Anal Cytology

8

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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

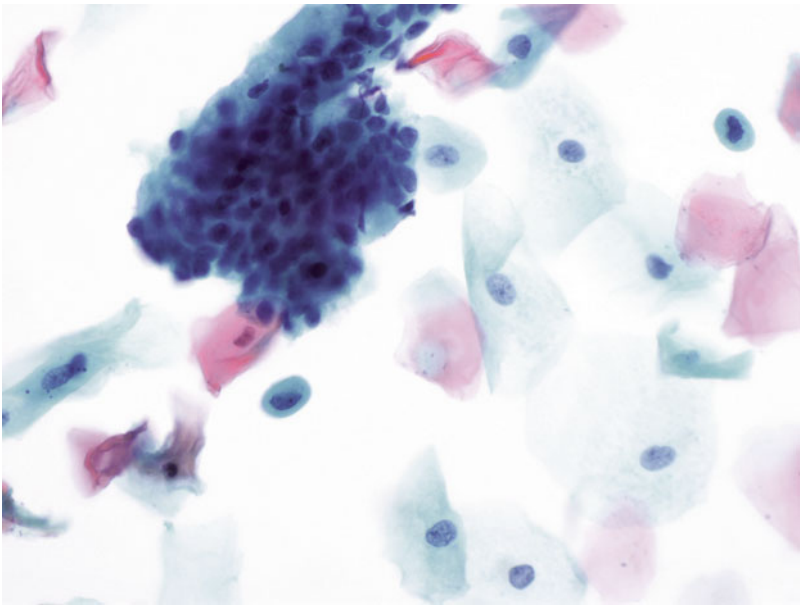

Fig. 8.1 Satisfactory specimen, negative for intraepithelial lesion (NILM) (*LBP, SurePath*). Benign intermediate type squamous cells, squamous metaplasia, and rectal columnar cells are present

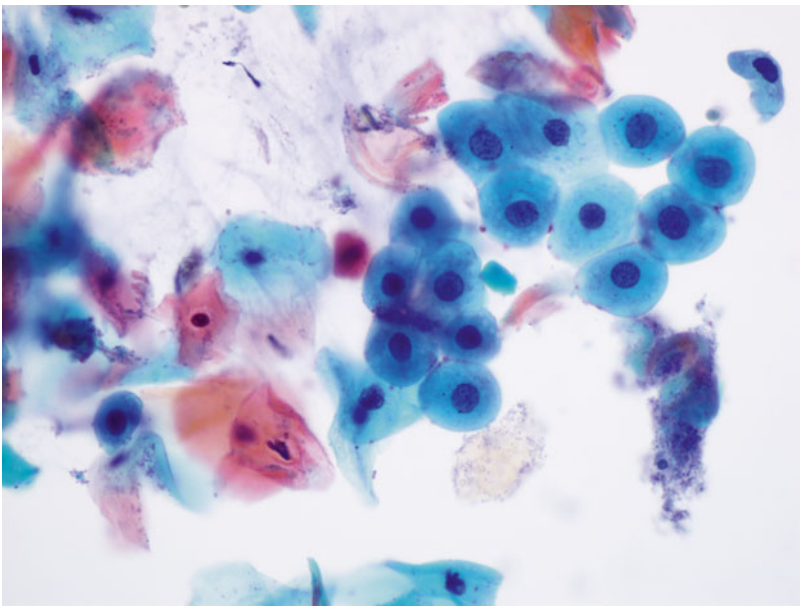

Fig. 8.2 Negative for intraepithelial lesion (*LBP, ThinPrep*). Several round squamous metaplastic cells with dense cytoplasm are present

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).

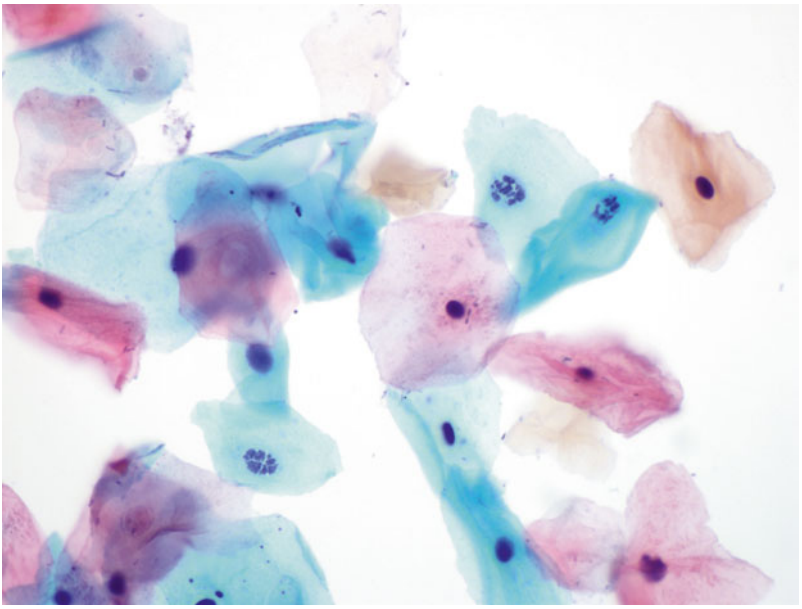

Fig. 8.3 Negative for intraepithelial lesion (*LBP, ThinPrep*). Benign squamous cells and anucleated squames. Nuclear karyorrhexis is present

