Epithelial Cell Abnormalities: Squamous

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Michael R. Henry, Donna K. Russell, Ronald D. Luff,
Marianne U. Prey, Thomas C. Wright Jr, and Ritu Nayar

5.1 Epithelial Cell Abnormalities

Squamous Cell

- Squamous Intraepithelial Lesion (SIL)
  - Low-grade squamous intraepithelial lesion (LSIL)
  - High-grade squamous intraepithelial lesion (HSIL)
    - With features suspicious for invasion (*if invasion is suspected*)
- Squamous cell carcinoma

M.R. Henry, MD (✉)
Department of Laboratory Medicine, Mayo Clinic, 200 1st Street SW, Rochester, MN 55905, USA
e-mail: henry.michael@mayo.edu

D.K. Russell, CT(ASCP)HT, MEd
Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, 601 Elmwood Avenue, 626, Rochester, NY 14642, USA
e-mail: donna_russell@urmc.rochester.edu

R.D. Luff, MD, MPH
Anatomic Pathology Division for Clinical Trials, Quest Diagnostics, Teterboro, NJ 07608, USA
e-mail: ronald.d.luff@questdiagnostics.com

M.U. Prey, MD
8829 Ladue Road, Ladue, Missouri 63124, USA
e-mail: marianne@extravirginoo.com

T.C. Wright Jr, MD
Department of Pathology and Cell Biology, Columbia University, 631 W 168th St, New York, NY 10032, USA
e-mail: tcw1@cumc.columbia.edu

R. Nayar, MD
Department of Pathology, Feinberg School of Medicine, Northwestern University, Northwestern Memorial Hospital, 251 East Huron Street, Galter Pavilion, 7-132B, Chicago, IL 60611, USA
e-mail: r-nayar@northwestern.edu

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5.2 Background

Squamous abnormalities encompass the spectrum of noninvasive cervical epithelial abnormalities associated with human papillomavirus (HPV), ranging from the cellular changes that are associated with transient HPV infection to those representing high-grade precursors, to invasive squamous cell carcinoma. It has now been well established that HPV is the main causal factor in the pathogenesis of virtually all cervical cancer precursors and invasive cancers [1]. The majority of invasive cervical cancers and their precursors contain HPV types referred to as "high-risk" HPVs (hrHPV), the most common being HPV 16 [2]. Our understanding of preinvasive HPV-associated squamous lesions supports only two conceptual divisions: HPV infection and true precancer. Transient infections generally regress over the course of 1–2 years [3, 4], and lesions with HPV persistence are associated with an increased risk of developing a cancer precursor (precancer) or invasive cancer [5–7]. This concept led to the introduction of the two-tiered nomenclature of low-grade squamous intraepithelial lesion (LSIL) and high-grade squamous intraepithelial lesion (HSIL), by the Bethesda System (TBS) in 1988.

In 2012, the Lower Anogenital Squamous Terminology Standardization Consensus Conference (LAST) adopted a two-tiered nomenclature, mirroring the Bethesda SIL classification, for the histologic diagnoses of HPV-associated squamous lesions of the lower anogenital tract [8]. Similarly, the 2014 WHO histopathology terminology for squamous cell precursors also advocated the use of a two-tiered classification system [9]. The basis of these recommendations was the fact that HPV-related lesions of the lower anogenital, both mucosal and cutaneous, have similar biology and accompanying risks for development of invasive carcinoma and should be managed similarly. In TBS for cytology and LAST/WHO for histopathology, LSIL encompasses the cellular changes associated with the older terms of koilocytosis, mild dysplasia, and CIN 1, while HSIL encompasses the more clinically significant lesions previously termed moderate and severe dysplasia, CIN 2, CIN 3, and carcinoma in situ.

At the 1988 Bethesda workshop, when the spectrum of SIL was subdivided into two categories, there were two main considerations. First was the desire to use morphologic categories that relate to the biology and clinical management of HPV-associated lesions as outlined above, and second was the acknowledged low inter- and intraobserver reproducibility with three- and four-grade classification systems [10, 11]. Then and since, it has been argued that a two-tiered system provides less information to clinicians than a three-tiered CIN terminology [12]. However, the cytologic distinction of CIN 2 and CIN 3 is poorly reproducible, and combining the cytologic correlates of biopsy-confirmed CIN 2 and CIN 3 into a single HSIL category was shown, in the ASCUS-LSIL Triage Study (ALTS), to have improved reproducibility (M. Schiffman, personal communication). Another concern voiced about the two-tiered classification is that the dividing line between low-grade and high-grade precursors should be set between CIN 2 and CIN 3 because the natural history of untreated CIN 2 is closer to that of CIN 1 than it is to CIN 3 [13]. In some European countries, CIN 1 and CIN 2 are grouped together for treatment purposes [12].

However, as a screening test, cervical cytology must emphasize sensitivity. Given the variability in the interpretation and biologic behavior of "cytologic CIN 2" [14], setting the cytologic threshold for low-grade and high-grade lesions between CIN 1 and CIN 2 is still considered appropriate. This cut point also demonstrated the best interobserver reproducibility using a dichotomous positive/negative result, based on data from ALTS (M. Schiffman, personal communication).

Even with only two categories of SIL, there is an overall 10–15 % inter-pathologist discrepancy rate between LSIL and HSIL interpretations on cervical cytology slides [15]. Cytology may also be discrepant with histology; 15–25 % of women with LSIL cytology are found to have histologic HSIL (CIN 2/CIN 3) upon further evaluation [16]. Benchmark data obtained from the College of American Pathologists (CAP) show that in 2006 the median rate for LSIL was 2.5 % for all preparation types and 2.9 % for liquid-based preparations. The median rate for HSIL was 0.5 % for all preparations types [17]. As of 2013, these rates have shown only minimal change.

The Bethesda System for reporting cervical cytology has been widely implemented, and current consensus management guidelines in the United States utilize the two-tiered LSIL/HSIL nomenclature to make clinical decisions regarding follow-up of abnormal cervical cytology test results [18]. There has been a shift in recent years with regard to the management of low-grade lesions especially in young women based on the recognition that most LSIL (CIN 1) represent a self-limited HPV infection [19]. The current emphasis of cervical cancer screening is therefore focused on detection and treatment of biopsy-confirmed high-grade disease [18].

Thus, the 2014 Bethesda update maintains the two-tiered reporting terminology of LSIL/HSIL.

5.3 Low-Grade Squamous Intraepithelial Lesion (LSIL) (Figs. 5.1–5.13)

Squamous cell changes associated with HPV infection encompass "mild dysplasia" and "CIN 1." Several studies have demonstrated that the morphologic criteria for distinguishing "koilocytosis" from mild dysplasia or CIN I vary among investigators and lack clinical significance. In addition, both lesions share similar HPV types, and their biologic behavior and clinical management are similar, thus supporting a common designation of LSIL [20–22].

5.3.1 Criteria

Cells occur singly, in clusters, and in sheets.

Cytologic changes are usually confined to squamous cells with "mature" intermediate or superficial squamous cell-type cytoplasm.

Overall cell size is large, with fairly abundant "mature" well-defined cytoplasm.

Nuclear enlargement more than three times the area of normal intermediate nuclei results in a low but slightly increased nuclear to cytoplasmic ratio (Fig. 5.1).

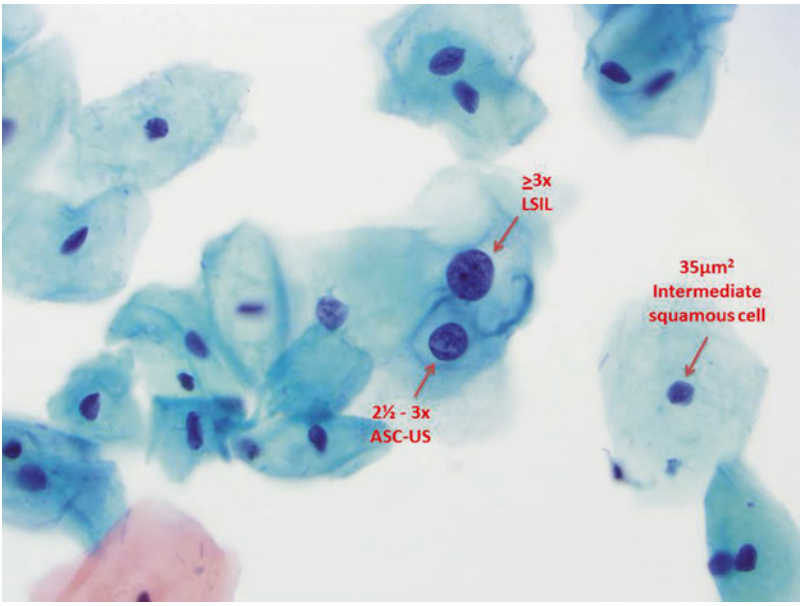

Fig. 5.1 Nuclear area (*LBP*, *ThinPrep*). The nuclear area of an intermediate squamous cell is approximately 35 µm^2. This is used as a reference to measure abnormal squamous cells such as ASC-US (approximately 100 µm^2) and LSIL (approximately 150–175 µm^2)

Nuclei are generally hyperchromatic but may be normochromatic.
Nuclei show variable size (anisonucleosis).
Chromatin is uniformly distributed and ranges from coarsely granular to smudgy or densely opaque (Fig. 5.2).
Contour of nuclear membranes is variable ranging from smooth to very irregular with notches (Fig. 5.2).
Binucleation and multinucleation are common (Fig. 5.3).
Nucleoli are generally absent or inconspicuous if present.
Koilocytosis or perinuclear cavitation consisting of a broad, sharply delineated clear perinuclear zone and a peripheral rim of densely stained cytoplasm is a characteristic viral cytopathic feature but is not required for the interpretation of LSIL (Figs. 5.4 and 5.6).
Cells may show increased keratinization with dense, eosinophilic cytoplasm with little or no evidence of koilocytosis.
Cells with koilocytosis or dense orangeophilia must also show nuclear abnormalities to be diagnostic of LSIL (Figs. 5.4–5.6); perinuclear halos or clearing in the absence of nuclear abnormalities does not qualify for the interpretation of LSIL (Fig. 5.7; see Fig. 2.36).

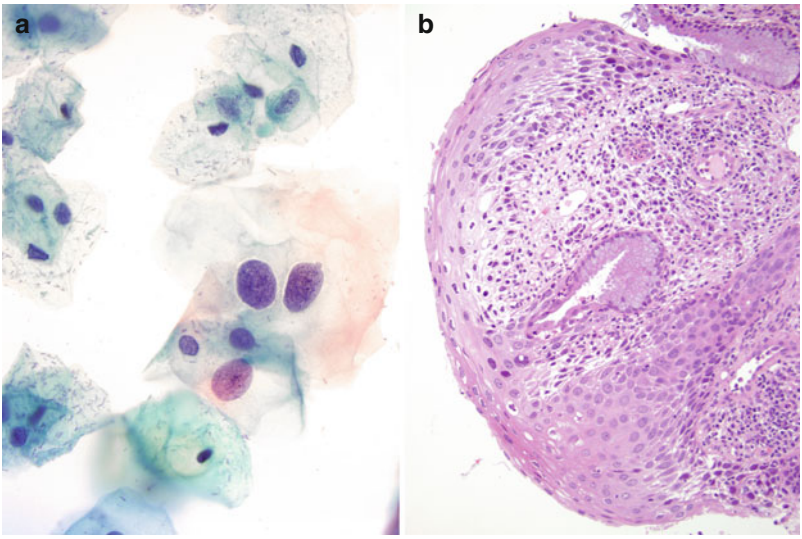

Fig. 5.2 Low-grade squamous intraepithelial lesion (LSIL) (**a**, *left*: *LBP*, *ThinPrep* and **b**, *right* cervix, H&E stain). Nuclear enlargement and hyperchromasia are of sufficient degree for the interpretation of LSIL (**a** & **b**). HPV-associated cytoplasmic changes are not a prerequisite for LSIL

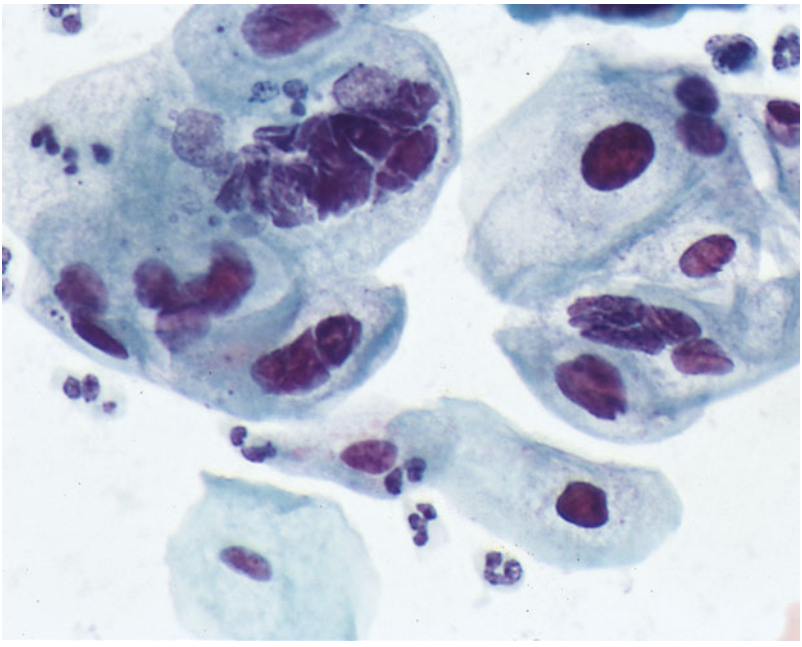

Fig. 5.3 LSIL (*LBP*, *ThinPrep*). A 32-year-old woman, day 15, routine cervical cytology screening. Note the overall large cell size, "smudged" nuclear chromatin, well-defined cytoplasm, and multinucleation

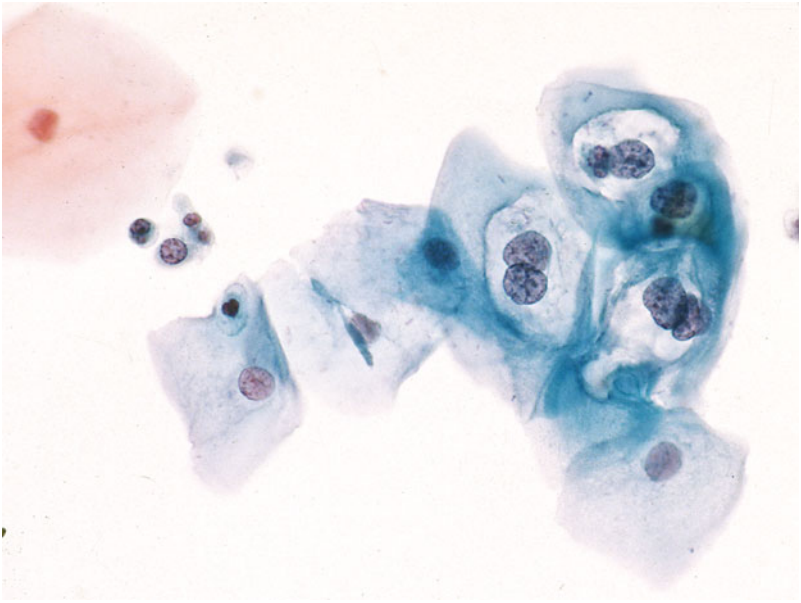

Fig. 5.4 LSIL (*LBP*, *ThinPrep*). Routine screen from a 32-year-old woman. Nuclear abnormalities are required to make an interpretation of LSIL. HPV cytopathic effect manifested by perinuclear cavitation often accompanies the nuclear abnormalities but is not required for an interpretation of LSIL

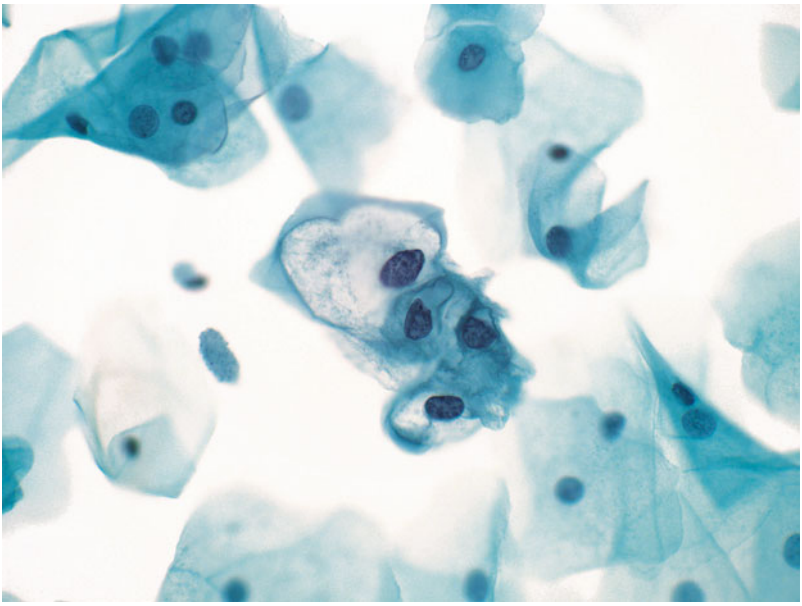

Fig. 5.5 LSIL (*LBP*, *SurePath*). Cells with diagnostic koilocytic features of LSIL have a sharply defined perinuclear cavity, condensation of cytoplasm around the periphery, and abnormal nuclear features including enlargement and nuclear membrane irregularity. In liquid-based samples, nuclear hyperchromasia may be less evident

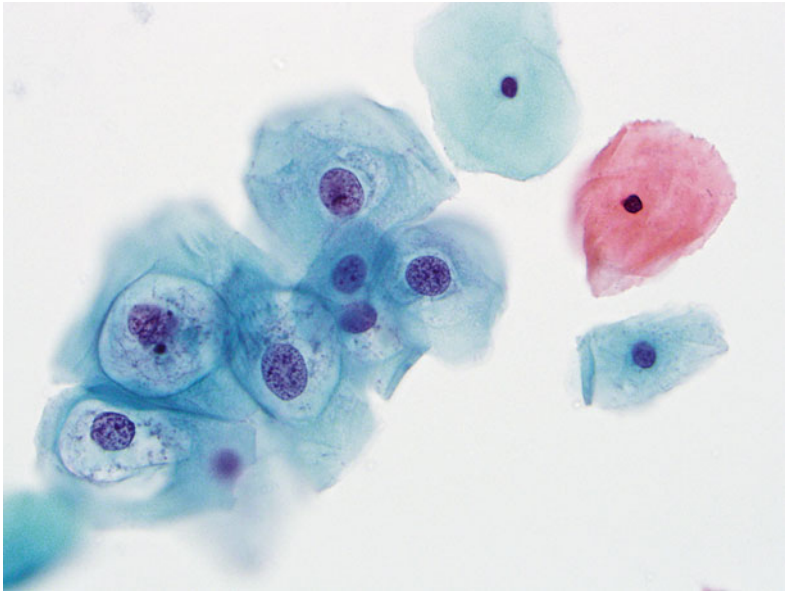

Fig. 5.6 LSIL (*LBP*, *ThinPrep*). A 28-year-old woman with a history of ASC-US and positive hrHPV testing. LSIL on cytology is characterized by mature squamous cells with enlarged nuclei with variable chromatin and nuclear membranes. Koilocytosis or perinuclear cavitation in the cytoplasm, a characteristic of HPV cytopathic effect is present, however it is not required for an interpretation of LSIL

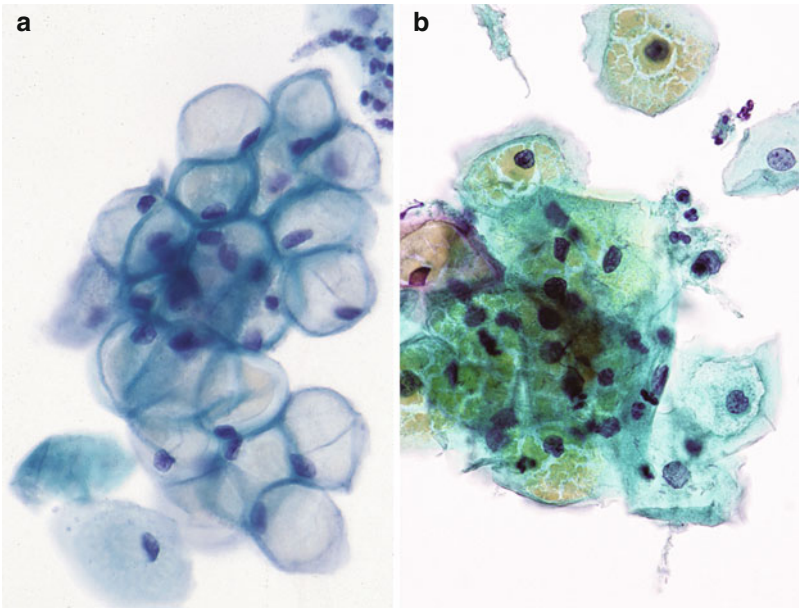

Fig. 5.7 Pseudokoilocytes (*LBP*, *ThinPrep*). Glycogen in squamous cells can give the appearance of "pseudokoilocytosis" (**a**). The halos associated with glycogen often have a yellow refractile appearance (**b**). The nuclear abnormalities required for an interpretation of LSIL are absent. Follow-up in both cases was NILM

Preparation-Specific Criteria

In LSIL, there are minimal differences between conventional preparations and liquid-based preparations.

The nuclei may show less hyperchromasia on LBPs, but overall the morphology of the cells is the same as in conventional preparations.

5.4 Problematic Patterns in LSIL

An interpretation of LSIL should be based on strict criteria to avoid unnecessary follow-up of women for nonspecific morphologic changes. By and large, the interobserver reproducibility of LSIL on cytology is far greater than LSIL (CIN 1) on histology [23]. A few pitfalls and gray areas should be kept in mind.

5.4.1 Keratinized Squamous Cells (Fig. 5.8)

Parakeratosis, as represented by miniature squamous cells with round to oval small, pyknotic nuclei and low nuclear to cytoplasmic ratios, is by itself not an

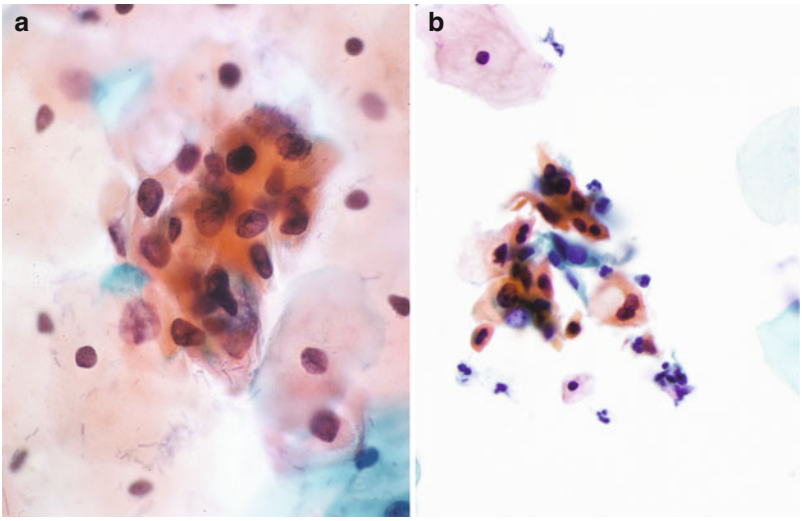

Fig. 5.8 ASC-US versus LSIL (**a** *left CP*, **b** *Right LBP*, *ThinPrep*). Atypical squamous cells with orangeophilic cytoplasm ("atypical parakeratosis"). These cells have some features of SIL; however, such keratinized lesions may be difficult to grade. hrHPV triage is helpful in determining follow-up

HPV-related entity (see Chap. 2). However, parakeratosis may be found as a background pattern in HPV-associated lesions and as such should elicit a careful search for classic HPV-related cytologic changes (see Figs. 2.15 and 2.16). Keratinized cells showing nuclear abnormalities and low N/C ratios should be categorized as "atypical squamous cells–undetermined significance" (ASC-US) (see Figs. 4.15 and 4.16) or higher, based on the degree of nuclear abnormality (Figs. 5.8 and 5.9).

