# The Bethesda System for Reporting Cervical Cytology

## Definitions, Criteria, and Explanatory Notes

Third Edition

Ritu Nayar
David C. Wilbur
*Editors*

Springer

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# Foreword

It is a privilege, a pleasure, and something of a surprise for me to write this Foreword to the third edition of the Cervical Cytology Bethesda System Atlas. I never imagined that a small meeting on the campus of the National Institutes of Health in Bethesda, Maryland, one snowy weekend in December 1988 would begin a process that has changed the practice of cervical cytology – in both the laboratory and the clinician's office – around the world. This third edition of the atlas continues that evolution, presenting the latest refinements to the Bethesda System (TBS) in a convenient easy-to-use reference designed to be accessible for cytopathologists and cytotechnologists regardless of laboratory setting.

The initial Bethesda System workshop was convened to address a well-recognized but seemingly intractable problem of variability in laboratory reports of Papanicolaou smears [1]. Different laboratories used a multiplicity of terms including, in many cases, Pap class numbers, with confusing and idiosyncratic modifications, or dysplasia terminology with multiple, poorly reproducible gradations including a biologically inaccurate distinction between changes induced by human papillomavirus (HPV) and what was considered "true dysplasia." Additionally, a non-reproducible distinction between severe dysplasia and carcinoma in situ was sometimes used clinically to decide if a hysterectomy should be performed.

The first Bethesda workshop, ably chaired by Dr. Robert Kurman, convened roughly three dozen laboratorians, clinicians, and research scientists with the goal of finding a better way. Over 2 days, the following fundamental principles emerged that have guided the Bethesda System from that day to this:

1. Terminology used by the laboratory must communicate appropriate and clinically relevant information to the clinician
2. Terminology should be consistent from one laboratory to another and reasonably reproducible in practice but at the same time be flexible enough to be adapted in a wide variety of laboratories and geographic settings
3. Terminology should be continuously updated to reflect the most current understanding of the pathobiology of cervical neoplasia and integrate advances in laboratory practice

With these principles in mind, the workshop participants developed terminology based on the underlying pathobiology of the morphologic changes of cervical

epithelial abnormalities. Squamous intraepithelial lesion (SIL) with only two gradations (low and high grade) reflected the different biologic states of productive HPV infections versus lesions with a higher risk of transitioning to precancer and ultimately cancer. In addition to the SIL terminology, TBS also introduced the concept of a "statement of adequacy" of the specimen as an integral part of the report and an important quality assurance element. The new terminology was named after the location of the workshop in Bethesda, Maryland.

Fast-forward 25 years:

Additional Bethesda System workshops were convened in 1991 and 2001, and the first two editions of this atlas were published in 1994 and 2004 [2, 3]. Each of these events was part of the continuing evolution of both scientific knowledge and clinical practice, in particular:

1. A major recommendation from the 1991 workshop was that criteria should be developed for the diagnostic terms and for the determination of specimen adequacy, which led to the publication of the first atlas [2].
2. The workshop in 2001 was the first to utilize the Internet in order to provide everyone an opportunity for input; over 2,000 comments were considered prior to the meeting, which then brought together over 400 participants including representatives from over two dozen countries [4].
3. Developments in laboratory practice and the transition for many to liquid-based cytology led to incorporating images and criteria specific to these preparations in the 2004 atlas [3].

Of all the changes introduced by TBS, none has been as controversial as "atypical squamous cells of undetermined significance" or ASC-US. ASC-US highlighted the inherent limitations of morphologic interpretation. Cytologic findings may be equivocal, resulting in frustration for clinicians who need to be able to make clear-cut management decisions. As ASC-US was (and still is) the most common cytologic abnormality reported for millions of women in the USA annually, this posed a significant clinical problem and threatened to overwhelm the available colposcopy services.

In response, the US National Cancer Institute sponsored a clinical trial, the ASCUS-LSIL Triage Study, or ALTS, to resolve the question of best practice [5]. The results of ALTS established molecular testing for HPV as the most cost-effective approach to clarify equivocal cytologic findings. HPV testing is now firmly integrated into algorithms both for primary cervical screening and cytology triage.

The results of ALTS and other clinical research have, in turn, informed the development of clinical management algorithms involving dozens of organizations and professional societies, spearheaded by the American Society for Colposcopy and Cervical Pathology, most recently in 2012 [6]. At a time when there were few test options for screening and evaluation of abnormal findings, management algorithms consisted of linear branch points based on a sequence of test results. With the multiplicity of testing options currently available, as well as additional assays on the horizon, various combinations of cytologic, molecular, and/or histopathologic test findings must now be integrated in order to determine an individual woman's risk for precancer/cancer and – based on that level of risk – her

appropriate management. A new chapter on a risk assessment-based management has been added to this atlas.

Beyond the field of cervical cytology, standardized terminology systems have now been developed for cytology of other body sites including thyroid [7] and pancreas [8], and most recently urine [9]. The two-tier terminology used in TBS has also been recommended for reporting histopathology of HPV-related squamous lesions of the lower anogenital tract [10, 11].

Terminology must evolve to keep pace with our insights into the basis of disease, to be responsive to the needs of the laboratory and clinician for clear communication, and ultimately to best serve women's health. True to the spirit of the underlying principles that guided the first Bethesda workshop, this third edition of the atlas refines the application of the Bethesda terminology based on experience gathered over the past decade, especially related to the morphology of liquid-based preparations and use of TBS in clinical practice.

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# Introduction

In the past decade, since the publication of the second edition of the Bethesda Atlas in 2004, considerable experience has been gained with the use and impact of the Bethesda terminology for cervical cytology in clinical practice. This includes additional experience with morphology on liquid-based preparations, further insights into HPV biology, implementation of HPV vaccination, and updated guidelines for cervical cancer screening and the management of abnormal cervical cytology and cancer precursors. Thus 2014 seemed to be the appropriate time for a review and update of the 2001 Bethesda System terminology and incorporation of revisions and additional information into this third edition of the Bethesda Atlas for cervical cytology.

Despite recent concern about the demise of the Papanicolaou test, as it gradually yields its role as a primary cervical cancer screening test to HPV and other biomarker testing, cervical cytology remains the most successful cancer prevention program ever devised. Its specificity will remain the cornerstone of future screening regimens, including those in women who have received HPV vaccination. Additionally, in many settings, cervical cytology will continue to be the first line screening test based on resources and local preferences. Hence, updating and further refinement of morphologic criteria for the great variety of entities seen in cervical cytology, both neoplastic and non-neoplastic, is an important function of this edition. Wide dissemination of this comprehensive and relatively inexpensive atlas will therefore serve to maximize the overall value of the test in all practice settings.

Since minimal changes were anticipated to the terminology recommended by the 2001 Bethesda System (TBS), there was no consensus workshop held in association with the 2014 Bethesda System update. Therefore, Dr. Ritu Nayar, President of the American Society of Cytopathology (ASC) in 2014, appointed a task force, chaired by Dr. David Wilbur (ASC President in 2002), which was comprised of a relatively small group of cytopathologists and clinicians/epidemiologists in order to expeditiously accomplish this task. Following literature review and formulation of the proposed new and expanded content for the atlas, a widely advertised Internet-based public open comment period was initiated within the international cytopathology community for a 3.5-month period lasting from March through mid-June of 2014. A total of 2454 responses were received from individuals in 59 countries spread over a broad demographic, on proposals from each of the atlas's 12 chapter-based

surveys. Excellent feedback was gathered on the proposed updates, which was compiled and reviewed by the chapter-based task force working groups. This process culminated in refinement of positions and content, which were then incorporated into the 2014 Bethesda System and this accompanying atlas.

This new edition of the atlas expands on the popular features of the prior editions [1, 2]. A portion of the text and images from the first and second editions have been retained for this edition, and credit is attributed to the individuals who participated in the 1988, 1991 and 2001 Bethesda Workshops and those who contributed to the resultant 1994 and 2004 Bethesda atlases (see Acknowledgments section). This edition has 12 chapters, 6 of which correspond to the major Bethesda interpretive categories, with the remainder being dedicated to other malignant neoplasms, anal cytology, reporting of adjunctive testing, computer-assisted screening, educational notes, and a new chapter on cervical cancer risk assessment. Each chapter consists of a background discussion, a description of definitions and cytologic criteria, brief explanatory notes that cover difficult morphologic patterns and mimics of epithelial lesions (where applicable), sample reports, and selected references. Cytologic criteria are described in general for all specimen types in every chapter, followed by any significant differences related to specific preparation types. (*Note that TBS does not endorse any particular methodology or manufacturer(s) for specimen collection, computer-assisted screening, adjunctive HPV or other testing).* New to this edition are increased content on basic disease biology as it pertains to each entity and discussions of the current clinical management guidelines.

Over 1000 images were evaluated for this atlas, including the 186 images from the second edition. The images went through a multistage review process; first by the relevant chapter group, and secondly by a cytopathologist/cytotechnologist subgroup of the Bethesda 2014 Task Force. Dr. Daniel Kurtycz is credited with the management of images collected for this edition of the atlas. The 370 illustrations in this third edition represent a spectrum of morphologic changes seen on both conventional smears and liquid-based preparations (LBPs); 56% are new images and 44% are from the prior two editions; 40% are conventional preparations and 60% are from LBPs. For LBP specimen illustrations, the figure legends specify which of the two commonly used methods is illustrated: ThinPrep™ (Hologic, Marlborough, MA) or BD SurePath™ (BD Diagnostics, Durham NC). Some images represent classic examples of an entity whereas others were selected to illustrate interpretive dilemmas or "borderline" morphologic features that may not be interpreted in the same way by all cytologists. A greater number and variety of "normal" findings as well as mimics of classic epithelial abnormalities are included in the third edition in order to provide a more complete representation of the morphologic variations that can be appreciated in cervical cytology specimens.

Prior to the publication of the second edition [2], selected images were posted on a website open to cytopathologists and cytotechnologists worldwide. This process was designed to evaluate inter-observer variability and to provide an educational tool for cytologists. Results of the Bethesda Interobserver Reproducibility Study (BIRST) can be viewed online and have also been published [3, 4]. To build on the information gathered from our experience with the BIRST project in 2003, we posted 85 of the

images from this atlas as "unknowns" on a website open to the cytopathology community. Data from this effort, in which over 850 participants submitted their answers online prior to the publication of this atlas, provides a realistic gauge of interpretive reproducibility. Information regarding the results of this exercise is available on the ASC website at www.cytopathology.org. While knowledge of normal morphology, its variations and epithelial abnormalities is essential, some degree of interobserver and interlaboratory variability in interpretation will always remain a reality [4, 5].

In parallel with the development of this third edition, a Bethesda 2014 website resource has also been developed by an ASC Bethesda Website Task Force under the direction of Drs. Daniel Kurtycz and Paul Staats. In addition to displaying all the illustrations that are used in this atlas, the website will contain many other examples of presentations and entities that could not be provided in this print version. The website group will also be exploring new avenues for delivery of the content which has been assembled during this update process. For further information on the Bethesda web atlas please go to the educational resources page on the American Society of Cytopathology website [6].

Although the Bethesda System was developed primarily for cervical cytology, specimens from other sites in the lower anogenital tract, such as the vagina and anus, may be reported using similar terminology. As in the 2001 Bethesda System, the terms "interpretation" or "result" are recommended instead of "diagnosis" in the heading of the cervical cytology report. This terminology is preferred because cervical cytology should be viewed primarily as a "*screening test, which in some instances may serve as a medical consultation by providing an interpretation that contributes to a diagnosis*." A patient's final diagnosis and management plan integrate not only the cervical cytology result but also the history, clinical findings, and other laboratory results such as molecular/biomarker testing and biopsy interpretations [2].

As in prior editions, the current editors and authors have committed to making the third edition affordable, and hence, widely accessible to all including practitioners in low resource environments. No honoraria or royalties will be accepted by the editors/authors for this work. The editors, the 2014 Bethesda System Task Force members, and all the dedicated cytologists who have contributed to this wonderful project over the past quarter of a century are delighted to come together to thank Drs. Diane Solomon and Robert Kurman for their pioneering vision in initiating the organization and implementation of the Bethesda System in 1988 [7, 8]. Indeed Bethesda's contributions and impact on the field of cervical cancer go far beyond just standardized reporting terminology. The Bethesda System formed the bedrock for the furthering of our understanding of HPV biology and provided the framework necessary for the development of systematic and evidence-based cervical cancer screening and management guidelines [8]. And finally, Bethesda brought the world together with one cytologic voice – now able to effectively communicate scientific and clinical data where previously such was difficult, if not impossible. Because of Bethesda, the interpretation of a high grade squamous intraepithelial lesion in the United States is based on exactly the same criteria as in India or anywhere else. On behalf of the American Society of Cytopathology, we, as a group are pleased to be

a part of this ongoing process and hope that the 2014 Bethesda System update and this corresponding expanded atlas will prove useful in your practice.

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# The 2014 BETHESDA SYSTEM FOR REPORTING CERVICAL CYTOLOGY

**SPECIMEN TYPE:**
*Indicate conventional smear (Pap smear) vs. liquid-based preparation vs. other*

**SPECIMEN ADEQUACY**

- Satisfactory for evaluation (*describe presence or absence of endocervical/transformation zone component and any other quality indicators, e.g., partially obscuring blood, inflammation, etc.*)
- Unsatisfactory for evaluation . . . (*specify reason*)
  - Specimen rejected/not processed (*specify reason*)
  - Specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormality because of (*specify reason*)

**GENERAL CATEGORIZATION** (optional)

- Negative for Intraepithelial Lesion or Malignancy
- Other: See Interpretation/Result (*e.g., endometrial cells in a woman ≥45 years of age*)
- Epithelial Cell Abnormality: See Interpretation/Result (*specify* 'squamous' *or* 'glandular' *as appropriate*)

**INTERPRETATION/RESULT**

**NEGATIVE FOR INTRAEPITHELIAL LESION OR MALIGNANCY**
(W*hen there is no cellular evidence of neoplasia, state this in the General Categorization above and/or in the Interpretation/Result section of the report--whether or not there are organisms or other non-neoplastic findings*)

NON-NEOPLASTIC FINDINGS (*optional to report optional to report; list not inclusive*)

- Non-neoplastic cellular variations
  - Squamous metaplasia
  - Keratotic changes
  - Tubal metaplasia
  - Atrophy
  - Pregnancy-associated changes

- Reactive cellular changes associated with:
  - Inflammation (includes typical repair)
    - Lymphocytic (follicular) cervicitis
  - Radiation
  - Intrauterine contraceptive device (IUD)
- Glandular cells status post hysterectomy

ORGANISMS
- *Trichomonas vaginalis*
- Fungal organisms morphologically consistent with *Candida* spp.
- Shift in flora suggestive of bacterial vaginosis
- Bacteria morphologically consistent with *Actinomyces* spp.
- Cellular changes consistent with herpes simplex virus
- Cellular changes consistent with cytomegalovirus

**OTHER**

- Endometrial cells (*in a woman ≥45 years of age*)
  (*Specify if "negative for squamous intraepithelial lesion"*)

**EPITHELIAL CELL ABNORMALITIES**

SQUAMOUS CELL

- Atypical squamous cells
  - of undetermined significance (ASC-US)
  - cannot exclude HSIL (ASC-H)
- Low-grade squamous intraepithelial lesion (LSIL)
  (*encompassing: HPV/mild dysplasia/CIN 1*)
- High-grade squamous intraepithelial lesion (HSIL)
  (*encompassing: moderate and severe dysplasia, CIS; CIN 2 and CIN 3*)
  - with features suspicious for invasion (*if invasion is suspected*)
- Squamous cell carcinoma

GLANDULAR CELL

- Atypical
  - endocervical cells (NOS *or specify in comments*)
  - endometrial cells (NOS *or specify in comments*)
  - glandular cells (NOS *or specify in comments*)
- Atypical
  - endocervical cells, favor neoplastic
  - glandular cells, favor neoplastic

- Endocervical adenocarcinoma in situ
- Adenocarcinoma
  - endocervical
  - endometrial
  - extrauterine
  - not otherwise specified (NOS)

**OTHER MALIGNANT NEOPLASMS:** *(specify)*

**ADJUNCTIVE TESTING**

*Provide a brief description of the test method(s) and report the result so that it is easily understood by the clinician.*

**COMPUTER-ASSISTED INTERPRETATION OF CERVICAL CYTOLOGY**

*If case examined by an automated device, specify device and result.*

**EDUCATIONAL NOTES AND COMMENTS APPENDED TO CYTOLOGY REPORTS (*optional*)**

*Suggestions should be concise and consistent with clinical follow-up guidelines published by professional organizations (references to relevant publications may be included).*

# Acknowledgements

**Bethesda System Committee Members and Contributors to Bethesda Atlas, First edition**
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# Contents

# Abbreviations

| | |
|---|---|
| ACOG | American College of Obstetricians and Gynecologists |
| ACS | American Cancer Society |
| AGC | Atypical glandular cells |
| AIN | Anal intraepithelial neoplasia |
| AIS | Adenocarcinoma in situ |
| ALTS | ASCUS–LSIL Triage Study |
| APK | Atypical parakeratosis |
| ASC | Atypical squamous cells |
| ASCCP | American Society for Colposcopy and Cervical Pathology |
| ASC-H | Atypical squamous cells cannot exclude an HSIL |
| ASC-US | Atypical squamous cells of undetermined significance |
| ASIL | Anal squamous intraepithelial lesions |
| CAP | College of American Pathologists |
| CDC | Centers for Disease Control |
| CIN | Cervical intraepithelial neoplasia |
| CMV | Cytomegalovirus |
| cNPV | Complement of the negative predictive value |
| CP | Conventional preparation |
| DARE | Digital anorectal exam |
| DES | Diethylstilbestrol |
| ECA | Epithelial cell abnormality |
| EC/TZ | Endocervical/transformation zone |
| FDA | Food and Drug Administration |
| FOV | Fields of view |
| HCG | Hyperchromatic crowded groups |
| hpf | High–power field |
| HPV | Human papillomavirus |
| HRA | High–resolution anoscopy |
| hrHPV | High–risk human papillomavirus |
| HSIL | High–grade squamous intraepithelial lesions |
| IUD | Intrauterine contraceptive device |
| LAST | Lower Anogenital Squamous Terminology |
| LBP | Liquid–based preparation |
| LEEP | Loop electrosurgical excision procedure |

| | |
|---|---|
| LMP | Last menstrual period |
| LSIL | Low–grade squamous intraepithelial lesion |
| LUS | Lower uterine segment |
| MMMT | Malignant Müllerian mixed tumor |
| N/C | Nuclear/cytoplasmic |
| NILM | Negative for intraepithelial lesion or malignancy |
| NOS | Not otherwise specified |
| nsc | Nucleated squamous cells |
| PNET | Ewing/primitive neuroectodermal tumors |
| PPV | Positive predictive value |
| SCC | Squamous cell carcinoma |
| SCJ | Squamocolumnar junction |
| SIL | Squamous intraepithelial lesion |
| TBS | The Bethesda system |
| UCSF | University of California–San Francisco |
| USPSTF | United States Preventive Services Task Force |

# Specimen Adequacy

# 1

George G. Birdsong and Diane Davis Davey

**Adequacy Categories**

***Satisfactory***

*Satisfactory for evaluation*

*(describe presence or absence of endocervical/transformation zone component and any other quality indicators, e.g., partially obscuring blood, inflammation, etc., as appropriate)*

***Unsatisfactory***

For unsatisfactory specimens, indicate whether or not the laboratory has processed/evaluated the slide. Suggested wording:

A. *Rejected specimen:*
*Specimen rejected (not processed) because _____ (specimen not labeled, slide broken, etc.)*
B. *Fully evaluated, unsatisfactory specimen:*
*Specimen processed and examined but unsatisfactory for evaluation of epithelial abnormality because of _____ (obscuring blood, etc.)*

Additional comments/recommendations, as appropriate

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## 1.1 Background

Evaluation of specimen adequacy is considered by many to be the single most important quality assurance component of the Bethesda system. The first two versions of the Bethesda terminology included three categories of adequacy: satisfactory, unsatisfactory, and a "borderline" category initially termed "less than optimal" and then renamed "satisfactory but limited by" in 1991. The 2001 Bethesda system eliminated the borderline category, in part, because of confusion among clinicians as to the appropriate follow-up for such findings and also due to the variability in criteria used to report "satisfactory but limited by" among laboratories [1]. To provide a clearer indication of adequacy, specimens are now designated as either "satisfactory" or "unsatisfactory."

Prior to the 2001 Bethesda system (TBS), criteria for determining adequacy were based entirely on expert opinion and the few available studies in the literature. Laboratory implementation of some of these criteria was shown to be poorly reproducible [2–4]. In addition, the increasing use of liquid-based cytology necessitated developing criteria applicable to these preparations. The 2001 Bethesda adequacy criteria were based on published data to the extent possible and were tailored to both conventional and liquid-based preparations. For this edition of the TBS atlas, data and clinical experience regarding specimen adequacy since 2001 were reviewed, leading to the offering of additional guidance for special situations, such as assessing cellularity in specimens obtained from postradiation patients, interfering substances and human papillomavirus testing.

### 1.1.1 Explanatory Notes

For satisfactory specimens, information on transformation zone sampling and other adequacy qualifiers should also be included in the report. Providing clinicians/specimen takers with regular feedback on specimen quality promotes heightened attention to specimen collection with consideration for the use of improved sampling devices and preparation technologies.

Any specimen with abnormal cells (atypical squamous cells of undetermined significance (ASC-US), atypical glandular cells (AGC), or worse) is by definition satisfactory for evaluation. If there is concern that the specimen is compromised, a note may be appended indicating that a more severe abnormality cannot be excluded.

Unsatisfactory specimens that are processed and evaluated require considerable time and effort on the part of the laboratory. Although an epithelial abnormality cannot be excluded in such specimens, reporting of information such as the presence of organisms, or endometrial cells in women 45 years of age or older, etc. (see Chap. 3), may help direct further patient management [5]. Note that the presence of benign endometrial cells at any age does not make an otherwise unsatisfactory specimen satisfactory.

Longitudinal studies looking at both conventional and liquid-based preparations found that unsatisfactory specimens that were processed and evaluated were more often from high-risk patients, and a significantly greater number of these were followed by a squamous intraepithelial lesion (SIL) or cancer when compared to a cohort of satisfactory index specimens [6–8]. Unsatisfactory cases which are hrHPV positive have been reported to have a much higher risk for precancerous lesions than those that are hrHPV negative [8].

## 1.2 Minimum Squamous Cellularity Criteria

### 1.2.1 Cellularity

There is no further evidence since the last Bethesda System update in 2001, to support adjustment of the minimum cellularity requirements for routine cervical cytology screening and follow-up. However, published literature and laboratory practice experience since the 2001 Bethesda workshop demonstrates ongoing confusion regarding the minimum cellularity estimates in special circumstances. Cytologists have often applied rigid minimum cellularity estimates to vaginal and postradiation or post-chemotherapy specimens, leading to a high unsatisfactory rate in these settings [9]. Quiroga-Garza found that almost half of 276 women with unsatisfactory results were over 50, and 85 % of these women had a history of gynecologic cancer. The most common cause for the unsatisfactory specimens was low squamous cellularity [10]. Women who have received radiation, chemotherapy, hysterectomy, or trachelectomy for invasive cancer often develop atrophic and reparative cellular changes, and when a cervix remains, there is frequently stenosis and altered anatomy [11]. There is little scientific evidence that a minimum cell threshold of 5,000 is required in these circumstances; some investigators recommend a lower threshold of 2,000 cells in these patients [12]. The 2001 Bethesda atlas stated that minimum cellularity criteria were developed for use with all cervical cytology specimens, but it is emphasized in this update that a 5,000 cell threshold should not be rigidly applied in vaginal and post-therapy specimens.

**Liquid-Based Preparations** (Figs. 1.1–1.11):

An adequate liquid-based preparation (LBP) from a woman with a cervix should have an estimated minimum of at least 5,000 well-visualized/well-preserved squamous or squamous metaplastic cells. This range applies only to squamous cells. Endocervical cells and completely obscured cells should be excluded from the estimate. Women who have had chemo- or radiation therapy, who are postmenopausal with atrophic changes, or who are post-hysterectomy may have samples with fewer than 5,000 cells, and such specimens may still be considered adequate at the discretion of the laboratory. The patient history must be taken into consideration in such cases. Samples with less than 2,000 cells, however, should be considered unsatisfactory in most circumstances.

Some have advocated that LBPs with 5,000–20,000 cells are of borderline or low squamous cellularity. In specimens with suspected low cellularity, an estimation of total cellularity can be obtained by performing representative field cell counts. A minimum of ten microscopic fields, usually at 40×, should be assessed along a diameter that includes the center of the preparation and the average number of cells per field estimated. When there are holes or empty areas on the preparation, the percentage of the hypocellular areas should be estimated, and the fields counted should reflect this proportion. Although both LBPs have similar numbers of cells overall, SurePath™ (BD Diagnostics, Durham, NC) slides have a higher cell density than do ThinPrep™ (Hologic, Inc., Bedford, MA) slides because of the smaller preparation diameter with SurePath™ (see Table 1.1). Siebers et al. evaluated several different protocols for estimation of low cellularity ThinPrep™ specimens and found that counting five fields along a horizontal diameter and five fields along a vertical diameter (SKML protocol) at 10× had the best correlation with a reference method that utilized image analysis software for counting cells [13]. However, when all of their measurements at different objective powers were merged, the differences between the SKML and the Bethesda protocols (as noted above) were not statistically significant.

Table 1.1 provides the average number of cells per field required to achieve a minimum of 5,000 cells on an LBP given the preparation diameter and field number of the eyepiece (ocular). For individuals using eyepieces and preparations not shown, the formula is: number of cells required per field = 5,000/(area of preparation/area of field). The diameters of SurePath and ThinPrep preparations are 13 and 20 millimeters (mm), respectively. The diameter of a microscopic field in millimeters is the field number of the eyepiece divided by the magnification of the objective. The area of the field is then determined by the formula used to calculate the area of a circle [pi × radius squared, $\pi r^2$]. The magnification power of the ocular does not affect this calculation [14, 15]. For additional explanation of the pertinent optical principles, see http://www.microscopyu.com/articles/formulas/formulasfieldofview.html.

Figures 1.1, 1.2, 1.3, 1.4, and 1.5 show cell coverage or density in unsatisfactory, borderline satisfactory, and satisfactory liquid-based preparations. These are *not* reference images, as they do not represent an entire microscopic field; thus, the cell density shown in the images cannot be compared directly to Table 1.1 for estimation of squamous cellularity.

In some instances, the cellularity on the prepared slide may not be representative of the collected sample. Slides with fewer than 5,000 cells should be examined to determine if the reason for the scant cellularity is a technical problem related to slide preparation such as an excessively bloody specimen. If a technical problem is identified and corrected, a repeat preparation may yield adequate cellularity (Fig. 1.6a, b). However, the adequacy of each slide should be determined separately and not cumulatively. Attempts to determine cellularity cumulatively by summing the cellularity of multiple inadequate slides may be confounded by uncertainty regarding the true cellularity of the specimen (not the slide), which might be substantially less than in a specimen with normal slide cellularity. This matter is in need of more research, and hence this guideline may be subject to change in the future. Given the relatively low minimum criterion for adequate cellularity, caution is warranted in borderline cases. The report should clarify whether blood, mucus, lubricant, inflammation, or technical artifact contributed to an unsatisfactory sample or whether the problem was simply low squamous cellularity.

**Table 1.1** Guidelines for estimating cellularity of liquid-based preparations

| | | FN20 eyepiece/10× objective | | FN20 eyepiece/40× objective | | FN22 eyepiece/10× objective | | FN22 eyepiece/40× objective | |
|---|---|---|---|---|---|---|---|---|---|
| Prep. diameter (mm) | Area ($mm^2$) | Number of fields at FN20, 10× | Number of cells/field for 5K total | Number of fields at FN20, 40× | Number of cells/field for 5K total | Number of fields at FN22, 10× | Number of cells/field for 5K total | Number of fields at FN22, 40× | Number of cells/field for 5K total |
| 13 | 132.7 | 42.3 | 118.3 | 676 | 7.4 | 34.9 | 143.2 | 559 | 9.0 |
| 20 | 314.2 | 100 | 50.0 | 1,600 | 3.1 | 82.6 | 60.5 | 1,322 | 3.8 |

*FN* field number

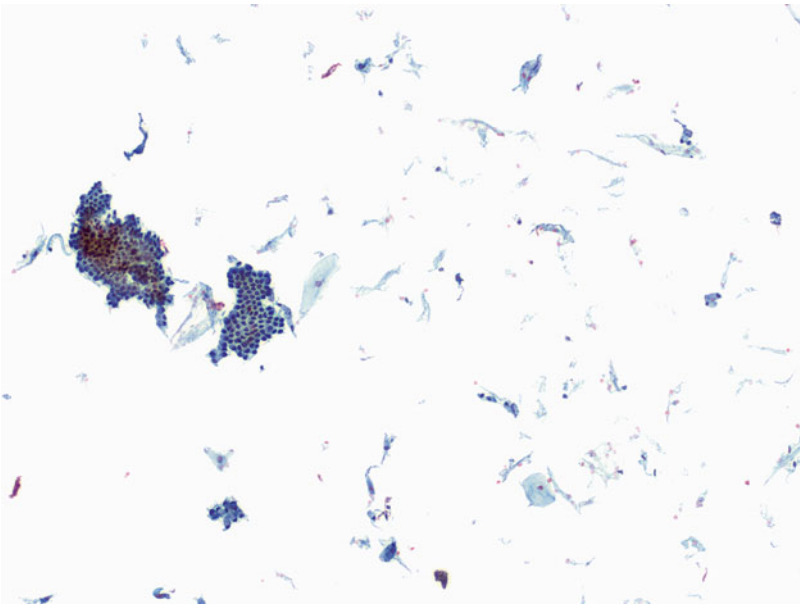

**Fig. 1.1** Unsatisfactory due to scant squamous cellularity. Endocervical cells are seen in a honeycomb arrangement (*LBP*, *ThinPrep* at 10× magnification)

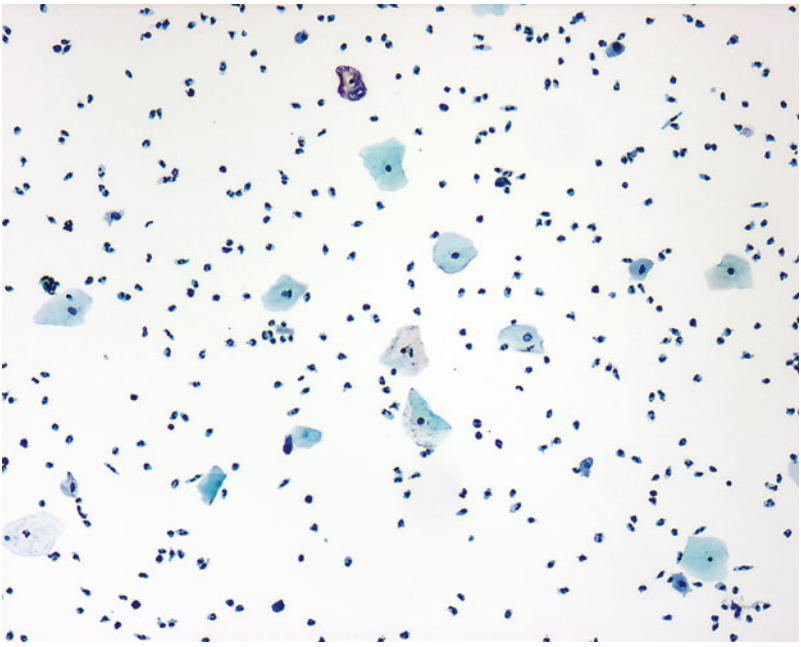

**Fig. 1.2** Unsatisfactory – scant cellularity (*LBP, SurePath*). Although this image cannot be directly compared to a microscopic field, this *SurePath* slide had fewer than 8 cells per 40× field. A *SurePath* specimen with this level of cellularity throughout the preparation would have fewer than 5,000 cells

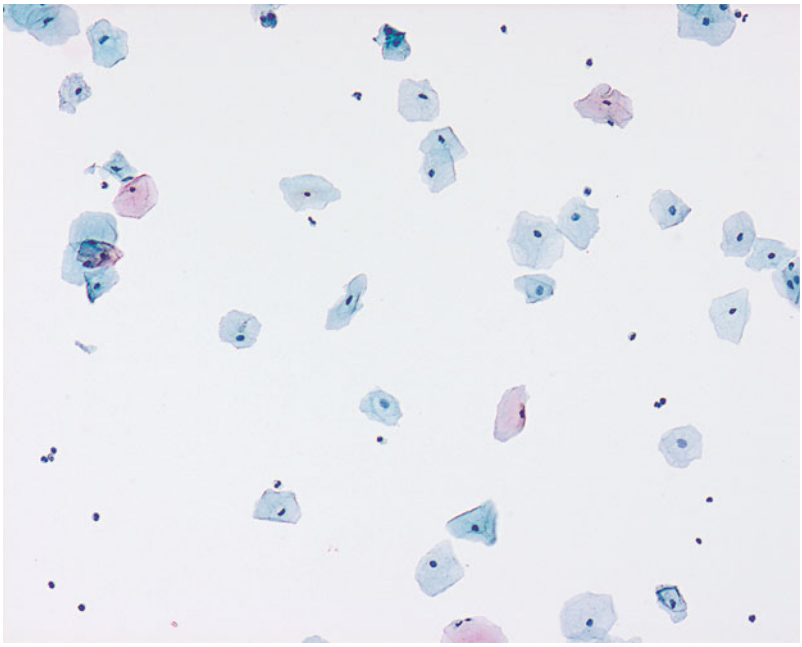

**Fig. 1.3** Satisfactory, but borderline squamous cellularity (*LBP*, *SurePath*). At 40×, there were approximately 11 cells per field when ten microscopic fields along a diameter were evaluated for squamous cellularity; this would give an estimated total cell count between 5,000 and 10,000

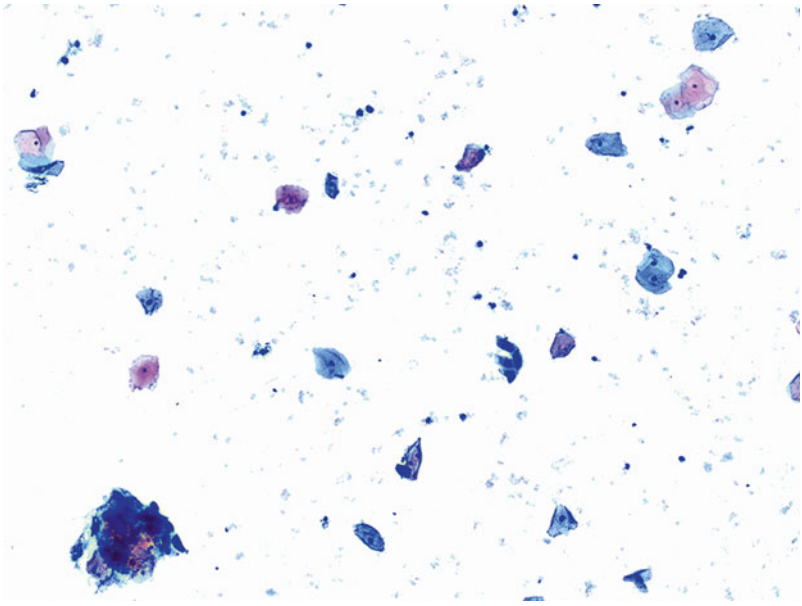

**Fig. 1.4** Satisfactory, but borderline squamous cellularity (*LBP*, *ThinPrep*): 10× fields of a *ThinPrep* specimen should have at least this level of cellularity to be considered satisfactory. At 40× magnification of this *ThinPrep* specimen, there were approximately four cells per field, which would correspond to slightly over 5,000 cells. Note that this level of cell density would be unsatisfactory in a *SurePath LBP* (see Fig. 1.2), corresponding to less than 5,000 cells because of the smaller preparation diameter

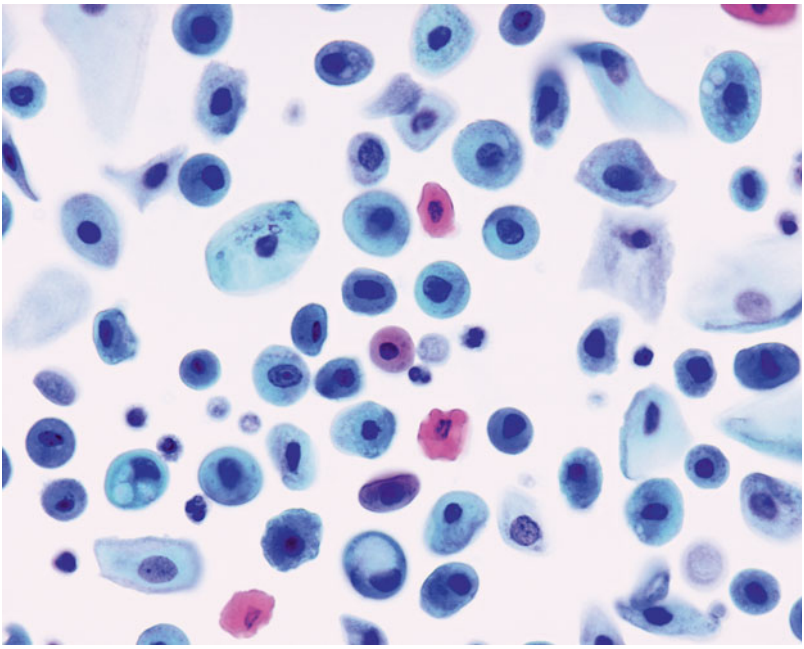

**Fig. 1.5** Squamous cellularity is satisfactory in this *LBP* from a 70-year-old woman with an atrophic cell pattern (*LBP*, *SurePath*). *LBP*s may show less nuclear enlargement than conventional preparations due to fixation in the suspended state. The transformation zone component(s) may be difficult to assess in atrophy

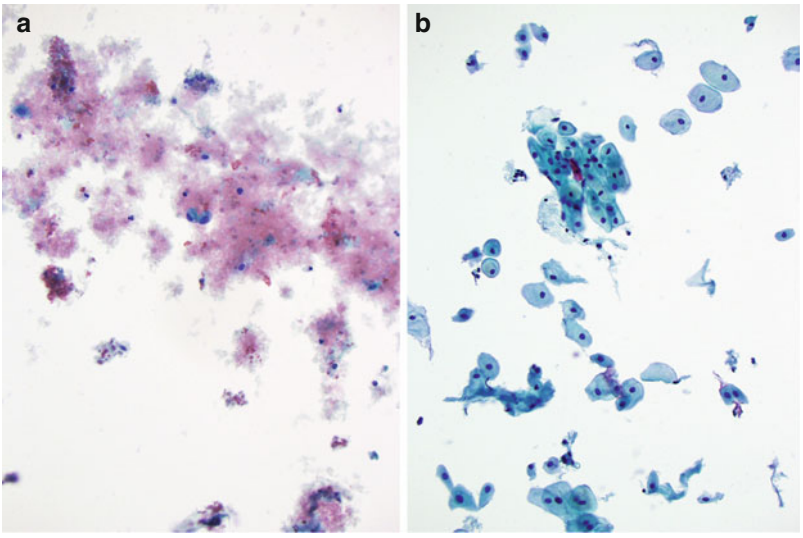


**Fig. 1.6** Unsatisfactory specimen reprocessing. Original preparation (**a**, *left*) from a 54-year-old woman was unsatisfactory due to scant squamous cellularity and excessive blood (*LBP*, *ThinPrep*). Reprocessing with glacial acetic acid resulted in a satisfactory sample (**b**, *right*)

**Fig. 1.7** Satisfactory vaginal cytology from a 56-year-old, status post total hysterectomy (with no cervix remaining) for endometrial adenocarcinoma (*LBP*, *ThinPrep*). Cellularity was estimated to be <5,000 but it was considered satisfactory since the source was vaginal

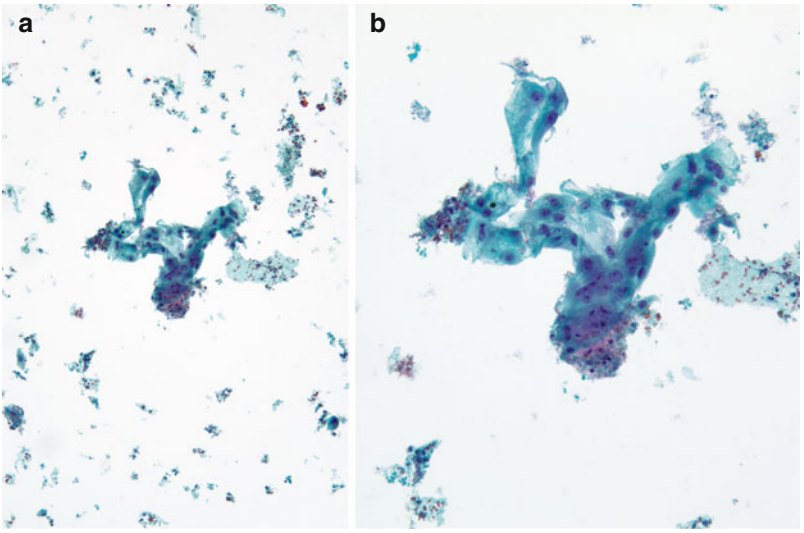


**Fig. 1.8** (**a**, **b**) Low-cellularity but satisfactory specimen in woman with history of radiation (*LBP*, *ThinPrep*; contributed by Fang Fan, MD)

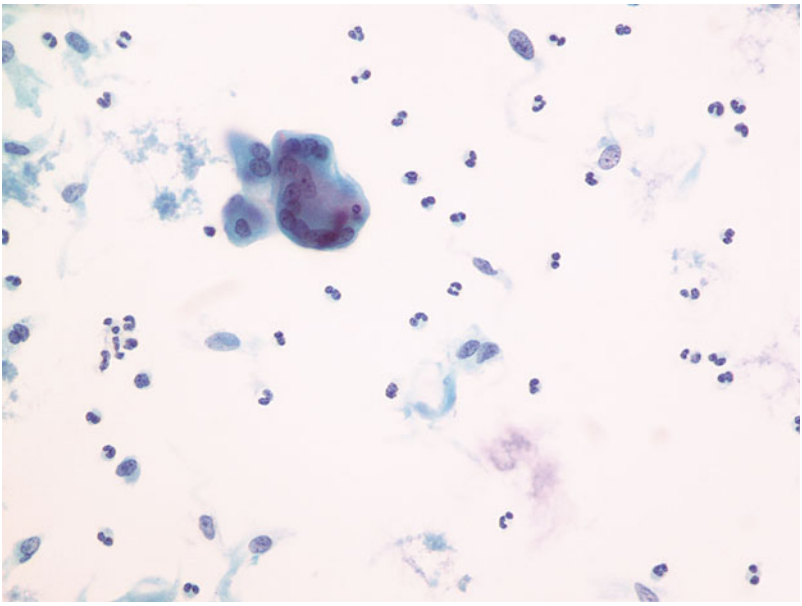

**Fig. 1.9** Low-cellularity but satisfactory specimen from a woman with history of pelvic radiation (*LBP*, *SurePath*)

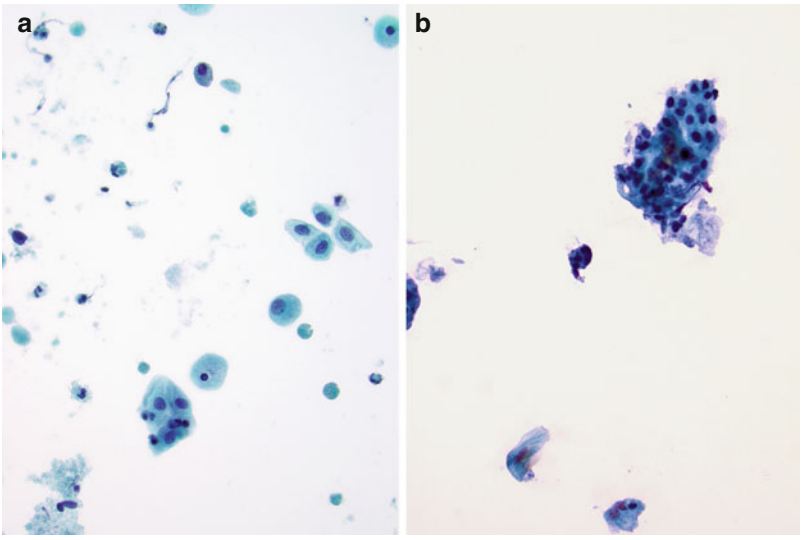


**Fig. 1.10** Atrophy: borderline cellularity in *LBP* preparations from two different postmenopausal women (*LBP*, *ThinPrep*). Parabasal cells can be seen isolated (**a**, *left*) or in clusters (**b**, *right*). It may be difficult to distinguish parabasal-type cells from squamous metaplastic cells in specimens showing atrophy due to a variety of hormonal changes including menopause, postpartum changes, and progestational agents

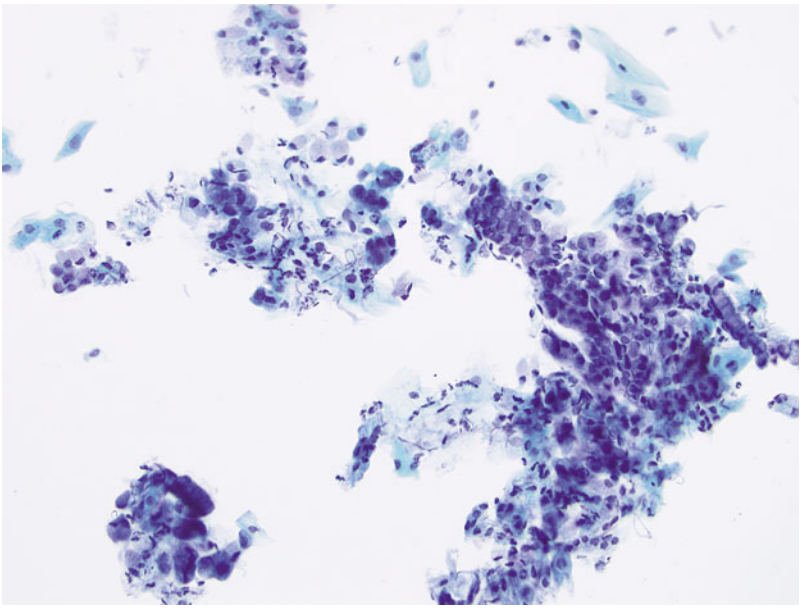

**Fig. 1.11** Unsatisfactory specimen from a 39-year-old woman (*LBP*, *ThinPrep*). Abundant endocervical cells and mucus are seen; however, the squamous component is inadequate

**Conventional Preparations** (Figs. 1.12–1.16):

An adequate conventional cervical specimen should contain an estimated minimum of approximately 8,000–12,000 well-preserved and well-visualized squamous epithelial cells. As was noted above for liquid-based preparations, this minimum cell range should be estimated, and laboratories should not count individual cells in conventionally prepared slides. This cellularity range should not be considered a rigid threshold and comments related to lower cellularity in post-therapy and vaginal specimens also apply to conventional preparations. "Reference images" of known cellularity are illustrated in Figs. 1.12, 1.13, 1.14, 1.15, and 1.16. These reference images have been computer edited to simulate the appearance of 4× fields on conventional preparations. Cytologists should compare these images to specimens in question to determine if there are a sufficient number of fields with approximately equal or greater cellularity than the reference images. For instance, if an image corresponding to a 4× field with 1,000 cells was used as the reference, a specimen would need to have at least eight such 4× fields to be deemed to have adequate cellularity.

## 1.2.2 Explanatory Notes

Strict objective criteria may not be applicable to every case. Some slides with cell clustering, atrophy, or cytolysis are technically difficult to count, and there may be clinical circumstances in which a lower cell number may be considered adequate.

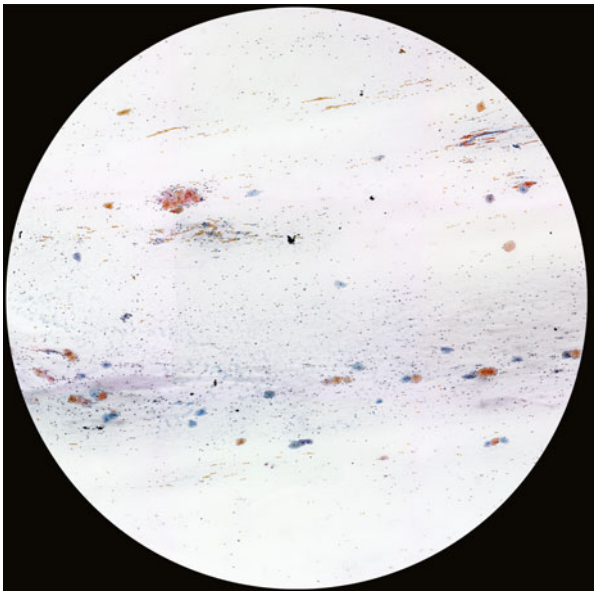

**Fig. 1.12** Squamous cellularity: this image depicts the appearance of a 4× field of a conventional preparation with approximately 75 cells. The specimen is unsatisfactory if all fields have this level, or less, of cellularity. It is to be used as a guide in assessing the squamous cellularity of a conventional smear (Used with permission, © George Birdsong, 2003)

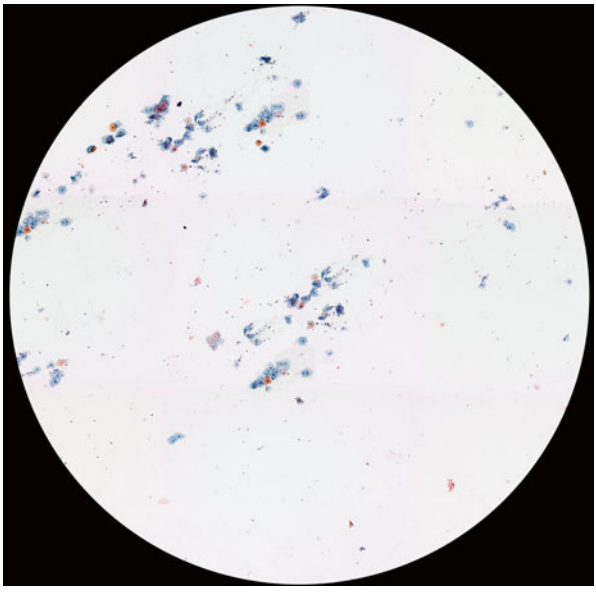

**Fig. 1.13** Squamous cellularity: this image depicts the appearance of a 4× field of a conventional preparation with approximately 150 cells. If all fields have this level of cellularity, the specimen will meet the minimum cellularity criterion, but by only a small margin (Used with permission, © George Birdsong, 2003)

Laboratories should apply professional judgment and employ hierarchical review when evaluating these uncommon borderline adequacy cases. It should also be kept in mind that *the minimum cellularity criteria described here were developed for use with cervical cytology specimens*.

The recommendation for a minimum cellularity of 5,000 cells for an LBP is based on relatively limited scientific evidence [16, 17]. This threshold is lower

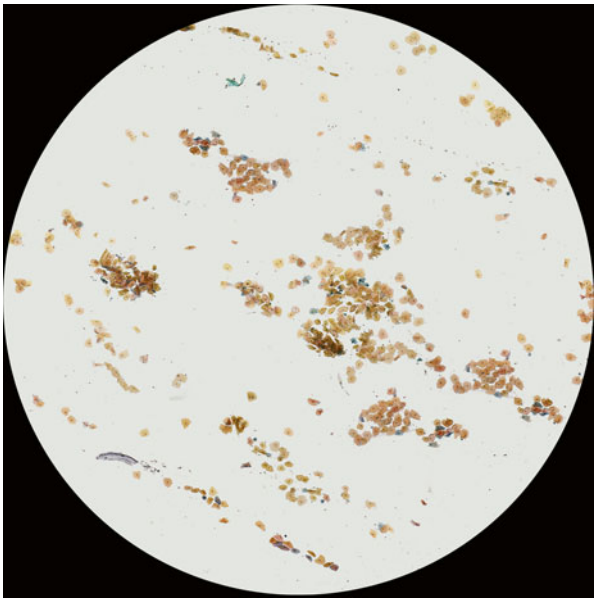

**Fig. 1.14** Squamous cellularity: this image depicts the appearance of a 4× field of a conventional preparation with approximately 500 cells. A minimum of 16 fields with similar (or greater) cellularity are needed to call the specimen adequate (Used with permission, © George Birdsong, 2003)

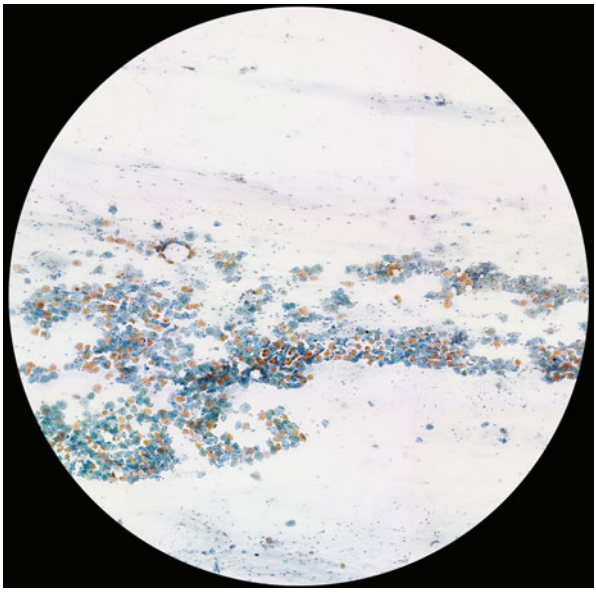

**Fig. 1.15** Squamous cellularity: this image depicts the appearance of a 4× field of a conventional preparation with approximately 1,000 cells. A minimum of eight fields with similar (or greater) cellularity are needed to call the specimen adequate (Used with permission, © George Birdsong, 2003)

than the 8,000–12,000 minimum cellularity for conventional preparations, because LBPs, by virtue of the preparation methodology, present a more random (and presumably more representative) sample of the collected cervical material. Although there are significant differences between ThinPrep and SurePath, there are not sufficient data to justify different minimum cellularities for the LBPs currently on the market.

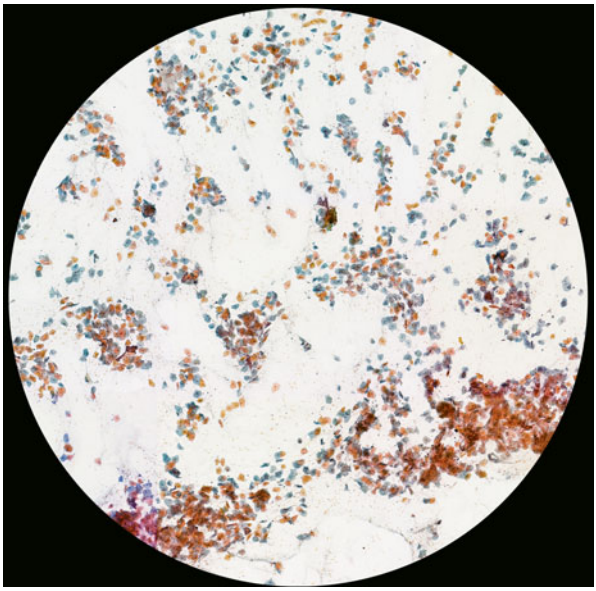

**Fig. 1.16** Squamous cellularity: this image depicts the appearance of a 4× field of a conventional preparation with approximately 1,400 cells. A minimum of six fields with similar (or greater) cellularity are needed to call the specimen adequate (Used with permission, © George Birdsong, 2003)

The relationship of number of cells present on a slide and the detection sensitivity for epithelial lesions has only been rarely investigated. One study reported a higher rate of detection of high-grade lesions when cellularity on LBPs exceeded 20,000 [18]. However, this study did not assess false-negative rates vs. cellularity. Investigators have attempted to perform seeding experiments to determine if there are minimal cellularity requirements for successful identification of abnormal cells in LBPs; however, no conclusions were reached, leading the authors to suggest that a pragmatic approach be maintained with minimum cellularity being set at 5,000–10,000 squamous cells [19]. Kitchener et al, in a recent, very thorough study involving 56 laboratories in the United Kingdom, assessed the relationships between cellularity, abnormal cell counts, and detection of abnormalities in liquid based cervical cytology preparations. They concluded that a minimum acceptable cell count of 15,000 and 5000 for SurePath and ThinPrep, respectively, would probably achieve the best balance between maintaining low levels of inadequate slides and not compromising the chances of detecting abnormalities. Although these suggested cell counts differ for the two preparation types, the proportion of slides which fell under the respective cutoffs were similar for the two preparations, and actually tended to be lower with SurePath [62]. Laboratories may choose to append a quality indicator comment such as "borderline or low squamous cellularity" for specimens that meet minimal criteria for satisfactory cellularity but have only 5,000–20,000 cells.

Cellularity can be quickly and reproducibly estimated in LBPs [16, 20]. Some manufacturers include estimation of LBP cellularity during training. Preliminary studies show that reference image methodology for conventional preparations is quickly learned and has better interobserver reproducibility than the previous Bethesda 10 % slide coverage criterion [21]. Additional studies relating sensitivity to cell number would be useful for all preparation types.

The College of American Pathologists (CAP) survey data shows that the 50th percentile rates for unsatisfactory specimens in US laboratories are 1.0, 1.1, and 0.3 % for conventional, ThinPrep, and SurePath preparations, respectively [22]. Unsatisfactory rates that significantly differ from these thresholds in an individual laboratory should prompt careful evaluation for the possible causes relating to sampling methodology, preparation technique, patient population, or interpretation thresholds.

## 1.3 Endocervical/Transformation Zone (EC/TZ) Component (Figs. 1.17–1.22)

The presence of transformation zone sampling is not necessary for an adequate specimen – only squamous cellularity, as noted above, is necessary. However, laboratories should report the presence or absence of a transformation zone component as it may be a useful quality assurance measure. For both conventional and liquid-based preparations, an adequate transformation zone sample requires at least ten well-preserved endocervical or squamous metaplastic cells, singly or in clusters (Figs. 1.17, 1.18, 1.19, 1.20, 1.21, and 1.22). The presence or absence of a transformation zone component is reported in the Specimen Adequacy section unless the woman has had a total hysterectomy. Degenerated cells in mucus and parabasal-type cells should not be counted in assessing transformation zone sampling. In such cases, the laboratory may elect to make a comment about the difficulty of assessing the transformation zone component. See Fig. 1.22 for discussion regarding difficulties in differentiating metaplastic and parabasal cells.

### 1.3.1 Explanatory Notes

In the past, there was concern that the squamocolumnar junction had not been adequately sampled when the cytology specimen lacked an EC/TZ component, implying that the region at greatest risk of showing SIL might not have been well represented. A negative cervical cytology test lacking cellular evidence of transformation zone sampling was thought to be at increased risk of being falsely negative. However, data on the importance of the endocervical/transformation zone (EC/TZ) component are conflicting. Cross-sectional studies show that SIL cells are more likely to be present on specimens in which EC/TZ cells are present [23–25]. Conversely, longitudinal studies have failed to show that women with negative cytology tests which lack an EC/TZ component have a higher risk of high-grade squamous intraepithelial lesions (HSIL) over time than women with negative tests that have an adequate EC/TZ component [26–31]. In one of these studies, a random sample of those with negative screening cytology and HPV tests (as well as women with any cytologic abnormalities or high-risk HPV) were offered repeat cytology, colposcopy, and biopsy. The follow-up results did not show a significant difference in detection of HSIL between patients who were EC/TZ negative in the initial screening evaluation and those who were EC/TZ positive [26]. Finally, retrospective case-control studies have failed to show an association between false-negative interpretations of specimens and lack of EC [32, 33]. A recent Canadian review

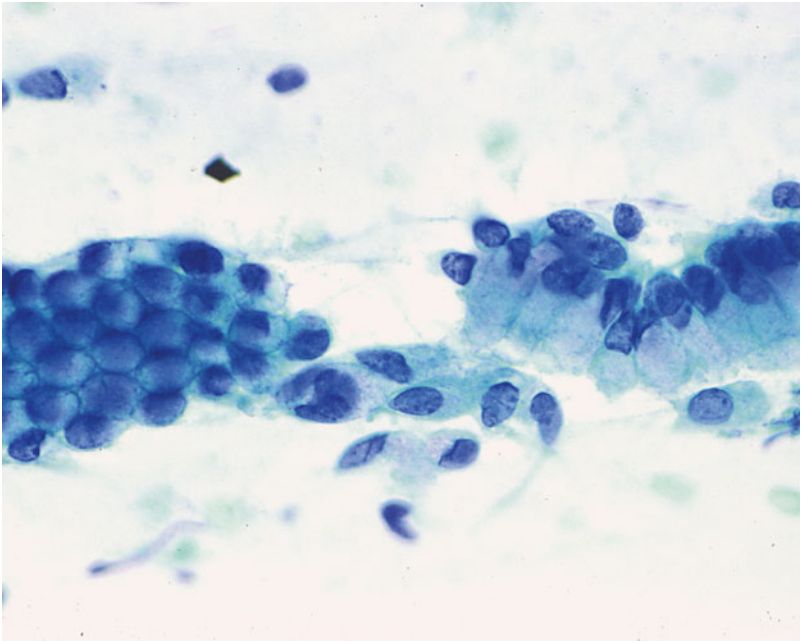

**Fig. 1.17** Endocervical cells (*CP*). Distinct cytoplasmic borders are seen in the cluster of cells on the *left*, giving a "honeycomb" appearance. The cell cluster on the *right* is seen from a side view, giving the "picket fence" appearance

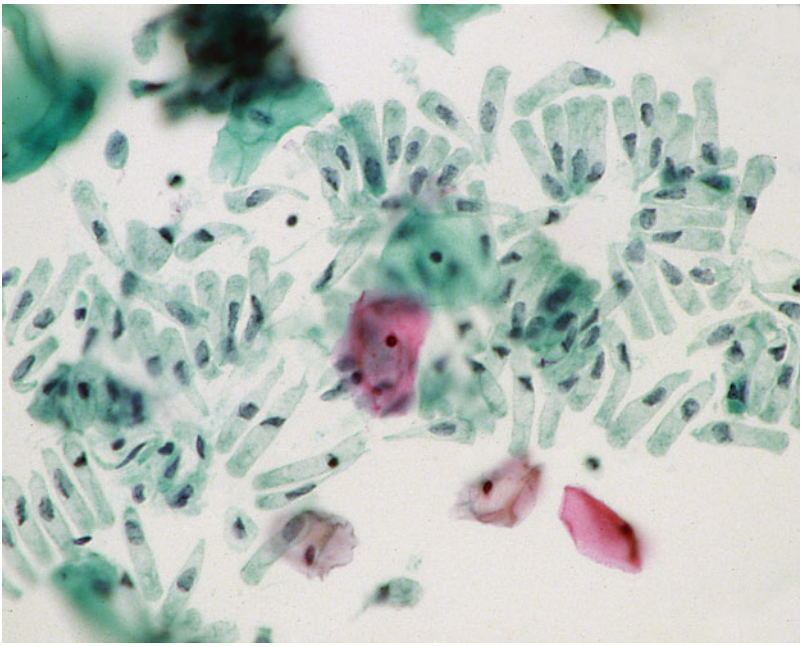

**Fig. 1.18** Endocervical cells (*LBP*, *SurePath*). Cellular dissociation is more frequent in liquid-based preparations than in conventional smear preparations

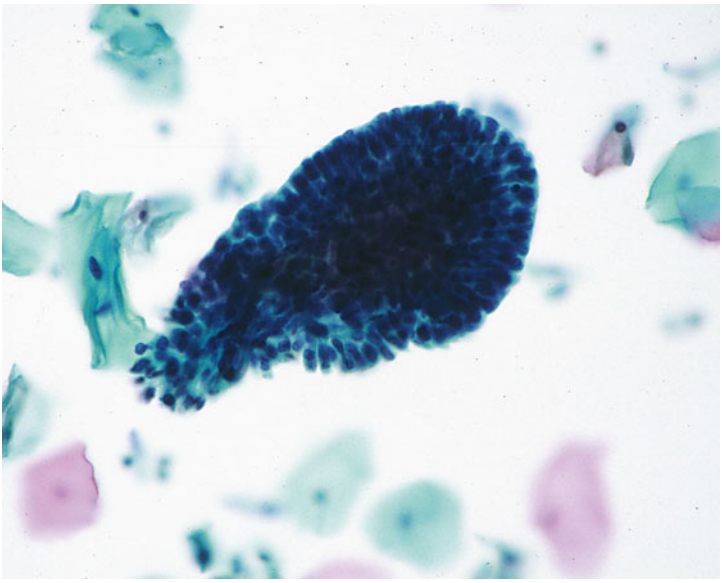

**Fig. 1.19** Endocervical cells (*LBP*, *SurePath*). Routine screening, 27-year-old woman, NILM on follow-up. Normal endocervical cells may appear in large hyperchromatic fragments, often in the center of some *LBP*s. The thickness of the fragment may give the appearance of architectural disarray; however, note normal appearing cells at the periphery of the fragment. Additionally, focusing up and down through the fragment reveals normal spacing of cells, distinct cytoplasmic borders, and bland nuclear chromatin. Normal endocervical cell groups with this appearance should not be confused with neoplastic clusters that show more crowding (even within a single layer of cells), nuclear enlargement, nuclear membrane irregularity, and abnormal chromatin pattern

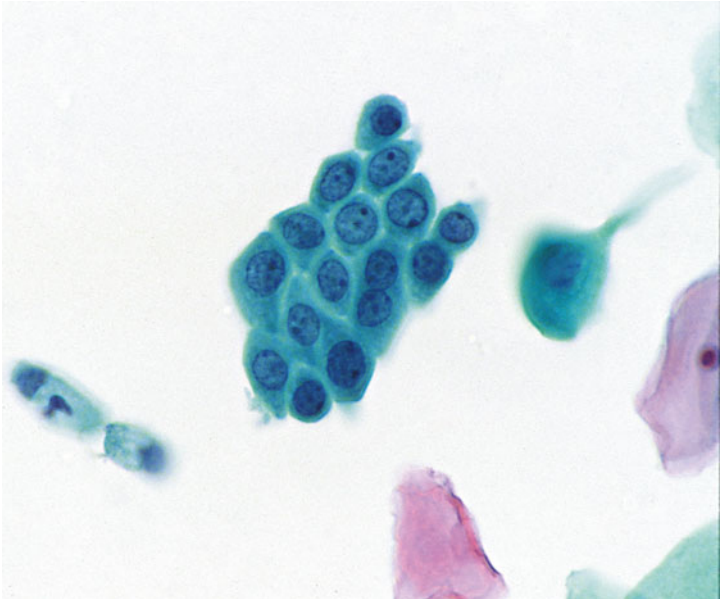

**Fig. 1.20** Transformation zone component (*LBP*, *SurePath*). Normal endocervical cells from the *upper* region of the endocervical canal can closely mimic squamous metaplastic cells

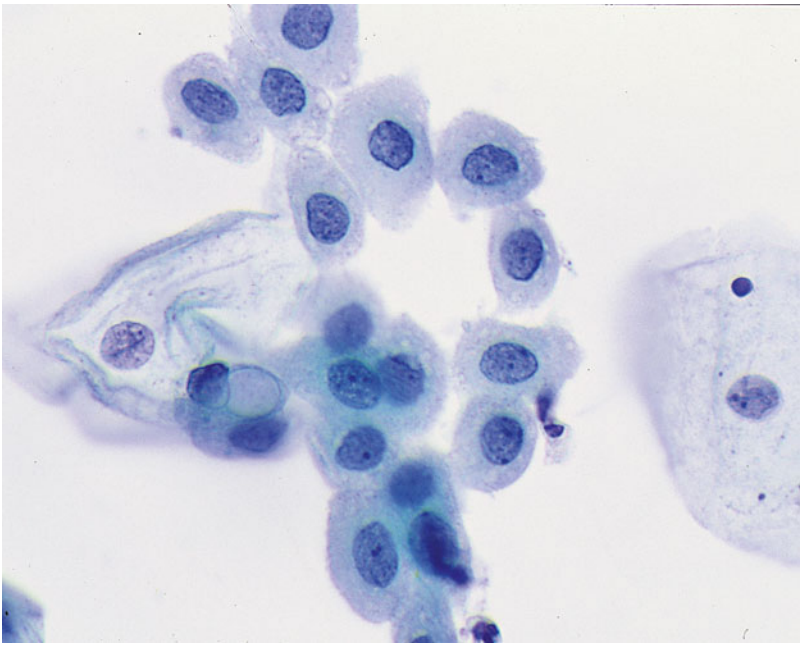

**Fig. 1.21** Normal squamous metaplastic cells (*LBP*, *SurePath*). Routine screening. Twenty-eight-year-old woman

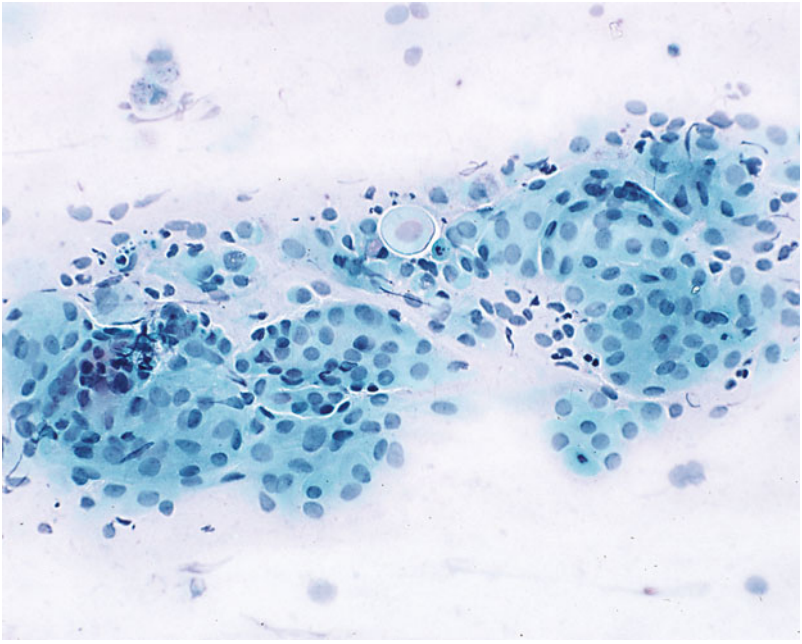

**Fig. 1.22** Atrophy (*CP*). Degenerated cells in mucus and parabasal-type cells should not be counted in assessing transformation zone sampling. It may be difficult to distinguish parabasal-type cells from squamous metaplastic cells in specimens showing atrophy due to a variety of hormonal changes including menopause, postpartum changes, and progestational agents. In such cases, the laboratory may elect to make a comment about the difficulty of assessing the transformation zone component

concluded that women should not be scheduled for early repeat testing because of lack of transformation zone sampling unless an abnormality was suspected [34, 61].

HPV testing appears to be independent of transformation zone sampling, and thus the addition of HPV co-testing in women aged 30 years and older will provide some reassurance that no lesion is present in women lacking EC/TZ who are also HPV negative, and may additionally provide increased sensitivity for lesions arising high in the endocervical canal (e.g., adenocarcinoma in situ and adenocarcinoma) [35, 36]. One case-control study found no difference in the rate of adenocarcinoma in situ (AIS) after negative cervical cytology with or without endocervical cells [37].

This 2014 TBS update still recommends reporting the presence or absence of EC/TZ component as a quality indicator even though the absence of an EC/TZ component should not lead to early repeat screening. If a clinical care provider only rarely or never obtains an EC/TZ component in a diversified population of women including those of childbearing age, this may indicate an issue with sampling quality. In addition, the presence of EC/TZ component may provide valuable information in women with a history of atypical glandular cells, early adenocarcinoma, trachelectomy for early-stage cancer, or other high-risk processes.

Automated screening devices often include EC/TZ components in the fields selected for review. When manually screening, cytotechnologists should look carefully for endocervical cells as they can be easily overlooked [38]; however, if an EC/TZ component is not visible on an automated screen and the specimen appears to be NILM and satisfactory, there may be limited value in extensive manual rescreening to locate a EC/TZ component in routine screening situations. Laboratories should develop policies on how to handle and report negative specimens with no EC/TZ component that have undergone automated screening. Education of clinical providers concerning the management recommendations related to specimen adequacy may be useful.

## 1.4 **Obscuring Factors** (Figs. 1.23 and 1.24)

Specimens in which more than 75 % of squamous cells are obscured should be termed unsatisfactory, assuming that no abnormal cells are identified (Fig. 1.23). When 50–75 % of the cells are obscured, a statement describing the specimen as partially obscured should follow the satisfactory term. *The percentage of cells obscured, not the slide area obscured, should be evaluated, although minimal cellularity criteria should also be applied*. Nuclear preservation and visualization are of key importance, and changes such as cytolysis and partial obscuring of cytoplasmic detail may not necessarily interfere with specimen evaluation. Abundant cytolysis may be mentioned as a quality indicator, but most such specimens do not qualify as "unsatisfactory" unless nearly all nuclei are devoid of cytoplasm. The criteria are similar for liquid-based and conventional preparations. In LBPs with obscuring factors and borderline cellularity (see Figs. 1.3 and 1.4), laboratories should estimate whether minimum numbers of well-visualized squamous cells are present as described above. When particular cells or areas of diagnostic interest are obscured, a report comment can be added: e.g., "air-drying of possible atypical cells" or "severe acute inflammation" (Fig. 1.24).

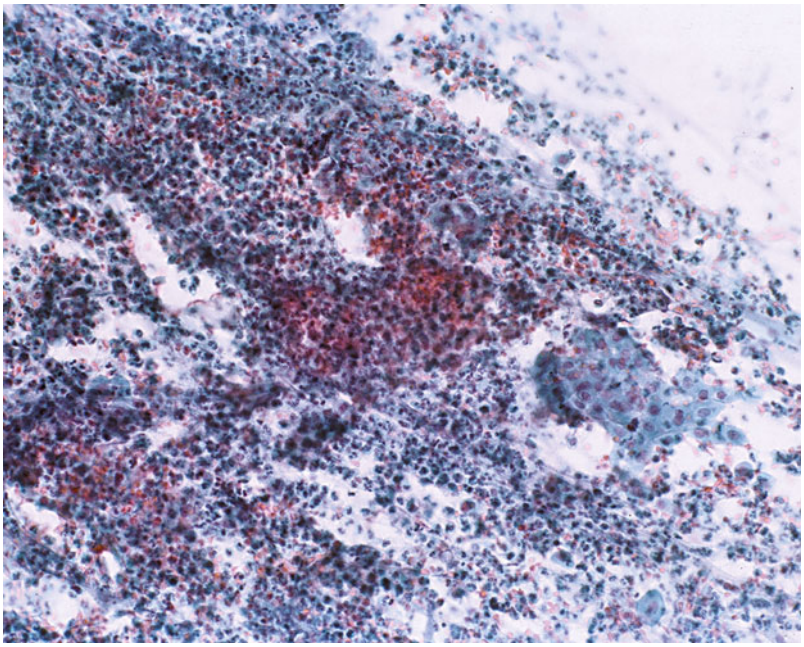

**Fig. 1.23** Unsatisfactory due to obscuring white blood cells (*CP*). If 50–75 % of the epithelial cells are covered, obscuring inflammation should be mentioned in the Quality Indicators section of the report (>75 % obscuring is considered unsatisfactory if no abnormal cells are identified). In assessing the adequacy of a slide with respect to obscuring factors and cellularity, one should keep in mind that the minimum cellularity criteria outlined above refer to well-visualized cells

### 1.4.1 Explanatory Notes

Reporting obscuring factors may be indicated because of patient care or quality concerns. The adequacy assessment of specimens with partial obscuring factors has been shown to have fair interobserver reproducibility [39]. Although retrospective case-control studies [32, 33] fail to show that partial obscuring factors indicate risk for a false-negative interpretation, prospective studies have not been done. Liquid-based preparations have been shown to be relatively free from obscuring factors when compared to conventional preparations [40].

## 1.5 Interfering Substances (Figs. 1.6 and 1.25)

### 1.5.1 Lubricants (Fig. 1.25)

Studies of the impact of lubricants on ThinPrep Pap tests have shown varying results. Some have shown minimal impact with water-based lubricants [41, 42], while others have shown a significant effect on adequacy rates [43–45]. Lubricants containing

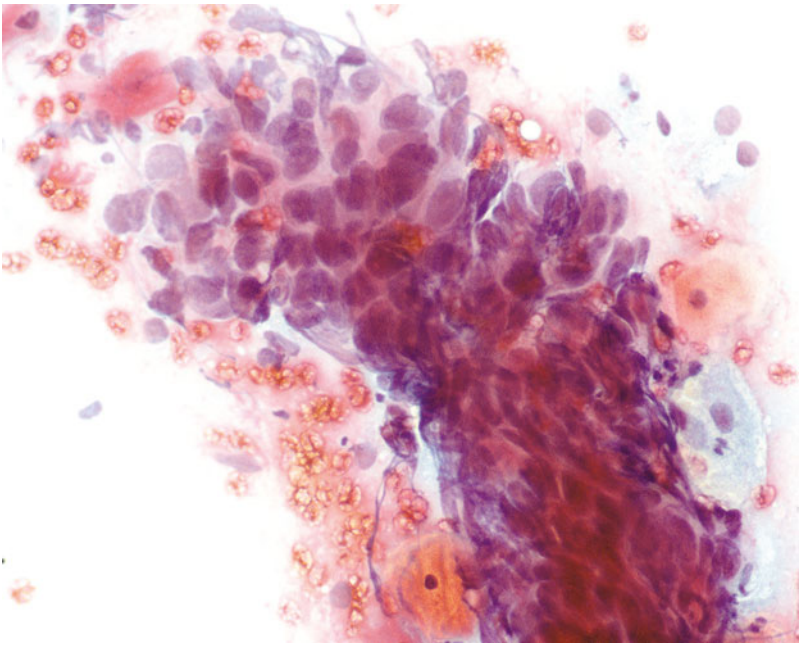

**Fig. 1.24** Satisfactory for evaluation; extensive air-drying artifact present. Atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion (ASC-H) (*CP*). Enlarged, pale nuclei with indistinct chromatin. The nuclei are crowded and lack an orderly architectural arrangement. Note that if the interpretation is atypical cells or worse, then the specimen cannot be considered "unsatisfactory" regardless of specimen squamous cellularity or overall quality. Histologic follow-up in this case was HSIL/CIN 2

carbomers or carbopol polymers have a marked adverse impact on the cellularity of ThinPreps [42, 45] (Fig. 1.25), and the manufacturer recommends against their use. Reprocessing is less likely to be successful in specimens with lubricant material [43]. Some laboratories have successfully reprocessed such specimens using a modified SurePath preparation technique [46]. Most studies have not found an adverse impact of lubricants on conventional preparations [47–50].

Interfering substances have little to no effect on the unsatisfactory rates of SurePath specimens [51–53], SurePath specimens generally show the lowest unsatisfactory rates among the liquid-based preparations [22, 54]. As of this writing, no "recovery" procedure for SurePath specimens with interfering substances has been published, and there does not appear to be a need for such a procedure.

### 1.5.2 Excessively Bloody Specimens (Fig. 1.6)

Abundant blood in a ThinPrep vial may interfere with liquid-based processing by clogging the filter. Several studies have documented that bloody specimens that

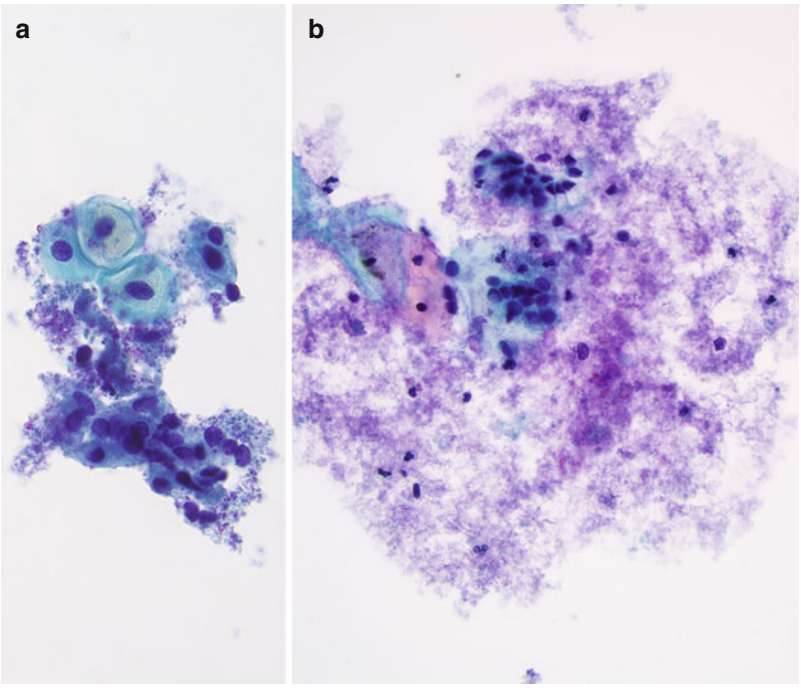


**Fig. 1.25** Unsatisfactory due to excess lubricant; 59-year-old woman (*LBP*, *ThinPrep*). Lubricant may mimic blood or mucus at low magnification (**a**, *left*). On higher magnification, the material is granular and lysed RBC or RBC "ghosts" are not seen (**b**, *right*). Practitioners who have a high unsatisfactory rate due to lubricant interference should be advised of manufacturer recommendations regarding lubricant usage as part of laboratory quality assurance

initially have unsatisfactory preparations can be successfully reprocessed by utilizing a diluted glacial acetic acid wash [55, 56] (Fig. 1.6a, b). The ThinPrep unsatisfactory cervical cytology rate can be decreased by over half with a glacial acetic acid wash assuming that the original sample included sufficient squamous cells [55–58]. However, laboratories should be aware that some studies have documented an impact on HPV testing; this may vary with the HPV test used and the type of processing or reprocessing procedures used by the laboratory [57, 59, 60].

## 1.6 Human Papillomavirus Testing on Unsatisfactory Specimens

The 2012 ASCCP Consensus Guidelines for the Management of Abnormal Cervical Cancer Screening Tests included adequacy management guidelines vetted by a national consensus conference [61]. The role of high-risk human papillomavirus (hrHPV) triage and co-testing was specifically considered. Some HPV assays do not utilize a nucleic acid sequence control to indicate the presence of cells in the

sample, and the negative control DNA in other HPV tests may not be specific for cells of epithelial origin. In these scenarios, a negative HPV test could be falsely negative and cannot be relied upon when the cervical cytology is unsatisfactory. If HPV testing is done in unsatisfactory specimens and is positive, the woman will still require some additional follow-up.

## 1.7 Management Guidelines Related to Adequacy

The 2012 ASCCP consensus guidelines for management of patients with unsatisfactory cervical cytology specimens are listed below [61].

**Management of Women with Unsatisfactory Cytology**

1. Repeat cytology in 2–4 months is recommended for women with unsatisfactory cytology. hrHPV triage testing is not recommended. Women with unsatisfactory cytology may receive treatment to resolve atrophy or obscuring inflammation (when a specific infection is noted) prior to repeat cytology. If a Pap test is unsatisfactory due to low cellularity in a woman with a recent negative screening history (i.e., the current, unsatisfactory Pap was taken at a shorter interval than suggested in the screening guidelines), the timing of the repeat Pap test triggered by the current unsatisfactory Pap test can be adjusted to a longer time interval.
2. Colposcopy is recommended when a woman has had two consecutive unsatisfactory cytology tests. Colposcopy can also be performed if the woman is known to be HPV16 or HPV18 positive by genotyping or if she is age 30 or greater and is hrHPV positive.

**Management of Women with Cytology Reported as Negative but with Absent or Insufficient EC/TZ Component**

1. For women aged 30 years and older, hrHPV testing is preferred when cytology is reported as negative with absent EC/TZ component. If the hrHPV test is negative, routine screening interval is recommended. Repeat cytology in 3 years is acceptable if hrHPV testing is not performed.
2. For women aged 21–29 years, routine screening is recommended when cytology is reported as negative with absent EC/TZ component. hrHPV co-testing is not indicated in women aged 25–29 years; however, some women in this age group may elect to undergo hrHPV primary screening using FDA-approved testing methods (for patients in the United States).

## 1.8 Report Formatting

The Bethesda system recommends that specimen adequacy be reported in a discrete section of the report. If a specimen is unsatisfactory, the reason(s) may also be reported in this section as quality indicators.

Nothing should be reported with respect to intraepithelial lesions or neoplasia in the Interpretation section for an unsatisfactory specimen because by definition the specimen is unsuitable for evaluation. Statements regarding inflammation, organisms, or other causes for an unsatisfactory specimen may be reported in the Interpretation section instead of in the Specimen Adequacy section if that is the preference of the laboratory or clinician.

Laboratories that utilize the optional General Categorization section in their reports may elect to leave this section blank or report it as "Unsatisfactory, see Adequacy (or Interpretation) section" or something similar. Having a General Categorization statement on the report even though a specimen is unsatisfactory may facilitate computerized or manual sorting of reports. It is suggested but not mandatory that the Adequacy section be listed first in the report.

## 1.9 Sample Reports

**Example 1**
*Specimen Adequacy:*
Satisfactory for evaluation; endocervical/transformation zone component present.
*Interpretation:*
Negative for intraepithelial lesion or malignancy.

**Example 2**
*Specimen Adequacy:*
Satisfactory for evaluation; endocervical/transformation zone component absent/insufficient.
*Interpretation:*
Negative for intraepithelial lesion or malignancy.

*Optional Note:*
Follow-up recommendations:
Age 21–29 or ≥30 and HPV negative: routine screening.
Age ≥30 and HPV unknown: HPV testing (preferred) or repeat cytology in 3 years.
Age ≥30 and HPV positive: repeat cytology and HPV in 1 year or HPV genotyping.

*Reference:* Massad LS, Einstein MH, Huh WK, Katki HA, Kinney WK, Schiffman M, et al. 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. J Low Genit Tract Dis. 2013;17:S1

**Example 3**
*General Categorization:*
Unsatisfactory for evaluation; see Specimen Adequacy section.
*Specimen Adequacy:*
Unsatisfactory for evaluation; specimen processed and examined but unsatisfactory for evaluation of epithelial abnormality because of obscuring inflammation.

*Comment:*
*Trichomonas vaginalis identified.* Consider repeat cervical cytology after treatment of *Trichomonas.*

**Example 4**
*Specimen Adequacy:*
Unsatisfactory.
*General Categorization:*
Unsatisfactory.
*Interpretation:*
Specimen processed and examined but unsatisfactory for evaluation of epithelial abnormality because of insufficient squamous cellularity. Partially obscuring blood identified.

*Comment:*
Endometrial cells present consistent with day 5 of LMP (last menstrual period) as provided.

**Example 5**
*Specimen Adequacy:*
Unsatisfactory for evaluation; specimen rejected because vial was received unlabeled.

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# Non-Neoplastic Findings

# 2

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## 2.1 Negative for Intraepithelial Lesion or Malignancy

When there is no cellular evidence of neoplasia, this is stated in the General Categorization and/or in the Interpretation/Result section of the report. Organisms or other nonneoplastic findings are optional to report, in addition to this statement.

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**Normal Cellular Elements**
- Squamous cells
- Endocervical cells
- Endometrial cells
- Lower uterine segment cells

**Nonneoplastic Findings (Optional to Report)**
- Nonneoplastic cellular variations
  - Squamous metaplasia
  - Keratotic changes
  - Tubal metaplasia
  - Atrophy
  - Pregnancy-associated changes
- Reactive cellular changes associated with:
  - Inflammation (includes typical repair)
  - Lymphocytic (follicular) cervicitis
  - Radiation
  - Intrauterine contraceptive device (IUD)
- Glandular cells status post hysterectomy

**Organisms**
- *Trichomonas vaginalis*
- Fungal organisms morphologically consistent with *Candida* spp.
- Shift in flora suggestive of bacterial vaginosis
- Bacteria morphologically consistent with *Actinomyces* spp.
- Cellular changes consistent with herpes simplex virus
- Cellular changes consistent with cytomegalovirus

## 2.2 Background

The category "negative for intraepithelial lesion or malignancy" is used for specimens that show a spectrum of nonneoplastic changes, including those associated with protective and reactive responses to inflammation, hormonal alterations, and colonizing or infectious organisms.

Cervical cytology is a screening test primarily for the detection of squamous cell carcinoma of the cervix and its precursors. Due to the wide spectrum of reactive cytomorphologic changes, criteria are not well-defined and may lack reproducibility [1–5]. The reporting of specific nonneoplastic findings is optional and at the discretion of the laboratory. Reasons for continuing to report certain nonneoplastic findings in a cervical cytology report include the following:

1. Utility as a triage tool and as documentation for laboratory regulations regarding referral for hierarchical review.
2. Fostering a discipline in applying cytomorphologic criteria during screening and sign-out.
3. Documentation of morphologic findings to explain differences in interpretation on review [6].
4. Facilitation of clinical-cytologic correlation. For example, the cytologic finding of hyperkeratosis and parakeratosis may correlate with the colposcopist's assessment of the uterine cervix.
5. Documentation of reactive cellular changes in the report to spot trends in a series of cervical cytology specimens from one woman. Studies have reported a slight increase in the incidence of squamous intraepithelial lesion (SIL) in cases interpreted as reactive compared to those interpreted as within normal limits [7, 8]. This may relate to the concept that tissues which are more frequently subjected to infection, inflammation, and other traumatic stimuli may either be more subject to high-risk HPV infection or that mutational changes occur more frequently in traumatized tissues undergoing repair [9].
6. Documentation of findings that allow education of both laboratorians and clinical practitioners as to biologic processes relevant to the patient and to cytomorphology in general.
7. Marked reactive and/or reparative changes may cause concern for or be overinterpreted as squamous or glandular neoplastic entities. These more worrisome examples of reactive changes and repair should be subject to additional hierarchical review.

Note that the list of nonneoplastic findings in TBS is not comprehensive. Additionally, these interpretive categories do not necessarily correspond to regulatory requirements for hierarchical supervisory review; within the parameters of government regulation, it is up to the laboratory to specify findings that trigger additional review.

**Negative for Intraepithelial Lesion or Malignancy (NILM)**
Specimens for which no epithelial abnormality is identified are reported as "negative for intraepithelial lesion or malignancy" (NILM). If optional nonneoplastic findings are reported, NILM should still be included as the primary interpretation or as the General Categorization to avoid ambiguity.

## 2.3 Normal Cellular Elements

It is important that persons analyzing cervical samples have an understanding of both the nuclear morphology and sizes of the cellular constituents. Pioneers of cervical cytology derived basic understanding of benign and neoplastic processes from careful measurements in conventional cytologic samples [10]. While there is no contemporary literature on such measurements in liquid-based preparations, size relationships remain important in defining diagnostic entities and functional states.

### 2.3.1 Squamous Cells

#### 2.3.1.1 Superficial Cell

Derived from the outermost layer of the cervical epithelium and usually seen in the proliferative phase of the menstrual cycle and in the presence of irritation. The nucleus is highly condensed (pyknotic) and 10–15 μm$^2$ in cross-sectional area. The cytoplasm is abundant, usually eosinophilic. Keratohyaline granules may be found in the cytoplasm, reflecting elaboration of high molecular weight keratin protein (Fig. 2.1).

#### 2.3.1.2 Intermediate Cell

Generally present in the middle or intermediate layer of the squamous epithelium. In the secretory phase, this cell type may compose both the middle and superficial layers of the normal cervical epithelium. It is particularly prominent in pregnancy and with the use of progestational agents. The nucleus is larger than that of the superficial cell, with a cross-sectional nuclear area of 35 μm$^2$ and a finely granular chromatin pattern. The nucleus is often elongate with a longitudinal nuclear groove (Fig. 2.2). *The intermediate cell nucleus serves as the basic size reference for other cells in cervical cytology specimens*. Naked intermediate cell nuclei are seen in the second half of the cycle secondary to bacterial cytolysis (Fig. 2.59).

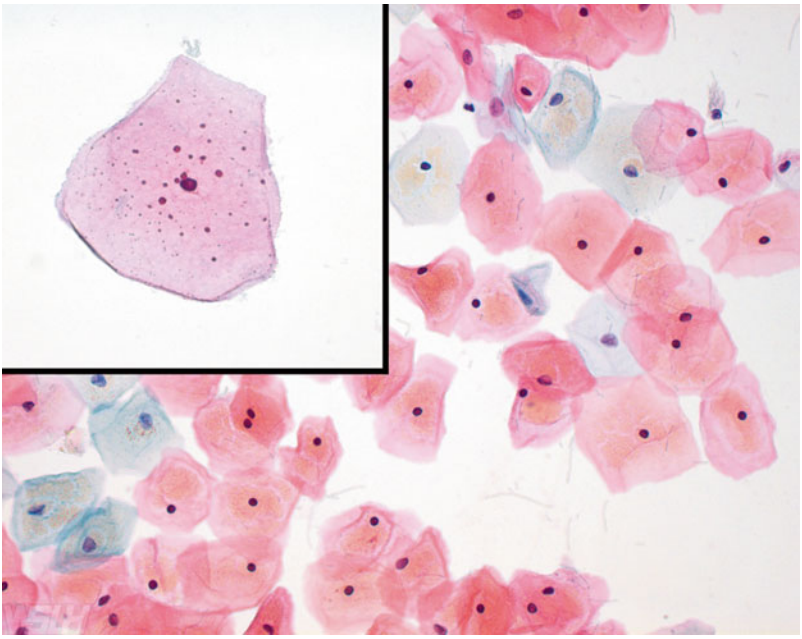

**Fig. 2.1** Superficial squamous cells (*LBP*, *ThinPrep*). Admixture of superficial and intermediate squamous cells. The superficial cells have smaller condensed (pyknotic) nuclei. Light brown glycogen is present in the cytoplasm of both cell types. The inset reveals a characteristic superficial cell at high magnification. Note the polygonal cytoplasmic profile, cytoplasmic keratohyaline granules, and pyknotic nucleus with a cross-sectional area of approximately 10 μm$^2$. The dense nucleus is opaque to light

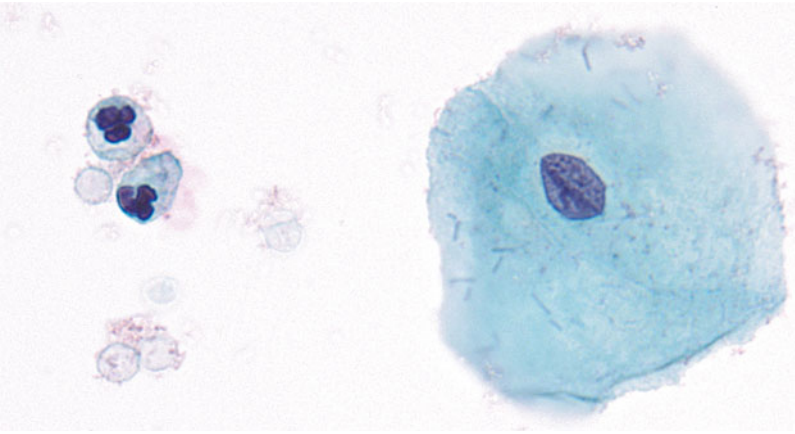

**Fig. 2.2** Intermediate squamous cell (*LBP*, *ThinPrep*). A typical intermediate cell with a polygonal cytoplasmic profile. The nucleus possesses finely granular chromatin with a longitudinal groove. The cross-sectional area of the intermediate nucleus is approximately 35 μm$^2$ and is generally used as the internal reference for size comparison. Light can pass through the intermediate nucleus due to the chromatin being more open than that of a superficial cell

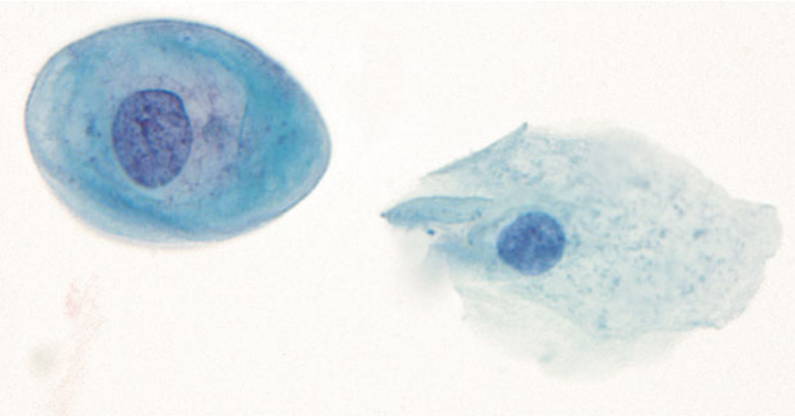

**Fig. 2.3** Parabasal cell (*LBP*, *ThinPrep*). A parabasal cell is contrasted with an intermediate cell. The parabasal cell exhibits typical features with an oval nucleus, fine chromatin, and a cross-sectional area of approximately 50 μm$^2$. The cytoplasm is dense relative to the intermediate cell, because the intermediate cell cytoplasm flattens out next to the nucleus, whereas in the parabasal cell, the cytoplasm is heaped up. If the cells were viewed from the side, an intermediate cell would be a flattened saucer with a central nuclear heap. The parabasal cell would resemble a hill with sloping sides

#### 2.3.1.3 Parabasal Cell

Along with immature squamous metaplastic cells, parabasal cells are the least mature cells in a cervical cytology sample. They are generally not present in specimens from a hormonally mature epithelium as they are derived from deep cell layers not typically sampled in cervical cytology specimens from premenopausal women. In the absence of hormonal stimulation, this cell type comprises layers of a relatively thin and atrophic epithelium. Parabasal cells may predominate in postmenopausal and postpartum states. The nuclei are larger than in intermediate cells with an area of 50 $\mu m^2$. The cytoplasmic area is smaller and the nuclear to cytoplasmic ratio is higher than in intermediate or superficial cells; and the cytoplasmic texture is more granular and dense (Fig. 2.3).

