ONCOLOGY

# Visual appearance of the uterine cervix: correlation with human papillomavirus detection and type

Jose Jeronimo, MD; L. Stewart Massad, MD; Mark Schiffman, MD; for the National Institutes of Health/American Society for Colposcopy and Cervical Pathology (NIH/ASCCP) Research Group

**OBJECTIVE:** Infection with carcinogenic human papillomaviruses (HPVs) is necessary for cervical precancer and cancer, but the effects of type-specific HPV infection on cervical appearance are poorly understood.

**STUDY DESIGN:** Twenty expert colposcopists evaluated a total of 939 digitized cervigrams that were obtained during the ASCUS (atypical squamous cells of undetermined significance)-LSIL (low-grade squamous intraepithelial lesion) Triage study after the application of 5% acetic acid. Each reviewer rated the number and severity of lesions in 112 pictures that were matched on histologic diagnoses and HPV typing results so that ≥2 reviewers rated each image. We used standard tests of association and correlation to relate HPV type and visual appearance.

**RESULTS:** Pairs of reviewers were significantly ($P < .05$) more likely to agree that a definite lesion was present when HPV DNA was found, particularly HPV16, regardless of histologic diagnosis. However, the link between infection status and visual appearance was weak for each individual reviewer. Interestingly, many women with multiple HPV infections had no visible lesions and vice versa.

**CONCLUSION:** HPV16 causes more definite visual abnormalities than other HPV types, regardless of eventual histologic diagnosis. Otherwise, the associations between HPV infection and lesion recognition are weak.

**Key words:** colposcopy, cervical cancer, human papillomavirus (HPV), intraepithelial lesion

Cite this article as: Jeronimo J, Massad S, Schiffman M, et al. Visual appearance of the uterine cervix: correlation with human papillomavirus detection and type. Am J Obstet Gynecol 2007;197:47.e1-47.e8.

Cervical cancer has a progressive evolution that starts when women are infected with 1 of the carcinogenic types of human papillomavirus (HPV).[1,2] Multiple concurrent infections are frequent due to common sexual infection, and most infections clear within a few months.[3] A small percentage of women become chronically infected, with a greatly increased risk of progression to precancer and eventual cancer.

Colposcopy is the current standard triage test that is used to determine which women with abnormal screening results require treatment. Colposcopy identifies epithelial lesions on the cervix and guides the biopsy of those abnormal areas.[4] If large or multiple lesions are detected during the colposcopic evaluation, examiners must evaluate characteristics such as acetowhitening, vascular pattern, and margins to forecast the severity of the underlying disease and take biopsy samples from the "worst looking" areas that appear to contain high-grade disease. However, recent evidence suggests that the accuracy of colposcopy may be suboptimal.[5-9]

We are attempting to study colposcopy rigorously, in part by correlation to HPV typing. Some HPV types, particularly HPV16, are associated more strongly with cervical cancer than others.[10,11] HPV typing might someday be useful in different phases of cervical cancer screening and clinical management of abnormalities.[12] To integrate HPV testing and typing into clinical management requires a better understanding of how HPV testing, cytologic condition, and colposcopy relate. We already know that, among HPV-infected women, HPV type influences the frequency and severity of cytologic abnormality. However, we do not know whether particular HPV types cause more clearly defined colposcopic lesions.

Given the central etiologic role of HPV infection and the critical clinical importance of colposcopy, our objective was to evaluate the correlation between HPV infection and the visual appearance of the cervix using a recently developed web-based software to collect the evaluations of expert colposcopists.[13]

## Materials and Methods

### Study design and population

This was a substudy of the ASCUS (atypical squamous cells of undetermined significance)-LSIL (low-grade squamous

From the Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD (Drs Jeronimo and Schiffman), and the Department of Obstetrics and Gynecology, Southern Illinois University School of Medicine, Springfield, IL (Dr Massad).

Received Oct. 24, 2006; accepted Feb. 27, 2007.

Reprints: Jose Jeronimo, MD, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 6120 Executive Blvd, MSC 7234, Bethesda, MD 20892; guibovij@mail.nih.gov

This is an ancillary study of the National Cancer Institute (NCI) funded ASCUS-LSIL Triage Study (ALTS). The results do not necessarily reflect the opinions of NCI or the ALTS Investigators.