5.4.2 Borderline Changes (Figs. 5.9–5.11)

Specimens with borderline nuclear changes that fall short of a definitive LSIL interpretation may be categorized as "atypical squamous cells–undetermined significance" (ASC-US) (Figs. 5.9–5.11).

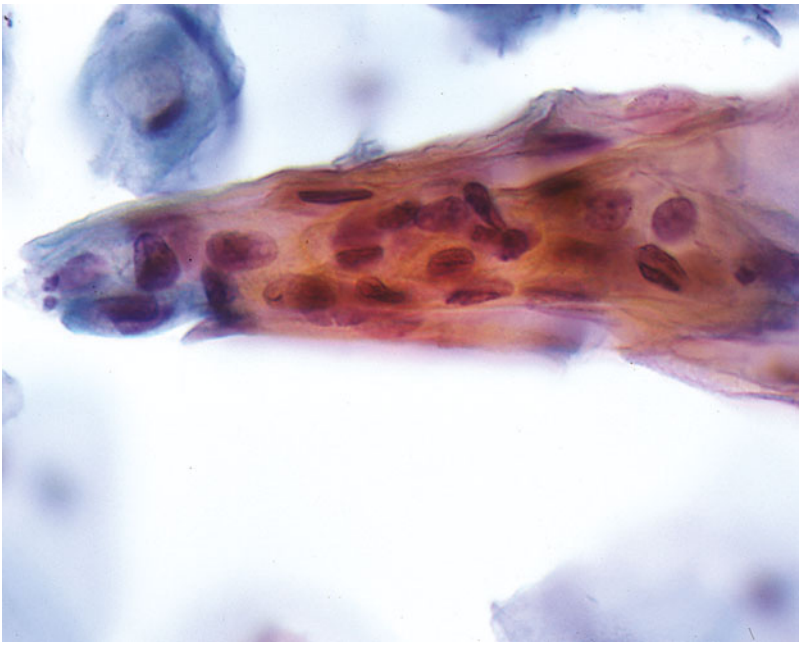

Fig. 5.9 ASC-US versus LSIL (*LBP*, *ThinPrep*). A 32-year-old woman. Clusters of squamous cells may be seen in "spikelike" aggregates; such clusters should be classified based on the degree of nuclear abnormalities. This patient had an LSIL interpretation on a conventional smear 2 months before this cytology which was interpreted as ASC-US. hrHPV test was positive

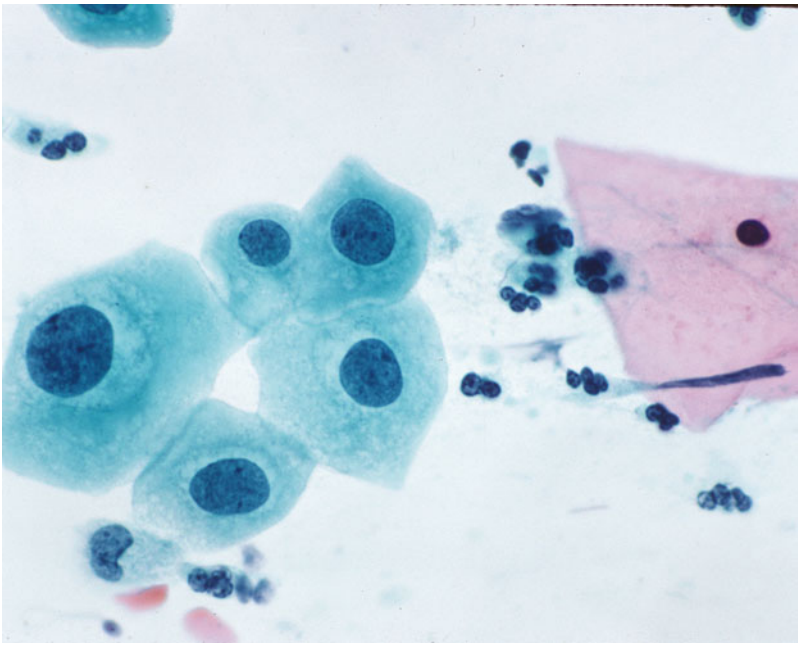

Fig. 5.10 ASC-US versus LSIL (*CP*). Nuclear features are borderline between those required for ASC-US and LSIL. Cases such as this will no doubt have poor interobserver reproducibility as demonstrated in various studies including the Bethesda 2001 BIRST project

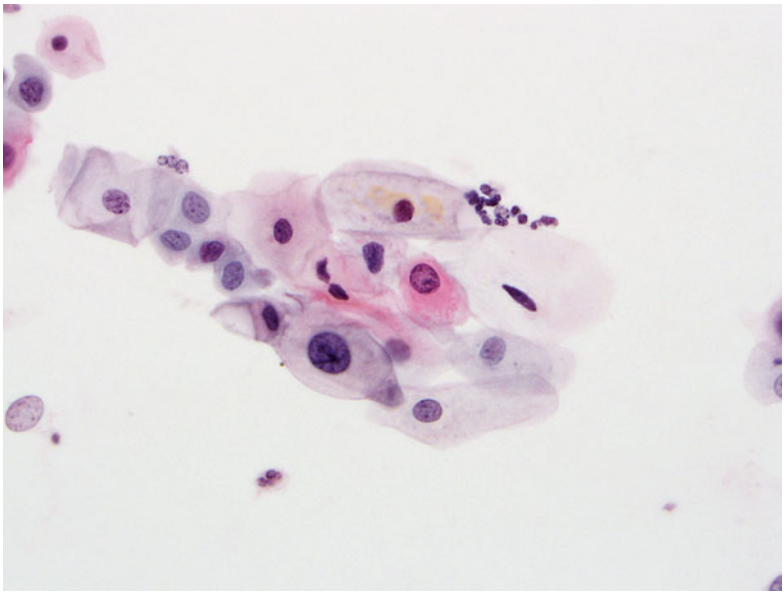

Fig. 5.11 ASC-US versus LSIL (*LBP*, *ThinPrep*). Abnormal nuclear enlargement without concomitant HPV cytopathic change is identified in this Pap test from a 32-year-old woman. The hallmark of LSIL is an enlarged nucleus, often as much as four to six times the area of a normal intermediate cell nucleus. The N/C ratio is low and hyperchromasia varies, especially in liquid-based preparations

5.5 Mimics of LSIL

5.5.1 Pseudokoilocytosis (Fig. 5.7)

Cytoplasmic perinuclear clearing without accompanying atypical nuclear features should not be considered as LSIL (Fig. 5.7a). Small indistinct perinuclear halos are often seen in *Trichomonas* infections or in other reactive processes (see Figs. 2.36 and 2.52). Cytoplasmic vacuolization due to glycogen often takes on a yellow refractile, "cracked" appearance (Fig. 5.7b).

5.5.2 Herpes Cytopathic Effect (Fig. 5.12)

Classical herpes cytopathic effect, with multinucleated cells showing nuclear molding, margination of chromatin, and clear, ground glass nuclei, does not typically pose a differential diagnostic problem in comparison to LSIL. However, early herpes cytopathic effect may lack diagnostic nuclear features. Given the nuclear enlargement and degenerative chromatin, which may be hyperchromatic, such cases may be mistaken for LSIL (Fig. 5.12b). These cells lack the other changes of HPV cytopathic effect such as koilocytosis, and often other cells in the preparation will show more classic diagnostic changes of herpes. Occasionally, herpetic changes may also mimic HSIL (Fig. 5.12a).

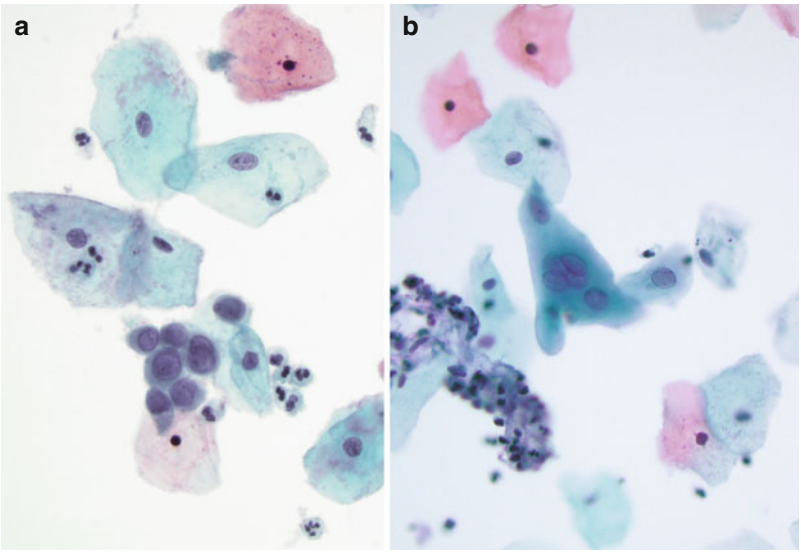

Fig. 5.12 Herpes (*LBP*, *ThinPrep*). Routine cervical cytology. A 25-year-old woman. Endocervical cell (**a**) and intermediate cells (**b**) showing herpes virus cytopathic effect with clearing of chromatin. These cells can be mistaken for ASC-US or LSIL (**b**) or occasionally HSIL (**a**) when obvious nuclear changes associated with herpes virus infection are not seen. Looking elsewhere on the same slide will usually clarify that the changes are due to herpes cytopathic effect

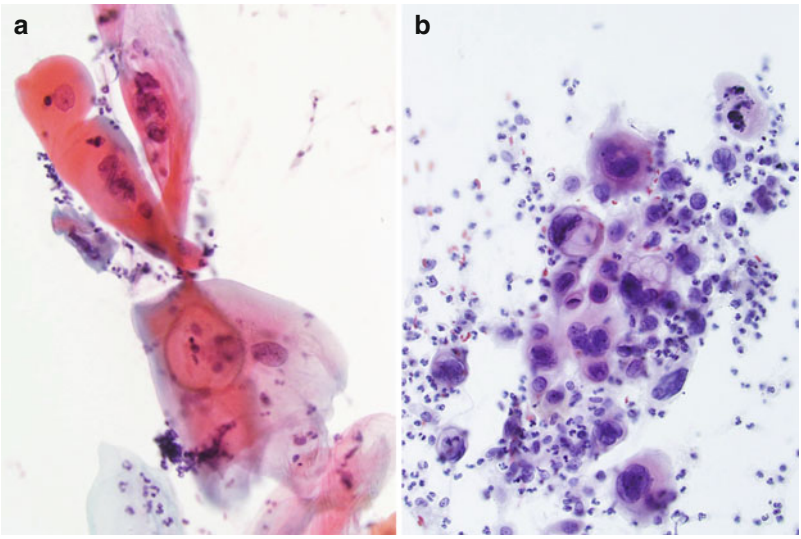

Fig. 5.13 Radiation change versus squamous cell carcinoma (*CP*). (**a**) A 61-year-old woman with a history of squamous cell carcinoma and radiation. Mature squamous cell showing cytomegaly, low N/C ratios, intracytoplasmic vacuoles with neutrophils. The mild enlargement of the nucleus should not be mistaken for LSIL. (**b**) Patients radiated for squamous cell carcinoma may also show tumor cells with radiation effect. These changes should be distinguished from radiation changes in benign cells (**a**)

5.5.3 Radiation Changes (Fig. 5.13)

Cells showing the effects of ionizing radiation have a low nuclear to cytoplasmic ratio with large nuclei which are often the same size as those seen in LSIL. The cytoplasm of these cells is usually quite distinctive with a two-toned, vacuolated appearance that lacks the perinuclear clearing and peripheral condensation present in a typical koilocyte (Fig. 5.13a; see Fig. 2.43). Patients radiated for squamous cell carcinoma may also show tumor cells with radiation effect (Fig. 5.13b), and these changes should be distinguished from radiation changes in benign cells.

5.6 Management of LSIL

In the data from the ASCUS-LSIL Triage Study (ALTS), hrHPV types were detected in 85 % of LSIL cases, with the conclusion being that HPV testing is not a useful triage strategy for cytologic LSIL, particularly in young women because of the high

prevalence of HPV infection in this age group [24]. On the contrary, reflex HPV testing is acceptable for LSIL in postmenopausal women due to higher specificity in this population.

With the advent of HPV co-testing in women over the age of 30, many women with an interpretation of LSIL will have concurrent HPV testing. Thus, the 2012 ASCCP management guidelines recommend that women under the age of 25 with a cytologic interpretation of LSIL be followed up with cytology at 12 months. Women 25 years and older can be cotested in 3 years if they are HPV negative, but colposcopic examination is recommended if HPV positive. Women of unknown HPV status should have a repeat cytology in 12 months [18].

5.7 High-Grade Squamous Intraepithelial Lesion (HSIL) (Figs. 5.14–5.48)

5.7.1 Criteria

The cells of HSIL are smaller and show less cytoplasmic maturity than cells of LSIL (Fig. 5.14).

Cells occur singly, in sheets, or in syncytial-like aggregates (Figs. 5.15 and 5.16).

Syncytial aggregates of dysplastic cells may result in hyperchromatic crowded groups. (HCG) of immature cells which should always be carefully assessed for nuclear abnormalities (Fig. 5.15, 5.16, and 5.17).

While overall cell size is variable, in general, the cells of HSIL are smaller than those of LSIL. Higher-grade lesions often contain quite small basal-type cells (Figs. 5.28, 5.40, and 5.45).

Degree of nuclear enlargement is more variable than that seen in LSIL. Some HSIL cells have the same degree of nuclear enlargement as in LSIL, but the cytoplasmic area is decreased, leading to a marked increase in the nuclear to cytoplasmic ratio (Figs. 5.18 and 5.19). Other cells have very high nuclear/cytoplasmic ratios, but the actual size of the nuclei may be considerably smaller than that of LSIL, at times even as small as a normal intermediate cell nucleus (Fig. 5.21).

Nuclear to cytoplasmic ratio is higher in HSIL compared to LSIL.

Nuclei are generally hyperchromatic but may be normochromatic or even hypochromatic (Fig. 5.22).

Chromatin may be fine or coarsely granular and is evenly distributed.

Contour of the nuclear membrane is quite irregular and frequently demonstrates prominent indentations (Figs. 5.20 and 5.23) or grooves (Fig. 5.24).

Nucleoli are generally absent, but may occasionally be seen, particularly when HSIL extends into endocervical gland spaces or in the background of reactive or reparative change (Fig. 5.25).

Appearance of the cytoplasm is variable; it can appear “immature,” lacy, and delicate (Fig. 5.19) or densely metaplastic (Fig. 5.20); occasionally, the cytoplasm is “mature” and densely keratinized (keratinizing HSIL) (Figs. 5.26 and 5.43).

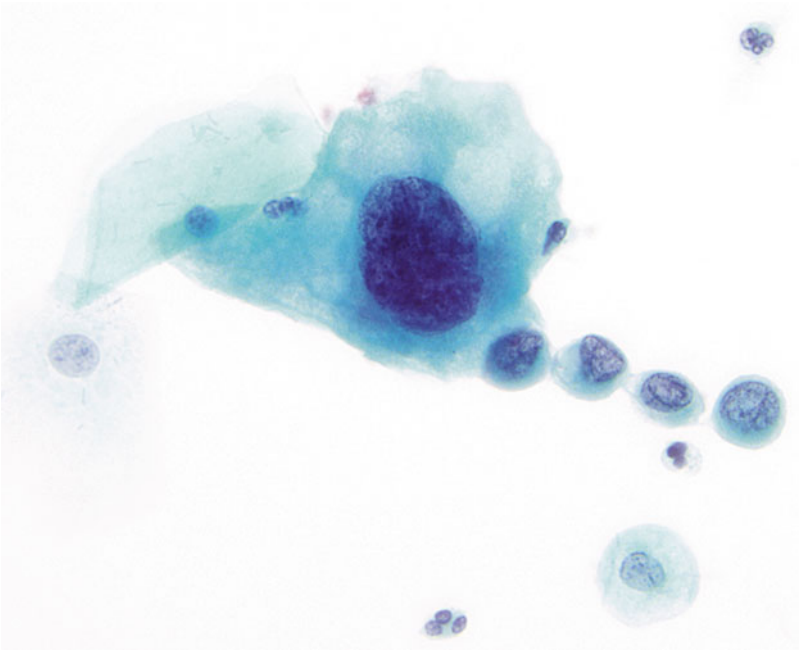

Fig. 5.14 High-grade squamous intraepithelial lesion (HSIL) (*LBP*, *ThinPrep*). There is a mixture of dysplastic cells here, one large LSIL cell, and four adjacent, small, high N/C ratio cells with nuclear features consistent with HSIL

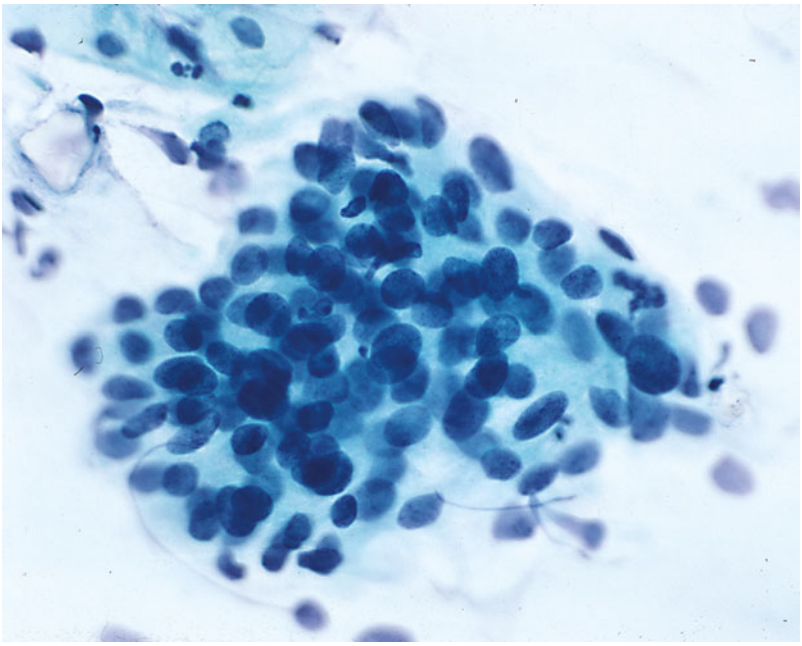

Fig. 5.15 High-grade squamous intraepithelial lesion (HSIL) (*CP*). The dysplastic cells are seen here in a syncytial cluster or hyperchromatic crowded group

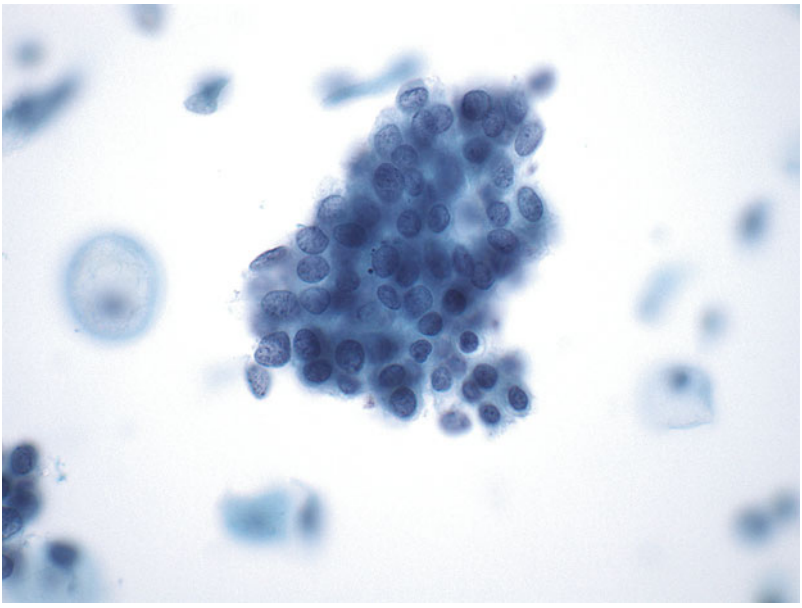

Fig. 5.16 HSIL-syncytial cluster (*LBP*, *SurePath*). As in conventional smears, crowded hyperchromatic cell groups should be examined with care. If a squamous abnormality is suspected, a thorough search for single dysplastic cells in the background is warranted. Follow-up showed HSIL (CIN 3) with endocervical gland involvement

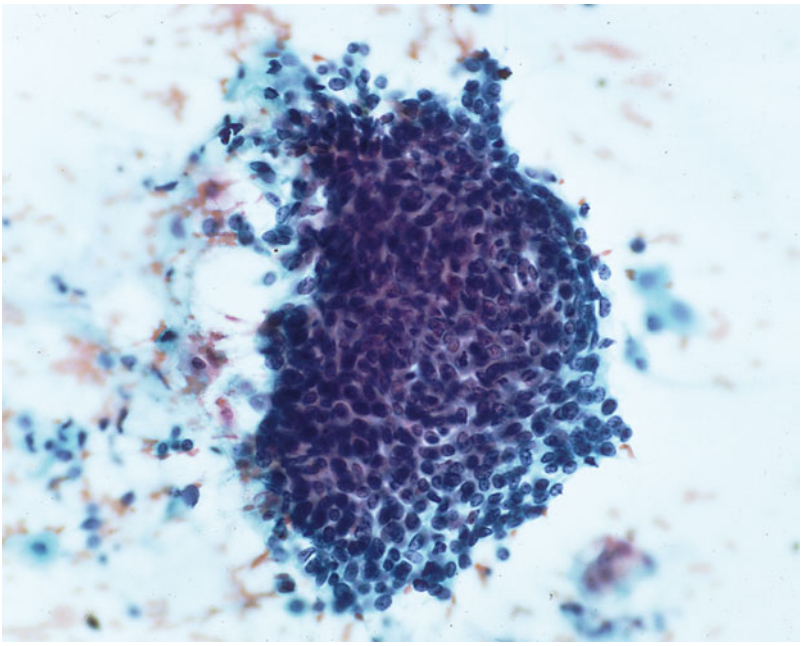

Fig. 5.17 HSIL (*CP*). A 58-year-old postmenopausal woman on hormone replacement therapy. Hyperchromatic crowded groups seen at low power require careful examination at higher magnification. Flattening at the edge of the cell cluster and whorling in the center are suggestive of HSIL over a glandular abnormality. Follow-up showed HSIL (CIN 3) with endocervical gland involvement

