Anal Intraepithelial Neoplasia from a Pathologists Point of View

Keegan M. Lyons, MD, MPH¹ Samantha L. Butler, MD¹

¹Department of Pathology and Laboratory Services, San Antonio Military Medical Center, Ft. Sam Houston, Texas

Address for correspondence Samantha L. Butler, MD, Department of Pathology and Laboratory Services, San Antonio Military Medical Center, Ft. Sam Houston, TX 78234 (e-mail: samantha.l.butler.mil@mail.mil).

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Abstract

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Anal squamous cell carcinoma is a relatively rare diagnosis, but its incidence has continued to rise. Anal squamous cell carcinoma and its precursor lesion, anal intraepithelial neoplasia (AIN), are human papillomavirus (HPV)-associated squamous neoplasias. High-risk HPV subtypes cause cellular proliferation in the anal transformation zone mucosa leading to similar dysplastic changes as seen in the cervix. Unified cytologic and histologic classification systems have emerged for all HPV-associated squamous lesions of the lower anogenital tract due to recent advancements in the understanding of these lesions. P16 immunohistochemical stain, a biomarker for HPV, is recommended in the diagnosis of HPV-associated lesions. The unity of terminology will aid in communication between pathologists and clinicians, ultimately leading to improved patient care.

The rate of anal squamous cell carcinoma (SqCC) in the U.S. population has increased by 2.2% per year for the last 10 years, and while still a relatively rare diagnosis (0.5% of all U.S. cancer) it is estimated to newly impact 8,081 persons and cause 1,080 deaths in 2016.¹ Diagnosis of anal SqCC is often made after local spread and is seen as a “lost opportunity” for screening, where early detection of a precursor lesion could have altered the course of the disease.² However, due to the lack of long-term prospective studies on the natural course and progression from precursor lesion to anal SqCC, the exact timing and nature of appropriate screening tests has yet to be determined.^{1,3} Retrospective cohort studies have determined that risk factors for anal SqCC include human immunodeficiency virus (HIV) infection, men who have sex with men (MSM), anal receptive intercourse, women with multicentric lower genital tract squamous intraepithelial lesion (SIL), organ transplant recipients, and human papillomavirus (HPV) persistence in the anal epithelium.^{4,5}

Many similarities exist between cervical SqCC and anal SqCC to include common risk factors, development in the transformation zone, histo- and cyto-morphologic similarities, and, most importantly, the causative agent HPV, with at

least 95% of anal SqCC thought to be HPV-related.^{6–10} HPV infects basal epithelial cells with a predilection for the transformation zone. The virus enters the cells and alters cellular function to produce viral proteins and replicate itself. High-risk HPV, (hrHPV) subtypes, specifically HPV 16 and HPV 18, produce viral proteins E6 and E7 which function like oncoproteins. In hrHPV subtypes, E6 and E7 viral proteins interact with cell cycle growth factors allowing for integration into the hosts DNA, leading to uncontrolled cellular reproduction and escape of programmed cell death. HPV-infected cells can be targeted by the normal immune system, with clearing of the infection in most cases. Studies on HPV clearance have demonstrated median clearance to be 150 days.^{11–13} However, some HPV can escape immune regulation and integrate into the host cells' DNA. With a persistent infection, there is potential for gene dysregulation resulting in increased cell growth and progression to a precancerous lesion and possibly invasive carcinoma.^{4,5,7} In the cervix, the development of cervical SqCC via persistence of HPV may take approximately 10 years, with known cytological correlates for precancerous and malignant conditions.^{6,7} HPV in anal SqCC may progress through similar cycles of infection, clearance, persistence, and eventual

progression leading to AIN and anal SqCC.^{6,14–16} Concordance between cervical and anal HPV types was found to be 74% in one study, and while the rate of progression from AIN to anal SqCC is unknown, it is lower than the rate of cervical cancer progression despite high HPV prevalence.^{2,11,17}

