Emerging Technologies and Cervical Cancer

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Human papillomavirus (HPV) has been demonstrated to be a necessary cause of invasive cervical cancer across epidemiologic and virologic studies worldwide *(1)*. However, the biologic mechanisms for malignant transformation by HPV were well established long before the epidemiologic studies showed any consistency among investigators or across study designs. Once sensitive biologic assays became available for the detection of HPV, correlational, case–control, and cohort studies demonstrated high relative risks, high attributable fractions, appropriate time sequence, differential risk among higher viral load and higher risk viral types, and consistency among investigators *(2)*. Now, with the relationship of HPV to cervical cancer established, attention has turned to how to use this information to decrease the morbidity of and mortality from cervical cancer.

Cervical cancer is the third most common cancer in women worldwide. Parkin et al. *(3)* estimated that 371 200 cases of cervical cancer were diagnosed worldwide in 1990, 80% of them in developing countries. In 1999, there were an estimated 12 800 cases of invasive cervical cancer and 4800 deaths from this cancer in the United States *(4)*. The natural history of cervical cancer is well understood; lesions progress from dysplasia to carcinoma *in situ* to cancer. The cervix can be sampled cytologically by use of the Pap smear. Although never subjected to a randomized clinical trial, the Pap smear has decreased mortality in all countries in which cervical cancer screening programs have been established.

Meanwhile, the natural history of HPV infections in relationship to the natural history of cervical cancer is less well understood. A study by de Villiers et al. *(5)* has demonstrated that sexually active women become infected and the prevalence of infection peaks between 18 and 25 years of age. Infection prevalence falls off as patients reach age 30 years, the age at which the incidence of high-grade lesions and invasive cancers begins to rise. The immunobiology of HPV is difficult to study given the epitheliotropic nature of the virus and the complicated function of the immune system. Both the antibody and cell-mediated immune system respond to HPV infection. In fact, until recently, studies on the exact relationship between viral antibody response to HPV and disease were producing very inconsistent results. The cell-mediated system also appears to be important in the control of HPV as it is in the control of the human immunodeficiency virus (HIV). Many studies [reviewed in *(6)*] are addressing its role in anticipation of vaccine development. The HPV prevalence data and the relationship of HPV infection to age suggest that patients who remain infected may be those most at risk for the development of high-grade lesions, but the serologic and cell-mediated immunobiologic data to support this concept are still lacking.

In the developing world, the resources for detecting and treating cervical cancer are limited. The goals of cancer eradication programs include finding better screening methods, reaching unscreened populations, and developing treatment programs for cancers that are detected. In developed countries with established screening and treatment programs, the challenge of cervical cancer presents itself differently. In such countries, many of the incident cases have not been screened at regular intervals. On the other end of the spectrum, 50 million Pap smears are performed annually in the United States. Of these smears, an estimated 2.5 million reveal low-grade lesions and atypias whose natural history in more than 80% of cases includes regression. More than 6 billion dollars is spent annually in the United States on their evaluation and management *(7)*. Thus, the challenge in developed countries is finding ways to reach the few women who are unscreened and identifying those lesions most likely to be or to become invasive cancer and using resources wisely to manage them.

The present article by the ALTS Group in this issue of the Journal *(8)* is the first of a series of articles reporting long-awaited findings from the Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesions (ASCUS/LSIL, or ALTS) Trial. Eligible patients included women 16 years old and older who had had a cervical Pap smear showing ASCUS or LSIL within 2 months priot to trial entry, had no history of cervical or other genital cancers, had no known HIV infection, were not pregnant, were able to give written informed consent, and were likely to commit to follow-up. All patients were to undergo a repeat Pap smear, undergo a cervigram, and have cervical specimens collected for HPV testing.

The study was originally designed to recruit 3600 women with ASCUS and 3600 women with LSIL from a representative sample of U.S. women into a randomized, multicenter clinical trial with three arms: an immediate-colposcopy arm, an HPV-testing arm, and a conservative-management arm wherein patients were followed with cytology. The end point for the sample size calculation—screening efficacy at 3 years—was based on the number of high-grade squamous intraepithelial lesions (i.e., “management failures”) expected on the HPV-testing and conservative-management arms. A two-tailed significance level of .05 was assumed, and a 0.1% failure rate on the immediate-colposcopy arms and 1%, 2%, and 3% failure rates on the conservative-management or HPV-testing arms would have yielded powers of .84, .98, and .999, respectively. The trial was to include 3 years of follow-up and as many ethnic groups as possible. Many safeguards were built into the original trial design, including central review committees for Pathology Quality Control, HPV Testing Quality Control, and Colposcopy Quality Control. In addition, several committees were created to provide oversight: a Steering Committee, an Ethics and Data Monitoring Committee, and a Coordinating Unit that would oversee all aspects of the trial at the four participating clinical centers. While

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the methods paper for the trial is pending, The ALTS Group's brief description suggests that the original trial design was followed.

The original study design called for the specimens in the HPV-testing arm to be used to manage patients. In brief, those patients positive for high-risk types of HPV were to be referred for colposcopy at a subsequent visit. In the original solicitation, it was expected that 50% of LSIL patients would be positive for high-risk HPV types. The original design also called for interim analysis of the trial data from each arm. In this article, the ALTS Group describes the interim analysis of 642 specimens from women referred for LSIL, which revealed that 79%–86% of the specimens were positive for high-risk HPV types across the four clinical centers, much higher than the anticipated 50% *(8)*. They conclude that the high prevalence makes HPV testing with the Hybrid Capture II (HCII) assay (Digene, Silver Spring, MD) not useful in the triage of these patients. While we await the methods paper for the trial and the results of more sophisticated analyses of emerging technologies, the conclusions reached in this article appear to be justified.