### 2.3.2 Glandular Cells

#### 2.3.2.1 Endocervical Cell

Endocervical glandular cells have nuclear sizes that are highly variable with a mean of 50 $\mu m^2$ which is slightly larger than that of an intermediate squamous cell. The nucleus shows a finely granular and evenly distributed chromatin pattern with small nucleoli. The cytoplasm is diffusely vacuolated or granular. Cells exhibit polarity with nuclei at one end of the cytoplasm and mucus present at the opposite end. The cytomorphology will differ depending on the orientation of the cells on the slide: when viewed from the side, there will be a "picket-fence" formation, whereas when viewed en face, they will have a classic "honeycomb" configuration (Fig. 2.4).

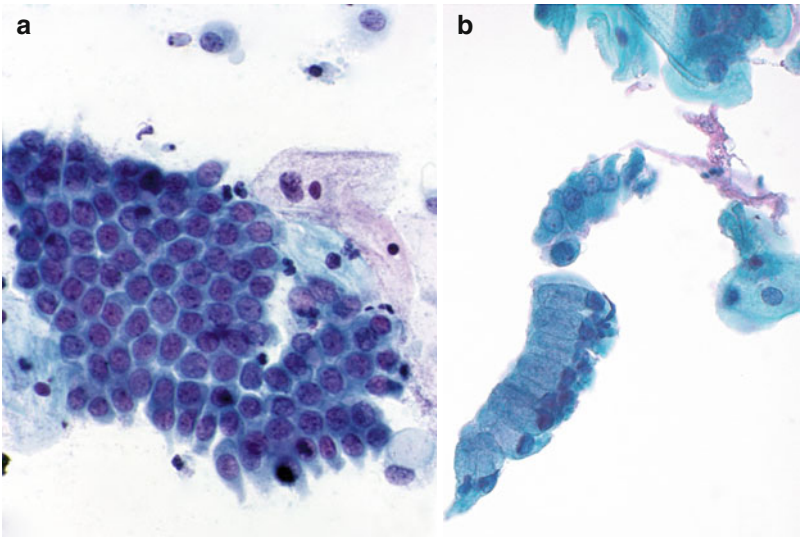


**Fig. 2.4** Endocervical cells (*LBP*, *ThinPrep*). Endocervical cells may be seen en face in a typical "honeycomb" arrangement of benign glandular epithelium (**a**). Alternatively, endocervical cells when viewed from the side present in a "picket-fence" configuration (**b**). There is normal nuclear polarity and ample evidence of apical mucin in these columnar cells

### 2.3.2.2 Endometrial Cell

Spontaneously exfoliated endometrial cells may be of epithelial or stromal origin and can occur as isolated cells or as aggregates. Endometrial glandular cells are typically smaller than endocervical cells, with a nuclear area equal to or slightly smaller than an intermediate cell nucleus (35 μm$^2$) and have a higher nuclear to cytoplasmic ratio. The nuclear chromatin tends to be dense, heterogeneous and may contain apoptotic debris due to degenerative changes. Nucleoli are generally not prominent, but may be observed in liquid-based preparations due to improved fixation. The cytoplasm is scant and may be dense or vacuolated. Exfoliated endometrial stromal cells are typically arranged in dense aggregates which often have a surrounding layer of glandular epithelium – a characteristic formation often referred to as an "exodus" ball because of its presence at the end of menstrual flow. Exfoliated endometrial stromal cells may also be isolated and have spindled tails of wispy cytoplasm. Exfoliated endometrial cells (Figs. 2.5 and 2.6, see Figs. 3.1, 3.2, and 3.4) present differently than do directly sampled lower uterine segment and endometrial cells, which are described below (Figs. 2.7, 2.8, and 2.9, see Fig. 3.5).

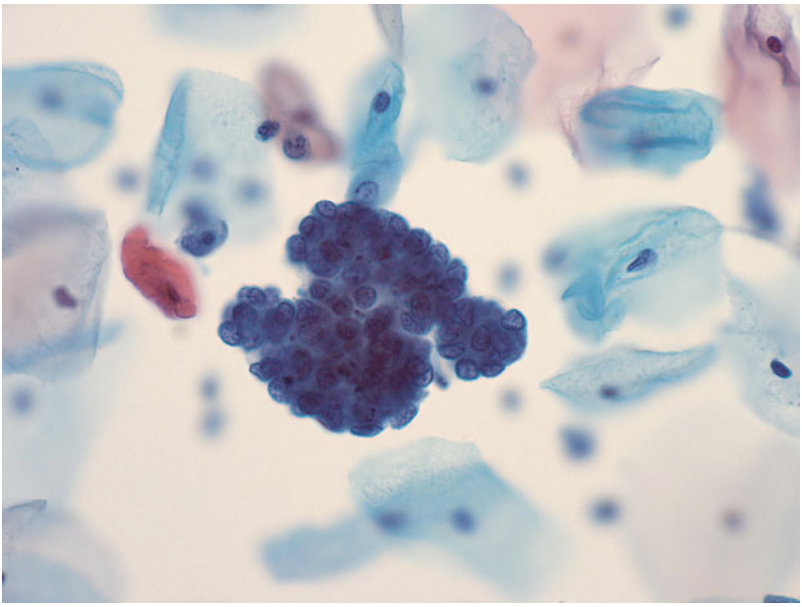

**Fig. 2.5** Endometrial cells (*LBP*, *SurePath*). A tight cluster of endometrial glandular cells with nuclei having cross-sectional areas slightly smaller than the 35 μm$^2$ of intermediate cells. Endometrial cell nuclear to cytoplasmic ratios are high and the cells tend to form three-dimensional groups. The small and monotonous nuclear size should prevent overinterpretation as a squamous, or glandular abnormality

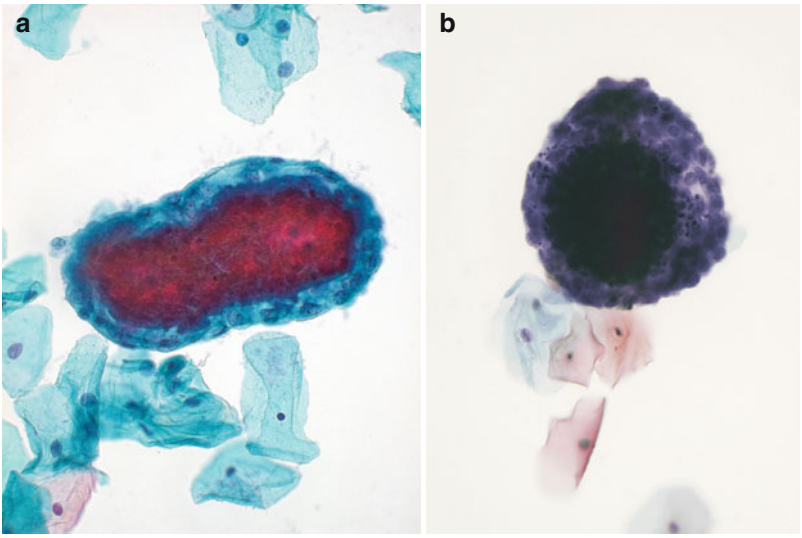


**Fig. 2.6** Endometrial cells, exodus (*LBP*, *ThinPrep*). Collections of peripheral glandular and central stromal endometrial cells (exodus ball) are typically seen between day 6 and 10 of the menstrual cycle. These clusters are among the last remnants of endometrial shedding and the cells may show degenerative changes. Both images show exodus balls from two different cases. On the left (**a**) is an intermediate magnification from a conventional preparation. More nuclear structure is observed in cells on the periphery of the exodus ball. In (**b**), from a liquid based preparation, physical forces have accentuated the rounding up of cells during fixation. The resultant three dimensional cell ball obstructs more light, is darker and may be over interpreted as a glandular abnormality

## Preparation-Specific Criteria for Normal Cellular Elements

*Liquid-Based Preparations*:
Fixation is generally improved and these preparations remove much of the background bacteria, debris, and inflammatory material that can obscure the cells of interest. Glandular cells may form three-dimensional structures, as cellular fixation occurs during suspension in liquid as opposed to preparations in which fixation occurs after smearing on a slide. Rounded benign groups can be more densely cellular and hyperchromatic. Observation of cells near the borders of the group becomes more important to determine the true origin and nature of such cell groupings. Nucleoli may be better preserved and more prominent.

*Conventional Preparations*:
Bacteria, inflammatory cells, and debris are more prominent in the background. Degenerative changes, "air-drying artifact", mechanical artifact, and other limiting factors associated with sample collection and preparation are more common. Cells may be larger as they are flattened out on the slide.

### 2.3.3 Lower Uterine Segment and Directly Sampled Endometrial Cells (Figs. 2.7–2.9)

#### 2.3.3.1 Criteria

Cells directly sampled from the lower uterine segment or endometrial cavity may present as large, cellular, hyperchromatic groups composed of both endometrial glandular and stromal cells (Fig. 2.7, see Fig. 3.5). Branching glands can be seen in some groups, with surface gland openings and palisading of nuclei in the interior of the fragments (Fig. 2.8). The glands are surrounded by stroma, which may contain small vessels that can appear to protrude from the surface of the groups in a spindled or "feathered" pattern. Smaller fragments may contain only glandular or stromal cells. Nuclear crowding and overlap are present in both epithelial and stromal components.

Directly sampled endometrial and lower uterine segment glandular cells are columnar and have round to oval, variably hyperchromatic nuclei, with moderately coarse but evenly distributed chromatin and smooth nuclear borders (Fig. 2.8).

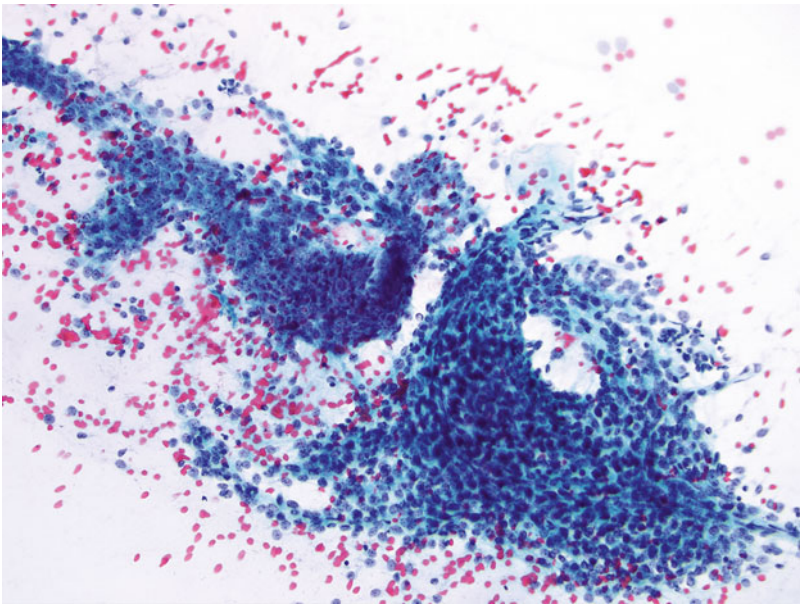

**Fig. 2.7** Lower uterine segment sampling (*CP*). Lower uterine segment sampling with ill-defined glandular cells near the upper left aspect and stromal cells loosely adherent to the glandular cells. Several blood vessels can be seen protruding from the group. Stromal and glandular components are not always easy to distinguish on cervical cytology

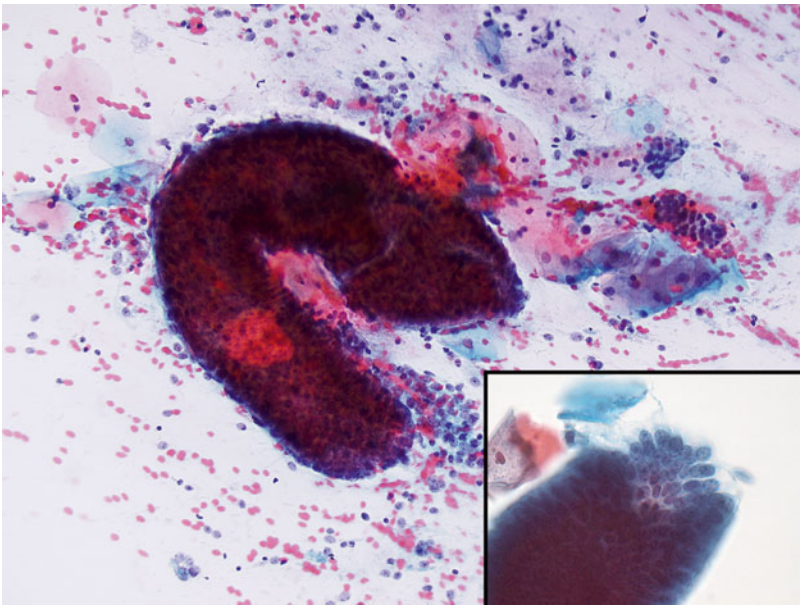

**Fig. 2.8** Lower uterine segment sampling (*CP*). A well-preserved endometrial gland presenting as a tubular structure. A stromal component is also visible at the lower right side of the epithelial tube. The inset shows columnar endometrial glandular cells that have round to oval, variably hyperchromatic nuclei, with moderately coarse but evenly distributed chromatin and smooth nuclear borders (*CP*)

Nucleoli are inconspicuous and mitotic figures may be seen, particularly during the proliferative phase. Ciliated cells may be present in the case of coexistent tubal metaplasia.

Stromal cell groupings are arranged in a disorganized pattern (Fig. 2.9). The cells have oval to elongate nuclei and scant, spindled cytoplasm. Nuclei have smooth contours and an evenly distributed, finely granular chromatin pattern. Nucleoli are inconspicuous and mitotic figures are rare.

### Preparation-Specific Criteria

In liquid-based preparations, lower uterine segment and directly sampled endometrium tends to exhibit small dense cellular groups containing only epithelium or stroma (Fig. 2.8). In conventional preparations, large cellular groups may have a "stretched" configuration and glands and blood vessels are more commonly noted (Fig. 2.7, see Fig. 3.5).

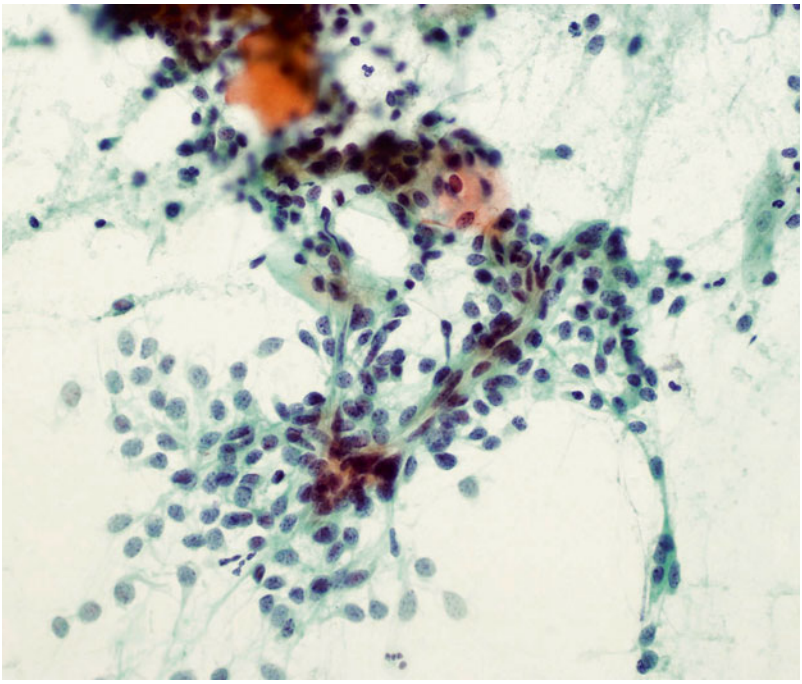

**Fig. 2.9** Lower uterine segment sampling (*CP*). Endometrial stromal cells adherent to blood vessels and flattened against the slide in a fanlike pattern

### 2.3.3.2 Explanatory Notes

Sampling of the lower uterine segment and endometrium may occur because of closer proximity to the cervical os following an excisional procedure (loop electrosurgical excision or conization) that shortens the endocervix [11] or a trachelectomy (a fertility-sparing resection of the cervix, upper vagina, and adjacent tissue, for minimally invasive squamous cell carcinoma) [12, 13]. Direct endometrial sampling can occasionally be present in women with an intact cervix secondary to the vigorous use of an endocervical brush or broom sampling device.

Directly sampled endometrial tissue may mimic glandular neoplastic abnormalities or rarely high-grade squamous lesions due to the presence of hyperchromatic crowded groups with nuclear crowding, nuclear overlap, and high nucleus to cytoplasmic ratios. In contrast to spontaneously exfoliated endometrial cells, direct brushing of endometrial tissue may yield large cellular fragments that can recapitulate their native in situ architecture (so-called organoid differentiation). This appearance may include branching tubular glands amid stroma composed of round to spindle-shaped cells. Peripheral palisading may be evident. The low-power recognition of branching glands and glandular-stromal complexes can avoid confusion with

atypical glandular cells (AGC) or glandular neoplasia. In liquid-based preparations, smaller rounded groups may have only one visible component. The most helpful clues in this situation are small nuclear size (approximating that of an intermediate nucleus); smooth, regular nuclear contours; and evenly distributed chromatin. In addition, groups of endometrial stromal cells may contain small vessels that protrude from the surface of the organoid groups, a feature not seen in neoplastic epithelial abnormalities.

## 2.4 Nonneoplastic Cellular Variations

### 2.4.1 Squamous Metaplasia (Figs. 2.10–2.13)

#### 2.4.1.1 Criteria

Squamous metaplastic cells which show a range of cytoplasmic differentiation from immature parabasal-like cells to those that approximate the appearance of differentiated intermediate/superficial cells (Fig. 2.10). The mean nuclear area is larger than that of the intermediate cell and similar to the parabasal cell at 50 $\mu m^2$.

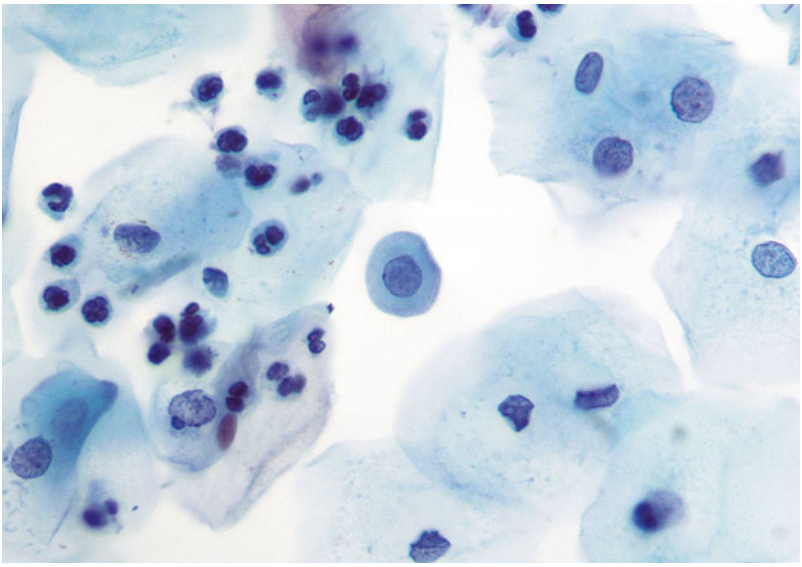

**Fig. 2.10** Squamous metaplasia (*LBP*, *SurePath*). A characteristic metaplastic cell is found in the center of the field. The nucleus is round to oval with fine, evenly distributed chromatin. The nuclear to cytoplasmic ratio is variable, and in this instance, it approaches one to one. These cells should not be overinterpreted as ASC-H or HSIL

### Preparation-Specific Criteria

Cells having spindled cytoplasmic projections ("spider cells") are often seen in conventional preparations due to disruption of the cohesion of cellular attachments by the force of the smearing procedure (Fig. 2.11).

### 2.4.1.2 Explanatory Notes

The process of metaplasia represents the replacement of one type of epithelium (in this case endocervical) with another (squamous) as a protective response. Squamous metaplastic cells can exhibit a spectrum of morphology from relatively undifferentiated small round cells to highly differentiated intermediate/superficial squamous cells. In metaplasia, stimuli such as infection, inflammation, or other type of trauma cause an alteration in the pathway of development of new cells replacing those lost by wear and tear. The newly generated cells become progressively more

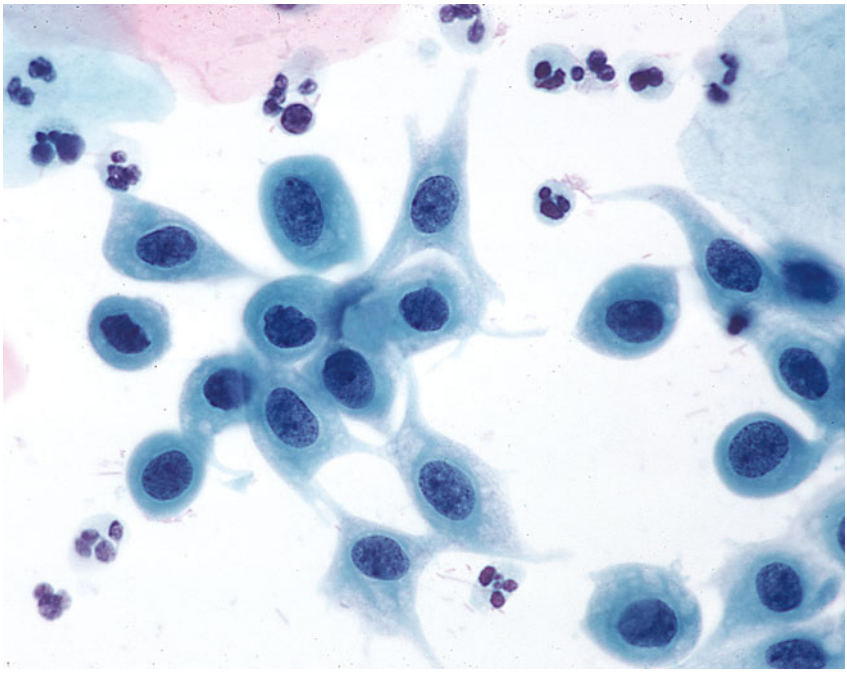

**Fig. 2.11** Squamous metaplasia (*CP*). Routine screening from a 27-year-old woman, day 8 of menstrual cycle shows reactive metaplastic cells with "spidery" cytoplasmic processes, a feature that is seen more often in conventional smears. Follow-up cytology was NILM

differentiated along the squamous pathway in response to the noxious stimulus. The metaplastic surface epithelium may eventually become indistinguishable from other squamous mucosa; however, the histologic finding of glandular spaces filled by endocervical or metaplastic squamous cells beneath the surface is a marker of the cervical transformation zone and an indication that the overlying epithelium was once glandular (Fig. 2.12).

One of the most difficult tasks in day-to-day cytologic practice is the evaluation of metaplastic cells, especially those with high nuclear to cytoplasmic ratios. Nuclear enlargement without other nuclear abnormalities in squamous metaplastic cells should lead to cautious evaluation, so as not to overinterpret the sample. One should evaluate single nuclei in intact cells. A nuclear to cytoplasmic ratio of less than 50 %, smooth nuclear contours, and even distribution of chromatin all favor benign squamous metaplasia (Fig. 2.13). A higher nuclear to cytoplasmic ratio in conjunction with hyperchromasia and/or nuclear contour irregularities, such as notching or grooving, should prompt consideration of a HSIL or ASC-H designation.

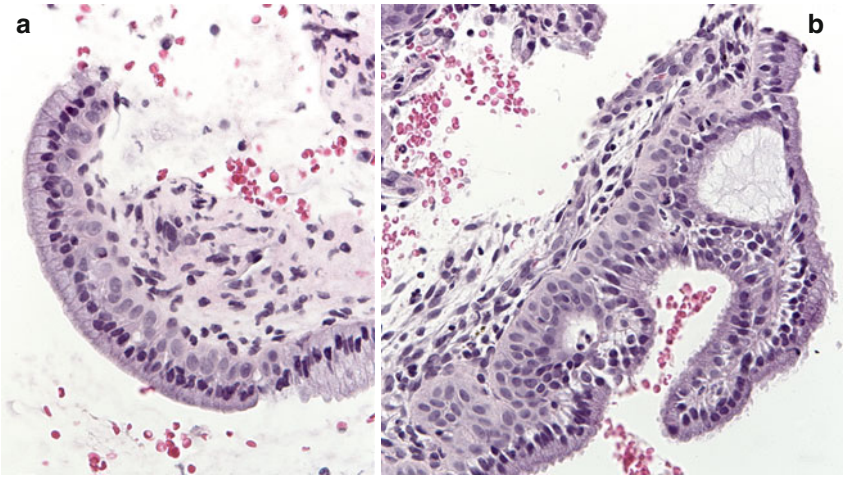


**Fig. 2.12** Squamous metaplasia (histology, H&E). (**a**, *left*) Early squamous metaplasia in an endocervical sample. A variety of stimuli can trigger an altered pathway of differentiation in the stem cell population that was committed to generating endocervical cells. The cells underneath the mucus secreting epithelial cells have rounded up, lost their ability to secrete mucin, and assumed a protective role, increasing the thickness of barrier between the stimulus and the underlying tissue. (**b**, *right*) A later stage in squamous metaplasia where multiple layers of metaplastic cells are seen under the surface epithelium

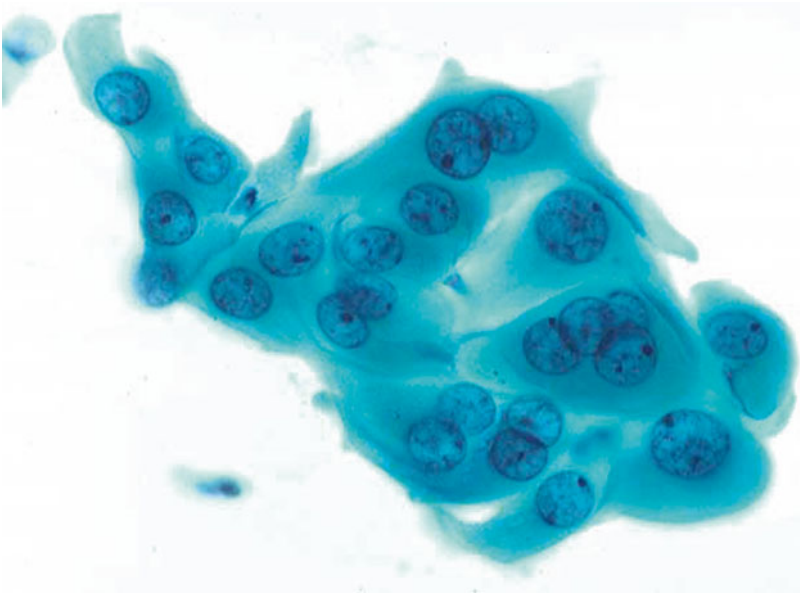

**Fig. 2.13** Squamous metaplasia (*CP*). Squamous metaplastic cells show nuclear size similar to parabasal cells. This cohesive group of cells also shows some modest nucleolar prominence that is consistent with reactive/reparative changes

### 2.4.2 Keratotic Cellular Changes (Figs. 2.14–2.17)

Normally, the cervix is a nonkeratinizing, stratified squamous epithelium. Keratotic changes usually occur as a protective reactive phenomenon or in association with human papillomavirus (HPV)-induced cell changes. Both of these processes lead to hypermaturation of the native squamous epithelium, more closely approximating the normal appearance of skin. Keratotic changes can be considered a second-order protective reaction for subepithelial tissues with metaplasia being the first-order reaction.

"Keratosis," "hyperkeratosis," "parakeratosis," and "dyskeratosis" are descriptive terms for keratotic cellular changes which have been used inconsistently in the past. These terms are not specifically listed in Bethesda terminology due to lack of consensus definitions. They are included parenthetically for clarification only. Although some cytologists may choose to include such terms to describe a morphologic feature that may correlate with leukoplakia on colposcopy, they should not be used as an interpretive category in cytology reports.

After metaplastic conversion, continued trauma may lead to formation of cytoplasmic keratohyaline granules (Fig. 2.14). In rare examples, the epithelium may come to resemble skin with a granular layer.

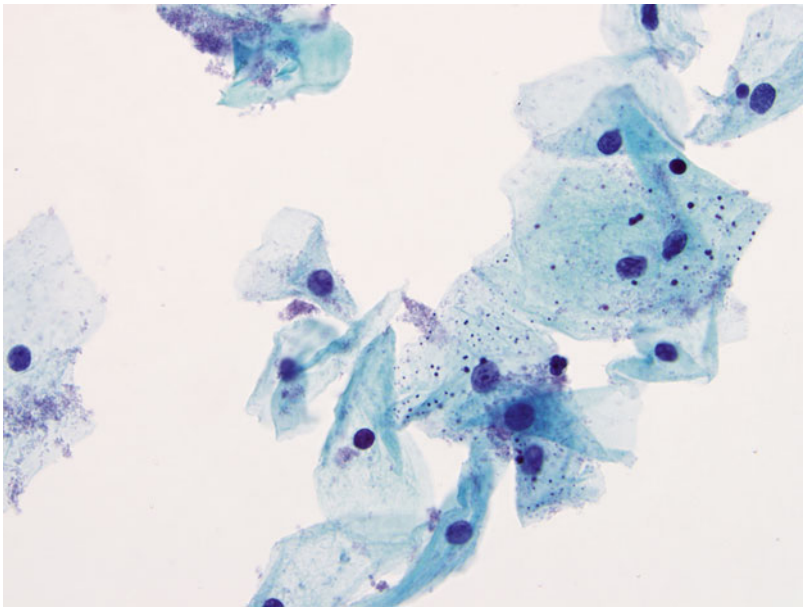

**Fig. 2.14** Keratotic cellular changes (*LBP*, *ThinPrep*). Intermediate squamous cells showing prominent cytoplasmic keratohyaline granules, a precursor to full keratinization

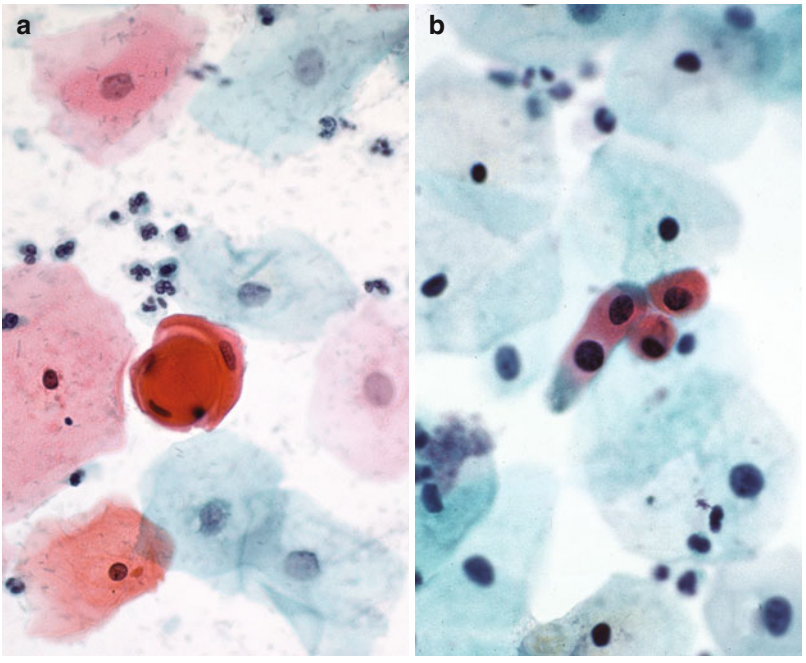


**Fig. 2.15** Keratotic cellular changes (*CP*). Keratotic cellular changes, "typical parakeratosis." On the left side (**a**), note the "squamous pearl" formation in this specimen from a 49-year-old woman being followed up after treatment for SIL. On the right side (**b**) is a small cluster of miniature squamous cells. Both are examples of "typical parakeratosis" showing miniature squamous cells with small bland, pyknotic nuclei

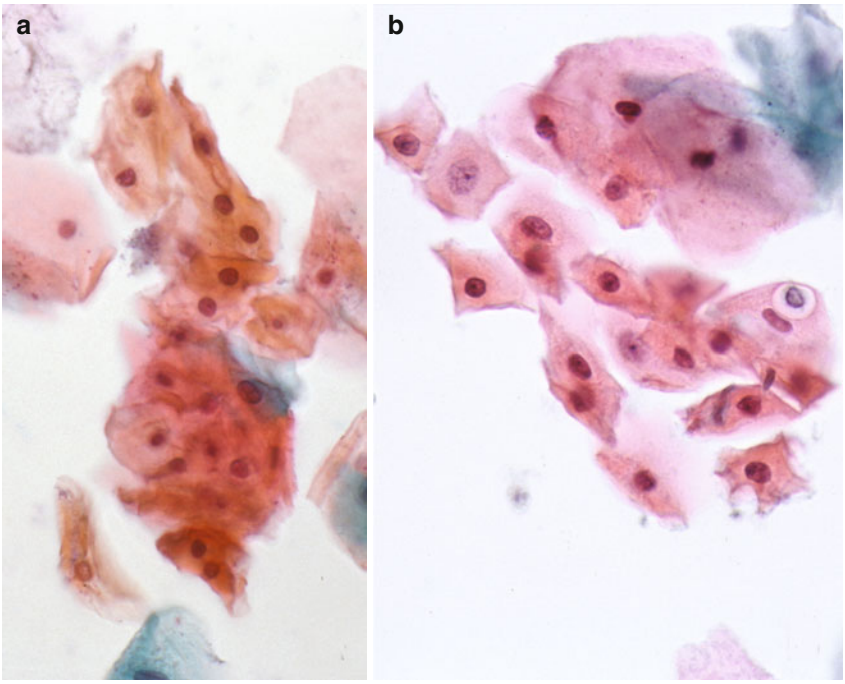


**Fig. 2.16** Keratotic cellular changes. Keratotic cellular changes, "typical parakeratosis". On the left (**a**, *CP*) is an orangeophilic cluster, and on the right (**b**, *LBP*, *ThinPrep*) are more eosinophilic squamous cells with small, opaque nuclei. Human papillomavirus (HPV) testing, performed as part of co-testing on the liquid-based specimen, was negative

### 2.4.2.1 Typical Parakeratosis (Figs. 2.15 and 2.16)

#### 2.4.2.1.1 Criteria

Miniature superficial squamous cells with dense orangeophilic or eosinophilic cytoplasm. Cells may be seen in isolation, in sheets, or in whorls; cell shape may be round, oval, polygonal, or spindle shaped.

Nuclei are small (approximately 10 μm$^2$ in cross-sectional area) and dense (pyknotic).

If atypical nuclear changes are present, an atypical squamous cell (ASC-US/ASC-H) or SIL interpretation should be considered, but if nuclei are round, regular, and resemble neighboring nuclei, a designation as abnormal is not warranted.

### 2.4.2.2 Hyperkeratosis (Fig. 2.17)

#### 2.4.2.2.1 Criteria

Anucleate but otherwise unremarkable mature polygonal squamous cells, often associated with mature squamous cells showing keratohyaline granules.

Empty spaces or "ghost nuclei" may be noted.

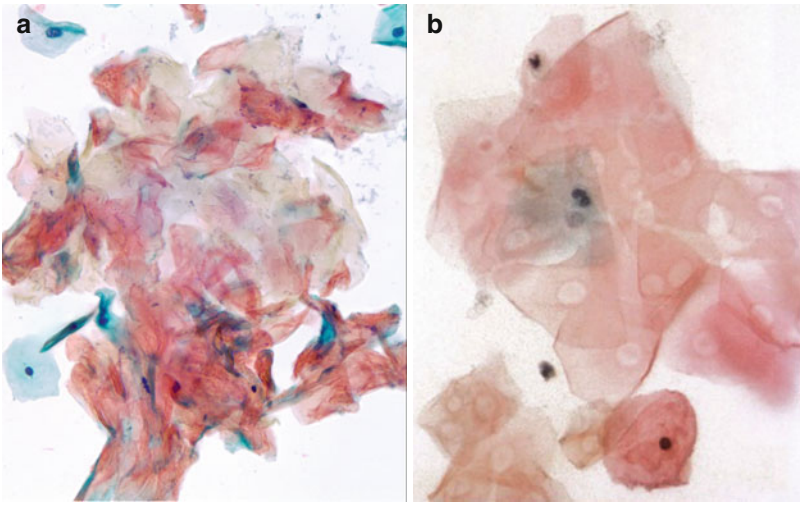


**Fig. 2.17** Keratotic cellular changes, "hyperkeratosis." On the *left* (**a**, *LBP*, *ThinPrep*) is a group of anucleate squames at low power. On the *right* (**b**, *LBP*, *ThinPrep*) are anucleate, mature polygonal squamous cells with ghostlike "nuclear holes" ("**b**" is reprinted with permission from Williamson et al. [15])

#### 2.4.2.3 Explanatory Notes

The Bethesda classification and interpretation of such keratotic changes depends on the nuclear alterations present. Miniature squamous cells with small pyknotic nuclei and orangeophilic to eosinophilic cytoplasm ("parakeratosis") are a nonneoplastic reactive cellular change. However, single cells or cell clusters that demonstrate pleomorphism of nuclear shape and/or increased nuclear size and/or chromasia ("atypical parakeratosis," "dyskeratosis," or "pleomorphic parakeratosis") are representative of an epithelial cell abnormality. Such findings should be categorized as atypical squamous cells (ASC) or as a squamous intraepithelial lesion (SIL), depending on the degree of cellular abnormality identified (see Figs. 4.15, 4.16, 5.8, 5.9, 5.26, 5.42, 5.43, 5.44, 5.56, and 5.59) [14].

Anucleate, but otherwise unremarkable mature, squamous cells ("hyperkeratosis") constitute a nonneoplastic cellular change. Inadvertent contamination of the specimen with vulvar material may also introduce anucleate squamous cells into the cervical cytology specimen. When extensive hyperkeratosis is present, an underlying neoplastic or nonneoplastic process may be associated and should be considered when evaluating such cytologic preparations [15]. Thick plaques of pleomorphic anucleate squamous cells with irregular contours may rarely be the only clue to an underlying squamous cell carcinoma [16]. Similar to parakeratosis, hyperkeratosis alone does not constitute a specific interpretive category.

### 2.4.3 Tubal Metaplasia (Figs. 2.18–2.21)

#### 2.4.3.1 Definition

Tubal metaplasia is a metaplastic phenomenon in which the normal endocervical epithelium is replaced by an epithelium that recapitulates that of the normal fallopian tube. This metaplastic epithelium includes several cell types (ciliated cells, peg cells, and goblet cells) [17] (Fig. 2.18). Tubal metapalsia is a frequent finding in the upper endocervical canal/lower uterine segment.

#### 2.4.3.2 Criteria

Columnar ciliated endocervical cells that may occur in small groups or as pseudostratified crowded groups (Figs. 2.19 and 2.20).

Nuclei are round to oval and may be enlarged, pleomorphic, and often hyperchromatic.

Chromatin is evenly distributed and nucleoli are usually not seen.

Nuclear to cytoplasmic ratio can be high.

The cytoplasm may show discrete vacuoles or goblet cell change (Fig. 2.21).

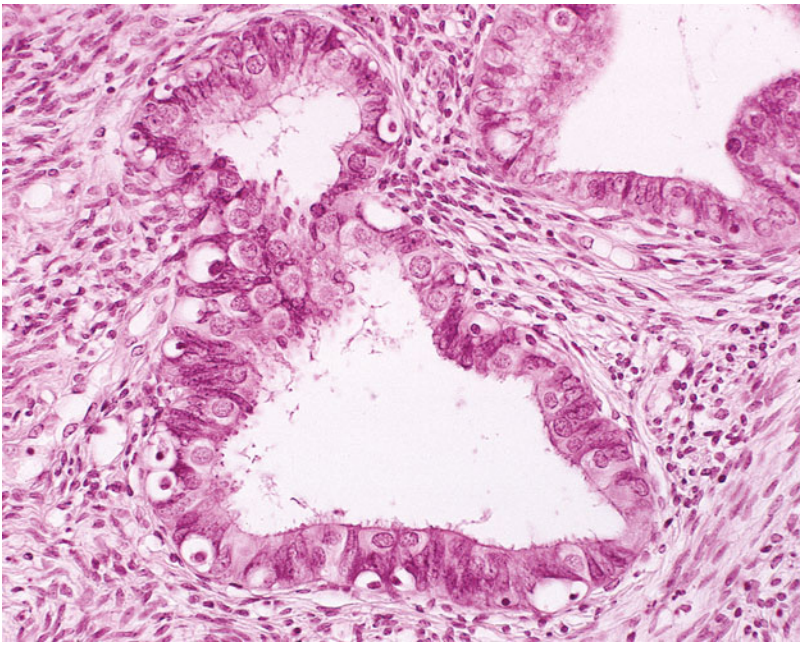

**Fig. 2.18** Tubal metaplasia (histology, H&E). Endocervical gland with tubal metaplasia amid cervical stroma. The ciliated cells of tubal metaplasia show prominent terminal bars at the base of the cilia

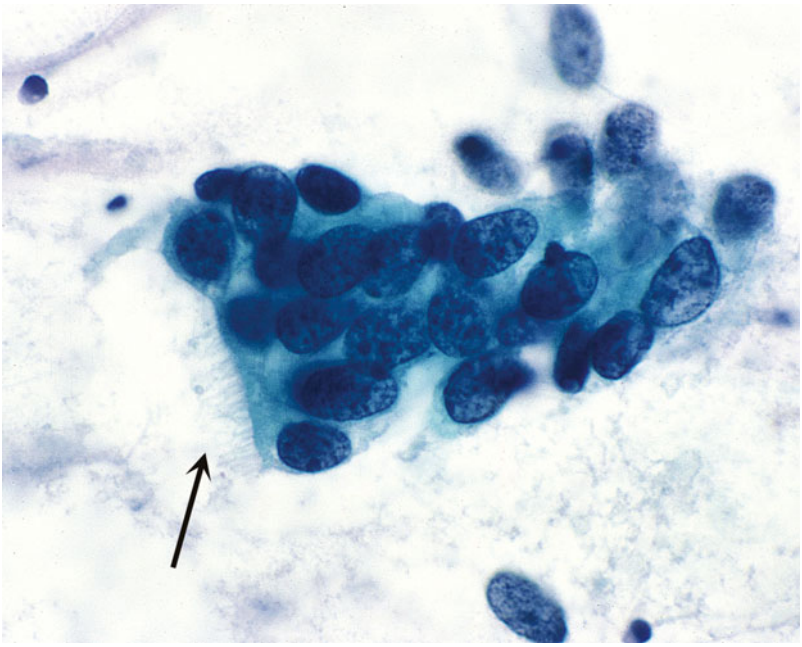

**Fig. 2.19** Tubal metaplasia (*CP*). Ciliated cells derived from tubal metaplasia. Note terminal bar and cilia at left edge (*arrow*). Tubal metaplasia shows prominent pseudostratification and can have enlarged nuclei that make it a look-alike for endocervical adenocarcinoma in situ

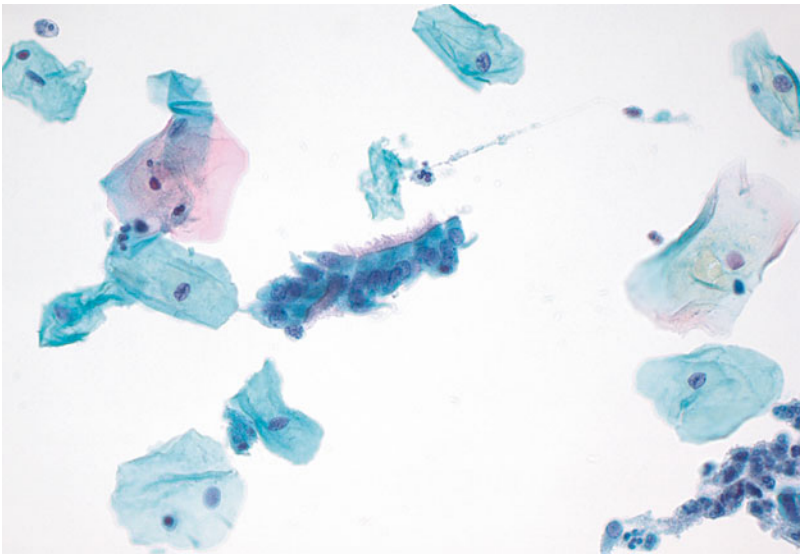

**Fig. 2.20** Tubal metaplasia (*LBP*, Thin Prep). A linear array of cells showing tubal metaplasia

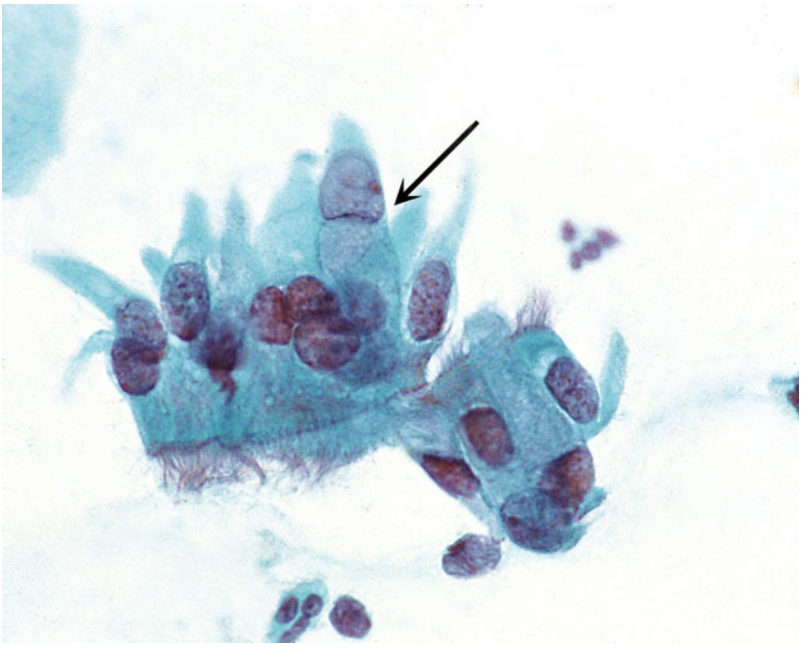

**Fig. 2.21** Tubal metaplasia (*CP*). Ciliated columnar endocervical cells. A goblet cell is seen at the center with its nucleus closer to the top of the image (*arrow*)

Presence of cilia and/or terminal bars is characteristic, but single ciliated cells in isolation are not sufficient for the designation.
Mitoses may be present.

#### 2.4.3.3 Explanatory Notes

Tubal metaplasia is among the most common benign processes to be misinterpreted as endocervical atypia or neoplasia. This is due to the tendency toward enlarged nuclei, crowded nuclei, and nuclear stratification. However, terminal bars and cilia establish a benign interpretation (see Figs. 6.12, 6.13, and 6.14).

### 2.4.4 Atrophy (Figs. 2.22–2.27)

#### 2.4.4.1 Definition

Atrophy is a normal aging phenomenon associated with lack of hormonal stimulation that leads to thinned epithelium consisting of only immature basal/parabasal cells (Fig. 2.22).

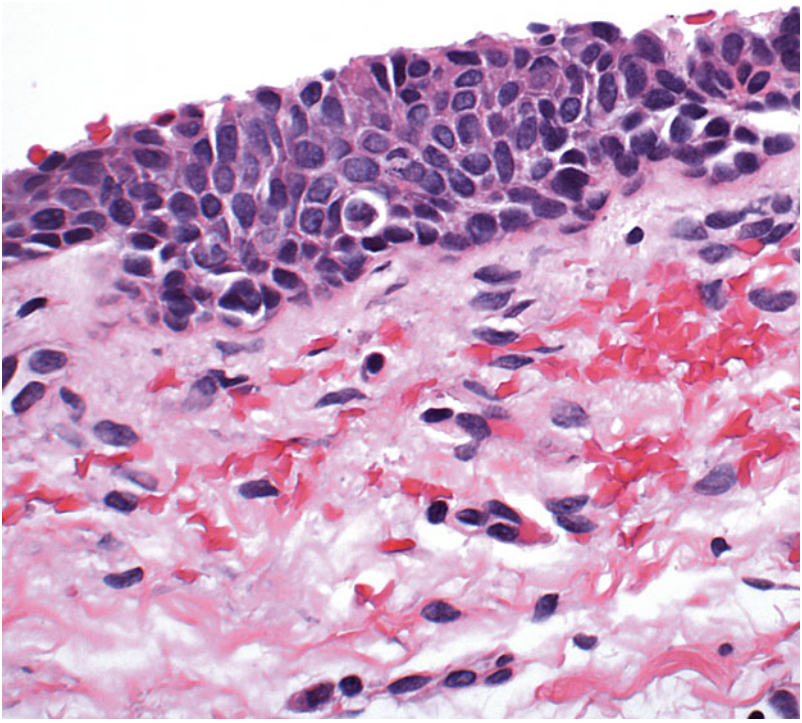

**Fig. 2.22** Atrophy (histology, H&E). The cervical squamous epithelium is remarkably thinned and made up entirely of parabasal cells. This is a consequence of waning hormonal support. In such cases, p16 immunostain would be negative

### 2.4.4.2 Criteria

Flat, monolayer sheets of parabasal-like cells with preserved nuclear polarity and little nuclear overlap in individual focal planes (Fig. 2.23).

Dispersed parabasal-type cells may predominate.

Generalized nuclear enlargement may occur with a slight increase in nuclear to cytoplasmic ratio.

Intermediate cells tend to be normochromatic, but parabasal-type cells may have mild hyperchromasia and tend to have more elongated nuclei.

Chromatin is uniformly distributed and nuclear contours are regular.

Autolysis may result in the presence of stripped nuclei.

An abundant inflammatory exudate and basophilic granular background that resembles tumor diathesis may be present in examples of extreme atrophy (atrophic vaginitis) (Figs. 2.24 and 2.25).

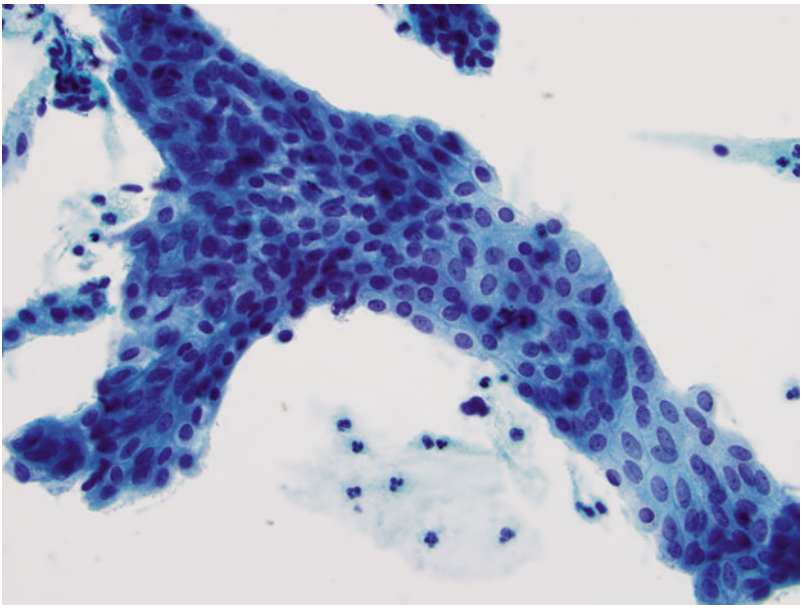

**Fig. 2.23** Atrophy (*LBP*, *ThinPrep*). Note flat, monolayer sheet of parabasal-type cells, with preserved nuclear polarity

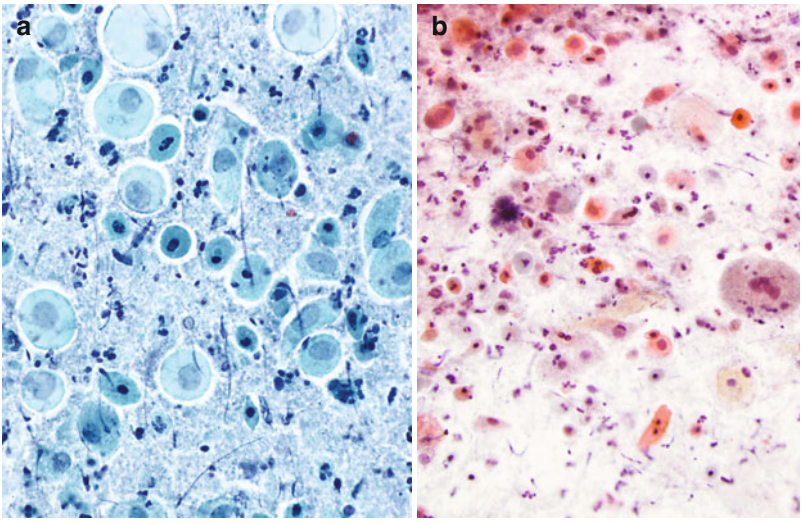


**Fig. 2.24** Atrophy with inflammation ("atrophic vaginitis") (*CP*). Note the classic finding of granular debris in background, degenerating parabasal cells, and polymorphonuclear leukocytes. (**a**) "Blue blobs" and pseudoparakeratosis are also seen in atrophic vaginitis, the former being more prominent in conventional preparations (**b**)

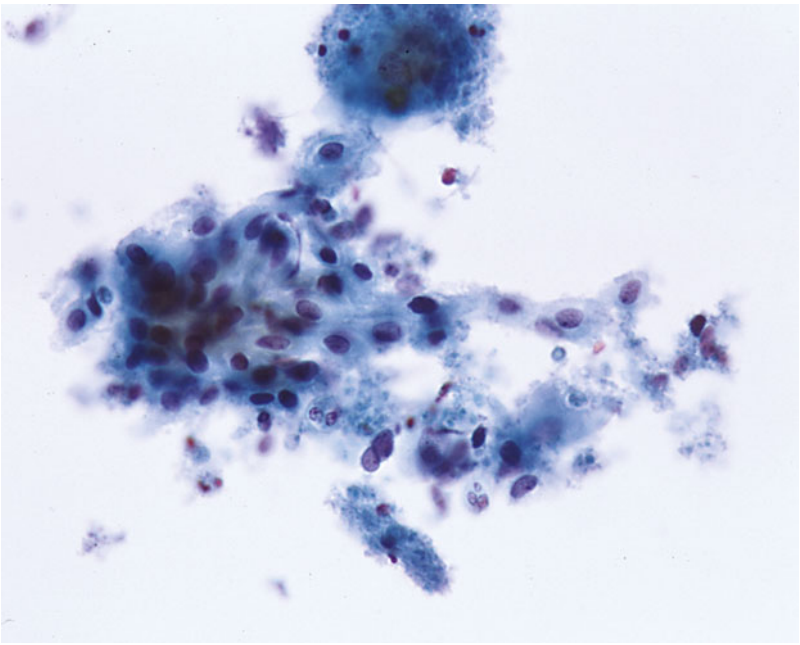

**Fig. 2.25** Atrophy with inflammation (atrophic vaginitis) (*LBP*, *ThinPrep*). In liquid-based preparations, the granular debris is often clumped and adheres to atrophic cell clusters in a pattern that may mimic "clinging tumor diathesis" (see Fig. 5.58). Attention to cellular features is crucial to avoid overinterpretation

Globular collections of basophilic amorphous material (blue blobs) reflect either degenerated parabasal cells or inspissated mucus.

Degenerated orangeophilic or eosinophilic parabasal cells with nuclear pyknosis resembling "parakeratotic" cells may be present ("pseudoparakeratosis") (Fig. 2.26).

Histiocytes varying in size and shape and containing multiple, round to epithelioid nuclei and foamy or dense cytoplasm may be seen (Fig. 2.27).

### Preparation-Specific Criteria

*Liquid-Based Preparations*:

Less nuclear enlargement than in conventional preparations due to immediate fixation, rounding up, and a lack of flattening on the slide.

Naked nuclei from autolysis may be reduced in number.

Granular background material tends to clump rather than be dispersed, yielding a "cleaner" background (Fig. 2.26); however, the clumps may "cling" to the cells and make it difficult to visualize individual cells (Fig. 2.25).

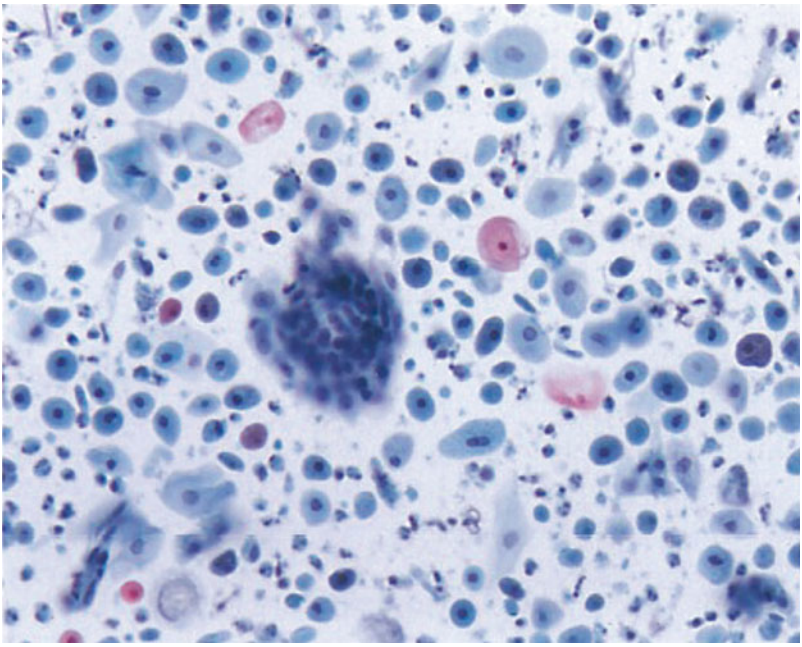

**Fig. 2.26** Atrophy (*LBP*, *SurePath*). Note more dissociation of parabasal cells in a relatively clean background

*Conventional Preparations*:
Air-drying artifact may result in more prominent cellular enlargement.
Granular basophilic "dirty" background of debris, with more "blue blobs" (Fig. 2.24).

### 2.4.4.3 Explanatory Notes

Atrophic changes are due to decreased hormonal support of epithelial tissues. The degree of atrophic change is thus highly variable, reflecting the differing levels of hormonal support that may be present. Cytomorphology can range from intermediate cell predominant to parabasal predominant to deeply atrophic (atrophic vaginitis) patterns in postmenopausal women. These differences may reflect alternate sources of endogenous estrogen or the presence of exogenous estrogenic substances.

Reporting of atrophic changes is variable and poorly reproducible [18]. Atypical cellular changes associated with atrophy warrant an interpretation of atypical squamous cells (ASC). Although cytology should be judged on its own morphologic merits, a patient is more likely to have significant disease in face of a history of previous cervical abnormality or a prior positive high-risk HPV test. In addition, atrophy may coexist with dysplasia or neoplasia, and the diffusely increased

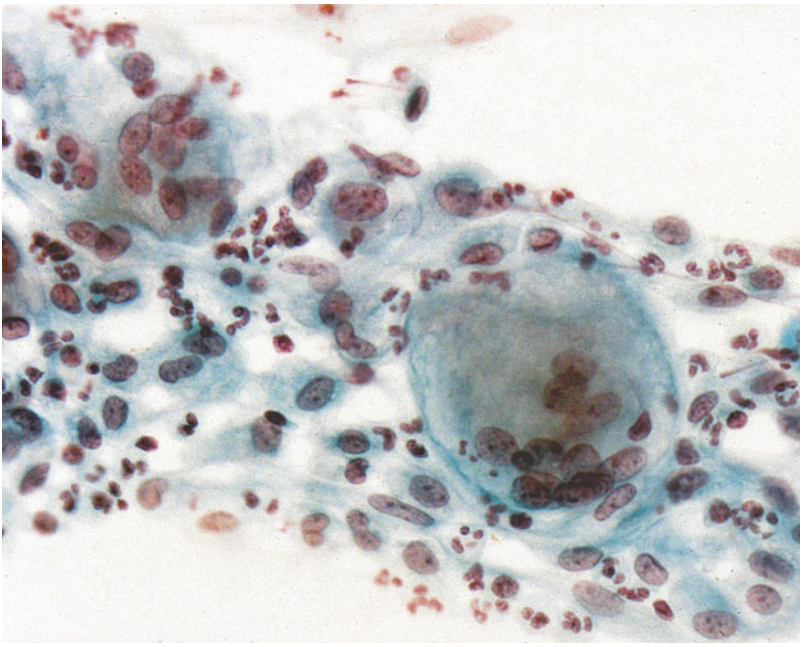

**Fig. 2.27** Atrophy with multinucleated giant cells (*CP*). Multinucleated histiocytic giant cells are a nonspecific finding and are often seen in postmenopausal and postpartum specimens. They differ from other giant cells such as syncytiotrophoblast (Fig. 2.29b) and multinucleated cells in herpes infection (Fig. 2.63)

nuclear to cytoplasmic ratio of background parabasal/basal squamous cells can make identification of true abnormalities more challenging. As such, these cases should be reviewed with care. "Atrophic" changes may also be seen for weeks after parturition and other situations where estrogen and progesterone levels have decreased.

In postmenopausal and postpartum states, multinucleated histiocytes (giant cells) are often found in cervical samples associated with chronic inflammatory processes [19] (Fig. 2.27).

### 2.4.5 Pregnancy-Related Cellular Changes (Figs. 2.28–2.30)

During pregnancy, a variety of epithelial and non-epithelial cell changes can be identified in cervical cytology specimens. These changes can be misinterpreted as representing neoplastic abnormalities.

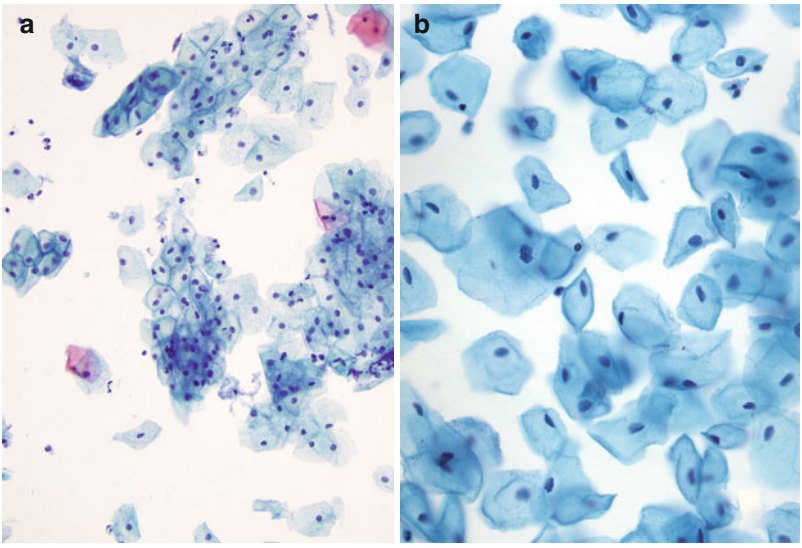


**Fig. 2.28** Pregnancy-related hormonal changes – navicular cells. In pregnant patients, squamous cells become laden with glycogen, and have a vaguely "boatlike" shape referred to as "navicular" cells (**a**) *left*, *LBP*, *ThinPrep*, and (**b**) *right*, *LBP*, *SurePath*

### 2.4.5.1 Hormonal Changes (Fig. 2.28)

The altered hormonal stimulation in pregnancy leads to incomplete maturation of the squamous epithelium resulting in an intermediate cell – dominant pattern. In association with this pattern, a particular appearance of the intermediate squamous cell showing prominent glycogen with a flattened "boatlike" appearance is common. This appearance is referred to as "navicular" cells. When progesterone secretion is prolonged (as in pregnancy), the navicular cells have greatly thickened borders and can form dense clusters (Fig. 2.28).

#### 2.4.5.1.1 Criteria

Boat-shaped intermediate cells.
Abundant basophilic to clear cytoplasm, rich in glycogen.
Nuclei are vesicular and have a delicate chromatin structure.

### 2.4.5.2 Decidua (Fig. 2.29a)

Decidual cells are present in pregnancy and during the postpartum period. These cells are derived from hormonally stimulated endocervical or endometrial stroma.

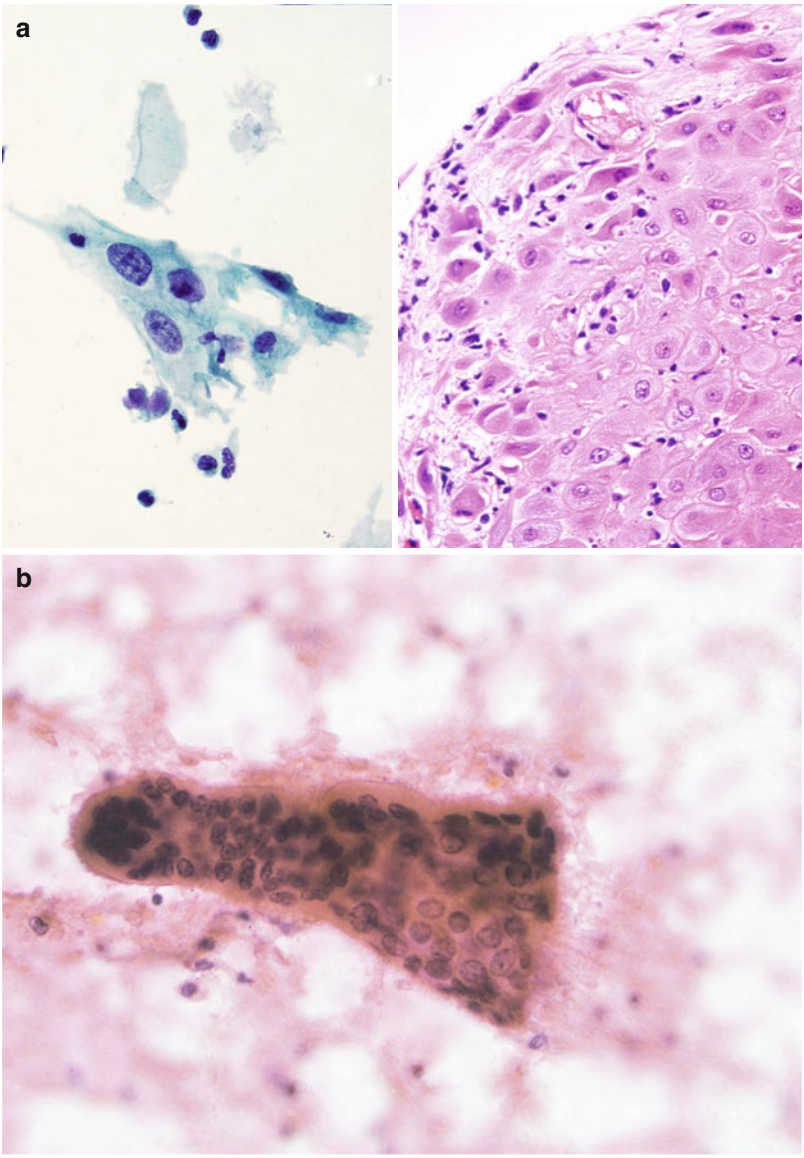


**Fig. 2.29** (**a**) Pregnancy-related cellular changes, decidua. Decidual change involving the cervical stroma can be sampled and resemble epithelial cell abnormalities, both LSIL and HSIL (See Fig. 5.53). *On the upper left* (*LBP*, *ThinPrep*) are cells that are loosely cohesive, approximately the size of mature squamous cells, with soft, ill-defined cytoplasm, and nuclei that have nucleoli and pale, finely granular, evenly distributed chromatin. They can be misinterpreted as reactive squamous cells or LSIL if one is not aware of the history of pregnancy or recent delivery. On the upper right (histology, H&E) is the corresponding histology showing decidual change. Note the resemblance to the cytology *on the upper left*. Pregnancy-related cellular changes, syncytiotrophoblast (*CP*). (**b**) The placental-derived syncytiotrophoblast is a unique cell that can have 50 or more nuclei and tends to be elongated with granular cytoplasm. Other multinucleated cells that can be seen in cervical cytology include multinucleated histiocytes in postmenopausal and postpartum women and cells infected with herpes virus

#### 2.4.5.2.1 Criteria

Cells occur singly and rarely in small clusters.
Cytoplasm is abundant, granular, or finely vacuolated and there may be cytoplasmic processes.
Nuclei are 35–50 $\mu m^2$ in area and may be lobulated or multinucleated.
Chromatin is fine, evenly distributed, and normochromatic to hyperchromatic.
Nuclear membranes are generally smooth.
Nucleoli are usually prominent and basophilic [20, 21].

### 2.4.5.3 Cytotrophoblast

Cells of cytotrophoblastic origin are derived from the placenta in late pregnancy and in the postpartum period. Rarely, they can be present for months after delivery. Cytotrophoblast are rarely identified as such. They may resemble small squamous metaplastic or endometrial cells, as well as high-grade squamous intraepithelial lesion cells. When recognized, the background often has either findings of exodus or other elements of pregnancy, e.g., decidua or syncytiotrophoblasts, which gave a clue to their identity [20].

#### 2.4.5.3.1 Criteria

Typically single cells, occasionally in small clusters.
Cells are small with enlarged nuclei, high nuclear to cytoplasmic ratios, and hyperchromasia. Chromatin is evenly distributed.
Cytoplasm is scant and may have prominent vacuoles.
Background often highly inflamed and sometimes bloody.

### 2.4.5.4 Syncytiotrophoblast (Fig. 2.29b)

Syncytiotrophoblastic cells are derived from fusion of cytotrophoblastic cells. They can be identified in cervical cytology specimens in late pregnancy and postpartum periods. They can rarely be present for months after delivery.

#### 2.4.5.4.1 Criteria

Large, multinucleated cells with up to 50 or more nuclei (Fig. 2.29b).
Nuclei are normochromatic with even chromatin distribution but often have irregular nuclear contours.
Tapering of granular cytoplasm at one end of cell.

### 2.4.5.5 Arias-Stella Reaction (Fig. 2.30)

Arias-Stella reaction is a benign process which involves glandular epithelial cells (either endocervical or endometrial) and is found in association with pregnancy or occasionally

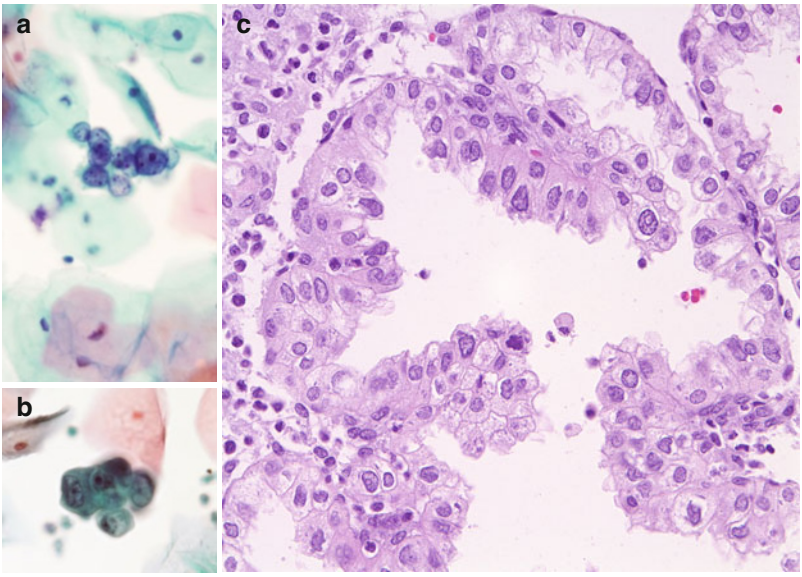


**Fig. 2.30** Pregnancy-related cellular changes, Arias-Stella reaction. The upper and lower left images (**a**, **b**, *LBP*, *SurePath*) show groups of stimulated endometrial glandular epithelium that could be mistaken for a glandular epithelial abnormality. The histology (**c**, *right*, H&E) demonstrates the exuberant variation in epithelial nuclear morphology due to hormonal stimulation during pregnancy

in nonpregnant hormonally stimulated individuals. In histologic specimens, Arias-Stella reaction manifests as pleomorphism of size and shape in glandular cell nuclei, often with bizarre forms, in association with a characteristic smudgy chromatin pattern.

#### 2.4.5.5.1 Criteria

Glandular cells, singly or in clusters.
Cytoplasm is of variable quantity and may be vacuolated.
Nuclear to cytoplasmic ratio variable, but often high.
Nuclei are large, hyperchromatic with contour irregularities (grooves and pseudoinclusions), and granular to smudgy chromatin.
Multiple prominent nucleoli.
Background is usually inflammatory, often with leukophagocytosis [22].

### 2.4.5.6 Explanatory Notes

The changes seen in pregnancy can be misinterpreted as being of preneoplastic or neoplastic origin, primarily because they may show concerning nuclear features [20]. It is important to be aware of the patient's pregnant or postpartum status to avoid overinterpretation of these findings. Even if the clinician does not provide this

information, the finding of one or more of the characteristic features noted above should elicit query regarding pregnancy or postpartum state, particularly if only a few cells with the changes are present and if the features noted are not classic for epithelial neoplasia.

Squamous alterations are common during pregnancy. Reactive and metaplastic squamous changes are often present. In addition, increased glycogenation can result in cytoplasmic clearing in intermediate (navicular) cells that may mimic koilocytic change; however, the clearing due to glycogenation is typically diffuse, involving all or most of the cell, and lacks the sharp "cookie cutter" edges of koilocyte vacuoles (See Figs. 5.4, 5.5, and 5.6). More importantly, the cells lack nuclear atypia, necessary for the interpretation of a squamous preneoplastic abnormality. Reactive glandular cell alterations are also commonly encountered in cervical cytology specimens from pregnant women and have features similar to reactive/reparative endocervical alterations from other causes.

Decidual cells can be misinterpreted as ASC-US or LSIL when cytoplasm is abundant or ASC-H or HSIL when there is a high nuclear to cytoplasmic ratio. However, at low magnification, these cells are typically larger than dysplastic squamous cells, particularly those of high-grade lesions. Additionally, the nuclear contours are typically smooth, the chromatin is finely granular and evenly distributed, and nucleoli are usually prominent [20, 21].

Cytotrophoblast cells most commonly resemble reactive squamous cells but can occasionally be mistaken for HSIL or ASC-H, as the nuclei are large and hyperchromatic and the nuclear to cytoplasmic ratios are often high. However, chromatin texture is fine and evenly distributed. Nucleoli, when present, also support a benign interpretation. Syncytiotrophoblast is most likely to be mistaken for herpes infection, but the nuclei lack the ground-glass inclusions seen in herpetic cytopathic effect and show some heterochromatin. The tapering of the cytoplasm at one end (where the cell was attached to the placenta) and "bunching up" of nuclei may be helpful in distinguishing syncytiotrophoblast from other multinucleated cells.

## 2.5 Other Nonneoplastic Findings

### 2.5.1 Reactive/Reparative Cellular Changes

#### 2.5.1.1 Definition

Reactive cellular changes which are associated with inflammation, physical or chemical trauma, radiation, IUD irritation, or other nonspecific causes.

### 2.5.2 Reactive Cellular Changes Associated with Inflammation (Includes Typical Repair) (Figs. 2.31–2.40)

#### 2.5.2.1 Criteria

Nuclear enlargement of a variable degree (Figs. 2.31).
Nuclei are typically nonoverlapping.
Endocervical cells may show greater nuclear enlargement (Figs. 2.32 and 2.33).
Occasional binucleation or multinucleation may be observed.
Nuclear outlines are smooth, round, and uniform.
Nuclei may appear vesicular and hypochromatic (Figs. 2.34).
Mild hyperchromasia may be present, but the chromatin structure and distribution remain uniformly finely granular (Fig. 2.35).
Prominent single or multiple nucleoli may be present.
Cytoplasmic boundaries are well defined.
Cytoplasm may show polychromasia, vacuolization, or perinuclear halos but without peripheral thickening (Figs. 2.36 and 2.37).
Enlarged cells often form cohesive sheets that interdigitate in a classic "school of fish" architecture or may be mechanically distorted by sampling and elongate to form "taffy pull" cytoplasmic appendages (Figs. 2.38, 2.39, and 2.40).

**Preparation-Specific Criteria**

*Liquid-Based Preparations:*

Both squamous and endocervical reparative groups are more rounded and three-dimensional and thus darker due to light having to pass through more cytoplasmic and nuclear material. The edges of cells are better fixed and show less streaming relative to conventional preparations (Fig. 2.38).

*Conventional Preparations:*

Reparative changes may be more pronounced as cells flatten out against the slide. Inflammatory background tends to be more pronounced.

#### 2.5.2.2 Explanatory Notes

Reparative changes ("typical repair") may involve mature squamous, squamous metaplastic, or columnar epithelium. Cognizance of criteria for reactive/reparative changes is important for stratifying the boundaries between NILM and epithelial abnormalities. Reactive and reparative processes can show wide variation in nuclear area. This size variability can range from the normal area of squamous or endocervical cell nuclei to markedly enlarged, often within the same cellular

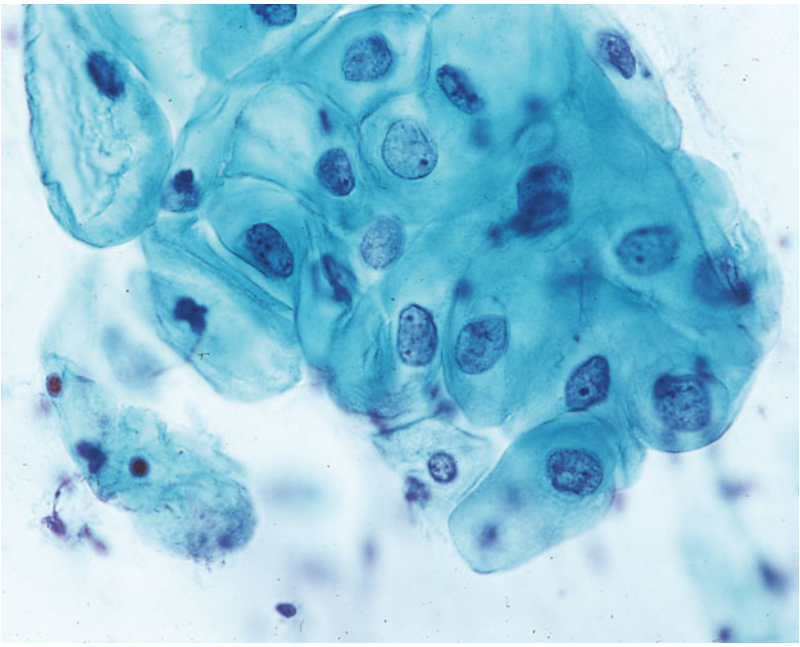

**Fig. 2.31** Reactive-reparative cellular changes (*CP*). These reactive squamous epithelial cells display mild nuclear enlargement without any significant chromatin abnormalities (Reprinted with permission from Kurman RJ. [39])

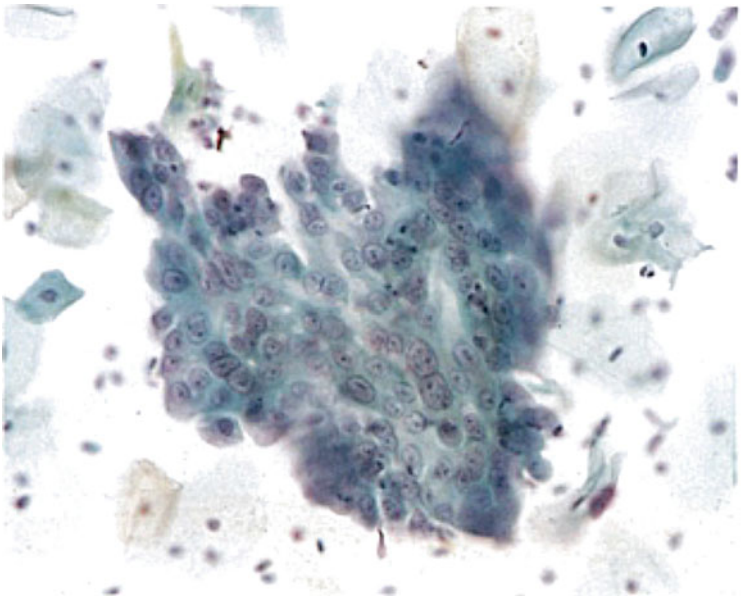

**Fig. 2.32** Reactive-reparative cellular changes: reactive endocervical cells (*LBP*, *SurePath*). Thirty-two-year-old woman. Variation in nuclear size, prominent nucleoli, and rare intracytoplasmic polymorphonuclear leukocytes are seen; these features are consistent with endocervical repair. Follow-up cytology was NILM

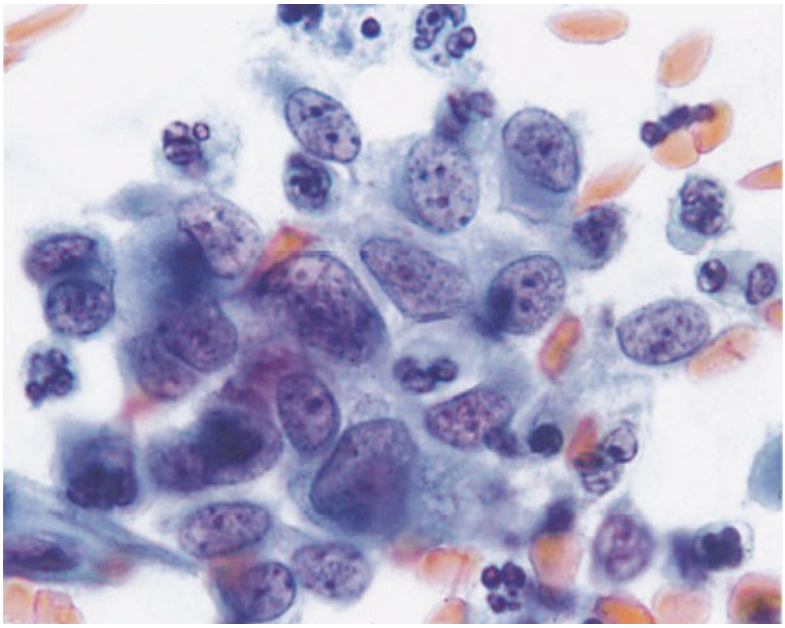

**Fig. 2.33** Reactive-reparative cellular changes: reactive endocervical cells (*CP*). A 22-year-old woman status post loop electrosurgical excision procedure (LEEP) 6 months earlier for high-grade cervical intraepithelial neoplasia (CIN). Endocervical cells show variable increase in nuclear size, prominent nucleoli, and fine chromatin. Concurrent biopsy was benign

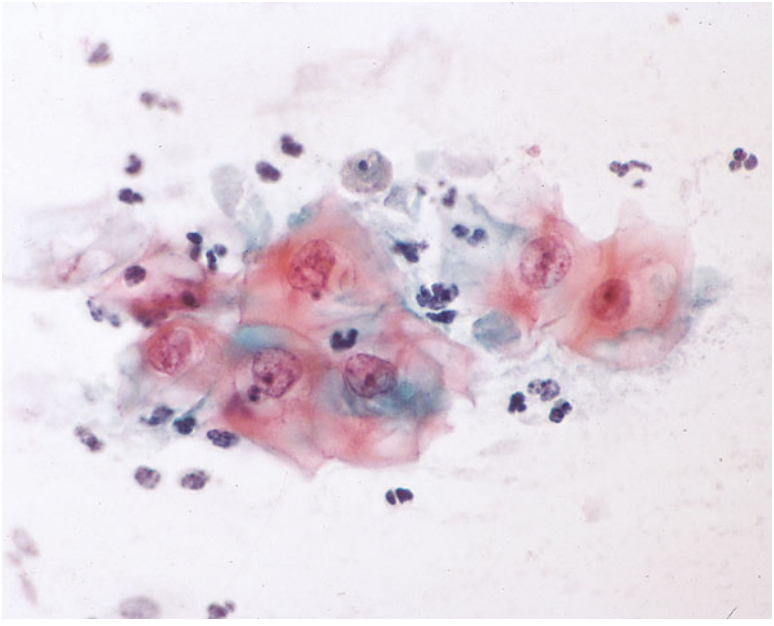

**Fig. 2.34** Reactive-reparative cellular changes: reactive squamous cells (*CP*). A 26-year-old woman, day 14 of menstrual cycle with mild vaginal discharge. Squamous cells show mild nuclear enlargement with nuclear hypochromasia, perinuclear halos, and cytoplasmic polychromasia resulting in a "moth-eaten" appearance. Trichomonads are seen in the background. Follow-up was NILM

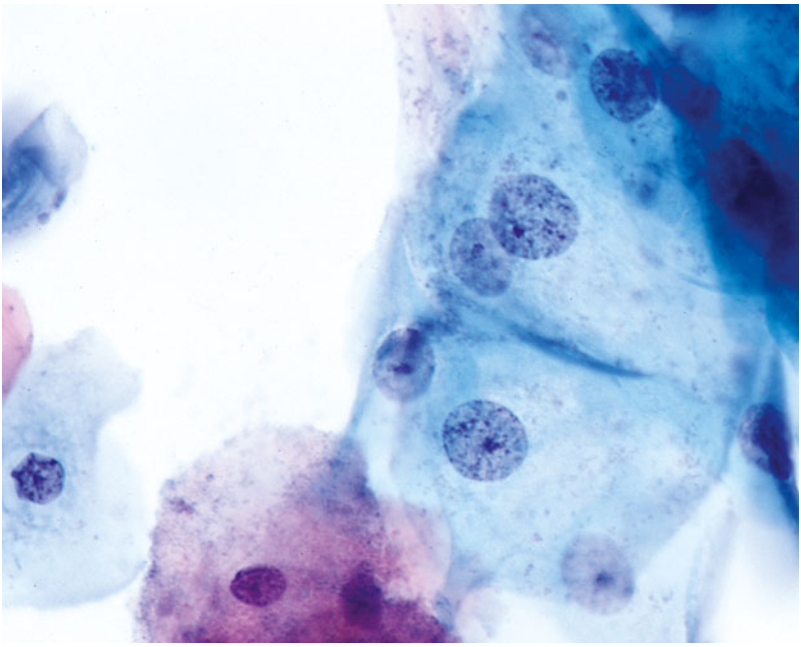

**Fig. 2.35** Reactive-reparative cellular changes: reactive squamous cells (*LBP*, *ThinPrep*). Routine screen of a 32-year-old woman. Although there is nuclear enlargement in the cells on the *right side*, the smooth nuclear contours and finely distributed chromatin favor reactive change over ASC-US

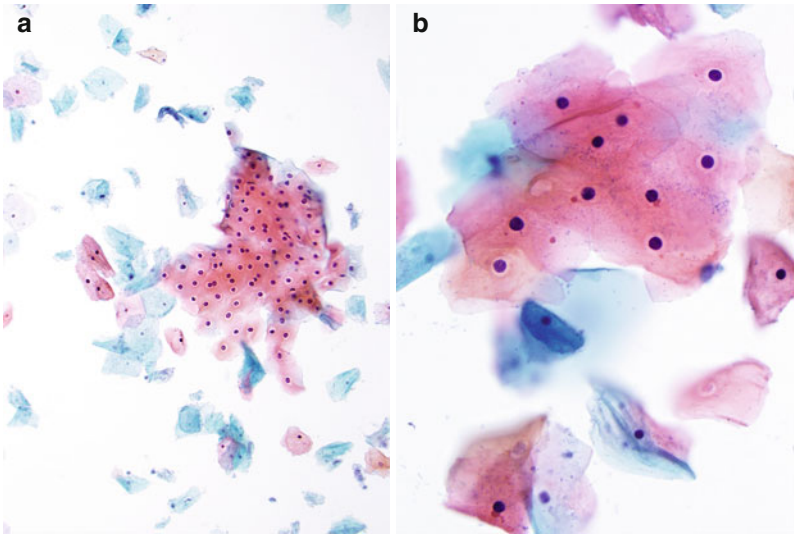


**Fig. 2.36** Reactive-reparative cellular changes: inflammatory halos. Examples of reactive perinuclear halos induced by organisms/inflammation such as seen in trichomonas infection. The images demonstrate reactive squamous cells showing small perinuclear halos that should be differentiated from koilocytic clearing seen in HPV cytopathic effect. *On the left* (**a**) is a low power from an *LBP*, *ThinPrep* and *on the right* (**b**) is a higher-power image obtained from a conventional preparation

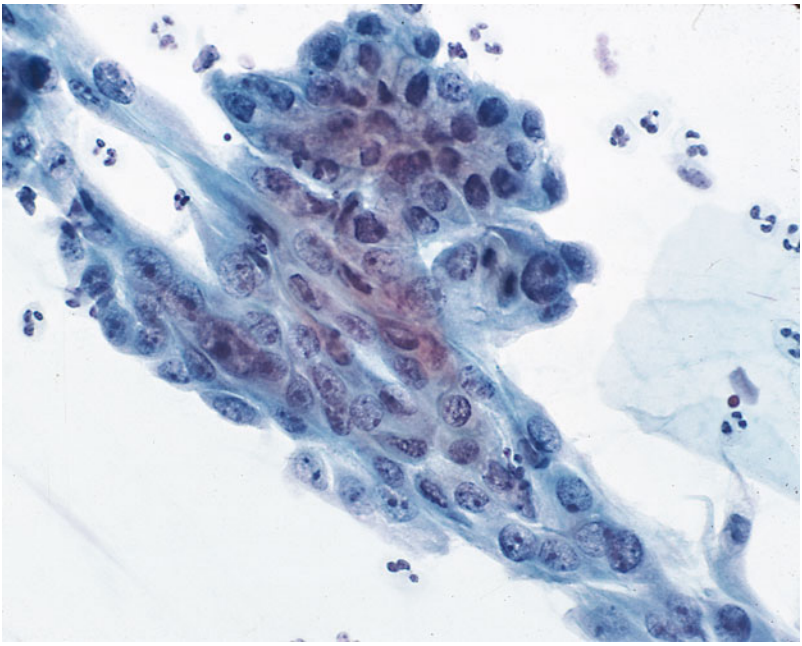

**Fig. 2.37** Reactive-reparative cellular changes: repair (*CP*). A 67-year-old woman with uterine prolapse. Flat, monolayer sheet of reparative cells with distinct cytoplasmic borders, streaming nuclear polarity, and a prominent nucleolus in almost every cell. Reactive group of endocervical cells seen at top center

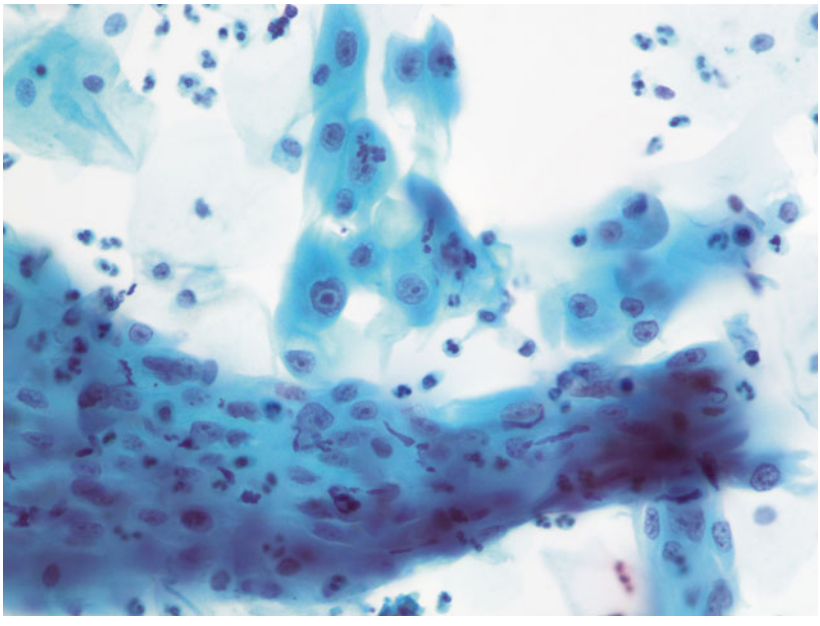

**Fig. 2.38** Reactive-reparative cellular changes: repair (*LBP*, *SurePath*). Thirty-two-year-old woman. Changes are similar to those seen on *CPs*, but cell streaming may be less apparent due to rounding of cell clusters. Note the intracytoplasmic polymorphonuclear leukocytes, another feature seen in repair. Compare to Figs. 2.39 and 2.40

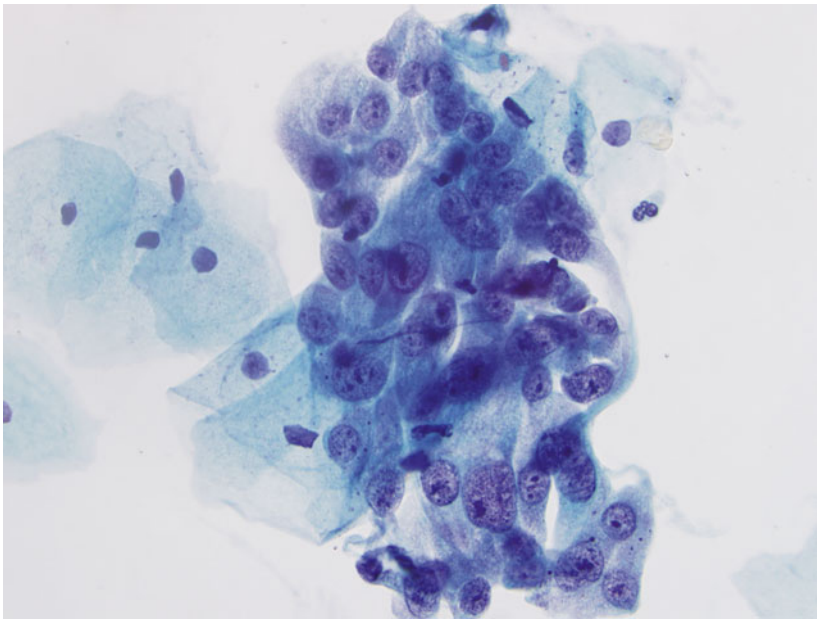

**Fig. 2.39** Reactive-reparative cellular changes: repair (*LBP*, *ThinPrep*). Cohesive group of reactive endocervical cells stimulated by factors related to inflammation and infection. Nucleoli are prominent

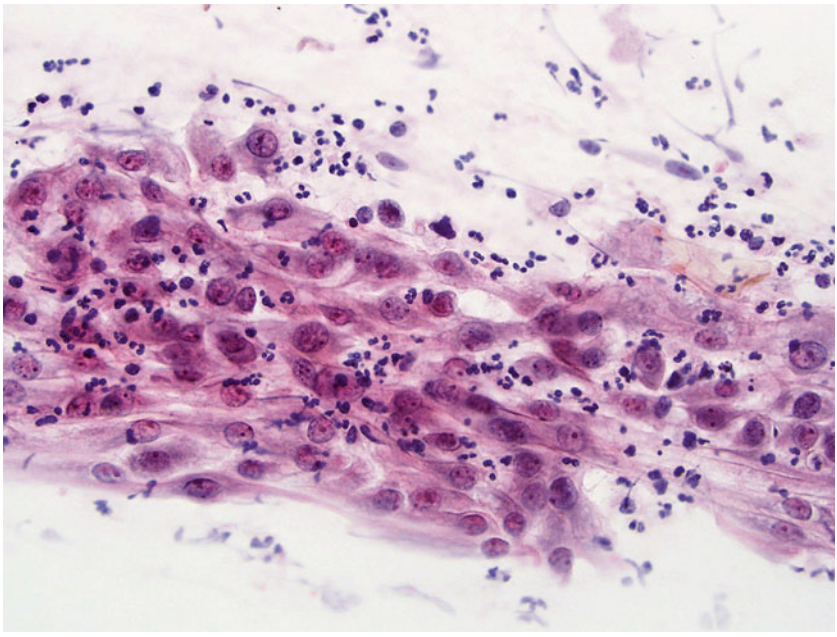

**Fig. 2.40** Reactive-reparative cellular changes: repair (*CP*). Example of cytoplasmic cohesion and streaming in repair. Note intracytoplasmic polymorphonuclear leukocytes. The streaming and interdigitation of cells has been likened to a "school of fish." Also seen in Fig. 2.37

group. In some instances, the nuclear size may even fall into the range noted in SIL or cancer. In general, round nuclear contours, even chromatin distribution, nucleoli, cellular cohesion with "school of fish" or "taffy pull" cytoplasmic features, and overall uniform cellular morphology favor a nonneoplastic process. In any preparation type, repair should have a paucity of isolated cells. When a combination of anisonucleosis, irregularities in chromatin distribution, nuclear contour irregularities, or variation in size and shape of nucleoli are present – features of so-called atypical repair – the differential diagnosis widens to include not only reactive conditions but also squamous intraepithelial lesions and even invasive cancers. When present such changes may be better categorized as "atypical glandular cells" (AGC) or "atypical squamous cells" (ASC-US or ASC-H) (see Figs. 5.66, 4.17 and 4.18).