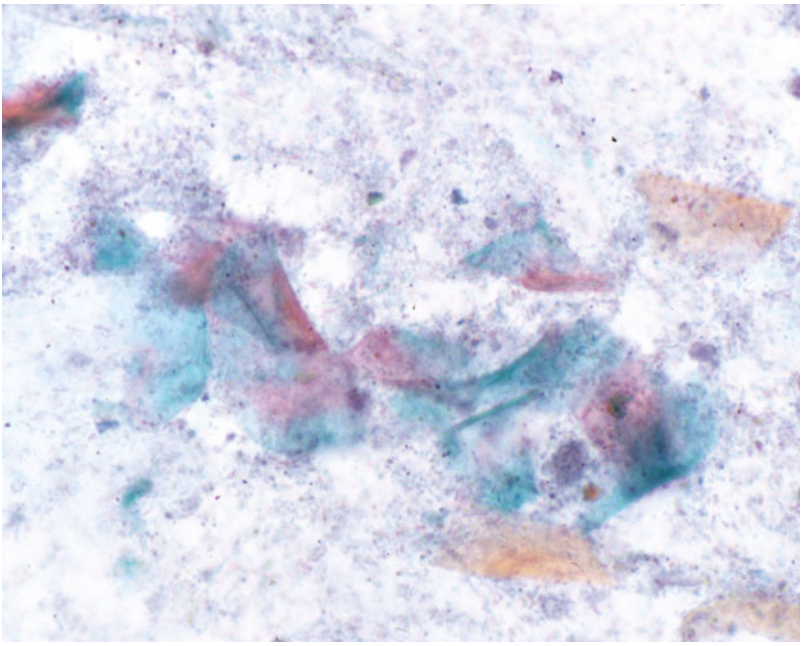

Fig. 8.4 Unsatisfactory specimen (*conventional preparation*). Particularly on conventional anal smears, bacteria and fecal material can predominate and obscure cellular detail

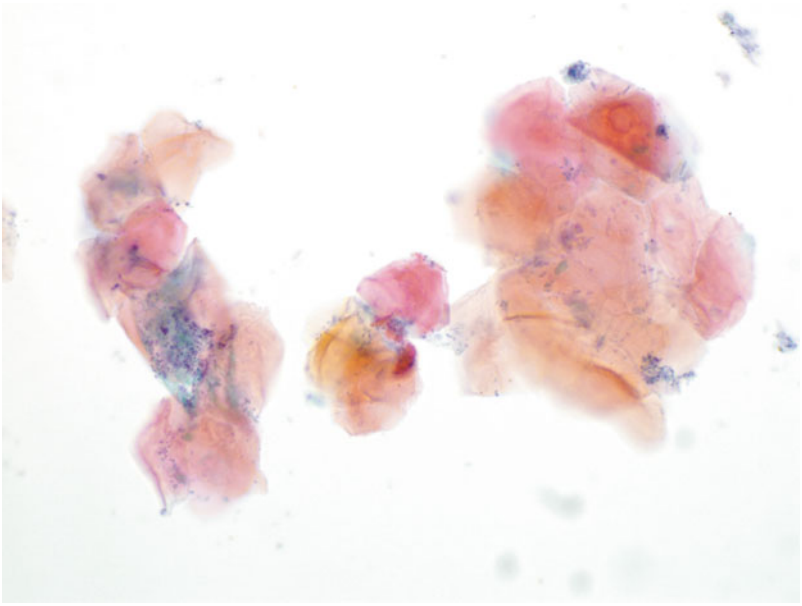

Fig. 8.5 Unsatisfactory specimen (*LBP, ThinPrep*). Anucleated squames only. On *ThinPrep* anal cytology, an average of 1–2 nucleated squamous cells per high-power field are needed for adequacy

