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p16 Immunohistochemistry Is Not Always Required For Accurate Diagnosis Of Grade 2 Squamous Intraepithelial Lesions

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Abstract

Objectives—Preinvasive squamous neoplasms of the lower genital tract are currently classified using a two-tier system (LSIL or HSIL) as directed by the Lower Anogenital Squamous Terminology (LAST) guidelines, but may also be subclassified as -IN1, -IN2 or -IN3. LAST recommended that all diagnoses of -IN2 be supported by immunohistochemistry (IHC) for p16. We examined whether p16 and Ki-67 IHC are necessary to diagnose -IN2 when the lesion has obvious high-grade histology.

Methods—p16 and Ki67 IHC were performed prospectively and retrospectively on vulvar, vaginal and cervical specimens with an initial diagnosis of -IN2 based on H&E morphology, and a final diagnosis was made after consensus review.

Results—5/46 prospective and 4/38 retrospective cases were p16 negative. The diagnosis of -IN2 was maintained in 8 of these 9 cases owing to compelling HSIL histology. Overall, p16 and Ki67 IHC altered the -IN2 diagnosis to a lower grade in only 1/84 cases (1.2%, <0.01-7.1%). Moreover, p16 was positive in all cases where the preanalytic impression was of -IN2/3 (13/13).

Conclusions—p16 IHC lacks utility in cases of morphologically obvious -IN2, as the stain is positive in a majority of cases. The LAST recommendation to use p16 IHC to support all diagnoses of -IN2 will result in performing the immunostain in many circumstances where it is not medically necessary. Among cases that are p16 negative, many have compelling high-grade morphology. The LAST perspective that the stain trumps histology may allow false-negative IHC results to prevail.

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Keywords

Lower genital tract disease; Intraepithelial neoplasia; Immunohistochemistry; Cyclin-dependent kinase inhibitor p16; Surgical pathology; Diagnostic techniques

Introduction

Squamous cell carcinomas of the lower anogenital tract are a significant cause of morbidity and mortality worldwide, and many of these carcinomas can be linked to human papillomavirus (HPV) infection^{1,2}. Colposcopy-directed biopsy of women with positive screening by cervical cytology, and visually directed biopsy of vulvar and vaginal lesions, aims to distinguish between transient HPV-associated epithelial changes and precancerous lesions with malignant potential^{1–3}. Current evidence supports a dichotomous classification of high-grade and low-grade squamous intraepithelial lesions (HSIL and LSIL), which subsumes the older 3-tiered system (intraepithelial neoplasia grade 1, 2 and 3; -IN1, -IN2, -IN3) correlating with the presence of dysplasia in the bottom third only, extending into the middle third, or extending into the top third of the epithelium^{3–6}. The treatment threshold in the United States is HSIL (-IN2 and -IN3)⁷. In our institution, we use both reporting systems (e.g., “HSIL [CIN 2]”), since a diagnosis of -IN2 as opposed to -IN3 can convey to clinicians that there may be a margin of safety for observation. This is especially important in younger women with desired future fertility, as repeated excision can predispose to cervical insufficiency⁸.

To improve reproducibility in grading intraepithelial lesions, multiple ancillary studies have been proposed. The p16^{INK4A} cyclin-dependent kinase inhibitor is upregulated in cervical precancers as a result of pRb inactivation by viral E7 protein. Strong, diffuse p16 staining has been significantly associated with high-risk HPV infection and high lesion grade in multiple studies (24–100% of -IN2 lesions and 54–100% of -IN3 lesions)^{9–11}. Ki-67 immunohistochemistry, too, can be useful in classification of cervical squamous neoplasia, although the data supporting its diagnostic use is still equivocal^{12,13}. This antibody reacts with the nuclei of cells that are in nonresting phases of the cell cycle. Ki-67 particularly has a role in differentiating reactive conditions (with proliferation restricted to parabasal cells) from dysplastic ones. The presence of ectopic proliferating cells in the lower, middle or upper third of mature squamous epithelium supports a diagnosis of -IN1, 2 or 3 respectively¹⁴.

