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Authors

Nayar, Ritu
Chhieng, David C
Crothers, Barbara
[et al.](#)

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Moving forward—the 2019 ASCCP Risk-Based Management Consensus Guidelines for Abnormal Cervical Cancer Screening Tests and Cancer Precursors and beyond: implications and suggestions for laboratories

Ritu Nayar, MD^{a,*}, David C. Chhieng, MD^b, Barbara Crothers, DO^c,
Teresa M. Darragh, MD^d, Diane D. Davey, MD^e, Carol Eisenhut, MD^f,
Robert Goulart, MD^g, Eric C. Huang, MD, PhD^b, Sana O. Tabbara, MD^h

^a Department of Pathology and Northwestern Memorial Hospital, Northwestern University, Feinberg School of Medicine, Chicago, Illinois

^b Department of Pathology, University of Washington, School of Medicine, Seattle, Washington

^c Joint Pathology Center, Silver Spring, Maryland

^d University of California, San Francisco, California

^e Department of Clinical Sciences, University of Central Florida College of Medicine, Orlando, Florida

^f DCL Pathology, Indianapolis, Indiana

^g New England Pathology Associates, Trinity Health of New England, Springfield, Massachusetts

^h Department of Pathology, The George Washington University, Washington, District of Columbia

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Pathology Society Representation* to the 2019 ASCCP Guidelines Effort: Cytopathology Education and Technology Consortium (CETC) - Ritu Nayar. American Society of Cytopathology (ASC) - Ritu Nayar, Sana O. Tabbara. College of American Pathologists (CAP) - Barbara Crothers, Teresa M. Darragh, Diane D. Davey. American Society for Clinical Pathology (ASCP) - Robert Goulart, Carol Eisenhut. Papanicolaou Society of Cytopathology (PSC) - Eric C. Huang, David C.

Chhieng. Pathologists on ASCCP Guidelines Steering Committee - Teresa M. Darragh, Ritu Nayar.

*Note: The content of this paper represents the authors opinions, not necessarily that of the societies they represent.

*Corresponding author: Ritu Nayar, MD; Northwestern Memorial Hospital, Galter 132-B 251 East Huron Street, Chicago, IL 60611; Tel.: 312 926 7008; Fax: 312 926 6037.

E-mail address: r-nayar@northwestern.edu (R. Nayar).

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The 2019 ASCCP Risk Based Management Consensus Guidelines for prevention of cervical cancer promote clinical management recommendations aligned with our increased understanding of HPV biology and cervical carcinogenesis. They employ HPV-based testing as the basis for risk estimation, allow for personalized risk-based management by incorporating knowledge of current results with prior results, and streamline incorporation of new test methods as they are validated. They continue to support the principles of “equal management for equal risk” and “balancing harms and benefits” adopted in the 2012 version of the guidelines. These updated guidelines will be able to adjust for decreasing CIN3+ risks as more patients who received HPV vaccination reach screening age. Pathology organizations were closely involved in the development of these guidelines. Herein the pathologists who served as representatives to the 2019 ASCCP guidelines steering committee and workgroups, summarize the changes that are relevant to laboratories, pathologists, and cytotechnologists. Prior relevant screening and reporting recommendations that have not been widely and/or inconsistently adopted by laboratories are also discussed and considerations for modification of laboratory practices offered.

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Introduction

In 2012, screening and management guidelines for the prevention of cervical cancer adopted the principle of “equal management for equal risk”. Despite the variety of individual test results from different points in the screening and management of an individual, those with similar risks for precancer or cancer can be managed similarly.¹ Since 2012, further risk data relevant to cervical cancer have accumulated and show that an individual’s risk of precancer is not dependent solely on their current test results, but may be significantly modified by other factors such as prior screening history, duration and genotype of a human papillomavirus (HPV) infection, and previous treatment for precancer.²⁻⁶ Newer approaches to screening and triage have been approved in the United States (eg, primary HPV screening; p16/Ki67 dual staining),^{7,8} and additional methods and new technologies are either in advanced development or seeking regulatory approval.

The 2012 ASCCP guidelines included 19 management algorithms. The guidelines were complex and did not fully incorporate the increasing understanding of an individual’s risk for cervical precancer. In 2017, the ASCCP, formerly the American Society of Colposcopy and Cervical Pathology, and the National Cancer Institute (NCI) entered into a memorandum of understanding to undertake the development of new risk-based management guidelines that would do just this—incorporate the ability to consider individualized risk by taking into account current test results and past history when managing each patient.⁹⁻¹¹ The 2019 ASCCP guidelines highlight that detection and treatment of precancer (high-grade squamous intraepithelial lesion [HSIL]/cervical intraepithelial neoplasia [CIN]3) remains the main aim of cervical cancer prevention in the United States and that the management of patients with abnormal screening results can be optimized by identifying those with the highest risk while minimizing procedures with potential harms for those with low risk.¹²

A major goal of the 2019 ASCCP guidelines effort was to simplify the management guidelines, thereby clarifying appropriate tests and testing intervals. Risks were estimated by NCI statisticians using large population data sets in the United States.¹³ Kaiser Permanente Northern California is the largest, most comprehensive data set in the United States, although for the 2019 guidelines, additional databases were analyzed to ensure that results are applicable to patients of diverse racial, ethnic, and socioeconomic strata. Risk estimates were compared using screening and follow-up data from clinical trials (BD Onclarity registrational trials), the New Mexico State HPV Pap Registry, and the US Centers for Disease Control and Prevention's National Breast and Cervical Cancer Early Detection Program, a national program that includes many low-income and minority patients.^{14,15}

The risk strata (ranges of risk for CIN 3+) for the guidelines are defined by clinical action thresholds (CATs) that

were determined through the consensus process under the principle of “equal management for equal risk”. The immediate and 5-year cumulative risks of CIN 3+ were estimated for each combination of current test results and screening/surveillance history (including unknown history). To determine the next step in management, the estimated risk for a given patient is compared with the proposed CATs (Fig. 1). Management options include return to routine screening, a shortened surveillance interval (1 year or 3 years), colposcopy, or treatment. Providers can use the 2019 ASCCP guidelines to manage their patients by using the tables in Egemen et al¹³ or by inputting patient results and history into either a new ASCCP mobile app or Web site designed to facilitate navigation of the tables available at <http://www.asccp.org>, including a no-cost version. Although there have been requests for integration of the 2019 ASCCP risk-based guidelines with the electronic medical record and laboratory reports, these options are not currently available.

Figure 1 This figure demonstrates how patient risk is evaluated. For a given current results and history combination, the immediate CIN 3+ risk is examined. If this risk is 4% or greater, immediate management via colposcopy or treatment is indicated. If the immediate risk is less than 4%, the 5-year CIN 3+ risk is examined to determine whether patients should return in 1, 3, or 5 years. Adapted with permission from Perkins et al.¹⁵

The following are examples of potential change to the 2012 “equal management for equal risk” principle of management that will resonate with pathologists as well as providers.