### 2.5.3 Lymphocytic (Follicular) Cervicitis (Figs. 2.41 and 2.42)

Lymphocytic cervicitis (follicular cervicitis) is a form of chronic cervicitis that results in the formation of mature lymphoid follicles in the subepithelium of the cervix. These subepithelial lymphocytes may be sampled in the course of obtaining a cervical specimen.

#### 2.5.3.1 Criteria

Polymorphous population of lymphocytes with or without tingible body macrophages.

**Preparation-Specific Features**

*Liquid-Based Preparations*:

Lymphocytes more often appear as loosely aggregated clusters or scattered single lymphocytes in the background due to separation during processing (Fig. 2.41).

*Conventional Preparations*:

Lymphocytes are seen in clusters or streaming in strands of mucus (Fig. 2.42).

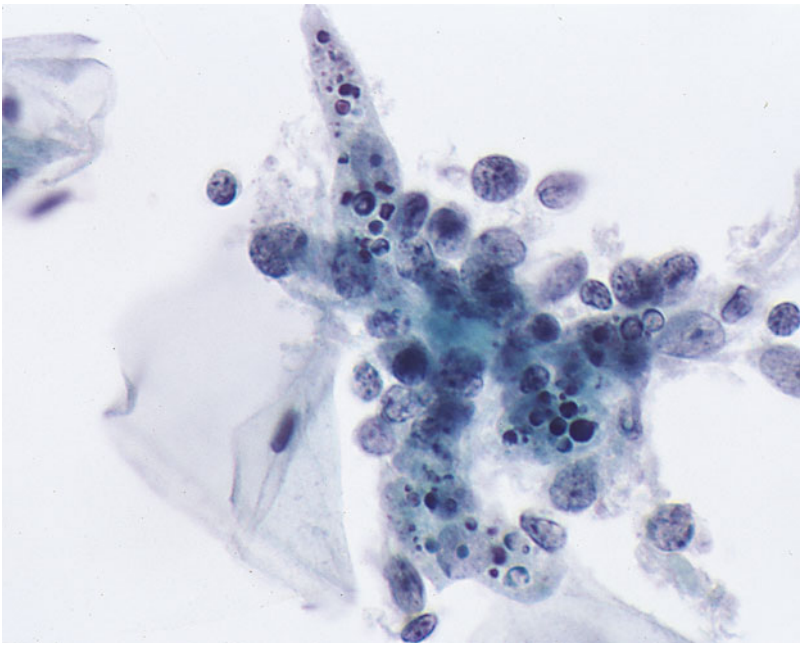

**Fig. 2.41** Reactive-reparative cellular changes: lymphocytic (follicular) cervicitis (*LBP*, *ThinPrep*). Note polymorphous population of lymphoid cells and tingible body macrophages; the lymphoid cells may clump on liquid-based preparations

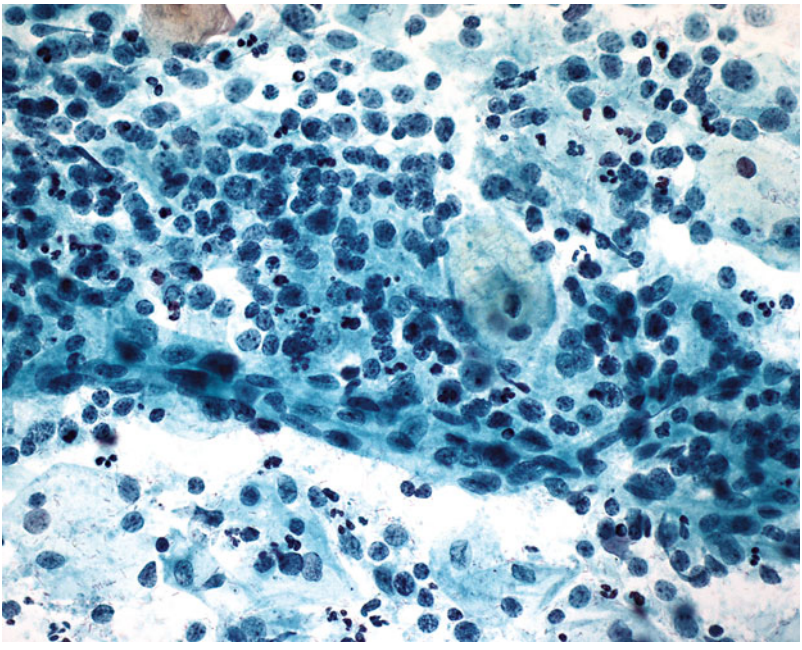

**Fig. 2.42** Reactive-reparative cellular changes: lymphocytic (follicular) cervicitis (*CP*). Abundant lymphoid cells with a tingible body macrophage located centrally

### 2.5.4 Reactive Cellular Changes Associated with Radiation (Figs. 2.43 and 2.44)

The effects of ionizing radiation on cells can lead to cytologic features which may be mistaken for neoplastic or preneoplastic conditions.

#### 2.5.4.1 Criteria

Cell size is markedly increased without a substantial increase in the nuclear to cytoplasmic ratio (Figs. 2.43 and 2.44).

Bizarre cell shapes may occur.

Nuclei may vary in size, with some cell groups having both enlarged and normal-sized nuclei.

Binucleation or multinucleation is common.

Mild nuclear hyperchromasia may be present.

Enlarged nuclei may show degenerative changes including nuclear pallor, wrinkling or smudging of the chromatin, and nuclear vacuolization.

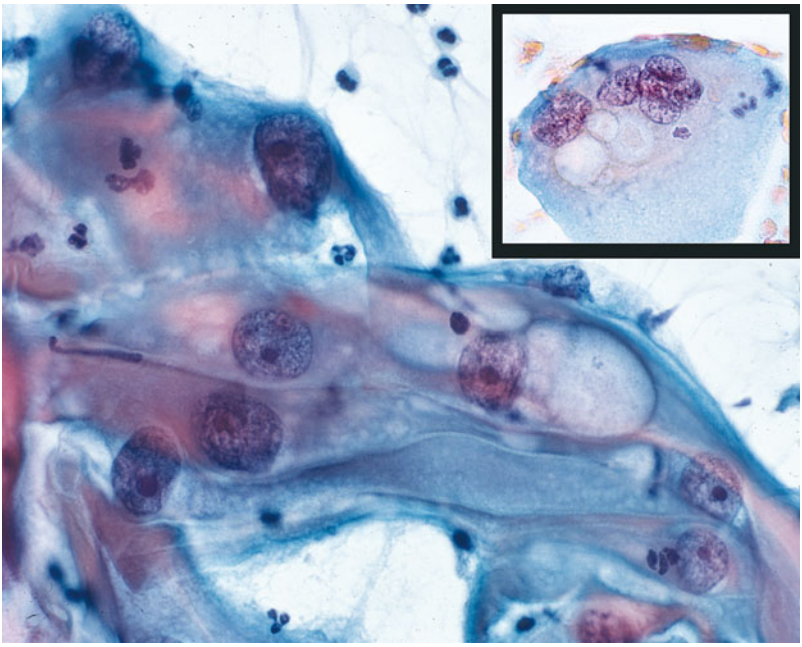

**Fig. 2.43** Reactive-reparative cellular changes: radiation (*CP*). Reactive cellular changes associated with radiation (*CP*). A 40-year-old woman with history of squamous cell carcinoma of the cervix who completed radiation therapy 8 weeks earlier. Cells with enlarged nuclei, abundant vacuolated polychromatic cytoplasm, mild nuclear hyperchromasia without coarse chromatin, and prominent nucleoli. Note multinucleation (*upper right corner inset*)

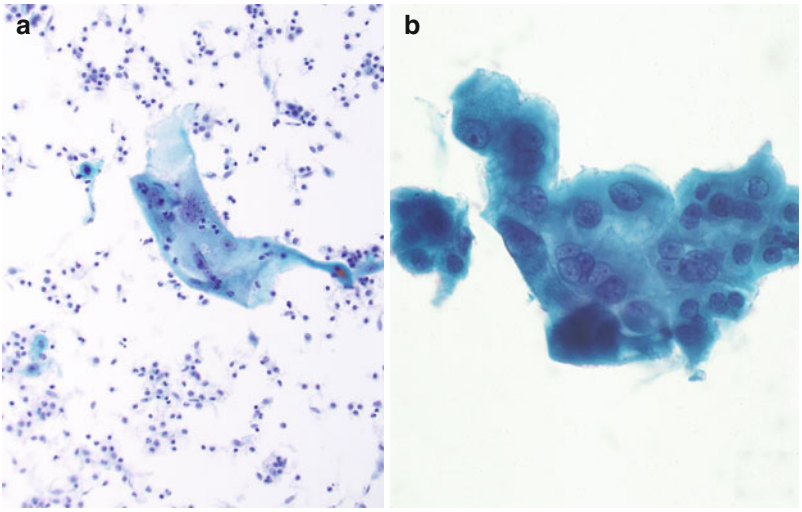


**Fig. 2.44** Reactive-reparative cellular changes: radiation. Low-power image of radiation changes in a squamous cell (**a**, *CP*). Note the irregularly shaped abundant cytoplasm and the streaming or "windblown" edges of the cell in a conventional preparation. Nuclei are typically enlarged and may be pale or become hyperchromatic as nuclear material condenses. Nucleoli are typically seen. In this case, numerous polymorphonuclear leukocytes are seen in the background. On the *left* (**b**, *LBP*, *ThinPrep*) radiated cells in liquid-based preparations do not tend to show the streaming and the cytoplasm is typically more dense. Nuclear degeneration and cytoplasmic vacuolization are common in both preparation types

Prominent single or multiple nucleoli may be seen if coexisting repair is present.
Cytoplasmic vacuolization and/or cytoplasmic polychromatic (two-color, amphophilic) staining and intracytoplasmic polymorphonuclear leukocytes may be seen.

#### Preparation-Specific Criteria

*Liquid-Based Preparations*
Cytoplasmic rounding, with less streaming.
Better preservation may attenuate the finding of bizarre cytoplasmic morphology.
Nucleoli may be more prominent.
Nuclei are often degenerated and may resemble the findings of low-grade squamous intraepithelial lesion [23].

### 2.5.4.2 Explanatory Notes

Acute radiation-induced changes, consisting of degenerated blood, bizarre cell forms, and cellular debris, generally resolve within 6 months following therapy. However, in some patients, chronic radiation-induced cellular changes may persist indefinitely. These chronic changes can include increases in cytoplasmic amount (cytomegaly), nuclear enlargement (karyomegaly) without nuclear to cytoplasmic ratio alteration, mild hyperchromasia, neutrophil invasion of cytoplasm (so-called engulfment), and

persistent polychromatic cytoplasmic staining. Certain chemotherapeutic agents may produce changes in cervical epithelial cells similar to those seen with acute and chronic radiation effects. It is important to note that bona fide squamous intraepithelial lesions in patients who have received pelvic radiation therapy will appear identical to such lesions in non-radiated patients. Care must be taken to not overinterpret specimens from radiated patients, especially in the face of perceived low-grade lesions associated with degenerated cells. Pelvic examinations and colposcopic procedures are more difficult in an irradiated pelvis which can complicate overall management.

### 2.5.5 Reactive Cellular Changes Associated with Intrauterine Contraceptive Device (Figs. 2.45–2.47)

The reactive glandular cell clusters occasionally seen in women with intrauterine devices (IUD) may represent either endometrial or endocervical columnar cells exfoliated as a result of chronic irritation by the device.

#### 2.5.5.1 Criteria

Glandular cells may be present singly or in clusters, usually of 5–15 cells, present in a clean background (Fig. 2.45, see Fig. 6.5).

The amount of cytoplasm varies, and frequently large vacuoles may displace the nucleus, creating a signet-ring appearance (Fig. 2.46).

Occasional single epithelial cells with increased nuclear size and high nuclear to cytoplasmic ratio may be present, which can be mistaken for HSIL/ASC-H (Fig. 2.47).

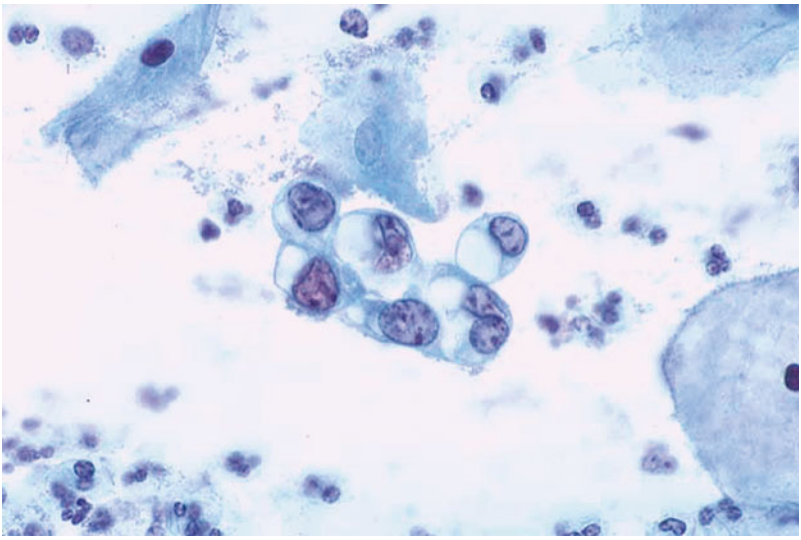

**Fig. 2.45** Reactive-reparative cellular changes: IUD (*CP*). Reactive cellular changes associated with intrauterine contraceptive device (IUD). Note small cluster of glandular cells with cytoplasmic vacuoles displacing nuclei

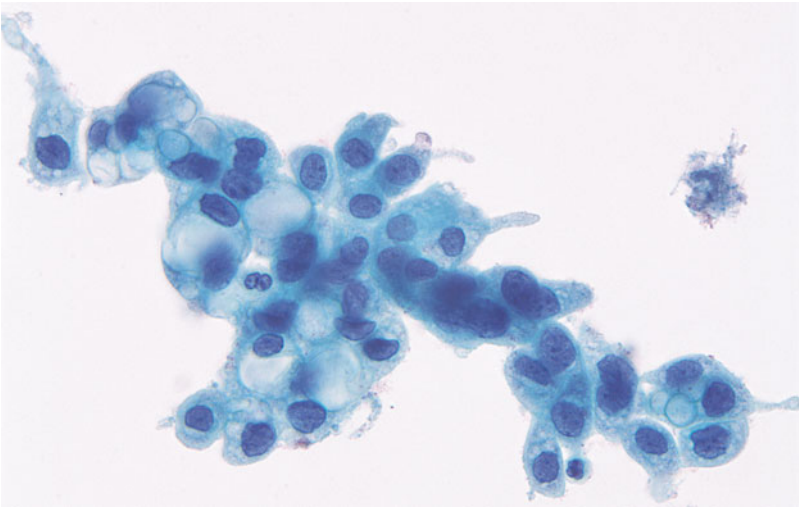

**Fig. 2.46** Reactive-reparative cellular changes: IUD (*LBP*, Thin Prep). In liquid-based preparations, cellular groups tend to be tighter but the same features of cytoplasmic vacuolization and reactive nuclear changes are present as are noted in *CP*

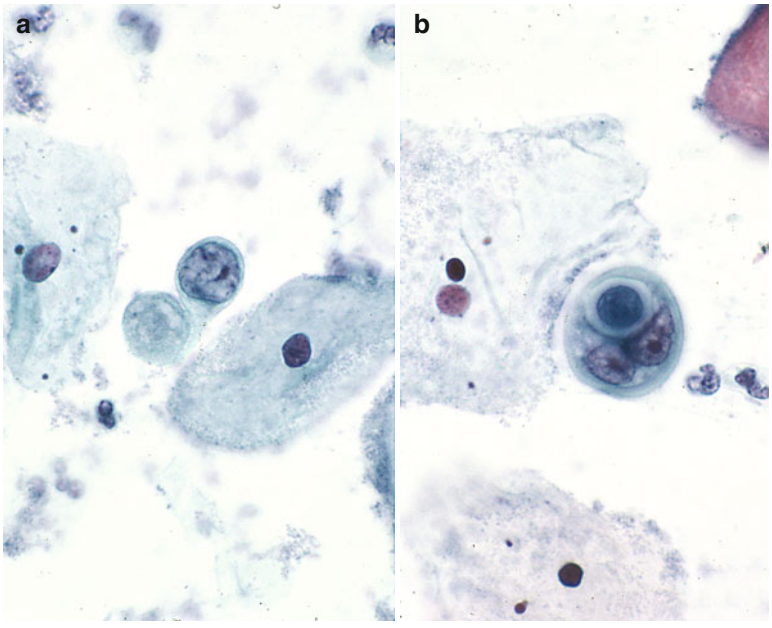


**Fig. 2.47** Reactive-reparative cellular changes: IUD (*CP*). Epithelial cells with a high nuclear to cytoplasmic ratio may mimic high-grade squamous intraepithelial lesion (HSIL) (*left*, **a**); however, the morphologic spectrum of abnormalities usually present with squamous intraepithelial lesions is absent. Presence of nucleoli in isolated cells with a high N/C ratio as seen in this cell (*right*, **b**) is not typical of HSIL. Obtaining a history of the presence of an IUD is important in the face of this type of abnormal morphology

Nuclear degeneration with a "wrinkled" chromatin appearance or nuclear "cracking" may be present.
Nucleoli may be prominent.
Calcifications resembling psammoma bodies are sometimes present.
Actinomyces-like organisms may be present in up to 25 % of cases (see Figs. 2.60 and 2.61).

#### 2.5.5.2 Explanatory Notes

Cells associated with the presence of an IUD may persist for several months after removal of the device. The characteristic changes fall into two distinct patterns. When present as three-dimensional clusters with vacuolated cytoplasm and nuclear changes, IUD-associated cells may resemble clusters of cells derived from adenocarcinoma of the endometrium, fallopian tube, or ovary (see Figs. 6.46, 6.47, 6.48, 6.49, 6.50, 6.51, 6.55, 6.56, and 6.57). When present as single atypical cells with higher nuclear to cytoplasmic ratios, IUD-associated cells mimic a high-grade squamous intraepithelial lesion. In general, the interpretation of adenocarcinoma should be made only with great caution in the presence of an IUD. In cases where the differential diagnosis includes HSIL or ASC-H, hrHPV testing may be helpful. If there is any doubt as to the significance of the cellular abnormalities, the cytopathologist should consider recommending removal of the IUD followed by repeat cervical cytology sampling.

## 2.6 Glandular Cells Status Post Hysterectomy (Figs. 2.48 and 2.49)

Occasionally benign-appearing glandular cells can be present in cervical cytology specimens from women who have undergone prior hysterectomy. While the origin of these benign cells may be obscure, the morphology should not be of concern for neoplasia [24].

### 2.6.1 Criteria

Benign-appearing endocervical-type glandular cells that cannot be differentiated from those routinely sampled from the endocervix (Figs. 2.48 and 2.49).
Goblet cell or mucinous metaplasia may be noted.
Round to cuboidal cells may resemble endometrial-type cells.

#### Preparation-Specific Criteria

In liquid-based preparations, there is more rounding up, formation of three-dimensional groups, and a hyperchromatic appearance.

### 2.6.2 Explanatory Notes

There are a number of explanations for this phenomenon, including the existence of glandular rests adjacent to vaginal mucosa, development of adenosis after

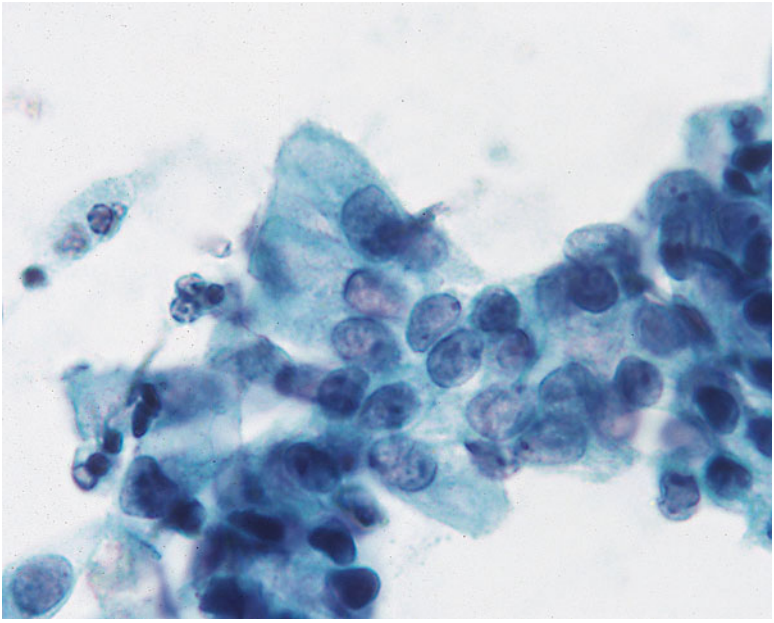

**Fig. 2.48** Glandular cells status post hysterectomy (*CP*). Vaginal smear from a 49-year-old woman status post total hysterectomy for squamous cell cancer of the cervix, showing benign, endocervical-like cells. If benign-appearing, these are of no clinical consequence and reporting is optional

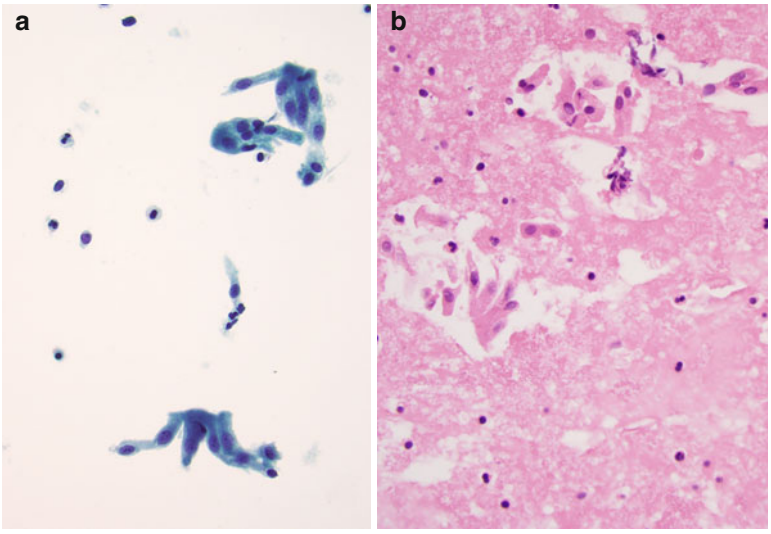


**Fig. 2.49** Glandular cells status post hysterectomy (*LBP*, *ThinPrep*). Columnar glandular cells are seen in a vaginal sample from a 68-year-old woman status post hysterectomy (**a**). She had a recto-vaginal fistula; however, a cell block (**b**) was made and the glandular cells were negative for CDX2 immunostain, making colonic origin unlikely

trauma [25, 26], mucinous or goblet cell metaplasia in response to atrophy [27], or prolapse of the remaining fallopian tube after simple hysterectomy. Following supracervical hysterectomy, an increasingly common procedure, benign endocervical-type glandular cells should be expected. The most important task is to exclude adenocarcinoma, particularly when the hysterectomy was performed for glandular neoplasia. If not atypical, post hysterectomy glandular cells have no clinical significance and reporting them is optional, since they do not change management [28].

## 2.7 Organisms

In the evaluation of reports of cervical samples with organisms, clinical management is dictated by signs and symptoms in most instances, rather than the mere presence of an organism. Clinicians and laboratories should communicate with one another about their expectations for reporting organisms and the format in which they would like to see such reports. In the absence of specific communication regarding this issue, the organisms listed in TBS should generally be reported, if identified.

Cervical cytology has relatively high specificity for most of the organisms discussed in the following sections, thus reporting them can be helpful in alerting clinicians to a potential new diagnosis, although a confirmatory test is often merited. The literature indicates that the Papanicolaou test has low sensitivity for most organisms, so it is rarely the ideal method for primary screening or diagnosis [29]. On the other hand, some laboratories are using the same liquid-based cytology vial for both morphology and microbiologic testing. Testing menus currently include *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in addition to well-established hrHPV tests.

### 2.7.1 *Trichomonas vaginalis* (Figs. 2.50–2.53)

#### 2.7.1.1 Criteria

Pear-shaped, oval, or round cyanophilic organism ranging in area from 15 to 30 $\mu m^2$ (Fig. 2.50).
Nucleus is pale, vesicular, and eccentrically located.
Eosinophilic cytoplasmic granules are often evident.
Flagella are sometimes observed.
Leptothrix may be seen in association with *T. vaginalis* (Fig. 2.51).
Associated background changes include mature squamous cells with small perinuclear halos ("trich change") and 3-dimensional clusters of neutrophils ("polyballs") (Fig. 2.52).

#### Preparation-Specific Criteria

*Liquid-Based Preparations*
Organisms tend to be smaller due to fixation in solution and rounding.
Nuclei and cytoplasmic eosinophilic granules are often better visualized.

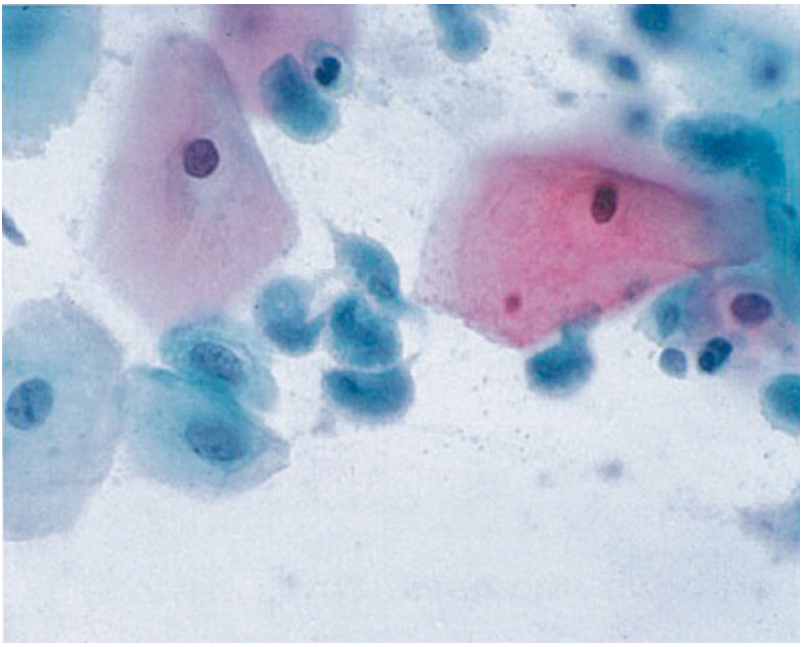

**Fig. 2.50** *Trichomonas vaginalis* (*CP*): trichomonads. Pear-shaped organism with eccentrically located nucleus and eosinophilic cytoplasmic granules. Presence of a nucleus and cytoplasmic granules distinguishes trichomonads from cytoplasmic fragments

Flagella may be better preserved and therefore identified more readily.
Occasional kite-shaped forms may be seen, especially on SurePath preparations (Fig. 2.53).

*Conventional Smears*
Increased neutrophilic infiltrate is common.
Flagella are less often identifiable.

### 2.7.1.2 Explanatory Notes

At times degenerated fragments of cytoplasm (especially in cytolysis) or inflammatory cells can be mistaken for trichomonads. Therefore, at least one of the following – good nuclear detail, eosinophilic cytoplasmic granules, or flagella – should be present to make an accurate interpretation of trichomonas. In most cases, trichomonad organisms are plentiful. Therefore, a rare fragment of cyanophilic debris is not likely to be a true trichomonad. When cervical *Leptothrix* (a gram-positive anaerobic rod, which is longer than lactobacilli, but shorter and thinner than *Candida pseudohyphae*) are present, one should search for the possible presence of trichomonads.

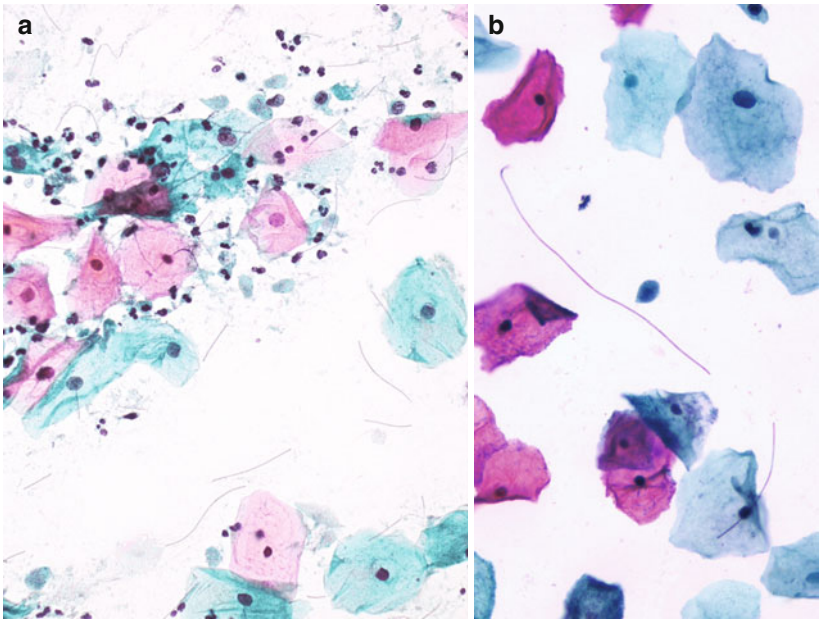


**Fig. 2.51** *Trichomonas vaginalis* and *Leptothrix. Leptothrix* (***a***, *left*, *CP*) may be seen in association with *T. vaginalis*; finding *Leptothrix* alone is not sufficient for a diagnosis of *Trichomonas,* but suggests the presence of trichomonads. On the right (**a**) is an example from a liquid-based (*SurePath*) preparation

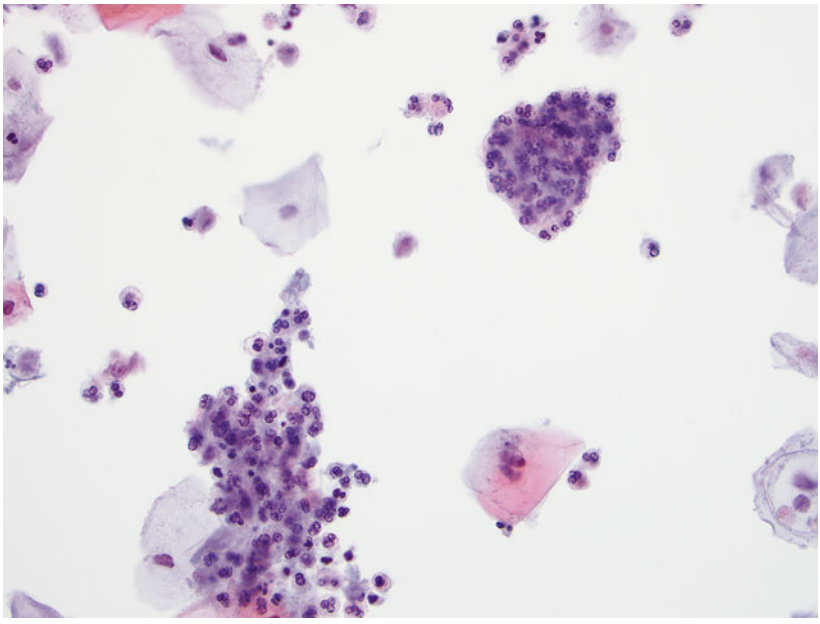

**Fig. 2.52** *Trichomonas vaginalis* (*LBP*, *ThinPrep*): "polyballs." A clue to the presence of trichomonads in a sample is the presence of aggregates of neutrophils or "polyballs." These are seen here along with a few trichomonads in the background

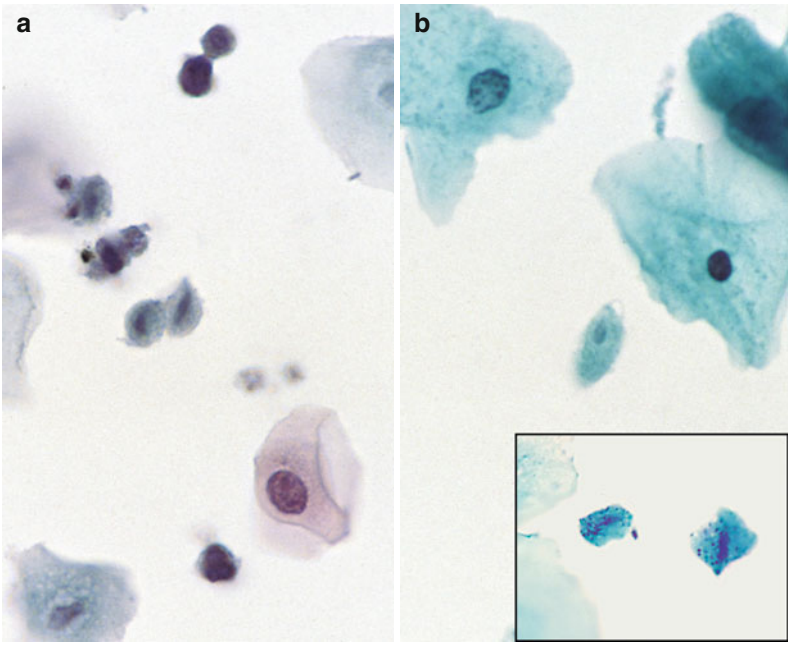


**Fig. 2.53** *Trichomonas vaginalis* (*LBP*, *SurePath*): a 32-year-old woman with vaginal discharge. The organism's nucleus, cytoplasmic granules, and flagella (*right*) may be better visualized on liquid-based preparations. Note the kite shape and granules (*bottom right inset*)

## 2.7.2 Fungal Organisms Morphologically Consistent with *Candida* Species (Figs. 2.54–2.56)

### 2.7.2.1 Criteria

Budding yeast (3–7 μm) and/or pseudohyphae; pseudohyphae can be quite long, spanning many cells, and are eosinophilic to gray brown on the Papanicolaou stain.

Pseudohyphae, formed by cytoplasmic extension of budding yeasts, lack true septations but show complete constrictions along their length that indicate the formation of new cells (Fig. 2.54).

Fragmented leukocyte nuclei and groups of squamous epithelial cells "speared" by pseudohyphae and held together in a rouleaux are often seen (Fig. 2.55).

#### Preparation-Specific Criteria

*Liquid-Based Preparations*

"Spearing" of epithelial cells is more common and can be seen at low power even if the pseudohyphae are not prominent ("shish kebab" effect) (Fig. 2.55).

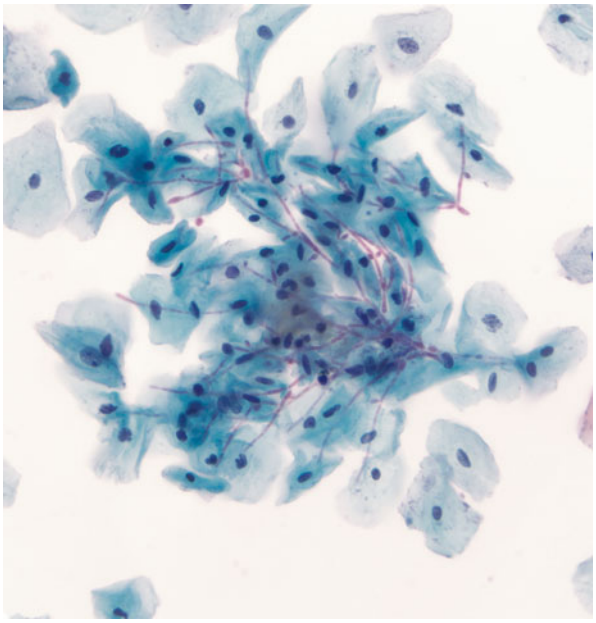

**Fig. 2.54** *Candida species*. (*LBP*, *ThinPrep*): pseudohyphae. Fungal organisms morphologically consistent with *Candida* spp. Note pseudohyphae and modest number of yeast forms

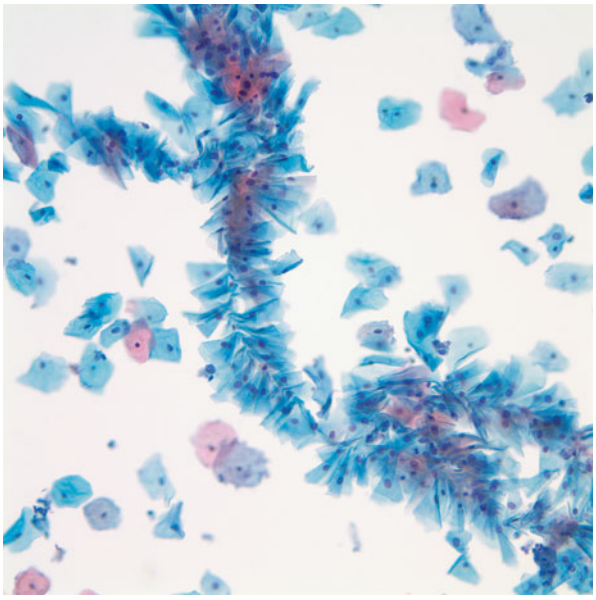

**Fig. 2.55** *Candida species*. (*LBP*, *ThinPrep*): spearing. Fungal organisms morphologically consistent with *Candida* spp. Forty-five-year-old woman. Note "spearing" or a "shish kebab" appearance of squamous cells. This feature is readily appreciated at low power, even when the pseudohyphae are not prominent. Follow-up cytology was NILM

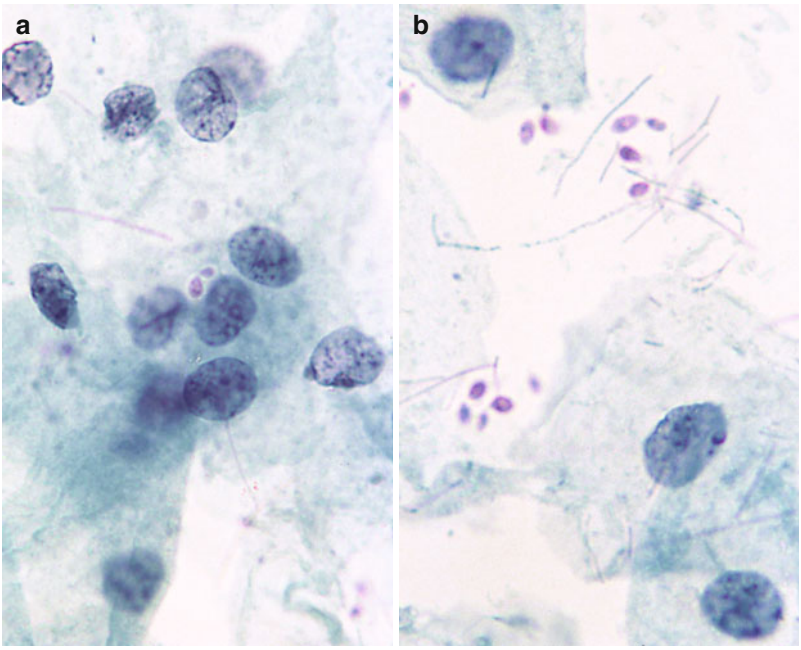


**Fig. 2.56** Candida species (*CP*): *Torulopsis*. Routine screening of a 63-year-old woman. Fungal organisms morphologically consistent with *Candida glabrata* (previously known as *Torulopsis glabrata*). Note clear halos surrounding the yeast forms (*left*). Bacteria, not pseudohyphae, are also seen in the background. This organism does not form pseudohyphae and may be pathogenic in immunocompromised individuals

#### 2.7.2.2 Explanatory Notes

*Candida (Torulopsis) glabrata* shows small uniform, round budding yeast forms surrounded by clear halos on Papanicolaou stain. Unlike other *Candida* species, it does not form pseudohyphae in vivo or in culture (Fig. 2.56).

### 2.7.3 Shift in Flora Suggestive of Bacterial Vaginosis (Figs. 2.57 and 2.58)

#### 2.7.3.1 Criteria

Individual squamous cells are covered by a layer of coccobacilli that obscure the cell membrane, forming the so-called clue cells (Fig. 2.57). Large numbers of inflammatory cells indicate a vaginitis rather than a vaginosis. There is a conspicuous absence of lactobacilli.

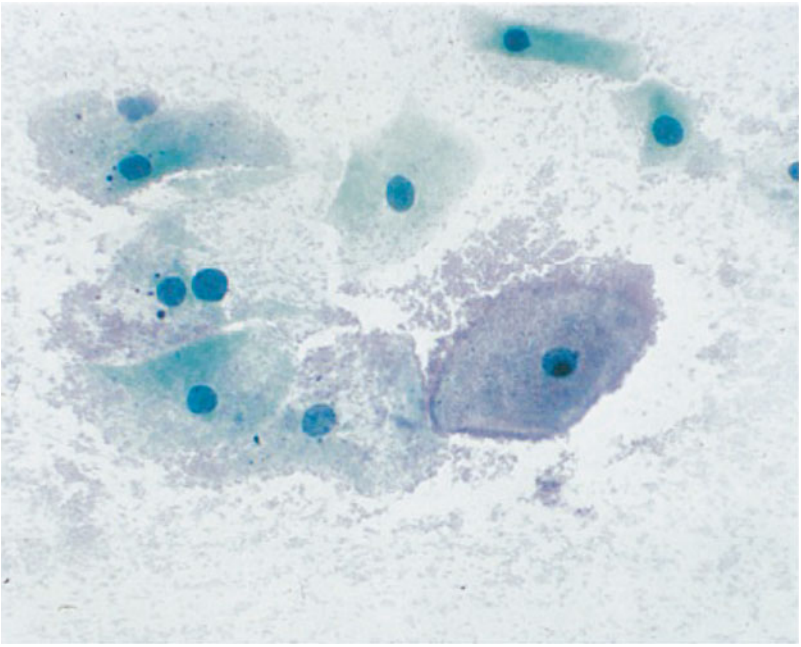

**Fig. 2.57** Bacteria – coccobacilli (*CP*). Shift in flora suggestive of bacterial vaginosis. Note the "clue cell" and filmy background due to large numbers of coccobacilli

### Preparation-Specific Criteria

*Liquid-Based Preparations*:

Squamous cells are covered with coccobacilli; however, the background is clean (Fig. 2.58).

*Conventional Preparations*:

A generalized film of coccobacilli covers cells and the background, usually without a significant neutrophilic response.

## 2.7.3.2 Explanatory Notes

*Lactobacillus* spp. (Döderlein's bacilli) are gram-positive facultative anaerobic rod-shaped bacteria that constitute a major component of the normal vaginal flora (see Fig. 2.59). Predominance of coccobacilli represents a shift in vaginal flora from lactobacilli to a polymicrobial process involving several types of obligate and facultative anaerobic bacteria, including but not limited to *Gardnerella vaginalis*, *Peptostreptococcus*, *Bacteroides*, and *Mobiluncus* spp. [30, 31]. This shift in flora, with or without accompanying clue cells, is not sufficient for the clinical diagnosis of bacterial vaginosis because specimens obtained from any single site are not necessarily representative of the entire flora of the cervix and vagina [32]. However,

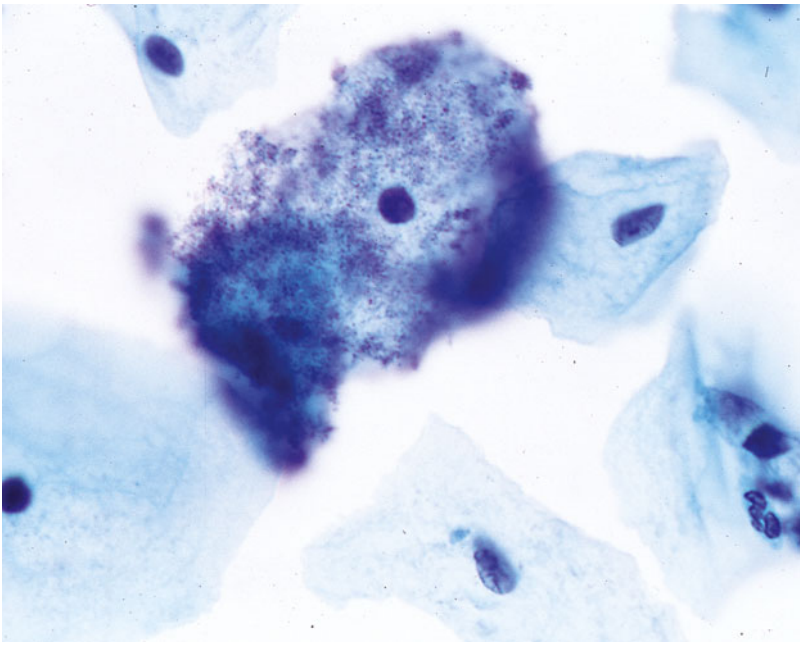

**Fig. 2.58** Bacteria – coccobacilli (*LBP*, *SurePath*). Shift in flora suggestive of bacterial vaginosis. Twenty-five-year-old woman. Note clue cell and the relatively clean background compared to that in *CPs* (see also Fig. 2.57)

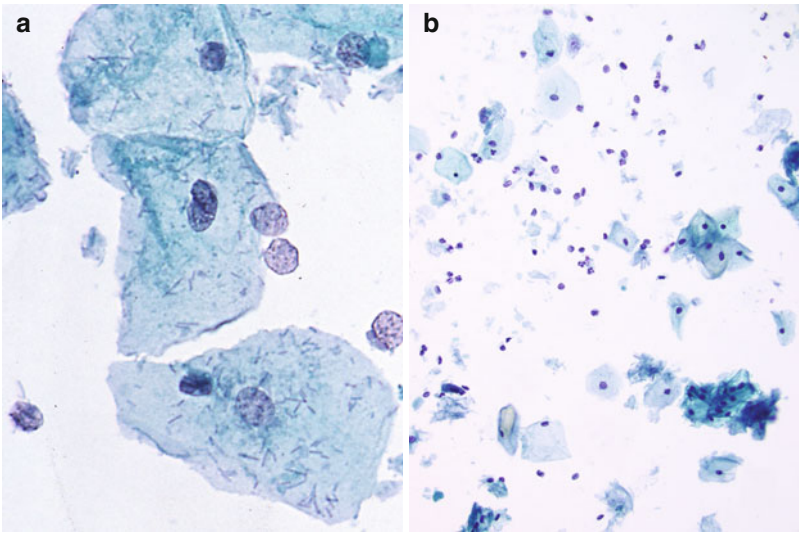


**Fig. 2.59** Bacteria: lactobacilli and cytolysis (**a**, *left*, *CP*). Lactobacilli are typically seen on the cell surfaces in liquid-based preparations and not dispersed in the background as in conventional preparations. Contrast with coccobacilli in Figs. 2.57 and 2.58 in **b**, *right*, *LBP*, *ThinPrep*) note the presence of a cytolytic background with cell debris and numerous stripped nuclei of intermediate cells

the presence of coccobacilli and absence of lactobacilli do correlate with gram-stained smears of vaginal secretions and in the proper clinical context are suggestive of the clinical diagnosis of bacterial vaginosis [33]. Bacterial vaginosis has been associated with pelvic inflammatory disease, preterm birth, postoperative gynecologic infections, and abnormal cervical cytology [34, 35]. Consultation with clinical services is suggested before routinely reporting findings of vaginitis/vaginosis so as to tailor reports to meet clinical needs.

### 2.7.4 Bacteria Morphologically Consistent with *Actinomyces* (Figs. 2.60–2.62)

#### 2.7.4.1 Criteria

Tangled clumps of filamentous organisms, often with acute angle branching, are recognizable as "cotton ball" clusters on low power (Fig. 2.60).

Filaments sometimes have a radial distribution or have an irregular "woolly body" appearance.

Masses of leukocytes adherent to microcolonies of the organism with swollen filaments or "clubs" at the periphery may be identified.

An acute inflammatory response with polymorphonuclear leukocytes is often present.

**Preparation-Specific Criteria**

*Liquid-Based Preparations*:

The strands of actinomycotic organisms tend to be finer and more delicate since the coating proteinaceous material is washed away during processing (Fig. 2.61).

The number of background neutrophils is decreased.

*Conventional Preparations*:

Aggregation of proteinaceous material tends to form a coating or "club" at the periphery of actinomyces filaments.

#### 2.7.4.2 Explanatory Notes

The presence of *Actinomyces* species in cervical cytology has an association with the presence of an intrauterine contraceptive device (IUD) and may be associated with chronic endometritis (up to 25 % of IUD patients will have *Actinomyces* organisms in cervical specimens). Detection of *Actinomyces* in cervical cytology specimens along with clinical evidence of pelvic infection can help alert clinicians to the possibility of a significant Actinomycotic infection [36]. The mere presence of *Actinomyces* in a cervical smear in an asymptomatic IUD user does not appear to constitute grounds for IUD removal [37]. Therefore, the implications of finding *Actinomyces* on a cervical cytology specimen should be considered in conjunction with the clinical findings. In liquid-based preparations, lactobacilli may aggregate to form "clumps" and mimic *Actinomyces* (Fig. 2.62).

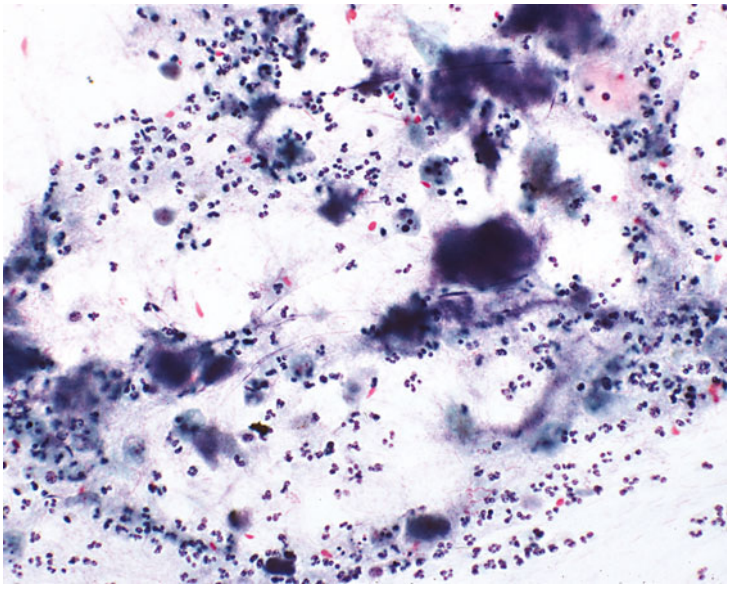

**Fig. 2.60** Bacteria morphologically consistent with *Actinomyces* (*CP*). Forty-one-year-old woman. Low power shows "cotton ball" appearance of tangled clumps of filamentous organisms. An acute inflammatory response is also apparent

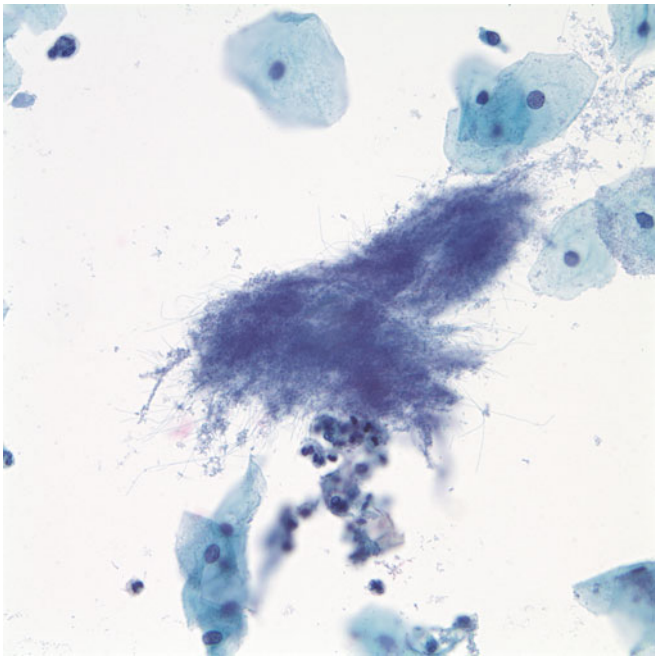

**Fig. 2.61** Bacteria morphologically consistent with *Actinomyces* (*LBP*, *ThinPrep*). Note that the clumps of protein usually seen in conventional preparations tend to be washed away in liquid-based preparations leaving only fine thin bacterial filaments. These are much thinner than the pseudohyphae of *Candida* spp

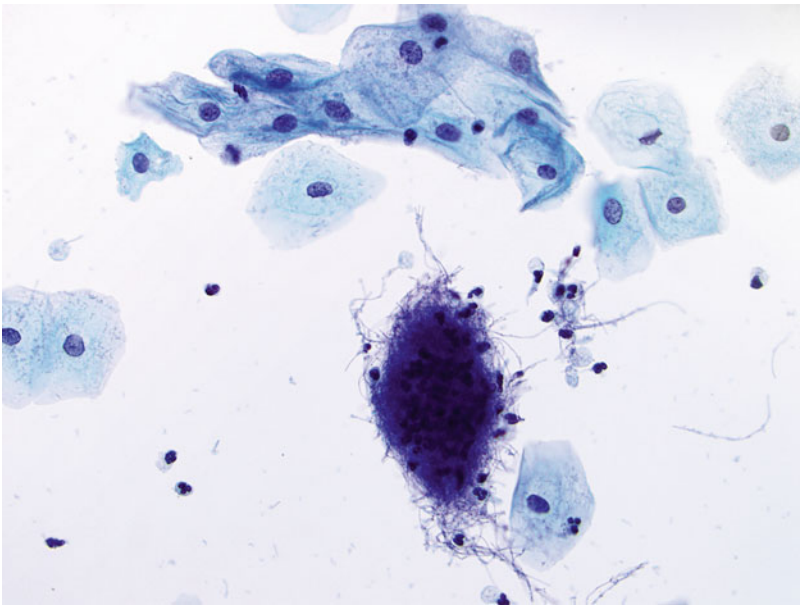

**Fig. 2.62** Bacteria: lactobacilli (*LBP*, *ThinPrep*). In liquid-based preparations, lactobacilli may aggregate to form "clumps" that may resemble *Actinomyces* species and should be distinguished by the presence of similar isolated bacilli in the background and absence of characteristic features of actinomyces

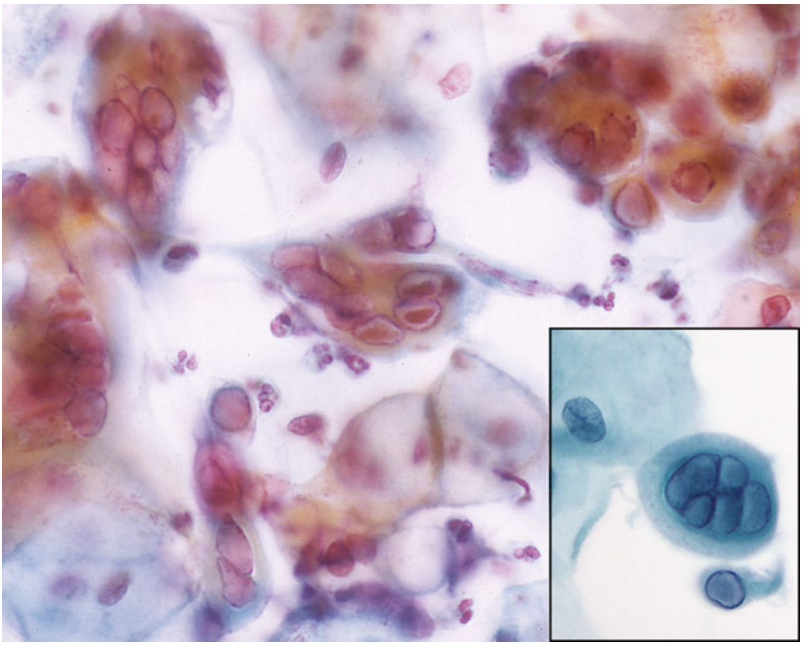

**Fig. 2.63** Cellular changes consistent with herpes simplex virus (*CP*). Note the eosinophilic intranuclear "Cowdry A-type" inclusions. The "ground-glass" appearance of the nuclei is due to accumulation of viral particles leading to peripheral margination of chromatin. The *inset* shows a *SurePath* liquid-based preparation with a typical multinucleated herpetic cell showing "ground-glass" appearance of the nuclei

### 2.7.5 Cellular Changes Consistent with Herpes Simplex Virus (Fig. 2.63)

#### 2.7.5.1 Criteria

Nuclei have a "ground-glass" appearance due to intranuclear viral particles and enhancement of the nuclear envelope caused by peripheral margination of chromatin.

Dense eosinophilic intranuclear (Cowdry) inclusions surrounded by a halo or clear zone are variably present and can be seen in both primary and recurrent infections.

Large multinucleated epithelial cells with molded nuclei are characteristic but may not always be present; mononucleate cells with the nuclear features described above may be the only finding.

#### 2.7.5.2 Explanatory Notes

Herpes cytopathic effect shows 3 "Ms" – multinucleation, molding, and margination of chromatin. Multinucleated cells have a limited differential diagnosis that includes multinucleated endocervical cells, multinucleated histiocytes, and syncytiotrophoblast cells. Herpes infection is distinguished from all of these by ground-glass (hyaline) intranuclear inclusions. The mononuclear cells of herpes infection have been shown to be overinterpreted as both LSIL and HSIL (see Fig. 5.12), especially in testing situations, although herpes is a relatively reproducible interpretation in clinical practice. Distinguishing the intranuclear herpetic inclusion from the hyperchromatic chromatin of SIL is the key to making this distinction [38].

### 2.7.6 Cellular Changes Consistent with Cytomegalovirus (Fig. 2.64)

The cytopathic effect of cytomegalovirus (CMV) affects mostly the endocervical glandular cells but can also be present in stromal cells.

#### 2.7.6.1 Criteria

Cellular and nuclear enlargement.

Large eosinophilic intranuclear viral inclusions with a prominent halo.

Small cytoplasmic, basophilic inclusions can also be present.

#### 2.7.6.2 Explanatory Notes

CMV cytopathic effect is most commonly seen in immunocompromised individuals. The large CMV infected cells may sometimes be confused with bizarre tumor cells; however, the inclusions have characteristic central eosinophilic bodies and marginated material, creating a prominent halo around the central inclusion. In contrast to herpes viral effect, CMV can also show cytoplasmic, in addition to nuclear, viral inclusions.

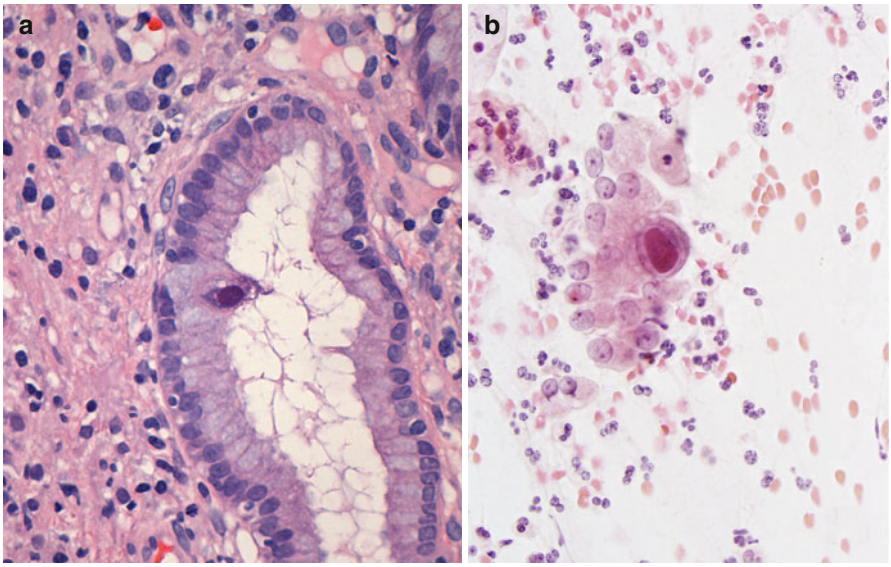


**Fig. 2.64** Cytomegalovirus (CMV). The histologic image on the left (**a**, H&E) shows CMV cytopathic effect in an endocervical cell with the typical lilac-red-colored large intranuclear inclusion. Smaller basophilic cytoplasmic inclusions adjacent to the nucleus are also apparent. On the right (**b**, *CP*) CMV inclusions are seen in an endocervical cell. CMV infection is usually not seen in squamous cells; however, it can infect a wide range of other epithelial, mesenchymal, lymphoid, and hematopoietic cells

## 2.8 Sample Reports

**Example 1**
*Specimen Adequacy:*
Satisfactory for evaluation; endocervical/transformation zone component present.
*Interpretation*:
Negative for intraepithelial lesion or malignancy.

**Example 2**
*Specimen Adequacy:*
Satisfactory for evaluation; endocervical/transformation zone component present; partially obscuring inflammation present.
*Interpretation*:
Negative for intraepithelial lesion or malignancy.
Trichomonas vaginalis identified.
Reactive squamous cells associated with inflammation (includes typical repair).

**Example 3**
*Specimen Adequacy:*
Satisfactory for evaluation; endocervical/transformation zone components absent.
*Interpretation*:
Negative for intraepithelial lesion or malignancy.
Reactive cellular changes associated with radiation.

**Example 4**
*Specimen Adequacy:*
Satisfactory for evaluation; endocervical/transformation zone component cannot be assessed because of severe atrophy.
*Interpretation*:
Negative for intraepithelial lesion or malignancy.
Fungal organisms morphologically consistent with Candida species.
Atrophy.

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# Endometrial Cells: The How and When of Reporting

# 3

Edmund S. Cibas, David Chelmow, Alan G. Waxman, and Ann T. Moriarty

## 3.1 Other

- Endometrial cells in a woman ≥45 years of age (*Specify if negative for squamous intraepithelial lesion*)

## 3.2 Background

Exfoliated endometrial cells are a normal finding in cervical cytology preparations from women of reproductive age and are commonly seen during menses and the proliferative phase of the menstrual cycle. In postmenopausal women, exfoliated endometrial cells are considered abnormal and raise the possibility of endometrial

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neoplasia [1–7]. Although most women with endometrial cancer present with bleeding symptoms [2], some are asymptomatic. In these women, benign-appearing exfoliated endometrial cells on a cytology specimen may be the only abnormal finding [1, 7]. For these reasons, the 1991 Bethesda System recommended that cytologically benign-appearing endometrial cells be reported in postmenopausal women. This posed an unforeseen problem for laboratories, because menopausal status is often unclear, inaccurate, or unknown to the laboratory. The median age of final menstrual period is 51 years in the United States, but the coefficient of variation is large [8].

To resolve this dilemma, the 2001 Bethesda System recommended that benign-appearing exfoliated endometrial cells be reported in all women 40 years of age or older, and it was suggested that this interpretation be qualified by an educational note [9]. This age was chosen to maximize the likelihood of including all postmenopausal women. It was intended that the woman's care provider, who knows her menstrual history and risk factors for endometrial carcinoma, would determine if further evaluation is indicated. Not surprisingly, the rate of reporting benign appearing endometrial cells increased with the transition from the 1991 to 2001 Bethesda System [10]. This led many to investigate the predictive value of the 2001 approach [10–18]. A meta-analysis of studies prior to 2001 indicates that the risk of biopsy-proven endometrial hyperplasia and cancer in the presence of benign endometrial cells on exfoliative cytology was 12 and 6 %, respectively (Table 3.1); these risks dropped to 2.0 and 1.1 % after the implementation of the 2001 Bethesda System (Table 3.2) [19].

**Table 3.1** Benign-appearing endometrial cells in postmenopausal women: predictive value for endometrial hyperplasia and carcinoma (Data pre-2001)

| Authors, year | Definition of postmenopausal | Cases with biopsy, *n* | Hyperplasia, *n* (%) | Cancer, *n* (%) | Hyperplasia or cancer, *n* (%) |
|---|---|---|---|---|---|
| Cherkis et al. (1988) [1] | ≥40 | 179 | 23 (13) | 20 (11) | 43 (24) |
| Gomez-Fernandez et al. (1999) [2] | Unknown | 84 | 6 (7) | 6 (7) | 12 (14) |
| Gondos and King (1977) [3] | ≥40 | 147 | 23 (16) | 2 (1) | 25 (17) |
| Ng et al. (1974) [4] | ≥40 | 501 | 52 (10) | 23 (5) | 75 (15) |
| Sarode et al. (2001) [5] | >55 | 81 | 4 (5) | 4 (5) | 8 (10) |
| Yancey et al. (1990) [6] | Unknown | 74 | 9 (12) | 0 | 9 (12) |
| Zucker et al. (1985) [7] | Unknown | 23 | 10 (43) | 6 (26) | 16 (70) |
| **Total** | | **1,089** | **127 (12 %)** | **61 (6 %)** | **188 (17 %)** |

With permission from Cibas and Ducatman [19]

**Table 3.2** Benign-appearing endometrial cells in women over 40: predictive value for endometrial hyperplasia and carcinoma (Data post-2001)

| Authors, year | Cases with biopsy, *n* | Hyperplasia, *n* (%) | Cancers, *n* (%) | Hyperplasia or cancer, *n* (%) |
|---|---|---|---|---|
| Browne et al. (2005) [11] | 211 | 1 (0.5) | 6 (2.8) | 7 (3.3) |
| Thrall et al. (2005) [12] | 159 | 9 (5.7) | 0 | 9 (5.7) |
| Bean et al. (2006) [13] | 140 | 2 (1.4) | 0 | 2 (1.4) |
| Kapali et al. (2007) [14] | 499 | 4 (0.8) | 4 (0.8) | 8 (1.6) |
| Moroney et al. (2007) [15] | 370 | 9 (2.4) | 6 (1.6) | 15 (4.0) |
| Li et al. (2012) [16] | 739 | 13 (1.8) | 7 (0.9) | 20 (2.7) |
| Moatamed et al. (2013) [18] | 186 | 10 (5.4) | 4 (2.1) | 14 (7.5) |
| **Total** | **2,394** | **48 (2.0)** | **27 (1.1)** | **75 (3.1)** |

Modified with permission from Cibas and Ducatman [19]

The clinical management proved to be a source of confusion to healthcare providers, especially non-gynecologists. To clarify this, if a woman aged 40 years or older has endometrial cells on a cervical cytology test, the American Society for Colposcopy and Cervical Pathology (ASCCP) specifically recommended in the 2012 management guidelines that histologic endometrial assessment only be performed if the woman is postmenopausal [20].

Studies in the 2001 Bethesda System era found little evidence to support the role of cervical cytology in uncovering endometrial cancer in women under the age of 45 [10, 11, 17, 21]. To improve the predictive value of exfoliated endometrial cells, it is now recommended that *benign-appearing endometrial cells be reported in women 45 years of age or older*. This revised recommendation is made with the understanding that it is not feasible for a screening test to detect every malignancy. Moreover, it bears emphasis that cervical cytology is primarily a screening test for squamous lesions; it is not intended to screen for endometrial lesions and should not be used to evaluate suspected endometrial abnormalities.

Atypical endometrial cells should still be reported under the general category "epithelial cell abnormality" and managed as such.

## 3.3 Exfoliated Endometrial Cells (Figs. 3.1–3.4)

### 3.3.1 Criteria

Cells are small and often arranged in tight, ball-like clusters, rarely as isolated cells (Figs. 3.1 and 3.2).

Nuclei are small, similar in area to a normal intermediate squamous cell nucleus.

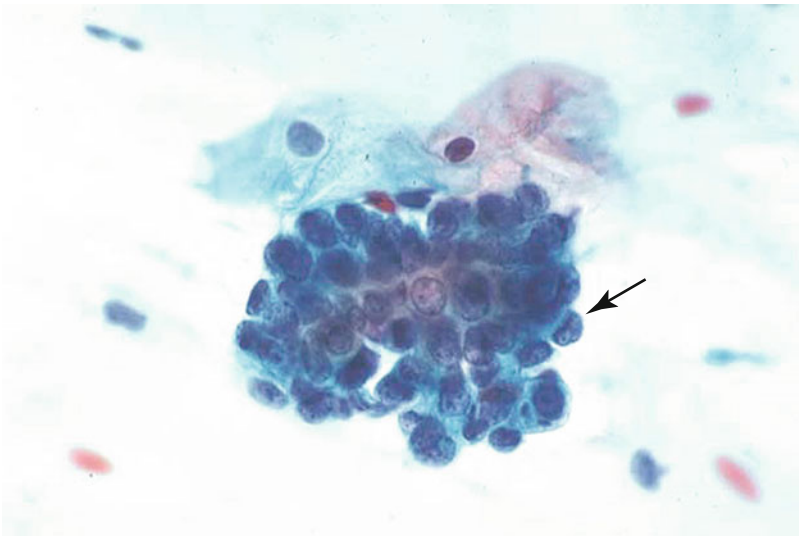

**Fig. 3.1** Exfoliated endometrial cells (*conventional preparation*, *CP*). Cells are arranged in three-dimensional clusters. Nuclei are small and similar in size to an intermediate squamous cell nucleus. Nucleoli are inconspicuous. Cytoplasm is scant, and cell borders are indistinct

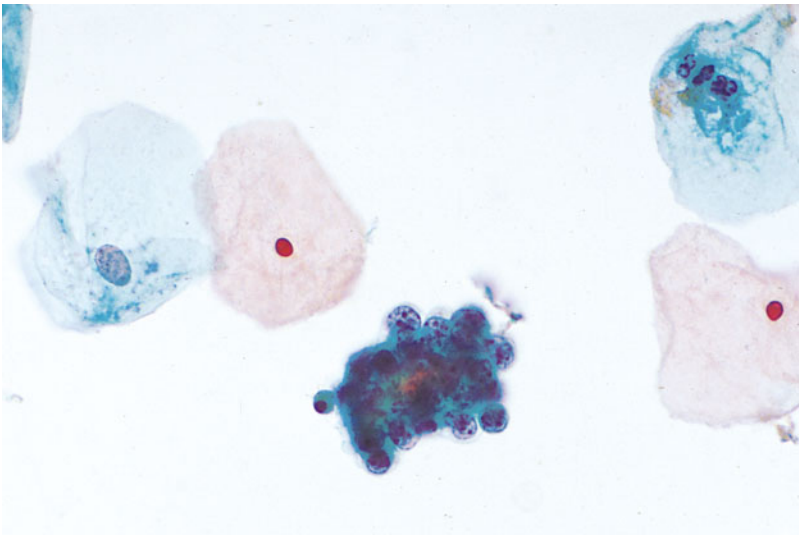

**Fig. 3.2** Exfoliated endometrial cells (liquid-based preparation (*LBP*), *ThinPrep*)

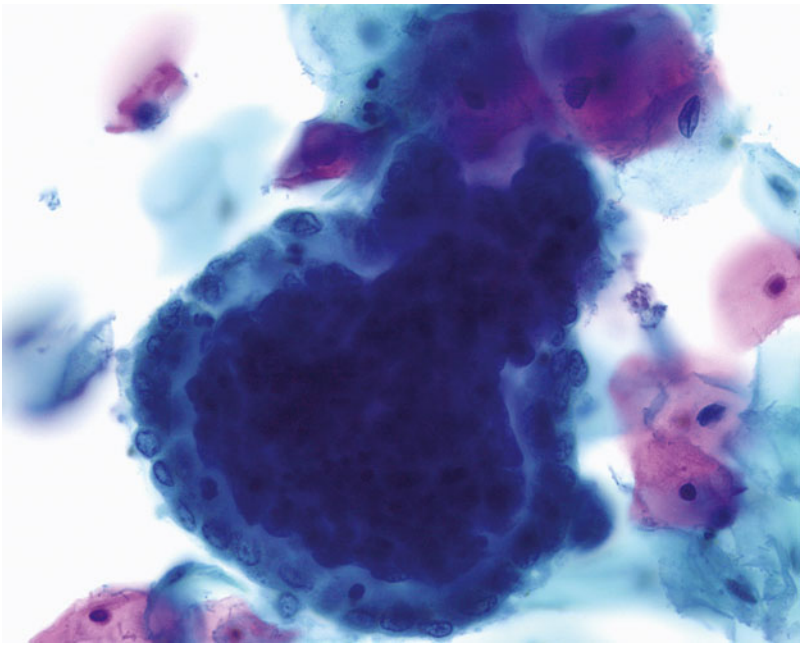

**Fig. 3.3** Double-contoured cluster of exfoliated endometrial cells (*LBP*, *ThinPrep*). Endometrial glandular cells surround a dark core of stromal cells. Note the cleaner background typical of LBP menstrual specimens

Some nuclei around the edge of clusters may have a cup-shaped appearance (Fig. 3.1, arrow).
Nuclei are dark, but the chromatin pattern is often difficult to discern because of overlapping cells.
Nucleoli are inconspicuous.
Karyorrhexis is often present.
Mitoses are absent.
Cytoplasm is scant, occasionally vacuolated.
Cell borders are ill defined.
Double-contoured clusters of endometrial cells may be seen (Fig. 3.3).

### Preparation-Specific Criteria

*Liquid-Based Preparations*:
Cell groups may appear "above the plane" of squamous epithelial cells with gradient-based liquid-based preparations.
Isolated cells may be more evident.

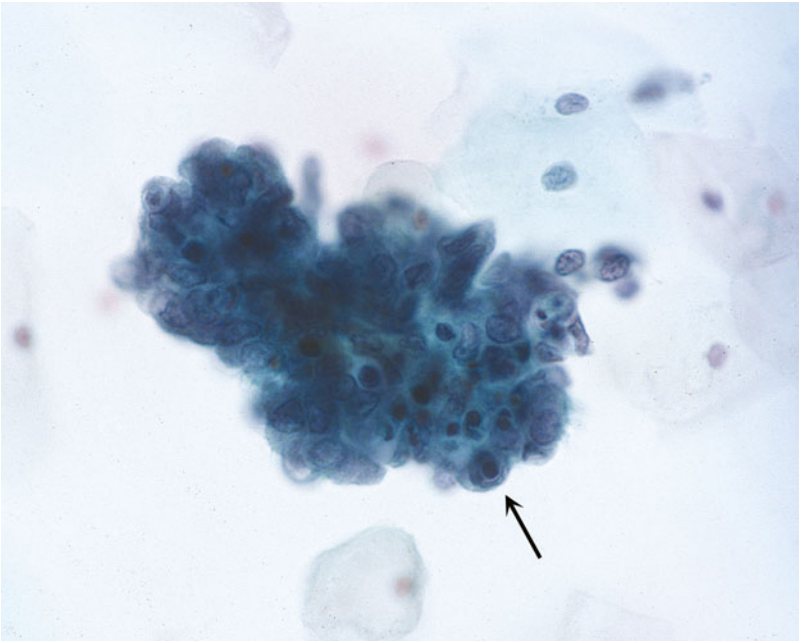

**Fig. 3.4** Exfoliated endometrial cells (*LBP*, *SurePath*). Single cell necrosis (apoptosis) can be seen in exfoliated endometrial cell clusters (*arrow*)

Nucleoli and chromatin detail may be more apparent (Fig. 3.2); intracytoplasmic vacuoles are more common and easily visible.
Karyorrhexis is easily seen (Fig. 3.4).
Background appears cleaner, especially in menstrual smears (Fig. 3.3).

## 3.4 Explanatory Notes

In the 2014 Bethesda System, exfoliated endometrial cells should be reported in a woman 45 years of age or older. Benign-appearing endometrial cells in women under 45 years of age need not be reported, even if they are seen during the luteal phase ("out of cycle"), because they have little if any predictive value for endometrial neoplasia.

Exfoliated endometrial cells are normally present in cervical cytology specimens from day 1 to day 12 of the menstrual cycle, with the specific pattern of "exodus" noted from day 6 to day 10. The term "exodus" is used for a distinctive arrangement of benign, spontaneously exfoliated endometrial stromal and glandular cells that are arranged in three-dimensional, double-contoured groups, with central small, dark stromal cells rimmed by larger, paler glandular cells. Exfoliated endometrial cell clusters are comprised of epithelial cells, stromal cells, or both; morphologic

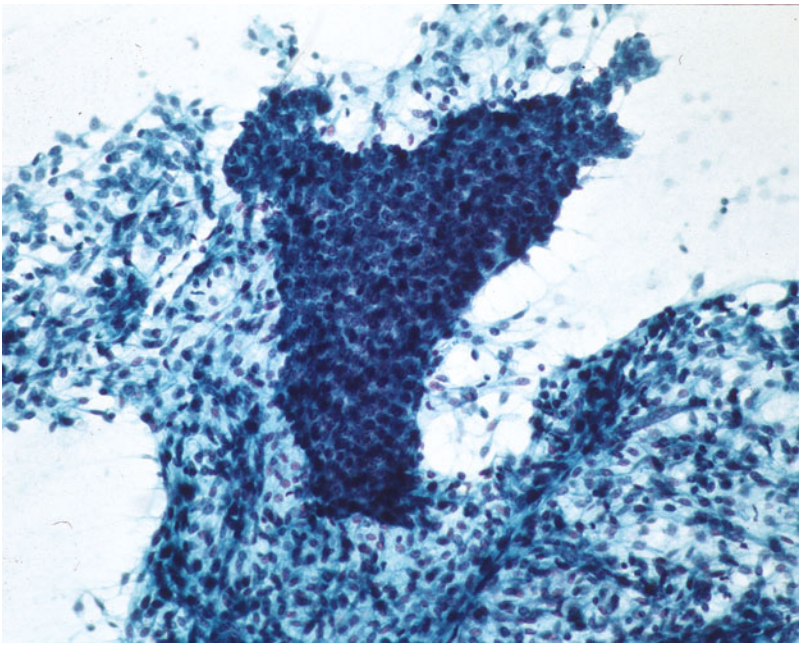

**Fig. 3.5** Abraded lower uterine segment (LUS) fragment (*CP*). A large fragment of epithelium is associated with vascular stroma composed of tightly packed spindle-shaped cells. Abraded LUS/endometrium does not carry the same implications as exfoliated endometrial cells

distinction between these two cell types is not reliable with the Papanicolaou stain alone, except for double-contoured "exodus" groups (Fig. 3.3) [22].

Benign-appearing endometrial cells in a woman with endometrial neoplasia likely represent the endometrial stromal and glandular breakdown that is commonly associated with neoplasia.

In liquid-based preparations, exfoliated endometrial cells may be slightly larger, with more easily visible nucleoli and enhanced chromatin detail compared to conventional smear preparations. These features may be worrisome to those unfamiliar with the appearance of endometrial cells in liquid-based preparations.

Abraded – as opposed to exfoliated – endometrium and lower uterine segment (LUS) fragments are not associated with an increased risk of endometrial cancer and therefore do not generally warrant reporting [23]. Abraded LUS and endometrium is a result of inadvertent sampling beyond the endocervix and is often seen in women who have undergone a cervical excision (e.g., LEEP/LLETZ, cone biopsy, trachelectomy). Directly-sampled LUS and endometrium is characterized by biphasic tissue fragments: a densely packed stromal component, comprised of spindle-shaped cells, sometimes with visible vessels, and a sharply distinct glandular component arranged in a sheet or as simple or branching tubules [23]. The two components may be spatially connected (Fig. 3.5,

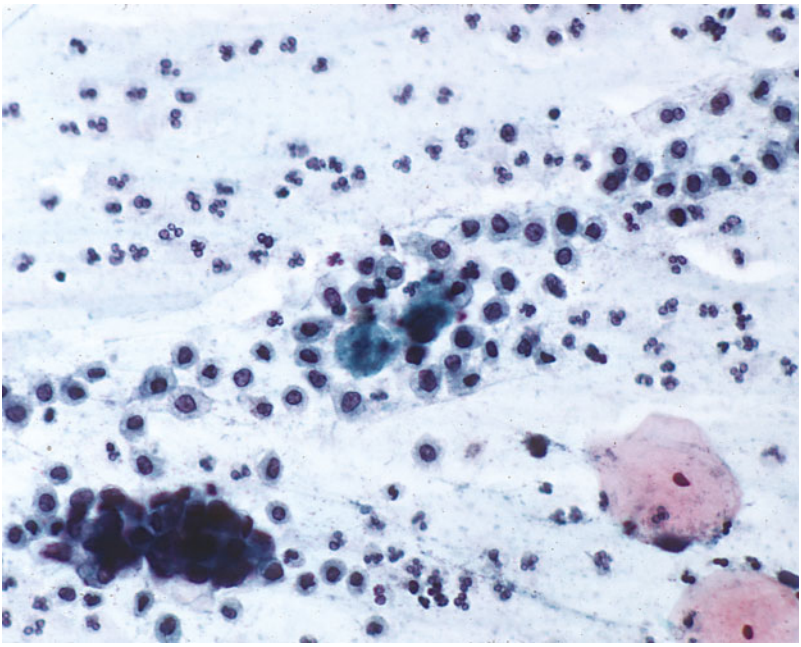

**Fig. 3.6** Histiocytes (*CP*). Histiocytes have a round to reniform nucleus and a moderate amount of finely vacuolated cytoplasm. They are often seen in association with exfoliated endometrial cells. Histiocytes alone have no significance in predicting the presence of endometrial carcinoma

see Fig. 2.7) or separated (see Figs. 2.8 and 2.9). Glandular and stromal cells inadvertently directly-sampled from endometrium during the proliferative phase can have abundant mitoses.

Unlike exfoliated endometrial cells, histiocytes are more often dispersed as isolated cells, although small, usually loose clusters are sometimes seen. Histiocytes are recognized on the basis of their frequently folded, grooved, or kidney-shaped nucleus and moderate amount of vacuolated cytoplasm (Fig. 3.6). They are often seen along with exfoliated endometrial cells but by themselves carry no significant association with endometrial neoplasia [7, 24, 25].

Clusters of naked nuclei are a common mimic of exfoliated endometrial cells but are distinguished by the complete absence of cytoplasm. These cells have smooth nuclear contours and evenly distributed granular chromatin, sometimes with conspicuous molding (Fig. 3.7). The incidence of these so-called small blue cells increases with age. At one time, their presence was associated with tamoxifen treatment, but the frequency of small blue cells is no higher than in women who are not taking tamoxifen [26]. The naked nuclei are likely of

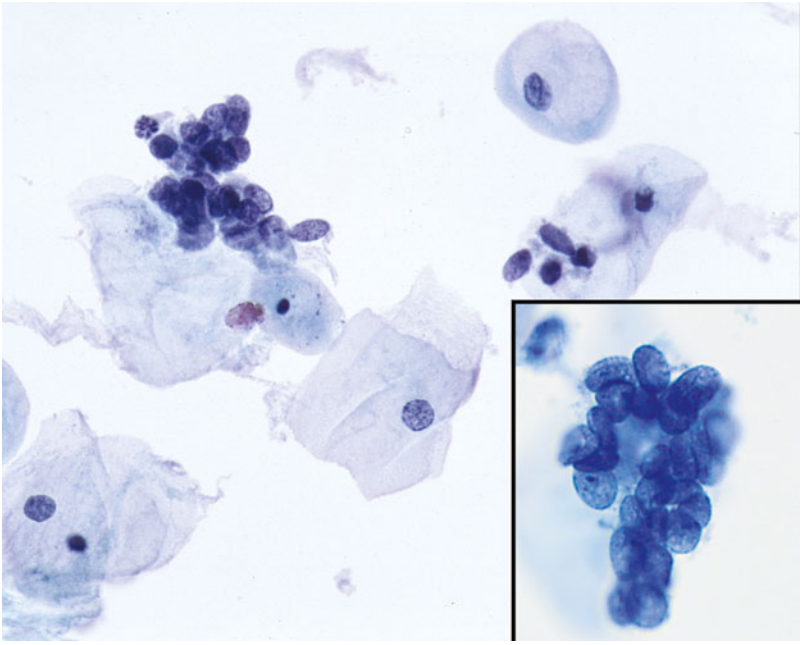

**Fig. 3.7** "Small blue cells" (*LBP*, *ThinPrep*). Naked nuclei are clustered and demonstrate molding. The *insert* (*lower right*) shows a higher magnification of a grapelike cluster of nuclei with finely textured chromatin. Such clusters should not be mistaken for endometrial cells

parabasal squamous or reserve cell origin and should not be mistaken for endometrial cells.

Clusters of lymphoid cells, mostly small round lymphocytes, occasionally accompanied by plasma cells and/or tingible body macrophages, are uncommonly encountered in cervical Pap slides (Fig. 3.8; see Figs. 2.41 and 2.42) and correlate with follicular cervicitis on histologic sections. They have no diagnostic significance. Because lymphocytes are the same size as endometrial cells, these lymphoid cell clusters may mimic exfoliated endometrial cells.

An educational/explanatory comment can be useful when reporting exfoliated endometrial cells in a woman who is 45 years or older. The comment should stress that exfoliated endometrial cells are usually derived from a benign process and that only a small proportion of women with this finding have endometrial abnormalities (see Sample report Example 1). If the date of the last menstrual period (LMP) is provided and the specimen was obtained in the first half of the cycle, the laboratory may wish to append a comment indicating that the finding of endometrial cells correlates with the menstrual history (see Sample report Example 2).

This "Other" Bethesda interpretive category does not mandate hierarchical review. It is up to the laboratory to have a policy specifying the circumstances under which endometrial cells without cytologic atypia are referred for a pathologist's review.

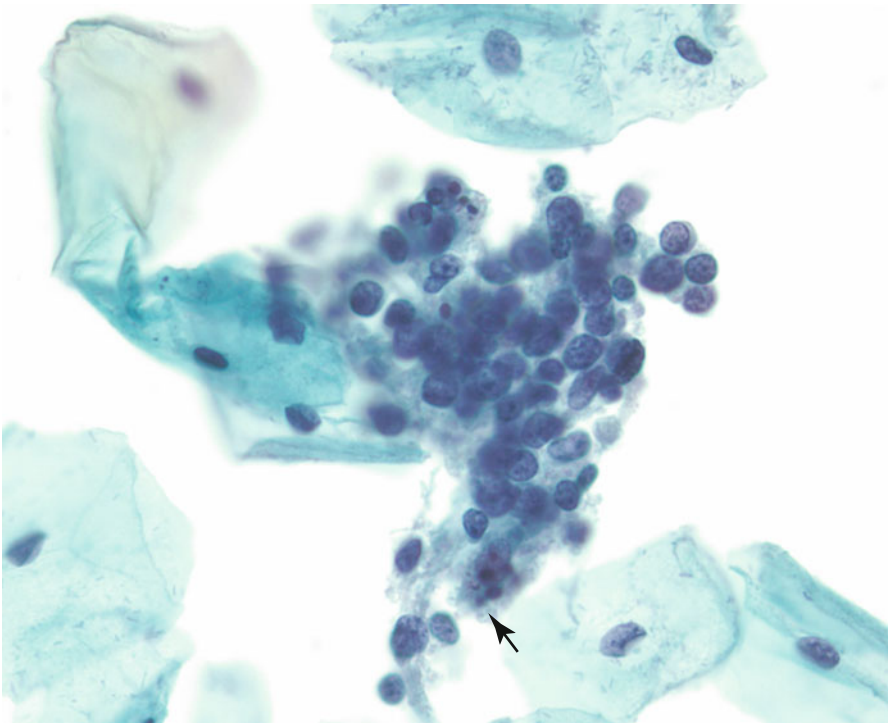

**Fig. 3.8** Follicular cervicitis (*LBP*, *ThinPrep*). The lymphocytes of a lymphoid follicle may aggregate into three-dimensional clusters. Tingible body macrophages (*arrow*) mimic the apoptosis of exfoliated endometrial cells. In contrast to exfoliated endometrial cell clusters, lymphoid aggregates are looser and more irregularly shaped, and small mature lymphocytes have coarser chromatin than endometrial cells

## 3.5 Sample Reports

**Example 1**
**Using a General Categorization**
*General Categorization*:
Other: see Interpretation/Result.
*Interpretation/Result*:
Endometrial cells present in a woman ≥45 years of age (see note).
Negative for squamous intraepithelial lesion.

**Example 2**
**Without Use of the General Categorization ("Other")**
Endometrial cells are present in a woman ≥45 years of age (see note).
Negative for squamous intraepithelial lesion.