Despite the connection between hrHPV and anal SqCC, HPV testing does not have the same impact and cost-effectiveness in anal cytology screening as in cervical cytology. In high-risk populations for anal dysplasia (HIV-positive MSM), HPV has been detected in up to 86% of all patients and 60% of non-HIV patients with other risk factors.^{16,18} HrHPV types have been found in a third of these patients and multiple coinfecting HPV types are common. HPV is nearly ubiquitous in anal SqCC and therefore the specificity of screening is very low. In a 2010 review article, HPV testing was found to be nearly insensitive for diagnosing or predicting anal SIL (21% PPV).¹⁸ With low sensitivity, low predictive value, and high HPV prevalence, the role of HPV testing is limited to conditions where negative result may impact clinical decision making. Negative HPV screening tests with a correlating normal cytology examination may provide a better assessment of short-term risk for anal dysplasia than cytology alone, but further studies are required for validation.² It has been suggested that HPV 16 genotyping alone may have a more important role in anal SqCC screening.¹⁹

Anal SqCC cytology screening has grown out of the successful cervical cytological screening pathway due to similarities in risk factors and anatomy.^{5,6,10} Both anal and cervical SqCC are overwhelmingly associated with HPV, and similar hrHPV types (primarily but not limited to HPV 16).^{6,14,16} The anal mucosa undergoes a similar transformation from glandular to squamous mucosa, resulting in more mitotically active basal cell exposure to potential infectious agents at this location. The transformation zone of both anal and cervical mucosa is exposed to micro abrasions during receptive intercourse and to chemical factors from exogenous and commensal microbiological organisms.^{8–10,14,16} The pathways to cancer are theorized to be similar and therefore the terminology and cytological findings from cervical cancer screening have been utilized for anal cytology screening.^{5,6,10}

Cytology Classification System for Anal Pap Tests: The Bethesda System

The Bethesda Classification System, better known as The Bethesda System (TBS), for cervical cytology is used as the basis for describing cytology findings from the anal canal. TBS is broken into categories of low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), atypical squamous cells (ASC) that include unknown significance (ASCUS), and cannot exclude high grade (ASC-H), and negative for intraepithelial lesion or malignancy (NILM). In the cervix, there is good correlation between cervical cytology and follow-up biopsy findings; however, in the anal mucosa this does not hold true. The value of anal cytology LSIL, ASCUS, and HSIL diagnoses is in predicting “any degree of SIL” and not a specific low- or high-grade finding on biopsy.^{3,16,18} The value of a single anal

smear to detect changes of a similar dysplastic category on follow-up histology is poor, ranging from 20 to 40%.^{3,16} It is unknown how this will change with repeated samples over time in a single patient, improved provider training, and increased screening of high- and moderate-risk patients.

Specimen Factors (Collection and Complications)

Cytology of the anal canal is complicated by sampling technique differences, the relative inexperience of many pathologists performing anal cytology evaluations, and contamination of the specimen with normal rectal flora and feces.³ Pathologist’s interpretations are sensitive to changes in quality of specimen’s received.⁹ To ensure the highest quality of anal cytology interpretation, consistent standardized collection technique is essential. The New York State Department of Health Aids Institute (NYSDHAI) guidelines indicate best performance with a Dacron swab inserted to at least 3 to 4 cm past the anal verge and continued wall pressure on the anal vault during swab withdrawal.¹⁴ The swab should be inserted at least to the point of the anal transition zone, and several authors recommend insertion to 6 cm or until resistance against the rectum is encountered.^{14,20,21} Rotating the collection device upon withdrawal may provide improved sampling by potentially sampling a larger portion of the anal canal. Sample transfer into the collection fluid should occur immediately to prevent specimen drying and limit specimen preparation artifacts. Several sources suggest twirling or swirling the collection device into the appropriate collection fluid (Thin Prep or Sure Path vials) with the collection device remaining in the fluid for 15 to 60 seconds.^{3,14} Dacron material for collection is preferred over wood or cotton devices, as it reliably releases more sample material into cytology fluid.^{14,16,20}

