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The LAST Project and the diagnostic bottom line

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The LAST Project and the diagnostic bottom line

The LAST Project's consensus recommendations for terminology and biomarker use to improve the accuracy and reproducibility of histopathological diagnoses for HPV-associated squamous intraepithelial lesions of the lower anogenital tract are reviewed.

Keywords: squamous intraepithelial lesions, HPV, terminology, p16

The bottom line of pathology reports – the histopathological diagnosis – is the gold standard that informs clinical management. However, morphological diagnoses are subject to interpretive variability and diagnostic uncertainty. The black and white print on a pathology report can fail to capture the subjective nature inherent in morphological interpretation. Historically, the various terminologies that have been employed by pathologists and clinicians from differing specialties for the same histopathological entity also contribute to potential perplexity. The Lower Anogenital Squamous Terminology (LAST) Project, jointly sponsored by the College of American Pathologists (CAP) and the American Society for Colposcopy and Cervical Pathology (ASCCP) sought to address these dilemmas for HPV-associated squamous lesions of the lower anogenital tract. The LAST Project's consensus recommendations and supporting information were published in three journals.[1–3] Its recommendations for HPV-associated squamous intraepithelial lesions are reviewed here.

As with the Bethesda System for reporting gynaecological and anal cytology, the recommendations from the LAST Project were based on three fundamental principles regarding terminology.[4] Terminology should:

1 Communicate clinically relevant information from the laboratory to the patient's health care provider.
2 Be uniform and reasonably reproducible across different pathologists and laboratories and also flexible enough to be adapted to a wide variety of lab settings and geographical locations.
3 Reflect the most current understanding of the disease process.

Our current understanding of HPV-associated squamous lesions is that the virus can affect squamous epithelia in two ways. HPV can produce a productive infection that resolves spontaneously in the majority of immunocompetent individuals and results in a low-grade morphological lesion. In those with persistence of oncogenic HPV, the expression of viral oncoproteins results in cellular transformation and the morphological derangements recognised as high-grade pre-cancer. When high-grade lesions are left untreated, some will progress over time to invasive disease. These HPV-mediated cellular alterations are morphologically similar in squamous epithelia of all sites of the lower anogenital tract, in either sex. Indeed, over 25 years ago, Dr Ralph Richart, who coined the three-tiered CIN classification system, urged that the terminology of the HPV-related precursor lesions be modified to a dichotomous, two-tiered system that would best satisfy the requirements of both science and clinical care.[5]

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The LAST Project's recommendations for HPV-associated lower anogenital tract squamous intraepithelial lesions

1 A unified histopathological nomenclature with a single set of diagnostic terms is recommended for all HPV-associated pre-invasive squamous lesions of the lower anogenital tract.
2 A two-tiered nomenclature is recommended for non-invasive HPV-associated squamous proliferations of the lower anogenital tract that may be further qualified with the appropriate -IN terminology.
   - -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 -IN3 lesion:
     - *Cervix = CIN3*
     - *Vagina = VaIN3*
     - *Vulva = VIN3*
     - *Anus = AIN3*
     - *Perianus = PAIN3*
     - *Penis = PeIN3*
3 The recommended terms for HPV-associated squamous lesions of the lower anogenital tract are Low Grade Squamous Intraepithelial Lesion (LSIL) and High Grade Squamous Intraepithelial Lesion (HSIL), which may be further classified by the applicable -IN subcategorisation.

Additionally, morphological interpretation is subject to interobserver variability.[6] Clearly, interpretive variability can be improved by limiting the number of diagnostic categories employed. Additionally, the interpretation of a haematoxylin and eosin (H&E) slide can remain challenging, even to the most experienced pathologist – we deliberate and give our most informed educated guess, but diagnostic uncertainty remains. This variability and uncertainty are not reflected in the diagnostic bottom line. The histopathological diagnosis directly impacts patient management. When high-grade SIL is in the differential diagnosis on H&E, these issues can be alleviated by the use of more objective biomarkers, such as p16 immunohistochemistry (IHC), as recommended by the LAST Project.

The LAST Project's recommendations for biomarkers in HPV-associated lower anogenital squamous intraepithelial lesions

1 p16 immunohistochemistry is recommended when the H&E morphological differential diagnosis is between pre-cancer (-IN2 or -IN3) and a mimic of pre-cancer (e.g. processes known to be not related to neoplastic risk such as immature squamous metaplasia, atrophy, reparative epithelial changes, tangential cutting, etc.). Strong and diffuse block positive p16 results support a categorisation of pre-cancerous disease.
2 If the pathologist is entertaining an H&E morphological interpretation of -IN 2 [under the old terminology; that is a biologically equivocal lesion falling between the morphological 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 categorisation of pre-cancer. Negative or non-block positive staining strongly favours 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 histological specimen interpretation, with the caveat that the differential diagnosis includes a pre-cancerous lesion (-IN2 or -IN3).
4 LAST recommends against the use of p16 IHC as a routine adjunct to the histological assessment of biopsy specimens with morphological interpretations of negative, -IN1, and -IN3.
   - *Special circumstance*: p16 IHC is recommended as an adjunct to morphological assessment of biopsy specimens interpreted as ≤-IN1 that are at high risk for missed high-grade disease, which is defined as a prior cytological interpretation of HSIL, ASC-H, ASC-US/HPV16+, or AGC(NOS). Any identified p16 positive area must meet H&E morphological criteria for a high-grade lesion to be re-interpreted as such.

To date, the biomarker, p16, has the most robust published literature on its utility to help make H&E morphological diagnoses of HPV-associated squamous lesions more objective and reproducible. A recent meta-analysis indicates improved interobserver agreement for the diagnosis of high-grade cervical lesions with the adjunctive use of p16 compared with H&E morphology alone.[7] Additionally, individual studies published since the LAST Project's recommendations have shown that p16 immunostaining is useful for the diagnosis of high-grade lesions in a variety of anogenital sites including the cervix, anus and genital skin.[8–11] However, p16 is not a panacea; LSIL and benign squamous

mucosa can be p16 block positive.[12] Thus, the judicious use of p16 is recommended by the LAST Project for diagnostically challenging situations, as noted above. In the future, other biomarkers may provide more of a magic bullet and help discriminate those lesions destined to become invasive if left untreated.

For cervical disease, CIN2 is the clinical threshold for treatment in most situations.[13] The use of a two-tiered classification system, as recommended by the LAST Project, does not change that. In circumstances where the potential harms of timely treatment may outweigh its benefits, the decision to treat or observe is rationally based on the lesion's size and distribution rather than on its histological grade alone.[14]

As with the Bethesda System for cervical and anal cytology, widespread implementation of a new nomenclature takes time. Summary recommendations for the LAST Project are available for download on the CAP website.[15] The LAST Project's recommendations have recently been adopted by the World Health Organization for the cervix, vagina and vulva.[16] For HPV-associated squamous intraepithelial lesions of the lower anogenital tract, the LAST Project provides rational recommendations for the judicious use of tissue-based biomarkers to improve diagnostic accuracy and reproducibility. The diagnostic bottom line and optimal patient management depend on it.

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