In 2012, a consensus group sponsored by the ASCCP presented the “Lower Anogenital Squamous Terminology (LAST)” recommendations for histopathology reporting of HPV-related squamous lesions of the lower anogenital tract¹⁵. Working Group 4 (WG4) of this panel called for “recommended” use of ancillary p16 immunohistochemistry (IHC) when a diagnosis of -IN2 is being considered, based on the rationale that -IN2 diagnoses have poor reproducibility^{16,17}, and that the use of p16 IHC has been shown to reduce interobserver variability^{18–21}.

While this recommendation was intended to reduce over-diagnosis of HSIL when the appropriate diagnosis is LSIL, it raises several concerns. Up to 35% of histologic -IN2

lesions are p16-negative (not diffusely positive) while up to half of -IN1 lesions are diffusely p16 positive^{10,11,22,23}. A shift to a p16-driven definition of -IN2 would thus result in over-diagnosis of p16-positive LSIL as HSIL, and under-diagnosis of p16-negative HSIL as LSIL. This would result in overtreatment in some cases, with unnecessary expense and avoidable risks, and undertreatment in others, with the possibility of progression of disease. In addition, constant reliance on ancillary immunostains would increase cost and turnaround time. An alternative to universal p16 staining is consensus assessment of biopsies, which may improve diagnostic validity but may not be generalizable to all practices.

We hypothesized that cases with an H&E impression unequivocally favoring -IN2 would not be revised to a lower-grade diagnosis after the performance of p16 and Ki-67 IHC. To test this hypothesis, we reviewed a series of lower anogenital tract specimens for which a histologic impression of -IN2 was formed based on H&E findings alone. We performed p16 and Ki-67 IHC and documented the effects of these stains on the ultimate diagnosis.

Materials and Methods

IRB Approval

This study, including a waiver of consent, was approved by the Human Research Protection Office at Washington University School of Medicine.

Cases and Cohorts

Patients eligible for inclusion were women undergoing biopsy or excision of a squamous lesion of the lower genital tract in our institution between 7/27/15 and 4/30/17. Overall, we intended to include all cases for which LAST WG4 Recommendation 2 would mandate p16 IHC. These are cases for which the pathologist “is entertaining an H&E morphologic interpretation of -IN2 (under the old terminology)”¹⁵. Cases were excluded if grading of dysplasia in the subject tissue was precluded by 1) minuscule tissue; 2) detached epithelium without underlying stroma; 3) marked cautery and crush artifact; 4) focus of dysplasia exhausted on deeper levels or IHCs. Cases were also excluded if definite histologic CIN3 or above was identified (in which case LAST guidelines would not recommend p16) or if the H&E differential included benign or LSIL (in which case WG4 recommendation 1, not 2, would be more pertinent, and would call for performing the stain). When more than one specimen within a case met eligibility criteria including possible -IN2, the one with the largest area of dysplasia was selected as representative of the case.

Two cohorts of patients were enrolled. The prospective cohort consisted of women for whom a diagnosis of -IN2 was considered apparent by the case pathologist when reviewing initial H&E-stained slides. The impression of -IN2 was considered a “preanalytic” determination, since it was recorded before performing p16 and Ki-67 IHC, which were the interventions under study. The site (cervix, vagina, vulva, or perineum/perianus) and type of specimen (biopsy or excision) were also recorded. The preanalytic impression for each case was sustained at a daily gynecologic pathology quality assurance conference, attended by a subset of nine subspecialty gynecologic pathologists; when disagreement occurred, a consensus interpretation was reached.

