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Foreword

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Consulting Editor

One of the most remarkable improvements in women's health care is in the primary and secondary prevention of cervical carcinoma. Although the incidence and mortality from cervical cancer decreased substantially in the past several decades in the United States, it remains the third most common gynecologic malignancy. When cervical cytology screening programs were introduced to communities, a marked reduction in cervical cancer incidence followed. In countries where cytologic screening is not widely available, cervical cancer remains common.

This issue of *Obstetrics and Gynecology Clinics* guest edited by Alan Waxman, MD, MPH, provides a comprehensive review of cervical cancer screening and prevention techniques. Matters addressed include the recommended timing and frequency of screening with cytology, and the role of human papillomavirus (HPV) DNA testing in cervical screening. The contributors offer a comparison of liquid and conventional Pap tests, and describe how cytology specimens are processed and interpreted in the laboratory. The increasing use of computer-assisted technologies in the interpretation of Pap tests is particularly exciting.

Colposcopy with directed biopsy is still the standard of care for initial management of most cytologic abnormalities. Readers will find in this monograph a comprehensive review of the histologic basis of colposcopy and the uncertain role of endocervical curettage. The intricacies of management of both the abnormal Pap test and abnormalities proven on biopsy were revamped by the second American Society for Colposcopy and Cervical Pathology (ASCCP) Consensus Conference, whose recommendations were published in the Fall of 2007. The authors provide several articles written by participants of that conference to give readers a comprehensive understanding of the new guidelines and the evidence that supports them.

We now know that infection with HPV is necessary in the development of cervical neoplasia. Factors that determine which high-risk types of HPV infections will develop into squamous intraepithelial lesions remain poorly identified. Although it is estimated that up to 100% of women with histologic cervical intraepithelial neoplasia (CIN) 2 or CIN 3 will test positive for a high-risk type of HPV, many women harbor the virus in their lower genital tracts without cytologic or histologic changes. Primary prevention is now available thanks to new anti-cancer vaccines using a virus-like particle produced from

Obstet Gynecol Clin N Am 35 (2008) xiii–xiv
doi:10.1016/j.ogc.2008.10.004 **obgyn.theclinics.com**
0889-8545/08/$ – see front matter

the L1 gene of the HPV. Dr. Waxman opens this issue with an in-depth discussion of the natural history of HPV infection, its role in the pathophysiology of cervical cancer, and the promise of the new vaccines.

It is our desire that this issue will attract the attention of providers caring for the millions of women undergoing cervical cancer screening. Practical information provided herein by this distinguished panel of contributors will hopefully aid in the development and implementation of more specific and individualized treatment plans. Views expressed here are not absolute, however, and should be considered as guidelines based on advice from experts such as these contributors.

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Preface

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Guest Editor

Those of us involved in the healthcare of women have seen a remarkable transformation in screening techniques for cervical cancer and its precursors since the mid 1990s.

- The staid old Pap smear technique of scraping cells from the cervix with a wooden spatula and cotton-tipped applicator and smearing them onto a glass slide is a thing of the past in most practices. We now use plastic collection devices to transfer cells from the cervix into a preservative which is sent to the lab for liquid-based cytology and reflex human papillomavirus (HPV) testing.
- The work of the cytotechnologist is often assisted and in some cases, replaced by electronic screening that employs software-driven intelligence.
- Dysplasia and cervical intraepithelial neoplasia-based terminology gave way to the Bethesda System (TBS) in 1988. TBS has undergone periodic revision, most recently in 2001. We now have atypical glandular cells (AGC) and atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H). Atypical squamous cells of undetermined significance (ASC-US) has been hyphenated.
- In 2002 and 2003, The American Cancer Society and the American College of Obstetricians and Gynecologists moved away from the old dogma of a yearly Pap for every woman starting at age 18 or the onset of intercourse. This empiric regimen has been replaced by data-driven, age-specific screening guidelines.
- The mysteries of the class II Pap have been unraveled, and the National Cancer Institute's Atypical Squamous Cells of Undetermined Significance / Low-grade Squamous Intraepithelial Lesion Triage Study (ALTS) provided data to clarify the role of HPV in ASC-US (the class II Pap's latest incarnation).
- The American Society for Colposcopy and Cervical Pathology (ASCCP) built on the data provided by the many papers generated from ALTS as well as research from around the world, to derive practice guidelines for the management of the abnormal Pap test. These were most recently revised in 2006 based on emerging data.
- The etiology of cervical cancer has been revealed. The disease which epidemiologists had known for decades to result from a sexually transmitted oncogenic

Obstet Gynecol Clin N Am 35 (2008) xv–xvi
doi:10.1016/j.ogc.2008.10.001 **obgyn.theclinics.com**
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agent, has now definitively been shown to be caused by high-risk types of HPV. The discovery led to a Nobel Prize for Harald Zur Hausen in 2008.
- The HPV genome has been largely decoded, and the mechanisms of its ability to cause malignant transformation of host cell lines are becoming understood.
- Adding a test for HPV DNA to the Pap test has been shown to increase the sensitivity and negative predictive value of cervical screening. Furthermore, use of the HPV test has become the mainstay in the triage of an ASC-US cytology result.
- The L1 gene of the HPV has been harnessed to produce a virus-like particle which has, in turn, become the antigenic component of an anti-cancer vaccine. Secondary prevention in the form of Pap testing has given way to primary prevention by vaccination.

We've come a long way!

In this issue of *Obstetrics and Gynecology Clinics*, an outstanding group of teachers, researchers, and clinicians has come together to discuss all of the above-mentioned and more. In the articles that follow, you will find a review and update in the many aspects of colposcopy and cervical cancer prevention. In addition to reviews of Pap guidelines, and what really happens in the cytology lab, by myself and Dr. Nancy Joste, respectively, the histologic basis of colposcopy is reviewed by Dr. Dennis O'Connor and the controversies surrounding the endocervical curettage are elaborated upon by Drs. Rita Driggers and Chris Zahn. Because our examination of the lower genital tract is not limited to the cervix, an article on colposcopy of the vulva and vagina was contributed by Dr. Hélène Gagné. Several articles are devoted to aspects of the management of the abnormal Pap test and resulting biopsies. These articles incorporate discussion of the 2006 ASCCP Consensus Guidelines and were written by experts, most of whom participated in the Consensus Conference in which they were developed: Drs. Lori Boardman and Colleen Kennedy, Charlie Dunton, Kathy McIntyre-Seltman and Jamie Lesnock, Anna-Barbara Moscicki, and Meggan Zsemlye. Finally, information about the nature of human papillomavirus and the status of the HPV vaccine was contributed by Dr. Cosette Wheeler, one of the world's foremost HPV virologists. Anal neoplasia is also HPV mediated and is on the rise in immunocompromised patients. Drs. Joel Palefsky and Mary Rubin are contributing a discussion of this spectrum of diseases which will appear in the March 2009 issue of this journal.

Cervical cancer prevention is a very dynamic field. It seems that new research on HPV, the management of the abnormal Pap test and the HPV vaccine is being reported almost daily. Much of what worked in 2000 is now obsolete. This issue will bring you up to date as the first decade of the 21st century nears its end, but don't blink - by 2020 today's cutting-edge practices will undoubtedly have been replaced by technologies and practices that we can only begin to imagine.

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Natural History of Human Papillomavirus Infections, Cytologic and Histologic Abnormalities, and Cancer

Cosette Marie Wheeler, PhD

KEYWORDS

- HPV • Natural history of HPV infection
- Abnormal cervical cytology • Abnormal cervical histology
- Cervical intraepithelial neoplasia
- HPV vaccination and screening

PAPILLOMAVIRUS INFECTIONS OF THE HUMAN GENITAL TRACT

Papillomaviruses (PVs) form the family *Papillomaviridae*, a diverse taxonomic group of DNA tumor viruses that coevolved with a variety of animal hosts over millions of years.[1] PVs have similar or colinear genomic organizations but their nucleotide sequences can differ by greater than 50%. PV infections can be asymptomatic, cause benign hyperplasias (eg, warts) or malignancies.

Human papillomaviruses (HPVs) are part of the family *Papillomaviridae*, and those viruses infecting the human genital tract are in the genus *Alphapapillomavirus*.[2] A phylogenetic tree representing the relationships between a subset of *Alphapapillomavirus* is shown in **Fig. 1**. Over 100 HPV types have been identified to date, of which over 40 infect the genital tract. A new PV isolate can be established if the complete genome has been cloned and the DNA sequence of the L1 open reading frame (ORF) differs by more than 10% from the closest known PV type. Differences between 2% and 10% nucleotide sequence homology define an HPV subtype and less than 2% a variant. HPVs primarily target infections of the basal cells in the stratified squamous epithelium and metaplastic cells within squamocolumnar junctions. In the squamous

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Obstet Gynecol Clin N Am 35 (2008) 519–536
doi:10.1016/j.ogc.2008.09.006 **obgyn.theclinics.com**
0889-8545/08/$ – see front matter

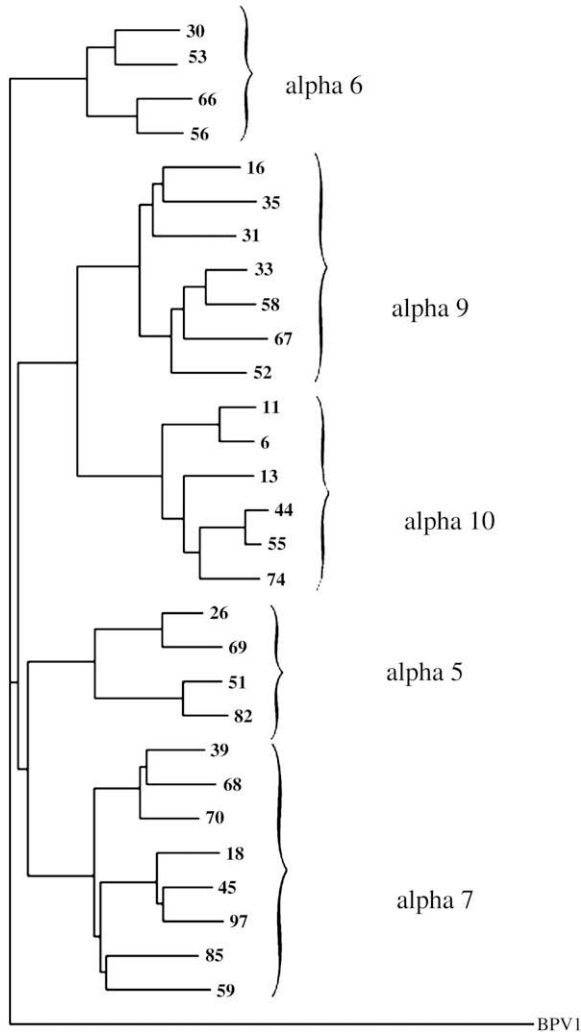

Fig. 1. Phylogenetic tree representing a subset of *Alphapapillomaviruses* based on L1 amino acid sequence similarities. A consistency based multiple sequence aligner, PROBCONS[36] was used to align the amino acid sequences for the complete L1 open reading frames of the HPV genotypes displayed. HPV types assigned to species groupings alpha 5, 6, 7, 9 and 10 are displayed. NJplot,[37] a tree drawing program, was used to draw the phylogenetic tree. Amino acid sequences were derived from GENBANK as follows: A5 HPVs (HPV26 NC001583, HPV69 AB027020, HPV51 M62877, HPV82 AB027021), A7 HPVs (HPV59 X77858, HPV18 NC_001357, HPV45 DQ080002, HPV97 DQ080080, HPV85 AF131950, HPV70 U21941, HPV39 PPHT39, HPV68 DQ080079), A9 HPVs (HPV52 X74481, HPV67 D21208, HPV33 M12732, HPV58 D90400, HPV16 AY686581, HPV31 J04353, HPV35 M74117), A10 HPVs (HPV6 AF092932, HPV11 M14119, HPV13 X62843, HPV74 U40822, HPV44 U31788, HPV55 U31791).

epithelium, their life cycles are linked closely to differentiation factors expressed within various layers of infected cells, although the biology of infections in other cell types, including glandular cells that do not have multiple stratified layers, has not been described.

HPV genomes generally encode eight ORFs. The E6 and E7 ORFs encode what have been described as the primary HPV transforming or oncoproteins.[3,4] The retinoblastoma tumor suppressor protein (pRB) and p53 are the two host proteins whose role in the transformation process has been the focus of a number of studies. During the infectious process, HPV E6 and E7 inactivate or interfere with a number of requisite host regulatory functions, including those served by pRB and p53. In women who have persistent HPV infections, over expression of HPV E6 and E7 and associated host cell genomic instability can occur. It is unknown what triggers this outcome and the necessary cofactors in the process to this day are not well understood. Early dogma proposed that in some women, HPV infected cells were lethally deregulated as a result of disruption or deletion of the HPV E2 protein during integration of HPV genomes. Integrated HPV forms commonly detected in HPV-related malignancies often demonstrated E2 ORF disruption at the viral integration insertion site. One function of E2 is to act as a transcriptional regulator of HPV E6 and E7 expression.[5]

Over time, our understanding of HPV-related host cell transformation has revealed a complexity beyond the simplistic view of requirements for HPV integration associated with E2 loss, subsequent E6 and E7 over-expression, and a resultant host genomic instability from which a clonal malignancy could arise. For example, not all HPV-related malignancies have integrated viral forms detected.[6] Even if HPV integrants are detectable, most HPV-related severe abnormalities, including cancers, harbor many HPV episomes (ie, extrachromosomal HPV genomes) with intact E2 ORFs.[6,7] Model in vitro systems have now demonstrated that even low copy numbers of HPV episomes have the ability to express E2, which can regulate E6 and E7 expression in trans on integrated HPV genomes.[8] Furthermore, HPV proteins have been found to interact with a wide spectrum of host regulatory proteins beyond p53 and pRB.[3,4] Ultimately, many complex HPV-induced changes within infected host cells, including genetic and epigenetic alterations (eg, methylation) can, when infection persists, result in overall genetic instability and clonal malignancy. It is likely that viral integration of oncogenic HPV genomes in cervical lesions is a consequence rather than the cause of chromosomal instability induced by deregulated HPV E6-E7 oncogene expression. Data support differences in the induction of chromosomal instability by various high-risk carcinogenic HPV types, which is reflected by their integration frequencies in advanced lesions and the transit time for lesions to progress to invasive cancer.[6]

GENITAL HPV INFECTION

Genital HPV infection is estimated to be the most common sexually transmitted infection; an estimated 6.2 million persons are newly infected every year in the United States.[9] Infections with multiple HPV types (coinfections) are common (approximately 50%) principally because of their shared primary route of sexual transmission. The many different genital HPV types appear to infect, resolve, or persist, and cause cervical intraepithelial neoplasia (CIN) including low- and high-grade CIN ($\geq$CIN 2), and in some cases cancer, independent of each other (ie, in general infections with multiple HPV types do not seem to affect type-specific outcomes in a positive or negative manner).[10] Sexual intercourse is not the only means for transmission of genital HPV, although other modes are believed to be very uncommon. Neonatal transmission has been reported, although detection of genital HPV infections in children beyond times closely related to actual birth and delivery remains controversial. Most studies have not detected genital HPV infections routinely in either the oral cavity or genital areas of children.[11,12] In a longitudinal study, virginal women were shown to have a 2-year

cumulative HPV infection rate of 2.4%, and among those in those engaging in nonpenetrative sexual contact, approximately 10% were positive for HPV.[13]

In Northern Europe[14,15] and the United States,[16,17] peak genital HPV prevalence appears generally under age 25 and decreases with increasing age. In these same regions, studies of young women who have recently become sexually active have detected a very high cumulative incidence of HPV infection (eg, about 50% in 3 years).[13,18] It has thus been generally presumed that the vast majority of HPV infections are acquired in the first few years after sexual debut and that HPV prevalence steadily declines thereafter as a result of spontaneous clearance of prevalent infections. In a few studies, a second peak of HPV infection has been observed in older women, raising the possibility that the age distribution of HPV infection might vary within different populations.[19,20] The distribution of HPV prevalence in representative samples of women from 15 areas in four continents has in fact revealed substantial variation in the shape of age-specific curves of HPV prevalence.[21] In surveys conducted by investigators at the International Agency for Research on Cancer (IARC), steady declines in HPV prevalence were observed with increasing age in the highest-income countries. In contrast, a flat age curve was observed in the lowest-income areas of Asia and in Nigeria, where HPV prevalence was similar across age groups. Three areas in Latin America (Chile, Colombia, and Mexico) revealed a U-shaped curve of age-specific prevalence (ie, a second peak of HPV infection was observed in older women). Further research is needed to understand the role of screening and other reasons for the differences in age-related HPV prevalence observed in different settings.

Longitudinal studies have consistently shown that most HPV infections are no longer detectable within 1 to 2 years following initial observation.[10] About 50% of HPV infections in women with normal cytology will have resolved in less than 1 year, and approximately 90% of women with either normal or CIN 1 diagnoses will ultimately resolve on their own.[22,23] In fact, most HPV infections are asymptomatic and so transient that most individuals have no idea that they are infected.

For clinical purposes, HPV infections associated with normal cervical cytology and those associated with low-grade CIN (CIN 1) are considered essentially the same.[24] Resolution or clearance of any HPV type appears to result in immunity to that type, at least based on available evidence from ongoing prospective cohort studies. It is unknown whether HPV infections can become dormant in basal cells and if so, whether future downstream reactivation of so called ''latent HPV'' genomes occurs. At present it is impossible to distinguish reactivation from newly acquired HPV infections and, therefore, any contribution of potential HPV reactivation to disease outcomes remains unclear.

Although cumulative HPV exposure is difficult to quantify because nearly all HPV infections are transient and HPV serology is inaccurate (ie, only about 60% of women with known HPV infections ever develop detectable HPV-specific antibodies), a substantial proportion of HPV DNA-negative, seronegative women have been exposed. A majority of women in the world are probably infected with at least one if not several types of HPV during their sexual lifetime; however, only few will progress to high-grade disease, including cancer.

In the subset of women who are diagnosed with invasive cervical cancer, the cause is virtually all attributable to persistent cervical infection with 1 of approximately 15 carcinogenic HPV types.[25] HPVs are a necessary cause of both squamous cell carcinoma and adenocarcinoma, although HPV genotype distributions and the role of nonviral cofactors seem to differ by histologic type.[26–28] Rapidly invasive cancers are rarely diagnosed in young women, as the transit time from initial HPV infection to

invasion is believed to be on average greater than two decades. Nevertheless, prevention strategies in a number of countries are often formulated to prevent these cases in young women. Well-organized cervical cancer screening programs in many developed countries have reduced the incidence of squamous cell carcinoma of the cervix over the past few decades, although adenocarcinoma of the cervix has been increasing in some countries[29,30] for reasons that have not been fully defined.

HPV type 16 is the most common carcinogenic HPV type and is detected in approximately 50% of high-grade squamous intraepithelial lesions (HSIL) and invasive cervical cancers worldwide.[31–33] The risk of a severe CIN 3 and cancer outcome is remarkably greater for HPV type 16 infections when compared with risk estimates for all other carcinogenic HPV types.[34] HPV types 16 and 18 are detected in about 50% and 10% to 20% of invasive cervical cancers,[31–33] respectively. HPV 18 is found in a greater proportion of adenocarcinomas than squamous-cell cervical carcinomas.[28] Other carcinogenic HPV types contributing to the global burden of cervical cancer include types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, and 82. Each of these HPV types contributes 5% or less to the cumulative incidence of HPV-associated cervical cancers worldwide. A number of additional HPV types infecting the genital tract are considered low-risk or noncarcinogenic. These include HPV types 6 and 11, which are responsible for over 90% of anogenital warts. Because noncarcinogenic HPV types cause cytologic and histologic abnormalities, detecting infections with carcinogenic HPV types is more important than detecting the presence or absence of equivocal or low-grade cytologic or histologic abnormalities.

For the past few decades, cervical cancer prevention has primarily been based on screening by cytology, evaluation of the cervix with colposcopy, and biopsy of potentially abnormal tissues. Biopsy-proven high-grade abnormalities are treated by excision or ablation of the cervical transformation zone. Despite enormous expenditures on cervical cancer screening and over 60 million Papanicolaou (Pap) tests performed each year, the American Cancer Society estimates that in 2008, approximately 11,070 cases of invasive cervical cancer will be diagnosed in the United States.[35]

A brief overview of the major abnormal cytology and histology diagnostic categories and their relationship to HPV infections is provided below. Clinical management of various diagnostic categories is not detailed here but guidelines recommending clinical management strategies have been published elsewhere.[24]

HPV AND ABNORMAL CYTOLOGY

Carcinogenic and noncarcinogenic HPV types result in abnormal cervical cytology. A cytologic diagnosis of atypical squamous cells (ASC) is the most common of all cytologic categories, but ASC is also the least reproducible among pathologists. Atypical squamous cells of undetermined significance (ASC-US) and atypical squamous cells cannot exclude HSIL (ASC-H) represent the two subcategories of ASC. The proportion of high-risk HPV-positive women reported among these two categories ranges from 40% to 51% for ASC-US, and from 74% to 88% for ASC-H.[36–41] Similarly, the prevalence of CIN 2 and 3 is higher among women with ASC-H than among women with ASC-US.[42] ASC-H is typically considered equivocal HSIL and a productive HPV infection. A 2004 meta-analysis reported that the pooled estimate of the sensitivity of HPV testing for detecting women with CIN 2 and 3 in women with atypical or equivocal cytology is considerably higher than that of a single repeat cytology.[43] The overall prevalence of invasive cervical cancer is low among women with ASC.[44]

Low-grade squamous intraepithelial lesions (LSIL) have previously been described using a number of terms, including HPV effects, koilocytosis, parakeratosis, mild

dysplasia, and CIN 1. Cytologic LSIL are, however, not equivalent to histologic CIN 1. LSIL is highly correlated with HPV infection. For example, in the United States National Cancer Institute's (NCI) ASCUS/LSIL Triage Study (ALTS) trial, when testing for 38 possible HPV types, HPV DNA positivity among women with LSIL diagnoses was 85%.[45] The risk of CIN 2 or 3 and the clinical management of women with LSIL is the same for women with ASC-US who are positive for carcinogenic HPV DNA.[46] The prevalence of CIN 2 and 3 or cancer among women with LSIL has been reported to be between 12% and 17%.[47,48] In contrast to other cytology diagnoses, which have generally remained constant, the prevalence of LSIL diagnoses in the United States has nearly doubled over the past decade.[49] The increase has been largely attributed to an increase in liquid-based cytology.

HSIL have previously been described as moderate dysplasia, severe dysplasia, carcinoma in situ, CIN 2, and CIN 3. Cytologic HSIL are not equivalent to histologic CIN 2 or 3. An HSIL cytology result is highly correlated (>85%) with HPV infection[33] and indicates a high risk for significant cervical disease, with 53% to 66% of women having a CIN 2 or 3 or cancer diagnosis following biopsy.[48,50,51] An estimated 2% of women with HSIL have invasive cancer.[52]

Cytologic abnormalities of glandular cells that are less severe than adenocarcinoma are divided into three categories: atypical glandular cells (AGC; endocervical, endometrial, or "glandular cells" not otherwise specified); AGC, either endocervical or "glandular cells" favor neoplasia (AGC favor neoplasia); and endocervical adenocarcinoma in situ (AIS). AGC results are overall uncommon. By comparison to ASC, LSIL, and HSIL, which are common in younger women, AGC is more common in women over age 40.[53] AGC is frequently caused by benign conditions, such as reactive changes, but a fair number of women with AGC have significant intraepithelial neoplasia (CIN 2 or 3, AIS, or cancer), and 3% to 17% have invasive cancer.[54–57]

It is worth commenting on the psychosocial morbidity of the previously described abnormal cytology diagnoses. Research has shown that distress and anxiety are reported by a majority of women (59%) after having even a low-grade abnormal Pap test.[58] Women also report negative impacts on their sexuality, fear about developing cancer, and wondering if the abnormalities could interfere with their ability to bear children.[59–61] The significant psychosocial morbidity and health care expenditures associated with abnormal Pap tests requires improved identification of those HPV infections that are destined to persist and progress, as very few women with abnormal cytology will ever develop invasive cervical cancer.

HPV-RELATED HISTOLOGY OUTCOMES

Among women of reproductive age, abnormal histology or CIN is a relatively common diagnosis. It has been estimated that in the United States, greater than 1 million women are diagnosed each year with CIN 1 and that approximately 500,000 are diagnosed with high-grade cervical cancer precursor lesions that include both CIN 2 and 3.[49] The histologic diagnosis of CIN represents the standard for determining clinical management. **Fig. 2** provides a schematic diagram to show the disease continuum of CIN development following HPV infection.

High rates of spontaneous regression, ranging from 70% to 90%, have been reported for CIN 1 lesions that remain untreated, and thus progression of CIN 1 to CIN 2 or worse is rarely observed. In the NCI ALTS trial, the risk for having a CIN 2 or 3 lesion during 2 years of follow-up after initial colposcopy was nearly identical in women with a histologic diagnosis of CIN 1 (13%) and in women whose initial colposcopy and biopsy were negative (12%).[46] CIN 1 lesions are associated with high-risk

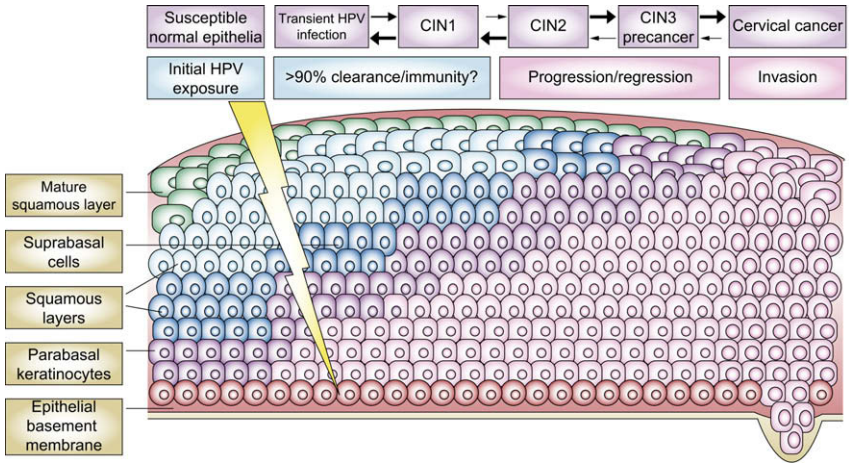

Fig. 2. Schematic diagram to show the disease continuum of cervical neoplasia development following HPV infection. Infection of the cervical transformation zone with genital HPV can be cleared relatively rapidly through innate and adaptive immunity or other mechanisms not yet defined. Established HPV infections can sometimes be recognized as cytologic or histologic abnormalities, most often CIN 1. Most of these cellular abnormalities will be resolved, presumably by host immunity. When carcinogenic HPV infections persist, cervical precancers, such as CIN3, can arise from genetic instability and ultimately clonal expansion of highly transformed cells. The events associated with and necessary for invasion of the basement membrane remain unknown. The following factors lead to HPV persistence: HPV type (greatest risk = HPV 16), increasing age, smoking, mutagens, immunosuppression, inflammation, hormones, and genetic factors. (*From* Wheeler CM. Advances in primary and secondary interventions for cervical cancer: human papillomavirus prophylactic vaccines and testing. Nat Clin Pract Oncol 2007;4(4):225; with permission.)

carcinogenic types of HPV, but the distribution of HPV types in women with normal cytology and CIN 1 is markedly different than what is detected in CIN 2 and 3 [33,62–64] and invasive cervical cancer, as shown in **Table 1**.

In designing cervical cancer prevention strategies, precancer or CIN 3 or worse is a reasonable surrogate for invasive cervical cancer, as numerous studies demonstrate essential equivalence on a molecular basis. By comparison, CIN 2 is a highly heterogeneous entity where the biologic importance varies greatly. Therefore, for a number of reasons, CIN 2 has severe limitations when included with CIN 3 as a surrogate endpoint for cancer. For example, there are a number of noncarcinogenic HPV types that can cause CIN 2 but which rarely if ever cause invasive cancer.[65,66] There is even direct evidence that CIN 2 lesions have an intermediate cancer risk when compared with CIN3.[67] A review of the literature found 43% of untreated CIN 2 lesions regressed in the absence of treatment, 35% persisted, and 22% would progress to carcinoma in situ or become invasive.[68] The rates of regression, persistence, and progression for CIN 3 were 32%, 56%, and 14%, respectively. Furthermore, CIN 2 is not a reproducible diagnosis among pathologists because of an overall lack of agreement on specific cytomorphologic criteria. In the NCI ALTS trial, only 43% of CIN 2 diagnosed among community center pathologists was accepted as CIN 2 by the expert consensus panel.[69] Many continue to debate whether a CIN 2 diagnosis should be considered a low-grade or high-grade lesion, as there is good evidence demonstrating CIN 2 often represents acute HPV infection with worrisome microscopic features that will

Table 1
HPV genotype distribution reported in various large studies

	New Mexico USA[a]	IARC Pool[b]	LSIL[c]	HSIL[d]	ICC[d]
HPV Genotype	% of All HPV Positives				
16	19.2	19.7	26.6	55.2	45.3
18	5.9	7.2	8.6	6.9	6.9
45	6.0	5.6	4.9	2.3	2.3
31	7.7	7.5	11.7	3.8	8.6
33	2.5	5.8	7.6	3.7	7.3
52	7.6	5.3	8.8	2.9	5.1
58	6.5	7.6	8.5	2.8	7.0
35	2.4	5.9	5.9	1.5	3.8
59	5.4	2.9	6.0	1.1	0.8
51	7.5	4.0	10.9	1.0	3.6
56	6.3	7.1	9.7	1.0	2.9
39	8.5	4.3	7.8	0.9	2.0
68	3.0	2.1	NR	0.5	1.1
66	2.4	4.1	8.5	0.4	1.9
53	13.3	1.2	10.1	NR	NR
70	2.3	NR	NR	0.1	1.3
73	2.6	2.3	NR	0.4	1.8
82[d]	2.0	0.8	NR	0.1	1.2
6	5.2	1.4	NR	0.5	2.2
11	1.3	1.4	NR	0.1	1.3
Total sample, *n*	3,863	15,613	8,308	7,094	14,595
Any HPV positive	1,515	1,429	5,910		
(% of total sample)	(39.2)	(9.2)	(71.1)	(84.9)	(87)

Abbreviation: ND, not reported.

[a] Clinic-based study enrolled women ages 18 to 40 with no past history of cytologic abnormality in past year, no history of ever having high-grade cervical diagnosis, cervical treatment or hysterectomy. *Data from* Peyton CL, Gravitt PE, Hunt WC, et al. Determinants of genital human papillomavirus detection in a US population. J Infect Dis. 2001;183(11):1554–64. Epub 2001 May 9.

[b] IARC population-based survey in 11 countries enrolling an age-stratified sample of women ages 15 to 74 without cytologic abnormalities. *Data from* Clifford GM, Gallus S, Herrero R, et al. Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. Lancet. 2005;366(9490):991–8.

[c] LSIL cases (n = 8,308) from 55 published studies were included in a meta-analysis. Regional distribution of included cases: Europe 46.5%, North America 32.9%, South/Central America 14.8%, Africa 3.0%, and Asia 2.9%. *Data from* Clifford GM, Rana RK, Franceschi S, et al. Human papillomavirus genotype distribution in low-grade cervical lesions: comparison by geographic region and with cervical cancer. Cancer Epidemiol Biomarkers Prev 2005;14(5):1157–64.

[d] Includes HPV IS39, now designated as a variant of HPV82.

inevitably regress. None-the-less, a significant proportion of CIN 2 lesions associated with high-risk carcinogenic HPV types harbor incipient precancers, with a high risk of invasive outcome. As such, in the United States, CIN 2 is combined with CIN 3 and represents the clinical threshold requiring ablative or excisional therapy.[70] Although treatment of CIN 2 may currently be appropriate to insure a high degree of safety, given the high prevalence of CIN 2 in reproductive-aged women, the potential for negative-reproductive outcomes associated with loop electrosurgical excision

procedures must be considered. Loop electrosurgical excision has been reported to double the risk for subsequent preterm delivery, premature rupture of membranes, and of having a low birth-weight infant.[71] Identification of biomarkers to predict which CIN cases represent true precancers requiring treatment remains an important area for further discovery work.

The immediate precursors of invasive cervical cancer are squamous cell carcinoma in situ (CIS) and adenocarcinoma in situ. AIS is much less commonly observed than are CIN 2 and 3 and CIS. The earliest form of invasive cancer is histologically recognized as microinvasive carcinoma: cancers that have invaded no more than 5-mm deep and 7-mm wide into the underlying cervical stroma. Early invasive cancers appear as a tiny bud of invasive cells that have penetrated through the basement membrane and pushed into the underlying stroma. Histologically, approximately 90% to 95% of invasive cervical cancers arising from the uterine cervix in developing countries are squamous cell cancers, and about 5% are adenocarcinomas. Adenocarcinoma arises in the endocervical canal from the glandular epithelium. Virtually all of squamous- and adenocarcinomas of the uterine cervix are caused by high-risk carcinogenic HPV genotypes. The most widely used staging system for invasive cervical cancer is based on tumor size and the extent of disease spread into the vagina, parametrium, urinary bladder, rectum, and distant organs. Clinical stage of disease at presentation is the single most important predictor of survival from invasive cervical cancer.

UNDERSTANDING COFACTORS OF HPV PERSISTENCE AND PROGRESSION TO HIGH-GRADE CERVICAL ABNORMALITIES

Several factors are implicated in enhancing HPV persistence and HPV-related disease progression to high-grade cervical abnormalities and cancer; however, it is difficult to disentangle persistence from HPV-related disease progression. Persistence can be defined as the detection of the same HPV genotype two or more times with a specific time interval between samples. There is currently no agreed upon definition of an appropriate interval (eg, 6, 12, 18 months) to define ''meaningful'' persistence. Data demonstrate that the longer an HPV infection has persisted, the more likely it is to remain persistent. Additionally, some data indicate that HPV 16 persists longer than other genotypes.[72]

Studies have demonstrated that older women with HPV infections are more likely to persist longer than infections in younger women.[73,74] Because these studies were cross-sectional, it is probable that the older women already had these persistent HPV infections for some time, and thus it should not be presumed that new infections in older women by nature have an increased risk of longer persistence. Long-term persistence (>5 years) is not a strict correlate of carcinogenicity. Noncarcinogenic HPV types can also persist for long periods.[72]

Studies assessing the risk of CIN 3 or cervical cancer among HPV-positive women have been consistent in finding smoking as a cofactor, but this association is less clear for persistence of HPV.[75,76] In women infected with high-risk carcinogenic HPV genotypes, long-term oral contraceptive use can significantly increase the risk of developing high-grade cervical lesions including cancer.[77] Some sexually transmitted infections have been suggested as cofactors for HPV outcomes. The majority of studies examining *Chlamydia trachomatis* in HPV-positive women have demonstrated an association with high-grade cervical lesions and invasive cancer.[78] *Chlamydia trachomatis* has also been associated with increased HPV persistence.[79,80] Studies of other sexually transmitted infections as cofactors for HPV-related outcomes, including herpes simplex virus and *Trichomonas vaginalis*, have reported inconsistent results.[78]

Nutrients, intake of fruits and vegetables, and alcohol intake have also been implicated inconsistently. Genetic and immunologic host factors, such as HLA class I and II genes[81] and viral factors, such as HPV variants, viral load, and viral integration, appear important in determining risks for HPV-related cervical disease outcomes, although a great deal of work is needed to further clarify specific roles of these factors.

Natural immunity has been implicated as an important modifier of HPV infection and HPV-related disease; however, because HPVs have evolved to evade host immune recognition, specific immune responses have been difficult to characterize. Extremely low-level responses are often not measurable by existing immunologic methods. Cell-mediated immune responses are often barely above background measures, and detectable HPV-specific antibodies are only detectable in about 60% of infected women, although this varies somewhat among different HPV types studied.[82] Women with transient HPV infections are less likely to develop detectable HPV-specific antibodies or cell-mediated responses than women with persistent HPV infections.[83] Thus, innate immunity may have an important role in the elimination of many HPV infections. HPV-specific antibody is associated with prior HPV exposure but does not appear to provide protection against HPV persistence or disease.[84] In longitudinal cohort studies, once clearance of any HPV type is observed, it is very uncommon to detect that specific HPV type again,[85] giving support to the notion that some aspect of natural immune protection is generated.

INTEGRATING PRIMARY AND SECONDARY CERVICAL CANCER PREVENTION STRATEGIES

Given the discovery of carcinogenic HPVs as a single primary cause of invasive cervical cancer, numerous opportunities for developing targeted primary and secondary interventions have been realized. In those countries where high coverage has already been achieved for cervical screening, improving the sensitivity of the screening test has become a primary goal. In a number of studies, HPV DNA testing alone has emerged over the past decade as a more sensitive primary screening test in women who are at least 30 years of age.[85] The IARC has stated there is sufficient evidence indicating that the efficacy of HPV testing using a validated system as the primary screening modality can be expected to be at least as good as that of conventional cytology.[65] In comparison to cytology, HPV testing is objective and amenable to automation and it can be performed in a more reproducible and accurate manner. As HPV testing costs are reduced, and if lower cost HPV tests are made available to developing countries, a variety of HPV-based cervical screening programs can be envisioned throughout the world. It is further possible that HPV tests capable of distinguishing specific, individual HPV genotypes will find utility in classifying women at greatest risk of disease outcome: those with persistent HPV infections. Some of the most common HPV types found in cancer, including HPV 16, 18, 31, 33 and 45, are currently being considered in longitudinal studies that will assess the clinical utility of algorithms employing multiple HPV genotype-specific measurements.

In addition to improvements expected in secondary cervical cancer prevention through HPV testing, two manufacturers have developed prophylactic HPV vaccines that have demonstrated high efficacy in populations that are naïve to the HPV vaccine types.[86–88] The vaccines are composed of noninfectious, recombinant HPVviral-like particles (VLPs) that target reductions in the two HPV types, HPV 16 and 18. HPV 16 and 18 are responsible for approximately 70% of invasive cervical cancer worldwide. One of the vaccines[86,87] also includes VLP immunogens for HPV types 6 and 11, which cause the majority of anogenital warts. However, for cervical cancer incidence to be reduced, women will require both screening and vaccination, as

first-generation HPV vaccines do not provide protection against a number of carcinogenic HPVs. Thus, cervical cancer screening programs must continue, and the relative roles of HPV vaccination in young women and HPV testing in older women (alone or in conjunction with cytology) will be determined over the next decades. Presently, no change in current screening is planned in vaccinated or unvaccinated women.[89]

As HPV vaccines are implemented, there are certain reductions in screening diagnoses that can be anticipated, primarily because of reductions in circulating HPV16. A small impact on ASC-US and LSIL diagnoses is expected, and the number of HSIL and cancer diagnoses will diminish to a greater extent. However, HSIL and cancer diagnoses represent a very small proportion of the overall abnormalities encountered. The positive-predictive value of an abnormal cytology for predicting CIN 3 and cancer will therefore decrease. The same decrease in the positive-predictive value will apply to current high-risk carcinogenic HPV assays, as the primary value of this testing lies in the detection of HPV 16 and 18. Vaccination will, in effect, eliminate some of the intrinsic value of cervical cytology programs.

The addition of HPV vaccination will therefore require adjustments in the associated cervical cancer screening programs, particularly because HPV vaccines are costly and will add billions of dollars to the estimated $5 to $6 billion already spent each year in the United States on current cervical screening programs. For example, if HPV vaccines achieve high coverage, then removal of HPV 16 and 18 from the circulating HPV pool will most likely justify increasing the age of first cervical screening. Other carcinogenic HPV types are less common in precancer and cancers detected in younger women, and cost-effectiveness analyses support increasing the age of first cervical screening to approximately 25 years.[90] Over time, as more data become available, extension of screening intervals in vaccinated populations may also be warranted. This would be particularly important if HPV testing is routinely used in screening. The cost-effectiveness of HPV vaccination will depend on the duration of vaccine immunity and will be optimized by achieving high coverage in presexually active adolescent girls, targeting initial catch-up efforts to women up to 18 or 21 years of age and revising screening policies.[91]

To enable the appropriate and timely integration of HPV vaccination and screening, it will be important to conduct surveillance in populations for which any coordinated modifications are under consideration. This may be particularly relevant in settings such as the United States, where there are no national cervical screening programs with call and recall support and where HPV vaccination may take several years to achieve high population coverage. In the short term, population-based registries and information systems collecting longitudinal data on cervical screening (Pap tests and $\geq$CIN 1), treatment, and vaccination will be needed to inform appropriate decision-making and to determine the population-based effectiveness or lack thereof for these interventions.[92]

SUMMARY

There are over 40 common genital HPV types that are primarily sexually transmitted. The vast number of women will be infected with one or more HPV types in their sexual lifetime. Persistent infection with HPV types can cause abnormal cytology (Pap tests) including diagnoses of ASC, AGC, LSIL, and HSIL, as well as abnormal histology identified following biopsy diagnosis as CIN 1 to 3, AIS, and cancer. Only a small subset of women infected with high-risk carcinogenic HPV will develop invasive cervical cancer. Although carcinogenic HPV is a necessary cause of invasive cervical cancer, a number of cofactors have been associated with HPV persistence and HPV-related disease

progression, including: (1) viral factors such as genotype (eg, HPV 16) and variant; (2) tobacco and long-term oral contraceptive use; and (3) genetic and immunologic host factors including innate immunity. About 15 carcinogenic HPV types are responsible for the global burden of invasive cervical cancer with HPV type 16 demonstrating the greatest risk. Given the identification of carcinogenic HPV as a necessary cause of cervical cancer, primary and secondary interventions have been highly successful. HPV testing has been used in cervical screening and may one day be used as a primary cervical screening test at least in women greater than or equal to 30 years. Prophylactic HPV vaccines based on VLPs have demonstrated high efficacy in sexually naïve populations. For cervical cancer incidence to be reduced, however, women will require both screening and vaccination, as first-generation HPV vaccines do not provide protection against a number of carcinogenic HPVs. Thus, cervical cancer screening programs must continue and the relative roles of HPV vaccination in young women and HPV testing in older women (alone or in conjunction with cytology) will be determined over the next decades. Population-based registries and information systems collecting longitudinal data on cervical screening (Pap tests and $\geq$CIN 1), treatment, and HPV vaccination will be needed to inform appropriate decision-making and to determine the population-based effectiveness or lack thereof for these interventions.

