Chapter 14: Role of Triage Testing in Cervical Cancer Screening

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The classic model of cervical cancer prevention—primary screening with cytology, followed by diagnostic colposcopically directed biopsy, and finally treatment of cancer precursors—is undergoing dynamic change. The introduction of human papillomavirus (HPV) DNA testing and other new modalities provides more options but increases complexity in the sequence of screening, triage, diagnosis, and patient management. This chapter will focus on the role of triage and risk stratification in management. The utility of HPV testing has been established for triage of cytologic findings of atypical squamous cells of undetermined significance but not for low-grade squamous intraepithelial lesions or worse. Countries without established cytology services may consider alternative screening, triage, and treatment programs that may be more readily implemented than a resource-rich “cytology followed by colposcopy” paradigm requiring an infrastructure of highly trained personnel. The diagnostic step of colposcopy and directed biopsy is not completely sensitive in the detection of cervical intraepithelial neoplasia (CIN) 2 or 3 as is sometimes assumed. The partial insensitivity of this diagnostic step results in a population of women with negative colposcopically directed–biopsy findings but at increased risk for missed prevalent disease: these women may require additional triage rather than resumption of routine screening. As more efficient cytology screening, triage, and diagnosis increase the sensitivity of detection of even very small CIN2 or CIN3, overtreatment of lesions that might otherwise regress becomes a concern and highlights the need to identify accurate markers of risk of progression to cancer. Markers of molecular events further along the pathway from HPV infection to development of cancer may ultimately provide more specificity in triage and diagnosis. [J Natl Cancer Inst Monogr 2003;31:97–101]

CLASSIC MODEL

The classic construct of cancer prevention consists of three steps: 1) screening asymptomatic individuals to identify those at risk of disease, 2) diagnosis of the disease state, and 3) treatment of those with cancer or a cancer precursor (Fig. 1).

Screening

A screening test must be safe, easily performed on large populations of asymptomatic individuals, acceptable to the patient, and relatively inexpensive. Ideally, a screening test should have both high sensitivity and high specificity; however, sensitivity is the more important consideration in the context of screening. Cervical cytology, also known as the Pap test after its originator Dr. George Papanicolaou, is an example of a successful screening test that has dramatically reduced the incidence of and mortality from cervical cancer where screening has been implemented.

Diagnosis

Diagnosis establishes the severity of disease and forms the basis for treatment. Colposcopy combined with directed biopsy is the standard diagnostic procedure for evaluating the cervix and has been presumed to be highly sensitive and accurate.

Treatment

Treatment of cancer precursor lesions consists of removal or ablation of involved tissue by one of several methods: loop electrosurgical excision procedure (LEEP), cryotherapy, or cold-knife conization.

Triage

Triage is an additional step (Fig. 1) interposed between screening and diagnosis to further stratify individuals with positive primary screening results according to risk for the disease state.

 Certain procedures may overlap several of the categories diagrammed in Fig. 1. Under certain circumstances, LEEP or conization may be both a diagnostic and a therapeutic procedure. As another example, colposcopy combined with cervical biopsy is viewed as diagnostic, but colposcopy is used in some settings, particularly in Europe, as an adjunctive screening test to improve cytology sampling. Alternatively, colposcopy after an abnormal screening test result may function as a form of triage that determines whether or not a diagnostic biopsy is obtained. In addition to the varying functional role of colposcopy, there is a broad range of examiner expertise that affects the accuracy of colposcopy. Guidelines and classification schemes for reporting colposcopic interpretations have been proposed; however, validation and reproducibility assessments are generally not available. Since colposcopy has not been shown to be adequate for primary screening and is not by itself considered to be diagnostic, it is categorized as a triage modality in Fig. 1.

This chapter focuses on the role of triage and risk stratification in cervical cancer prevention.

UTILITY OF TRIAGE

The utility of a triage test in the context of a screening program will depend not only on the performance characteristics of the test itself but also on the target screening population, the prevalence of disease, the screening test employed, the costs of
follow-up, the available resources (logistical and monetary), and patient compliance. Triage is of most value when the screening test lacks specificity and/or the diagnostic procedure is expensive or a limited resource. An efficient triage test should reduce overtreatment, patient anxiety and inconvenience, and overall management costs, usually by reducing the number of diagnostic procedures performed—all without sacrificing sensitivity for detection of disease. However, if the initial screening test is highly specific and/or the triage test is positive in the vast majority of cases and/or the triage test results in a decrement of sensitivity, then the triage test has questionable utility. Cost-effectiveness analyses are critical to evaluating the interplay of population prevalence, sensitivity for disease detection, specificity, and overall management costs with various screening and triage strategies.