0002-9378/$32.00

doi: 10.1016/j.ajog.2007.02.047

**TABLE 1**
**Characteristics of the study sample**

| Cervigram result | HPV | | | |
|---|---|---|---|---|
| | HPV negative | Noncarcinogenic types only | ≥1 Carcinogenic type | Total |
| Negative | 98 | 36 | 123 | 257 |
| A-P1 | 114 | 69 | 387 | 570 |
| P2-P3 | 10 | 10 | 92 | 112 |
| Total | 222 | 115 | 602 | 939 |

*A*, atypical, lesion of doubtful significance; *P1*, compatible with CIN 1; *P2*, compatible with CIN 2-CIN3; *P3*, compatible with cancer.

intraepithelial lesion) Triage study (ALTS). The design of ALTS and characteristics of the population have been described previously.[14] The study was approved by the National Cancer Institute and local institutional review boards. Briefly, 5060 women were enrolled because they had received a community-based cytologic diagnosis of ASCUS (n = 3488) or LSIL (n = 1572). They were assigned randomly to 1 of 3 treatment strategies (immediate colposcopy, triage based on HPV results and liquid-based cytologic results, or triage based on cytologic results only). The study took place in 4 clinical settings: Magee-Women's Hospital of the University of Pittsburgh Medical Center Health System (Pittsburgh, PA), the University of Oklahoma (Oklahoma City, OK), the University of Alabama (Birmingham, AL), and the University of Washington (Seattle, WA). Written informed consent was obtained from each woman. Using a broom sampler, we collected cervical samples into PreservCyt (Cytyc Corporation, Boxborough, MA) for liquid-based cytologic results (ThinPrep; Cytyc Corporation) and Hybrid Capture 2 (Digene Corporation, Gaithersburg, MD) HPV detection of a pool of 13+ carcinogenic HPVs. For the HPV typing that is reported in this substudy, we also collected a Dacron-swab specimen placed into Specimen Transport Medium (Digene Corporation). Finally, the cervix was washed with 5% acetic acid and 2 cervigrams (National Testing Laboratories, Fenton, MO) were taken.

Women were followed for 2 years with an aggressive exit strategy to maximize safety (ie, detection of cervical intraepithelial neoplasia 2+ [CIN2+] and especially CIN3+). In ALTS, the demonstrated imperfect sensitivity of the first colposcopy to detect many cases of CIN3 that, in retrospect, were present at enrollment led the investigators to classify final disease status as CIN3+, CIN2, CIN1, or less than CIN1 on the basis of the worst histologic evidence that was found during the trial. The final diagnosis during ALTS, rather than the provisional diagnosis at the time of colposcopy, was used for these analyses.[15]

## HPV testing

HPV genotyping was performed on the specimen transport medium specimen with an L1-based polymerase chain reaction assay that uses a primer set that is designated PGMY09/11. Amplimers were subjected to reverse-line blot hybridization (Roche Molecular Systems, Alameda, CA) for detection of types 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51-59, 61, 62, 64, 66-73, 81, 82, 82v, 83, 84, and 89. For this analysis, 13 types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) were considered carcinogenic HPV types, which recognized that the strength of association with cancer varied widely. The samples for HPV testing were collected during the same visit when the cervigrams were taken.

We explored the influence of different groups of HPV types on the presence of definite or equivocal acetowhite lesions. To stratify the levels of certainty of definite lesions, we created 3 categories of visual diagnosis: (1) If both evaluators who reviewed the image agreed to a diagnosis of normal cervix or benign changes (metaplasia), then the patient was considered to be normal. (2) If both evaluators agreed that the diagnosis was low-grade lesion (LGL) or worse, the patient was considered to be LGL+. (3) If the pair of evaluators disagree in the diagnosis (1 evaluator said normal, and the other evaluator said LGL+), the case was categorized as equivocal. We evaluated the frequency of normal, equivocal, and LGL+ cases in the total sample of subjects that were stratified by HPV status: HPV16 regardless of other types, other carcinogenic types without HPV16, noncarcinogenic HPV types only, and negative.

## Image evaluation

We randomly selected a sample of 1000 women who were evaluated at enrollment of ALTS, stratified by severity of cervigram interpretation (normal, atypical, positive 1, positive 2 and positive 3) and HPV type, to ensure adequate numbers of different combinations (Table 1). Of note, cervigram interpretations were used only to select a varied sample of images.

Twenty-one women did not have a cervigram at enrollment, and 40 women had a cervigram considered to be inadequate for evaluation, which left a final sample of 939 women. The cervigrams from a random selection of 20 women of the sample were assigned for evaluation by all the expert colposcopists of the study. The cervigrams of the remaining 919 women were distributed randomly among the evaluators in such a way that each evaluator had a set of 112 cervigrams that had been selected randomly from each level of cervigram severity and HPV group, and all images were evaluated by at least 2 evaluators.