*Educational Note(s)* (optional):

A. For all reports with endometrial cells in women 45 years or older:
*Endometrial cells in women 45 years or older may be associated with benign endometrium, hormonal alterations, and, less commonly, endometrial or uterine abnormalities. Endometrial evaluation is recommended in postmenopausal women.*

B. Additional note to consider when a woman's LMP is provided and endometrial cells are seen in the first half of the menstrual cycle:
*Endometrial cells correlate with the menstrual history provided.*

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# Atypical Squamous Cells

# 4

Fadi W. Abdul-Karim, Celeste N. Powers, Jonathan S. Berek, Mark E. Sherman, Sana O. Tabbara, and Mary K. Sidawy

## 4.1 Epithelial Cell Abnormalities

**Squamous Cell**

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

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R. Nayar, D.C. Wilbur (eds.), *The Bethesda System for Reporting Cervical Cytology: Definitions, Criteria, and Explanatory Notes*,
DOI 10.1007/978-3-319-11074-5_4

## 4.2 Background

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

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

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

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

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

## 4.3 Atypical Squamous Cells

### 4.3.1 Definition

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

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

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

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

### 4.4.1 Definition

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

### 4.4.2 Criteria

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

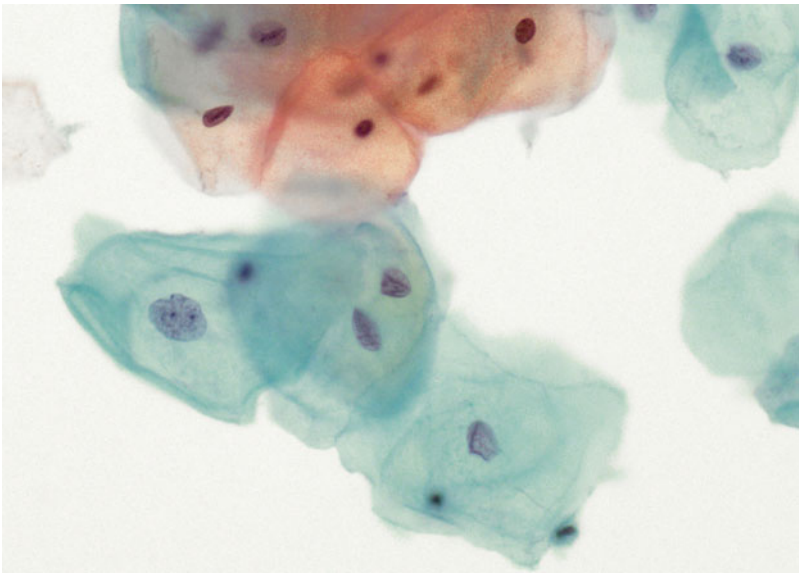

**Fig. 4.1** ASC-US (*LBP, ThinPrep*). A 32-year-old woman. Atypical intermediate squamous cells with a nucleus 2–3× the area of a normal intermediate squamous cell nucleus and mild irregularity of nuclear contour. This isolated cell has some features suggestive of HPV infection. hrHPV was positive.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

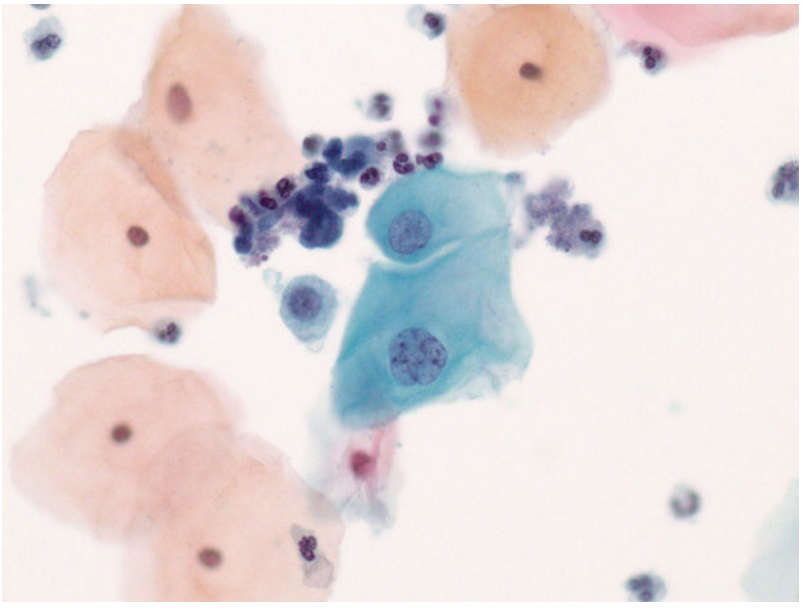

**Fig. 4.2** ASC-US (*LBP, ThinPrep*). A 28-year-old woman. An intermediate squamous cell with an enlarged nucleus and slight nuclear membrane irregularity. The atypical features do not meet the criteria for LSIL. hrHPV was positive. Follow-up biopsy revealed LSIL (CIN1)

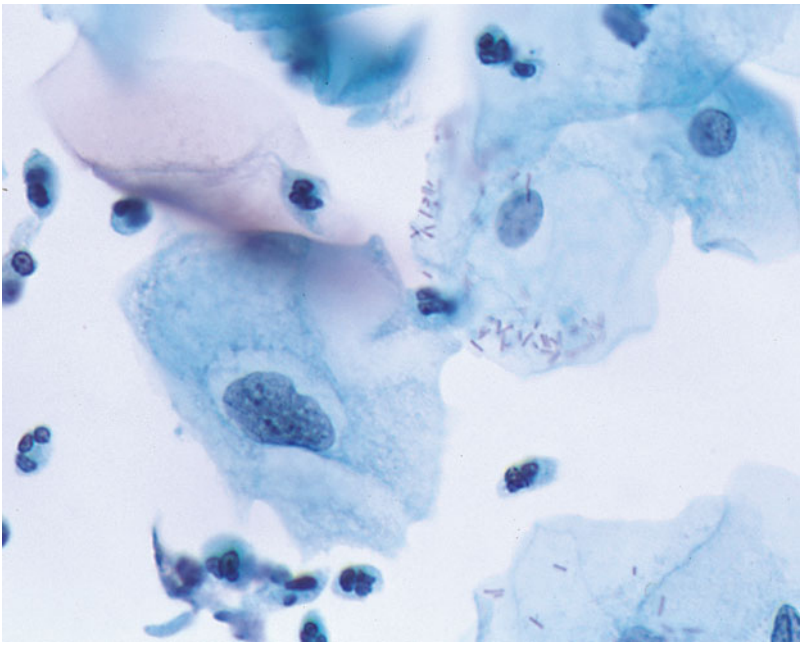

**Fig. 4.3** ASC-US (*LBP, SurePath*). Routine screen from a 32-year-old woman. Single atypical squamous cell with ill-defined cytoplasmic halo in a background of inflammation. Adjacent squamous cell shows adherent lactobacilli. HPV testing was not performed on this sample

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

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

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

### Preparation Specific Criteria

*Conventional Preparations*:

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

*Liquid-Based Preparations*:

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

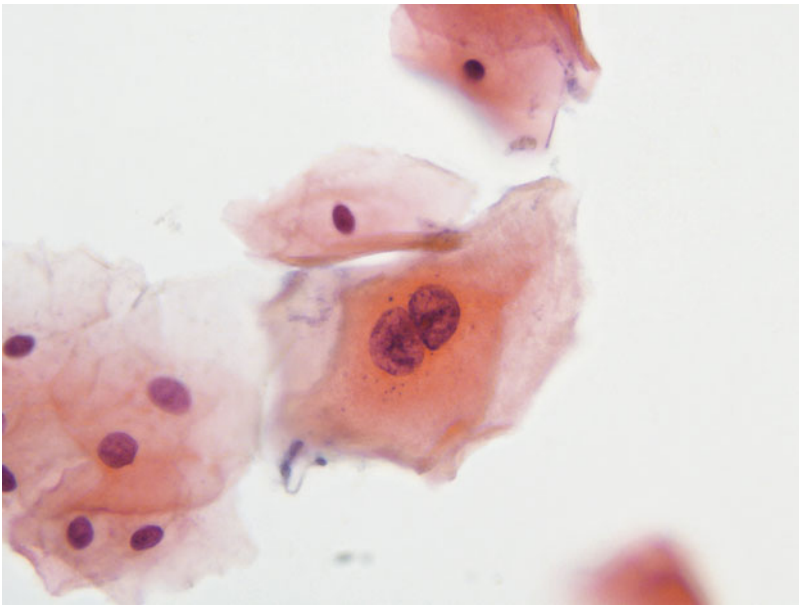

**Fig. 4.4** ASC-US (*LBP, ThinPrep*). A 28-year-old female. An atypical binucleated intermediate cell with molded nuclei and orangeophilic cytoplasm suggestive but not diagnostic of LSIL. hrHPV was positive. Follow-up biopsy revealed LSIL (CIN1)

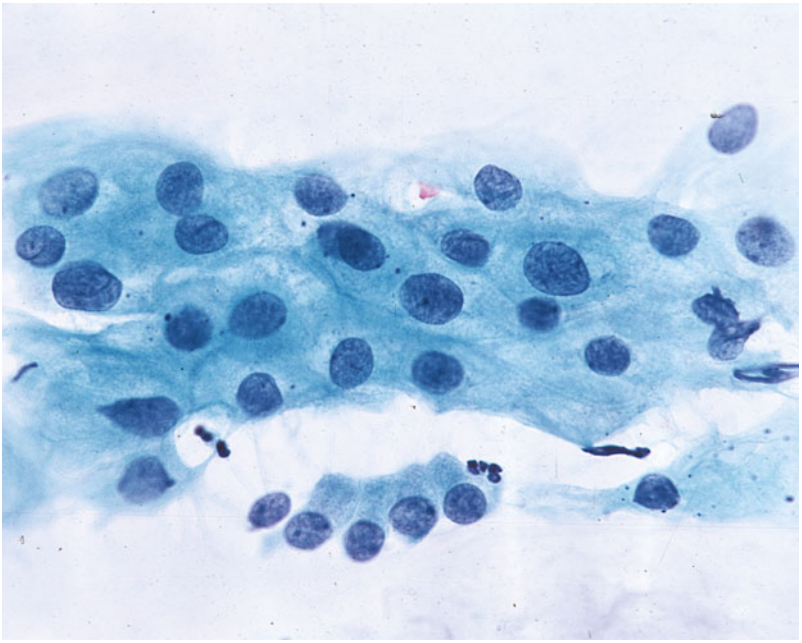

**Fig. 4.5** Negative for intraepithelial lesion or malignancy (NILM) versus atypical squamous cells – undetermined significance (ASC-US) (*CP*). Perimenopausal woman. Mature squamous cells show mild nuclear enlargement, binucleation, and even chromatin distribution. Note benign endocervical cells at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

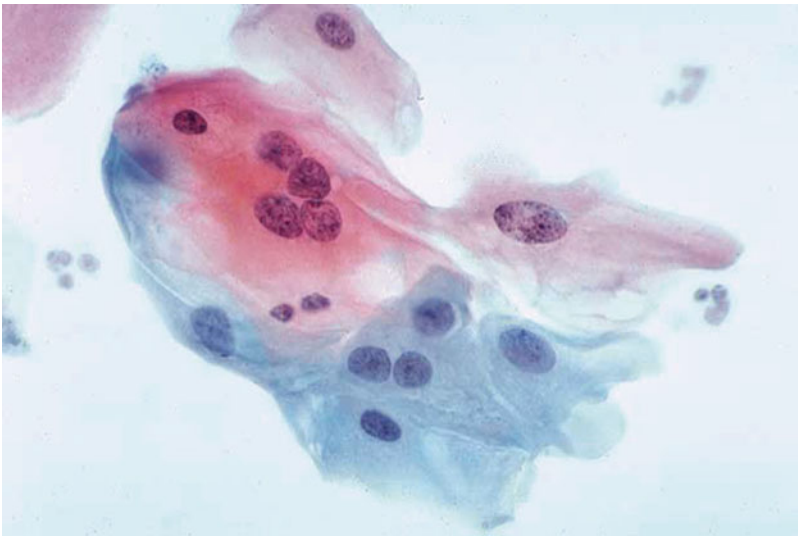

**Fig. 4.6** ASC-US (*CP*). Cells with multinucleation, nuclear enlargement, and air-drying artifact, possibly representing LSIL (CIN1)

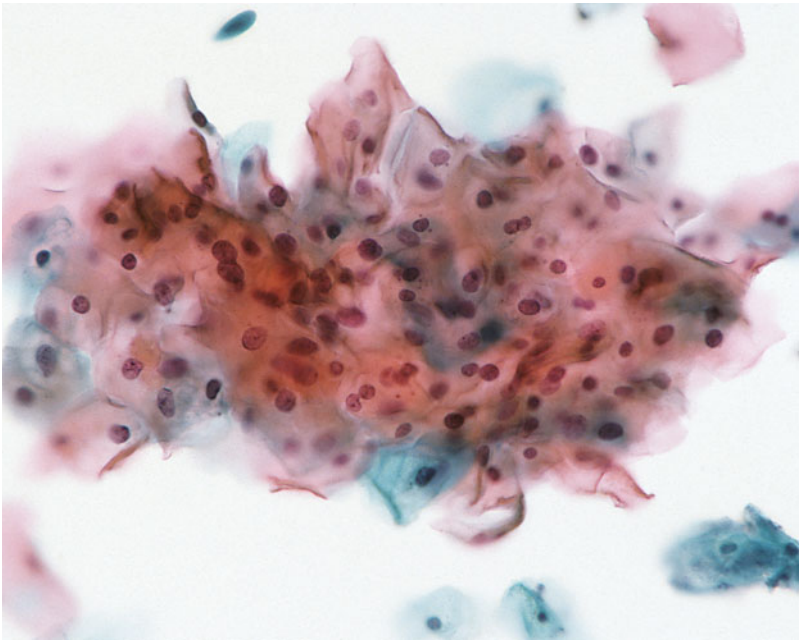

**Fig. 4.7** ASC-US (*LBP, SurePath*). A 21-year-old woman. Thick cohesive sheet of cells with focal nuclear enlargement, orangeophilic cytoplasm, poorly formed cytoplasmic vacuoles, and binucleation. Follow-up biopsy was LSIL (CIN1)

### 4.4.3 Explanatory Notes

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

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

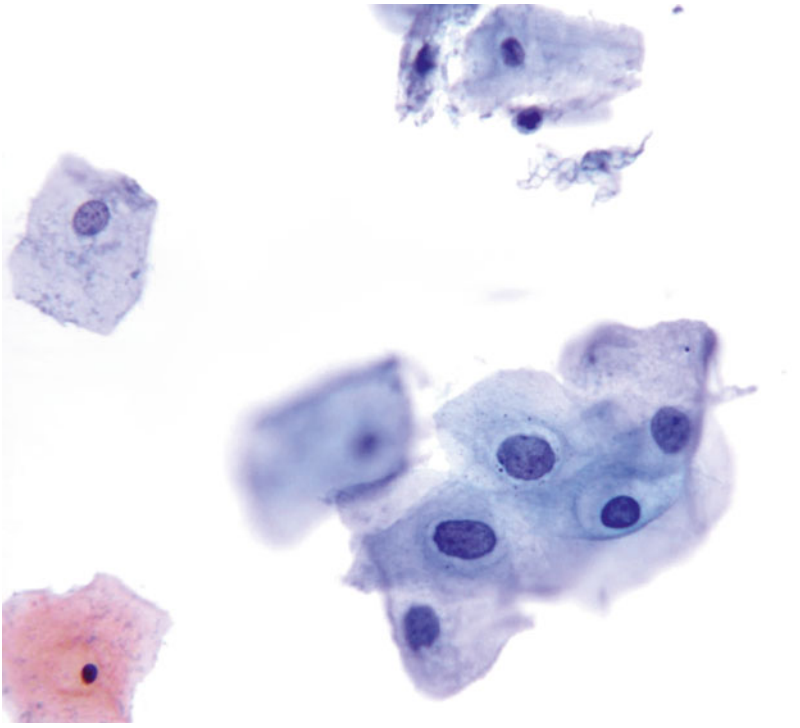

**Fig. 4.8** ASC-US (*LBP, ThinPrep*). A 35-year-old woman. A group of cells featuring mild nuclear enlargement, slight nuclear membrane irregularity and mild hyperchromasia in a clean background. The cytologic features do not meet the criteria for LSIL. hrHPV was positive. Follow-up biopsy revealed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

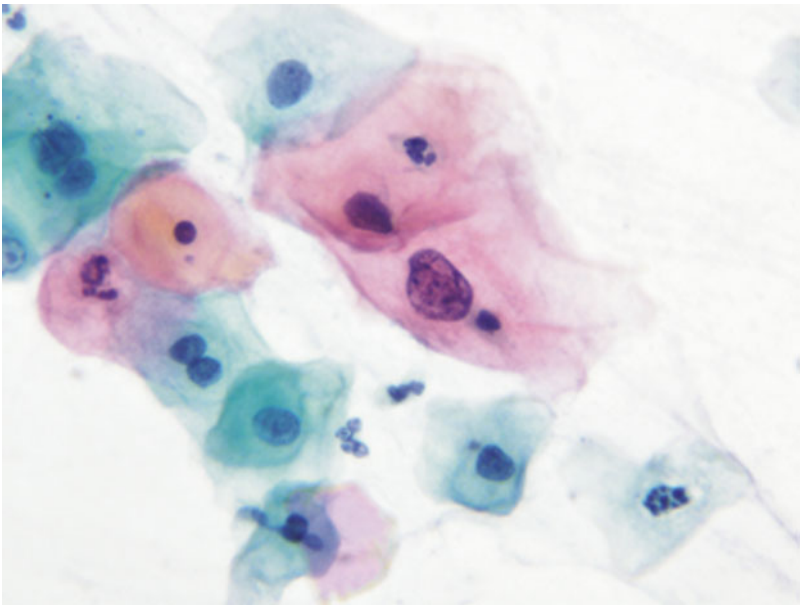

**Fig. 4.9** ASC-US (*LBP, ThinPrep*). A 25-year-old woman. Intermediate cells with nuclear enlargement ×2–3 that of normal intermediate squamous cell nucleus. There are rare binucleated cells. Slight nuclear irregularity and hyperchromasia are present that do not meet the diagnostic criteria for LSIL.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

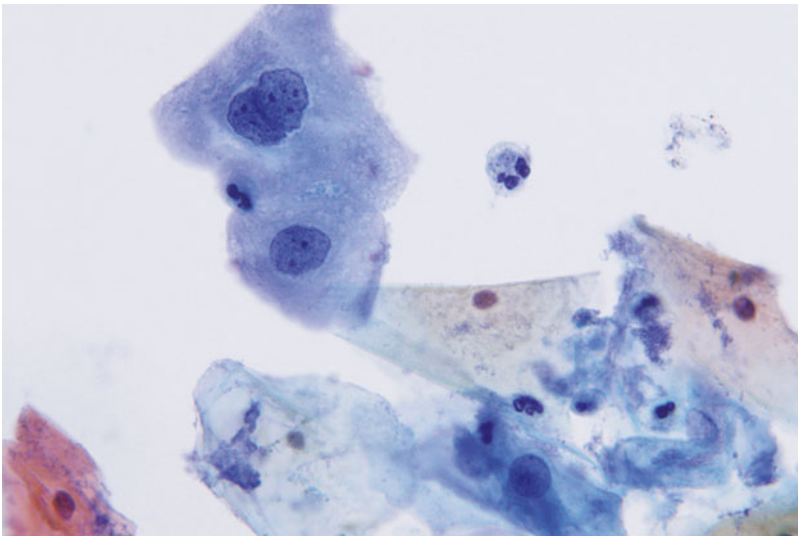

**Fig. 4.10** ASC-US (*LBP, ThinPrep*). A 40-year-old woman. Binucleated atypical intermediate squamous cell with slightly enlarged irregular nuclei in an inflammatory background. hrHPV was positive. Follow-up biopsy showed LSIL (CIN1)

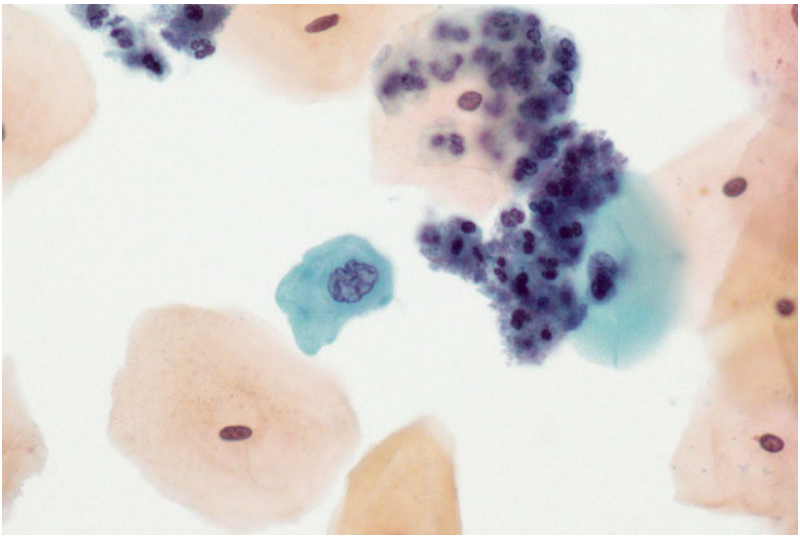

**Fig. 4.11** ASC-US (*LBP, ThinPrep*). A 40-year-old woman. A single atypical intermediate squamous cell with a nucleus that is 2 to 3 times the area of a normal intermediate squamous nucleus and an irregular nuclear contour. The background shows acute inflammation. The cytologic features do not meet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

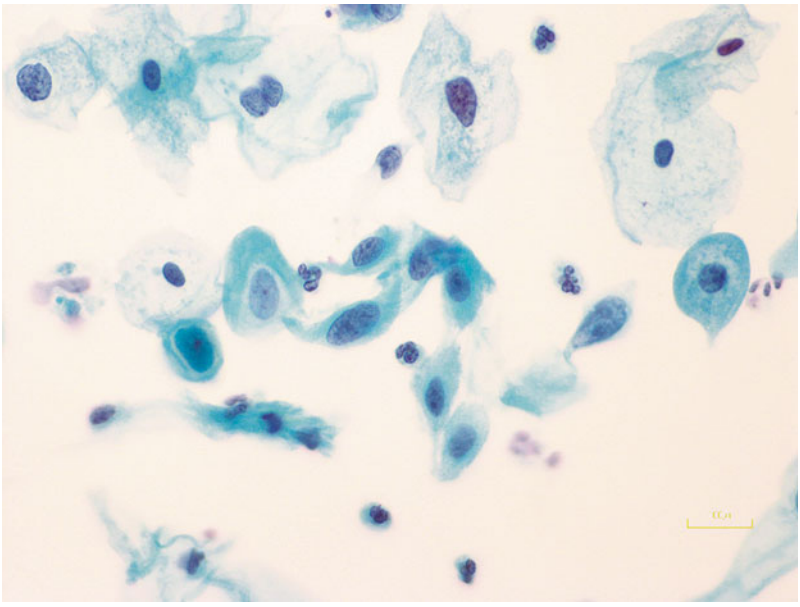

**Fig. 4.12** ASC-US (*LBP, SurePath*). Routine screening in a perimenopausal woman. Several cells showing slightly increased nuclear hyperchromasia and nuclear to cytoplasmic ratios. Occasional bi-nucleation and cytoplasmic halos are seen. These features may be seen in a reactive/infectious process;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

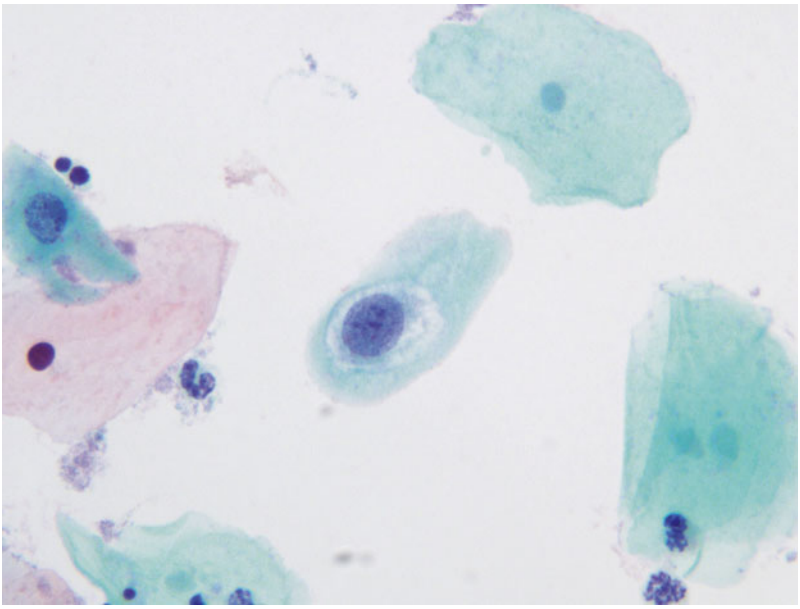

**Fig. 4.13** ASC-US (*LBP, ThinPrep*). A 23-year-old woman. An atypical intermediate squamous cell with a mildly enlarged nucleus and a poorly-formed perinuclear halo. The atypical features are suggestive but not diagnostic of LSIL. hrHPV was positive. Follow-up biopsy revealed LSIL (CIN1)

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

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

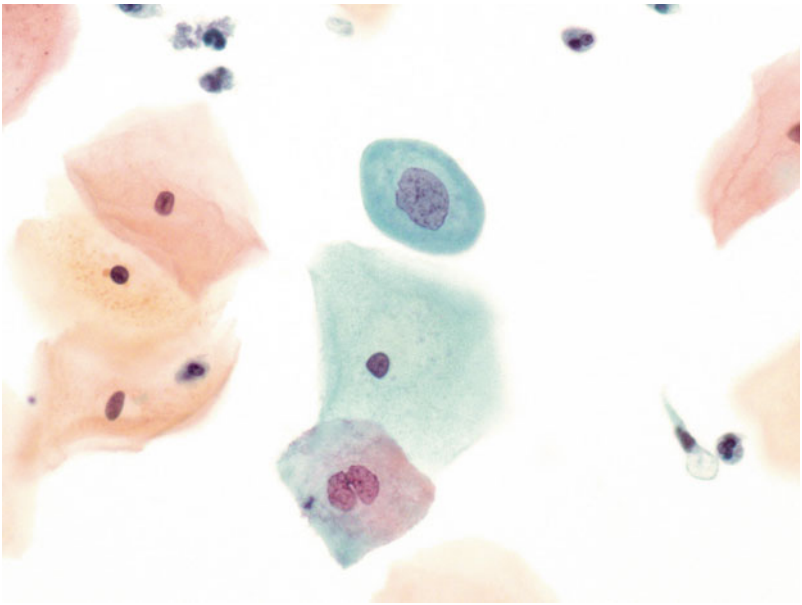

**Fig. 4.14** ASC-US (*LBP, ThinPrep*). A 30-year-old woman. A metaplastic cell with dense cytoplasm, slightly enlarged nucleus and mild nuclear membrane irregularity is seen in the center. Below it is a binucleated intermediate squamous cell with irregular nuclear contour. The cytologic features are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

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

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

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

### 4.5.2 Atypical Repair (Figs. 4.17 and 4.18)

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

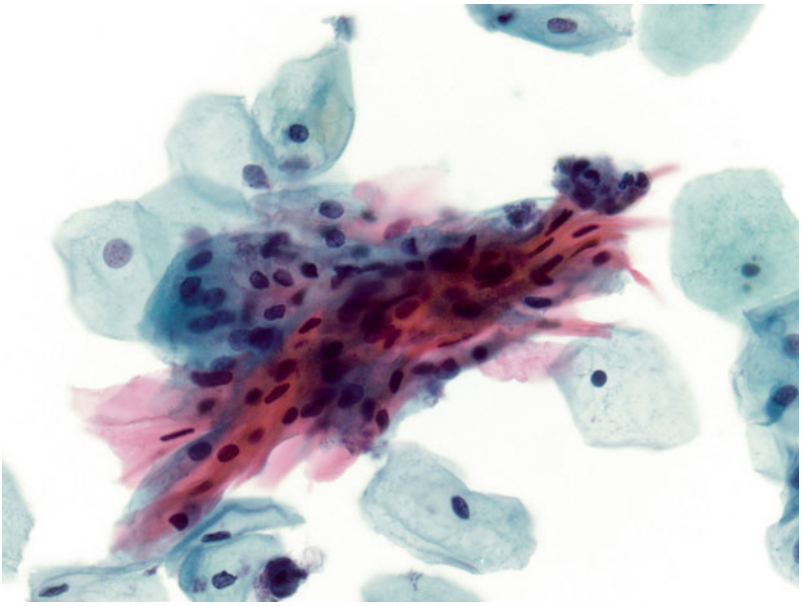

**Fig. 4.15** ASC-US – atypical keratinized cells (*LBP, ThinPrep*). A 25-year-old woman. A cohesive sheet of spindled keratotic cells with nuclear enlargement, hyperchromasia and orangeophilic cytoplasm. hrHPV was positive. Follow-up biopsy revealed LSIL with prominent keratinization

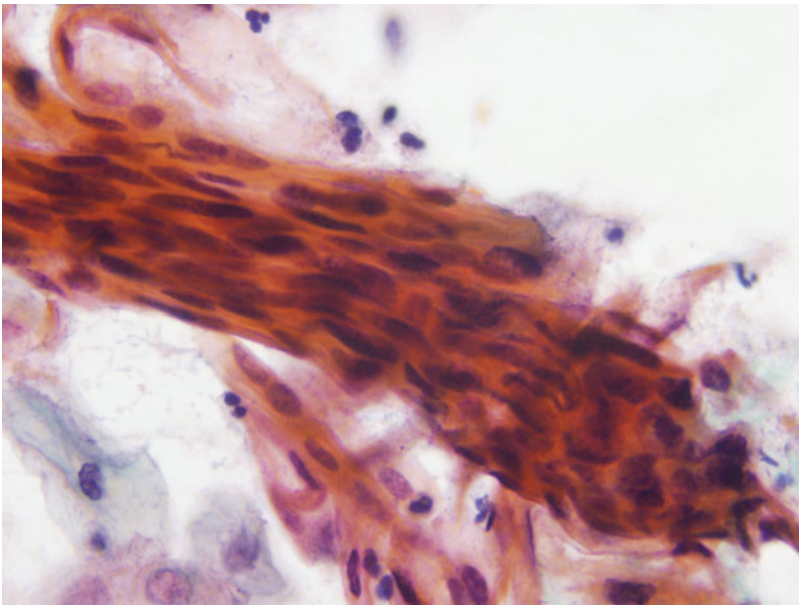

**Fig. 4.16** ASC-US – atypical keratinized cells (*LBP, ThinPrep*). A 32-year-old woman. Cohesive sheet of atypical squamous cells with orangeophilic cytoplasm and elongated, hyperchromatic crowded nuclei. hrHPV was positive. Follow-up biopsy revealed HSIL (CIN 2) with prominent keratinization

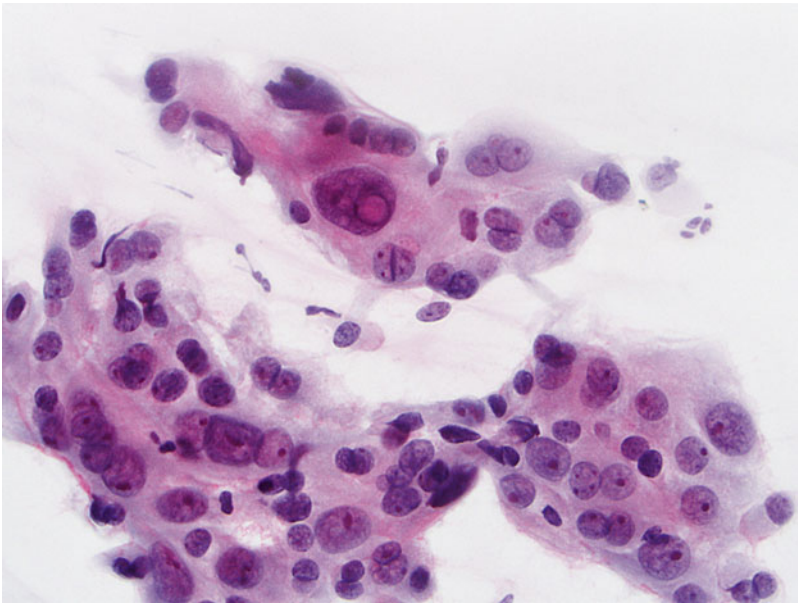

**Fig. 4.17** ASC-US – atypical repair (*CP*). In this image, cells are arranged in two-dimensional sheet with abundant cytoplasm showing a "pulled-out" or streaming effect. Nuclei show pleomorphism of size and shape with some cells having multiple nuclei. Most nuclei show prominent nucleoli. These changes,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

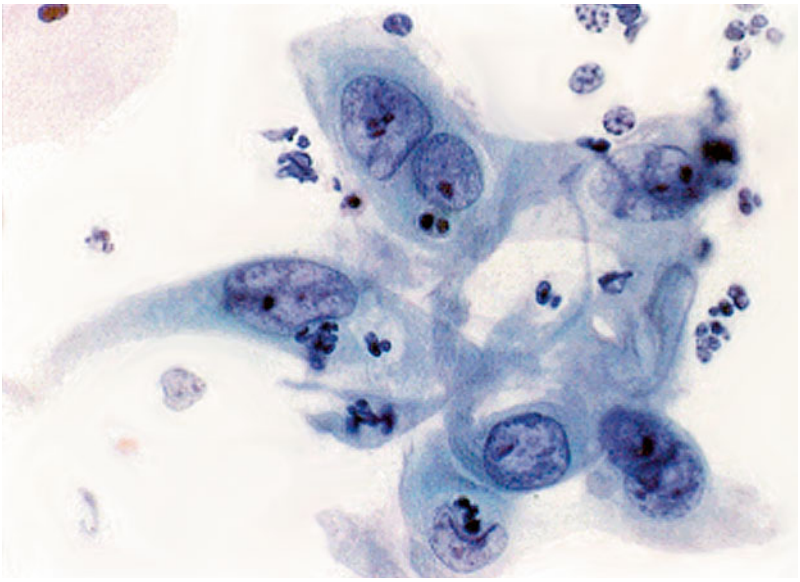

**Fig. 4.18** ASC-US – atypical repair (*CP*). Group of cells with features of repair; however, the presence of irregular chromatin distribution and the increased nucleus to cytoplasmic ratio are not typical (see Figs. 2.38 and 2.39). Atypical reparative squamous cells may be classified as ASC-US, or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

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

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

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

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

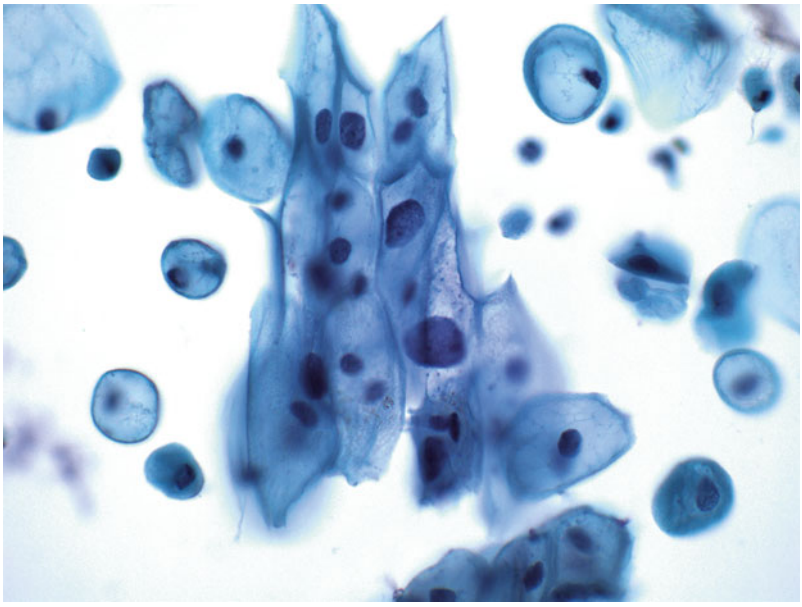

**Fig. 4.19** Postmenopausal atypia (*LBP, SurePath*). Postmenopausal woman with an atrophic cell pattern, predominantly comprised of parabasal cells. The presence of occasional enlarged nuclei is a characteristic feature of postmenopausal atypia and is often overcalled as ASC-US. hrHPV testing is usually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

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

### 4.5.4 Other Patterns

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

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

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

### 4.6.1 Definition

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

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

## 4.7 Common ASC-H Patterns

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

#### 4.7.1.1 Criteria

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

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

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

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

### Preparation Specific Criteria

*Liquid-Based Preparations*:

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

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

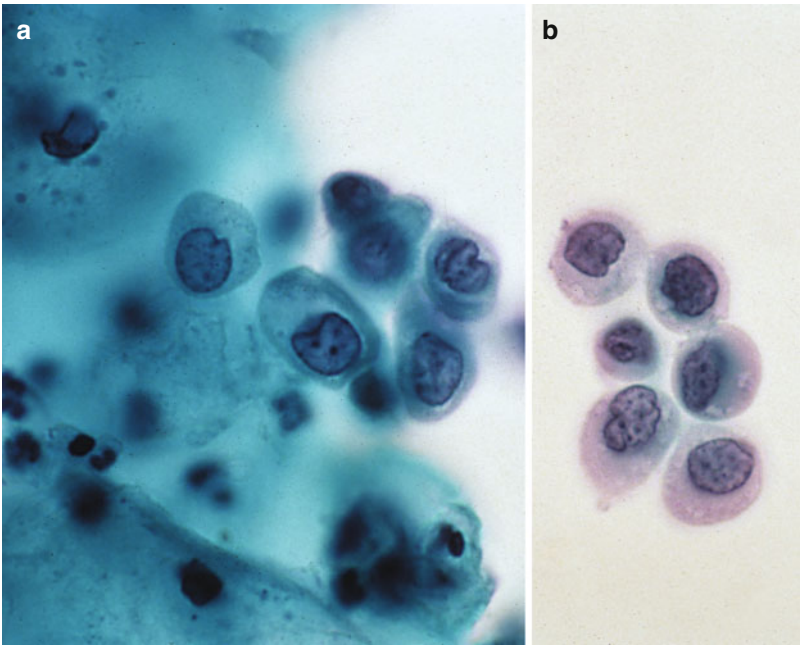


**Fig. 4.20** ASC-H (*LBP, ThinPrep*). A 27-year-old woman. (**a**) On the *left* are isolated small cells with variable N/C ratios and some cells displaying prominent nuclear irregularity. (**b**) On the *right* is a high-magnification view of six small cells with enlarged and irregular, but degenerated,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

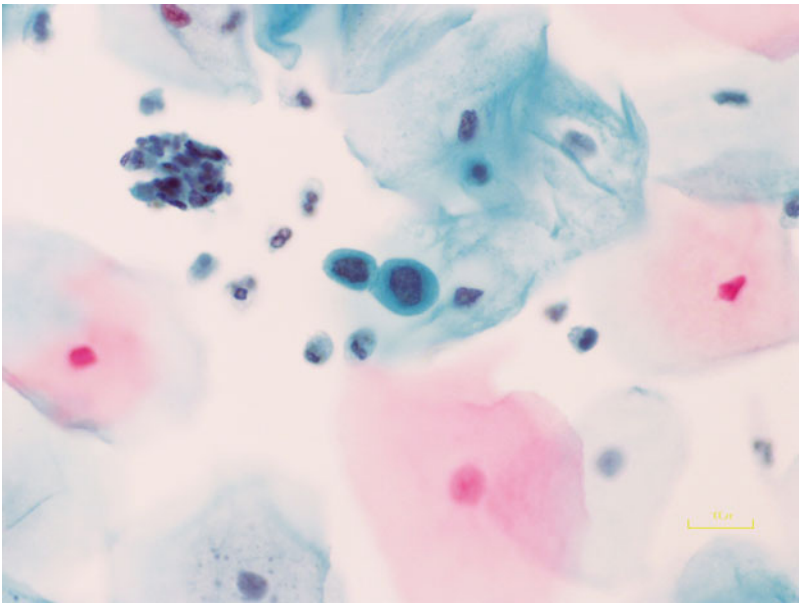

**Fig. 4.21** ASC-H (*LBP, SurePath*). Routine cytology for a 30-year-old woman. Rare metaplastic cells with dense cytoplasm and nuclear enlargement with hyperchromasia are present in a background of scattered acute inflammation. An interpretation of ASC-H was rendered. Follow-up cervical biopsies revealed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

#### 4.7.1.2 Explanatory Notes

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

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

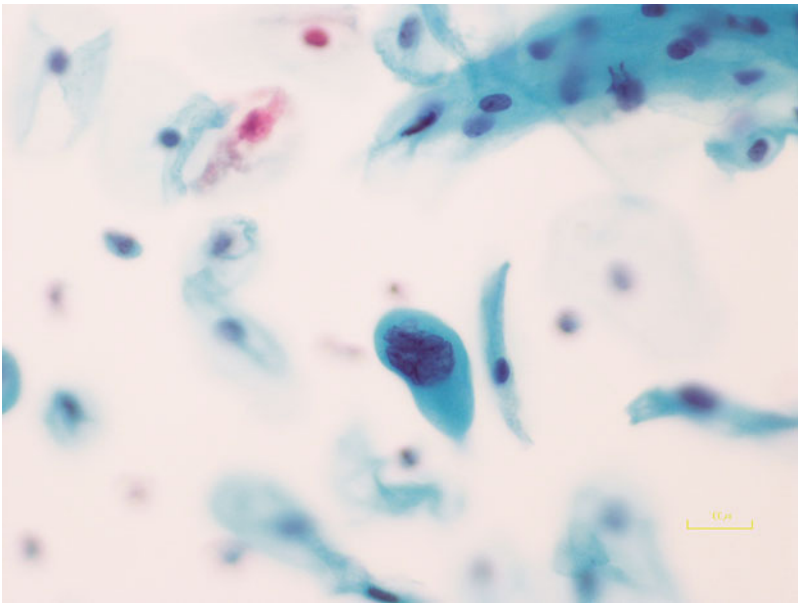

**Fig. 4.22** ASC-H (*LBP, SurePath*). Perimenopausal woman with history of LSIL. Unremarkable slide with only a single large atypical cell in a clean background. The nuclear irregularity and hyperchromasia were worrisome but not definitive for SIL. Cervical biopsies were performed and showed tubal metaplasia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

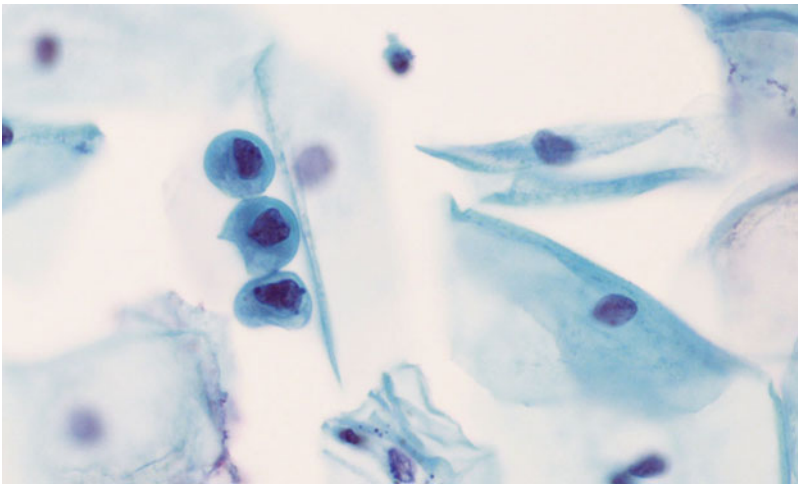

**Fig. 4.23** ASC-H (*LBP, SurePath*). Perimenopausal woman with history of atypical cytology (ASC-US). Three small atypical metaplastic cells with hyperchromatic nuclei and irregular nuclear membranes are identified. The interpretive considerations included immature metaplasia; however, a high-grade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

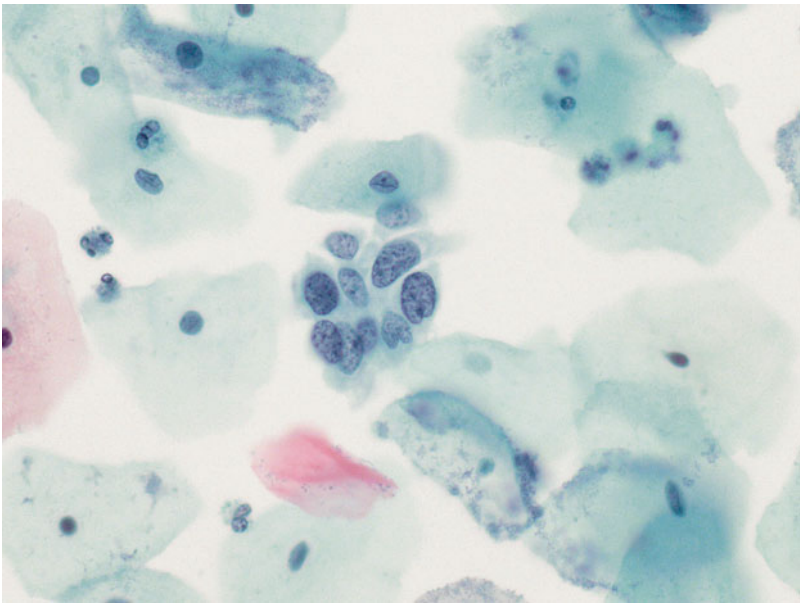

**Fig. 4.24** ASC-H (*LBP, SurePath*). A group of atypical immature metaplastic cells with enlarged nuclei, high nuclear to cytoplasmic ratio, coarse chromatin and irregular nuclear contour. The cytologic features are worrisome but insufficient for an interpretation of HSIL. Follow-up biopsy revealed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

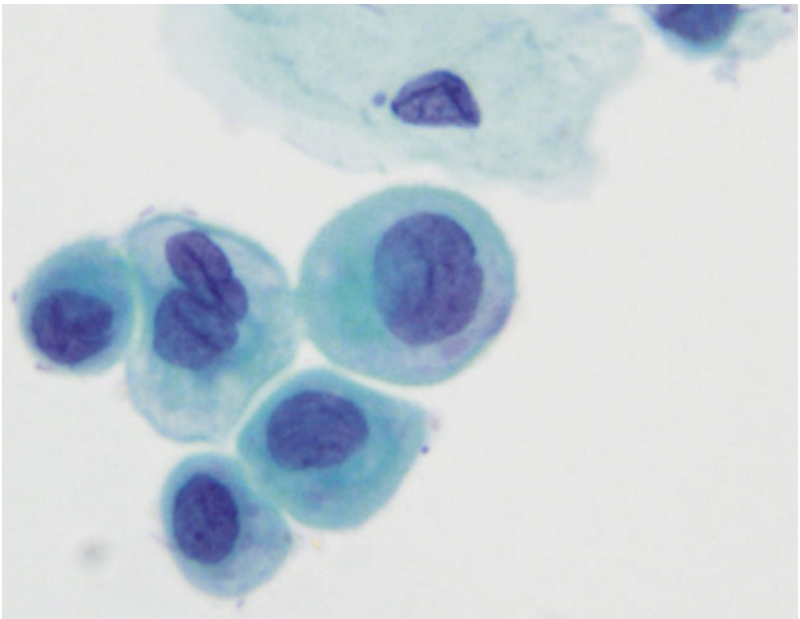

**Fig. 4.25** ASC-H (*LBP, ThinPrep*). A 35-year-old woman. An isolated group of atypical immature metaplastic cells with dense cytoplasm, high nuclear to cytoplasmic ratio, enlarged nuclei, irregular nuclear contour and nuclear grooves. Follow-up biopsy revealed HSIL (CIN2)

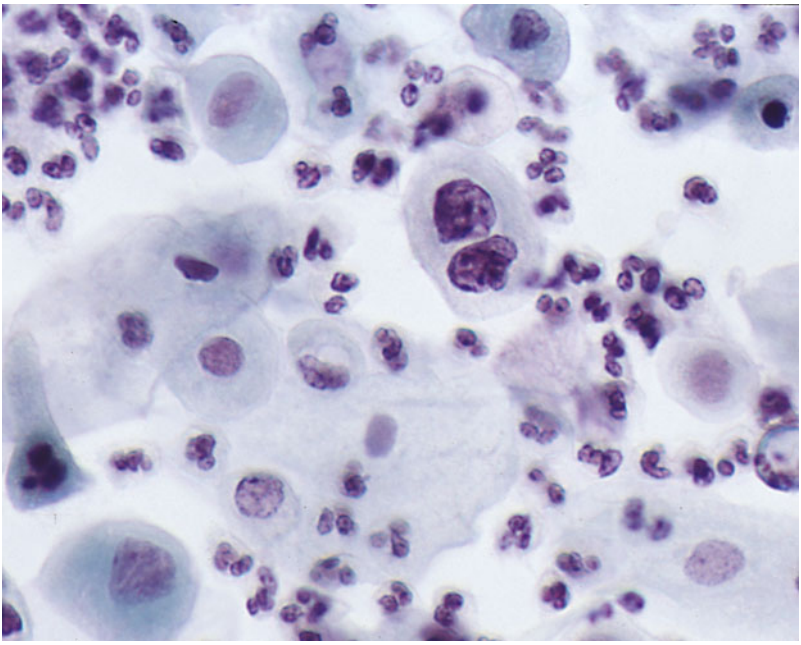

**Fig. 4.26** ASC-H (*LBP, ThinPrep*). Vaginal specimen obtained from patient with prior history of vaginal HSIL (VAIN 3) and endometrial carcinoma. Cells present show degenerated, markedly hyperchromatic nuclei, worrisome for HSIL. Follow-up histology was HSIL (VaIN 3)

### 4.7.2 "Crowded Sheet Pattern" (Fig. 4.27)

#### 4.7.2.1 Criteria

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

**Preparation Specific Criteria**

*Conventional Preparations*:

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

#### 4.7.2.2 Explanatory Notes

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

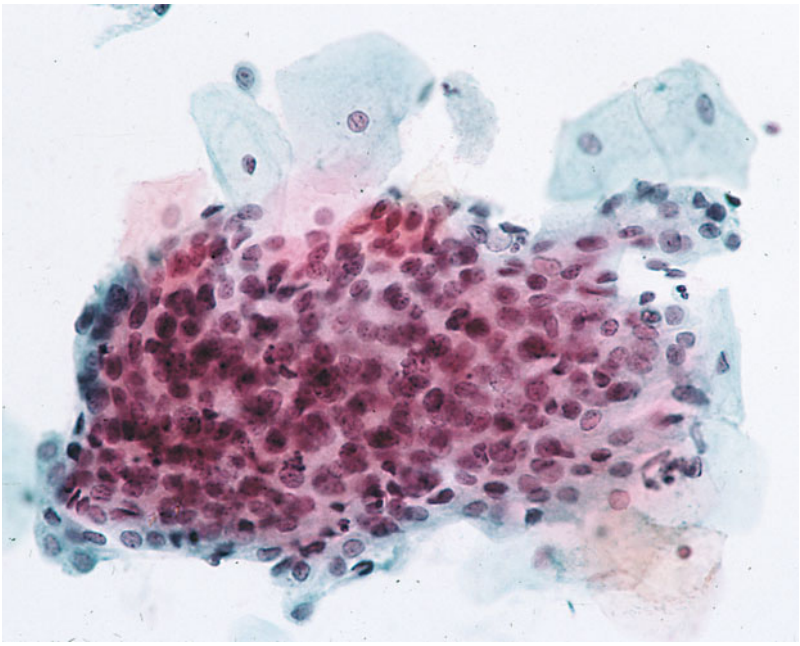

**Fig. 4.27** ASC-H (*CP*). Thick aggregate of cohesive, air-dried, overlapping cells containing nuclei with even chromatin and regular borders. The thickness of the cluster makes it difficult to determine if the cells are squamous or glandular. The disorganization of the cells within the group is suggestive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

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

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

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

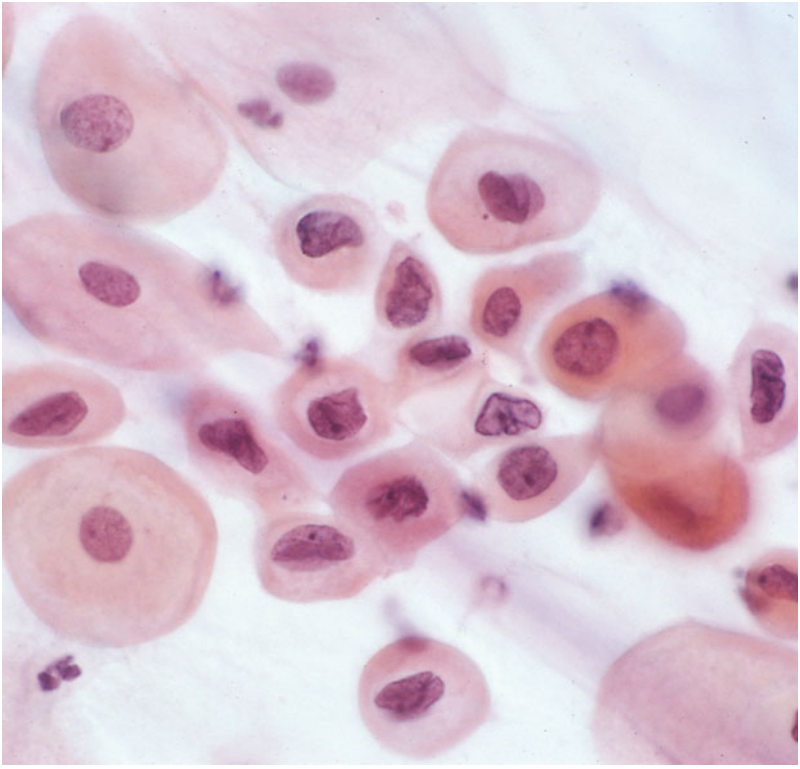

**Fig. 4.28** ASC-H (*CP*). Smear from postmenopausal patient containing ovoid cells with irregular poorly preserved nuclei. Possible interpretations include NILM (atrophy), ASC-H and HSIL

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

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

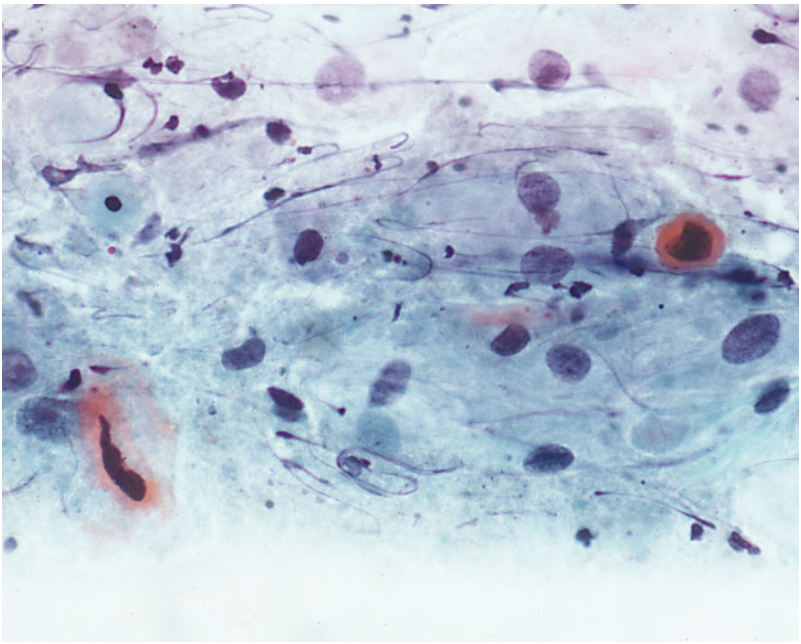

**Fig. 4.29** ASC-H (*CP*). A 50-year-old postmenopausal woman with prior abnormal cytology. Two cells with extremely hyperchromatic, degenerated nuclei, and orangeophilic cytoplasm, in a background of atrophy with lysed cells and debris. Follow-up demonstrated HSIL (CIN 2)

## 4.8 ASC-H Mimics

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

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

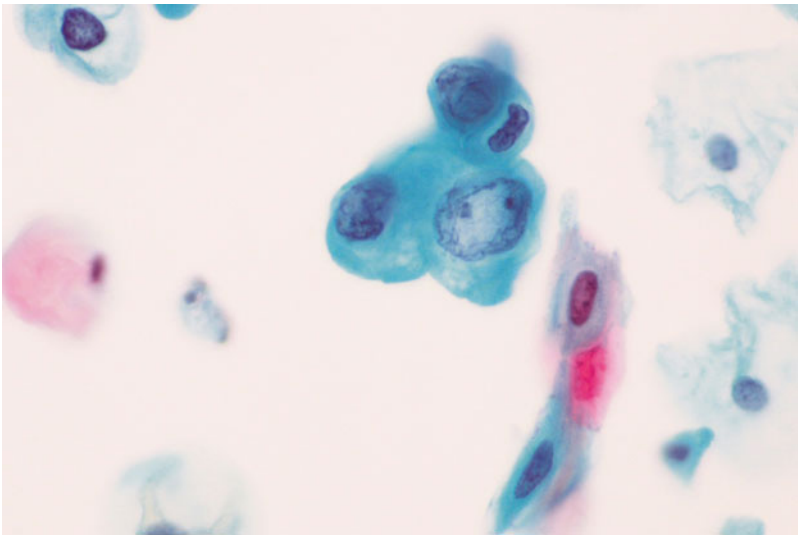

**Fig. 4.30** ASC-H (*LBP, SurePath*). Routine cervical cytology from a perimenopausal woman. A group of metaplastic cells with increased nuclear to cytoplasmic ratios is identified in a relatively clean background. In addition to slightly increased nuclear size, the cells also show some nuclear clearing.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

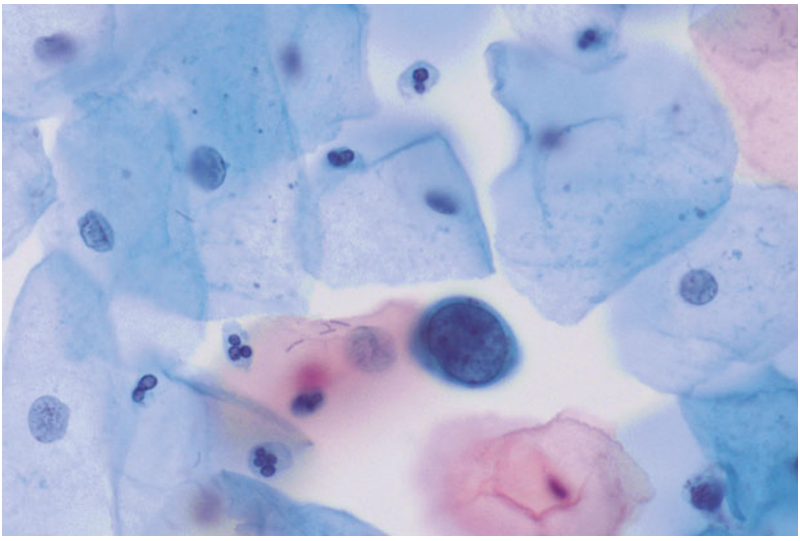

**Fig. 4.31** ASC-H (*LBP, SurePath*). Perimenopausal woman with no significant medical history. Cervical cytology was unremarkable with the exception of a single enlarged cell with scant cytoplasm, a distinct, regular nuclear membrane and evenly distributed chromatin. An interpretation of ASC-H was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

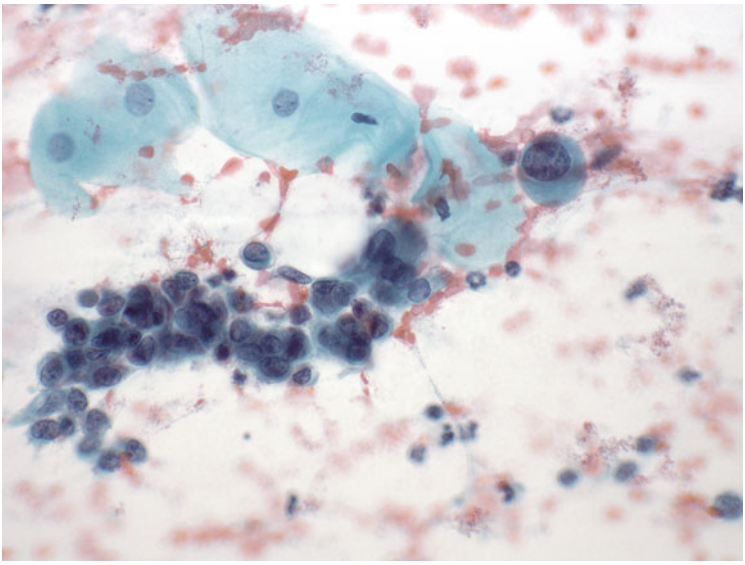

**Fig. 4.32** Endometrial cells mimicking HSIL (*CP*). A crowded group of poorly preserved endometrial cells featuring small cells with hyperchromatic nuclei and high nuclear to cytoplasmic ratios

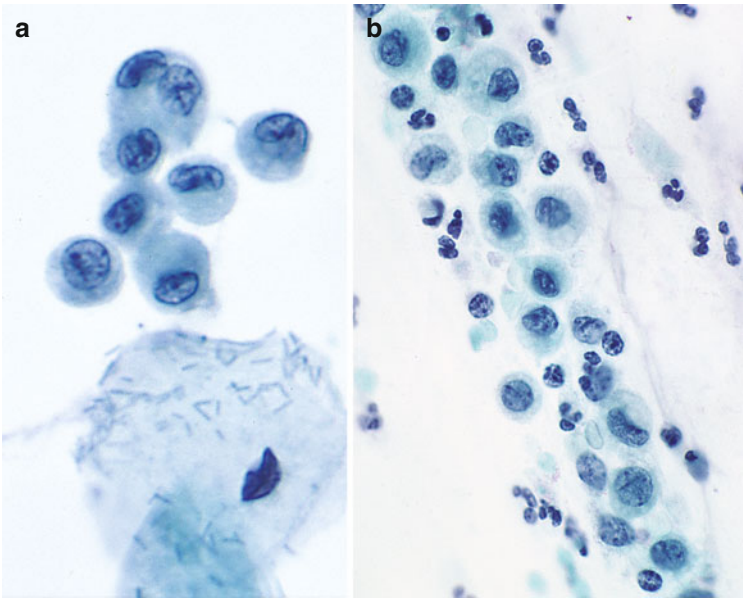


**Fig. 4.33** Histiocytes: appearance on liquid based and conventional preparations. (**a**) *Left panel*. NILM, histiocytes (*LBP, ThinPrep*). Routine screen from a 32-year-old woman. Cells possess eccentric oval and round nuclei and foamy cytoplasm. The rounder shape of most cells in *LBP* as compared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

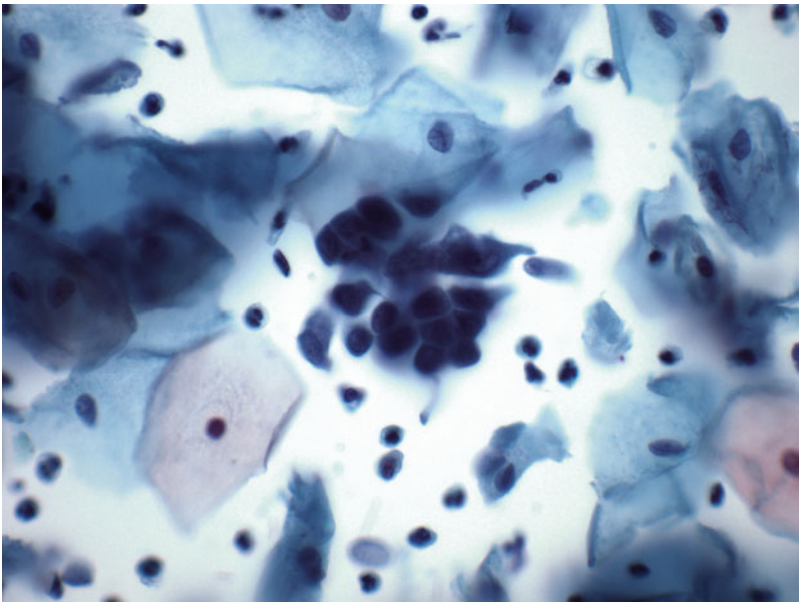

**Fig. 4.34** NILM, Endocervical cell grouping (*LBP, SurePath*). Endocervical cells, when viewed on end, may mimic ASC-H, showing high nuclear to cytoplasmic ratios, and a configuration reminiscent of metaplastic cells. Maintenance of a "honey-comb" structure, and a mucus cap when focusing above the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

### 4.8.2 Artifacts (Fig. 4.34)

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

## 4.9 Management

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

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

These guidelines are as follows [27]:

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

## 4.10 Quality Assurance

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

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

## 4.11 Sample Reports

**Example 1**
*Adequacy*:
Satisfactory for evaluation; transformation zone components identified
*Interpretation*
*Epithelial cell abnormality*, *squamous*:
Atypical squamous cells – undetermined significance (ASC-US)

*Comment*:
Suggest high-risk HPV testing if clinically warranted (if reflex testing not ordered or if conventional preparation and no co-collection sample was received)
OR
Specimen sent for reflex HPV testing per clinician request.

**Example 2**
*Adequacy*:
Satisfactory for evaluation; transformation zone component identified
*Interpretation*
*Epithelial cell abnormality*, *squamous*:
Atypical squamous cells – cannot exclude a high-grade squamous intraepithelial lesion (ASC-H).

*Comment*:
Suggest colposcopy/biopsy as clinically indicated.

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

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# Epithelial Cell Abnormalities: Squamous

# 5

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## 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

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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).

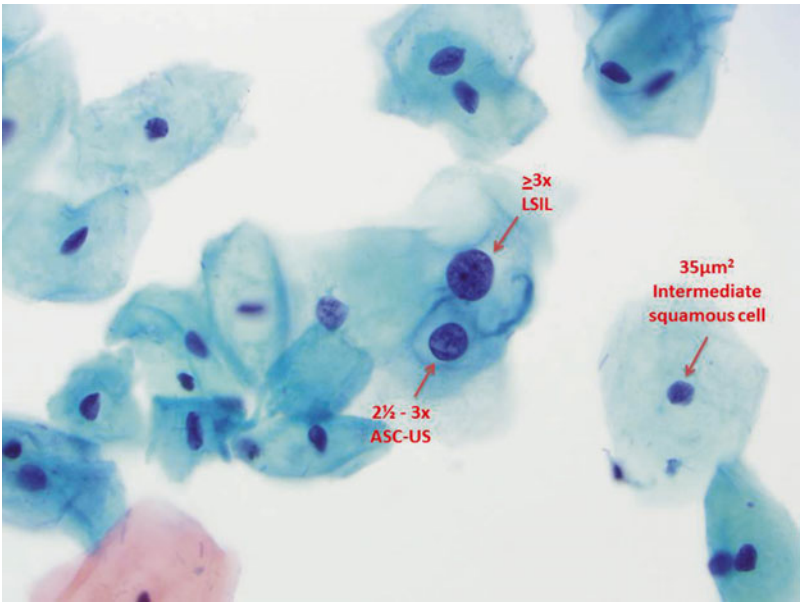


**Fig. 5.1** Nuclear area (*LBP*, *ThinPrep*). The nuclear area of an intermediate squamous cell is approximately 35 µm$^2$. This is used as a reference to measure abnormal squamous cells such as ASC-US (approximately 100 µm$^2$) and LSIL (approximately 150–175 µm$^2$)

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).

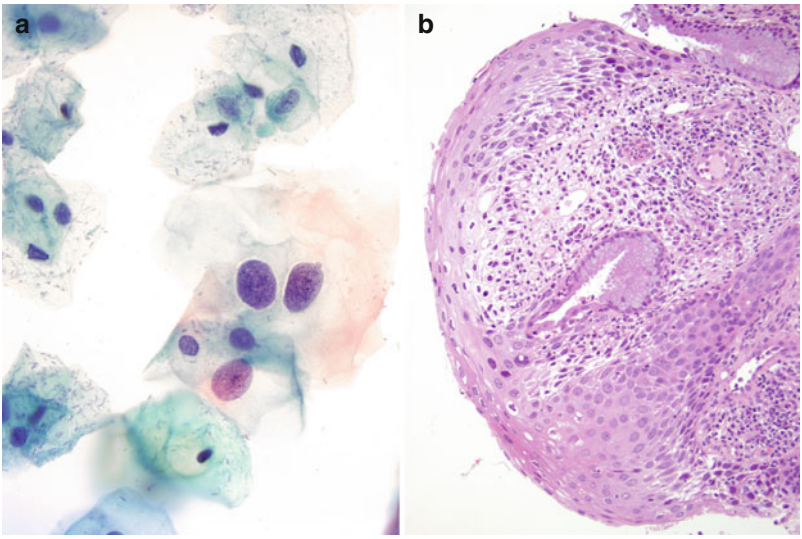


**Fig. 5.2** Low-grade squamous intraepithelial lesion (LSIL) (**a**, *left*: *LBP*, *ThinPrep* and **b**, *right* cervix, H&E stain). Nuclear enlargement and hyperchromasia are of sufficient degree for the interpretation of LSIL (**a** & **b**). HPV-associated cytoplasmic changes are not a prerequisite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

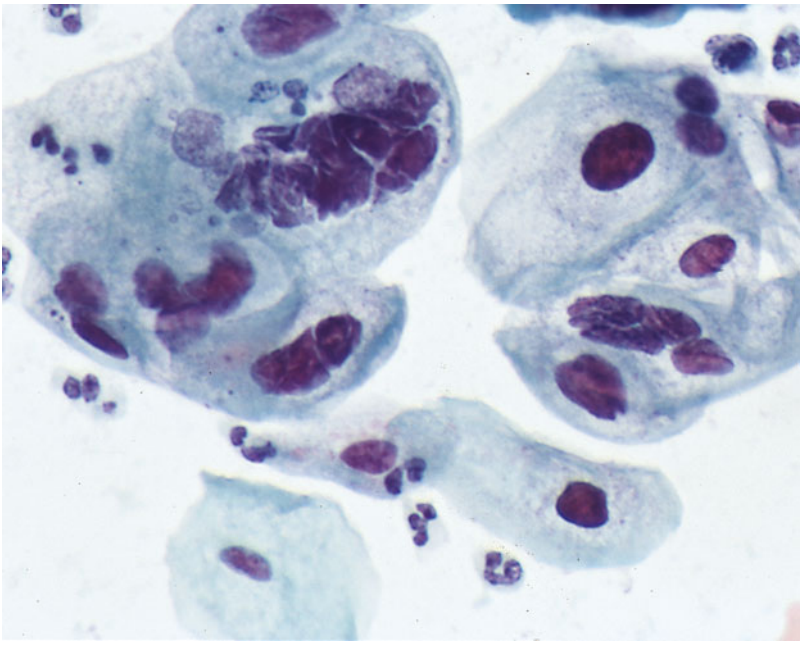

**Fig. 5.3** LSIL (*LBP*, *ThinPrep*). A 32-year-old woman, day 15, routine cervical cytology screening. Note the overall large cell size, "smudged" nuclear chromatin, well-defined cytoplasm, and multinucleation

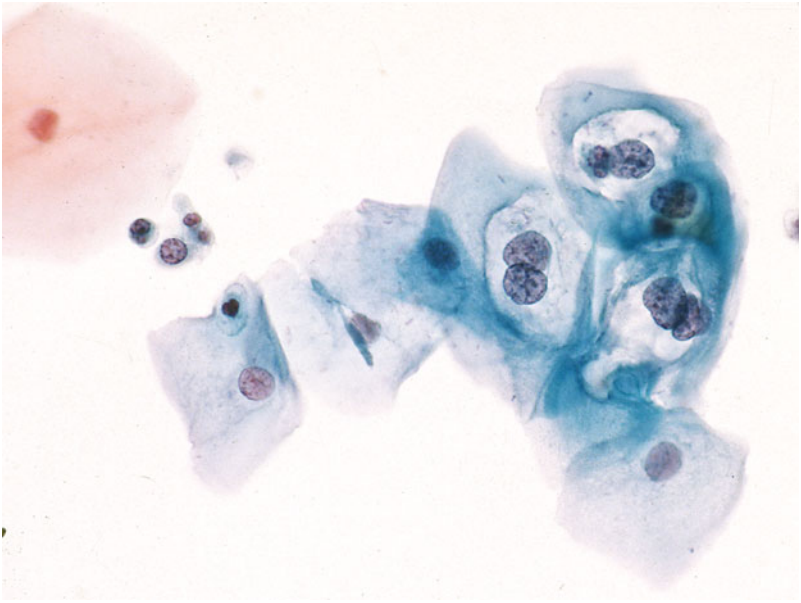

**Fig. 5.4** LSIL (*LBP*, *ThinPrep*). Routine screen from a 32-year-old woman. Nuclear abnormalities are required to make an interpretation of LSIL. HPV cytopathic effect manifested by perinuclear cavitation often accompanies the nuclear abnormalities but is not required for an interpretation of LSIL

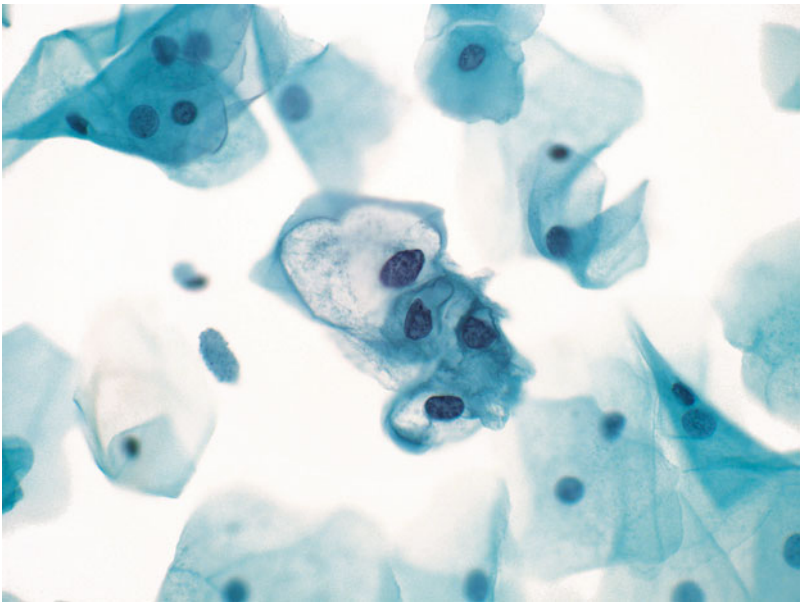

**Fig. 5.5** LSIL (*LBP*, *SurePath*). Cells with diagnostic koilocytic features of LSIL have a sharply defined perinuclear cavity, condensation of cytoplasm around the periphery, and abnormal nuclear features including enlargement and nuclear membrane irregularity. In liquid-based samples, nuclear hyperchromasia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

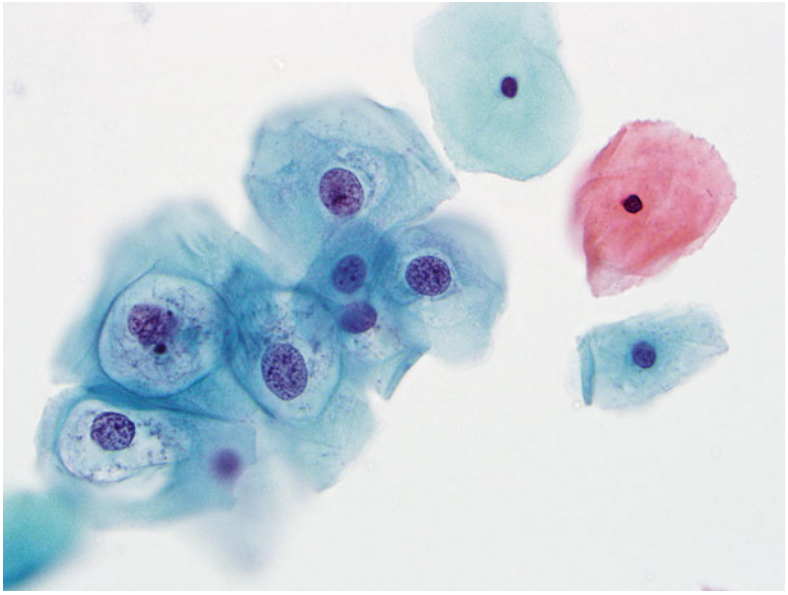

**Fig. 5.6** LSIL (*LBP*, *ThinPrep*). A 28-year-old woman with a history of ASC-US and positive hrHPV testing. LSIL on cytology is characterized by mature squamous cells with enlarged nuclei with variable chromatin and nuclear membranes. Koilocytosis or perinuclear cavitation in the cytoplasm, a characteristic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

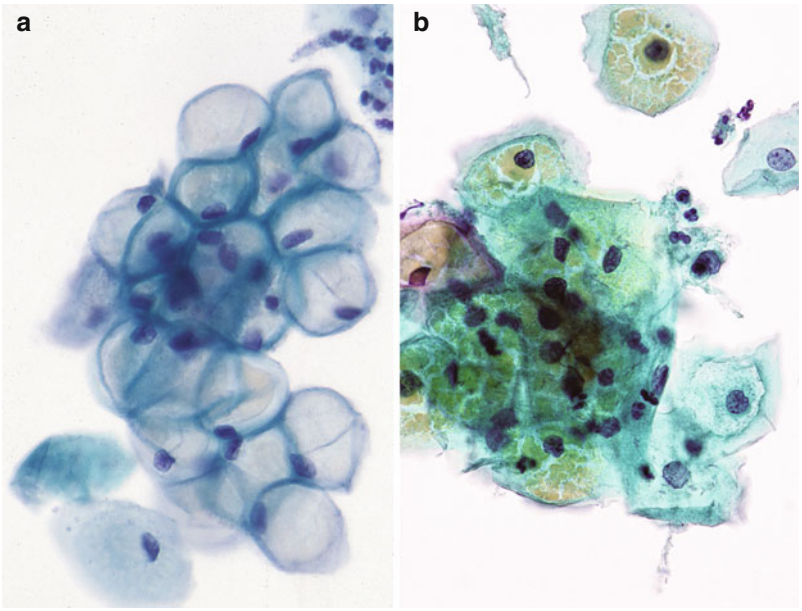


**Fig. 5.7** Pseudokoilocytes (*LBP*, *ThinPrep*). Glycogen in squamous cells can give the appearance of "pseudokoilocytosis" (**a**). The halos associated with glycogen often have a yellow refractile appearance (**b**). The nuclear abnormalities required for an interpretation of LSIL are absent. Follow-up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

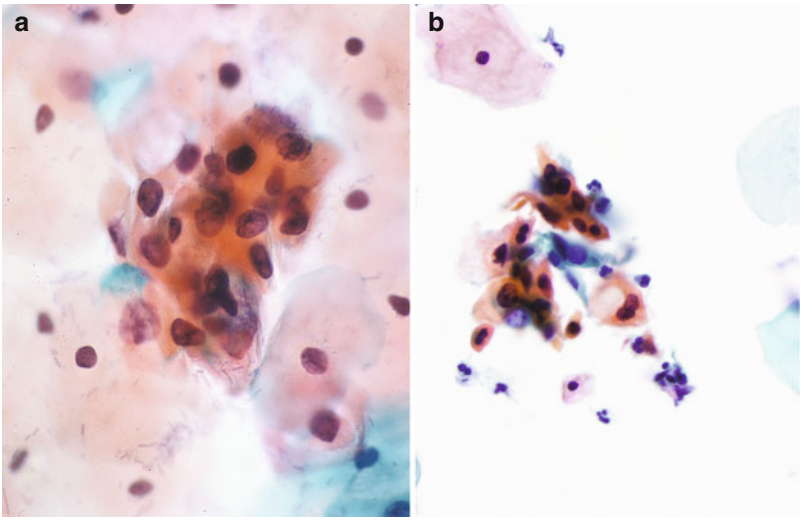


**Fig. 5.8** ASC-US versus LSIL (**a** *left CP*, **b** *Right LBP*, *ThinPrep*). Atypical squamous cells with orangeophilic cytoplasm ("atypical parakeratosis"). These cells have some features of SIL; however, such keratinized lesions may be difficult to grade. hrHPV triage is helpful in determining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

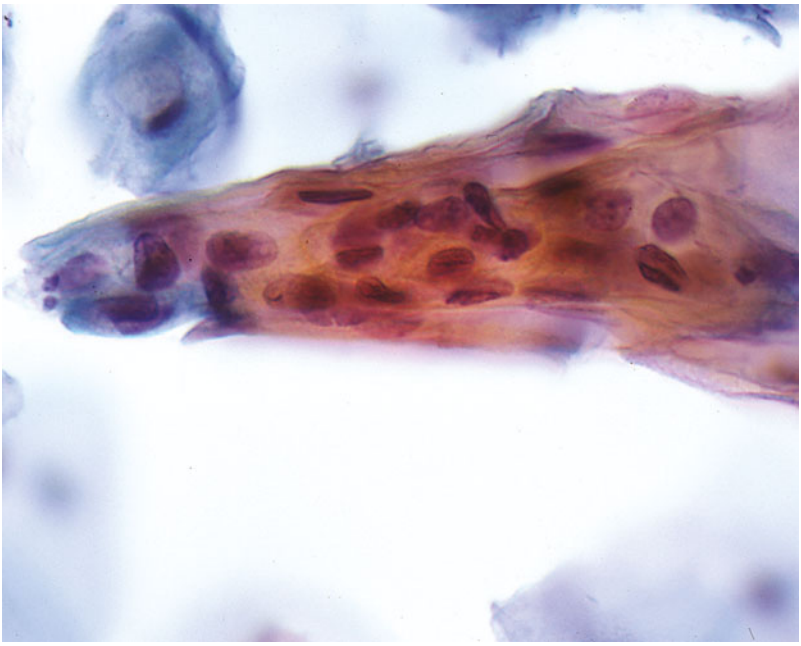

**Fig. 5.9** ASC-US versus LSIL (*LBP*, *ThinPrep*). A 32-year-old woman. Clusters of squamous cells may be seen in "spikelike" aggregates; such clusters should be classified based on the degree of nuclear abnormalities. This patient had an LSIL interpretation on a conventional smear 2 months before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

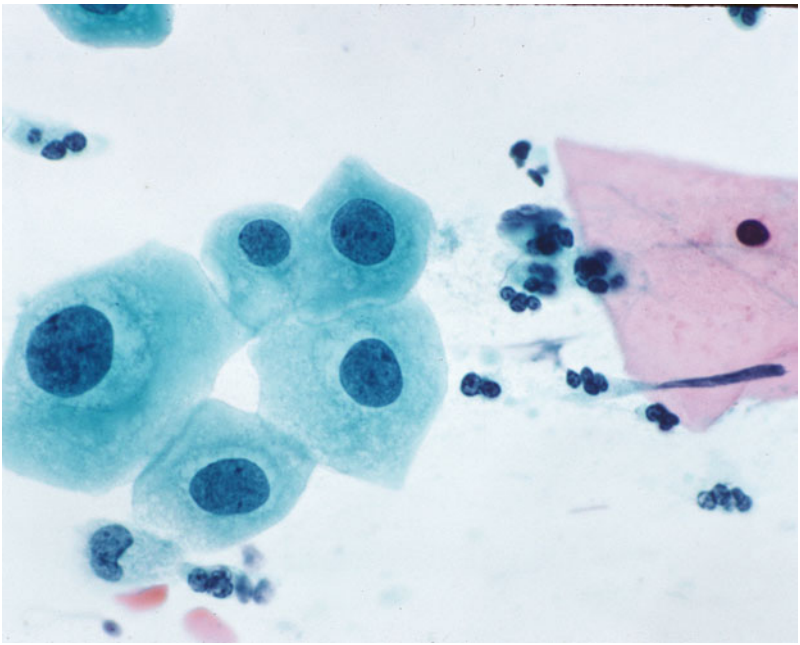

**Fig. 5.10** ASC-US versus LSIL (*CP*). Nuclear features are borderline between those required for ASC-US and LSIL. Cases such as this will no doubt have poor interobserver reproducibility as demonstrated in various studies including the Bethesda 2001 BIRST project

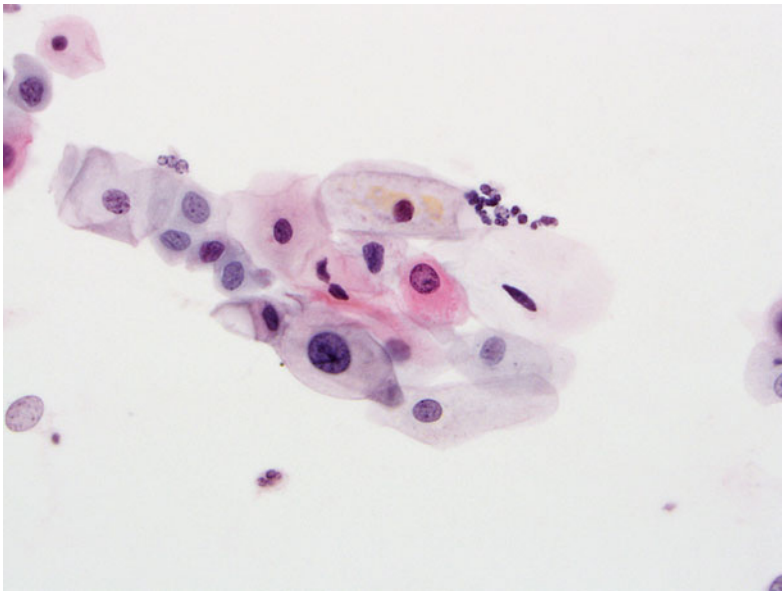

**Fig. 5.11** ASC-US versus LSIL (*LBP*, *ThinPrep*). Abnormal nuclear enlargement without concomitant HPV cytopathic change is identified in this Pap test from a 32-year-old woman. The hallmark of LSIL is an enlarged nucleus, often as much as four to six times the area of a normal intermediate cell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

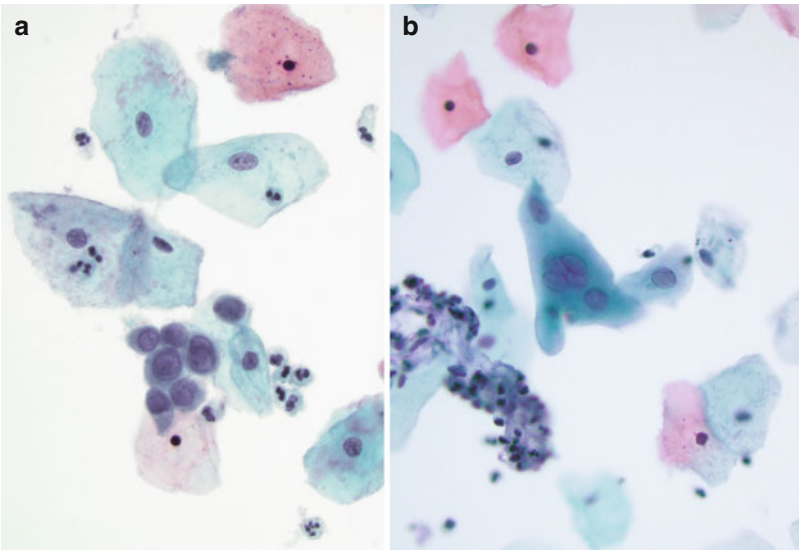


**Fig. 5.12** Herpes (*LBP*, *ThinPrep*). Routine cervical cytology. A 25-year-old woman. Endocervical cell (**a**) and intermediate cells (**b**) showing herpes virus cytopathic effect with clearing of chromatin. These cells can be mistaken for ASC-US or LSIL (**b**) or occasionally HSIL (**a**) when 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

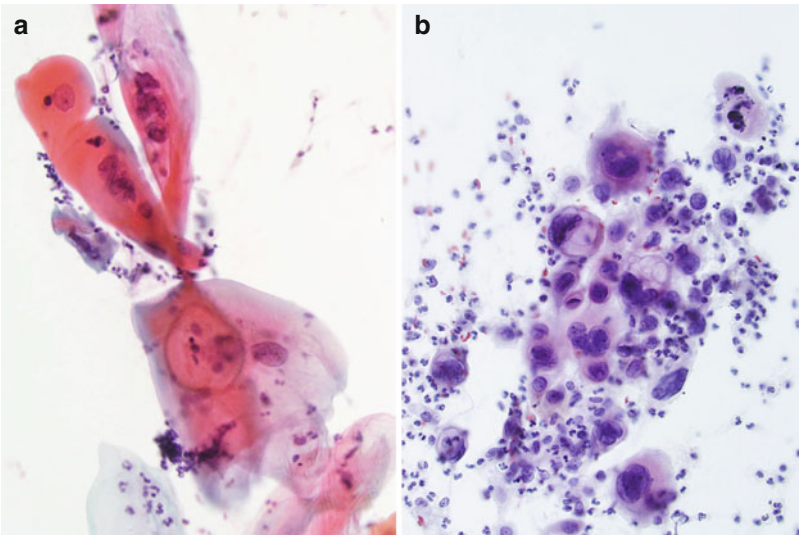


**Fig. 5.13** Radiation change versus squamous cell carcinoma (*CP*). (**a**) A 61-year-old woman with a history of squamous cell carcinoma and radiation. Mature squamous cell showing cytomegaly, low N/C ratios, intracytoplasmic vacuoles with neutrophils. The mild enlargement of the nucleus should not 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).

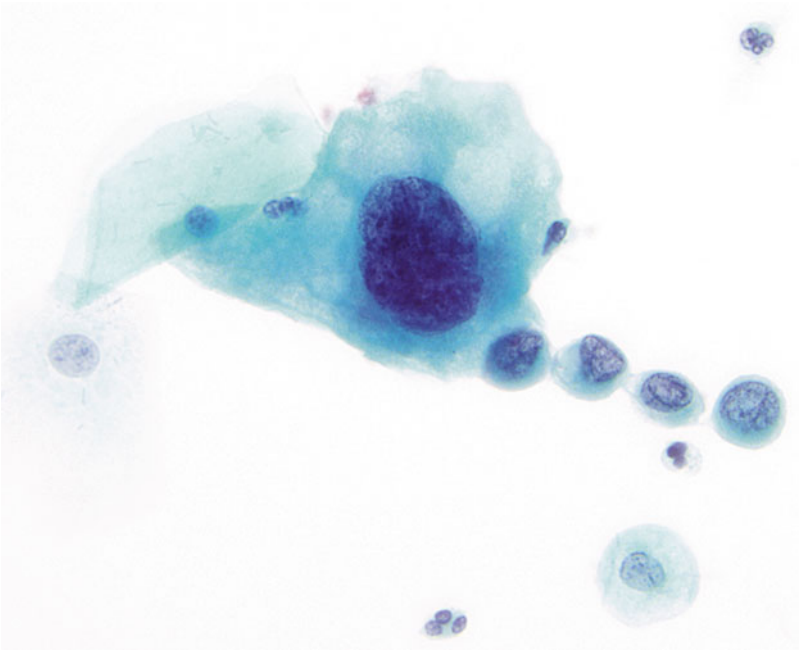

**Fig. 5.14** High-grade squamous intraepithelial lesion (HSIL) (*LBP*, *ThinPrep*). There is a mixture of dysplastic cells here, one large LSIL cell, and four adjacent, small, high N/C ratio cells with nuclear features consistent with HSIL

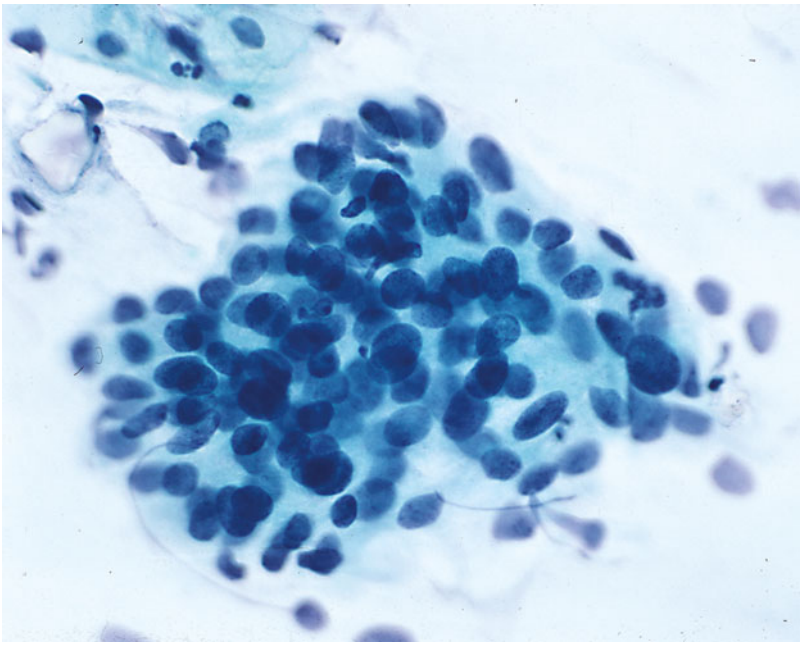

**Fig. 5.15** High-grade squamous intraepithelial lesion (HSIL) (*CP*). The dysplastic cells are seen here in a syncytial cluster or hyperchromatic crowded group

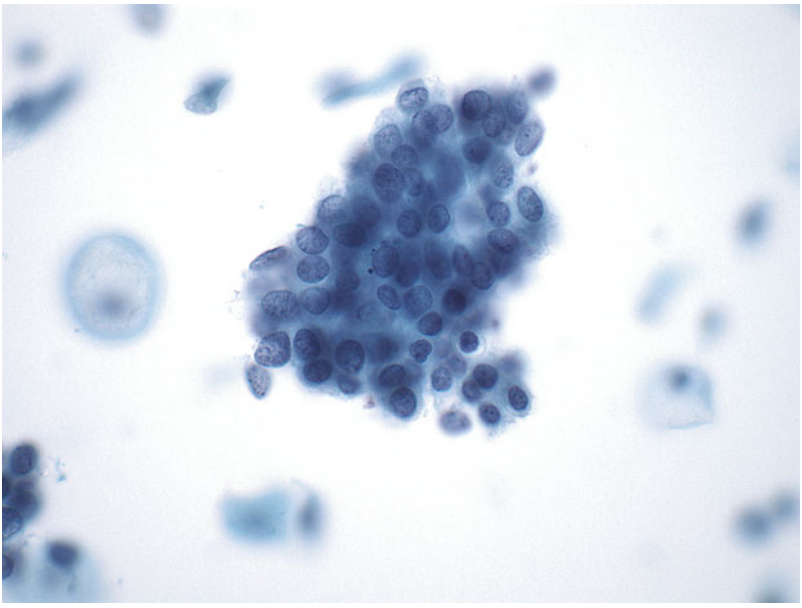

**Fig. 5.16** HSIL-syncytial cluster (*LBP*, *SurePath*). As in conventional smears, crowded hyperchromatic cell groups should be examined with care. If a squamous abnormality is suspected, a thorough search for single dysplastic cells in the background is warranted. Follow-up showed HSIL (CIN 3) with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

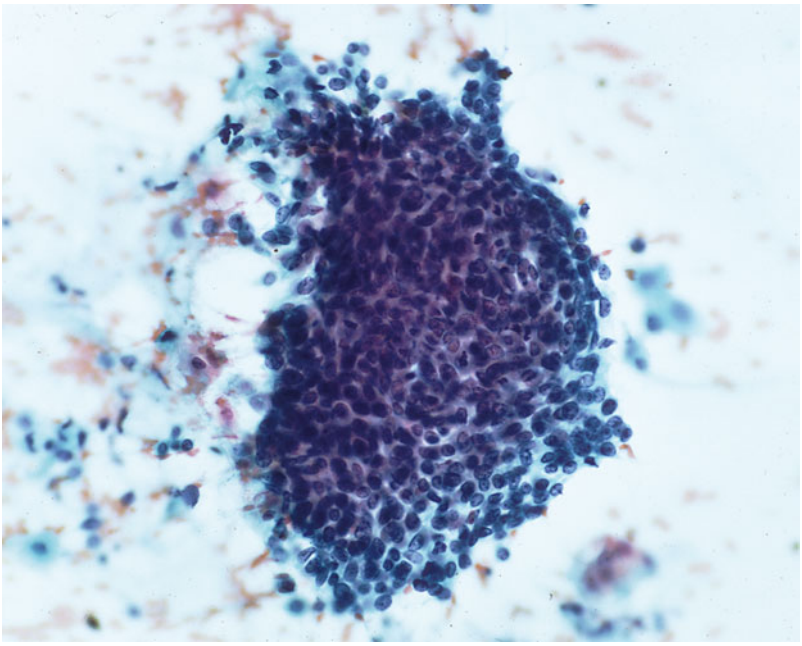

**Fig. 5.17** HSIL (*CP*). A 58-year-old postmenopausal woman on hormone replacement therapy. Hyperchromatic crowded groups seen at low power require careful examination at higher magnification. Flattening at the edge of the cell cluster and whorling in the center are suggestive of HSIL over a glandular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

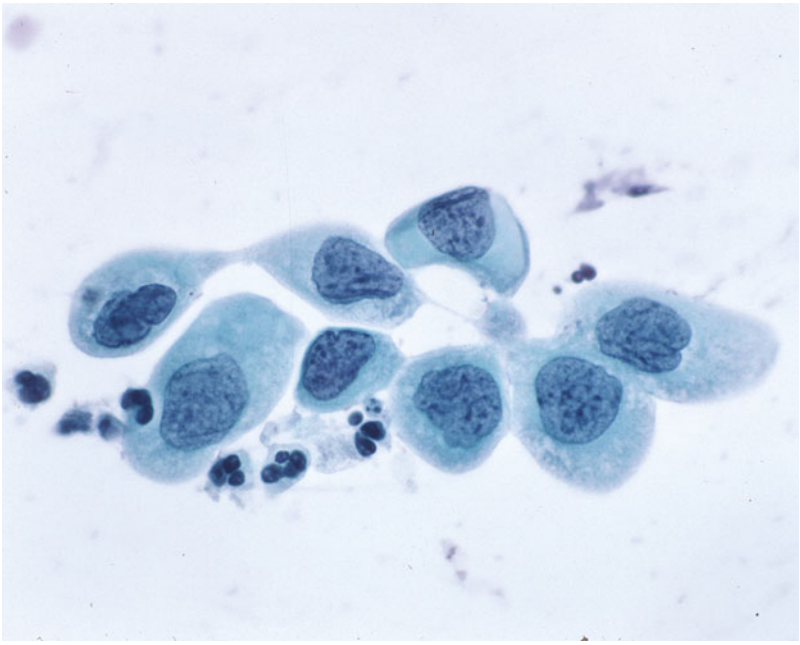

**Fig. 5.18** HSIL (*CP*). Nuclear changes are HSIL; however, the nuclear/cytoplasmic (N/C) ratio is on the low end for HSIL

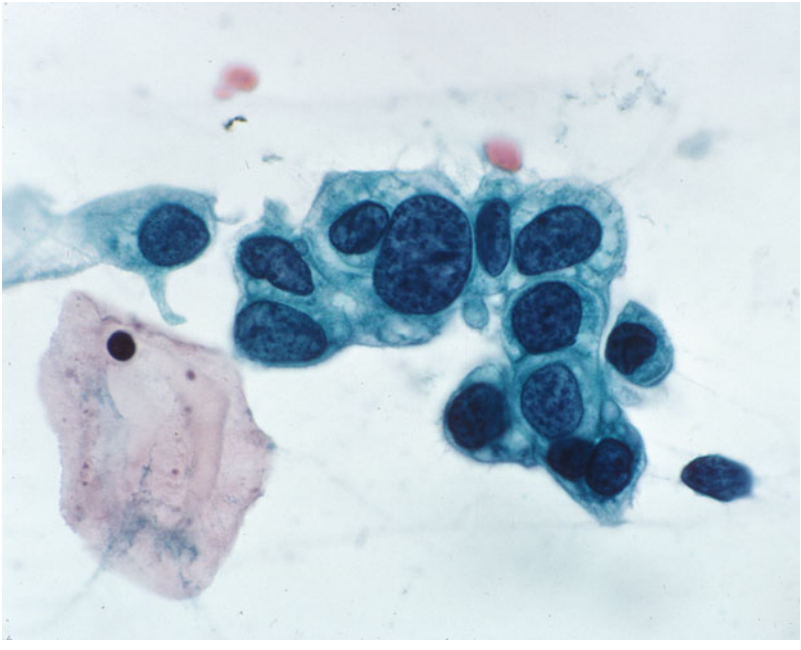

**Fig. 5.19** HSIL (*CP*). There is variation in nuclear size and shape, and the cells have delicate cytoplasm

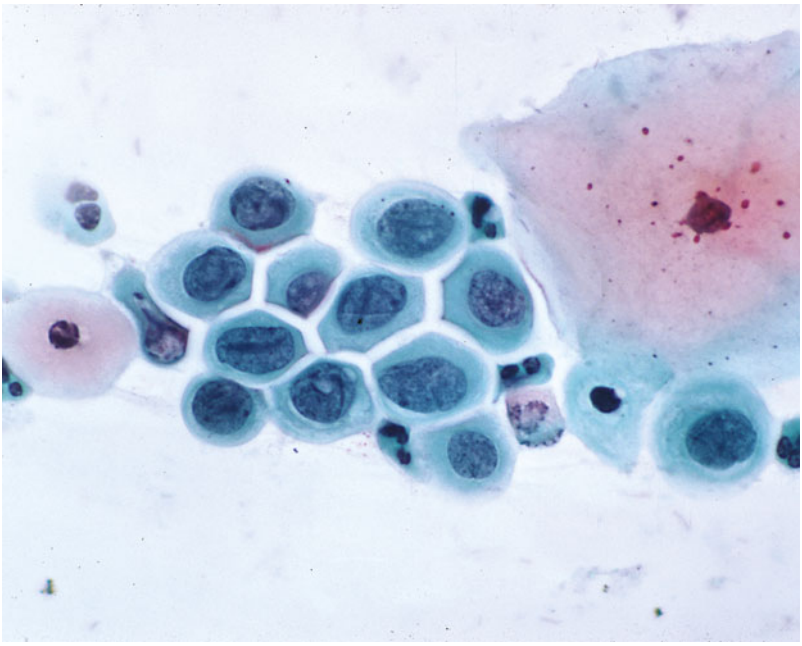

**Fig. 5.20** HSIL (*CP*). HSIL with "metaplastic" or dense cytoplasm, in contrast to that seen in the syncytial groups of HSIL (Fig. 5.19)

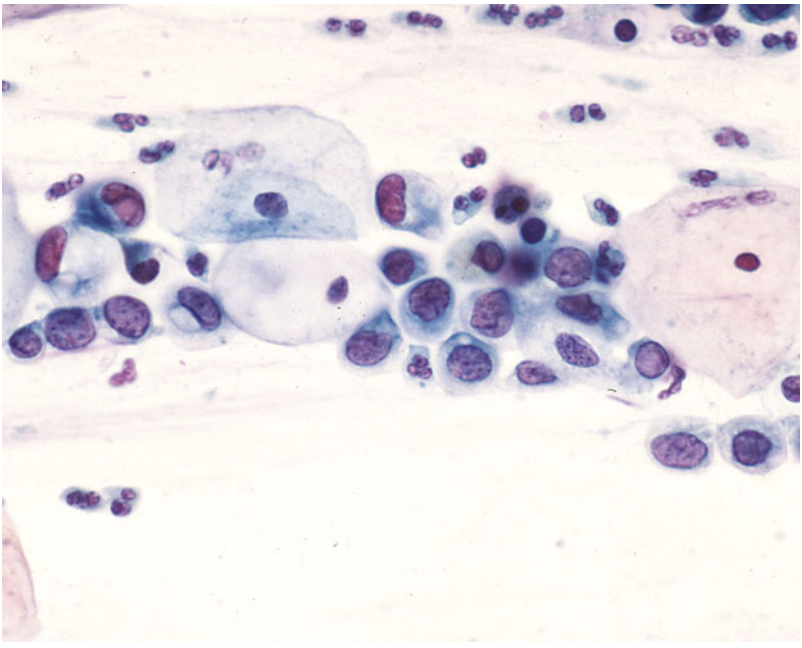

**Fig. 5.21** HSIL (*CP*). HSIL cells with some variation in cell size and N/C ratios. A cluster such as this may be misinterpreted as squamous metaplastic cells if examined only under lower magnification. Follow-up showed HSIL (CIN 3)

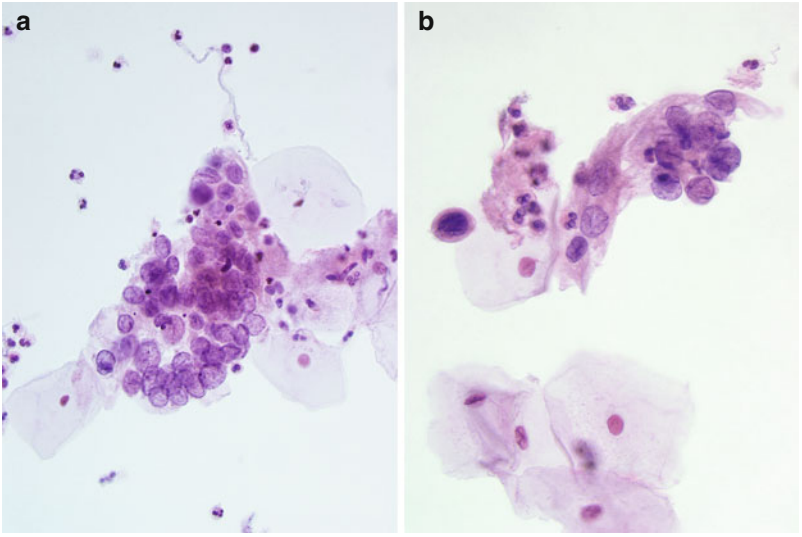


**Fig. 5.22** HSIL (**a**, **b** *LBP*, *ThinPrep*). HSIL that is markedly hypochromatic. A diligent search may reveal more classic cells elsewhere on the same slide. (**a**) On the *left* side, note syncytial arrangement and nuclear grooves. (**b**) On the *right* side, abnormal naked nuclei and a hyperchromatic,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

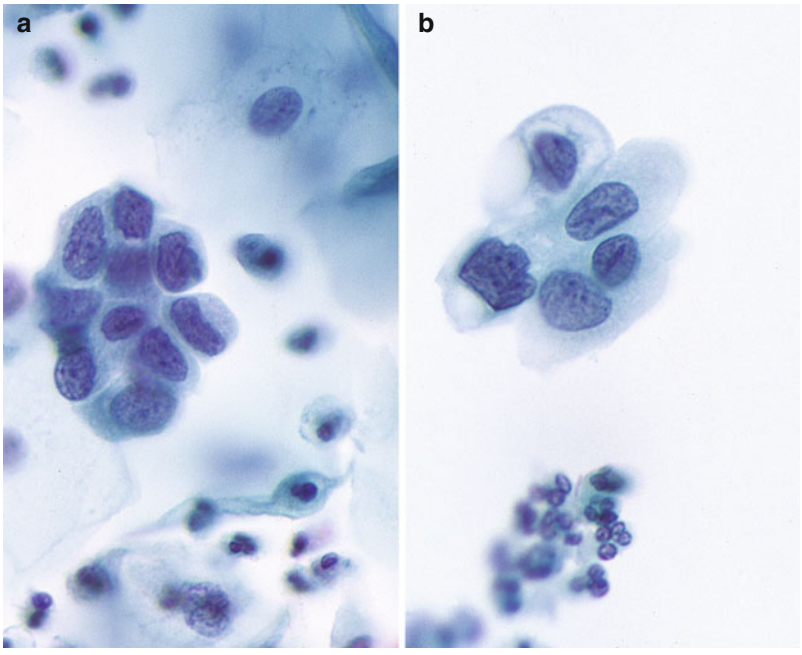


**Fig. 5.23** HSIL (**a**, **b** *LBP*, *SurePath*). Note the nuclear envelope irregularities and abnormal chromatin. As seen here in *LBPs*, hyperchromasia may not be as prominent as in conventional smears

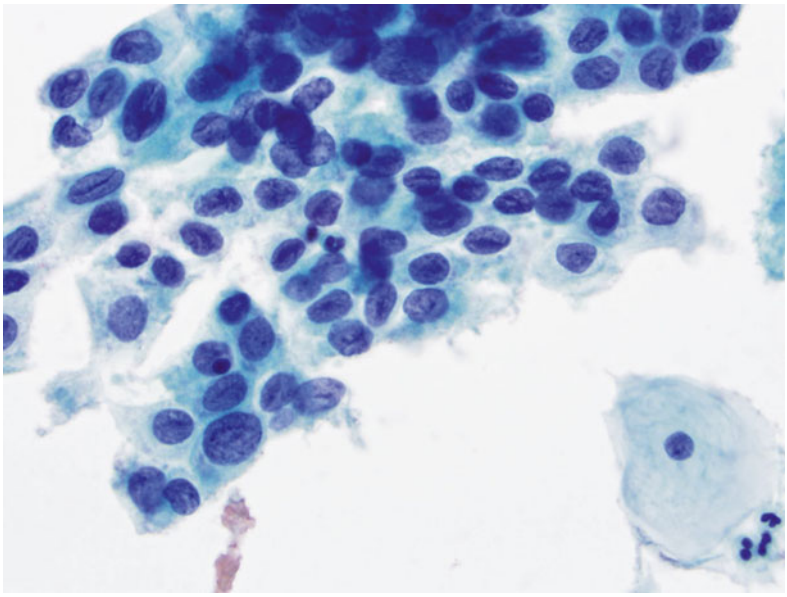

**Fig. 5.24** HSIL (*LBP*, *ThinPrep*). Cells showing variably sized, ovoid nuclei with prominent nuclear grooves. In this case, the chromatin is not particularly hyperchromatic, and cytoplasm has ill-defined borders

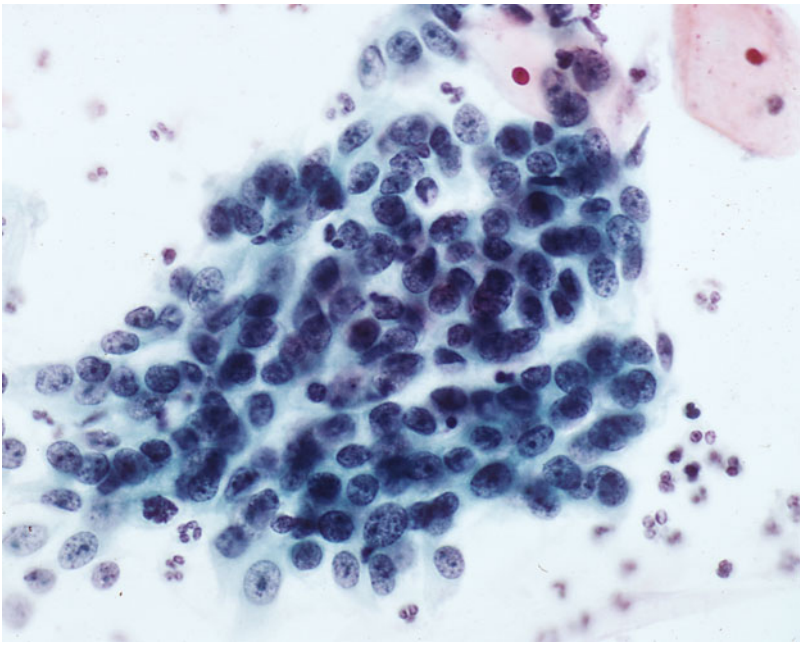

**Fig. 5.25** HSIL (*CP*). A 42-year-old woman. Although uncommon, nucleoli may be seen in HSIL, especially with extension into endocervical gland spaces. The chromatin may appear less coarsely granular

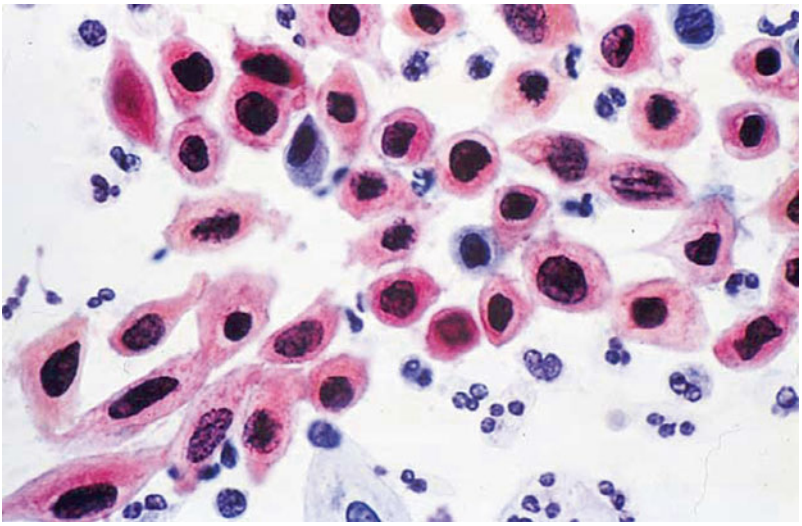

**Fig. 5.26** HSIL-keratinizing lesion (*CP*). The criteria of nuclear to cytoplasmic ratio and degree of nuclear abnormalities used for grading SIL may be more difficult to apply to keratinizing lesions. The extent of abnormality here qualifies for an interpretation of HSIL (contrast with Figs. 5.8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

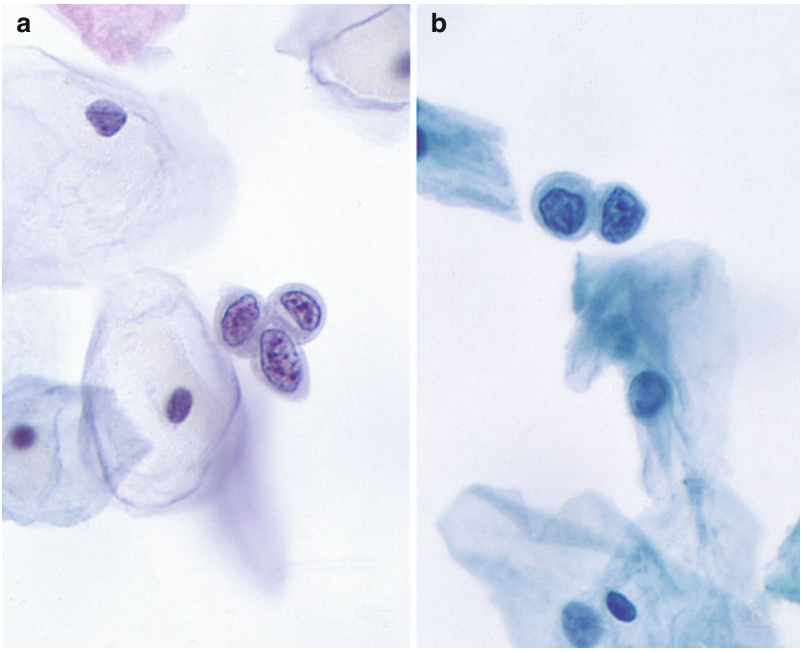


**Fig. 5.27** HSIL (**a, b**: *LBP*, *ThinPrep*). A 29-year-old woman from a high-risk clinic. Close attention to isolated cells is required when screening *LBPs* because the abnormal isolated cells may not be as apparent as clusters of HSIL cells and may lie between benign cell clusters or in "empty 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).