Immunostaining and Scoring

In the prospective cohort, immunostains for p16 and Ki-67 were performed after H&E examination. p16 (MTM Laboratories, clone E6H4, prediluted) and Ki-67 (Ventana, clone 30-9, prediluted) were used for staining on a Ventana IHC autostaining platform. The results of the p16 IHC were visually coded by an attending pathologist for intensity (block/diffuse positive, patchy positive, focal positive, or failed/lost) and distribution (confined to lower 1/3, extending to middle 1/3, extending to upper 1/3). p16 staining was considered positive if there was continuous strong nuclear ($\pm$ cytoplasmic) staining involving at least 1/3 of the epithelial thickness. The results of the Ki-67 IHC were also coded for intensity (increased, not increased, or failed/lost) and distribution (confined to lower 1/3, extending to middle 1/3, extending to upper 1/3). All completed cases (including the IHC stains and H&E) were reviewed at consensus conference, and a postanalytic diagnosis was reached.

Due to varying pathologist practices, not all eligible cases occurring during the study period were entered into the prospective cohort. To give a complete series, a retrospective cohort was constructed to recapture the remaining cases. We retrieved consecutive cases for which a diagnosis of squamous intraepithelial neoplasia was rendered, with or without ancillary immunostaining, and that had not been entered into the prospective series. These cases were reviewed by all of the authors in a consensus conference setting, blinded to the original diagnosis, to identify those with a preanalytic (H&E only) impression of definite -IN2. IHC for p16 and Ki-67 was then performed on these cases, as previously described, if it had not been previously done. The H&E and IHC slides were then reviewed by the authors in consensus (L.S., L.Z., and one or both of I.S.H. or H.R.K.) to reach a postanalytic diagnosis incorporating IHC results.

Statistics

Statistical analyses were performed using Excel version 14.3.9 (Microsoft Corp., 2013, Redmond, Wash.) and Prism 7 (Graphpad Software, 2016, San Diego, Calif.).

Role of the Funding Source

This research was supported by a grant from the Anatomic Pathology Fund at Washington University School of Medicine, supporting immunostains, with no influence on study design or data analysis.

Results

Prospective cohort

For the prospective cohort, 46 cases were identified with a preanalytic (H&E) impression of at least -IN2 and with documentation by the case pathologist that immunostains did not appear medically necessary to establish a diagnosis of -IN2 (Table 1). By design, cases where the histologic differential included diagnoses lower than -IN2 were excluded. The most common site was cervix (36/46=78%) and the majority of specimens were biopsies (32/46=70%). Of cases for which HRHPV status had been ascertained, 25/27=93% were positive, while 19 had unknown HRHPV status. The two cases that were HRHPV negative had final diagnoses of -IN2 and -IN3, supported by IHC results.

In all prospective cases, p16 and Ki-67 were performed and used to support or refute the presumptive diagnosis. 5/46 cases=11% had negative p16 staining (two focal/patchy positive and five with no reaction) (Table 2 and Figure 1). In four of these cases, the final diagnosis remained -IN2 based on morphology and Ki67 staining. In the other case, in addition to negative p16 staining, the Ki67 staining was not increased, and the final diagnosis was downgraded to -IN1. There were three cases in which p16 staining supported a change in the final diagnosis to -IN3. Examples of positive and negative p16 staining patterns are shown in Figure 2.

Similar results were obtained with Ki-67 staining of the prospective cases. Among cases with a preanalytic impression of definite -IN2 or -IN2/3, the Ki-67 supported an HRHPV-driven lesion in 43/46=93% of cases (i.e., with evidence of increased proliferation extending above the parabasal layer), but in 3/46=7% of cases, reactivity was only present in the lower third (Table 2). In one of these cases, the p16 staining was also negative, and the diagnosis was downgraded to -IN1. In the other two, the histologic features together with positive p16 caused the diagnosis of -IN2 to be sustained.

The IHC results were integrated with the H&E findings to arrive at a final diagnosis that was supported by consensus interpretation. The correlation between preanalytic and postanalytic diagnoses is shown in Figure 3A. There was only one case (1/46=2%) for which a diagnosis lower than -IN2 was ultimately rendered after integration of p16 and Ki-67 data.