Previously, women with test results of atypical squamous cells of undetermined significance (ASC-US)/HPV-positive were managed with colposcopy—however, based on the 2019 ASCCP guidelines, management may differ depending on prior history/HPV status.¹⁵ The 2019 ASCCP guidelines aim to decrease unnecessary procedures in low-risk women. An example of risk-based management for the same result is illustrated here: An ASC-US HPV-positive result will have an immediate risk of CIN 3+ of 4.4% (exceeding the 4% colposcopy threshold) when the prior history is unknown. However, if the prior result was a negative HPV primary screen or cotest, the immediate CIN 3+ risk is 2% if, resulting in a 1-year HPV-based follow-up (Table 1A, Egemen D, et al).¹³ If the ASC-US/HPV-positive was followed by colposcopy with biopsy showing CIN1 the prior year, the negative colposcopy reduces the risk of CIN 3+ within the next 2 years, and the immediate risk of CIN 3+ would be 3.1%, also resulting in a 1-year HPV-based follow-up (Table 4A, Egemen D, et al).¹³

The New Technology Committee will continue to assess and implement updates as new tests become approved and available, providing an enduring platform for new developments to be incorporated and the ability for the 2019 ASCCP guidelines to be updated on a more regular basis, without a large consensus conference effort. Additional risk modifiers, such as HPV vaccination status, will also be added, as vaccinated cohorts in the United States enter screening age, and enough data accumulates.

A deliberative method of stakeholder engagement was utilized in the development of these consensus guidelines, with the inclusion of representatives from 19 stakeholder organizations in the guideline working groups. Input from a number of surveys of providers and patients, and feedback from an open public comment period on draft guidelines, were considered before finalizing the 2019 ASCCP guidelines.¹⁶ Pathologists were well represented by major professional organizations, with 11 pathologists distributed among the working groups and 2 on the steering committee. The 2019 ASCCP Risk-Based Consensus Management Guidelines rolled out on April 2, 2020 during the virtual ASCCP annual meeting.¹⁵

As laboratorians, how can we support cervical cancer prevention in view of the new screening and management guidelines? Herein we summarize the changes in the 2019 ASCCP guidelines that are relevant to laboratories, pathologists, and cytotechnologists. Prior relevant screening and reporting recommendations that have not been widely and/or consistently adopted by laboratories are also discussed. We also offer considerations for modification of laboratory practices as we transition to these updated practice guidelines.

Human papillomavirus (HPV) testing

HPV has long been established as a necessary cause of cervical cancer.¹⁷ Over the past 3 decades, advances in our understanding of the pathobiology of HPV have clarified that it involves the anogenital tract epithelium in 2 distinct ways: HPV infection and true precancer. In the context of HPV testing for cervical cancer prevention, our goal is to detect those infections that may progress to precancer or cancer; hence, only testing platforms detecting high-risk or oncogenic types of HPV should be used. Low-risk types of HPV infection can cause external genital warts and are rarely, if ever, linked to cervical cancer or high-grade precursor lesions. Testing for low-risk HPV infections in the cervix does not provide information with regard to a patient's risk status and/or impact patient management. In fact, identification of low-risk HPV infection may lead to additional unnecessary testing and procedures, which can result in patient harm. Thus, we recommend against testing for low-risk HPV types.

HPV testing was introduced into the repertoire for cervical cancer screening and management in the United States in the mid 1990s. The indications for HPV testing vary based on the specific clinical situation for each patient and include screening, triage, and surveillance. Use of high-risk HPV testing was endorsed in screening and management guidelines, initially as a triage test for the cytologic result of ASC-US (2001). Regulatory approvals for cotesting (2003), for post-colposcopic/post-treatment follow-up and risk stratification using partial genotyping (2006), and as a stand-alone primary screening option (2014) followed.

In 2018, the US Preventive Services Task Force endorsed primary HPV screening for women aged 30 to 65 years.⁷ The updated American Cancer Society guidelines, due to be released in summer 2020, are also likely to endorse primary HPV screening in the United States. Although the United States has not widely adopted primary HPV testing as a screening modality, this transition is currently underway. These ongoing updates to cervical cancer screening guidelines, both in the United States and internationally, have been based on the higher sensitivity of HPV-based screening, modeling studies, and data from a number of trials, both in the United States and internationally. Comparison of screening methods is not the primary subject of this publication; however, additional communications, including from the Cytopathology Education and Technology Consortium, discuss concerns regarding the approval and implementation of primary HPV screening as the preferred screening strategy, without the option of cotesting, in the current US opportunistic screening program.¹⁸⁻²²

There are currently 5 HPV tests approved by the US Food and Drug Administration (FDA) for cytology and HPV cotesting and reflex indications: Qiagen Hybrid Capture (Gaithersburg, MD), Hologic Cervista and Hologic Aptima (Marlborough, MA), Roche cobas (Indianapolis,

Table 1 Comparison of the 5 FDA-approved HPV testing platforms.

Test	Hybrid capture II	Cervista	Cobas	Aptima	BD Onclarity
Manufacturer	Qiagen	Hologic	Roche	Gen Probe (Hologic)	Becton Dickinson
Year FDA approved for reflex HPV testing and HPV/Papanicolaou cotesting	2001	2009	2011	2011	2018
Year approved for primary screening	N/A	N/A	2014 (ThinPrep only) 2018 (ThinPrep and SurePath)	N/A	2018 (SurePath only)
Method	DNA (non-PCR based) Signal amplification: full genome probe	DNA (non-PCR based) Signal amplification: L1, E6, and E7 genes	DNA (PCR based); Target amplification: L1 gene target	mRNA (PCR based); Target amplification: E6/E7 gene target	DNA (PCR based); Target amplification: E6/E7 gene target
Genotypes detected	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 with genotyping of 16 and 18	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68; genotyping as separate test (16, 18/45)	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68; simultaneous, discrete identification of 16, 18, and 45
Clinical trial	ASC-US/LSIL Triage Study (ALTS), 2006 CAP	Cervista HPV HR	ATHENA	CLEAR trial	Onclarity trial (baseline phase)
Clinical validation	Extensive	Limited	Limited	Limited	Limited
Sensitivity for CIN 2/3	63.6%-100%	92.8%-100%	71.1%-99%	55.3%-100%	85.7%-100%
Specificity for CIN 2/3	6.2%-98.4%	—	24%-86.2%	28.8%-99.2%	17%-98.8%
Built-in internal control	No	Yes (HIST2H2BE)	Yes (β-globin)	Yes, an internal control transcript (HPV16 E6/7 transcript) is added to each reaction at the target capture step	Yes (β-globin)

Abbreviations: N/A, not applicable; PCR, polymerase chain reaction.

Adapted with permission from Salazar et al.²⁴

IN), and Becton Dickinson Onclarity (Franklin Lakes, NJ). All of these tests, except Aptima, utilize DNA hybridization and amplification or signal amplification. The Aptima test uses RNA amplification and some studies suggest that it is slightly more specific for high-grade precancers and cancers.²³ All, except Qiagen's HC2, have an internal control; however, none of the internal controls specifically identify epithelial cells. Only 2 of these platforms are approved for primary screening: cobas and Onclarity. The 2015 ASCCP interim and new (2019) guidelines, along with the Cytopathology Education and Technology Consortium, recommend that only platforms approved for primary screening be used when HPV testing alone is performed as a follow-up test strategy for women with a history of abnormal screening tests.^{15,22} Table 1 summarizes the characteristics of the FDA-approved HPV testing platforms currently available in the United States.²⁴

In the 2019 ASCCP risk-based management consensus guidelines, HPV-based testing is the basis for risk estimation and laboratories will likely see a larger volume of HPV testing requests.^{13,14} The term HPV-based testing is used in the 2019 ASCCP guidelines to refer to use of either primary HPV testing alone or HPV testing in conjunction with cervical cytology (cotesting).¹⁵ Previously, HPV testing had mostly been used for cotesting or as a reflex test for an ASC-US cytology.

