Journal of Clinical and Translational Pathology **2023** vol. 3(2) | 75–83
DOI: 10.14218/JCTP.2023.00004

Review Article

Updates in Cervical Cancer Screening Guidelines, The Bethesda System for Reporting Cervical Cytology, and Clinical Management Recommendations

Tiannan Wang[1*], Huina Zhang[2], Yang Liu[3] and Chengquan Zhao[4*]

[1]Department of Pathology, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA; [2]Department of Pathology, University of Rochester Medical Center, Rochester, NY, USA; [3]Department of Medicine, Department of Bioengineering, University of Pittsburgh Hillman Cancer Center, 5117 Centre Ave, Pittsburgh, PA, USA; [4]Department of Pathology, Magee-Womens Hospital, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

Received: February 13, 2023 | **Revised:** March 2, 2023 | **Accepted:** March 20, 2023 | **Published online:** April 21, 2023

Abstract

Over the past decades, cervical cancer has been a worldwide public health problem. Population-based early cancer risk detection and prevention approaches, including vaccination, cytology screening and human papilloma virus (HPV) detection, with the aligned clinical management, have formed a well-rounded high-quality implementation system for cervical cancer control, and revolutionarily improved the quality of life of women: (1) the success of cervical cancer screening practices, (2) standardization of The Bethesda system for reporting cervicovaginal cytology, (3) improvement in the understanding of HPV pathogenesis in cervical cancer, and (4) the development of appropriate management approaches have significantly decreased the disease burden of cervical cancer worldwide. This scoping review aimed to understand the evolvement of cervical cancer screening and management guidelines, describe the Bethesda cervical cytology reporting system, and HPV vaccines and tests, and highlight the key information of present policies and practices.

Citation of this article: Wang T, Zhang H, Liu Y, Zhao C. Updates in Cervical Cancer Screening Guidelines, The Bethesda System for Reporting Cervical Cytology, and Clinical Management Recommendations. J Clin Transl Pathol 2023;3(2):75–83. doi: 10.14218/JCTP.2023.00004.

Keywords: Bethesda cervical cytology reporting system; HPV testing; HPV vaccine; Cervical cancer screening; Cervical cancer management.

Abbreviations: ACS, The American Cancer Society; AGC, atypical glandular cells; ASCCP, American Society for Colposcopy and Cervical Pathology; ASC-H, Atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion; ASC-US, atypical squamous cells of undetermined significance; CAP, The College of American Pathologists; CIN, cervical intraepithelial neoplasia; HPV, human papilloma virus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; TBS, The Bethesda system.

[*]**Correspondence to**: Chengquan Zhao, Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA 15213, USA. ORCID: https://orcid.org/0000-0001-7524-3048. Tel: +1 412-641-6678, Fax: +1 412-641-1675, E-mail: zhaoc@upmc.edu; Tiannan Wang, Department of Pathology, Keck School of Medicine, University of Southern California, Los Angeles, CA 90033, USA. ORCID: https://orcid.org/0000-0002-2983-7168. Tel: +1323-409-4606, Fax: +1323-441-8183, E-mail: Tiannan.Wang@med.usc.edu

Historical review of cervical cancer screening guidelines, the cytologic reporting system, and clinical management recommendations

Cervical cancer was previously one of the leading causes of cancer death in women worldwide. However, its incidence, mortality and survival have markedly declined in the past several decades due to the following: (1) the success of cervical cancer screening practices, (2) the standardization of The Bethesda system (TBS) for reporting cervicovaginal cytology, (3) improvement in the understanding of the human papilloma virus (HPV) pathogenesis in cervical cancer, and (4) the development of appropriate management approaches.

Dr. George Papanicolaou introduced cervical cytology to the world in his landmark work, "Diagnosis of Uterine Cancer by the Vaginal Smear", in 1943[1] through the untiring efforts of Dr. Diane Solomon, Dr. Robert Kurman and various authors. The first version of TBS was formulated in 1988 to provide a universally accepted reporting system for cervical cytology.[2] This visionary contribution in the field of cervical cancer provided fundamental knowledge for the later development of evidence-based cervical cancer screening and management guidelines. This system was the first to incorporate "statement of adequacy" as a component of the cytology report, and recommended a two-tiered reporting system for squamous intraepithelial lesions: low-grade and high-grade. The aim of this initial system was to effectively communicate cervical cytology findings to clinicians. Then, in 1991, the interim evidence-based, consensus guidelines for the management of women with cervical cytological abnormalities sponsored by the American Society for Colposcopy and Cervical Pathology (ASCCP) were finalized.[3]

As the knowledge of cervical carcinogenesis advanced, a few changes, including the terminology and recognition of glandular lesions, were introduced in the TBS 2001 update.[4–6] During this time, liquid-based cytology and HPV tests were introduced. In the subsequent 13 years, the TBS 2001 print (2nd edition) became the fundamental advisory in the international cytopathology community, and produced a significant impact on education and practice. Then, the revision for the alignment of management guidelines with the terminology update was published in 2006 and 2012.[7,8] In

2001 to 2002, the American Cancer Society (ACS) was the first to convene a multidisciplinary expert panel to publish the cervical cancer screening recommendations, in which screening was recommended to started no later than 21 years of age, and that a HPV DNA test should be initially incorporated.[9,10] In 2012, ACS, ASCCP and the American Society for Clinical Pathology published updates on the screening guidelines. The new screening recommendations addressed the age-appropriate screening strategies, the age at which to exit the screening, and screening strategies for women vaccinated against HPV16 and HPV18 infections.[11]

The latest revision of TBS was finished and formalized in 2014.[12] The updated TBS system had minimal changes in terminology, and continued to recommend a uniform and reproducible cytology report, which has five components: specimen type, specimen adequacy, general category, interpretation/result, and adjunctive testing. The major changes in TBS 2014 included the following: the reporting of benign appearing endometrial cells for women ≥45 years old, instead of women ≥40 years old; "low-grade squamous intraepithelial lesion (LSIL), cannot exclude high-grade intraepithelial squamous lesion (HSIL)" is not recommended, etc. The US Preventive Services Task Force in 2018 updated the recommendations for the screening of cervical cancer. The major change in these guidelines was the addition of an alternative option of every five years with a high-risk HPV (hrHPV) test alone for women within 30–65 years old.

In 2020, ACS published its most recent cervical cancer screening guidelines update. The new ACS recommendation differs in four important aspects, when compared to the 2012 version: (1) the recommended age to start screening is 25 years old, rather than 21 years old; (2) a primary HPV test, and co-testing or cytology alone are recommended starting at 25 years old, rather than at 30 years old; (3) the preferred screening strategy is a primary HPV test every five years, with co-testing and cytology alone, which are acceptable in areas where access to US Food and Drug Administration (FDA)-approved primary HPV tests were not yet availa.[13] This new ACS screening guidelines was immediately aligned with the Risk-Based Management Consensus Guidelines update by the 2019 ASCCP.[14] This guidelines were the first to clearly define that risk thresholds based on the results of HPV tests, alone or in adjunct with cytology, which can be used to guide its management (more or less frequent surveillance, colposcopy, or treatment; or return to routine screening). Cervical intraepithelial neoplasia 3+ (CIN3+) risk-stratified decision making, but not results-based management, was largely emphasized. The patient's age was also a consideration in the management for reproductive desire. The risk was determined by combining the present results and history. Expedited treatment is preferred for HPV16 positive HSIL cytology, or immediate risk of CIN3+ ≥60% in women of 25 years old or older. Excisional treatment for HSIL (CIN2 or CIN3) or cervical adenocarcinoma is preferred, while observational follow-up is recommended for CIN1. If the primary screening test presents a positive HPV16 or 18, colposcopy with biopsy is necessary, even when the cytology results are negative. Lifetime surveillance at 3-year intervals is recommended for the initial post-management of histologic high-grade lesions.