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

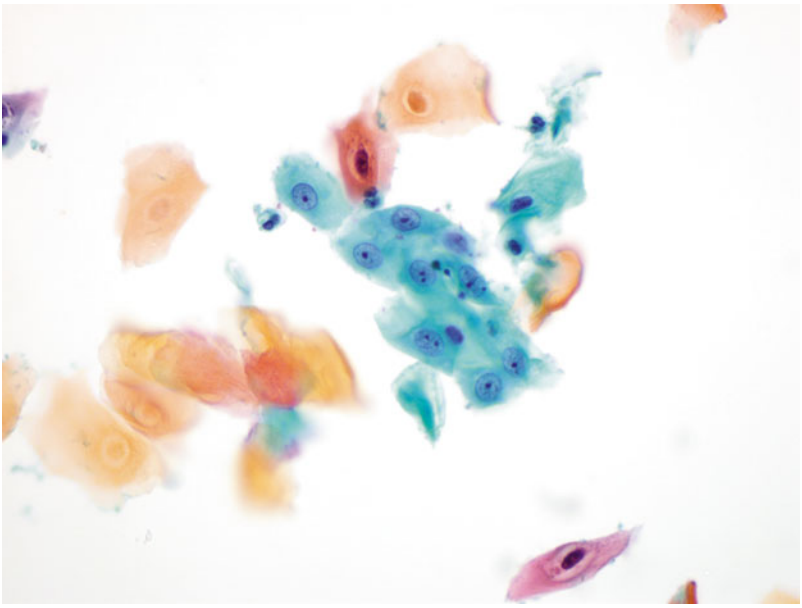

Fig. 8.6 Squamous cells with reactive nuclear changes including nuclear enlargement, hypochromasia, and nucleoli. Other cells have narrow perinuclear halos

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].

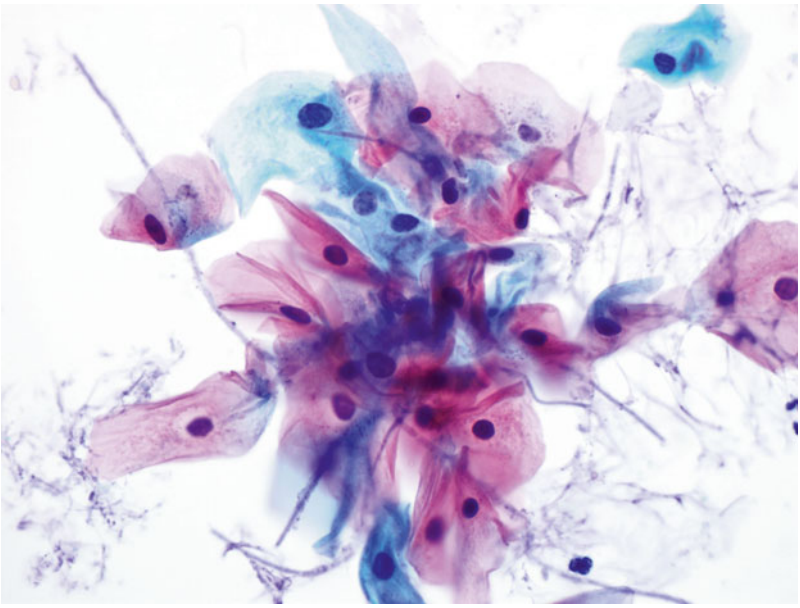

Fig. 8.7 Candida (*LBP, ThinPrep*). Fungal pseudohyphae are threading through the cluster of squamous cells

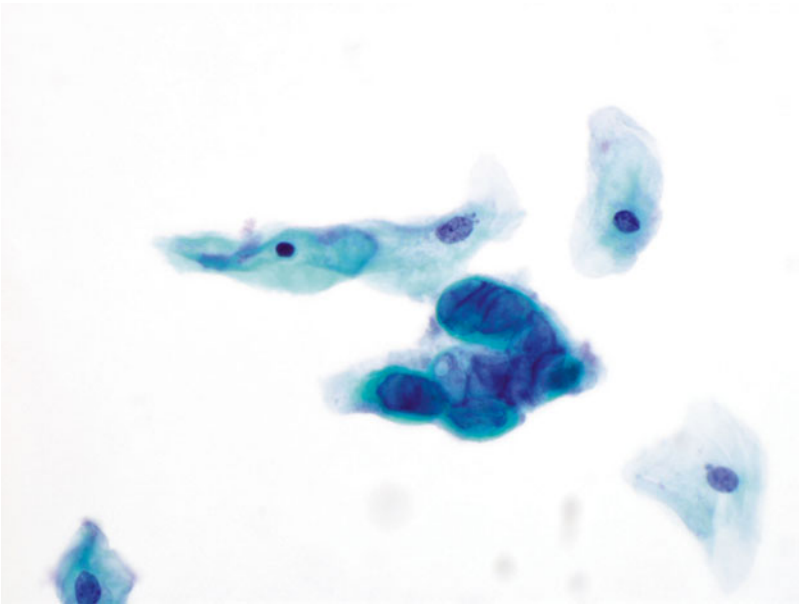

Fig. 8.8 HSV (*LBP, SurePath*). Molded nuclei with "ground-glass" appearance are present