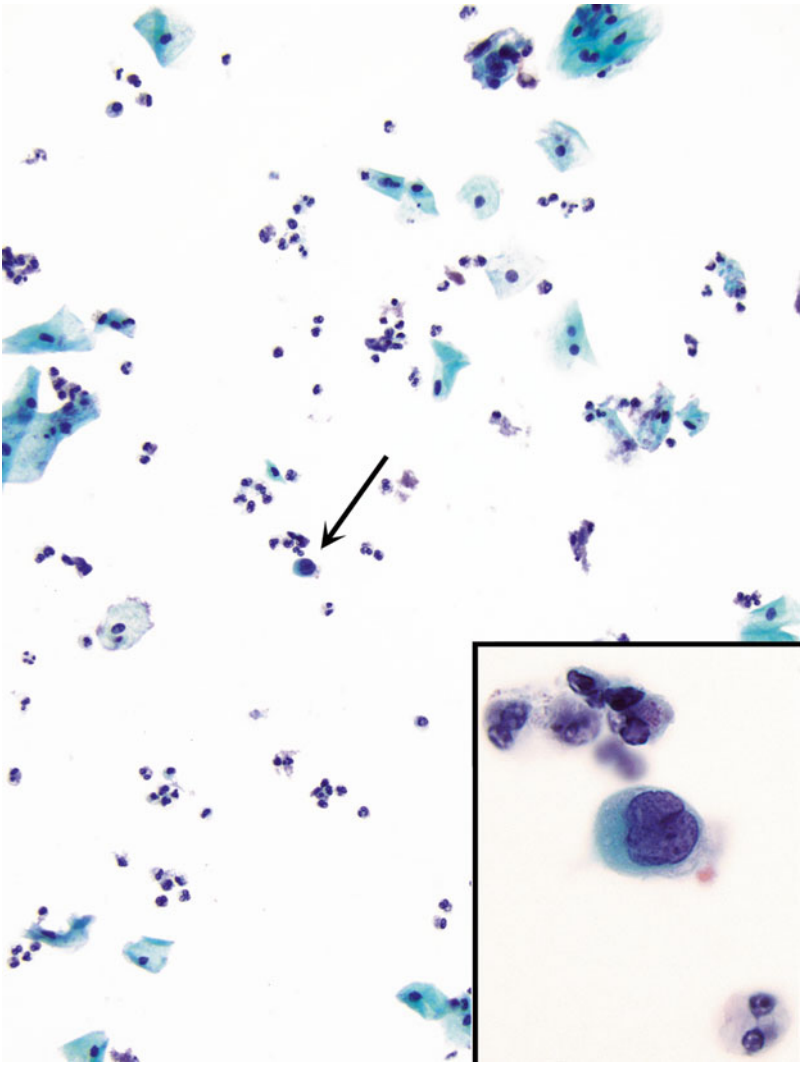

**Fig. 5.28** HSIL (*LBP*, *ThinPrep*). Isolated single abnormal cells (*arrow*) are more often seen in *LBPs*. These small cells may be seen in the spaces between cells as seen here and may be easily missed on screening. The inset magnifies the cell indicated by the *arrow*, which shows abnormal features 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).

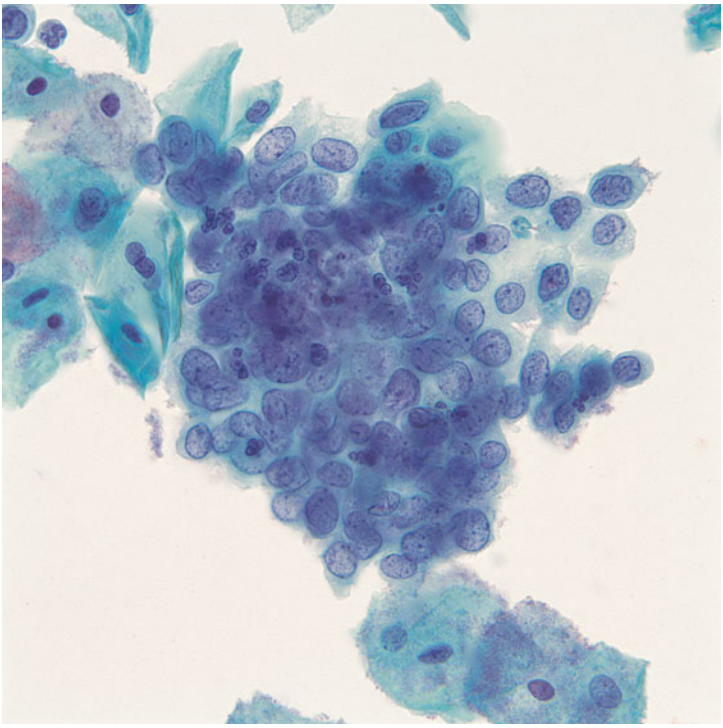

**Fig. 5.29** HSIL (*LBP*, *ThinPrep*). A 32-year-old woman with a history of abnormal Pap tests and positive hrHPV testing. A syncytial cluster of cells with overlapping of hypochromatic nuclei are seen. The nuclei are often less hyperchromatic in liquid-based preparations. Follow-up cone biopsy revealed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

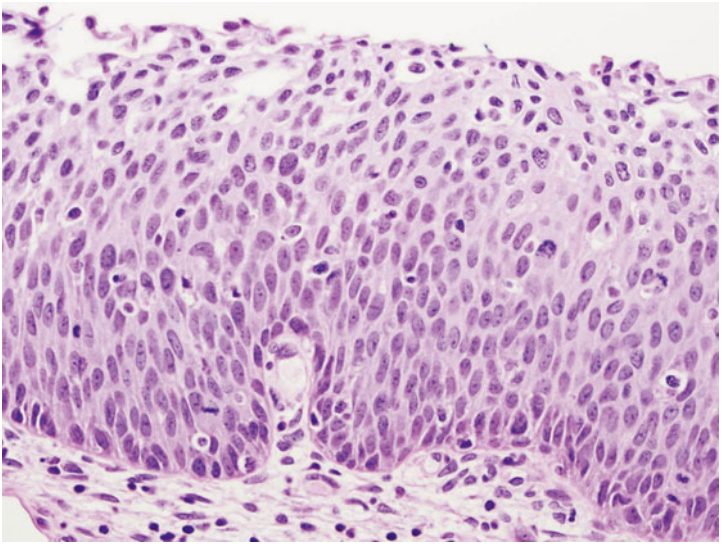

**Fig. 5.30** HSIL (CIN 3) (*cervix*, *H&E stain*). The histology of HSIL (CIN 3) reflects the findings seen in clusters of HSIL seen on cytology. The abnormal immature cells show minimal maturation from the base of the epithelium to the surface with nuclear size and shape variation

### 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

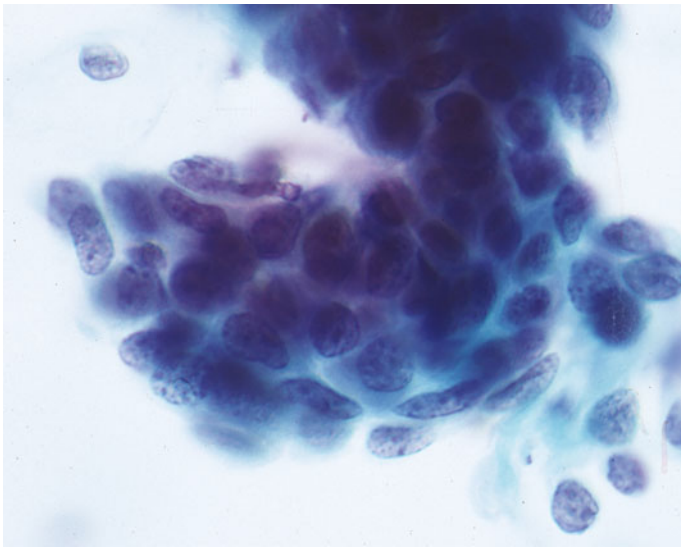

**Fig. 5.31** HSIL with extension into endocervical gland space (*LBP*, *SurePath*). Note flattening of cells at the edge of the cluster, a feature that favors HSIL over a glandular lesion

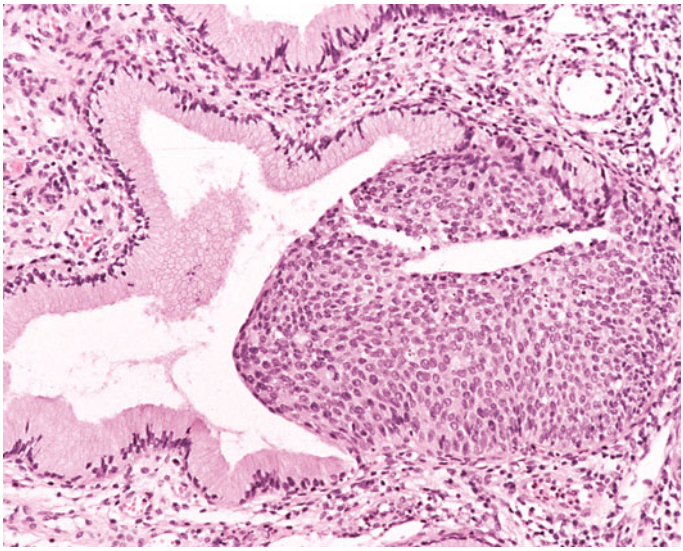

**Fig. 5.32** HSIL (CIN 3) with extension into endocervical glands (*cervix*, *H&E stain*). Squamous dysplasia, especially high-grade lesions, often extends into endocervical glands replacing the normal endocervical glandular cells

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).

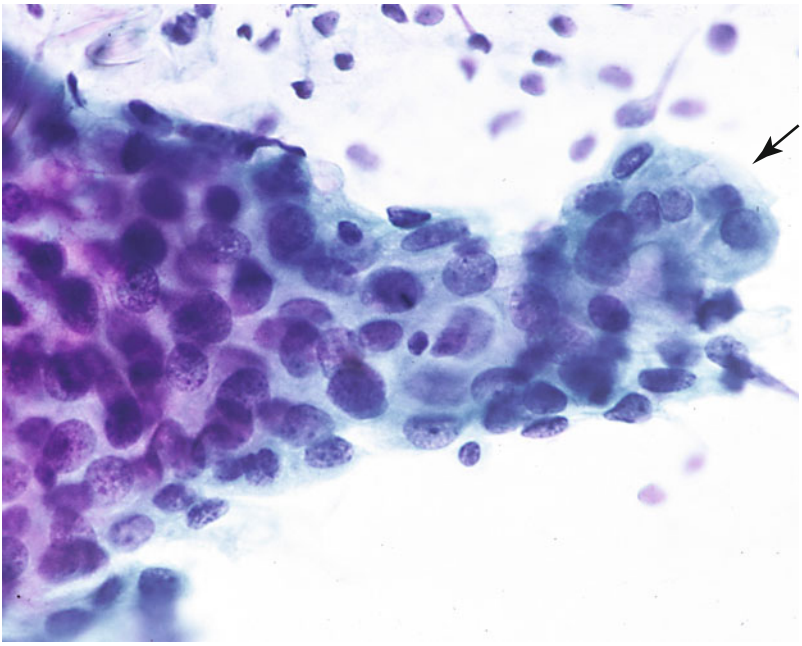

**Fig. 5.33** HSIL (*CP*). A 30-year-old woman with atypical glandular cells on a prior Pap test. When HSIL lesions involve endocervical glands, they may show features that overlap with those of adenocarcinoma in situ (AIS). Note normal columnar cells with residual mucin at the *right upper edge* of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

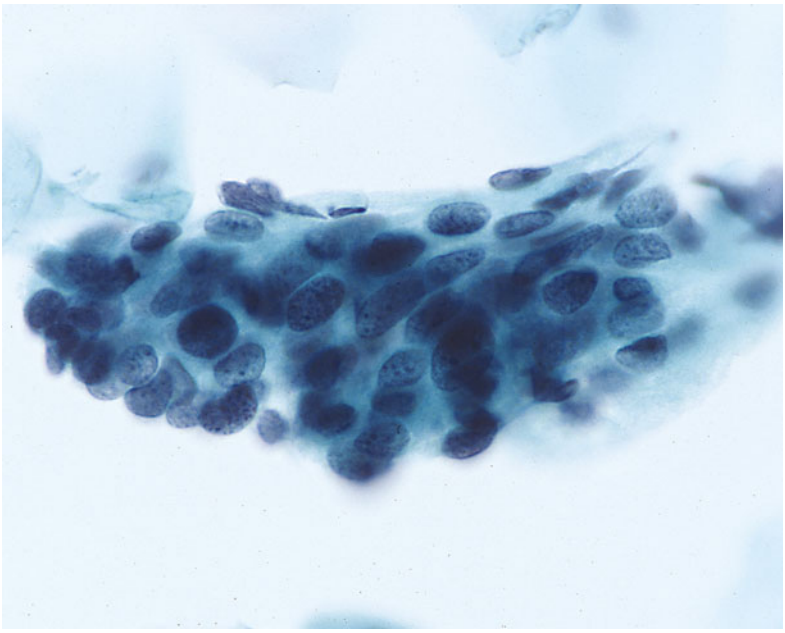

**Fig. 5.34** HSIL (*LBP*, *SurePath*). A 44-year-old woman. Syncytial cluster of HSIL cells with features of endocervical gland extension. Such "hyperchromatic crowded groups" may raise a wide differential diagnosis under low magnification; attention to architectural pattern and cellular detail are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

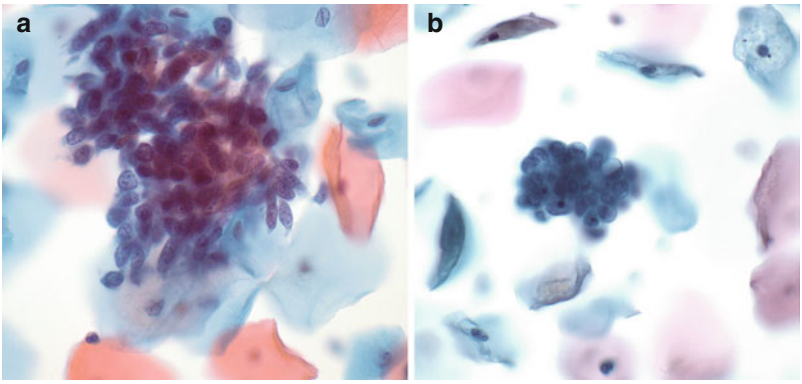


**Fig. 5.35** HSIL (**a** and **b** *LBP*, *SurePath*). This rare example of HSIL (**a**) shows a loosely aggregated group of dysplastic cells having a spindled appearance reminiscent of endometrial stromal cells. The cells at the margins of the group show tapered cytoplasmic ends. The nuclei show atypical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

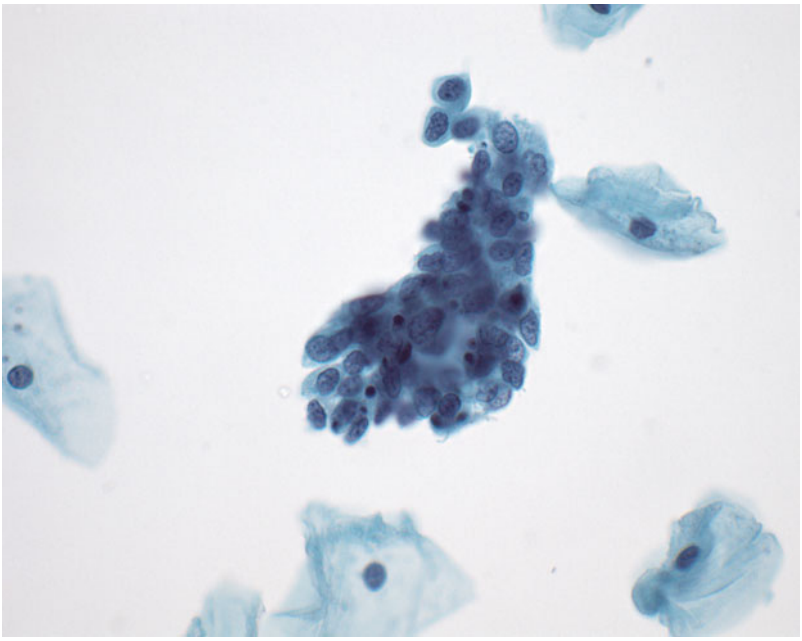

**Fig. 5.36** HSIL (*LBP*, *SurePath*). HSIL can present in three-dimensional groups that closely mimic shed endometrial cells. In this example, the nuclei are smaller that might be expected for the typical HSIL; however, they do show atypical chromatin and irregular contours. Apoptotic debris is present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

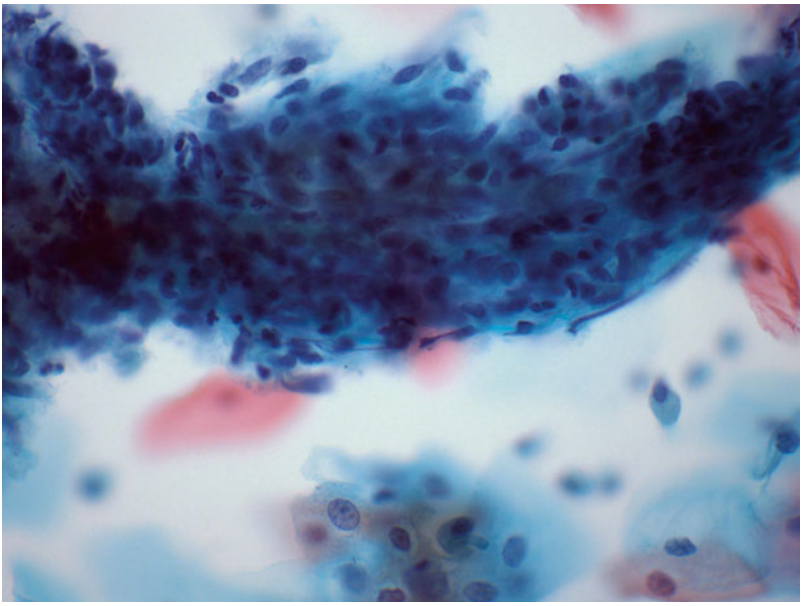

**Fig. 5.37** HSIL (*LBP*, *SurePath*). In some cases of HSIL, more voluminous amounts of cytoplasm with cytoplasmic appendage formation reminiscent of repair can be present. Note also the presence of intermixed inflammatory cells within the group, another feature that overlaps with reparative changes.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

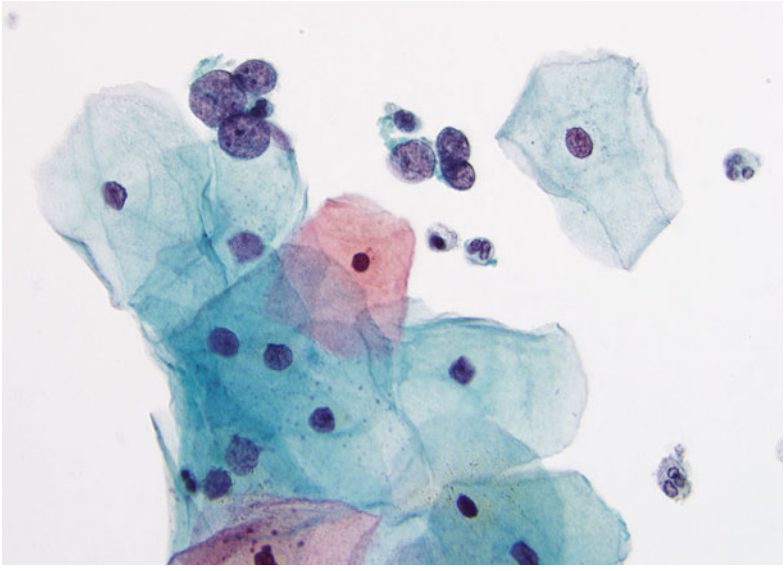

**Fig. 5.38** HSIL (*LBP*, *ThinPrep*). Abnormal, large stripped nuclei are seen that are considerably bigger than the intermediate cell nuclei. Such cells should elicit a search for classic, intact HSIL cells elsewhere on the same preparation. These stripped nuclei should be distinguished from endometrial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

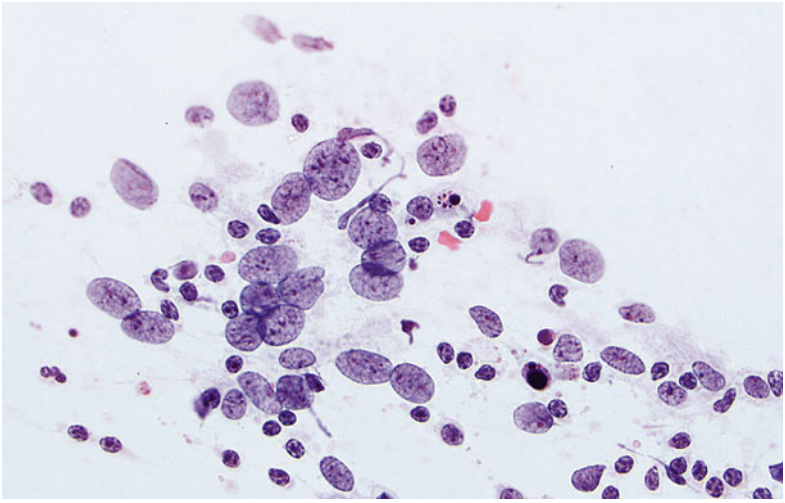

**Fig. 5.39** HSIL-stripped nucleus pattern (*CP*). A 38-year-old woman with a history of LSIL. These abnormal stripped nuclei are often a useful diagnostic clue that other abnormal cells may be identified on the same slide. They should be distinguished from the bare intermediate cell nuclei seen in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

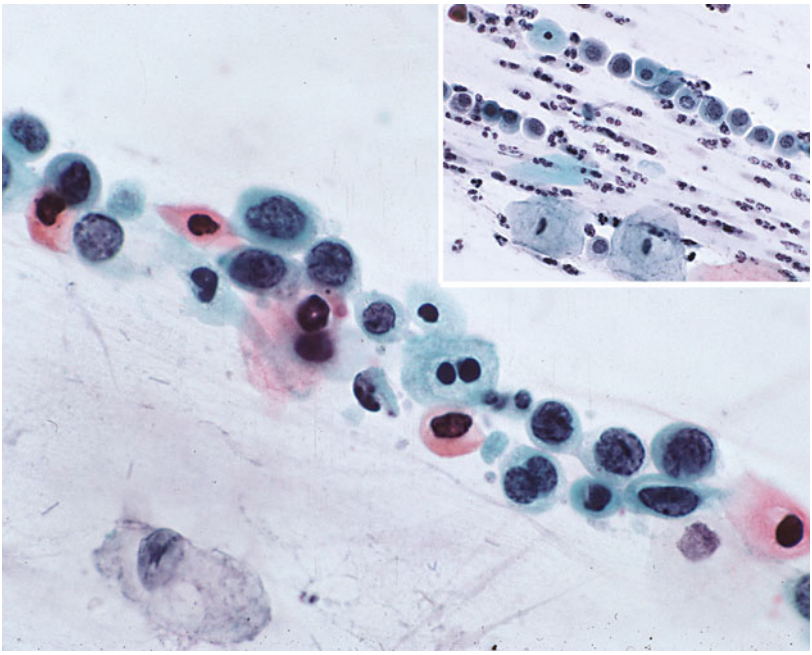

**Fig. 5.40** HSIL (*CP*). At low magnification (*right upper inset*), the pattern of HSIL cells streaming within mucus can mimic histiocytes and endocervical/metaplastic cells. At high power, HSIL can be readily distinguished (see also Figs. 5.35, 4.33, and 4.34)

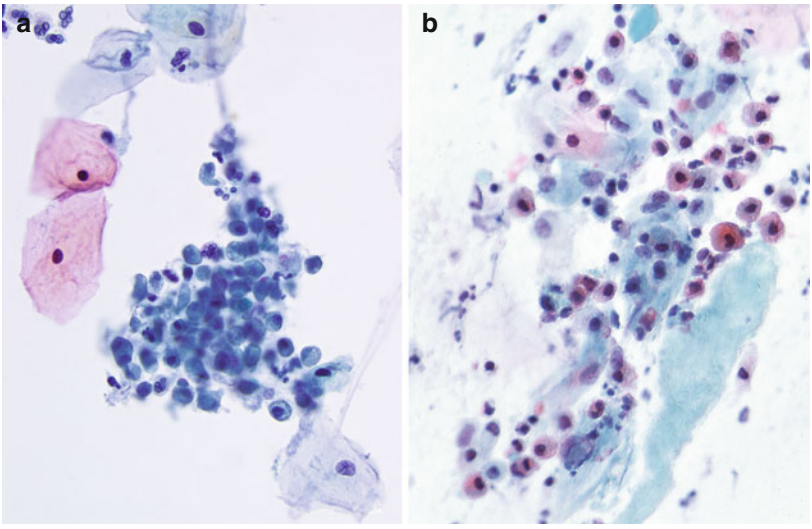


**Fig. 5.41** NILM; endocervical microglandular hyperplasia (**a** *LBP*, *ThinPrep*, **b** *CP*). A 34-year-old woman on day 19 of menstrual cycle. Degenerated endocervical cells, seen in a streaming pattern along with thick mucus, is a pattern that has been associated with microglandular hyperplasia (**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

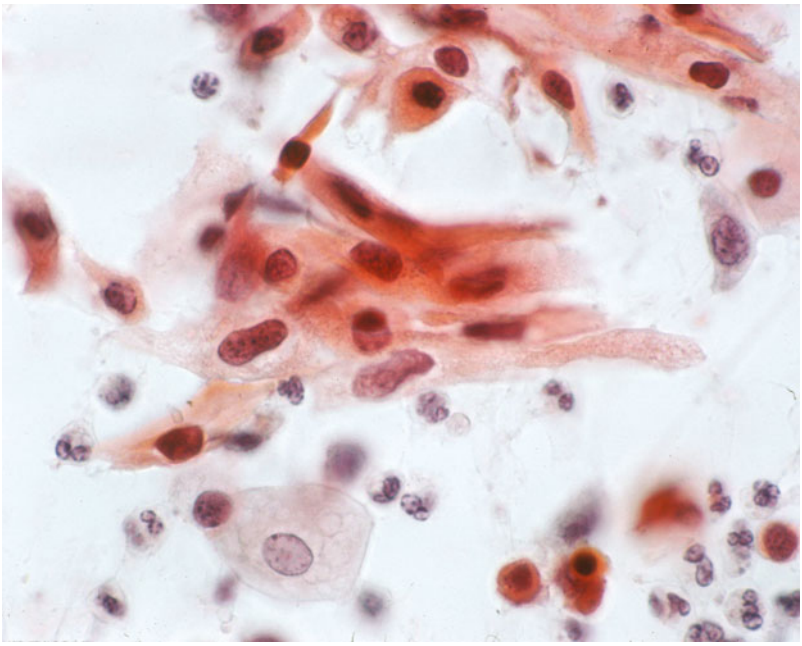

**Fig. 5.42** HSIL (*CP*). Classification of atypical keratinized cells depends on the degree of nuclear abnormality, the N/C ratio, and to some extent on the pleomorphism of the abnormal cells. This image shows a range of cells from the LSIL cells seen in the center to the HSIL cells seen around the 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)

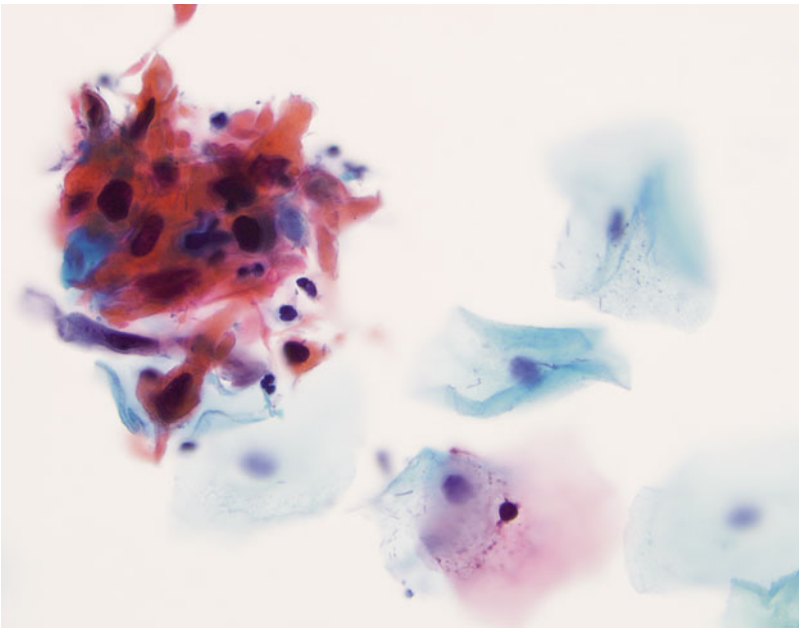

**Fig. 5.43** HSIL (*LBP*, *ThinPrep*). These cells demonstrate marked pleomorphism of the nuclei and keratinized cytoplasm. The marked variation in shape and the presence of cells with a high N/C ratio is consistent with an interpretation of HSIL

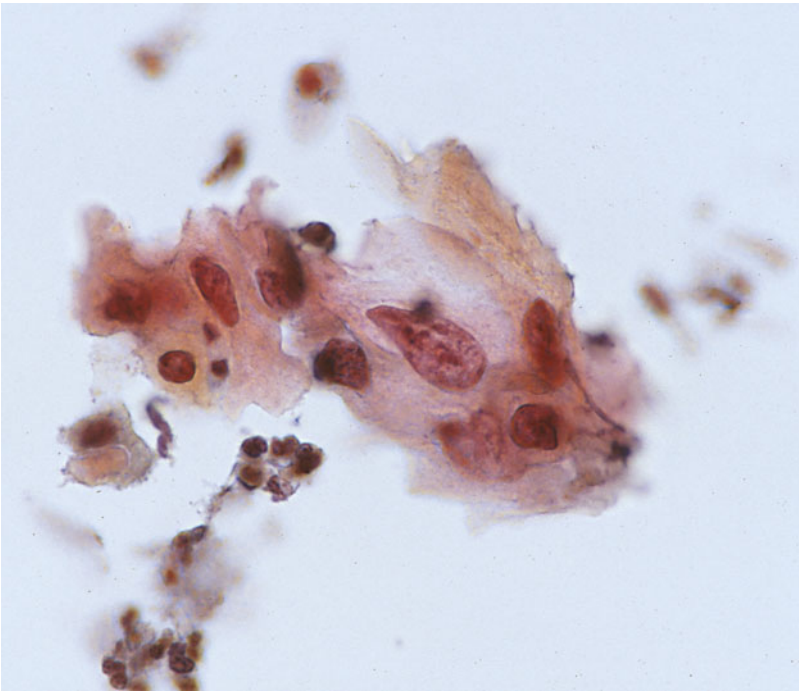

**Fig. 5.44** HSIL (*LBP*, *ThinPrep*). A 42-year-old woman. Keratinized dysplastic cells with nucleoli and angulated or "carrot"-shaped nuclei that may raise suspicion for invasion and qualify for an interpretation of HSIL cannot rule out invasion. Follow-up showed only HSIL (CIN 3) that was keratinizing

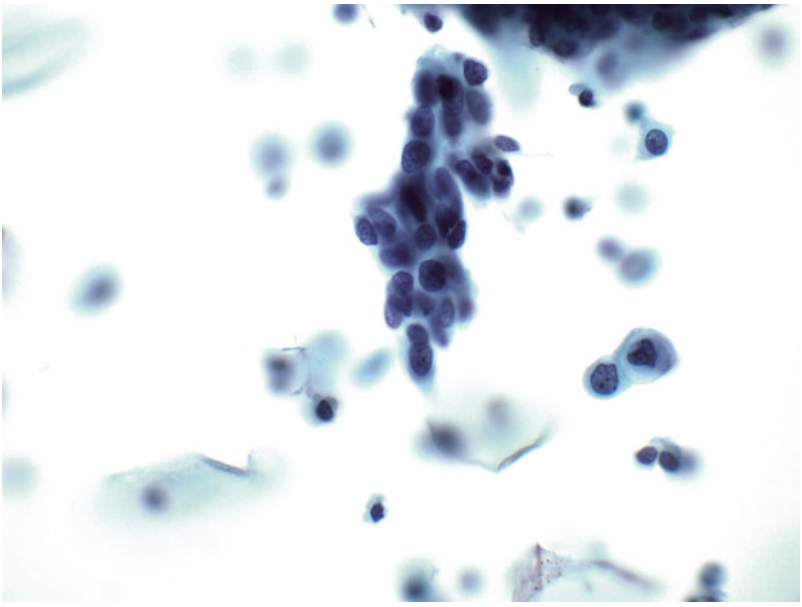

**Fig. 5.45** HSIL (*LBP*, *SurePath*). HSIL in atrophy may be difficult to distinguish from clusters of benign atrophic squamous cells. In HSIL, as seen here, the cells show a syncytial arrangement, and looking at these clusters by focusing in different planes allows one to better distinguish them from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

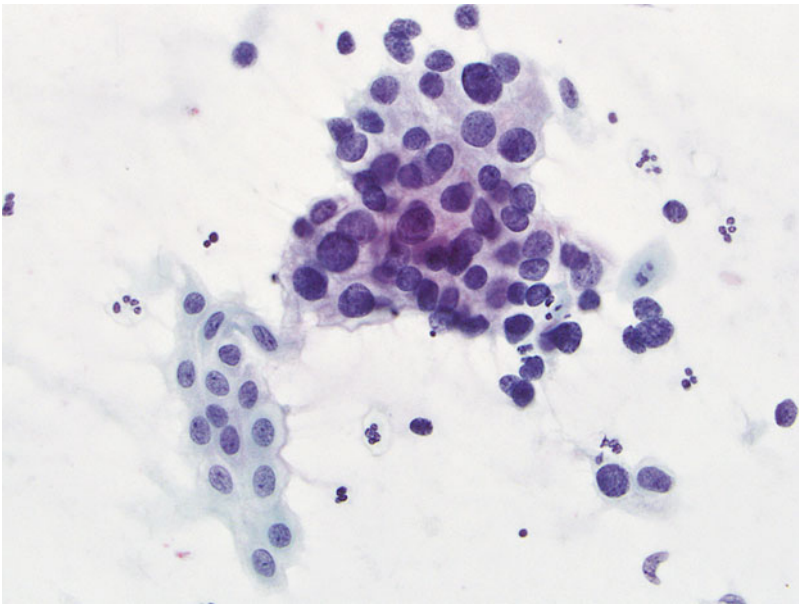

**Fig. 5.46** HSIL (*CP*). Clusters of parabasal cells are commonly identified in the background of HSIL in atrophy. The HSIL illustrated here shows a sheet-like arrangement, a pattern commonly seen in HSIL, with significant nuclear size variation and a loss of polarity with overlapping of the nuclei.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

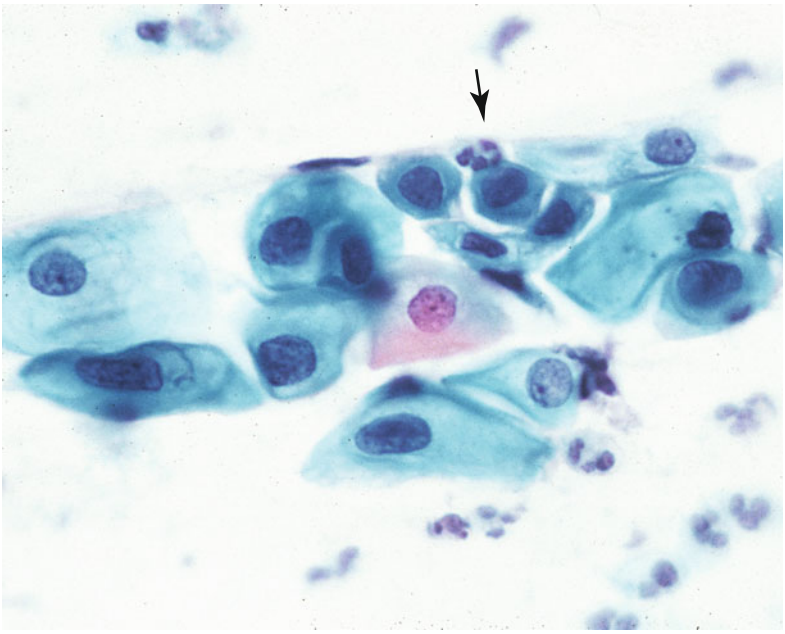

**Fig. 5.47** LSIL with some cells suggesting the possibility of a concurrent HSIL (*CP*). Routine screen from a 28-year-old woman. Most of these cells qualify as LSIL; however, there are three atypical metaplastic cells at the top center (*arrow*) that raise the possibility of a high-grade lesion. Cases 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)

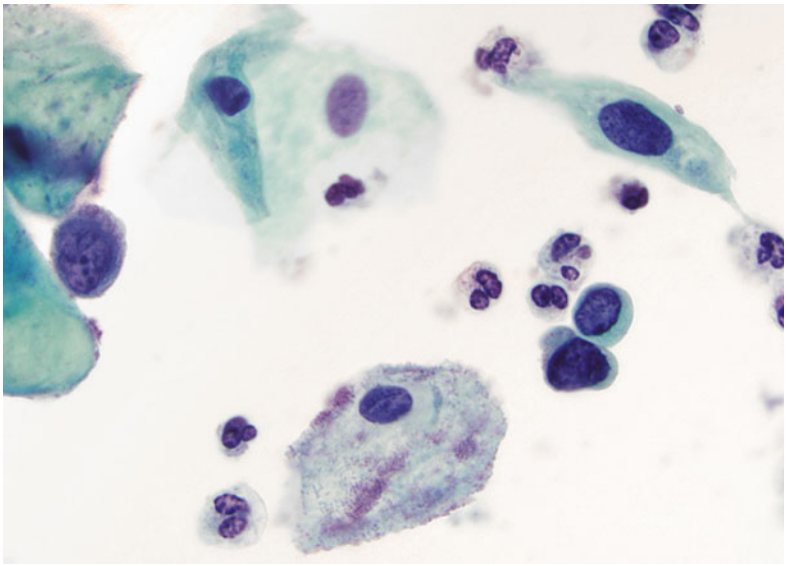

**Fig. 5.48** HSIL (*LBP*, *ThinPrep*). In this case, diagnostic HSIL cells are present. Even if these cells are seen in the background of a majority of LSIL elsewhere on the slide, the final interpretation should be HSIL

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

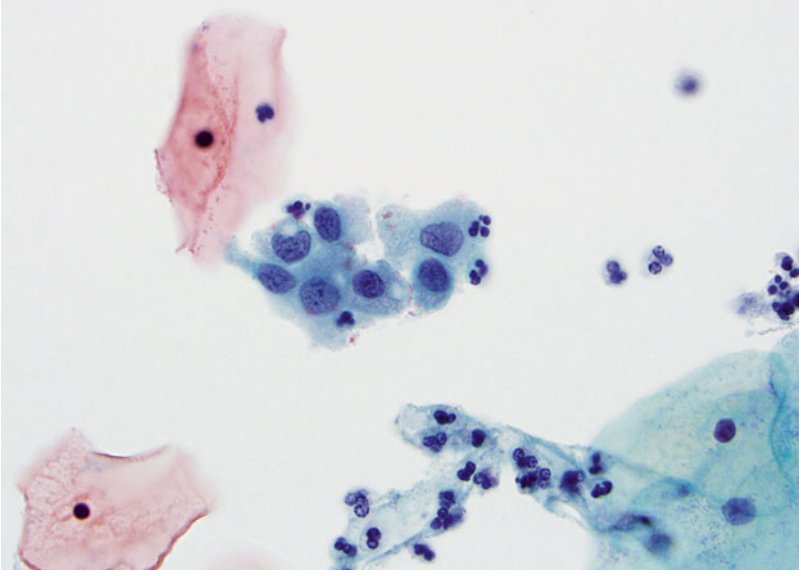

**Fig. 5.49** Immature squamous metaplasia (*LBP*, *ThinPrep*). Immature metaplastic cells can mimic dysplastic cells. Degenerative and reactive changes in these small squamous cells can be confused with dysplasia or carcinoma. Cytologic features that support a benign interpretation include nuclear uniformity,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

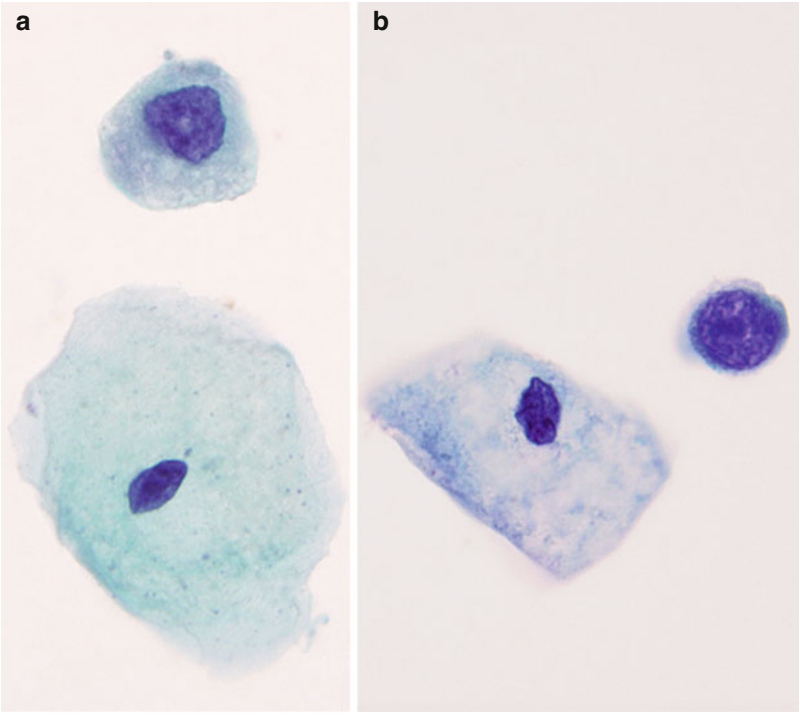


**Fig. 5.50** HSIL versus benign endocervical cells (*LBP*, *ThinPrep*). Single cells are randomly distributed in liquid-based preparations. Single benign endocervical cells are prone to cytoplasmic lysis and (**b**) may mimic single cells of HSIL. The common cellular features of HSIL (**a**), such as 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

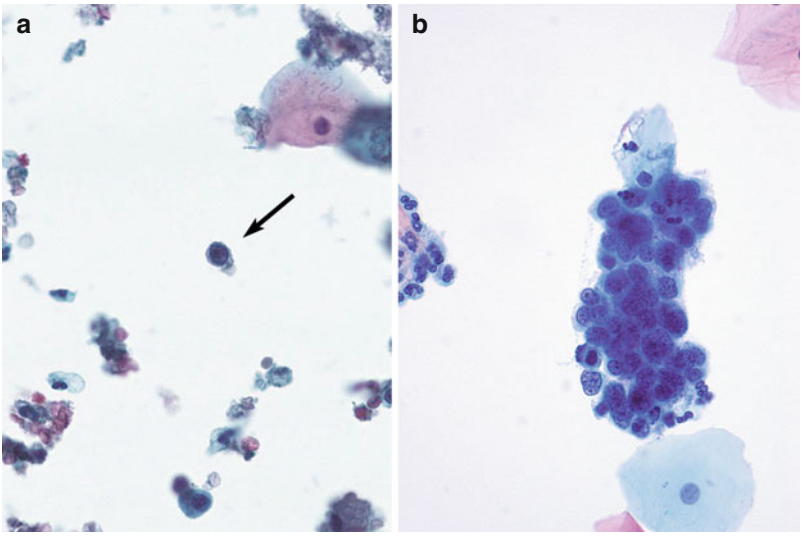


**Fig. 5.51** NILM, endometrial cells (*LBP*, *ThinPrep*). Single endometrial cells (**a** *arrow*) may be mistaken for HSIL. The small round nucleus with smooth nuclear membranes helps to classify this as benign. Comparison to more classic clusters of endometrial cells from the same slide (**b**) is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

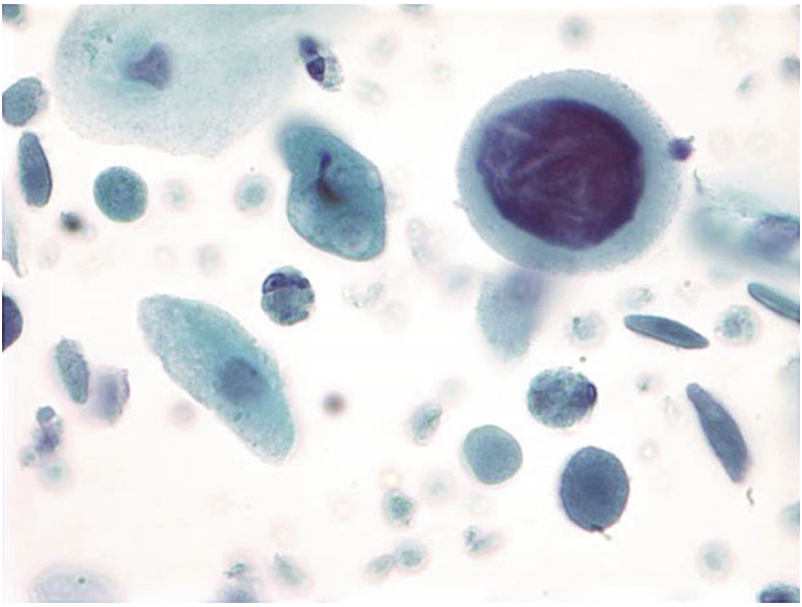

**Fig. 5.52** ASC-US (*LBP*, *SurePath*). Large bizarre cells may be seen in atrophic preparations. Because of the increased N/C ratio, these cells raise the possibility of HSIL, but the degenerative nuclear features and background atrophy make a benign process more likely. An interpretation of ASC-US 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).

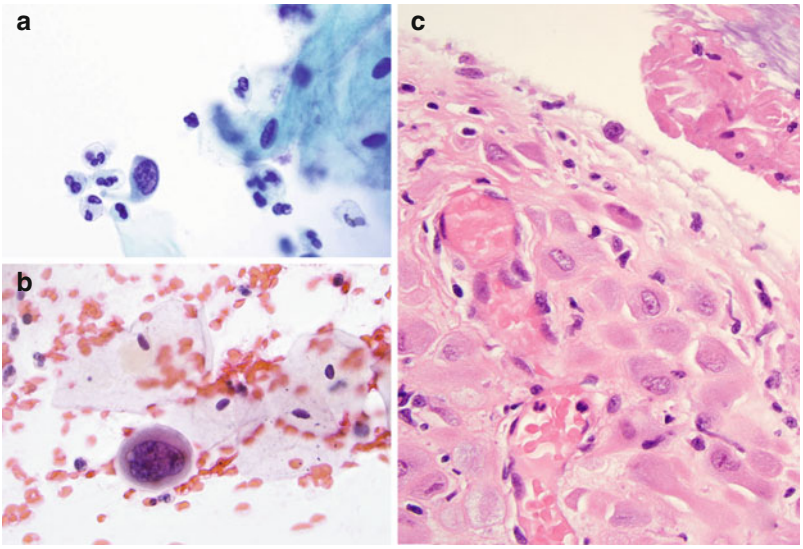


**Fig. 5.53** NILM (**a**, **b** *LBP*, *ThinPrep*, **c** cervix *H&E stain*). A young woman in the late second trimester of pregnancy. These single cells (**a**, **b**) with an increased N/C ratio and nuclear hyperchromasia are worrisome for HSIL. Features suggesting the true stromal decidual nature 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**)

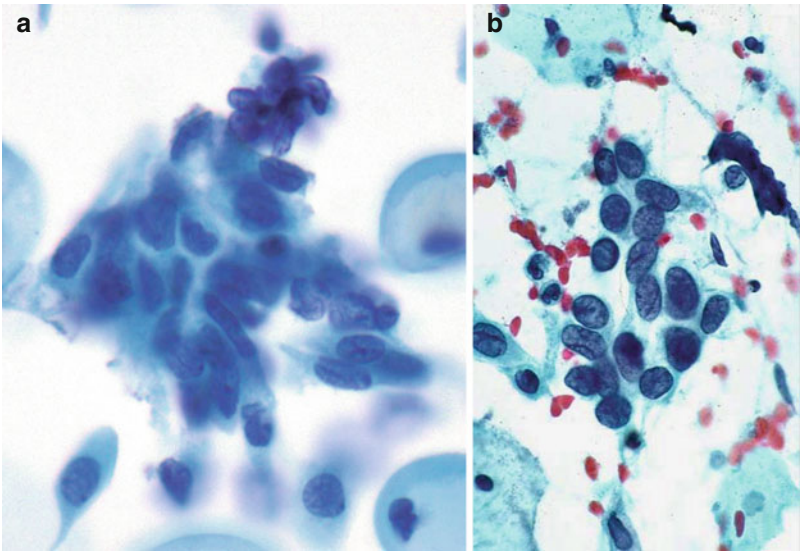


**Fig. 5.54** NILM (**a** *LBP*, *ThinPrep*) versus HSIL (**b** *CP*). Both dysplastic and benign squamous cells can demonstrate longitudinal nuclear grooves. The benign cells seen in (**a**) are derived from transitional cell metaplasia and show distinct nuclear grooving without any of the other dysplastic 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).

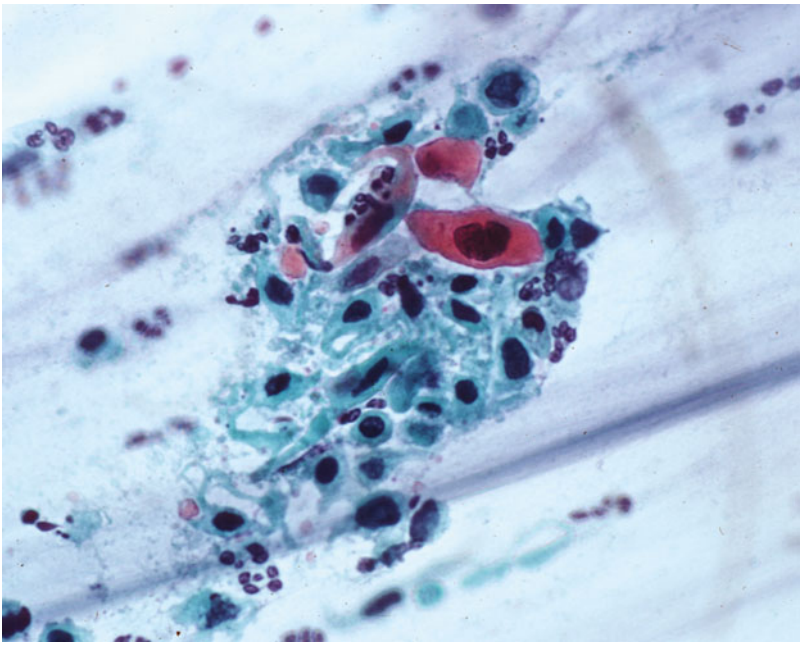

**Fig. 5.55** HSIL with features suspicious for invasion (*CP*). A 71-year-old postmenopausal woman. HSIL filling endocervical glands may undergo focal necrosis that can mimic the tumor diathesis associated with invasive lesions. Follow-up showed HSIL (CIN 3) extending into glands with focal epithelial 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.

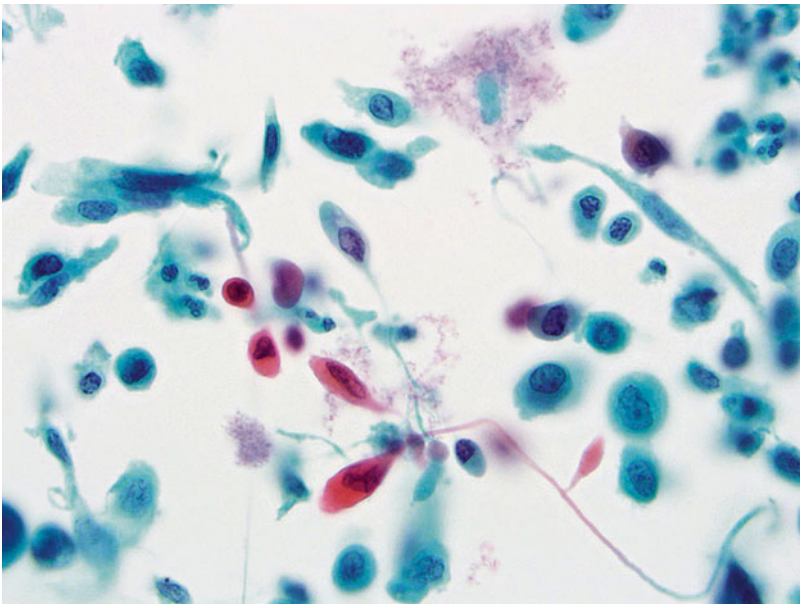

**Fig. 5.56** Squamous cell carcinoma, keratinizing (*LBP*, *SurePath*). The malignant cells have variable shapes and sizes and show some keratinized "tadpole cells." Nuclei vary from vesicular with irregular nuclear contours and nucleoli to pyknotic in the keratinized cells. The cytoplasm is dense and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

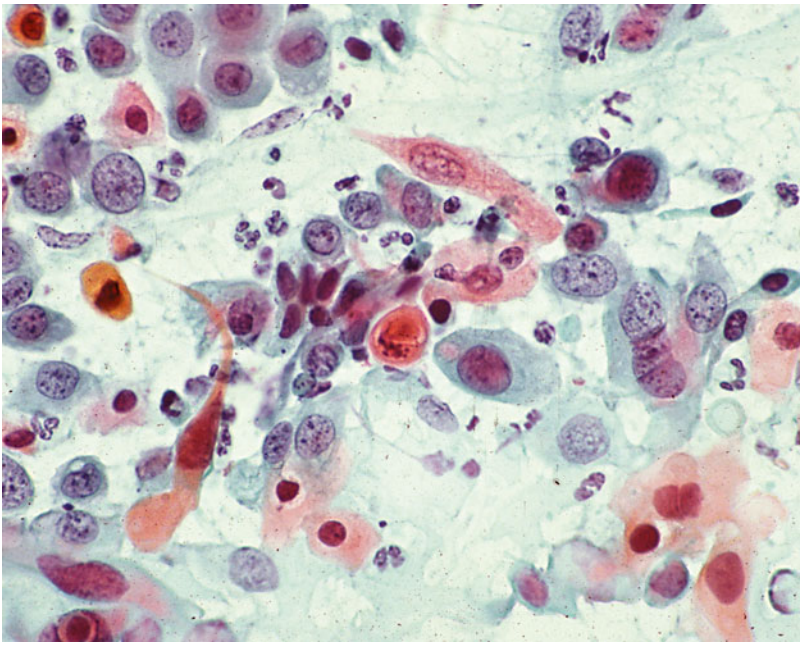

**Fig. 5.57** Squamous cell carcinoma, keratinizing (*CP*). There is marked pleomorphism of cell size and shape, cytoplasmic keratinization, and tumor diathesis in the background

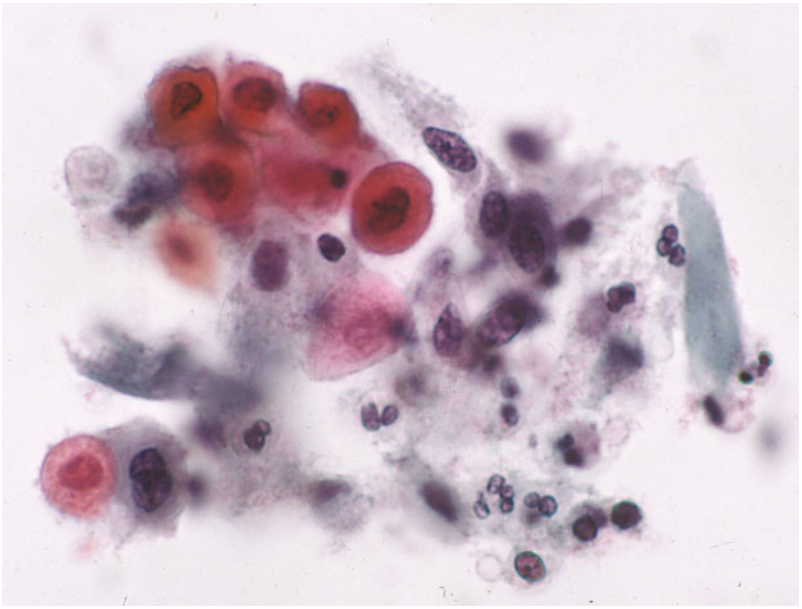

**Fig. 5.58** Squamous cell carcinoma, keratinizing (*LBP*, *ThinPrep*). A 68-year-old woman. Diathesis may be more subtle in *LBPs* and often tends to collect at the periphery of cell groups, a pattern that has been referred to as "clinging diathesis." Follow-up showed squamous cell carcinoma

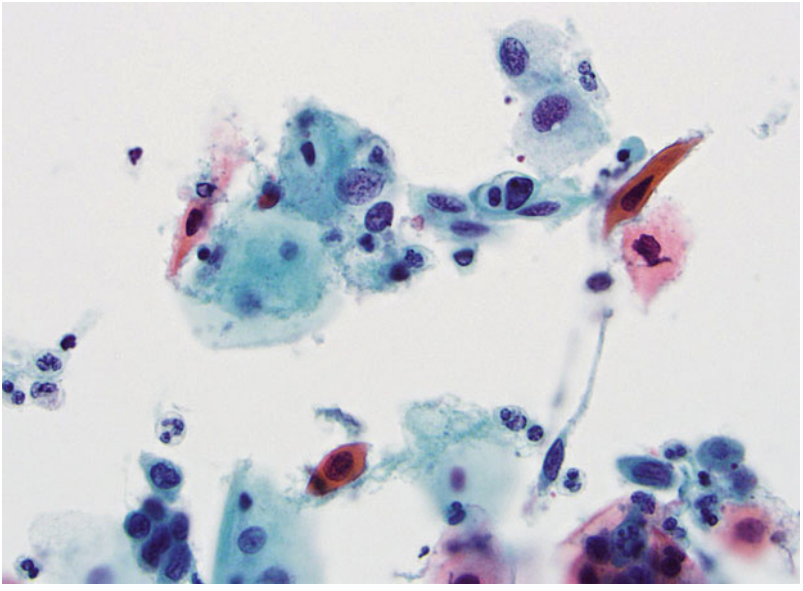

**Fig. 5.59** Squamous cell carcinoma, keratinizing (*LBP*, *ThinPrep*). A 57-year-old woman. Note the tumor diathesis, abnormal keratinized cells, and spindle cells. Follow-up biopsy revealed invasive squamous cell carcinoma

### 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).

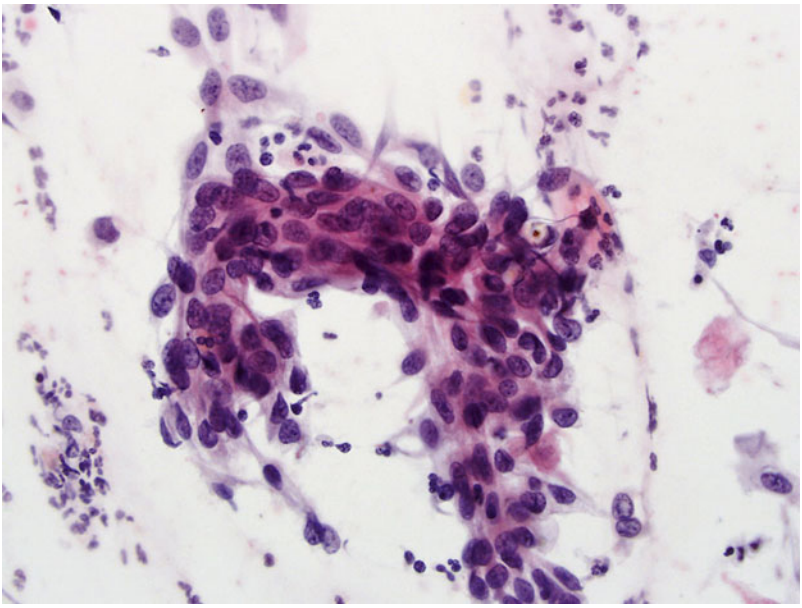

**Fig. 5.60** Squamous cell carcinoma, nonkeratinizing (*CP*). These dysplastic cells demonstrate nuclear features of HSIL. Pleomorphic cell shapes should raise concern for invasion even though prominent nucleoli and tumor diathesis are absent in this field. Follow-up cervical biopsy revealed an invasive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

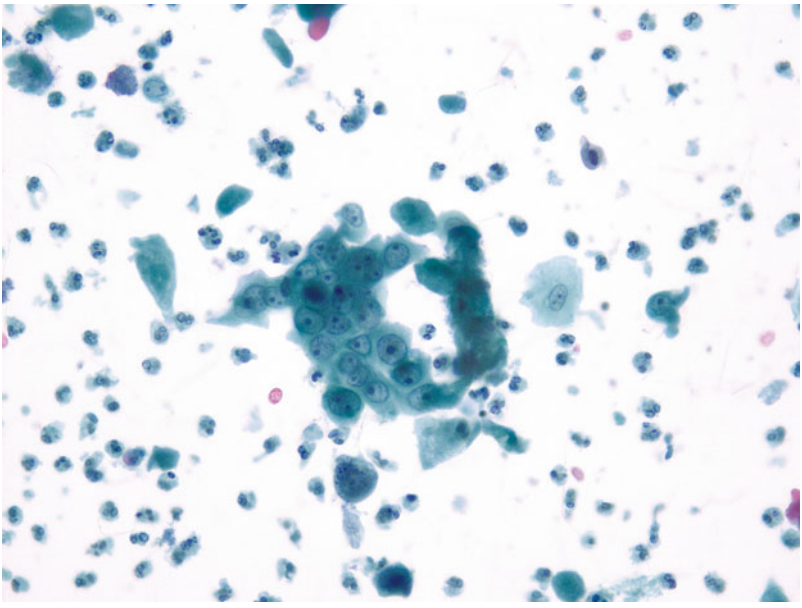

**Fig. 5.61** Squamous cell carcinoma, nonkeratinizing (*LBP*, *SurePath*). A 59-year-old woman with postmenopausal bleeding. Abnormal nuclei are present with prominent nucleoli and irregular chromatin distribution. Single abnormal cells are also seen. There is a tumor diathesis present in the background.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

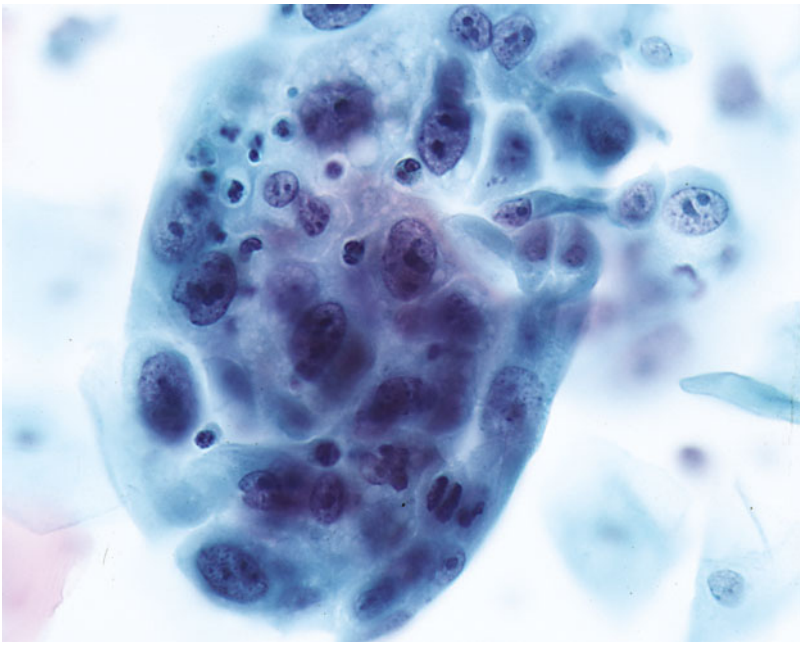

**Fig. 5.62** Squamous cell carcinoma (*LBP*, *SurePath*). Malignant cell clusters tend to show more rounding on *LBPs*, and distinction between a squamous and glandular lesion may be difficult. Attention should be given to looking for isolated neoplastic cells in the background

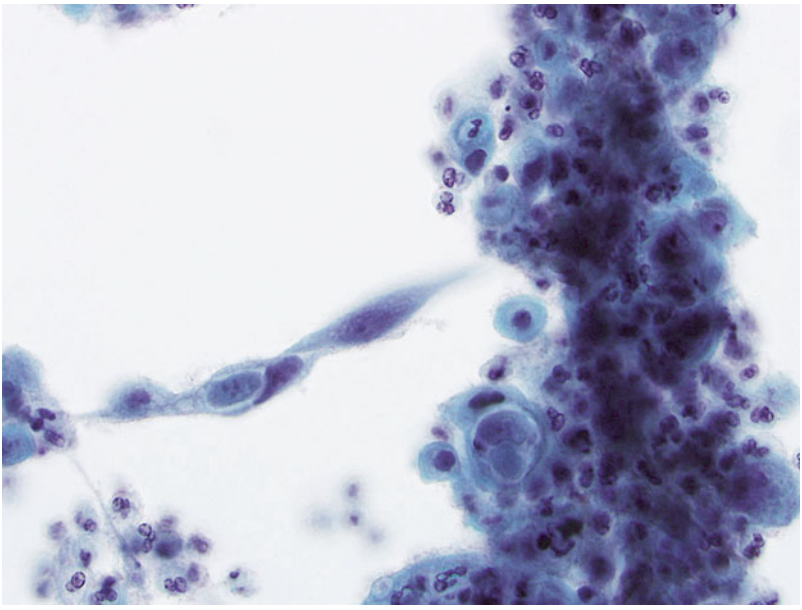

**Fig. 5.63** Squamous cell carcinoma, nonkeratinizing (*LBP*, *ThinPrep*). A 63-year-old woman with postmenopausal bleeding. Clusters of cells and single abnormal cells are identified with a background of inflammatory cells. Follow-up revealed a nonkeratinizing squamous cell carcinoma of the uterine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

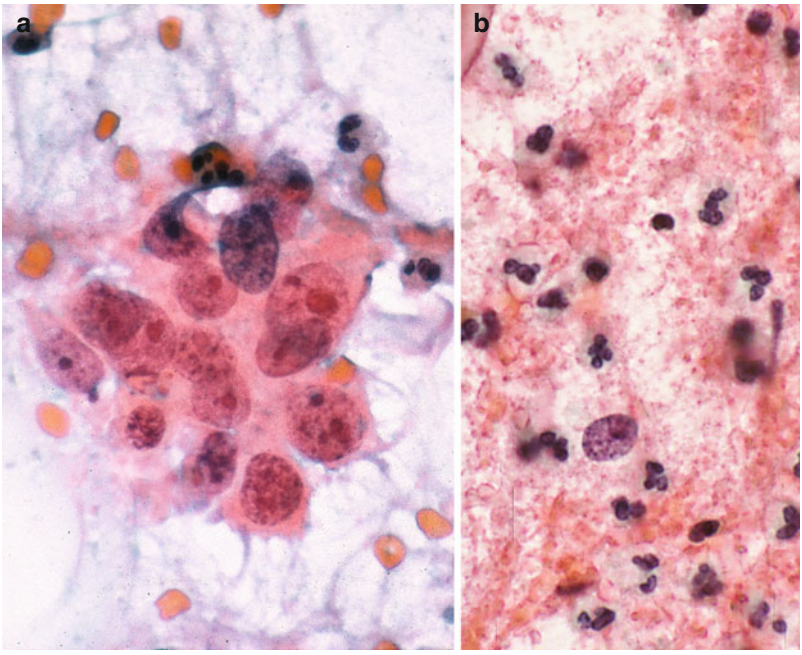


**Fig. 5.64** Squamous cell carcinoma (*CP*). There is tumor diathesis in the background and prominent nucleoli in the malignant cells (*left*). On the right, from a different case, tumor diathesis is prominent, and only a naked nucleus is seen in this field (*right*)

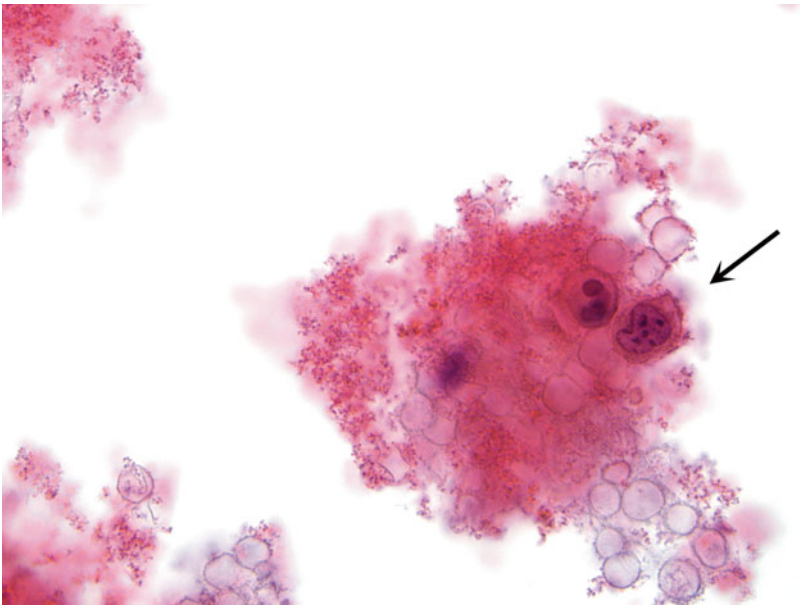

**Fig. 5.65** Squamous cell carcinoma (*LBP*, *ThinPrep*). Bloody samples are often seen in the presence of squamous carcinoma. Because the blood may clog the filter of the *ThinPrep*, these samples may be very scantly cellular and technically unsatisfactory. Bloody unsatisfactory specimens should still 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

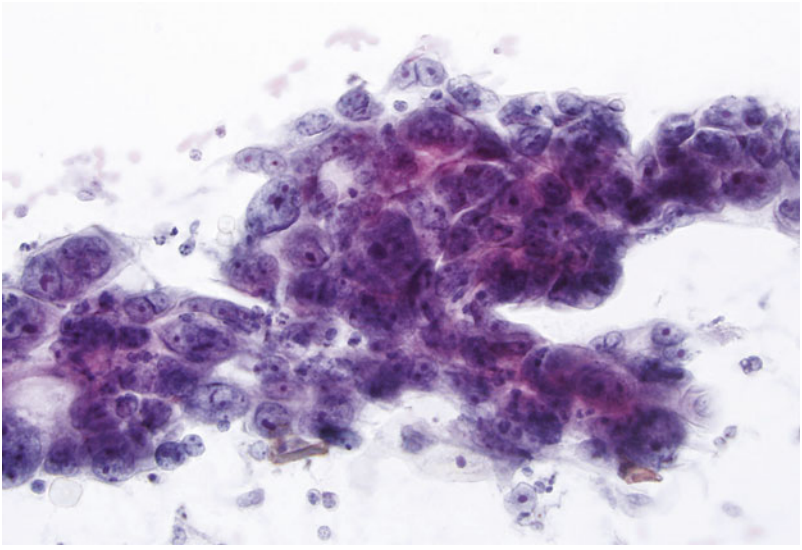

**Fig. 5.66** Atypical repair (*CP*). A 48-year-old woman with a normal screening history. Prominent nucleoli are identified in virtually every nucleus. The cells are cohesive and lack irregular chromatin distribution. Atypical repair is a differential in the diagnosis of carcinoma of the cervix

(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

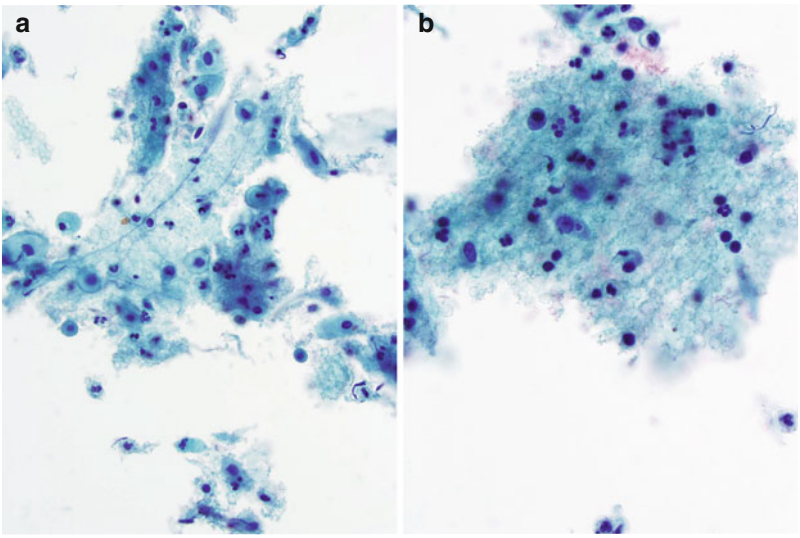


**Fig. 5.67** Diathesis look-alike (*LBP*, *ThinPrep*). (**a**) *Left*, a 66-year-old postmenopausal woman. Routine cervical cytology. (**b**) *Right*, a 39-year-old woman on day 12 of menstrual cycle. The background of atrophy (*left*) and inflammatory debris (*right*) can mimic tumor diathesis. Lack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

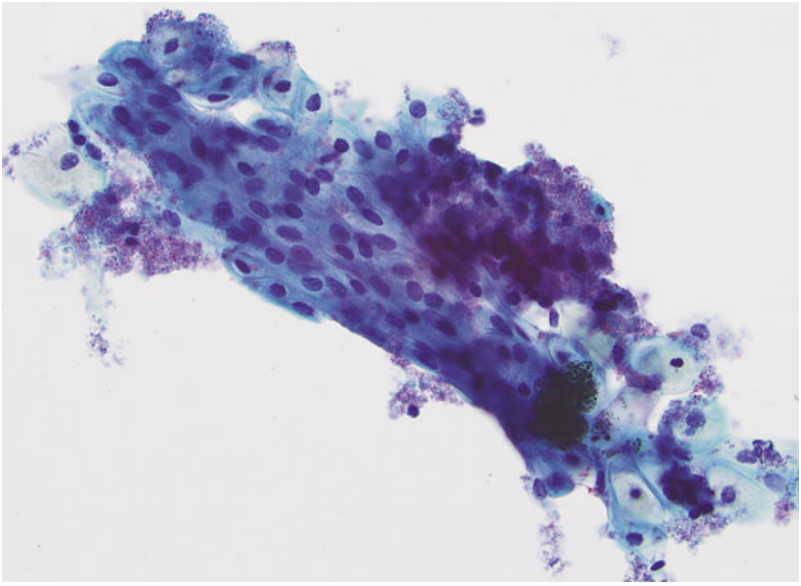

**Fig. 5.68** Diathesis look-alike (*LBP*, *ThinPrep*). A 63-year-old postmenopausal woman. Lubricant may be used in Pap test collection and presents as granular debris that may mimic tumor diathesis

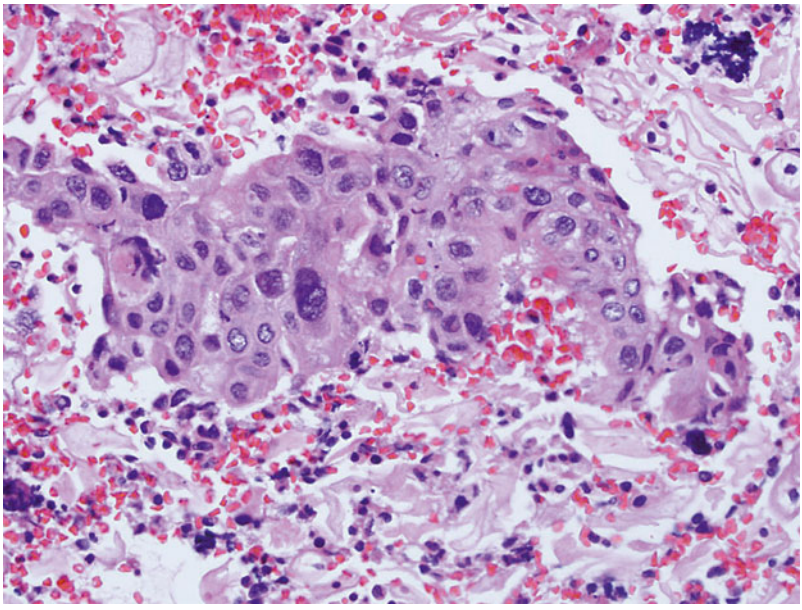

**Fig. 5.69** Squamous cell carcinoma, cell block (*cell block*, *H&E stain*). A 57-year-old postmenopausal woman with irregular bleeding. A cell block preparation was made from the residual *ThinPrep* vial. An abnormal cluster of cells with dense pink cytoplasm and abnormal nuclei is seen. Follow-up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

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# Epithelial Abnormalities: Glandular

# 6

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## 6.1 Epithelial Cell Abnormalities

**Glandular Cell**

- Atypical
  - Endocervical cells (NOS or specify in comments)
  - Endometrial cells (NOS or specify in comments)
  - Glandular cells (NOS or specify in comments)
- Atypical
  - Endocervical cells, favor neoplastic
  - Glandular cells, favor neoplastic

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- Endocervical adenocarcinoma in situ (AIS)
- Adenocarcinoma
  - Endocervical
  - Endometrial
  - Extrauterine
  - Not otherwise specified (NOS)

## 6.2 Background

Continued advancement of the understanding of cervical glandular carcinogenesis and refinement of the cytomorphologic criteria has led to greater sensitivity and precision in interpretation of these lesions. Improved communication among laboratories and clinicians has ensued, thereby facilitating the appropriate management of patients [1]. As is well known, cervical cytology is primarily a screening test for squamous intraepithelial lesions and squamous cell carcinoma; the relative sensitivity of the test for the detection of glandular lesions can be limited by issues related to both sampling and interpretation [2].

Endocervical adenocarcinoma in situ is considered to be the glandular counterpart of high-grade squamous intraepithelial lesion (HSIL) and the precursor to invasive endocervical adenocarcinoma. Similar human papillomavirus (HPV) types have been demonstrated in most, but not all, invasive endocervical adenocarcinomas and adenocarcinomas in situ (AIS) [3, 4]. The proportion of adenocarcinomas associated with HPV 18 is larger than for squamous cell carcinoma. Using well-defined criteria, the cytologic interpretation of AIS correlates well with histologic outcome. However, a low-grade endocervical glandular entity analogous to low-grade squamous intraepithelial lesion (LSIL) has not been well established either histologically or cytologically. A significantly lower rate of detection of high-risk HPV in so-called histologic "glandular dysplasia" suggests that most may be unrelated to cervical carcinogenesis, representing reactive mimics in a significant percentage of cases. Therefore, terms such as "endocervical glandular dysplasia" or "low-grade glandular intraepithelial lesion" are not included in the Bethesda terminology [1]. The interpretation of "atypical—endocervical, endometrial, or glandular—cells" defines an increased level of risk, as opposed to a specific neoplastic precursor entity.

Additional highlights of this "atypical" category include the following:

- The term "atypical glandular cells *of undetermined significance*" is not utilized in order to avoid confusion with the terminology for squamous cell abnormalities (ASC-US).
- Atypical glandular cells should be categorized as to the favored site of origin (endocervical or endometrial) whenever possible, as the clinical workup and management for patients with glandular abnormalities may vary significantly depending upon the cell type; otherwise, the generic "atypical glandular cells" (AGC) terminology is used.

- "Atypical endocervical cells" and "atypical glandular cells" may be further qualified as "favor neoplastic." The qualifier "favor reactive" is considered to be potentially misleading and, therefore, is not included in the Bethesda terminology. If not further qualified, the designation "not otherwise specified" (NOS) may be used.
- "Atypical endometrial cells" are not further qualified as to "NOS" or "favor neoplasia," reflecting the difficulty in reliable further subclassification of this category.

## 6.3 Atypical Endocervical Cells

### 6.3.1 Definition

Endocervical-type cells that display nuclear atypia that exceeds obvious reactive or reparative changes but lack unequivocal features of endocervical adenocarcinoma in situ or invasive adenocarcinoma.

The interpretation of "atypical glandular cells" (AGC) should be qualified, if possible, to indicate whether the cells are thought to be of endocervical or endometrial origin. If the origin of the cells cannot be determined, the generic "glandular" term is used. Atypical endocervical cells should be further qualified when a particular entity, including neoplasia, is favored.

### 6.3.2 Atypical Endocervical Cells: NOS (Figs. 6.1–6.7)

#### 6.3.2.1 Criteria

Cells occur in sheets and strips with some cell crowding, nuclear overlap, and/or pseudostratification (Figs. 6.1, 6.2, and 6.4).

Nuclear enlargement, up to three to five times the area of normal endocervical nuclei (Fig. 6.4).

Some variation in nuclear size and shape (Fig. 6.3 and 6.5).

Mild nuclear hyperchromasia (Fig. 6.7).

Mild degrees of chromatin irregularity.

Occasional nucleoli (Fig. 6.6).

Mitotic figures are rare.

Cytoplasm may be fairly abundant, but the nuclear to cytoplasmic ratio is increased.

Distinct cell borders are often discernible.

**Preparation-Specific Criteria**

*Liquid-Based Preparations*:

Groups are more rounded and three-dimensional with piled-up layers of cells, making individual cells in the center difficult to visualize.