Cervigrams were digitized and compressed following parameters that have been described previously to assure optimal resolution and visual quality.[16] The evaluations were performed with a novel software boundary marking tool (BMT) that was developed by staff members at the National Library of Medicine,[13] which was accessed through the world-wide web (Figure 1). Equipment that was used for viewing was not standardized. A

protocol for rating and marking images was provided to all, and web-based practice images were used to allow evaluators to become familiar with the BMT; however, centralized training was not conducted, and common rating systems were not imposed. All the evaluators were masked to any clinical data, including HPV status and cervigram diagnosis.

Evaluators were asked to identify and draw a boundary around any acetowhite lesion, then they scored the punctation, mosaicism, borders, and color of the lesions using parameters similar to the Reid index.[17] Evaluators first determined whether each cervical image that was displayed was adequate for diagnosis or obscured by blood, poor focus, vaginal wall prolapse, or other factors. If the image was satisfactory and a lesion was present, evaluators rated each lesion to be not evaluable, metaplasia, LGL, high-grade lesion, or cancer. Additionally, they selected a diagnosis for the whole cervix by considering the worst area that could be seen. For this analysis, we were concerned with how sure reviewers were that a lesion was present and how many lesions they believed to be present, not colposcopic grading of individual lesion diagnosis; therefore, we combined the visual rating into categories of normal, equivocal, or LGL+ for the whole image.

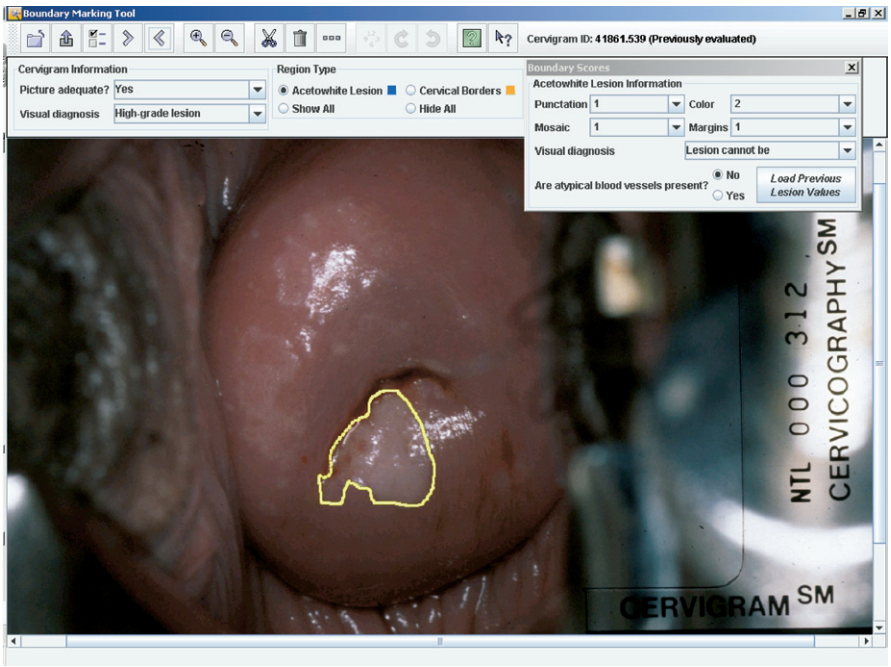
Clinicians with expertise in colposcopy were identified by members of the Board of Directors of the American Society for Colposcopy and Cervical Pathology and by staff at the National Cancer Institute. The evaluators included 20 expert colposcopists: 12 general gynecologists and 8 gynecologist oncologists. Eighteen of the experts work in academic settings, and 2 of the experts are in private practice.