Retrospective cohort

38 cases were identified for the retrospective cohort. The majority were from the cervix (35/38=92%) and were biopsies (22/38=58%; Table 1). Of cases for which HRHPV status had been determined, 23/25=92% were positive.

After IHC, 1/38=3% of cases had negative p16 staining, but Ki-67 showed full-thickness reactivity, and the case received a postanalytic diagnosis of -IN3 (Table 2 and Figure 1). There were three cases with patchy p16. Two had increased Ki-67; one had no increase in Ki-67. For all three, the postanalytic consensus opinion was that the findings overall still substantiated a diagnosis of -IN2. Staining failed for two cases due to tissue depletion. Thus, altogether, in the retrospective series, no diagnoses were revised downward to less than HSIL with the addition of IHC results (Figure 3B).

Discussion

This study tested the utility of routine immunohistochemistry in supporting morphologic diagnoses of -IN2 in a practice employing consensus review by specialist gynecologic pathologists. The LAST consensus statement is widely accepted as expert opinion in the United States and worldwide. WG4 of the LAST consensus panel recommended that, “if the pathologist is entertaining an H&E morphologic interpretation of -IN2... p16 IHC is recommended to help clarify the diagnosis. Strong and diffuse block-positive p16 results support a categorization of precancer. Negative or non-block-positive staining strongly favors an interpretation of low-grade disease or a non-HPV associated pathology.”¹⁵ This recommendation is based upon the rationale that -IN2 diagnoses are poorly reproducible,

representing a mixed cohort of both precancerous and transient lesions. The authors cite evidence that p16 contributes to more robust diagnoses by serving as a surrogate for clinically relevant infection by high-risk HPV. This may be helpful in cases with a bona fide differential diagnosis of LSIL vs. HSIL. However, neither -IN2 nor HSIL is defined by either HPV or p16, so we contend that negative HPV or p16 are not *prima facie* obstacles to diagnosing -IN2/HSIL. Furthermore, false-negative p16 results can occur²⁴.

We undertook the present study to determine the circumstances under which p16 contributes usefully to the diagnostic process. We coupled p16 with Ki-67, a marker of cellular proliferation, since the presence of proliferating cells above the parabasal layer can provide adjunctive information for classifying squamous dysplasia^{12,13}. The LAST recommendations do not advocate routine use of Ki-67 due to insufficient supporting literature; our results here support the conclusion that Ki-67 IHC adds minimal benefit in diagnosis^{15,22}. We included cases for which the pathologist initially judged the diagnosis sufficiently clear that IHC would not be required, but for which p16 would be mandated by the LAST guideline. Of these, 9/84=10% of total prospective and retrospective cases were p16 negative, and only one case (1/84=1.2%, 95% confidence interval <0.01–7.1% by modified method of Wald) was downgraded out of the -IN2+ range as a result of p16 IHC. Of some note, this case had a concurrent biopsy at a separate site in which -IN2 (supported by positive p16 and Ki-67 IHC) was present.

In reviewing cases for inclusion, we identified many for which the H&E diagnosis was sufficiently uncertain as to merit IHC. These are cases for which p16 and Ki-67 would be performed out of medical necessity. We wish to emphasize that in these cases of diagnostic uncertainty, these ancillary studies would be useful, and they should be employed at the pathologists' discretion. However, in unambiguous cases, p16 would be extraneous.

Of the 13/84=15% of cases that were initially graded -IN2/3, 100% stained positively for p16. While the LAST recommendations explain that p16 is not recommended in cases of morphologically unequivocal -IN3, there is no explicit recommendation for cases where the initial differential diagnosis is between -IN2 and -IN3. While our study included only a small number of such -IN2/3 cases, this would seem to be a group in which p16 could particularly be spared. In the face of limited resources for health care delivery, it seems unnecessary to mandate the use of an immunostain in unambiguous cases.