**Triage After Cervical Cytology Screening**

Cytology results are not dichotomous—positive or negative—but are based on the degree of the morphologic abnormality observed (atypical squamous cells, low-grade squamous intraepithelial lesions [LSILs], high-grade squamous intraepithelial lesions [HSILs], and cancer) (1). These diagnostic gradations have different performance characteristics. A cytologic interpretation of HSIL or cancer, for example, has extremely high specificity (high positive predictive value), obviating the need for a triage test. However, in an effort to maximize sensitivity and negative predictive value, atypical squamous cells of undetermined significance (ASCUS) is used as the threshold for referral for additional follow-up in the United States. This lower threshold greatly increases sensitivity for identifying histologic cervical intraepithelial neoplasia (CIN) 2 or 3 (2) but at the cost of referring millions of women for colposcopy and biopsy, the majority of whom do not have prevalent CIN2 or CIN3 and who are not destined to develop it in the immediate future. In this setting of lower specificity, a triage test that could further stratify women according to cancer risk would be useful. One concern, however, is that the costs associated with developing novel triage tests may make them unaffordable to most countries.

**Triage of ASCUS and LSILs**

A multicenter, randomized clinical trial was conducted by the National Cancer Institute (NCI), Bethesda, MD, to compare different strategies for managing the 2–3 million women with ASCUS and the 1.25 million women with LSIL cytology results in the United States each year (3). The Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesions Triage Study (ALTS) (4–6) have shown that cytologic interpretations of LSIL are so highly associated with human papillomavirus (HPV) that an HPV triage test is not useful. In the setting of an ASCUS interpretation, however, approximately 40%–50% of the women are HPV positive (7,8). The proportion of ASCUS that is HPV positive depends on the patient population and the cytomorphologic threshold used by the cytologist. Importantly, virtually all of the occult CIN2 or CIN3 associated with ASCUS is found in the HPV-positive fraction. Therefore, in the context of an ASCUS cytology, HPV triage saves approximately 50% of the women from unnecessary colposcopy without compromising sensitivity. Follow-up by repeat cytology with a low threshold of ASCUS as a trigger for colposcopy is also a safe triage strategy; however, the trade-off of sensitivity and referral percentage is not as favorable for cytology as with HPV triage (9).

A recently published modeling analysis (10) comparing four management strategies for ASCUS—immediate colposcopy, HPV testing, repeat cytology, and no action—confirms that HPV triage is more cost-effective than repeat cytology, colposcopy, or no action. Even so, only about one quarter of the women who are ASCUS and HPV positive (HPV+ ASCUS) who are referred to colposcopy will have CIN2 or CIN3 over the course of 2 years. How can we further improve specificity? Sherman et al. (11) evaluated using viral load and age parameters as potential ways to improve specificity of HPV testing in the context of an ASCUS or LSIL cytology. Compared with a 1-pg HPV-DNA/mL cut point for referral to colposcopy, a higher 10-pg/mL threshold decreased referral percentages from 59.0% to 41.7% but also decreased HPV testing sensitivity for CIN3 from 96.1% to 91.5%. The overall sensitivity of HPV testing at 1.0 pg/mL varied minimally with age (range, 93.9%–97.8%), but strikingly, only 31.2% of women aged 29 years or older would be referred compared with more than 65% of younger women. Thus, the positive predictive value of HPV testing for triage of ASCUS improved in older women. However, using an age-restricted strategy of HPV triage would not address the majority of ASCUS cases, which occur in younger women. In addition, in contrast with ASCUS, for women with LSIL the percentage referred on the basis of a positive HPV test did not decline dramatically with age. We clearly need to inves-
tigate other innovative strategies to triage ASCUS in younger women and all women with LSIL to reduce referrals to colposcopy while maintaining sensitivity for CIN3. In addition, studies focusing on older, perimenopausal and postmenopausal women may be helpful in refining triage strategies specific for this age group.

International variation in cytology terminology, compounded by the use of different morphologic criteria for similarly termed diagnoses, might suggest that ALTS results cannot be generalized to settings outside the United States (12). However, using an atlas of cytology images, with known HPV status and disease outcome, the performance of HPV triage could be predicted for any classification system or screening program. A web-based collection of cytology images and cervigrams (high-resolution photographs), derived from ALTS and other NCI-funded studies, is currently in development. This could reduce the need for additional randomized clinical trials for different terminologies and/or different locales.

Consensus management guidelines for follow-up of an ASCUS cytology result developed under the sponsorship of the American Society for Colposcopy and Cervical Pathology (ASCCP) include repeat cytology, immediate colposcopy, or HPV testing as options (13). However, if liquid-based collection was used for the initial cytologic sample, then reflex HPV testing is considered to be the preferred approach because it obviates the need for a repeat office visit. With conventional cytology smears, there is no residual sample available for HPV testing. Dual collection of a cytology and an HPV sample at the initial visit, or a self-collected HPV sample done later, may be ways to perform triage HPV testing without the need for an additional office visit if using conventional smears.