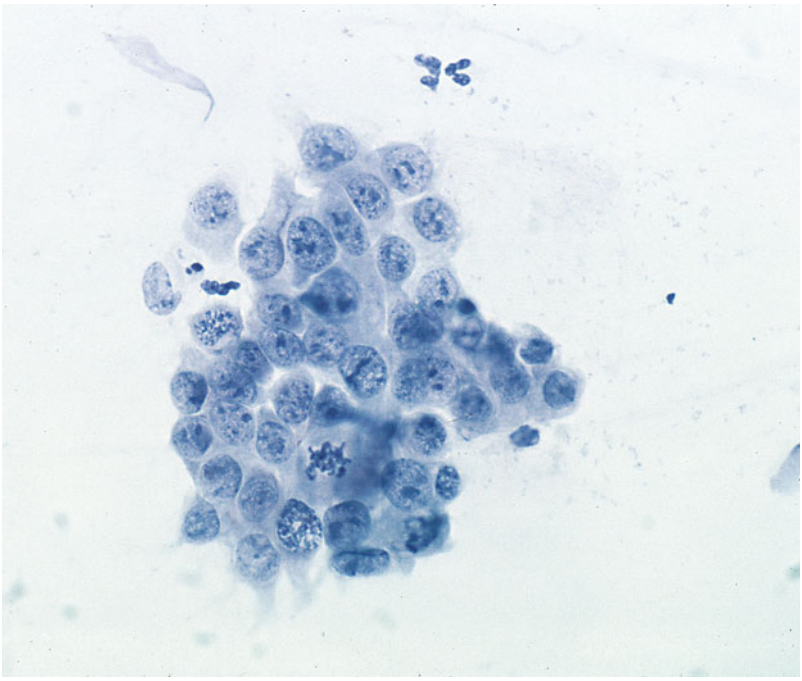

**Fig. 6.1** Atypical endocervical cells, most likely from a reparative process (*CP*). Routine screen from a 39-year-old woman. Sheet of cells that demonstrate nuclear enlargement, increased nuclear to cytoplasmic (N/C) ratios, prominent, sometimes multiple nucleoli, and mitotic activity. Three-year follow-up showed NILM cytology

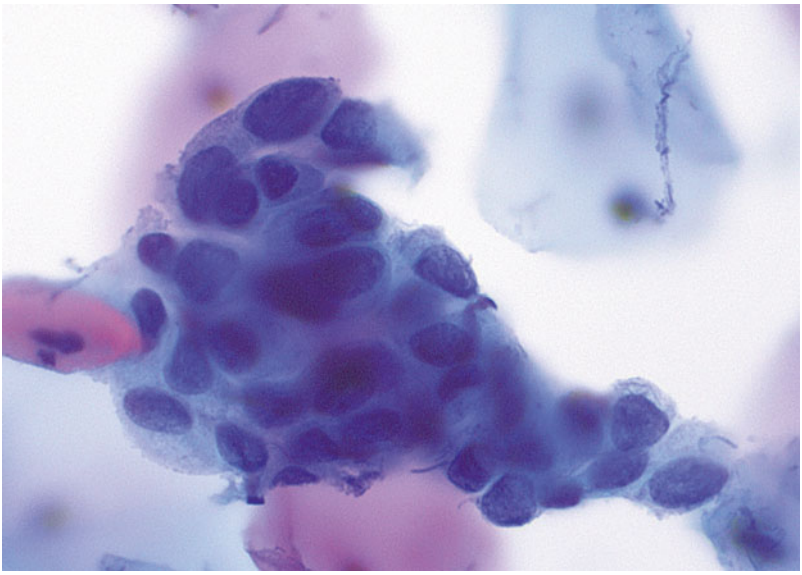

**Fig. 6.2** Atypical endocervical cells, not otherwise specified (NOS) (*LBP*, *ThinPrep*). Cluster of slightly crowded endocervical cells with some nuclear crowding and round to oval nuclei showing washed-out chromatin. Follow-up showed tubal metaplasia. The terminal bars and cilia were difficult to visualize in this case. The fine granularity of the chromatin pattern is an important feature of cases that are derived from tubal metaplasia

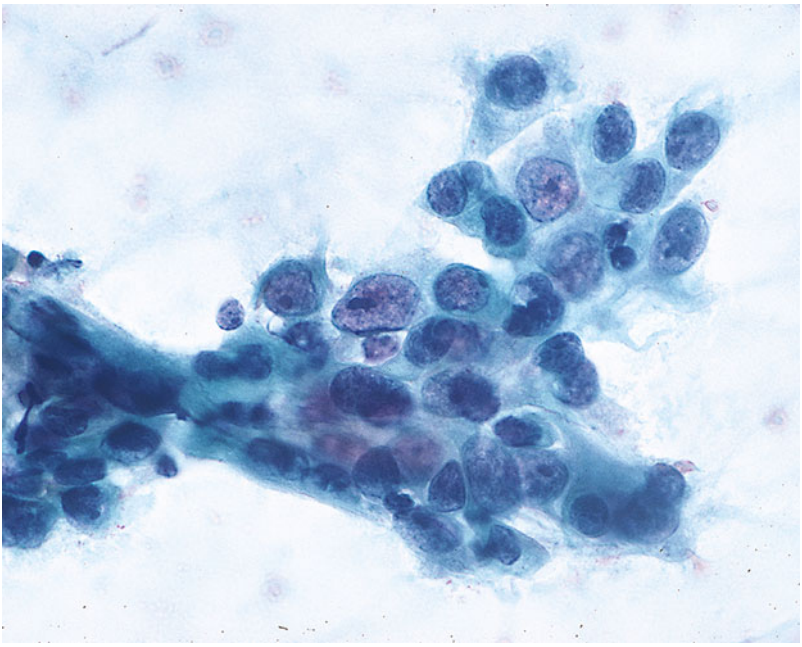

**Fig. 6.3** Atypical endocervical cells, most likely related to ionizing radiation therapy (*CP*). A 54-year-old woman, 4 months status post radiation therapy for cervical carcinoma. Sheet of glandular cells showing nuclear enlargement, marked variation in nuclear size, prominent nucleoli, and distinct cell borders. Follow-up showed NILM

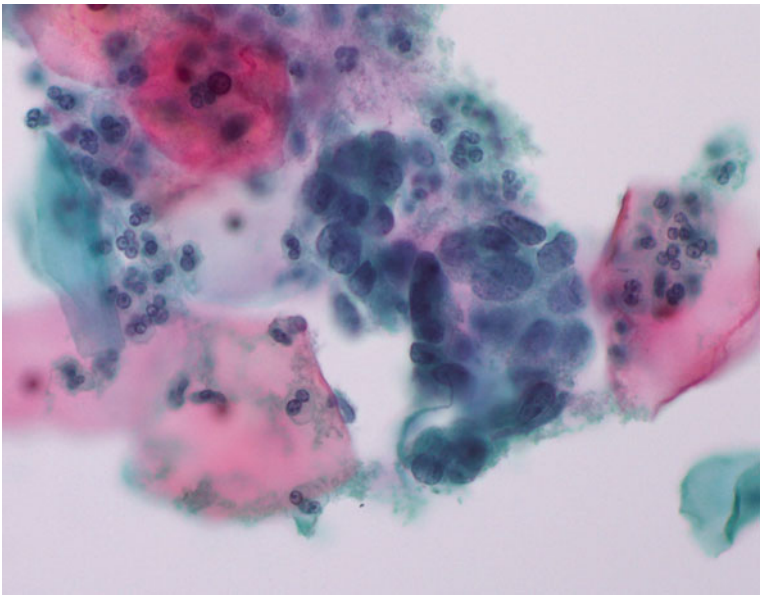

**Fig. 6.4** Atypical endocervical cells, NOS (*LBP*, *ThinPrep*). Cluster of crowded endocervical cells with nuclear enlargement, overlap and some nuclear irregularity. An ill-defined rosette is present at 11 o'clock. Follow-up showed AIS and HSIL. The cells in this image represent the three to four clusters of atypical endocervical cells present on the slide that correlate with AIS in the tissue biopsy. The rest of the slide showed cells diagnostic of HSIL which are not shown in this image

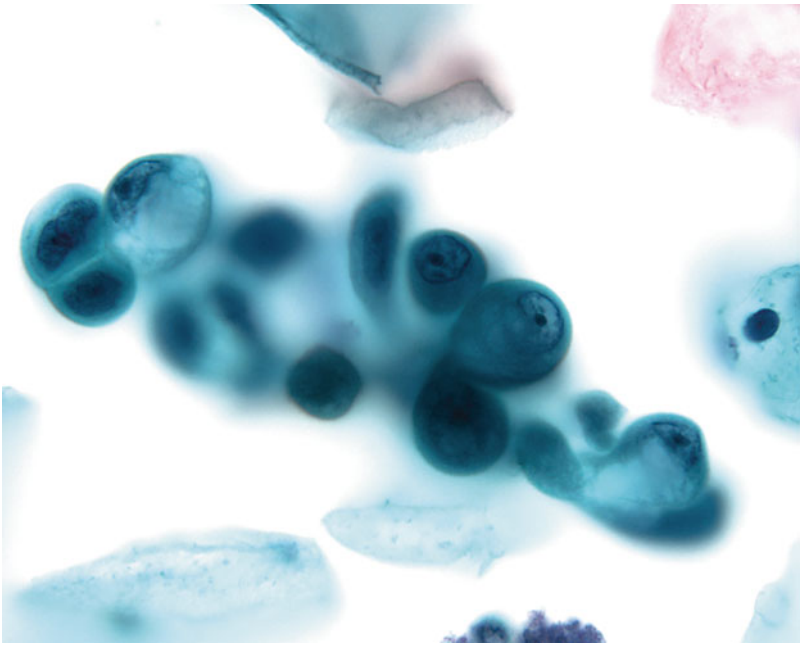

**Fig. 6.5** Reactive glandular cells associated with IUD (*LBP*, *SurePath*). A 45-year-old woman with an intrauterine device (IUD). The presumed endocervical cells demonstrate nuclear enlargement, nucleoli, and cytoplasmic vacuolization, consistent with changes associated with presence of an IUD. In the absence of a clinical history of IUD, such changes may be reported as atypical glandular cells, NOS

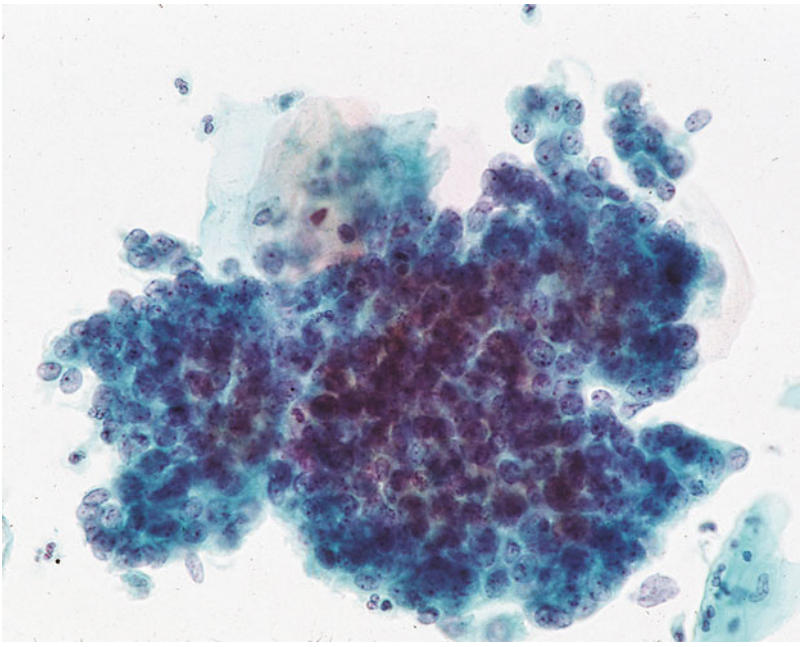

**Fig. 6.6** Atypical endocervical cells, NOS (*CP*). Cluster of cells with crowding and overlapping of nuclei, nuclear enlargement, chromocenters, and small nucleoli. Follow-up biopsies showed high-grade squamous intraepithelial lesion (HSIL) with extension into endocervical glands

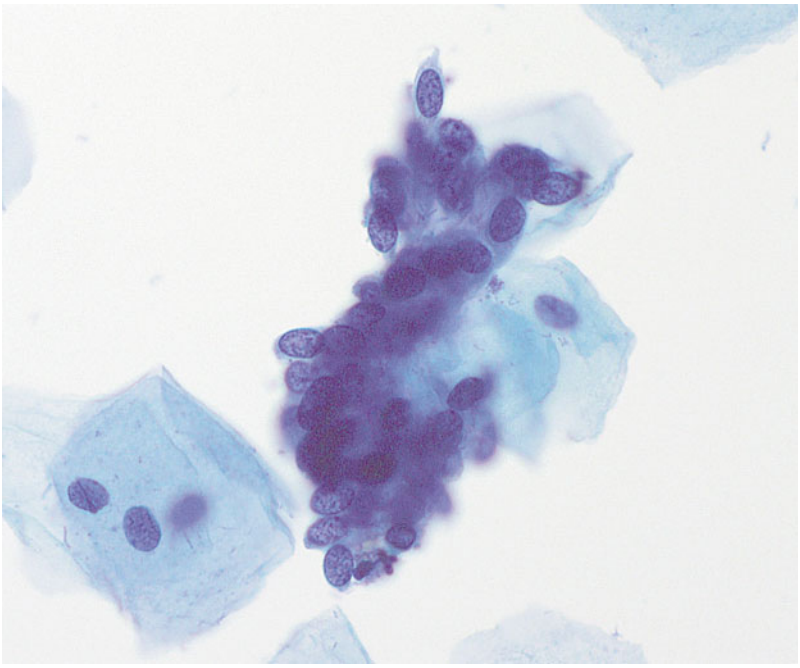

**Fig. 6.7** Atypical endocervical cells, NOS (*LBP*, *ThinPrep*). *ThinPrep* imager stained cluster of endocervical cells with dark nuclei and some focal feathering with minimal nuclear overlap which was initially interpreted as atypical endocervical cells, NOS. Follow-up was normal. In retrospect dark imager staining, mimicking hyperchromasia, resulted in the overinterpretation

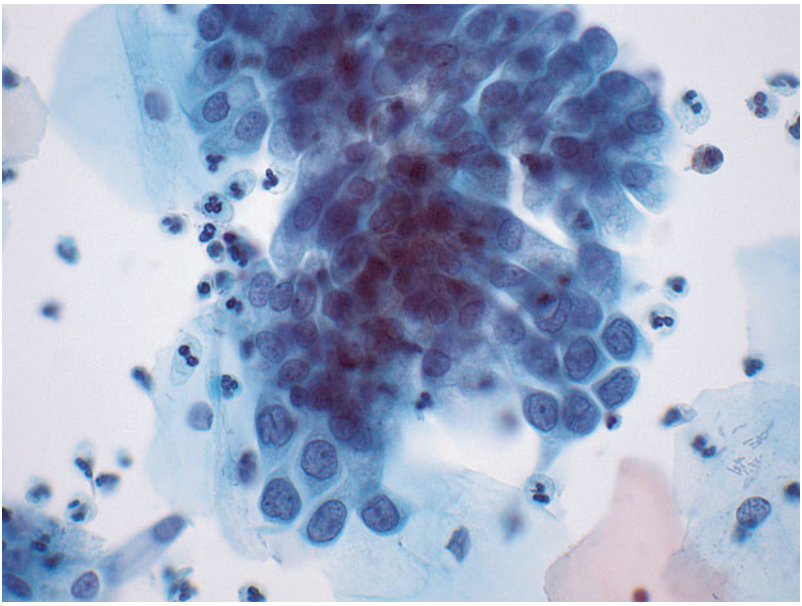

**Fig. 6.8** Normal endocervical cell "brush effect" (*LBP*, *SurePath*). Pictured is one of many such groups present on this slide, resulting from vigorous sampling with an endocervical "broom" device. The endocervical cells show uniform, evenly distributed, finely granular chromatin, and well-defined cytoplasmic boundaries consistent with a benign etiology

### 6.3.3 Atypical Endocervical Cells, Favor Neoplastic (Figs. 6.9–6.11)

#### 6.3.3.1 Definition

Cell morphology, either quantitatively or qualitatively, falls just short of an interpretation of endocervical adenocarcinoma in situ or invasive adenocarcinoma.

#### 6.3.3.2 Criteria

Abnormal cells occur in sheets and strips with nuclear crowding, overlap, and/or pseudostratification (Figs. 6.9 and 6.10).
Rare cell groups with rosettes (gland formations) or feathering (Fig. 6.11).
Nuclei are enlarged and often elongated with some hyperchromasia.
Coarse chromatin with heterogeneity.
Occasional mitoses and/or apoptotic debris.
Nuclear to cytoplasmic ratios are increased.
Cell borders may be ill-defined.

#### Preparation-Specific Criteria

*Liquid-Based Preparations*:
Cell groups may be three-dimensional, very densely packed, with layers of cells obscuring central nuclear detail.

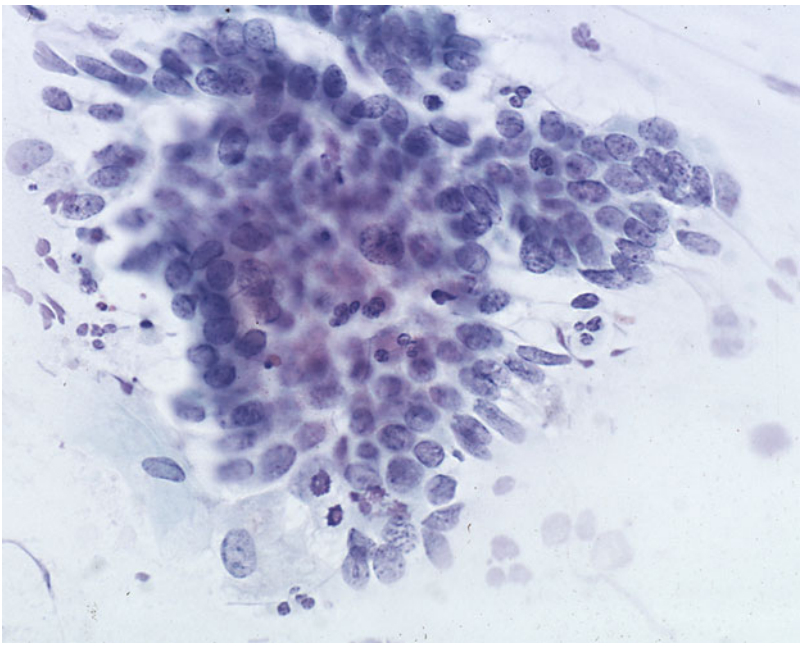

**Fig. 6.9** Atypical endocervical cells, favor neoplastic (*CP*). Routine screen from a 29-year-old woman. Sheet of crowded cells with increased N/C ratios and mitotic activity. Note feathering at the edges of the sheet. Follow-up showed endocervical AIS

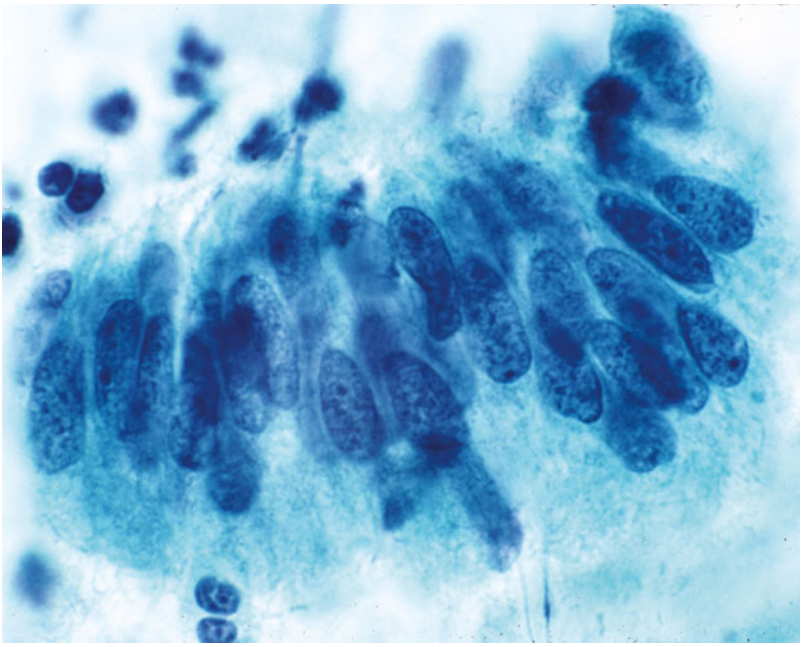

**Fig. 6.10** Atypical endocervical cells, favor neoplastic (*CP*). Pseudostratified strip of endocervical cells with enlarged, elongated nuclei and evenly distributed chromatin granularity

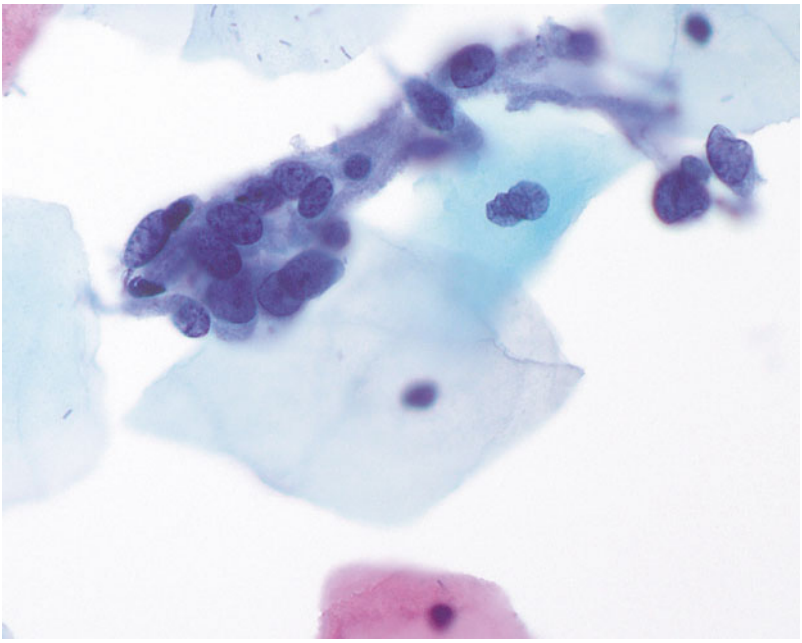

**Fig. 6.11** Atypical endocervical cells, favor neoplastic (*LBP*, *ThinPrep*). Atypical endocervical cells characterized by round or oval nuclei with nuclear enlargement, crowding, disordered arrangement, and occasional nucleoli. A rosette-like cellular arrangement is present. Follow-up showed endocervical AIS

### 6.3.4 Explanatory Notes

Endocervical and endometrial glandular cells may show a variety of cellular changes associated with various benign processes in the endocervical canal and endometrium [5]. Many of these reactive changes are not specific for any particular disease entity, but are of significance as mimics of glandular neoplasia in cervical cytology [6]. Reactive endocervical cells are characterized by the presence of a honeycomb or sheetlike arrangement with abundant cytoplasm, well-defined cell borders, and minimal nuclear overlap. Some degree of pleomorphism of cell size and nuclear enlargement may be noted; however, the nuclei remain round or oval with smooth contours and finely granular and evenly distributed chromatin. Nucleoli may be prominent, and multinucleation can occur, especially in cases of repair and inflammation. Cytoplasmic mucin may be diminished, giving the cell cluster a more hyperchromatic appearance. This constellation of reactive changes should be considered as "negative for intraepithelial lesion or malignancy" and not included in the AGC category (see Figs. 2.4, 2.32, and 2.33) [1].

"Atypical endocervical cells" may be used for cases demonstrating some, but not all, of the criteria necessary for endocervical adenocarcinoma in situ (AIS) or invasive adenocarcinoma. These features may include nuclear enlargement, crowding, variation in size, hyperchromasia, chromatin heterogeneity, and/or evidence of proliferation. Some nonneoplastic processes that may show atypical cellular changes and lead to interpretive difficulty include lower uterine segment sampling, tubal metaplasia, repair, endocervical polyps, microglandular hyperplasia, Aria–Stella change, and effects of ionizing radiation [5, 7–10].

Vigorous sampling using an endocervical brush may transfer large hyperchromatic groups of intact normal endocervical cells to the slide, resulting in so-called brush artifact (see Fig. 6.8). Such hyperchromatic groups may cause concern due to the inability to visualize centrally placed cells. These groups should be carefully evaluated for nuclear and architectural features of glandular or squamous neoplasia before rendering an "atypical" interpretation.

Tubal metaplasia is usually categorized as "negative for intraepithelial lesion or malignancy" (NILM). However, it is also a significant pitfall in the interpretation of glandular changes [10]. Only when the findings are sufficiently atypical to raise concern for neoplasia should the interpretation "atypical endocervical cells" be used. The nuclei of cells from tubal metaplasia are often enlarged, hyperchromatic, and pseudostratified, resembling those features seen in endocervical adenocarcinoma in situ (AIS) (Figs. 6.12, 6.13, and 6.14). Although some architectural and cytologic features overlap with AIS, the nuclei in tubal metaplasia tend to be round or oval and display more finely granular, evenly dispersed chromatin. Feathered edges, rosette formation, and mitoses may be seen, but they are less common compared to classic AIS. The most helpful criterion is the presence of cilia or terminal bars that may require high-powered microscopic evaluation of cell clusters to be appreciated. Although the presence of rare ciliated abnormal cells has been described in glandular neoplasia, terminal bars and cilia are indicative of a benign origin in the vast majority of cases. In addition, intermixed goblet cells and slender "peg" cells may be identified in tubal metaplasia (see Figs. 6.14, 2.19, 2.20, and 2.21)

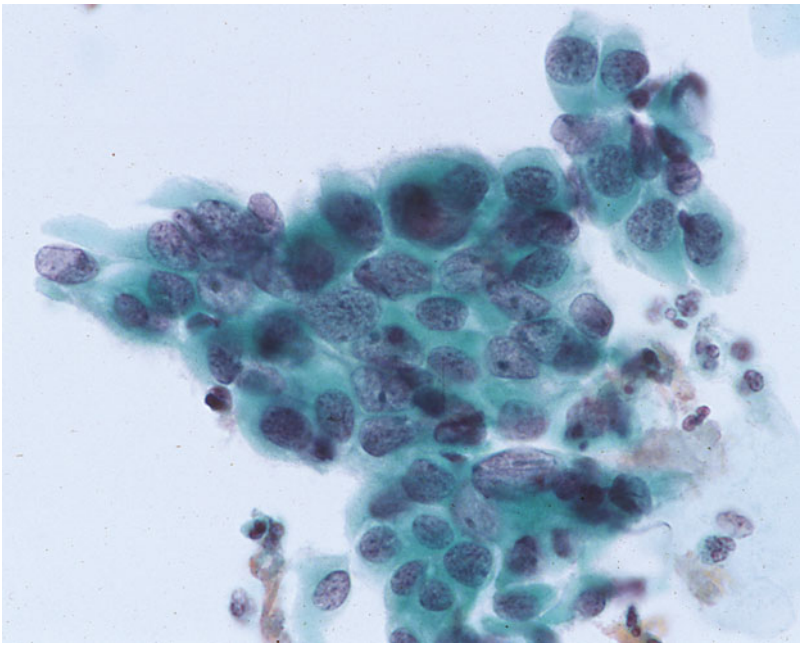

**Fig. 6.12** Atypical endocervical cells, most likely associated with tubal metaplasia (*CP*). Routine screen from a 38-year-old woman. Sheet of cells having enlarged, variably sized nuclei with some nuclear crowding and overlap. Note cilia at upper edge of sheet. Follow-up biopsy showed only tubal metaplasia

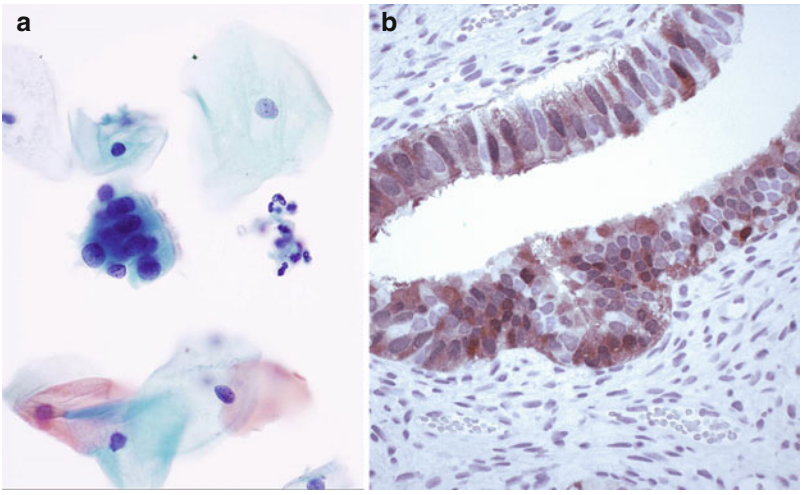


**Fig. 6.13** Tubal metaplasia. (**a**) Tubal metaplasia showing pseudostratified nuclei in a cellular strip (*LBP*, *ThinPrep*). Note the prominent terminal bars and cilia on the cells. (**b**) p16 immunostaining of tubal metaplasia (biopsy H&E) can show some positivity of the cells; however, not all cells in the epithelium are stained, in contrast to the diffuse staining typically noted in AIS (see Fig. 6.20)

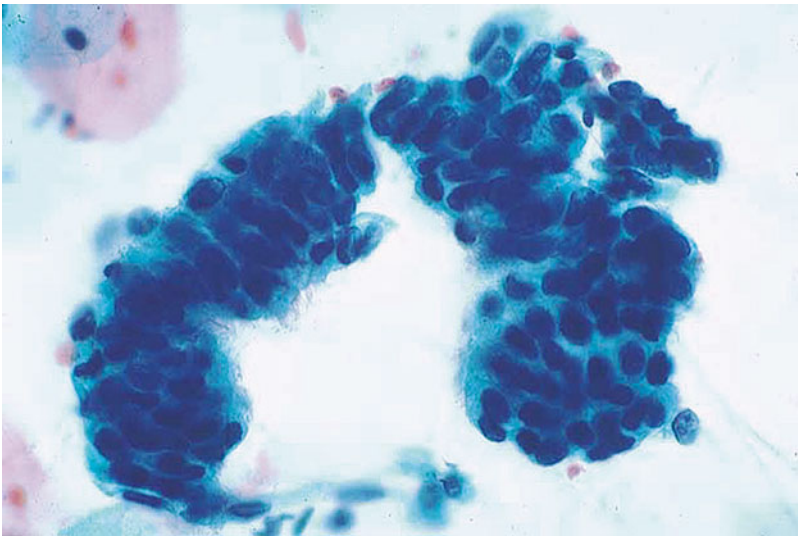

**Fig. 6.14** Atypical endocervical cells, probably derived from tubal metaplasia (*CP*). Cell groups from tubal metaplasia may raise the differential diagnosis of endocervical adenocarcinoma in situ (AIS). It is useful to note that due to the presence of mucin in goblet cells overlying some nuclei, and the variety of cell types (goblet, ciliated, and peg) in tubal metaplasia, scattered nuclei demonstrate relative hypochromasia or a "washed-out" appearance and lack the monotony of changes characteristic of AIS (contrast with Fig. 6.21)

(Table 6.1). However, it must be remembered that because tubal metaplasia is very common in the high endocervical canal and lower uterine segment, it may coexist with endocervical neoplasia, and hence its presence should not dissuade an atypical or neoplastic designation if other types of atypical cells are present in the same specimen.

### 6.3.5 Mimics of Atypical Glandular Cells (Fig. 6.12–6.14)

#### 6.3.5.1 High-Grade Squamous Intraepithelial Lesion (Figs. 5.15–5.17, 5.25, 5.29, 5.31, 5.33, and 5.34)

HSIL involving gland spaces may present as contoured clusters mimicking the appearance of a glandular lesion (see Fig. 6.6). Groups are composed of tightly packed cells with high nuclear to cytoplasmic ratios and hyperchromatic nuclei with coarsely granular chromatin. In addition to classic morphologic descriptions, HSIL involving endocervical glands may also show the presence of nucleoli. The cytoplasm often has no specific differentiation. Flattening of cells at the periphery of the cluster, loss of cell polarity within the clusters, and the presence of isolated dysplastic squamous cells in the background can be very helpful features to suggest HSIL (see Figs. 5.15, 5.16, 5.17, 5.25, 5.29, 5.31, 5.33, and 5.34). HSIL involving gland spaces also lacks specific architectural features of AIS such as feathering, rosettes, and pseudostratified strips of columnar cells. Endocervical gland involvement by HSIL can lead to maintenance of cellular polarity within groups—a feature more

**Table 6.1** Adenocarcinoma in situ/AGC and mimics

| Cytologic criteria | AIS | HSIL | Repair | Tubal metaplasia | Directly sampled endometrium/ endometriosis |
|---|---|---|---|---|---|
| Cellularity | Cellular | Usually cellular | Rare fragments | Rare event | Few groups/variable |
| HCGs | Many | Can be many | Absent | Rare | Present/can be numerous |
| Sheets/strips | Many with feathering/3D | Syncytia | Flat sheets | Absent/rare | Present, 3D |
| Nuclear crowding/overlap | Present | Present | Absent | Present but mild | Present |
| Perpendicular nuclear polarization | Present | Absent | Absent | Present | Can be present |
| Hyperchromasia | Present | Present | Absent | Mild | Mild |
| Nuclear shape | Oval/elongate | Round/irregular | Round | Oval/cigar shaped | Oval/cigar shaped |
| Feathering | Present | Absent/focal | Absent | Rare | Absent/rare |
| Strips | Present | Absent | Absent | Present | Present |
| Rosettes | Present | Absent | Absent | Absent | May be present/gland openings/ tubules |
| Terminal bars/cilia | Absent | Absent | Absent | Present/diagnostic | Rare/may be present |
| Spindled stroma | Absent | Absent | Absent | Absent | Present |
| Mitosis/apoptosis | Present | May be seen | Rare | Rare | May be present |
| p16 pattern | Block positive | Block positive | Negative | Patchy positive | Patchy, focal to rare glandular cells |

Abbreviated from Mody [11]

commonly noted in glandular lesions and not characteristically present in the classic presentation of HSIL (see Table 6.1 and Figs. 6.23, 6.24, 6.26) [5, 11–13].

## 6.4 Atypical Endometrial Cells (Figs. 6.15–6.18)

### 6.4.1 Definition

The distinction of cytologically benign from atypical endometrial cells is based primarily on the criterion of increased nuclear size.

Atypical endometrial cells are generally not further qualified as favor neoplastic since this is a difficult and poorly reproducible distinction. However, specific comments can be appended if clinical findings/history is available (e.g., presence of IUD, polyp) [1].

### 6.4.2 Criteria

Cells occur in small groups, usually 5–10 cells per group (Figs. 6.15 and 6.18).
Nuclei are slightly enlarged compared to normal endometrial cells.
Mild hyperchromasia.
Chromatin heterogeneity.

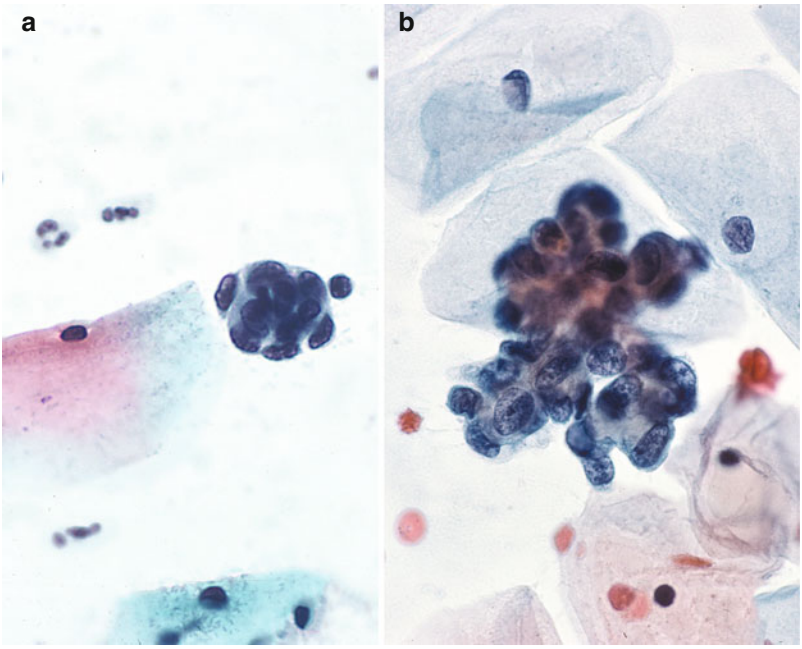


**Fig. 6.15** Atypical endometrial cells (*CP*). An 82-year-old woman with postmenopausal bleeding. Three-dimensional groups of small cells with mildly hyperchromatic nuclei, small nucleoli, and occasionally vacuolated cytoplasm. (**a**) shows a very tight cluster, while (**b**) shows a more loosely aggregated group. Follow-up showed endometrial hyperplasia

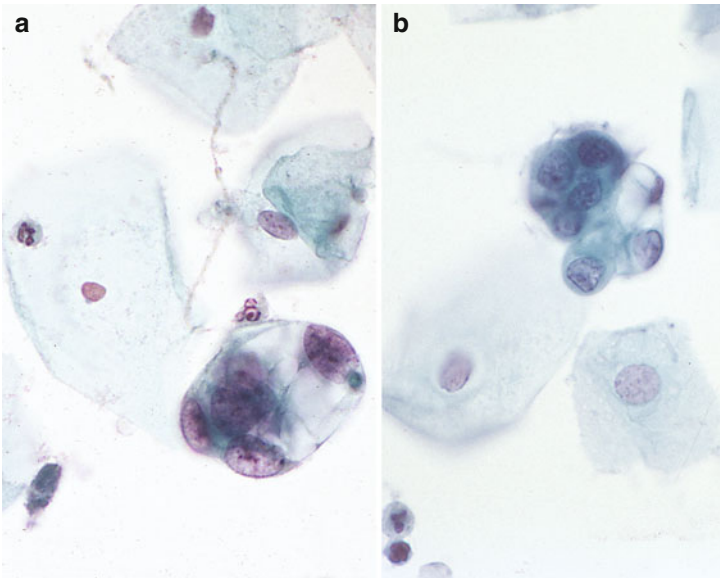


**Fig. 6.16** Atypical endometrial cells (*LBP*, *ThinPrep*). Small groups of cells with slightly enlarged nuclei, small nucleoli, and vacuolated cytoplasm. (**a**) A 63-year-old woman. Follow-up showed endometrial adenocarcinoma grade 1. (**b**) A 55-year-old woman. Follow-up showed endometrial hyperplasia

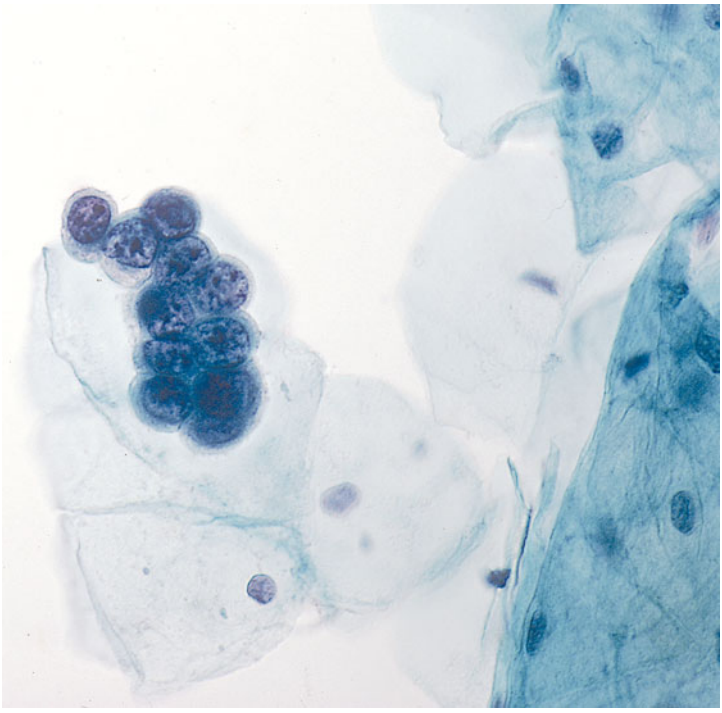

**Fig. 6.17** Atypical endometrial cells (*LBP*, *ThinPrep*). A 63-year-old woman with postmenopausal bleeding. Aggregate of small cells with slightly enlarged round or oval nuclei, small nucleoli, and finely vacuolated cytoplasm. Follow-up showed endometrial adenocarcinoma grade 1

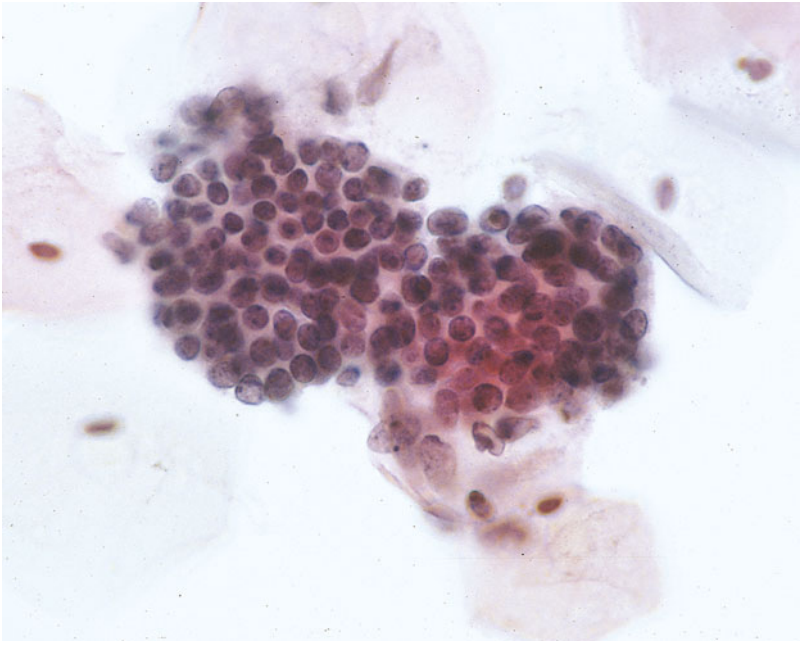

**Fig. 6.18** Atypical endometrial cells (*LBP*, *ThinPrep*). A 52-year-old woman on hormone replacement therapy. Three-dimensional grouping of small cells with crowded round or oval nuclei. Follow-up showed endometrial hyperplasia

Occasional small nucleoli (Fig. 6.16).
Scant cytoplasm is occasionally vacuolated (Fig. 6.17).
Cell borders are ill defined.

**Preparation-Specific Criteria**
*Liquid-Based Preparations*:
Nuclear hyperchromasia may be more prominent.
Nucleoli may be more prominent.

### 6.4.3 Explanatory Notes

Atypical endometrial cells, like their cytologically bland counterparts, may be associated with the presence of endometrial polyps, chronic endometritis, an intrauterine device (IUD), endometrial hyperplasia, or endometrial carcinoma (see Fig. 6.16). Caution should be used in the interpretation of atypia in endometrial material on liquid-based preparations because shed/menstrual endometrial cells may show significantly greater pleomorphism of nuclear size and shape than is seen in conventional preparations (see Figs. 3.2 and 3.4). These changes are likely due to improved

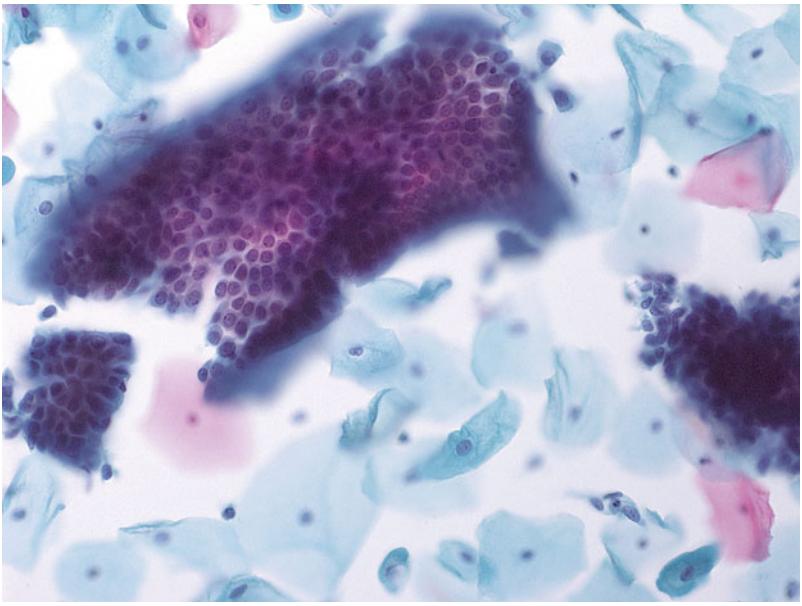

**Fig. 6.19** Directly sampled tubular endometrial glands with adjacent stromal elements seen post trachelectomy (*LBP*, *SurePath*). The geometric/tubular shapes of the endometrial glands should clue one in to the possibility of directly sampled endometrium. The stromal fragments consist of spindled cells but may be separated from the glands in liquid-based preparations

visualization of degenerating endometrial cells resulting from clearing of blood, inflammation, and debris in liquid-based preparations from menstrual specimens and should not be overinterpreted as "atypical." Clinical information can also be helpful in correctly categorizing such cases [5, 14]. The presence of "exodus" type of arrangements and a background containing endometrial stromal cells can be helpful in this discrimination.

Endometrial/endocervical cells derived from post-trachelectomy specimens (Figs. 6.19 and see Figs. 2.7, 2.8, 2.9, 3.5) may elicit an atypical glandular cell interpretation, especially when the history is not known. Helpful features include the presence of tubular glandular structures closely associated with bipolar endometrial stromal cells. In the absence of stromal cells, the geometric shape of the glandular clusters without feathering along the periphery is a helpful feature which is appreciated on low magnification [15–17].

Residual liquid-based cytology specimens can be used to make cell blocks to help resolve the origin of atypical glandular cells, including mimics, such as menstrual or directly sampled endometrium, and tubal metaplasia. Hematoxylin and eosin-stained sections and immunocytochemical stains, such as p16, may clarify the nature of densely crowded cell groups [18–21] (Fig. 6.20).

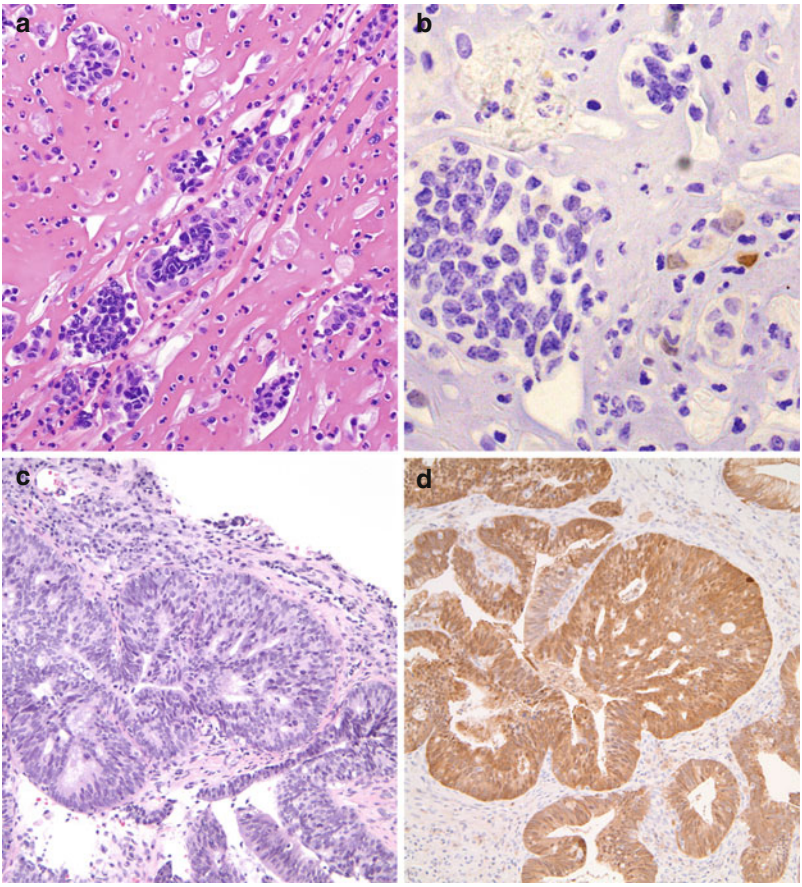


**Fig. 6.20** Comparison between benign endometrial tissue and endocervical AIS stained with the p16 immunostain. (**a**) Shed endometrium (cell block H&E), (**b**) shed endometrium (cell block p16), (**c**) AIS (biopsy H&E), and (**d**) AIS (biopsy p16). p16 is diffusely positive in AIS and essentially negative in shed endometrium. Cell blocks of residual material from cytologic specimens can be useful for the application of biomarkers (Compare to Fig. 6.13 for p16 staining pattern in benign tubal metaplasia)

## 6.5 Management of AGC

The 2012 consensus guidelines from the American Society for Colposcopy and Cervical Pathology (ASCCP) include recommendations for the initial workup and subsequent management of women with glandular abnormalities based on the 2001 Bethesda terminology [22].

Initial management of all categories of AGC, except atypical endometrial cells is colposcopy with endocervical sampling. Women 35 years and older or at risk for endometrial neoplasia should also have endometrial sampling. Those with atypical

endometrial cells should have endometrial and endocervical sampling; colposcopy may be deferred if endometrial pathology is identified. Subsequent management of AGC depends on the findings from the initial sampling and cytologic interpretation. Triage using repeat cytology is not an option for the AGC category as it is high risk, and may harbor significant squamous and glandular preinvasive and invasive disease. If invasive disease is not identified on initial evaluation, a diagnostic excisional procedure is recommended for women with a cytologic interpretation of atypical glandular or endocervical cells, favor neoplasia or endocervical adenocarcinoma in situ.

The 2012 ASCCP guidelines do not recommend hrHPV triage for initial presentations of AGC. Based on two recent large studies from the United States, 25 % of cases in the AGC category test positive for hrHPV [23–25]. The most prevalent hrHPV genotypes detected are 18/45, followed by 16 [26, 27]. Overall, HPV genotype 16 and/or 18 accounts for 20–53 % of all AGC that are positive for hrHPV [26–28]. Few studies have addressed hrHPV positivity among the subcategories of AGC. Overall, approximately 50 % of AGC cases that test positive for hrHPV are found to be associated with significant lesions (e.g., HSIL, AIS or endocervical adenocarcinoma), whereas less than 5 % of AGC cases, which are negative for hrHPV, are found to be associated with significant HPV-associated precancer/cancer diseases [22, 23, 29]. In summary, hrHPV-positive AGC is more likely to have cervical pathology, such as adenocarcinoma in situ, endocervical adenocarcinoma, squamous intraepithelial lesion, or squamous carcinoma. hrHPV-negative AGC is more likely to show cancer which is endometrial or extrauterine in origin, or a benign reactive condition such as an endocervical or endometrial polyp.

## 6.6 AGC Reporting Rates and Outcomes

Reporting rates for AGC are published by the College of American Pathologists (CAP) for benchmarking purposes for cytology labs enrolled in their Laboratory Accreditation Program. The 50th percentile of the reporting rates ranges from 0.1 to 0.2 % for conventional and liquid-based technologies. The rates range from 0 % at the low end to 0.8 % at the high end for the reporting of AGC [30]. Follow-up of AGC cytologic interpretations shows that high-grade lesions are identified in 10–40 % of cases and are more often squamous (HSIL/CIN 2–3) than glandular [5, 11, 23]. In addition, HSIL frequently coexists with AIS.

## 6.7 Endocervical Adenocarcinoma In Situ (AIS) (Figs. 6.21–6.32)

### 6.7.1 Definition

A noninvasive high-grade endocervical glandular lesion that is characterized by nuclear enlargement, hyperchromasia, chromatin abnormality, pseudostratification, and mitotic activity.

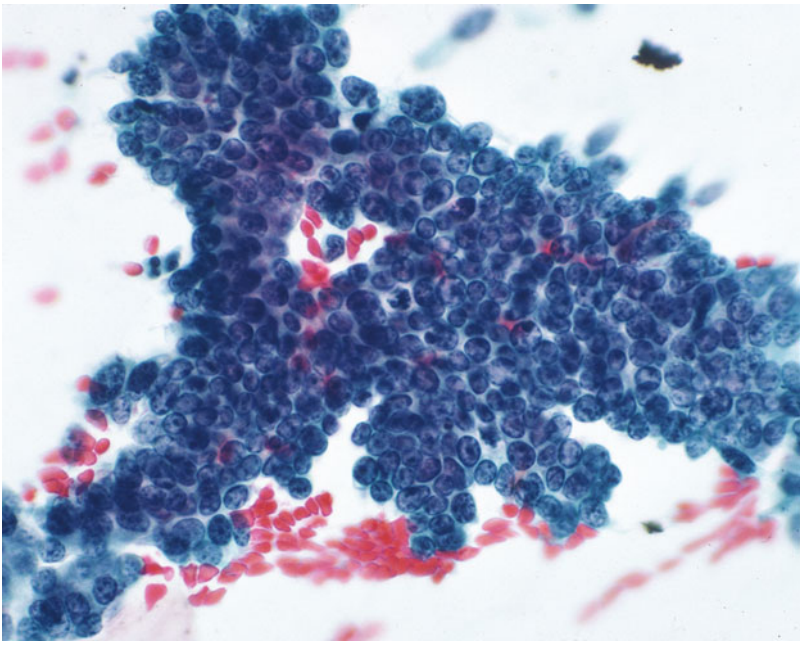

**Fig. 6.21** Endocervical adenocarcinoma in situ (*CP*). Sheet of crowded cells with enlarged, hyperchromatic nuclei, increased nuclear to cytoplasmic ratios, and feathering at the periphery of the sheet. Note the monotony of the hyperchromatic nuclei as contrasted with the more variable nuclear changes in tubal metaplasia (see Figs. 6.12 and 6.14 for comparison)

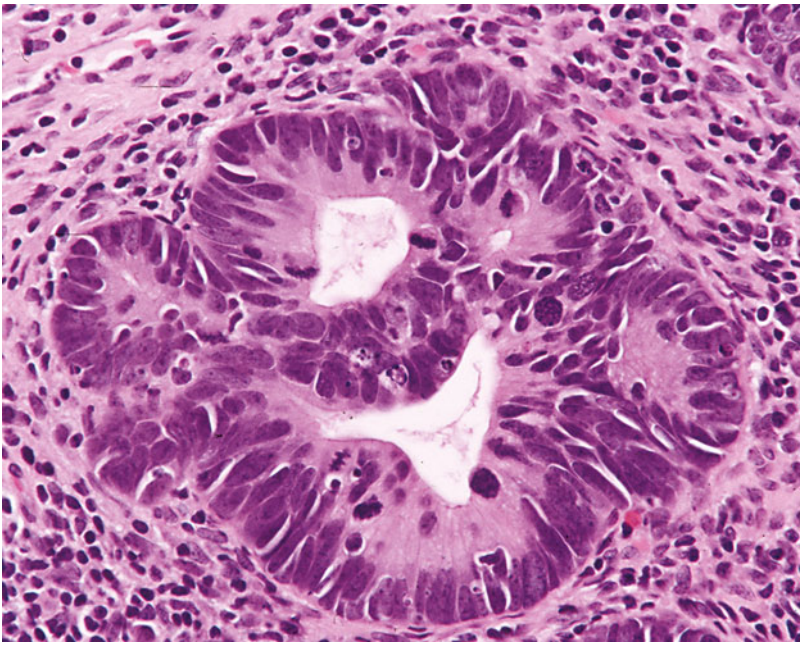

**Fig. 6.22** Endocervical adenocarcinoma in situ (histology, H&E)

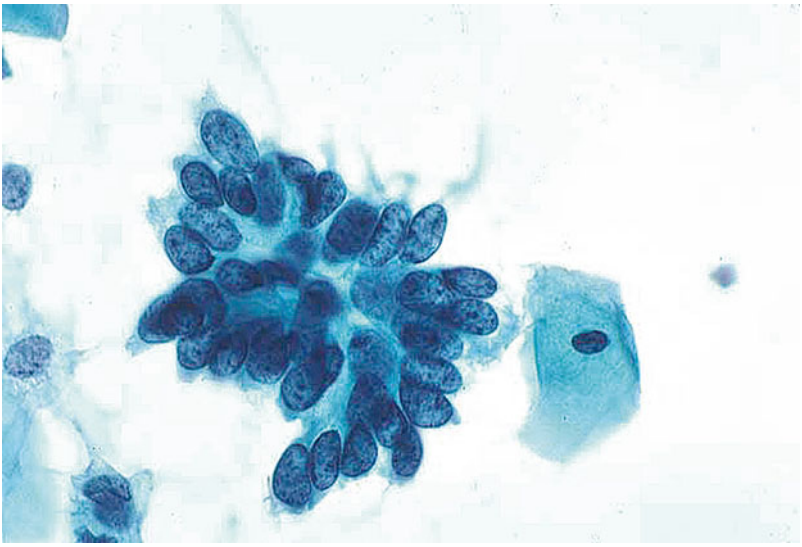

**Fig. 6.23** Endocervical adenocarcinoma in situ (*CP*). The typically oval nuclei are crowded with nuclear overlapping and show hyperchromasia with evenly distributed but coarsely granular chromatin. Note the prominent gland-like configuration (rosette)

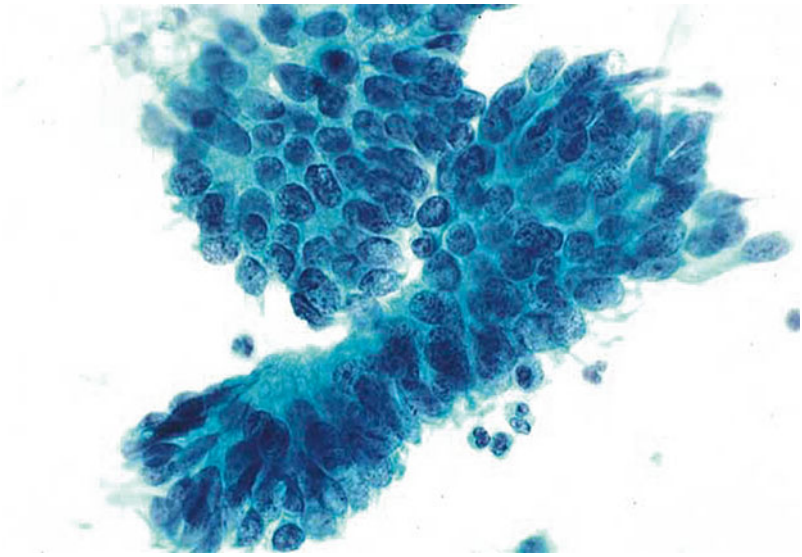

**Fig. 6.24** Endocervical adenocarcinoma in situ (*CP*). Pseudostratified strip of cells demonstrating crowding, nuclear enlargement, and peripheral feathering

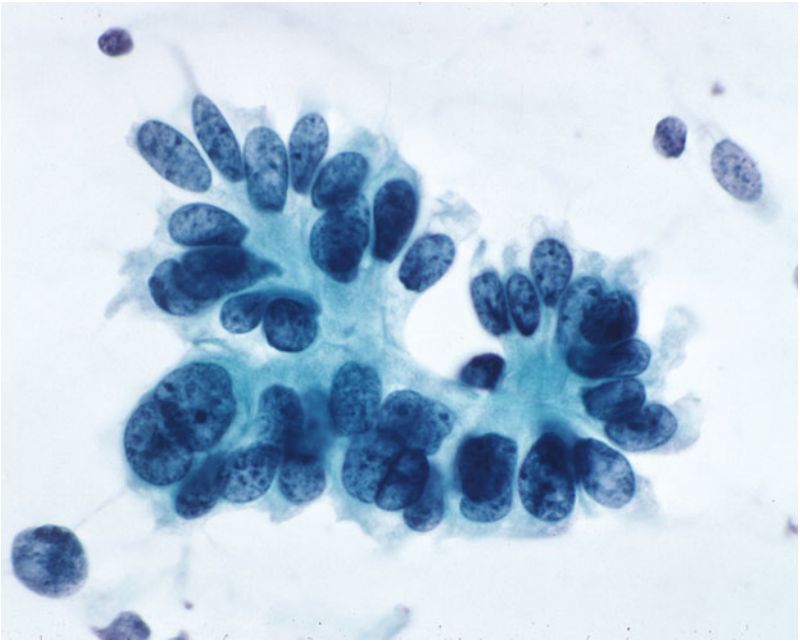

**Fig. 6.25** Endocervical adenocarcinoma in situ (*CP*). Cell group in a rosette-like arrangement. Nuclei are oval or elongated, are hyperchromatic, and have granular, evenly distributed chromatin

### 6.7.2 Criteria

Cells occur in sheets, clusters, pseudostratified strips, and rosettes with nuclear crowding and overlap and loss of a well-defined honeycomb pattern. Single abnormal cells may be present but are uncommon (Figs. 6.21, 6.23, 6.24, 6.25, 6.29, and 6.30).

Some cells show a definite columnar appearance.

Cell clusters have a palisading nuclear arrangement with nuclei and cytoplasmic tags protruding from the periphery ("feathering").

Nuclei are enlarged, variably sized, and oval or elongated.

Nuclear hyperchromasia with evenly dispersed, coarsely granular chromatin.

Nucleoli are usually small or inconspicuous.

Mitoses and apoptotic bodies are common.

Nuclear to cytoplasmic ratios are increased; the quantity of cytoplasm, as well as cytoplasmic mucin, is diminished.

Background is typically clean (no tumor diathesis, although inflammatory debris may be present).

Abnormal squamous cells may be present if there is a coexisting squamous lesion.

**See Table 6.1 for summary of the criteria for AIS and its mimics.**

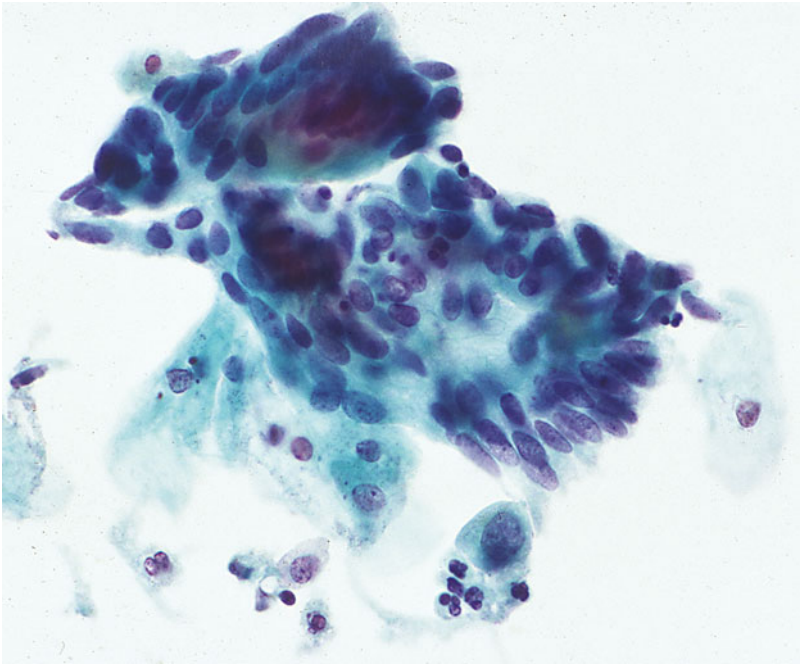

**Fig. 6.26** Endocervical adenocarcinoma in situ (*LBP*, *ThinPrep*). A 64-year-old woman with prior abnormal cytology. Cell groups in *LBP*s may be more three-dimensional with sharper, smoother margins, and feathering may have a more subtle presentation. Follow-up showed AIS with a small focus of invasion

#### Preparation-Specific Criteria

*Liquid-Based Preparations*:

Single intact cells are more easily found.

Hyperchromatic crowded groups are smaller, denser, and more three-dimensional with smoother, sharper margins.

Pseudostratified strips of cells, often presenting as short "bird tail"-like arrangements (especially on SurePath), may be the most prominent architectural feature (Figs. 6.26 and 6.27).

Architectural features of peripheral feathering, rosettes, and cell strips have a more subtle presentation.

Nuclear chromatin may be coarse or finely granular.

Nucleoli may be more readily visible (Fig. 6.28).

### 6.7.3 Explanatory Notes

The cytologic interpretation of endocervical adenocarcinoma in situ can be difficult and should only be made in cases where sufficient criteria are present. In problematic cases, the interpretation of "atypical endocervical/glandular cells, favor neoplastic" is justified [1].

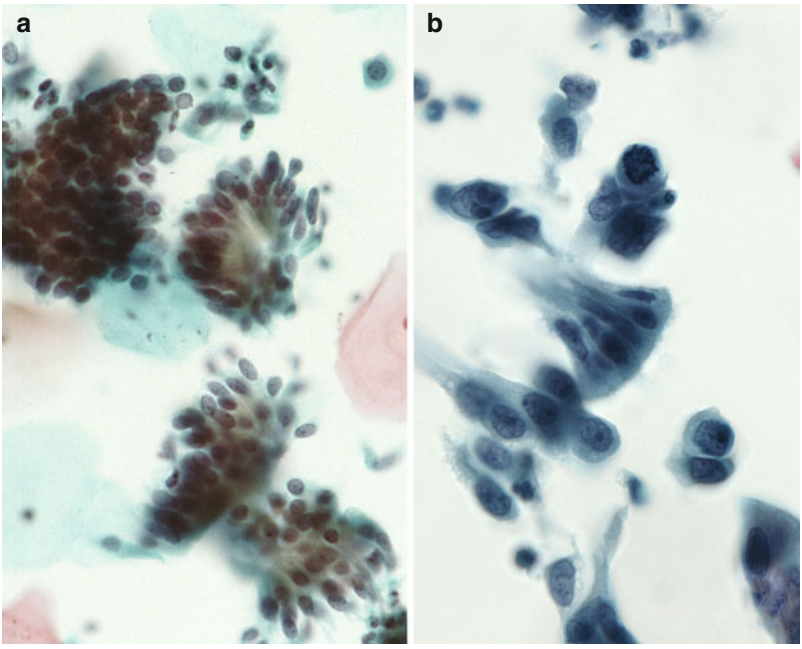


**Fig. 6.27** Endocervical adenocarcinoma in situ (*LBP*, *SurePath*). Routine screen from a 25-year-old woman. Pseudostratified strips of cells often present as short "bird tail-like" arrangements in SP as seen on the *right side* of this image (**b**). Feathering, although less prominent than in conventional smears, is demonstrated on the *left* (**a**). Follow-up showed AIS

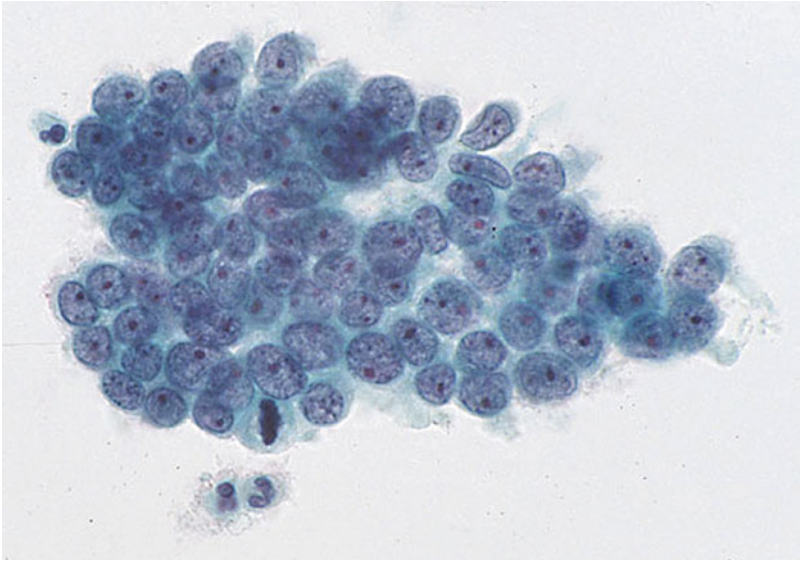

**Fig. 6.28** Endocervical adenocarcinoma in situ (*LBP*, *ThinPrep*). AIS may occasionally demonstrate nucleoli, raising the differential of invasive endocervical carcinoma (see Fig. 6.40)

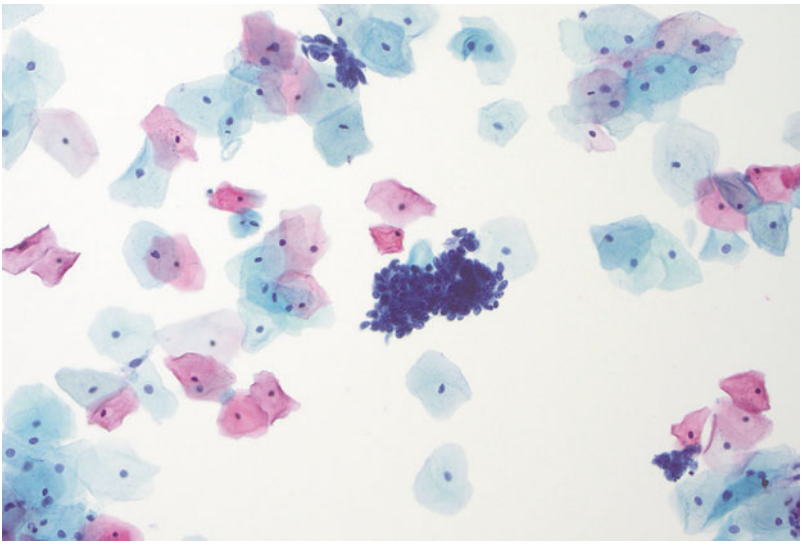

**Fig. 6.29** Endocervical AIS on low magnification (*LBP*, *ThinPrep*). Hyperchromatic crowded groups of cells characterized by sheets with nuclear crowding with peripheral feathering as seen in the *center* of image. A strip of cell with nuclear crowding, overlapping, and hyperchromasia is seen near the upper edge of the image. Dense cellular groups present on low-magnification scans may be the first clue to the presence of a glandular lesion

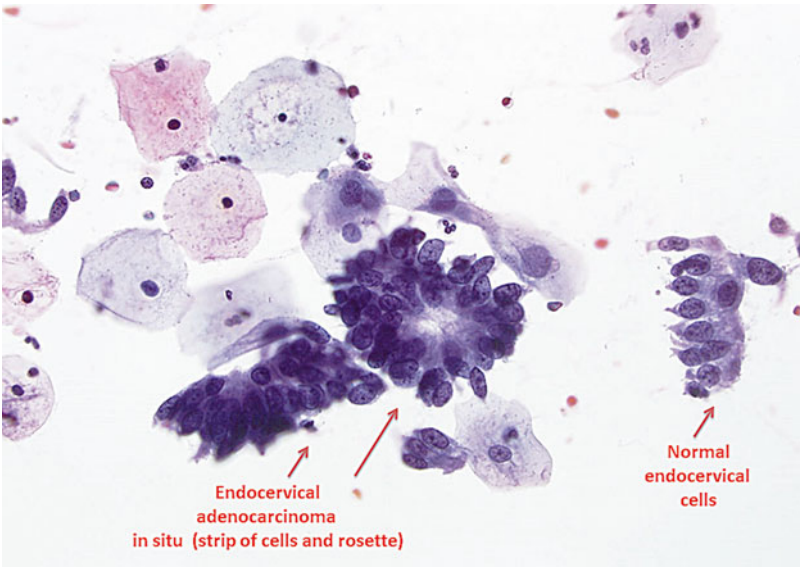


**Fig. 6.30** Benign and neoplastic endocervical cells (*LBP*, *ThinPrep*). The group on the *right side* of the image shows a strip of normal endocervical cells with low nuclear to cytoplasmic ratios and lack of overlapping contrasted with the groups on the *left side* of the image which show strips and rosettes of AIS with high nuclear to cytoplasmic ratios, nuclear hyperchromasia, crowding, feathering and overlapping

In liquid-based preparations, density of cell groups can be difficult to interpret, and visualization of the cell nuclei is more problematic. Close and careful scrutiny, particularly of the cells at the group margins, is essential to correctly categorize these clusters as glandular in origin. Criteria described for AIS are the features for the most common endocervical form [5, 11, 31–34]. Although uncommon, variant forms of AIS exist e.g., mucinous, intestinal (Fig. 6.31), endometrioid (Fig. 6.32), and clear cell, that may show other morphologic features [5, 11, 35–38]. The endometrioid variant, although rare, has been shown to be a commonly missed form of AIS. The cells in the endometrioid variant are smaller than the usual form, and groups are erroneously interpreted as being of benign endometrial origin [36].

### 6.7.4 Management of Endocervical Adenocarcinoma in situ

The 2012 consensus guidelines from the American Society for Colposcopy and Cervical Pathology (ASCCP) include recommendations for the initial workup and subsequent management of women with glandular abnormalities based on the 2001 Bethesda terminology [22].

The initial management of AIS is colposcopy and endocervical sampling. HPV testing for triage is not recommended due to the possibility of HPV negative lesions and inadequate sampling. Therefore, an associated negative HPV test should not alter the initial evaluation. If the patient is greater than 35 years of age or exhibits symptoms which may suggest an endometrial lesion (e.g. vaginal

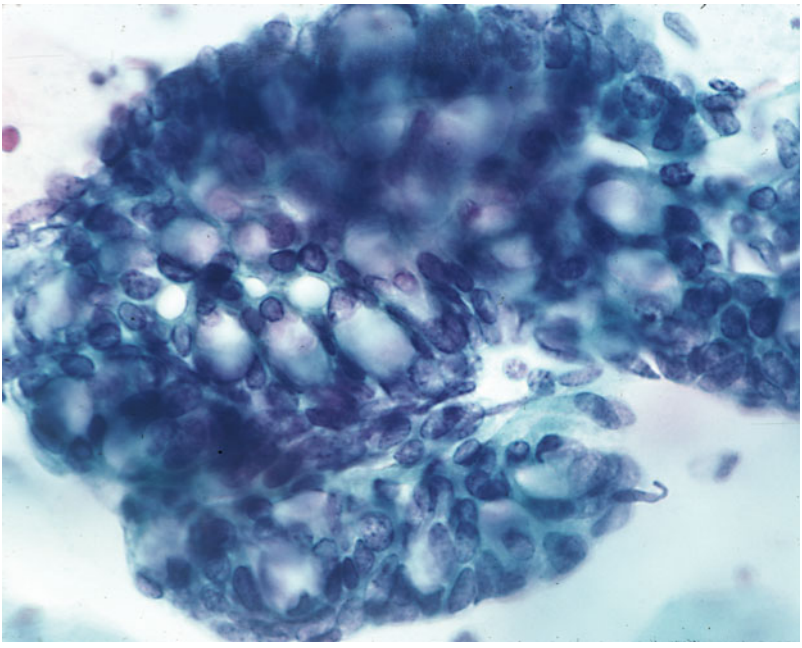

**Fig. 6.31** Endocervical adenocarcinoma in situ, intestinal type (*CP*). Cells show nuclear crowding and overlap and have elongated nuclei. Note numerous goblet-type cells

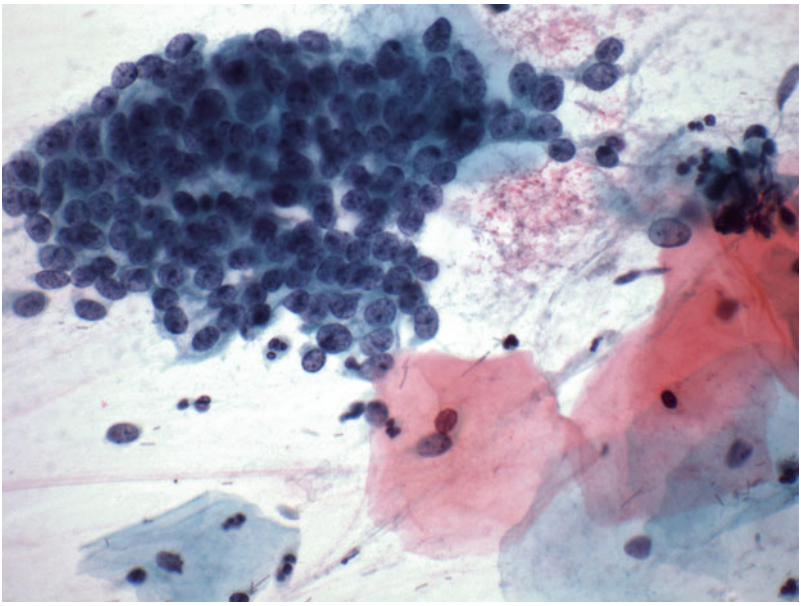

**Fig. 6.32** Endocervical adenocarcinoma in situ, endometrioid variant (*CP*). Endometrioid AIS has similar features to the usual type of AIS but shows much smaller average nuclear area (compare to intermediate cell nucleus in the image). Because of this size difference, endometrioid AIS can be mistaken for directly sampled benign endometrium. Attention to overall architecture and lack of stromal cells can be helpful in differentiation

bleeding or symptoms of chronic anovulation), endometrial sampling should be added. A diagnostic excision is recommended if no evidence of invasive disease is identified on initial evaluation. If histologic AIS is present on colposcopic biopsy, a total hysterectomy is the treatment of choice. If conservative management to preserve child bearing is desired an excisional procedure (either cold knife or LEEP biopsy) with evaluation of margins is recommended. With margins positive for AIS, a re-excision is recommended. Because AIS may be multifocal in a small percentage of cases, negative margins do not insure complete excision and hence continued follow-up is important. With negative margins, repeat co-testing, colposcopy, and endocervical sampling are recommended at 6 months. In women not having a hysterectomy continued long term follow-up is recommended.

For women with AIS, there is no difference in the management of the disease in special populations, such as in pregnancy and in women aged 21–24 years.

## 6.8 Coexisting Squamous and Glandular Lesions (Figs. 6.33 and 6.34)

The possibility of coexisting glandular and squamous lesions in the cervix should always be considered when making an interpretation of endocervical AIS (Figs. 6.33 and 6.34) [5, 11]. In some studies, up to half of AIS lesions have a coexisting

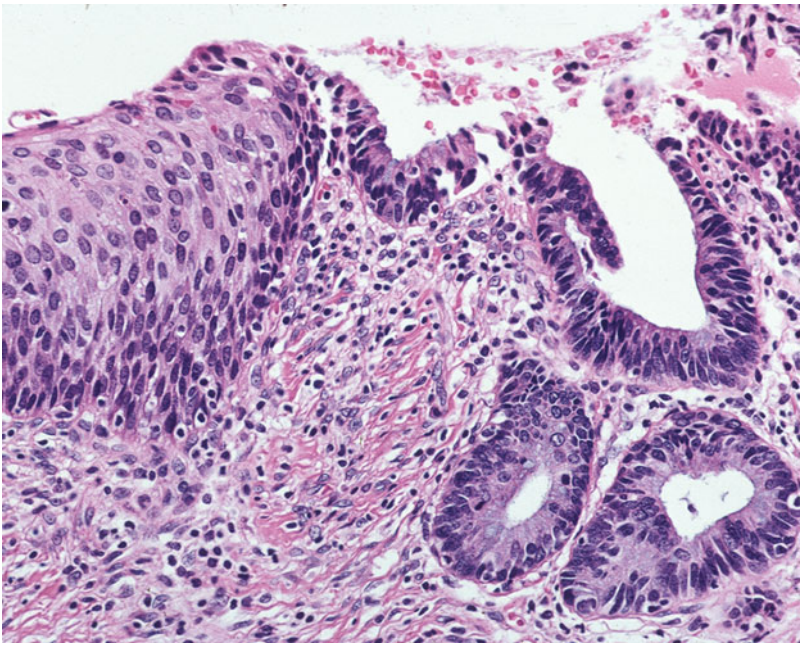

**Fig. 6.33** AIS and HSIL (histology, H&E). Glandular and squamous lesions may coexist. HSIL is present on the squamous epithelial surface on the *left side* of this image, and endocervical adenocarcinoma in situ is present in gland spaces on the *right* (© 2001 American Society for Clinical Pathology Reprinted with permission)

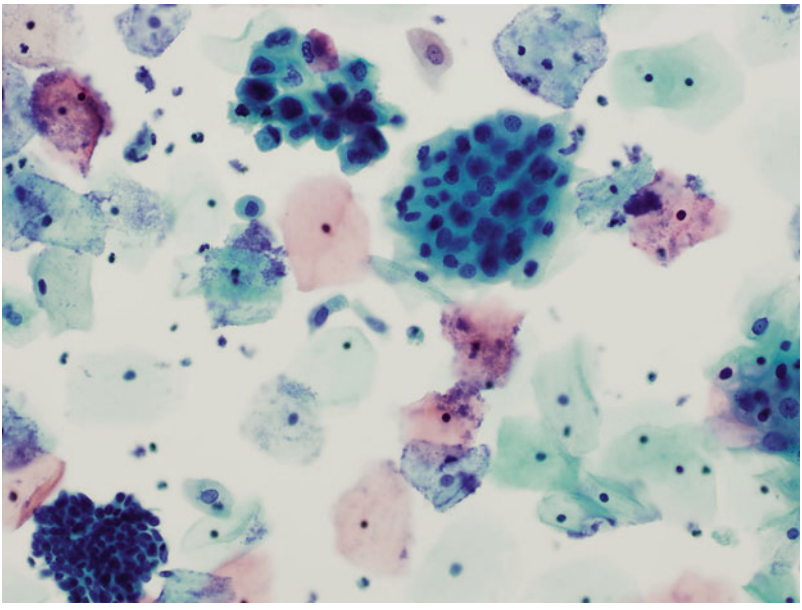

**Fig. 6.34** AIS and HSIL (*LBP*, *ThinPrep*). The preparation showing HSIL (11–12 o'clock), LSIL (3 o'clock), and AIS (endometrioid type, 7–8 o'clock) all in one medium-magnification field. Note the smaller size of the cells in the cluster of AIS characterized by some peripheral feathering. Follow-up showed HSIL as well as AIS

squamous intraepithelial lesion, usually of high grade. Often, the cytoplasmic features and the cell arrangements differentiate the two neoplastic processes.

## 6.9 Adenocarcinoma

### 6.9.1 Endocervical Adenocarcinoma (Figs. 6.35–6.45)

Cytologic criteria overlap those outlined for AIS, but may show additional features indicative of invasion (Fig. 6.35).

#### 6.9.1.1 Criteria

Abundant abnormal cells, typically with columnar configuration.

Single cells, two-dimensional sheets or three-dimensional clusters, and syncytial aggregates (Fig. 6.37).

Enlarged, pleomorphic nuclei demonstrate irregular chromatin distribution, chromatin clearing, and nuclear membrane irregularities (Fig. 6.36).

Macronucleoli.

Cytoplasm is usually finely vacuolated.

Necrotic tumor diathesis is common.

Abnormal squamous cells may be present, representing a coexisting squamous lesion or the squamous component of an adenocarcinoma showing partial squamous differentiation.

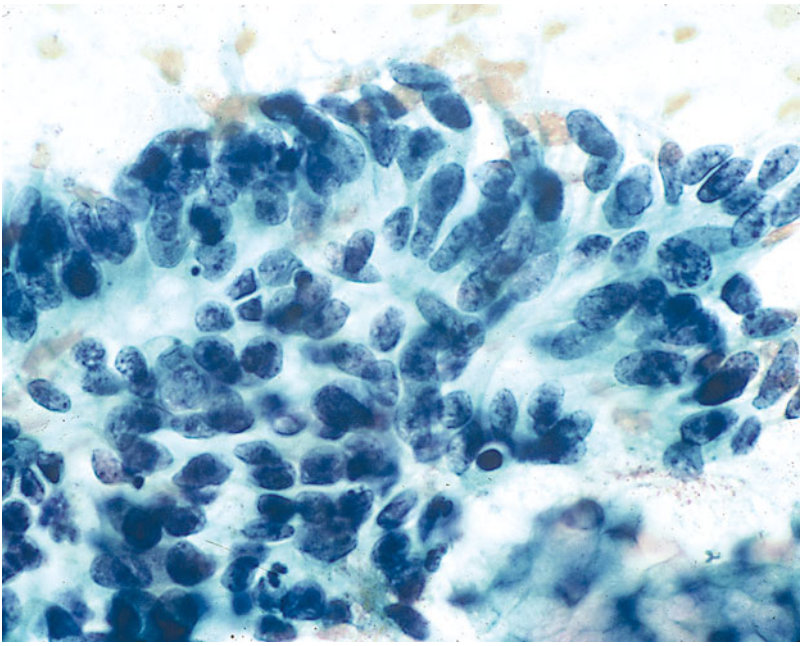

**Fig. 6.35** Adenocarcinoma, endocervical (*CP*). A 32-year-old woman with abnormal cervix on pelvic exam. Cytologic features may overlap with those of endocervical adenocarcinoma in situ. Follow-up showed invasive endocervical adenocarcinoma

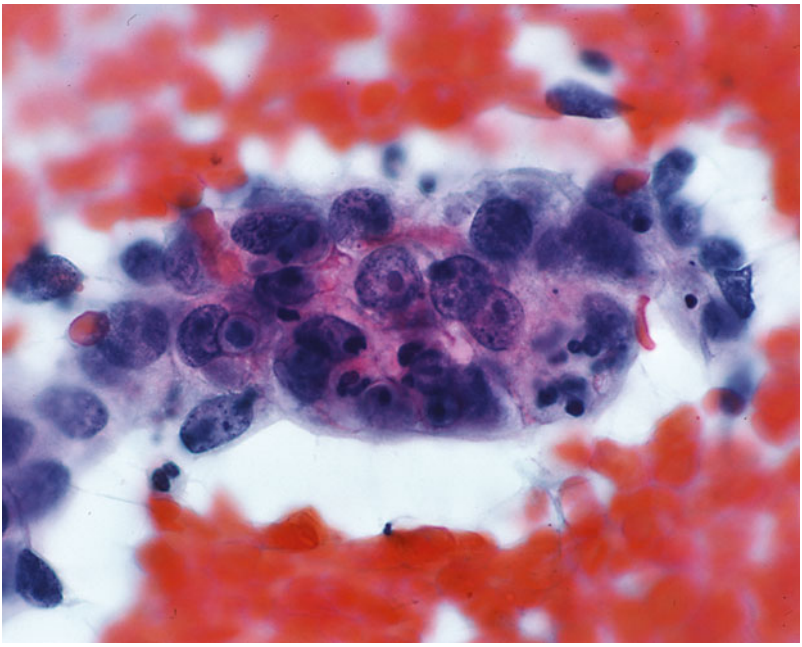

**Fig. 6.36** Adenocarcinoma, endocervical (*CP*). Nuclei are enlarged and pleomorphic with irregular chromatin distribution and prominent or macronucleoli. Cytoplasm is finely vacuolated. Note the prominent blood-filled background

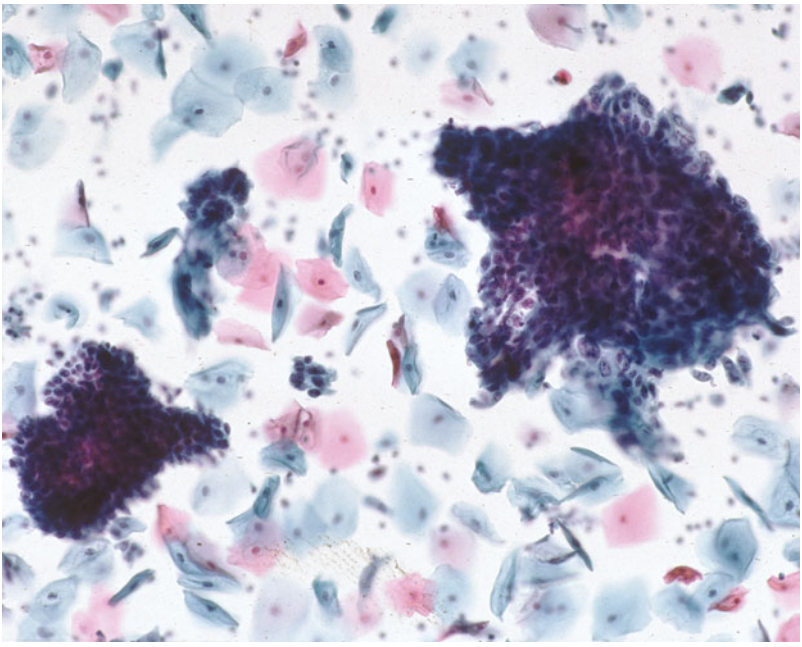

**Fig. 6.37** Adenocarcinoma, endocervical (*LBP*, *SurePath*). Large cell groups may be thick and three-dimensional, making architecture more difficult to interpret and visualization of cell nuclei more problematic

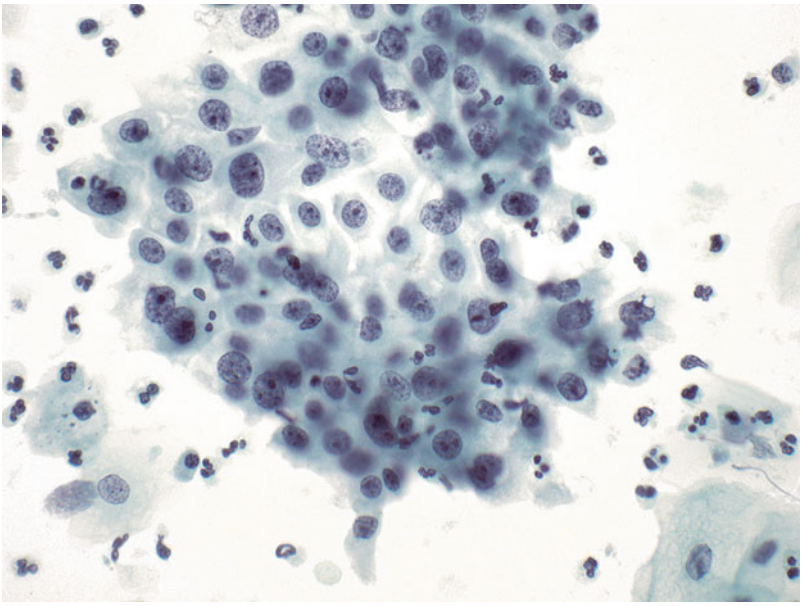

**Fig. 6.38** Adenocarcinoma, endocervical (*LBP*, *SurePath*). Cell group demonstrates glandular architecture and large nuclei, irregular chromatin distribution, and prominent macronucleoli. This group shows well-defined cytoplasmic boundaries mimicking reparative change, which can often be a problematic differential diagnosis

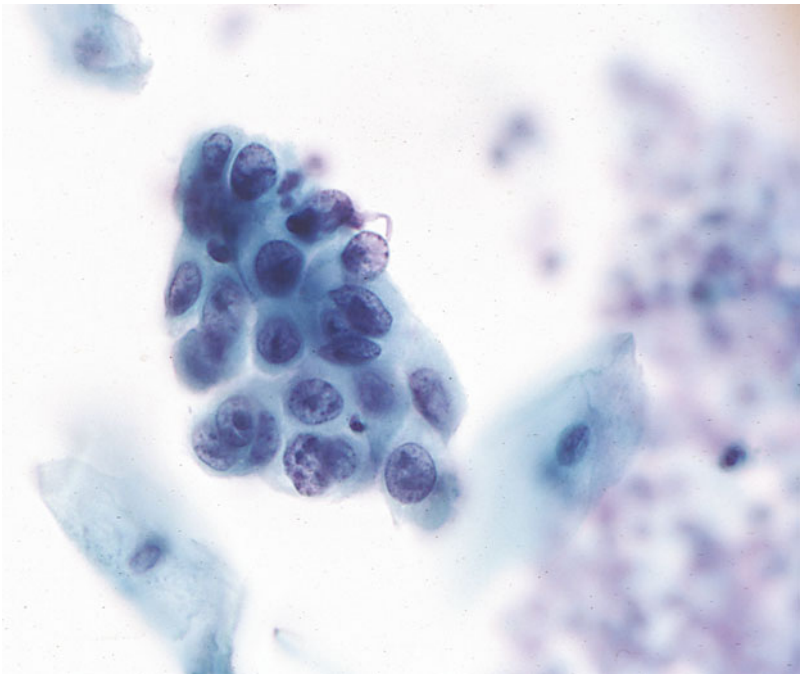

**Fig. 6.39** Adenocarcinoma, endocervical (*LBP*, *ThinPrep*). A 46-year-old woman. Cell nuclei may have more vesicular chromatin with irregular distribution and chromatin clearing as well as macronucleoli. Follow-up showed invasive endocervical adenocarcinoma

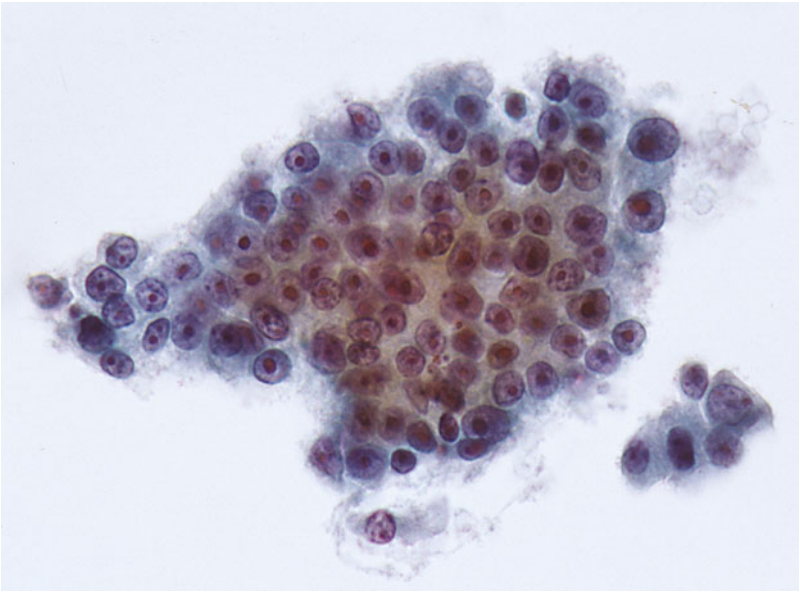

**Fig. 6.40** Adenocarcinoma, endocervical (*LBP*, *ThinPrep*). A 39-year-old woman on day 12 of menstrual cycle. Tumor diathesis may be less prominent and seen as debris clinging to the periphery of the abnormal cell clusters in *LBP*s. Follow-up showed invasive endocervical adenocarcinoma

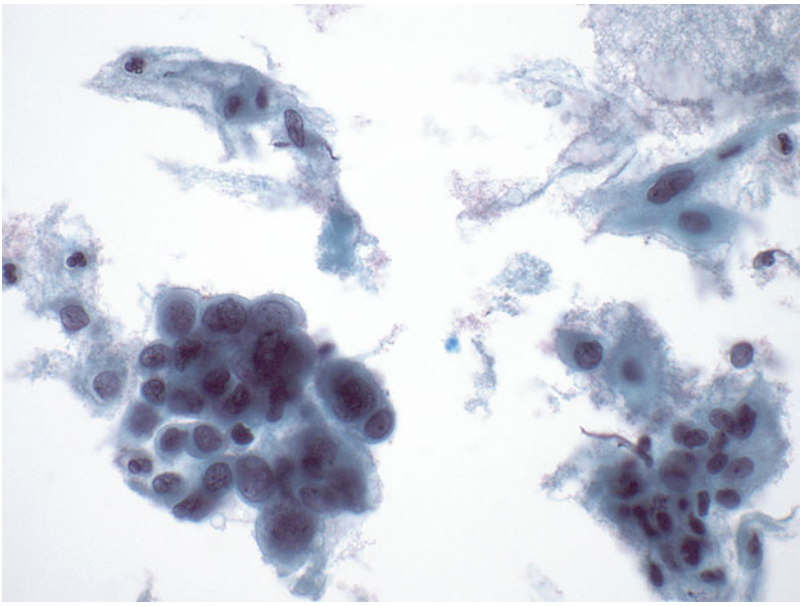

**Fig. 6.41** Adenocarcinoma, endocervical (*LBP*, *ThinPrep*). Note the prominent wispy or frothy diathesis surrounding the malignant cells and present as a coagulum in the background. This type of diathesis is common in liquid-based preparations due to the immediate fixation of material

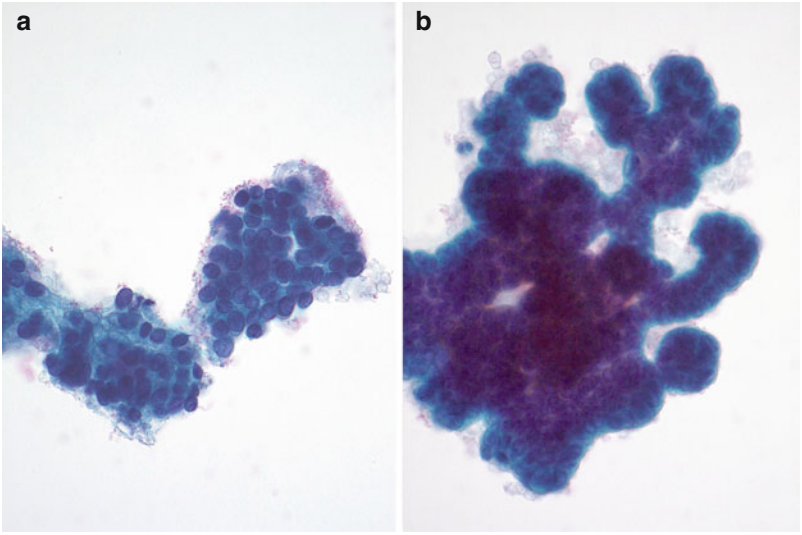


**Fig. 6.42** Adenocarcinoma, endocervical villoglandular (*LBP*, *ThinPrep*). A rare neoplasia of the cervix, villoglandular carcinoma may demonstrate large cohesive groups of endocervical cells with nuclear crowding and loss of normal honeycomb pattern, with true papillary clusters being characteristic. (**a**) Cytologic atypia is often minimal, emphasizing the importance of appreciating the low-power architectural abnormalities of this neoplasm (**b**)

### Preparation-Specific Criteria

*Liquid-Based Preparations* [33, 34]:

Cell groups tend to be denser, spherical, and three-dimensional; nuclei within the central portions of groups may be completely obscured.

Isolated abnormal cells are more frequently seen.

Chromatin is more vesicular with irregular chromatin distribution and chromatin clearing (Fig. 6.39).

Nucleoli are more prominent (Fig. 6.38).

Tumor diathesis is less apparent, consisting of aggregates of proteinaceous and inflammatory debris often found clinging to the surface of individual cells or cell clusters in a pattern that has been referred to as "clinging diathesis" (Fig. 6.40). SurePath specimens have a finer "cotton candy" diathesis (Fig. 6.41).

## 6.9.1.2 Explanatory Notes

An invasive adenocarcinoma should be strongly considered in the presence of tumor diathesis, nuclear clearing with uneven distribution of chromatin, or macronucleoli, [5, 6, 11] although in well-differentiated cases, tumor diathesis and macronucleolus formation may be minimal. The cytologic presentations of various histologic types of invasive endocervical adenocarcinoma have been

described [5, 11, 36–39]. Villoglandular adenocarcinomas are important because they arise in younger women than do the usual type and because they are often only superficially invasive. This combination allows for conservative management in low-stage tumors for women who still desire childbearing, and thus they are important to recognize. Villoglandular adenocarcinomas present as well-differentiated lesions showing pseudostratified strips of epithelium which are often arranged as large branching tissue fragments or as bulbous groups [39] (Fig. 6.42).

Mucinous carcinomas (minimal deviation adenocarcinoma or well-differentiated mucinous adenocarcinoma (adenoma malignum)) may be difficult to recognize in cytologic specimens. These tumors show gastric-type differentiation and are not typically associated with HPV. Hence, hrHPV testing and p16 immunostains will be negative. Adenoma malignum shows cells with bland nuclear features and low nuclear to cytoplasmic ratios. The cytoplasm shows abundant mucin or goblet cell differentiation and in some cases has a characteristic yellowish tinge resembling gastric foveolar epithelium [37, 38]. The large abnormally configured sheets of cells, with nuclear crowding, diathesis, background mucin, and the presence of rare groups of highly atypical cells aid in arriving at the correct interpretation (Figs. 6.43, 6.44, and 6.45).