ACKNOWLEDGMENTS

It is with great appreciation and gratitude that I extend my thanks to the hundreds of clinicians and scientists who have contributed to our understanding of HPVs as necessary etiologic agents of invasive cervical cancer. In addition, I would like to thank the many members of my laboratory and clinical research group at the University of New Mexico, who have worked with me toward this same goal over the past 20 years. Your efforts have enabled major advances in primary and secondary cervical cancer prevention. Special thanks to Cheri Peyton-Goodall, who conducted the sequence alignments and prepared the phylogenetic tree shown in **Fig. 1**.

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Cervical Cancer Screening in the Early Postvaccine Era

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KEYWORDS

- Pap test • Cervical cytology
- Cervical cancer screening • Cervical cytology guidelines
- HPV DNA test • Liquid-based Pap test

The Pap test is the foundation of cervical cancer screening in North America and most industrialized countries. It has been widely used in the United States since the 1950s.[1] The regimen of repeated cytologic screenings, follow-up of abnormal results using colposcopy and biopsy, and treatment or continued close follow-up has resulted in a dramatic decline in the incidence of, and mortality from, cervical cancer over the past 50 years. It is an often-cited example of a successful program of secondary prevention.[2] It has lead to a reduction in the incidence of invasive cervical cancer in the United States from 14.8 per 100,000 women in 1975 to 6.7 in 2005. Over the same 30-year period, mortality from the disease has declined from 5.6 to 2.4 per 100,000 women.[3] Recently, primary prevention has become available in the form of immunization against human papillomavirus (HPV) types 16 and 18, the two types of HPV responsible for 65% to 76% of invasive cervical cancer worldwide.[4] Although vaccination affords 98% to 100% protection from high-grade dysplasia or cervical cancer caused by these two HPV types in women who have not been previously exposed,[5,6] the efficacy declines by about half if women who had prior HPV 16 or 18 infections are included in the calculation. Preadolescent girls who have not yet started sexual activity are the ideal cohort for vaccination; however, it may take up to 20 years after widespread immunization of this group to see the vaccine's full impact on cervical cancer rates. Therefore, at least during the early postvaccine era, some form of cervical screening will continue to be necessary for most women. But are our current screening guidelines still justified? In this article, the author reviews the current recommendations for cervical cancer screening by the American Cancer Society (ACS) and the American College of Obstetricians and Gynecologists (ACOG), reviews the relative efficacy of liquid-based cytology versus the conventional Pap smear, and discusses the role of HPV DNA testing in primary screening.

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Obstet Gynecol Clin N Am 35 (2008) 537–548
doi:10.1016/j.ogc.2008.09.005 **obgyn.theclinics.com**
0889-8545/08/$ – see front matter

WHEN SHOULD A WOMAN BEGIN CERVICAL SCREENING?

The ACOG and the ACS recommend waiting about 3 years after coitarche or until age 21 before performing a young woman's first Pap test. This recommendation is based on the low rate of cancer in this age group, despite high rates of sexual activity and high acquisition of HPV infections.

HPV is acquired efficiently by adolescents. Data from the National Center for Health Statistics' National Health and Nutrition Examination Survey[7] showed 24.5% of teens aged 14 to 19 to be HPV positive on self-sampling, and 17.5% had one or more high-risk types. The proportion of women who tested positive for HPV, and specifically for high-risk HPV types, increased to 44.8% and 28%, respectively, in women aged 20 to 24. Longitudinal studies have demonstrated rapid acquisition of HPV among sexually active adolescents and young women.[8–10]

Winer and colleagues[11] followed 130 college women aged 18 to 24 (mean 19.4 years) from within 3 months of their first act of heterosexual vaginal intercourse. By 4 months after coitarche, 20% had had at least one HPV infection. This number increased to 28.5% by 12 months, 39.2% by 24 months, and 49.1% by 36 months.

Most HPV infections are transient, with median durations of 6 to 8 months. Ho and colleagues,[8] in a study of 608 college women (average age 20), found the average duration of an HPV infection to be 8 months, with 70% reverting to negative within 12 months and 81% within 24 months. Infections with high-risk HPV types appear to take about twice as long to clear as do those with low-risk HPV types.[9,12] Insinga and colleagues[12] found a mean duration of incident HPV 6 and 11 to be 8.3 and 8.4 months, respectively, where the mean duration of infections with HPV 16 and 18 were 18.2 and 16.4 months, respectively.

Cervical dysplasia is also common in sexually active adolescents and young women. Fortunately, most dysplasias are low grade. Wright and colleagues[13] reported the findings of 10,090 Pap tests of adolescent girls aged 12 to 18 read at a hospital-based laboratory from 1997 to 2003. Four hundred twenty two (5.7%) were reported as low-grade squamous intraepithelial lesions (LSIL), but only 55 (0.7%) were high-grade squamous intraepithelial lesions (HSIL). The HSIL Paps, however, included 12 girls younger than 16; the youngest was aged 12.

When high-grade dysplasia develops, how long does it take? Winer and colleagues[14] followed 602 women aged 18 to 20 with cytology and HPV testing every 4 months for a mean duration of 38.8 months. Among those who acquired a new HPV infection, 47.2% developed cervical dysplasia within 3 years, with a median time from HPV infection to squamous intraepithelial lesions of 4.0 months, and 11.1% developed cervical intraepithelial neoplasia (CIN) 2,3. Ninety-four percent or those who developed CIN 2,3 did so within 40 months of a new HPV infection, and in that group, median interval from HPV infection to diagnosis of CIN 2,3 was only 14.1 months. The risk for CIN 2,3 was highest (27.2%) among those who had HPV 16 or 18. Not only do most HPV infections clear spontaneously in adolescents and young women, but most squamous intraepithelial lesions also regress without treatment. Moscicki and colleagues[15] prospectively followed 187 women aged 18 to 22 and diagnosed with LSIL. By the end of the first year of follow-up, 61% had reverted to normal without treatment; by 3 years, 91% had reverted to normal. Only 3% progressed to CIN 3. No cancers were reported. Two smaller studies followed women aged 21 and younger who had CIN 2 on biopsy.[16,17] After a median follow-up of 12 and 18 months, respectively, most had reverted either to CIN 1 or negative. Few progressed to CIN 3 and, again, no cancers developed.

Although the rate of CIN 2,3 in adolescents who have abnormal cytology does not differ greatly from adults who have abnormal Pap tests, the risk for cancer in the

adolescent age group is exceedingly low. Only 0.1% of all cervical cancers occur in women under age 20, a rate of 0.26 per 100,000 women. By contrast, 15.2% of invasive cancers are diagnosed between the ages of 20 and 34. The age-specific incidence rates per 100,000 women aged 20 to 24, 25 to 29, and 30 to 34 years, respectively are 1.90, 6.45, and 11.37.[3]

Therefore, although HPV infections are widespread in sexually active adolescents, and LSIL and even HSIL are not uncommon, given the estimated 10-year transit time from CIN 3 to invasive cancer based on the difference in average age at diagnosis of the two conditions,[18] the strategy of waiting until about 3 years after the onset of intercourse to perform the first Pap test seems conservative.

This approach allows for the acquisition and spontaneous clearance of most HPV infections, and the acquisition and spontaneous clearance of most CIN lesions. It permits the timely detection and treatment of persistent high-grade CIN lesions before invasive cancer can develop, while minimizing morbidity from unnecessary interventions including colposcopy and treatment procedures.[19,20]

SCREENING INTERVALS: HOW OFTEN TO SCREEN WITH THE PAP TEST

Current ACS and ACOG guidelines recommend a decade or so of intensive screening once a young woman has had her initial Pap test.[19,20] ACOG recommends annual examinations until age 30. The ACS guidelines are in agreement if the conventional Pap smear is used; the ACS suggests screening every other year until age 30 if a liquid-based cytology preparation is used. Both agree that after age 30, a woman who has had three consecutive satisfactory negative Pap tests may space her screening interval to 2 to 3 years. The US Preventive Services Task Force makes no age distinction in its recommendation of triennial Pap testing.[21] Recent studies support the practice of intensively screening women under 30 and spacing Pap tests out in well-screened women after age 30.[22,23]

Multiple studies have shown that in older cohorts of women, comparable sensitivity can be achieved from cervical cytology, despite increasing screening intervals. Some studies suggest that this may not be the case in younger women.[23–26] Sasieni and colleagues[26] reported on the development of invasive cervical cancer (stage 1B and worse) in 1305 British women aged 20 to 69 screened with conventional Pap tests. These cases were compared with 2532 controls. The investigators assessed the odds of acquiring cancer as a function of the time from the last negative screening Pap test to the diagnosis of cancer. They found that the risk for cancer increased with increasing time since the last negative test, but this trend lessened with advancing age. The percent of cancers prevented by screening at various intervals in women aged 20 to 39 declined from 76% if the prior negative Pap was 1 year previously, to 61% and 30% at 3- and 5-year intervals, respectively. In women aged 40 to 54, screening at 1- and 3-year intervals showed little difference (ie, 88% and 84% with a decline to 73% at 5 years). Among women in the 55-to-69 age group, protection increased only slightly, even at the 5-year interval. The comparable rates of protection were 87%, 87%, and 83% for screening at 1, 3, and 5 years, respectively.

Although repeated negative screening tests confer a greater degree of protection than one-time or infrequent testing, it appears that younger women get less relative protection from a single negative Pap test than do older women; hence, the recommendation for more intensive screening before age 30. In a retrospective review of 455 women diagnosed with invasive cervical cancer enrolled in a large health maintenance organization,[22] one half had not had a Pap test within the 3 years before diagnosis. Among those who had had recent Paps, the small number (11) diagnosed under

age 30 were far more likely to have had a negative screening Pap test within the previous 3 years than were women in older age groups.

In an analysis of data from the National Breast and Cervical Cancer Early Detection Program, investigators found the incidence of dysplasia of all grades was highest in women under age 30.[23] Except for women younger than 30, the rates of dysplasia decreased with the number of previous Pap tests recorded in the program. In women younger than 30, the prevalence of dysplasia of all grades did not differ whether they had had one or two prior negative tests. The rates did decline in this age group after three or more previous negative tests. Using modeling techniques, they calculated the risk for developing invasive cancer for women after three consecutive negative Paps who had their next Pap test in 3, as opposed to 1, year. Lengthening the screening interval was associated with five additional cancers per 100,000 women aged under 30, compared with three additional cancers for women aged 30 to 44, and one in those aged 45 to 59. They then showed the cost effectiveness of more frequent screening in younger women by estimating the number of additional tests needed to detect each cancer prevented by annual, rather than triennial, screening. In women younger than 30, averting a single cancer with annual screening would require the addition of 42,621 Pap tests and 2364 colposcopies. The ''cost'' increases with age to 209,324 Pap tests and 11, 502 colposcopies in the 45-to-59 age group.

A subsequent study reported a more formal cost-effectiveness analysis of Pap screening interval using data from the same national program.[27] With increasing age and increasing number of prior screening Pap tests, the cost of each year of life saved rose substantially. The investigators concluded that annual screening is never cost effective. They suggested that the most cost-effective screening strategy for women younger than 30 is to screen those who had zero or one prior negative Pap test every 2 or 3 years. Women in all other age groups should be screened no more often than every 3 years.

WHEN SHOULD SCREENING BE STOPPED?

The ACS, the ACOG, and the US Preventive Services Task Force have long advised against cytology screening in women who have undergone hysterectomy with removal of the cervix for benign indications. (CIN 2,3 is not considered a benign indication).[19–21] In women who have undergone hysterectomy for benign indications, the object of continued screening is the prevention of primary vaginal carcinoma. Vaginal cancer of any type is rare, with an incidence of only 2210 new cases projected for 2008. These cancers represent about 2.8% of all incident gynecologic cancers.[28] Continued cytologic testing in this group will uncover vaginal intraepithelial neoplasia (VAIN), mostly VAIN 1, which is not felt to be precancerous.[29] Although data are limited, the malignant potential of VAIN is thought to be less than that of CIN.

The risk for vaginal neoplasia after a total hysterectomy in women who have a history of high-grade dysplasia, although low, is significantly higher than in those whose hysterectomy was for benign indications and not preceded by high-grade CIN.[29–32] Stokes-Lampard and colleagues[29] reviewed 40 years of literature on Pap tests after hysterectomy. They found nine papers of good methodologic quality. They reported that of 6543 women who had had a hysterectomy for benign indications, 117 (1.8%) had an abnormal Pap; 8 (0.12%) had VAIN on biopsy. No cancers were reported. By contrast, among the 5037 who had CIN 3 before hysterectomy, 14.1% had an abnormal Pap and 1.7% had VAIN on biopsy, and a single cancer developed. A more sobering report from a single hospital in Belgium[32] found two vaginal cancers in a series of 94 women followed after hysterectomy with CIN 2 or worse. Because of

the increased risk for vaginal neoplasia in women who have undergone total hysterectomy with prior high-grade CIN, it is recommended that testing continue until three consecutive negative Pap test are documented within 10 years.

The ACS recommends discontinuing screening altogether in women older than 70 with well-documented negative screening histories (ie, at least three consecutive satisfactory negative Pap tests in the previous 10 years). (Continued screening is recommended for women who have a history of in utero diethylstilbestrol exposure or who are immunocompromised**).**[19] The US Preventive Services Task Force makes a similar recommendation at age 65.[21]

Although the age-specific incidence of cervical cancer peaks in the late 30s and early 40s and begins to decline after the mid-50s, new cases are diagnosed into the 80s and beyond.[3] Women aged 65 and older represent only 14.3% of the United States population[33] but have 19.7% of incident cases of cervical cancer.[3] The presumed cause of this disproportion is poorer screening among those older women who get cervical cancer.[22] Multiple negative Pap tests offer more protection in older women than in women younger than age 30. Furthermore, as women age, the prevalence of high-risk HPV infections declines,[7] and the area of the cervix at risk for neoplastic transformation (ie, the active transformation zone) is likely to be reduced in size and in a protected location within the endocervical canal.

What's the harm in continuing to do periodic Pap tests in low-risk populations, such as women who have had hysterectomies for benign indications or in previously well screened older women? Screening in these populations is likely to result in higher false-positive rates, with accompanying increases in expenditures, anxiety, and unnecessary additional testing. An ingenious example of the additional testing that accompanies overscreening was reported by George Sawaya[34] using data from the Heart and Estrogen/Progesterone Replacement Study. He tracked 2561 postmenopausal women, with an average age of 67 years, after a negative Pap test. In the next 1 to 2 years, all had had a repeat Pap. One hundred ten were abnormal. The follow-up of these 110 women involved 231 interventions, including numerous repeat Pap tests, 33 colposcopies, 33 cervical or vaginal biopsies, 35 endocervical curettages, eight endometrial biopsies, four dilation and curettages, and nine cone biopsies or loop excisions. The ultimate yield was a single case of mild-to-moderate dysplasia.

LIQUID-BASED CYTOLOGY VERSUS THE CONVENTIONAL PAP TEST

The first liquid-based Pap test was approved by the Food and Drug Administration for clinical use in 1996. Two products are currently in use in the United States, ThinPrep and SurePath, with a third, Monoprep, that is FDA approved and soon to go on the market. In the first few years after its introduction, the liquid-based Pap test rapidly became the preferred Pap technology in the United States. By 2003, nearly 90% of obstetrician/gynecologists used liquid-based cytology for cervical screening.[35] With the liquid-based technology, cells scraped from the cervix are suspended in a liquid transport medium and, in the laboratory, a thin layer of cells is displayed on a glass slide. With the "conventional Pap smear," on the other hand, cervical cells are transferred directly to a glass slide by the provider and are fixed with ethyl alcohol or spray fixative. Liquid-based cytology minimizes or eliminates artifact that in conventional Pap smears may interfere with accurate interpretation. These include air drying, clumping of epithelial cells, and presence of inflammatory cells and noncellular debris. The uniform layer of cells in the liquid-based cytology prep is easier to interpret, although cytotechnologists and pathologists, once trained to consider inflammatory cells and background debris in their diagnoses, can no longer use these clues to render diagnoses.[36]

The liquid-based Pap test was marketed as a more sensitive screening test than the conventional smear. In addition, it offers the option of performing reflex HPV DNA testing for atypical squamous cells of uncertain significance (ASC-US) triage and for screening with the combination of cytology plus the HPV DNA test. These uses of HPV DNA testing from cells in the liquid medium have also been found to be effective and cost effective.[37,38] The use of concomitant HPV testing can be done with conventional Paps, but it requires cocollection of the HPV DNA specimen separately; many laboratories find such cocollection logistically cumbersome.

The question of whether liquid-based cytology is more sensitive than conventional cytology remains unanswered. Early studies cited an apparent increased sensitivity in the liquid-based preparations when compared with the conventional Pap. Their methodologies, however, were less than ideal.[39–42] More recent research has called into question whether the liquid-based Pap is truly more sensitive.[43–46]

This issue was recently addressed in an article that reviewed 56 published studies.[46] The investigators identified flaws in study design in all of them. In the ideal study, women should be randomly assigned to one or the other type of cytology, and all slides, or at least all positive tests, should be verified by colposcopy with biopsy. None of the studies they reviewed met both of these criteria. Five studies were considered to be of high quality, although not ideal. Four were paired-sample studies and the fifth took separate specimens from each patient, selecting conventional and liquid-based cytology at random. Among these five studies, the liquid-based Pap tests classified more slides as ASC-US or LSIL, but the conventional Pap smear classified more as HSIL. Only four studies allowed valid estimates of sensitivity and specificity. The investigators concluded that the available literature does not allow for the conclusion that the liquid-based Pap test is more accurate than the conventional Pap smear. In addition, they did not feel that the studies justified the claim that liquid-based cytology yields a lower rate of unsatisfactory specimens than conventional Paps.

A subsequent large study in Italy[44] used a screening population (N = 45,174) and randomized women to liquid-based or conventional cytology. All abnormal slides were reviewed by a supervisor or team of cytotechnologists. All abnormals were sent for colposcopy. At the cytology threshold of ASC-US, they found the liquid-based Pap was significantly more sensitive in detecting CIN 1 or worse, but was no different from the conventional Pap in detecting CIN 2 or worse. The positive predictive value to detect CIN 2 or worse or CIN 3 or worse was significantly higher with the conventional Pap (ie, the liquid Pap would result in a higher false-positive rate, leading to more unnecessary colposcopies without diagnosing more high-grade dysplasia or cancer).

Most recently, Arbyn and colleagues[36] performed a meta-analysis of eight studies that compared liquid-based and conventional cytology. Of these, only two appeared in Davey's previous analysis.[46] Each of the studies subjected all women to a reference standard of colposcopy. In addition, these investigators included the large randomized Italian study cited above.[44] The Italian study had, by far, the largest sample size of the eight studies and may have exerted undue influence over the meta-analysis results. Given that caveat, this meta-analysis also found no significant difference between the two technologies in terms of sensitivity or specificity to diagnose CIN 2 or worse at a cytology threshold of either LSIL or HSIL. When the threshold of ASC-US was used, the specificity was lower for the liquid-based method.

So, is the liquid-based technique a better Pap test? Arguably not, but it does have advantages that, despite its increased cost, are likely to keep it widely used in the United States. The primary attraction of the liquid pap is the ability to use the residual fluid, after the cytology preparation, to test for HPV DNA and also gonorrhea and chlamydia. The 2001 and 2006 American Society for Colposcopy and Cervical Pathology

(ASCCP) Consensus Guidelines state that, where possible, reflex HPV is the "preferred" triage modality for the ASC-US Pap result.[37] Although screening with cytology and the HPV DNA test is not as widely used as reflex HPV,[35] it may become more the norm in the future. It is unlikely that the conventional Pap smear will return to dominance in the United States.

CAN WE REPLACE THE PAP TEST WITH HUMAN PAPILLOMAVIRUS DNA TESTING?

The causative role of high-risk types of HPV in the development of CIN 2,3 and cervical cancer is well established. The place for HPV DNA testing in cervical cancer screening has been the subject of extensive research over the past decade. A clinically validated solution hybridization test, has been found to be a sensitive and cost-effective test for triage of a cytology diagnosis of ASC-US.[37,38,47] In 2003, this test was FDA approved for use with cytology for primary cervical screening in women of at least age 30. Since then, several studies have confirmed the efficacy of combined screening with Pap plus HPV, and others have evaluated HPV DNA testing alone as a screening test. Studies from around the world have been published comparing screening with cytology alone versus HPV DNA testing alone or HPV in combination with cytology. Findings vary in degree, but are consistent in direction. Compared with cytology alone, HPV DNA testing is more sensitive, less specific, and has a higher negative predictive value. The combined cytology plus HPV DNA testing has the highest sensitivity, the lowest specificity, and the highest negative predictive value.[48,49]

Currently, in the United States, HPV DNA testing is FDA approved for screening only when used in conjunction with cytology. The ACS[19] and the ACOG[20] have approved this combination as appropriate for use in women aged 30 and older. These professional organizations and the ASCCP[37] recommend that if screening results on cytology and HPV DNA testing are negative, the interval until the next screening should be no shorter than 3 years.

A large-scale National Cancer Institute study conducted in a large health maintenance organization in Portland, Oregon, justifies this practice.[50] Conventional Pap smear and HPV DNA testing by solution hybridization were performed at baseline on 20,810 women who were then followed with routine cytology and customary follow-up for the next 10 years. The negative predictive value for CIN 3 or worse after 45 months of follow-up for the combination of a negative HPV DNA test and a negative Pap test was 99.84%. This finding compared with 99.47% for cytology alone and 99.76% for a negative HPV test alone. Recently published studies from Sweden[51] and The Netherlands[52] confirmed the efficacy of screening with the combination of HPV DNA and cytology and compared it with screening using cytology alone. These studies used conventional Pap testing and HPV DNA testing with a polymerase chain reaction–enzyme immunoassay test that used G5P+ /6+ HPV primers. This latter test includes the same 13 high-risk HPV types as the commonly used solution hybridization test plus HPV 66. Both studies compared the rate of diagnosis of precancerous CIN lesions at an initial testing session with the diagnosis at a second round of testing 4 or 5 years later. The subjects in the Swedish study were aged 32 to 38; in the Dutch study, the women were 29 to 56. In the initial round of testing in the Swedish group, combined testing found 31% more CIN 3 or worse than did cytology alone. Combined testing in the Dutch study diagnosed 70% more CIN 3 or worse. In the second round of testing, combined HPV plus cytology found 47% and 55% less CIN 3 or worse in the Swedish and Dutch studies, respectively. In both studies, the total number of lesions with CIN 3 or worse diagnosed over the two rounds of testing did not differ between the two screening groups. This finding suggests that the combination of cytology and HPV DNA testing allows earlier diagnosis

of existing high-grade lesions and may justify prolonging the screening interval with the use of the combined testing.

Cuzick and colleagues[53] reviewed eight studies from Europe and North America that compared the efficacy of the Pap test with that of HPV DNA testing used alone for primary screening. More than 60,000 women, mostly aged 30 to 60, were included. Six study sites used the solution hybridization test; two sites used consensus polymerase chain reaction with G5P+ / 6+ primers. All used a split sample methodology in which Pap and HPV testing were done from the same cervical sample. Most used conventional cytology. All studies were performed on screening populations and most excluded women who had had a recent abnormal Pap test. The sensitivity of HPV DNA testing to detect CIN 2 or worse based on colposcopic biopsy was homogeneous across studies, at 96.1% The sensitivity remained constant across age groups. The specificity for CIN 2 or worse was 90.7% but was lower in studies with younger women. The positive predictive value of a positive HPV DNA test was 15.5% and was higher in younger women than in women older than 35 years of age.

In contrast to screening with HPV DNA testing, the sensitivity of cytology varied considerably among the various studies reviewed by Cusick and colleagues[53] and was consistently and significantly lower than HPV testing. The overall sensitivity of the Pap test for CIN 2 or worse was 53.0%. The positive predictive value of cytology was higher than that of HPV testing, 20.3%. Cytology had the higher specificity, 96.3%, although in women older than age 35, the differences in specificity were small.

A recently published study from Eastern Canada[54] used a unique study design. Instead of using a split sample, patients were randomized into groups undergoing the Pap test first followed by HPV DNA testing or the HPV DNA test first followed by cytology. A total of 10,154 women aged 30 to 69 were screened. Conventional cytology or HPV DNA testing by solution hybridization was used. Women were sent for colposcopy and biopsy for a Pap result of greater than or equal to ASC-US or a positive HPV DNA test at a threshold of 1 pg/mL. An additional random sample of women who had negative results was evaluated colposcopically. The relative sensitivities of HPV testing versus Pap test to detect CIN 2 or worse were 94.6% and 55.4%, respectively. Corresponding specificities were 94.1% and 96.8%, respectively.

A large, recently published Italian study[55] included more than 49,000 women, one half of whom were randomized to screening with conventional cytology and one half to screening with solution hybridization HPV testing. Patients screened with cytology were triaged to colposcopy at the ASC-US threshold; those in the HPV arm were referred to colposcopy for a positive result of at least 1 pg/mL. Results were published as a ratio of the sensitivities of HPV DNA test to cytology. In women aged 36 to 60, the relative sensitivity of the HPV test to detect CIN 2 or worse on biopsy was 1.92 (95% CI 1.28–2.87) compared with cytology. For women aged 25 to 34, it was 3.50 (95% CI 2.11–5.82).

A study from Hammersmith Hospital in London[53] evaluated the duration of protection offered by a negative screening Pap smear versus negative HPV by solution hybridization. The investigators followed 2516 women aged 35 and older for a median of 6.4 years. Women who had negative cytology at baseline had a risk for developing CIN 2 or worse of 0.33%, 0.83%, and 2.20% in 1, 5, and 9 years, respectively. For those who had negative HPV by solution hybridization at baseline, the risks were 0.19%, 0.42%, and 1.88%, respectively. The investigators observed that it takes 6 years for the rates of high-grade dysplasia in women who are initially negative by HPV DNA test to reach the same level seen after 3 years in women whose initial test was a negative cytology. Therefore, they argue, it would be reasonable to extend the screening interval to 6 years if HPV DNA is used for primary screening.

CERVICAL CANCER SCREENING IN THE POSTVACCINE ERA

The final chapter in the story of cervical cancer screening has yet to be written. Currently, only one HPV test is FDA approved. Others will follow, as will type-specific tests approved for clinical use. It has been suggested that if HPV DNA testing continues to prove more sensitive than cytology, it may ultimately replace cytology as the principal cervical cancer screening modality.[2] Combined HPV DNA testing and cytology has already taken the dominant role in some practices in the United States. Cytology, with its reliance on specialized laboratories, cytotechnologists, and pathologists, is too costly for most resource-poor countries, including those where cervical cancer is most prevalent. An inexpensive, sensitive HPV test would be appealing as the primary screening test of the future. Before this can happen, however, large-scale studies will be needed to derive algorithms for the clinical application of the various HPV molecular tests that may soon hit the market. Standardization of the results of the various technologies will be needed so that different tests can lead to common follow-up pathways. These pathways have yet to be defined. Will a positive HPV DNA screening test trigger a repeat HPV test, or should the Pap test become the measure that triages an HPV-positive woman to colposcopy? At what age should we start screening with HPV DNA? Current evidence suggests not before age 30. How then should we screen the woman younger than 30 in a future age of screening HPV tests? And how about the woman who has been immunized against HPV types 16 and 18? The preadolescents targeted in the initial rollout of the HPV vaccine, and who stand to gain the most protection from immunization, will not reach the age of greatest risk for high-grade dysplasia for at least another decade. They will not reach the age of highest risk for cancer for another 2 decades or more. Until then, it is clear that periodic screening must continue for them and for older newly vaccinated women. Once most vaccinated women reach their 20s and 30s, how will we screen? Will cervical cancer caused by non-16 and non-18 HPV types increase? By that time, a vaccine may be able to protect against additional HPV types; how will that change our practice? These questions are not purely rhetorical. Research will continue and practice guidelines will follow. It is truly an exciting time to be involved in the prevention of cervical cancer, and the only sure statement that can be made is that our practices will continue to change with new developments. Our patients will undoubtedly benefit.

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Overview of the Cytology Laboratory: Specimen Processing Through Diagnosis

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KEYWORDS

• Cytology laboratory • Pap tests
• Squamous dysplasia • Cervical cancer
• HPV test • Computer-assisted screening

The initial step of cervical cancer screening of women by a Papanicolaou (Pap) test leads to a report generated by the pathology laboratory. Much relies on this Pap test result and subsequent laboratory results. Most importantly, does the patient return to her usual screening schedule or does this result prompt consideration of other management options? The laboratory and pathologist may generate other relevant lab results: human papilloma virus (HPV) test, cervical biopsy and endocervical curettage, leep biopsy, and hysterectomy results all potentially follow from that initial Pap test.

There are a number of important steps undertaken as a Pap test navigates its way from the clinic to the laboratory, involving numerous laboratory personnel, including pathologists, different test options, a variety of instruments, diagnostic criteria and terminology, and always quality assurance. The laboratory and pathologist's primary goal is to produce clearly stated high-quality results for subsequent management of women with both normal and abnormal Pap results. Essential to this goal is effective communication between pathologist and clinician, starting with the information on the Pap test requisition form and extending to the final pathology report. A clear understanding by clinicians of how testing occurs within the laboratory can bolster this effective communication and is important background for understanding results.

This article provides a basic overview of the cytology laboratory to clarify specifics on how Pap test specimens are handled, diagnosed, and quality assured. Discussed is the flow of Pap test processing, including the task of actual Pap screening, computer-assisted screening, diagnosis generation, Pap test diagnostic terminology and criteria, reflex testing, and the steps taken by laboratories for quality assurance.

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Obstet Gynecol Clin N Am 35 (2008) 549–563
doi:10.1016/j.ogc.2008.09.003 **obgyn.theclinics.com**
0889-8545/08/$ – see front matter

TYPES OF PAP TESTS

From the initial acquisition of a Pap test in the office or clinic, the clock starts in the delivery of a reliable result to a clinician and patient. Pap test sensitivity begins when the Pap test is obtained and cervical dysplasia is sampled from a woman's cervix. Despite best efforts at obtaining a Pap test, it is clear that cells of a dysplasia do not always make it onto the resulting Pap slide. Inadequate sampling of dysplasia is beyond the control of the cytology laboratory and so the laboratory focuses on the steps after the Pap test has been collected to produce the highest quality result.

In these days of extensive market penetration of liquid-based Pap tests, the days of conventional Pap smears with poor preservation and air-drying artifact are gone in much of the country. Liquid-based Pap tests, with their discrete circle of a thin layer of cells, were developed largely to reduce or eliminate the specimen-quality problems that have plagued conventional Pap smears. They have the additional advantage of ancillary testing for HPV, gonorrhea, and chlamydia. There are two liquid-based Pap tests currently on the market, the ThinPrep Pap Test (Cytyc Corporation, Boxboro, MA), and the SurePath Pap Test (Becton, Dickinson and Company, Franklin Lakes, NJ).

The actual type of liquid-based Pap test varies between laboratories and between gynecologists, each of whom may have their own test preferences based on experience, marketing, and other factors. Features that may lead a gynecologist to prefer one test over another may be different than those of the cytology laboratory director, who has other factors to consider. The laboratory makes its decision about which liquid-based Pap test to use based on ease of specimen processing and automation, processing instrument costs and reliability, costs of test disposabes (specimen filters), specimen unsatisfactory rate for particular instrument types, company service support, and availability of computer-assisted screening.

From the standpoint of the gynecologist, the manufacturers of liquid-based Pap tests have made it relatively simple to use either type of test. The ThinPrep Pap Test and the SurePath Pap Test differ as to whether the collection device is agitated to dislodge cervical cells into the preservative vial and then discarded in the clinic (ThinPrep) or the device is simply deposited into the vial, with both sent to the laboratory for processing (SurePath). Because of the very large expense of processing instruments, most laboratories will have chosen one manufacturer over the other and clinicians will be encouraged to use that particular test. Very large laboratories can afford different types of processing instruments, so in that setting there may be a choice of liquid-based Pap tests and clinician preference can be accommodated.

ThinPrep and SurePath use very different technologies in specimen processing. ThinPrep uses a filter-transfer technology, where each specimen uses a relatively expensive nonreusable filter to capture cells and transfer to a slide. To make a second ThinPrep test slide from a patient for any reason necessitates use of another filter and effectively doubles the cost for the laboratory. SurePath uses a gradient centrifugation technique that produces a cellular pellet, which is resuspended and an aliquot of sample is applied to a slide. The SurePath disposables are less expensive than ThinPrep filters, so there is a lower cost to generation of a second slide should one be needed. Both technologies produce a circle of well-preserved cervical cells, with minimal cell overlap and little obscuring debris or blood, permitting improved visualization of cells and any infectious organisms.

PAP TEST PROCESSING AT THE CYTOLOGY LABORATORY

The flow of a Pap smear through the cytology laboratory involves many steps: sample accessioning into the laboratory information system, aliquoting of liquid-based sample for ancillary testing (gonorrhea, chlamydia, and HPV), preparation of the sample for staining (conventional smears) or processing and then staining (liquid-based Pap tests), computer-assisted screening, cytotechnologist screening, review of abnormal Pap tests by a pathologist, and finally, results entry into laboratory information system with report delivery to the requesting physician or clinic.

On a Pap specimen's arrival at the cytology laboratory, the slide or vial with its test requisition form and attendant information will be entered or "accessioned" into the laboratory's information system and given a unique accession number. The computer will check to see if the patient has had any previous Pap smears evaluated by the laboratory and if so, what those results were. If the patient is noted to have a history of previous abnormal Pap tests she is considered high risk and her Pap test will receive additional attention, such as a second screening. If ancillary tests are requested from a liquid-based Pap test, these will usually be ordered at this point.

The Pap requisition form contains the patient's name, date of birth and other identifiers, and other pertinent clinical information. This labeling and identification requirement on both the specimen and requisition is absolute; its absence is cause for a specimen to be rejected, necessitating a woman returning for a repeat Pap test.

Additional information needed on the requisition form includes the specimen source (cervix or vagina), a woman's last menstrual period or, if postmenopausal, history of previous abnormal Pap tests and treatment, history of HPV tests, and an indication of being at high risk for dysplasia because of early age at sexual debut, multiple sexual partners, and a history of sexually transmitted diseases, such as gonorrhea, chlamydia, or syphilis. Also important are any history of gynecologic malignancy and any treatment with radiation and chemotherapy. Hormonal and contraceptive history are relevant as to birth-control type (oral contraceptives, injectable or implants, intrauterine devices), if pregnant or postpartum, and use of estrogen replacement therapy. In the future, information regarding prior HPV vaccination may be requested on a Pap test requisition.

A number of the above patient information items are important for the cytology personnel to know while they are reviewing the Pap test, as they may produce changes in the cells themselves and fore-knowledge of these features may be critical for correct interpretation.[1,2] Knowledge of patient age is critical, as specimens from postmenopausal women may have characteristic changes, such as cell immaturity, histiocytes, multinucleated cells, and parakeratotic changes, and these changes would be quite unusual in a reproductive-age woman. Hormonal history is important, whether from exogenous hormones for contraception, recent pregnancy, or menopausal, as cellular changes result from hormones that can be interpreted as atypical unless viewed in the context of hormone history. Contraceptive hormones from implants and injections, medroxyprogesterone acetate in particular, impart changes to cells, such as nuclear enlargement and increased nucleus-to-cytoplasm ratio.

Pregnancy-related hormone changes can lead to squamous and glandular atypia and can have a confusing picture with decidual cells, Arias-Stella cells, and even trophoblasts. A postpartum Pap tends to look atrophic with histiocytes and can have atypical features related to the hormonal milieu of the postpartum state. This postpartum pattern generally resolves in 6 to 8 weeks or persists if a woman is lactating. The date of the last menstrual period allows assessment of whether endometrial cells are present during the proliferative phase, when they would normally be

expected, or need further explanation. Cervix and vaginal sources differ as to whether endocervical cells would be expected.

Intrauterine devices are well known for producing atypical endocervical or endometrial cells with nuclear enlargement, multinucleation, prominent nucleoli, and vacuolization. These changes can be explainable in the context of intrauterine device use but can be worrisome for a dysplasia or adenocarcinoma without that knowledge. This is also true for previous treatment for malignancy, with both radiation and chemotherapy. Radiation change in particular, can cause nuclear enlargement, multinucleation and nuclear hyperchromasia.

ANCILLARY TESTING

The requisition form allows requests for ancillary testing from liquid-based Pap tests. For ancillary testing of any type, an aliquot of the residual media is typically sent from cytology to the laboratory's microbiology area for those specific tests. Following the Center for Disease Control and Prevention's guidelines, gonorrhea and chlamydia testing can be done from an aliquot of the preservative. A separate report is generally issued with those results, although some laboratories might generate a composite report with both Pap and microbiology results.

Reflexive HPV DNA testing following a Pap test with the diagnosis of ''atypical squamous cells of undetermined significance'' (ASCUS) has increased substantially over the past 6 to 7 years. It is now estimated that 85% of United States women with ASCUS Pap results undergo reflex HPV testing.[3] Most laboratories try to combine Pap test and HPV DNA test results in a single report, although this may lead to a slight delay in report generation in awaiting the completion of both tests.

HPV DNA testing is performed using residual fluid from a liquid-based Pap test with Digene HPV Hybrid Capture 2 (hc2) test (Qiagen Incorporated, Valencia, CA). This test is currently the only HPV DNA test that is FDA-approved and only for use with the ThinPrep Pap test. HPV testing with the SurePath Pap test has not yet obtained FDA-approval; however, many laboratories offer hc2 with SurePath after they have performed in-house validation of the test. A validation study can be done by double collection of a number of patient specimens in both SurePath and ThinPrep media and performing hc2 testing on both specimen sets to ensure HPV-test result concordance. There are several other HPV DNA tests that are currently undergoing testing and in a few years there should be other HPV test options.

HPV DNA testing by the Digene hc2 can also be performed in women with conventional Pap tests; however, it requires an additional cervical sample taken either at the same time as the Pap test or on a return visit. That sample can be placed into Digene's Standard Transport Media and sent to the laboratory for HPV DNA testing.

PROCESSING AND STAINING OF PAP TESTS

The vials with liquid-based Pap tests then undergo special processing with either ThinPrep or SurePath instruments that convert the cell-liquid suspension to glass slide with a discrete circle of cervical cells, with little cellular overlap. The slide will be labeled with its unique accession number associated with the patient's name. The next step in the processing of both conventional and liquid-based Pap tests is staining by the Pap stain. With the Pap stain, the nucleus takes on varying shades of blue, allowing analysis of nuclear size, color, and contour details. The cytoplasm stains orange, pink, or blue, varying with degrees of cytoplasmic keratinization.

COMPUTER-ASSISTED SCREENING

The stained slides are then ready either for primary screening by a cytotechnologist or computer-assisted screening. Computer-assisted Pap test screening detects cervical cell abnormalities by having a computer analyze every square millimeter of a Pap test slide. There are currently two such devices that are FDA-approved and in use in the United States. One is the FocalPoint device (Becton, Dickinson and Company, Franklin Lakes, NJ). The other is the ThinPrep Imaging System (Cytic Corporation, Boxboro, MA).

The ThinPrep Imaging System is called a ''location-guided'' screening system for use only with ThinPrep Pap test slides. The imaging system scans a slide and selects 22 areas on the slide that are the most worrisome for a squamous intraepithelial lesion (SIL). These areas are noted by coordinates, allowing a cytotechnologist to review those 22 areas and decide if abnormal cells are present or not. If abnormal cells are present, the slide then goes to a pathologist for a final decision as to diagnosis. The manufacturers of FocalPoint are also developing a location-guided system like the ThinPrep system, but it is not yet approved by the FDA.

The FocalPoint system works in a different manner and is called a ''primary screening'' instrument. It scans SurePath slides, as well as conventional slides, but is not approved for ThinPrep slides. At the end of the computer scanning a given set of slides, it can declare up to 25% of the slides in the set to be normal and need no further review by cytotechnologists or pathologists and can be auto-archived (or filed away). The remaining 75% of Pap test slides in a given set (considered the ''most abnormal'') are ranked into quintiles from 1 to 5 (number 1 quintile being the most abnormal) and also receive a score related to the percent chance of having an abnormality compared with the other Pap slides in that set. This quintile and percentage information accompanies the slide to the cytotechnologist, who then completely screens the slide as usual. Because of the computer's prescreening and ranking capabilities, review of slides by cytotechnologists and pathologists can pay additional attention to slides considered at higher risk for abnormalities.

While the FDA approval of FocalPoint allows for up to 25% of Pap tests to have no further human review (auto-archived), many laboratories have these slides screened by cytotechnologists, nonetheless. This allows the reassurance of all Pap tests being reviewed at least twice: by both a computer and one or two human beings. From a laboratory management standpoint, a primary screening instrument can also effectively reduce a laboratory's workload by 25% if the FocalPoint instrument is used to screen then auto-archive slides with no further human review.

The manufacturers of both instruments claim that their computer-assisted screening can reduce the incidence of a laboratory's false-negative Pap tests. Laboratories using these instruments may not all see a huge reduction in false-negative rate simply because their rate was initially low. Laboratories with problems with a higher rate of false-negative Pap tests may be the most likely to benefit from this technology.