The Bethesda system for reporting cervical cytology

The three guiding principles (Nayar R et al.[15])

1. The classification terminology of the cervical smear report should be uniform and reproducible in different laboratories worldwide, and at the same time, this should be flexible to suit local population requirements;
2. The cervical smear report should provide clinically appropriate and relevant information to the treating clinician;
3. The terminology used in the report must be periodically updated, in order to reflect the present understanding of cervical cancer.

Preparation method

Liquid-based preparation (LBP): the two FDA approved liquid-based systems.

1. ThinPrep™ by Hologic (Marlborough, MA, USA): requires an instrument and a special polycarbonate filter. Cells are transferred from the filter to a 20 mm diameter circle on the slide.
2. SurePath™ by BD Diagnostics (Durham, NC, USA): requires a computer-controlled robotic pipette and a centrifuge after multiple steps of processing. The cells form a circle of 12.5 mm in diameter on the slides.

Conventional preparation: The direct spreading makes a cytological smear of the specimen onto the slide.

Specimen adequacy[12]

Unsatisfactory: This category includes the following: (1) specimen rejected/not processed by providing a specific reason; (2) specimen processed and examined, but unsatisfactory for the evaluation of epithelial abnormality. The latter represents the most common clinical scenario.

Adequate criteria: women with a cervix should have an estimated minimum of 5,000 (LBP) and 8,000–12,000 (CP; 1,000 cells/4× field) well-visualized/well-preserved squamous or squamous metaplastic cells. A rough guide to estimate the number of cells: ThinPrep, 50 cells/10× field in 100 fields, 1,600 cells/10× field in 50 fields; SurePath, 118 cells/10× field in 42 fields, 676 cells/10× field in 118 fields. In addition, the presence or absence of the endocervical/transformation zone (EC/TZ) component should be included in the report. Unsatisfactory is often observed in more than 75% of squamous cells, which are obscured. The report should clarify whether the presence of blood, mucus, lubricants, inflammation, or technical artifacts contributed to the unsatisfactory sample, or whether the problem was simply due to low squamous cellularity.

The College of American Pathologists (CAP) reported the 50th percentile rate for unsatisfactory specimens in US laboratories: 1.0% (CP), 1.1% (ThinPrep) and 0.3% (SurePath) were reported in the 2006 survey, and 1.1% (CP), 1.6% (ThinPrep) and 0.3% (SurePath) were reported in the 2022 survey.[16,17] The ASCCP Risk-Based Management Consensus Guidelines update in 2019 recommended a repeat cytology after 2–4 months for unsatisfactory cytology. Colposcopy is recommended for two consecutive unsatisfactory cytology results, or women who are known to be HPV16/18 positive with unsatisfactory cytology.[18] For women who received radiation, chemotherapy, hysterectomy, or trachelectomy for cervical cancer, these patients often develop atrophy, and the cervix may become stenotic. A lower threshold of 2,000 cells in these patients can be considered.

Interpretations/results[12]

Negative for intraepithelial lesion or malignancy (NILM): Since cervical cytology is primarily performed to detect squamous cell carcinoma or precancerous lesions, this category includes a broad spectrum of non-neoplastic changes, such as inflammation (e.g. lymphocytic cervicitis), physiological (e.g. endometrial cells, atrophy, metaplastic changes, and keratotic squamous) or chemical trauma, radiation,

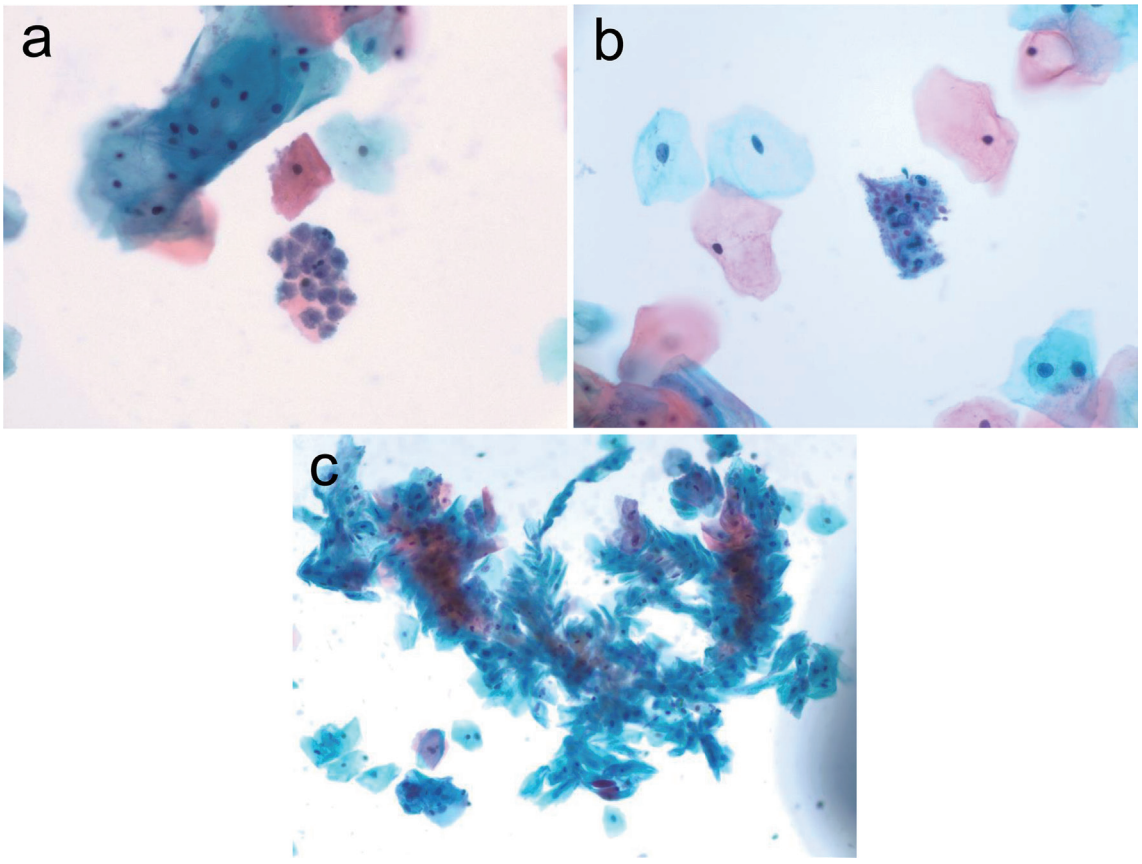

Fig. 1. Infection showing the hyphae with "shish kabob" cytomorphologic features. (a) Trichomonas infection; (b) Candida *spp.* Infection with spores; (c) Candida *spp.*

and hormonal alterations (e.g. pregnancy related, colonizing or infectious viral/bacterial/fungal organisms, and IUD-associated irritation; Fig. 1). As forementioned, in this category, exfoliated endometrial cells are normal findings (Fig. 2). However, the reporting of endometrial cells in women of ≥45 years old is required. A meta-analysis of studies indicated that the risk of biopsy-proven endometrial hyperplasia and cancer in the presence of benign endometrial cells on the exfoliative cytology of women of ≥40 years old was 12% and 6%, respectively, and this risk dropped to 2.0% and 1.1%, respectively, after the implementation of TBS 2001.[19] In order to improve the predictive value of exfoliated endometrial cells, TBS 2014 modified the age to 45 years old or older.