The 2019 ASCCP management guidelines¹⁵ specifically state:

- Human papillomavirus assays that are Food and Drug Administration (FDA)-approved for screening should be used for management according to their regulatory approval in the United States. (Note: all HPV testing in this document refers to testing for high-risk HPV types only).
- For all management indications, HPV mRNA and HPV DNA tests without FDA approval for primary screening alone should only be used as a cotest with cytology, unless sufficient, rigorous data are available to support use of these particular tests in management.
- Surveillance with cytology alone is acceptable only if testing with HPV or cotesting is not feasible. Cytology is less sensitive than HPV testing for detection of precancer and is therefore recommended more often.

HPV genotyping

The 2019 ASCCP guidelines incorporate partial genotyping (HPV 16/18) information, if available, in the risk assessment profile for patients who test positive for high-risk HPV types. This guidelines consensus effort acknowledged that not all laboratories may currently have genotyping capability; however, when available, this additional information further stratifies risk.¹⁵ Large data sets analyzed as part of the guidelines review process show that the identification of HPV 16 clearly mandates consideration in clinical management of new abnormal screening results. HPV 18

positivity must be considered as a special situation because of an established disproportionate risk of invasive cancer.²⁵

When HPV-based screening is performed, the 2019 ASCCP guidelines recommend that:

- All positive primary HPV screening tests, regardless of genotype, should have additional reflex triage testing performed from the same laboratory specimen (eg, reflex cytology). This is a change from the 2015 ASCCP interim guidance for management of primary HPV screening results.²²
- Additional testing from the same laboratory specimen is recommended because the findings may inform colposcopy practice. For example, the CAT for expedited treatment is met for patients who test HPV-16 positive with HSIL cytology; this approach bypasses the intermediate step of colposcopic biopsy confirmation of high-grade disease.
- If HPV 16 or 18 testing is positive, and additional laboratory testing of the same sample is not feasible, the patient should proceed directly to colposcopy.
- HPV 16 or 18 infections have the highest risk for CIN 3 and occult cancer, so additional evaluation (eg, colposcopy with biopsy) is necessary even when cytology results are negative *or unavailable*.

Laboratory requisitions and electronic medical records/laboratory information system ordering options used at this time are likely to have been developed predominantly for HPV cotesting and reflex testing. Cytopathology professionals and laboratories should be prepared to facilitate testing strategies recommended in the 2019 ASCCP guidelines by assessing the following:

- (a) Ability to identify and confirm primary versus cotest HPV testing requests in a clinical context.
- (b) Ability to offer FDA-approved primary HPV screening and genotyping platforms.
- (c) Testing options/order sets and workflow for HPV based screening/management options within the cytopathology laboratory and other locations (eg, molecular diagnostics/referred testing).
- (d) Integrated reporting of results (preferred), when both HPV and cytology are utilized, whether as cytology/reflex HPV, cotesting, or primary HPV/reflex cytology and irrespective of the test indication (screening, triage or management).
- (e) Electronic medical record clinical decision support implementation, especially passive clinical decision support, to guide age-compliant screening and triage options and support clinical providers.

Clinical providers are equally obligated to provide accurate and sufficient patient history on the requisition form to allow the laboratory to make certain that HPV test orders are consistent with the 2019 ASCCP guidelines. Providers should also ascertain that the HPV testing platform being utilized by their laboratory is FDA-approved for the specific test indication. Cytopathology professionals and laboratories should discuss

HPV testing platforms with clinicians and monitor compliance with age-specific screening and risk-based management guidelines and provide feedback to their clinician groups.

Reporting and management of anatomic pathology results

Cytopathology

Atypical glandular cells (AGC) and adenocarcinoma in situ (AIS)

The Bethesda System (TBS) 2001 update replaced the term “atypical glandular cells of undetermined significance” (AGUS) with the term atypical glandular cells (AGC), and recommended a 2-tiered subcategorization: first, as to cell type/origin, if possible (ie, endocervical versus endometrial, or glandular when uncertain), and second, as favor neoplasia or not otherwise specified (NOS) for AGC and atypical endocervical cells (AEC). The rubric of AGC also includes atypical endometrial cells. Although cervical cytology is not a screening test for endometrial pathology, lesional cells can sometimes be seen. Atypical endometrial cells are not further subcategorized in terms of risk of neoplasia. TBS 2001 also introduced a specific cytologic interpretation of adenocarcinoma in situ (AIS).²⁶ There were no changes made to the reporting categories for glandular epithelial cell abnormalities in TBS 2014.²⁷

The overall category of AGC, albeit rare, may correspond histologically to several different entities ranging from benign (eg, polyps, tubal metaplasia) to intraepithelial lesions and invasive cancer, and include both glandular and squamous lesions (eg, HSIL involving endocervical glands, AIS, invasive adenocarcinoma of the Mullerian tract or other sites).²⁸ AGC is a much higher-risk cytologic interpretation than ASC-US. Even with established TBS diagnostic criteria, cytologic glandular abnormalities remain poorly reproducible and diagnostically challenging; this results in both under- and over-interpretation by cytotechnologists and cytopathologists.^{29,30} Some laboratories target AGC cases for consensus review by additional pathologists prior to issuing a final report.

The 2019 ASCCP guidelines for glandular cytologic abnormalities remain similar to the 2012 guidelines. Clinicians and cytologists often incorrectly think of cytologic AGC as a group with the same management. Triage of any category of AGC by HPV testing continues to be unacceptable in the 2019 ASCCP guidelines. All cytologic glandular abnormalities will lead to initial colposcopic evaluation with endocervical sampling except when atypical endometrial cells are specified. In that case, the preferred initial evaluation is endometrial and endocervical sampling. Additional endometrial sampling is also recommended when the patient is 35 years of age or older, symptomatic, or at high risk for endometrial neoplasia

based on risk factors. The 2019 ASCCP guidelines emphasize that although endometrial cancer is rare in premenopausal patients without risk factors, the prevalence of premenopausal endometrial cancer is increasing, underscoring the importance of endometrial sampling when indicated. Subsequent management for cytologic AEC/AGC, favor neoplasia and AIS is a diagnostic excision procedure, even if a high-grade abnormality is not confirmed on colposcopic biopsy with endocervical sampling, and even if HPV testing is negative. As endocervical lesions are difficult to visualize clinically, colposcopists rely on pathologists to provide appropriate categorization of cell origin and level of suspicion for neoplasia so that appropriate management is possible.³¹

Atypical squamous cells, cannot exclude HSIL (ASC-H) and combinations of cytology results