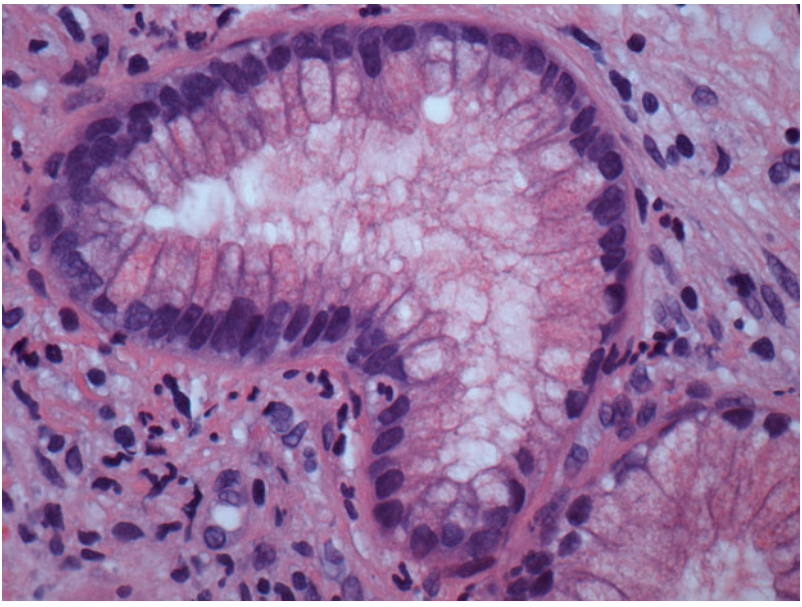

**Fig. 6.43** Mucinous carcinoma, gastric type (adenoma malignum) (H&E biopsy). Note the bland nuclear morphology and the similarity to normal mucinous endocervical epithelium

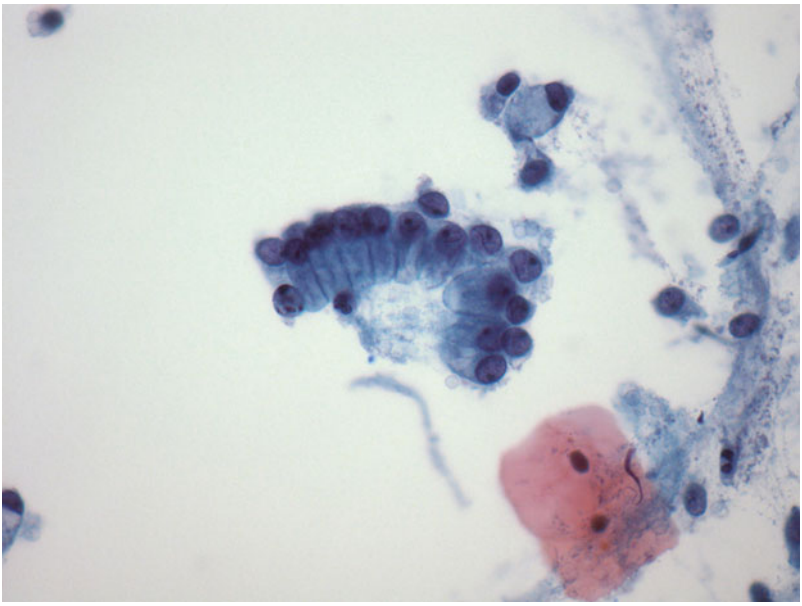

**Fig. 6.44** Mucinous carcinoma, gastric type (adenoma malignum) (*LBP*, *SurePath*). Abundant mucinous cytoplasm and occasional goblet cells are present. Note bland nuclear morphology similar to what is noted in the histology (see Fig. 6.43)

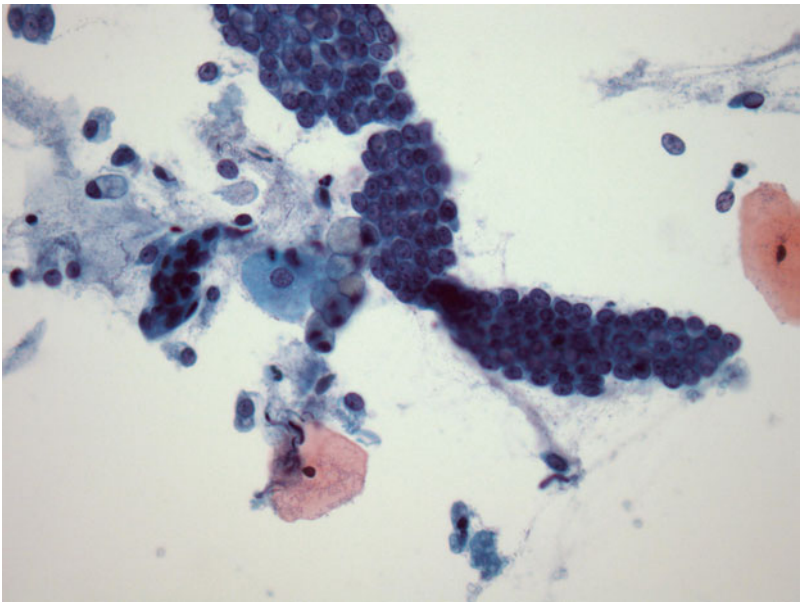

**Fig. 6.45** Mucinous carcinoma, gastric type (adenoma malignum) (*LBP*, *SurePath*). Note the centrally located goblet cells with typical brown/yellow hue to the mucin, consistent with pyloric differentiation

### 6.9.2 Endometrial Adenocarcinoma (Figs. 6.46–6.54)

#### 6.9.2.1 Criteria

Cells typically occur singly or in small, tight clusters (Fig. 6.46).

In well-differentiated tumors, nuclei may be only slightly enlarged compared to nonneoplastic endometrial cells, becoming larger with increasing grade of the tumor (Fig. 6.49).

Variation in nuclear size and loss of nuclear polarity.

Nuclei display moderate hyperchromasia, irregular chromatin distribution, and chromatin clearing, particularly in high-grade tumors (Fig. 6.48).

Small to prominent nucleoli; nucleoli become larger with increasing grade of tumor.

Cytoplasm is typically scant, cyanophilic, and often vacuolated.

Isolated cells or small groups of tumor cells may show intracytoplasmic neutrophils, often with the appearance of a "bag of polys" (Fig. 6.54).

A finely granular or "watery" tumor diathesis is variably present, most commonly in conventionally prepared specimens (Fig. 6.47).

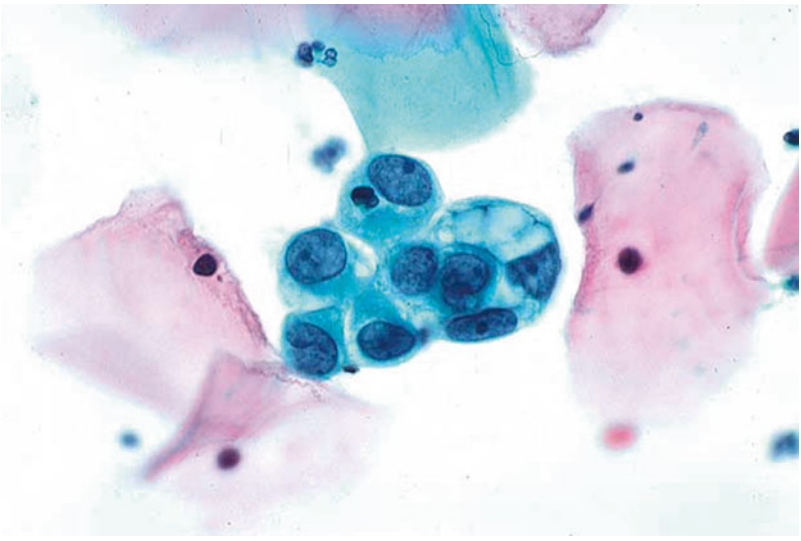

**Fig. 6.46** Adenocarcinoma, endometrial, low grade (*CP*)

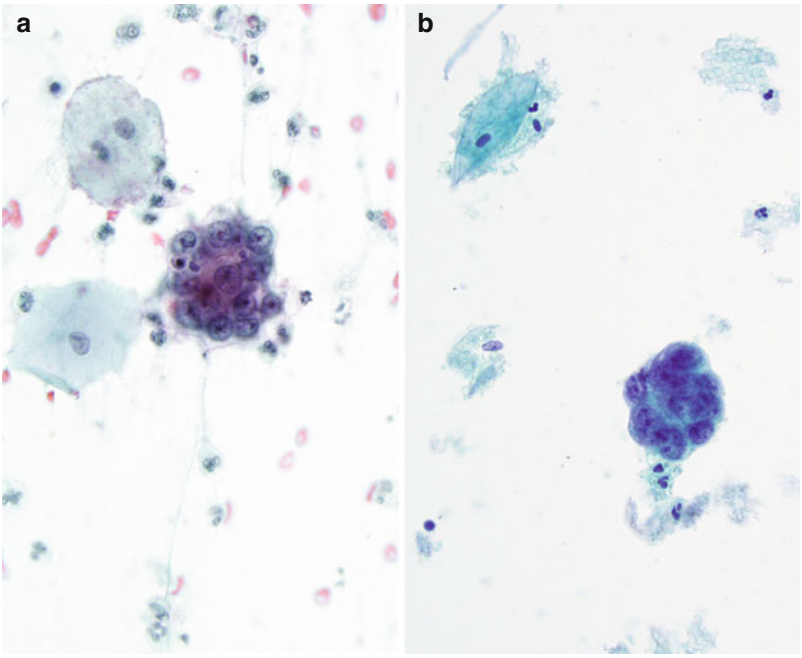


**Fig. 6.47** Endometrial adenocarcinoma, high grade. (**a**) A 61-year-old woman with postmenopausal bleeding (*CP*). (**b**) A 57-year-old woman with PM bleeding (*LBP*, *ThinPrep*). High-grade endometrial adenocarcinoma is characterized by tight clusters of glandular endometrial type cells with enlarged hyperchromatic nuclei and a clinging granular diathesis as well as a precipitate of acellular diathesis material in the background. Nucleoli are prominent, and chromatin is coarse and irregularly distributed. As grade increases, larger numbers of cells are shed and present in the cervical cytologic specimen. Both cases seen here had histologic follow-up of endometrial adenocarcinoma FIGO grade 3

### Preparation-Specific Criteria

*Liquid-Based Preparations*:

Three-dimensional groups and clusters or papillary configurations are more common (Fig. 6.50).

Nuclei tend to be larger with finely granular chromatin.

Tumor diathesis may be less prominent and seen as finely granular debris clinging to the periphery of clusters of abnormal cells or as coagulated debris (Fig. 6.53).

*Conventional Preparations*:

Diathesis presents as granular debris throughout the background ("watery" diathesis) (Fig. 6.52).

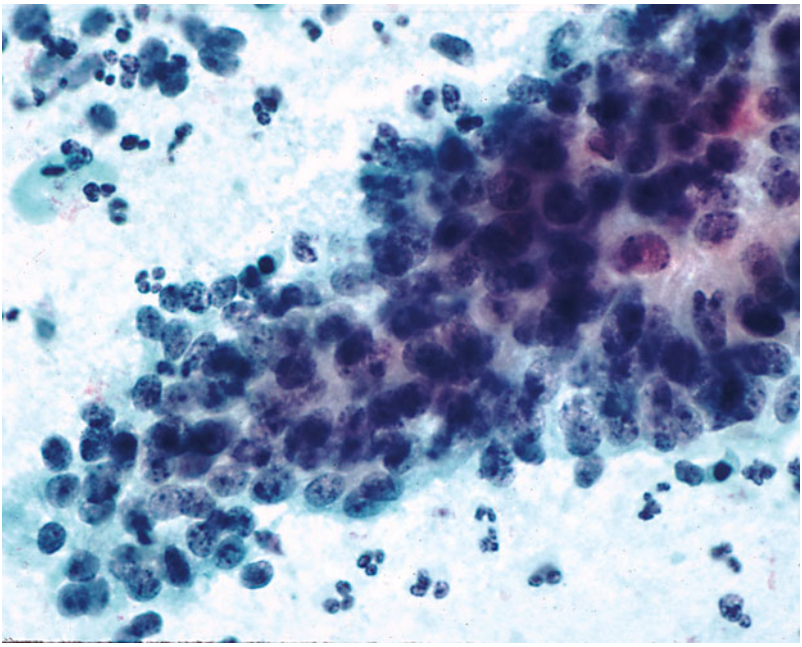

**Fig. 6.48** Adenocarcinoma, endometrial, high grade (*CP*). A 58-year-old woman with postmenopausal bleeding. Nuclei in higher-grade tumors are larger and display moderate hyperchromasia with irregular chromatin distribution. Note finely granular diathesis in background. Follow-up showed high-grade endometrial adenocarcinoma

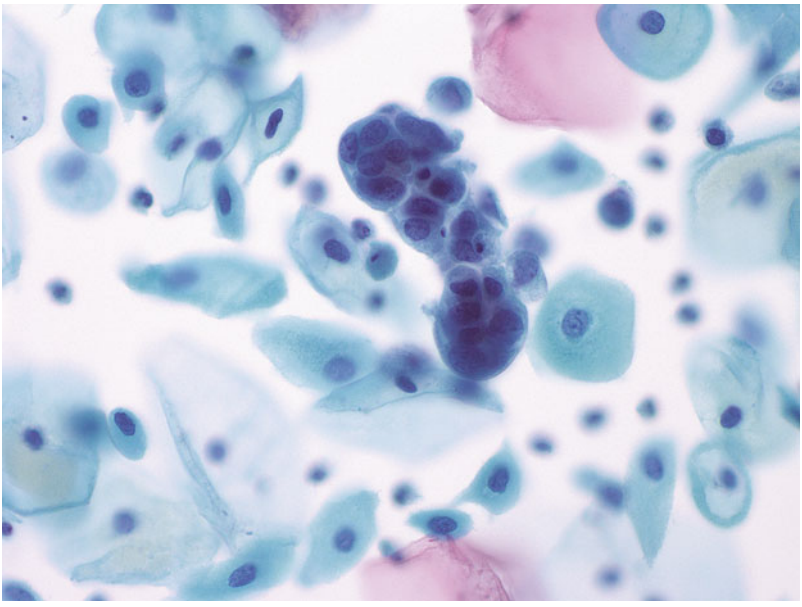

**Fig. 6.49** Endometrial adenocarcinoma on (*LBP*, *SurePath*). Large tight cluster of hyperchromatic and enlarged endometrial cells with some maturation of background normal cells. Endometrial biopsy showed a FIGO grade 2 endometrioid adenocarcinoma

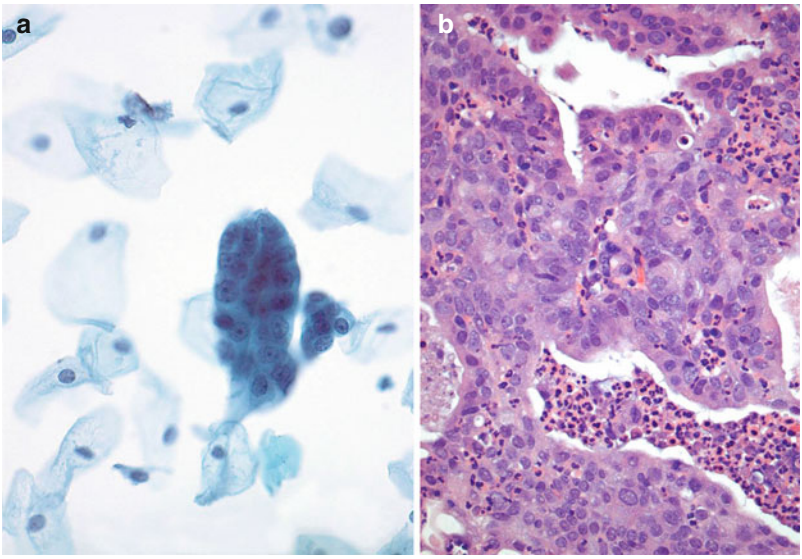


**Fig. 6.50** Adenocarcinoma, endometrial. A 67-year-old woman with postmenopausal bleeding. (**a**) Three-dimensional cell group with papillary configuration (*LBP*, *SurePath*). (**b**) Follow-up histology (biopsy H&E) showed endometrial adenocarcinoma grade 1–2

### 6.9.2.2 Explanatory Notes

The cytologic findings in endometrial adenocarcinoma are largely dependent upon the grade of the tumor. Grade 1 tumors tend to shed few abnormal cells with minimal cytologic atypia and would typically be interpreted as atypical endometrial cells (see Figs. 6.15, 6.16, 6.17, and 6.18). Cytologic detection of endometrial adenocarcinoma, especially well-differentiated tumors, in cervical specimens is limited by the small number of well-preserved abnormal cells and the subtlety of their cellular alterations. In contrast to endocervical adenocarcinomas that are directly sampled, the detection of endometrial carcinomas in cervical cytology depends on exfoliated cells being present in the collected specimen. Thus, there are generally fewer abnormal cells present as compared to endocervical cancers (Figs. 6.46 and 6.50). In addition, the malignant cells from endometrial carcinomas generally have a smaller cell and nuclear area, nucleoli are less prominent, and tumor diathesis if present is "watery" or finely granular and more difficult to appreciate [5, 6, 11, 14]. High-grade endometrial serous carcinomas morphologically resemble their ovarian counterpart with papillary fragments, large cell size, and prominent nucleoli (Fig. 6.54). Endometrial cancers are hrHPV negative.

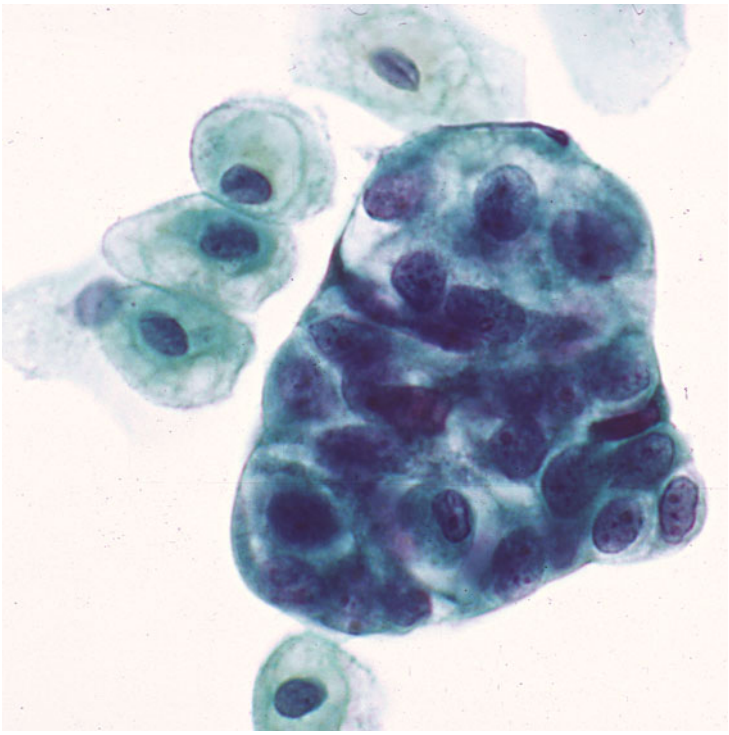

**Fig. 6.51** Adenocarcinoma, endometrial (*LBP*, *ThinPrep*). A 64-year-old woman. Papillary serous carcinomas may resemble their ovarian counterparts and present with papillary groups, large cell size, and prominent nucleoli. Follow-up showed papillary serous adenocarcinoma of the endometrium

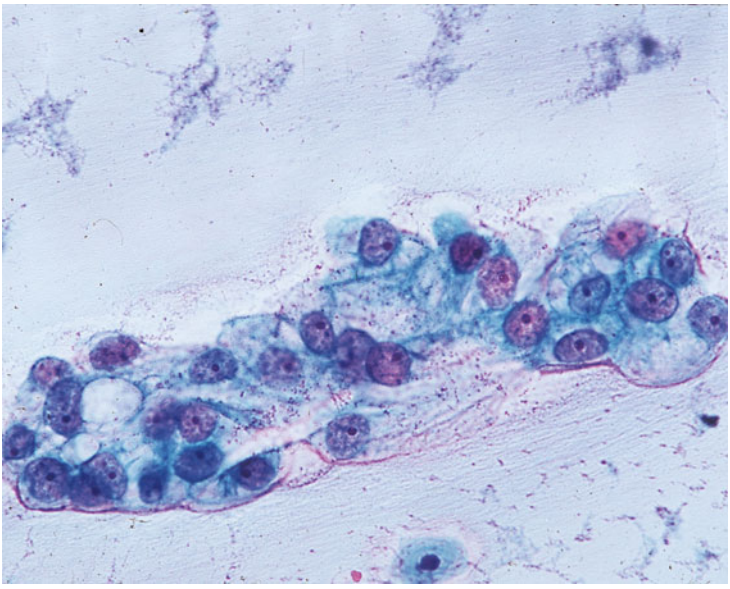

**Fig. 6.52** Adenocarcinoma, endometrial, high grade (*CP*). Tumor diathesis, if present, is watery and may be difficult to appreciate (Reprinted with permission from Kurman [40])

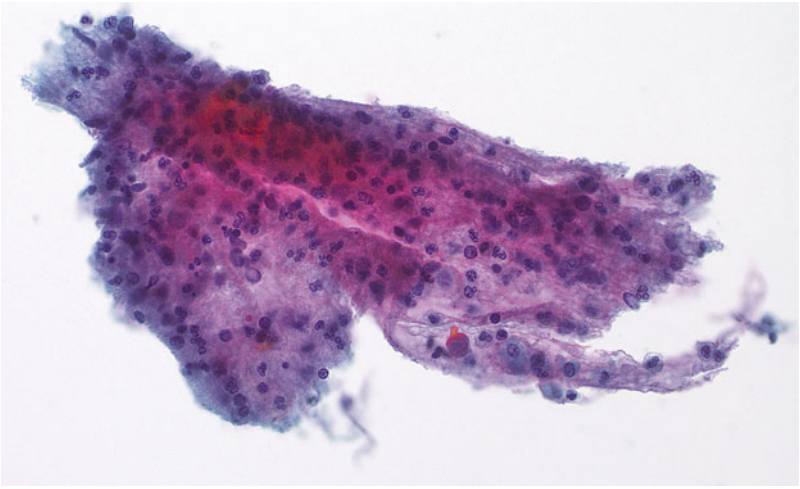

**Fig. 6.53** Adenocarcinoma, endometrial (*LBP*, *ThinPrep*). Amorphous, finely granular ("wrinkled tissue paperlike") diathesis. The malignant and inflammatory cells may be trapped in the diathesis. There is usually a clear space surrounding this type of diathesis as there is shrinkage once fixed in alcohol after the *ThinPrep* is prepared

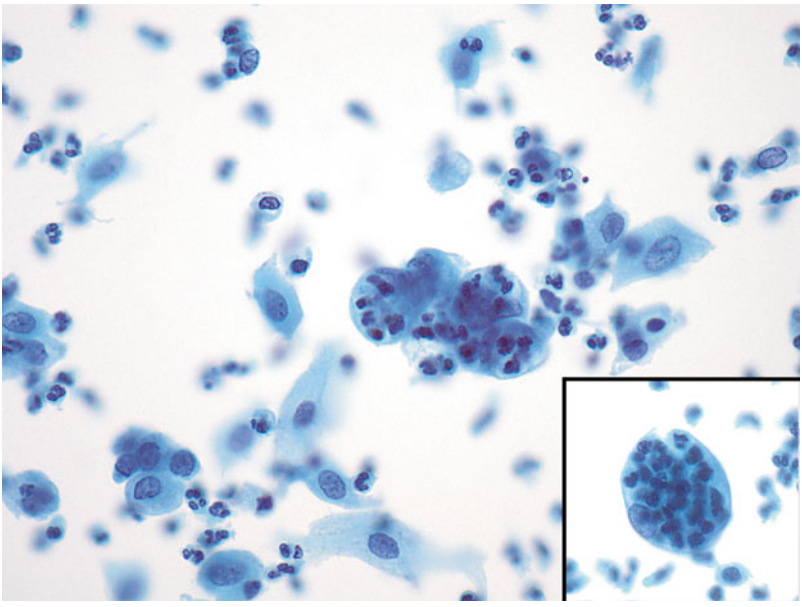

**Fig. 6.54** Endometrial adenocarcinoma (*LBP*, *SurePath*). Endometrial adenocarcinoma often shows cells with prominent cytoplasmic vacuoles full of neutrophils ("bag of polys cells") (*inset*—high magnification)

### 6.9.3 Extrauterine Adenocarcinoma (Figs. 6.55–6.59)

When cells diagnostic of adenocarcinoma occur in association with a clean (no diathesis) background or with morphology unusual for tumors of the uterus or cervix, an extrauterine neoplasm should be considered. Sources still within the female genital tract include the ovary and fallopian tube [6, 11]. Although not specific, the presence of papillary clusters and psammoma bodies suggests a Mullerian carcinoma (Figs. 6.55, 6.56, and 6.57). Because they are exfoliated and travel from distant sites, the malignant cells may show degenerative changes. When diathesis is present with a suspected extrauterine tumor, it is usually associated with metastasis or direct extension to the uterus or vagina, most commonly from the colon or bladder [11] (Fig. 6.58). Breast cancer may also present in cervical cytologic specimens. Lobular carcinomas that present in a background of atrophy can be particularly problematic to identify (Fig. 6.59). Other tumors metastatic to the cervix or uterus are considered in Chap. 7.

**A synopsis of the different cytologic presentations of glandular malignancies is presented in Table 6.2.**

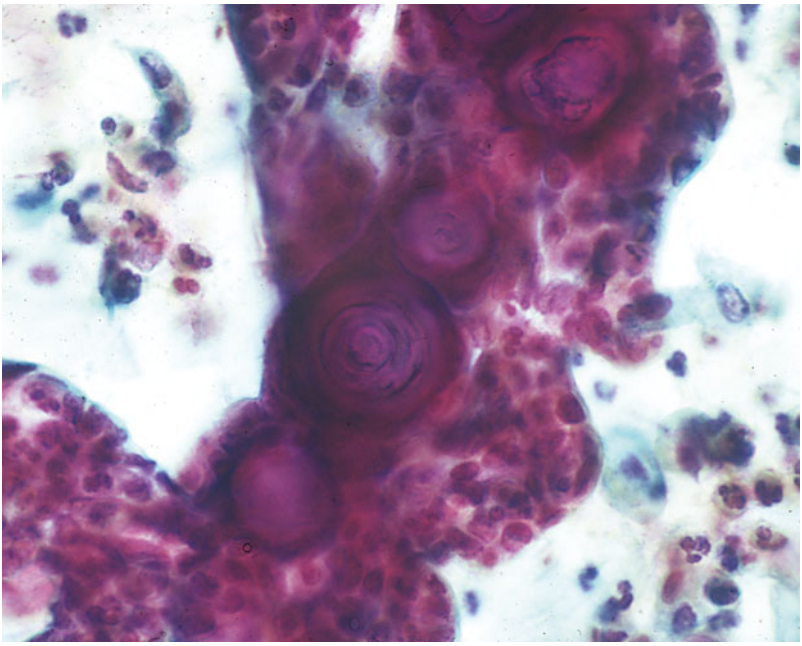

**Fig. 6.55** Adenocarcinoma, extrauterine (*CP*). A 70-year-old woman with large pelvic mass and ascites. Ovarian/tubal/peritoneal carcinoma may be characterized by papillary configurations and psammomatous calcifications (psammoma bodies). Follow-up showed an ovarian primary

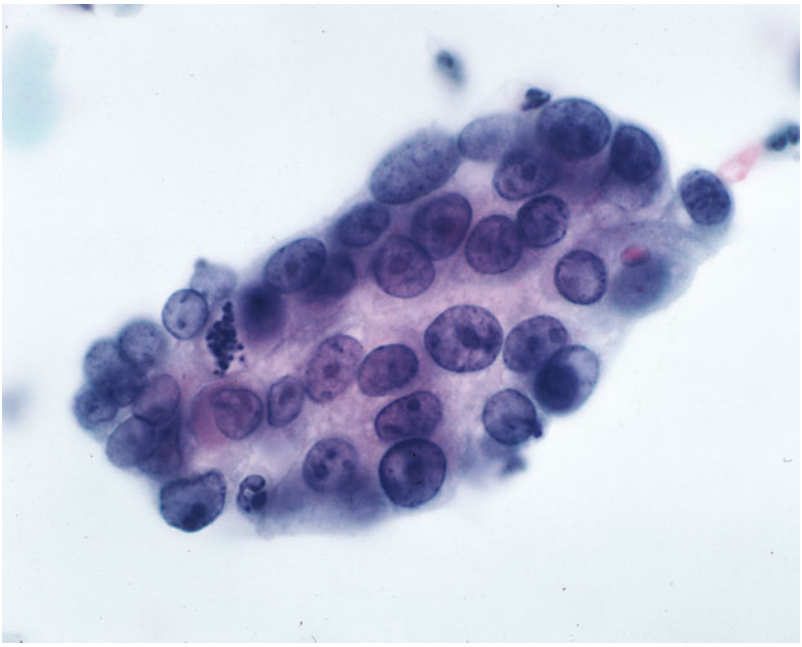

**Fig. 6.56** Adenocarcinoma, extrauterine (*CP*). Clusters of cells from ovarian carcinoma have enlarged, variably sized round or oval nuclei with prominent macronucleoli. The background is typically clean

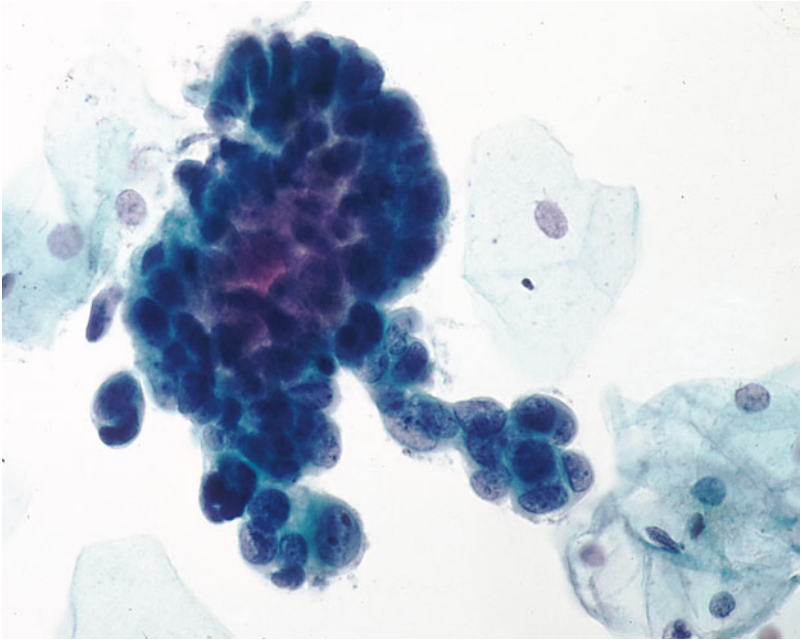

**Fig. 6.57** Adenocarcinoma, extrauterine (*LBP*, *ThinPrep*). A 66-year-old woman with pelvic mass and ascites. Papillary clusters from ovarian carcinoma may be three-dimensional, making evaluation of the component cells difficult. Follow-up showed intra-abdominal dissemination of ovarian carcinoma

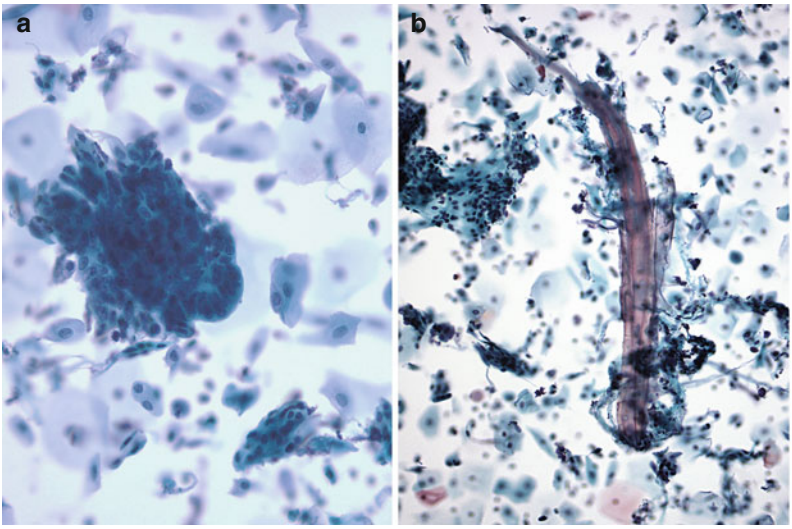


**Fig. 6.58** Colonic adenocarcinoma (*LBP*, *SurePath*). Adenocarcinoma of the colon typically involves cervical specimens by direct invasion. (**a**) A columnar architecture can closely mimic endocervical adenocarcinoma. (**b**) The presence of background vegetable material (fecal material) is a clue to the diagnosis

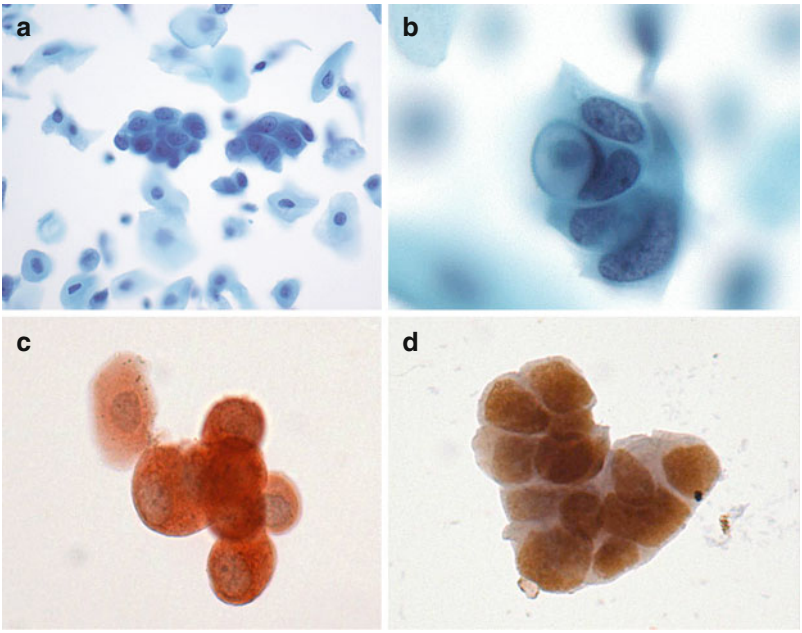


**Fig. 6.59** (**a–d**) These are images of lobular breast carcinoma (*LBP*, *SurePath*). Lobular breast cancers presenting in an atrophic background can be challenging. (**a**) Small clusters of cells and (**b**) individual cells with mucin vacuoles contrast with a background of parabasal cells. Confirmation with immunostains can be helpful, including (**c**) gross cystic disease fluid protein 15 and (**d**) estrogen receptor immunocytochemistry

**Table 6.2** Cytologic distinction between endocervical, endometrial, and extrauterine cancers

| Features | Endocervical Ca | Endometrial Ca | Extrauterine Ca |
|---|---|---|---|
| Cellularity | Hypercellular | Low cellularity usually | Rare cells (unless direct extension/mets) |
| Pattern | Strips, rosettes, sheets with feathering, single malignant cells | Small clusters, rarely papillae, single cells | Varies depending upon primary and mode of spread |
| Diathesis | Visible, type varies by preparation | Variable, watery or subtle or absent | Usually absent unless direct spread or mets |
| Cell shapes | Oval, columnar, pleomorphic | Round, irregular, usually smaller | Variable, do not belong |
| Nuclei | Oval, elongated, pleomorphic, vesicular | Round, irregular in higher grade | Variable |
| Cytoplasm | Mucin + | Degenerative vacuoles | Variable |
| SIL or Sq Ca | Present in >50 % | Absent | Absent |
| High-risk HPV | Positive in most | Negative | Negative |
| p16 | Block positive | Patchy/focal except in high grade/serous | Variable, depends on type |

Adapted from Mody [11]

## 6.10 Sample Reports

**Example 1**
*Adequacy:*
Satisfactory for evaluation; endocervical/transformation zone present
*General Categorization:*
Epithelial cell abnormality, glandular
*Interpretation:*
Atypical endometrial cells present (not otherwise specified)

**Example 2**
Satisfactory for evaluation; endocervical/transformation zone present
Epithelial cell abnormality, glandular
Atypical endocervical cells present, not otherwise specified. See note.

*Note:* The findings may represent benign tubal metaplasia; however, an endocervical neoplastic lesion cannot be excluded. Further investigation is recommended if clinically indicated.

**Example 3**
Satisfactory for evaluation; endocervical/transformation zone present
Epithelial cell abnormality, glandular
Atypical glandular cells present, favor neoplastic. See note.

*Note*: Suggest colposcopy (with endocervical sampling) and endometrial sampling (if >35 years old or abnormal bleeding) as clinically indicated.

*Reference*: Massad LS, Einstein MH, Huh WK, et al. 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. J Low Genit Tract Dis. 2013;17:S1–27.

**Example 4**
Satisfactory for evaluation
Epithelial cell abnormality, glandular
Endocervical adenocarcinoma in situ

**Example 5**
*Adequacy:*
Satisfactory for evaluation
*Interpretation:*
Epithelial cell abnormality, glandular
Adenocarcinoma, favor endometrial origin

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# Other Malignant Neoplasms

# 7

Sana O. Tabbara and Walid E. Khalbuss

## 7.1 Background

Malignant neoplasms, other than squamous and adenocarcinoma, infrequently involve the uterine cervix but nevertheless present in cervical cytologic preparations [1–4]. Most often these tumors are uncommon gynecologic primaries arising in the uterine corpus or adnexa that appear in the cervical preparation, either as exfoliated cells, or via direct sampling of tumors that involve the cervix or vagina by direct extension. Secondary or metastatic tumors to the uterine cervix are seen rarely, owing to the nature of the lymphatic drainage and low vascularity of the cervix [2, 5]. In general, a definitive classification of the tumors described in this chapter may not be possible on cytologic preparations alone because of limited sampling and cytomorphologic overlap with other entities, creating interpretation pitfalls. However, familiarity with these entities is useful when unusual tumor morphology is encountered. Recognition of these rare tumors may help decrease the potential for misinterpretation and allow for more appropriate patient management.

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DOI 10.1007/978-3-319-11074-5_7

## 7.2 Uncommon Primary Tumors of the Cervix and Uterine Corpus (Figs. 7.1–7.9)

### 7.2.1 Carcinomas

#### 7.2.1.1 Spindle Squamous Cell Carcinoma (Fig. 7.1)

Spindle squamous cell carcinoma is a poorly differentiated variant of squamous cell carcinoma characterized by pleomorphic, spindled nonkeratinizing cells with high mitotic activity [6, 7]. The differential considerations include sarcoma and malignant melanoma with spindle cell features. An immunocytochemical panel for vimentin, S100 protein, and cytokeratin with positivity for cytokeratin may be helpful to demonstrate an epithelial origin.

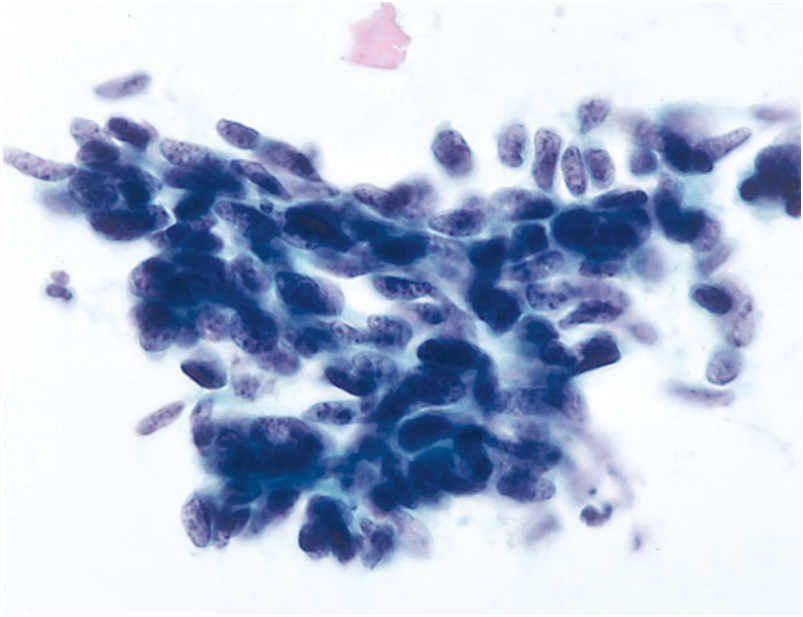

**Fig. 7.1** Spindle cell carcinoma (*CP*). Spindle-shaped nonkeratinizing cells displaying variability in nuclear size, nuclear membrane irregularity, coarse granular chromatin, and conspicuous nucleoli are arranged in a loosely cohesive cluster. The cytologic features are not specific and could be compatible with sarcoma, spindle cell carcinoma, or malignant melanoma

#### 7.2.1.2 Poorly Differentiated Squamous Carcinoma with Small Cells (Fig. 7.2)

Poorly differentiated squamous carcinoma with small cells morphologically resembles a high-grade squamous intraepithelial lesion of small cell size and may also be confused with true small cell (neuroendocrine) carcinoma [6] (see below). The cells have more cytoplasm, greater cytoplasmic density, better definition of cell borders, coarsely granular chromatin, and less crush artifact than do those of small cell carcinoma [3]. The lack of definitive nuclear molding and background necrosis and the identification of a squamous component favor squamous cell carcinoma [4]. Ancillary studies (see below) can be helpful in arriving at the correct interpretation. While neuroendocrine markers are negative in squamous carcinoma, p63 and p40 will show some positvity.

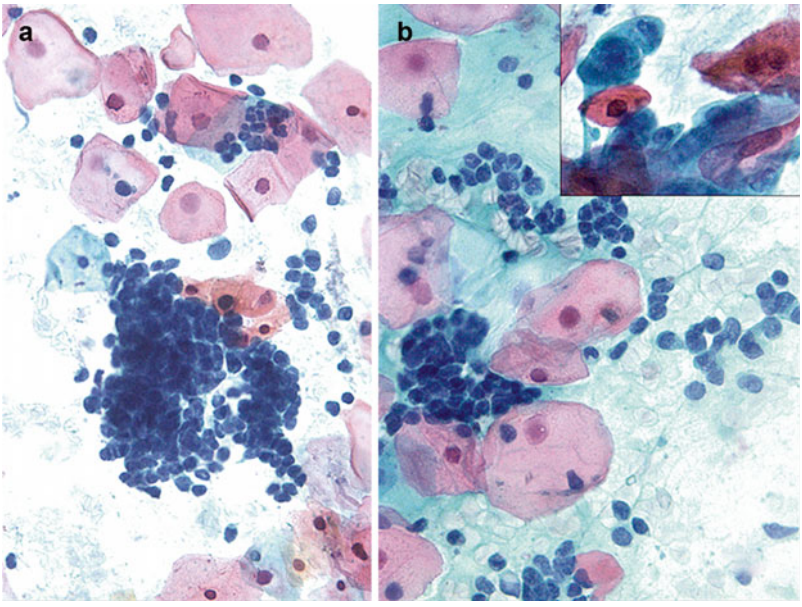


**Fig. 7.2** (**a**, **b**) Squamous cell carcinoma with small cells (*CP*). Clusters of small cells with scant cytoplasm and small cell morphology with crowded nuclei and attempt at nuclear molding. The *inset* shows single cells with characteristic squamous cell features and dense cytoplasm

### 7.2.2 Neuroendocrine Tumors

These uncommon tumors may occur over a wide age range and account for 1–5 % of cervical malignancies. Neuroendocrine tumors are classified in the 2014 World Health Organization terminology as low-grade neuroendocrine tumors (carcinoid and atypical carcinoid) and high-grade neuroendocrine carcinomas (small cell neuroendocrine carcinoma and large cell neuroendocrine carcinoma) [8].

#### 7.2.2.1 High-Grade Neuroendocrine Carcinoma (Small Cell Carcinoma) (Fig. 7.3)

This malignant neoplasm comprises a small minority of all cervical carcinomas [1, 6]. As at other body sites, this tumor is highly aggressive and is treated differently from other malignancies of the cervix. Small cell carcinoma is composed of relatively uniform small, cells with scant cyanophilic cytoplasm. Characteristically, the cells are seen singly and in loosely cohesive groups with nuclear molding and "crush artifact" being frequent findings. The nuclei are angulated, hyperchromatic with granular or stippled chromatin and inconspicuous nucleoli. Background necrosis and mitotic figures are common. Although the cytologic features of small cell carcinoma of the cervix are similar to those described in the lung and other body sites [6, 9–11], in the cervix these tumors are strongly associated with human papillomavirus (HPV) 16 and 18, an association not found at other primary sites [12].

The differential diagnosis includes poorly differentiated squamous carcinoma with small cells, poorly differentiated adenocarcinoma, low-grade endometrial stromal sarcoma, and lymphoma. The interpretation of small cell carcinoma should be reserved for

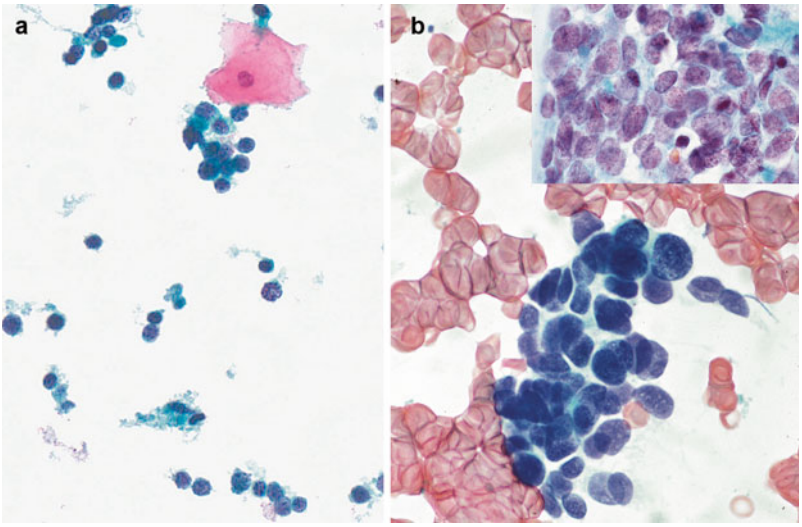


**Fig. 7.3** (**a**, **b**) Small cell undifferentiated carcinoma. (**a**) The malignant cells are dispersed in loosely cohesive clusters. They show nuclear pleomorphism and more conspicuous nucleoli. Nuclear molding although present is less prominent and crush artifact is absent (*left*, *LBP*, *ThinPrep*). (**b**) Small to medium-sized cells with minimal cytoplasm, high nuclear/cytoplasmic ratio, hyperchromatic nuclei, inconspicuous nucleoli, and prominent nuclear molding. The *upper right inset* shows a characteristic finely granular, stippled "neuroendocrine" chromatin pattern (*right*, *CP*)

tumors composed of small cells in which squamous or glandular differentiation is absent or minimal [6]. The presence of abnormal keratinized cells would favor an interpretation of poorly differentiated squamous cell carcinoma. If residual material from a liquid-based specimen is available, immunocytochemical staining for neuroendocrine markers, CD56, synaptophysin, chromogranin, and rarely TTF-1, can be useful to demonstrate neuroendocrine features. Other entities in the differential diagnosis include unusual malignant neoplasms including small cell primitive neuroectodermal tumor [3, 14], myeloid sarcoma [15], melanoma, and undifferentiated sarcoma or undifferentiated carcinoma.

#### 7.2.2.2 Large Cell Neuroendocrine Carcinoma (Fig. 7.4)

This is an extremely rare and aggressive poorly differentiated cancer. It may occur during pregnancy and may also arise from a cervical polyp. The cytomorphology can be mistaken for squamous or adenocarcinoma. Cervical cytology preparations show large cells dispersed singly or arranged as loosely cohesive sheets or hyperchromatic crowded groups or gland-like aggregates. Tumor cells have moderately abundant cytoplasm with small to large angulated hyperchromatic nuclei. The nuclei are mildly pleomorphic with coarse chromatin and prominent nucleoli [16]. Mitotic figures are common, and karyorrhectic debris can be identified with no keratinization seen. Ancillary studies can be performed on cell block material and will show positive immunostaining for neuroendocrine markers, similar to small cell carcinoma.

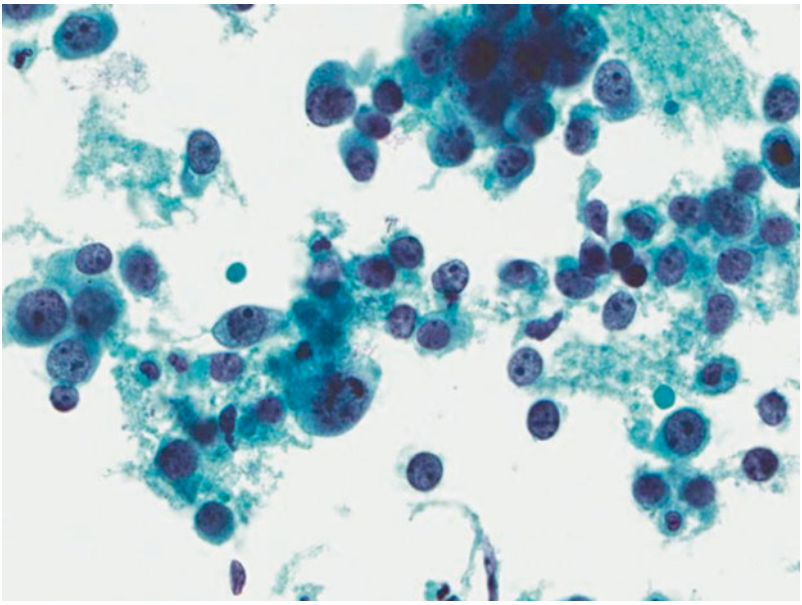

**Fig. 7.4** Large cell neuroendocrine carcinoma (*LBP*, *ThinPrep*). The malignant cells are larger than those of small cell carcinoma with more cytoplasm and are arranged in loosely cohesive clusters. The nuclei are only mildly pleomorphic with one or more prominent nucleoli and coarser chromatin. No crush artifact or nuclear molding is observed

#### 7.2.2.3 Low-Grade Neuroendocrine Tumor (Carcinoid Tumor)

These are rare primary tumors of the cervix. The small cells with high nuclear to cytoplasmic ratio resemble those in small cell carcinoma but lack nuclear molding, necrosis, and frequent mitoses [5, 12]. More abundant granular cytoplasm and areas of "organoid" architectural differentiation are more commonly present when compared to high-grade neuroendocrine neoplasms. Adenocarcinomas of the cervix may occasionally demonstrate "carcinoid-like" features [8].

### 7.2.3 Glassy Cell Carcinoma (Fig. 7.5)

Glassy cell carcinoma of the cervix is a rare variant of poorly differentiated adenosquamous carcinoma that affects younger patients [4, 17] and is associated with HPV types 18 and 16 [18]. Characteristically, tumor cells are arranged in sheets and clusters with large abundant granular (ground glass-like) cytoplasm and large pleomorphic nuclei. The nuclei have coarse irregular chromatin and distinctive prominent nucleoli [17] that may be mistaken for inclusions of herpes virus or Reed-Sternberg cells in Hodgkin disease. Cytoplasmic vacuolization and bizarre cells with multinucleation can be seen. The tumor may be associated with an eosinophilic lymphoplasmacytic infiltrate in the background. Dyskeratosis and intracellular glycogen may not be appreciated. The differential diagnosis is with other

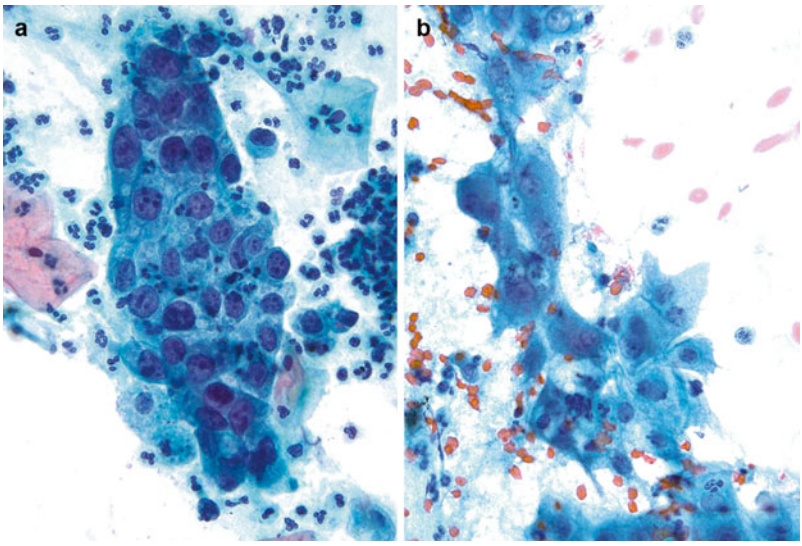


**Fig. 7.5** (**a**, **b**) Glassy cell carcinoma (*LBP*, *ThinPrep*). The tumor cells are arranged in sheets with abundant granular, ground glass-like cytoplasm. Large pleomorphic nuclei, coarse irregular chromatin, and prominent (inclusion-like) nucleoli are characteristic. An inflammatory cell infiltrate is present

poorly differentiated neoplasms involving the cervix including nonkeratinizing squamous cell carcinoma, poorly differentiated adenocarcinoma, and clear cell carcinoma, or a metastasis/extension from the colon, endometrium, vagina, or urethra where glassy cell carcinomas have been reported.

### 7.2.4 Mucinous Carcinoma, Gastric Type (Minimal Deviation Adenocarcinoma, Adenoma Malignum) (Fig. 7.6)

This tumor comprises about 1 % of endocervical carcinomas, although higher prevalence rates have been reported in the Japanese literature [19]. It is, for the most part, hrHPV DNA negative [20–22]. Cervical cytology specimens show a large number of glandular cells that closely resemble benign endocervical cells arranged in clusters, strips, and isolated cells. Pseudostratified glandular strips, loss of polarity within clusters, a disorganized "drunken" honeycomb sheet arrangement, and a spectrum of atypical nuclear changes are the key diagnostic features. The individual cells are cuboidal to columnar and have abundant lacy, golden-yellow vacuolated cytoplasm containing neutral gastric/pyloric type mucin [23]. Marked nuclear enlargement (two to three times the size of intermediate squamous nuclei), nuclear

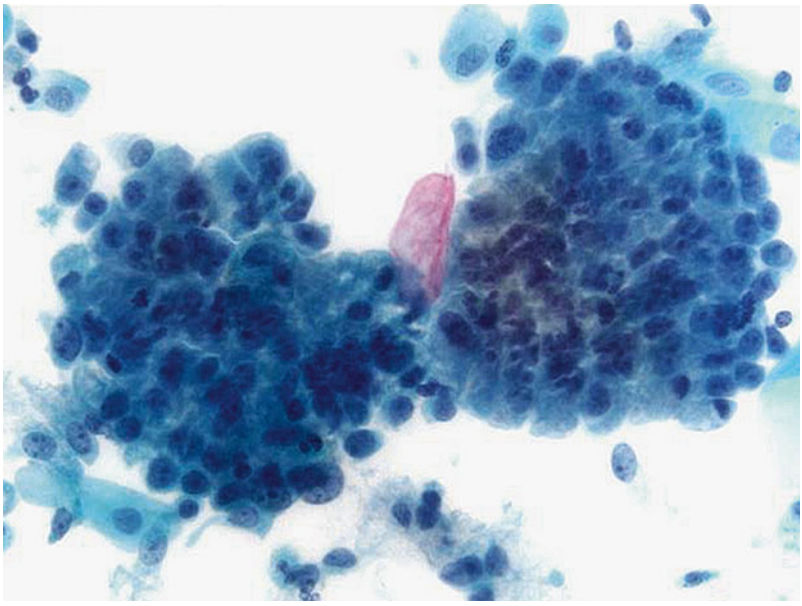

**Fig. 7.6** Minimal deviation adenocarcinoma/adenoma malignum (*LBP*, *SurePath*). Large numbers of glandular clusters with overall bland cytologic features, resembling benign endocervical cells show subtle nuclear pleomorphism, crowding, and loss of polarity. Cells have abundant, occasionally yellow/golden vacuolated cytoplasm. The nuclei are enlarged and may have visible nucleoli

pleomorphism, and visible nucleoli are seen only in a minority of these cell groups. This tumor is also positive for CEA, Ki67 (>50 % of tumor nuclei), and p53 while it is negative for estrogen and progesterone receptors.

The differential diagnostic considerations include benign endocervical glands, atypical glandular cells, adenocarcinoma in situ (AIS), and endometrial adenocarcinoma [20]. AIS shows loss of mucin and lacks abnormal single cells, which are both present in mucinous carcinomas. Tightly crowded sheets of glandular cells with overlapping nuclei, "ragged edged" borders, and feathering are distinctive features of AIS which are not present in mucinous carcinoma. Endometrial adenocarcinoma displays three-dimensional groupings with nuclear overlap or papillary architecture, pleomorphic hyperchromatic nuclei with irregularly distributed chromatin, and scant vacuolated cytoplasm containing intracytoplasmic neutrophils (see Figs. 6.46, 6.47, and 6.48).

### 7.2.5 Malignant Müllerian Mixed Tumor (MMMT) or Carcinosarcoma (Figs. 7.7 and 7.8)

MMMT is an uncommon and highly aggressive carcinosarcoma (<5 % of malignant neoplasms of the uterine corpus) that arises in the endometrium, but which may extend as a fungating mass into the cervical os. By definition, the tumor is

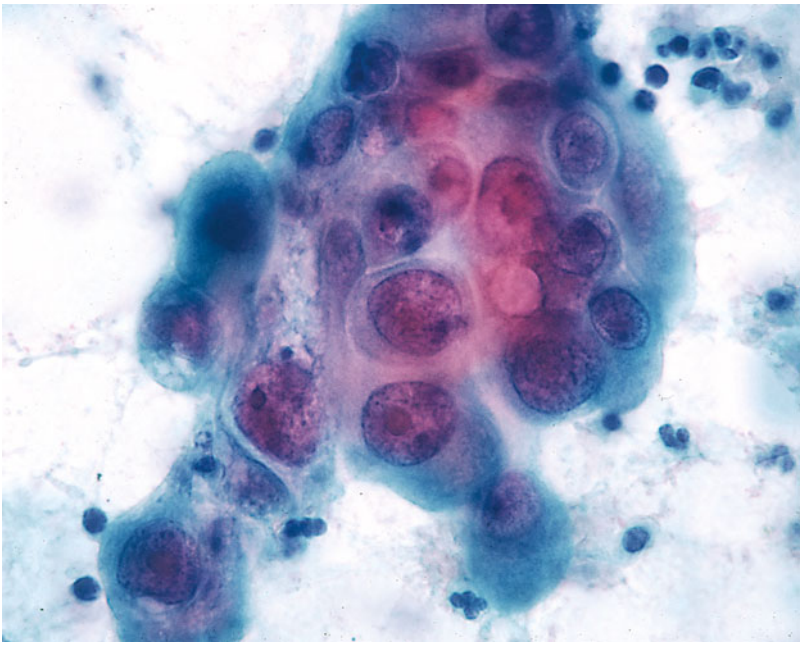

**Fig. 7.7** Malignant Müllerian mixed tumor (MMMT) (*CP*). Three-dimensional cluster of large epithelioid cells with round but pleomorphic nuclei, coarse granular chromatin, macronucleoli, and a moderate amount of cytoplasm

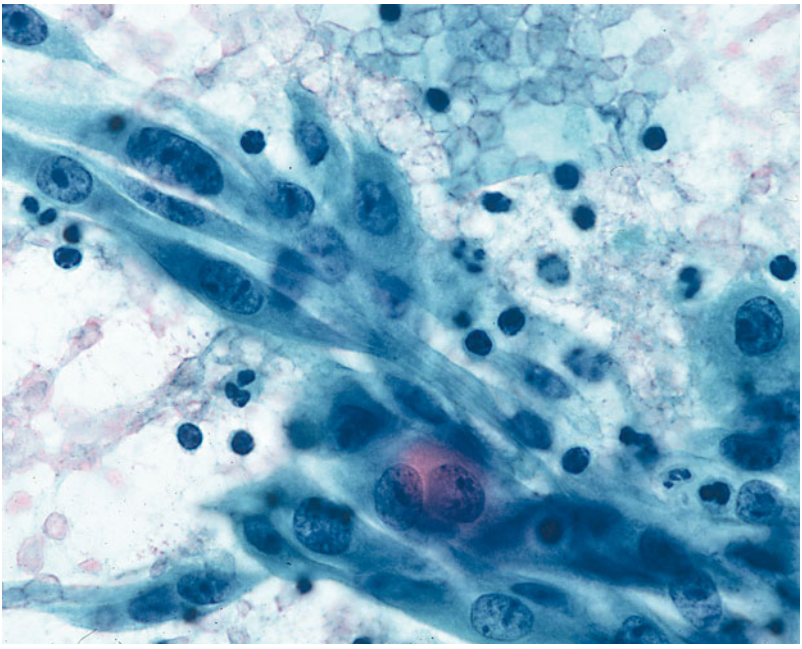

**Fig. 7.8** Malignant mixed mesodermal tumor (MMMT) (*CP*). Spindle cells with pleomorphic nuclei, coarse granular chromatin, macronucleoli, and a moderate amount of cytoplasm constitute the "sarcomatous" component of the same tumor depicted in Fig. 7.7

biphasic, being composed of malignant epithelial and mesenchymal components. The malignant epithelial component morphologically most often resembles poorly differentiated endometrioid adenocarcinoma; clear cell and serous differentiation are less frequent. Mesenchymal (sarcomatous) elements are usually endometrial stromal, fibroblastic, or leiomyosarcomatous. Occasional heterologous elements may include rhabdomyosarcoma, chondrosarcoma, or osteosarcoma. Recent clinicopathologic, immunohistochemical, and molecular genetic studies have provided strong evidence that MMMTs are best classified as variants of carcinoma.

Exfoliated malignant cells from the endometrium or direct sampling of extension of an MMMT to the cervix/vagina may yield malignant cells in a cervical cytology sample. Morphologic presentations of MMMT on cytologic preparations are usually hypercellular and show high-grade malignant tumor cells. The presence of both malignant epithelial (Fig. 7.7) and sarcomatous components (Fig. 7.8) suggests the possibility of MMMT. However, degeneration or limited sampling of poorly differentiated malignant cells may lead to interpretive difficulties [24, 25]. The differential diagnosis includes endometrial adenocarcinoma, pure sarcoma, botryoid rhabdomyosarcoma (seen in children/adolescents), and other poorly differentiated or undifferentiated tumors.

### 7.2.6 Clear Cell Adenocarcinoma (Fig. 7.9)

Clear cell adenocarcinoma of the cervix or the vagina is a rare tumor of Müllerian origin that [26] occurs most commonly in daughters of women who received diethylstilbestrol (DES), a nonsteroidal estrogen, during pregnancy. The peak age for DES-associated clear cell carcinoma is between 14 and 22 years and for non-DES-associated cases it ranges between 13 and 80 years. Cervical cytology specimens show cells that are arranged in sheets, clusters, or papillae [27]. The tumors contain cells with delicate, vacuolated, glycogen-rich cytoplasm, naked nuclei and a "tigroid" background similar to that seen in other glycogen-rich tumors. The nuclei are large, pale, and round with prominent nucleoli. HPV has been detected only in 40 % of clear cell carcinomas in both DES- and non-DES-associated cases [28].

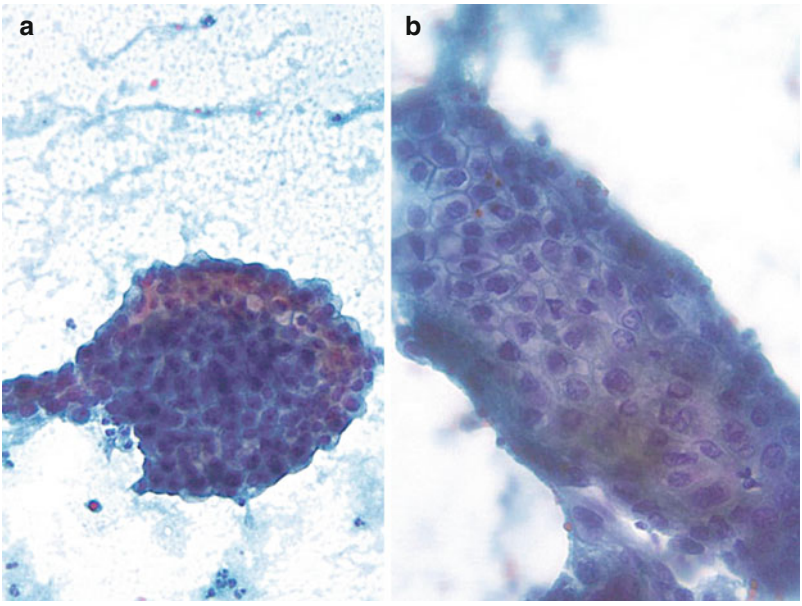


**Fig. 7.9** Clear cell adenocarcinoma (*CP*). Tumor cells with delicate finely granular cytoplasm and large pleomorphic nuclei are arranged in sheets and papillae (**a**, **b**). A "tigroid" background and stripped nuclei are also seen (**a**, *left*)

### 7.2.7 **Sarcomas** (Figs. 7.10–7.12)

Primary sarcomas of the female genital tract are rare; these can originate from the vagina, cervix, uterus, fallopian tubes, or ovaries, but most commonly arise in the uterine corpus. Sarcomas may be pure or mixed with epithelial components and usually present with degenerated, sparse, or isolated tumor cells in the cervical sample [1–3].

Pure sarcomas include leiomyosarcoma, rhabdomyosarcoma, fibrosarcoma [29], endometrial stromal sarcoma [30], Ewing/primitive neuroectodermal tumors (PNET) [13, 14], and myeloid sarcoma [15]. Most pure sarcomas present with undifferentiated, pleomorphic, multinucleated, and/or bizarre cells and cannot be further subtyped. If present, characteristic cytologic features such as spindle or strap cells or round blue cell cytomorphology may suggest the specific type of sarcoma [1–3, 8]. When sufficient cytologic material is available, immunohistochemistry may help further subcategorize the sarcoma.

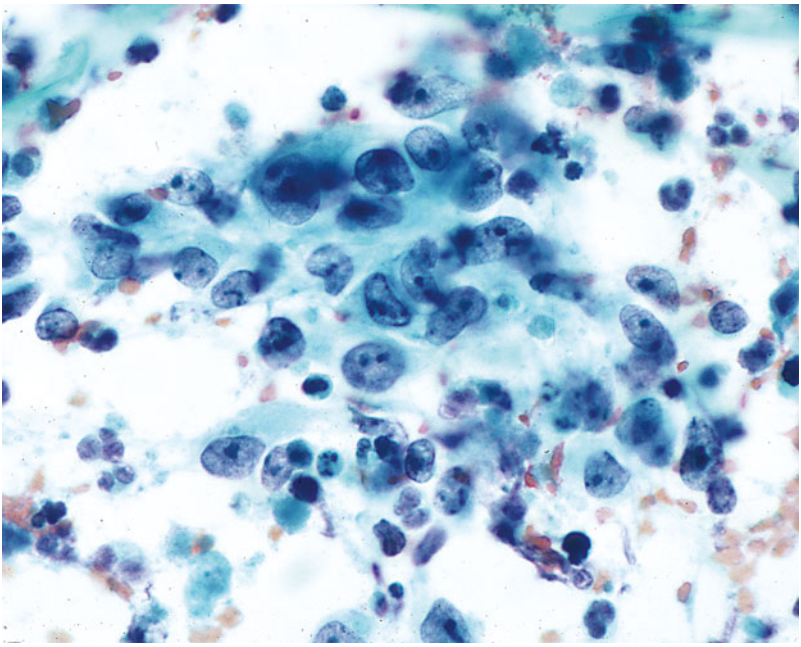

**Fig. 7.10** Sarcoma not otherwise specified (NOS) (*CP*). A loosely cohesive group of haphazardly arranged malignant cells with enlarged irregular nuclei and prominent nucleoli. Distinctive epithelial or mesenchymal differentiating features are not seen

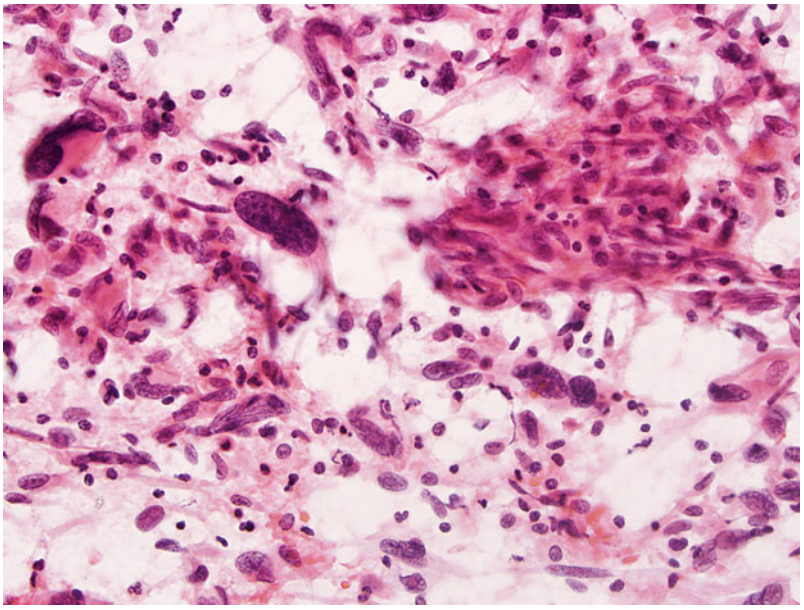

**Fig. 7.11** Leiomyosarcoma (*CP*). Spindle cells with delicate ill-defined cytoplasm and elongated pleomorphic nuclei are arranged in groups and as single cells. Usually, the scant number of exfoliated cells from this tumor is reflected by scattered single cells. Nuclear membrane irregularity, coarse irregular chromatin, and prominent nucleoli separate the spindle-shaped cells of leiomyosarcoma from those of reactive reparative changes characterized by round nuclei and smooth nuclear membranes

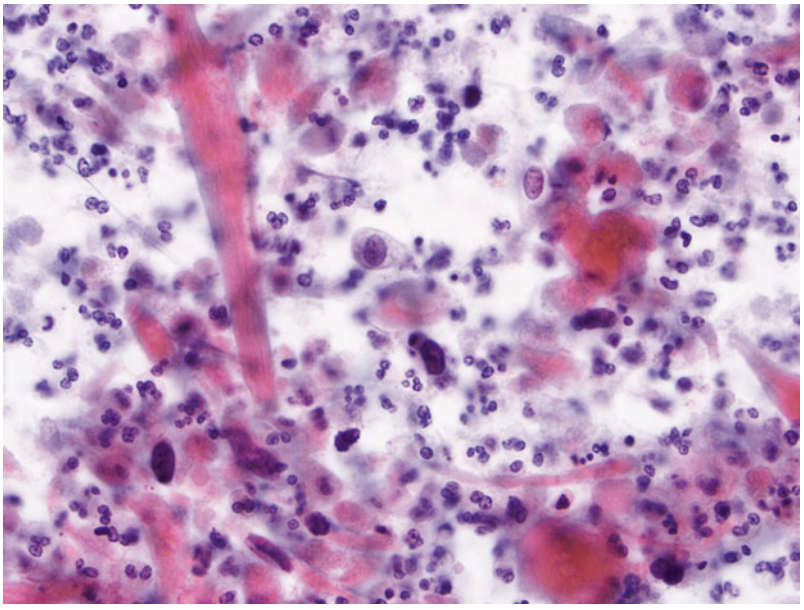

**Fig. 7.12** Rhabdomyosarcoma (*CP*). Spindle/strap cells with cytoplasmic cross striations are an indication of skeletal muscle differentiation. Some bizarre-shaped cells are present in the background. Nuclei may vary from oval to elongated and display membrane irregularity and coarsely granular chromatin

### 7.2.8 Other Primary Tumors

Primary cervical germ cell tumors have been described, including choriocarcinoma, yolk sac tumor, and teratomas [6]. Leukemia/lymphoma and malignant melanoma rarely can be primary in the cervix.

## 7.3 Secondary or Metastatic Tumors

### 7.3.1 Extrauterine Carcinomas (Figs. 7.13–7.18)

Extrauterine carcinomas may spread to the cervix, or be present in a cervical cell sample, in one of three ways. Direct extension from a primary tumor in the pelvis, such as endometrium, bladder, and rectum, is the most common source of cervical involvement by secondary carcinoma [6]. Lymphatic and/or hematogenous metastases to the cervix are less frequent, with the most common primary sites being the gastrointestinal tract (Figs. 7.13, 7.14, and 7.15; see Fig. 6.58), the breast (Fig. 7.16a, b; see Fig. 6.59), and the ovary (Fig. 7.17a, b; see Figs. 6.55 and 6.56) [6]. Lastly, exfoliated cells from an ovarian tumor or from malignant ascites may pass through the fallopian tubes, endometrial cavity, and endocervical os, to present in the cervical sample.

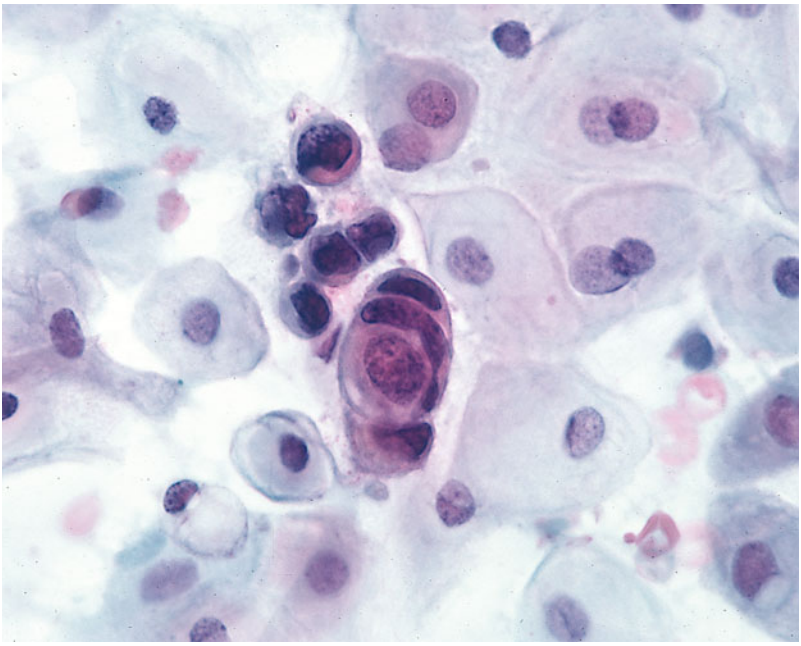

**Fig. 7.13** Metastatic gastric carcinoma (*CP*). A small cluster of cells with malignant nuclear features displays the "cell in cell" arrangement often seen in gastric carcinoma. A cytoplasmic vacuole is present in one of the single cells. The background is free of tumor diathesis, a feature that favors metastatic rather than a primary origin of the tumor

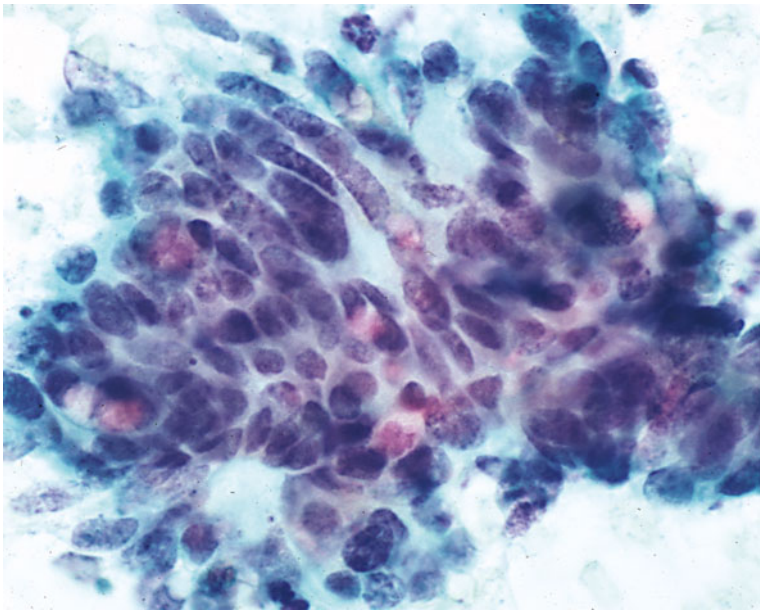

**Fig. 7.14** Metastatic colon carcinoma (*CP*). A group of tall columnar glandular cells demonstrates nuclear pleomorphism, hyperchromasia, cellular overlap, and loss of polarity within the cell group. These morphologic features would lead to an interpretation of malignancy. The columnar cell shape, palisading cigar-shaped nuclei, and scattered goblet cells containing distended mucin-filled vacuoles seen in this image are distinctive morphologic features of colonic adenocarcinoma, as is "dirty necrosis" (not shown here)

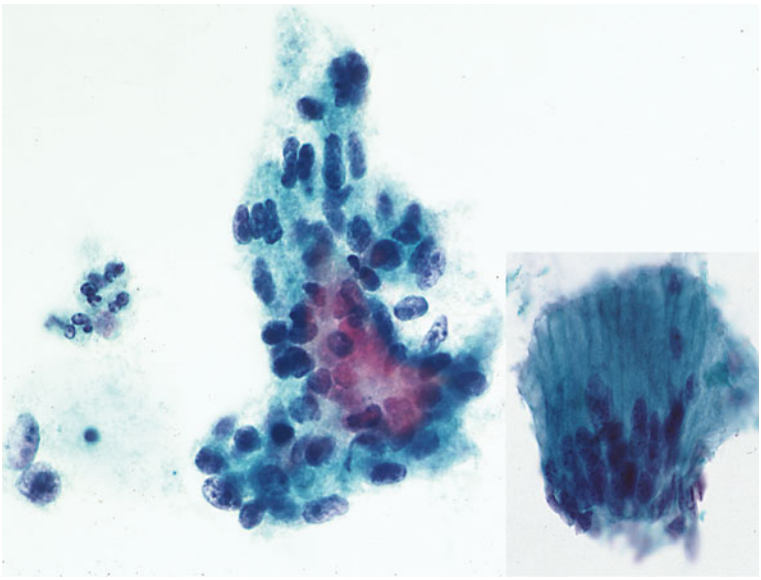

**Fig. 7.15** Metastatic colon cancer (*LBP*, *ThinPrep*). A cluster of malignant cells from metastatic colon carcinoma shows tall columnar cells with elongated nuclei at the upper edge and a glandular lumen in the center. Goblet cells are not identified in this group, and a mild degree of degeneration is noted. A fragment of normal colonic epithelium is shown in the *lower right inset* for comparison with the tumor cells in this figure and Fig. 7.14

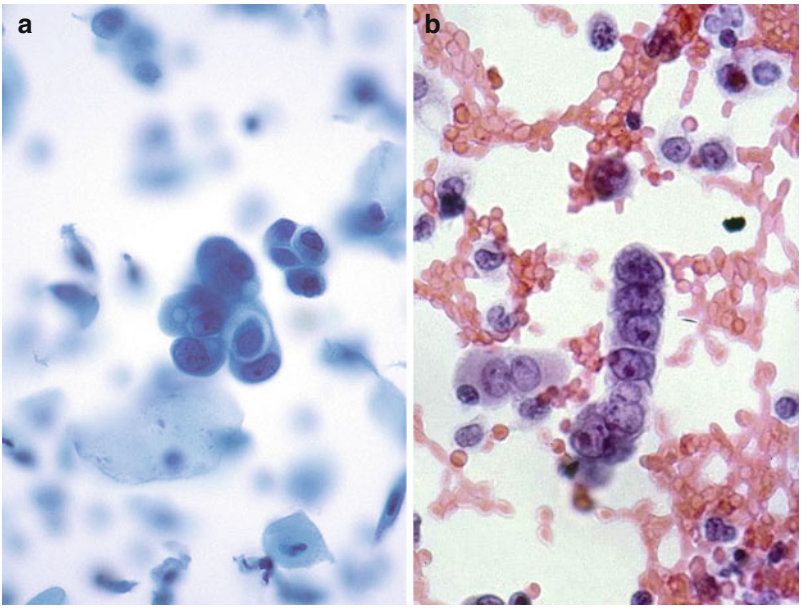


**Fig. 7.16** (**a, b**) Metastatic breast carcinoma. Clusters of small cells with scant to moderate amounts of vacuolated cytoplasm, including an intracytoplasmic lumen, show a cell within cell arrangement similar to gastric carcinoma. Nuclei are round with minimal variation in size (**a** *left*, *LBP*, *SurePath*). A single file of small monotonous cells with scant cytoplasm, round nuclei, and prominent nucleoli is a feature that is highly suggestive of breast carcinoma (**b** *right*, *CP*)

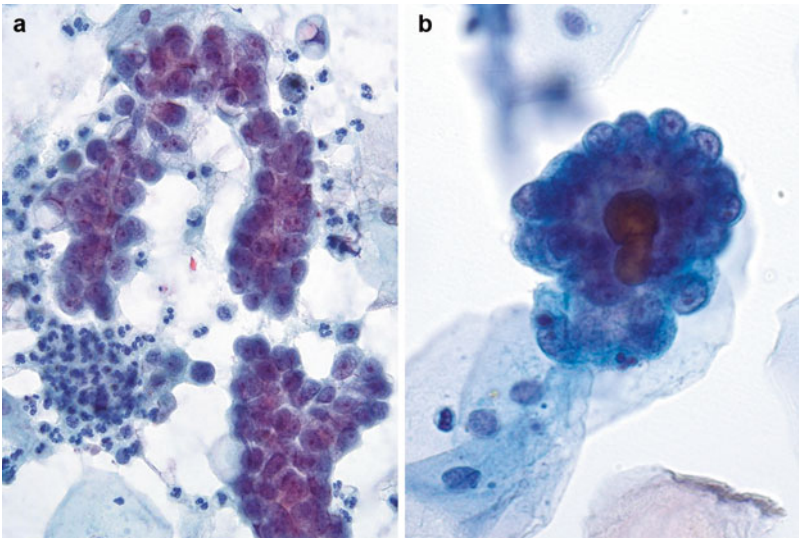


**Fig. 7.17** Ovarian carcinoma. Papillary clusters with scalloped border consisting of large overlapping cells with round nuclei, prominent nucleoli, and moderate amounts of cytoplasm showing eccentrically placed vacuoles (**a** *left*, *CP*). Similar papillary clusters comprise cells with enlarged nuclei with finely granular chromatin and prominent nucleoli. Occasional psammoma bodies are seen in ovarian carcinoma (**b** *righ*t, *LBP*, *ThinPrep*)

**Table 7.1** Frequency and morphologic features of selected extrauterine carcinomas presenting in cervical cytology specimens

| Primary site (frequency %) | Cytologic features | Immunohistochemistry expression |
|---|---|---|
| Breast (12 %) | Signet ring cells | GATA-3, ER, PR |
| | Intracytoplasmic lumens | |
| | Single cell arrangement | |
| | Cell in cell arrangement | |
| Stomach (15 %) | Signet ring cells | CK7, CK20, MUC2 |
| | Single cell arrangement | |
| | Cell in cell arrangement | |
| Ovary and tubes (36 %) | Large cells | WT1, p53, ER |
| | Tight papillary clusters | |
| | Psammoma bodies | |
| Colon (30 %) | Tall columnar cells with mucin | CK20, CDX2 |
| Kidney (3 %) | Large cells | RCC, CD10, PAX8 |
| | Large round nuclei with macronucleoli | |
| | Abundant delicate cytoplasm | |
| | Clear cytoplasm | |
| Bladder (3 %) | Cells similar to squamous metaplastic epithelium | CK20, p63, GATA-3 |
| | Dense cytoplasm | |
| | Tadpole or racket cells, cercariform cells | |

The majority of patients with metastatic tumors in a cervical sample have a prior history of a malignancy that leads to the correct interpretation [3, 6]. Very rarely, cervical involvement is the first manifestation of disease. The metastasis may be recognized by its unique cytologic features or because the cells appear foreign to the preparation (Table 7.1) [31–42]. The majority of metastatic tumors are characterized by a clean background or absence of tumor diathesis (see Fig. 6.41). However, when there is direct extension of tumor to the cervix/vagina, the associated tissue invasion and destruction can produce a tumor diathesis. Urothelial/transitional cell carcinoma may involve the vagina by intraepithelial spread, and in such cases it may potentially be confused with squamous intraepithelial lesions and/or invasive squamous carcinoma (Fig. 7.18).

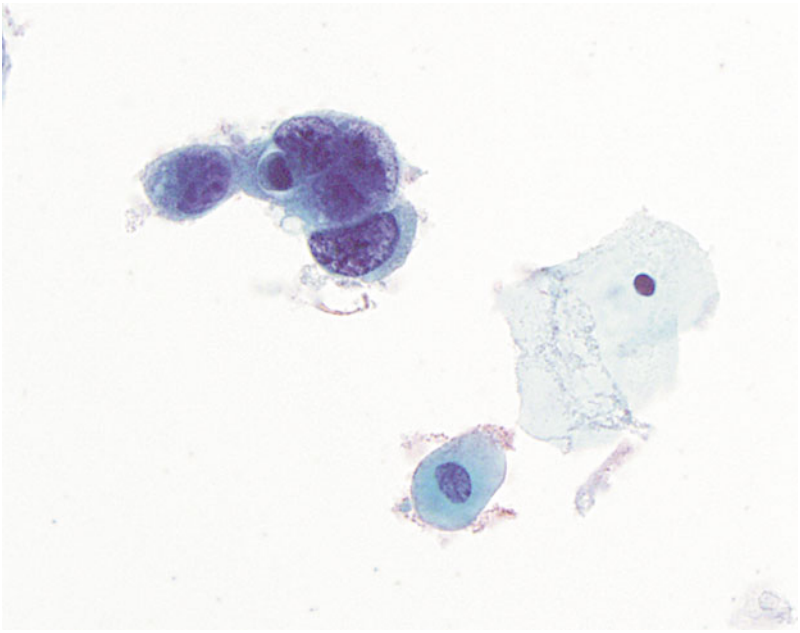

**Fig. 7.18** Urothelial carcinoma (*LBP*, *ThinPrep*). Small clusters and single cells with markedly atypical nuclei showing hyperchromasia, nuclear irregularity and prominent nucleoli, and dense cytoplasm have cytologic features overlapping with squamous metaplastic cells and high-grade squamous intraepithelial lesion. Identification of racket-shaped or cercariform cells in the presence of a history of an urothelial primary can contribute to making the correct interpretation

### 7.3.2 Malignant Melanoma (Figs. 7.19 and 7.20)

Five to 10 % of malignant melanoma in females arises in the genital tract on the vulva or vagina. Primary cervical melanoma is exceedingly rare, but metastatic melanoma is relatively more common [6, 43, 44]. The cytologic features are those common to melanoma from other sites. The cervical cytology preparation is cellular; cells are typically pleomorphic, dissociated, and round, oval, or spindled, with large nuclei and prominent nucleoli. Binucleation and intranuclear pseudoinclusions may be identified. The cytoplasm is well defined with or without cytoplasmic melanin pigment. Melanophages and tumor diathesis may be present. The differential diagnosis includes many poorly differentiated malignant neoplasms (primary or metastatic). Immunocytochemical stains for S100 protein, HMB45, and Mart1 may be used to substantiate the interpretation.

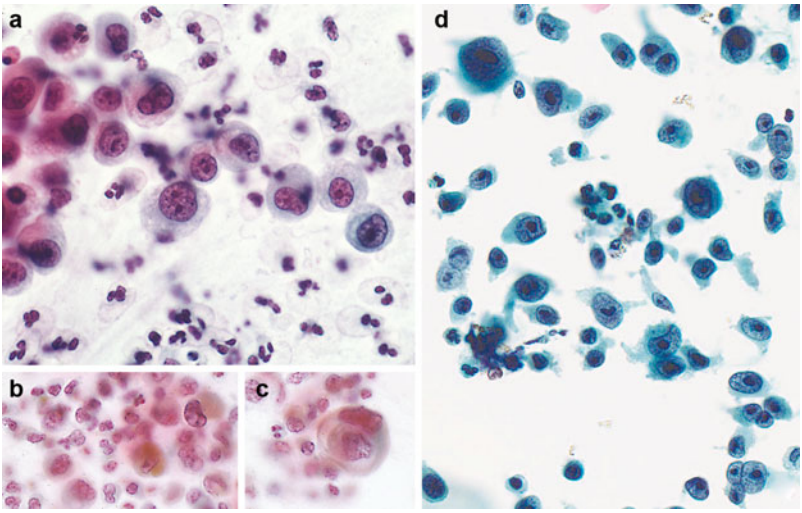


**Fig. 7.19** Malignant melanoma. Dispersed and loosely cohesive large cells have a moderate amount of cytoplasm, round nuclei, irregular nuclear membranes, coarsely clumped, irregularly distributed chromatin, and prominent nucleoli (**a** *left*, *CP*). Cytoplasmic pigment consistent with melanin is a helpful finding but is not always present (**b**, **c** *left lower inset panels*) Mostly single cells, some binucleated, with scant to moderate amounts of dense, well-defined cytoplasm. Nuclei are round with prominent nucleoli (**d** *right*, *SurePath*)

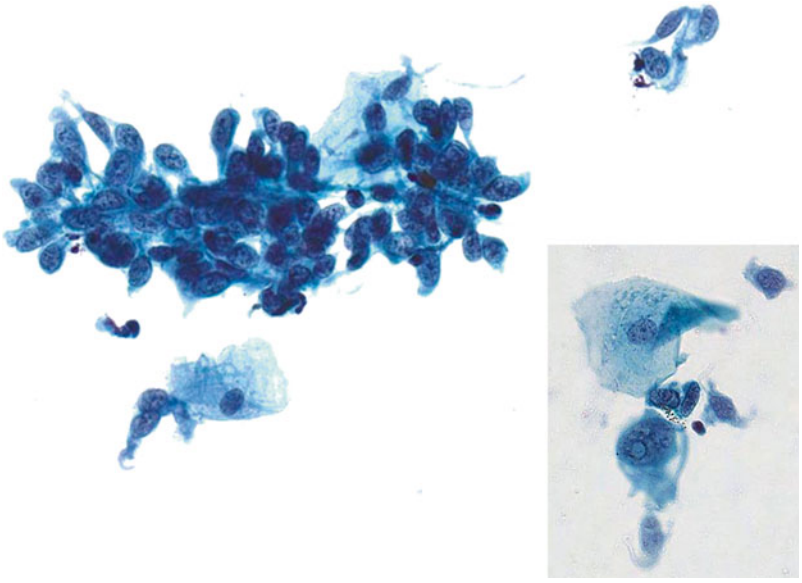

**Fig. 7.20** Malignant spindle cell melanoma (*LBP*, *ThinPrep*). Clusters of spindle-shaped cells with elongated atypical pleomorphic nuclei and irregular chromatin that can mimic a sarcoma, such as stromal sarcoma, or a spindle cell carcinoma. The presence of an intranuclear pseudoinclusion in the *inset* provides a clue to the correct interpretation which can be confirmed by immunocytochemistry

### 7.3.3 Malignant Lymphoma (Fig. 7.21)

Malignant lymphoma may uncommonly involve the cervix in the context of disseminated disease or as a primary site [45]. The lymphoma cells are individually scattered or in loose groups and often show nuclear abnormalities including membrane irregularities and coarse uneven chromatin. An abnormal lymphoid population is generally more monotonous as compared to reactive chronic inflammatory processes; however, the specific morphology depends on the type of lymphoma. The differential diagnosis includes chronic/follicular (lymphocytic) cervicitis and small cell undifferentiated carcinoma. If a liquid-based specimen is available, immunocytochemistry can be helpful in identifying a monoclonal lymphoid population.

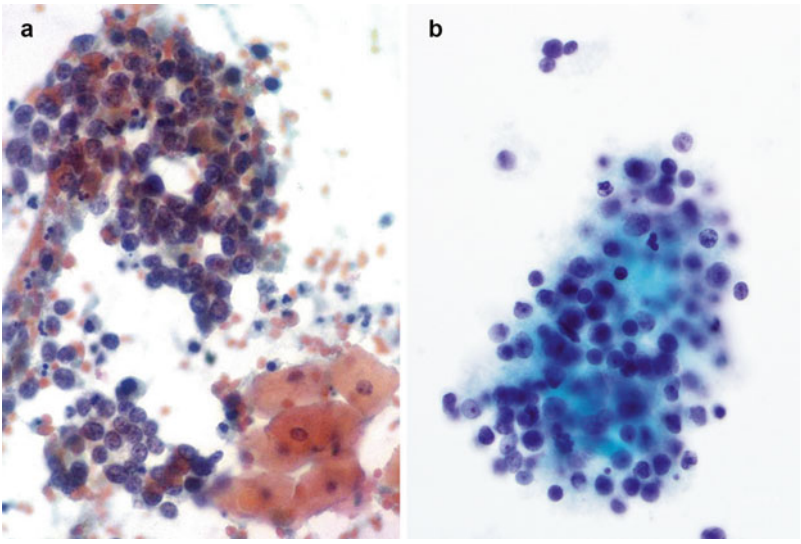


**Fig. 7.21** Malignant non-Hodgkin lymphoma. A monotonous population of lymphoid cells with scant cytoplasm forms loose groups. The absence of tingible body macrophages and lack of a range of maturation of the lymphocytes, which are seen in chronic follicular cervicitis, should raise the possibility of a malignant lymphoma (**a** *right*, *CP*; **b** *left*, *LBP*, *SurePath*)

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# Anal Cytology

# 8

Teresa M. Darragh and Joel M. Palefsky

## 8.1 Background

Anal cytology was first included in the 2001 Bethesda System Atlas. It has gained acceptance as a tool for anal cancer screening in conjunction with high-resolution anoscopy (HRA) and biopsy – in a role similar to the Pap test [1–4]. Recommendations in TBS 2001 included guidance on sampling, adequacy, use of Bethesda terminology for anal cytology, and basic morphologic characteristics of anal squamous intraepithelial lesions (ASIL). This 2014 update to the chapter incorporates a brief review of the epidemiology of anal cancer, additional images, and expands information on the performance characteristics of anal cytology, the role of HPV testing and biomarkers, and briefly addresses clinical management.

## 8.2 Anal Cancer

Anal squamous cell carcinoma is an uncommon cancer. Over 90 % of anal cancers are attributable to persistent HPV infections with HPV16 predominating [5]. The 2014 American Cancer Society [6] estimates for anal cancer in the United States are approximately 7,210 new cases (4,550 in women and 2,660 in men) and 950 deaths (580 in women and 370 in men). However, rates of anal squamous cell carcinoma have been increasing over the last several decades, especially in high-risk groups.

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Patient groups at high risk include men who have sex with men (MSM), HIV-positive men and women, organ transplant recipients, and women with a history of multicentric lower genital tract neoplasia. The incidence of anal cancer in HIV-infected adults is about 30-fold higher than in the general population [7]. Among HIV-infected MSM in the United States, the anal cancer incidence rates are estimated at 131 per 100,000 person-years [8], far exceeding the rates of cervical cancer in women in the United States prior to initiation of screening.

As with cervical disease, histologic anal high-grade squamous intraepithelial lesion (HSIL) is a cancer precursor [9]. There are no direct estimates of the progression rate of HSIL to anal cancer. Machalek et al. calculated the theoretical progression rate to be 1 in 377 per year in HIV-infected MSM, compared with 1 in 4,196 per year in HIV-uninfected MSM [10]. These rates are lower than estimates of the rate of progression of cervical HSIL (CIN3) to cancer estimated at around 1 % per year in HIV-uninfected women [11].

## 8.3 Anal Cytology

Anal cytology is used as a screening test for ASIL, mirroring the use of the Pap test in cervical cancer screening. An essential component of the anal examination is the digital anorectal exam (DARE). This is the primary anal cancer screening test. Cancers may be palpable, with the lesions feeling hard or indurated; they are often painful to the patient. When screening is directed to the populations at high risk for anal cancer, cytologic abnormalities are common. Sensitivity and specificity of a single anal cytologic specimen are comparable to that of a single cervical cytology test [12]. In a recent meta-analysis, the sensitivity and specificity of anal cytology for HSIL were comparable to that of Pap tests with sensitivity ranging from 69 to 93 %, and the specificity ranging from 32 to 59 % [13]. However, these metrics are different for HSIL in HIV-positive and HIV-negative MSM due to higher disease prevalence in those with HIV infection [14].

There is relatively poor correlation between the cytological and histological grade of ASIL found on HRA-directed biopsy. Cytology often underestimates the grade of ASIL compared with the corresponding biopsy [1, 12, 15, 16]. In a study comparing the results of anal cytology with biopsy, more than one-third of all specimens with low-grade squamous intraepithelial lesions (LSIL) on anal cytology showed HSIL on biopsy [17]. However, the positive predictive value of HSIL on anal cytology is high and can be used as a quality assurance monitor for performance of HRA in populations with an increased prevalence of ASIL such as HIV-positive MSM [18]. A large proportion of patients with any level of abnormal anal cytology have histopathologically verifiable HSIL [15].

