## Title
Letter to the Editor – Reply



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grade, there is a possibility of infection by HR-HPV that could be detected by p16 IHC.

According to LAST, LSIL are typically self-limited HPV infections that will resolve spontaneously [1]. It is also claimed that CIN 2 is an equivocal diagnosis of cervical precancers and includes both CIN 1 and HPV effects as well as some precancerous lesions [2, 4]. This supports the recommendation of a 2-tier classification, but it also begs the question whether the "equivocal" CIN 2 belongs to the high-grade category. LAST also seems to underestimate the progressive potential of low-grade lesions. Because the hallmark of potential progression is HR-HPV infection, it might be preferable to implement p16 IHC to all low- and high-grade lesions. This would help select patients in need of a closer follow-up.

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**In Reply:**

As co-chairs of the steering committee for the CAP-ASCCP LAST Project and first and senior authors on the published article [1], we thank Dr. van Bogaert for his perceptive and prescient comments and hope to add a few points of clarification.

One source of confusion from the article may be a recently detected typographical error in which the word 'NOT' was omitted in the following sentence: "A positive p16 stain does NOT exclude CIN 1; at least 30% of adjudicated CIN 1 cases are p16-positive."

The LAST Project makes specific recommendations, supported by the literature review, for the use of the biomarker, p16, to clarify and augment H&E morphologic diagnoses in specific situations. If a pathologist's diagnosis on H&E is unequivocal histologic low-grade squamous intraepithelial lesion (LSIL) or histologic high-grade squamous intraepithelial lesion (HSIL), p16 is not recommended.

If the histologic impression is less certain, p16 may be used to support a specific diagnosis over others in the differential. This applies to the relatively common differential of a high-grade lesion versus a histologic mimic of precancer where diffuse p16 immunostaining supports a diagnosis of precancer. In addition, the LAST Project recommends the use of p16 to clarify an H&E interpretation of "–IN2"; here, diffuse p16 staining supports a diagnosis of HSIL, whereas a negative p16 stain supports a diagnosis of LSIL. Rather than being contradictory, these LAST recommendations enhance our diagnostic accuracy and reproducibility.

It is the persistence of high-risk HPV infection that is the risk factor for cervical precancer and cancer, not the mere presence of the virus. The cellular tumor suppressor protein, p16, can help distinguish transforming from productive HPV infections. In general, nondysplastic squamous epithelia infected with either low-risk or high-risk HPV do not diffusely stain for p16 [2].

Normally, p16 blocks the activity of an important cell cycle checkpoint regulator, the cyclin-dependent kinase CDK4/6. Checkpoint dysregulation induces cells to enter into a noncontrolled proliferative cycle. In a transforming HPV infection, the viral oncogene, E7, interferes substantially with cell cycle regulation. E7 disrupts the protein of retinoblastoma (pRb) from its binding to E2F transcription factor and thereby promotes cell cycle progression, a molecular switch that is usually activated by CDK4/6. Cells transformed by persistent infection with high-risk HPV strongly express p16 to counteract the irregular cell cycle activation; however, since E2F is not released through CDK4/6 action, but by E7, p16 expression has no effect on cell cycle activation. Over time, p16 accumulates in the nucleus and cytoplasm of affected cells and is indicative of a transforming high-risk HPV infection that has altered normal cell cycle regulation [2–4]. Along with the viral oncogene E6, the long-term expression and overexpression of E6 and E7 lead to an accumulation of genetic errors, which may ultimately lead to the progression of HSIL to cancer.

Initially, the relatively low levels of E6 and E7 present in histologic LSIL do not significantly compromise the functions of their cellular targets sufficiently to facilitate cancer progression [4]. However, some recent studies have shown that p16-positive low-grade lesions have a higher risk of progression than p16-negative lesions [5–7], suggesting that p16 could be used as a marker to discriminate low-grade lesions with a higher progression risk from those that will most likely regress spontaneously. This intriguing information is acknowledged in the LAST Project and awaits further confirmation by additional studies to see if p16 staining in histologic LSIL is sufficiently predictive of future progression to warrant a change in the clinical management of these lesions.

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