CYTOTECHNOLOGIST SCREENING OF PAP TESTS

Specimen Adequacy

After computer-assisted screening, if performed, a Pap test is screened by a cytotechnologist who microscopically examines the Pap slide, aided by microscope devices to allow systematic review of the entire slide. While it is obviously impossible to look at every cell on a slide, the vast majority of cells will be viewed and assessed by the cytotechnologist. For a conventional Pap smear, this ranges between 100,000 and 300,000 cells, while liquid-based Pap tests will have somewhat fewer cells: about 75,000 cells are typically contained within their cellular circles.

While screening, a cytotechnologist will assess specimen adequacy by several criteria: Are there enough squamous cells present? Was the transformation zone sampled, as seen by endocervical or squamous metaplastic cells? Are there obscuring factors hindering diagnostic interpretation, such as blood, inflammation, or thickly smeared slides?

Cytotechnologists use well-defined criteria for specimen adequacy that are specific for both conventional and liquid-based Pap tests, as found in The Bethesda System 2001, a publication resulting from National Cancer Institute (NCI)-sponsored conferences in Bethesda, Maryland, outlining adequacy and diagnostic criteria.[4] Well over 90% of Pap slides are usually acceptable in terms of cellularity and cell visibility.

Conventional smears tend to have more adequacy problems because of thickly smeared slides, obscuring blood, inflammation, and poor specimen fixation. Rapid fixation of conventional Pap smears remains absolutely critical to having a sample that is reliably interpreted, as poor fixation can result in enlarged and irregular nuclei, both features of abnormal cells but also the result of inadequate fixation. Liquid-based Pap tests are also assessed for adequate cellularity and presence of obscuring blood. Bloody specimens can still present problems with ThinPrep Pap tests, which can be ameliorated by the addition of a small amount of glacial acetic acid to lyse red blood cells. ThinPrep Pap tests may rarely produce slides with low cellularity, despite collection of adequate cells, particularly if certain lubricants are used or if a specimen contains abundant mucus or sperm. Based on this adequacy analysis, the decision may be made that a Pap smear is of marginal value for detecting cervical precancers and is deemed unsatisfactory.

By Bethesda System criteria, Pap tests with some adequacy problems may still be considered satisfactory, with the report listing the specific ''quality indicators'' that are an issue. Quality indicators include scant cellularity, partially obscuring blood or inflammation, poor fixation, thickly smeared slides, and absence of endocervical or transformation zone (EC/TZ) component.

Until 2001, endocervical cells or squamous metaplasia cells indicating transformation zone were required for a Pap test to be considered satisfactory for evaluation. The changes in the Bethesda System in 2001 no longer necessitated these cells for a satisfactory test. The rationale for this change is that while squamous dysplastic cells are more likely to be seen on Pap tests where an EC/TZ component is present, other studies have shown that Pap tests lacking the cells are no more likely to have squamous dysplasia than those where such cells are absent.[5,6] There does not seem to be a correlation between false-negative Pap tests and lack of an EC/TZ component.

There is a general impression among cytopathologists that endocervical cells may be more difficult to see on liquid-based Pap tests because of cell orientation and fragmentation of endocervical groups. In an effort to reduce having their tests lack the EC/TZ component, some laboratories implement additional rescreening of any Pap test that on initial review is thought to lack endocervical or transformation zone cells. The current practice of assessing EC/TZ adequacy may change in the future with regards to endocervical adenocarcinoma, which appears to be on the increase.

Identification of Abnormal Cells

During screening, a cytotechnologist highlights with a marking pen abnormal cells, both squamous cells and endocervical, for future review and diagnosis by a pathologist. Specific infections, such as candida, trichomonas, bacterial vaginosis, and herpes simplex virus, are noted and recorded during this review.

At the conclusion of this examination, the cytotechnologist will render a diagnosis and generate a report. Over 90% of the slides will generally be diagnosed ''negative

for intraepithelial lesion or malignancy.'' All of the cytotechnologist's observations on normal Pap tests will result in the formal negative Pap smear report, which will either be mailed, faxed, or computer delivered to the originating physician office or clinic. Negative Pap tests are not reviewed by a pathologist unless the cytotechnologist diagnoses an accompanying benign reactive or reparative process, which sometimes can be diagnostically challenging.

A Pap test with abnormal cells detected by a cytotechnologist is given a diagnosis and sent to a pathologist for review, final diagnosis, and report generation. The pathologist may agree with the cytotechnologist's diagnosis, they may downgrade it to a negative diagnosis, or upgrade to a higher grade lesion. Diagnosis criteria and terminology for Pap tests are discussed later in this article.

SOURCES OF ERROR IN THE CYTOLOGY LABORATORY

At any one of these steps in a Pap test's trip through the laboratory, there are opportunities for errors. Much of the daily work in the cytology laboratory revolves around keeping the number of errors to a minimum. Unfortunately, they still occur and the **Box 1** shows some of their sources, grouped into three general types: prelaboratory, laboratory, and postlaboratory errors.

''Prelaboratory errors'' occur at the clinic or doctor's office and relate to Pap test collection, preparation, slide labeling, delivery to the laboratory, and preparation of a Pap requisition form. ''Laboratory errors'' are those relating to entry into the laboratory computer system, slide staining, laboratory labeling, cytotechnologist screening, and pathologist interpretation. ''Postlaboratory errors'' are those relating to generating the final report with final delivery of that report to the clinic and ultimately to the patient.

Included in prelaboratory errors are poorly collected or prepared conventional Pap smears that are not promptly fixed in alcohol or thickly smeared. Also problematic are incomplete Pap smear requisition forms that omit the important patient history discussed previously. Unlabeled or mislabeled Pap tests, leading to specimen rejection by the laboratory, also fall into this category. The specimens themselves can be compromised before receipt by the laboratory, with glass slide breakage beyond repair and leakage of liquid-based Pap test media from failure to secure the vial lid. These situations will also require a test to be repeated.

After the specimen's arrival at the cytology laboratory, ''accessioning'' errors are possible where any of the information listed on each patient's Pap test requisition form may be entered incorrectly into the laboratory's computer. Potential misentries include the wrong patient name, date of birth, past medical history, date of last menstrual period, the ordering physician, and billing information. Most laboratories have quality-assurance procedures for quickly catching and correcting identification errors.

Errors occurring during Pap test screening by cytotechnologists are typically cited as a cause of false-negative Pap tests and the reduced sensitivity of Pap tests. As mentioned above, failure to sample a dysplasia at the time the Pap is performed is also a substantial contributor to the test's false-negative rate. Pap test screening by cytotechnologists is a difficult and demanding job. Conventional Pap smears contain on average 200,000 individual cells, while liquid-based Pap tests have fewer cells (about 75,000), but they are often more concentrated so that it may take as much attention and time to screen as a conventional Pap smear. Cytotechnologists may screen 60 to 80 Pap tests a day, and some abnormal cells are occasionally missed. The laboratory's quality-assurance procedures are designed to minimize these false-negatives and are discussed later in this article.

Box 1
Sources of error encountered in Pap test evaluation

Prelaboratory errors (at clinic)

Poorly prepared Pap smears

- Poor fixation in alcohol
- Cells smeared too thickly
- Transformation zone not sampled
- Bloody smear taken during menses
- Inflamed smear taken during infection
- Unlabled slide or vial

Inadequate information on the requisition form

- Incorrect or missing patient name
- Incorrect or missing patient identifier
- Incorrect or missing patient birthdate

Laboratory errors (at cytology laboratory)

Incorrect entry of patient information into laboratory computer system:

- Patient name, birthdate, last menstrual period, ordering physician, numerical identifiers, billing information

Screening errors by cytotechnologists:

- Abnormal cells not identified when present

Interpretative errors by cytotechnologists or pathologists:

- Abnormal cells are identified but incorrectly interpreted

Postlaboratory errors (laboratory to clinic)

Pap smear completed and reported at laboratory but no report generated (computer system failure)

Report mailed or faxed to physician but never received

Report delivered to physician but results not relayed to physician

Results mailed to patient but never received

Lastly, a pathologist's interpretation of abnormal cells identified by the cytotechnologist can be exceedingly difficult and subjective. Pathologists are sometimes called upon to make a diagnosis on as few as one or two cells out of the hundreds of thousands of cells on a slide. This difficult task can rarely result in interpretive errors, whereby abnormal cells are felt to be normal (false-negative). Conversely, normal cells may be over-interpreted to be abnormal (false-positive), which is why all atypical reports must be followed by further examination and testing.

Postlaboratory errors revolve around ensuring each woman gets her Pap result. Errors may result from any problems with a laboratory's report-printing operation, mail delivery service, fax machines, or office personnel who do not relay results to doctors or to patients. Most cytology laboratories send to each doctor's office or clinic a monthly list of all of their patients who have received an atypical Pap test report. The doctor's office can then verify receipt of those Pap reports. Some cytology

laboratories will also call a clinic or office with the results from any Pap test showing cervical cancer or a serious precancerous lesion.

LABORATORY QUALITY-ASSURANCE PRACTICES

The Clinical Laboratory Improvement Act of 1988, otherwise known as CLIA '88, outlines the procedures laboratories must undertake to ensure Pap test quality. While directed at pathology laboratories as a whole, many of the provisions of this legislation are quite specific for Pap tests. The following describes some of the CLIA '88 provisions that are in place in every cytology laboratory in this country and are required for laboratory accreditation by the College of American Pathologists, an organization with deemed status by the Joint Commission.

Ten-Percent Slide Rescreen

The laboratory must rescreen a minimum of 10% of the slides diagnosed as normal by having a second cytotechnologist rescreen the entire slide. This must be done before the final release of a Pap-test report. In general, Pap tests from all high-risk women are selected to be rescreened as part of that 10%. If a laboratory uses FocalPoint computer-assisted screening, the FDA requires 15% of cases rescreened taken from the pool of normal cases in the first quintile (the most abnormal) of the computer's assessment. FocalPoint also has built-in criteria to select additional cases to rescreen for "quality control review," also from the first and second quintiles. Ten percent of slides rejected by the computer for poor quality will also be rescreened.

Through all these additional rescreening mechanisms, laboratories may routinely rescreen over 20% of their negative cases. Not only does this practice serve to reduce false-negative Pap tests, but it also allows the laboratory to keep close tabs on cytotechnologist performance. Any cytotechnologist who may not be screening with a high degree of accuracy can be recognized and dealt with quickly by increased oversight, additional in-service training or retraining, and proficiency testing.

Five-Year Look-Back

This CLIA '88-mandated practice requires a review of all previously diagnosed negative Pap tests for any woman who has a newly diagnosed cervical cancer or high-grade squamous intraepithelial lesion (HSIL). In this way, a laboratory can recognize screening or interpretation errors (again, false-negative Pap tests), and perhaps potentially identify problem cytotechnologists and pathologists. A laboratory is not required to report any previous false-negative Pap tests revealed by this review. CLIA '88 stipulates that laboratories need to report or amend reports only if a changed diagnosis will result in patient management changes.

Pap Test and Cervical Biopsy Correlation

This provision from CLIA '88 requires that the laboratory compare a woman's original abnormal Pap test with the resulting cervical biopsy to ensure the diagnoses of these two samples agree. In this way, laboratories can detect Pap tests that are incorrectly diagnosed as dysplasia when they are actually normal (false-positive Pap tests). This also ensures cervical biopsies are correctly diagnosed. Laboratories generally see approximately 10% discordant Pap test/cervical biopsy pairs, whereby a dysplasia is seen on the Pap test but the cervical tissue samples are negative. When reviewed, occasional false-positive Pap tests are detected and occasionally deeper tissue sections of a cervical biopsy are necessary to demonstrate a dysplasia. Any changes in diagnoses of either Pap tests or cervical biopsies generally leads to notifying a clinician of

the change and an amended report, as patient management may change. Most commonly, the review shows that both Pap tests and cervix specimens have been diagnosed correctly and it must be assumed that the dysplasia was missed by colposcopy or that a dysplasia has resolved over time.

Pap Test Proficiency Testing

Also part of the original CLIA '88 legislation is the requirement for cytology laboratories to conduct regular competency testing of their cytotechnologists and pathologists involved in Pap screening and diagnosis. Since 2006, proficiency testing occurs as an annual test administered to all physicians and cytotechnologists involved in diagnosing Pap tests in the United States. They must successfully pass the test to continue their work in diagnostic gynecologic cytopathology.

DIAGNOSTIC TERMINOLOGY FOR CERVICAL CYTOLOGY

The Bethesda System (TBS) 2001 is the currently accepted terminology for cervicovaginal abnormalities that is widely in use in the United States (**Box 2**).[3] It is a modification of TBS from 1988 and the result of NCI-sponsored meetings of expert cytopathologists, gynecologic pathologists, and those involved in management of patients with cervical abnormalities. TBS is a two-tiered system for squamous dysplasia, with squamous cervical precancers placed into low- or high-grade categories: low-grade squamous intraepithelial lesion (LSIL) and HSIL. It is designed to replace the several other older terminology systems (**Box 3**) and appears to have increased reproducibility amongst pathologists. TBS relates clinically relevant information for appropriate management and reflects current understanding of cervical neoplasia with respect to HPV biology. **Table 1** lists the diagnostic criteria of squamous abnormalities through HSIL and the following discusses some issues specific to each diagnostic category. **Fig. 1** illustrates the squamous morphologies seen in normal and dysplastic Pap tests.

NEGATIVE FOR INTRAEPITHELIAL LESION OR MALIGNANCY

The diagnosis of "negative for intraepithelial lesion or malignancy" is used for Pap tests that show normal squamous and endocervical cells lacking nuclear and cytoplasmic features of HPV infection. From TBS 2002, this diagnostic category also includes normal tests with benign endometrial cells in women greater than or equal to 40 years of age. Since being implemented in 2002, this practice has prompted an increase in endometrial biopsies in women in this age group, which includes both premenopausal and postmenopausal women.[7]

Recent studies indicate that routine endometrial sampling of women with normal Pap smears and endometrial cells present is clinically significant and cost-effective only in postmenopausal women or in premenopausal women 40 years and older only if they are symptomatic with abnormal bleeding or are otherwise at risk for endometrial hyperplasia (eg, with polycystic ovarian syndrome).[8] Some laboratories now have added a comment in this setting that states the significance of endometrial cells is uncertain and needs to be evaluated in the context of other clinical data, such as age, menstrual status, pattern of bleeding, health history, hormone-replacement therapy (including tamoxifen), and contraception.

Negative Pap tests from a vaginal source after hysterectomy may occasionally contain a comment about the presence of benign glandular cells. There are a several explanations for this, such as vaginal endometriosis, Bartholin glands, periurethrual and perivaginal glands, vaginal adenosis, remnants after surgical or ablative therapies, or prolapsed fallopian tube.[2] The glandular cells must look completely benign to be

Box 2
Pap smear diagnoses: the 2001 Bethesda System for classifying cervicovaginal smears

Negative for intraepithelial lesion or malignancy

Infection

- *Trichomonas vaginalis*
- Fungal infection such as *Candida* (yeast)
- Bacterial vaginosis
- Herpes simplex virus
- *Actinomyces*

Other findings

- Reactive cellular changes:
  - Inflammation (includes repair changes)
  - Radiation effects
  - Intrauterine contraceptive device (IUD)
  - Atrophy
  - Glandular cells present after hysterectomy

Epithelial cell abnormalities

Squamous cells

- Atypical squamous cells of undetermined significance
- Atypical squamous cells of undetermined significance, favor HSIL
- Low-grade squamous intraepithelial lesion
- High-grade squamous intraepithelial lesion
- Squamous cell carcinoma

Glandular cells

- Atypical glandular cells of undetermined significance (endocervical, endometrial, or not otherwise specified)
- Atypical glandular cells of undetermined significance, favor neoplasia
- Endocervical adenocarcinoma in-situ
- Endocervical adenocarcinoma
- Endometrial adenocarcinoma

considered negative, as the presence of any glandular atypia in such smears would warrant a diagnosis of atypical glandular cells.

ATYPICAL SQUAMOUS CELLS OF UNDETERMINED SIGNIFICANCE

The reality of cervical cytology is that not all squamous abnormalities seen on Pap smears are clearly defined as either LSIL or HSIL. TBS includes an equivocal category of ASCUS, which reflects the difficulties that cytopathologists can have in confidently placing all cervical squamous abnormalities into two categories. There are causes of atypical squamous cells other than HPV infection, such as atrophy, exuberant repair, or reactive processes. Approximately 25 million women each year receive an ASCUS diagnoses on their Pap tests. The current management guidelines have increased the

Box 3
Comparison of diagnostic terminology for cervical squamous precursor lesions

Low-grade squamous intraepithelial lesion

Mild or slight dysplasia

Cervical intraepithelial neoplasia 1 (CIN 1)

High-grade squamous intraepithelial lesion

Moderate or severe dysplasia

Cervical intraepithelial neoplasia 2 and 3 (CIN 2 and 3)

Carcinoma in-situ

utility of this diagnosis when it is coupled with an HPV test to determine women with high-risk HPV and those truly at risk for a precancer.[9] Between 10% and 20% of women with ASCUS Pap results will prove to have HSIL on a subsequent tissue biopsy,[10,11] and because there is a larger number of ASCUS Pap tests, they ultimately detect more HSIL than HSIL Pap tests.[12]

Management of women with an ASCUS diagnoses was addressed by the NCI-sponsored multi-center ASCUS/LSIL Triage Study (ALTS). This study led to two management documents, one in 2002 and the second modified in 2007.[9] ALTS confirmed the wisdom of the two-tiered Bethesda system in diagnosis of cervicovaginal lesions and the validity of using HPV test results in women with ASCUS as a more sensitive way to detect underlying HSIL than repeat Pap tests.

Table 1
Diagnostic criteria for squamous abnormalities in cervical Pap tests

ASCUS	ASCUS-H	LSIL	HSIL
Nuclei 2.5–3 times increase over normal intermediate cell	Atypia in immature cells	Atypia in mature cells	Atypia in immature cells
Slight increase in N:C	Nuclei 1.5–2.5 times increase over normal metaplastic cell	Nuclei 3× increase	Variability in cell size including small cells
Mild nuclear hyperchromasia and chromatin irregularity	Increase in N:C similar to HSIL	Slight increase in N:C	Marked increase in N:C
Mild variation in nuclear shape	Variations in nuclear size and shape	Nuclear hyperchromasia and coarsely granular chromatin	Marked nuclear hyperchromasia with fine to course chromatin
Atypical parakeratosis	Nuclear membrane irregularity	Variations in nuclear size and shape	Variations in nuclear size and shape
		Binucleation and multinucleation	Marked nuclear membrane irregularity
		Variably irregular nuclear membranes	Syncytial groups possible
		Perinuclear cavitation or koilocytosis (cytoplasmic halo)	

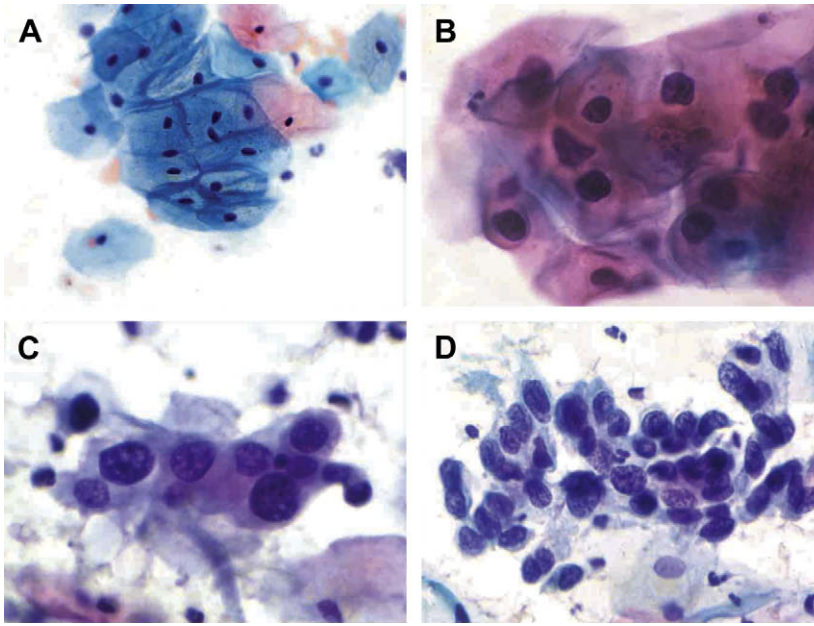

Fig. 1. Papanicolaou test squamous cytology. (*A*) Normal squamous and endocervical cells. (*B*) Low-grade squamous intraepithelial lesion. (*C*) High-grade squamous intraepithelial lesion; moderate dysplasia/CIN 2. (*D*) High-grade squamous intraepithelial lesion; severe dysplasia/CIN 3.

ATYPICAL SQUAMOUS CELLS OF UNDETERMINED SIGNIFICANCE CANNOT EXCLUDE HSIL

This diagnostic category (called the ASCUS-H) can be used for situations when there is a high suspicion of HSIL, yet the atypical cells are too scant or poorly visualized for a firm diagnosis. The Pap tests tend to show nuclear atypia in immature squamous metaplastic cells. The usefulness of this subcategory of ASCUS is very clear, with the positive predictive value of the ASC diagnosis for HSIL being close to 50%.[13,14] The current management recommendation for this diagnostic category is colposcopy followed by HPV DNA testing or serial cytology, if no HSIL is identified on tissue sampling.[9]

LOW-GRADE SQUAMOUS INTRAEPITHELIAL LESION

ALTS also investigated HPV DNA testing for high-risk HPV in women with LSIL Pap tests, and found that over 80% of them were positive for high-risk HPV.[15] It appears that LSIL is a low-risk lesion caused by a large number of different HPV types, both low and high risk. Some women with LSIL will have an underlying and unsampled HSIL, and the current management guidelines addresses this possibility with colposcopy, with tissue biopsy now being the recommended action rather than repeat Pap testing.[16] Occasionally, an LSIL Pap report may include a comment that HSIL cannot be excluded if the test shows predominantly LSIL with only rare cells suggesting HSIL. Approximately 18% of women with LSIL Pap test diagnoses later prove to have HSIL on tissue biopsy.[10]

HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESIONS

Women with the diagnosis of HSIL have a cervical lesion that has a significant risk for cervical cancer. ALTS demonstrated that 97% of women with this diagnosis test positive for high-risk HPV DNA.[11] Management of women with HSIL is based on colposcopy findings, with the most recent management recommendations taking into account a woman's age and whether the lesion is CIN 2 or CIN 3 by biopsy.[16] CIN 2 dysplasias in adolescents appear to have a different behavior than CIN 2 in older women, which allows a management option of observation rather than surgery in young women 20 years and younger.

SQUAMOUS CELL CARCINOMA

Pap tests suspicious for or diagnostic of invasive squamous cell carcinoma are fortunately rare compared with diagnoses of precancers. The morphology of squamous cell carcinoma can vary in the degree of differentiation and presence and absence of keratinization. Some appear identical to HSIL on Pap tests, particularly microinvasive squamous cell carcinoma. Because of the immediate need for tissue confirmation, Pap tests suspicious for squamous cell carcinoma may be diagnosed as HSIL (CIN 3), with a comment that invasive squamous cell carcinoma cannot be excluded. False-positive Pap diagnoses of squamous cell carcinoma are possible because of some overlap in key morphologic features. Markedly atypical keratinization can be seen in invasive tumors but can also overlie a keratinizing dysplasia. Invasive squamous cell carcinoma can demonstrate the presence of prominent nucleoli; however, that feature can also occasionally be seen in CIN 3, a reason to be cautious with a firm diagnosis of cancer.

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A Tissue Basis for Colposcopic Findings

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KEYWORDS

• Cervix • Colposcopy
• Dysplasia • Acetowhite • Punctation • Mosaicism
• Atypical vessels

Colposcopy was developed by Dr. Hans Hinselmann as a means to examine occult cervical preinvasive and invasive lesions. Working in collaboration with the Leitz optical company, his final working model consisted of a stereoscopic lens system mounted on a moveable stand with a white-light source. The lenses had variable magnifications of 10×, 20×, 30×, and 40×. A screw could be used for fine-magnification adjustment. Hinselmann tried various liquid agents to remove mucus and to act as a light contrast. After trying substances such as saline, cedarwood oil, dilute silver nitrate, iodine, and dilute acetic acid, he decided that the latter two were optimal for visualization of small lesions. Presently, acetic acid is universally used as an adjunct to colposcopic examination.[1,2]

As a tool for examination of the cervix and vagina, colposcopy is based on the variable absorption and reflection of white light off different tissue interfaces. Mucosal tissue color depends on the amount of hemoglobin viewed at the tissue surface, which gives the tissue different degrees of redness. The degree of redness depends on the distance between the underlying vasculature and the surface, which indirectly implies the amount of cellular material (stroma and epithelium) between the vessels and surface.[3]

How acetic acid works as a contrast agent is unclear. Although acetic acid can improve the surface light reflection by dissolving mucus, it can also modify cellular proteins, including cytokeratins and nuclear proteins.[2] Confocal microscopy before and after the application of acetic acid has demonstrated an increased nuclear signal, which implies increased light scattering by nuclear material.[4] Lastly, it is believed (but not yet proved) that acetic acid dehydrates the cell, which removes most of the cytoplasm. After dehydration, the cell is left with organelles, cytoskeleton filaments, and nuclear proteins. The effects of acetic acid are transitory: when rehydration of the cell cytoplasm occurs, any protein alterations revert to their normal state.[3]

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Obstet Gynecol Clin N Am 35 (2008) 565–582
doi:10.1016/j.ogc.2008.09.009 **obgyn.theclinics.com**
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Because acetic acid specifically modifies cell cytoplasm and nuclear proteins, the contrast created by its application to the cervical and vaginal mucosa depends on the number of surface epithelial cells, the amount of cytoplasm in these cells, and the amount of nuclear material in each cell. It would follow that more light would be absorbed and little light would be reflected if there were few surface cells with small nuclei and large amounts of cytoplasm. The effects of acetic acid on these cells would require frequent reapplications to maintain the dehydrated state. The opposite (more light reflection) would occur if the surface interface were to consist of numerous cells with large nuclei and small amounts of cytoplasm. The affects of acetic acid would last longer because these cells would have little cytoplasmic fluid to rehydrate.

THE NORMAL CERVIX

Ectocervix and Endocervix

The normal cervix consists of the *ectocervix* (exocervix) present on the portio, which is lined by mature squamous epithelial cells, and the *endocervix*, lined by columnar cells. The squamous epithelium contains multiple cell layers that vary due to age and the ratio of the hormones estrogen and progesterone. An increased amount of estrogen leads to cornification of the squamous cells into pseudolayers (basal, parabasal, intermediate, and superficial cells). Near the basement membrane, these cells are immature, with an increased nuclear-to-cytoplasmic ratio. In contrast, the superficial cells have small punctate nuclei and large amounts of glycogenated cytoplasm. Although they vary depending on the cell type, the overall amount of cytoplasm and nuclear material is moderate in well-estrogenized squamous epithelium. The blood vessels supplying these cells consist of small capillary arcades deep within the underlying stroma, with branching feeder vessels that extend into approximately one-third the thickness of the epithelium. Specifically, these vessels supply the immature basal and parabasal cells.

The endocervix is covered by a single layer of columnar cells. The cells have small nuclei and a moderate amount of cytoplasm at the cell base. The vessel loops supplying the endocervix can be found directly underneath the columnar cells (**Fig. 1**).[5,6]

The application of acetic acid to the ecto- and endocervix in a reproductive-age woman has contrasting effects. Dehydration and alteration of the nuclear proteins in the squamous epithelial cells results in equivocal amounts of light absorption and refection such that the surface coloration is typically pink. The postmenopausal woman has fewer squamous cells that are mature. In this case, slightly more light may be reflected than absorbed such that the surface color may be less pink and more gray. In addition, because there are fewer cells between the capillary arcades and the surface, these vessels are commonly visible. The endocervix, with its single layer of columnar cells and minimal nuclear material, has minimal light reflection and maximal light absorption. Therefore, the surface color of the endocervix is pink-red. Because the superficial squamous and columnar cells have relatively large amounts of cytoplasm, these color changes require frequent reapplications of acetic acid (**Fig. 2**).

THE TRANSFORMATION ZONE

Squamous metaplasia develops from subcolumnar reserve cells that develop in the region of the original or native squamocolumnar junction that, for various reasons, migrates to the portio. These reserve cells subsequently divide to form immature metaplastic cells that replace the columnar cells on the surface and in the endocervical crypts (glands). Over time, these metaplastic cells evolve into mature squamous

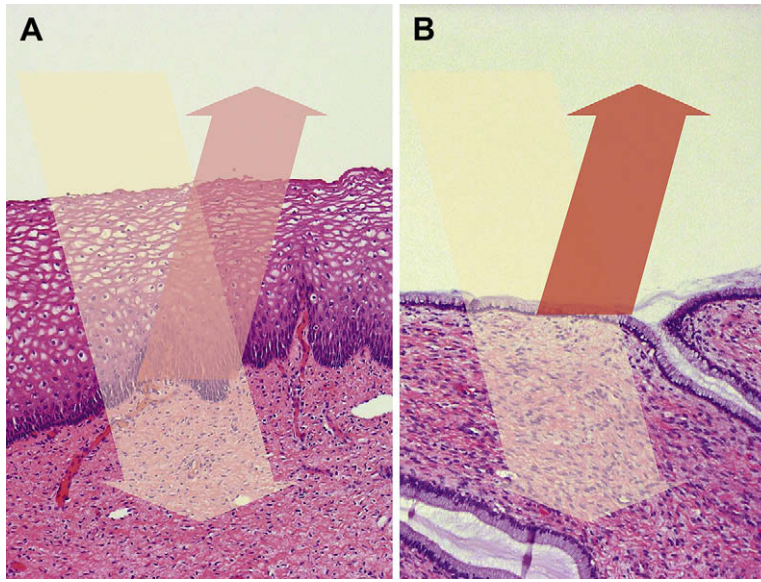

Fig. 1. Histology of the normal cervix. The ectocervix (*A*) has multilayered squamous epithelium with superficial vessels that extend into to basal portion. Although some light is absorbed through the surface to the underlying tissues after acetic acid application, a considerable amount is reflected back by the nuclear material in the squamous cells, which results in a pink coloration. The endocervix (*B*) has a single layer of columnar cells. Considerably more light is absorbed than reflected after acetic acid application, resulting in a deeper red coloration (hematoxylin and eosin, intermediate magnification).

cells that merge into the original squamous epithelium.[7,8] Viewed microscopically, individual metaplastic cells show nuclei that are larger than those present in mature squamous and columnar epithelium. In addition, these nuclei are uniform in size throughout the thickness of the metaplastic area (**Fig. 3**). Thus, the area of metaplasia

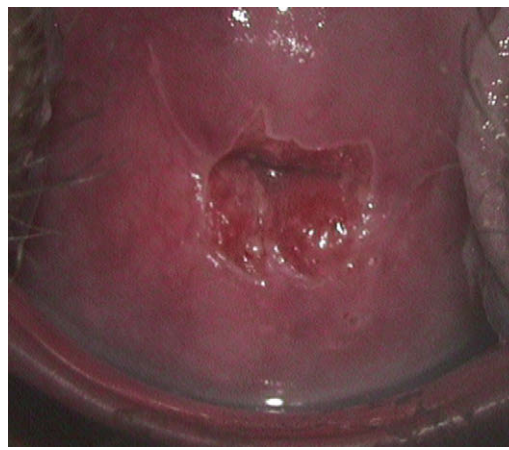

Fig. 2. Colpophotograph of a normal cervix. 5% acetic acid has been applied. The ectocervix appears pink relative to the more red endocervix. (*Courtesy of* A. Waxman, MD, MPH, Albuquerque, NM)

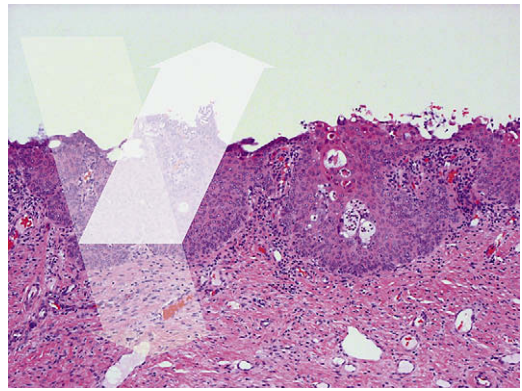

Fig. 3. Histology of the transformation zone. The metaplastic cells are more uniform in size and have nuclei that are larger than the nuclei of mature squamous epithelial cells. More light is reflected than absorbed after acetic acid application, which results in a white coloration. Chronic inflammatory cells are also present, which causes production of capillary vessel loops that extend to the surface of the metaplastic cells (hematoxylin and eosin, intermediate magnification).

reflects relatively more light than mature estrogenized squamous epithelium and appears flocculent or snow white. The comparative degree of color change varies with the developmental evolution of the metaplasia at a particular point in time. The cells adjacent to the original squamous epithelium have minimal morphologic differences with those cells, and their color change is similar. The least mature metaplastic cells adjacent to the newly formed squamocolumnar junction, however, demonstrate a prominent white coloration next to the red endocervix, resulting in a distinct linear edge at the inner aspect of the transformation zone (**Fig. 4**).[3,8]

As is typical in areas of high cell destruction and replacement, the transformation zone shows areas of inflammation, which are occasionally acute and erosive but

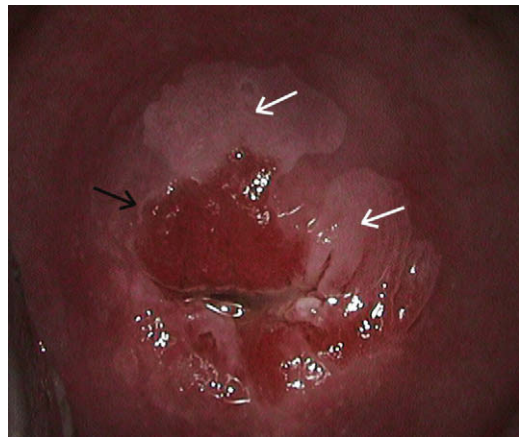

Fig. 4. Colpophotograph of metaplasia. The linear acetowhitening at the squamocolumnar junction typical of squamous metaplasia (*black arrow*). The larger, well-defined acetowhite lesions (*white arrows*) are also manifestations, though less common, of immature squamous metaplasia. (*Courtesy of* A. Waxman, MD, MPH, Albuquerque, NM)

are usually chronic. The inflammation and the immature metaplastic cell growth generates an influx of feeder vessels that grow from the stroma through the metaplastic epithelium to the surface. These fine vessel loops appear on end as small uniform punctate dots to the colposcopist.

Although the metaplastic cells have less cytoplasm and larger nuclei than the adjacent mature squamous and columnar cells, the overall change is still small such that multiple reapplications of acetic acid are required to see the white color change in the metaplastic area.[3,8]

THE ABNORMAL TRANSFORMATION ZONE

The abnormal colposcopic changes that occur in the transformation zone are divided into two general categories: cellular and vascular (angiogenic). Cellular changes include those color changes that occur without (leukoplakia) and with (acetowhite) the application of the contrast agent acetic acid. Vascular changes include those seen with the influx of capillary loops (punctation), further arborization and coalescence of these intraepithelial vessels (mosaicism), and nonuniform growth (atypical vessels) (**Box 1**). Although most of these changes are seen in different degrees of squamous and glandular intraepithelial lesions, they can also occur in benign conditions (eg, the fine punctation seen in areas of metaplasia with inflammation).[3]

ACETOWHITE CHANGES

Low-Grade Intraepithelial Lesions

Persistent infection by human papillomavirus (HPV) eventually results in productive growth of the virus in an immature (basal or parabasal) epithelial cell. As the epithelial cells mature, shed, and lyse, they release their intranuclear viral particles that have the potential to reinfect other sites on the cervicovaginal mucosal surfaces.[6] Thus, lesions related to these viral infections can vary in size and shape. They are not necessarily confined to the transformation zone and can involve any site on the portio or vagina. Although often flat, these lesions can be papillary (wartlike), which is consistent with the viral nature of the infection.

Box 1
Classification of the abnormal transformation zone

- Cellular changes
  - Leukoplakia
  - Acetowhite
- Vascular (angiogenic)
  - Punctation
    - Fine
    - Coarse
  - Mosaicism
    - Fine
    - Coarse
  - Atypical vessels

The histologic lesions categorized as cervical intraepithelial neoplasia (CIN) 1 contain cells indicative of human papillomavirus infection. Specifically, after the HPV DNA enters the basal cell's nucleus, stimulation of viral DNA replication causes these cells to proliferate to a point whereby they occupy approximately one third of the lower surface epithelium. As the intranuclear HPV reacquire their protein coats, signals from the viral DNA cause these cells to mature, resulting in intermediate and superficial cells with enlarged abnormal nuclei.[6] Many of these cells have perinuclear clearing, signifying aggregation of cytoplasmic organelles and filaments toward the cell periphery (koilocytosis) (**Fig. 5**). Due to the proliferation of basal cells and their nuclei, along with the increase in nuclear size in the intermediate and superficial cells, there is an overall increase in the amount of nuclear material compared to noninvolved squamous epithelium and, to a lesser degree, less mature metaplastic epithelium. The interface (border) between the HPV-involved epithelium and the benign epithelium varies in prominence depending on the cell type. Specifically, immature metaplasia and CIN 1 differ only in the presence of koilocytes, whereas the border between CIN 1 and normal endocervix, the latter with its single layer of columnar cells, is more prominent.[6,9]

After the application of acetic acid, the increased nuclear material along the epithelial surface reflects comparatively more light than normal and metaplastic squamous and glandular epithelium. Viewed colposcopically, CIN 1 is a snow to bright white compared with the translucent or flocculent white of metaplasia. As with metaplasia, however, repeated applications of acetic acid may be required to maintain this acetowhite change, and in some cases, the difference in the degree of whiteness between an immature metaplasia and CIN 1 can be hard to distinguish. Nevertheless, in contrast with metaplasia, CIN 1 lesions are multiple and not necessarily confined to the transformation zone. They are often large and geographic, with borders that may fade into areas of metaplasia (**Fig. 6**).[3,10,11]

High-Grade Intraepithelial Lesions

Whereas low-grade lesions equate to infection by HPV, high-grade lesions represent viral DNA modifications resulting from integration into the immature host cell DNA. The resultant overproduction of various oncoproteins leads to structural alterations,

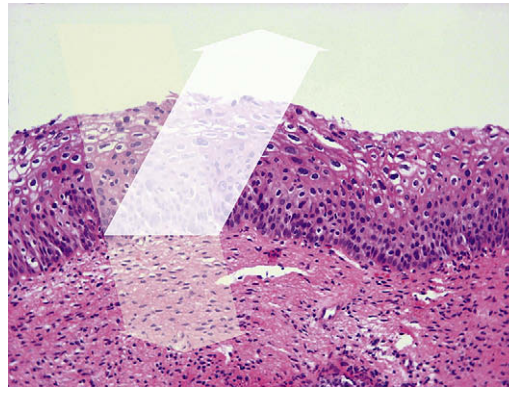

Fig. 5. Histology of CIN 1. The squamous cells show proliferation of cells near the base of the epithelium; the superficial cells show markedly enlarged nuclei with ballooning (clearing) of the cytoplasm. The increase of nuclear material results in more light reflection and a brighter white coloration after acetic acid application compared with that seen in metaplasia (hematoxylin and eosin, intermediate magnification).

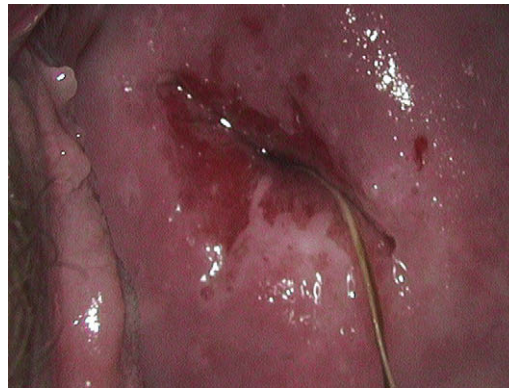

Fig. 6. Colpophotograph of a low-grade lesion. The posterior lip of this cervix shows a pale acetowhite lesion with geographic borders. (*Courtesy of* A. Waxman, MD, MPH, Albuquerque, NM)

unregulated cell replication, and immortality. Histologically, these abnormal (dysplastic) basaloid cells replace normal cells in the surface epithelium. The number of dysplastic cells that are present dictate the degree of squamous abnormality. Lesions with dysplastic cells that occupy between one third and two thirds of the surface epithelium are consistent with CIN 2. Lesions characterized by dysplastic cells that involve greater than two thirds of the surface epithelium (eventually replacing the entire surface epithelium) represent CIN 3. Nevertheless, the basement membrane remains intact.[6,9]

Cytologically, these cells reflect the size and shape of a basal cell or immature metaplastic cell. They tend to be small and round to oval. The major difference is related to the size of the nucleus, which is enlarged with irregular nuclear borders. In addition, the nuclei are hyperchromatic, reflecting alterations in the nuclear chromatin. As the dysplasia worsens, the cell size decreases in relation to the enlarged nucleus such that (in CIN 3) the cell consists of a darkened nucleus with a small rim of cytoplasm (**Fig. 7**).[12]

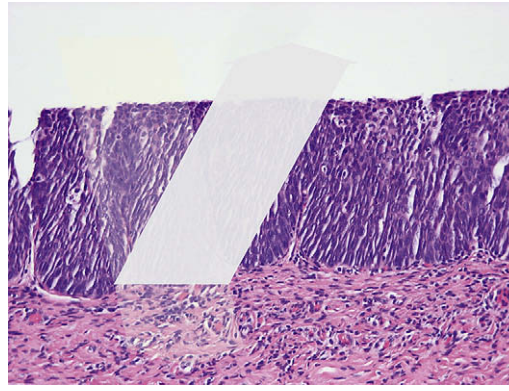

Fig. 7. Histology of CIN 3. There is an abundance of nuclear material in cells with very little cytoplasm, which exists throughout the surface epithelium. Consequently, after acetic acid application, essentially all light is reflected back and little is absorbed, resulting in a dull gray coloration (hematoxylin and eosin, high magnification).