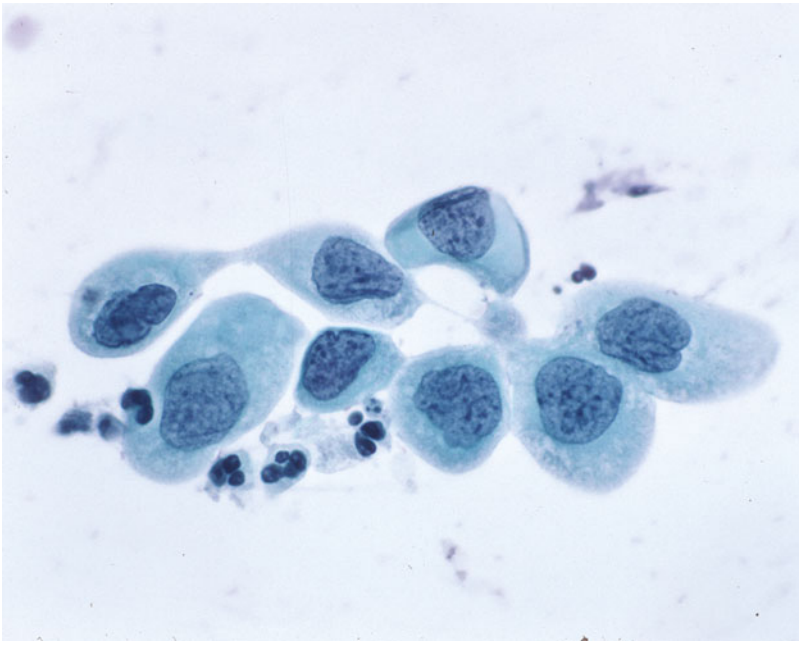

Fig. 5.18 HSIL (*CP*). Nuclear changes are HSIL; however, the nuclear/cytoplasmic (N/C) ratio is on the low end for HSIL

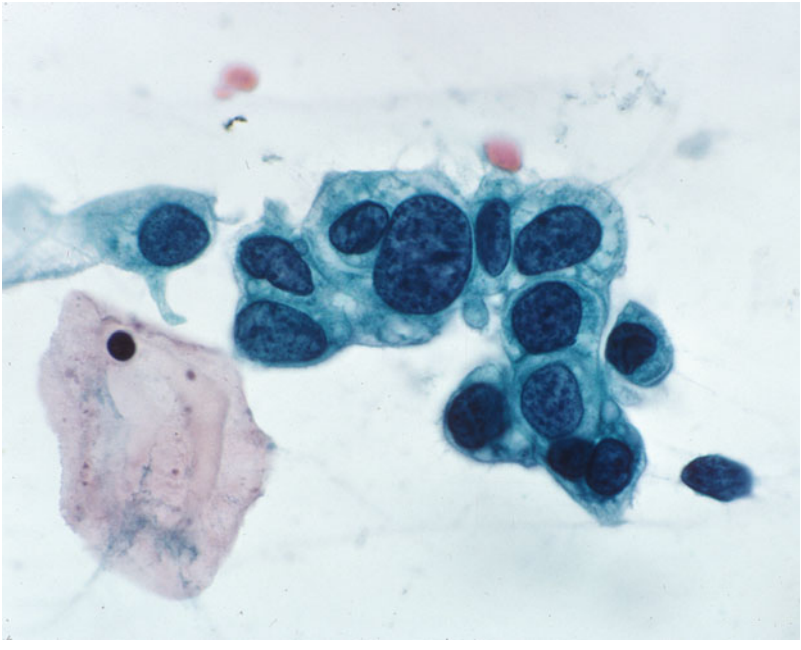

Fig. 5.19 HSIL (*CP*). There is variation in nuclear size and shape, and the cells have delicate cytoplasm

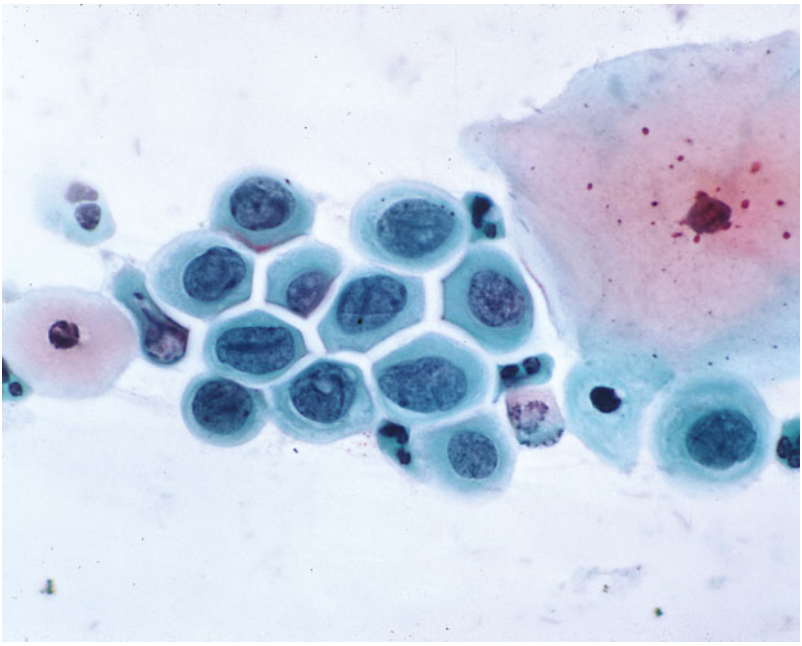

Fig. 5.20 HSIL (*CP*). HSIL with "metaplastic" or dense cytoplasm, in contrast to that seen in the syncytial groups of HSIL (Fig. 5.19)

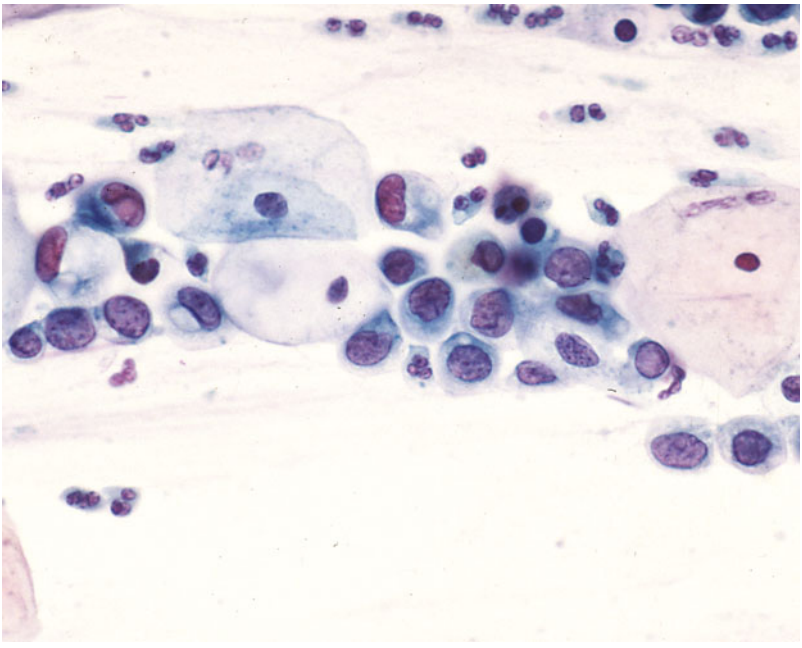

Fig. 5.21 HSIL (*CP*). HSIL cells with some variation in cell size and N/C ratios. A cluster such as this may be misinterpreted as squamous metaplastic cells if examined only under lower magnification. Follow-up showed HSIL (CIN 3)

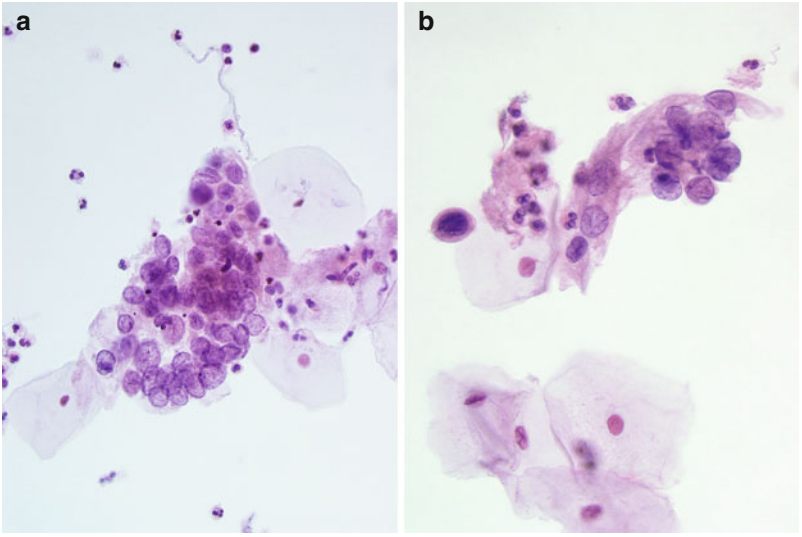

Fig. 5.22 HSIL (**a**, **b** *LBP*, *ThinPrep*). HSIL that is markedly hypochromatic. A diligent search may reveal more classic cells elsewhere on the same slide. (**a**) On the *left* side, note syncytial arrangement and nuclear grooves. (**b**) On the *right* side, abnormal naked nuclei and a hyperchromatic, high N/C ratio single HSIL cell are seen

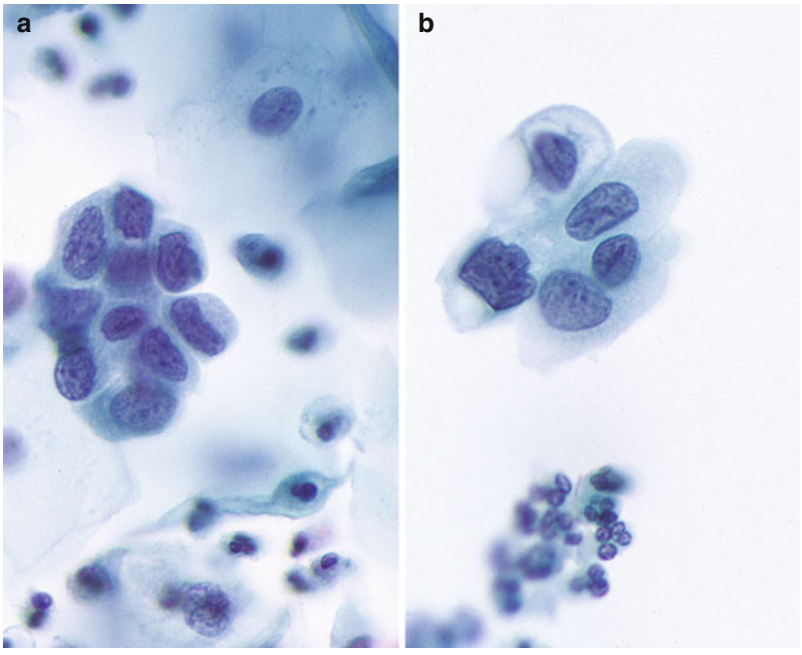

Fig. 5.23 HSIL (**a**, **b** *LBP*, *SurePath*). Note the nuclear envelope irregularities and abnormal chromatin. As seen here in *LBPs*, hyperchromasia may not be as prominent as in conventional smears

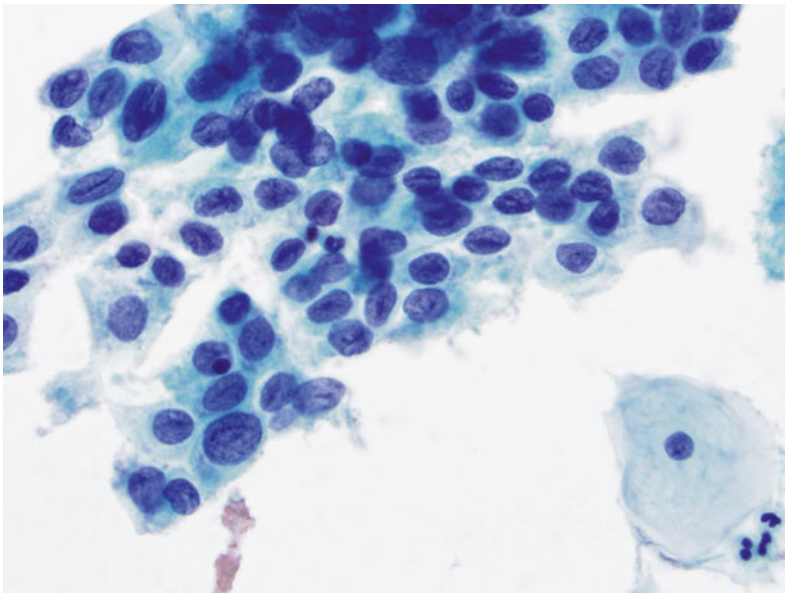

Fig. 5.24 HSIL (*LBP*, *ThinPrep*). Cells showing variably sized, ovoid nuclei with prominent nuclear grooves. In this case, the chromatin is not particularly hyperchromatic, and cytoplasm has ill-defined borders

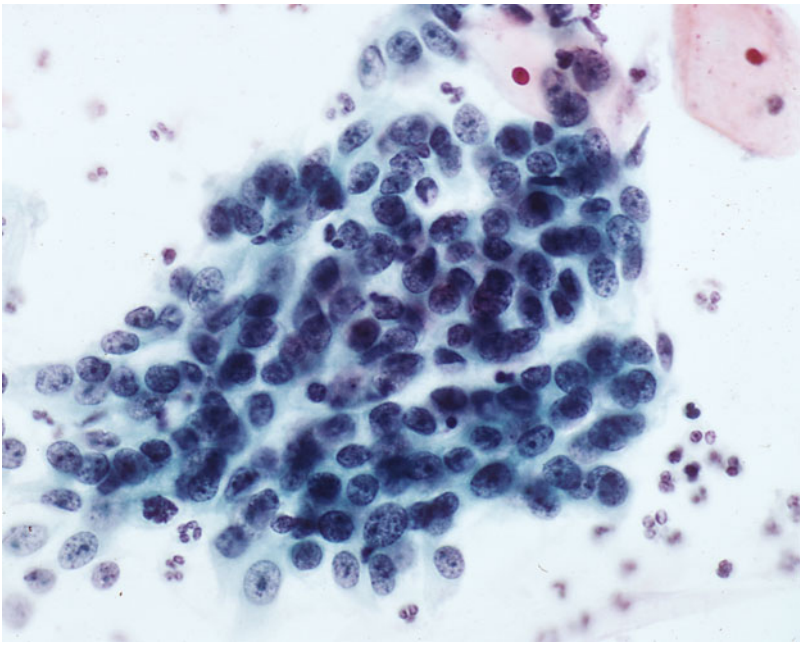

Fig. 5.25 HSIL (*CP*). A 42-year-old woman. Although uncommon, nucleoli may be seen in HSIL, especially with extension into endocervical gland spaces. The chromatin may appear less coarsely granular

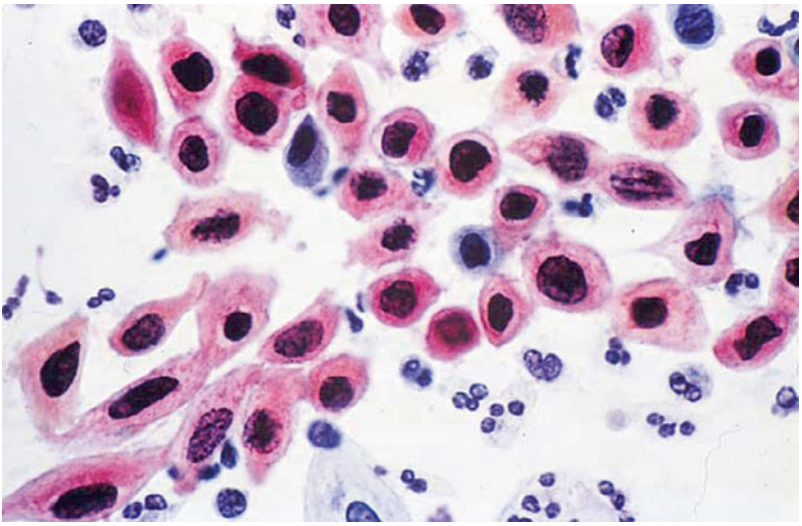

Fig. 5.26 HSIL-keratinizing lesion (*CP*). The criteria of nuclear to cytoplasmic ratio and degree of nuclear abnormalities used for grading SIL may be more difficult to apply to keratinizing lesions. The extent of abnormality here qualifies for an interpretation of HSIL (contrast with Figs. 5.8 and 5.9)

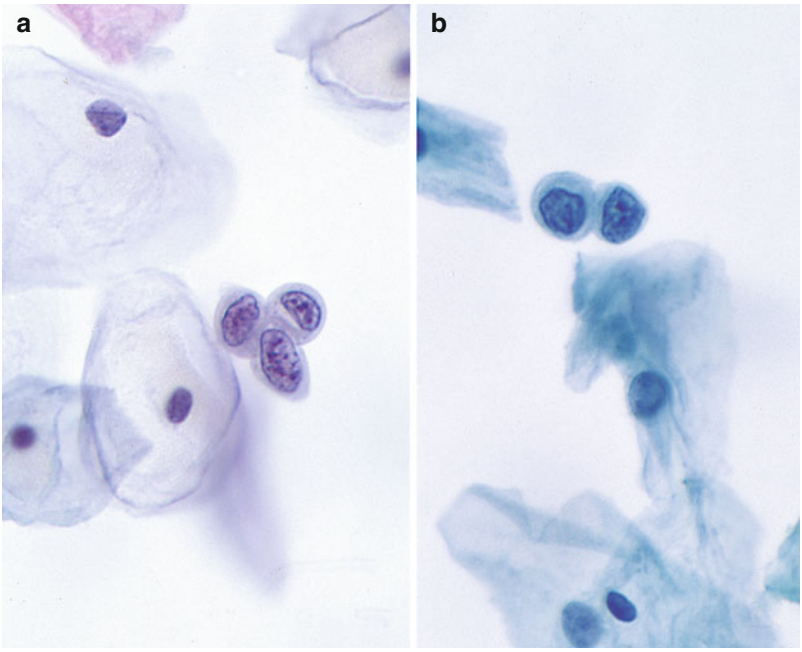

Fig. 5.27 HSIL (**a, b**: *LBP*, *ThinPrep*). A 29-year-old woman from a high-risk clinic. Close attention to isolated cells is required when screening *LBPs* because the abnormal isolated cells may not be as apparent as clusters of HSIL cells and may lie between benign cell clusters or in "empty spaces" on the preparation. When the criteria for HSIL are met, such cells should be interpreted as HSIL and not ASC-H. Both images (**a** and **b**) demonstrate such cells. Follow-up showed HSIL (CIN 3)

Preparation-Specific Criteria

Liquid-Based Preparations:

Dispersed abnormal single cells are seen more often than sheets and syncytial aggregates, and isolated cells may be present in the empty spaces between cell clusters (Figs. 5.27 and 5.28).

Relatively fewer abnormal cells may be present.

Cells may be quite small and can be mistaken for histiocytes or endometrial cells.

Nuclei may be normochromatic or even hypochromatic, but other cytologic features of HSIL (high nuclear to cytoplasmic ratio and irregular nuclear membrane) are present [25] (Figs. 5.22 and 5.23).

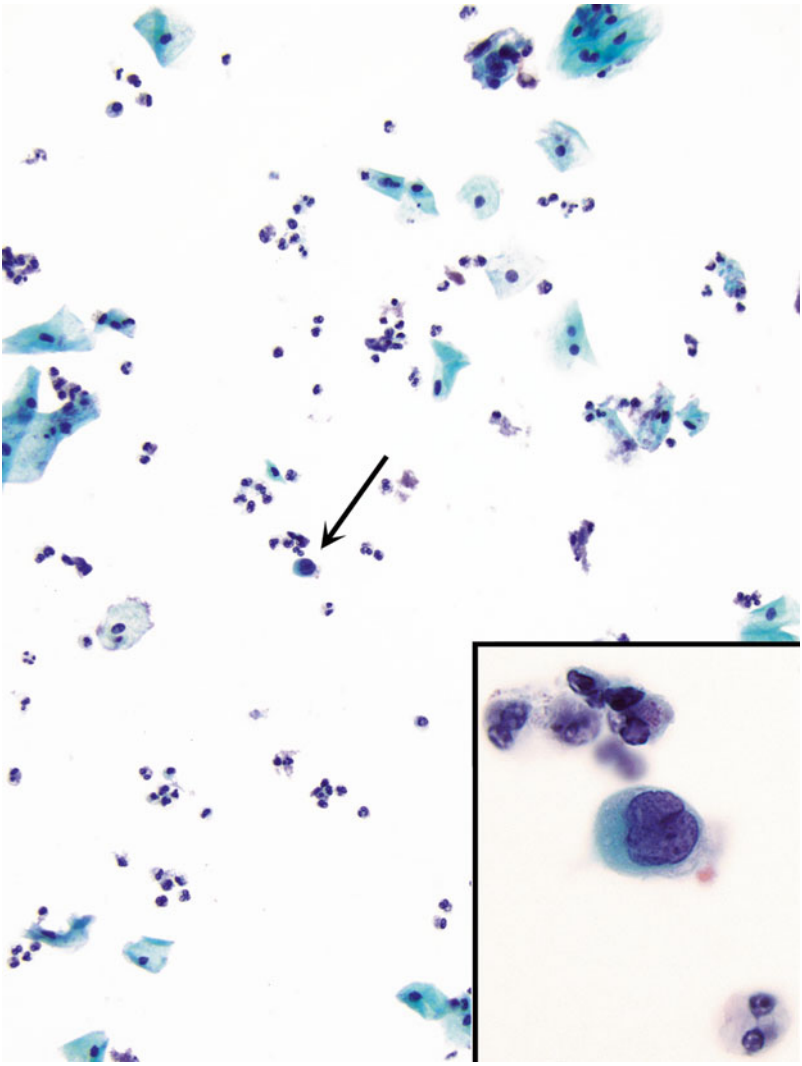

Fig. 5.28 HSIL (*LBP*, *ThinPrep*). Isolated single abnormal cells (*arrow*) are more often seen in *LBPs*. These small cells may be seen in the spaces between cells as seen here and may be easily missed on screening. The inset magnifies the cell indicated by the *arrow*, which shows abnormal features including a large hyperchromatic nucleus with irregular nuclear membranes and increased N/C ratio

5.8 Problematic Patterns in HSIL

5.8.1 Syncytial Aggregates/Hyperchromatic Crowded Groups (Figs 5.15–5.17 and 5.29)

Cellular aggregates of high-grade squamous lesions in conventional smears often have a syncytial-like appearance with no visually discernable cytoplasmic borders between the cells and loss of nuclear polarity within the groups. Specimens collected using modern sampling devices and prepared using liquid-based methodologies often demonstrate tight clusters which appear to be hyperchromatic due to a three-dimensional arrangement of cells showing scant cytoplasm and variable chromasia of the nuclei. These clusters should be closely examined for the presence of abnormal features which justify an interpretation of HSIL [26].