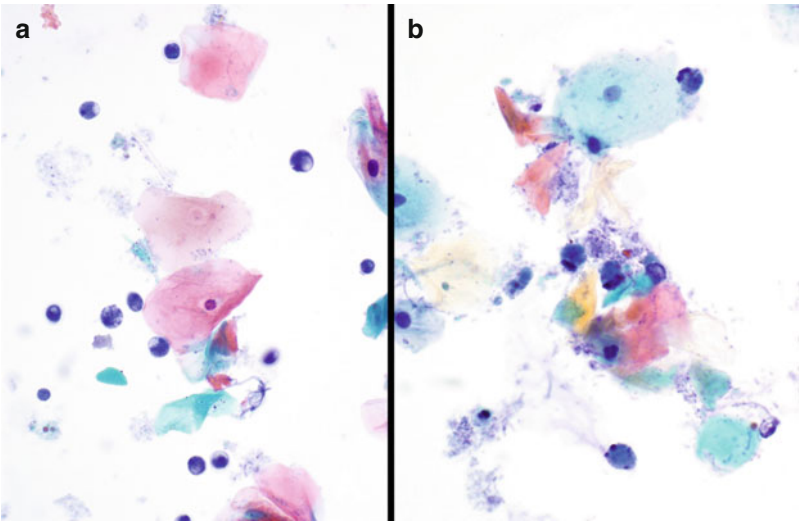

Fig. 8.9 (**a**) Numerous amebic cysts are present (*LBP, ThinPrep*). Internal structure and refractile cyst wall help differentiate ameba from HSIL. (**b**) Macrophages (*LBP, ThinPrep*) may be seen on anal cytology, particularly after ablative treatment and need to be distinguished from ameba. Note the cytoplasmic cellular debris

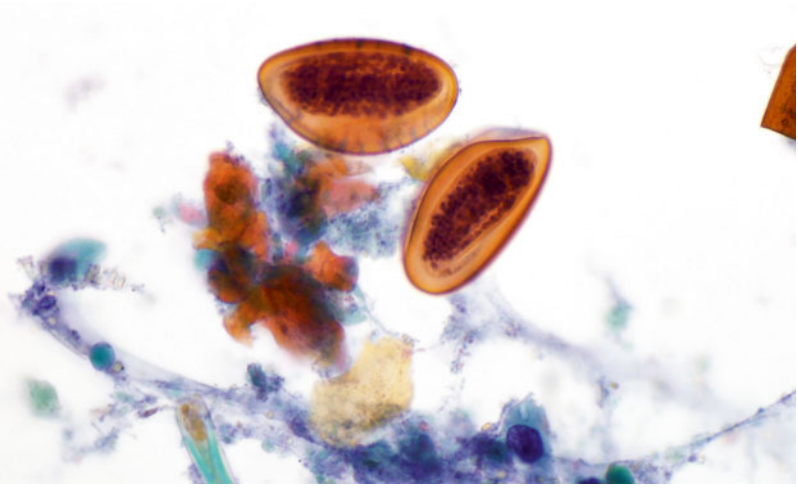

Fig. 8.10 Pinworm eggs (*LBP, ThinPrep*)

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.

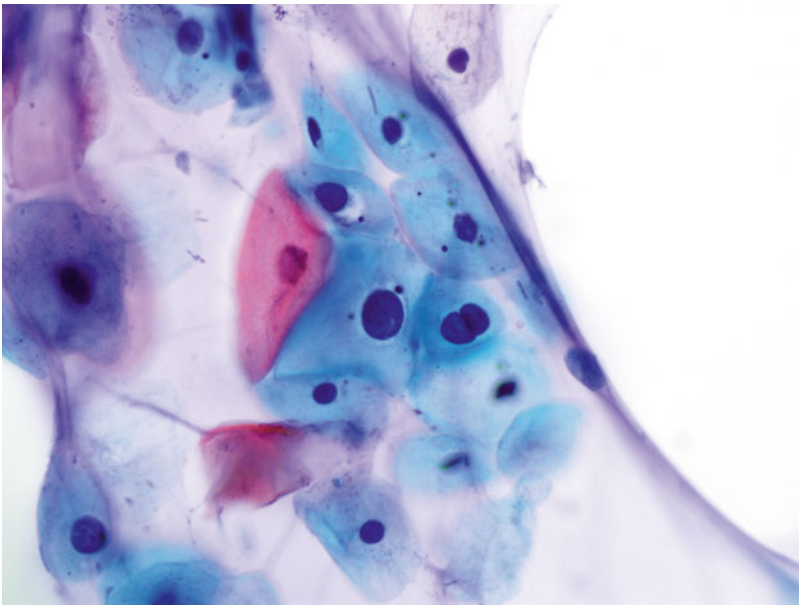

Fig. 8.11 ASC-US (*LBP, ThinPrep*). Atypical squamous cells with enlarged but smooth nuclear contours with smudgy chromatin and narrow perinuclear clearing. One cell is binucleated