According to the Kaiser Permanente Northern California data, HPV-negative atypical squamous cells, cannot exclude HSIL (ASC-H) and HPV-positive ASC-H had very different CIN 3+ rates, but similar cancer rates. HPV-positive ASC-H had an immediate CIN 3+ risk of 26% and a cancer risk of 0.92%, whereas HPV-negative ASC-H had an immediate CIN 3+ risk of 3.4%, but an immediate cancer risk of 0.69%. Because the immediate cancer risk for ASC-H is disproportionately high compared with the CIN 3+ risk, the 2019 guidelines carried forward the 2012 recommendations of colposcopy for all patients with ASC-H, regardless of HPV test results.¹⁵

HSIL found in the background of atrophy is often difficult to appreciate because of the lack of maturation of squamous cells and the similarity between small atrophic cells and the dysplastic cells. Additionally, as illustrated in the 2014 Bethesda Atlas, isolated highly atypical squamous cells, with very large nuclei, a characteristic smudgy or degenerative chromatin pattern, and a very high nucleus to cytoplasmic ratio can be occasionally identified in deeply atrophic specimens.²⁶ Such cells can elicit morphologic concern for HSIL but are observed, often in older patients with few or no risk factors. A conservative approach, such as interpretation as ASC-US with follow-up/reflex HPV testing, rather than ASC-H, may be more appropriate in such cases. In cases of atrophy with abnormal cells meeting criteria for HSIL, however, an interpretation of HSIL should be made.

When reporting squamous intraepithelial lesions (SIL) according to Bethesda terminology, it is important to recognize that the concurrent presence of LSIL cells is not necessary to make an interpretation of HSIL and the presence of even a small population of definitive HSIL cells in the background of a predominance of LSIL cells should result in an interpretation of HSIL.²⁷ In rare cases, cytology specimens may exhibit squamous cells with features that lie between low- and high-grade SIL. These include cases that exhibit predominantly LSIL and a few cells suggestive, but not diagnostic, of HSIL (ASC-H) or cases that display

keratinized cells with dense eosinophilic cytoplasm that give an impression of higher nucleus to cytoplasmic ratio than in classic LSIL, but without specific features of classic HSIL.²⁷ Per TBS 2014, such cases should be reported using well-established TBS categories, specifically “ASC-H in a background of LSIL”, and not in other terms such as “LSIL, cannot exclude a high-grade lesion”.²⁷

Non-TBS reporting terms do not provide clear management direction to the clinical provider, since they are not linked to the ASCCP management guidelines as are the TBS categories. Management under the 2019 ASCCP guidelines is based on the highest-risk cytologic interpretation, for example, ASC-H, when both ASC-H and LSIL are reported on the same sample. In other rare combinations of cytologic results such as HSIL and AGC, or ASC-US and AGC, management is also determined by the highest risk abnormality.^{15,27}

Histopathology

Reporting of HPV-Associated squamous intraepithelial lesions

Both the Lower Anogenital Squamous Terminology (LAST) Project³² and the World Health Organization³³ recommend the use of a 2-tiered nomenclature for HPV-associated squamous cervical lesions: LSIL, the morphologic representation of HPV infection, and HSIL, the histopathological correlate of precancer. Rather than the spectrum of disease that is implied by the 3-tiered CIN classification system, the 2-tiered system of LSIL/HSIL better reflects the known biology of HPV disease. Both LAST and WHO also permit the CIN grade to be designated in parenthesis after the SIL nomenclature. Adoption of this terminology by pathologists has not been universal.

The 2019 ASCCP guidelines include the following specific statement regarding the use of p16 immunohistochemistry (IHC) and a 2-tiered nomenclature for reporting histopathology of HPV-associated squamous lesions of the lower anogenital tract.¹⁵

- It is important to use p16 immunohistochemical staining according to the guidance provided by the College of American Pathologists (CAP)-ASCCP Lower Anogenital Squamous Terminology (LAST) Project. p16 immunohistochemistry should be used for specific indications as recommended by the LAST guidelines when interpreting the hematoxylin and eosin (H&E) slide. A positive p16 immunostain supports the diagnosis of histologic HSIL if the morphological assessment of H&E slides is consistent with CIN 2 or CIN 3. There is a risk of overcalling cervical histology findings when p16 is used incorrectly. Most importantly, a morphologic CIN1 on H&E should not be upgraded to histologic HSIL (CIN 2), even if p16 positive.
- For epidemiologic and clinical management purposes, it is strongly recommended to qualify a histologic HSIL

result by CIN 2 or CIN 3, according to the options given by the LAST guidelines (eg, histologic HSIL [CIN 2]).

Rationale. This CIN qualification can have clinical importance (eg, to identify cases of CIN 2 in patients for whom conservative management is an acceptable option). It is also important for post-vaccine surveillance studies and quality control assessments of cervical precancer that have historically relied on CIN 2 and CIN 3 endpoints. Furthermore, it is important for future research efforts to distinguish diagnoses of histologic HSIL (CIN 2) from HSIL (CIN 3) so that diagnostic categories are compatible with the histologic endpoints used for current guidelines.

Note. Strong and diffuse block staining for p16, as defined by LAST³² is “*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 p16 immunostain positive. Focal or patchy staining is nonspecific and can be seen with reactive squamous metaplasia, as well as low-grade disease (LSIL, CIN 1). All other staining patterns, described as cytoplasmic only, wispy, scattered, single cells, and others, are defined as negative.

It is important for pathologists to remember that p16 IHC is not a highly specific test³⁴ and thus to use p16 only as suggested by the LAST recommendations to avoid overusage. As with other immunostains, correct interpretation of p16 IHC is also needed to avoid over interpretation of positivity. The LAST recommendations are undergoing an update, starting in 2020, and additional biomarkers could possibly further refine morphologic interpretations.

The 2019 ASCCP guidelines¹⁵ recommend treatment of histologic HSIL in most circumstances in non-pregnant women. Treatment is specifically recommended for HSIL (CIN 3), for any HSIL if the proximal extent of the lesion is in the endocervical canal or the entire squamocolumnar junction cannot be visualized, or if endocervical sampling shows HSIL or ungraded CIN. Treatment is also recommended for HSIL not further qualified (ie, CIN 2/3). Observation is preferred only when HSIL is specifically qualified as CIN 2 and only in specific situations: for women <25 years or for women whose concerns about pregnancy-related treatment complications outweigh concerns about cancer. Thus, a pathology report of cervical HSIL, regardless of CIN grade or unqualified, serves as the foundation for treatment decisions in most situations.

Given that background, how can pathologists give the most reliable histopathology diagnoses for cervical biopsies to their clinical colleagues to facilitate sound patient management recommendations? Any morphologic interpretation is subject to interobserver variation. Pathologists give more consistent diagnoses on cervical biopsies with the judicious

use of p16 IHC. Improved interobserver agreement for the diagnosis of CIN 2+ with the conjunctive use of hematoxylin and eosin (H&E) morphology and p16 IHC compared with H&E morphology alone has been shown in several studies,³⁵⁻³⁸ including in a systematic review and meta-analysis.³⁹ Because histologic HSIL is the usual trigger for treatment, the use of p16 per the LAST and WHO guidelines provides our clinical colleagues more reproducible diagnoses upon which to base management recommendations.