The cytomorphologic features of HSIL include significant anisonucleosis, coarsely granular chromatin, irregular nuclear membranes, and increased nuclear to cytoplasmic ratios. The presence of mitoses within these clusters is also suggestive of an epithelial abnormality. While the center of such clusters is often difficult to evaluate due to the dense and dark nature of these groups, close examination of the periphery of the cluster will usually allow for better evaluation of the cells.

The differential diagnosis for syncytial groups includes a variety of benign entities such as immature squamous metaplasia, atrophy, and benign endocervical or endometrial cells. If the cells are abnormal squamous cells, but not diagnostic of HSIL, the appropriate interpretation would be ASC-H. If the cells are abnormal but with glandular features, the differential considerations would include endocervical adenocarcinoma in situ or endocervical or endometrial adenocarcinoma. Flattening at the edges of the cell cluster, whorling of cells in the center, and lack of glandular architectural features (feathering, rosettes, and pseudostratified strips) favor HSIL over a glandular abnormality (see Table 6.1 for differential diagnosis of HSIL and AIS) (Figs. 5.15–5.17, 5.29–5.30).

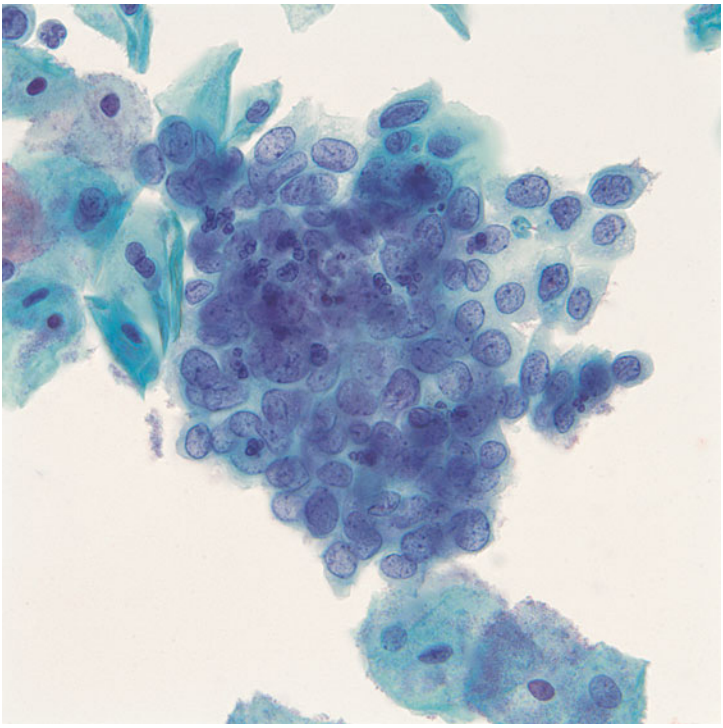

Fig. 5.29 HSIL (*LBP*, *ThinPrep*). A 32-year-old woman with a history of abnormal Pap tests and positive hrHPV testing. A syncytial cluster of cells with overlapping of hypochromatic nuclei are seen. The nuclei are often less hyperchromatic in liquid-based preparations. Follow-up cone biopsy revealed HSIL (CIN 3)

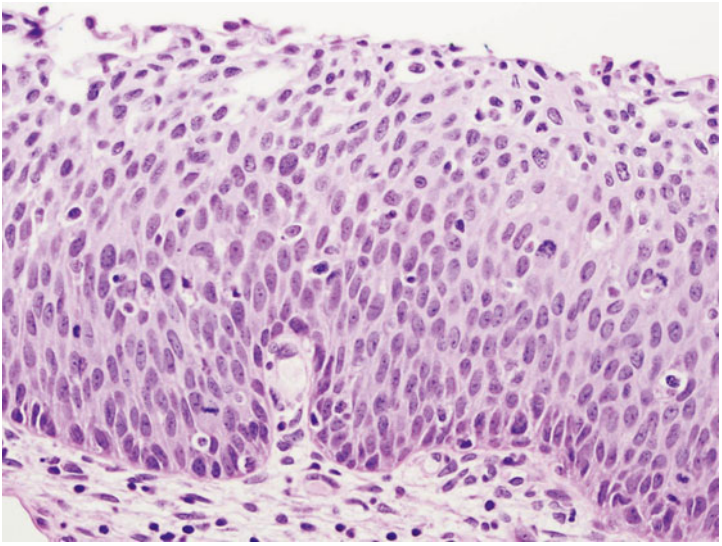

Fig. 5.30 HSIL (CIN 3) (*cervix*, *H&E stain*). The histology of HSIL (CIN 3) reflects the findings seen in clusters of HSIL seen on cytology. The abnormal immature cells show minimal maturation from the base of the epithelium to the surface with nuclear size and shape variation

5.8.2 SIL with Endocervical Gland Involvement (Figs. 5.31–5.34)

When SIL, especially HSIL, extends into the endocervical glands, resultant cell clusters may be misinterpreted as being of glandular origin. Clues that the lesion is actually of squamous origin include centrally located cells showing spindling or

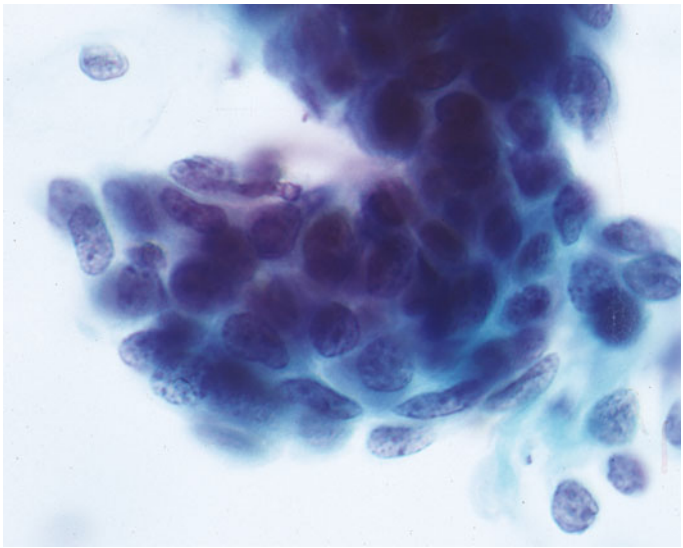

Fig. 5.31 HSIL with extension into endocervical gland space (*LBP*, *SurePath*). Note flattening of cells at the edge of the cluster, a feature that favors HSIL over a glandular lesion

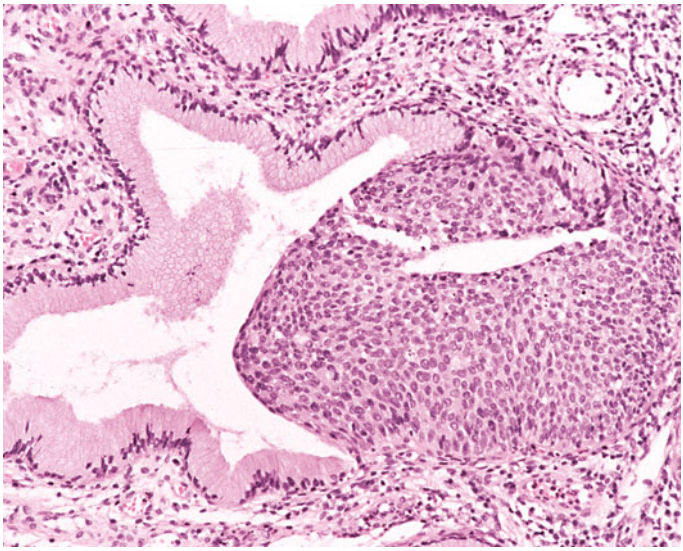

Fig. 5.32 HSIL (CIN 3) with extension into endocervical glands (*cervix*, *H&E stain*). Squamous dysplasia, especially high-grade lesions, often extends into endocervical glands replacing the normal endocervical glandular cells

whorling with flattening of the nuclei at the periphery of the cluster, giving a smooth, rounded border (Figs. 5.17, 5.31–5.34). However, in distinction from the syncytial groups of HSIL mentioned above, HSIL in endocervical glands may demonstrate peripheral palisading of cells and nuclear pseudostratification, features that are usually associated with glandular cervical lesions [25, 27].

On LBPs, loss of central cell polarity and piling within cell groups is observed in HSIL involving glands but not in AIS. Also, in contrast to conventional smears, nucleoli may be visualized in HSIL within glands on liquid-based preparations, but are not as prominent as in AIS (Fig. 5.17) [28]. However, it must always be remembered that HSIL and AIS can coexist in a single specimen [29] (see Figs. 6.33 and 6.34).

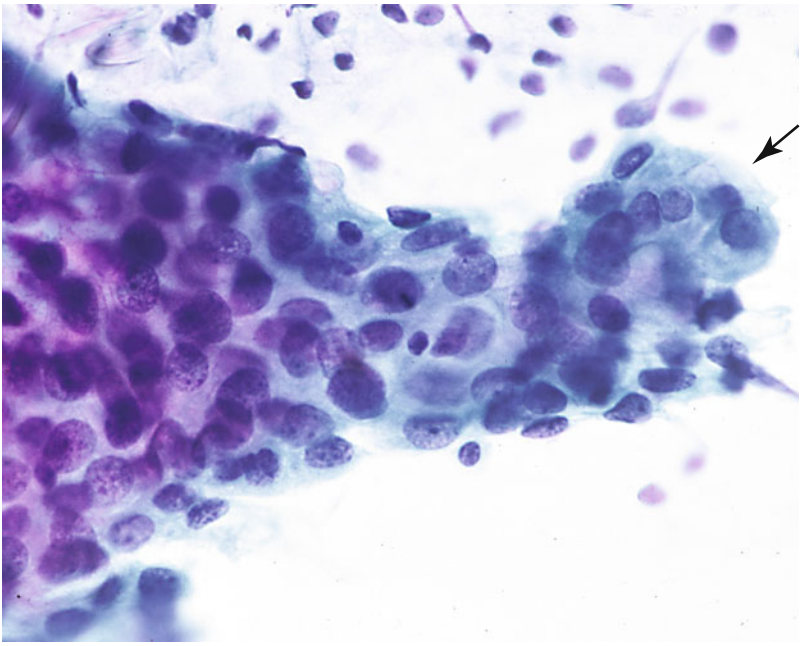

Fig. 5.33 HSIL (*CP*). A 30-year-old woman with atypical glandular cells on a prior Pap test. When HSIL lesions involve endocervical glands, they may show features that overlap with those of adenocarcinoma in situ (AIS). Note normal columnar cells with residual mucin at the *right upper edge* of the cell cluster (*arrow*). Follow-up showed CIN with endocervical gland involvement

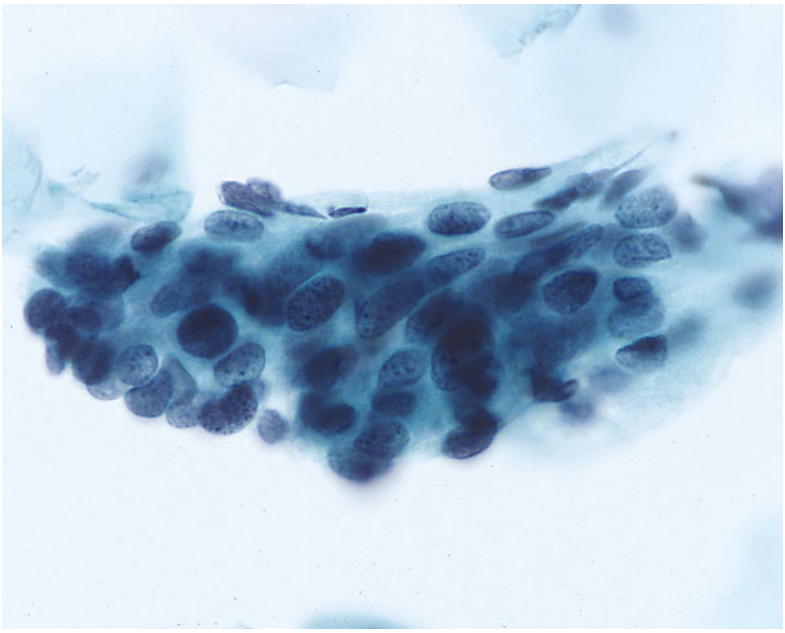

Fig. 5.34 HSIL (*LBP*, *SurePath*). A 44-year-old woman. Syncytial cluster of HSIL cells with features of endocervical gland extension. Such "hyperchromatic crowded groups" may raise a wide differential diagnosis under low magnification; attention to architectural pattern and cellular detail are necessary for correct interpretation. Follow-up showed HSIL (CIN 3) with endocervical gland involvement

5.8.3 HSIL: Pattern Resembling Endometrial Cells and Repair (Figs. 5.35–5.37)

HSIL may rarely present in cervical specimens in a pattern which resembles endometrial stromal or glandular cells or as squamous repair. The identification of the endometrial-like pattern is often made more difficult by the concurrent presence of blood or broken-down blood in the background, which can simulate the background features of menses or a concurrent inflammatory reaction. In this pattern, individual cells are small, often with degenerated nuclei showing pyknosis, and scant cytoplasm that can show tapered ends (Figs. 5.35 and 5.36). These features may closely simulate shed endometrial cells, leading to misinterpretation as such. In the repair-like pattern, HSIL cells show more abundant cytoplasm and may have elongated, "taffy-pull" cytoplasmic appendages, enlarged nuclei, and prominent nucleoli. The latter features simulate the classic features of reparative changes (see Chap. 2 and Figs. 5.66 and 5.37). In most cases showing either of these patterns, cells with more

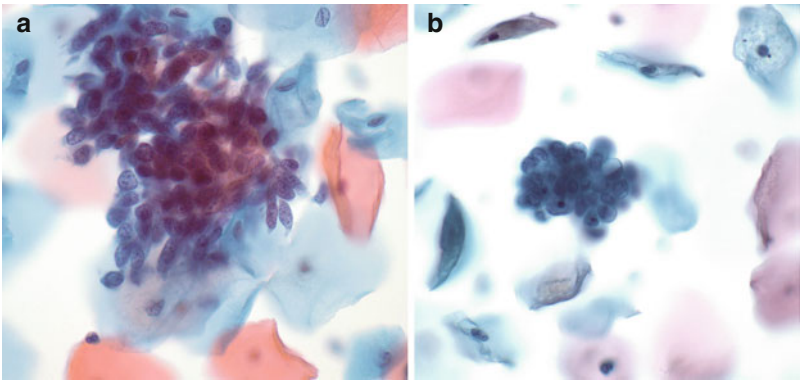

Fig. 5.35 HSIL (**a** and **b** *LBP*, *SurePath*). This rare example of HSIL (**a**) shows a loosely aggregated group of dysplastic cells having a spindled appearance reminiscent of endometrial stromal cells. The cells at the margins of the group show tapered cytoplasmic ends. The nuclei show atypical chromatin and irregular nuclear contours that are more in keeping with the high-grade squamous lesion. Compare the cytologic features with shed endometrium (**b**)

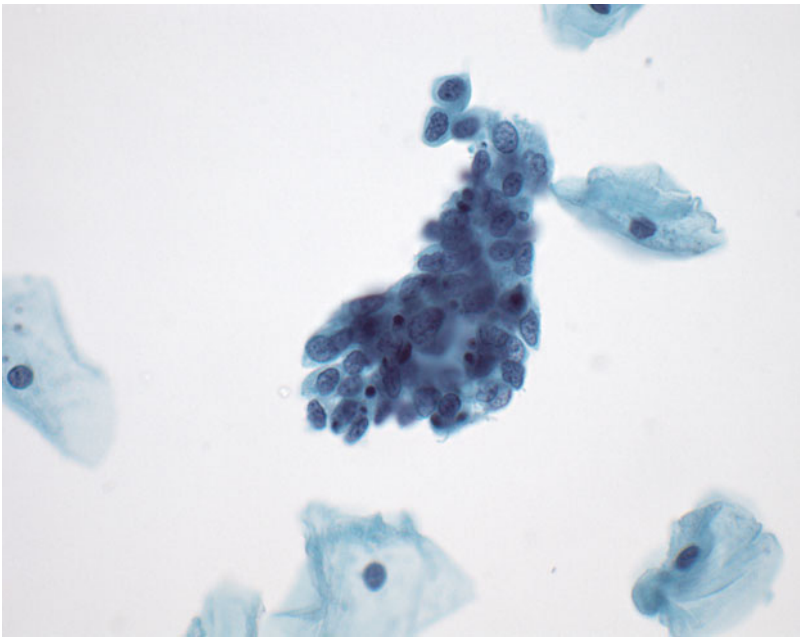

Fig. 5.36 HSIL (*LBP*, *SurePath*). HSIL can present in three-dimensional groups that closely mimic shed endometrial cells. In this example, the nuclei are smaller that might be expected for the typical HSIL; however, they do show atypical chromatin and irregular contours. Apoptotic debris is present within the groups, a feature that is commonly present in shed endometrium

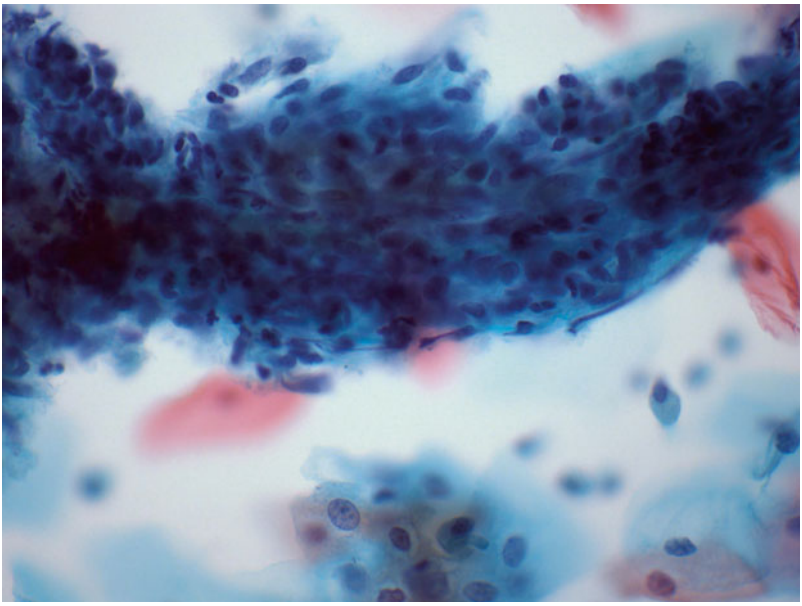

Fig. 5.37 HSIL (*LBP*, *SurePath*). In some cases of HSIL, more voluminous amounts of cytoplasm with cytoplasmic appendage formation reminiscent of repair can be present. Note also the presence of intermixed inflammatory cells within the group, another feature that overlaps with reparative changes. Such samples should be interpreted cautiously, with an attempt to find more typical HSIL cells

classic features of HSIL will be present on the same slide and should be carefully looked for if suspicion of an HSIL is under consideration. These patterns may be difficult in isolation and are therefore often discovered only on retrospective review of cases found to be precancer on follow-up material.

5.8.4 Single and Rare Small HSIL Cells (Figs. 5.27 and 5.28)

The cells of HSIL are often single with fewer sheets and clusters than are seen in LSIL. Specimens with rare, small, high nuclear to cytoplasmic ratio HSIL cells may be problematic with regard to identifying the cells (screening/location) as well as categorizing the abnormality accurately (interpretation) [30]. There is a higher probability of a false-negative result when there are relatively few detached neoplastic cells or when only a few large groups of neoplastic cells are present [31]. Liquid-based preparations frequently have fewer diagnostic cells compared to conventional preparations, although the cells may be better visualized. Close attention should be paid to small, single cells with increased N/C ratios, which

may be found in the "empty spaces" between cells. In HSIL, closer examination of these cells will show nuclear membrane and chromatin abnormalities. If rare abnormal cells are identified but the findings fall short of an interpretation of HSIL, the specimen should be reported as ASC-H (see Figs. 4.20–4.26).

The differential diagnosis of isolated cells with high nuclear to cytoplasmic ratios includes immature squamous metaplasia, cellular changes associated with intrauterine device use (see Figs. 2.47 and 6.5), and isolated cells of endocervical or endometrial origin (see Fig. 5.50).

5.8.5 HSIL: Abnormal Stripped Nuclei (Figs. 5.22b, 5.38 and 5.39)

Stripped nuclei which are cytologically abnormal should be differentiated from those seen in cytolysis (Fig. 2.62) and the "small blue cells" seen in atrophy/tamoxifen therapy [32] (Fig. 3.7). The finding of abnormal stripped nuclei in a specimen should prompt a thorough review for more classic HSIL cells.