Given that the possibility of -IN2 can only be ascertained after the review of H&E slides, mandatory use of p16 IHC would add a delay (of one day, in our laboratory) to the turnaround time of these cases, potentially delaying patient care and impinging on a metric that is important to payors and providers.

In addition, we caution against too much reliance on ancillary immunostains. Using p16 positivity alone to define -IN2 would result in over-diagnosis of p16-positive LSIL as HSIL, and under-diagnosis of p16-negative HSIL as LSIL. This is in concordance with findings from WG4 that “there is insufficient evidence to prospectively determine high-grade versus low-grade disease based solely on a p16 result.”¹⁵ For example, in a consensus-based, prospective cohort, it was demonstrated that p16 staining in a strong and diffuse block

pattern was more sensitive than the combined performance of multiple pathologist reviews (86.7% compared to 68.9%, respectively) but much less specific (82.8% vs. 97.2%)²⁵. By those authors' calculations, using p16 IHC positivity as a threshold to treat would result in overtreatment of 39% of women with consensus-diagnosed CIN1. This observation supports the LAST recommendation that p16 should not be used in cases with unequivocal morphology of -IN1. Unnecessary treatment adds to physical and psychological stress, escalates health care expenses, and incurs risks of side effects and adverse events. Conversely, in our study, 8/84 = 9% of total cases with negative p16 staining remained classified as -IN2 after consensus expert review. Reclassifying these as LSIL based on the p16 staining alone would result in significant under-diagnosis of lesions that do not fit within the current definition of LSIL. Our percentage of p16-negative -IN2 lesions is lower than those in previous reports, which ranged from 19 to 35%^{10,23,26}. This difference might be accounted for by consensus review of our cases by gynecologic pathologists.

To some extent, the present question would be obviated by full implementation of the LAST project's fundamental recommendation to classify squamous lesions of the lower anogenital tract using a two-tier system (LSIL and HSIL). Under this system, the distinction between -IN2 and -IN3 would vanish. Immunohistochemistry would remain an ancillary test that could be useful for differentiating LSIL vs. HSIL, and HSIL vs. reactive conditions, but could be omitted for unambiguous cases. While LAST recommends a two-tier system, and this has been implemented in our laboratory and others, we agree with the LAST authors that maintaining an -IN2 category may be useful in clinical management; for example, by allowing for conservative treatment of cervical lesions in young women of reproductive age³. It thus remains relevant to study the validity and criteria for diagnoses in the three-tier system.

Limitations of the present series include its small size and its reliance upon practices at a single institution. Results may not be generalizable to practices that do not include pathologists specializing in gynecologic pathology. In addition, while we treat a consensus panel of expert gynecologic pathologists as the gold standard for diagnosis, expert interpretation may not reach the "true" diagnosis in all cases, and microscopic diagnosis of histopathology, including -IN2, remains an "imperfect science" prone to interpretive variance. In this regard, follow-up data would be clinically informative. It is currently not clear whether p16-negative -IN2 lesions consistently behave like precancer (with progression) or like transient HPV-induced lesions (with regression). Guedes et al. found no significant difference in regression between p16-positive and p16-negative CIN2 lesions, further undermining p16 positivity as a threshold for treatment²⁶.

Conclusion

Our results show that diagnoses of -IN2 can be made reliably on squamous specimens of the lower female genital tract using H&E alone in many cases, at least in practice settings where subspecialty expertise is available.

First, we identified a group of patients for whom a diagnosis of -IN2 was made after an initial differential of -IN2/3; 100% of these cases were p16-positive in our series, and this is

a population for which ruling out a lower diagnosis is not medically necessary and p16 can be spared. It may be beneficial to add a comment to pathology reports to clarify when this situation arises (e.g., “This specimen has definite histologic features of HSIL, and on a three-tier scale is best graded as -IN2”).

