At LAST: Consensus Recommendations for Histopathology Reporting of HPV-Associated Squamous Lesions of the Lower Anogenital Tract

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Dr. Ritu Nayar, well known to readers of The ASC Bulletin as a past Chair of the Scientific Program Committee and Co-Editor of The Bethesda System "Blue Book," has provided a summary of the recommendations of the LAST Conference concerning a unified classification of HPV-driven lower anogenital tract squamous lesions. As you will read, surgical pathology has finally "caught up" with cytopathology by categorizing these lesions into a two-tiered system and has even adopted the cytologic terminology of LSIL and HSIL so familiar to all of us. This conference and the new terminology it has produced promise to improve the reproducibility and clinical utility of pathologic diagnosis of HPV-driven lesions. This will help all of us make our daily efforts to identify and treat precancerous lesions before they can threaten patients' lives all the more effective.

Michael J. Thrall, MD, Editor, The ASC Bulletin

The terminology used for HPV-associated lesions of the anogenital tract has varied historically over time as ideas and hypotheses of pathogenesis have changed. Terminology has also varied on the basis of the training and clinical expertise of the reporting pathologist (cytopathologist,
surgical pathologist, dermatopathologist, etc.). The resultant plethora of terms has resulted in potential communication issues between the laboratory and the patient’s health care provider. Additionally, over the past few decades, new and valuable insights into the biology of HPV-associated neoplasia and a clearer understanding of the limitations of both pathologist diagnostic reproducibility and colposcopy have been realized.1,2 In cytopathology, the principles underlying The Bethesda System for reporting cervical cytology are communicating clinically relevant information from the laboratory to the provider, using terminology that is reasonably reproducible across different pathologists and laboratories, and that reflects the most current understanding of the disease process.3

More recently, the concept of risk assessment to guide patient management, such that those with similar risk get comparable follow-up and/or intervention, and the concept of balancing of benefits and harms have been increasingly incorporated into consensus guideline development.4 However, as a starting point for the effective utilization of management guidelines, it is imperative that clear, comparable and reproducible communication of diagnosis exists between and among pathologists and clinicians.

The Lower Anogenital Squamous Terminology (LAST) Standardization Project for HPV-Associated Lesions, co-sponsored by the College of American Pathologists (CAP) and the American Society of Colposcopy and Cervical Pathology (ASCCP), was undertaken to propose histopathology terminology for HPV-associated squamous lesions of the lower anogenital tract (LAT) that would be more reflective of their unified biology and help address the variability and reproducibility issues, much in the way that The Bethesda System did for gynecologic cytopathology.

The process was lead by a steering committee (SC) and five work groups (WG) that consisted of experts in the field including surgical pathologists, gynecologic pathologists, dermatopathologists, and medical and surgical specialists including gynecologists, gynecologic oncologists, dermatologists, infectious disease specialists, and representatives from government organizations. The WGs performed an extensive literature review on the key questions assigned to them and posted draft recommendations on line for a public comment period, followed by the LAST Consensus Conference held March 13 -14,

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2012 in San Francisco. Thirty-five participating organizations sent representatives to review, discuss, and revise the recommendations. Each recommendation required a two-thirds majority (66% or higher) to pass; those not achieving consensus on the first vote were revised by the WGs and submitted for a revote. All final recommendations achieved the required majority votes.

The LAST project addressed intraepithelial and minimally invasive squamous lesions and the recommendations were published in 2012. For the purposes of this review, only the recommendations and rationale for the intraepithelial lesion terminology (WG2) and biomarker usage (WG4) are summarized below.

LAST Recommendations and Rationale
Squamous Intraepithelial Lesions

1. A unified histopathological nomenclature with a single set of diagnostic terms is recommended for all HPV-associated preinvasive squamous lesions of the lower anogenital tract (LAT).

2. A two-tiered nomenclature is recommended for non-invasive HPV-associated squamous proliferations of the LAT which may be further qualified with the appropriate –IN terminology.

Note: -IN refers to the generic intraepithelial neoplasia terminology, without specifying the location. For a specific location, the appropriate complete term should be used. Thus for an –IN 3 lesion: cervix=CIN 3, vagina=VaIN 3, vulva=VIN 3, anus=AIN 3, perianus=PAIN 3, and penis=PeIN 3.

3. The recommended terminology for a two-tier system is Low Grade Squamous Intraepithelial Lesion (LSIL) and High Grade Squamous Intraepithelial Lesion (HSIL), which may be further classified by the applicable –IN subcategorization.