The overall HPV prevalence in the US is 42.5% (29% for hrHPV and 28.5% for low-risk HPV [lrHPV]) for females within 14–59 years old.[20] A meta-analysis of studies conducted for HPV detection in 1,016,719 women with normal cytologic findings worldwide revealed that the crude and adjusted HPV

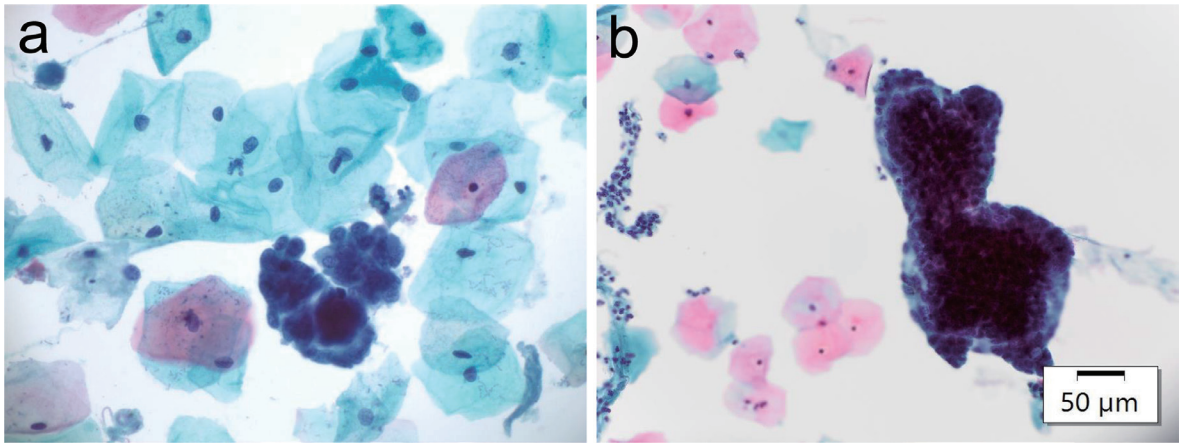

Fig. 2. Clusters of benign endometrial cells forming a 3-dimention (3D) glandular configuration (a–b).

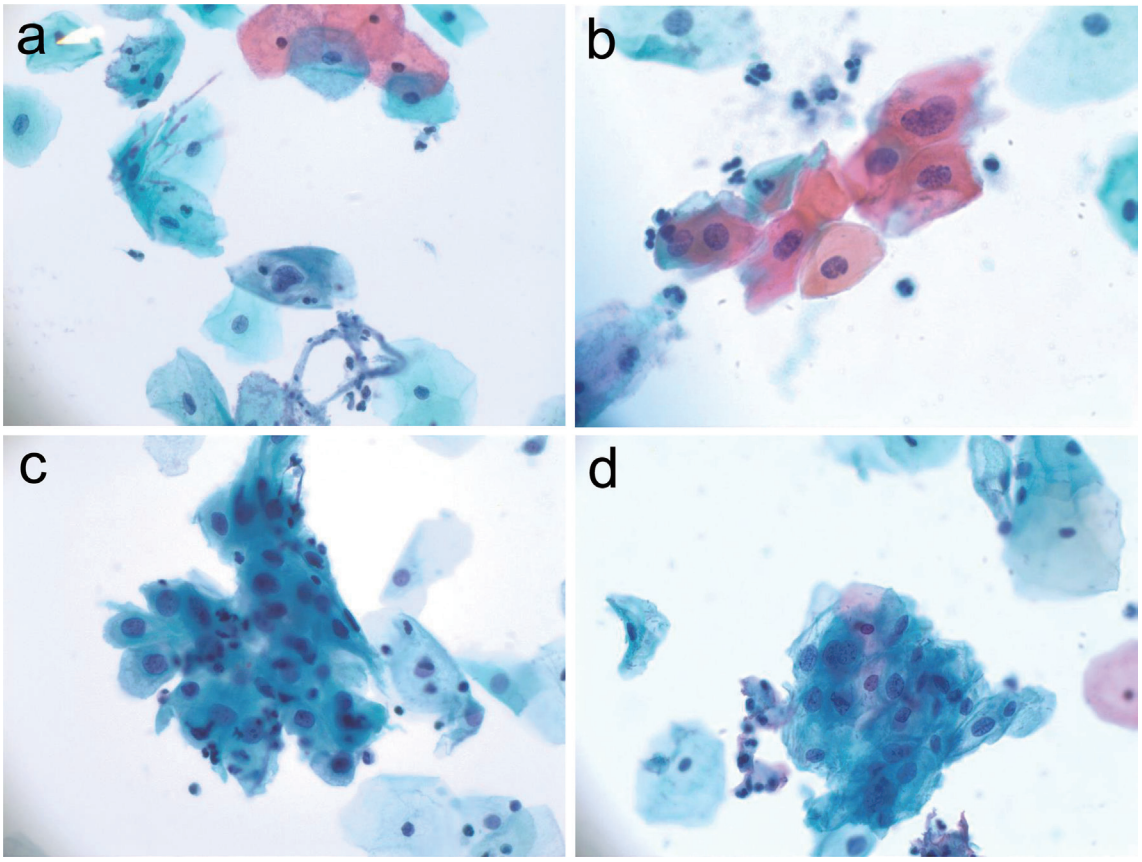

Fig. 3. ASC-US cells with enlarged nuclei, fine chromatin, and a smooth nuclear membrane (a–d). Candida *spp*. is also identified in (a).

prevalence was 72.0% and 11.7% by PCR or HC2, respectively.[21] The most common HPV types in women with normal cytological findings were, as follows: HPV16 (3.2%), HPV18 (1.4%), HPV52 (0.9%), HPV31 (0.8%), and HPV58 (0.7%). For women of 30 years old and older, with negative cytological screening, the prevalence of HPV ranged within 3.4–8.2%.[22] The detection rate for hrHPV was higher in women younger than 30 years old (8.0%), but the hrHPV positive rate was only 1.9% (490 of 25,259) in women of 30 years old and older, with negative cytology test results.[23]

The 2019 ASCCP risk-based management guidelines reported that women with positive HPV and NILM cytology have an immediate risk of 2.1%. Therefore, a follow-up after one year was recommended, except for women with HPV16 or 18 positive NILM, in which a colposcopy should be considered.[18]

Atypical squamous cells of undetermined significance (ASC-US): The ASC-US category is the one of dichotomous reporting terminologies for atypia in the atypical squamous cell (ASC) category, accounting for 90% for ASCs. This represents a finding suggestive of the possible presence of an underlying LSIL. However, the definitive interpretation remains insufficient. Furthermore, 10–20% of women with ASC-US were identified to have biopsy-proven HSIL.[24] The three cytologic features for the diagnosis of ASC-US are, as follows: nuclear enlargement with increased nuclear-to-cytoplasmic ratio (N:C ratio), and minimal nuclear changes (e.g. hyperchromasia, chromatin clumping, irregularity, smudging, and multinucleation; Fig. 3).

The cytologic criteria for ASC-US is often subjective with poor reproducibility. The nuclei are 2–3 times the area of a nucleus of a normal intermediate squamous cell, or twice the size of a nucleus of a squamous metaplastic cell. Atypical parakeratosis or incomplete koilocytosis should be reported as ASC-US (Fig. 1).