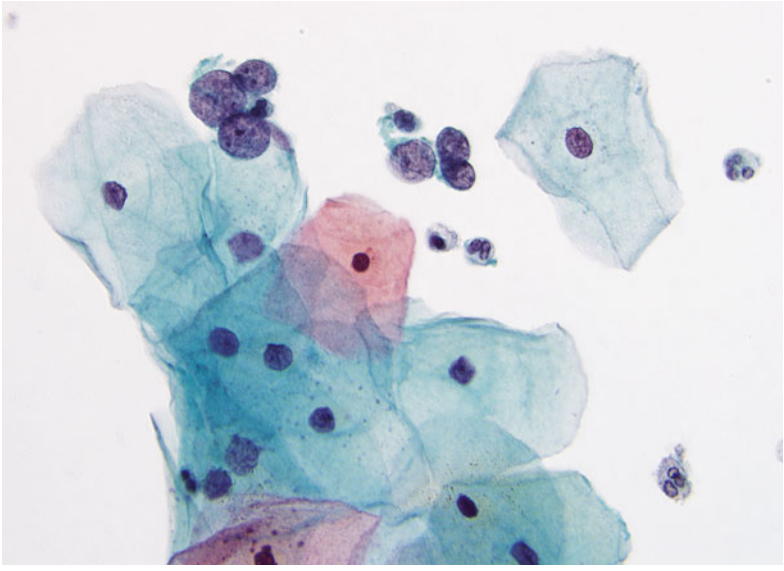

Fig. 5.38 HSIL (*LBP*, *ThinPrep*). Abnormal, large stripped nuclei are seen that are considerably bigger than the intermediate cell nuclei. Such cells should elicit a search for classic, intact HSIL cells elsewhere on the same preparation. These stripped nuclei should be distinguished from endometrial cells or the stripped clusters of atrophic nuclei that are often seen in *LBPs* in the background of atrophy

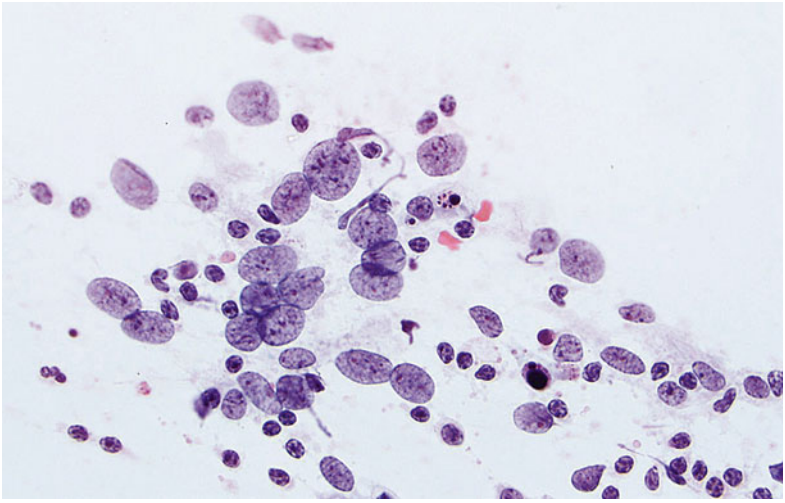

Fig. 5.39 HSIL-stripped nucleus pattern (*CP*). A 38-year-old woman with a history of LSIL. These abnormal stripped nuclei are often a useful diagnostic clue that other abnormal cells may be identified on the same slide. They should be distinguished from the bare intermediate cell nuclei seen in cytolysis (Fig. 2.62) and from "small blue cells" (see Fig. 3.7)

5.8.6 Streams of HSIL Cells, Usually Within Mucus (Figs. 5.40 and 5.41)

In conventional preparations, HSIL in mucus strands can resemble histiocytes/superficial endometrial stromal cells or degenerated endocervical cells as in microglandular hyperplasia (Figs. 5.40 and 5.41). The low-magnification pattern of small cells in a streak of mucus warrants evaluation at higher magnification. This pattern is rarely observed in liquid-based preparations since mucus is dispersed and the cells randomized as to their location on the slide.

5.8.7 Keratinizing High-Grade Lesions (Figs. 5.26, 5.42–5.44)

Although most HSILs are characterized by cells with a high nuclear to cytoplasmic ratio, some high-grade lesions are composed of cells with more abundant, but abnormally keratinized, cytoplasm (Figs. 5.26, 5.42–5.44). Such cells may be shed singly or in three-dimensional clusters and have enlarged hyperchromatic nuclei, often with dense or opaque chromatin that obscures other nuclear features. In addition, these cells are often pleomorphic with marked variation of nuclear size (anisokaryosis) and cellular shape, including elongate, spindle, caudate, and tadpole cells.

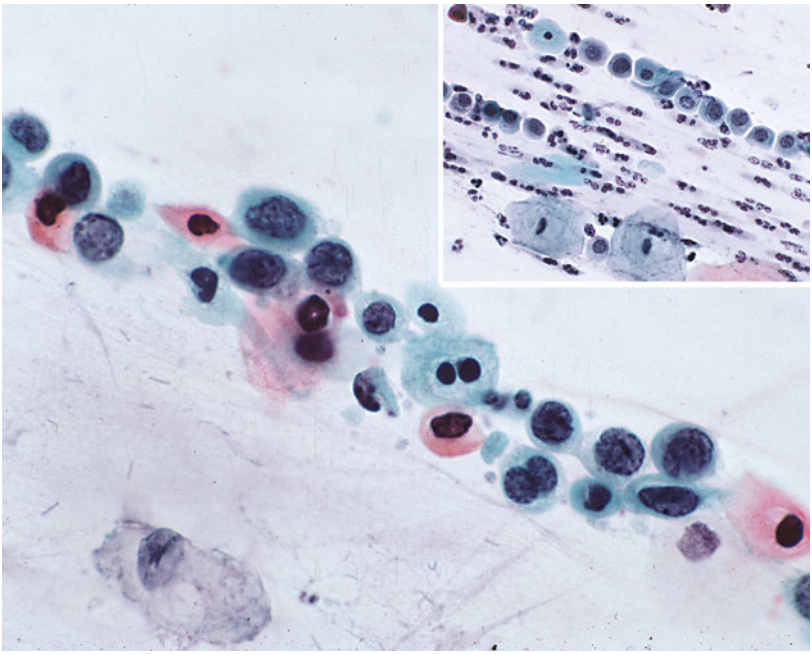

Fig. 5.40 HSIL (*CP*). At low magnification (*right upper inset*), the pattern of HSIL cells streaming within mucus can mimic histiocytes and endocervical/metaplastic cells. At high power, HSIL can be readily distinguished (see also Figs. 5.35, 4.33, and 4.34)

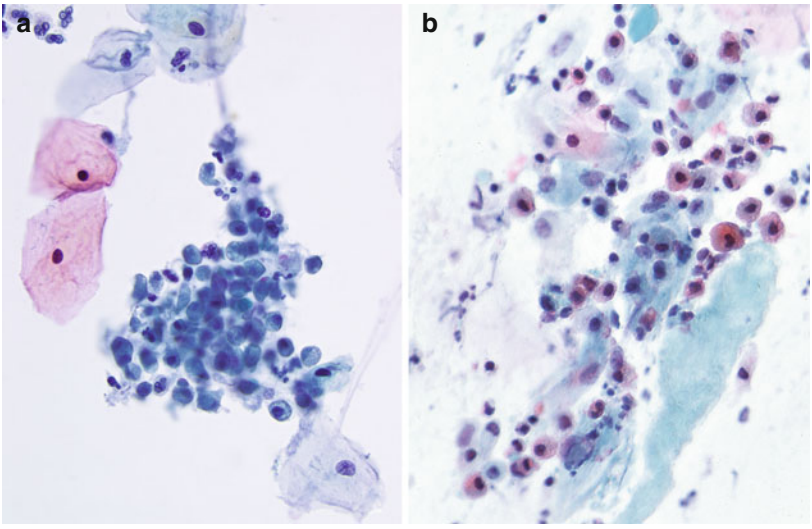

Fig. 5.41 NILM; endocervical microglandular hyperplasia (**a** *LBP*, *ThinPrep*, **b** *CP*). A 34-year-old woman on day 19 of menstrual cycle. Degenerated endocervical cells, seen in a streaming pattern along with thick mucus, is a pattern that has been associated with microglandular hyperplasia (**b**). The appearance is more subtle in liquid-based preparations (**a**). When identified, it is typically during the second half of the menstrual cycle, often in women taking oral contraceptives, and may mimic HSIL at low magnification. Follow-up cytology showed NILM

In contrast to invasive squamous carcinoma, nucleoli and tumor diathesis are generally absent. Such lesions have been variously termed "atypical condyloma," "keratinizing dysplasia," and "pleomorphic dysplasia." However, these terms should not be used as these lesions are most often HSIL. Keratinized lesions may be indistinguishable from invasive carcinoma, especially in samples with a relatively scant number of abnormal cells. In these instances, an explanatory note may be appended to indicate that the differential diagnosis includes an invasive squamous cell carcinoma, or the interpretation of *HSIL with features suspicious for invasion* can be used (Fig. 5.44).

5.8.8 HSIL in Atrophy (Figs. 5.45 and 5.46)

HSIL found in the background of atrophy is often difficult to appreciate because of the lack of maturation of squamous cells and the similarity between small atrophic cells and the dysplastic cells. Cells of HSIL in atrophy are generally small, often the size of parabasal cells or immature squamous metaplastic cells. In general, atrophic cells will maintain a lower nucleus to cytoplasmic ratio and lack the nuclear membrane irregularities seen in HSIL (Fig. 5.45). The nuclei of atrophic cells may be

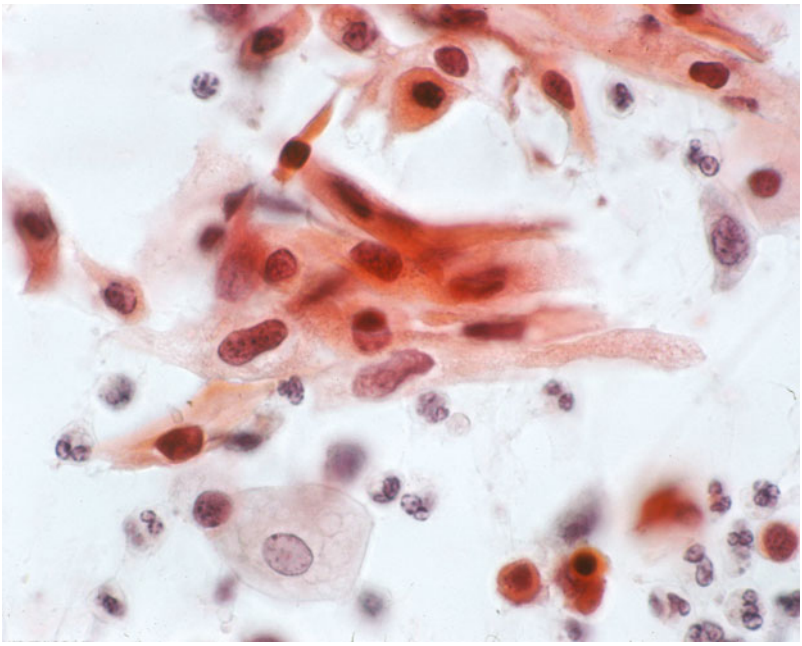

Fig. 5.42 HSIL (*CP*). Classification of atypical keratinized cells depends on the degree of nuclear abnormality, the N/C ratio, and to some extent on the pleomorphism of the abnormal cells. This image shows a range of cells from the LSIL cells seen in the center to the HSIL cells seen around the periphery. The high-grade cells have an increased N/C ratio as well as more marked variability in cytoplasmic shape (see also Figs. 5.8 and 5.26)

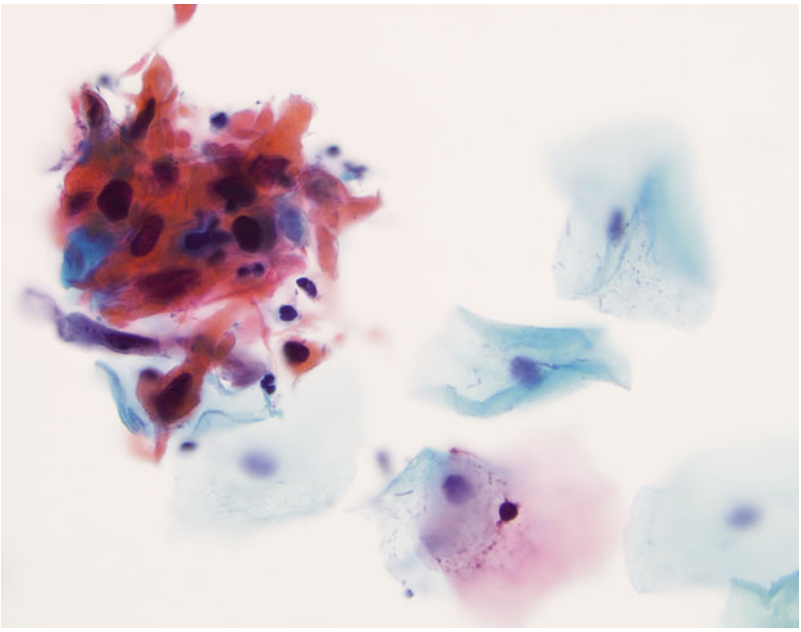

Fig. 5.43 HSIL (*LBP*, *ThinPrep*). These cells demonstrate marked pleomorphism of the nuclei and keratinized cytoplasm. The marked variation in shape and the presence of cells with a high N/C ratio is consistent with an interpretation of HSIL

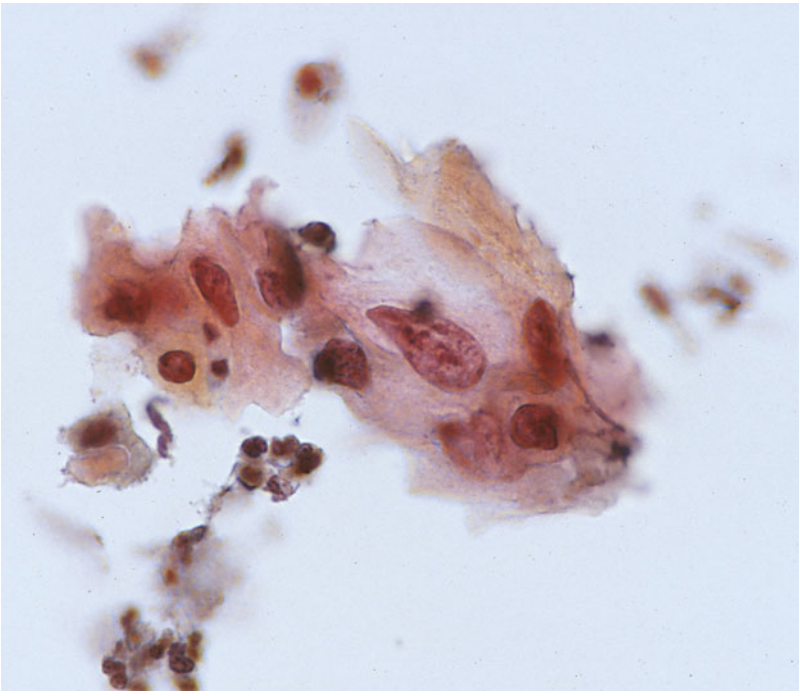

Fig. 5.44 HSIL (*LBP*, *ThinPrep*). A 42-year-old woman. Keratinized dysplastic cells with nucleoli and angulated or "carrot"-shaped nuclei that may raise suspicion for invasion and qualify for an interpretation of HSIL cannot rule out invasion. Follow-up showed only HSIL (CIN 3) that was keratinizing

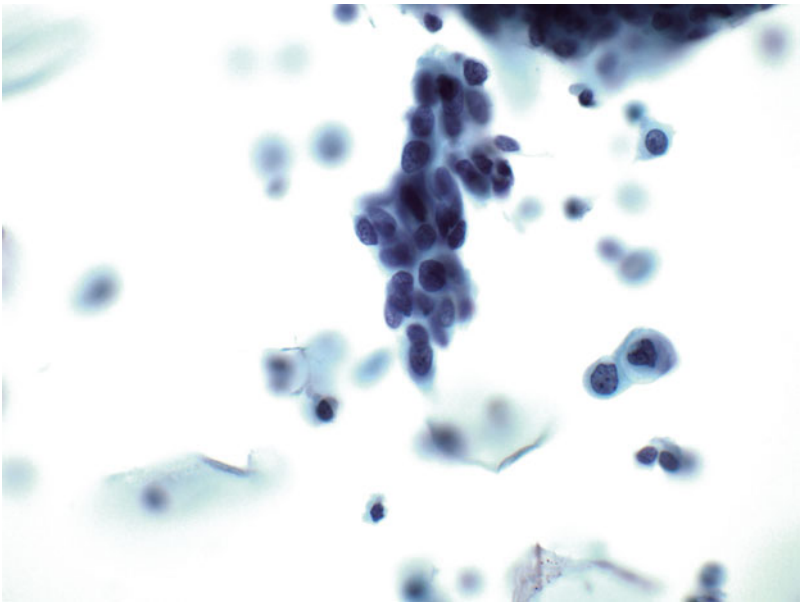

Fig. 5.45 HSIL (*LBP*, *SurePath*). HSIL in atrophy may be difficult to distinguish from clusters of benign atrophic squamous cells. In HSIL, as seen here, the cells show a syncytial arrangement, and looking at these clusters by focusing in different planes allows one to better distinguish them from the parabasal cells in the background

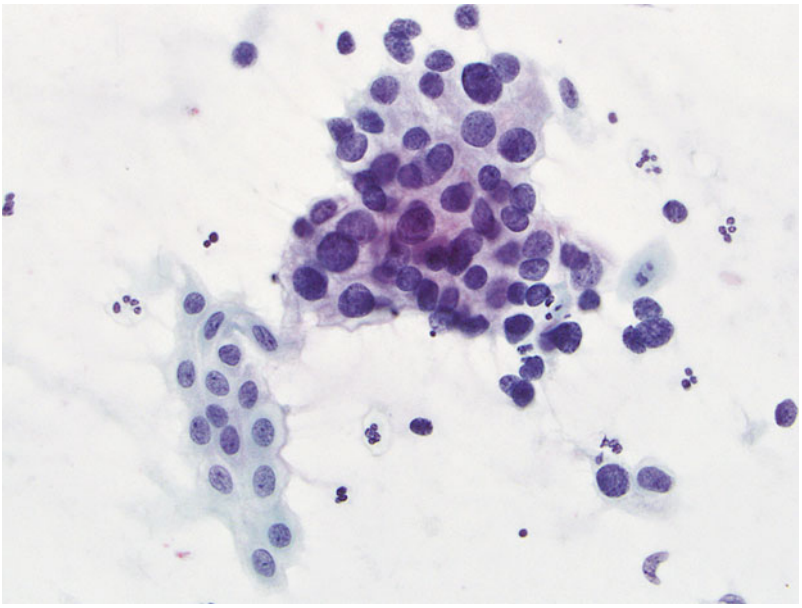

Fig. 5.46 HSIL (*CP*). Clusters of parabasal cells are commonly identified in the background of HSIL in atrophy. The HSIL illustrated here shows a sheet-like arrangement, a pattern commonly seen in HSIL, with significant nuclear size variation and a loss of polarity with overlapping of the nuclei. HSIL in the background of atrophy can be a diagnostic challenge

quite hyperchromatic due to degeneration, but the chromatin is more often smudgy than coarse. One maneuver that can be helpful in the detection of HSIL presenting as dense groups in atrophic specimens is to observe the cells in the group within a single high-magnification focal plane. If the nuclei are noted to overlap in single planes, the group is most likely a syncytial arrangement of HSIL. If the nuclei do not overlap in the single focal plane, the group is more likely to be normal parabasal cells.

5.8.9 LSIL with Some Features Suggestive of the Presence of a Concurrent HSIL (Figs. 5.42, 5.47, and 5.48)

Some specimens may have cytologic features that lie between low- and high-grade SIL. Such cases often have keratinized cells with dense eosinophilic cytoplasm that give an impression of higher nucleus to cytoplasmic ratio than in classic LSIL, but without specific features of classic HSIL (Fig. 5.42). Another pattern is one in which the predominant cell type favors an LSIL but in which a few cells show immature cytoplasmic features with a higher nucleus to cytoplasmic ratio than what is typical for LSIL (Fig. 5.47). In such cases, attention to morphologic features usually supports classification as either LSIL or HSIL. Note that in HSIL cases that meet cytomorphologic criteria for this interpretation, the presence of concurrent

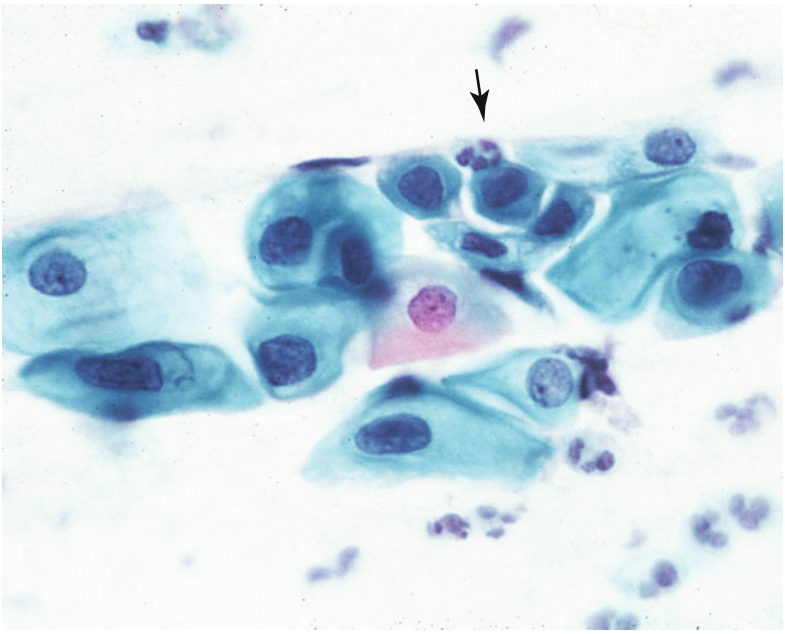

Fig. 5.47 LSIL with some cells suggesting the possibility of a concurrent HSIL (*CP*). Routine screen from a 28-year-old woman. Most of these cells qualify as LSIL; however, there are three atypical metaplastic cells at the top center (*arrow*) that raise the possibility of a high-grade lesion. Cases such as this are may be interpreted as LSIL with a comment explaining the possibility of HSIL or as LSIL with an additional interpretation of ASC-H. The presence of a few diagnostic HSIL cells in the background of a predominant LSIL pattern should be interpreted as HSIL. Follow-up in this case showed HSIL (CIN 2)

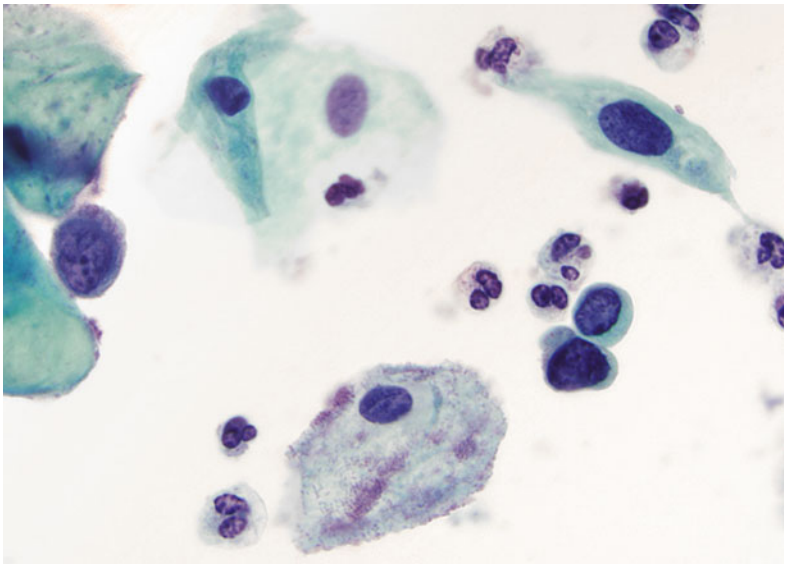

Fig. 5.48 HSIL (*LBP*, *ThinPrep*). In this case, diagnostic HSIL cells are present. Even if these cells are seen in the background of a majority of LSIL elsewhere on the slide, the final interpretation should be HSIL

LSIL cells is not necessary to make an interpretation of HSIL. It is also important to recognize that the presence of even a small population of definitive HSIL cells in the background of a predominance of LSIL cells should result in an interpretation of HSIL (Fig. 5.48).