When exposed to acetic acid, areas of high-grade CIN show minimal cytoplasmic effects relative to nuclear chromatin alterations, which reflects the high nuclear-to-cytoplasmic ratios seen in these cells. Thus, areas of high-grade CIN reflect considerably greater amounts of white light than is absorbed. The amount of acetowhite change seen in high-grade CIN is increased over that present in CIN 1 lesions and is considerably more prominent than the change seen in nondysplastic metaplasia. Descriptions for these color changes include bright white to dull oyster gray. In addition, these changes occur almost immediately after the application of acetic acid and require fewer reapplications to maintain them. Although there are cytologic and histologic differences between CIN 2 and 3, with relatively greater numbers of smaller abnormal cells for the latter, the disparity is not enough to distinguish degrees of whiteness between the two. From a colposcopic standpoint, it is probably best to limit the impressions regarding intraepithelial abnormalities to low- and high-grade intraepithelial lesions, which combines (as does the Bethesda System nomenclature for cytology) CIN 2 and CIN 3.[3,10,11]

Because the difference between normal and high-grade abnormalities is more pronounced than the interface between normal and low-grade lesions, the border between a high-grade acetowhite lesion and normal mucosa is markedly distinct. Typically, it is straight and smooth, lacking the irregular or geographic edges of low-grade lesions. This border is most striking in areas in which high-grade dysplasia ends at the new squamocolumnar junction. The dense cellularity in the thick dysplastic lesion, with little dehydration after application of acetic acid, tends to roll over the single cell layer of adjacent columnar cells, leading to raised or rolled borders (**Fig. 8**). When high-grade lesions are found adjacent to low-grade lesions, the difference in cellularity may appear as an internal border or margin.[3]

Other features particular to high-grade colposcopic lesions include their location high in the transformation zone at the new squamocolumnar junction. These lesions tend to be flat and smooth compared with low-grade lesions, which are often raised and papillary (**Fig. 9**). **Table 1** summarizes the acetowhite changes in colposcopic low- and high-grade intraepithelial lesions.[3,11]

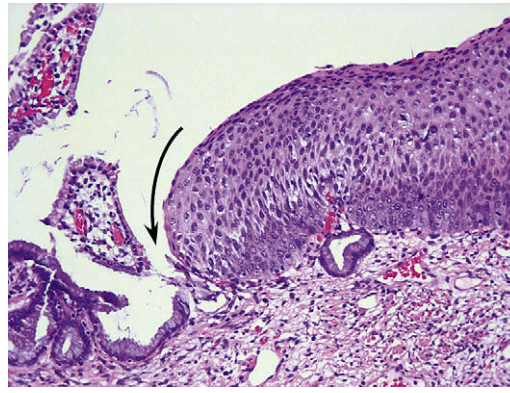

Fig. 8. Histologic interface between high-grade CIN (CIN) and normal endocervix. The sharp contrast between the multicellular CIN with its abundant nuclear material and the single nuclear layer of the adjacent columnar cells results in a raised and rolled border (*arrow*) after dehydration of cytoplasm from acetic acid application (hematoxylin and eosin, high magnification).

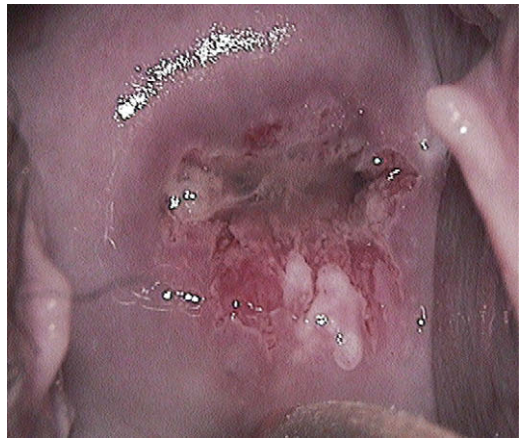

Fig. 9. Colpophotograph of a high-grade lesion. A distinct high-grade lesion is seen with straight borders and a "pasted-on" appearance. The lesion appears quickly and fades slowly after application of 3–5% acetic acid. (*Courtesy of* A. Waxman, MD, MPH, Albuquerque, NM)

LEUKOPLAKIA

Leukoplakia is defined as areas on mucosal surfaces that appear white on initial examination with or without magnification. No application of any contrast agent is required to generate this change. Leukoplakia has been described at various sites, including the mouth and tongue. It implies a surface interface that reflects most of the light directed toward the area. The condition that is invariably associated with leukoplakia is the production of abnormal amounts of keratin, a protective material usually present on the skin surface. Hyperkeratosis is defined as an overproduction of keratin, leading to a thickened surface layer. Parakeratosis indicates retained nuclei in the keratin, which is usually acellular. Because the cervix and vagina are mucosal tissues, keratin is not commonly present. Therefore, any keratin noted histologically on the cervix is considered hyperkeratosis. Reasons for abnormal keratin production on the cervix include CIN and irritation and reactive change such as that seen with prolapse (**Fig. 10**).

Table 1
Acetowhite changes in low- and high-grade colposcopic lesions

Feature	Low-Grade Lesion	High-Grade Lesions
Color	Snow white to bright white	Bright white to dull (oyster) gray
Lesion size and shape	Relatively large and geographic; raised and papillary	Relatively small; smooth and flat
Location	Throughout the ectocervix	In the upper transformation zone at or near the new squamocolumnar junction
Time interval to color change; number of reapplications	Slow to change; requires numerous reapplications to maintain color differential	Rapid change; requires few reapplications to maintain color differential
Border	Irregular; relatively indistinct	Straight, raised or rolled; prominent

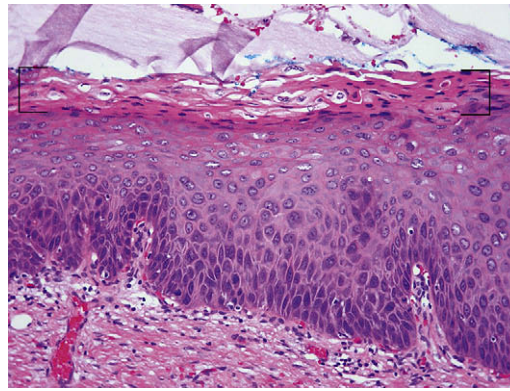

Fig. 10. Histology of CIN with hyperkeratosis and parakeratosis. A thickened layer of pink-red keratin (*brackets*) overlays a high-grade dysplasia. The presence of nuclei in this layer is indicative of parakeratosis (hematoxylin and eosin, high magnification).

Depending on the amount of surface keratin present, the cervix can have a glossy surface or a distinct white change when exposed to light (**Fig. 11**). Because the keratin is a barrier, application of acetic acid does not alter this appearance. It is unfortunate that the degree of whiteness with hyper- and parakeratosis only reflects the amount of keratin present and not the condition of the cells underneath. The only way to document whether an abnormality is present with abnormal keratin production is to perform a biopsy.[6,13]

VASCULAR (ANGIOGENIC) CHANGES IN INTRAEPITHELIAL LESIONS

Punctation

By its nature, CIN in the transformation zone represents an area of high cell replication and turnover, which invariably leads to production of angiogenic factors that generate

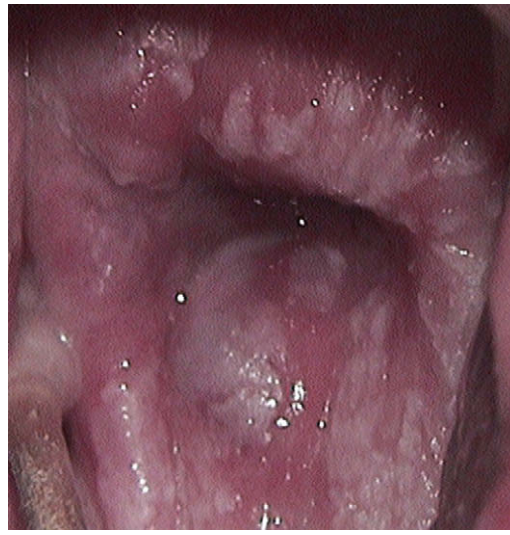

Fig. 11. Colpophotograph of hyperkeratosis. This cervix shows shiny raised plaques of hyperkeratosis and parakeratosis even before the application of acetic acid. Multiple biopsies found no evidence of dysplasia. (*Courtesy of* A. Waxman, MD, MPH, Albuquerque, NM)

an influx of numerous feeder vessels into the surface epithelium.[14,15] These capillary loops are seen on end by the colposcopist as punctate dots. In the presence of dysplasia, continued production of these factors leads to greater vascular growth and intrusion into the surface epithelium. The loops become larger, which leads to an increase in the size of the dots. In some cases, the loops extend beyond the superficial epithelium, and the punctate dots appear to "float" above the surface (**Fig. 12**). Continued vascular growth results in variably sized capillary loops, which is represented by irregularly sized surface dots.[3,6,13]

The distance between the capillary loops (intracapillary distance) increases as the amount of cell proliferation increases. As such, the higher the grade of CIN, the greater the distance between the capillary loops. This pattern occurs for two reasons. Although the dysplastic cells propagate from the basement membrane to the superficial epithelial surface, they also multiply along the various pseudolayers (lateral growth). This proliferation pushes the capillary loops apart and increases the intracapillary distance between each loop. In addition, as the overall cell numbers increase around each loop, they tend to compress the smaller loops. In the end, the colposcopist only recognizes the larger ones, which become irregularly spaced.[13,16]

The vascular pattern of punctation is subcategorized as fine or coarse depending on the size of the punctate dots and the spacing between the dots. Fine punctation, present in reactive metaplasias and low-grade CIN, is characterized by small uniformly sized dots with a decreased, consistently spaced intracapillary distance. Coarse punctation, seen in high-grade CIN, is characterized by large irregularly sized dots that may appear above the epithelial surface. The intracapillary distance is increased and the spacing is uneven (**Fig. 13**).[3]

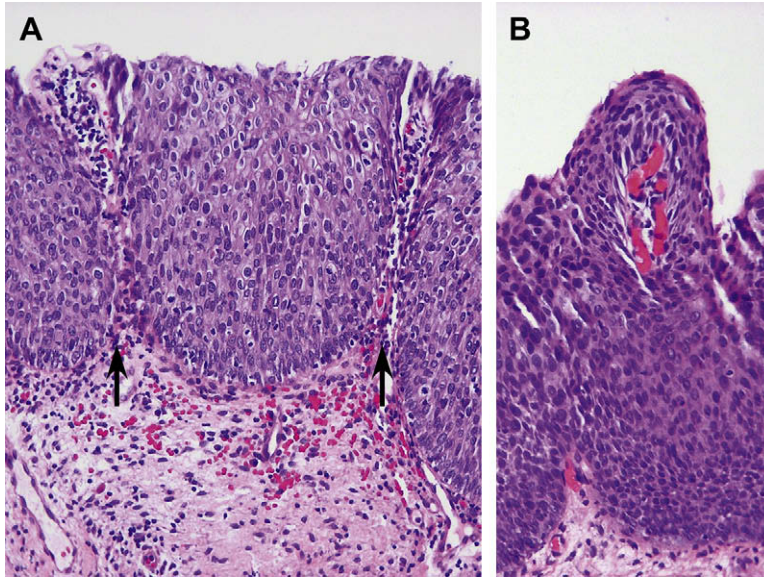

Fig. 12. Histology of punctation. (*A*) Prominent capillary loops (*arrows*) extend to the surface of a high-grade CIN. These loops can be so conspicuous (*B*) that they may occasionally appear to float above the surface (hematoxylin and eosin, high magnification).

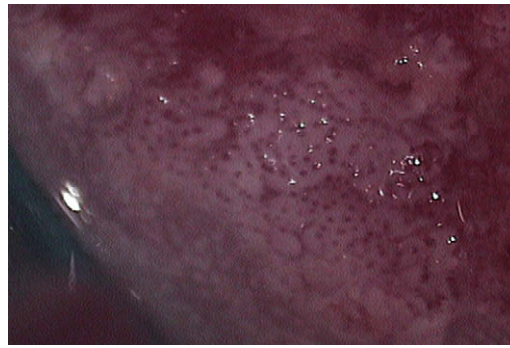

Fig. 13. Colpophotograph of punctation. Course punctation is seen on this cervix with high-grade CIN. The light reflexes suggest that the vessels are raised above the surface of the acetowhite epithelium. Mosaic changes can be seen at the periphery of this lesion. (*Courtesy of* A. Waxman, MD, MPH, Albuquerque, NM)

Mosaicism

Continued production of angiogenic factors in the presence of persistent cell production results in further vascular growth. Specifically, the capillary loops begin to arborize and coalesce, which produces a lateral vessel growth that surrounds and isolates the surface cells into individual nests. Histologically, the overall pattern resembles a mosaic, with the cellular material representing the individual tiles and the vessels symbolizing the surrounding grout (**Fig. 14**). Mosaicism is a natural progression from punctation, and it is common to see evidence of punctate dots adjacent to or within an area of mosaicism.[6,13]

Continued vascular growth within mosaicism results in changes similar to the coarse punctation seen in high-grade CIN. The tiles show irregular shapes and varying sizes. The vessel caliber also fluctuates. As with punctation, these changes are categorized as fine or coarse. Fine mosaicism, usually seen in reactive metaplasias and low-grade CIN, is characterized by small, regularly shaped tiles with uniformly sized surrounding vessels. Coarse mosaicism, usually seen in high-grade CIN, is characterized by larger tiles that vary in size and shape; the surrounding vessels are also nonuniform in size (**Fig. 15**).[3]

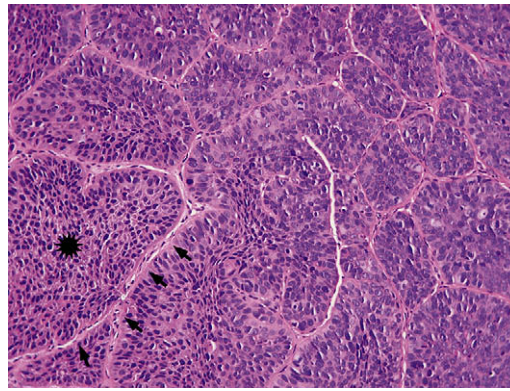

Fig. 14. Histology of mosaicism. Islands of high-grade CIN (*star*) are separated by coalescing lateral vessels (*arrows*) (hematoxylin and eosin, intermediate magnification).

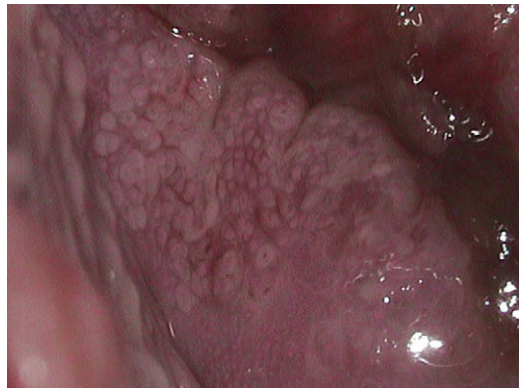

Fig. 15. Colpophotograph of mosaicism. Large caliber vessels, some with wide intercapillary distance and central "umbilicated" punctation are characteristic of high-grade CIN. (*Courtesy of* A. Waxman, MD, MPH, Albuquerque, NM)

The vascular changes seen in punctation and mosaicism are also modulated by the application of acetic acid. After the cells dehydrate, residual altered nuclear material causes compression of smaller capillary loops, and it is possible to lose lesser degrees of punctation or mosaicism in high-grade CIN. Because of this transient compression, many colposcopists examine the cervix after an initial application of saline to look for subtle angiogenic changes, then reexamine after application of acetic acid to see whether these changes are lost in the presence of an acetowhite transformation. In some traditional scoring systems, this transient loss of fine vascular growth patterns is considered more significant than persistence of these changes after acetic acid is applied.[17] **Table 2** summarizes the vascular changes seen in low- and high-grade colposcopic lesions.

CARCINOMA

The presence of squamous or glandular carcinoma is histologically characterized by disruption of the basement membrane barrier and infiltration of the underlying stromal tissues by malignant epithelial cells. Depending on the degree of differentiation, the

Table 2
Angiogenic changes associated with low- and high-grade colposcopic lesions

Feature	Low-Grade Lesion	High-Grade Lesion
Acetic acid change	Persistence of fine punctation/ mosaicism	Loss of fine punctation/ mosaicismPersistence of coarse punctation/mosaicism
Punctation	Predominately fine (uniformly sized, relatively small dots confined to the surface epithelium; uniformly close intracapillary distance)	Predominately coarse (variably sized dots may float above the surface epithelium; variable intracapillary distance is increased overall)
Mosaicism	Predominately fine (uniformly sized small tiles encased by uniformly sized small vessels)Fine punctation is often present	Predominately coarse (variably sized large tiles encased by enlarged vessels that are of nonuniform caliber)Coarse punctation is often present

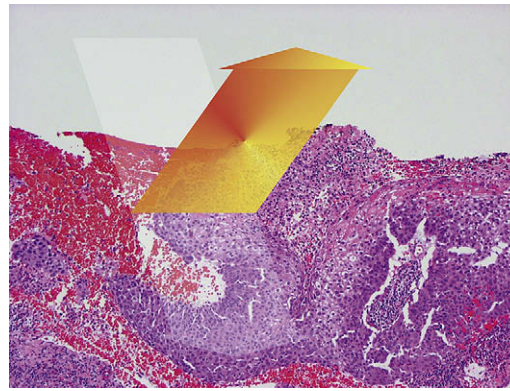

Fig. 16. Histology of invasive carcinoma. The surface is a mixture of malignant squamous cells, surface necrosis, and hemorrhage. Because of this, any reflected light has a yellow-red coloration (hematoxylin and eosin, low magnification).

proliferation of these cells can be pronounced and extensive. The growth is uneven and can result in irregular surface contours. Continued production of angiogenic factors results in an influx of more vessels to sustain growth and expansion of the cancer. Nevertheless, other areas, depending on their location, may outgrow and lose their blood supply. When this happens, necrosis occurs. Widespread necrosis, particularly near the surface, can result in erosion and vascular disruption, the latter leading to hemorrhage (**Fig. 16**).[6,18]

Extension of the malignancy beyond the basement membrane creates a reaction within the surrounding stroma known as desmoplasia. In addition to an influx of inflammatory cells, the fibromuscular stromal cells are replaced by fibroblasts and collagen, resulting in an overall dense fibrous effect.[6] Paradoxically, this fibrosis can result in a focal adjacent decrease in vascularity, and there may be minimal bleeding at a biopsy site.

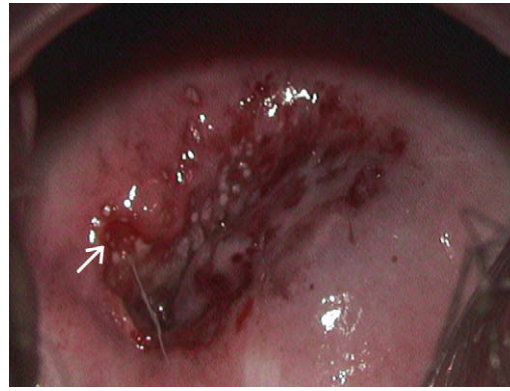

Fig. 17. Colpophotograph of occult cancer. This lesion is characterized by acetowhite epithelium extending into the endocervical canal. There is a faint orange coloration from necrosis (*arrow*). Ulceration is seen in the lower left aspect of the cervical os. The acetowhite line that characterizes the squamocolumnar junction is absent helping to identify this as an ulcer. (*Courtesy of* A. Waxman, MD, MPH, Albuquerque, NM)

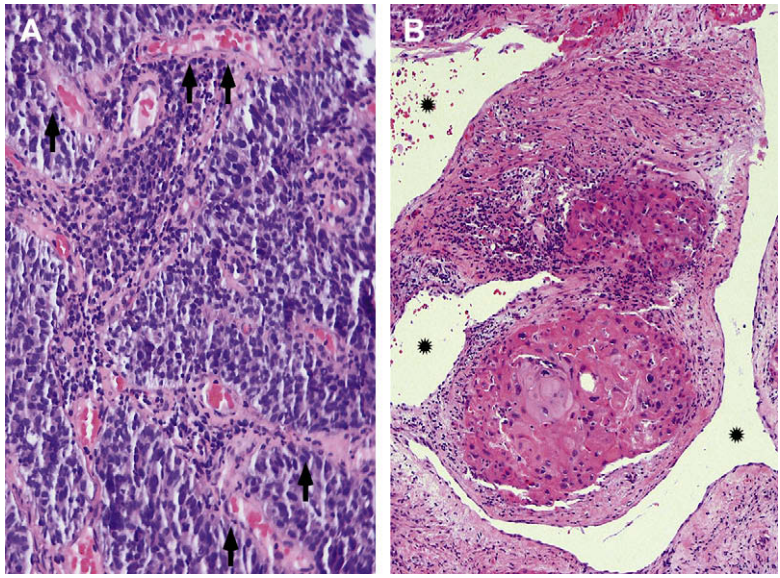

Fig. 18. Histology of atypical vessels. Malignant squamous cells (*A*) are surrounded by haphazardly arranged vessels with no uniform direction or branching (*arrows*). (*B*) Dilated vascular spaces (*stars*) separate small islands of invasive squamous cancer (hematoxylin and eosin, intermediate magnification).

When a small occult cancer is examined colposcopically after the application of acetic acid, the appearance varies depending on the size of the tumor. The lesions are usually large and often cover multiple quadrants. The color varies from dull oyster gray, which might indicate a microinvasive lesion within a high-grade CIN, to yellow (indicating the presence of necrosis), and to red (indicating hemorrhage). The surface topography is often raised and irregular (simulating an aerial view of a mountain range), cerebroid (brainlike), or depressed (indicating the presence of erosion or ulceration) (**Fig. 17**).[6]

Because carcinoma demonstrates an accelerated cellular growth above that seen in intraepithelial neoplasia, the production of tumor angiogenic factors is increased to allow continued neovascular growth and development. To keep up with continued tumor expansion, the newly established vessels lose their consistent branching patterns and are now arranged haphazardly (**Fig. 18**). The term used to describe this nonuniform appearance is *atypical vessels*. Atypical vessels are subcategorized on the basis of their general appearance and are grouped with other items that have similar characteristics; for example, ''glyphs'' (pictographs) and ''noodlelike,'' ''rootlike,'' and ''hair-pin'' vessels. Another common feature is the ability to branch abnormally. As normal vessels divide, their caliber progressively decreases in size. Atypical vessels can paradoxically increase in size as they separate (**Fig. 19**).[6,13]

Although the word ''atypia'' or ''atypical'' has various connotations depending on its use, the term, when applied colposcopically, is very specific. One must be careful when commenting on vascular changes; if unusual angiogenic patterns are seen that do not necessarily imply malignancy, then other descriptors should be used. The term *atypical* should be avoided unless there is a colposcopic impression of carcinoma.

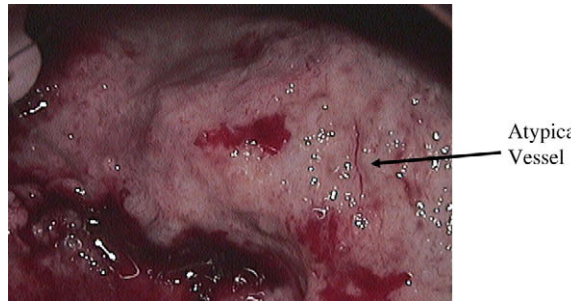

Fig. 19. Colpophotograph of atypical vessels. A straight "noodle-like" vessel is seen on this area of invasive cervical cancer. The vessel is not large, but starts and stops abruptly with no branches. (*Courtesy of* A. Waxman, MD, MPH, Albuquerque, NM)

SUMMARY

This discussion represents a somewhat simplistic explanation of how the topographic changes seen colposcopically can be explained by changes at the cellular level. Although these explanations may have some credence, there is much we do not understand regarding interactions between HPV and immature squamous cells in the lower genital tract. Recent analysis of cervigram images that have documented biopsy results and HPV data seems to suggest that lesions with HPV DNA type 16 are more readily identified.[19]

It is also clear that although acetowhite and angiogenic changes may vary among low-grade intraepithelial lesions, high-grade intraepithelial lesions, and carcinoma, there is considerable overlap. Analysis of lesions with neovascular growth showing increased intracapillary distance and atypical vessels demonstrates that although these features are most commonly seen in high-grade intraepithelial lesions and carcinoma, they can be present in low-grade and benign lesions.[16] In addition, many of these features, such as different degrees of whiteness and changes in vessel caliber, represent subjective observations. Because of this, the reproducibility of colposcopic impressions is not ideal, as documented by review of cervigram images and colposcopic impressions by colposcopic experts.[20,21] In hopes of improving the specificity of overall colposcopic impressions, various grading systems have been developed using different colposcopic features. It is unfortunate that these are also disappointing.[22,23] Nevertheless, it would still seem that there is a better degree of agreement in the presence of the higher-grade abnormalities.[24] In the end, the best results seem to occur when more biopsy samples are taken.[25]

Thus, it appears that changes in the surface cells of the cervix, particularly in the transformation zone, whether they are physiologic or pathologic, can result in alterations in light absorption and reflection. The ability to see different intensities in hemoglobin pigment and new vessel growth suggests the presence of surface abnormalities. These features, however, are not as discriminating as one would like. Documentation of disease can be accomplished only by tissue biopsy and histologic examination. The old adage "if it's white, take a bite" may be more reasonable than originally thought. If any abnormality is present, a biopsy is the most prudent course of action. On the other hand, as a corollary, when presented with a patient who has overt cervical carcinoma, it is recommended that the tumor mass be evaluated colposcopically. Although the examination will not contribute toward the eventual biopsy,

the opportunity to evaluate colposcopic features not commonly seen should be considered too good to ignore.

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To ECC or Not to ECC: The Question Remains

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KEYWORDS

• Endocervical curettage • ECC

Approximately 14 years ago, an article entitled, ''Endocervical Curettage Has No Place in the Routine Management of Women With Cervical Intraepithelial Neoplasia: Debate,'' and another entitled, ''Endocervical Curettage: A Technique in Search of an Indication?: Debate,'' were published in the same edition of a clinical monograph.[1,2] Despite significant advances in research involving cervical cancer and preinvasive disease screening, evaluation, and treatment, particularly involving the use of human papillomavirus (HPV) testing, controversy surrounding the usefulness of endocervical curettage (ECC) remains. Nonetheless, two American Society for Colposcopy and Cervical Pathology (ASCCP)-sponsored conferences with resultant publications of practice guidelines include ECC as a component of the clinician's armamentarium in the evaluation of women who have abnormal cervical testing. The objective of this article is to summarize evidence addressing ECC in the evaluation of women who have abnormal cervical tests in an attempt to provide readers with some background in assessing the usefulness of this procedure.

To consider the role of ECC, it is appropriate to consider squamous and glandular neoplasia independently. Therefore, evidence addressing ECC is summarized separately for each of these conditions as is the use of ECC as a postprocedure evaluation tool. Subsequently, the technique of performing the procedure is addressed, followed by a brief summary of the current guidelines regarding ECC. Finally, a significant yet often overlooked consideration—that of reproducibility of an ECC-based diagnosis—is addressed.

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Obstet Gynecol Clin N Am 35 (2008) 583–597
doi:10.1016/j.ogc.2008.09.007 **obgyn.theclinics.com**
0889-8545/08/$ – see front matter. Published by Elsevier Inc.

SQUAMOUS ABNORMALITIES

Four possible arguments regarding the use of ECC have been promulgated, including[3]

- Perform ECC regardless of colposcopic findings, as ECC may improve diagnostic accuracy and reduce the risk for occult cancer.
- Perform ECC in the setting of satisfactory colposcopy only, as an excisional procedure may be performed in a woman who has an unsatisfactory colposcopy anyways.
- Perform ECC in the setting of unsatisfactory colposcopy only, to detect occult cancer and potentially avoid unnecessary conization.
- Do not perform ECC at all as omission of the ECC does not reduce colposcopic accuracy.

It is, therefore, apparent that data regarding the usefulness of ECC in the evaluation of women who have squamous abnormalities are mixed based on the number of conclusions that have been offered.

Presented in **Table 1** is a compilation of studies reporting ECC results in women who have had squamous lesions, including the status of the colposcopic examination.[4–16] Interpretation of this data demonstrates that the prevalence of an abnormal ECC result is greater in women who have had an unsatisfactory colposcopy, and

Table 1
Compilation of studies reporting rates of positive (abnormal) endocervical curettage results relative to colposcopic status (adequacy) and the general conclusion of these studies

Study	N	Positive Endocervical Curettage: Satisfactory Colposcopy	Positive Endocervical Curettage: Unsatisfactory Colposcopy	Conclusion Regarding Performance of Endocervical Curettage
Drescher et al[4]	540	17.9%	48.7%	Perform always
Urcuyo et al[5]	259	8.6%	57.3%	Not if satisfactory colpo
Oyer and Hanjani[6]	518	1.4%	25.7%	Not if satisfactory colpo
Saltzman et al[7]	207	15%	NR	Perform always
Spirtos et al[8]	261	4.7%	NR	+ECC if satisfactory colpo is due to contamination
Hatch et al[9]	2304	13.9%	53%	Perform always
Granai et al[10]	278	11.5%	42%	Perform always
Krebs et al[11]	177	5%	45%	Not if satisfactory colpo
Naumann et al[12]	341	1.4%	NR	Omit ECC
Grainger et al[13]	712	17.6%	NR	Perform always
Moniak et al[14]	2126	10%	33%	Perform always
Massad and Collins[15]	2068	12%	27%	ECC: no benefit in 94% of patients
Soisson et al[16]	1500[a]	8%	NR	Perform always

Abbreviations: colpo, colposcopy; N, number of patients in the study for which colposcopic status regarding adequacy was reported; NR, not reported.
[a] Colposcopic status was not delineated for Soisson and colleagues' study.

most authorities agree that performance of an ECC in a woman who has an unsatisfactory colposcopic examination is appropriate, although it may be unnecessary if an excisional procedure would be performed regardless of ECC result.

Although a comprehensive review of studies addressing ECC in the evaluation of squamous abnormalities is beyond the scope of this article (and a systematic review has not been done), several investigations warrant further discussion. Krebs and colleagues[11] identified a higher rate of cancer diagnosed in women who had an unsatisfactory colposcopy (9% versus 1.5%), but even with a 5% positive ECC rate in women who had a satisfactory colposcopy, no lesions were deeper in the endocervical canal than predicted by colposcopy. Therefore, ECC was considered unnecessary in women who had a satisfactory colposcopic examination. In a study by Hatch and colleagues,[9] ECC was compared to biopsy. These investigators concluded that ECC, when correlated with cytology, colposcopy, and biopsy results, may eliminate the need for conization in most women who have unsatisfactory colposcopy or no lesion identified and they recommend performing the procedure in all evaluations.[9] The investigators suggest, however, that ECC would reduce the number of conizations if all women who have unsatisfactory colposcopy or no lesions seen undergo conization. In current practice, it is unlikely that these same groups of women would uniformly undergo conization; therefore, these conclusions may not be as relevant.

Another consideration is the grade of dysplasia. Saltzman and colleagues[7] noted that ECC was positive in 260% more cases in which cervical intraepithelial neoplasia (CIN) 3 was present than in those of CIN 1 or 2. Spitzer and coworkers analyzed ECC prior to performance of cold knife conization, and reported that if the preoperative ECC was high grade, the conization specimen contained a high-grade lesion in 86% of cases, but high-grade lesions were present on the cone specimen in only 17% of those in whom the ECC was low grade.[17] It also has been reported that a positive ECC is related directly to the severity of the cytologic smear. Williams and colleagues[18] studied women who had atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesion (LSIL) cervical cytology, and normal colposcopy. In this study, the ECC was positive in only 2.5% of cases, and none of these women subsequently was found to have greater than CIN 1. Pretorius and colleagues[19] reported higher rates of positive ECC in ASCUS and LSIL cytology compared to Williams and colleagues' results (15.6% and 14.8% with $\geq$CIN 2, respectively) but noted a much higher rate of positive ECCs in the setting of high-grade squamous intraepithelial lesion (HSIL) on referral cervical cytology (38% with $\geq$CIN 2).

Opponents of Endocervical Curettage

Considering "opposition" to performing an ECC, most of the support for this approach relates to false-positive and false-negative ECCs, which reduce the usefulness of this procedure. Massad and Collins[15] reported that a positive ECC was associated with serious lesions at follow-up; however, of all ECCs performed, only 1.4% were diagnosed with CIN 2 or 3 based solely on ECC result. These investigators concluded that ECC failed to provide a benefit in 94% of patients and considered it unnecessary in nulliparous women who had a satisfactory colposcopic examination (recommendations regarding multiparous women were not specified in this study, although multiparous women were included in the analysis).[15] Andersen and coworkers[20] reported that ECC missed 45% of lesions in the endocervical canal identified on subsequent conization specimens and also reported a 25% false-positive rate. Irvin and colleagues,[3] in a study of 304 women, reported that ECC was positive in 6.4% of study cases but that the ECC would have altered the evaluation plan in only 4.3% of women.

Additionally, no occult cancer would have been missed if the ECC were omitted, and the only case of invasive cancer was found in a woman who had a negative ECC.[3] In a study by el-Dabh and coworkers, ECC was positive in 96 women who had a satisfactory colposcopic examination.[21] The investigators measured the extent of the abnormality into the endocervical canal in the conization specimens; greater than or equal to 5 mm was considered a true-positive result relative to the ECC. In this study, the false-positive rate was 82%, and the investigators concluded that ECC was not necessary.[21]

Related to the concept of false-positive ECCs, Spirtos and colleagues[8] performed a study of 210 women who had satisfactory and 51 women who had unsatisfactory colposcopic examinations and reperformed colposcopy after ECC to determine if an ectocervical lesion was disrupted during the performance of the ECC. In those who had satisfactory colposcopy, all those who had a positive ECC (9.5%) had a disrupted ectocervical lesion. Of those women with unsatisfactory colposcopy in whom cold knife conization was performed (39 women), 4 (10%) were diagnosed with squamous carcinoma. These investigators concluded that if ECC was performed in a woman who had a satisfactory colposcopic examination, that colposcopy should be performed after the ECC, and if a lesion was disrupted, a more conservative follow-up as opposed to an excisional procedure for a positive ECC should be considered.[8]

Proponents of Endocervical Curettage

The ''proponent'' argument for performing routine ECC relates to the potential for missing serious lesions, including cancer, if the ECC had not been performed. Moniak and colleagues[14] correlated ECC result with disease location on subsequently performed excisional specimens. In women in whom the ECC was the only specimen positive for high-grade disease (CIN 2 or 3), ectocervical lesions were present in 69%; 12.5% had high-grade endocervical lesions. These investigators concluded that ECC should be performed in all women because of the ability to detect lesions that otherwise might be missed by colposcopy and biopsy, although the investigators acknowledged that ECC is most likely to detect ectocervical disease.[14] These same investigators also considered ECC results in the setting of normal colposcopically directed biopsies or if a biopsy was not done (**Table 2**), supporting the concept that the ECC may be the only manifestation of intraepithelial neoplasia.[14] The concept that abnormalities may be identified with further sampling also is supported by Pretorius and colleagues.[19] In their study of 364 women, all who had satisfactory colposcopy and ECC performed, colposcopically directed biopsies identified 57% of those who had lesions, but the addition of a random biopsy identified an additional 37% of women who had lesions greater than or equal to CIN 2. Performance of ECC identified an additional 5.5% of women who had greater than or equal to CIN 2 disease. These

Table 2
Endocervical curettage results compared to colposcopically directed biopsy results (if done)

	Endocervical Curettage Result		
Colposcopically Directed Biopsy	**HPV/CIN 1**	**CIN 2/3**	**Total**
Normal	12.5%	8.6%	21.1%
Biopsy not done (colposcopy normal)	13.0%	29.6%	42.6%

Data from Moniak CW, Kutzner S, Adam E, et al. Endocervical curettage in evaluating abnormal cervical cytology. J Reprod Med 2000;45(4):285–92.

investigators concluded that ECC should be performed and random biopsy considered if the referral cytologic interpretation is high grade.[19]

Further supporting the concept of ECC as the only manifestation of cervical disease, Soisson and coworkers reported that in women who had positive ECCs, 9% had otherwise negative biopsies and another 9% had no other biopsy performed. In these situations, the majority of women (>80%) had dysplasia on the conization specimen, supporting the concept that ECC should be performed as the ECC was the only evidence of neoplastic epithelium.[16] These investigators, however, did not distinguish low- from high-grade intraepithelial neoplasia on the conization specimen; therefore, it cannot be determined whether or not the ECC was the only indication for subsequent evaluation leading to diagnosis of high-grade disease.

Others also have described ECC as the only evidence of disease, particularly leading to an eventual diagnosis of carcinoma. In the study by Hatch and coworkers, discussed previously, of 15 women diagnosed with squamous carcinoma, ECC was the only evidence of invasion in seven (47%) of these women, although the investigators noted that the ECC was useful mostly in detecting disease in women who had an unsatisfactory or normal colposcopic examination.[9] Fine and colleagues[22] reported that 6 of 17 women diagnosed with carcinoma and a positive ECC had normal colposcopy, and in 5 of these 6 women, positive ECC was the only indication to perform a cone biopsy. Ferenczy reported that 19 women who had unsuspected carcinoma were identified with ECC, although 18 of these 19 women also had unsatisfactory colposcopy.[1]

With the number of studies that have addressed ECC, there is one meta-analysis, published in 1992.[23] Using the criterion of microinvasion, this analysis considered predictive values of ECC. The positive and negative predictive values of ECC in women who had satisfactory colposcopy were 2.4% and 99.4%, respectively. In women who had unsatisfactory colposcopy, the positive and negative predictive values were 22.4% and 96.7%, respectively. The negative predictive values were similar regardless of the adequacy of the colposcopic examination. The conclusion of this meta-analysis was that the impact of ECC was limited; although, in those who had satisfactory colposcopy, performance of an ECC may have reduced underdiagnosis. In women who had unsatisfactory colposcopy, however, the predictive value for invasive disease was increased.[23]

In summary, for squamous abnormalities, data are mixed. The high false-positive and -negative rates argue against ECC as having significant benefit in evaluating women who have abnormal cervical cytology; however, the reports of ECC as the only evidence leading to the eventual diagnosis of dysplasia or carcinoma may pressure clinicians into performing an ECC, even with the potential for a false-positive result. The data are conclusive regarding the value of ECC in the setting of unsatisfactory colposcopy, but for women who have satisfactory colposcopy, there is evidence to defend either approach (performing or not performing an ECC).

GLANDULAR ABNORMALITIES

Although for squamous lesions the usefulness of ECC may be debatable, performance of an ECC in the setting of potential glandular lesions generally is more accepted, although data are limited. Furthermore, the procedure may not be as useful as might be hoped in identifying women who have glandular abnormalities. Poynor and colleagues[24] reported that ECC was positive in only 43% of women who had glandular lesions on conization specimens. Similarly, Wolf and coworkers[25] noted that ECC was positive in only 35% of women subsequently diagnosed with glandular lesions.

In a study by Lea and colleagues,[26] ECC was compared to margin status on an excisional specimen for predicting residual adenocarcinoma in situ (AIS). These investigators found that ECC had a higher positive and negative predictive value compared to margin status for residual AIS; however, even with a negative ECC and negative margins, 11% of the women in the study had residual AIS. The potential lack of assurance of a negative ECC and negative margin in excluding residual AIS also has been reported by others.[27–29]

Despite the few studies addressing ECC in glandular lesions, it generally is recommended for the evaluation of women who have atypical glandular cells (AGC) on cervical cytology.[30,31] Because high-grade squamous abnormalities are not uncommonly found in women who have AGC cytology, if a clinician's practice is to perform ECC in women who have potentially high-grade disease, such as evaluation of HSIL cytology, then performance of an ECC in women who have AGC seems reasonable.

ENDOCERVICAL CURETTAGE: POST PROCEDURE

Another consideration for the use of ECC in the evaluation of women who have cervical disease is postprocedural performance of the ECC after conization or loop electrosurgical excision procedures (LEEP). In general, ECC correlates with margin status for prediction of residual disease, although it is not well established that ECC is an independent predictor of postprocedure residual abnormalities, and the usefulness of ECC in this setting is controversial.

In a study of margin status versus ECC for prediction of residual disease, Kalogirou and colleagues[32] found that ECC was predictive and margins were not, although gland involvement, cytology, and the pathology of the LEEP specimen also were predictive. It was, therefore, not clear whether or not the ECC was an independent predictive factor. In another study of women post LEEP or post conization, the strongest predictor of residual disease was margin status, followed by ECC.[33] In this investigation, positive margins predicted 38% with residual disease, but the combination of positive margins and a positive ECC identified 67% of women who had residual disease.[34] Others also have demonstrated that the combination of a positive ECC and positive margins were the most predictive for identification of subsequent dysplasia or cancer; however, residual dysplasia was present in 18% of women in whom both margins and ECC were negative.[34]

Alternatively, several investigators have suggested that postprocedure ECC is of minimal value in following these women. In a study of postconization prediction of residual disease, ECC and margins were considered poor predictors; residual disease was identified in 23% of women who had a negative ECC.[35] Vierhout and de Planque[36] found that margin status had much greater sensitivity and nearly the same specificity as ECC in identifying residual disease after conization. Moniak and colleagues[14] compared follow-up cytology to ECC after various procedures, including ablative and excisional techniques. In this study, the likelihood of a positive ECC was far greater if the cytologic specimen was abnormal, in particular HSIL. These investigators concluded that the ECC was of little value in this setting compared to follow-up cytology. In a study of postconization surveillance in which all women had positive margins, Fine and colleagues[22] also identified a low rate of ECC positivity if follow-up cytology was normal and concluded that cytologic follow-up was adequate.