Precollection and postcollection factors can also affect the quality of an anal cytology specimen. Gel- or ointment-based lubrication for specimen collection is not endorsed due to its potential to obscure cytological findings in liquid-based cytology. No precollection rectal cleaning or patient preparation is required for anal cytology collection. However, patients should be advised against anal intercourse for 24 to 48 hours.¹⁴ Vigorous examination techniques have been associated with trauma and result in hemorrhage that limits cytology evaluation in some cases.²⁰ Post-collection, the experience and training of the reading pathologist has been directly related to changes in interpretation of cytology specimens.²

Adequacy Criteria

As in cervical cytology, the assessment of anal cytology specimens utilizing TBS begins with an estimation of specimen adequacy. Adequacy is based on overall specimen cellularity, presence or absence of obscuring factors (blood, feces), and the actual detection of an abnormality.⁶ The “adequate” rectal specimen should provide at least 2,000 to 3,000 nucleated squamous cells for review by the

pathologist.^{6,8} The squamous cells should be clear of obscuring artifacts such as fecal contaminants, excess bacteria, and overlying red blood cells. Cytological evaluation can be made on direct smear specimens from the rectal mucosa; however, several authors have indicated better reproducibly and reduced artifact for liquid-based cytology specimen collection and processing.^{3,16,20} Lack of clearly interpretable squamous cells and/or obscuring blood, bacteria, and fecal material are reasons for a diagnosis of “unsatisfactory for evaluation.”⁶ A quality indicator of adequate specimen collection, but not a cause for unsatisfactory specimens, is the presence of glandular (columnar) intestinal-type mucosa. The presence of glandular mucosal cells indicates that the transformation zone has been sampled and the specimen is therefore representative of the highest risk area for anal SIL.^{6,8} Any specimen with clearly identifiable SIL is deemed adequate for evaluation and will be interpreted by the pathologist.^{4,6,14,16}

Bethesda System Reporting Categories

NILM cytology interpretations are indications that only normal cells were found on cytology examination. The normal anal squamous cellularity is composed of superficial cells, intermediate cells, parabasal cells, and basal cells. The glandular component is composed of mucous secreting columnar or squamous metaplastic cells. These cells are mixed with normal bacterial flora of the lower gastrointestinal track, fecal material, and a minor component of red blood cells, white blood cells, and mucous. In the normal state, superficial squamous cells are shed into the fecal stream and replaced by maturing cells from deeper levels of the mucosa. The diagnosis of NILM is based on finding well-preserved superficial squamous cells, which have small, round, or pyknotic nuclei, and smooth nuclear membranes.^{4,6,8,9,21} Glandular cells are columnar to cuboidal in shape with cytoplasmic vacuoles and mucin^{4,6,18} (► Fig. 1).

NILM results from a single anal cytology specimen have been associated with up to 16% prevalence of SIL on follow-

up anal biopsy specimens by high-resolution anoscopy (HRA).^{16,18} This may be the result of failure to sample the dysplastic lesion by blind cytological collection. Following a NILM cytology result, the NYSHDAI recommends annual screening without lengthening subsequent screening interval.¹⁴ Based on current data, NILM results are encouraging, but continued clinical vigilance and testing are required.

LSIL anal cytology changes are characterized by nuclear and cytoplasmic changes within the anal squamous cells. The superficial squamous cells display enlarged nuclei, nuclear membrane irregularities, and chromatin clumping. Cytoplasmic changes can include perinuclear clearing (koilocytes) characteristic of HPV infection, but this finding is more common in cervical than anal cytology.^{6,8,9} Nucleoli are usually not prominent, and the nuclear-to-cytoplasmic ratio is increased (► Fig. 2).