The CAP survey data revealed the 50th percentile rate for ASC-US specimens in US laboratories: 2.4% (CP), 4.9% (ThinPrep) and 4.1% (SurePath) were reported in the 2006 survey; 1.7% (CP), 5.4% (ThinPrep) and 4.7% (SurePath) were reported in the 2022 survey.[16,17] The prevalence of hrHPV in women with ASC-US cytology varies within 31.2–34.5% in the literature.[25–27] The CAP survey revealed a median rate of 36.6% hrHPV positivity, and 21% of laboratories reported rates of less than 25%. The hrHPV rate for the ASC-US cytology was 48% in the ASC-US/Low-Grade Squamous Intraepithelial Lesion Triage Study (ALTS),[28] and 57.1% in the phase III trial of the Cervista HPV HR test.[29] On histologic follow-up, the reported prevalence of CIN2/3 in women with ASC-US cytology and positive hrHPV DNA results widely ranged within 4.3–26.7%. According to the 2019 ASCCP risk-based management guidelines, HPV-positive ASC-US results in an immediate CIN3+ risk of 4.5%. Therefore, colposcopy is recommended. However, women with an HPV-negative ASC-US screening result in the setting of an unknown history can return after three years (estimated 5-year CIN3+ risk of 0.40%).[18]

Atypical squamous cells cannot exclude an HSIL (ASC-H): ASC-H accounts for 10% of ASCs, and is often sparse, representing cytologic changes suggestive of HSIL. The two common cytologic patterns of ASC-H that include small cells with high N/C ratios are, as follows: the atypical

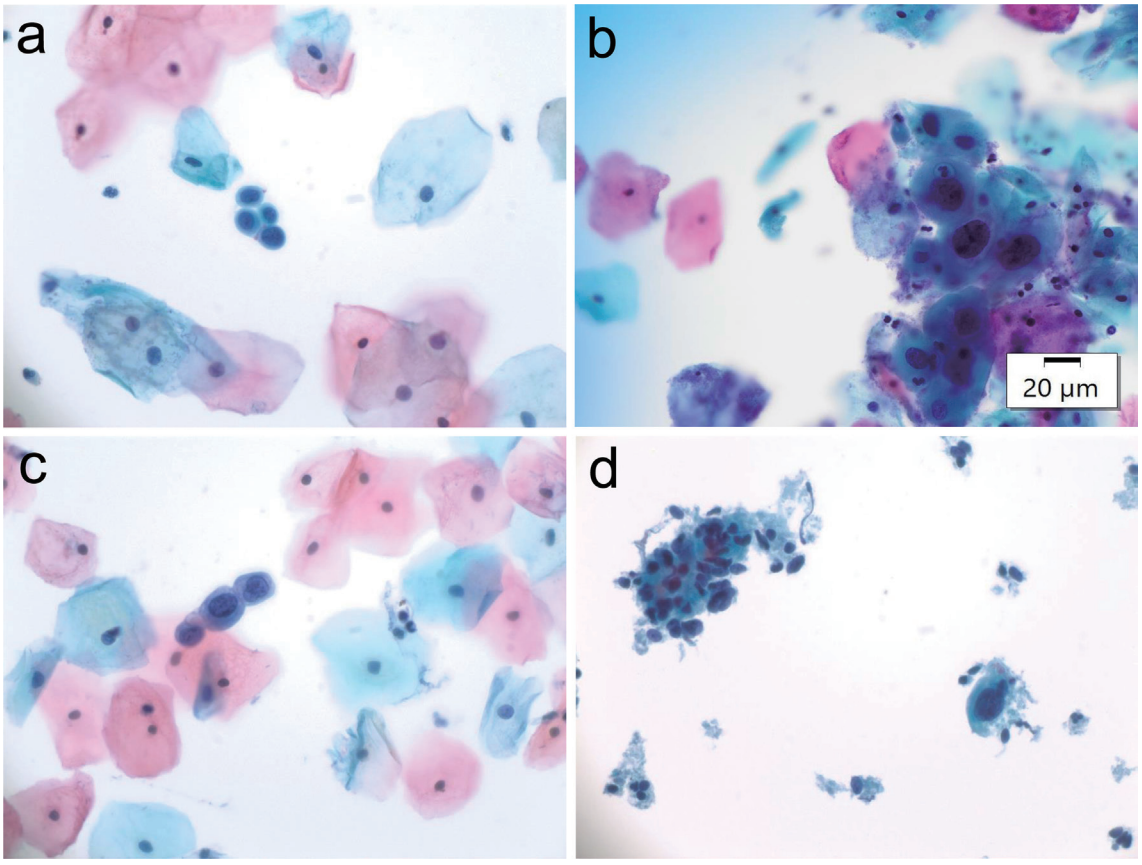

Fig. 4. ASC-H cells with enlarged nuclei, and the relatively remaining N/C ratio (a–d).

immature metaplasia pattern and crowded sheet pattern.

The cytologic criterion for the atypical immature metaplasia pattern is singly dispersed or small groups of metaplastic cells with 1.5–2.5 times larger nuclei than normal, while the criteria for the crowded sheet pattern are crowded squamous cells that contain nuclei that are difficult to visualize, a dense cytoplasm, and a polygonal cell shape (Fig. 4).

The CAP survey revealed the 50th percentile rate for ASC-H specimens in US laboratories: 0.2% (CP), 0.3% (ThinPrep) and 0.3% (SurePath) were reported in 2006; 0.1% (CP), 0.4% (ThinPrep) and 0.3% (SurePath) were reported in 2022.[16,17] The Magee-Womens Hospital data[18] and ASCUS-LSIL Traige Study data[24] reported prevalence rates for hrHPV DNA in ASC-H cases that widely ranged within 33.3–85.6%, with a mean of 55%. According to the 2019 ASCCP guidelines, the immediate CIN3+ risk in women with ASC-H cytology is >25%, which is significantly higher, when compared to the 4% threshold. In addition, for HPV-positive ASC-H cytology, the immediate CIN2+ and CIN3+ risk increases to 50% and 26%, respectively. The CIN3+ rates for HPV-negative vs. HPV-positive ASC-H are quite different. If HPV is positive, the CIN3+ rate is 26%, and the cancer rate is 0.92%. This contrasts with the respective 3.4% and 0.69% rate when HPV is negative. Therefore, a colposcopy examination should be considered for women with ASC-H cytology, with HPV triaging.[18,30–32]

High-grade intraepithelial neoplasia (HSIL): In general, HSIL cells are smaller, and show less cytoplasmic maturity. Furthermore, the N/C ratio markedly increases with no visually discernible cytoplasm. Moreover, the contour of the nuclear membrane is quite irregular, and frequently demonstrates prominent indentations. The nuclei are generally hyperchromatic. There are a few problematic HSIL patterns in the cytology diagnosis: syncytial aggregates/hyperchromatic crowded groups, endocervical gland involvement, patterns that resemble endometrial cells and repair, single and rare small HSIL cells, keratinizing high-grade lesions, HSIL in atrophy, and LSIL with some features of HSIL (Fig. 5).