Second, we also noted that, after an initial impression of -IN2, 8/9 of the cases that were p16-negative still had compelling -IN2 morphology and did not fit into current definitions of LSIL (-IN1). It is not completely clear how such cases should be reported, since this group meets the morphologic criteria on which -IN2 (with its risk of progression) was defined, and will include any false-negative p16 cases (true-positive HSIL which should be diagnosed and treated). We concede that this will be controversial among those whose opinion is that the stain provides accuracy superior to morphology. The LAST group itself agrees that “adjudicated pathology panel interpretations [are] the best surrogate available for biologic accuracy.”¹⁵ We contend that it is not always true that “adding a p16 result to the H&E morphologic assessment leads to a more accurate prediction of the risk of the patient for having a precancerous lesion,”¹⁵ when this statement pertains to obvious histologic -IN2 that is p16-negative. The concern is that strict reliance on p16 to adjudicate histologic -IN2 may permit under-diagnosis of high-grade lesions. This situation is not fully addressed in the current LAST recommendations, and should perhaps be reconsidered in future updates. Pathologists and clinicians should remember that p16 and Ki-67 IHC remain useful as ancillary tools for resolving a differential diagnosis between -IN2 and a lower diagnosis when a differential exists.

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List of abbreviations and acronyms

H&E	Hematoxylin and eosin
HPV	Human papillomavirus
HRHPV	High-risk human papillomavirus
HSIL	High-grade squamous intraepithelial lesion
IHC	Immunohistochemistry
-IN	Intraepithelial neoplasia
IRB	Institutional review board
LAST	Lower Anogenital Squamous Terminology
LEEP	Loop electrosurgical excision procedure