Studies evaluating self-collected cervicovaginal samples for HPV testing have found good agreement with clinician-collected specimens (14); however, the sensitivity of HPV testing for CIN2 or CIN3 is slightly lower (15). Self-collection may be more acceptable to certain groups of women who may be reluctant to undergo pelvic examination (16). Additional studies are needed that compare clinician-directed sampling and self-collected specimens with regard to sensitivity and specificity performance characteristics for both cytologic assessment and HPV testing.

Triage of Atypical Glandular Cells

Atypical glandular cells (AGC), the glandular counterpart of atypical squamous cells, is a much less common cytologic interpretation with a higher positive predictive value for CIN2 or CIN3 compared with ASCUS. Current ASCCP guidelines recommend colposcopic evaluation with endocervical sampling for all women with AGC (13). One study (17) suggests the possible utility of HPV triage in the setting of an AGC result. If these findings could be confirmed in a larger study, HPV triage may have a role in AGC. However, considering the overall small number and the apparently elevated risk of women with AGC, reducing colposcopic referral for these women is not the most critical research priority.

Other Molecular Markers in Triage

HPV infection is a necessary but early step in the pathogenesis of CIN2 or CIN3 and cancer. Markers of molecular events further along the path toward development of a cancer precursor may provide greater specificity for triage. For example, women with LSIL or HPV+ ASCUS screening results have an approximately 25% risk of CIN2 or CIN3 over 2 years (18). According to ASCCP guidelines, all such women are referred to colposcopy (13). An assay that could distinguish the quarter of women with high-grade disease would allow the majority of women with LSIL and HPV+ ASCUS screening results to avoid the worry and costs associated with colposcopy and further follow-up. At this writing, no such markers have been validated, but research efforts should focus on this critical question.

Primary Screening Using HPV-DNA Testing Affects the Role of Triage

For women aged 30 years and over, the use of HPV as a primary screening test in conjunction with cytology was approved by the U.S. Food and Drug Administration in March 2003 and included in the American Cancer Society guidelines (19). Women who are dual negative (HPV negative and cytology negative) are at very low risk of CIN3 or cancer and can safely be screened every 3 years (20). However, strategies to manage women who are HPV positive and cytology negative need to be defined. Do these women need more intensive follow-up? At what interval? By what test?

In the United States, given the current medicolegal climate and societal expectations that emphasize sensitivity at the expense of specificity, it will require intensive patient and clinician education to accept a 6- to 12-month follow-up (rather than colposcopy) of women with HPV-positive and cytology-negative findings. If management includes repeat HPV testing, a type-specific HPV assay might be of value in this context to distinguish persistence of a specific viral type as opposed to sequential infection with different HPV types. The time interval between HPV testing events will be determined by balancing the woman’s desire for action and the need to allow time for regression of the virus.

It is also imperative to restrict HPV screening to women aged 30 years and older to avoid excessive referral and unnecessary procedures and treatment. The high prevalence of HPV in younger women and the low risk of invasive cancer argue against using HPV as a primary screening technique in the younger age group.

The combination of HPV and cytology may be an interim strategy in an evolution that ultimately leads to primary screening by HPV with triage by cytology. In fact, many suggest that HPV followed by cytology is the more rational approach for older women, given the higher sensitivity of HPV testing and the greater specificity of cytology. The performance of cytology as a triage test may be very different compared with its characteristics as a screening test. Currently, the vast majority (>90%) of the 55 million screening Pap tests performed each year in the United States are negative. With a 10:1 ratio of negative-to-abnormal specimens, cytotechnologists face problems of visual boredom. Microscopic review of Pap specimens is the pathology equivalent of looking for the needle in the haystack and requires exceptional vigilance. In some cases, false-negative cytology results are due to the limitations inherent in this subjective process. If cytology shifted from functioning as a primary screening test to a triage test, there would be a dramatic reduction in the number of tests overall and a marked increase in the yield of positive results, altering the negative-to-abnormal ratio of specimens. It is unclear how this would impact the sensitivity and specificity of Pap testing. Would the higher proportion of
abnormal specimens improve the cytotechnologist’s performance by reducing visual boredom? Or would knowledge of the HPV-positive screening result bias the interpretation of the subsequent cytologic sample, leading to more false-positive results? Even so, the slightly increased false-positive fraction might be acceptable if sensitivity is maintained.