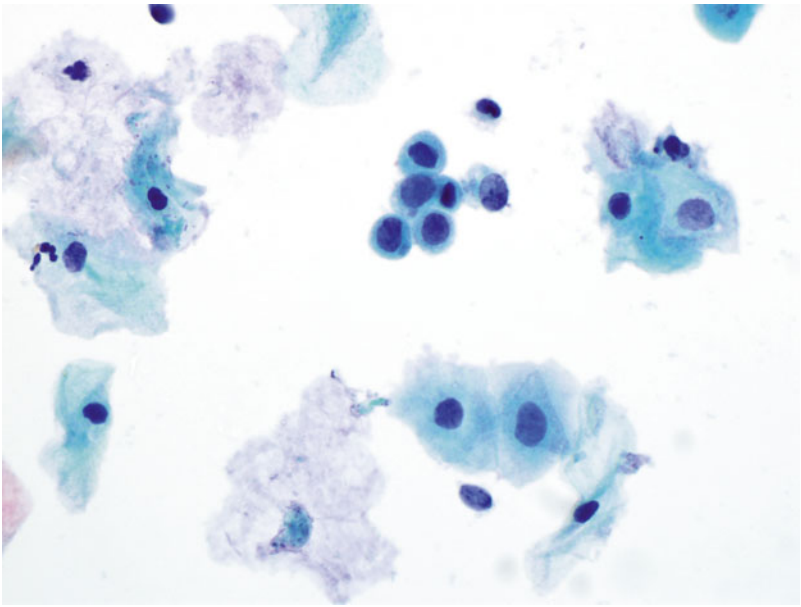

Fig. 8.12 ASC-H (*LBP, ThinPrep*). Small immature squamous metaplastic cells with dark but smudgy nuclear chromatin

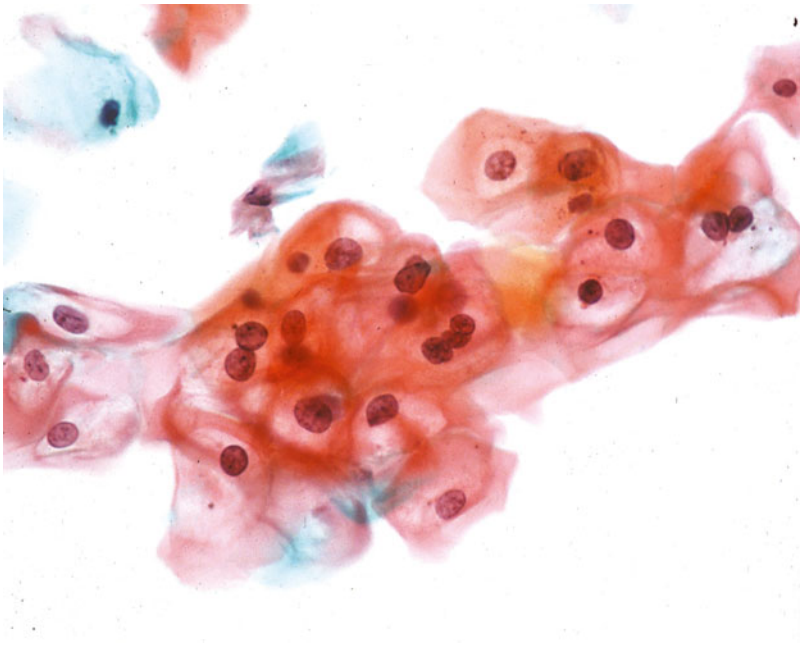

Fig. 8.13 LSIL (*LBP, ThinPrep*). Criteria for interpretation of SIL are similar to cervical specimens

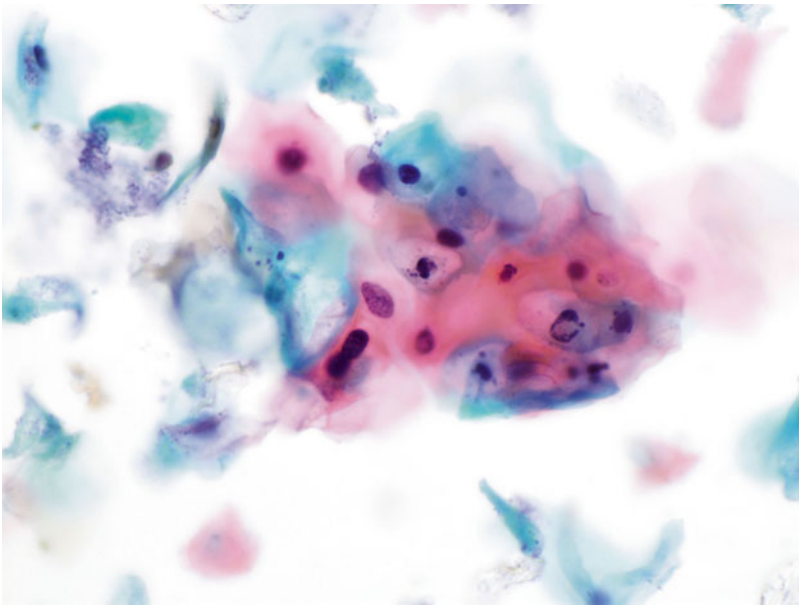

Fig. 8.14 LSIL with karyorrhectic nuclei (*LBP, SurePath*)

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).