LSIL	Low-grade squamous intraepithelial lesion
WG4	Working Group 4

References

- Schiffman M, Castle PE, Jeronimo J, et al. Human papillomavirus and cervical cancer. *Lancet*. 2007; 370(9590):890–907. DOI: 10.1016/S0140-6736(07)61416-0 [PubMed: 17826171]
- Crosbie EJ, Einstein MH, Franceschi S, Kitchener HC. Human papillomavirus and cervical cancer. *Lancet*. 2013; 382(9895):889–899. [PubMed: 23618600]
- Nucci MR, Crum CP. Redefining Early Cervical Neoplasia: Recent Progress. *Adv Anat Pathol*. 2007; 14(1):1–10. DOI: 10.1097/PAP.0b013e31802e0de7 [PubMed: 17198305]
- Egawa N, Egawa K, Griffin H, Doorbar J. Human papillomaviruses; Epithelial tropisms, and the development of neoplasia. *Viruses*. 2015; 7(7):3863–3890. DOI: 10.3390/v7072802 [PubMed: 26193301]
- Doorbar J, Quint W, Banks L, et al. The biology and life-cycle of human papillomaviruses. *Vaccine*. 2012; 30(SUPPL. 5):doi: 10.1016/j.vaccine.2012.06.083
- Doorbar J, Egawa N, Griffin H, Kranjec C, Murakami I. Human papillomavirus molecular biology and disease association. *Rev Med Virol*. 2015; 25(S1):2–23. DOI: 10.1002/rmv.1822 [PubMed: 25752814]
- Santesso N, Mustafa RA, Schünemann HJ, et al. World Health Organization Guidelines for treatment of cervical intraepithelial neoplasia 2-3 and screen-and-treat strategies to prevent cervical cancer. *Int J Gynecol Obstet*. 2016; 132(3):252–258. DOI: 10.1016/j.ijgo.2015.07.038
- Kyrgiou, M., Mitra, A., Arbyn, M., et al. Fertility and early pregnancy outcomes after conservative treatment for cervical intraepithelial neoplasia. In: Kyrgiou, M., editor. *Cochrane Database of Systematic Reviews*. Chichester, UK: John Wiley & Sons, Ltd; 2015. p. CD008478
- O'Neill CJ, McCluggage WG. p16 expression in the female genital tract and its value in diagnosis. *Adv Anat Pathol*. 2006; 13(1):8–15. DOI: 10.1097/01.pap.0000201828.92719.f3 [PubMed: 16462152]
- Genoves J, Alameda F, Mancebo G, et al. Human papillomavirus detection and p16INK4a expression in cervical lesions: A comparative study. *Hum Pathol*. 2014; 45(4):826–833. DOI: 10.1016/j.humpath.2013.10.035 [PubMed: 24656093]
- Tsoumpou I, Arbyn M, Kyrgiou M, et al. p16(INK4a) immunostaining in cytological and histological specimens from the uterine cervix: a systematic review and meta-analysis. *Cancer Treat Rev*. 2009; 35(3):210–220. DOI: 10.1016/j.ctrv.2008.10.005 [PubMed: 19261387]
- Bulten J, van der Laak JA, Gemmink JH, Pahlplatz MM, de Wilde PC, Hanselaar AG. MIB1, a promising marker for the classification of cervical intraepithelial neoplasia. *J Pathol*. 1996; 178(3):268–273. DOI: 10.1002/(SICI)1096-9896(199603)178:3<268::AID-PATH482>3.0.CO;2-4 [PubMed: 8778331]
- Pirog EC, Baergen RN, Soslow RA, et al. Diagnostic Accuracy of Cervical Low-Grade Squamous Intraepithelial Lesions Is Improved With MIB-1 Immunostaining. *Am J Surg Pathol*. 2002; 26(1):70–75. [PubMed: 11756771]
- Kalof AN, Cooper K. Our approach to squamous intraepithelial lesions of the uterine cervix. *J Clin Pathol*. 2007; 60(5):449–455. DOI: 10.1136/jcp.2005.036426 [PubMed: 17046846]
- Darragh TM, Colgan TJ, Cox JT, Heller DS, Henry MR, Luff RD, McCalmont T, Nayar R, Palefsky JM, Stoler MH, Wilkinson EJ, Zaino RJ, Wilbur DC. M of LPWG The Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. *Arch Pathol Lab Med*. 2012; 136(10):1266–1297. [PubMed: 22742517]
- Castle PE, Stoler MH, Solomon D, Schiffman M. The relationship of community biopsy-diagnosed cervical intraepithelial neoplasia grade 2 to the quality control pathology-reviewed diagnoses: An alts report. *Am J Clin Pathol*. 2007; 127:805–815. DOI: 10.1309/PT3PNC1QL2F4D2VL [PubMed: 17439841]