LSIL is the most common anal cytology diagnosis among high-risk populations, which varies greatly from the cervix (>40% for anal vs. <3% for cervical).^{6,16,18} The finding of anal LSIL may indicate a transient HPV infection, but longitudinal studies have demonstrated hrHPV types in up to 30% of LSIL anal cytology patients.¹⁸ LSIL anal cytology does not correlate directly to low-grade dysplasia on follow-up anal biopsy histology, as up to half of patients were found to have high-grade dysplastic changes (AIN 2 or greater).^{16,18,20} LSIL cytology should be used as an indicator that significant dysplasia may be found on follow-up biopsy, and that the patient has significant long-term risk for AIN.¹⁴

HSIL anal cytology is characterized by markedly elevated nuclear-to-cytoplasmic ratio, nuclear and chromatin irregularity (► Fig. 3). These changes occur in less mature cells within the deeper anal mucosa and mitotic activity is a frequent finding.^{6,8,20,21} HSIL interpretation on anal cytology is meant to convey significant risk for follow-up high-grade dysplasia on biopsy. However, the sensitivity for HSIL anal cytology is poor for the finding of high-grade dysplasia on anal histology. In a retrospective review of high-grade dysplasia (AIN) patients, only approximately 25% were found to have a prior HSIL cytology determination.^{3,16} HSIL cytology was specific for

Fig. 1 (A) NILM cytology (Papanicolaou stain, x600) normal squamous intermediate cell (arrow) with small round nucleus surrounded by superficial squamous cells and hyperkeratosis. (B) Anal transformation zone (Papanicolaou stain, x600). Group of benign glandular (intestinal-type) mucosa representative of transformation zone sampling.

Fig. 2 (A, B) Low-grade squamous intraepithelial lesion (LSIL) cytology (Papanicolaou stain, x600). Squamous cells with enlarged nucleus, granular chromatin distribution, clearing around nucleus (halo effect), and cytoplasmic condensation at the edge of the halo consistent with LSIL koilocytic change. A single binucleated cell is also present.

high-grade dysplasia on histology and has moderate negative predictive value despite low overall positive predictive value.^{3,14,16} There are ongoing studies to determine long-term significance of HSIL anal cytology and high-grade anal dysplasia, with early findings suggesting progression in approximately 5% of patients over 13 years.^{4,14,15,20}

Perhaps the most clinically controversial interpretation in anal cytology is ASC and the subcategories of ASCUS and ASC-H. The diagnosis of ASCUS by anal cytology requires mild squamous cell nuclear enlargement and/or nuclear irregularities.^{6,8,9} ASCUS has been found in approximately 12% of anal cytology specimens from high-risk populations, and has a poor positive predictive value for high-grade dysplasia on retrospective biopsy correlation.¹⁸ ASCUS anal cytology has been found to precede high-grade SIL on follow-up biopsy in up to 10% of high-risk patients, so it should not be dismissed as insignificant.^{16,18}

ASC-H is similar in cytological features to HSIL, with similar nuclear changes and size variation. The primary difference between ASC-H and HSIL is the limited quantity of cellular findings, leading pathologists to question the underlying degree of dysplasia.^{6,16,18} In a 10-year study of HIV-positive patients, Johnson et al found ASC-H had a positive predictive value for SIL of any grade of 100%, but

limited sensitivity of 7% on subsequent biopsy.¹⁶ ASC-H is a significant finding that requires close clinical follow-up, but is not a reliable precursor lesion for high-grade SIL on biopsy.

While beyond the scope of AIN, cytology of the anal canal can be used to screen for nonneoplastic anal conditions as well as for SqCC. Organisms such as *Trichomonas*, *Entamoeba*, fungi, and some viral changes (CMV and HSV) can be reliably detected by anal pap smear.^{6,8} SqCC is suggested by markedly ASC with nuclear and chromatin irregularity, altered shapes, and a necrotic and inflammatory background.⁹ The definitive diagnosis of SqCC can only be made after the documentation of invasion by biopsy and there are no reliable features in anal cytology that are unique to distinguish HSIL, carcinoma in situ, and invasion.^{8,9} Cytology of the anal canal is limited in its ability to detect anal and sigmoid glandular dysplastic changes.^{16,20}

Implications of Cytological Categorization

Despite TBS categorization (NILM, LSIL, HSIL, and ASC), the diagnosis of any grade of dysplasia is of greater importance than category. Several studies have documented a high prevalence of SIL on follow-up anal biopsy for all cytology specimens diagnosed as “greater than or equal to ASC.”^{16,20}

Fig. 3 (A) High-grade squamous intraepithelial lesion (HSIL) cytology (Papanicolaou stain, x600). Squamous cells with high nuclear-to-cytoplasmic ratios, irregular nuclear membranes, hyperchromas, and coarse chromatin consistent with HSIL. Adjacent normal intermediate cells are present for size comparison. (B) HSIL cytology (Papanicolaou stain, x600). Squamous cells with similar morphology and more pronounced hyperchromasia.