Littenberg *(9)* proposes a particularly appropriate five-part paradigm for the evaluation of emerging technologies such as HCII: biologic plausibility, technical feasibility, intermediate effects, patient outcomes, and societal outcomes. Biologic plausibility asks if the current understanding of the biology and pathology of a disease support the technology. Technical feasibility asks whether the level of assessment will allow the safe and reliable delivery of the technology to the target population. Intermediate effects address the performance of the test in multicenter trials (specifically, the sensitivity, specificity, positive and negative predictive values, likelihood ratios, and receiver–operating characteristic [ROC] curve analysis against a rigorous gold standard). Patient outcomes address whether the technology improves health from the patient's point of view. Societal outcomes address the cost-effectiveness and ethical use of technologies.

What test are we using in the ASCUS/LSIL trial, and what has the trial shown us so far in terms of the Littenberg paradigm? As mentioned above, the test being used is the commercially available, U.S. Food and Drug Administration-approved, HCII assay. There is good consensus among national HPV experts that this is the best test to use, and the threshold for positivity has been set to equal that of the polymerase chain reaction (PCR) assay of Gravitt et al. *(10)*. In the study under review, the ALTS Group validated the HCII assay with PCR in 210 patients: the kappa value she obtained, 0.65, indicated good but not excellent agreement. Is it feasible then to use PCR in its current labor-intensive form? No, it isn't. Could the HCII test be improved so that, when compared with PCR its kappa statistic is in the excellent range (0.8–1.0)? It is possible that a more sensitive test could be developed, but, at present, it would not be more cost-effective. In summary, the best test is being tested.

As for the Littenberg paradigm, the trial's biologic plausibility has been established. Technical feasibility has been established. Clinical effectiveness is being tested in this first multicenter National Cancer Institute-funded study of its kind. The ALTS Group reports a high prevalence of HPV infection in this population of referred LSIL patients. We do not yet know the results of the colposcopically directed biopsies, the rigorous gold standard, and we await the data on sensitivity, specificity, positive and negative predictive value, likelihood ratios, ROC curves, sensitivity analysis, and cost-effectiveness that will appear in future publications. Patient outcomes are typically assessed through patient interviews, and we do not know if such interviews were included in the study design from solicitation onward. This information may appear in the methods paper. Societal outcomes cannot be fully evaluated until the sensitivity analyses and cost-effectiveness analyses are performed, but the use of the test is clearly ethical if it does not induce too much anxiety. In summary, the ALTS Group's interim analysis suggests that, while the HCII test is biologically plausible and technically feasible, it may not be clinically useful as a primary triage tool in the LSIL population in a developed country like the United States. Kaufman and Adam *(11)* reached the same conclusion in their review of several reported trials; we do not, however, have any information about patient acceptance or cost-effectiveness yet.

The results of the ALTS Group's study beg three questions: 1) Could there be a better test, 2) could the HCII assay be clinically effective in a different carefully selected population, and 3) could a different technology be more clinically effective, acceptable to patients, and cost-effective? These questions require that more be known about the relationship among HPV, immunobiology, and genetic susceptibility.

As for a better test, it appears that viral persistence, viral load, and messenger RNA expression might be more useful biologic markers than HCII. They are all plausible biologic measures whose measurement is technically feasible. But, will tests for these be clinically useful and cost-effective? We do not know. In the meantime, will other markers of genetic susceptibility or immunotolerance be developed and evaluated? Will any of these tests be better than the Pap smear or its quantitative assessment?

As for using the HCII assay in different populations, we first need to understand more of the immunobiology and natural history of HPV infections in the cervix in women at different ages. We need to know in whom HPV infections persist and in whom the virus integrates into the host DNA and why. Should we set a different threshold once we understand more about the natural history of HPV infections? Should we test for HPV only in those women older than 30 years and only worry about those with persistent infections with high-risk types? Should we test for HPV as a second and not a primary measure? Are there other populations in which the HCII test will be useful? Preliminary data from Wright et al. *(12)* and Schiffman et al. *(13)* suggest that the test might be very useful in screening in developing countries *(12,13)*. Manos et al. *(14)* have demonstrated the clinical usefulness and cost-effectiveness of the HCII assay as a second test in the triage of ASCUS Pap smears.

If HPV testing is not clinically useful or cost-effective, can other technologies such as optical technologies be helpful? Many optical technologies are under study: the polar probe, fluorescence spectroscopy, reflectance spectroscopy, optical coherence tomography, and confocal imaging. However, they will have to be evaluated rigorously following the paradigm of Littenberg *(15–17)*.

Clearly, the well-designed and provocative ASCUS/LSIL Trial on which the ALTS Group is reporting will yield answers to some of these questions in future publications. Meanwhile, cervical cancer screening, detection, and treatment will continue to pose different problems in developing versus developed countries. Hopefully, additional studies will lead to a better understanding of the immunobiologic aspects of HPV infection, tease

out the important role of an individual's genetic susceptibility, and rigorously evaluate new and emerging technologies.

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