The LAST Project recommends that p16 IHC, when positive, should be used to support the diagnostic interpretation of HSIL in times of diagnostic uncertainty (such as with atypical squamous metaplasia on H&E) and to support a diagnosis of HSIL (CIN 2). CIN 2 has historically been and remains the threshold for treatment in most circumstances. However, among pathologists, CIN 2 is the least reproducible of the CIN categories.^{40,41} If a diagnosis of CIN 2 is entertained based on solid morphologic criteria, a p16 should be performed to support a high-grade lesion. If negative, the diagnosis should be downgraded to LSIL or a benign process depending on the differential diagnosis on H&E. Judicious use of p16, in conjunction with H&E morphology is needed, however. When the morphologic diagnosis is LSIL (CIN 1), a p16 should not be performed. Morphologic LSIL (CIN 1) is frequently p16-positive; thus if p16 is positive, LSIL should not be upgraded to HSIL. There is insufficient data to indicate that the risk profile of p16-positive LSIL meets the CAT for treatment. Given diagnostic uncertainty encountered in tissue diagnoses of challenging histomorphology and the inherent interobserver variability of morphologic diagnosis, p16 IHC, when positive, is a useful tool to support a diagnosis of precancer.

More high-grade lesions may be identified as clinicians adopt the ASCCP colposcopy standards published in 2017.⁴² These standards recommend that colposcopists take multiple biopsies of all areas with acetowhitening, metaplasia, or higher colposcopic abnormalities. As a result, many colposcopic procedures will have at least 2 and up to 4 targeted biopsies from distinct lesions. This recommendation should help reduce sampling issues related to colposcopic-directed biopsies and interobserver variability of colposcopic impression of lesion grade, but it may also detect more small lesions that are morphologically high-grade.

An important and potentially overlooked issue is that of the size of the cervical HSIL. Lesion size is not a component of the treatment algorithms; treatment recommendations are based on the histopathology diagnosis of a potential precancer regardless of lesion size. In the only known and now infamous “Unfortunate Experiment”, an observational study of untreated CIN 3, the long-term risk of developing invasive cancer was approximately 30% over a 30-year period.⁴³ As Schiffman and Rodriguez accurately point out in an accompanying commentary, these were large, prevalent lesions in women with a median age of 38 years.⁴⁴ The

natural history of early, small lesions, potentially detected only because of increased sampling and screening efforts, remains unknown.

Another issue is the inability to accurately grade a fragmented squamous intraepithelial lesion (SIL), which is often reported as SIL, ungraded. Newer cervical biopsy collection devices, such as the SoftBiopsy, (Histologics Anaheim, CA; <https://histologics.com/softbiopsy.html>) and endocervical curettage specimens “denude” the cervical epithelium for tissue examination rather than provide a full-thickness tissue biopsy. This can result in problems with tissue orientation that make the lesion difficult to grade. These cases are more likely to result in a diagnosis of “SIL, ungraded”. A recent study by Lee et al⁴⁵ found that histologic SIL, ungraded, although representing only 1.9% of their total cases, resulted in positive results (HSIL, AIS, or carcinoma) in 41% of patients with histologic follow-up. They found that a combination of p16 and ki-67 helped to correctly classify these lesions. Many clinical care providers do not understand that ungraded SIL represents a high-risk category for precancer and patients may not undergo sufficient follow-up; thus, laboratories are encouraged to communicate the possibility of HSIL or other significant abnormality in their reports.

Reporting and management of histologic glandular lesions

Adenocarcinoma in situ (AIS)

The Society of Gynecologic Oncology (SGO) recently completed guidelines on the management of AIS and the 2019 ASCCP risk-based management consensus guidelines for glandular lesions are harmonized with the SGO guidelines.⁴⁶ The only additional SGO-specific recommendation for management of an abnormal HPV-based screen result is that endocervical sampling is acceptable for any patient who tests positive for HPV 18 because of the high rate of HPV 18-positive AIS.

A diagnostic excisional procedure is recommended for all patients with a diagnosis of AIS on cervical biopsy to rule out invasive adenocarcinoma, even if hysterectomy is planned. The guidelines do not specifically recommend cold knife conization over loop electrocautery excisional procedure (LEEP), but do state that excisional procedures should aim to remove an intact specimen for evaluation of margin status by the pathologists. Hence, performance of a LEEP followed by a “top hat” endocervical excision is not acceptable. Ultimately, hysterectomy remains the preferred management for all patients with histologic AIS, but a fertility-sparing procedure is considered acceptable in select patients. Hysterectomy is preferred for AIS because it is often located within the endocervical canal, and is difficult to recognize on colposcopy, thus making decision on the extent of excision difficult. AIS is also known to have a higher risk of being multifocal, so negative margins on an

excisional specimen do not ensure complete excision of disease. After hysterectomy, surveillance is recommended per the 2019 ASCCP guidelines for treated CIN 2+.^{15,46}

Newer glandular entities

Clinical providers are often not familiar with newer histologic entities, such as the stratified mucin-producing intraepithelial lesion (SMILE), a form of intraepithelial lesion distinct from conventional squamous and glandular counterparts.⁴⁷ An invasive form of SMILE has recently been reported.^{48,49} In the largest retrospective cytology study to date, Backhouse et al found that almost 90% of SMILE were interpreted as squamous lesions on initial cytology and only about 10% were classified as glandular lesions.⁵⁰ Pathologists should consider providing a short description of the significance of newer entities in their reports and/or discussing with the clinical provider the significance of newer terminology, so as to provide guidance on management.

Pregnancy-related considerations

Pregnancy is a special management consideration that weighs the risk of harm to fetus and mother against the risk of missing cancer. Although these should not be ignored, the evidence suggests that diagnostic procedures are safe in the hands of experienced colposcopists and that the rates of CIN 3+ progression are not higher in the pregnant woman.⁵¹ The goal of colposcopy during pregnancy is to exclude cancer, rather than to find high-grade disease. Adding to the complexity, however, are the challenges that colposcopists have detecting and recognizing lesions on the pregnant cervix. Visual recognition of CIN 3+ may be compromised by physiologic changes and cervical hyperemia of pregnancy, leading to underdiagnosis of CIN 3+. Colposcopically, decidual change on the cervix can be overinterpreted as suspicious for cancer, and cellular changes such as Arias-Stella changes and decidua maybe over interpreted as AGC, ASC-H, ASC-US, and LSIL by cytologists.²⁶

Social issues also complicate treatment timing decisions. Patients may not attend postpartum follow-up because of employment issues and demands of a new baby; in addition, an estimated 11% of women lose their health insurance in the postpartum period.⁵² For these reasons, some clinicians may determine that prepartum diagnostic procedures are appropriate. The 2019 ASCCP guidelines recommend the same clinical action thresholds for management, surveillance, and colposcopy for pregnant women as for non-pregnant women, except that endocervical curettage, endometrial biopsies, and expedited treatment (eg, conization or LEEP without histologic confirmation) are not recommended. Although colposcopy, biopsy, and cytology with age-appropriate HPV testing should occur during pregnancy, treatment for CIN 2/3 is postponed until postpartum.