The CAP survey reported the 50th percentile rate for HSIL specimens in US laboratories: 0.3% (CP), 0.6% (ThinPrep) and 0.3% (SurePath) were reported in 2006; 0.2% (CP), 0.4% (ThinPrep) and 0.3% (SurePath) were reported in 2022.[16,17]

The Kaiser Permanente Northern California (KPNC) study in 2010 revealed a 92.2% hrHPV positive rate for women of 30 years old and older, with HSIL cervical cytology.[25] Magee-Womens Hospital reported an overall hrHPV detection rate of 95.7% for women with HSIL cytology, and that the hrHPV DNA detection rates slightly decline with the increase in age.[33] In general, HSIL cytology warrants an expedited treatment. For non-pregnant patients of 25 years old or older, with HSIL cytology and concurrent positive HPV genotype 16, when the immediate risk of CIN3+ is ≥60%, an expedited treatment is preferred. For patients with HSIL cytology without HPV16/18 infection, and with a completely normal colposcopy impression, untargeted biopsies are not recommended. These patients should be observed without biopsy, according to the 2019 ASCCP risk-based management guidelines. The 2020 ACS cancer screening guidelines addressed a concern on the low-risk patient management recommenda-

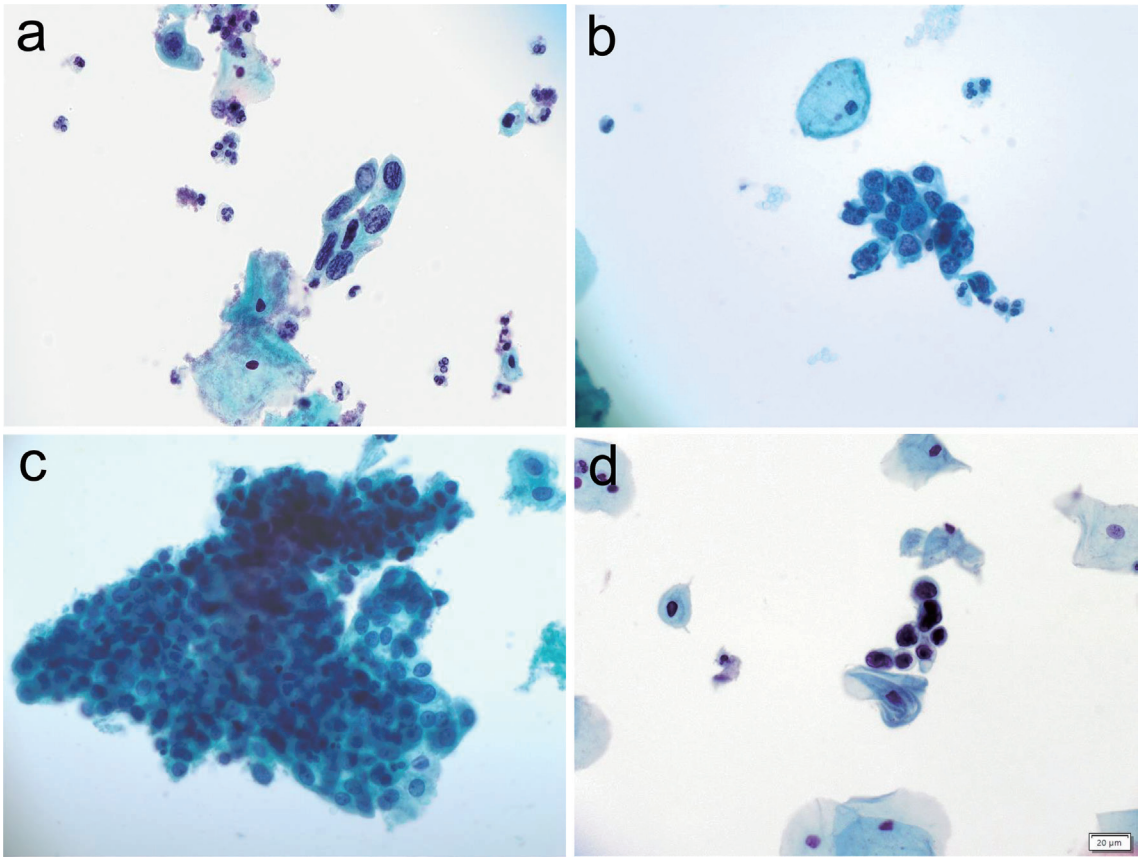

Fig. 5. HSIL cells with a high N/C ratio, hyperchromatic nuclei, and occasionally dispersed single cells (a–d).

tion in the 2019 ASCCP guidelines: "It cannot be stressed too emphatically that the updated ASCCP management guidelines should be regarded as integral to the success of this screening guideline, because failure to follow-up a positive screening test in a manner that is adherent to the ASCCP management guidelines undermines what was achieved with the screening, which can result in harm to the patient".[13]

Atypical glandular cells: The pap test was not designed to primarily screen for glandular lesions of the cervix, because the abnormalities are increasingly difficult to sample, and the sensitivity is limited. This category includes the following: (1) atypical-endocervical cells, NOS or specify in comments; (2) atypical-endometrial cells, NOS or specify in comments; (3) glandular cells, NOS or specify in comments; (4) atypical-endocervical cells, favor neoplastic; (5) atypical glandular cells, favor neoplastic; (6) endocervical carcinoma *in situ*; (7) adenocarcinoma, endocervical or endometrial, or extrauterine or NOS (Fig. 6).

The CAP survey reported the 50th percentile rate for AGC specimens in US laboratories: 0.1% (CP), 0.2% (ThinPrep) and 0.2% (SurePath) were reported in both 2006 and 2022.[16,17]

Atypical endocervical cells: NOS on cytology is a poorly reproducible diagnostic category. The diagnostic criteria include the following: glandular cells crowding, nuclear overlap and enlargement (3–5 times of normal endocervical cells), occasional nucleoli, and rare mitotic figures. Atypical endocervical cells-favor neoplastic have endocervical cells with cell morphology, either quantitatively or qualitatively, and falls short of the interpretation for endocervical adenocarcinoma *in situ* (AIS) or invasive adenocarcinoma. Cells present with the following: form rosette or glands with an increased N/C ratio, nuclear crowding/pseudo-stratification, enlargement and nuclear hyperchromasia, and occasional mitoses. Atypical endometrial cells-NOS vs. favor neoplasia on cytology are poorly reproducible, and the distinction of cytologically benign from atypical endometrial cells is primarily based on the criterion of increased nuclear size. In interpreting atypical endometrial cells, clinical findings and history, the IUD in place or polyp are important. The typical cytomorphologic features of endocervical AIS are, as follows: columnar cells with nuclear enlargement, hyperchromasia, chromatin abnormality, pseudo-stratification, and mitotic activity without tumor diathesis.

The prevalence of positive hrHPV in AGC cytology ranges within 20–40%. KPNC documented a 21.3% hrHPV rate for women of 30 years old and older. The Magee-Womens Hospital data revealed a positive hrHPV rate of 24.3% (75/309),[34] which is similar to the rate of 29% reported by Derchain *et al*.[35] Furthermore, Rabelo-Santos *et al*. reported an hrHPV rate of 43% in women with AGC or AIS cytology, and HPV16 was the most prevalent type, followed by HPV18. HPV16 was significantly associated with both squamous and glandular neoplasia, and HPV18 was more correlated to glandular neoplasia.[36] The hrHPV types were detected in 20.3% of AGC cytology (33 of 161) in the study conducted by Mulhem E *et al*. Among these, HPV16 and/or 18 were detected in 8% of the AGC cytology.[37]

The AGC cytologic results are associated with the histologic diagnosis of AIS (3–4%), CIN2+ (9%), and invasive can-