Recently it has been suggested that these intermediate morphologic patterns be designated with a diagnostic term other than LSIL or HSIL. Terms such as LSIL cannot exclude HSIL or LSIL-H have been suggested [33–36]. Not surprisingly, on follow-up colposcopy and biopsy, these lesions have an increased incidence of HSIL (CIN 2+) compared to that of routine LSIL cytology [37–39]. In preparation for this update to TBS, opinions regarding this topic were openly solicited with consensus achieved that formal TBS nomenclature should be limited to the original LSIL and HSIL, two-tier classification. Adding terminology such as LSIL-H would lead to a de facto three-tier system negating the beneficial aspects of the two-tier TBS nomenclature. Current management guidelines use LSIL and HSIL nomenclature without an intermediate category and the current recommendations also encourage reporting histology as LSIL/HSIL [8, 9]. Likely poor reproducibility and overutilization of any indeterminate cytology terminology could easily lead to confusion among clinicians and to inappropriate management [19].

In occasional specimens where it is not possible to grade a SIL as clearly low or high [23, 40], a comment explaining the nature of the uncertainty may be appropriate (see Figs. 5.32 and 5.47). In some cases, an interpretation of ASC-H may be made in addition to an LSIL interpretation. This would indicate that definite LSIL is present as well as some cells that suggest the possibility of HSIL. In general, follow-up guidelines for these interpretations are for colposcopy and

biopsy, but in cases (such as in young women) where the guidelines differ between LSIL and ASC-H, the addition of the ASC-H interpretation should then lead to colposcopy.

It must be emphasized that intermediate interpretations should comprise only a small minority of cases in any laboratory, as classification into either LSIL or HSIL is possible in most instances following careful overall evaluation of the cellular morphology (Fig. 5.48).

5.9 Mimics of HSIL

5.9.1 Isolated Cells

There are many types of isolated cells which may mimic HSIL in cervical cytology. These include:

5.9.2 Isolated Epithelial Cells (Figs. 5.49–5.52)

Isolated epithelial cells which may mimic HSIL include reserve cells, parabasal cells, and immature squamous metaplastic cells (Fig. 5.49). These cells closely resemble each other and may be distinguished from HSIL by lower nuclear to

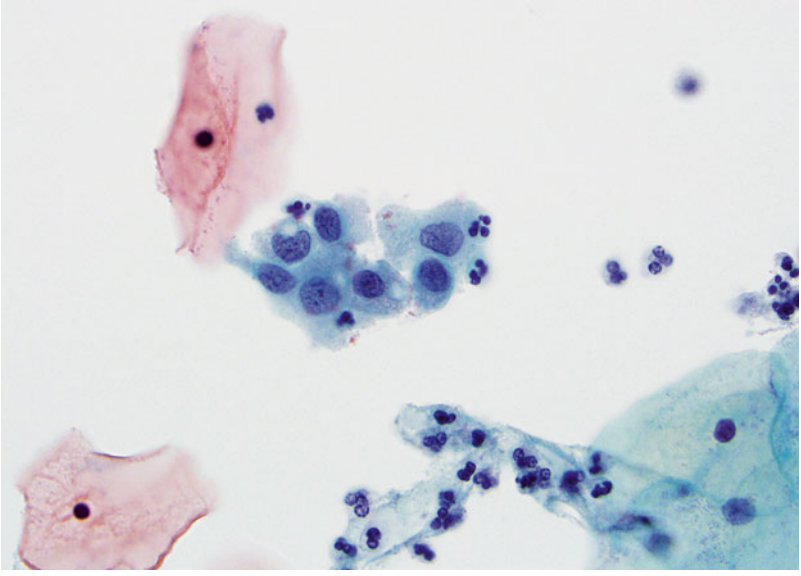

Fig. 5.49 Immature squamous metaplasia (*LBP*, *ThinPrep*). Immature metaplastic cells can mimic dysplastic cells. Degenerative and reactive changes in these small squamous cells can be confused with dysplasia or carcinoma. Cytologic features that support a benign interpretation include nuclear uniformity, smooth nuclear borders, and fine and evenly distributed chromatin

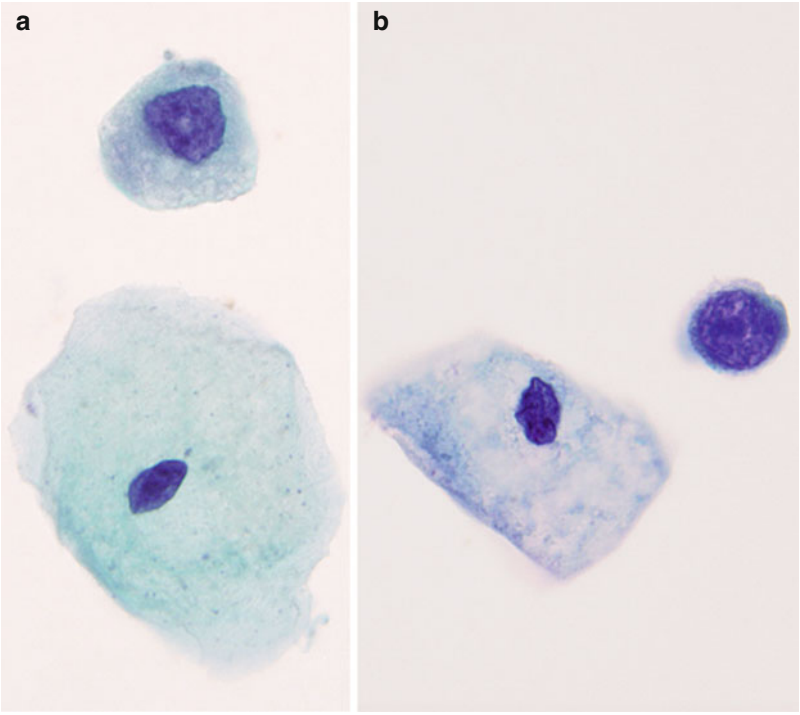

Fig. 5.50 HSIL versus benign endocervical cells (*LBP*, *ThinPrep*). Single cells are randomly distributed in liquid-based preparations. Single benign endocervical cells are prone to cytoplasmic lysis and (**b**) may mimic single cells of HSIL. The common cellular features of HSIL (**a**), such as irregular nuclear membranes, absence of nucleoli, and hyperchromasia, help to make the correct interpretation

cytoplasmic ratios, lack of nuclear membrane irregularities, and/or lack of hyperchromasia. Endocervical cells which have been exfoliated and sampled from the endocervical mucus can mimic HSIL because of their "rounded up" appearance and high nuclear to cytoplasmic ratio (Fig. 5.50). The keys to a correct interpretation of benign endocervical origin are the presence of small nucleoli, finely granular and evenly distributed chromatin, smooth nuclear contours, and granular cytoplasm which may show some elongation. Reactive high endocervical cells associated with irritation from an IUD may also mimic HSIL as discussed in Chap. 2 (see Fig. 2.47). Exfoliated endometrial cells can occasionally be mimics of HSIL, particularly when appearing in a single-cell pattern. Their very small size, degenerated nuclei, and the presence of more typical three-dimensional endometrial cell groups elsewhere on the slide are the keys to proper interpretation (Fig. 5.51a, b).

Isolated highly atypical squamous cells can be occasionally identified in deeply atrophic specimens (Fig. 5.52). These cells may have very large nuclei with a characteristic smudgy or degenerative chromatin pattern and a very high nucleus to cytoplasmic ratio. Because of the concern for HSIL that such cells can engender, often in patients with few or no risk factors, conservative approaches, such as

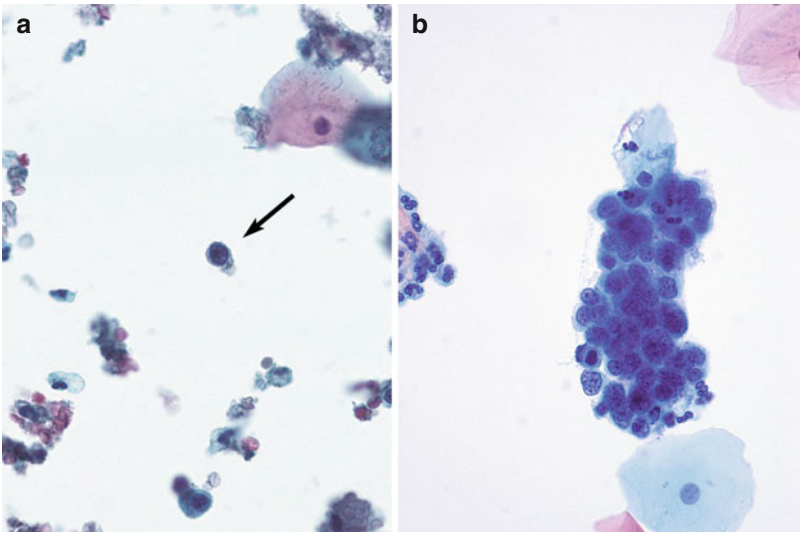

Fig. 5.51 NILM, endometrial cells (*LBP*, *ThinPrep*). Single endometrial cells (**a** *arrow*) may be mistaken for HSIL. The small round nucleus with smooth nuclear membranes helps to classify this as benign. Comparison to more classic clusters of endometrial cells from the same slide (**b**) is also useful

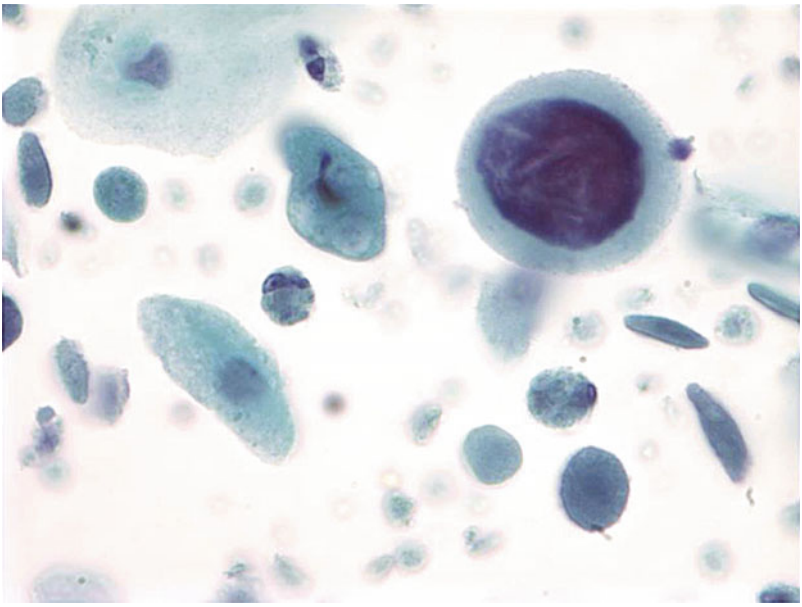

Fig. 5.52 ASC-US (*LBP*, *SurePath*). Large bizarre cells may be seen in atrophic preparations. Because of the increased N/C ratio, these cells raise the possibility of HSIL, but the degenerative nuclear features and background atrophy make a benign process more likely. An interpretation of ASC-US may be more appropriate than ASC-H in this case. In this case, follow up hrHPV testing was negative and no abnormality was identified with colposcopic biopsy and subsequent repeat cytology

designation as ASC-US with follow-up hrHPV testing, may be appropriate. In cases of atrophy with abnormal cells meeting criteria for HSIL (see Fig. 5.45), an interpretation of HSIL should be made.

5.9.3 Inflammatory Cells Such as Histiocytes or Lymphocytes (Figs. 2.41, 2.42, 3.6, and 3.8)

Histiocytes have small oval- to coffee bean-shaped nuclei, occasionally with a prominent longitudinal groove (Fig. 3.6). Small lymphocytes have small round nuclei with dense, coarsely granular chromatin and only minimal cytoplasm (Figs. 2.41, 2.42, and 3.8). Larger reactive lymphocytes, or even more rarely lymphoma, may be mistaken for abnormal epithelial cells. Reactive lymphocytes present in loose clusters with accompanying tingible body macrophages (Fig. 2.41). These cells lack the nuclear membrane notching and irregularity of HSIL.

5.9.4 Decidualized Stromal Cells (Figs. 2.28 and 5.53)

Decidual cells can mimic LSIL or HSIL. Most often these cells are isolated, large cells with low nucleus to cytoplasmic ratio similar to the appearance of LSIL. Unlike LSIL these cells have a more granular, less dense cytoplasm, prominent basophilic nucleolus, and lack any evidence of HPV cytopathic effect (Fig. 2.28). Occasionally, decidual cells are smaller with high nucleus to cytoplasmic ratios mimicking HSIL. The history of pregnancy and lack of HSIL features and HPV cytopathic effect should allow for appropriate classification (Fig. 5.53).

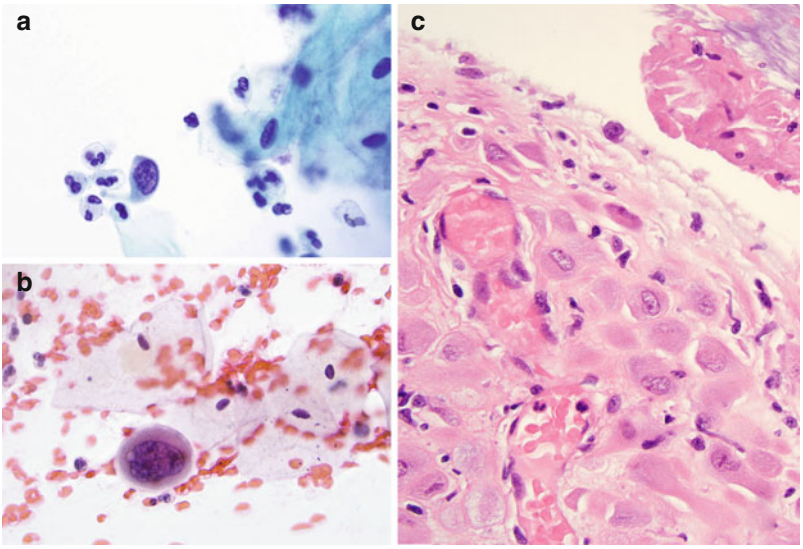

Fig. 5.53 NILM (**a**, **b** *LBP*, *ThinPrep*, **c** cervix *H&E stain*). A young woman in the late second trimester of pregnancy. These single cells (**a**, **b**) with an increased N/C ratio and nuclear hyperchromasia are worrisome for HSIL. Features suggesting the true stromal decidual nature of the cells include the smudgy chromatin and the presence of a nucleolus. Similar cells can be seen in a follow-up cervical biopsy (**c**)

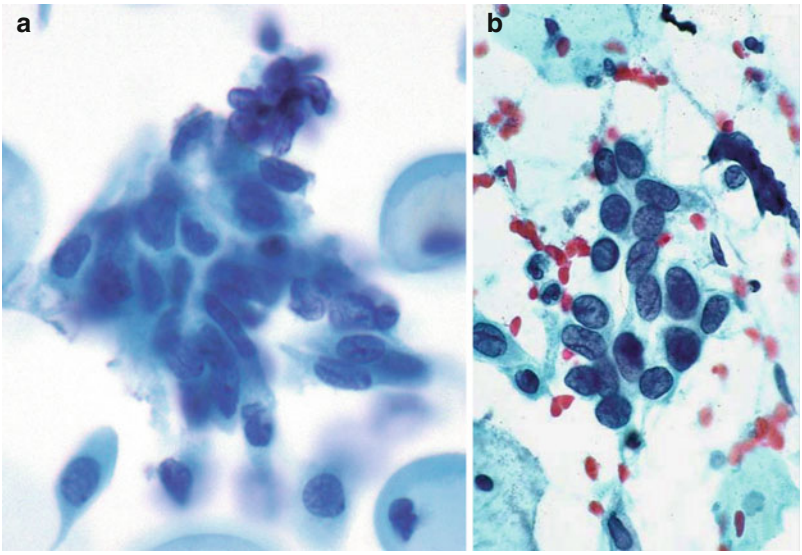

Fig. 5.54 NILM (**a** *LBP*, *ThinPrep*) versus HSIL (**b** *CP*). Both dysplastic and benign squamous cells can demonstrate longitudinal nuclear grooves. The benign cells seen in (**a**) are derived from transitional cell metaplasia and show distinct nuclear grooving without any of the other dysplastic features. In (**b**), the HSIL cells show other features of dysplasia including significant nuclear size variation and nuclear membrane notching, as well as grooves

5.9.5 Hyperchromatic Crowded Groups (HCGs)

Many entities, both benign and neoplastic, may present as hyperchromatic crowded groups of cells that mimic the classic syncytial arrangements of HSIL. Densely cellular groups may be comprised of tissue fragments derived from squamous, endocervical, or endometrial epithelial cells. Lack of ability to see into the central areas of the groups can raise concern that a neoplastic lesion is present due to the hyperchromasia resulting from nuclear overlap. When examining these groups, close attention to the cells at the group margins, where nuclear features are better discerned, is important.

In contrast to HSIL, atrophic or immature metaplastic squamous cells presenting as HCGs will show no alteration in nuclear to cytoplasmic ratios, minimal pleomorphism of size and shape, and smooth nuclear contours. Nuclear overlap in single focal planes will be minimal (see Fig. 2.23). Dense groups of transitional cell metaplasia (a benign metaplasia of the squamous epithelium commonly present in atrophy) can also mimic HSIL. Transitional cell metaplasia has characteristic nuclear morphology showing longitudinal grooves and smooth nuclear contours (Fig. 5.54).

Endocervical or endometrial cells presenting as HCGs may mimic either squamous or glandular high-grade precancers. Groups of benign endocervical cells retain a columnar cytoplasmic configuration with eccentrically placed nuclei, and granular or finely vacuolated cytoplasm (see Fig. 2.4). Groups from endocervical epithelium with tubal metaplasia can be particularly challenging due to the

Table 5.1 Key differential features of HSIL/ASC-H and their mimics

	Single cells and individual cells in groups	Clusters and sheets
HSIL/ASC-H	Variable N:C ratio; may be very high	Significant anisonucleosis
	Nuclear membrane notching and marked irregularity	Syncytial arrangement
	Generally hyperchromatic nuclei but may be normo- or hypochromatic	Occasional mitosis
	Coarse evenly dispersed chromatin	Loss of nuclear polarity
	Lack of nucleoli	Horizontal arrangement of cells at periphery of clusters
Squamous		
Squamous metaplasia	Lower N:C ratio	Minimal variability in nuclear size
	Smooth nuclear membranes or single groove	Polygonal cells with cytoplasmic borders
	May have nucleoli if reactive	Repair may have normal mitoses
		Generally maintains nuclear polarity
Atrophy	N:C ratio varies	Minimal variability in nuclear size
	Degenerated nuclear chromatin with smooth membranes	No mitoses
	Spectrum of changes from obvious benign to problematic	
Glandular		
Benign endocervical	Low N:C ratio	Parallel nuclear arrangement
	Basally placed nucleus	Nucleoli may be prominent
	Smooth nuclear membranes	Maintains nuclear polarity
	Normochromatic	
	Vacuolated cytoplasm	
Exfoliated endometrial	Small nuclei with high N:C ratios	Minimal anisonucleosis
	May have small nucleoli	Syncytial arrangement with minimal cytoplasm
	Few single cells	
Directly sampled endometrium	Nuclei slightly larger than intermediate nuclei	Minimal anisonucleosis
	Lower N:C ratio	Maintains nuclear polarity
	Smooth nuclear membranes	Mitosis may be seen in proliferative phase
		May form tubules associated with stromal cells
Tubal metaplasia	Apical terminal bar and cilia	May form crowded groups but tends to maintain polarity
	Nuclei same size as squamous metaplastic nuclei	Parallel nuclear arrangements
	Basally placed nucleus	
	Smooth nuclear membranes	
	N:C ratios higher than normal endocervical cells	

Table 5.1 (continued)

	Single cells and individual cells in groups	Clusters and sheets
IUD changes	N:C ratio varies generally low but may be quite high	Small clusters of cells
	Nuclei are degenerative with smudgy dark chromatin	May be endocervical or endometrial in origin
	Cytoplasm often vacuolated	
AIS	Hyperchromatic nuclei with fine to coarse chromatin	Parallel nuclear arrangements
	Nuclear membranes may show irregularity or notches	Nuclei tend to have basal or palisaded arrangement
	Increased N:C ratios	
Other cell types		
Lymphocytes	Small nuclei in mature cells to larger nuclei in germinal center cells	Not seen in cohesive groups but may be in loose clusters
	Chromatin is coarse to open in larger cells	May be accompanied by tingible body macrophages
Histiocytes	Small to medium-sized oval-kidney bean nuclei with longitudinal groove	Not seen in cohesive groups but may be in loose clusters
	Normochromatic	May be associated with endometrial cells in exodus
	Foamy to vacuolated cytoplasm	

pseudostratification of nuclei which can lead to a significantly greater degree of crowding than is present in otherwise normal endocervical cell groupings (see Fig. 6.2). Shedding endometrial groups will show characteristic degenerative changes, including nuclear opacity and pyknosis and the presence of apoptotic bodies within the groups (see Fig. 3.4). Abraded endometrial groups will have the typical organoid architectural configurations and the presence of associated endometrial stromal cells (see Figs. 2.7, 2.8, and 3.5; Table 5.1).

5.10 HSIL with Features Suspicious for Invasion (Figs. 5.44 and 5.55)

In rare cases of HSIL, invasive carcinoma is difficult to exclude. This situation may occur when there are highly pleomorphic HSIL cells with keratinized cytoplasm present that are not accompanied by the characteristic background features of invasion (necrosis or tumor diathesis; see Fig. 5.44). Conversely, the slide may contain features suggesting tumor diathesis (blood, necrosis, or granular proteinaceous debris in the background), but overtly malignant cells may not be identified. Occasionally, HSIL without invasion but extending into glands may be associated with focal epithelial cell necrosis and micronucleoli. In such cases, the necrosis is associated with the cell group in an otherwise clean background and is not admixed with broken-down blood and inflammation as is typically noted in an invasive tumor diathesis [41] (Fig. 5.55 and 5.56).

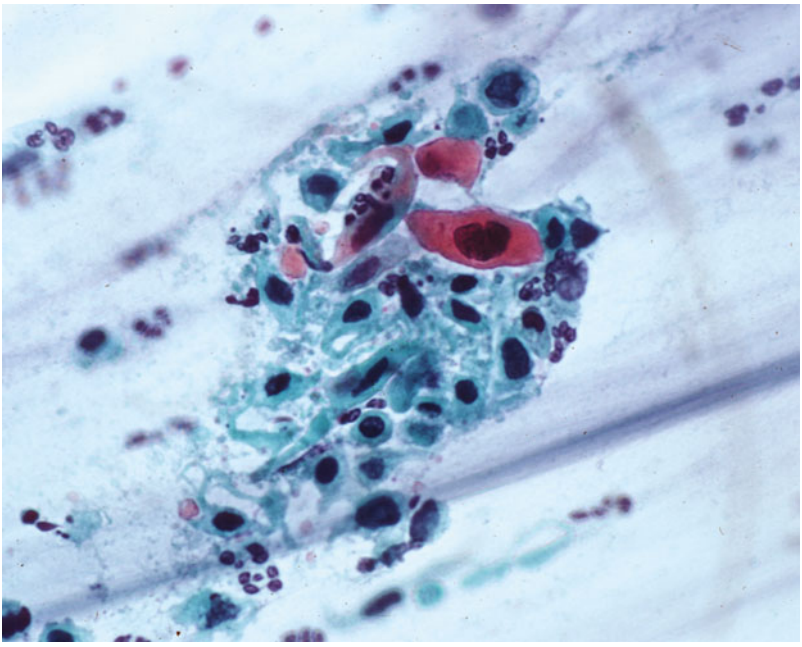

Fig. 5.55 HSIL with features suspicious for invasion (*CP*). A 71-year-old postmenopausal woman. HSIL filling endocervical glands may undergo focal necrosis that can mimic the tumor diathesis associated with invasive lesions. Follow-up showed HSIL (CIN 3) extending into glands with focal epithelial necrosis, but no invasion

5.11 Management of HSIL

Most women with a cytologic result of HSIL will have biopsy-confirmed HSIL (CIN 2+) identified at the time of colposcopy [42]. Therefore, the 2012 ASCCP consensus guidelines state that for women aged 25 years and older with cytologic HSIL, immediate excisional procedure may be performed at the time of colposcopy if a lesion is identified. Also, if biopsy-confirmed HSIL is not identified at colposcopy in a woman with a cytologic interpretation of HSIL, review of cytologic and histologic material, with additional recuts and p16 immunohistochemistry, may reveal the lesion [8].