In summary, for postprocedural evaluation, there does not seem to be a consensus as to the usefulness of ECC. Nonetheless, as summarized later in this monograph recent guidelines recommend the performance of an ECC in the follow-up of women treated for high-grade intraepithelial neoplasia in certain situations.[37]

ENDOCERVICAL CURETTAGE: TECHNIQUE

Although not the intent of this article, it is not surprising if readers are frustrated with the lack of evidence to clearly establish the benefit, or lack thereof, of ECC in most situations despite recommendations or opinions regarding its performance. Nonetheless, if ECC is to be performed, one must also consider if there are differences in technique that may impact interpretation. Providers who have performed ECC understand that the yield of material grossly present on the instrument used to obtain a specimen ranges considerably, which translates into the amount of material present on a slide for a pathologist to evaluate. Adequacy of a specimen might have an impact on sensitivity and specificity of ECC in evaluation of women who have abnormal cervical cytology.

Several investigators have evaluated specimen adequacy, mostly comparing methods for obtaining specimens. The most commonly compared techniques involve curette and brush; findings of several studies comparing curette and brush relative to specimen adequacy are presented in **Table 3**.[20,38–40] As demonstrated, use of the brush generally was associated with lower rates of inadequate specimens, although Klam and colleagues[40] did not identify a significant difference between the two approaches but did note that the inadequacy rate decreased over time, possibly because of a learning curve in performing the procedure.

In a separate study, the method of collecting the specimen, not the sampling technique, was evaluated.[41] In this investigation, ECC was performed with a curette, but the method of specimen collection (brush versus curette) was compared. Specimen collection with the curette was associated with a significantly greater inadequacy rate compared to collecting the specimen with a brush (10% versus 0% respectively, $P = 0.01$).[41] These investigators concluded that use of the brush may aid in obtaining specimens and reduce the potential for inadequate specimens.

Further comparison of brush versus curette addresses sensitivity, specificity, and predictive values (**Tables 4** and **5** show data from several studies,[20,39,42] including a randomized trial by Klam and colleagues[40]). As demonstrated, sensitivity generally is greater with the brush, but specificity and positive predictive value data are mixed. Negative predictive values generally are higher with the brush. Sensitivity, specificity, and predictive values also vary depending on the nature of the colposcopic examination (see **Table 5**).[42] A study by Hoffman and colleagues[42] concluded that the curette was less specific and, therefore, considered less than optimal for disease in the

Table 3
Comparison of inadequate specimen rates between brush and curette for endocervical sampling

	Inadequate/Scant Specimen Rates	
Study	**Curette**	**Brush**
Boardman et al[38]	22%	2% (sleeved)
Mogensen et al[39]	12%	0%
Klam et al[40]	2.5% (scant)	7.6% (scant)
	0% (inadequate)	0.6% (inadequate)
Andersen et al[20]	20% (scant)	NR
	8% (inadequate)	NR

In Boardman and colleagues' study, a sleeved cytobrush was used. In Klam and colleagues' and Andersen and colleagues' investigations, both inadequate and scant specimen rates were reported.
Abbreviation: NR, not reported.

Table 4
Comparison of brush versus curette for endocervical sampling

Study	Statistic	Curette	Brush	*P* Value
Andersen et al[20]	Sensitivity	55%	92%	<0.001
	Specificity	75%	38%	0.02
	FP rate	25%	63%	0.02
	FN rate	45%[a]	8%	<0.001
	PPV	91%	87%	0.48
	NPV	27%	50%	0.14
Mogensen et al[39]	Sensitivity	82%	96%	0.08
	Specificity	88%	95%	0.78
	FP rate	NR	NR	N/A
	FN rate	NR	NR	N/A
	PPV	90%	98%	NR
	NPV	79%	91%	NR
Hoffman et al[42,b]	Sensitivity	49%	93%	<0.001
	Specificity	86%	26%	<0.001
	FP rate	NR	NR	N/A
	FN rate	NR	NR	N/A
	PPV	69%	52%	0.99
	NPV	65%	82%	0.004
Klam et al[40]	Sensitivity	64%	77%	NR
	Specificity	97%	97%	NR
	FP rate	NR	NR	N/A
	FN rate	3.6%	2.1%	NR
	PPV	69%	71%	NR
	NPV	96.4%	97.9%	NR

Abbreviations: FN, false negative; FP, false positive; NPV, negative predictive value; NR, nor reported; PPV, positive predictive value.

[a] Curette FN rate decreased to 16.7% if abundant volume of tissue was present.

[b] Overall data including satisfactory and unsatisfactory colposcopy; data separated for satisfactory versus unsatisfactory colposcopy are depicted in **Table 5**.

endocervical canal. In contrast, the brush was more sensitive for endocervical disease, and a negative result more meaningful. Based on the randomized trial, Klam and coworkers[40] concluded that there was no significant difference in yield or discomfort but because the brush had lower false-positive results, the brush was an acceptable alternative to a curette.

Unquestionably, use of a brush to sample the cervical canal is supported by the previously summarized data; however, it could be argued that specificity and predictive values could be enhanced. To enhance specificity, a sleeved cytobrush has been described; the sleeve is used to prevent contamination of the brush by ectocervical lesions.[43] Gosewehr and colleagues[43] compared a sleeved to an unsleeved cytobrush for endocervical sampling and demonstrated slight improvement in sensitivity (89% to 95%) and a marked increase in specificity (60% to 90%) with the addition of the sleeve. Accordingly, the investigators noted increased positive and negative predictive value with the sleeved brush. In a split-sample study comparing a sleeved cytobrush to a curette with randomization of the order in which the procedures were

Table 5
Comparison of brush versus curette for endocervical sampling, demonstrating data for satisfactory and unsatisfactory colposcopy

	Satisfactory Colposcopy (N = 65)			Unsatisfactory Colposcopy (N = 36)		
	Curette	Brush	*P* Value	Curette	Brush	*P* Value
Sensitivity	25%	100%	<0.001	64%	89%	0.008
Specificity	81%	20%	<0.01	83%	62%	0.97
PPV	33%	34%	0.45	94%	89%	0.76
NPV	74%	100%	<0.001	35%	62%	0.01

Abbreviations: NPV, negative predictive value; PPV, positive predictive value.
Data from Hoffman MS, Sterghos Jr. S, Gordy LW, et al. Evaluation of the cervical canal with the endocervical brush. Obstet Gynecol 1993;82(4):573–7.

performed, the rate of inadequate samples was significantly lower with the sleeved brush.[38] Analysis of the two approaches unmatched according to the order in which they were performed revealed similar sensitivities and specificities for the brush and curette, but analysis matched according to order of performing the procedures demonstrated increased sensitivity for the brush compared to the curette. The investigators concluded that although sensitivities and specificities generally were similar, the lower rate of specimen inadequacy for the brush supported the brush as a reasonable alternative to the curette.[38]

CURRENT GUIDELINES

The ASCCP, along with participating organizations, sponsored two consensus conferences, in 2001 and 2006, with resultant publications of guidelines for management of women who have abnormal cervical cytology and histology.[30,37,44,45] Readers are referred to these publications and the ASCCP Web site (www.asccp.org) for detailed information and background regarding these guidelines (see articles by Boardman and Kennedy, Zsemlye, and Dunton elsewhere in this issue addressing LSIL, HSIL, and glandular abnormalities). This article briefly summarizes ECC performance. Generally, indications for performance of an ECC did not change significantly between the publication of the 2001 and 2006 guidelines.

Abnormal Cytology

In women who have ASCUS, further evaluation is recommended only for those testing positive for high-risk HPV (HRHPV). In this setting, evaluation is the same as for LSIL cytology, which described ECC as ''preferred'' for women in whom no lesions are present or those who have unsatisfactory colposcopy and ECC as ''acceptable'' for women who have adequate colposcopy and a lesion in the transformation zone. The level of the evidence listed for performing ECC with satisfactory colposcopy is C, acknowledging the lack of clearly established evidence for ECC in this setting.

For women who have atypical squamous cells–cannot exclude HSIL (ASC-H), colposcopy is recommended but ECC is not addressed specifically. This situation presents a clinical dilemma. The risk for CIN 2 or 3 in the setting of ASC-H cytology is greater than for those who have ASCUS and is considered an equivocal HSIL.[30] The rate of CIN 2 or 3 in women who have ASC-H may be higher than for women who have LSIL, which is estimated to range from 12% to 16%.[30,46–48] Therefore, with the risk for identifying high-grade disease approaching that of women who have HSIL cytology, should the indication for ECC in women who have ASC-H be

the same as for women who have HSIL? The decision is left to clinicians, as recommendations are not supplied in current guidelines.

For HSIL cytology, colposcopy with endocervical assessment is acceptable (except in certain populations). In those in whom CIN 2 or 3 is not identified and observation is used for follow-up, colposcopy and endocervical sampling is to be performed.

For cytology interpreted as AGC, it is recommended that initial evaluation include several modalities, including colposcopy, endocervical sampling, and endometrial sampling, depending on patient age. The guidelines acknowledge poor sensitivity of these tests in the evaluation of women who have AGC; nonetheless, endocervical sampling is recommended. Postcolposcopy management uses primarily cytology and HRHPV testing with referral for colposcopy if follow-up cytology is greater than or the same as ASCUS or if HRHPV testing is positive; endocervical sampling at the time of follow-up colposcopy is not addressed specifically. Presumably, clinicians base the indication for ECC on the subsequent cytologic interpretation or colposcopic impression, although this is not described in the guidelines.

Abnormal Histology

In the recently updated guidelines, recommendations for management of women who have CIN 1 depend on the referral cytologic interpretation. For women who have CIN 1 preceded by ASCUS, ASC-H, or LSIL cytology, follow-up recommendations are described, including cytology, HRHPV testing, and referral for colposcopy if positive follow-up results are found. The first mention of endocervical sampling is not provided until the recommendation that a diagnostic excisional procedure be performed if follow-up colposcopic examination is unsatisfactory, patient was treated previously, or endocervical sampling is positive. Prior to this recommendation, endocervical sampling is not addressed. It seems, therefore, that it is up to individual clinicians to perform endocervical sampling if follow-up colposcopy is indicated.

For women in whom CIN 1 persists for at least 2 years, however, treatment is considered acceptable, and excision or ablation is considered acceptable if the colposcopic examination is satisfactory. There is no specific reference to endocervical sampling prior to ablation in the recent guidelines. Others have strongly recommended endocervical sampling prior to cryotherapy based on missed cancers and higher recurrence rates of dysplasia in women who have positive ECCs; it seems appropriate to perform endocervical sampling prior to ablative therapy.[45,49–51]

For women who have CIN 1 preceded by HSIL or AGC, options for follow-up are described, with conservative follow-up permissible provided colposcopy is adequate and endocervical sampling is negative. Considering that endocervical sampling is recommended for referral HSIL or AGC cytology, the recommendation for endocervical sampling in this case is consistent.

For women who have CIN 2 or 3 (except for certain situations), excision and ablation are considered acceptable if colposcopy is satisfactory, although, again, endocervical sampling is not referenced specifically relative to ablative therapy. Follow-up may include colposcopy, cytology, or HRHPV testing, and endocervical sampling is recommended for those referred for colposcopy if follow-up cytology is abnormal or HRHPV testing is positive. Additionally, endocervical sampling is recommended during follow-up if the immediate postprocedure ECC is positive or if the excision specimen margins is positive for CIN 2 or 3.

For women who have AIS, if excisional procedures are used as opposed to hysterectomy, endocervical sampling is recommended, although, as discussed previously, the relative importance of margin status and ECC at the time of excision varies. In either situation, close surveillance of conservatively treated patients is critical.

Pregnancy

The guidelines reference ''special populations'' in considering evaluation and treatment options, notably adolescents, immunocompromised patients, and pregnant women. Detailed recommendations are provided in the guidelines. Recommendations regarding pregnant patients, primarily related to endocervical sampling, are highighted. ECC is considered ''unacceptable'' in pregnant patients.[30] Fortunately, colposcopy most often is adequate in pregnant women. If endocervical sampling is considered important in the evaluation of a pregnant woman who has abnormal cervical cytology, however, two small series demonstrated that use of the cytobrush was safe in pregnancy and associated with a much greater yield than the traditionally used cotton swab in pregnant women.[52,53]

REPRODUCIBILITY

One of the concerns relative to cervical cytologic interpretation and cervical histopathologic diagnoses is reproducibility of cytologic or histologic interpretations. In the large ASCUS/LSIL Triage Study, the most common abnormal cervical cytologic interpretation, ASCUS, was downgraded 39% of the time when the cervical cytology specimen was reviewed by the study pathology group.[54] Regarding histopathlogic diagnoses, low-grade squamous lesions (encompassing HPV-related changes and CIN 1) are poorly to moderately reproducible; however, interobserver agreement in the setting of high-grade squamous histology is greater.[54–62]

Although poor interobserver agreement seems to be acknowledged for atypical and low-grade cervical cytology and histology, there essentially are no data regarding reproducibility of diagnoses rendered on ECC specimens. Even more concerning related to reproducibility is the small amount of tissue on ECC specimens, which often is not oriented, making diagnosis even more challenging. Significant clinical decisions, however, including the potential for LEEP and cold knife conization, may be based on ECC interpretations. The only data even loosely related to reproducibility come from a study of women who developed cervical cancer after cryotherapy.[49] In this investigation, 7 of 10 ECC specimens initially interpreted as negative subsequently were considered dysplastic after review.[49] Although not addressed specifically, it is possible that the relatively high false-positive and false-negative rates associated with ECCs (discussed previously) in part may be the result of interpretative difficulties. It seems there is a need for study of the reproducibility of ECC diagnoses, particularly because important clinical interventions may hinge on ECC interpretation.

SUMMARY

The debate referenced in the introduction to this article continues. Data regarding performance of ECC unquestionably are mixed. There are no well-done randomized trials regarding the performance of ECC, only the technique. Generally, the yield on ECC seems to be increased in the setting of unsatisfactory colposcopy; in this situation, there likely is less controversy regarding performance of ECC. The yield on ECC also is greater with severe lesions, although the usefulness of ECC in the setting of high-grade dysplasia and adequate colposcopy as a separate diagnostic tool is debatable. As discussed previously, reproducibility also is a concern. Although guidelines exist to aid clinicians in management decisions, additional data are sorely needed to further define the role of ECC in the evaluation of women who have cervical disease.

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Management of Atypical Squamous Cells, Low-Grade Squamous Intraepithelial Lesions, and Cervical Intraepithelial Neoplasia 1

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KEYWORDS

• ASCUS • ASC-H • LSIL • CIN 1 • Management

The goal of this article is to review the American Society for Colposcopy and Cervical Pathology 2006 Consensus Guidelines (ASCCP), elaborating on the changes and providing the rationale for management decisions.

ATYPICAL SQUAMOUS CELLS

Although often incorrectly considered a diagnosis of exclusion, atypical squamous cells (ASC) indicate cytologic findings suggestive, but not diagnostic, of a squamous intraepithelial lesion. The 2001 Bethesda System further classifies ASC into two categories: ASC of undetermined significance (ASC-US) and ASC cannot exclude high-grade squamous intraepithelial lesion (HSIL) (ASC-H).[1] Of the two, ASC-US smears predominate, representing 90% of smears interped as ASC,[2] and comprise approximately one half of all abnormal Pap smear diagnoses in American women undergoing cervical cytologic screening.[3] ASC-US remains the most common Pap smear

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Obstet Gynecol Clin N Am 35 (2008) 599–614
doi:10.1016/j.ogc.2008.09.001 **obgyn.theclinics.com**
0889-8545/08/$ – see front matter

abnormality preceding a diagnosis of cervical intraepithelial neoplasia (CIN) 2 or worse; approximately 39% of high-grade disease occurs among women presenting with ASC-US,[4] although serious disease among the 2.75 million women annually diagnosed with ASC-US occurs rarely. Estimates of moderate to severe CIN range from 5% to 16% among women who have this cytologic diagnosis,[3,5–10] and invasive cancer is exceedingly rare (0.1%–0.2%).[11] In contrast, women who have ASC-H have a much higher risk for developing CIN 2 or worse as compared with women who have ASC-US. In several studies, reported rates of CIN 2+ range, in general, from 40% to 50%,[10,12–14] findings that provide the rationale for the differences in management of these two cytologic diagnoses.

Because of the lack of evidence surrounding the optimal management of women who have mild cytologic abnormalities (including low-grade squamous intraepithelial lesions [LSIL] and ASC-US), the National Cancer Institute implemented a multicenter randomized trial: the ASC-US/LSIL Triage Study (ALTS).[15] The ALTS trial was designed to compare the sensitivity and specificity of three different management strategies (colposcopy, repeat cytology, oncogenic human papillomavirus [HPV] testing) in detecting severe histologic disease (CIN 3) in women who had mild cytologic abnormalities. As such, ALTS was intended to clarify the management of women who have ASC-US and LSIL, and its results have led to significant differences in how such abnormalities are followed.

Although data initially appeared to support the role of oncogenic HPV testing in women who have LSIL, the March 2000 publication from the ALTS trial demonstrated the opposite.[16] In this analysis, oncogenic HPV was confirmed in 83% of the population who had LSIL. Based on the high prevalence of infection, the costs of HPV testing in women who have LSIL were determined to outweigh the savings gained from avoiding colposcopy in a few cases. For ASC-US, however, the addition of reflex HPV testing resulted in a referral rate of only 56%.[3,17] Furthermore, the sensitivity of oncogenic HPV typing for the detection of CIN 3 or worse was 96.3% (95% CI 91.6–98.8). Although the authors of the 2001 ASCCP Consensus Guidelines concluded that repeat cervical cytologic testing, colposcopy, and DNA testing for high-risk HPV types were all acceptable methods for managing women who have ASC-US, reflex HPV testing emerged as the preferred method for providers using liquid-based cytologic screening methods or performing co-collection for HPV DNA testing.[18]

After the 2001 Guidelines, additional evidence continued to accumulate to demonstrate that clinical outcomes and cost effectiveness favored reflex HPV testing for ASC-US. In a meta-analysis of 20 studies conducted worldwide to assess the usefulness of HPV triage for ASC-US, Arbyn[19] demonstrated that the combined sensitivity of HPV DNA testing in the setting of ASC-US for the detection of CIN 2 or worse was 92.5% (95% CI 90.1–94.9). In a separate meta-analysis, Arbyn[20] found that repeat cytologic testing using an ASC-US cutoff for referral to colposcopy resulted in a combined sensitivity of 81.8%. Similarly, specificity for HPV testing was found to be 62.5% (95% CI 57–67), whereas a program of repeat cytology resulted in a slightly lower specificity of 57.6% (95% CI 50–66). Cost-effectiveness studies have also shown the economic viability of HPV triage for ASC-US.[21,22] In a cost-effectiveness analysis based on ALTS data, Kulasingam found that immediate colposcopy and conservative management with up to three repeat cytology visits detected fewer cases of CIN 3 or worse and were more costly than HPV DNA testing in the management of ASC-US.[22]

Atypical Squamous Cells of Undetermined Significance in Special Populations

With the 2006 Consensus Guidelines, prior recommendations for the management of ASC-US remained largely unchanged, with several important exceptions, one of the

most significant of which involved adolescents. Management of mild cytologic abnormalities in adolescents, defined as young women aged 20 and under, differs significantly from that for adult women and is discussed in further detail in Moscicki's article, ''Management of Adolescents with Abnormal Cytology and Histology.'' In brief, adolescents found to have ASC-US are recommended to undergo follow-up with annual cytologic testing, with colposcopy only in the presence of HSIL or worse at 1 year or persistent ASC-US or greater at 2 years. Neither HPV triage nor immediate colposcopy for the management of this population is recommended because the prevalence of HPV infection is high[23,24] and the risk for cancer exceedingly low.[25]

The 2006 Guidelines were therefore modified to clarify this distinction. For the management of ASC-US in adult women (defined as women over the age of 20), the guidelines again preferentially call for the addition of HPV DNA testing when liquid-based cytology is used or if the HPV test is co-collected at the time of cytologic screening. As with the 2001 Guidelines, repeat cervical cytologic testing or immediate colposcopy in the adult populations are also acceptable management options.[26] These recommendations hold for pregnant women older than 20 who have ASC-US, with the addition of one exception, deferral of colposcopy until at least 6 weeks postpartum. Evidence suggests that antepartum management of women who have ASC-US (and LSIL) does not significantly alter management and thus could be deferred until the pregnancy is completed.[27]

Also different from the prior guidelines is the removal of the recommendation for postmenopausal women who have ASC-US to undergo a course of intravaginal estrogen therapy followed by repeat cervical cytology. Because of the lack of evidence to support this suggested management, the recommendation was revised in 2006. Postmenopausal women who have ASC-US, then, should be managed in the same manner as adult women in the general population. Because the prevalence of HPV infection declines with age (only 20% of women aged 40 and older who had ASC-US were found to be HPV positive in ALTS[17]), and histologic high-grade disease approximates that seen in younger women who have ASC-US, triage of ASC-US in postmenopausal women with HPV testing should prove effective from clinical and cost standpoints.[28–30]

Lastly, for immunosuppressed women who have ASC-US, previous recommendations called for referral to colposcopy for further evaluation. This recommendation extended to all HIV-infected women, irrespective of the degree of immunosuppression or the use of antiroviral therapy.[18] The 2001 Guidelines were based on reports demonstrating a high prevalence of oncogenic HPV infection in HIV-infected women who had ASC-US and a similarly high prevalence of high-grade histologic disease. In one cross-sectional study of HIV-negative and HIV-infected women referred to a colposcopy clinic, cytologic and histologic characteristics were found to be highly correlated in the HIV-negative population, a finding not seen in the HIV-positive women. Biopsy results revealed that 49% of the HIV-infected women had histologic characteristics more severe than their cytology indicated, compared with 27% of the HIV-negative patients.[31]

Other investigators, however, found no significant impact of HIV infection on either cytologic-histologic discrepancy or the accuracy of abnormal Pap smears. In a recent prospective study following 189 HIV-infected women and 95 uninfected women enrolled in the Baltimore HIV Epidemiology Research study site, Anderson and colleagues[32] demonstrated a high level of concordance between cytology and colposcopic and histologic findings. Several studies have also demonstrated rates of CIN 2 or worse among HIV-infected women who have ASC-US to be similar to HIV-negative women who have the same cytologic diagnosis.[33,34] For example, in one cross-sectional cohort study, the frequency of high-grade histologic disease was comparable

between HIV-infected and HIV-negative women who had mildly abnormal cervical cytology, and approximated 15%.[33] Accordingly, the 2006 Guidelines were revised to recommend that immunosuppressed women who have ASC-US be managed in the same manner as women in the general population.

Recommended Management of Women who have Atypical Squamous Cells of Undetermined Significance

Women who have ASC-US who are HPV DNA negative can undergo repeat cytologic testing at 12 months, given evidence of the low absolute risk (1.4%) of histologically significant disease at either the time of the initial cytology or in the subsequent 2 years of follow-up (**Fig. 1**). Indeed, this risk approximates that of women who have negative cytology in the absence of HPV testing.[35] High-risk HPV-negative CIN 3 cases do occur and were encountered in approximately 5% of ALTS participants. Fewer than one half of these lesions, however, remained HPV negative in follow-up, thus reducing the risk that such lesions would go undetected if clinical guidelines are followed.[36]

Women who have ASC-US who are found to be HPV DNA positive should undergo colposcopic evaluation. The authors of the 2006 Guidelines specifically commented that such women should be managed in the same fashion as women found to have LSIL, based on evidence that LSIL and HPV-positive ASC-US pose similar risks for significant cervical disease. For example, using data from ALTS, Cox and colleagues[37] demonstrated that the cumulative 2-year risk for CIN 2 or 3 was equivalent for LSIL (27.6%) and HPV-positive ASC-US (26.7%).

Given these findings, the current recommendations for colposcopic management of HPV-positive ASC-US were made consistent with the recommendations for LSIL. Previously, no specific recommendation existed to address the need for endocervical sampling during colposcopy for women who have ASC-US. With the 2006 Guidelines, for both HPV-positive ASC-US and LSIL, endocervical sampling (either by way of curettage or brush) is preferred for both nonpregnant women who have no visible colposcopic lesions or those with an unsatisfactory colposcopy. Such sampling is acceptable for nonpregnant women with satisfactory colposcopy and a lesion identified in the transformation zone.

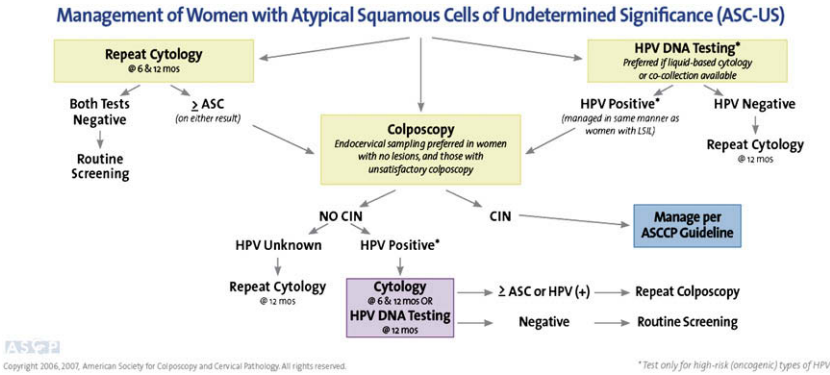

Fig. 1. Management of women who have ASC-US. (*Reprinted from* The Journal of Lower Genital Tract Disease Vol. 11 Issue 4, with the permission of ASCCP © American Society for Colposcopy and Cervical Pathology 2007. No copies of the algorithms may be made without the prior consent of ASCCP.)

If CIN 2 or worse is found on biopsy in the nonpregnant patient, appropriate treatment should then follow, based on the degree of abnormality. If histologic material (biopsy or endocervical sampling), however, reveals CIN 1 or less, HPV DNA testing at 12 months, or repeat cytologic testing at 6 and 12 months, is recommended. Both strategies are associated with high sensitivity (92% for HPV testing at 1 year and 88% for repeat semi-annual cytology with referral to colposcopy at an ASC-US threshold), although the latter scheme resulted in a higher rate of referral to colposcopy (64% versus 55% for HPV testing). The addition of cytology to HPV testing did not improve sensitivity, decreased specificity, and resulted in 10% more referrals to colposcopy, and thus, is not recommended.[38] The authors of the 2006 Guidelines emphasize that HPV DNA testing should not be done at intervals shorter than 12 months.

In circumstances in which reflex HPV testing is not available or cannot be performed, two repeat cytologic examinations at 6-month intervals is an effective means of follow-up. The negative predictive value of two consecutive ''negative for intraepithelial lesion or malignancy'' results obtained at 6-month intervals following ASC-US is high, and women thus followed can subsequently be returned to routine cytologic screening. However, should an intervening cytologic result be abnormal (ie, $\geq$ASC-US), colposcopy is indicated. If colposcopy is used initially to manage women who have ASC-US, and CIN is not confirmed, repeat cytologic testing at 12 months is recommended. Routine use of diagnostic excisional procedures is unacceptable as initial management for ASC-US in the absence of biopsy-confirmed CIN 2 or 3.[39]

Atypical Squamous Cells Cannot Exclude High-Grade Squamous Intraepithelial Lesion

Because the prevalence of CIN 2,3 is higher in the setting of ASC-H as compared to ASC-US, women who have ASC-H should be referred for colposcopic evaluation (**Fig. 2**). If CIN 2 or worse is not identified, follow-up with HPV DNA testing at 12 months, or cytologic testing at 6 and 12 months, is recommended.[39] Women who subsequently test positive for HPV DNA or have ASC-US or greater on their repeat cytologic tests should be returned to colposcopy for evaluation. If the HPV test is negative, or both repeat cytologic tests are negative for intraepithelial lesion or

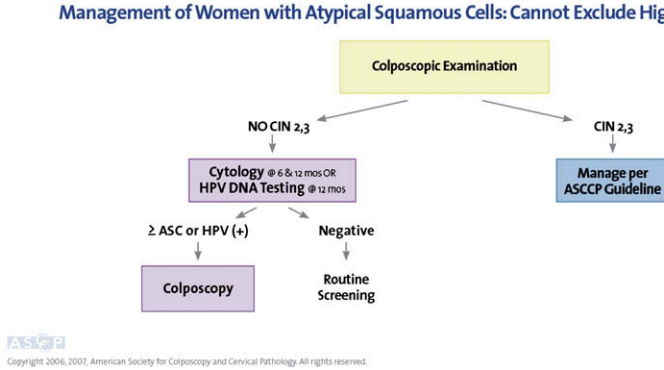

Fig. 2. Management of women who have ASC-H. SIL, squamous intraepithelial lesion. (*Reprinted from* The Journal of Lower Genital Tract Disease Vol. 11 Issue 4, with the permission of ASCCP © American Society for Colposcopy and Cervical Pathology 2007. No copies of the algorithms may be made without the prior consent of ASCCP.)

malignancy, the patient can be returned to routine cytologic screening.[39] The usefulness of reflex HPV DNA testing in ASC-H has not been well established. Recent studies have shown varying rates of positive high-risk HPV DNA (37%–100%) among women who have ASC-H.[40–42] Given the rates of high-grade cervical neoplasia found in women who have ASC-H, and the high prevalence of oncogenic HPV, initial management of ASC-H with HPV DNA testing is not advised.

LOW-GRADE SQUAMOUS INTRAEPITHELIAL LESIONS

Approximately 2% of abnormal cervical cytology reveals LSIL, making this diagnosis the second most common abnormal cytology report in the United States. With the use of liquid-based cytology, the reporting of LSIL, although not ASC or HSIL, has increased over the last decade.[2] The impact of such increases on the detection of significant histologic disease, however, remains unclear.[33–45] Biopsy-confirmed CIN is common in the setting of LSIL, with most women (approximately 70%) found to have CIN 1. Based on earlier studies and ALTS data, LSIL is associated with CIN 2,3 in approximately 12%–18% of women on initial colposcopy and rarely is it associated with the finding of cervical cancer.[16,37,46,47]

Unlike ASC-US, however, most women who have LSIL will test HPV positive. In ALTS, more than 80% of women evaluated were found to harbor oncogenic HPV infection,[48] a result that was confirmed in a recent meta-analysis where the pooled estimate of high-risk HPV positivity among women who had LSIL was 76.6%.[19] Postmenopausal women are a notable exception because the prevalence of high-risk HPV infection in this population is lower.[12,17]

LSIL and high-risk HPV DNA-positive ASC-US are managed with colposcopic evaluation based on their similar risks for high-grade histologic disease,[37] except in special populations (adolescents, postmenopausal women, and pregnant women). Excluding those special populations, managing women who have LSIL with repeat cytology is insensitive and necessitates multiple follow-up visits. Similarly, HPV triage is not useful given the high prevalence of infection in premenopausal women who have LSIL; the addition of such testing would escalate costs and spare only approximately 20% of women from colposcopy.

Low-Grade Squamous Intraepithelial Lesions in Special Populations

In the 2006 Guidelines, different management schemes for certain groups of women are now emphasized. Adolescents, for example, often clear LSIL without intervention. Rates of regression among those who have clinically evident and mild manifestations of infection (ie, LSIL) are high and progression is rare.[49] In a population of young women who had LSIL, Moscicki[50] found that more than 60% experienced regression of LSIL within 12 months, a proportion that increased to more than 90% by 3 years. The high prevalence of HPV DNA positivity in teens, as with other premenopausal women, precludes the use of such testing in the context of LSIL. As with ASC-US, teens who have LSIL should undergo follow-up with annual cytologic testing, with colposcopy only in the presence of HSIL or worse at 1 year or persistent ASC-US or greater at 2 years. For a more extensive discussion of adolescents who have mild cytologic abnormalities, please refer to Moscicki's article, "Management of Adolescents with Abnormal Cytology and Histology."

For postmenopausal women, studies have shown that oncogenic HPV infection and CIN 2,3 decline with age in women who have LSIL,[28–30] suggesting less aggressive management for this population. Among pregnant women who have mildly abnormal cytology, the goal of colposcopy is to identify invasive cancer. High-grade histologic

disease (ie, CIN 2 or 3) is not treated during pregnancy. In several published series of nearly 200 pregnant women who had LSIL, more than 90% had CIN 1 or less, and no invasive cancers were detected during the antepartum period.[23,24,51–53]

Recommended Management of Low-Grade Squamous Intraepithelial Lesions

The guidelines for colposcopic examination for women who have LSIL are the same as those for women who have HPV-positive ASC-US (**Fig. 3**). In the nonpregnant woman who has no identifiable lesion, or in circumstances in which the colposcopic examination is unsatisfactory, endocervical sampling is preferred because sampling may identify an area of CIN that would have been overlooked. Endocervical sampling is acceptable when the colposcopy is satisfactory and one or more lesions are identified. If CIN is identified, patients should be managed according to the appropriate 2006 Consensus Guideline for CIN.[39] Management no longer differs by whether or not the colposcopy was satisfactory, but rather, depends on the results of the endocervical sampling.

As with HPV-positive ASC-US, acceptable postcolposcopy management options for women who have LSIL in whom CIN 2 or worse is not found include testing for high-risk HPV at 12 months or repeat cervical cytologic testing at 6 and 12 months. Women may return to routine cytologic screening if the HPV test is negative or if two consecutive cytologic tests are negative for intraepithelial lesion or malignancy. If the HPV test is positive, or if repeat cytology is found to be ASC-US or greater, colposcopy is recommended.

If, on colposcopic evaluation, CIN 2 or worse is not identified but rather, CIN 1 is found, observation should follow (**Fig. 4**). Previously, if CIN 1 was detected in a woman who had unsatisfactory colposcopy, then a diagnostic excisional procedure was recommended; in 2006, this recommendation was removed.[18,39] In the absence of CIN 2 or 3, then, women who have LSIL and are found to have CIN 1 or less on histology should be observed. Diagnostic excisional or ablative procedures are unacceptable in the initial management of LSIL.[39]

For pregnant, nonadolescent women who have LSIL, colposcopy is preferred. In 2006, however, the Consensus Guidelines were revised to include as an acceptable option postponement of initial colposcopy until at least 6 weeks postpartum. If

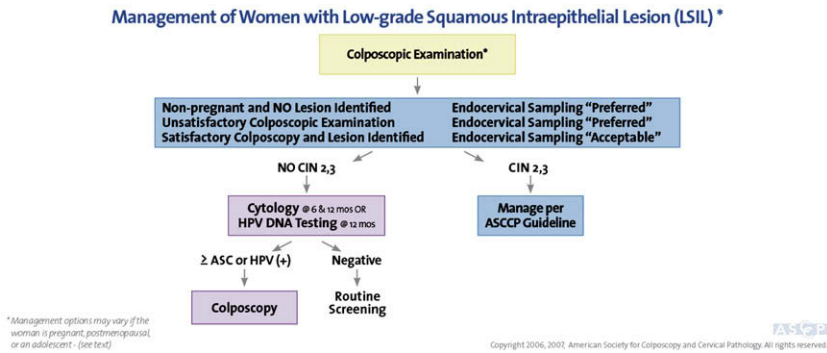

Fig. 3. Management of women who have LSIL. (*Reprinted from* The Journal of Lower Genital Tract Disease Vol. 11 Issue 4, with the permission of ASCCP © American Society for Colposcopy and Cervical Pathology 2007. No copies of the algorithms may be made without the prior consent of ASCCP.)

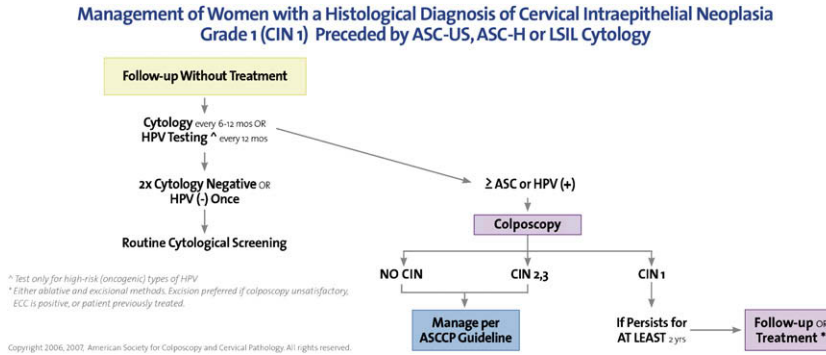

Fig. 4. Management of women who have a histologic diagnosis of CIN 1 preceded by ASC-US, ASC-H, or LSIL cytology. (*Reprinted from* The Journal of Lower Genital Tract Disease Vol. 11 Issue 4, with the permission of ASCCP © American Society for Colposcopy and Cervical Pathology 2007. No copies of the algorithms may be made without the prior consent of ASCCP.)

colposcopy is undertaken during pregnancy, endocervical cutage should not be performed secondary to the potential for bleeding and rupture of the amniotic membranes. For women who do not have cytologic, histologic, or colposcopic evidence of CIN 2 or worse, postpartum follow-up is recommended. Repeating cytology or colposcopy during the pregnancy is unacceptable for pregnant women initially referred for either HPV-positive ASC-US or LSIL[39] because doing so will not lead to alterations in management during the pregnancy.

For postmenopausal women who have LSIL, the authors of the 2006 Consensus Guidelines modified prior recommendations to state that this population should be managed in the same fashion as premenopausal women who have ASC-US (immediate colposcopy, triage with high-risk HPV testing, or serial cytology). The prior option of observation with Pap testing following a course of intravaginal estrogen was also dropped from the 2006 Guidelines. The rationale for these changes stems from multiple observations. For instance, more than 75% of the population who had LSIL in ALTS was between 18 and 27 years of age, whereas only 9% were 35 or older.[15] Mildly abnormal cervical cytology in postmenopausal women not only occurs less frequently but also poses several diagnostic dilemmas. Several epithelial and cellular changes characterize the postmenopausal cervix. Such changes, including prominent perinuclear halos (koilocytosis), variations in nuclear size, and multinucleation, can mimic HPV-related neoplasia.[54,55] The source of such cellular changes has often been attributed to atrophy related to estrogen deficiency. Although topical estrogen was previously recommended to correct atrophy-related cellular changes (and thus result in the interpation of the repeat Pap smear as normal), evidence that this is the case is far from definitive. On the other hand, the use of HPV testing will allow for differentiation between true precancerous lesions and atrophy-related changes.

Application of the new recommendations to postmenopausal women will result in several potential outcomes. If the HPV DNA test is negative or if CIN is not identified at the time of colposcopy, repeat cytology in 12 months should be obtained. If the HPV DNA test is positive, or if repeat cytology reveals ASC-US or greater, then colposcopy is recommended. If two consecutive repeat cytologic tests are negative for intraepithelial lesion or malignancy, then return to routine cytologic screening is advised.[39]

CERVICAL INTRAEPITHELIAL NEOPLASIA I

The management of CIN 1 has changed drastically over the last decade.[56] Historically, CIN was believed to represent a disease continuum, with progression from CIN 1 to CIN 3. Thus, treatment was considered for women who had CIN 1 to prevent the progression of neoplasia and ultimately prevent the development of cervical cancer. More recently, however, this theory has been challenged in that women who have normal immune function typically suppress HPV-induced low-grade lesions, although with current screening tools it is impossible to predict the minority who may progress.[57] Additionally, studies have identified a lack of histologic reproducibility among even expert pathologists, with diagnostic variability noted for all types of histologic specimens.[58] Given the histologic variability of CIN 1 lesions, variability in interpretation, and nonspecific cause leading to the abnormality, CIN 1 is felt to represent a heterogeneous group of lesions with low malignant potential.[59]

Regarding premalignant potential, CIN 1 carries the same risk as HPV infection alone; both are likely to regress as a result of activation of the host immune system. Thus, CIN 1 should be followed expectantly rather than treated, given the consequences of treatment (especially repeat procedures) and the high likelihood of regression over 2 to 4 years in the absence of treatment.[59] Furthermore, recent data found that progression to CIN 2,3 is uncommon within the first 2 years of diagnosis. Findings from ALTS indicate that the risk for having a CIN 2,3 lesion identified during 2-year follow-up of an LSIL or HPV + ASC-US Pap test, after initial colposcopy, was nearly identical for both histologically confirmed CIN 1 and for women whose initial colposcopy and biopsy were negative, about 11% to 13%.[37] Bansal and colleagues[60] followed 1001 women who had CIN 1 and identified 64 (6%) who progressed to a high-grade lesion during 1-year follow-up, data that again support prolonged conservative follow-up in women who have CIN 1.

The exception to the low risk potential of CIN 1 is among women whose initial colposcopy (detecting CIN 1) was preceded by HSIL or atypical glandular cells (AGC) cytology. In women who had initial HSIL cytology, CIN 2/3 or worse was identified in 97% of women undergoing "see and treat loop electrosurgical (LEEP)."[61] Similar rates of CIN 2,3 have been identified among women who had initial AGC cytology.[59] Given the identified risk for high-grade neoplasia, separate recommendations were made for women who have CIN 1 preceded by HSIL or AGC cytology in the 2006 Consensus Guidelines.

Additionally, persistent CIN 1 likely reflects not only persistent HPV infection but, more specifically, infection with an oncogenic subtype or subtypes. HPV type distribution varies among sites and grades of disease and can have an important effect on disease.[62,63] Castle and colleagues[64] found that among women who had LSIL and infection with HPV 16, the 2-year risk for CIN 3 or worse was 39% compared with 10% with any other oncogenic HPV type. HPV viral load also appears to play a role in the progression from low-grade to high-grade lesions.[65] Thus, although CIN 1 typically represents transient infection (which should not be treated), persistent disease is more often associated with oncogenic HPV infection and subsequent high-grade neoplasia, underscoring the heterogeneous mix of CIN 1 and the initial role for surveillance, with treatment reserved for those who have persistent neoplasia.

Recommended Management of Women who have Cervical Intraepithelial Neoplasia 1

Several changes to the guidelines for management of CIN 1 were made at the 2006 Consensus Conference. In the new guidelines, all CIN 1 preceded by ASC-US, ASC-H, or LSIL cytology is followed, with no option for treatment in most cases for

2 years. Observation may be prolonged beyond 2 years (see **Fig. 4**). However, management is more aggressive when a diagnosis of CIN 1 follows HSIL or AGC cytology.