17. Stoler MH, Schiffman M, Atypical Squamous Cells of Undetermined Significance-Low-grade Squamous Intraepithelial Lesion Triage Study (ALTS) Group. Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL Triage Study. *JAMA*. 2001; 285(11):1500–1505. <http://www.ncbi.nlm.nih.gov/pubmed/11255427>. Accessed April 27, 2016. [PubMed: 11255427]
18. Dijkstra MG, Heideman DAM, de Roy SC, et al. p16(INK4a) immunostaining as an alternative to histology review for reliable grading of cervical intraepithelial lesions. *J Clin Pathol*. 2010; 63:972–977. DOI: 10.1136/jcp.2010.078634 [PubMed: 20924035]
19. Klaes R, Benner A, Friedrich T, et al. p16INK4a immunohistochemistry improves interobserver agreement in the diagnosis of cervical intraepithelial neoplasia. *Am J Surg Pathol*. 2002; 26:1389–1399. DOI: 10.1097/00000478-200211000-00001 [PubMed: 12409715]
20. Bergeron C, Ordi J, Schmidt D, Trunk MJ, Keller T, Ridder R. Conjunctive p16INK4a testing significantly increases accuracy in diagnosing high-grade cervical intraepithelial neoplasia. *Am J Clin Pathol*. 2010; 133:395–406. DOI: 10.1309/AJCPXSVCDZ3D5M2M [PubMed: 20154278]
21. Horn L-C, Reichert A, Oster A, et al. Immunostaining for p16INK4a used as a conjunctive tool improves interobserver agreement of the histologic diagnosis of cervical intraepithelial neoplasia. *Am J Surg Pathol*. 2008; 32:502–512. DOI: 10.1097/PAS.0b013e31815ac420 [PubMed: 18223479]
22. Galgano MT, Castle PE, Atkins KA, Brix WK, Nassau SR, Stoler MH. Using biomarkers as objective standards in the diagnosis of cervical biopsies. *Am J Surg Pathol*. 2010; 34(8):1077–1087. DOI: 10.1097/PAS.0b013e3181e8b2c4 [PubMed: 20661011]
23. Miyamoto S, Hasegawa J, Morioka M, Hirota Y, Kushima M, Sekizawa A. The association between p16 and Ki-67 immunohistostaining and the progression of cervical intraepithelial neoplasia grade 2. *Int J Gynecol Obstet*. 2016; 134(1):45–48. DOI: 10.1016/j.ijgo.2015.12.005
24. Guedes AC, Brenna SMF, Coelho SAS, Martinez EZ, Syrjänen KJ, Zeferino LC. p16INK4a Expression does not predict the outcome of cervical intraepithelial neoplasia grade 2. *Int J Gynecol Cancer*. 2007; 17:1099–1103. DOI: 10.1111/j.1525-1438.2007.00899.x [PubMed: 17367324]
25. Galgano MT, Castle PE, Atkins KA, Brix WK, Nassau SR, Stoler MH. Using biomarkers as objective standards in the diagnosis of cervical biopsies. *Am J Surg Pathol*. 2010; 34(8):1077–1087. DOI: 10.1097/PAS.0b013e3181e8b2c4 [PubMed: 20661011]
26. Guedes AC, Brenna SMF, Coelho SAS, Martinez EZ, Syrjanen KJ, Zeferino LC. p16 INK4a Expression does not predict the outcome of cervical intraepithelial neoplasia grade 2. *Int J Gynecol Cancer*. 2007; 17(5):1099–1103. DOI: 10.1111/j.1525-1438.2007.00899.x [PubMed: 17367324]

Figure. 1.

Figure. 2.

Figure. 3.

Table 1

Clinicopathologic characteristics of the study population.

	Prospective cohort n=46	Retrospective cohort n=38
Mean age, y (SD)	37.5 (12.7)	35.4 (12.8)
Site, n (%)		
Cervix	36 (78)	35 (92)
Perineum/perianus	1 (2)	1 (3)
Vagina	6 (13)	1 (3)
Vulva	3 (7)	1 (3)
Procedure, n (%)		
Biopsy	32 (70)	22 (58)
Excision	14 (30)	16 (42)
HRHPV status, n (%)		
Unknown/Not done	19 (41)	13 (34)
Positive	25 (54)	23 (61)
Negative	2 (4)	2 (5)
Preanalytic impression, n (%)		
-IN2	39 (85)	32 (84)
-IN2/3	7 (15)	6 (16)
Final diagnosis, n (%)		
-IN1	1 (2)	0 (0)
-IN2	34 (74)	33 (87)
-IN2/IN3	8 (17)	3 (8)
-IN3	3 (7)	2 (5)

Table 2

Results of p16 and Ki-67 immunostaining results in prospectively and retrospectively collected cases of histologic -IN2.

	Prospective cohort	Retrospective cohort
p16, n (%)	46 (100)	38 (100)
Block positive	40 (87)	32 (84)
Focal/patchy positive	2 (4)	3 (8)
No reaction	3 (7)	1 (3)
Focus lost	1 (2)	2 (5)
Ki67, n (%)	46 (100)	38 (100)
Increased		
Middle 1/3	10 (22)	10 (26)
Top 1/3	29 (63)	26 (68)
Not increased	3 (7)	1 (3)
Failed/focus lost	4 (9)	1 (3)