The overall sensitivity of “greater than or equal to ASC” anal cytology is documented as 93% for the detection of SIL on biopsy. High sensitivity is contrasted with low specificity for predicting the severity or grade of dysplasia on subsequent biopsy (50%).^{14,16} Based on the high specificity and low sensitivity for anal cytology, clinical follow-up and consideration of biopsy is warranted for any patient with cytology findings of “greater than or equal to ASC.” Long-term studies have documented that 67% of all anal cytology specimens in high-risk patients will be abnormal, resulting in a large number of biopsies and follow-up requirements.¹⁶

Overall Statistical Analysis of Anal Cytology

Anal cytology screening programs are intended to find anal dysplasia prior to progression to cancer, and overall cytology performs well in select patient groups. The sensitivity for finding dysplasia ranges from 47 to 90% in published data, while specificity ranges from 16 to 92%.^{17,20} The positive predictive value for finding a high-grade dysplastic lesion on subsequent histology ranges from 47% for LSIL cytology to 92% for ASC-H cytology in a published study by Johnson et al.¹⁶ Greater than 60% of high-risk MSM were found to have abnormal cytology overall and 87% were HPV positive.¹⁶ The best statistical results for a dysplasia diagnosis result from combining Bethesda categories into “greater than or equal to ASCUS,” with negative predictive values ranging from 82 to 88% overall.²² As a screening tool, anal cytology performs well, but should not be used to follow up or surveil dysplastic lesions due to low sensitivity and sampling variability.

Interobserver Variability

The training and experience of the community pathologist is limited with anal Pap smears and may be causal to moderate interobserver variability.⁴ Pathologist’s agreement with a standard “reference determination” on educational material was documented as 61% for anal cytology versus 85% for cervical cytology.²³ In a recent review by Darragh et al, agreement between two observers was 66% (moderate agreement) using the individual Bethesda Categories and 86% (good agreement) for stratification of “ASCUS + .”²⁴ In a study of four pathologists independently reviewing anal cytology, Zhao et al documented moderate agreement with a Kappa statistics of 0.54.^{7,25} Anal cytology has not yet achieved the consistent high interobserver agreement that cervical cytology has demonstrated.

Histologic Terminology for HPV-Associated Lesions in the Lower Anogenital Tract: The LAST Project

TBS standardization of lower anogenital tract terminology was ahead of its time when first published in 1988, with later revisions in 1991, 2001, and most recently in 2014. Universal utilization of the TBS first in the United States and then internationally bridges the communication gap between