Cervical biopsies may be obtained during pregnancy in cases of suspected invasive carcinoma.

Quality assurance: cytology-histology correlation and more

Converting to a risk-based management strategy with primary HPV screening, especially in vaccinated individuals, introduces potential future diagnostic problems. HPV tests are highly sensitive but not specific—they identify the presence of the virus but don't define the disease. Colposcopy has its own set of limitations and risks, as will the use of cervical cytology and biopsy as diagnostic tests. That is why the 2019 ASCCP guidelines recommend that all positive HPV tests receive cytology as a reflex test to aid with management, and recommend collection of a cytology sample at the time of colposcopy if reflex cytology from the screening sample is not possible.

Pathologist concordance with a particular cervical cytology or cervical biopsy result varies depending on the lesion. Overall, diagnostic reproducibility between general surgical pathologists and gynecologic pathologists regarding cervical biopsies are typically moderate, at best.⁵³⁻⁵⁵ Pathologists are most concordant at the far ends of the diagnostic spectrum (negative and CIN 3)⁵⁶ but agree less often regarding CIN 1 and CIN 2.⁵⁷ The histologic distinction between CIN 1 and CIN 2 is often nebulous, presenting a problem if the trigger for treatment is at CIN 2. The ASCUS-LSIL Triage Study demonstrated that diagnostic concordance for CIN 1/LSIL was greater for cervical cytology than for cervical biopsies.⁵³ The diagnosis of CIN 1 on biopsies has only fair to moderate concordance. In a study by Basu et al, the lowest agreement among pathologists was for CIN 1 whereas the highest was for squamous cell carcinoma.⁵⁶ Even when pathologists use a 3-tiered system of cervical biopsy interpretation, such as negative, LSIL, and HSIL, there is still difficulty separating normal cervical biopsies from LSIL and separating LSIL from HSIL.⁵⁸ There is a tendency for pathologists to overcall normal epithelium as CIN 1 and to overcall CIN 1 as CIN 2,3.⁵⁴ The use of p16 may be helpful in equivocal cases, as discussed above. A negative p16 is useful in excluding HSIL, but p16 positivity only indicates an HPV-associated lesion and does not differentiate HSIL from LSIL. Although cytology is more accurate than histology in diagnosing CIN 1, the distinction of CIN 2 from CIN 3 is problematic in both tissue and cytology, with poor to moderate reproducibility in both.^{57,59} In educational settings, such as the College of American Pathologists Educational Glass Slide Program, both pathologists and cytotechnologists are more likely to undercall HSIL as LSIL on cytology than the reverse.⁶⁰ Papanicolaou tests with mixed LSIL and HSIL do not perform well in these circumstances either.⁶¹ These diagnostic tendencies may continue to confound management algorithms, skew future

data, and serve as a reminder that clinical management should not be solely reliant on test results alone.

The 2019 ASCCP guidelines continue to encourage clinical provider and pathologist interaction with review of the specimens when there is a significant cytologic, histologic, or colposcopic discrepancy. For example: “when CIN 2+ is not identified histologically after an ASC-H or HSIL cytology result, it is acceptable to review the cytologic, histologic, and colposcopic findings; if the review yields a revised interpretation, management should follow guidelines for the revised diagnosis”.¹⁵ Optimal practice includes real-time cytologic-histologic correlation with efforts to resolve discrepancies, such as obtaining deeper levels and/or performing p16 staining on histology if the preceding cytology result is of a higher-grade lesion. Making note of the review of cases, action taken, and resolution in the histology report is ideal.

Diagnostic competence in surgical pathology and cytopathology depends upon exposure; that is, seeing an appropriate volume of cases and making comparisons with other clinical and laboratory data. As a quality assurance metric, cytologic-histologic correlation plays a vital role in providing feedback on accuracy and “fine tuning” of diagnostic criteria.^{62,63} The opportunity for cytologic-histologic correlation will decrease as cytology is used primarily as triage or follow-up test. In an era where fewer cytology tests are reviewed, and most of these are HPV-positive, the proportion of atypical cytology results is likely to increase because of reviewer bias that knowledge of HPV status introduces.^{64,65} The consequence may be more colposcopy referrals for women without disease.

The complex and highly regulated quality environment federally mandated by the Clinical Laboratory Improvement Amendments⁶⁶ (CLIA) for the practice of gynecologic cytopathology resulted in a robust system of quality assurance that is fully-developed and highly successful.⁶⁷ Most surgical pathology quality systems in place are not as robust, and this may affect the interpretation of cervical biopsies. For example, a minimum of 2 individuals (a cytotechnologist and a pathologist) usually reviews all abnormal cervical cytology cases, but a single pathologist may solely diagnose cervical biopsies. There is no required peer review, interpreter monitoring, or correlation with outcomes for cervical biopsies similar to that mandated for cervical cytology. With higher stakes for missing a cervical abnormality due to extended screening intervals and follow-up, it may become a future best practice that more cervical biopsies receive a second review.

Conclusion

The landscape of cervical cancer prevention is rapidly changing. HPV immunization is reducing infections caused by targeted high-risk genotypes in vaccinated populations and reducing the incidence of high-grade lesions, caused

by these types, whose morphology is most familiar to pathologists. Molecular approaches to screening with HPV tests are supplanting our familiar, but decades-old, morphology-based screening approach, the Papanicolaou test. New biomarkers, such as dual staining for screening, have recently received FDA approval.⁸ Additional molecular tests, such as extended HPV genotyping and type-persistence data from HPV tests that include partial and extended genotyping, need to be incorporated into the assessment of progression risk for those with histologic HSIL. New molecular tests, such as viral and host methylation, are on the horizon and promise to provide more objective and precise assessments of the risk for true precancer. In the future, pathologists may also have additional biomarkers for histopathology that more accurately reflect a lesion’s true risk for cancer progression. The clinical management guidelines will continue to evolve and, hopefully, more accurately balance the benefits and potential harms of cervical cancer prevention efforts. The World Health Organization has set targets for the elimination and eradication of cervical cancer.⁶⁸ As laboratorians, we must keep abreast of these new developments and guidelines and be prepared to proactively participate in and facilitate multidisciplinary secondary prevention of cervical cancer.