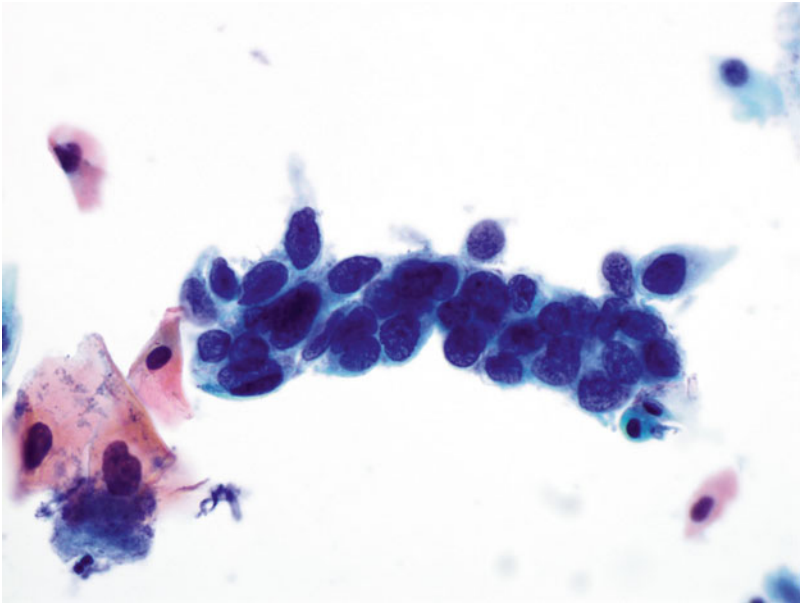

Fig. 8.15 HSIL (*LBP, ThinPrep*). Hyperchromatic group with altered chromatin pattern and irregular nuclear contours

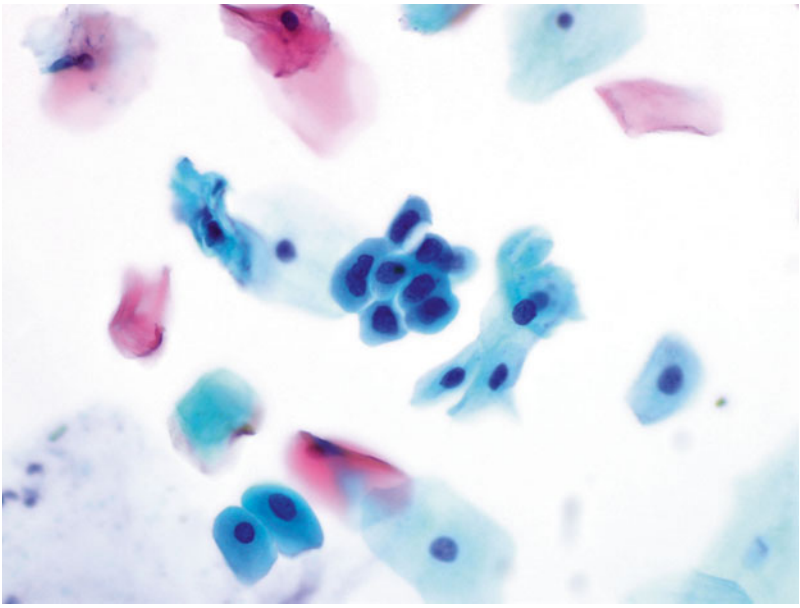

Fig. 8.16 HSIL (*LBP, SurePath*). Dysplastic cells with metaplastic cytoplasm and irregular nuclear contours