5.12 Squamous Cell Carcinoma

5.12.1 Definition

As defined in the 2014 WHO terminology, squamous cell carcinoma is "an invasive epithelial tumor composed of squamous cells of varying degrees of differentiation" [9].

The Bethesda System does not subdivide squamous cell carcinoma; however, for descriptive purposes, nonkeratinizing and keratinizing carcinomas are discussed separately.

5.12.2 Keratinizing Squamous Cell Carcinoma (Figs. 5.56–5.59)

5.12.2.1 Criteria

Presents predominantly as isolated, single cells and less commonly in cellular aggregates.

Marked variation in cellular size and shape is typical, with caudate and spindle cells that frequently contain dense orangeophilic cytoplasm.

Nuclei vary markedly in area, nuclear membranes may be irregular, and numerous dense opaque nuclei are often present.

Chromatin pattern, when discernible, is coarsely granular and irregularly distributed with chromatin clearing.

Macronucleoli may be seen but are less common than in nonkeratinizing squamous cell carcinoma.

Associated keratotic changes (hyperkeratosis or parakeratosis) may be present but are not sufficient for the interpretation of carcinoma in the absence of nuclear abnormalities.

A tumor diathesis may be present but is usually less than that seen in nonkeratinizing squamous cell carcinomas.

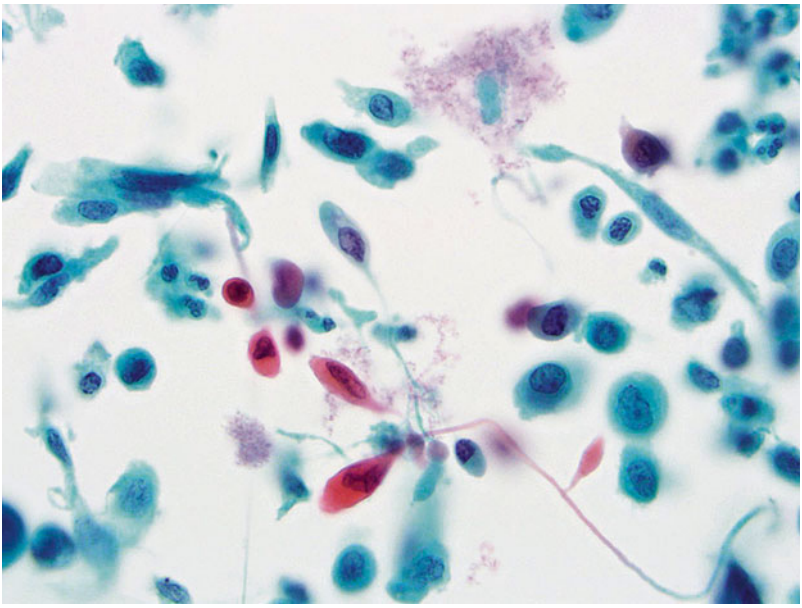

Fig. 5.56 Squamous cell carcinoma, keratinizing (*LBP*, *SurePath*). The malignant cells have variable shapes and sizes and show some keratinized "tadpole cells." Nuclei vary from vesicular with irregular nuclear contours and nucleoli to pyknotic in the keratinized cells. The cytoplasm is dense and may be deeply eosinophilic or cyanophilic. Cervical biopsy revealed an invasive squamous cell carcinoma

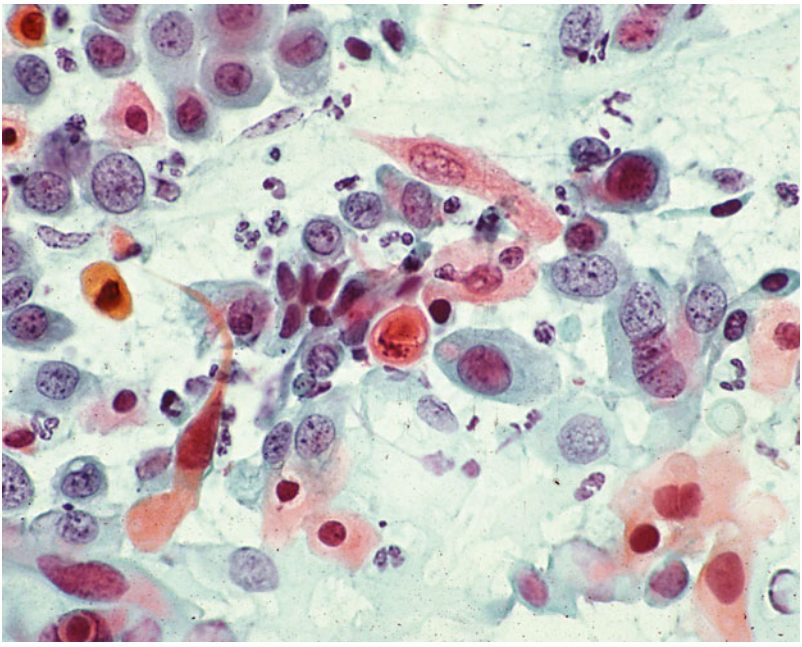

Fig. 5.57 Squamous cell carcinoma, keratinizing (*CP*). There is marked pleomorphism of cell size and shape, cytoplasmic keratinization, and tumor diathesis in the background

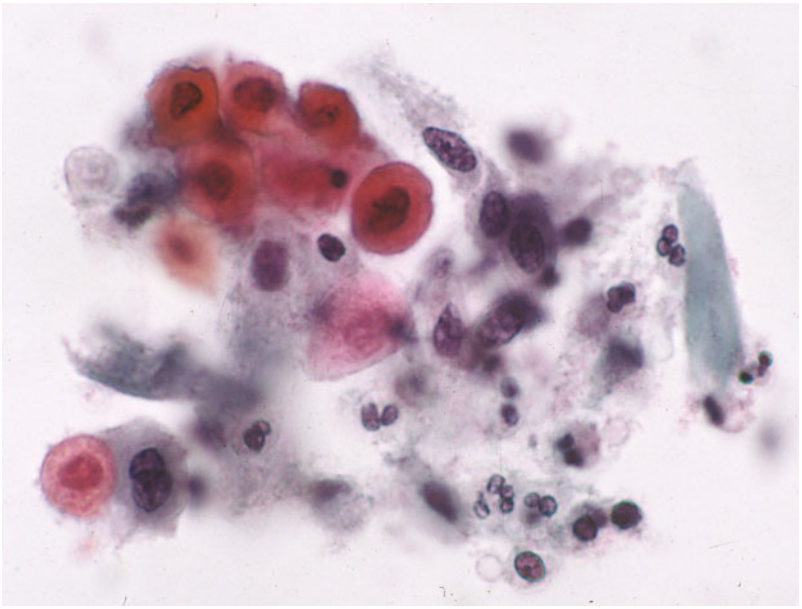

Fig. 5.58 Squamous cell carcinoma, keratinizing (*LBP*, *ThinPrep*). A 68-year-old woman. Diathesis may be more subtle in *LBPs* and often tends to collect at the periphery of cell groups, a pattern that has been referred to as "clinging diathesis." Follow-up showed squamous cell carcinoma

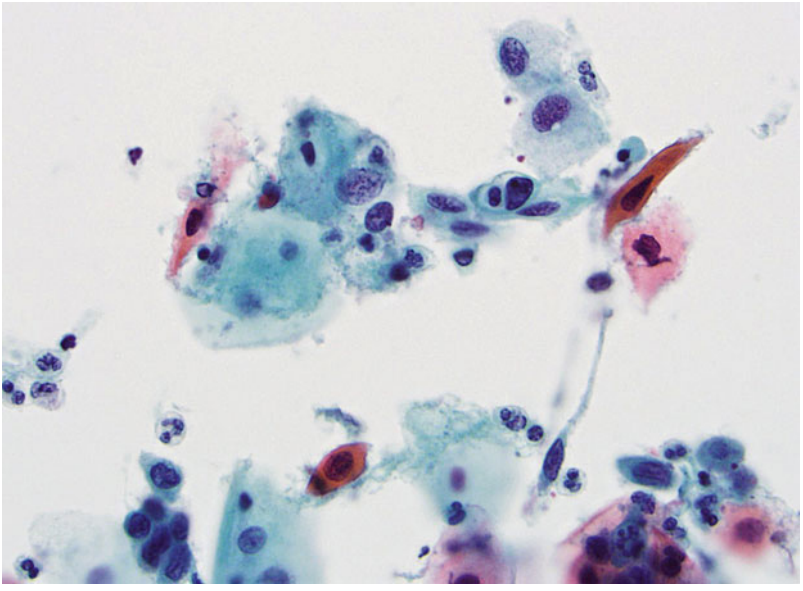

Fig. 5.59 Squamous cell carcinoma, keratinizing (*LBP*, *ThinPrep*). A 57-year-old woman. Note the tumor diathesis, abnormal keratinized cells, and spindle cells. Follow-up biopsy revealed invasive squamous cell carcinoma

5.12.3 Nonkeratinizing Squamous Cell Carcinoma (Figs. 5.60–5.63)

5.12.3.1 Criteria

Cells occur singly or in syncytial aggregates with poorly defined cell borders (Fig. 5.60).

Cells may be somewhat smaller than those of many HSIL, but display most of the features of HSIL.

Nuclei demonstrate markedly irregular distribution of coarsely clumped chromatin with chromatin clearing.

Nucleoli may be prominent (Fig. 5.61).

A tumor diathesis consisting of necrotic debris and broken-down blood elements is often present.

Preparation-Specific Criteria

Liquid-Based Preparations:

Often characterized by lower tumor cellularity [43].

Rounding up of individual cells and cell groups in LBPs may impart a glandular appearance to squamous tumors, leading to a misinterpretation of adenocarcinoma (Figs. 5.62 and 5.63).

Diathesis is usually identifiable, but can be subtle compared to conventional smears; necrotic material often collects at the periphery of the cell groups, referred to as "clinging diathesis," as opposed to being distributed in the background as is seen in conventional preparations [44, 45] (Fig. 5.58).

5.12.4 Explanatory Notes

Invasive squamous cell carcinoma is the most common malignant neoplasm of the uterine cervix. The 2014 WHO terminology classifies squamous cell carcinoma into keratinizing, nonkeratinizing, papillary, basaloid, warty, verrucous, squamotransitional, and lymphoepithelioma-like categories [9]. These divisions are defined by histologic patterns which are often not clearly distinctive on cytologic specimens. In addition, the prognosis does not vary between the variants, but is defined predominantly by the stage of the disease; hence, these distinctions are not necessary in the cytopathology report.

Historically, "small cell carcinoma" comprised a heterogeneous group of neoplasms, including poorly differentiated squamous cell carcinoma, as well as tumors demonstrating neuroendocrine features (often of the small cell or "oat cell" type). Current classifications limit the use of the term "small cell carcinoma" to non-squamous tumors with evidence of high-grade neuroendocrine differentiation. Such tumors, similar to their counterparts in the lung, are categorized separately from squamous cell carcinoma in the 2014 WHO terminology [9] (see Chap. 7).

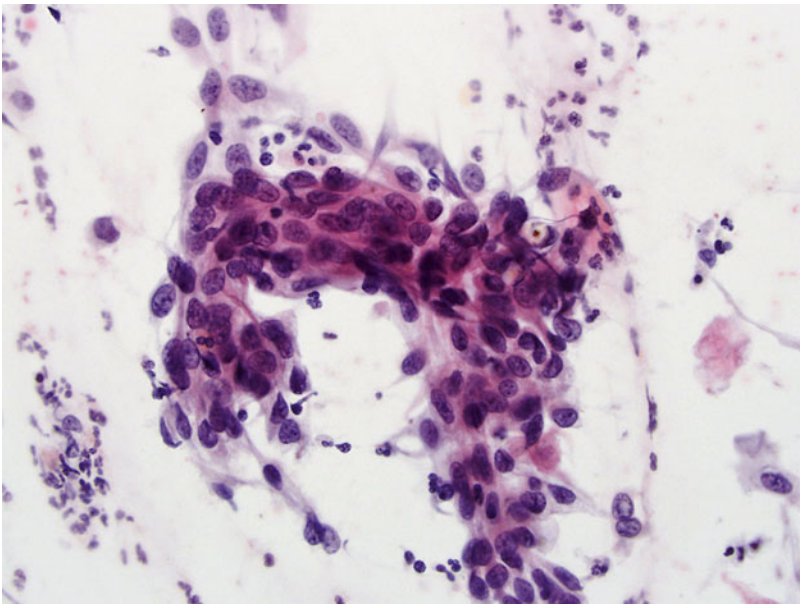

Fig. 5.60 Squamous cell carcinoma, nonkeratinizing (*CP*). These dysplastic cells demonstrate nuclear features of HSIL. Pleomorphic cell shapes should raise concern for invasion even though prominent nucleoli and tumor diathesis are absent in this field. Follow-up cervical biopsy revealed an invasive squamous cell carcinoma

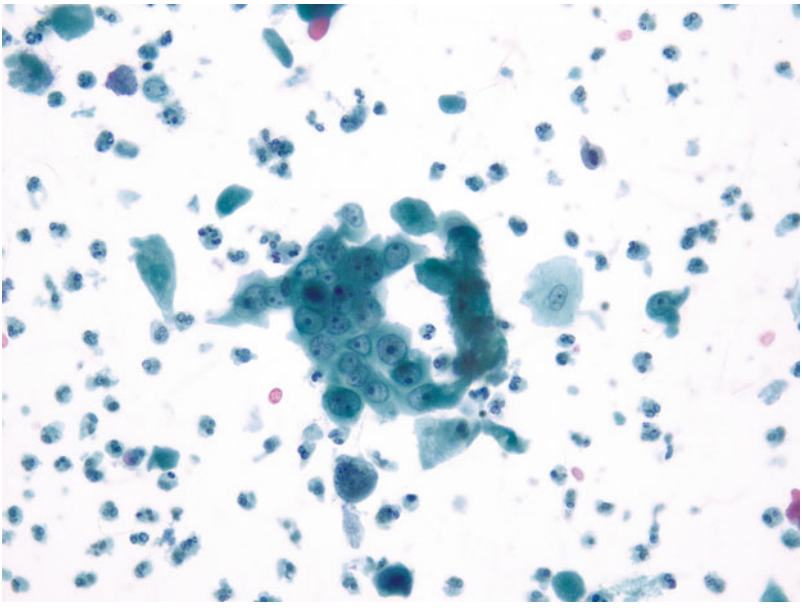

Fig. 5.61 Squamous cell carcinoma, nonkeratinizing (*LBP*, *SurePath*). A 59-year-old woman with postmenopausal bleeding. Abnormal nuclei are present with prominent nucleoli and irregular chromatin distribution. Single abnormal cells are also seen. There is a tumor diathesis present in the background. Follow-up revealed a nonkeratinizing squamous cell carcinoma of the cervix

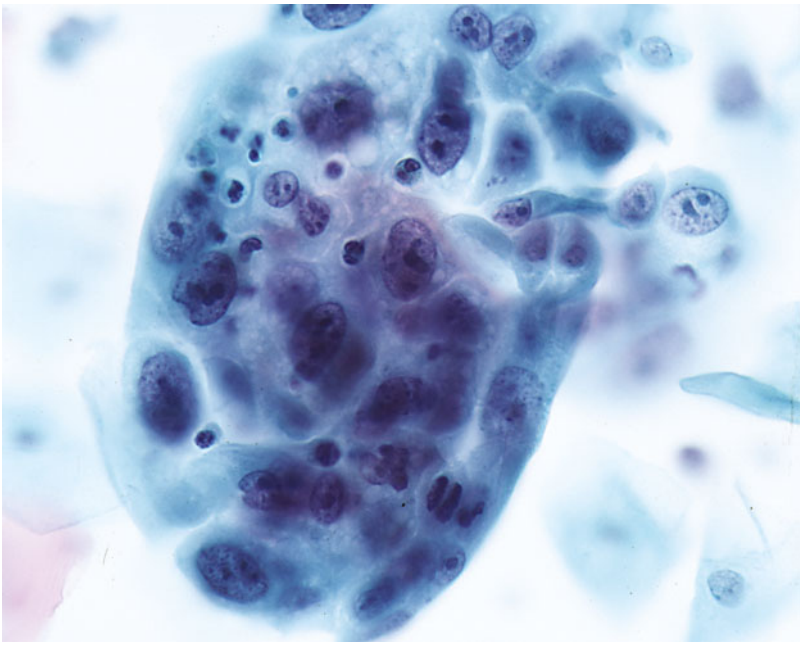

Fig. 5.62 Squamous cell carcinoma (*LBP*, *SurePath*). Malignant cell clusters tend to show more rounding on *LBPs*, and distinction between a squamous and glandular lesion may be difficult. Attention should be given to looking for isolated neoplastic cells in the background

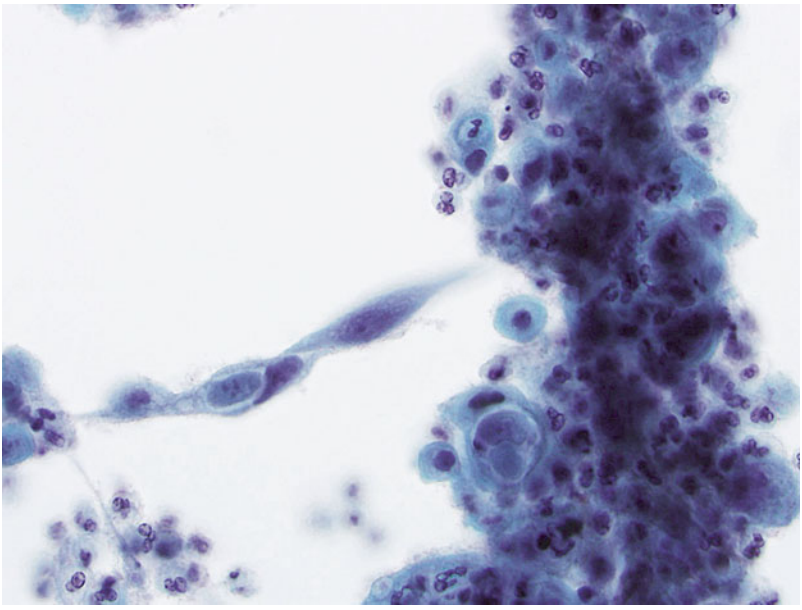

Fig. 5.63 Squamous cell carcinoma, nonkeratinizing (*LBP*, *ThinPrep*). A 63-year-old woman with postmenopausal bleeding. Clusters of cells and single abnormal cells are identified with a background of inflammatory cells. Follow-up revealed a nonkeratinizing squamous cell carcinoma of the uterine cervix

5.12.5 Problematic Patterns and Pitfalls Associated with Squamous Cell Carcinoma

5.12.5.1 Low Cellularity Specimens and Cases with Obscuring Blood (Figs. 5.64 and 5.65)

Specimens from squamous carcinoma are often bloody and may be scantly cellular to the point of being technically unsatisfactory. It is always important to screen these unsatisfactory specimens carefully to make sure a significant lesion is not missed. Bloody ThinPrep samples may prematurely clog the filter resulting in essentially acellular preparations with large holes in the center of the circle. Bloody ThinPrep samples may be treated with glacial acetic acid which often results in a satisfactory sample [46] (Figs. 5.64 and 5.65).