References

1. Saslow D, Solomon D, Lawson HW, et al. American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer. *CA Cancer J Clin*. 2012;62:147–172.
2. Demarco M, Lorey TS, Fetterman B, et al. Risks of CIN 2+, CIN 3+, and cancer by cytology and human papillomavirus status: the foundation of risk-based cervical screening guidelines. *J Low Genit Tract Dis*. 2017;21:261–267.
3. Castle PE, Kinney WK, Xue X, et al. Role of screening history in clinical meaning and optimal management of positive cervical screening results. *J Natl Cancer Inst*. 2019;111:820–827.
4. Katki HA, Schiffman M, Castle PE, et al. Five-year risk of recurrence after treatment of CIN 2, CIN 3, or AIS: performance of HPV and Pap cotesting in posttreatment management. *J Low Genit Tract Dis*. 2013;17:S78–S84.
5. Katki HA, Schiffman M, Castle PE, et al. Five-year risks of CIN 2+ and CIN 3+ among women with HPV-positive and HPV-negative LSIL Pap results. *J Low Genit Tract Dis*. 2013;17:S43–S49.
6. Demarco M, Cheung LC, Kinney WK, et al. Low risk of cervical cancer/precancer among most women under surveillance postcolposcopy. *J Low Genit Tract Dis*. 2018;22:97–103.
7. US Preventive Services Task ForceCurry SJ, Krist AH, Owens DK, et al. Screening for cervical cancer: US preventive services task force recommendation statement. *JAMA*. 2018;320:674–686.
8. Roche. Roche receives FDA approval for CINtec PLUS cytology test to aid clinicians in improving cervical cancer prevention. Available at: <https://diagnostics.roche.com/global/en/news-listing/2020/roche-receives-FDA-approval-for-CINtec-plus-cytology-test.html>. Accessed April 29, 2020.
9. Schiffman M, Wentzensen N, Khan MJ, et al. Preparing for the next round of ASCCP-sponsored cervical screening and management guidelines. *J Low Genit Tract Dis*. 2017;21:87–90.

10. Perkins RB, Schiffman M, Guido RS. The next generation of cervical cancer screening programs: making the case for risk-based guidelines. *Curr Probl Cancer*. 2018;42:521–526.
11. Castle P, Feldman S, Perkins RB. The next generation of cervical cancer screening: should guidelines focus on best practices for the future or current screening capacity? *J Low Genit Tract Dis*. 2018;22:91–96.
12. Schiffman M, Wentzensen N, Perkins RB, Guido RS. An introduction to the 2019 ASCCP risk-based management consensus guidelines. *J Low Genit Tract Dis*. 2020;24:87–89.
13. Egemen D, Cheung LC, Chen X, et al. Risk estimates supporting the 2019 ASCCP risk-based management consensus guidelines. *J Low Genit Tract Dis*. 2020;24:132–143.
14. Cheung LC, Egemen C, Xiaojian C, et al. 2019 ASCCP risk-based management consensus guidelines: methods for risk estimation, recommended management, and validation. *J Low Genit Tract Dis*. 2020;24:90–101.
15. Perkins RB, Guido RS, Castle PE, et al. 2019 ASCCP Risk-Based Management Consensus Guidelines for abnormal cervical cancer screening tests and cancer precursors. *J Low Genit Tract Dis*. 2020;24:102–131.
16. Perkins RB, Fuzzell LN, Lake P, et al. Incorporating stakeholder feedback in guidelines development for the management of abnormal cervical cancer screening tests. *J Low Genit Tract Dis*. 2020;24:167–177.
17. IARC. Human Papillomaviruses. In: . Lyon, France: IARC; 1995: *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*. 64.
18. Nayar R, Goulart RA, Tiscornia-Wasserman PG, Davey DD. Primary human papillomavirus screening for cervical cancer in the United States—US Food and Drug Administration approval, clinical trials, and where we are today. *Cancer Cytopathol*. 2014;122:720–729.
19. Nayar R, Goulart RA, Davey DD. Primary HPV cervical cancer screening in the United States: are we ready? *J Am Soc Cytopathol*. 2018;7:50–55.
20. Davey DD, Goulart RA, Nayar R. An advocacy victory: final USPSTF cervical cancer screening recommendations revised to include cotesting option. *J Am Soc Cytopathol*. 2018;7:333–335.
21. Kaufman HW, Alagia DP, Chen Z, et al. Contributions of liquid-based (Papanicolaou) cytology and human papillomavirus testing in cotesting for detection of cervical cancer and precancer, in the United States. *Am J Clin Pathol*; 2020. (In press).
22. Huh WK, Ault K, Chelmow D, et al. Use of primary high risk papillomavirus testing for cervical cancer screening: interim clinical guidance. *Gynecol Oncol*. 2015;136:178–182.
23. Ge Y, Christensen P, Luna E, et al. Performance of Aptima and cobas HPV testing platforms in detecting high-grade cervical dysplasia and cancer. *Cancer Cytopathol*. 2017;125:652–657.
24. Salazar KL, Duhon DJ, Olsen R, Thrall M. A review of the FDA-approved molecular testing platforms for human papillomavirus. *J Am Soc Cytopathol*. 2019;8:284–292.
25. Demarco M, Egemen D, Raine-Bennett T, et al. A study of partial human papillomavirus genotyping in support of the 2019 ASCCP Risk-Based Management Consensus Guidelines. *J Low Genit Tract Dis*. 2020;24:144–147.
26. Solomon D, Nayar R, eds. *The 2001 Bethesda System for Reporting Cervical Cytology*. 2nd ed. New York, NY: Springer; 2004.
27. Nayar R, Wilbur DC, eds. *The Bethesda System for Reporting Cervical Cytology*. 3rd ed. New York, NY: Springer; 2015.
28. Ronnett BM, Manos MM, Ransley JE, et al. Atypical glandular cells of undetermined significance (AGUS): cytopathologic features, histopathologic results, and human papillomavirus DNA detection. *Hum Pathol*. 1999;30:816–825.
29. Lee KR, Darragh TM, Joste NE, et al. Atypical glandular cells of undetermined significance (AGUS): interobserver reproducibility in cervical smears and corresponding thin-layer preparations. *Am J Clin Pathol*. 2002;117:96–102.
30. Zhao C, Florea A, Onisko A, Austin RM. Histologic follow-up results in 662 patients with Pap test findings of atypical glandular cells: results from a large academic womens hospital laboratory employing sensitive screening methods. *Gynecol Oncol*. 2009;114:383–389.
31. Levine L, Lucci JA, Dinh TV. Atypical glandular cells: new Bethesda terminology and management guidelines. *Obstet Gynecol Surv*. 2003;58:399–406.
32. Darragh TM, Colgan TJ, Cox JT, et al. 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;136:1266–1297. *J Low Genit Tract Dis* 2012;16:205–42.
33. World Health Organization. *WHO Classification of Tumours of Female Reproductive Organs*. 4th ed. Lyon, France: International Agency for Research on Cancer; 2014.
34. Castle PE, Adcock R, Cuzick J, et al. Relationships of p16 immunohistochemistry and other biomarkers with diagnoses of cervical abnormalities: implications for LAST terminology. *Arch Pathol Lab Med*. 2019;144:725–734.
35. Bergeron C, Ordi J, Schmidt D, et al. Conjunctive p16INK4a testing significantly increases accuracy in diagnosing high-grade cervical intraepithelial neoplasia. *Am J Clin Pathol*. 2010;133:395–406.
36. Maniari KP, Sanchez B, Paintal A, Gursel DB, Nayar R. Role of the biomarker p16 in downgrading -IN 2 diagnoses and predicting higher-grade lesions. *Am J Surg Pathol*. 2015;39:1708–1718.
37. Stoler MH, Wright TC, Ferenczy A, et al. Routine use of adjunctive p16 immunohistochemistry improves diagnostic agreement of cervical biopsy interpretation: results from the CERTAIN Study. *Am J Surg Pathol*. 2018;42:1001–1009.
38. Thrall MJ. Effect of lower anogenital squamous terminology recommendations on the use of p16 immunohistochemistry and the proportion of high-grade diagnoses in cervical biopsy specimens. *Am J Clin Pathol*. 2016;145:524–530.
39. Reuschenbach M, Wentzensen N, Dijkstra MG, von Knebel Doeberitz M, Arbyn M. p16INK4a immunohistochemistry in cervical biopsy specimens: a systematic review and meta-analysis of the inter-observer agreement. *Am J Clin Pathol*. 2014;142:767–772.
40. Carreon JD, Sherman ME, Guillén D, et al. CIN2 is a much less reproducible and less valid diagnosis than CIN 3: results from a histological review of population-based cervical samples. *Int J Gynecol Pathol*. 2007;26:441–446.
41. 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.
42. Wentzensen N1, Massad LS, Mayeaux Jr EJ, et al. Evidence-based consensus recommendations for colposcopy practice for cervical cancer prevention in the United States. *J Low Genit Tract Dis*. 2017;21:216–222.
43. McCredie MR, Sharples KJ, Paul C, et al. Natural history of cervical neoplasia and risk of invasive cancer in women with cervical intraepithelial neoplasia 3: a retrospective cohort study. *Lancet Oncol*. 2008;9:425–434.
44. Schiffman M, Rodríguez AC. Heterogeneity in CIN3 diagnosis. *Lancet Oncol*. 2008;9:404–406.
45. Lee S, Sabourin J, Gage J, Franko A, Nation JG, Duggan MA. Squamous intraepithelial lesion in cervical tissue samples of limited adequacy and insufficient for grading as high or low grade: outcome, clinic-pathological correlation, and predictive role of p16INK4a and Ki67 biomarker staining. *J Low Genit Tract Dis*. 2015;19:35–45.
46. Teoh D, Musa F, Salani R, et al. Diagnosis and management of adenocarcinoma in situ: a Society of Gynecologic Oncology evidence-based review and recommendations. *Obstet Gynecol*. 2020;135:869–878.
47. Park JJ, Sun D, Quade BJ, et al. Stratified mucin-producing intraepithelial lesions of the cervix: adenosquamous or columnar cell neoplasia? *Am J Surg Pathol*. 2000;24:1414–1419.
48. Lastra RR, Park KJ, Schoolmeester JK. Invasive stratified mucin-producing carcinoma and stratified mucin-producing intraepithelial