CIN 1 preceded by ASC-US, ASC-H, or LSIL cytology should be followed by either HPV DNA testing every 12 months or repeat cervical cytology every 6 to 12 months, with referral for repeat colposcopy for positive high-risk HPV or ASC-US or greater cytology, regardless of whether or not the examination was satisfactory.[59] If the HPV test is negative or two consecutive repeat cytology tests are negative, return to routine cytologic screening is recommended.[59] This recommendation follows the findings by Spitzer and colleagues,[66] in which only 9% of women who had prior LSIL, CIN 1 on biopsy, and unsatisfactory colposcopy were found to have CIN 2 or greater on cone biopsy, and is designed to allow lesions to regress spontaneously because the risk for progression to cancer appears to be negligible.

Although follow-up of women who have low-grade CIN must take into account the potential for a high-grade lesion to develop, CIN 1 should not be treated unless it is persistent for at least 2 years. After that time, either continued follow-up or treatment is acceptable because regression rates decline substantially. Treatment is not required, and the decision about treatment should be based on thorough counseling and should incorporate the woman's desire for fertility and risk for preterm delivery, the lesion size, and other risk factors (eg, smoking). If treatment is elected (following 2 years of observation), a diagnostic excisional procedure is recommended if the colposcopic examination is unsatisfactory, the endocervical sampling contains CIN, or the patient has been treated previously. Otherwise, either excision or ablation is acceptable.[59] Although no medical treatments (eg, imiquimod, fluorouracil, or podophyllin-related products) are approved for treatment of CIN and they may cause systemic toxicity, randomized trials have shown similar efficacy for LEEP, laser ablation, and cryotherapy in the treatment of CIN. Thus, the choice of treatment (excluding hysterectomy as an option) should be determined by the judgment of the clinician.[59] The risk for procedure-related morbidity and mortality with hysterectomy outweigh the minimal risk for cancer in women who have CIN 1, and it is therefore considered unacceptable as a primary treatment option.[59]

Cervical Intraepithelial Neoplasia 1 Preceded by High-Grade Squamous Intraepithelial Lesion or Atypical Glandular Cell Cytology

Evaluation following HSIL or AGC cytology is likely to reveal CIN 2,3 or greater and warrants more aggressive management (**Fig. 5**). Thus, either a diagnostic excisional procedure or observation with colposcopy and cytology at 6-month intervals for 1 year (provided the initial colposcopic examination is satisfactory and endocervical sampling is negative in the latter) are acceptable.[59] However, a diagnostic excisional procedure is recommended for women in whom the colposcopic examination is unsatisfactory (except in pregnancy) because CIN 2,3, adenocarcinoma-in-situ, and cancer have not been excluded.[59] Review of cytologic, histologic, and colposcopic findings to help clarify the discrepancy is also acceptable, with management as per the revised interpretation.

Because of their higher risk for CIN 2,3 and lower probability of regression, women who are followed by observation with cytology and colposcopy should undergo a diagnostic excisional procedure for repeat results of HSIL or AGC cytology at either the 6- or 12-month visit, regardless of the colposcopic impression or biopsy.[59] Alternatively, regression is likely when serial cytology and colposcopy are negative at both follow-up visits. Thus, women who have two consecutive negative results can return to routine cytologic screening.[59]

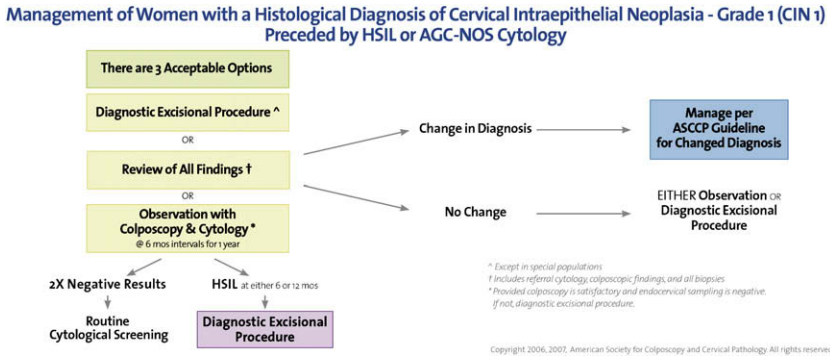

Fig. 5. Management of women who have a histologic diagnosis of CIN 1 preceded by HSIL or AGC–not otherwise specified cytology. NOS, not otherwise specified. (*Reprinted from* The Journal of Lower Genital Tract Disease Vol. 11 Issue 4, with the permission of ASCCP © American Society for Colposcopy and Cervical Pathology 2007. No copies of the algorithms may be made without the prior consent of ASCCP.)

Cervical Intraepithelial Neoplasia 1 in Special Populations

CIN 1 identified during adolescence and pregnancy should especially be managed conservatively. Natural history studies of HPV infection in healthy young women show that infection is prevalent but is typically transient and rarely progresses to invasive cervical disease.[67] The rate of resolution of CIN 1 approaches 90% in the adolescent population.[56] The high rate of regression and low likelihood of progression among these women warrant expectant management. Among adolescent women, follow-up with annual cytologic assessment is recommended. If HSIL or worse is detected at 1 year, or if ASC-US or worse is found at 2 years, the patient should be referred for colposcopy. HPV DNA should not be obtained in adolescents, as results are likely to be positive and do not reflect short-term risk for cancer. Furthermore, if HPV testing is inadvertently performed, the results should not influence management.[39]

Among pregnant women with CIN 1, follow-up is similarly recommended for the following reasons. A histologic diagnosis of CIN 1 during pregnancy essentially excludes the risk for development of invasive cancer during pregnancy and treatment carries a high risk for pregnancy loss and hemorrhage without benefit.[59] Rather, pregnant women who have CIN 1 should be followed without treatment until postpartum.

SUMMARY

The authors reviewed the 2006 Consensus Guidelines regarding management of ASC-US, LSIL, and CIN 1. Several changes in the management of mildly abnormal cervical cytology and histology were made. As summarized below (key points), the most notable changes involve the management of adolescents (defined as aged 20 and younger), pregnant women, and postmenopausal women. Previously, treatment of CIN 1 was offered to interrupt potential progression to high-grade neoplasia and cancer. Recent evidence has identified most CIN 1 as a transient infection with a high likelihood of regression. Thus, the guidelines for CIN 1 now focus on conservative

management with observation and no longer suggest ablative or excisional procedures for women who have CIN 1 in the setting of unsatisfactory colposcopy.

Take-home points are as follows:

- For adolescents, management of ASC-US and LSIL is conservative, eliminating the need for immediate colposcopy and relying instead on cytology for follow-up. High-risk HPV DNA is likely to be positive and is of no clinical use in the management of adolescents.
- For pregnant women, options have been made to allow for deferral of colposcopy until pregnancy completion because the risk for invasive cancer following ASC, LSIL, and CIN1 is unlikely.
- For postmenopausal women, the new guidelines call for management with HPV DNA testing or repeat cytology in the setting of mild cytologic abnormalities. A trial of local estrogen therapy is no longer suggested.
- Because the risk for high-grade neoplasia or cancer has been found to be similar to that in women in the general population, immunosuppressed women should be managed in the same manner.
- The guidelines for CIN 1 now focus on conservative management with observation and no longer suggest excisional procedures for women who have CIN 1 in the setting of unsatisfactory colposcopy.
- The role of high-risk HPV testing has been clarified in the screening, triage, and follow up of ASC, LSIL, and CIN 1.

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High-Grade Cervical Dysplasia: Pathophysiology, Diagnosis, and Treatment

Meggan Zsemlye, MD

KEYWORDS

- Cervical dysplasia • Cervical cancer
- Loop electrosurgical excision procedure
- Cold knife cone biopsy
- Human papillomavirus

PREVALENCE OF HUMAN PAPILLOMAVIRUS

Data from the 2003–2004 National Health and Nutrition Examination Survey show that the prevalence of human papillomavirus (HPV) infection in women in the United States during that time was 26.8% (95% CI, 23.3%–30.9%).[1] The prevalence was highest (44.8%) among women aged 20 to 24 (95% CI, 36.3%–55.3%) and was noted to decrease with age. Women aged 50 to 59 had a prevalence of only 19.6% (95% CI, 14.3%–26.8%). In this study the HPV types found in currently available vaccines were less common. HPV 16 was detected in 1.5% of participants (95% CI, 0.9%–2.6%), and HPV 18 was found in 0.8% (95% CI, 0.4%–1.5%) (**Box 1**).[1]As expected, independent risk factors for HPV positivity included young age, single marital status, and increased number of sexual partners.

PERSISTENCE OF HIGH-RISK HUMAN PAPILLOMAVIRUS INFECTION

Persistence of high-risk HPV infection is a key factor in the development of high-grade cervical lesions. Persistent HPV is defined as an infection lasting more than 6 to 12 months. Risk factors for persistent HPV infection include older age (>55 years), high-risk HPV type, and duration of infection.[2] The ALTS trial showed that the longer an infection is present, the longer it takes to clear. No consensus has yet been reached on the importance of measured viral load in predicting outcomes of HPV infections. Immunosuppression and cigarette smoking also are known to increase the risk for high-grade cervical disease.

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Obstet Gynecol Clin N Am 35 (2008) 615–621
doi:10.1016/j.ogc.2008.09.008 **obgyn.theclinics.com**
0889-8545/08/$ – see front matter

Box 1
Commonly found human papillomavirus types

Common high-risk (oncogenic) HPV types:
16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 69, 82
Common low-risk (nononcogenic) HPV types:
6, 11, 40, 42, 43, 44, 54, 61, 72, 81

A 7-year longitudinal study from Brazil illustrates the risk for persistent HPV causing high-grade cervical dysplasia. Women in the study initially were free of dysplasia and were followed with frequent HPV testing and Papanicolaou (Pap) testing. Patients were referred for colposcopy and biopsy if their Pap test result showed high-grade squamous intraepithelial lesions (HSIL). Results showed that the relative risk for HSIL over 5 years of follow-up was 3.85 if women had been positive for HPV 16 or 18 at one visit. If they were positive for HPV 16 or 18 at two visits (showing a persistent infection), the relative risk for HSIL over the same time period was 12.27.[3]

CYTOLOGY OF HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESIONS

A Pap test result that is interpreted as HSIL includes the changes consistent with a diagnosis of cervical intraepithelial neoplasia (CIN) 2,3. These include but are not limited to hyperchromatic nuclei, abnormal chromatin distribution, nuclear pleomorphism, increased nuclear/cytoplasmic ratio, and nuclear atypia. Mild manifestations of these changes warrant a diagnosis of low-grade squamous intraepithelial lesion (LSIL) on Pap test; severe or more dramatic changes are diagnostic of HSIL. As is true in all types of cervical dysplasia, the cytologic diagnosis of HSIL is not reliably reproducible. In the ALTS trial, reviewing pathologists agreed with an initial cytologic diagnosis of HSIL only 47% of the time. This is much greater reliability/agreement than is seen with lower-grade Pap tests but still higher than is desirable.[4]

MANAGEMENT OF HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESIONS PAPANICOLAOU TEST

Guidelines for the management of abnormal Pap test results have been put forth by the American Society for Colposcopy and Cervical Pathology (ASCCP). The ASCCP held its most recent consensus conference in September 2006, at which time close to 150 experts in the fields of pathology, microbiology, and women's health and oncology gathered to review data and come up with guidelines for management of abnormal cervical cytology and histology results. Previous guidelines had been issued in 2001 but the explosion of information about HPV, its pathophysiology, and its connection with cervical dysplasia and cancer necessitated another conference in 2006. The guidelines are evidence based and freely available. Whenever the term *guidelines* is used in this article, it is in reference to the ASCCP 2006 Consensus Conference guidelines.[5]

The guidelines for the management of a Pap test result showing HSIL are straightforward. Colposcopy with endocervical assessment is acceptable as is immediate treatment with a loop electrosurgical excisional procedure in most patients.[5] The exceptions to this rule are patients who are pregnant or adolescent and have an HSIL Pap test result. The follow-up for those groups is discussed later.

Immediate loop excision of the transformation zone after an HSIL Pap test result is considered acceptable treatment because of the high likelihood that CIN 2 or worse will be found on subsequent biopsy or excision of the cervix. Women who have an

HSIL Pap test result have a 60% to 75% chance of having CIN 2 or worse found on colposcopically directed biopsy.[6] The chance of finding CIN 2+ when evaluation occurs by diagnostic excisional procedure is even higher, up to 94%.[6] This method of see-and-treat (immediate loop excision of the transformation zone) has been found cost effective and also time saving for patients because they do not present for a colposcopy visit separately from the excisional procedure.[6]

Many or most patients, however, have a colposcopic examination and biopsies taken after an HSIL Pap test result. It is recommended that endocervical sampling be performed when a colposcopy is done for an HSIL Pap test result. Endocervical sampling more likely is positive for dysplasia with more severe abnormalities on Pap testing but the value of this part of the procedure is controversial. Endocervical sampling has a false-positive rate of 30% with a curette[7] and may not be necessary in patients who have a satisfactory colposcopy. In a study in 2004, endocervical sampling was found positive for dysplasia in 6.4% of 300 women. The ECC result, however, altered the plan of care in less than 5% of cases and no cancers would have been missed if endocervical sampling had not been performed.[8]

If the colposcopically directed biopsies taken after an HSIL Pap test result do not show CIN 2,3 or worse, there are three options for follow-up according to the ASCCP guidelines. A diagnostic excisional procedure may be performed because the risk for having a CIN 2 or worse lesion is high. The original Pap test result and the biopsies may be reviewed with a pathologist and if the diagnosis of either changes, patients should be managed according to the appropriate part of the guidelines. The other option is to follow patients with Pap tests and colposcopy every 6 months for a year. If they have a result of HSIL on either of the next two Pap tests, they should have a diagnostic excisional procedure. If the Pap test results and colposcopies are negative both times, they can return to routine cytologic screening.[5]

Nonpregnant women aged 21 or older who have a Pap test result showing HSIL and an unsatisfactory colposcopy should have a diagnostic excisional procedure performed.[5]

Pregnant women who present with a Pap test result showing HSIL should have colposcopy performed and biopsies taken if CIN 2 or worse is suspected. No studies to date have shown any risk to mother or fetus by performing a colposcopy with or without biopsies during pregnancy. Also, the colposcopic impression is notoriously inaccurate in pregnancy, with one study showing that 54% of pregnant women who had a negative colposcopic impression had dysplasia found on biopsy.[9] Cervical dysplasia should not be treated in pregnancy. There are two reasons for this: first, pregnancy does not hasten the course of cervical dysplastic disease, and second, the regression rate for CIN after pregnancy is high. Yost and colleagues[10] in 1999 found that the majority of patients who had cervical dysplasia found during pregnancy had some regression of disease after delivery. For example, of women who had CIN 3 diagnosed by biopsy during pregnancy, only 30% had CIN 3 after delivery—the rest had improvement in their histologic findings and there were no cases of progression to cervical cancer. The route of delivery does not seem to influence the regression of cervical dysplasia; those who delivered by cesarean section had identical regression rates compared with those patients who delivered vaginally. Another more recent study confirms this finding. In this study, women were followed with Pap tests, colposcopy, and biopsies during and after pregnancy. Again, the majority (59%) had regression of dysplasia after delivery and the route of delivery did not affect this regression.[11]

In the ASCCP 2006 guidelines, an adolescent is defined as a woman who is 20 years old or younger. The guidelines recommend that adolescents who have a Pap test result showing HSIL should have a colposcopy. The see-and-treat option of immediate

excisional procedure is not acceptable in adolescents.[5] If CIN 2,3 is not identified on colposcopy in an adolescent, follow-up may be accomplished by repeat colposcopy and cytology every 6 months for 2 years. If colposcopy after an HSIL Pap test result is not satisfactory or endocervical sampling is positive for dysplasia, a diagnostic excisional procedure is recommended. During follow-up, if dysplasia is identified, it should be managed according to the ASCCP 2006 guidelines. If no dysplasia is identified but the result of HSIL on cytology continues for a year, a diagnostic excisional procedure should be considered.[5] After two consecutive negative cytology results, adolescent patients may return to routine cytologic screening. The rationale behind the guidelines for adolescents is that although HPV infection is common in adolescents, cervical cancer is rare and the vast majority of dysplasia found in adolescents regress spontaneously.[12] This premise is discussed further in the section on management of CIN 2,3 histology.

PATHOLOGY DESCRIPTION OF CERVICAL INTRAEPITHELIAL NEOPLASIA 2,3

The designation CIN 2,3 includes lesions formerly referred to as moderate dysplasia (CIN 2), severe dysplasia (CIN 3), or carcinoma in situ CIS (CIN 3). In CIN 2,3 lesions, immature basal type cells occupy more than the lower one third of the epithelium but do not cross the basement membrane. Characteristic findings when observing the cellular nuclei include nuclear crowding, pleomorphism, and loss of polarity. Often, the nuclei are enlarged and chromatin is clumped and granular. Nuclei may be bilobed or otherwise shaped erratically. Many mitotic figures, normal and abnormal, may be seen. The relative amount of cytoplasm (compared with nucleus) is reduced in the cells and perinuclear clearing is observed.

The most common lesions that can be mistaken for CIN 2,3 are immature squamous metaplasia and atrophy. These can be differentiated from dysplasia by the lack of nuclear pleomorphism present and the maintenance of cellular polarity.

INCIDENCE OF CERVICAL INTRAEPITHELIAL NEOPLASIA 2,3/PROGRESSION FROM HUMAN PAPILLOMAVIRUS INFECTION TO DISEASE

The incidence of CIN 2,3 in the United States is 1.5 per 1000 women.[13] It usually is diagnosed in women 25 to 35 years of age. Generally, progression from persistent HPV infection to CIN 2,3 to cervical cancer is slow, averaging 15 years.[14] Progression to CIN 2,3 is somewhat more rapid, with one study showing that 27% of women who have an initial infection with HPV 16 or 18 progressed to CIN 2,3 within 36 months.[15] Another large study showed that of women over age 30 who contracted HPV 16, 20% developed CIN 3 within 120 months.[16] This timeline of the progression of cervical HPV disease allows health care providers to identify high-grade dysplasia during screening and, ideally, prevent progression to invasive cancer.

REPRODUCIBILITY OF CERVICAL INTRAEPITHELIAL NEOPLASIA 2,3 ON HISTOLOGY

Many studies from as long as 40 years ago show that interobserver variability in the histologic diagnosis of cervical dysplasia is high. The variability decreases as the severity of the lesion increases. In the ASCUS/LSIL Triage Study, independent pathologists (quality-control group) evaluated histologic slides that had been interpreted at various clinical centers as negative, atypical squamous cells of undetermined significance (ASCUS), LSIL (CIN 1), or HSIL (CIN 2,3). The study found that the reviewing pathologists agreed with the diagnosis of CIN 2,3 more than 76% of the time, downgrading only a quarter of the specimens they looked at. Reproducibility was

decreased in lower-grade lesions; LSIL histology was agreed on only 42% of the time, an equal percentage to those histologic specimens believed normal by the reviewers.[4] If the diagnosis of CIN 2,3 is split into its two parts, the diagnosis of CIN 2 is less reproducible than that of CIN 3. Another study, again using independent pathologists evaluating slides from clinical centers, found that on review, the independent evaluators agreed with the original diagnosis of CIN 3 more than 80% of the time although agreeing only 30% of the time (at most) if the original diagnosis was CIN 2.[17] There is no obvious way to reconcile this interobserver variability. Even having subspecialist pathologists (gynecologic pathologists) grade lesions does not improve the reliability of histologic diagnoses of cervical dysplasia when compared with general pathologists.[18]

MANAGEMENT OF CERVICAL INTRAEPITHELIAL NEOPLASIA 2,3

Women who have CIN 2,3 on histology have a 1.44% chance of having cervical cancer diagnosed in the next 24 months. Regression to normal occurs in only 35%.[19] Because of the rate of progression of CIN 2,3 to invasive cervical cancer, the ASCCP 2006 consensus guidelines recommend that women who have this histologic diagnosis receive treatment.[5] Treatment options include ablative and excisional techniques. Further discussion of these techniques occurs in the next section. Hysterectomy is not a recommended therapy for simple CIN 2,3. Ablative treatment (by laser, cryotherapy, or electrofulguration) is not an acceptable option if patients have an unsatisfactory colposcopy when they are diagnosed with CIN 2,3. Women who are pregnant or adolescents (20 years old or less) at the time of diagnosis and have CIN 2,3 have different follow-up recommendations and generally should not have excisional or ablative therapy. Treatment and follow-up for these patients are discussed later.

The decision of which of the treatment options to use for nonpregnant women over age 20 who have a histologic diagnosis of CIN 2,3 is up to the provider as long as certain criteria are met. A commonly quoted trial comparing cryotherapy, laser ablation, and loop electrode excision found that all three methods had a high success rate for treating dysplasia. There were no significant differences in complication rate or rate of persistence or recurrence. Failure of therapy (persistent disease) was more likely with a large lesion. Recurrent disease was more likely in patients over age 30, those positive for HPV 16 or 18, or those who had had prior treatment.[20] Ablative therapies are not appropriate if colposcopy is unsatisfactory, if the entire squamocolumnar junction or the entire lesion is not seen, if the lesion is large (covers 3 or more quadrants of the cervix), or if invasive cancer is suspected. Each of the most commonly used types of treatment has advantages and disadvantages. Cryotherapy is easy to perform and the equipment needed is inexpensive. There are essentially no risks for acute bleeding with cryotherapy and it is a frequently used treatment in areas where resources are limited. The World Health Organization supports the use of cryotherapy in appropriate circumstances. Disadvantages of cryotherapy are that patients have a profuse watery discharge for 2 to 4 weeks after the procedure and many patients may experience cramping or vasovagal symptoms during the procedure. The other potential disadvantage is that no tissue specimen is obtained for further histologic evaluation of the cervix. Other forms of ablative therapy (laser vaporization, for example) are used less frequently but have similar indications and disadvantages. Loop electrode excisional procedures and cone biopsies (via laser or cold knife cone [CKC]) are the excisional procedures used most often. Loop excision is contraindicated if invasive cancer is suspected. Loop excision generally is performed as an outpatient procedure under local anesthesia and patients who are unable to receive local anesthesia or tolerate

an outpatient procedure may be served better with another therapy. Complications of loop excision include acute bleeding and unintentional burns (rare) and thermal artifact at the edge of the removed specimen, which may make pathologic interpretation of margins difficult. This can occur up to 31% of the time.[21] CKC does not leave thermal artifact but the blood loss is higher on average (although still small at 16 mL)[21] and the procedure usually is performed in an operating room with general anesthesia. CKC is the procedure of choice if there is a suspicion of microinvasion. All forms of excision have the advantage of removal of a specimen that can be examined histologically after the procedure.

An important consideration in deciding whether or not to treat cervical dysplasia is the effect that treatment may have on future fertility and pregnancy outcomes. The peak incidence of CIN 2,3 (hence, treatment of same) occurs during the reproductive years. There are no good large-scale studies looking at infertility after treatment for cervical dysplasia but there has been interest in recent years in the possible risk for preterm delivery after treatment. There are no published randomized controlled trials on the topic and most have been case series or retrospective cohort studies. Understandably, the method of treatment studied depends on the type of treatment used most commonly at the time. Therefore, earlier studies examined the effect of CKC whereas later studies were more likely to look at the effect of loop excision or laser excision. A review and meta-analysis in *The Lancet* looked at 27 studies from 1979 to 2004.[22] Overall, the conclusion was that there is similar pregnancy-related morbidity for all types of excisional procedures examined (loop excision, CKC, and laser). A statistically significant increase in the risk for preterm delivery and low birth weight were noted for loop excision and CKC. The relative risk for preterm delivery was 1.70 (95% CI, 1.24–2.35) for pooled data. The relative risk for low birth weight was 1.82 (95% CI, 1.09–3.06) for pooled data. There was no significant difference, however, with respect to perinatal mortality, neonatal ICU admission, or cesarean delivery (for loop excision).[22] Some of the studies included in this analysis tried to examine the association between obstetric outcomes and the amount of tissue removed. These were difficult to compare although there was a risk for preterm delivery associated with increasing depth of tissue excised, especially greater than 10 mm. The pooled relative risk for preterm delivery in this group was 2.5 (95% CI, 1.5–5.3).[22] In the studies evaluated, fertility did not seem to be affected. Various endpoints, including total pregnancies, ability to conceive within a specified time period, and time elapsed to pregnancy, were looked at. Because of dissimilar outcomes measured, no meta-analysis could be performed. The general consensus is that pregnancy outcomes are affected by treatment for dysplasia, especially with respect to preterm delivery and low birth weight. Although these effects are not severe enough to warrant foregoing treatment altogether, providers should warn patients of the risks and consider carefully whether or not treatment is warranted.

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Management of Atypical Glandular Cells and Adenocarcinoma in Situ

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KEYWORDS

- Adenocarcinoma in situ • Cervical cancer screening
- Cytology guidelines • Endometrial neoplasia
- Atypical glandular cells

Glandular abnormalities of the cervix continue to present clinicians with difficult management decisions. Glandular abnormalities represent a small percentage of all abnormal Pap smears.[1] The rarity of this finding, coupled with a high underlying rate of neoplasia, may lead to significant underdiagnosis and missed opportunities for care. Additionally, clinicians fail to comply with published guidelines in the management of these abnormalities at a higher than desirable rate.[2]

This article highlights the definitions of glandular abnormalities, reviews current published guidelines for clinical management, and discusses the underlying rates of neoplasia associated with these cytology reports. It reviews proper follow-up of patients found not to have neoplasia and current treatment options for patients who have significant neoplasia. It also discusses the diagnosis of associated endometrial lesions and the use of human papillomavirus (HPV) DNA testing in the management of glandular lesions of the lower genital tract.

DEFINITIONS OF ABNORMAL GLANDULAR CYTOLOGY

Glandular abnormalities are described as ''atypical glandular cells (specify endocervical, endometrial or not otherwise specified), atypical glandular cells, favor neoplastic (specify endocervical cells or not otherwise specified).''[3] Additionally, separate categories of endocervical adenocarcinoma in situ (AIS) and adenocarcinoma are reported.

The term ''atypical glandular cells'' (''AGC'') has replaced the previous term ''atypical glandular cells of undetermined significance'' (''AGUS''). The differentiation

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Obstet Gynecol Clin N Am 35 (2008) 623–632
doi:10.1016/j.ogc.2008.09.002 **obgyn.theclinics.com**
0889-8545/08/$ – see front matter

of ''favor reactive'' and ''probably neoplastic'' has been dropped from the terminology. This change was made based on evidence that a high rate of underlying pathology could still be seen in the favor reactive category. Changes were also made in terminology because of confusion of AGUS with atypical squamous cells of undetermined significance (ASC-US).[3]

A cytologic finding of glandular lesion occurs in less than 0.5% of Pap smears. However, the underlying significant neoplasia rate ranges from 9% to 50%. In some studies, more than 10% of patients may have an underlying cancer.[4,5] Studies have shown that the reproducibility of AGC is difficult for pathologists.[6]

The most common neoplastic histologic finding in patients who have AGC is actually squamous dysplasia. A significant number of patients will have AIS or adenocarcinoma of the cervix. Invasive squamous cell carcinoma and endometrial lesions are also found. Other common findings, which are nonneoplastic, include adenosis, polyps, inflammation, and reactive changes. Findings of upper genital tract neoplasia, such as fallopian tube or ovarian carcinomas, have been reported. Therefore, complete evaluation of patients presenting with glandular abnormalities may be necessary in certain situations.

A recent review of a compilation of 24 studies demonstrated a 0.29% rate of glandular abnormalities in almost 2.4 million smears. Evaluation showed that 11.1% of patients had high-grade squamous lesions, 2.9% had AIS, 1.4% had endometrial hyperplasia, and 5.2% had malignancy. In patients who had malignancy, most were endometrial carcinoma (57.6%), cervical adenocarcinoma (23.6%), and squamous cell carcinoma of the cervix (5.4%). In these series of patients who had malignancy, 6.4% had an ovarian or fallopian tube primary carcinoma and other malignancies were found 6.9% of the time.[7] More significant clinical abnormalities occur in women older than 40 who have glandular cytologic findings than in younger women. Therefore, the clinician should have a high index of suspicion in the management of older women who have this finding.[8]

All modalities for the detection of glandular lesions lack sufficient sensitivity for detection of lesions by themselves. Many women who have AIS have normal colposcopic examinations.[9] Additionally, repeat cytology does not improve detection of disease.[10] Limitations of testing may be due to the location of these lesions, which are in gland crypts and may escape detection by sampling devices and visual methodology. Therefore, multimodality testing is necessary to detect these lesions. Management algorithms based on the 2006 American Society for Colposcopy and Cervical Pathology (ASCCP) Consensus Guidelines are available at www.asccp.org.

ATYPICAL GLANDULAR CELLS (FAVOR ENDOCERVICAL OR NOT OTHERWISE SPECIFIED)

Current management for this cytologic report includes colposcopy, directed biopsies, endocervical curettage, and HPV DNA testing (**Fig. 1**). The relative rarity of this report should not overburden clinicians in the management of these patients. A complete workup is necessary because of significant findings during initial and subsequent evaluations. In women older than 35, and women of any age who have abnormal bleeding, endometrial evaluation is also necessary. Women of any age at risk for endometrial lesions (ie, polycystic ovarian disease, obesity) should also have evaluation for endometrial pathology. After complete evaluation, depending on which studies are quoted, 50% or more of patients will have a negative initial evaluation.[11]

Although HPV DNA testing has not been shown to be sensitive enough to triage patients who have glandular abnormalities, it has been demonstrated that patients who have an initial negative complete evaluation and are HPV DNA negative are at

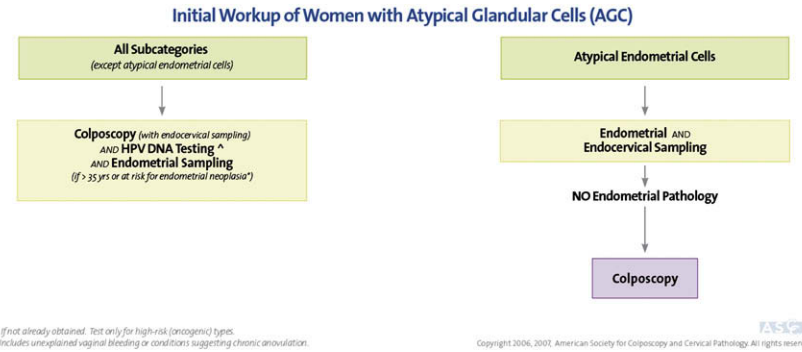

Fig. 1. Initial work-up of women who have AGC. (*Reprinted from* The Journal of Lower Genital Tract Disease Vol. 11 Issue 4, with the permission of ASCCP © American Society for Colposcopy and Cervical Pathology 2007. No copies of the algorithms may be made without the prior consent of ASCCP.)

low risk for neoplasia. The initial evaluation, however, is important to direct appropriate follow-up. If patients have negative evaluations and are HPV DNA negative, recommendations are for a repeat cytology and HPV DNA test in 12 months.[12] However, if HPV DNA testing is positive and initial evaluation is negative, closer observation is indicated because of a significant rate of undiagnosed neoplasia. Repeat testing with cytology and HPV DNA testing is recommended at 6 months in this situation. Complete evaluation is necessary if either is abnormal. If neoplasia is found, clinicians should follow guidelines for treatment of histologic lesions (**Fig. 2**).[11]

ATYPICAL GLANDULAR CELLS (FAVOR ENDOMETRIAL)

If the pathologist interprets the abnormality as atypical endometrial cells, an endometrial biopsy can be preformed first (see **Fig. 1**), Current recommendations also call for endocervical curettage, along with endometrial biopsy. However, if significant neoplasia is not found, a complete cervical evaluation is necessary.[11] Pathologic interpretation

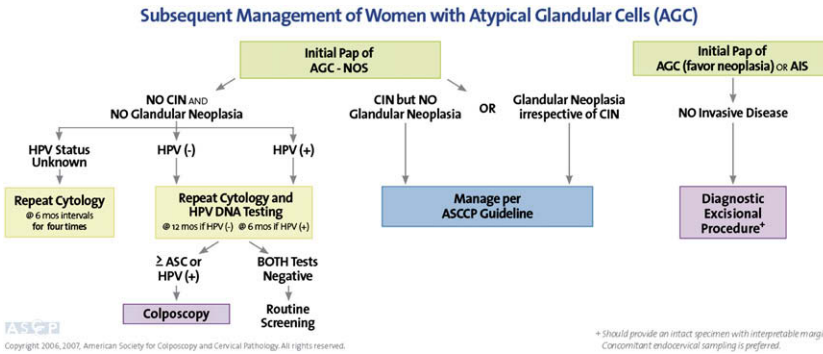

Fig. 2. Subsequent management of women who have AGC. (*Reprinted from* The Journal of Lower Genital Tract Disease Vol. 11 Issue 4, with the permission of ASCCP © American Society for Colposcopy and Cervical Pathology 2007. No copies of the algorithms may be made without the prior consent of ASCCP.)

may not be able to differentiate endocervical from endometrial abnormalities on a consistent basis.[13] It would seem prudent if a patient is recalled for an endometrial biopsy to perform colposcopy at the same time as the endometrial evaluation.

HPV DNA testing could also be preformed. However, endometrial neoplasia is typically HPV DNA negative, which is why complete evaluation for AGC is necessary and another reason why triage with HPV DNA testing cannot be used.

Ultrasound evaluation for atypical endometrial cells has not been investigated. Endometrial thickness on ultrasound has proved to be a valuable tool if office endometrial biopsy cannot be performed. Thickness of less than 5 mm has a high negative predictive value in postmenopausal women.[14] It is logical to use this modality for endometrial evaluation if necessary.

If endometrial neoplasia is discovered, it should be treated according to standard protocols for endometrial lesions. If endometrial biopsy or ultrasound does not reveal neoplasia and abnormal bleeding is persistent, a dilation and curettage (D&C) and hysteroscopy may be necessary.

ATYPICAL GLANDULAR CELLS (FAVOR NEOPLASIA) OR ADENOCARCINOMA IN SITU

A report of AGC favor neoplasia carries a higher underlying neoplasia rate. Initial evaluation is the same as AGC not otherwise specified. If a significant lesion is found, treatment should be directed by histology. However, if no lesions or only cervical intraepithelial neoplasia (CIN) 1 are found, it is necessary to perform an excision biopsy. The relative insensitivity of testing to determine underlying lesions with this diagnosis necessitates excision biopsy.[11] In a recent study, AGC favoring neoplasia had a statistically significant higher prevalence of AIS and malignancy compared with AGC alone. The underlying rates of low- or high-grade CIN showed no difference.[7]

The management of a cytology specimen showing AIS is the same as that of AGC favor neoplasia. With the specimen showing this degree of abnormality, the underlying rate of cancer is higher. Attempts to introduce the term ''glandular dysplasia'' with subdivisions of mild, moderate, and severe have not met with widespread acceptance. If the pathologic diagnosis of glandular dysplasia is encountered, secondary pathologic review may be indicated.[15]

ADENOCARCINOMA

Occasionally, clinicians will be presented with a report stating that adenocarcinoma present. If an invasive lesion is not found in the endometrium or in the cervix, it is prudent to investigate the upper genital tract. Most series reviewing glandular abnormalities of the cervix will report fallopian tube and ovarian malignancies among the diagnoses. Other malignancies, such as colonic or pancreatic disease, although rare, have been reported.[5]

If excisional biopsy and D&C/hysteroscopy are negative, ultrasound imaging of the pelvis should be performed. If clinical suspicion based on cytology is confirmed by a second review, it may be necessary to perform abdominal imaging such as a CT scan. CA125 testing may also be necessary. However, rarely do clinical situations require this type of diagnostic workup.[16]

OTHER ATYPICAL GLANDULAR CELLS

Benign-appearing endometrial cells, endometrial stromal cells, or histiocytes in asymptomatic premenopausal woman are not associated with significant neoplasia. Therefore, further evaluation of these findings has not been recommended.[11]

In postmenopausal patients, endometrial evaluation is suggested for a cytologic finding of endometrial cells, regardless of symptoms. An underlying rate of hyperplasia or malignancy has been found in up to 7% of these cases.[17]

Greenspan and colleagues[18] have published an excellent review of this topic. The investigators note that most women who have endometrial cells on Pap tests have benign findings. However, up to 40% may have polyps. The incidence of hyperplasia has been reported to be as high as 20%, whereas the incidence of atypical hyperplasia has been reported to be 8%, and that of carcinoma, 15%.

Thrall and colleagues[19] reported their experiences in patients who had endometrial cancer and the preceding cytology. In women who had a diagnosis of endometrial cancer, 38% had a glandular abnormality (AGC or adenocarcinoma) preceding the diagnosis and an additional 5.5% had a record of benign-appearing endometrial cells. Patients who have uterine papillary serous carcinoma have a high rate of positive cervical cytology.[20] For patients who have had a hysterectomy, a cytologic report of benign glandular cells has not been shown to have significance and no further evaluation is recommended.[11,18]

HUMAN PAPILLOMAVIRUS DNA TESTING

Perhaps the most significant change in the evaluation of glandular abnormalities is the addition of HPV DNA testing. Several studies have a documented sensitivity for HPV DNA triage ranging from 85% to 95%.[21–24] The 2006 ASCCP Consensus conference proposed that, given the underlying significant rate of disease, this sensitivity was not high enough to allow for appropriate triage. Therefore, a negative HPV DNA test with a finding of AGC does not obviate the need for complete evaluation, including directed biopsies and endometrial evaluation if necessary.

The value of HPV DNA testing with this cytologic finding relates to subsequent evaluation. Clinicians can be assured that if, after a comprehensive evaluation, no significant lesions are found, patients who have negative HPV DNA testing are at extremely low risk for neoplasia. Fetterman and colleagues[12] reported on more than 1100 patients who had AGC Pap smears and HPV DNA results. After initial evaluation, 396 women who were HPV DNA negative were followed for 12 months or more. None of these women were found to have a diagnosis of high-grade neoplasia in subsequent evaluations. Initial evaluation did reveal neoplasia in some women who were HPV DNA negative. An important finding from this study was that women who had a negative initial evaluation and were HPV DNA positive had a 24% risk for having high-grade CIN or AIS in the follow-up.

In women who are followed after initial evaluation, repeat colposcopy, biopsy, and endocervical curettage is recommended if either cytology or HPV DNA testing is positive. Women who are HPV DNA negative can be followed by routine screening. If HPV DNA testing is not available, the 2001 ASCCP guidelines' recommendation for four negative cytologies over 2 years has continued.[11]

PREGNANCY

Evaluation of AGC in the pregnant patient is no different than in the nonpregnant patient except that endocervical curettage is contraindicated. Treatment of any neoplasia would be managed according to the guidelines, with consideration for severity of the lesion and the gestational age. In general, only invasive disease needs treatment during pregnancy. AIS and CIN 3 can be treated in the postpartum period.[25]

ADOLESCENTS

Little data are available on the evaluation of adolescents who have AGC. In one study, only 8 of 1678 adolescent women aged 14 to 21 had a diagnosis of AGC. Current recommendations for management of adolescents are no different than those for older women.[26]

CYTOLOGY PREPARATIONS

Some studies have compared liquid-based preparations with conventional smears. Data suggest that liquid-based techniques may have increased sensitivity for detection of glandular lesions.[27,28] Attempts have been made with immunohistochemical preparations such as p16, Ki-67, and MN antigen to improve the accuracy of cytology with glandular lesions. At the current time, these remain research tools.[29–32]

HISTOLOGY

AIS of the cervix is a distinct histologic entity. Pathologic findings demonstrate glands showing stratification, nuclear abnormalities, and lack of invasion of the basement membrane. The concept of microinvasion for adenocarcinoma has been proposed. This area is controversial and review of pathologic material by the clinician would be necessary to treat patients properly.[33] If invasion is seen, referral to a gynecologic oncologist is appropriate.

If AIS is diagnosed on a cervical biopsy, excisional biopsy is necessary to rule out an invasive lesion. If a diagnosis of AIS is confirmed on excision biopsy, several options exist, based on desire for future fertility. Several articles have described conservative management in patients desiring future child bearing if margins are free of neoplasia on excisional specimens.[34–36] Studies have also shown that if margins are involved, the risk exists for persistent disease or invasive cancer. Most experts recommend repeat excision if margins are positive.[37,38]

In patients who are not interested in child bearing, the consensus is that hysterectomy is indicated for AIS. Simple hysterectomy is the treatment of choice.[39]

Recently, a series of 42 women undergoing conservative treatment of AIS were described. Follow-up consisted of Pap smear biopsy and HPV testing at 6-month intervals. Persistent or recurrent disease was found in 19% of patients who had free margins. Of patients who had involved margins on initial excision, 65% had disease. In this study, HPV DNA testing after treatment significantly predicted disease persistence or clearance. The investigators reported 100% negative predictive value if two Pap smears and HPV tests were negative.[40]

If the patient elects not to undergo hysterectomy, long-term follow-up is suggested. In the author's opinion, this follow-up would consist of cytology and HPV DNA testing. Conservative therapy should not be offered unless margins are free of disease.[41]

Data are not available on long-term follow-up of women treated after hysterectomy for AIS. However, because AIS is a high-grade cancer precursor and recent publications have highlighted the increased risk for neoplasia and women treated for CIN, it would seem prudent to continue long-term surveillance.[42]

TREATMENT

In the management of glandular lesions, the term ''excisional biopsy'' is used. Debate continues concerning the use of thermal techniques, such as loop excision or laser, in the diagnosis of glandular abnormalities.[43] In the past, cold knife cone biopsy was recommended. Several studies showed a higher recurrence rate and higher margin

positivity than with thermal techniques. However, other trials did not show a difference between the techniques.[44,45] Consensus has been reached on evidence that an intact specimen with interpretable margins is key to direct therapy in glandular abnormalities. Therefore, clinicians should choose the modality they feel will most likely yield the best pathologic specimen. Endocervical curettage is recommended at the time of excisional biopsy in suspected glandular abnormalities. Data suggest that this is predictive of residual disease.[46]

VACCINATION

Glandular neoplasia has increased over the last several decades. Proportions of cervical cancers that are now adenocarcinoma are higher than previously, most likely because of increased detection and elimination of squamous lesions by screening programs. However, beside the relative increase in adenocarcinoma, the number most likely due to HPV also appears to have increased.[47] Currently available HPV vaccines prevent infection with HPV 16 and 18. Adenocarcinoma and AIS appear to be related to HPV 18 in most cases.[48] Data from vaccine trials have shown prevention of AIS and squamous lesions. Widespread vaccination should decrease glandular neoplasia.[49]

SUMMARY

Glandular abnormalities of the cervix remain a difficult clinical problem. It is a challenge for the clinician to manage and follow this unusual cytologic finding properly. The rarity and high underlying neoplasia rate make proper management important. Full evaluation, including colposcopy, directed biopsies, endocervical curettage, and endometrial evaluation, are necessary as the initial management step. Subsequent evaluations can be shortened in HPV DNA–negative patients. Close surveillance is necessary even if initial evaluation is negative in HPV DNA–positive patients. Excisional biopsy is necessary in many cases, especially if cytologic results favored neoplasia or AIS. Conservative therapy is possible for women desiring child bearing if invasion is not detected and a complete excision of glandular abnormalities has been performed. It is to be hoped that new guidelines will simplify management for clinicians and aid them in the detection of neoplasia with these diagnoses.