In many developing countries without established cytology services for comprehensive screening or for triage, other approaches that do not rely on an extensive infrastructure of highly trained personnel must be considered. It may be more feasible to combine an inexpensive HPV test for primary screening with triage modalities other than cytology, e.g., direct visual assessment by nonphysician providers (Jeronimo J, Castle PE, Herrero R, Burk RD, Hildesheim A, Bratti MC, et al.: unpublished data). Visual-assessment thresholds could be calibrated to maximize sensitivity if screening was done only once or twice in a woman’s lifetime. Screening, triage, and even treatment services could be combined in the same visit and thereby reduce loss to follow-up in areas remote from health clinics.

**MANAGEMENT AFTER COLPOSCOPY: RETURN TO TRIAGE**

Colposcopically directed biopsy has been used as the gold standard for diagnosis. However, findings from ALTS indicate that colposcopy and biopsy misses about one quarter of prevalent CIN2 or CIN3 (5,9). Women with less than CIN2 after colposcopy are at approximately 10% risk of CIN2 or CIN3 within 2 years. This risk is similar, regardless of whether the colposcopy and directed biopsy result was negative or CIN1 (18).

Incomplete sensitivity of colposcopy and directed biopsy results in a group of women with negative results but at increased risk of CIN3 relative to the general screening population. These women may require additional triage rather than returning to routine screening (Fig. 1). Using ALTS data for women who were less than CIN2 after initial colposcopy, various retriage strategies combining follow-up cytology and HPV testing were compared (21). A single HPV test at 12 months demonstrated the best trade-off of sensitivity and referral percentage. Alternatively, semiannual cytology sampling could be considered if HPV testing is not available. Further studies are needed to find assays or strategies that more efficiently identify women with occult CIN2 or CIN3 and allow the majority of women to safely return to routine screening.

**DIAGNOSIS AND RISK CLARIFICATION—FUTURE DIRECTIONS**

Diagnosis and risk clarification is conceptually a step between diagnosis and treatment (Fig. 1) that includes efforts to refine the histologic diagnosis and/or to further stratify individuals according to risk of invasive cancer.

**Diagnosis Clarification**

Histologic diagnosis is generally the basis for determining patient management and treatment, and in research settings, it is often used to establish definitive determinations of disease. However, histologic interpretation is associated with significant interobserver variability (22). To improve disease determination and risk assessment, we need to identify molecular markers of neoplasia to augment light microscopy and morphologic diagnosis. As an example, p16INK4a, a cyclin-dependent kinase inhibitor, is highly expressed in CIN2 and CIN3 tissues and in CIN1 associated with high-risk HPV, but not in normal cervical tissues. The use of ancillary immunohistochemistry with a monoclonal antibody directed to p16INK4a improved interobserver diagnostic reproducibility for CIN among a group of experts by highlighting small CIN lesions (often a source of false-negative results) and reduced false-positive interpretations associated with equivocal CIN1 (23). However, the prognostic value of p16INK4a remains to be determined.

**Risk Clarification**

The essence of cervical cancer prevention is detection and ablation of true precursor lesions (CIN3) that would otherwise progress to cancer. However, because screening and management strategies are acknowledged to be less than completely sensitive and effective for detecting and treating CIN3, screening and treatment thresholds are set lower to increase sensitivity and safety. Lower screening thresholds increase costs of referral for additional follow-up, and lower treatment thresholds result in unnecessary procedures. Current standards in the United States generally require treatment of histologically confirmed CIN2 or CIN3. More sensitive screening and triage strategies that translate into increased detection of early and often very small CIN2 or CIN3 lesions may lead to earlier treatment of high-grade lesions; however, the impact on cancer outcomes is probably minimal. The vast majority of small high-grade lesions would probably not progress to invasive cancer if detected later, when larger but still intraepithelial (24). In addition, there is a greater likelihood of overtreatment of lesions, particularly CIN2, that might otherwise regress. Identifying markers of risk of progression to cancer is a priority to reduce unnecessary treatment and attendant complications and costs associated with treating all CIN2 or CIN3.

**MONITORING AFTER TREATMENT**

Ablative and excisional procedures are very effective (>90%) in treating CIN2 or CIN3. However, in the United States, in approximately 5%–10% of the cases, there is residual or recurrent disease after initial treatment that necessitates additional therapeutic procedures (25). In addition, women treated for CIN2 or CIN3 remain at increased risk of cervical cancer for at least 8 years compared with the general population (26). Generally, cytology and/or colposcopy have been used for post-treatment surveillance (27). HPV testing merits investigation as another possible strategy to monitor the efficacy of treatment. Several small studies (28,29) have shown that HPV DNA clearance is associated with low risk of subsequent CIN after treatment, and persistent HPV positivity predicts increased risk of treatment failure. However, the possibility of vaginal recolonization with HPV or a new HPV infection with another HPV type must be considered. Type-specific HPV testing may be more informative in this context than a cocktail probe such as the Hybrid Capture 2 assay to distinguish the persistence and recurrence of the same lesion as opposed to newly acquired infection.

**REFERENCES**