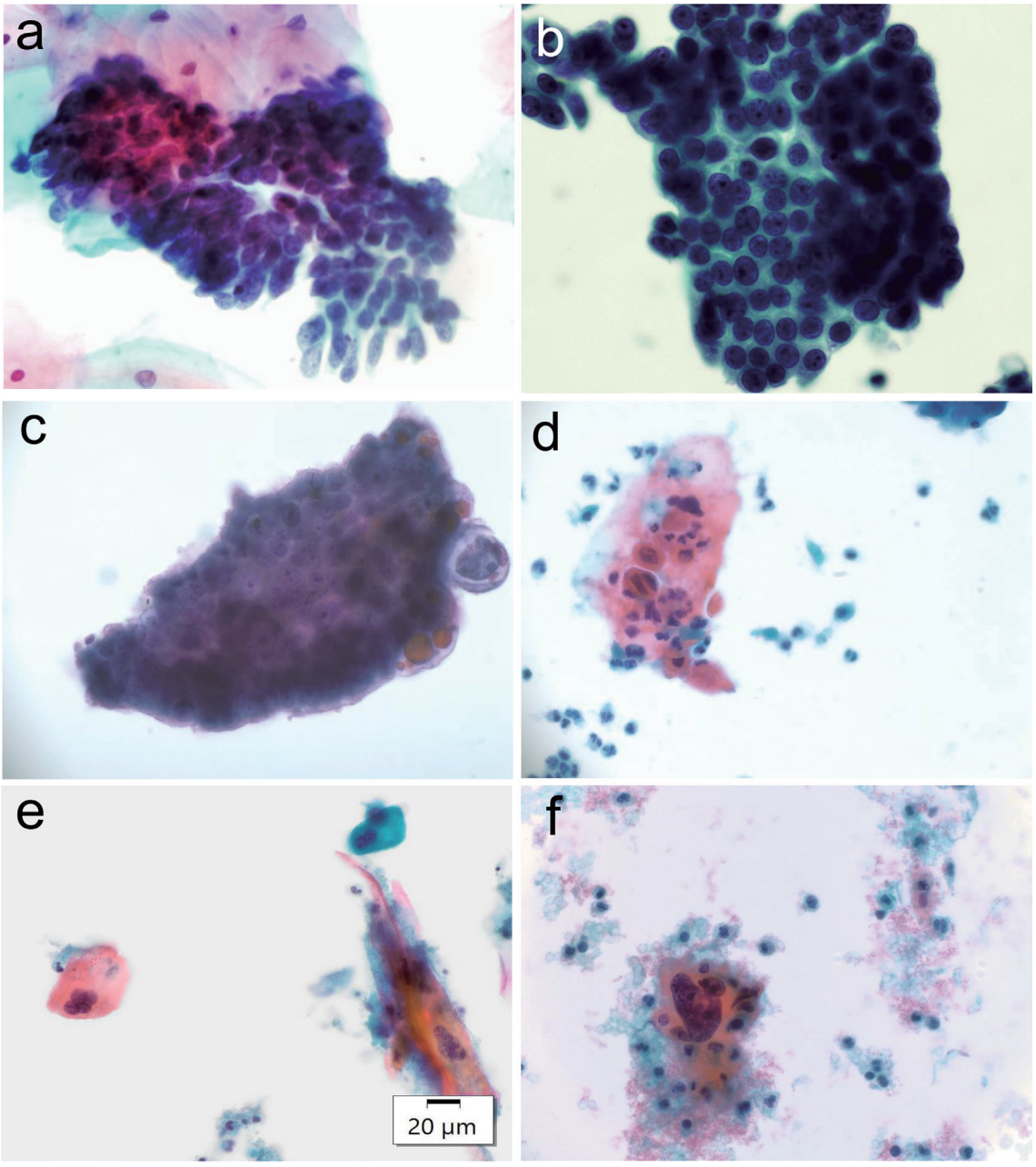

Fig. 6. (a) Adenocarcinoma *in situ* with feathering endocervical glandular arrangements. (b–c) Adenocarcinoma: NOS cells forming a glandular architecture with nuclear overlapping crowding, and hyperchromasia. (d–f) Squamous cell carcinoma presenting orangeophilic tumor cells with an irregular nuclear contour, hyperchromasia, and tadpole features in the background of the tumor diathesis.

cer (2–3%).[38–40] The immediate CIN2+/CIN3+ risk in HPV positive AGC patients is 40% and 26%, respectively, while the immediate CIN3+ risk in HPV negative AGC patients is 1.1%. For non-pregnant patients of all ages with AGC and AIS, except for atypical endometrial cells, colposcopy is recommended, regardless of the HPV result. Endocervical sampling is recommended at initial colposcopy. Furthermore, endometrial sampling is recommended for women of 35 years old and older, and women younger than 35 years, who are at risk of endometrial neoplasia. For AGC without identifiable histologic HSIL or AIS/carcinoma on biopsy, co-testing after one and two years is recommended. If there are any abnormal test results, a colposcopy is recommended.[18]

HPV testing

Over 100 subtypes of HPV have been classified as high-risk and low–risk type, depending on the oncogenic capability.

The FDA-approved platform for primary HPV screening includes the following: (1) Roche Cobas HPV, approved by the FDA in 2014 to test HPV genotype 16,18, and other 12 high risk HPV types for the primary screening of women of 25 years old and above. This is the only HPV test in the ThinPrep liquid-based cytology vial approved in the US. (2) Onclarity

HPV by Becton Dickinson, approved in 2018, and this was expanded in 2020 to test for HPV genotypes 16, 18, 45, 31, 51, 52, 33+58, 35+39+68, and 56+59+66 in the BD SurePath liquid-based cytology vial, and the Hologic PreservCyt® solution (not approved in the US).

For the co-test and triage, there are presently six FDA approved assays: (1) Digene Hybrid Capture 2 HPV DNA test (Qiagen, Hilden, Germany; approved in 2001), for the detection of 13 HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68); (2) Cervista HPV HR test (Hologic, Marlborough, Massachusetts, USA; approved in 2009), a hrHPV DNA-based assay for detecting high-risk HPV DNA from 14 hrHPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68); (3) Cervista HPV 16/18 test (approved in 2009), a hrHPV DNA-based genotyping assay, approved for the reflex testing of patients with positive hrHPV Cervista test results (Cervista HPV HR test and Cervista HPV 16/18 test were not applied in the US market); (4) Cobas 4800 HPV test (Roche, Basel, Switzerland; approved in 2011), a hrHPV DNA-based PCR screening assay for specifically identifying HPV types 16 and 18, while concurrently detecting the 12 remaining high-risk types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) by real-time PCR; (5) Aptima HPV assay (Hologic, San Diego, California, USA; approved in 2012) for detecting the E6 and E7 mRNA transcripts of 14 hrHPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68), and Aptima HPV 16 18/45 genotype assay.[41]

HPV vaccine in brief

"HeLa cells" are the basis of HPV vaccine development. This is an *in vitro* immortal cervical cancer cell line obtained from an African American woman, Henrietta Lack, who died from cervical cancer.[42]

The US Advisory Committee on Immunization Practices routinely recommends HPV vaccination at the age of 11 or 12 years old. To date, three prophylactic HPV vaccines are licensed in the US, which are all noninfectious, and comprise of viral-like particles: 9-valent (9vHPV, Gardasil 9, Merck & Co. 2014), Quadrivalent (4vHPV, Gardasil, Merck & Co. 2006), and bivalent (2vHPV, Cervarix, GlaxoSmithKline, 2007). The 9vHPV targets HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58, the 4vHPV targets HPV 6, 11, 16 and 18, and the 2vHPV only targets hrHPV 16 and 18. At present, in the US, 9vHPV is the only marketed product.

The safety of the vaccine is robust, and has consistently exhibited no concerns or significant adverse events from the clinical trials, except for a sporadic report on motor impairment in a vaccinated girl.[43–47] The HPV vaccine was initially introduced to girls within 9–14 years old in 2009 by the World Health Organization (WHO) worldwide. The HPV vaccine and its safety were endorsed by the WHO and the Global Safety Vaccine Advisory Group. The updated recommendations for the HPV vaccination schedule by the WHO are, as follows: (1) a one- or two-dose schedule for girls within 9–14 years old; (2) a one- or two-dose schedule for girls and women within 15–20 years old; (3) two doses with a 6-month interval for women older than 21 years old. The purpose of this schedule is, as follows: "The primary target of the vaccination is girls within 9–14 years old, prior to the start of sexual activity. The vaccination of secondary targets, such as boys and older females, is recommended where feasible and affordable".[48]

What is the present and what is in the future

As mentioned in the updates for cervical cancer screening guidelines and management guidelines by several societies, laboratories across the US are undergoing a shift in testing methodology, from cytology to hrHPV testing. However, issues, such as the low cost-effectiveness of HPV testing, have limited the availability of FDA-approved HPV primary screening platforms, and insignificant and low specificity HPV positive results need to be addressed. Nevertheless, the most informed decision should be made by a multitude of efforts from the clinician, laboratorian and patient, or on a case to case basis.