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Management of Adolescents Who Have Abnormal Cytology and Histology

Anna-Barbara Moscicki, MD

KEYWORDS

- Adolescence • Human papillomavirus
- Low-grade and high-grade squamous intraepithelial lesions
- Cervical intraepithelial neoplasia I, II, and III

Clearly, cytology screening programs have resulted in decreased cancer rates worldwide. However, the cost of these current programs in the United States and other developed countries reaches into the millions.[1] Cytology was first used to detect early cervical invasive cancer and targeted adult women at the age of cervical cancer. Epidemiology studies began to unfold the natural history of cervical cancer, showing that cancers were preceded by the development of preinvasive lesions that, if treated, could prevent cancer development. Consequently, programs expanded referral diagnosis to encompass a much broader group of abnormalities. With the development of new molecular techniques, human papillomavirus (HPV) was defined as the causative agent.[2] Epidemiology studies embraced these molecular techniques in the 1980s and quickly showed that HPV was common in sexually active women and extremely common in adolescents. In particular, studies targeted ''at-risk'' youth, defined as those who have multiple partners, are pregnant, or are infected with sexually transmitted infections, which showed unprecedented rates of HPV and abnormal cytology. These findings, with all good intention, were interpreted as uncovering an unrecognized glacier of women at risk for cancer. The numbers of young women finding their way into colposcopy clinics began to escalate. Several important epidemiologic findings began to elucidate the natural history of HPV, underscoring its benign nature and oncogenic potential. These studies were critical in formulating the more recent guidelines for triage and treatment of HPV-associated disease in adolescents. This article discusses the prevalence and natural history of HPV, cytologic squamous intraepithelial

Dr. Moscicki is supported in part by National Institutes of Health/National Cancer Institute (NIH/NCI) 2 R37 CA51323 and NCI 3 R01 CA87905.
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Obstet Gynecol Clin N Am 35 (2008) 633–643
doi:10.1016/j.ogc.2008.09.004 **obgyn.theclinics.com**
0889-8545/08/$ – see front matter

lesions (SIL), and histologic cervical intraepithelial neoplasia (CIN) in adolescents and the biologic factors associated with vulnerability to HPV and its consequences in this age group. Finally, the article covers new guidelines in the United States that are based on these observations.

ADOLESCENTS AND HUMAN PAPILLOMAVIRUS

Repeated studies have shown that adolescents remain one of the highest risk groups for HPV infection. A recent meta-analysis of studies throughout the world showed that most countries demonstrate the same pattern, with a peak in women younger than 25 years of age and a steady decline afterwards.[3] Underscoring the vulnerability of young women to cervical HPV infection, studies of young women who recently began sexual intercourse show that one half will acquire HPV within 2 to 3 years.[4–6] Rates of HPV in adolescents, however, do vary; some populations show rates as low as 5% in adolescents, with no decline or increase over time.[3] The high rates of HPV reported in most adolescent populations have been attributed to either sexual behavior or biologic vulnerability. It remains unclear whether adolescents are more vulnerable to HPV because of their risk behaviors or if a true biologic vulnerability exists. Likely, both contribute.

Certainly, risks for HPV in adolescents are similar to those of adult women and include new sexual partners and lack of condom use.[5,7,8] Most studies show that adolescents have more sexual partners than adult women and are less frequent users of condoms.[9] One study also showed herpes simplex virus (HSV) infection as an independent predictor of HPV acquisition.[7] It is plausible that inflammation associated with HSV may contribute to the risk but the presence of HSV may also reflect risky behavior.

Structurally, the adolescent cervix is different from the adult's in that is has greater areas of immaturity, described as a predominance of columnar and metaplastic epithelium (**Fig. 1**). This topography starts during embryologic development.[10] To review briefly, the cervix is initially lined by Müllerian columnar epithelium and later replaced by urogenital squamous epithelium from the vagina toward the endocervical os in utero, which results in an abrupt squamous-columnar junction located on the ectocervix in the neonate. This junction remains intact until puberty, when hormonal changes trigger uncommitted generative cells of the columnar epithelium to transform themselves into squamous epithelium in a process referred to as squamous metaplasia.

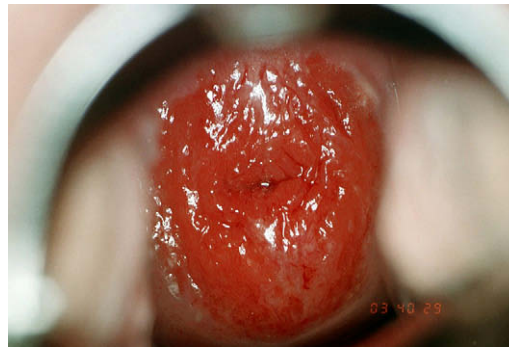

Fig. 1. Typical adolescent cervix. The cervix is primarily covered by a mixture of columnar and metaplastic tissue.

Eventually, the replacement results in a new squamocolumnar junction occurring well into the os, as seen in older women. This area of transition is referred to as the transformation zone and is also the site most vulnerable to cancer development.

It is thought that this epithelium may itself be vulnerable to HPV. First, columnar epithelium is a single-layer thick; hence, basal cells, which are the presumed target for HPV, are accessible. An example of the fragility of this area is the common presence of blood when Pap smears are obtained in adolescents who have large areas of ectopy. Studies of HPV comparing age groups show that incident infections remain more common in young women even when controlling for recent sexual behavior. Munoz and colleagues[11] examined the incidence of HPV in women who were normal cytologically and HPV negative at entry. The incidence of HPV was highest in adolescents aged 15 to 19, with a cumulative incidence of 17% at 1 year and 35.7% at 3 years. The rates declined with age: For women in the 20- to 24-year-old group, the 3-year incident rate was 24.1% and for women aged 45 and older it fell to 8.1%. Although these data support the notion that adolescents may be biologically vulnerable, they may also suggest that the male sexual partner of the older women is less likely to carry HPV, decreasing the chance of infection. Because of either behavior or biologic vulnerability, repeated infections in adolescents and young women are also common.[12,13]

Second, the process of metaplasia itself may support viral replication. HPV requires cell replication and differentiation for it to complete its life cycle.[14] Metaplasia, by definition, is a process of cell replication and differentiation and therefore a perfect environment for HPV replication. Hence, exposure to HPV during times of active metaplasia is more likely to result in an established infection. In one study, adolescents who had evidence of active metaplasia were more likely to show low-grade SIL (LSIL) if infected with HPV.[15] The high rates of squamous metaplasia in young women are likely the explanation for the high rates of LSIL seen in this population. LSIL is found most commonly in adolescents.[16] This finding is supported by the observation that some countries see a second peak in HPV prevalence in women older than 55, yet LSIL does not reflect this second peak, with rates remaining under 1% in the older women.[16]

Differences in immune responses may also explain these differences. Unfortunately, little is known about the adult, let alone the adolescent, cervical mucosal immune response to HPV and questions remain as to whether these differ. One study found that levels of interleukin (IL)-10 were much higher in adolescents who had large areas of ectopy compared with those who had mature cervixes.[17] IL-10 is considered a T-helper-2–type cytokine, which may favor HPV infection and persistence. Hormonal differences may also play a role.[18] Because adolescents have frequent anovulatory menstrual cycles, unopposed estrogen may also have effects on the immune response. The consequences of these high rates of HPV in adolescents may be concerning. Several studies have shown that initiating sex at a young age is a risk for cervical cancer.[19,20] Whether the risk is reflective of a high-risk partner or whether it is related to a biologic risk remains unknown. Other factors such as *Chlamydia trachomatis* infections may play a role in increasing adolescent vulnerability. *C trachomatis*, which is also most common in the adolescent age group, has been shown to enhance HPV persistence.[21]

NATURAL HISTORY OF SQUAMOUS INTRAEPITHELIAL LESIONS AND CERVICAL INTRAEPITHELIAL NEOPLASIA

Because cytology and histology are overlapping but unique entities, studies that reflect SIL and CIN outcomes are discussed separately.

Squamous Intraepithelial Lesions

Although some insist that all HPV infections result in LSIL, the rates of LSIL are, in general, much lower than those found for HPV DNA.[3,16] Certainly, LSIL is the manifestation of HPV replication and protein expression. However, studies have found that risk factors for LSIL are different from those for HPV acquisition.[7] An example is cigarette smoking, which is commonly associated with SIL but not HPV acquisition.[7] These findings may be explained if the risk factor is associated with acceleration of the lesion, causing it to become larger, quicker. Certainly, larger lesions are more likely to be detected by cytology than smaller lesions.

The natural history of LSIL parallels that of HPV, with rapid regression in most cases. As with HPV, more than 90% of LSIL has been shown to regress in adolescent and young women populations within 3 years.[22–24] These observations differ from those of adult studies, where regression rates are much lower.[25] Most likely, many of the LSIL detected in adults reflect persistent infections, with underlying CIN 2 or 3 helping to explain these differences.

High-grade SIL (HSIL) is also a reflection of HPV infection. However, because it represents CIN 2,3 lesions, it is thought to be further along the natural history of HPV. On the other hand, studies have shown that HSIL arises as rapidly as LSIL, possibly bypassing LSIL development.[26,27] The rate of HSIL in adolescents is similar to that found in older women. Mount and colleagues[16] reported that 0.7% of cytologies from 15- to 19-year-olds had HSIL compared with 0.8% of women aged 20 to 29 and 0.7% in 30- to 39-year-olds. Some speculate that these HSIL cases are just ''bad'' cases of HPV with more cellular changes than seen in LSIL. Certainly, the reproducibility of HSIL is less than desirable.[28] One study found that only 50% of adolescents referred to colposcopy for HSIL had confirmed CIN 2 or 3, suggesting that many cases of HSIL are ''overcalls.''[29] For those who perform colposcopy on adolescents, colposcopic interpretation can be challenging because atypical squamous metaplasia (**Fig. 2**), a common finding in this age group, has features similar to CIN, misguiding the colposcopist to biopsy metaplastic tissue instead of neoplastic.[30]

Cervical Intraepithelial Neoplasia 1, 2, and 3

Although cytology and colposcopy have their limitations, they both reflect current tools that guide providers to obtain histology. Histology remains the gold standard for diagnosis. Unfortunately, even histology is not perfect. Providers have long known that experience and the number of areas biopsied increase the chance of CIN 2 or 3

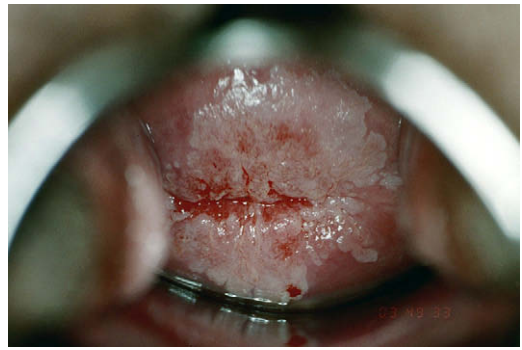

Fig. 2. Atypical squamous metaplastic tissue in adolescent (after application of 3% acetic acid).

diagnosis.[31] The reproducibility of CIN 1, 2, and 3 is also problematic. All of these diagnoses often have less than 50% agreement among pathologists.[28] Most studies agree, however, that more than 80% of CIN 1 diagnoses are likely to regress across all ages.[28] CIN 2 regression rates are more controversial. Syrjanen[25] made the observation that CIN 2 behaves more similarly to CIN 1 than to CIN 3. A recent study of adolescents based on chart review reported that 65% of adolescents who had CIN 2 showed regression over an 18-month period.[23] Most studies show that CIN 2 is more common than CIN 3 in adolescents who have HSIL.[16,29,32] Most importantly, the incidence of invasive cancer in women younger than 20 years of age in the United States is rare, with only 0 to 3 cases reported per one million women in this age group.[33] The low rates of invasive cancer suggest that even in those who have a CIN 2 or 3 diagnosis, progression to cancer as an adolescent is rare. On the other hand, the incidence of invasive cancer sees its first rise at 25 years of age, supporting more aggressive triage starting at age 25. Cancer rates in United States adolescent age groups have been stable over the last few decades, despite the lowering of the age of sexual debut.

SCREENING

One of the strategies for avoiding overtreatment and overreferral in adolescents is to avoid obtaining the Pap smear, which triggers intervention. Several groups, including the American Cancer Society, recommend initiating cervical cytology screening after 3 years of the onset of vaginal intercourse but at no later than 21 years of age.[2] These recommendations were based on the notion that HPV is commonly acquired after sexual intercourse is initiated, most of these infections are likely to be transient, and cancer development during this short period almost never occurs.[34,35]

MANAGEMENT OF ABNORMAL CERVICAL CYTOLOGY

The overall rationale for changes in management of abnormal cytology[36,37] in adolescents was based primarily on the following: (1) Because HPV is commonly acquired shortly after the onset of sexual intercourse, adolescents have high rates of HPV and its associated LSIL; (2) Most of these infections and their corresponding LSIL will spontaneously regress; (3) Adolescents frequently have multiple partners or serial monogamy, resulting in frequent new infections; (4) The rare CIN 3 that does occur is unlikely to progress to cancer during this age period.[36,37] Consequently, with the comings and goings of HPV during this age period, observation remains our best mode of surveillance. Another rationale for conservative management is the risk of the procedure versus the perceived benefit. Studies have shown that preterm delivery, low birth weight, and premature rupture of membranes are risks for cervical excisional procedures.[38] These risks are of concern in adolescents, particularly because this age group is at increased risk for repeated HPV infections, repeated cytologic abnormalities, and, consequently, repeated treatments.

HUMAN PAPILLOMAVIRUS TESTING

Given the current Food and Drug Administration–approved test for HPV, which is not type specific, HPV testing for any reason (atypical squamous cells of undetermined significance [ASC-US], LSIL follow-up) is not recommended in adolescents.[36] The rationale is that the repeat acquisition of HPV appears to be extremely common, specifically in nonmonogamous young women. Most of these infections are transient. Hence, HPV detection (with or without abnormal cytology) in young women is likely to

reflect this transient infection. A study by Boardman and colleagues[39] showed that more than three quarters of adolescents who had ASC-US were positive for high-risk HPV. Certainly, these high rates of infection negate the cost effectiveness of using high-risk HPV DNA testing as triage in adolescents. Rather, future studies should focus on strategies that identify women who have type-specific HPV persistence.[40] Although HPV persistence is key to the development of HSIL and invasive cervical cancers, the length of persistence in this group requiring referral is yet to be established.

ATYPICAL SQUAMOUS CELLS OF UNDETERMINED SIGNIFICANCE AND LOW-GRADE SQUAMOUS INTRAEPITHELIAL LESIONS

Recommendations for ASC-US or LSIL include repeat cytology at 12-month intervals for 2 years.[36] During the 2 years of follow-up, a threshold of HSIL or greater is recommended before referral to colposcopy. After 2 years, a threshold of ASC-US or greater is recommended before referral to colposcopy. Because HPV testing is not recommended, triage for ASC-US using HPV testing is no longer recommended for this age group. If HPV testing is unintentionally obtained, ASC-US/high-risk HPV positive is treated identical to ASC-US or LSIL. HPV testing for follow-up is not recommended.[36]

The rationale is that ASC-US and LSIL have similar natural histories and therefore management guidelines for these have been combined. The justification for bypassing the requirement to colposcopy in adolescents who have LSIL is based on natural history studies of cytologic LSIL and histologic CIN 1. Prevalence studies of LSIL in adolescents show that these are predominantly CIN 1 lesions. This finding is different from that of adult women, where LSIL on screening reveals higher rates of CIN 2 or 3 on referral to colposcopy. Follow-up by cytology is recommended for up to 2 years based on the observation that only 60% of LSIL regressed at 1 year and 92% regressed by 3 years.[22]

ATYPICAL SQUAMOUS CELLS CANNOT EXCLUDE HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESIONS AND HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESIONS

No changes have been made in recommendations since the American Society for Colposcopy and Cervical Pathology 2001 Consensus Guidelines,[36] and they remain similar to those for adults. Immediate triage to colposcopy with biopsy is recommended for atypical squamous cells cannot exclude HSIL (ASC-H) and HSIL. The main difference between adolescents and adults is that immediate excisional treatment of HSIL is an option for adult women but it is not warranted in adolescents. In the case of HSIL, if the biopsy does not show CIN 2 or 3, it is suggested that the adolescent be observed with colposcopy and cytology at 6-month intervals up to 2 years. If HSIL persists by cytology or by colposcopy at 1 year, then repeat biopsy is recommended. If HSIL on cytology persists at the end of 2 years with or without CIN 2,3 diagnosis, a diagnostic excisional procedure is recommended at that time. The exception to this rule is the absence of a satisfactory examination. For adolescents who have an unsatisfactory examination or for whom the endocervical sampling is positive for HSIL, a diagnostic excisional procedure is recommended. Two consecutive negative Pap smears and no high-grade abnormality visible on colposcopy are criteria for a return to routine screening. These recommendations are summarized in **Fig. 3**.

ASC-H in adolescents is treated in a way similar to that of adults, with immediate referral to colposcopy. If no CIN 2,3 is identified, cytology at 6-month intervals is recommended. If the repeat Pap is ASC-US or greater, then the adolescent should be referred back to colposcopy. If the repeat Pap smears at 6 and 12 months are

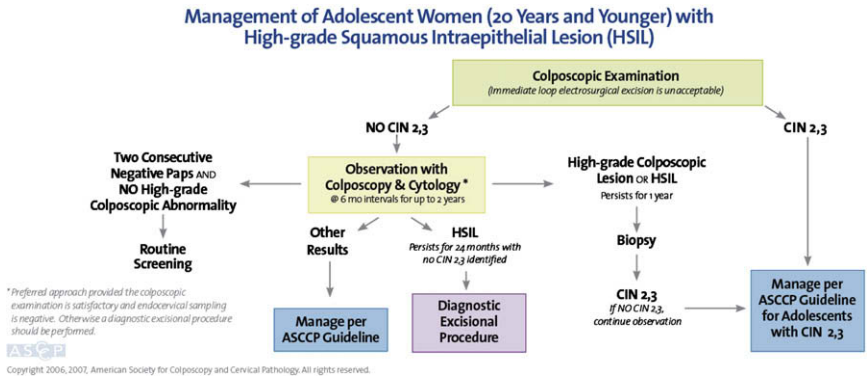

Fig. 3. Management of adolescent women (20 years and younger) who have high-grade SIL. ASCCP, American Society for Colposcopy and Cervical Pathology. (*Reprinted from* The Journal of Lower Genital Tract Disease Vol. 11 Issue 4, with the permission of ASCCP © American Society for Colposcopy and Cervical Pathology 2007. No copies of the algorithms may be made without the prior consent of ASCCP.)

negative, the adolescent may go back to routine screening. HPV testing in follow-up of ASC-H is not recommended in adolescents.

The rationale is that because a significant proportion of HSIL in adolescents is likely to be CIN 2 or less, referral to colposcopy rather than immediate treatment is justified. One study found that only 54% of loop electrosurgical excision procedure (LEEP) specimens in adolescents referred for histologic or cytologic HSIL had confirmed CIN 2.[41] This finding suggests that many of the lesions in adolescents, whether CIN 1, 2, or 3, regress spontaneously. On the other hand, this information may suggest that many HSILs are simply overcalled.

HISTOLOGIC CERVICAL INTRAEPITHELIAL NEOPLASIA 1

CIN 1 is considered benign in adolescents and adult women. In adolescents, triage of CIN 1 parallels that of cytologic ASC-US/LSIL for adolescents described earlier. Treatment of CIN 1 among adolescents is considered unwarranted.[37,42] In follow-up, itt is recommended that cytology be obtained at 12-month intervals. HSIL on repeat cytology at 1 year warrants rereferral. At 24-month follow-up, ASC-US or greater should be referred back to colposcopy. Two consecutive negative Pap tests are criteria for return to routine screening. If repeat biopsies are performed in follow-up, as long as CIN 1 remains the histologic diagnosis, observation is warranted. This recommendation holds for those who have endocervical CIN1.

The rationale is that CIN 1 remains a benign reflection of HPV. Repeated diagnosis of CIN 1 in young women may reflect new HPV infections rather than persistent ones. Because of its benign nature, treatment of CIN 1 in adolescents remains unwarranted.

HISTOLOGIC CERVICAL INTRAEPITHELIAL NEOPLASIA 2, 3

Recommendation for treatment of adults or adolescents is either excisional procedure or ablative. For those who have unsatisfactory colposcopy, excisional therapy is recommended. If the examination is satisfactory, either ablative or excisional therapy is recommended. Some suggest that focal LEEPs or cryotherapy are more suitable for adolescents who have smaller lesions because both of these have lower rates of

complications. Complications of excisional procedures include pelvic inflammatory disease, which underscores the importance of screening for sexually transmitted infections before treatment.[43]

The new treatment guidelines for CIN 2,3 have an additional option for adolescents. It is acceptable to not treat adolescents who have CIN 2 or lesions diagnosed CIN 2,3 and to consider observation. It is suggested that this recommendation be applied to those who are considered reliable candidates for follow-up.[37] Observation is similar to that described for HSIL without CIN 2,3. Follow-up with colposcopy and cytology are recommended at 6-month intervals. If the CIN 2,3 lesion persists by colposcopy or cytology (HSIL) at 1 year, repeat biopsy is recommended. If the lesion progresses to CIN 3 or greater, or if CIN 2 or greater persists at 2 years, treatment is recommended. Treatment is always recommended for a CIN 3 diagnosis. The recommendations are summarized in **Fig. 4**.

The rationale is that CIN 2, by many pathologists, is considered an equivocal diagnosis and, in reflection, is often recategorized as a CIN 1 or CIN 3. CIN 2 in adolescents is thought to reflect a lesion more similar to CIN 1 than CIN 3 because CIN 3 lesions are rare in adolescents and cervical cancers are extremely rare. Consequently, with time, lesions that would spontaneously regress will have the opportunity. In the same vein, lesions that are destined to progress are unlikely to undergo significant progression within short time periods. With close surveillance, the lesions can be diagnosed as persistent and still be treated in a timely and preventive manner. Observation, however, is for those adolescents in whom compliance is assured. The recommendations include CIN 2 or CIN 2,3 because, in the latter case, the histologic diagnoses of CIN 2 and 3 are often not distinguished on a pathology report. Because CIN 2 lesions are more common than CIN 3 in adolescent girls, it is recommended that lesions diagnosed as CIN 2,3 be treated similarly to CIN 2.

In summary, the new management guidelines for adolescents who have abnormal cytology and histology strongly favor observation. These guidelines are based on evidence that shows that HPV-associated lesions in adolescents are likely to regress and persistent lesions have a low probability of progression to cancer during adolescence. The guidelines recommend discussing the risks of treatment versus the risks for

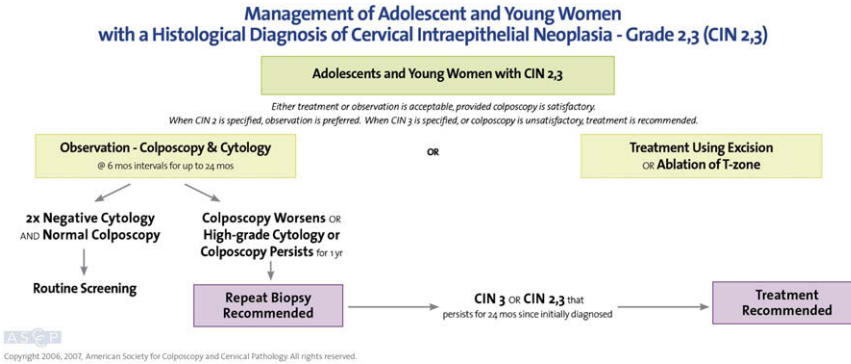

Fig. 4. Management of adolescent and young women who have a histologic diagnosis of CIN 2,3. (*Reprinted from* The Journal of Lower Genital Tract Disease Vol. 11 Issue 4, with the permission of ASCCP © American Society for Colposcopy and Cervical Pathology 2007. No copies of the algorithms may be made without the prior consent of ASCCP.)

progression with the adolescent. Because recurrent HPV infections are common in young women, follow-up using the current HPV tests is not recommended in adolescents. Current recommendations are for vaccinated and unvaccinated adolescents.

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Cervical Cancer Screening in Pregnancy

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KEYWORDS

- Cervical cancer • Cervical intraepithelial noeplasia
- Pregnancy • Colposcopy
- Management of cervical intraepithelial neoplasia in pregnancy

Cervical cancer is the most common malignancy diagnosed during pregnancy, with an incidence of 0.45 to 1 per 1000 live births in the United States.[1] Nearly 3% of cases of newly diagnosed cervical cancer occur in pregnant women, probably because it is one of the few cancers for which screening is part of routine prenatal care. The prevalence of abnormal Pap test results in pregnancy does not differ from the age-matched nonpregnant population. In some populations, up to 20% of pregnant women have an abnormal Pap result during pregnancy.[2] This article reviews the literature[3,4] regarding diagnosis and management of cervical dysplasia and cancer in pregnancy.

PHYSIOLOGIC CHANGES OF THE CERVIX IN PREGNANCY

Any clinician who cares for pregnant women is likely aware of the dramatic changes in the cervix as gestation progresses. The cervix undergoes hypertrophy and hyperplasia, with resulting eversion of endocervical epithelium. Increased blood flow leads to the familiar cyanotic hue of the cervix and vaginal walls. There is increased edema and fibromuscular relaxation of the cervix and vagina and copious thick mucus production, which make visualization of the cervix more difficult. Decidualization of the stroma often causes friability, polyps, and plaque-like changes that can be seen grossly and colposcopically (**Fig. 1**).

CYTOLOGIC APPEARANCE

Cytologic specimens are more difficult to interpret in pregnancy;[5–7] however, grade for grade, intraepithelial lesions are cytometrically identical to those in nonpregnant women. Hormonal changes in pregnancy cause changes in squamous and glandular epithelial cells, including hyperplasia and reactive atypia. The Arias-Stella reaction, a hyperplastic epithelial change that simulates malignancy, may cause confusion. Decidualization results in large cells with large nuclei that may be misinterpreted.

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Obstet Gynecol Clin N Am 35 (2008) 645–658
doi:10.1016/j.ogc.2008.10.003 **obgyn.theclinics.com**
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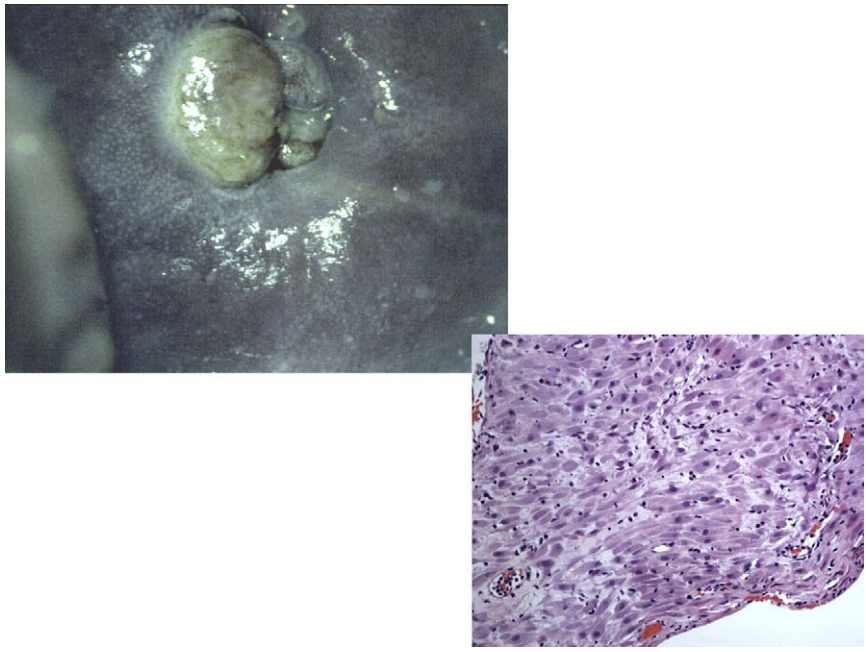

Fig. 1. Plaque-like decidual reaction in cervix. The cervix is seen through a "green" filter. On histology, note the stromal cells with plump polygonal cytoplasm characteristic of decidualization. (*Courtesy of* A. Waxman, MD, MPH, Albuquerque, NM.)

Cytotrophoblast and syncytiotrophoblast cells also may be seen in cytologic specimens. Immature metaplastic cells are often present in large numbers, which may appear similar to high-grade intraepithelial lesions, and there are more inflammatory cells. Despite these challenges, cervical cytology remains an effective screening tool for cervical cancer. Prenatal care provides an opportunity for screening because many women seek health care only when pregnant. It is appropriate to screen all pregnant women who are older than age 20 or sexually active more than 3 years when they present for their first prenatal visit.[8]

COLPOSCOPIC APPEARANCE

The colposcopic appearance of the cervix also changes dramatically throughout pregnancy. Even in the first trimester, edema, cyanosis, and friability can make colposcopy difficult. As pregnancy progresses, decidualization of the stroma often becomes prominent, appearing colposcopically as densely acetowhite plaque-like lesions with spidery superficial blood vessels. Cyanosis of the stroma causes a distinctly dusky appearance, and normal capillaries often have a ring of acetowhite decidualized stroma surrounding them, which causes a "starry sky" appearance. Active immature metaplasia often produces large areas of thin acetowhitening and may have fine mosaic and punctation vessels, making it difficult to distinguish from low-grade dysplasia.

Intraepithelial lesions are difficult to grade during pregnancy because the changes described previously tend to distort the colposcopic findings on the cervix that clinicians rely on to assess the grade of dysplasia. On one hand, edema of the cervix

makes acetowhite epithelium tend to look less intense, which makes the lesions appear less severe. On the other hand, vasodilation causes intraepithelial blood vessels to be larger, which makes lesions look more severe. In individual patients, these changes can be challenging to interpret (**Fig. 2**). More importantly, subtle signs of invasion are easy to miss within a high-grade intraepithelial lesion.

Colposcopic Technique

In early pregnancy, no changes in patient positioning are needed. As pregnancy progresses, patients may develop symptoms of supine hypotension during colposcopic examination, so folded sheets may be needed to wedge the right hip off the table. Visualization of the cervix can be difficult in pregnant women. Relaxation and redundancy of the vaginal walls, well known to all practitioners who care for pregnant women, can obscure the cervix even with a large speculum in place. It is important to use the largest (in width and depth) speculum a patient can tolerate. If separating the blades using the screw on the handle does not provide adequate exposure, a vaginal sidewall retractor can be used. The vaginal walls also can be retracted with a condom placed over the speculum and opened at the distal tip. Some clinicians advocate the use of a glove finger; however, this approach usually limits how far the speculum can be opened. If the cervix is displaced posteriorly, sometimes it can be coaxed between the blades of the speculum by flexing a patient's hips (modified McRobert's position).

Cervical mucus is usually thick, opaque, and tenacious, and pulling on the mucus is not usually successful because the cervix produces rapidly. Sometimes twisting the mucus around a dry cotton swab allows the twisted strand to be mobilized more easily. It often takes more time and more liberal application of acetic acid for the acetowhitening reaction to take place. The cervix is friable, so care must be taken to spray or dab the acetic acid rather than rub. Pregnant women often experience more burning sensation with application of acetic acid compared with nonpregnant

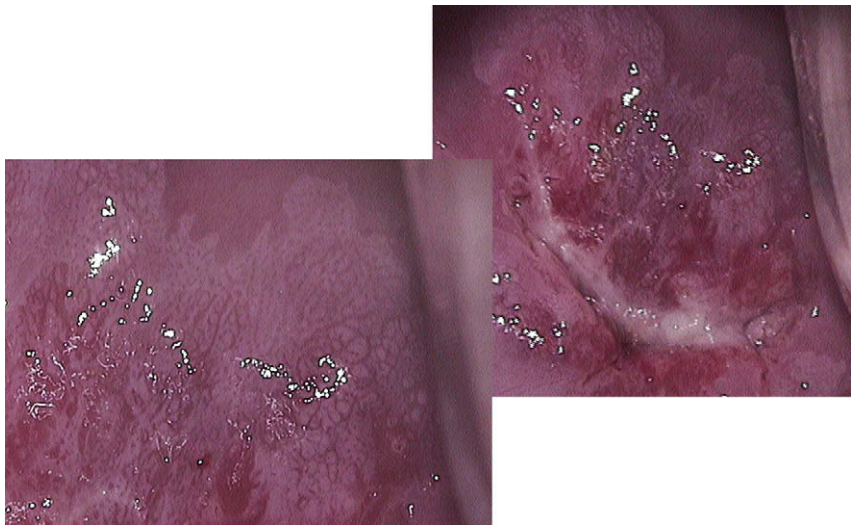

Fig. 2. Anterior lip of cervix on an 18-year-old woman at 20 weeks' gestation. Pap smear indicated low-grade squamous intraepithelial lesion. Inset shows prominent mosaic and punctation patterns. (*Courtesy of* A. Waxman, MD, MPH, Albuquerque, NM.)

women. Late in pregnancy, effacement and dilation of the cervix add to the challenges in visualizing dysplastic changes. Colposcopy in late gestation should be limited to patients most likely to have malignancy based on epidemiologic, cytologic, and gross findings.

Biopsy Technique

There is no evidence that biopsy of the pregnant cervix is any more risky than biopsy outside of pregnancy. Anecdotally, most clinicians note brisker bleeding, but there is no increase in the risk of clinically significant hemorrhage. Biopsies are indicated when the results could potentially impact a patient's management options. The American Society for Colposcopy and Cervical Pathology consensus guidelines recommend biopsy of lesions suspicious for cervical intraepithelial neoplasia (CIN) 2,3 or cancer.[9] A small, sharp biopsy forcep is especially important in pregnancy. It is helpful to prepare by placing an absorbent pad beneath the patient's buttocks extending beneath the speculum handle. Immediately after obtaining the biopsy specimen, pressure with a large swab prevents blood from welling up. After handing off the specimen, a second small swab with a hemostatic substance, such as Monsel's paste or silver nitrate, can be readied for application. Only then should the pressure be released. Both of these hemostatic agents are caustic, so care should be taken to apply as little as needed to the cut stroma, minimizing the amount on the epithelial surface. If bleeding is excessive, cautery, fine suture, or vaginal packing may be needed. Although this bleeding may result in anxiety on the part of the patient and the clinician, adverse effects are unlikely.

HISTOLOGIC APPEARANCE

Similar to cytologic changes, histologic changes in the cervix associated with pregnancy make interpretation of biopsies challenging. Glandular hyperplasia and atypia, decidual reaction, Arias-Stella reaction, and immature metaplasia may be present even in small punch biopsies. It is still appropriate to perform biopsies when indicated; it is incumbent on the clinician to notify the pathologist of a patient's gestational age. A small prospective study showed a high concordance between colposcopic prediction during pregnancy and the ultimate histologic diagnosis.[10] Several other larger retrospective studies confirmed a high correlation between antepartum colposcopic impression and histologic diagnosis.[11,12]

MANAGEMENT OF THE ABNORMAL PAP TEST RESULT IN PREGNANCY

Indications for colposcopic examination are similar for pregnant and nonpregnant women. The only exception in the American Society for Colposcopy and Cervical Pathology guidelines is that deferral of colposcopy until the postpartum period in women with low-grade squamous intraepithelial lesions or atypical squamous cells of uncertain significance (ASCUS) human papillomavirus (HPV)-positive status is acceptable.[9] Colposcopy is still preferred for women over age 20 and should be pursued in women with infrequent screening or women who may not access health care after pregnancy. Women with atypical glandular cells should undergo colposcopy, but endocervical and endometrial curettage are contraindicated because of concern about disrupting the gestation. Colposcopic examination is indicated for nonadolescents with all other intraepithelial lesion or neoplastic findings on Pap test (**Fig. 3**).

Biopsy should be considered if colposcopic findings suggest high-grade changes or worse. Because colposcopic appearances are difficult to interpret, biopsy documentation is particularly important in ruling out early invasive disease.

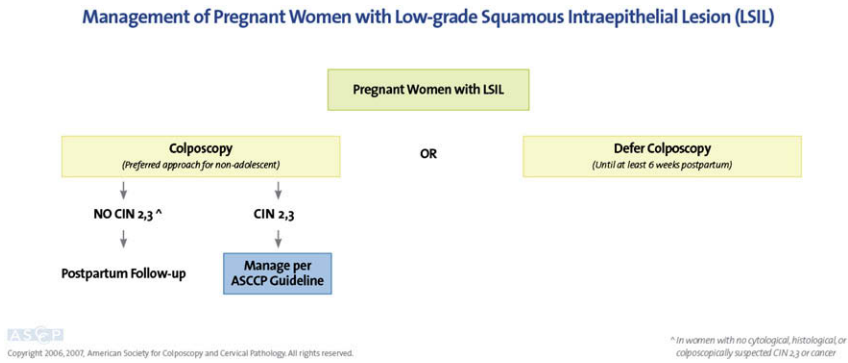

Fig. 3. Management of pregnant women with low-grade squamous intraepithelial lesion. (*Reprinted from* The Journal of Lower Genital Tract Disease Vol. 11 Issue 4, with the permission of ASCCP © American Society for Colposcopy and Cervical Pathology 2007. No copies of the algorithms may be made without the prior consent of ASCCP.)

MANAGEMENT OF COLPOSCOPIC FINDINGS

Low-Grade Lesions

Pregnant women with no cytologic or colposcopic evidence of high-grade disease may be safely managed without biopsy. Repeated colposcopy during pregnancy in women with no evidence of CIN 2,3 or cancer on cytology, colposcopy, or biopsy is unnecessary is termed "unacceptable" in the American Society for Colposcopy and Cervical Pathology guidelines.[9] Postpartum, these women can be evaluated with either repeat colposcopy and Pap test or with Pap test alone, with colposcopy being reserved for women with persistent abnormalities.

High-Grade Lesions

Pregnant women with high-grade lesions on cytology and correlating colposcopy, with or without biopsy, may be followed with repeat colposcopy at intervals no shorter than 12 weeks at the discretion of the clinician. There are no data to support the value of repeat evaluation, however. Women with high-grade cytologic findings but no colposcopic evidence of high-grade disease may undergo repeat colposcopy in an effort to locate the source of the abnormal cells. On the other hand, the American Society for Colposcopy and Cervical Pathology guidelines recommend that repeat evaluation with cytology and colposcopy be deferred until at least 6 weeks postpartum in the case of high grade squamous intraepithelial neoplasia (HSIL) cytology without colposcopic confirmation of CIN 2,3 or cancer. This recommendation, however, is based on expert opinion without strong evidence to support it.

Unsatisfactory Colposcopic Results

Most of the time, the transformation zone can be assessed readily in pregnancy. Eversion and gaping of the endocervical epithelium facilitate examination of the squamocolumnar junction. If the colposcopy is unsatisfactory early in gestation, it should be repeated in the second trimester. In almost all women, colposcopic examination becomes satisfactory by the end of the second trimester. In many cases, a ring forceps may be used successfully in place of an endocervical speculum. If the transformation zone still cannot be visualized in its entirety, the risk of diagnostic conization must be balanced against the likelihood of malignancy. In most women, it is appropriate to defer further evaluation until the postpartum period.

CONIZATION IN PREGNANCY

The indications for conization in pregnant women are different from those in nonpregnant women. Therapeutic conization is contraindicated in pregnancy. Diagnostic conization should be reserved for situations in which there is a significant risk of invasive cancer that cannot be diagnosed colposcopically and the finding of cancer would change the management of the patient. In younger women who have been screened regularly and in whom there is no cytologic, colposcopic, or histologic evidence of malignancy, conization can be deferred even if there is lack of correlation between Pap and biopsy results or an unsatisfactory colposcopy result. Indications for conization in pregnancy include microinvasion or adenocarcinoma in situ on a punch biopsy or strong colposcopic, cytologic, or histologic suspicion of invasion that cannot be confirmed. Conization in pregnancy is associated with significant morbidity, specifically hemorrhage, infection, and pregnancy loss or preterm delivery. The hemorrhage risk has been shown to correlate with trimester in which the conization is performed, with the greatest risk of more than 500 mL of blood loss approaching 10% in the third trimester.[13] Overall, fetal death rate is quoted at approximately 5% and most commonly results from chorioamnionitis or prematurity. The rate of preterm delivery is 10% to 15%.