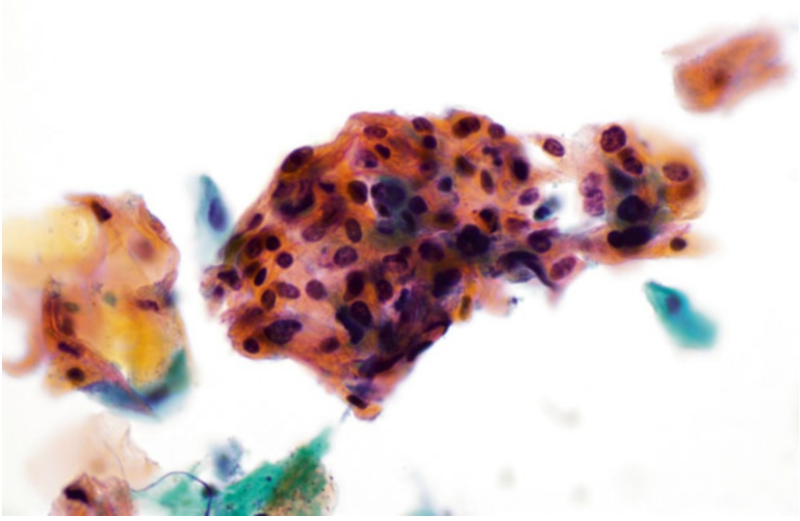

Fig. 8.17 HSIL (*LBP, ThinPrep*). High-grade keratinizing dysplasia

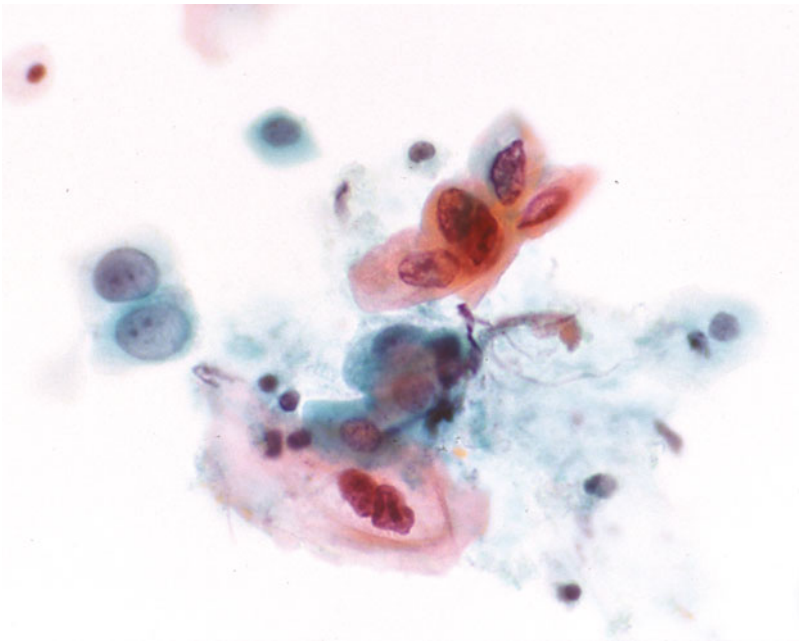

Fig. 8.18 Both HSIL and LSIL are present in this figure (*LBP, ThinPrep*). Note the cytoplasmic keratinization, a feature that is often more prominent in squamous lesions of the anal canal than in cervical lesions

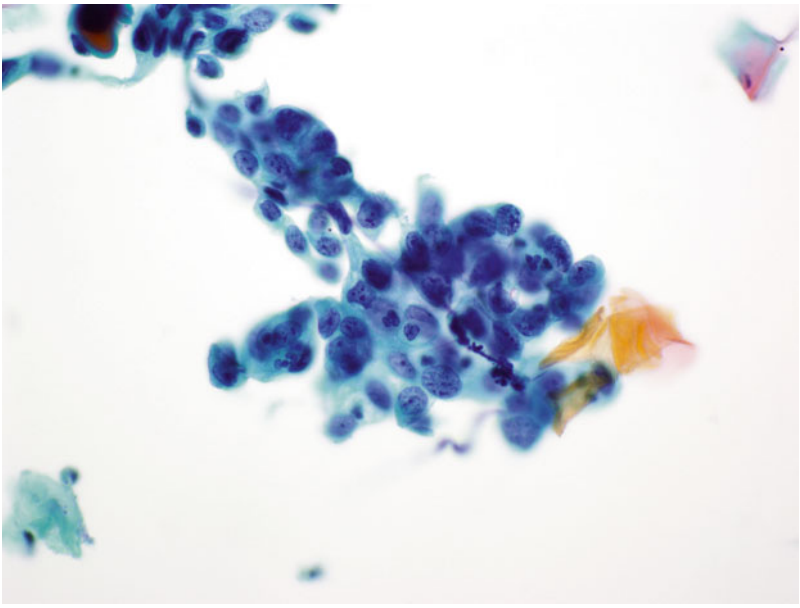

Fig. 8.19 HSIL (*LBP, ThinPrep*). Loose cluster of cells with dysplastic nuclei. Several nuclei have distinct nucleoli raising the possibility of an invasive process

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).

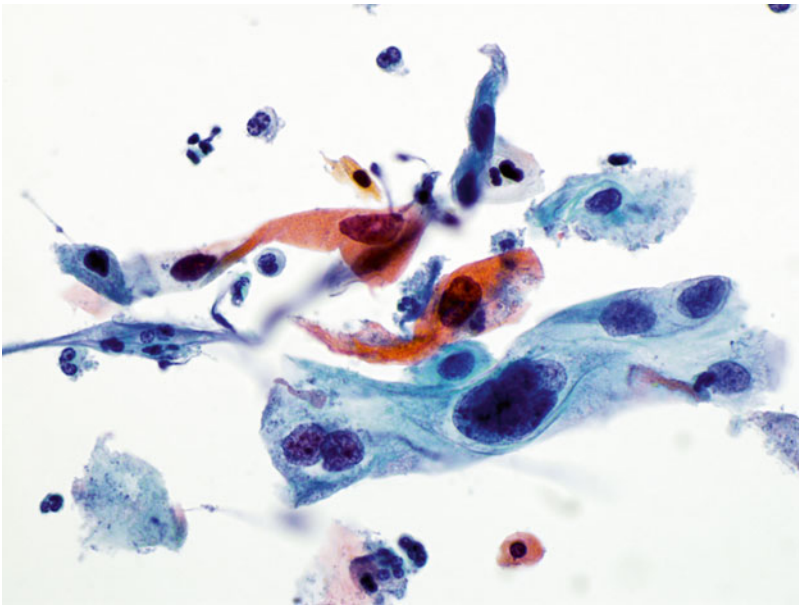

Fig. 8.20 SCC, keratinizing (*LBP, ThinPrep*). Marked pleomorphism of cell size and shape. Two tumor cells show cytoplasmic keratinization