Rationale:

We know now that HPV may have one of two interactions with squamous epithelium: (a) a transient, self limited, infectious or productive phase, which produces low grade lesions, mild dysplasia, condyloma or grade 1 intraepithelial neoplasia; or (b) viral oncogene overexpression that drives cell proliferation to produce a clonal expansion of relatively undifferentiated cells referred to as high grade lesions or “precancer”, which are characterized by persistent viral detection and carry a substantial risk of progressing to invasive cancer over time.

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The LAST literature review showed strong evidence of biologic and morphologic similarity of HPV-associated squamous lesions across the lower anogenital tract, supporting the use of similar nomenclature that is distinct and separate from non-HPV-associated squamous lesions, i.e. differentiated VIN in the vulva. Not only does our current understanding of HPV biology support a two-tier nomenclature, but there is also substantial evidence that for cervical disease, a two-tiered system of reporting is more reproducible (with higher kappa statistics) than a three-tiered system. Furthermore, CIN2 has the lowest reproducibility of the 3 tiers and is considered the “ASC-US” of histology. This is likely due to the fact that CIN2 is not a true biologic or distinct histologic entity, but rather represents a mixture of low-grade and high-grade lesions with borderline histopathologic features between classic CIN1 or condyloma and CIN3. Additionally, there are no current molecular marker-based studies to support a three-tiered biology, and the LAST WG4 found that the use of p16 to potentially upgrade or downgrade equivocal (CIN2) lesions effectively leads to a two-tiered classification system.

From the management perspective, a two-tiered approach is already in use for the most part—“watch and wait” for LSIL and “treat” for HSIL. The public comments obtained during the LAST project also strongly supported these recommendations. This is not surprising, considering that recent gynecologic pathology textbooks already use a two-tiered nomenclature for cervix/vagina lesions, and the most recent International Society for the Study of Vulvovaginal Disease (ISSVD) recommended terminology for vulvar HPV-related squamous lesions is essentially a two-tiered system. Furthermore, some pathology practices, both academic and private, have been using a two-tier reporting system for many years with no apparent compromise of patient care.

**Biomarkers in HPV-Associated Lower Anogenital Squamous Lesions**

1. **p16 IHC is recommended** when the H&E morphologic differential diagnosis is between precancer (–IN 2 or –IN 3) and a mimic of precancer (e.g., processes known to be not related to neoplastic risk such as immature squamous metaplasia, atrophy, reparative epithelial changes, and tangential cutting).

2. Strong and diffuse block-positive p16 results support a categorization of precancerous disease. If the pathologist is entertaining an H&E morphologic interpretation of –IN 2 (under the old terminology, which is a biologically equivocal lesion falling between the morphologic changes of HPV infection [low-grade lesion] and precancer), p16 IHC is recommended to help clarify the situation.
   - Strong and diffuse block positive p16 results support a categorization of precursor.
   - Negative or non-block-positive staining strongly favors an interpretation of low-grade disease or a non-HPV associated pathology.

3. **p16 is recommended** for use as an adjudication tool for cases in which there is a professional disagreement in histologic specimen interpretation, with the caveat that the differential diagnosis includes a precancerous lesion (–IN 2 or –IN 3).

4. **WG4 recommends against** the use of p16 IHC as a routine adjunct to histologic assessment of biopsy specimens with morphologic interpretations of negative, –IN1, or –IN3.

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4a) p16 IHC is recommended as an adjunct to morphologic assessment for biopsy specimens interpreted as ≤ –IN 1 that are at high risk for missed high-grade disease, which is defined as a prior cytologic interpretation of HSIL, ASC-IH, ASC-US/HPV16+, or AGC (NOS).
   - Any identified p16 positive area must meet H&E morphologic criteria for a high-grade lesion to be reinterpreted as such.

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Limiting the number of reporting tiers and the use of objective biomarkers has been shown to reduce diagnostic variation in histopathologic diagnoses.

Rationale:

Most studies in the literature that address biomarker usage in HPV-associated lesions focus on the cervix; however, given the similarity of the underlying biology, the recommendations from WG4 apply to all LAT sites. Clinicians were, and many still are, under the false premise that the biopsy is the gold standard and that everyone reads the biopsy in the same way. However there is substantial published data to the contrary. While the reproducibility of morphologic diagnoses using the current 3-tiered nomenclature (-IN1to -IN3) cannot be substantially improved by education of practitioners, limiting the number of reporting tiers and the use of objective biomarkers has been shown to reduce diagnostic variation in histopathologic diagnoses. A number of studies specifically address this issue with respect to the interpretation of LAT squamous lesions, and all conclude that there is substantial improvement in agreement between observers when p16 IHC is used. In fact, p16 IHC improves the accuracy of a single pathologist’s interpretation of high grade vs. low grade disease relative to an adjudicated pathology panel.6 Based on the high sensitivity of p16 for precancerous lesions, areas of small or equivocal high grade disease can be identified on histologic sections using p16, which were not readily identifiable on H&E sections alone – this is particularly relevant to cytologic-histologic correlation, when a high risk cytology interpretation (HSIL, ASC-H, ASCUS/HPV16+, AGC-NOS) is followed by a seemingly “negative” biopsy. It is important to note that the recommendations for p16 usage define “block positivity” as continuous strong nuclear or nuclear plus cytoplasmic staining of the basal cell layer with extension upward involving at least one third of the epithelial thickness. The latter height restriction is somewhat arbitrary but adds specificity. Full thickness staining or extension into the upper third or upper half is specifically not required to call a specimen positive. Additionally, any identified p16 positive area must meet H&E morphologic criteria for a high-grade lesion to be reinterpreted as such. (Figures 1–4)

At the current time, Ki67 and ProEx C show similar trends, but with less well-documented operating characteristics when compared to p16. If p16 is unavailable, technically inadequate, or equivocal, Ki67 and/or ProEx C may be considered for use in a similar fashion.

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Concerns from the public comment period and conference participants focused predominantly on two aspects.

1. **Impact on management:** Would a change to a 2-tiered system where most CIN2 lesions would fall into a high grade category lead to potential over-treatment of young women, since current clinical guidelines for treating cervical lesions in young women are based on a 3-tiered (i.e. CIN) system?

A two-tiered system using p16 for adjudication of equivocal high grade lesions results is a much more reproducible final diagnosis which will result in better clinical care, since it will focus the poorly reproducible diagnosis of CIN2 into real benign or precancerous categories. In general, the 2006 ASCCP guidelines were based on a 2-tiered system of diagnosis and allowed for the option to be more conservative with CIN2 and CIN2-3 in "young" women. Interim guidance for management of HSIL in the LAST terminology has been published recently. During transition to LAST terminology, and/or at the clinicians request, the LSIL/HSIL terminology may be further supplemented with current (-IN) terminology for each anogenital site. Since LAST terminology mirrors Bethesda cytologic terminology, laboratories must ensure that the report clearly states the specimen type. The 2012 ASCCP guidelines do not incorporate the LAST terminology, since delegates to the consensus process determined that this classification does not yet have a sufficiently robust outcomes evidence base to allow elucidation of risk-based management guidelines. However interim guidance is available, which states that when using 2012 LAST histopathology terminology, CIN1 is equivalent to histopathologic LSIL and CIN 2,3 is equivalent to histopathologic HSIL. It is emphasized that cytological LSIL is not equivalent to histopathological CIN 1 and cytological HSIL is not equivalent to histopathological CIN 2,3.

2. **Abuse of p16:** Pathologists will "overuse" p16 in cases where the differential is Negative/CIN1 and CIN1-2, and potentially call CIN1/p16-positive cases "HSIL," leading to unnecessary treatment.

At the current time, p16 is already widely used by many pathologists as an adjunct to cervical histopathology. The LAST recommendations specifically emphasize that p16 should not be used if the H&E morphologic differential diagnosis is between low grade disease (–IN 1) and negative, as –IN 1 can be either p16 negative or positive. If the pathologist’s histologic diagnosis
is “obvious” –IN 1, the WG does not recommend further IHC. There is insufficient evidence to determine whether there is a difference in the natural history between p16 positive and p16 negative –IN 1. Hence at the present time, it is recommended that clinical management of –IN 1 be based on the histologic diagnosis alone.

Implications and Implementation of LAST Recommendations

As with any new set of recommendations or guidelines, implementation can take time and concerted educational efforts. The LAST group is actively involved in efforts to disseminate LAST recommendations via publications, presentations at national/ international meetings, webinars, and development of web-based educational resources (http://www.cap.org; http://www.asccp.org/). We are also working with other stakeholders besides physicians - federal and state agencies, nurse practitioners, advocacy groups, and educational/certification bodies in order to address the implications of this terminology change on the work done by these groups. The World Health Organization, in conjunction with the International Society of Gynecologic Pathology, is currently updating reporting histopathology terminology for the cervix, vulva and vagina, and will also likely propose a two-tier reporting terminology. The 2012 Updated Consensus Guidelines for the Management of Abnormal Cervical Cancer Screening Tests and Cancer Precursors provide interim guidance for histopathology results reported using the LAST project’s two-tier SIL terminology.8

References