Anal cytologic interpretations have been reported to have moderate-to-good interobserver agreement [19, 20]. However, there was poor performance of anal cytology in the College of American Pathologists Interlaboratory Nongynecologic Cytology Glass Slide Comparison Program, especially with regard to correct identification of HSIL and squamous cell carcinoma – indicating a need for continued education and familiarization among cytologists [21].

## 8.4 Sampling

The target of sampling includes the entire anal canal – proximally to the distal rectal vault and distally to the anal verge. This includes the anal transformation zone and the nonkeratinized and keratinized squamous epithelium of the anal canal. The epithelium of the anal canal is opposed at rest by the tone of the anal sphincters.

Cytologic samples are usually obtained without direct visualization of the anal canal [22, 23], although some clinicians report using a small anoscope to introduce the collection device [24]. Obtaining an adequate sample can be a challenge. Some have tried to directly visualize the squamocolumnar junction (SCJ) for sampling but found that "blind" sampling was superior to directed sampling of the SCJ [25].

A variety of sampling devices have been used to collect cells from the anal canal for cytology. The most commonly used is a Dacron® or polyester synthetic fiber swab that has been moistened with tap water [22, 23]. The Dacron® swab is often recommended over a cotton swab because it releases its cellular harvest more readily and it has a plastic shaft that may be more appropriate for use with liquid-based sampling. Others have used cervical brushes [26–28] and flocked nylon swabs [24, 29]. The swab may be better tolerated by the patient than the cytobrush [22]. The type of device is probably less important than the skill of the operator in collecting an adequate sample [30].

Both conventional smears and liquid-based cytologic preparations are used. Some investigators have reported that liquid-based preparations increase cell yield and reduce compromising factors such as obscuring fecal material, air-drying, and mechanical artifacts [31, 32]. Others report that conventional and liquid-based cytology are equally effective in screening for ASIL [33]. Self-collection of anal cytology has also been investigated; in a community-based study of MSM, 80 % of men with limited or no experience with anal cytology screening were able to collect a sample on the first attempt that was sufficient for interpretation by a pathologist [34].

## 8.5 Adequacy (Figs. 8.1– 8.5)

The cellular harvest consists of superficial and intermediate types of nucleated squamous cells, squamous metaplastic cells, rectal columnar cells, and anucleated squames from the distal anal canal (Fig. 8.1). The presence of anal transformation zone components (rectal columnar cells and/or squamous metaplastic cells) should be reported as an indicator of sampling above the keratinized portion of the canal (Fig. 8.2). As with cervical cytology, the presence of transformation zone components is a quality indicator, not a measure of overall specimen adequacy. The presence of rectal columnar cells indicates that the anal swab collected cells up to and above the anorectal transformation zone. In a study using conventional smears, the performance characteristics of anal cytology were not affected by the presence or absence of rectal columnar cells; the absence of columnar cells did not significantly alter the sensitivity, specificity, or predictive value of anal cytology [1]. However, a more recent study using ThinPrep cytology found that negative samples with no transformation

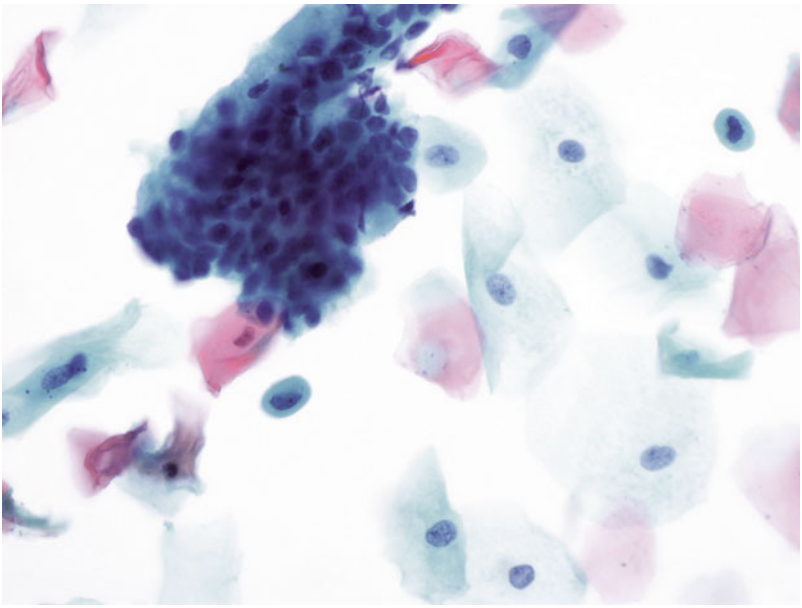

**Fig. 8.1** Satisfactory specimen, negative for intraepithelial lesion (NILM) (*LBP, SurePath*). Benign intermediate type squamous cells, squamous metaplasia, and rectal columnar cells are present

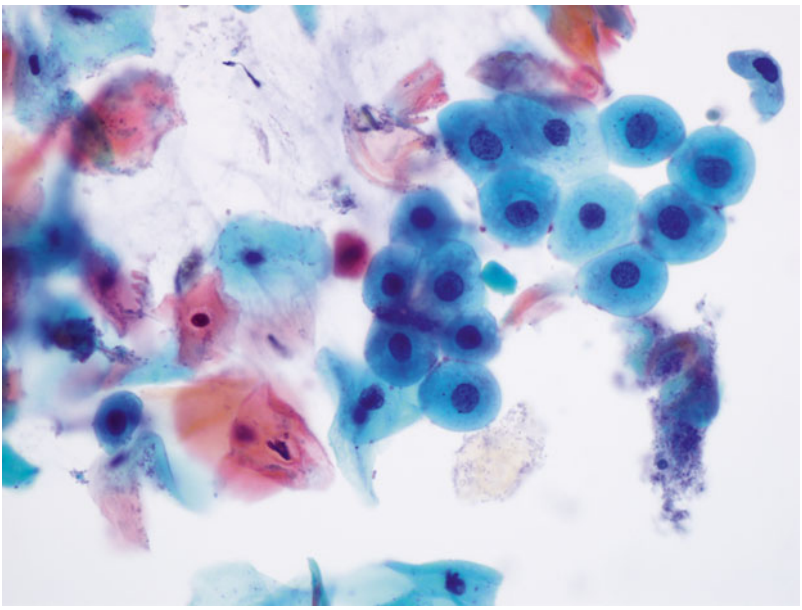

**Fig. 8.2** Negative for intraepithelial lesion (*LBP, ThinPrep*). Several round squamous metaplastic cells with dense cytoplasm are present

zone components were more likely to be false negative compared with those with transformation zone present [35].

There is a paucity of literature regarding what constitutes an adequate anal sample. The lower limits for adequate cellularity for anal cytology specimens have not been defined. Generally, the cellularity of adequate anal samples collected by experienced clinicians is similar to cervical samples. As a guide and based on expert opinion, the minimal cellularity for an adequate sample is approximately 2,000–3,000 nucleated squamous cells (nsc) for conventional smears. For liquid-based anal samples, this is equivalent to an average of 1–2 nsc per high-power field (hpf) for ThinPrep (with a diameter of 20 mm) and 3–6 nsc/hpf for SurePath (with a diameter of 13 mm), depending on the optical parameters of the microscope being used. Samples with no epithelial cell abnormality that contain fewer nsc than the above guidelines should be considered unsatisfactory due to scant cellularity. However, Arain et al. found that SurePath anal cytology samples averaging 6 or more nsc/hpf included abnormal cytologic diagnoses ranging from ASC-US through HSIL; SurePath samples averaging 5 or fewer nsc/hpf were either NIL or ASC-US [27].

Degenerative changes with nuclear karyorrhexis are frequently seen both in normal and abnormal samples (Fig. 8.3). Contamination with bacteria and fecal material may compromise evaluation (Fig. 8.4). A sample composed predominantly of anucleated squames or mostly obscured by fecal material is unsatisfactory for evaluation (Fig. 8.5).

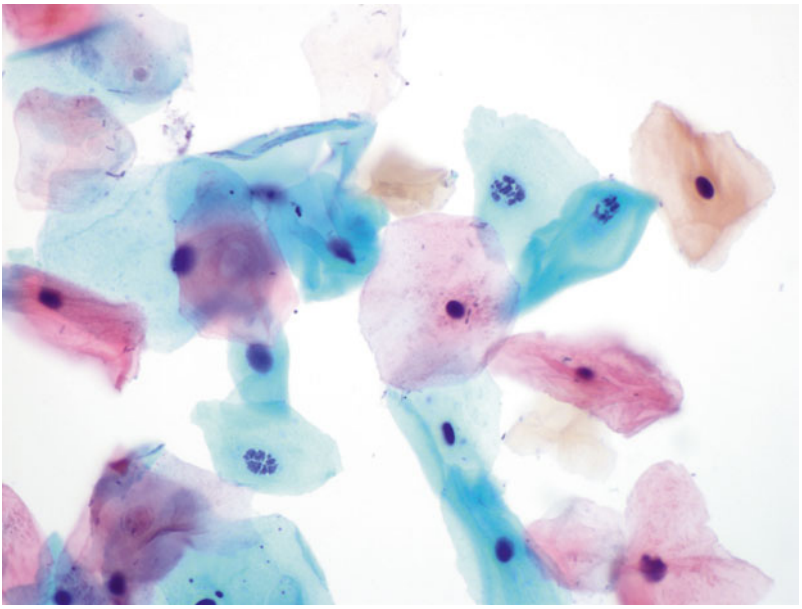

**Fig. 8.3** Negative for intraepithelial lesion (*LBP, ThinPrep*). Benign squamous cells and anucleated squames. Nuclear karyorrhexis is present

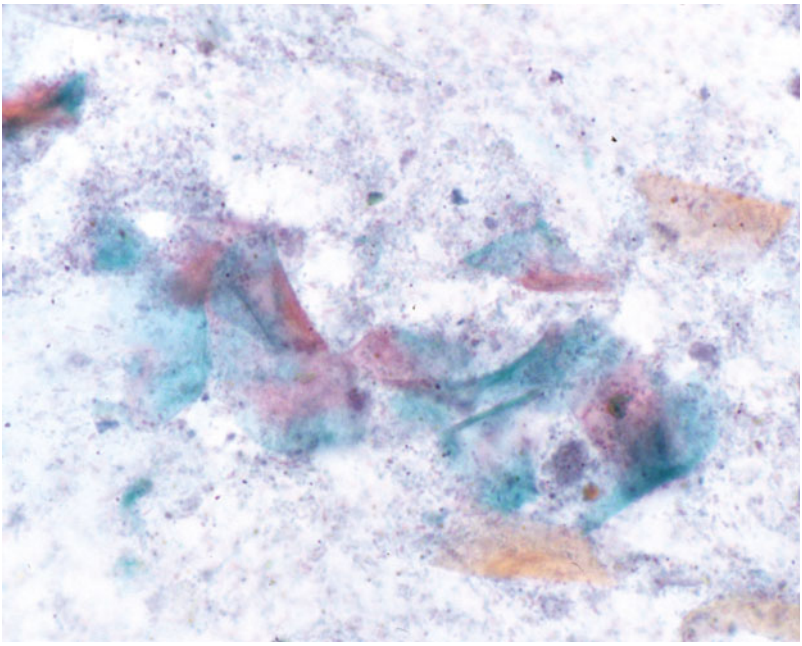

**Fig. 8.4** Unsatisfactory specimen (*conventional preparation*). Particularly on conventional anal smears, bacteria and fecal material can predominate and obscure cellular detail

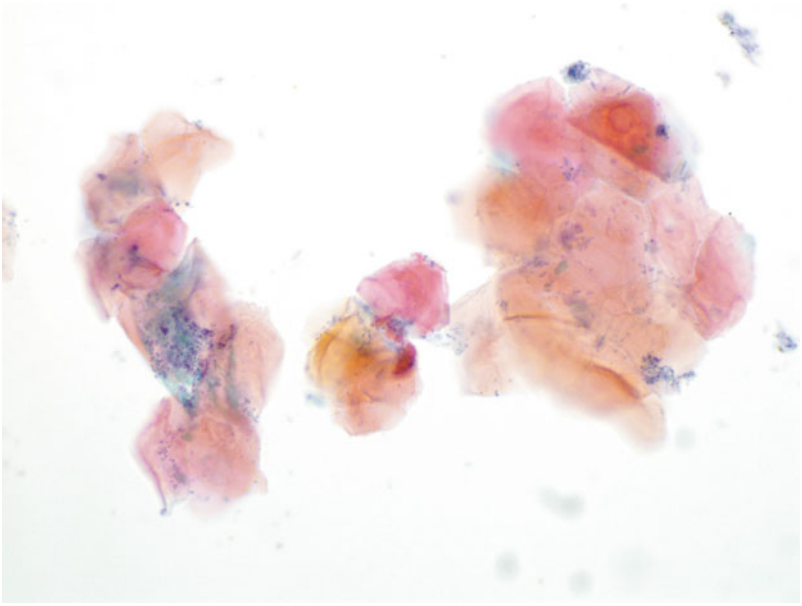

**Fig. 8.5** Unsatisfactory specimen (*LBP, ThinPrep*). Anucleated squames only. On *ThinPrep* anal cytology, an average of 1–2 nucleated squamous cells per high-power field are needed for adequacy

## 8.6 Interpretation

Terminology, morphologic criteria, and guidelines for the evaluation of anal cytologic specimens parallel those for cervical cytology. Bethesda terminology is used to report anal cytology and includes a cytologic interpretation and a statement of specimen adequacy. The Bethesda System is modified to reflect the particulars of this body site. For example, on the cytology report, rectal columnar cells are substituted for endocervical cells as a measure of transformation zone sampling.

### 8.6.1 Negative for Intraepithelial Lesion or Malignancy (Figs. 8.1 – 8.3, and 8.6)

A spectrum of benign findings can be seen on anal cytology; some are similar to cervical cytology, others are different. While reactive changes, such as tight perinuclear halos and small nucleoli, are frequently seen, typical reparative changes are not (Fig. 8.6). Keratotic changes are common on anal cytology since the keratinized and nonkeratinized portions of the anal canal are juxtaposed. Cytologic samples from the keratinized portion of the anal canal and hyperkeratosis due to a variety of causes both manifest as anucleated squames and are not distinguishable on anal cytology. Parakeratosis can be seen in both reactive changes and HPV-associated

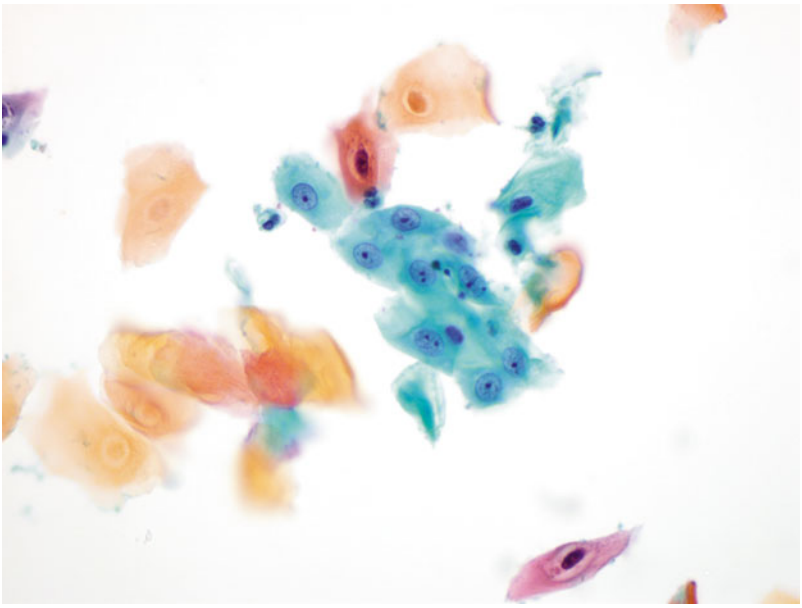

**Fig. 8.6** Squamous cells with reactive nuclear changes including nuclear enlargement, hypochromasia, and nucleoli. Other cells have narrow perinuclear halos

lesions. Atypical parakeratosis is abnormal and may be associated with cytologic interpretations ranging from ASC-US to SIL to cancer, depending on the degree of accompanying abnormalities.

### 8.6.2 **Organisms** (Figs. 8.7 – 8.10)

A variety of organisms can be encountered on anal cytology including viruses, protozoa, fungi, and helminthes. Some are identical to those encountered on Pap tests, such as Candida (Fig. 8.7) and herpes virus (Fig. 8.8). Others are unique to the gastrointestinal tract and are rare on gynecologic cytology. A large number of species of ameba can parasitize the human intestinal tract. Both amebic cysts and trophozoites are seen (Fig. 8.9a). All but *Entamoeba histolytica* are thought to be nonpathogenic commensals. The range of pathogens may be larger in immunocompromised patients who are at risk for opportunistic infections. Numerous macrophages can sometimes be seen on anal cytology, particularly in patients after ablative treatment (Fig. 8.9b). These need to be distinguished from amebic organisms. Various other intestinal parasites can be seen, including pinworms and their eggs (Fig. 8.10). The Centers for Disease Control (CDC) provides helpful information on the comparative morphology of intestinal parasites [36].

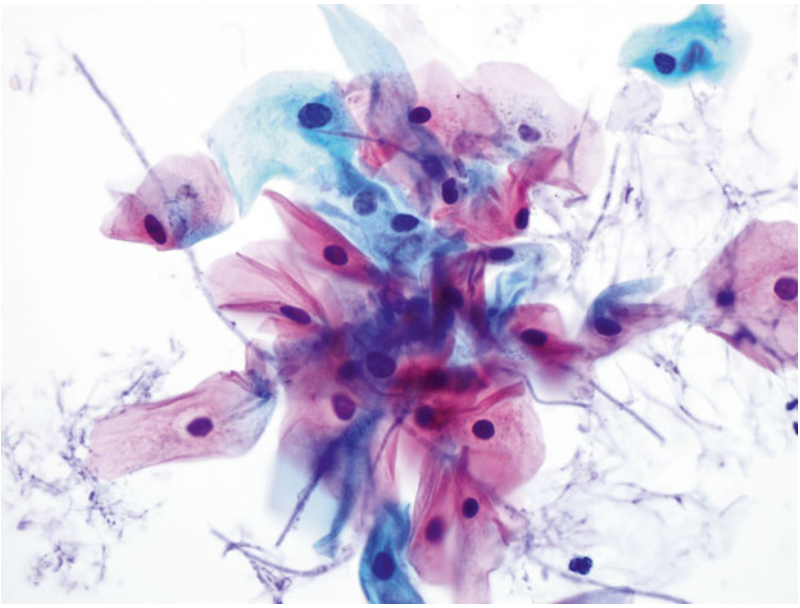

**Fig. 8.7** Candida (*LBP, ThinPrep*). Fungal pseudohyphae are threading through the cluster of squamous cells

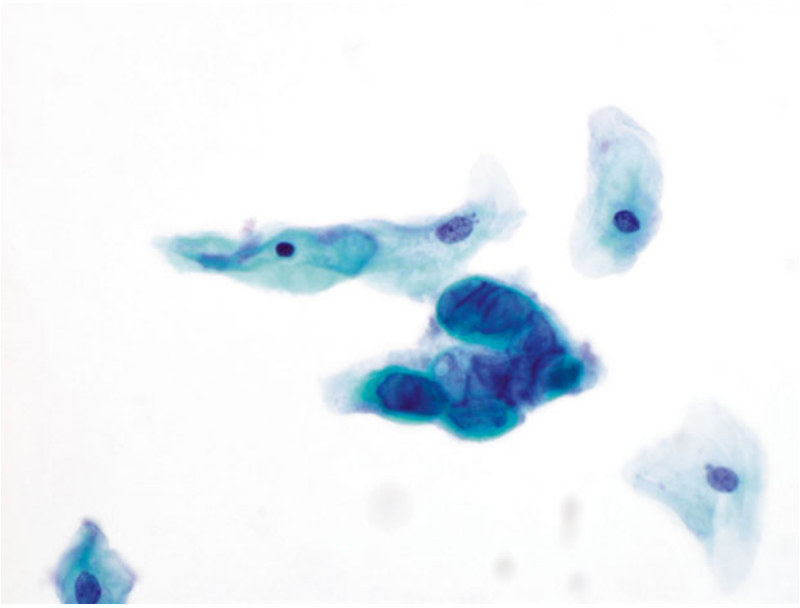

**Fig. 8.8** HSV (*LBP, SurePath*). Molded nuclei with "ground-glass" appearance are present

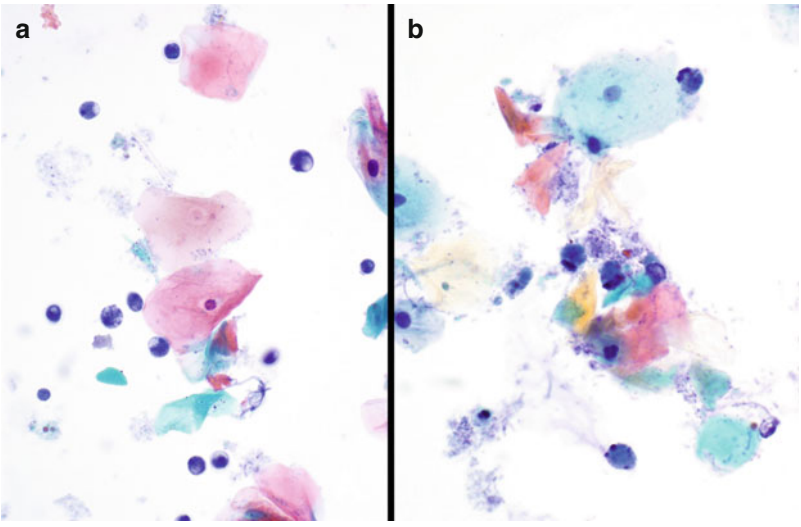


**Fig. 8.9** (**a**) Numerous amebic cysts are present (*LBP, ThinPrep*). Internal structure and refractile cyst wall help differentiate ameba from HSIL. (**b**) Macrophages (*LBP, ThinPrep*) may be seen on anal cytology, particularly after ablative treatment and need to be distinguished from ameba. Note the cytoplasmic cellular debris

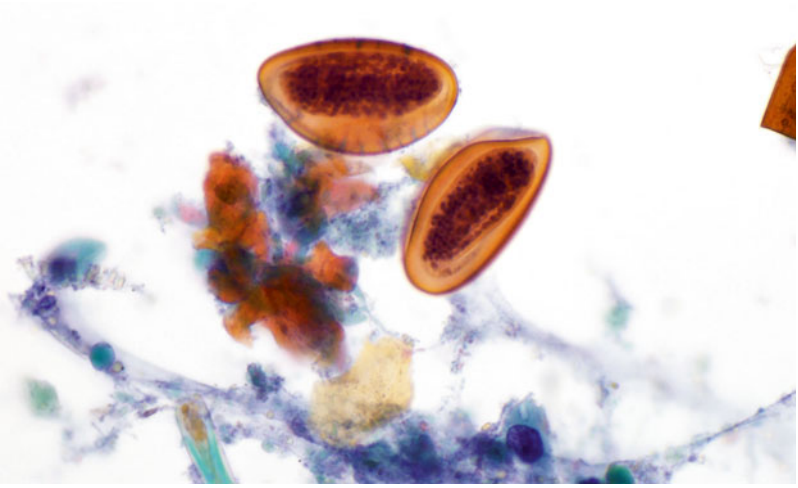

**Fig. 8.10** Pinworm eggs (*LBP, ThinPrep*)

### 8.6.3 Squamous Cell Abnormalities (Figs. 8.11 – 8.19)

#### 8.6.3.1 Atypical Squamous Cells (ASC) (Figs. 8.11 and 8.12)

The cytomorphologic criteria used for the evaluation of HPV-associated anal lesions are analogous to those seen on cervical cytology for ASC-US (Fig. 8.11), ASC-H (Fig. 8.12), LSIL (Figs. 8.13 and 8.14), and HSIL (Figs. 8.15, 8.16, 8.17, 8.18, and 8.19). Degenerative changes with nuclear karyorrhexis (Fig. 8.14) are more frequent than in cervical specimens. Squamous lesions with prominent orangeophilic cytoplasmic keratinization are common on anal cytology (Fig. 8.17).

#### 8.6.3.2 LSIL (Figs. 8.13 and 8.14)

LSIL is the cytologic manifestation of active HPV replication in superficial and intermediate type squamous cells. Similar to gynecologic cytology, both nuclear and cytoplasmic changes are observed. Nuclear changes include nuclear enlargement, hyperchromasia, and nuclear chromatin or membrane irregularities. Bi- and multinucleation are common. Cytoplasmic changes include broad perinuclear halos (koilocytosis) and keratinization.

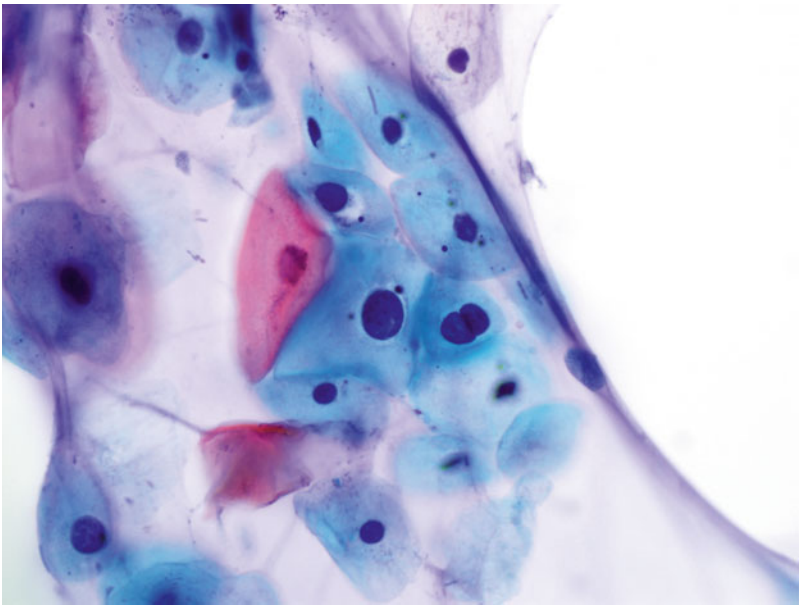

**Fig. 8.11** ASC-US (*LBP, ThinPrep*). Atypical squamous cells with enlarged but smooth nuclear contours with smudgy chromatin and narrow perinuclear clearing. One cell is binucleated

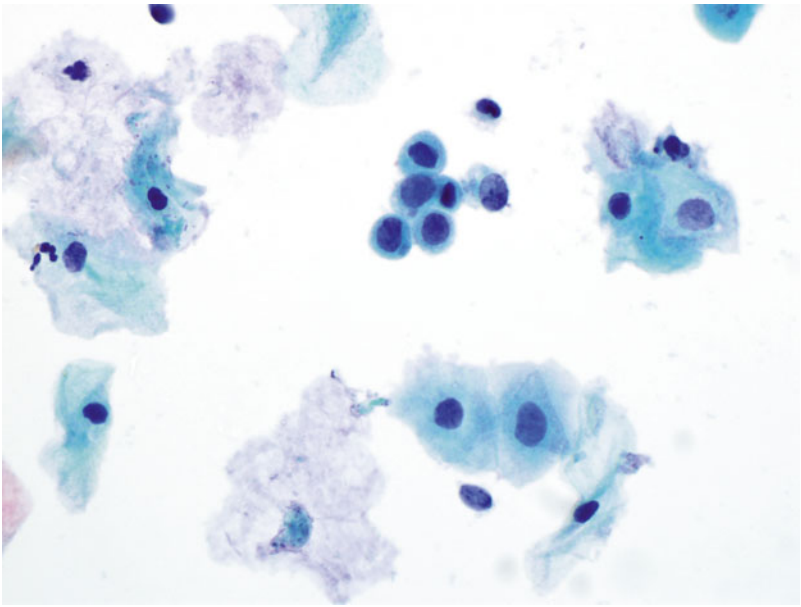

**Fig. 8.12** ASC-H (*LBP, ThinPrep*). Small immature squamous metaplastic cells with dark but smudgy nuclear chromatin

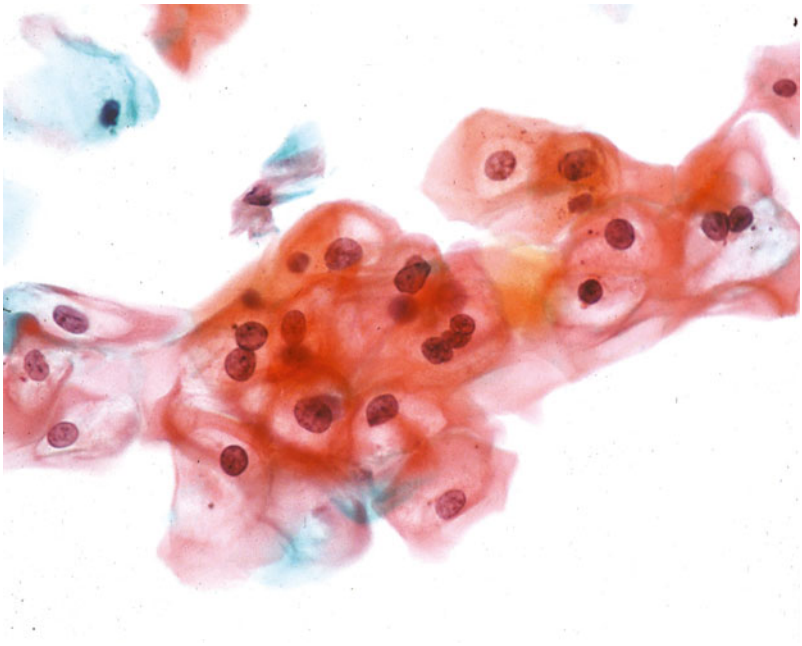

**Fig. 8.13** LSIL (*LBP, ThinPrep*). Criteria for interpretation of SIL are similar to cervical specimens

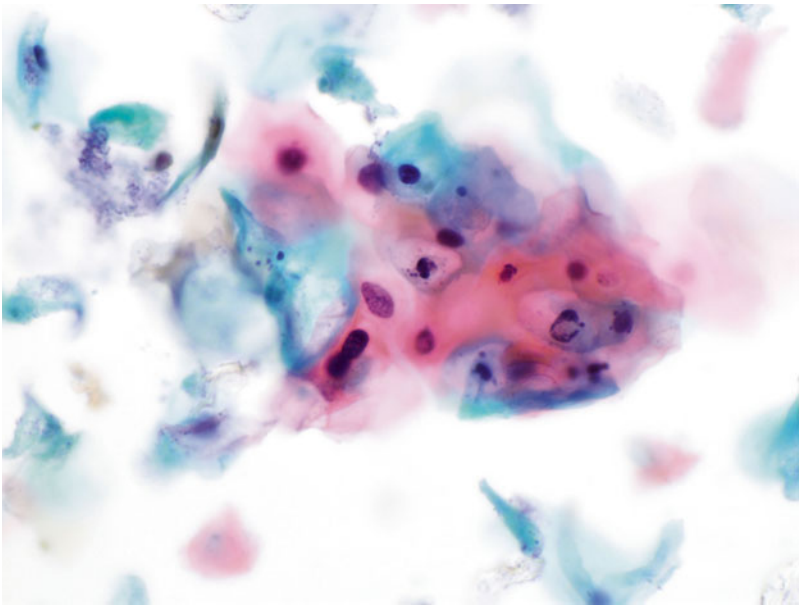

**Fig. 8.14** LSIL with karyorrhectic nuclei (*LBP, SurePath*)

### 8.6.3.3 HSIL (Figs. 8.15 – 8.19)

HSIL is a potential cancer precursor. The abnormal cells have a high nucleus-to-cytoplasmic ratio. Nuclear changes are similar to those seen in LSIL – enlargement, hyperchromasia, and nuclear chromatin and/or membrane irregularities – however, cytoplasm is scant, and it may be metaplastic or keratinized. The presence of a mixture of both LSIL and HSIL on the same sample is frequently seen on anal cytology, especially in the high-risk populations (Fig. 8.18). The presence of distinct nucleoli raises the possibility of invasive carcinoma (Fig. 8.19).

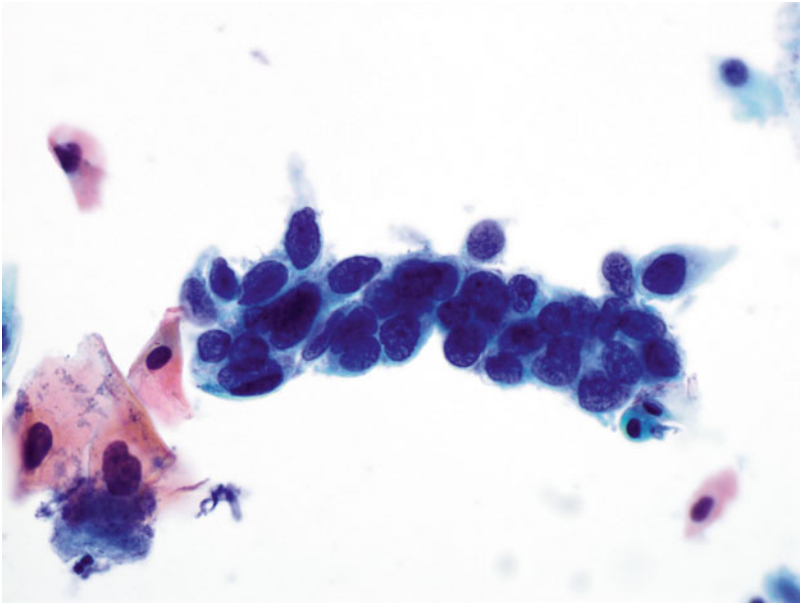

**Fig. 8.15** HSIL (*LBP, ThinPrep*). Hyperchromatic group with altered chromatin pattern and irregular nuclear contours

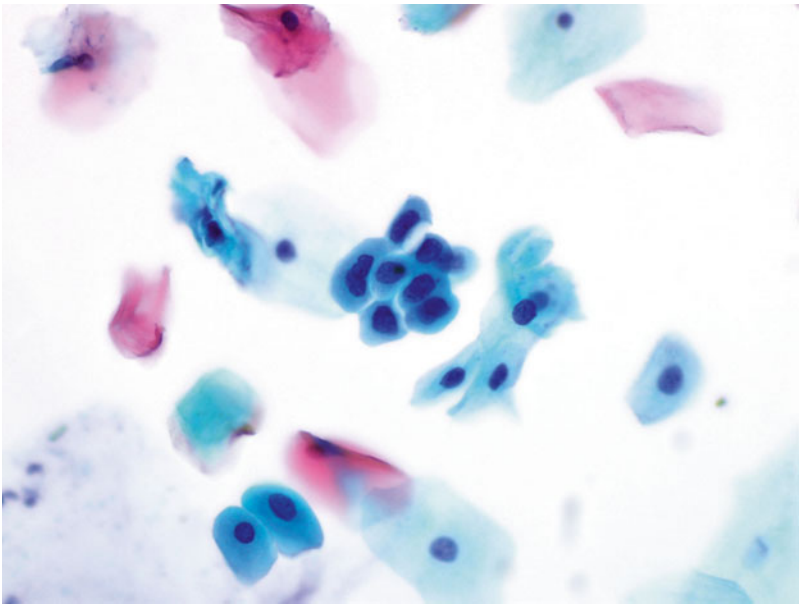

**Fig. 8.16** HSIL (*LBP, SurePath*). Dysplastic cells with metaplastic cytoplasm and irregular nuclear contours

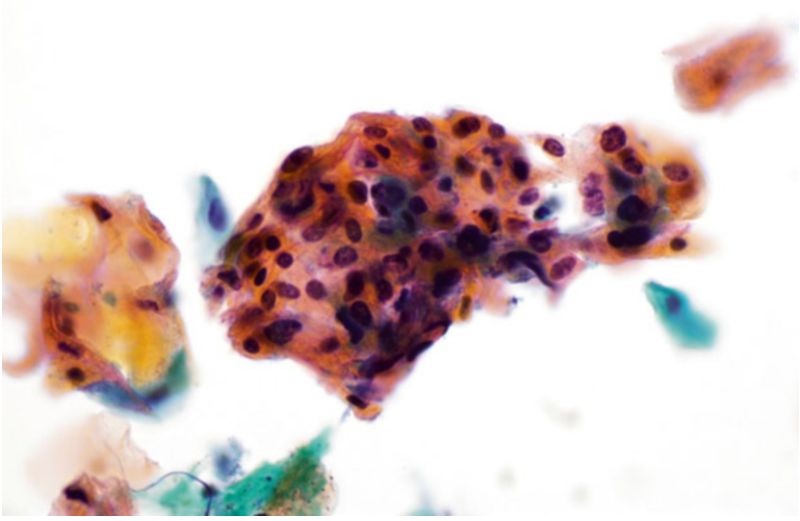

**Fig. 8.17** HSIL (*LBP, ThinPrep*). High-grade keratinizing dysplasia

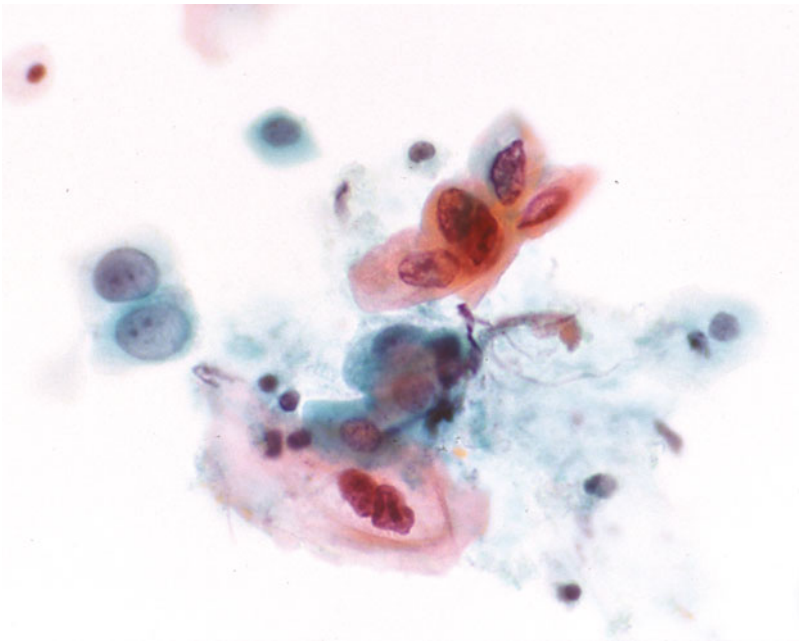

**Fig. 8.18** Both HSIL and LSIL are present in this figure (*LBP, ThinPrep*). Note the cytoplasmic keratinization, a feature that is often more prominent in squamous lesions of the anal canal than in cervical lesions

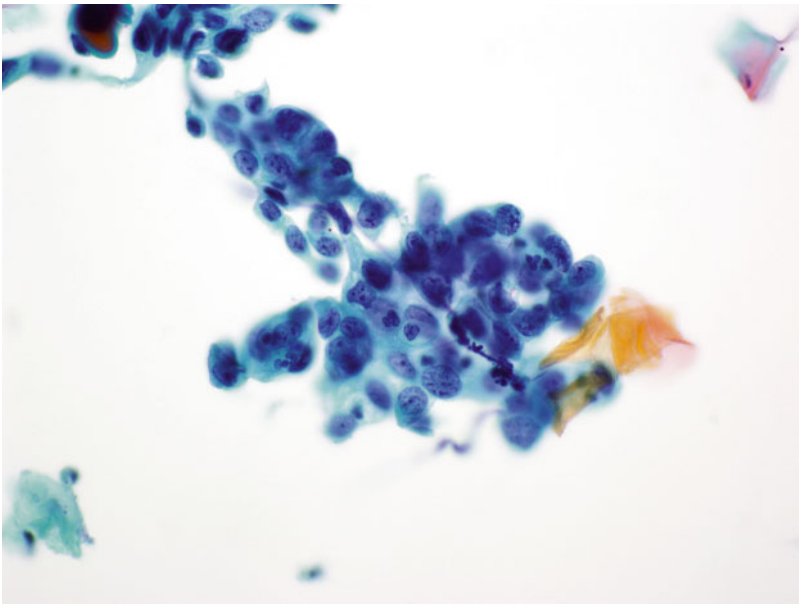

**Fig. 8.19** HSIL (*LBP, ThinPrep*). Loose cluster of cells with dysplastic nuclei. Several nuclei have distinct nucleoli raising the possibility of an invasive process

#### 8.6.3.4 Squamous Cell Carcinoma (SCC) (Figs. 8.20 – 8.22)

The cytologic diagnosis of anal squamous cell carcinoma can be challenging. Both keratinizing (Fig. 8.20) and nonkeratinizing SCC (Fig. 8.21) can be seen. Tumor diatheses may not be prominent and can be difficult to distinguish from fecal material. On liquid-based preparations, the diathesis is most apparent "clinging" to the malignant cells (Fig. 8.22).

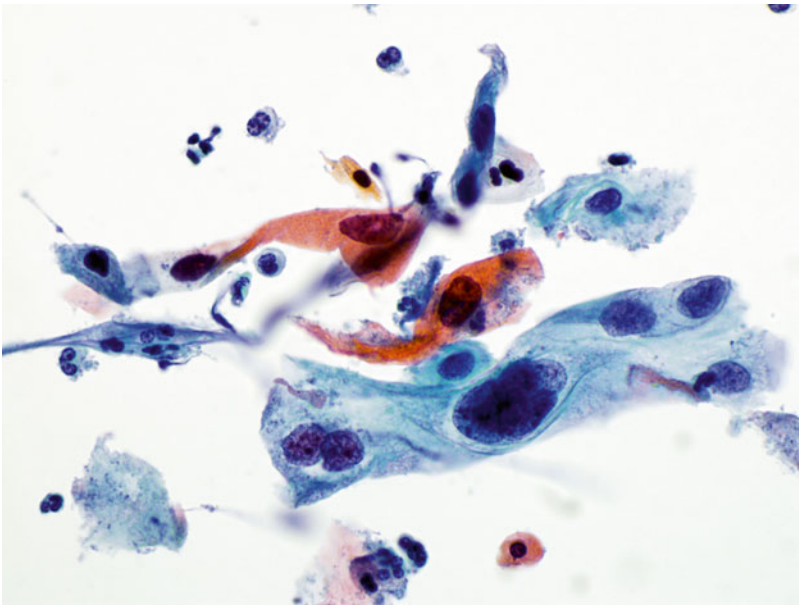

**Fig. 8.20** SCC, keratinizing (*LBP, ThinPrep*). Marked pleomorphism of cell size and shape. Two tumor cells show cytoplasmic keratinization

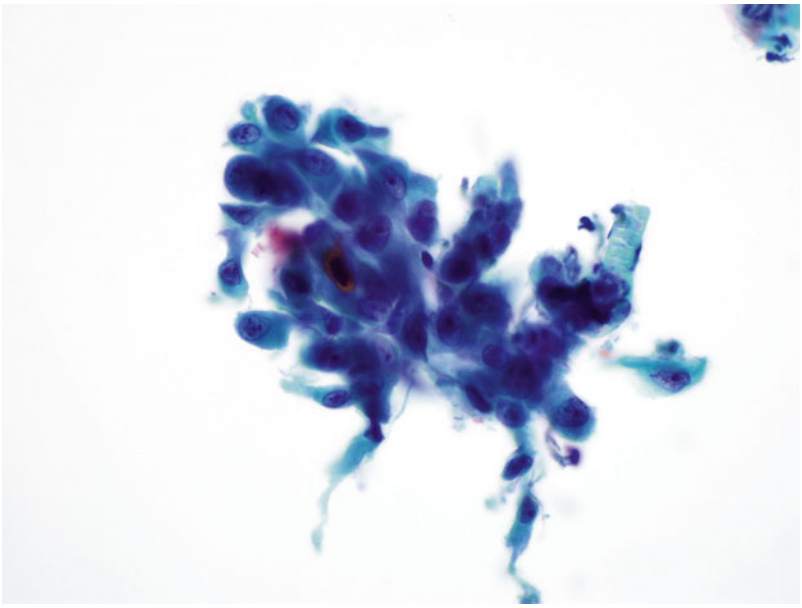

**Fig. 8.21** Squamous cell carcinoma, nonkeratinizing. Pleomorphic cell cluster *(LBP, ThinPrep)*. Some tumor cells have prominent nucleoli. A tumor diathesis is not prominent in this field

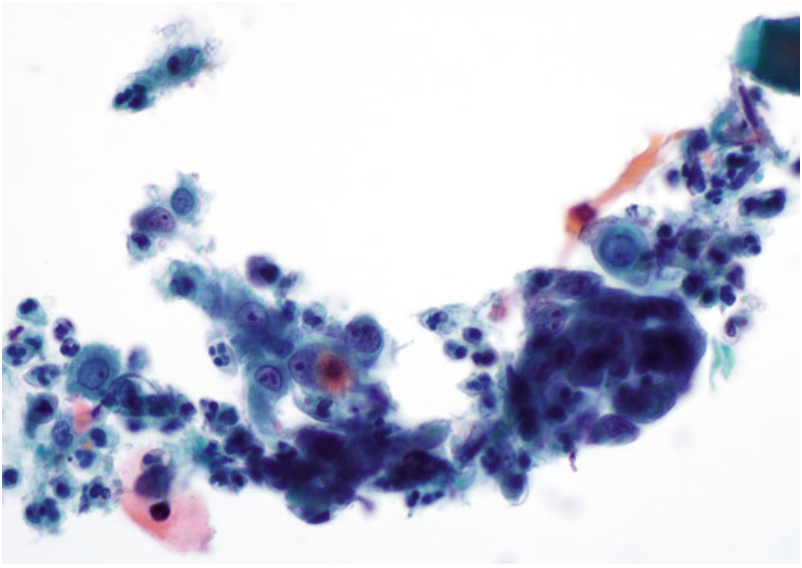

**Fig. 8.22** SCC with "clinging" diathesis (*LBP, ThinPrep*)

### 8.6.4 Glandular Cell Abnormalities

Glandular abnormalities are uncommon on anal cytology. HPV-associated glandular lesions of the anus – the counterpart to endocervical AIS – have not been convincingly described. Perianal Paget's disease can extend into the anal canal. Glandular abnormalities due to colonic lesions in the distal rectum such as colonic polyps and rectal adenocarcinoma (Fig. 8.23) are occasionally encountered on anal cytology.

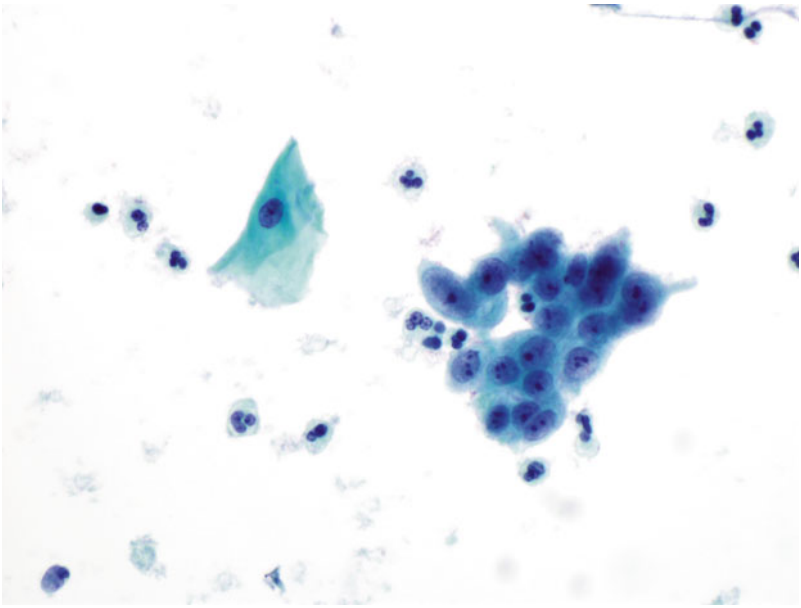

**Fig. 8.23** Rectal adenocarcinoma (*LBP, ThinPrep*). Malignant cells have vesicular nuclei with prominent nucleoli and finely vacuolated cytoplasm. This is a recurrence of a rectal adenocarcinoma

## 8.7 Anal Cytology Statistics

In the highest-risk populations targeted for anal cancer screening, abnormal anal cytology is common. At University of California-San Francisco (UCSF) in the United States, we have an active anal neoplasia clinic in which a large number of anal cytologies are reviewed, averaging over 2,500 samples per year for the last decade. The majority of the anal samples are collected from MSM and patients with HIV infection. HSIL or cancer is found in 10–15 % of samples and LSIL in approximately 30 %. ASC-US and ASC-H rates average 20 and 4 %, respectively. In this large and widely published practice, approximately 30 % of samples are negative and <5 % are unsatisfactory for evaluation.

## 8.8 Biomarkers

The optimal role of HPV testing for anal cancer screening and triage has yet to be defined [14]. At the time of this writing, none of the commercially available HPV tests are FDA-approved for use on anal specimens. Laboratories must validate the HPV test for this specimen type. Although some have found that reflex HPV testing may be helpful in triaging patients diagnosed with ASC-US [37], given the high prevalence of HPV in the populations targeted for screening, this is unlikely to be a cost-effective approach. Since most anal SCCs are associated with HPV16, HPV genotyping may have a more important role in anal cancer screening [38]. Nonetheless, a negative HPV test may be a clinically significant finding in high-risk groups because of the high negative-predictive value of a combined negative cytology and negative HPV [39].

In a study of the comparative performance of several biomarkers on anal samples, Wentzensen et al. found that HPV DNA testing had the highest sensitivity for biopsy-proven HSIL, followed by p16/Ki-67, HPV E6/E7 mRNA testing, and HPV16/18 genotyping. The best overall performance of the biomarkers, as measured by Youden's index, was observed for HPV E6/E7 mRNA testing, followed by HPV16/18 genotyping, p16/Ki-67 cytology, and HPV DNA testing. Increasing the threshold for positivity of p16/Ki-67 to five or more positive cells led to significantly higher specificity, but unchanged sensitivity for detecting anal intraepithelial neoplasia (AIN) 3 [40]. A recent study also found that the addition of p16 to anal cytology had greater specificity for HSIL and may improve diagnostic accuracy, especially for HSIL [41].

As reliance on the morphologic interpretation of cytologic samples diminishes with the increasing use of biomarkers, the type of collection device for anal specimens will need further investigation. Flocked swabs outperformed Dacron for cell count per slide based on slide imaging [29]. However, sample collection using Dacron swabs identified more human papillomavirus-positive patients and yielded higher relative light unit values than using the cervical brush [42].

## 8.9 Clinical Management

Among the high-risk populations that are the targets for anal cancer screening, those with any degree of abnormality on anal cytology are referred for HRA and biopsy, if resources allow. If resources for HRA are limited, then cytology can be used for triage: patients with HSIL or ASC-H cytology should be prioritized for HRA, followed by patients with LSIL, and finally by those with ASC-US [18]. However, anal cytology screening should only be instituted if treatment is available for individuals with HSIL. If expertise is not available to evaluate anal cytology, perform HRA and treat HSIL, then, at a minimum, high-risk patients should receive a DARE to palpate for masses in the anal canal [18].

## 8.10 Sample Reports

**Example 1**
*Specimen adequacy:*
Specimen adequate for evaluation; transformation zone component(s) present.
*Interpretation:*
High-grade squamous intraepithelial lesion (HSIL).

*Comment:*
Suggest high-resolution anoscopy.
*(Clinician's name)* notified of the results on *(month/day/year)* at *(time)* by *(pathologists name).*

**Example 2**
*Specimen adequacy:*
Unsatisfactory for evaluation due to scant nucleated squamous cells; anucleated squames predominate; transformation zone absent.
*Interpretation:*
Unsatisfactory for evaluation; see comment.

*Comment:*
Suggest repeat sample, as clinically indicated.

**Example 3**
*Specimen adequacy:*
Specimen adequate for evaluation; transformation zone components present.
*Interpretation:*
Negative for intraepithelial lesion or malignancy (NILM).
Reactive cellular changes.
Organisms present, see comment.

*Comment:*
Amebas are present. Both pathogenic and nonpathogenic amebas can be seen on anal cytology. Suggest clinical correlation and additional studies (e.g., stool examination for parasites) as indicated.

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# Adjunctive Testing

# 9

Mark H. Stoler, Stephen S. Raab, and David C. Wilbur

## 9.1 Background

Adjunctive testing is now commonly used in association with cervical cytology. HPV testing has become a mainstay of triage management for equivocal specimens and as a component or potential stand-alone test for primary screening. In addition, the near future may also include immunocytochemical testing as a method of triage and screening, using a number of newly discovered markers associated with the development of cervical cancer and precancer. If adjunctive assays are performed in association with cervical cytology, their results should become part of the final report. This chapter addresses considerations for the appropriate reporting of adjunctive tests in conjunction with cervical cytology.

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## 9.2 Adjunctive HPV Testing

### 9.2.1 Introduction

In 2004, at the time of the second edition of this Atlas, there was a single US Food and Drug Administration (FDA)-approved HPV test. Screening and management guideline writing at the time revolved around recognition of the differences in sensitivity for cervical intraepithelial neoplasia grade 2 or more severe (HSIL/CIN2+) interpretations between cytology and high-risk HPV (hrHPV) testing especially in the triage of ASC-US cytology or a combination of the two tests ("cotesting") [1–3]. The increased sensitivity of cotesting permitted professional societies to recommend screening at 3-year intervals for cotest-negative women. The sensitivity of hrHPV testing was perceived as the most important patient safety issue to permit screening interval extension without increasing cancer risk. Specificity was not considered to be a patient safety issue in either the regulatory or the clinical practice setting because the consensus at that time was that the morbidity of excision treatments such as loop electrosurgical excision procedure (LEEP) was minimal.

By contrast, the 2012 US cervical cancer-screening guidelines strongly emphasize the need for balancing the sensitivity and specificity of screening tests and to also balance the harms and the benefits of screening [4]. The guidelines governing the use of hrHPV testing in the clinical management of cervical screening and treatment are totally dependent on the *clinically validated* performance of the HPV tests used [5]. The difficulty with balancing analytical detection with the clinical trade-off of sensitivity and specificity for cervical precancer, cervical intraepithelial neoplasia grade 3 (HSIL (CIN3)), and cancer (HSIL+/CIN3+) has been amply documented by the numerous failures in the history of HPV test development, most of which have mistaken high analytic sensitivity for good clinical performance [6]. In contrast to other in vitro diagnostics where sensitivity is often the sole consideration, the goal of clinical HPV detection is *not* the detection of all HPV; it is the detection of clinically relevant levels of high-risk HPV types (i.e., HPV infections at analytical cutoffs that are highly correlated with the clinical detection of the majority (>90 %) of CIN3+/HSIL). Excessive analytic sensitivity results only in increasing the number of false-positive results (lowering clinical specificity) without the benefit of increasing clinical sensitivity. Expert opinions regarding what is or is not a good HPV test in the USA have been published, and similar criteria have been adopted by the European testing community as well. The above principles apply for all HPV tests whether the detected molecules are DNA or RNA [4, 6, 7].

The Bethesda System neither promotes nor discourages the use of any specific brand of HPV test. But current practice guidelines recognize that *clinically valid* HPV testing is an integral part of contemporary practice [4, 8, 9].

### 9.2.2 Applications of High-Risk Human Papillomavirus Testing (hrHPV) with or Without Genotyping

As of 2014, there are four hrHPV tests that are FDA approved for performance in association with cervical cytology. Three are DNA based and one is RNA based. The FDA approvals have all relied on data that meet the clinical validation concepts emphasized above. At least two more tests are currently undergoing clinical trials, and undoubtedly the number of tests available will continue to increase.

*Triage* of an abnormal cytology result by a hrHPV test effectively improves the balance of sensitivity vs. specificity for colposcopic referral and prevalent disease detection. While ASC-US is the largest TBS category in which this utility is established, selected utility has also been found for other TBS categories including ASC-H, LSIL in older patients, and AGC.

*Cotesting* refers to the performance of both an HPV test and a cervical cytology at the time of screening. Thus, combinations of HPV and cytology test results lead to algorithmic referral to colposcopy, with short-term follow-up or routine long-interval screening being based on the risk of precancer or cancer.

*Primary HPV screening* refers to screening with an HPV test and performing cytology only as part of the triage of a positive result. In 2014, a primary HPV testing algorithm that incorporates both genotyping and cytology as part of the triage was approved by the US FDA for a specific HPV test based on the safety and efficacy data submitted by the manufacturer, and subsequent interim management guidelines were developed [10, 11]. Other clinical trials that meet these validation criteria may well be expected in the near future.

*HPV genotyping* refers to the selective reporting of individual HPV types in conjunction with a positive pooled high-risk test. The concept is driven by the idea that the presence of selected types at clinically valid cutoffs (e.g., HPV 16 and 18) is so highly associated with an increased risk of precancer that such patients should be referred to colposcopy rather than followed over the short term. For instance, in association with an HPV 16-positive result, a woman with cytology reported as negative for intraepithelial lesion or malignancy (NILM) has a 10 % chance of having histologic HSIL (CIN3+), and this risk is more than 30 % if the concurrent cytology is abnormal. Both of these risks are above the current ASCCP threshold for colposcopy referral [9, 12]. Hence, the algorithms that use genotyping attempt to refine the balance of sensitivity vs. specificity compared to hrHPV testing without genotyping.

### 9.2.3 Description of Test Method and Results

The test method(s) should be briefly described (e.g., hybrid capture, polymerase chain reaction, RNA amplification, etc.), and the results reported in a clear and concise manner to the ordering clinician. For HPV testing, the specific types detected

by the assay should be reported. Testing should be restricted to a spectrum of oncogenic/high-risk types based on scientific consensus. There has been no clinical relevance shown for low-risk HPV testing in cervical cancer screening [13].

### 9.2.4 Sample Reports for HPV Testing

The following is a reporting schema that is generically applicable to *all* of the above applications and is usable with any HPV test. If genotyping (in this example for 16/18) is not used, the genotype-specific comments do not apply.
The HPV assay was performed using the [*Assay name*] [*Manufacturer name*, *City*, *State*]. The [*Assay name*] high-risk panel tests for HPV types: [*list HPV types*]. {if applicable} In addition, HPV genotyping results report the presence of the following specific types within the panel {list type}.

- High-risk HPV typing is **NEGATIVE**: None of the 13/14 high-risk HPV types are detected at the clinically validated threshold for HSIL detection of this assay.
- High-risk HPV typing is **POSITIVE**: One or more of the 13/14 high-risk HPV types are detected at the clinically validated threshold for HSIL detection of this assay (now pick one of the indented).
  - High-risk HPV typing is **POSITIVE**: Only **HPV type 16 detected**
  - High-risk HPV typing is **POSITIVE**: Only **HPV type 18 detected**
  - High-risk HPV typing is **POSITIVE**: **HPV types 16 and 18 detected**
  - High-risk HPV typing is **POSITIVE**: **High-risk HPV type 16** detected with additional high-risk HPV types detected, other than HPV 16 or HPV 18
  - High-risk HPV typing is **POSITIVE**: **High-risk HPV type 18** detected with additional high-risk HPV types detected, other than HPV 16 or HPV 18
  - High-risk HPV typing is **POSITIVE**: **High-risk HPV types 16 and 18** detected with additional high-risk HPV types detected, other than HPV16 or HPV 18
  - High-risk HPV typing is **POSITIVE**: High-risk HPV types detected, other than HPV 16 or HPV 18

*If using Educational Notes and Suggestions, one of the following coded comments may be appended to each HPV result*

- *Use this comment if HPV type 16 and/or type 18 are detected.*
  - Per the 2012 American Society of Colposcopy and Cervical Pathology (ASCCP) management guidelines, positive results that include HPV type 16 and/or 18 should be considered for immediate colposcopy, regardless of the concurrent cytology result.
- *Use this comment if high-risk HPV is detected but not identified as type 16 or type 18.*
  - Per the 2012 ASCCP management guidelines, positive results for HPV types other than 16 and 18 should be considered for immediate colposcopy when the concurrent cytology is abnormal at a threshold of ASC-US or above.

- *Use this comment if high-risk HPV is NOT DETECTED.*
  - Per the 2012 ASCCP management guidelines, a negative result for HPV testing when associated with an NILM cytology means the patient has significantly less than a 1 % chance of an HSIL (CIN3) lesion and repeat testing at decreased intervals is not warranted.

## 9.3 Immunochemical Assays

With better understanding of the molecular pathogenesis of HPV-associated neoplasia, a variety of related biomarkers have utility in the identification of high-grade squamous intraepithelial lesions. A recent consensus conference (LAST) developed recommendations for how these biomarkers could be incorporated into the practice of histopathology in order to increase the sensitivity and reproducibility of HSIL (CIN3) detection in tissue biopsies [14]. Although the data is not as well developed for cytology specimens, the same biomarkers have been shown to be useful in cytology, particularly for sensitive and specific detection of HSIL (CIN3) in the follow-up of equivocal specimens or in a primary screening role.

At present, the best-studied biomarkers are p16, ProExC, and Ki67. p16 and ProExC are both markers of an aberrant cell cycle which has been affected by the oncogenic effects of HPV. Ki67 is a marker of cellular proliferation. p16 stains both the nucleus and cytoplasm; ProExC and Ki67 stain the nucleus (Fig 9.1a, b). p16 has been shown to be effective for use in the triage of cervical cytology tests with

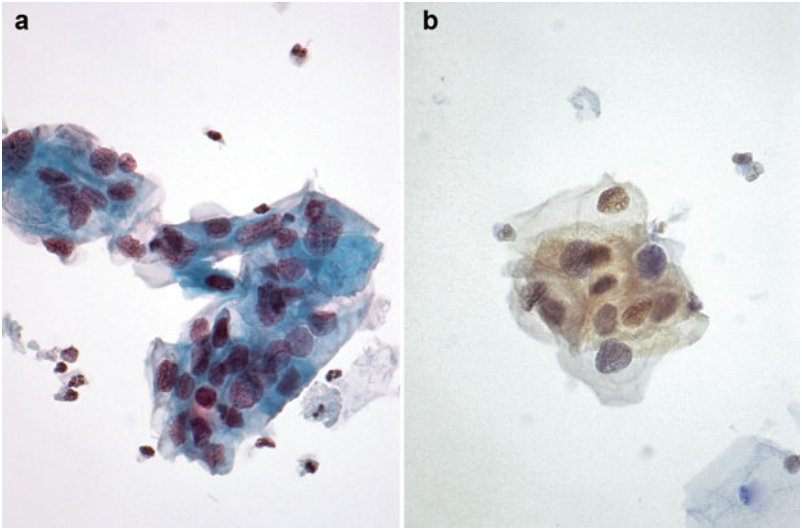


**Fig. 9.1** An example of cells from a high-grade squamous intraepithelial lesion from the same specimen: (**a**) Papanicolaou stain; (**b**) p16 immunocytochemical stain showing both nuclear and cytoplasmic staining. The presence of p16-positive cells is predictive of a precancerous lesion and may be useful in the screening and triage of cytologic specimens. (*LBP, SurePath*)

ASC-US and LSIL [15]. It has also been used in cell blocks of residual cervical liquid-based samples and shown to be sensitive in the detection of HSIL when compared to biopsy results [16]. ProExC has shown utility in the triage of atypical glandular cells [16, 17] and ASC-H [18] and as a follow-up immunocytochemical/cytology test after primary HPV screening [19]. When used as a dual immunostain, p16 and Ki67 have been shown to be as sensitive for HSIL as hrHPV testing and p16 alone [20, 21]. p16/ki67 is more sensitive with non-inferior specificity, for the detection of HSIL, as compared to cervical cytology, when used in a screening role. It has been suggested that dual-stained cytology screening may play a role in younger women where hrHPV testing has limitations [22] (Fig. 9.2).

It should be noted that as of this writing, none of the abovementioned immunocytochemical tests have been approved by the FDA for any of the uses noted. Therefore, any such use of these markers would require substantial validation in the user's laboratory prior to clinical implementation.

### 9.3.1 Reporting of Molecular/Immunochemical and Cytologic Results

It is preferable for cytology and adjunctive test results to be reported concurrently to facilitate communication and record keeping. In addition, correlation of

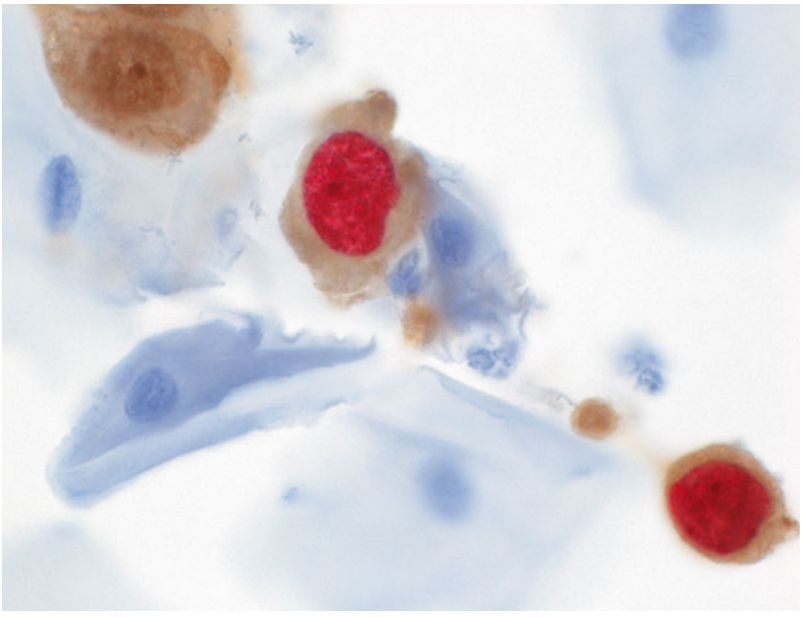

**Fig. 9.2** An immunocytochemical stain for p16 and Ki67 performed together on the same slide (dual stain): p16 stains both the cytoplasm and nucleus (*brown*), and Ki67 stains the nucleus (*red*). Cells that show combined staining are a strong predictor of the presence of a high-grade squamous intraepithelial lesion. (*LBP, ThinPrep*)

morphologic and adjunctive test results can be a valuable tool for pathology education and ongoing quality assurance. However, not all clinical practice settings allow for integrated reporting of cytology and molecular results. If integrated reporting is not feasible, then the report for each type of result should refer to the concurrent pending or previous report of the other test when possible.

### 9.3.2 Sample Report for Adjunctive Immunocytochemical Result

*Adequacy*:
Satisfactory for interpretation
*General categorization*:
Epithelial cell abnormality, squamous cell
*Interpretation*:
Atypical squamous cells – undetermined significance

*Note*: Immunocytochemical stains for p16 and Ki67 (performed in combination) show dual-stained positive cells.

*Comment*: The combination of p16 and Ki67 dual staining has been shown to correlate to the presence of HSIL in subsequent biopsy specimens.

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# Computer-Assisted Interpretation of Cervical Cytology

# 10

David C. Wilbur, Marianne U. Prey, and Ritu Nayar

## 10.1 Background

Early attempts to objectively quantify microscopic images began with simple cell and nuclear measurements. In the 1960s, computers allowed for automation of this process and also permitted analysis of numerous other cellular features. Limitations of computing power hampered significant advancement in the field until the 1980s, when technological developments in computer hardware, sophisticated algorithm development, and artificial intelligence rekindled interest in automating cervical cytology screening [1]. Automated screening devices have the potential to increase both the sensitivity and the specificity of the cervical cancer screening process. In addition, productivity gains may be achieved with their use [1–4]. In the era of HPV vaccine use, when prevalence of high-grade squamous intraepithelial lesions in the population is expected to decline, the sensitivity of manual screening will also decline [5]. Thus, automation with its potentially superior sensitivity for rare-event detection may play an important role in morphology-based screening and triage. The increase in the prevalence of disease as presented to the screener via focused selection of important fields of view, or via selection of high-risk slides for manual

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review, has the potential to maintain the level of sensitivity needed to continue effective manual-based morphologic screening.

## 10.2 Automated Devices

At present, there are several different methods for computer-assisted screening. These include automated screening with slide scoring and stratification based on risk of the presence of abnormality [2] and the so-called location-guided screening in which the computer screening process identifies areas (fields of view (FOV)) which have the highest potential to contain abnormal cells [3, 4]. For risk stratification devices, populations of slides may be designated as "no further review" (where risk is low and manual screening is not required) or "review" (where risk is higher and a full manual review is indicated). Risk stratification devices also allow for "targeted" quality control slide selection in which the highest scoring slides called negative for intraepithelial lesion or malignancy (NILM) on initial screening are reviewed again.

Location-guided screening devices are the most commonly used instruments at the time of writing of this edition. With these instruments the device-selected FOVs are reviewed, and if found to contain a potential abnormality, the slide receives a full manual screening. If no potentially abnormal cells or features are noted, the slide can be reported as NILM, without further review. Standard quality control is performed with these instruments as required by the manufacturers' FDA-approved device labeling, and included slides may be random/targeted or device-selected based on risk stratification.

Each device that is in use in the USA has FDA-approved labeling which details the maximum workload that can be performed when using instrument-screened slides. Issues have been raised over the sensitivity for abnormal slide detection with the indicated maximal workload limits. Recommendations have been put forth by a task force of the American Society of Cytopathology to address these issues. The proposed recommendations include limits for work hours and slide screening maximums; the use of a new measure referred to as the epithelial cell abnormality (ECA)-adjusted workload (which takes into account the prevalence of abnormality in specific laboratories) to determine the percentage of imaged slides that should undergo full manual review; and maintaining other measures of quality assurance as required [6]. These recommendations have been endorsed by most of the other United States national pathology organizations. The FDA issued a clarification in 2014 on how to record workload limits when using semiautomated gynecologic screening devices [7].

In addition to workload documentation, the use of automated screening instrumentation in the cytology laboratory should also be accompanied by robust laboratory-specified quality assurance measures which may include periodic reviews of device performance with regard to downtime and documentation of false-negative cases and the reasons for such cases.

## 10.3 Reporting the Results of Computer-Assisted Review

The preferred report format is to include a specific field designated for reporting appropriate information concerning the use of, and results from, the automated device. If this is not possible (e.g., because of laboratory information system constraints or local reporting convention), the automated screening information can be included as a comment or addendum. Some data resulting from automated review may not be intended for direct patient care but may be used for internal laboratory quality assurance (e.g., slide ranking data, quality control case selection data). Such data should not be included in the report, but can be kept for internal laboratory use.

The following information should be provided in the report:

1. Type of instrumentation used.
2. Whether or not the specimen was successfully processed by the device (regardless of the result).
3. Additional information depends upon whether there is manual screening/review of the specimen (the type of review may be indicated at the discretion of the laboratory (e.g., full manual screening, review of device identified fields of view only).

If the automated screening provides an interpretation of the specimen that replaces manual screening/review, then this result and any adequacy data derived from the computer assessment must be stated in the report. As with any automated laboratory instrument, the results generated by the instrument must be reviewed and verified by a laboratorian with appropriate training and authorization, even in the absence of manual screening/review. A record of who performed this data verification must be maintained as an internal laboratory record according to regulations issued pursuant to the Clinical Laboratory Improvement Amendments of 1988 [8]. In general, the name of the individual performing such verification should *not* be included in the cervical cytology report, so as to avoid giving the false impression that the individual examined the specimen. However, if local laboratory policy requires inclusion of the name, the report should indicate that the individual did not examine the slide. The name of the medical director may be included as part of the laboratory identification per local custom and where required by state regulations.

The name of anyone who examines a cervical cytology slide and renders an opinion for the final report should be documented in the report with the role of the person clearly stated.

## 10.4 Automated Review Summary

If a cervical cytology case is examined by an automated device, the report should specify the following:

1. Device utilized
2. Type of review
3. Result of the automated review process
4. The individual(s) involved in the process and their role stipulated

## 10.5 Sample Reports

**Example 1** Automated screening only – no manual review

| | |
|---|---|
| Test method | Liquid-based preparation (specify type) |
| Source | Cervix |
| Specimen adequacy | Satisfactory for evaluation, endocervical/transformation zone component present |
| Interpretation | Negative for intraepithelial lesion or malignancy |
| *Automated examination* | *Processed successfully, manual screening not required* [*Device name*] [*Manufacturer name, City, State*] |
| Verifying individual | Name |

**Example 2** Failure of automated screen, manual screen only

| | |
|---|---|
| Test method | Liquid-based preparation (specify type) |
| Source | Cervix |
| Specimen adequacy | Satisfactory for evaluation, endocervical/transformation zone component present |
| General category | Epithelial cell abnormality<br>See interpretation |
| Interpretation | High-grade squamous intraepithelial lesion (HSIL)<br>Fungal organisms morphologically consistent with *Candida* species |
| *Automated examination* | *Processing failed, manual screening required* [*Device name*] [*Manufacturer name, City, State*] |
| Educational note | Suggest further clinical investigation OR<br>Suggest colposcopy and endocervical assessment<br>(Massad LS, Einstein MH, Huh WK, et al. 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. J Low Genit Tract Dis. 2013;17:S1–27) |
| Cytotechnologist | CT (ASCP) |
| Pathologist | Doctor, M.D. |

**Example 3** Successful automated screen followed by manual screening

| | |
|---|---|
| Test method | Liquid-based preparation (specify type) |
| Source | Cervix |
| Specimen adequacy | Satisfactory for evaluation, endocervical/transformation zone component absent |
| General category | Epithelial cell abnormality<br>See interpretation |
| Interpretation | Atypical squamous cells of undetermined significance (ASC-US) |
| *Automated Scanning* | *Specimen processed successfully by automated locator device* [*Device name*] [*Manufacturer name, City, State*] |
| Educational note | Suggest high-risk HPV testing as clinically indicated<br>(Massad LS, Einstein MH, Huh WK, et al. 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. J Low Genit Tract Dis. 2013;17:S1–27) |
| Cytotechnologist | CT (ASCP) |
| Pathologist | Doctor, M.D. |

**Example 4** Automated screening and field of view-only interpretation

| | |
|---|---|
| Test method | Liquid-based preparation (specify type) |
| Source | Cervix |
| Specimen adequacy | Satisfactory for evaluation, endocervical/transformation zone component present |
| Interpretation | Negative for intraepithelial lesion or malignancy<br>Reactive cellular changes associated with inflammation |
| *Automated scanning* | *Specimen processed successfully by automated locator device – field of view examination only* [*Device name*] [*Manufacturer name, City, State*] |
| Cytotechnologist | CT (ASCP) |
| Pathologist | Doctor, M.D. |

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# Educational Notes and Comments Appended to Cytology Reports

# 11

Ritu Nayar, Dennis M. O'Connor, and Teresa M. Darragh

## 11.1 Background

Effective communication between laboratories and clinical providers is a key element of successful cervical cancer screening. Laboratorians and clinicians have a shared responsibility to remain current in their field and communicate significant changes in their respective disciplines to one another. When pathologists serve as consultants to health-care providers, giving appropriate advice on screening and follow-up tests, the patient is the beneficiary [1].

Communication takes many forms, both written and verbal. One effective means of written communication is to append educational notes or comments to the cytopathology report. The method of communication is left to the discretion of the laboratory and should be based on the individual practice setting and the content of the information to be conveyed.

Written comments regarding the significance and validity of cytologic results are the responsibility of the pathologist and should be directed to the health-care

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provider who requested the test. Optional educational notes provide additional information regarding the significance or predictive value of the cytologic findings and may be based on references to the medical literature or the laboratory's experience. Comments and educational notes should be carefully worded, concise, clear, and evidence based, whenever possible. In 2014, the United States Department of Health and Human Services issued a mandate to enable patients, or a person designated by the patient, the right to have direct access to the patient's completed laboratory test reports upon request [2]. This is part of ongoing efforts to encourage patients to be informed partners with their health-care providers. Direct access to laboratory results allows patients to track their health records, make decisions with their health-care professionals, and follow recommended treatment plans. Therefore, it should be kept in mind that patients may be reviewing their own cytology report and any accompanying notes or comments.

The format for appending educational notes and comments to the cytology report may vary depending on the preferences of the laboratory and the health-care providers it serves. The following examples highlight some circumstances in which comments could be helpful:

1. To improve the quality of a repeat specimen following receipt of an unsatisfactory specimen.
2. To identify patients with cytologic findings that may require further triage and management.
3. To indicate when further procedures would be helpful to clarify ambiguous morphologic findings.
4. To highlight the limitations of cervical cytology as a screening test (*previously referred to as "disclaimers"*).

Comments that alert clinicians to clinically significant or less commonly encountered results may be helpful. References to the appropriate clinical management guidelines published by professional organizations may be included. Examples of screening and clinical management guidelines pertinent to cervical cytology in the United States include those from the American Cancer Society (ACS) [3], the United States Preventive Services Task Force (USPSTF) [4], the American Society for Colposcopy and Cervical Pathology (ASCCP) [5], and the American College of Obstetricians and Gynecologists (ACOG) [6].

Direct notification of the health-care provider regarding a clinically significant cytologic result should be documented in the cytology report. Clarification of unusual or complex results may require specific detailed comments. If these points are discussed verbally with the provider, it is advisable to document this communication in the report. For example, "the significance of this result and possible management options were discussed with (clinician's name) by (pathologist's name) at (time) on (date)." If direct contact with the provider cannot be accomplished, general

statements such as "suggest follow-up as clinically indicated" or "further patient follow-up diagnostic procedures are suggested as clinically indicated" should be used, because the pathologist may be unaware of other pertinent clinical information.

## 11.2 Educational Notes and Comments: Summary

1. Educational notes and comments should be concise and relevant.
2. Suggestions for additional clinical follow-up should be evidence based and consistent with guidelines published by professional organizations.
3. Reference to relevant publications may be included.

## 11.3 Sample Reports

**Example 1**
*Specimen adequacy*:
Satisfactory for evaluation; transformation zone components absent.
*Interpretation:*
Negative for intraepithelial lesion or malignancy.

*Educational Note*:
Cervical cytology is a screening test primarily for squamous cancer and its precursors and has associated false-negative and false-positive results. Technologies such as liquid-based preparations may decrease but will not eliminate all false-negative results. Follow-up of unexplained clinical signs and symptoms is recommended to minimize false-negative results.

**Example 2**
*Specimen adequacy*:
Unsatisfactory for evaluation.
*Interpretation:*
Specimen processed and examined but unsatisfactory for evaluation of epithelial abnormality due to excessive air-drying artifact.

*Comment*:
Careful attention to rapid conventional slide fixation or the use of a liquid-based preparation is suggested to improve specimen quality. Per 2012 ASCCP management guidelines, a repeat cervical cytology test is indicated. (Massad LS, Einstein MH, Huh WK, et al. 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. J Low Genit Tract Dis. 2013;17:S1–27.)

**Example 3**

*Specimen adequacy*:

Specimen satisfactory for evaluation; transformation zone component present.

*Interpretation:*

Atypical endocervical cells, favor neoplastic.

*Educational Note*:

A significant percentage of patients with this cytologic interpretation have underlying high-grade squamous or glandular intraepithelial abnormalities. Further diagnostic follow-up procedures, such as colposcopy with endocervical sampling, are suggested as clinically indicated.

(Optional addition of appropriate reference or references, for example, Massad LS, Einstein MH, Huh WK, et al. 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. J Low Genit Tract Dis. 2013;17:S1–27.)

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# Risk Assessment Approach to Management

# 12

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## 12.1 Background

Human papillomavirus (HPV) infection is the primary cause of nearly all cervical cancer as well as other less common anogenital cancers. Recognition of this has motivated development and marketing of HPV tests, and their increasing incorporation into cervical screening algorithms. The optimal combination of high-risk HPV (hrHPV) testing and cytology has not been determined and recommendations for using these tests for screening and management are rapidly evolving. At present, both cytology (with hrHPV triage of ASC-US) and combined use of hrHPV testing

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and cytology (cotesting) are recommended in the United States. In 2014, one specific proprietary HPV test was FDA approved in the US for primary screening, with the use of cytology for triage of women testing positive for non-HPV16/18 high-risk types, creating a third potential screening strategy [1].

Updating accepted, successful screening and management strategies with new technologies requires a rational framework. The 2012 US consensus screening and management guidelines were developed using a risk assessment framework originally based on cytology [2]. The core principle was "similar management for similar risk." If two screening results have similar risk of cervical cancer (or its surrogate, a high-grade precursor lesion), the principle holds that they should have similar management. A good example is provided by cervical cytology results of low-grade squamous intraepithelial lesion (LSIL) and HPV-positive atypical squamous cells of undetermined significance (ASC-US). They have similar cancer risk, can be considered equivalent for management, and under current guidelines both are managed similarly, with referral for colposcopy. To use the risk assessment framework, the cervical cancer risk of each screening result (cytology result, HPV result, and combinations) must be calculated based on experiences in large, representative populations. Coherent guidelines are developed by grouping screening results that have similar risks to the same management, commensurate with the underlying risk. Cervical cancer prevention guidelines derived via risk estimation can serve as a paradigm for a rational and effective way to prevent cancer.

## 12.2 Principles of Risk Assessment

The risk assessment framework is a rational basis for clinical and public health decisions. A high risk of disease raises concern and indicates that more significant assessments or interventions may be required. A low risk of disease provides reassurance and usually implies that fewer or less invasive further testing or interventions are required.

Risk assessment is not unique to cervical cancer screening. It is commonly applied throughout clinical medicine. For example, elevated cholesterol levels indicate an increased risk of cardiovascular diseases and may lead to prescription of cholesterol-lowering drugs [3]. Detecting inherited mutations of BRCA indicates an increased risk of breast and ovarian cancer and may lead to recommendation of increased surveillance or prophylactic surgery [4]. Evaluation of tradeoffs between risk of breast cancer mortality and potential harm associated with false-positive mammography screening results have led to changes in breast cancer screening recommendations that are still the subject of controversy [5].

In assessing risk, it is very important to distinguish relative risk from absolute risk. While etiologic studies commonly report relative risk measures such as odds ratios, hazard ratios, or relative risks, clinical interventions are usually based on

absolute risk estimates. Importantly, large relative risks may not translate to large absolute risks for rare diseases [6].

Risk assessment is a process that updates a baseline, prior, or pretest risk of disease in a certain population to a posttest risk. For example, the risk of cervical cancer and CIN3 in the general population is low. Screening tests like cytology or HPV testing change the prior, baseline risk estimate to a higher risk in test-positive women and to a lower risk in those who are test negative (Fig. 12.1). The absolute risk of disease in test positives is equal to the positive predictive value (PPV), while the absolute risk of disease in test negatives is equal to the complement of the negative predictive value (cNPV or 1-NPV) [6]. The difference in absolute risk between the two posttest risk estimates (PPV-cNPV) is a measure of risk stratification for a specific test.

Risk stratification is only meaningful when different risk levels result in different clinical practice. For example, HPV testing of ASC-US changes management. HPV testing of HSIL, on the other hand, is not worth doing because colposcopy is indicated regardless of the result [7].

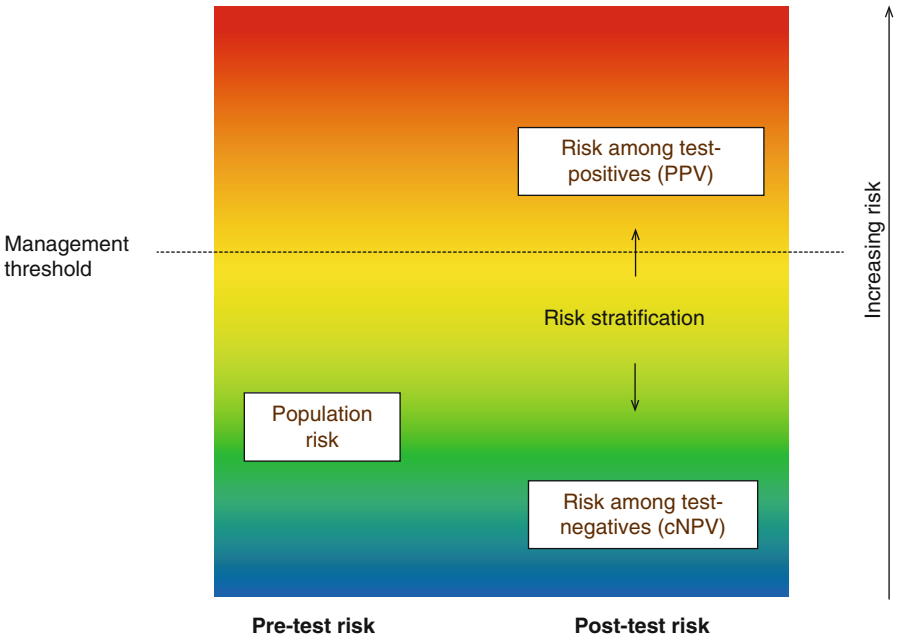


**Fig. 12.1** Risk stratification and risk-based management. The absolute risk of disease is shown on the *y*-axis. A test or biomarker stratifies the population with a pretest risk into two groups – one with a positive test and a higher risk of disease (positive predictive value, *PPV*) and a second group with a negative test and a lower risk of disease (complement of the negative predictive value, *cNPV*). The difference between PPV and cNPV is a measure of risk stratification. Risk stratification is only relevant when different risk levels lead to different management [6]

Absolute risk estimates have a temporal dimension. A risk can be estimated for disease present at the time of testing or for disease detected within several years after the initial test. The risk of future disease is important for selecting screening and management intervals. For example, compared to women with a negative cytology, women with a negative HPV test have a longer time interval before their risk rises to the threshold justifying rescreening [8]. Therefore, screening intervals can be safely extended to a longer time interval in women with negative HPV tests compared to women with negative cytology results.

These examples demonstrate that absolute risk levels should determine clinical management, rather than the result of a specific test. On a population level, different tests and various combinations of test results can have the same absolute risk of cervical cancer now or at a specified time interval in the future. This has led to the establishment of the principle of "similar management for similar risk." If two screening participants have the same risk of cancer, the principle holds that they should be managed similarly [9]. As new tests become available, they can be evaluated against specific risk thresholds, avoiding the need to develop recommendations specifically for each test modality.

## 12.3 Development of Risk Thresholds for Cervical Cancer Screening

Although risk is measured on a continuous scale, risk thresholds are important for clinical management. Importantly, the perception of risk may differ in different situations and societies. Therefore, risk thresholds are not absolute, but they are tied to a certain societal perception of risk and are often reflective of established clinical practice.

Cervical cytology has played an important role in defining risk thresholds for cervical cancer screening and for management of abnormal screening results. Traditionally, women with LSIL and high-grade squamous intraepithelial lesion (HSIL) cytology results have been referred for colposcopic evaluation (Fig. 12.2) [2]. ASCUS cytology results have posed a challenge to clinical management, since the interpretation of ASCUS is an abnormal cytology result, but with a lower aggregate risk of cervical precancer compared to LSIL or HSIL. Thus, the PPV or posttest risk of ASCUS is not high enough to refer women to colposcopy. In the ASCUS-LSIL Triage Study (ALTS), three management strategies for women with ASCUS cytology results were evaluated: immediate referral to colposcopy, repeat cytology, and triage with high-risk HPV (hrHPV) testing [10]. The trial demonstrated that hrHPV-positive ASCUS has a very similar risk to LSIL and led to recommending HPV testing for triage of ASCUS (ASC-US after the 2001 Bethesda update) cytology results [11, 12]. This is an early example of a systematic application of the "similar risk-similar management" principle in cervical cancer screening and management guidelines.

The risk of cervical precancer associated with a LSIL cytology result or an HPV-positive ASC-US cytology result is used as a risk benchmark for colposcopy referral. Other benchmarks have been defined accordingly. In the 2012 US screening guideline update, 3-year screening intervals were recommended for women with a negative cytology result [13]. Thus, the risk benchmark for 3-year rescreening is a

risk level equivalent to a negative Pap result [7]. Similarly, a 12-month repeat cytology is an accepted management for an ASC-US cytology result (with unknown HPV result). Consequently, the risk benchmark for a 6–12-month return is a risk level equivalent to an ASC-US Pap result (Fig. 12.2).

The same benchmarks can be used for primary screening and for management of abnormal screening results, since risk of cervical cancer is driving all clinical decisions. The risk benchmarks used for the 2012 American Society of Colposcopy and Cervical Pathology (ASCCP) updates to formulate the management guidelines for abnormal cervical cancer screening results were largely based on 5-year risk of histologic HSIL (CIN3) or greater observed in a cohort from the Kaiser Permanente Northern California, a large integrated health care organization with a population of over one million women screened with co-testing over 10 years [7].

While the absolute risk estimates for cervical cancer screening results may differ between populations, the relationship between the risk groups is very consistent, e.g., the aggregate risk for diagnosing histologic HSIL (CIN3+) in patients with cytologic LSIL is higher than that of those with cytologic ASC-US (HPV unknown) in most populations.

An important advantage of developing screening and management recommendations based on risk thresholds is that new assays can be integrated into current recommendations more easily based on risk equivalence studies. As noted above, the absolute risk thresholds among populations may vary; therefore, risks of precancer and cancer with new assays must either be specific to a population with established threshold-specific risks or risks at established benchmarks. For instance, the risk of precancer for a cytologic result of LSIL must be established for the population in which the new assay is validated.

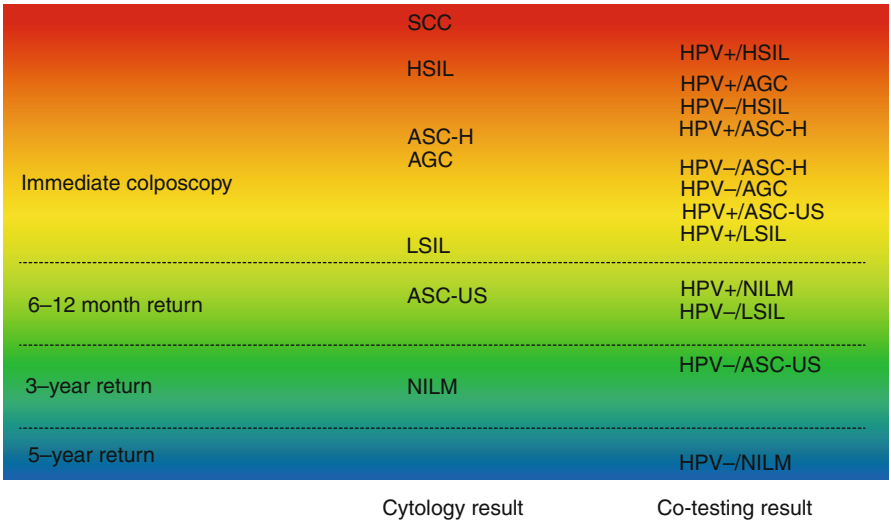


**Fig. 12.2** Risk benchmarks for 2012 ASCCP management guidelines. Absolute risk of cervical precancer is shown on the *y*-axis. Cytology results and co-testing results are grouped in their respective risk categories with different management strategies [2, 7]

## 12.4 Current Options for Cervical Cancer Screening

Cytology has been the mainstay for cervical cancer screening for decades and has led to substantial reduction in cervical cancer incidence in countries with screening programs. Our now remarkable understanding of HPV and cervical cancer natural history has brought new tools for cervical cancer prevention, including HPV vaccines for primary prevention, HPV testing for screening, and various molecular assays for detection of cervical precancers [14, 15]. These new options have been progressively introduced in the United States over the last decade. The first major change from cervical cytology-only screening came in the early 2000s with the addition of HPV reflex testing in cases interpreted as ASCUS [10, 16]. Another major change then occurred in 2002 when HPV testing in combination with cytology was proposed for the first time as a primary screening option compared to cytology alone [17] and was designated as the preferred method of screening in the over 30 years of age population in 2012 [13]. In 2014, the FDA approved an indication for primary HPV testing alone for a previously approved HPV test [1].

It is very instructive to evaluate the different cervical cancer screening options in the context of risk-based management (Fig. 12.3):

(a) Cytology-only screening has lower sensitivity for detection of cervical precancer and higher cNPV compared to the algorithms that include HPV testing; therefore, cytology-only screening needs to occur more frequently.
(b) The sensitivity of HPV-based screening is much higher compared to cytology and the cNPV is much lower, allowing safe extension of screening intervals.
(c) The further increase in sensitivity and decrease in cNPV of HPV and cytology co-testing compared to HPV alone is limited [18].

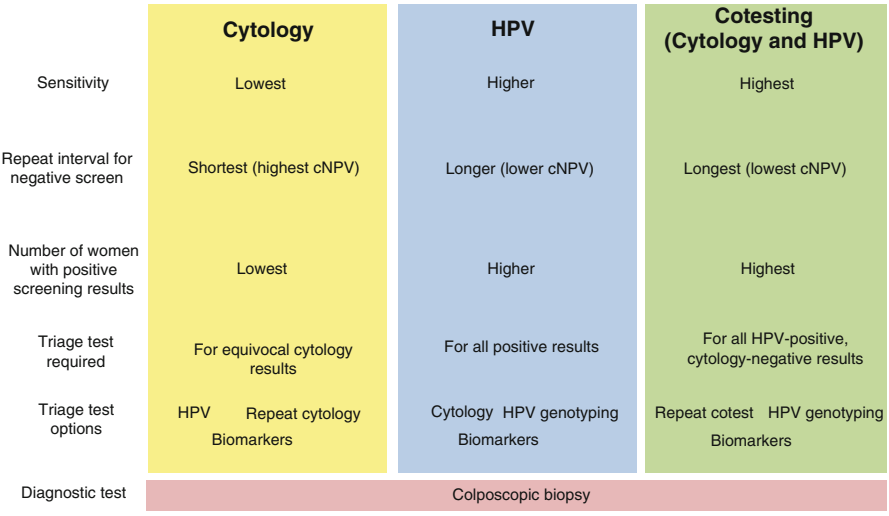


**Fig. 12.3** Current options for cervical cancer screening programs. The figure shows three currently available screening options with important characteristics such as sensitivity, screening interval, and requirement for triage tests [25]

All screening approaches require triage tests to identify women who need colposcopy. However, the extent to which triage tests are needed differs among the algorithms. In cytology-based screening, triage tests are needed only for women with ASC-US results. By contrast, for HPV-based screening, women testing positive for HPV need additional tests to decide who needs referral to colposcopy. The primary HPV screening algorithm approved by the US FDA in 2014 for one specific HPV test includes HPV genotyping for HPV16/18, with cytology referral for all women with other carcinogenic HPV types [1]. In HPV-cytology co-testing, two screening tests are performed for the whole population up front, reducing the need for triage strategies to HPV-positive, cytology-negative women.

hrHPV testing is used to triage women with ASC-US cytology results. Conversely, cytology has been proposed as a triage test for primary HPV screening. HPV genotyping has been evaluated for triage in HPV screening alone and in HPV-cytology co-testing [19]. Several other biomarkers, such as p16/Ki-67 cytology or host and viral methylation testing, are currently being evaluated and could become integral parts of screening and management algorithms in the future [20, 21]. The evaluation of any new triage tests would follow the same guiding principle of similar management for similar risk described above for primary screening. A triage test is evaluated based on its ability to stratify a population into higher- and lower-risk groups. The former requires further intervention/follow-up; the latter requires none or a lesser degree of intervention/follow-up (Fig. 12.1).

With so many options available for cervical cancer screening and management, choosing the optimal strategy can be a challenge. Decisions about cervical cancer screening must balance the benefit of preventing cervical cancer with the potential harms and cost of screening. Consideration must be given to the number of women screened to detect one with cancer, the number of screening tests over each woman's lifetime, the requirement for triage tests for an abnormal screen, unnecessary colposcopy referral, and the potential for overtreatment. The availability of many tested and proven choices for cervical cancer screening allows for designing new screening programs that adapt to specific needs in different healthcare systems, rather than just incrementally updating successful, but not necessarily efficient, programs. On the other hand, the number and complexity of options may be confusing to providers and could increase the risk that women may be lost to follow-up [22]. In a particular practice or geographic setting risk assessment, in conjunction with risk modeling and comparative effectiveness, research plays a central role in determining the optimal strategies for cervical cancer screening and management.

## 12.5 Conclusion

Cervical cancer screening programs, unchanged for decades, are now in flux. With different preventive options available, many countries are considering a variety of combinations, and no single "winning strategy" has yet emerged. The successful introduction of primary HPV testing into cervical cancer screening requires more than a sensitive screening test; robust triage tests are required to decide who among the HPV positives needs to be referred to colposcopy. Cervical cytology remains an

important component of current screening programs, as a stand-alone test, in co-testing, and for triage of women who test positive in HPV primary screening. In the future, cytology performed primarily for triage of HPV-positive women may have different test characteristics. For example, evaluating cytology with knowledge of HPV status can impact its performance compared to cytology conducted for the general population [23, 24]. Current risk-based benchmarks used in cervical cancer screening and management are largely based on established practice from cytology-based screening programs. It is conceivable that other risk thresholds will be explored in the future, weighing benefits and harms differently to address specific individual and public health needs. In cervical cancer screening, risk thresholds determine whether referral to colposcopy or treatment is needed and what time intervals should be chosen for different screening and management options. The risk scale described here is universal and independent of the test used. It can serve as a reference that allows making test-independent screening and management recommendations.

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