Conization Technique

In general, a full cone-shaped excision is not needed in pregnancy. Only the portion of cervical epithelium and stroma needed to make a diagnosis should be excised, with no attempt to remove all of the dysplastic tissue. Often, this approach results in either a wedge or a shallow disc- or coin-shaped specimen. Patients must be counseled that further therapy is needed in the postpartum period to address the remainder of the lesion. The complication rate of conization is related to gestational age, with the lowest rates of morbidity earlier in pregnancy. Conization is best avoided in the third trimester because there is an increased risk of hemorrhage and preterm delivery and possibly an increased risk of cervical laceration at the time of delivery.

Conization in pregnancy should be carried out in the operating room, preferably under regional anesthesia if feasible. If the fetus is at a gestational age of viability, continuous fetal monitoring is appropriate; for the previable fetus, intermittent auscultation with documentation of normal fetal heart tones before and after the procedure is adequate. Some clinicians have advocated prophylactic use of tocolytic agents, such as betamimetics and prostaglandin inhibitors, whereas others have recommended using such agents only if uterine contractions occur perioperatively. Intraoperative colposcopy is used to delineate the area to be excised. Acetic acid and Lugol's iodine are safe to use. It is generally recommended to perform excision with a knife. Several case reports recently discussed safe use of loop excision in pregnancy.[14–16]

There are multiple strategies for decreasing the risk of heavy bleeding. The use of intracervical vasoconstricting agents during pregnancy remains controversial. There is concern that resultant vasospasm of the uterine arteries may lead to fetal hypoxia. Transient abnormalities of the fetal heart rate tracing are not unusual, but their long-term significance is unclear. Sutures at 3 o'clock and 9 o'clock at the cervicovaginal junction can be placed before incision to ligate the descending cervical branches of the uterine artery. A McDonald-type cerclage suture also can be placed before incision high in the cervical stroma without advancing the bladder. This suture can be left untied so that the cervix is less distorted during the excision and it can be tied if bleeding is excessive. It may be left in place or removed at the end of the procedure if hemostasis is not a concern. Once the tissue has been excised, electrofulguration

can be used at the stromal bed to obtain hemostasis. A running, locking, delayed-absorbable suture also can be placed from side to side for a wedge-shaped excision or placed circumferentially for a wider, more superficial excision. Topical hemostatic agents, such as Monsel's solution, gelatin paste, oxidized regenerated collagen, microfibrillar collagen, or gelatin sponge soaked in thrombin, also can be applied. Vaginal packing can be used if needed as an adjunct to any of these techniques of hemostasis.

POSTPARTUM MANAGEMENT

The cervix undergoes tremendous remodeling and repair in the postpartum period, whether the infant was born by cesarean or vaginal delivery. Ideally, evaluation of dysplasia is best postponed until 8 to 10 weeks postpartum to allow adequate healing and decrease artifact. In some health care settings, it may be difficult for patients to return or have insurance coverage beyond the postpartum period, so earlier evaluation may be appropriate; however, it is best to wait a minimum of 6 weeks postpartum to perform cytology, colposcopy, and biopsies. Postpartum cytology, colposcopy, and biopsies as indicated should be performed in women who have had HSIL, atypical glandular cells, or ASC-H Pap tests during pregnancy, regardless of the findings during pregnancy. Women who have had cytologic and colposcopic evidence of low-grade dysplasia during pregnancy may be managed postpartum with Pap testing, deferring repeat colposcopy unless subsequent cytology is abnormal.[9] Women with low-grade squamous intraepithelial lesion or ASCUS HPV-positive status who have had colposcopy deferred during pregnancy should undergo colposcopy postpartum.

Management must be based on postpartum findings. Numerous studies have elucidated the natural history of CIN during pregnancy.[2,17–20] The collective body of literature suggests that at least half of women with dysplasia diagnosed during pregnancy have no evidence of disease postpartum. Of the remaining women, most have no change in the degree of dysplasia, with a substantial minority demonstrating worse disease. The rate of progression from high-grade preinvasive disease to carcinoma during pregnancy is probably on the order of 0.4%. Many of these studies are limited by the fact that follow-up was by cytology only and that a significant number of women were lost to follow-up. Many of these studies were conducted before the high rate of spontaneous regression of CIN in nonpregnant women was well understood. Although it was previously thought that remodeling and repair of the cervix after pregnancy contributed to disease regression, it may be that the high rates of postpartum regression simply reflect the natural history of HPV infection of the cervix.

Fetal Risks

It is well recognized that HPV infection can be transmitted vertically from mother to infant during the process of parturition, but the absolute risk of transmission is uncertain. Infants and children of mothers with HPV disease rarely are diagnosed with either genital HPV lesions or respiratory papillomatosis. There are no data about vertical transmission of high-risk HPV types. Genital HPV infections in children must prompt a careful investigation of the possibility of sexual abuse; however, cases of mother-to-child transmission in the apparent absence of sexual misuse are reported. Juvenile respiratory papillomatosis is a life-threatening disorder involving laryngeal papillomatosis, usually related to HPV 11. Almost all cases are associated with extensive condylomata of the maternal genital tract, but it is theoretically possible that maternal HPV carriage in the absence of overt warts may result in vertical transmission.[21] It is assumed that transmission occurs during vaginal delivery in settings with a high viral

load. Population registry-based studies suggest that the risk of juvenile respiratory papillomatosis is low.[22,23] Currently, it is reasonable to counsel pregnant women who have CIN but not extensive condylomata that there is negligible risk to their fetus.

Invasive Carcinoma

Invasive carcinoma of the cervix complicates approximately 0.5 to 1 per 1000 pregnancies in the United States. Diagnosis may be delayed because the symptoms of cervical carcinoma, such as bleeding or discharge, overlap with those of normal pregnancy. Once a malignancy is diagnosed by punch biopsy or conization, patients are best served by consultation with a multidisciplinary team, including specialists in gynecologic oncology, maternal-fetal medicine, neonatology, radiation oncology, and psychology and spirituality.

Staging

Patients must undergo appropriate staging before management decisions can be made. Cervical cancer staging is based on clinical examination, histologic findings of biopsies and conization specimens, and imaging studies of the chest and kidneys. These findings are classified according to the International Federation of Gynecology and Obstetrics scoring system, which is indicated in **Table 1**. The prognosis or survival rate depends on the stage of disease at the time of diagnosis, with 5-year rates of 99% for stage IA1, 95% to 98% for stage IA2, 90% for stage IB1, and 75% for stage IB2.[24] The prognosis of cervical cancer in pregnant patients is unchanged stage for stage.

Pregnant women should undergo staging with imaging, using strategies to limit the amount of ionizing radiation. CT is not absolutely contraindicated for the purpose of assessing for lymphadenopathy or hydronephrosis, because radiation exposure is within the clinically acceptable range, but other imaging modalities are available and should be considered. MRI has been shown to be fairly accurate in predicting parametrial involvement (up to 93%). It also has a high sensitivity and specificity in predicting nodal metastases for lymph nodes larger than 1 cm (88% and 91%, respectively).[25] (Of note, these data are based on the nonpregnant population.) Imaging of the urinary tract may be deferred in patients in whom extracervical disease is unlikely, such as stage IA or microscopic/ small stage IB (< 1 cm). For patients with higher stage disease or high-risk histology, ultrasonography or MRI may be considered in place of intravenous pylegram to rule out stage III disease.[25] A chest radiograph exposes pregnant patients to a minimal amount of radiation and should be obtained in patients with more than microscopic disease to evaluate for pulmonary metastases.

Management

The general principles regarding management of cervical cancer are altered during pregnancy. Definitive therapy results in pregnancy loss, but postponement of therapy may compromise maternal health. Decisions regarding the balance of fetal and maternal risks must be individualized depending on gestational age, cancer stage, and patient wishes.

Stage IA1

This diagnosis is confirmed after conization of the cervix shows only microinvasion. If the margins of the specimen are negative, several studies have demonstrated good outcomes with expectant management, with colposcopy and pelvic examinations every trimester. A small study of four patients with IA1 adenocarcinoma of the cervix diagnosed during pregnancy demonstrated no cases of invasive carcinoma in the postpartum period. All patients were followed without any interventions.[26] A slightly

Table 1
International Federation of Gynecology and Obstetrics staging and classification of cancer of the cervix

Stage	Classification
0	Carcinoma in situ
I	Carcinoma strictly confined to the cervix
IA	Carcinoma identified only microscopically, maximal stromal invasion depth of 5 mm and width of 7 mm
IA1	Maximal invasion depth of 3 mm and width of 7 mm
IA2	Invasion depth > 3 mm but $\leq$ 5 mm, maximal width of 7 mm
IB	Microscopic lesions > IA or clinical lesions confined to the cervix
IB1	Lesions $\leq$ 4 cm
IB2	Lesions > 4 cm
I	Involvement of upper two thirds of vagina or parametria (not extending to pelvic sidewall)
IIA	Involvement of upper two thirds of vagina
IIB	Involvement of parametria without extension to pelvic sidewall
III	Involvement of lower one third of vagina or extension onto pelvic sidewall or nonfunctioning kidney or hydronephrosis (unless attributable to other known causes)
IIIA	Involvement of lower one third of vagina
IIIB	Extension to pelvic sidewall, nonfunctioning kidney, or hydronephrosis
IV	Extension outside the reproductive tract
IVA	Involvement of bladder or rectal mucosa
IVB	Distant metastases

larger group of patients—eight—with squamous cell carcinoma were followed for up to 25 weeks, again with no cases of invasion.[27]

If conization margins are positive, the risk of residual disease is significant.[28] Invasive disease has not been completely ruled out in this situation. Repeat conization is absolutely necessary in the postpartum period for these reasons. During the pregnancy, patients may be followed with serial colposcopic examinations.

Stage IA2, IB, or Nonbulky IIA

The diagnosis of invasive cervical cancer in pregnancy poses a significant dilemma for patients and physicians. The health of the mother and fetus are essentially at direct juxtaposition. The possible detrimental effects of treatments on fetal health must be weighed against maternal desires to continue the pregnancy. The gestational age at the time of diagnosis has a great impact on management. When cervical cancer is diagnosed near term, treatment can be deferred until delivery, which should take place as soon as fetal lung maturity is demonstrated. If the patient is diagnosed before 20 weeks' gestation, termination of pregnancy is an option so that definitive management is not delayed. If cancer is diagnosed in mid-pregnancy, the decision whether to delay treatment until the postpartum period or terminate the pregnancy must be individualized. The definitive therapy is not different in pregnant patients—surgery and chemoradiation. What is different, however, is the presence of a fetus that may be harmed by those treatments and the timing of treatment. These specific treatment options are discussed later as they relate to pregnant patients.

LOCALLY ADVANCED DISEASE

The standard of care for patients with stages IIB to IVA disease is chemoradiation. The addition of cisplatin to primary radiation therapy in the treatment of cervical cancer has been shown to improve the 5-year survival rate by 12% over radiation therapy alone.[29,30] Regardless of gestational age at diagnosis, it is appropriate to consider prompt and definitive treatment. Radiation therapy results in fetal demise; however, passage of the products of conception may be delayed significantly. Several cases of administration of chemoradiation during pregnancy have been reported in the literature.[31,32] In one case report, two patients with stage IB2 squamous cell carcinoma were diagnosed during the second trimester and treated with whole pelvic irradiation concurrent with cisplatin radiosensitization.[31] Both cases required medical inductions of labor because of fetal demise without subsequent miscarriage.

Chemotherapy

Cisplatin is the most effective cytotoxic drug in the treatment of cervical cancer. Data examining the use of cisplatin during pregnancy are limited to case reports. One such report describes several cases, but with no direct causal effect elucidated. They reported on two infants with intrauterine growth restriction, two with moderate bilateral hearing loss, and one with idiopathic ventriculomegaly.[33] Chemotherapy during pregnancy is usually administered before late third trimester, which allows potential clearance of the chemotherapy and accounts for a known side effect of cisplatin—namely, transient neutropenia.[34] The earlier administration of therapy allows for bone marrow recovery.

Chemotherapy essentially has two roles in the treatment of cervical cancer: as neoadjuvant therapy or to prevent metastatic disease. Pregnant patients with metastatic disease need to be counseled extensively on the prognosis of their disease and the additional considerations with the presence of the pregnancy. In terms of neoadjuvant therapy, studies in nonpregnant women have demonstrated a survival benefit in patients treated with neoadjuvant chemotherapy followed by radical surgery compared to surgery only, radiation only, or sequential chemotherapy and radiation.[35,36]

Surgery

In patients with microinvasive disease, cesarean hysterectomy is an option, but because of the significant morbidity associated with this procedure, the potential benefits in the setting of good prognosis of this approach need to be explored thoroughly by patients. On the other hand, cesarean delivery is recommended for patients with stages IA2, IB, and IIA disease for reasons addressed previously. In this situation, radical hysterectomy and pelvic/para-aortic lymphadenectomy can be performed at the same time as delivery. This approach has the benefit of a single surgical procedure and no delay between delivery and definitive surgical management. The biggest complications associated with radical cesarean hysterectomy are increased blood loss and requirement for blood transfusion.[37–39]

In patients who desire to maintain their fertility but are candidates for surgical management, trachelectomy is the procedure of choice. There are some reports of radical trachelectomies performed during pregnancy, but they are associated with a high rate of fetal loss and should be avoided.[40,41] This technique is most beneficial for appropriate candidates during the postpartum period. Women with stage IA2 or small IB1 would be ideal candidates.

MODE OF DELIVERY

The decision for a vaginal delivery versus a cesarean delivery depends on the stage of the disease at time of diagnosis. Preinvasive disease has not been shown to be affected by the route of delivery.[42] Even microinvasive or early invasion is not a contraindication to vaginal delivery because maternal prognosis is not thought to be altered by it.[43] The presence of gross tumor of the cervix is a relative contraindication to vaginal delivery for several reasons. Gross tumor has a higher likelihood of bleeding with vaginal delivery. Several reports in the literature have discussed tumor cell implantation in the episiotomy site in women who delivered vaginally in the setting of cervical cancer diagnosis.[44] Nearly 50% of those patients ultimately died of their disease.[45]

OUTCOMES

The prognosis of women diagnosed with cervical cancer during pregnancy seems to be similar to nonpregnant women, stage for stage. Most studies that suggest this are retrospective. A cohort study that compared 40 women with pregnancy-associated cervical cancer to 89 nonpregnant women with cervical cancer demonstrated similar survival rates between both groups.[46] Another study that evaluated 53 women diagnosed with stage IB cervical cancer during pregnancy demonstrated similar 5-year survival rates, which were not changed by the administration of therapy during the pregnancy.[47] On the other hand, the prognosis of the pregnancy is often affected by the diagnosis of cancer. A large study from California showed that women diagnosed with cervical cancer during pregnancy or in the postpartum period have higher rates of spontaneous and iatrogenic prematurity and higher rates of low birth weight and very low birth weight infants.[48]

SUMMARY

Cervical intraepithelial lesions are common in pregnant women. Screening guidelines are no different in the pregnant population from the nonpregnant population. Colposcopic evaluation of women with an abnormal Pap test result should be performed during pregnancy, although it may be deferred until the postpartum period in women with low-grade squamous intraepithelial lesions or ASCUS HPV-positive status. Colposcopic diagnosis is challenging in pregnant women because of pregnancy-related changes in the appearance of the cervix and mechanical difficulties in visualization. The role of colposcopic evaluation is to rule out invasive cancer, because all other abnormalities can be safely managed expectantly until the pregnancy is over. When cancer is diagnosed during pregnancy, patients should undergo staging. Decisions regarding therapy, such as balancing risks to the fetus from therapy with potential risks to the mother from delaying therapy, must be individualized and are best addressed by a multidisciplinary team.

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Colposcopy of the Vagina and Vulva

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KEYWORDS

• Colposcopy • Vulvar intraepithelial neoplasia
• Vaginal intraepithelial neoplasia • VIN • VaIN • HPV

The vagina and vulva are less common sites than the cervix for development of cancer and cancer precursors. Vaginal intraepithelial neoplasia (VaIN) and vulvar intraepithelial neoplasia (VIN) are increasingly diagnosed, are often HPV related, and are known cancer precursors, however. Colposcopy of the vagina and vulva is an important component of the screening process for lower genital tract diseases.

COLPOSCOPY OF THE VAGINA AND DIAGNOSIS OF VAGINAL INTRAEPITHELIAL NEOPLASIA

Vaginal Colposcopic Indications

Limited vaginal colposcopy should be undertaken routinely with each cervical colposcopic examination with an evaluation of the proximal one third of the vagina after examining the cervix and during the withdrawal of the speculum. More detailed vaginal colposcopy is warranted in women with abnormal cervical cytologic results that are unexplained by cervical findings or incongruent with cervical colposcopic findings. Other indications include abnormal cytologic results in a woman with a previous hysterectomy or treatment of cervical dysplasia, palpable or visible vaginal lesions, unexplained postcoital or vaginal bleeding, coexisting human papillomavirus (HPV) disease in an immunosuppressed patient, and diethylstilbestrol exposure in utero.[1,2]

Vaginal Colposcopic Technique

Vaginal colposcopy can be challenging because of the anatomy and features of the vagina. The anterior and posterior walls of the vagina are usually obstructed by the speculum blades, and the anterior and posterior fornices can be difficult to access because of the position of the cervix. There is a large surface area involved. In women with a prior hysterectomy, the lateral aspects of the vaginal vault can be difficult to examine. The use of skin hooks, an angled mirror, or an endocervical speculum can improve access to these areas. The vaginal rugae can make examination of the vaginal

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Obstet Gynecol Clin N Am 35 (2008) 659–669
doi:10.1016/j.ogc.2008.10.002 **obgyn.theclinics.com**
0889-8545/08/$ – see front matter

walls challenging because the folds can obstruct the visualization of small lesions. The presence of abundant vaginal discharge can obscure the evaluation of the vaginal epithelium.

A systematic approach to the evaluation of the vagina includes vaginal cultures if indicated, cleaning off discharge with a saline-soaked, large, cotton-tipped applicator, application of 3% to 5% acetic acid to the fornices and lateral walls of the vagina, and careful inspection of these areas, which can be assisted by the use of cotton-tipped applicators to manipulate the cervix. Rotation of the speculum 90° (with collapsed blades) permits application of acetic acid and inspection of the anterior and posterior vaginal walls. Opening and closing the vaginal blades can help to smooth out the vaginal rugae for improved exposure. Application of Lugol's iodine also can be helpful in identifying any abnormal areas. Notes should be made of any areas with abnormal texture, lesions, cysts, acetowhitening, and Lugol's negative areas on a vaginal diagram. Punctation vascular changes are commonly seen in the vagina in the presence of dysplasia, atrophy, or inflammation, and mosaic vascular changes are uncommon in the vagina. The enhanced punctation is diffuse in the benign processes and localized in the presence of dysplasia. Should the vaginal visualization be significantly impaired because of atrophy, a short course of vaginal estrogen using 1 g of estrogen daily for 3 weeks can be used before repeat colposcopy.[2] Other benign causes of changes in the vaginal appearance include vaginal intercourse and use of tampons, diaphragms, vaginal spermicides, vaginal pessaries, and vaginal ring hormonal delivery systems.[3]

Vaginal Biopsy Technique

Colposcopically directed vaginal biopsies are indicated to evaluate any abnormal vaginal findings. Vaginal dysplastic changes are often less specific in appearance compared with cervical dysplasia and warrant histologic evaluation. The upper two thirds of the vagina have little sensation and often can be biopsied without anesthesia. The distal third usually requires injection of local anesthesia before biopsy. Pinching a neighboring normal area with a fine-tipped forceps can serve as a test to determine whether anesthesia is needed. It is essential that the biopsy forceps be sharp, and reducing the tension of the speculum blades sometimes can aid in obtaining the biopsy specimen by making the vaginal side wall bulge into view, which allows the perpendicular application of the biopsy forceps. Vaginal biopsies usually bleed little, and hemostasis is usually easily achieved with Monsel's solution or a silver nitrate stick. Biopsies should be labeled carefully to identify the distance from the cervix or introitus and the position on the clock face. If multiple biopsies are taken, they should be sent for pathologic evaluation in separate containers.

Vaginal Intraepthelial Neoplasia

VaIN occurs infrequently compared with cervical intraepithelial neoplasia (CIN), but its incidence is increasing,[4] likely because of increased awareness, increased frequency of cytologic and colposcopic screening, and an absolute increase in frequency. Most VaIN are HPV induced and can occur in conjunction with other HPV manifestations in the lower genital tract. In keeping with the nomenclature of CIN, VaIN is divided into three grades. VaIN 1 is diagnosed when atypia is present in the lower third of the epithelium; VaIN 2 has atypia present in the lower two thirds of the epithelium; VaIN 3 occupies more than two thirds of the epithelial thickness. VaIN 2/3 is often reported as high-grade VaIN consistent with the Bethesda system for reporting CIN. VaIN can be identified on cervical or vaginal vault cytology in women with a previous

hysterectomy. In a study of 31 women who underwent hysterectomy with a diagnosis of VaIN, 83% had vaginal cytologic results that demonstrated dysplasia.[5]

Low-Grade Vaginal Dysplasia (Vaginal Intraepithelial Neoplasia 1 and Vaginal Condyloma)

The colposcopic appearance of low-grade VaIN is similar to that of low-grade CIN, but lesions are often subtle and easily overlooked. This appearance includes acetowhitening, Lugol's nonstaining areas, and leukoplakia. Fine punctuation can be seen, but mosaic patterns are rare. Low-grade lesions are often multifocal. Vaginal condyloma can coexist with vulvar and perineal condyloma in approximately 30% of patients.[1] Because these lesions sometimes can be difficult to differentiate from high-grade dysplasia or neoplasia, any lesion that is persistent, atypical in appearance, or resistant to usual treatments should be biopsied.

High-Grade Vaginal Dysplasia (Vaginal Intraepithelial Neoplasia 2 and 3)

The colposcopic appearance of high-grade vaginal dysplasia is similar to that of high-grade cervical disease, except that the mosaic vascular pattern is not usually seen. Aceto-whitening, Lugol's nonstaining areas, and leukoplakia can be seen, but in a thicker, denser, and more sharply demarcated fashion (**Fig. 1**). Punctation is of a coarser nature than in low-grade lesions. Areas of erosion and surface irregularities also can be appreciated. VaIN 3 is rarely multifocal. Any suspicious area should be biopsied.

EPIDEMIOLOGY AND NATURAL HISTORY OF VAGINAL INTRAEPITHELIAL NEOPLASIA

Case series have evaluated the epidemiology of VaIN. In one group of 76 patients, the occurrence of VaIN was grade1, 53%, grade 2, 19%, and grade 3, 29%. There was a 15-year age difference between the women with VaIN 1 or 2 and women with VaIN 3.[6] Women with VaIN1, VaIN 2, and VaIN3 were found to have mean ages of 44.5, 47.8, and 61.8 years, respectively.[7] Twenty-three percent to 70% percent of patients had a previous hysterectomy, 25% to 87% in the presence of CIN or invasive cervical cancer.[4,7] Most VaIN lesions (84%–92%) occupy the upper third of the

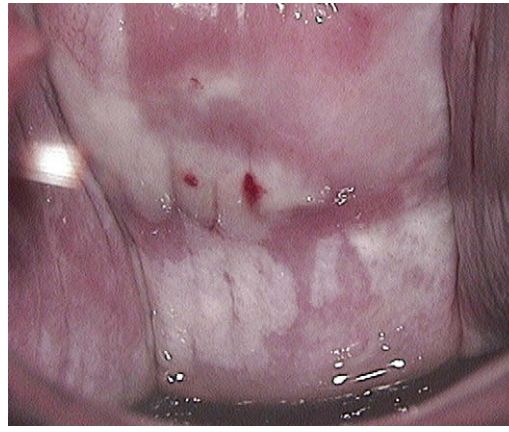

Fig. 1. VaIN 3 seen after application of 5% acetic acid in a 38-year-old woman with concomitant CIN 2. (*Courtesy of* A. Waxman, MD, MPH, Albuquerque, NM.)

vagina.[6,8] Another cohort found that 75% of VaIN lesions were associated with concurrent cervical disease, 8% by direct extension.[9] In an observational study of 23 patients who had VaIN and were observed for 3 years without treatment, 50% had multifocal lesions, one half of which were CIN or VIN. Two cases (9%) progressed to invasive vaginal cancer, 3 cases (13%) had persistence of VaIN, and VaIN regressed in 18 cases (78%).[10] In follow-up of 793 patients who had a hysterectomy with CIN, 41 (5%) presented with VaIN in a 10-year follow-up period. VaIN was found in the suture line and vault angles in 54% of these patients.[11] Extension from the cervix is likely in some cases of vault VaIN after hysterectomy in the presence of CIN. Many patients have de novo VaIN in the absence of cervical disease, which excludes extension from the cervix as its only etiology.

Other risk factors for vaginal dysplasia include HIV infection and diethylstilbestrol exposure. Compared to high-risk but HIV-negative women, HIV-positive women had an eightfold risk for developing VaIN, VIN, and perianal intraepithelial neoplasia over a 6-year observation period.[12] An increased occurrence of vaginal clear cell adenocarcinoma has been identified in women who were exposed to diethylstilbestrol in utero. These women have squamous metaplasia in the vagina and can be at increased risk for vaginal dysplasia.[13]

ROLE OF HUMAN PAPILLOMAVIRUS INFECTION IN VAGINAL INTRAEPITHELIAL NEOPLASIA

The association between VaIN and other types of intraepithelial neoplasia points to the multicentric nature of HPV infection in the lower genital tract. CIN occurs much more frequently than VaIN, probably because of the increased vulnerability of the cervical transformation zone to HPV infection, compared with the stratified squamous epithelium of the vagina.[14] Several HPV subtypes have been associated with VaIN. One hundred percent of vaginal dysplastic lesions in one study were found to be associated with HPV. VaIN 1 was 9% associated with high-risk HPV subtypes 16 and 18, VaIN 2 was 7% associated with subtypes 16 and 18, and VaIN 3 was 67% associated with subtypes 16 and 18. Other HPV subtypes were present in the remainder of the lesions. Vaginal condylomata were 100% positive for low-risk HPV types 6 and 11.[15] In another study, HPV DNA was found in 91% of VaIN 2/3 samples, and high-risk HPV subtypes 16 and 18 were found in 64% of samples.[16] The cervix does not seem to be the source of vaginal HPV because the prevalence of high-risk HPV subtypes is similar in women with and without hysterectomy.[17]

The advent of HPV vaccines for prevention of cervical dysplasia has opened the door for questions regarding the prevention of VaIN. Because a large number of high-grade VaIN lesions test positive for HPV 16 and 18, it is easy to project that HPV vaccines against these subtypes would be effective at reducing these lesions and, consequently, invasive vaginal cancer. The vaccine studies have not yet demonstrated a reduction in vaginal cancer incidence, probably because of the many years needed to develop this cancer, but they have shown a reduction in the incidence of HPV 16- and 18-associated VaIN. In HPV 16- and 18-naïve women, the reduction of HPV 16- and 18-associated VaIN 2/3 was 100% over 3 years. The reduction in all cases of VaIN 2/3 regardless of HPV typing was 49%.[18] Vaccination could significantly impact the incidence of VaIN and vaginal cancer in the future.

As more is understood about the role of HPV and the natural history of vaginal dysplasia, new screening regimens and modalities likely will evolve. Vaginal colposcopy remains the technique by which vaginal lesions are assessed and their severity evaluated, however.

COLPOSCOPY OF THE VULVA AND DIAGNOSIS OF VULVAR INTRAEPITHELIAL NEOPLASIA

Vulvar Colposcopic Indications

Careful examination of the vulva is indicated in the settings of vulvar complaints, cervical or vaginal dysplasia, or incidental findings of vulvar lesions or discolorations. In a preventive care and screening setting, a naked eye examination is sufficient. Being familiar with the normal anatomy of the vulva is essential for the interpretation of vulvar finding. Normal variants, such as vestibular papillomatosis and differing degrees of erythema, can be misinterpreted as pathologic conditions by a less-experienced clinician. Should any texture or color change or symptomatic area be identified, a more thorough examination with magnification is indicated.

Vulvar Colposcopic Technique

The use of colposcopy for examination of the vulva is controversial. Low-power magnification and good lighting are helpful in examining the vulvar skin, and they can be achieved with a colposcope, a hand-held magnifying glass, wearable loupes, or magnifying glass/light-emitting ring combination instruments.[19] The vulva should be examined in a systematic fashion to include the mons pubis and labia majora, the labia minora, clitoral prepuce, clitoris, perineum and anal areas. Attention should be given in the examination of the vestibule to the hymeneal ring or remnants, to the gland openings (Bartholin's and Skene's), and to the urinary meatus. Solutions have been used on the vulvar skin to assist in visualization. Acetic acid (3%–5%) can be used to help identify abnormal areas. Because much of the vulvar skin is keratinized, a prolonged application for 5 minutes using acetic acid–soaked compresses is needed to highlight abnormal areas. Acetic acid can cause acetowhitening of normal skin at the vestibule and the normal variant of vestibular papillomatosis, which can limit its usefulness in practice.[20] Any inflammatory condition of the vulva, including infection and trauma from intercourse, can cause acetowhitening. Historically, toluidine blue and Lugol's iodine solutions were used to stain the vulva and aid in identifying abnormal areas, but this practice has largely fallen out of favor because of high false-positive and false-negative rates.[19]

Vulvar Biopsy Technique

Vulvar biopsies are useful in differentiating between dermatologic and dysplastic conditions. They should be obtained before initiating treatment for vulvar diseases or, if initially certain of the diagnosis, should be obtained if a standard treatment proves ineffective. Areas suspicious for dysplasia or malignancy always should be biopsied. Ulcerated lesions should be biopsied at the raised edge, and multiple biopsies should be taken if the lesion has a complex appearance. The area to be biopsied should be infiltrated with 1% to 2% lidocaine using a fine-gauge needle.[21] Epinephrine with the lidocaine can help with hemostasis but can make the injection burn. Adding bicarbonate to the solution or using topical anesthesia before the injection can help to limit the discomfort. After a test to ensure adequate anesthetic effect using fine-tipped forceps, a biopsy can be obtained using a cervical biopsy forceps, a Keys punch, or a small scalpel blade, depending on the size and nature of the lesion. Small biopsy sites can be treated with Monsel's solution or silver nitrate to achieve hemostasis. Larger biopsy defects may need to be closed with interrupted absorbable sutures. Location of biopsies should be indicated on a vulvar diagram or photograph, and multiple biopsies should be sent separately for pathologic evaluation.

Vulvar Condyloma

Condylomata acuminata are known to be caused by HPV infection, 90% of which are by subtypes 6 and 11. Histologically they are found to contain koilocytes, which are the hallmark of HPV infection. They can be found on any area of the vulvar skin, including the urethra and anus. Vulvar condyloma may be flat or exophytic, and the exophytic condyloma can be papillary (filiform) or verrucous (flat-topped) (**Fig. 2**). Papillary condyloma can be differentiated from benign papillomatosis by observing the ratio of base to papillation. Condylomata have a single base and multiple papillae, whereas papillations have a one-to-one ratio of base to papillation. Sebaceous hyperplasia is another normal finding that can mimic condylomata. The skin on the inner aspect of the labia majora to the border of the vestibule (Hart's line) has multiple sebaceous glands that can enlarge and be seen as white dots or small exophytic lesions. They have a soft, smooth surface not in keeping with the diagnosis of condylomata.

Condylomata are usually seen in clusters and may be confluent, although single lesions are frequently seen. They can often be treated without pathologic confirmation because their appearance is usually characteristic. As with all vulvar lesions, if they do not respond as expected to standard treatments, a biopsy is indicated to rule out dysplasia or neoplasia.

Vulvar Intraepithelial Neoplasia

VIN was classified in 1986 by percentage of epithelial involvement—analogous to the Bethesda CIN system—as VIN1, 2, and 3. Before 1986, VIN was described in a variety of terms, including Bowen's disease and bowenoid papulomatosis.[22] Recently, the International Society for the Study of Vulvovaginal Disease reviewed the classification of VIN and recognized that there are two clinicopathologic types of vulvar squamous cell carcinoma (HPV positive and HPV negative), which have distinct precursors. The new classification discarded VIN 1 because it most often represents

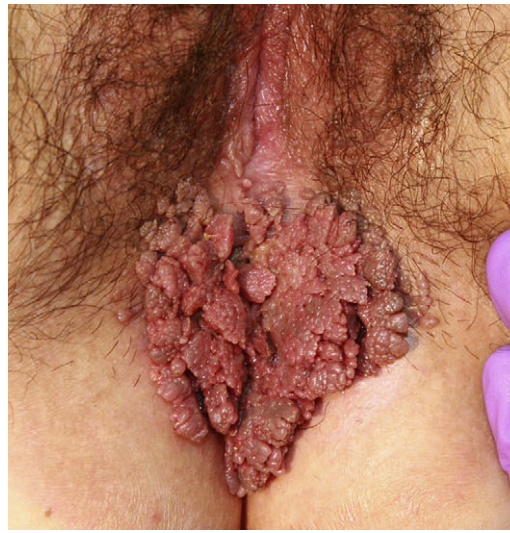

Fig. 2. Vulvar/perianal condylomata acuminata. (*Courtesy of* A. Waxman, MD, MPH, Albuquerque, NM.)

HPV-related changes, is poorly reproducible among pathologists, and no evidence exists that it is a cancer precursor.[23] The International Society for the Study of Vulvovaginal Disease 2004 terminology includes VIN usual type (HPV related) and VIN differentiated type (HPV unrelated). The VIN usual type can be further differentiated as warty, basaloid, or mixed. VIN differentiated type can be associated with vulvar skin conditions such as lichen sclerosus and lichen simplex chronicus. There has been some controversy regarding the dropping of VIN 1 as a significant lesion, because some of the lesions reported as VIN 1 do contain some high-risk virus types found in squamous cell cancer.[24] Many pathologists are still reporting VIN findings according to the 1986 system.

VIN lesions can have a diverse appearance and be symptomatic or completely asymptomatic. Lesions can be pigmented, white, or red or have a mix of colors. They can have a smooth surface or be warty in appearance (**Fig. 3**). Surface erosions may be present. Disease is often multifocal (50%) on the vulva and can include the anus and perineal areas. Most lesions are located in the non–hair-bearing areas of the vulvar skin. The main symptom is of localized pruritus, which is present in approximately 50% of patients.[25] Other complaints can be of texture change, pain, burning, dyspareunia, or dysuria. Symptomatic and asymptomatic vulvar infections can have signs and symptoms similar to dysplasia, and the integrity of the vulvar skin should be ascertained after treatment is completed. Signs and symptoms may include genital herpes erosions, molluscum contagiosum, chancre and condylomata lata of syphilis, chancroid, granuloma inguinale, lymphogranuloma venereum, tinea, and candidiasis with satellite lesions and excoriations.

Many nondysplastic lesions of the vulva can appear like VIN or can coexist with VIN. In patients with known vulvar diseases, increased vigilance is indicated because subtle dysplastic changes sometimes can be difficult to identify within the disease field. Special notice must be paid in women with inflammatory conditions of the vulva, such as lichen sclerosus, lichen simplex chronicus, and erosive lichen planus, which are associated with VIN differentiated type and pose an increased risk of squamous cell cancer of the vulva. Vulvar skin diseases tend to present with symmetric findings. Asymmetrical lesions should be biopsied, as should any areas not responding to treatment (**Fig. 4**). Lesions with a mixed appearance should have multiple biopsies to include all representative areas.

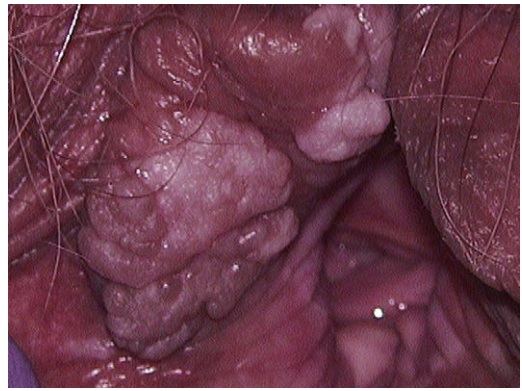

Fig. 3. VIN presenting as raised acetowhite plaques in a postmenopausal woman. (*Courtesy of* A. Waxman, MD, MPH, Albuquerque, NM.)

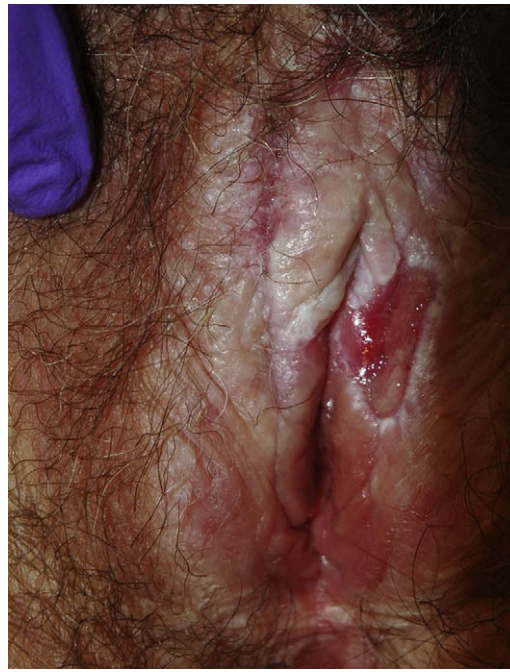

Fig. 4. Benign ulcer associated with lichen sclerosis. Biopsy taken at the ulcer margin is required to rule out cancer. This ulcer resolved after treatment with a topical super-potent corticosteroid ointment. (*Courtesy of* A. Waxman, MD, MPH, Albuquerque, NM.)

EPIDEMIOLOGY AND NATURAL HISTORY OF VULVAR INTRAEPITHELIAL NEOPLASIA

VIN is a precursor to squamous cell cancer of the vulva. In a study in which women with VIN 3 were observed rather than treated, seven out of eight cases (88%) progressed to invasive carcinoma.[26] The incidence of VIN has been increasing, especially in young women, probably related to the increasing prevalence of HPV infection. Several studies have demonstrated a two- to threefold increase in the occurrence of VIN over the last 20 to 30 years.[27] Multiple sexual partners, cigarette smoking, CIN, a history of genital warts, and HIV infection have been reported as risk factors for developing VIN. One series reported that 67.5% of 40 women with VIN were cigarette smokers compared with 12.5% of age-matched controls. Twenty-five percent of the women studied had a history of cervical dysplasia, and 67.5% were under age 50.[28] Women infected with HIV are known to develop more HPV disease than matched high-risk controls. In one study, 16 of 189 (8.5%) HIV-infected women developed vaginal, vulvar, or perianal dysplasia, of which only 13 had VIN. Only 1.1% of the high-risk controls developed VIN.[12] HPV disease is more likely to be multicentric and involve the vulva, vagina, and cervix in HIV-infected women compared with controls.[29]

Differentiated VIN is a disease of older women, some with the risk factor of chronic vulvar skin disease.[30] It seems to have a high rate of progression to squamous cell carcinoma compared with VIN usual type—on the order of 95%.[26]

The natural history of VIN is that it tends to recur over time, even when surgical margins appear clear of disease.[21] The disease is often multifocal on the vulva. Despite adequate treatment and follow-up, a small percentage of women with VIN develop invasive carcinoma of the vulva.[25] Approximately 1% of VIN cases regress on their own,

often when VIN is related to pregnancy.[31] Long-term follow-up and surveillance of women with a history of VIN are essential to identify and treat recurrences and prevent the development of invasive carcinoma.

ROLE OF HUMAN PAPILLOMAVIRUS INFECTION IN VULVAR INTRAEPITHELIAL NEOPLASIA

VIN usual type is thought to be a vulvar manifestation of genital HPV disease. In one series of VIN 3 cases, all patients had high-risk HPV subtypes present; 91% had HPV type 16.[24] Another study found that 99% of patients who had VIN usual type were HPV positive, of whom 98% tested positive for high-risk virus subtypes (16, 33, and 45). Forty-two percent of squamous cell carcinomas of the vulva in this series were HPV positive, 93% of which had high-risk subtypes.[32] In another series, 76% of VIN 2/3 samples tested positive for HPV 16 or 18, of which 134 of 139 samples tested positive for HPV 16. Sixty-six percent of the vulvar cancer specimens in that study were HPV 16 positive, and women with HPV-positive vulvar cancers were dramatically younger than the HPV-negative group. Younger women with vulvar cancer ($\leq$ 56 years) were 4.4 times more likely to be infected with HPV 16 than older women with vulvar cancer.[16]

High-risk HPV virus subtypes, particularly HPV type 16, seem to be the main players in the development of VIN usual type and HPV-associated squamous cell cancer of the vulva. Current HPV vaccines protect against HPV 16, which makes them likely to seriously impact the incidence of VIN and vulvar cancer in the future. In women naïve to HPV 16 and 18, the quadrivalent vaccine efficacy was 100% against HPV 16- and 18-associated VIN 2/3 over a 3-year follow-up period. In the total studied population, which included women already infected with HPV 16 and 18 at enrollment, the vaccine efficacy was 71% at preventing HPV type 16- and 18-associated VIN 2/3. The vaccine was 49% effective against VIN 2/3 regardless of the HPV status of the lesions.[18] These findings suggested that the implementation of wide-reaching HPV vaccination programs could result in a significant reduction in the incidence of VIN and vulvar cancer in the future.

SUMMARY

It is important for colposcopists to develop vaginal and vulvar colposcopic examination skills. Because HPV disease incidence is increasing, vaginal and vulvar dysplasia likely will become more common and occur in even younger women. Many of these women will require long-term follow-up because of the multicentric and multifocal nature of HPV disease. A high index of suspicion in high-risk patients and the judicious use of biopsies ensure maximal identification of disease with minimal harm to patients. Colposcopy continues to offer great challenges. The challenges of vaginal and vulvar colposcopy include difficult visualization and access in the vagina, recognition of normal variants, and the identification of a wide variety of disorders in the vulva. The future challenges of colposcopy include understanding the natural history of HPV disease at multiple genital sites, HPV type-specific clinical implications, and future directions regarding vaccination.

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