Acknowledgments

None.

Funding

Dr. Liu acknowledges the funding support obtained from the National Cancer Institute (R01CA232593).

Conflict of interest

Dr. Zhao has been an editorial board member of the *Journal of Clinical and Translational Pathology* since June 2021. The other authors have no conflict of interests related to this publication.

Author contributions

Dr. Tiannan Wang contributed in the manuscript writing, study design and technical support. Dr. Huina Zhang contributed in the manuscript critical revision and technical support. Dr. Yang Liu contributed in the critical funding and administration. Dr. Chengquan Zhao contributed in the study design, manuscript critical revision, and technical support. All authors made a significant contribution to the study, and approved the final manuscript.

References

[1] Papanicolaou GN, Traut HF. The diagnostic value of vaginal smears in carcinoma of the uterus. Am J Obstetr Gynecol 1941;42(2):193–206. doi:10.1016/S0002-9378(16)40621-6.
[2] The 1988 Bethesda System for reporting cervical/vaginal cytologic diagnoses. Developed and approved at the National Cancer Institute Workshop, Bethesda, Maryland, USA, December 12–13, 1988. Acta Cytol 1989;33(5):567–574. PMID:2781957.
[3] Kurman RJ, Solomon D. The Bethesda System for Reporting Cervical/Vaginal Cytologic Diagnoses. Definitions, Criteria, and Explanatory Notes for Terminology and Specimen Adequacy. New York: Springer; 1994. doi:10.1007/978-1-4684-0201-8.
[4] Nayar R, Wilbur DC, editors. The Bethesda System for Reporting Cervical Cytology. Definitions, Criteria, and Explanatory Notes. New York: Springer; 2004.
[5] Sherman ME, Dasgupta A, Schiffman M, Nayar R, Solomon D. The Bethesda Interobserver Reproducibility Study (BIRST). Cancer 2007;111(1):15–25. doi:10.1002/cncr.22423, PMID:17186503.
[6] Solomon D, Davey D, Kurman R, Moriarty A, O'Connor D, Prey M, *et al*. The 2001 Bethesda System: terminology for reporting results of cervical cytology. JAMA 2002;287(16):2114–2119. doi:10.1001/jama.287.16.2114, PMID:11966386.
[7] Wright TC Jr, Massad SL, Dunton CJ, Spitzer M, Wilkinson EJ, Solomon D, *et al*. 2006 consensus guidelines for the management of women with abnormal cervical cancer screening tests. Am J Obstet Gynecol 2007;197(4):346–355. doi:10.1016/j.ajog.2007.07.047, PMID:17904957.
[8] Massad LS, Einstein MH, Huh WK, Katki HA, Kinney WK, Schiffman M, *et al*. 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. J Low Genit Tract Dis 2013;17(5 Suppl 1):S1–S27. doi:10.1097/LGT.0b013e318287d329, PMID:23519301.
[9] Wright TC Jr, Cox JT, Massad LS, Twiggs LB, Wilkinson EJ, ASCCP-Sponsored Consensus Conference. 2001 Consensus Guidelines for the management of women with cervical cytological abnormalities. JAMA 2002;287(16):2120–2129. doi:10.1001/jama.287.16.2120, PMID:11966387.
[10] Saslow D, Runowicz CD, Solomon D, Moscicki AB, Smith RA, Eyre HJ, *et al*. American Cancer Society guideline for the early detection of cervical neoplasia and cancer. CA Cancer J Clin 2002;52(6):342–362. doi:10.3322/canjclin.52.6.342, PMID:12469763.