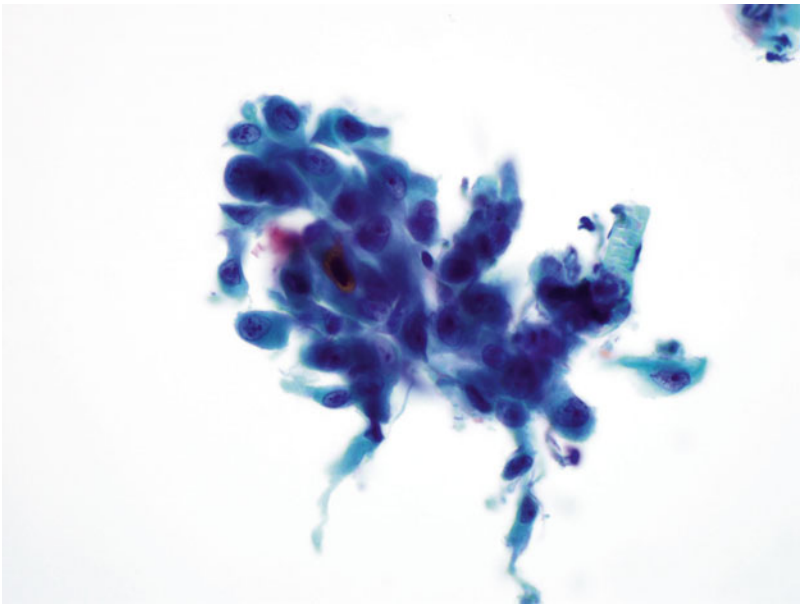

Fig. 8.21 Squamous cell carcinoma, nonkeratinizing. Pleomorphic cell cluster *(LBP, ThinPrep)*. Some tumor cells have prominent nucleoli. A tumor diathesis is not prominent in this field

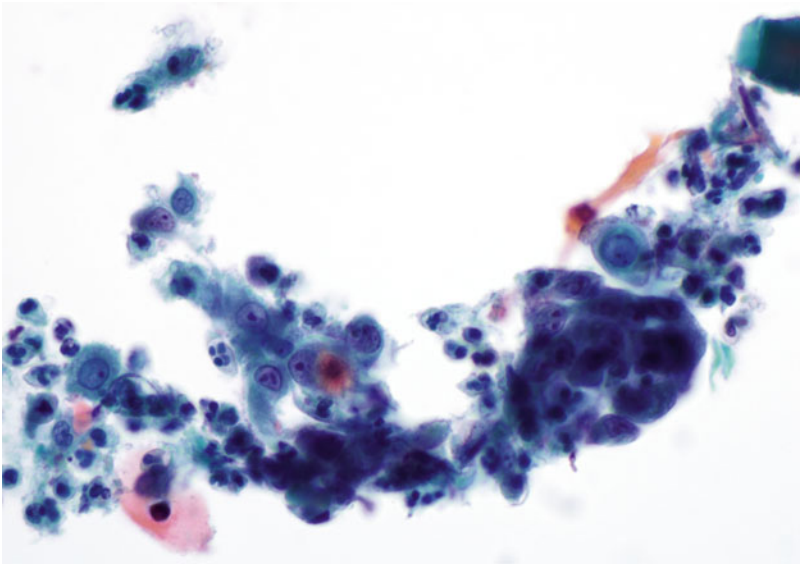

Fig. 8.22 SCC with "clinging" diathesis (*LBP, ThinPrep*)

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.

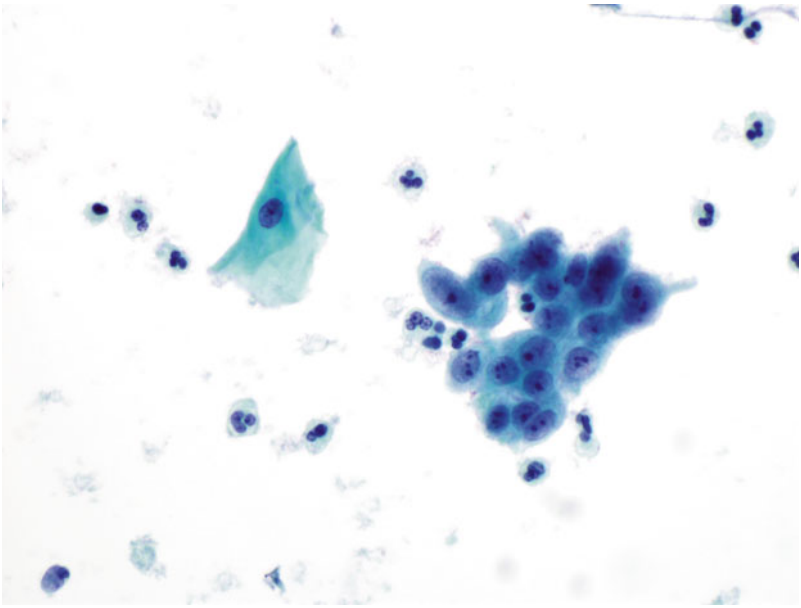

Fig. 8.23 Rectal adenocarcinoma (*LBP, ThinPrep*). Malignant cells have vesicular nuclei with prominent nucleoli and finely vacuolated cytoplasm. This is a recurrence of a rectal adenocarcinoma

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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