slide in an orientation that does not demonstrate the total cytoplasmic volume (Fig. 4.34). Comparison of nuclear features of the cells in question with normal-appearing metaplastic or endocervical cells is useful as is focusing through the cells in order to appreciate areas of cytoplasm that may be present in alternate focal planes.

4.9 Management

Overall more HSIL (CIN2+) is detected on follow-up of ASC results than those interpreted as HSILs [9], because ASC is a far more common cytologic interpretation than HSIL. For ASC-US/ASC-H interpretations having adjunctive hrHPV testing, the 5-year risks for histologic HSIL and cancer are as follows: ASC-US with

negative HPV, 1.1 %; ASC-US with positive HPV, 18 %; ASC-H with negative HPV, 12 %; and ASC-H with positive HPV, 45 %. These figures provided the basis for the risk-based 2012 ASCCP management guidelines [26].

These guidelines are as follows [27]:

- For ASC-US cytology, reflex HPV testing is preferred.
- Women with HPV-negative ASC-US, whether from reflex HPV testing or co-testing, should return for co-testing per 2012 ASCCP guidelines at 3 years.
- Women with HPV-positive ASC-US, whether from reflex HPV testing or co-testing, should be referred for colposcopy.
- When colposcopy does not identify CIN in women with HPV-positive ASC-US, co-testing at 12 months is recommended. If the co-test is HPV negative and cytology negative, return for age-appropriate testing in 3 years is recommended. If all tests are negative at that time, routine screening is recommended. It is recommended that HPV testing in follow-up after colposcopy not be performed at intervals of less than 12 months.
- For women with ASC-US cytology and no HPV result, repeat cytology at 1 year is acceptable. If the result is ASC-US or worse, colposcopy is recommended; if the result is negative, return to cytology testing at 3-year intervals is recommended.
- Endocervical sampling is preferred for women in whom no lesions are identified and for those with an inadequate colposcopy and is acceptable for women with an adequate colposcopy and a lesion identified in the transformation zone.
- Because of the potential for overtreatment, the routine use of diagnostic excisional procedures such as loop electrosurgical excision for women with an initial ASC-US in the absence of HSIL (CIN 2+) is unacceptable.
- The ASCCP management guidelines also address the initial management and follow-up of ASC-US in special populations: women aged 21–24 years, women aged 65 years and older, pregnant women, and postmenopausal women.
- For women with ASC-H cytology, colposcopy is recommended regardless of HPV result. Reflex HPV testing is not recommended.

4.10 Quality Assurance

Monitoring the relative frequency of atypical squamous cells (ASC) and squamous intraepithelial lesions (SIL) interpretations using ASC/SIL ratio and ASC-hrHPV positivity rates are commonly utilized quality assurance measures for cervical cytology [4, 28–30]. Comparison of overall laboratory statistics with benchmarking data collected by laboratory accrediting bodies such as the College of American Pathologists (CAP) can provide information regarding over- or under-use of the ASC category [14, 28, 31]. In addition, monitoring of individual ASC-hrHPV positive rates and ASC/SIL ratios has been shown to be an important quality assurance tool to help fine-tune daily usage by an individual practitioner.

The ALTS trial reported the rate of hrHPV positivity in ASC-US cases adjudicated by experienced pathologists to be 50.6 %; however, in general practice this rate has been found to be lower, generally ranging between 40 and 50 %, most likely due to conservatism and the bias that provides an objective test in equivocal cases [32, 33]. In the USA, the median reported ASC/SIL ratio is 1.5 [5, 32, 34–36]. For laboratories that serve high-risk populations, the ASC/SIL ratio should not exceed 3:1 [37]. A higher ratio suggests over use of ASC; however, over interpretation of both ASC and SIL can keep this ratio within accepted guidelines. Hence, it is important to note that neither the hrHPV+ rate for ASC-US nor the ASC/SIL ratio by themselves is a measure of diagnostic accuracy but is useful in detecting trends related to interpretation thresholds [29]. Correlation of cytology with follow-up biopsy provides another quality assurance tool, but it must be remembered that neither cytology, colposcopy, nor biopsy represents a diagnostic “gold” standard [38–42].

4.11 Sample Reports

Example 1

Adequacy:

Satisfactory for evaluation; transformation zone components identified

Interpretation

Epithelial cell abnormality, squamous:

Atypical squamous cells – undetermined significance (ASC-US)

Comment:

Suggest high-risk HPV testing if clinically warranted (if reflex testing not ordered or if conventional preparation and no co-collection sample was received)

OR

Specimen sent for reflex HPV testing per clinician request.

Example 2

Adequacy:

Satisfactory for evaluation; transformation zone component identified

Interpretation

Epithelial cell abnormality, squamous:

Atypical squamous cells – cannot exclude a high-grade squamous intraepithelial lesion (ASC-H).

Comment:

Suggest colposcopy/biopsy as clinically indicated.

For examples of reporting ASC-US in conjunction with HPV testing, see Chap. 9 on Adjunctive Testing.

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