- lesion (SMILE): 15 cases presenting a spectrum of cervical neoplasia with description of a distinctive variant of invasive adenocarcinoma. *Am J Surg Pathol*. 2016;40:262–269.
49. Stolnicu S, McCluggage WG. The evolving spectrum of endocervical adenocarcinoma in situ (AIS). *Virchows Arch*. 2020;476:485–486.
 50. Backhouse A, Stewart CJ, Koay MH, et al. Cytologic findings in stratified mucin-producing intraepithelial lesion of the cervix: a report of 34 cases. *Diagn Cytopathol*. 2016;44:20–25.
 51. He Y, Wu Y-M, Wang T, et al. Perinatal outcomes of pregnant women with cervical intraepithelial neoplasia. *Arch Gynecol Obstet*. 2013;288:1237–1242.
 52. McMorrow S, Kenney G. Despite progress under the ACA, many new mothers lack insurance coverage. Available at: <https://www.healthaffairs.org/doi/10.1377/hblog20180917.317923/full/>; 2018. Accessed April 29, 2020.
 53. Stoler MH, Schiffman M, for the 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;285:1500–1505.
 54. Stoler MH, Ronnett BM, Joste NE, et al. The interpretive variability of cervical biopsies and its relationship to HPV status. *Am J Surg Pathol*. 2015;205:729–736.
 55. Clark JL, Lu D, Kalir T, Lui Y. Overdiagnosis of HSIL on cervical biopsy: errors in p16 immunohistochemistry implementation. *Hum Pathol*. 2016;55:51–56.
 56. Basu P, Kamal M, Ray C, et al. Interobserver agreement in the reporting of cervical biopsy specimens obtained from women screened by visual inspection with acetic acid and hybrid capture 2. *Int J Gynecol Pathol*. 2013;32:509–515.
 57. Dalla Palma P, Rossi G, Buccoliero CG, et al. The reproducibility of CIN diagnoses among different pathologists: data from histology review from a multicenter randomized study. *Am J Clin Pathol*. 2009;132:125–132.
 58. McCluggage WG, Walsh MY, Thorton CM, et al. Inter- and intra-observer variation in the histologic reporting of cervical squamous intraepithelial lesions using a modified Bethesda grading system. *Br J Obstet Gynaecol*. 1998;105:206–210.
 59. Woodhouse SL, Stastney JF, Styer PE, Kennedy M, Praestgaard AH, Davey DD. Interobserver variability in subclassification of squamous intraepithelial lesions: results of the College of American Pathologists Interlaboratory Comparison Program in Cervicovaginal Cytology. *Arch Pathol Lab Med*. 1999;123:1079–1084.
 60. Crothers BA, Ghofrani M, Zhao C, et al. Low-grade squamous intraepithelial lesion or high-grade squamous intraepithelial lesion? Concordance between interpretation of low-grade squamous intraepithelial lesion and high-grade squamous intraepithelial lesion in Papanicolaou tests: results from the College of American Pathologists PAP Education Program. *Arch Pathol Lab Med*. 2019;142:81–85.
 61. Renshaw AA, Mody DR, Styer P, Schwartz M, Ducatman B, Colgan T. Papanicolaou tests with mixed high-grade and low-grade squamous intraepithelial lesion features: distinct performance in the College of American Pathologists Interlaboratory Comparison Program in Cervicovaginal Cytopathology. *Arch Pathol Lab Med*. 2006;130:456–459.
 62. Raab SS, Grzybicki DM. Cytologic-histologic correlation. *Cancer Cytopathol*. 2011;119:293–309.
 63. Crothers BA. Cytologic-histologic correlation: where are we now, and where are we going? *Cancer Cytopathol*. 2018;126:301–308.
 64. Moriarty AT, Nayar R, Arnold T, et al. The Tahoe Study: bias in the interpretation of Papanicolaou test results with human papillomavirus status is known. *Arch Pathol Lab Med*. 2014;138:1182–1185.
 65. Doxtader EE, Brainard JA, Underwood D, Chute DJ. Knowledge of HPV status biases cytotechnologist's interpretation of Pap tests originally diagnosed as negative for intraepithelial lesion or malignancy. *Cancer Cytopathol*. 2017;125:60–69.
 66. Department of Health and Human Services, Health Care Financing Administration. Clinical laboratory improvement amendments of 1988: final rule. *Fed Regist*. 1992;57:7146. §493.1–493.25; codified at 42 CFR §493.
 67. Tworek J, Nayar R, Savaloja L, et al. General quality practices in gynecologic cytopathology: College of American Pathologists Gynecologic Cytopathology Quality Consensus Conference working group 3. *Arch Pathol Lab Med*. 2013;137:190–198.
 68. World Health Organization. A global strategy for elimination of cervical cancer. Available at: <https://www.who.int/activities/a-global-strategy-for-elimination-of-cervical-cancer>. Accessed April 23, 2020.