5.12.5.2 Atypical Repair (Fig. 5.66)

Nucleoli are indicative of cellular metabolic activity and as such are commonly seen in the nuclei of both squamous carcinoma and in benign reparative or reactive epithelial cells. Carcinoma is distinguished from repair by less cellular cohesion and the presence of isolated cells, more marked nuclear abnormalities, irregular chromatin distribution with clearing, abnormal mitoses, and the presence of a tumor diathesis. In extreme cases of repair (so-called atypical repair), the similarity of morphologic features with invasive carcinoma may be striking

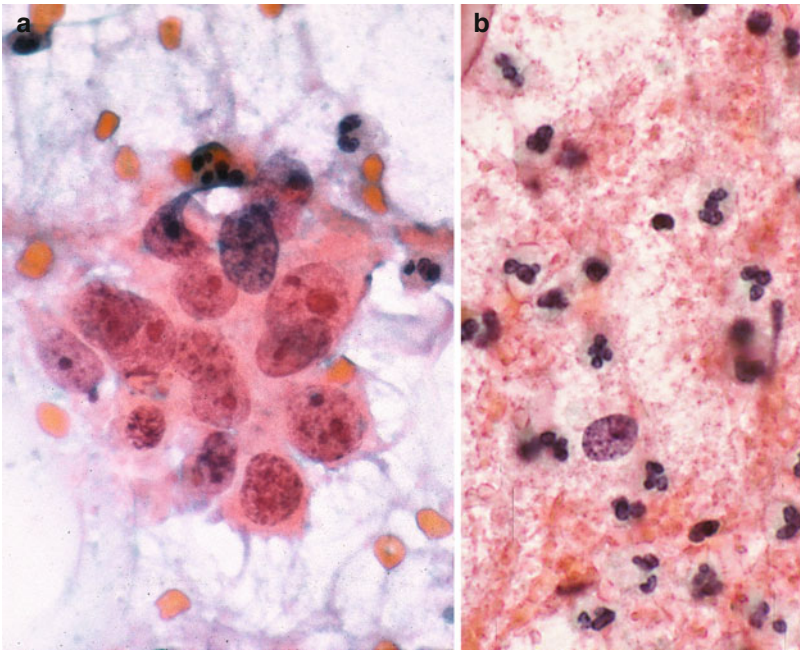

Fig. 5.64 Squamous cell carcinoma (*CP*). There is tumor diathesis in the background and prominent nucleoli in the malignant cells (*left*). On the right, from a different case, tumor diathesis is prominent, and only a naked nucleus is seen in this field (*right*)

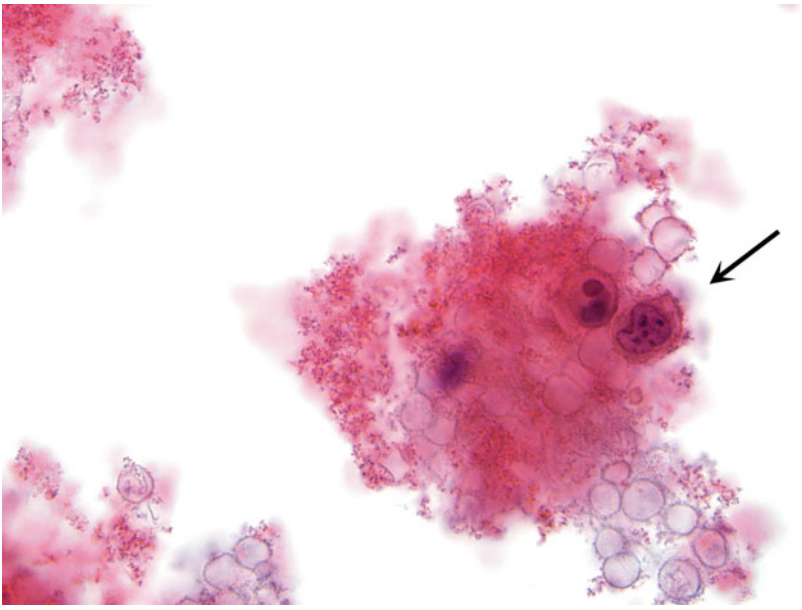

Fig. 5.65 Squamous cell carcinoma (*LBP*, *ThinPrep*). Bloody samples are often seen in the presence of squamous carcinoma. Because the blood may clog the filter of the *ThinPrep*, these samples may be very scantly cellular and technically unsatisfactory. Bloody unsatisfactory specimens should still be screened closely to look for rare abnormal cells buried in the blood as seen here (*arrow*). Re-prepping these samples with glacial acetic acid may yield a more cellular preparation

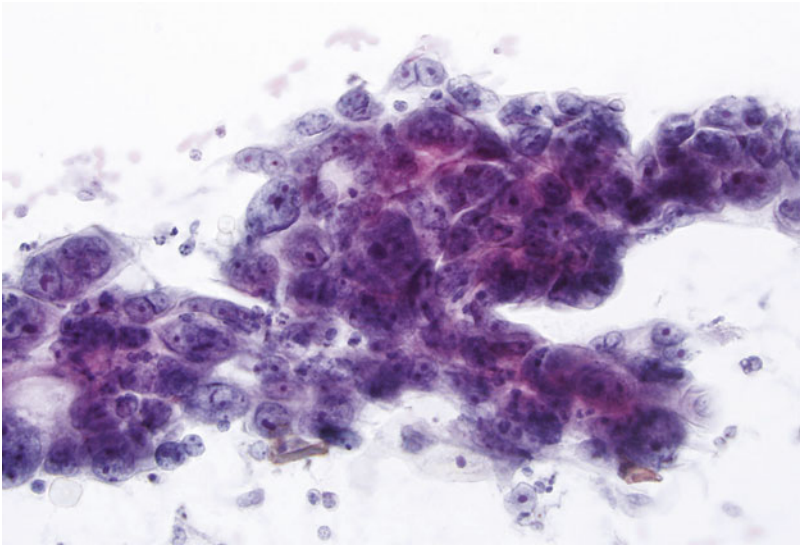

Fig. 5.66 Atypical repair (*CP*). A 48-year-old woman with a normal screening history. Prominent nucleoli are identified in virtually every nucleus. The cells are cohesive and lack irregular chromatin distribution. Atypical repair is a differential in the diagnosis of carcinoma of the cervix

(Fig. 5.66). Hence atypical repair warrants very close examination, along with clinicopathologic correlation and designation as abnormal so that appropriate follow-up can ensue [47].

5.12.5.3 Tumor Diathesis Mimics (Figs. 5.67 and 5.68)

Invasive carcinomas are often associated with tumoral and native tissue necrosis and its associated inflammatory reaction. Necrotic debris, inflammatory cells, and blood are therefore routinely present in the background of cytology specimens from invasive tumors (so-called tumor diathesis). In conventional preparations, diathesis material is spread evenly in the background of the slide. In liquid-based specimens, diathesis material tends to aggregate into balls or clings to the surfaces of cellular material (so-called "clinging" diathesis).

A variety of background patterns from nonneoplastic conditions can simulate tumor diathesis. Atrophic specimens often have a diffuse background of amorphous granular debris which may be associated with significant inflammation (Fig. 5.67, and see Fig. 2.24). Cases of irritated endocervical polyps, which often have areas of surface ulceration, may show necrotic and inflammatory debris that can be very difficult to distinguish from tumor diathesis. Lubricant material can simulate diathesis

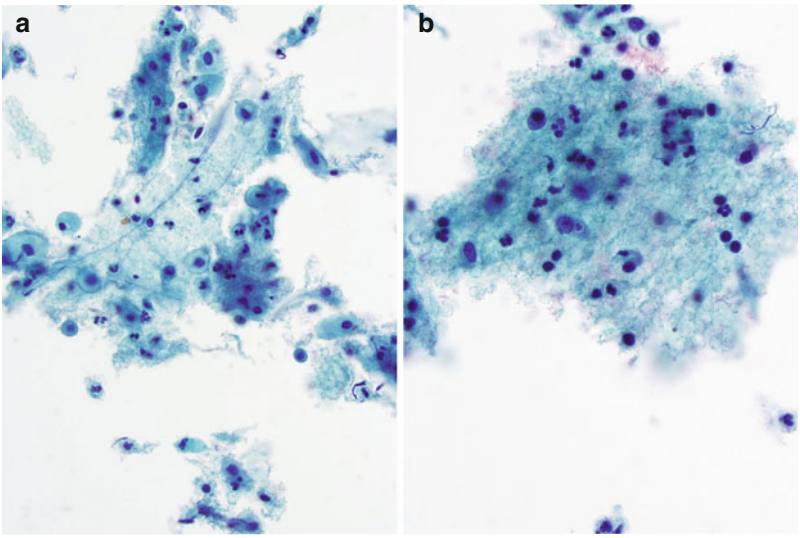

Fig. 5.67 Diathesis look-alike (*LBP*, *ThinPrep*). (**a**) *Left*, a 66-year-old postmenopausal woman. Routine cervical cytology. (**b**) *Right*, a 39-year-old woman on day 12 of menstrual cycle. The background of atrophy (*left*) and inflammatory debris (*right*) can mimic tumor diathesis. Lack of hyperchromatic crowded groups and atypical pleomorphic keratinized cells should aid in the correct interpretation

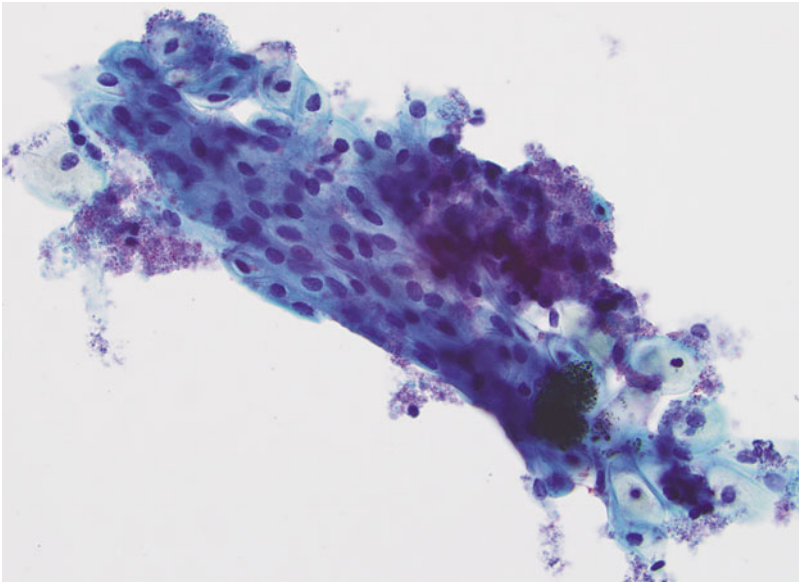

Fig. 5.68 Diathesis look-alike (*LBP*, *ThinPrep*). A 63-year-old postmenopausal woman. Lubricant may be used in Pap test collection and presents as granular debris that may mimic tumor diathesis

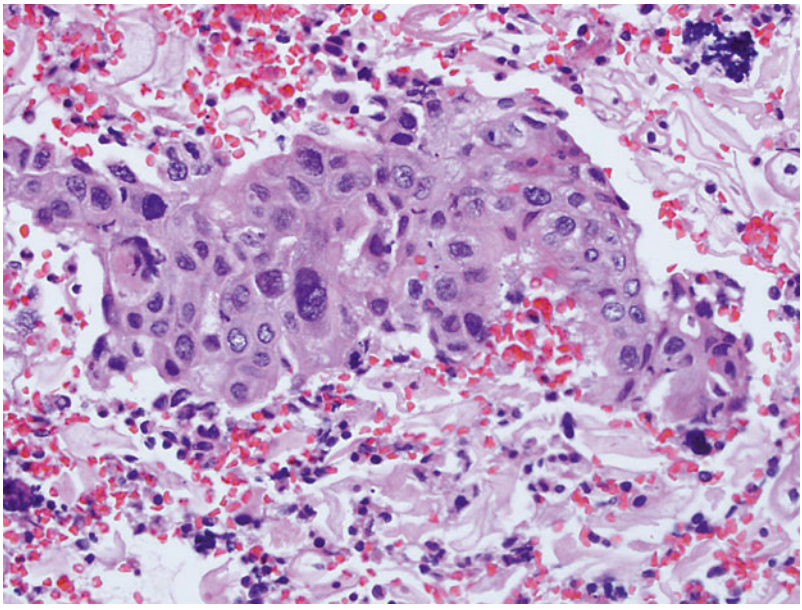

Fig. 5.69 Squamous cell carcinoma, cell block (*cell block*, *H&E stain*). A 57-year-old postmenopausal woman with irregular bleeding. A cell block preparation was made from the residual *ThinPrep* vial. An abnormal cluster of cells with dense pink cytoplasm and abnormal nuclei is seen. Follow-up cervical biopsy revealed an invasive squamous cell carcinoma

with granular material which can "cling" to cells similar to the diathesis pattern in liquid-based specimens (Fig. 5.68; see Fig. 1.25).

5.12.6 Squamous Cell Carcinoma Versus Adenocarcinoma (Fig. 5.69)

Nonkeratinizing squamous cell carcinoma can occasionally show features which make differentiation from adenocarcinoma (particularly of endocervical origin) difficult (see Figs. 5.61 and 5.62). In more poorly differentiated tumors which may present predominantly as hyperchromatic crowded groups lacking organoid architectural features, abundant dense cytoplasm, or evidence of overt keratinization, the use of cell blocks made from residual liquid-based material may be helpful. Histologic sectioning of the dense groups allows for better visualization of cytoplasmic features which are the key to differential diagnosis (Fig. 5.69) [48].

5.13 Sample Reports

Example 1
Adequacy Statement
Satisfactory for evaluation; endocervical/transformation zone present
Interpretation
Epithelial cell abnormality: squamous
Low-grade squamous intraepithelial lesion (LSIL)

Note: Further follow-up as clinically warranted (Massad LS, et al. 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. Obstet Gynecol. 2013;121:829–46)

Example 2
General Categorization
Epithelial cell Abnormality: squamous
Adequacy
Satisfactory for evaluation
Interpretation
High-grade squamous intraepithelial lesion (HSIL)

Note: Suggest colposcopic examination (with endocervical assessment) as clinically indicated (Massad LS, et al. 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. Obstet Gynecol. 2013;121:829–46).

Example 3
Report for a Postmenopausal Woman
Adequacy
Satisfactory for evaluation; endocervical/transformation zone not identified

Interpretation
Epithelial cell abnormality: squamous
Low-grade squamous intraepithelial lesion arising in an atrophic background

Note: Suggest colposcopy/biopsy, hrHPV testing or repeat cytology at 6 & 12 months. (Massad LS, et al. 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. Obstet Gynecol. 2013;121:829–46).

Example 4
Satisfactory for evaluation; endocervical/transformation zone present
Interpretation
Epithelial cell abnormality: squamous
Atypical squamous cells cannot exclude a high-grade squamous intraepithelial lesion (ASC-H). Background of low-grade squamous intraepithelial lesion (LSIL)
See Note.

Note: Predominantly LSIL with rare abnormal cells suggesting a high-grade lesion (HSIL). Suggest colposcopy/biopsy.

References

1. Bosch FX, Lorincz A, Munoz N, et al. The causal relation between human papillomavirus and cervical cancer. J Clin Pathol. 2002;55:244–65.
2. Munoz N, Bosch FX, de Sanjose S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med. 2003;348:518–27.
3. Castle PE, Gage JC, Wheeler CM, Schiffman M. The clinical meaning of a cervical intraepithelial neoplasia grade 1 biopsy. Obstet Gynecol. 2011;118:1222–9.
4. Ho GY, Bierman R, Beardsley L, et al. Natural history of cervicovaginal papillomavirus infection in young women. N Engl J Med. 1998;338:423–8.
5. Ylitalo N, Josefsson A, Melbye M, et al. A prospective study showing long-term infection with human papillomavirus 16 before the development of cervical carcinoma in situ. Cancer Res. 2000;60:6027–32.
6. Schlecht NF, Kulaga S, Robitaille J, et al. Persistent human papillomavirus infection as a predictor of cervical intraepithelial neoplasia. JAMA. 2001;286:3106–14.
7. Ellerbrock TV, Chiasson MA, Bush TJ, et al. Incidence of cervical squamous intraepithelial lesions in HIV-infected women. JAMA. 2000;283:1031–7.
8. Darragh TM, Colgan TJ, Cox JT, Heller DS, Henry MR, Luff RD, et al. For The Members of LAST Project Work Groups. The lower anogenital squamous terminology standardization project for HPV-associated lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. Arch Pathol Lab Med. 2012;136:1266–97.
9. Kurman RJ, Carcangiu ML, Herrington CS, Young RH. WHO classification of tumours of female reproductive organs. 4th ed. Lyon: IARC; 2014.
10. Ismail SM, Colelough AB, Dinnen JS, et al. Observer variation in histopathological diagnosis and grading of cervical intraepithelial neoplasia. Br Med J. 1989;298:707–10.
11. Robertson AJ, Anderson JM, Beck JS, et al. Observer variability in histopathological reporting of cervical biopsy specimens. J Clin Pathol. 1989;42:231–8.

12. Schneider V. Symposium part 2: should the Bethesda system terminology be used in diagnostic surgical pathology? Counterpoint. Int J Gynecol Pathol. 2002;22:13–7.
13. Syrjänen K, Kataja V, Yliskoski M, et al. Natural history of cervical human papillomavirus lesions does not substantiate the biologic relevance of the Bethesda system. Obstet Gynecol. 1992;79:675–82.
14. Mitchell MF, Tortolero-Luna G, Wright T, et al. Cervical human papillomavirus infection and intraepithelial neoplasia: a review. J Natl Cancer Inst Monogr. 1996;21:17–25.
15. Woodhouse SL, Stastny JF, Styer PE, et al. Interobserver variability in subclassification of squamous intraepithelial lesions: results of the College of American Pathologists Interlaboratory Comparison Program in cervicovaginal cytology. Arch Pathol Lab Med. 1999;123:1079–84.
16. Cox JT, Solomon D, Schiffman M. Prospective follow-up suggests similar risk of subsequent CIN 2 or 3 among women with CIN 1 or negative colposcopy and directed biopsy. Am J Obstet Gynecol. 2003;188:1406–12.
17. Eversole GM, Moriarty AT, Schwartz MR, Clayton AC, Souers R, Fatheree LA, et al. Practices of participants in the College of American Pathologists Interlaboratory Comparison Program in cervicovaginal cytology, 2006. Arch Pathol Lab Med. 2010;134:331–5.
18. Massad LS, Einstein MH, Huh WK, Katki HA, Kinney WK, Schiffman M, et al. For The 2012 ASCCP Consensus Guidelines Conference. 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. Obstet Gynecol. 2013;121:829–46.
19. Committee on Adolescent Health Care. ACOG committee opinion no. 436: evaluation and management of abnormal cervical cytology and histology in adolescents. Obstet Gynecol. 2009;113:1422–5.
20. Kadish AS, Burk RD, Kress V, et al. Human papillomavirus of different types in precancerous lesions of the uterine cervix: histologic, immunocytochemical and ultrastructural studies. Hum Pathol. 1986;17:384–92.
21. Willett GD, Kurman RJ, Reid R, et al. Correlation of the histological appearance of intraepithelial neoplasia of the cervix with human papillomavirus types. Int J Gynecol Pathol. 1989;8:18–25.
22. Wright TC, Ferenczy AF, Kurman RJ. Precancerous lesions of the cervix. In: Kurman RJ, editor. Blaustein's pathology of the female genital tract. 5th ed. New York: Springer; 2002. p. 253–354.
23. Stoler MH, Schiffman M. Atypical Squamous Cells of Undetermined Significance-Low-Grade Squamous Intraepithelial Lesion Triage Study (ALTS) Group. Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL Triage Study. JAMA. 2001;285:1500–5.
24. The ALTS Group. Human papillomavirus testing for triage of women with cytologic evidence of low-grade squamous intraepithelial lesions: baseline data from a randomized trial. J Natl Cancer Inst. 2000;92:397–402.
25. Hoda RS, Loukeris K, Abdul-Karim FW. Gynecologic cytology on conventional and liquid-based preparations: a comprehensive review of similarities and differences. Diagn Cytopathol. 2013;41:257–78.
26. Demay RM. Hyperchromatic crowded groups: pitfalls in pap smear diagnosis. Am J Clin Pathol. 2000;114(Suppl):S36–43.
27. Selvaggi SM. Cytologic features of squamous cell carcinoma in situ involving endocervical glands in endocervical cytobrush specimens. Acta Cytol. 1994;38:687–92.
28. Selvaggi SM. Cytologic features of high-grade squamous intraepithelial lesions involving endocervical glands on ThinPrep cytology. Diagn Cytopathol. 2002;26:181–5.
29. Zafar N, Balazs L, Benstein BD. Synchronous high-grade squamous intraepithelial lesion and adenocarcinoma in situ of cervix in a young woman presenting with hyperchromatic crowded groups in the cervical cytology specimen: report of a case. Diagn Cytopathol. 2008;36:823–6.
30. Frable WJ. Litigation cells: definition and observations on a cell type in cervical/vaginal smears not addressed by the Bethesda System. Diagn Cytopathol. 1994;11:213–5.

31. Bosch MM, Rietveld-Scheffers PE, Boon ME. Characteristics of false-negative smears tested in the normal screening situation. Acta Cytol. 1992;36:711–6.
32. Yang YJ, Trapkin LK, Demoski RK, Bellerdine J, Powers CN. The small blue cell dilemma associated with tamoxifen therapy. Arch Pathol Lab Med. 2001;125:1047–50.
33. Barron S, Li Z, Austin RM, Zhao C. Low-grade squamous intraepithelial lesion/cannot exclude high-grade squamous intraepithelial lesion (LSIL-H) is a unique category of cytologic abnormality associated with distinctive HPV and histopathologic CIN 2+ detection rates. Am J Clin Pathol. 2014;141:239–46.
34. Elsheikh TM, Kirkpatrick JL, Wu HH. The significance of "low-grade squamous intraepithelial lesion, cannot exclude high-grade squamous intraepithelial lesion" as a distinct squamous abnormality category in Papanicolaou tests. Cancer. 2006;108:277–81.
35. Ince U, Aydin O, Peker O. Clinical importance of "low-grade squamous intraepithelial lesion, cannot exclude high-grade squamous intraepithelial lesion (LSIL-H)" terminology for cervical smears 5-year analysis of the positive predictive value of LSIL-H compared with ASC-H, LSIL, and HSIL in the detection of high-grade cervical lesions with a review of the literature. Gynecol Oncol. 2011;121:152–6.
36. Zhou H, Schwartz MR, Coffey D, Smith D, Mody DR, Ge Y. Should LSIL-H be a distinct cytology category?: a study on the frequency and distribution of 40 human papillomavirus genotypes in 808 women. Cancer Cytopathol. 2012;120:373–9.
37. Nasser SM, Cibas ES, Crum CP, Faquin WC. The significance of the Papanicolaou smear diagnosis of low-grade squamous intraepithelial lesion cannot exclude high-grade squamous intraepithelial lesion. Cancer. 2003;99:272–6.
38. Thrall MJ, Galfione SK, Smith DA. The impact of LSIL-H terminology on patient follow-up patterns: a comparison with LSIL and ASC-H. Diagn Cytopathol. 2013;41:960–4.
39. Walavalkar V, Tommet D, Fischer AH, Liu Y, Papa DM, Owens CL. Evidence for increasing usage of low-grade squamous intraepithelial lesion, cannot exclude high-grade squamous intraepithelial lesion (LSIL-H) Pap test interpretations. Cancer Cytopathol. 2014;122:123–7.
40. Adams KC, Absher KJ, Brill YM, et al. Reproducibility of subclassification of squamous intraepithelial lesions: conventional versus ThinPrep Paps. J Lower Genital Tract Disease. 2003;7:203–8.
41. Covell JL, Frierson Jr HF. Intraepithelial neoplasia mimicking microinvasive squamous-cell carcinoma in endocervical brushings. Diagn Cytopathol. 1992;8:18–22.
42. Jones BA, Novis DA. Cervical biopsy-cytology correlation. A College of American Pathologists Q-Probes study of 22, 439 correlations in 348 laboratories. Arch Pathol Lab Med. 1996;120:523–31.
43. Clark SB, Dawson AE. Invasive squamous-cell carcinoma in ThinPrep specimens: diagnostic clues in the cellular pattern. Diagn Cytopathol. 2002;26:1–4.
44. Inhorn SL, Wilbur D, Zahniser D, Linder J. Validation of the ThinPrep Papanicolaou test for cervical cancer diagnosis. J Lower Genital Tract Disease. 1998;2:208–12.
45. Renshaw AA, Young NA, Colgan TJ, et al. Comparison of performance of conventional and ThinPrep gynecologic preparations in the College of American Pathologists gynecologic cytology program. Arch Pathol Lab Med. 2004;128:17–22.
46. Rowe LR, Bentz JS. A simple method to determine the need for glacial acetic acid treatment of bloody ThinPrep Pap tests before slide processing. Diagn Cytopathol. 2004;31:321–5.
47. Renshaw AA, Davey DD, Birdsong GG, Walsh M, Styer PE, Mody DR, et al. Precision in gynecologic cytologic interpretation: a study from the College of American Pathologists Interlaboratory Comparison Program in cervicovaginal cytology. Arch Pathol Lab Med. 2003;12:1413–20.
48. Sakamoto H, Takenaka M, Ushimaru K, Tanaka T. Use of Liquid-Based Cytology (LBC) and cell blocks from cell remnants for cytologic, immunohistochemical, and immunocytochemical diagnosis of malignancy. Open J Pathol. 2012;3:58–65.