[11] Saslow D, Solomon D, Lawson HW, Killackey M, Kulasingam SL, Cain J, *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. Am J Clin Pathol 2012;137(4):516–542. doi:10.1309/AJCPTGD94EVRSJCG, PMID:22431528.
[12] Nayar R, Wilbur DC. The Bethesda System for Reporting Cervical Cytology-definitions, Criteria and Explanatory Notes, 3rd ed. Switzerland: Springer International Publishing; 2015. doi:10.1007/978-3-319-11074-5.
[13] Fontham ETH, Wolf AMD, Church TR, Etzioni R, Flowers CR, Herzig A, *et al*. Cervical cancer screening for individuals at average risk: 2020 guideline update from the American Cancer Society. CA Cancer J Clin 2020;70(5):321–346. doi:10.3322/caac.21628, PMID:32729638.
[14] Perkins RB, Guido RS, Castle PE, Chelmow D, Einstein MH, Garcia F, *et al*. Erratum: 2019 ASCCP Risk-Based Management Consensus Guidelines for Abnormal Cervical Cancer Screening Tests and Cancer Precursors. J Low Genit Tract Dis 2021;25(4):330–331. doi:10.1097/LGT.0000000000000628, PMID:34542089.
[15] Nayar R, Wilbur DC. The Pap test and Bethesda 2014. Cancer Cytopathol 2015;123(5):271–281. doi:10.1002/cncy.21521.
[16] Eversole GM, Moriarty AT, Schwartz MR, Clayton AC, Souers R, Fatheree LA, *et al*. Practices of participants in the college of American pathologists interlaboratory comparison program in cervicovaginal cytology, 2006. Arch Pathol Lab Med 2010;134(3):331–335. doi:10.5858/134.3.331.
[17] CAP Cytopathology Checklist. Available from: https://www.cap.org/member-resources/councils-committees/cancer-topic-center/cytopathology-topic-center. Accessed October 24, 2022.
[18] Perkins RB, Guido RS, Castle PE, Chelmow D, Einstein MH, Garcia F, *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(2):102–131. doi:10.1097/LGT.0000000000000525, PMID:32243307.
[19] Cibas ES, Ducatman BS. Cytology: diagnostic principles and clinical correlates. 4th ed. Philadelphia: Elsevier; 2014. Available from: https://www.sciencedirect.com/book/9781455744626/cytology#book-info. Accessed March 20, 2023.
[20] Hariri S, Unger ER, Sternberg M, Dunne EF, Swan D, Patel S, *et al*. Prevalence of genital human papillomavirus among females in the United States, the National Health And Nutrition Examination Survey, 2003-2006. J Infect Dis 2011;204(4):566–573. doi:10.1093/infdis/jir341, PMID:21791659.
[21] Bruni L, Diaz M, Castellsague X, Ferrer E, Bosch FX, de Sanjosé S. Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. J Infect Dis 2010;202(12):1789–1799. doi:10.1086/657321, PMID:21067372.
[22] Saslow D, Runowicz CD, Solomon D, Moscicki AB, Smith RA, Eyre HJ, *et al*. American Cancer Society guideline for the early detection of cervical neoplasia and cancer. CA Cancer J Clin 2002;52(6):342–362. doi:10.3322/canjclin.52.6.342, PMID:12469763.
[23] Bansal M, Austin RM, Zhao C. High-risk HPV DNA detected in less than 2% of over 25,000 cytology negative imaged liquid-based Pap test samples from women 30 and older. Gynecol Oncol 2009;115(2):257–261. doi:10.1016/j.ygyno.2009.07.010, PMID:19665217.
[24] ASCUS-LSIL Traige Study (ALTS) Group. Results of a randomized trial on the management of cytology interpretations of atypical squamous cells of undetermined significance. Am J Obstet Gynecol 2003;188(6):1383–1392. doi:10.1067/mob.2003.457, PMID:12824967.
[25] Castle PE, Fetterman B, Thomas Cox J, Shaber R, Poitras N, Lorey T, *et al*. The age-specific relationships of abnormal cytology and human papillomavirus DNA results to the risk of cervical precancer and cancer. Obstet Gynecol 2010;116(1):76–84. doi:10.1097/AOG.0b013e3181e3e719, PMID:20567171.
[26] Stoler MH, Wright TC Jr, Sharma A, Apple R, Gutekunst K, Wright TL, *et al*. High-risk human papillomavirus testing in women with ASC-US cytology: results from the ATHENA HPV study. Am J Clin Pathol 2011;135(3):468–475. doi:10.1309/AJCPZ5JY6FCVNMOT, PMID:21350104.
[27] Sherman ME, Schiffman M, Cox JT, ALTS Study Group. Effects of age and human papilloma viral load on colposcopy triage: data from the randomized Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesion Triage Study (ALTS). J Natl Cancer Inst 2002;94(2):102–107. doi:10.1093/jnci/94.2.102, PMID:11792748.
[28] Castle PE, Solomon D, Schiffman M, Wheeler CM. Human papillomavirus type 16 infections and 2-year absolute risk of cervical precancer in women with equivocal or mild cytologic abnormalities. J Natl Cancer Inst 2005;97(14):1066–1071. doi:10.1093/jnci/dji186, PMID:16030304.
[29] Einstein MH, Martens MG, Garcia FA, Ferris DG, Mitchell AL, Day SP, *et al*. Clinical validation of the Cervista HPV HR and 16/18 genotyping tests for use in women with ASC-US cytology. Gynecol Oncol 2010;118(2):116–122. doi:10.1016/j.ygyno.2010.04.013, PMID:20488510.
[30] Bandyopadhyay S, Austin RM, Dabbs D, Zhao C. Adjunctive human papillomavirus DNA testing is a useful option in some clinical settings for disease risk assessment and triage of females with ASC-H Papanicolaou test results. Arch Pathol Lab Med 2008;132(12):1874–1881. doi:10.5858/132.12.1874, PMID:19061283.
[31] Sherman ME, Castle PE, Solomon D. Cervical cytology of atypical squamous cells cannot exclude high grade squamous intra-epithelial lesion (ASC-H): characteristics and histologic outcomes. Cancer 2006;108(5):298–305. doi:10.1002/cncr.21844, PMID:16544318.
[32] Salibay C, Chen Z, Ma B, Pan H, Hijazi M, Elatre W, *et al*. High-risk HPV testing improves accuracy in detection of CIN2+ lesions in ASC-H postmenopausal women? An academic hospital experiences. J Am Soc Cytopathol 2023;12(1):58–65. doi:10.1016/j.jasc.2022.08.004, PMID:36270913.
[33] Gao FF, Austin RM, Zhao C. Histopathologic follow-up and human papillomavirus DNA test results in 290 patients with high-grade squamous intraepithelial lesion Papanicolaou test results. Cancer Cytopathol 2011;119(6):377–86. doi:10.1002/cncy.20176, PMID:21774093.
[34] Zhao C, Florea A, Austin RM. Clinical utility of adjunctive high-risk human papillomavirus DNA testing in women with Papanicolaou test findings of atypical glandular cells. Arch Pathol Lab Med 2010;134(1):103–108. doi:10.5858/2008-0755-OAR1.1, PMID:20073612.
[35] Derchain SF, Rabelo-Santos SH, Sarian LO, Zeferino LC, de Oliveira Zambeli ER, do Amaral Westin MC, *et al*. Human papillomavirus DNA detection and histological findings in women referred for atypical glandular cells or adenocarcinoma in situ in their Pap smears. Gynecol Oncol 2004;95(3):618–623. doi:10.1016/j.ygyno.2004.08.033, PMID:15581973.
[36] Rabelo-Santos SH, Derchain SF, Villa LL, Costa MC, Sarian LO, do Amaral Westin MC, *et al*. Human papillomavirus-specific genotypes in cervical lesions of women referred for smears with atypical glandular cells or adenocarcinoma in situ. Int J Gynecol Pathol 2009;28(3):272–278. doi:10.1097/PGP.0b013e318190ed27, PMID:19620946.
[37] Mulhem E, Amin M, Copeland J, Hunter S. Type-specific human papillomavirus DNA detected in atypical glandular cell Pap tests. Acta Cytol 2012;56(2):155–159. doi:10.1159/000334199, PMID:22378077.
[38] Katki HA, Schiffman M, Castle PE, Fetterman B, Poitras NE, Lorey T, *et al*. Five-year risks of CIN 3+ and cervical cancer among women with HPV-positive and HPV-negative high-grade Pap results. J Low Genit Tract Dis 2013;17(5 Suppl 1):S50–S55. doi:10.1097/LGT.0b013e3182854282, PMID:23519305.
[39] Davey DD, Neal MH, Wilbur DC, Colgan TJ, Styer PE, Mody DR. Bethesda 2001 implementation and reporting rates: 2003 practices of participants in the College of American Pathologists Interlaboratory Comparison Program in Cervicovaginal Cytology. Arch Pathol Lab Med 2004;128(11):1224–1229. doi:10.5858/2004-128-1224-BIARRP, PMID:15504056.
[40] Schnatz PF, Guile M, O'Sullivan DM, Sorosky JI. Clinical significance of atypical glandular cells on cervical cytology. Obstet Gynecol 2006;107(3):701–708. doi:10.1097/01.AOG.0000202401.29145.68, PMID:16507944.
[41] 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(5):284–292. doi:10.1016/j.jasc.2019.06.001, PMID:31320315.
[42] Gey GO, Coffman WD, Kubicek MT. Tissue culture studies of the proliferative capacity of cervical carcinoma and normal epithelium. Cancer Res 1952;12:264–265.
[43] Gee J, Weinbaum C, Sukumaran L, Markowitz LE. Quadrivalent HPV vaccine safety review and safety monitoring plans for nine-valent HPV vaccine in the United States. Hum Vaccin Immunother 2016;12(6):1406–1417. doi:10.1080/21645515.2016.1168952, PMID:27029786.
[44] Descamps D, Hardt K, Spiessens B, Izurieta P, Verstraeten T, Breuer T, *et al*. Safety of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine for cervical cancer prevention: a pooled analysis of 11 clinical trials. Hum Vaccin 2009;5(5):332–340. doi:10.4161/hv.5.5.7211, PMID:19221517.
[45] Costa APF, Cobucci RNO, da Silva JM, da Costa Lima PH, Giraldo PC, Gonçalves AK. Safety of Human Papillomavirus 9-Valent Vaccine: A Meta-Analysis of Randomized Trials. J Immunol Res 2017;2017:3736201. doi:10.1155/2017/3736201, PMID:28812030.
[46] Shimabukuro TT, Su JR, Marquez PL, Mba-Jonas A, Arana JE, Cano MV. Safety of the 9-Valent Human Papillomavirus Vaccine. Pediatrics 2019;144(6):e20191791. doi:10.1542/peds.2019-1791, PMID:31740500.
[47] Phillips A, Patel C, Pillsbury A, Brotherton J, Macartney K. Safety of Human Papillomavirus Vaccines: An Updated Review. Drug Saf 2018;41(4):329–346. doi:10.1007/s40264-017-0625-z, PMID:29280070.
[48] WHO. WHO updates recommendations on HPV vaccination schedule. Available from: https://www.who.int/news/item/20-12-2022-WHO-updates-recommendations-on-HPV-vaccination-schedule. Accessed March 20, 2023.