pathologists and clinicians for these HPV-related lesions.^{6,26} A two-tiered system of low-grade intraepithelial lesion and high-grade intraepithelial lesion worked well when analyzing individual cell characteristics for features of dysplasia. However, biopsy diagnoses rendered for these squamous lesions were traditionally classified into a three-tier system. This three-tiered classification system, initially introduced by Richart in 1969, evolved from histopathologic changes seen in cervical mucosa, though similar changes were reported in the literature on anal mucosa.^{27,28} Richart and Barron proposed that cervical carcinogenesis was a continuum which began with mild dysplasia and evolved into cervical cancer, thus he conceived the term *cervical intraepithelial neoplasia* (CIN).^{29,30} The three tiers in intraepithelial neoplasia were defined as: mild dysplasia (-IN1), moderate dysplasia (-IN2), and severe dysplasia (-IN3). The idea that precancerous squamous lesions of the cervix lead to invasive cancer in a step-wise fashion led to aggressive clinical management of cervical lesions. It was not until progress was made with the understanding of the biology of HPV and cervical carcinogenesis that the biologic differentiation between CIN1, CIN2, and CIN3 emerged.^{12,13,31,32} It became clear that a two-tiered system of low-grade intraepithelial lesion (CIN1) and high-grade intraepithelial lesion (CIN2 and CIN3) was more biologically relevant and improved diagnostic reproducibility among pathologists.³³ Though the two-tiered system was successful in the reporting of cytological abnormalities, adoption of a two-tiered system for histologic diagnoses did not gain support among professional organizations. Thus, biopsy diagnoses from all regions within the lower anogenital tract, to include the anal canal, followed the paradigm set for the three-tiered system as reported in cervical histopathologic diagnoses. Anal preinvasive and invasive lesions of both cutaneous perianal lesions and lesions arising from the transformation zone of the anal canal were initially classified together until evidence emerged of the pathophysiologic and clinical differences between the lesions in these locations.^{27,28} Dysplasia of the anal canal, studied adjacent to anal canal carcinoma, was first described by Fenger and Bichel with follow-up publications linking the disease to the HPV carcinogenic pathway.^{34,35} The terminology for *intraepithelial neoplasia in the anal canal* (AIN) was introduced and divided into AIN1, AIN2, and AIN3, similar to corresponding cervical CIN terminology. For the purposes of this publication, the terminology discussion will focus on lesions arising in the anal canal. The anal canal is described surgically, by the American Joint Committee on Cancer, as extending from the apex of the anal sphincter complex, termed the anorectal bundle, to the palpable intersphincteric groove at the outermost boundary of the internal sphincter muscle, termed the anal verge.³⁶

The Lower Anogenital Squamous Terminology (LAST) Standardization Project, cosponsored by the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology published in 2012, was devised to standardize histopathologic terminology for HPV-associated squamous lesions from all sites in the lower anogenital tract. The LAST project highlights their common pathophysiology

while diminishing variability in diagnoses by pathologists. The recommendations from the intraepithelial lesion terminology working group focused on (1) creating a unified histopathologic nomenclature with a single set of diagnostic terms for all HPV-related preinvasive squamous lesions, and (2) recommending a two-tiered nomenclature for HPV-associated squamous lesions, designated as LSIL and HSILs. This two-tiered nomenclature may then be further classified with the appropriate -IN sub-categorization, with -IN1 corresponding with LSIL and -IN2 and -IN3 corresponding with HSIL.³⁷ Additionally, the LAST terminology harmonizes the cytopathology and histopathology diagnostic terminology for lower anogenital lesions, thus lessening confusion among clinicians.

LSIL, or AIN 1, reflects lesions where the biology of HPV translates into a transient active replication and are regarded as self-limited. Morphologically, these lesions result in proliferation of the basal cell layer (basal cell hyperplasia) to involve the lower third of the squamous epithelium with abnormal nuclear-to-cytoplasmic ratios, nuclear enlargement, and nuclear membrane irregularities. Maturation of the squamous cells then begins in the middle third of the epithelium. Additionally, mitotic figures rise above the basal cell layer and involve the lower third of the epithelium. Though not necessary for a diagnosis of LSIL, the presence of diagnostic cytopathologic effect of HPV (koilocytosis) is pathognomonic for this low-grade lesion. Koilocytes are defined as cells with enlarged nuclei, irregular nuclear membranes, and often irregular chromatin distribution accompanied by a perinuclear halo (→Fig. 4). Koilocytes can also be multinucleated. *Condyloma acuminatum*, an exophytic lesion that can be encountered in the perianal region, is a papillary proliferation with low-grade cytopathologic features generally caused by low-risk HPV types 6 and 11. According to LAST terminology, these lesions should be diagnosed as LSIL and can be further characterized as condyloma in parentheses.³⁷

HSILs encompass both AIN2 and AIN3. High-grade lesions have the potential to progress to invasive carcinoma. However, the -IN2 category was plagued with reproducibility issues among pathologists, along with unclear understanding of its biologic behavior and risk of progression to can-

cer.³⁸ It is thought to be a mixture of both low-grade lesions and precancerous lesions, which are unable to be further defined via light microscopy.³⁸ Additionally, many CIN2 lesions on biopsy were found to harbor CIN3 lesions on further follow-up.³¹ Thus, the two-tiered system allows for improved reproducibility of diagnoses among pathologists, while placing -IN2 in the appropriate category of high grade to represent the increased risk of this preinvasive lesion. HSIL lesions represent proliferation of squamous or metaplastic squamous cells up to the middle third and/or superficial thirds of the epithelium, along with abnormal nuclear features to include nuclear enlargement, nuclear membrane irregularities, and increased nuclear-to-cytoplasmic ratios. Mitotic figures are conspicuous and are often found in the upper half of the epithelium (→Fig. 5A).

Occasionally, reactive benign epithelium or metaplastic epithelium may emulate some features of HSIL lesions and thus can cause diagnostic dilemmas in small biopsy tissue. The LAST project was additionally tasked with evaluating the utility of biomarkers for HPV-associated lesions. Based on literature review and data extraction, the use of p16 immunohistochemical stain is recommended when the morphologic differential diagnosis includes both HSIL (-IN2 and -IN3) and a benign mimicker of precancerous lesions.³⁷ P16 biomarker expression reflects the presence of viral proteins E6 and E7, known cell cycle proliferation proteins integrated into the hosts DNA, which are associated with HSIL. Interpretation of p16 as positive requires strong, diffuse block positivity of the epithelium (→Fig. 5B). Studies have shown that more than 99% of CIN3 lesions are p16-positive.³⁹ Histologically benign biopsies were p16-positive in less than 5% of cases. On retrospective review, many of the p16-positive “benign” biopsies were reclassified as HSIL due to small HSIL lesions that were missed at initial diagnosis.^{39,40} Ki-67 immunohistochemical stain, a proliferative biomarker, can also be used in conjunction with p16 to detect increased proliferative activity. However, the literature evidence supporting the use of Ki-67 as a sensitive biomarker in the differential between HSIL and benign mimickers is not as strong as the evidence for the use of p16.³⁷

From a pathologist's point of view, the cytologic and histologic terminology for the lower anogenital region, to

Fig. 4 (A) Normal anorectal mucosa (H&E stain, x200). Normal squamous mucosa flanked by rectal glands consistent with anorectal transformation zone mucosa. (B) Low-grade squamous intraepithelial lesion (LSIL/AIN1) (H&E stain, x200). Squamous mucosa with basal cell hyperplasia, nuclear enlargement, and koilocytic change (arrow). Several of the nuclei are binucleated which is a feature seen associated with human papillomavirus changes.

Fig. 5 (A) High-grade squamous intraepithelial lesion (HSIL/AIN3) (H&E stain, x200). Squamous mucosa with full-thickness abnormal proliferation of nuclei with high nuclear-to-cytoplasmic ratios and loss of nuclear polarity. (B) P16/Ki-67 double immunohistochemical stain (x400). Diffuse, block staining with p16 immunohistochemical stain (pink) and Ki-67 demonstrating increased proliferation extending full thickness (brown).

include the anal canal, has evolved dramatically in recent years and now serves as an effective diagnostic communication tool for clinicians. The pathology community has come together to put forth a classification system which reflects the advancements in the biologic understanding of HPV-associated lesions of the lower anogenital tract, thus bridging the communication gap so often encountered between pathologists and clinicians. To provide the best diagnostic results, clinicians should use standardized collection techniques to ensure the highest quality specimen for anal cytology interpretation. Too frequently, anal pap tests that come across a pathologist's desk are diagnosed as unsatisfactory due to lack of an adequate number of squamous cells. Furthermore, the screening tools used for cervical neoplasia, such as pap tests and HPV molecular diagnostics, are not as effective in detecting anal squamous lesions despite similar HPV pathophysiology. Continued research on the pathophysiological differences between the two mucosal regions may shed light on enhanced or alternative screening methods which may be more useful to detect at least high-grade anal lesions which may progress to anal SqCC. Once better screening methods have been adopted, evidence-based consensus management algorithms can be developed to aid clinicians in appropriate management of these patients afflicted with anal squamous dysplastic lesions.

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