## Statistical analysis

The results from the evaluators were compared against the HPV test results overall and stratified by histologic diagnosis. The visual evaluation was focused on the presence or absence of discernible lesions, the number of lesions drawn, and the distinction between metaplasia and "true" (LGL+) lesions, with the use of standard contingency table methods (chi-square tests, odds ratios and the Spearman correlation coefficient). In this analysis, the grade of severity of the colposcopic impression was not considered. The chi-square statistic test revealed whether the categories of 2 variables were associated. To evaluate the strength of association between the presence of HPV (or oncogenic HPV) and lesions, as evaluated by individual raters, we calculated odds ratios as estimates of relative risks. There were 20 reviewers, and we present the pooled odds ratio to summarize these associations. We used the odds ratio to quantify the strength of the association between the categories as direct (>1), inverse (>0 to <1), or null (1). Correlation coefficients were computed to reveal the strength of association when 2 variables had many categories (or were continuous); the coefficients could range from −1 (perfectly but inversely correlated) through 0 (noncorrelated) to 1 (perfectly and directly correlated). To measure the correlation between the numbers of different HPV genotypes and the numbers of lesions for the 112 images that were evaluated by each individual rater, we chose the Spearman correlation coefficient because the variables that we were correlating (number of lesions vs number of HPV types) were not normally distributed.

Previous ALTS analyses demonstrated that HPV presence and type rather than subtle cytologic or biopsy (histologic) differences were of primary importance for the prediction of subsequent diagnosis of CIN3.[18,19] Specifically, among women with the same types of HPV infection, histologic diagnosis of CIN1 vs negative were poorly reproducible and conferred the same risk of subsequent diagnosis of CIN3. Therefore, all diagnoses of ≤ CIN1, which were controlled for HPV, were combined in this analysis, except as noted.

FIGURE
**Boundary-marking tool**




## RESULTS

Digitized pictures of the uterine cervix of 939 women that were evaluated at enrollment of ALTS were selected for review; the mean age of the subjects was 26.2 ± 7.8 (SD) years, and the median age was 24 years (range, 18-73 years). The result of the cytologic evaluation that referred the patient to the study was ASCUS in 577 women (61.5%) and LSIL in 362 women (38.8%). All the participants had a polymerase chain reaction

TABLE 2
**Visual appearance of HPV types**

| | Negative | | Noncarcinogenic HPV | | Non-16 carcinogenic HPV | | HPV16 | | |
|---|---|---|---|---|---|---|---|---|---|
| Appearance | n | % | n | % | n | % | n | % | Total |
| Total sample* | | | | | | | | | |
| Normal† | 91 | 42.9 | 36 | 31.3 | 104 | 27.1 | 23 | 11.1 | 254 |
| Equivocal‡ | 63 | 29.7 | 22 | 19.1 | 107 | 27.9 | 41 | 19.8 | 233 |
| LGL+§ | 58 | 27.4 | 57 | 49.6 | 173 | 45.1 | 143 | 69.1 | 431 |
| Total | 212 | 100 | 115 | 100 | 384 | 100 | 207 | 100 | 918‖ |
| Women with final diagnosis of normal¶ | | | | | | | | | |
| Normal† | 40 | 37.7 | 13 | 33.3 | 37 | 31.1 | 2 | 6.9 | 92 |
| Equivocal‡ | 29 | 27.4 | 4 | 10.3 | 28 | 23.5 | 9 | 31.0 | 70 |
| LGL+§ | 37 | 34.9 | 22 | 56.4 | 54 | 45.4 | 18 | 62.1 | 131 |
| Total | 106 | 100 | 39 | 100 | 119 | 100 | 29 | 100 | 293 |
| Women with histologic diagnosis of CIN1# | | | | | | | | | |
| Normal† | 7 | 53.8 | 2 | 16.7 | 16 | 21.6 | 4 | 17.4 | 29 |
| Equivocal‡ | 3 | 23.1 | 3 | 25.0 | 20 | 27.0 | 4 | 17.4 | 30 |
| LGL+§ | 3 | 23.1 | 7 | 58.3 | 38 | 51.4 | 15 | 65.2 | 63 |
| Total | 13 | 100 | 12 | 100 | 74 | 100 | 23 | 100 | 122 |
| Women with histologic diagnosis of CIN2** | | | | | | | | | |
| Normal† | 2 | 50.0 | 1 | 11.1 | 7 | 15.2 | 1 | 4.2 | 11 |
| Equivocal‡ | 1 | 25.0 | 3 | 33.3 | 22 | 47.8 | 5 | 20.8 | 31 |
| LGL+§ | 1 | 25.0 | 5 | 55.6 | 17 | 37.0 | 18 | 75.0 | 41 |
| Total | 4 | 100 | 9 | 100 | 46 | 100 | 24 | 100 | 83 |
| Women with histologic diagnosis of CIN3†† | | | | | | | | | |
| Normal† | 1 | 11.1 | 0 | 0 | 4 | 7.8 | 7 | 6.7 | 12 |
| Equivocal‡ | 4 | 44.4 | 0 | 0 | 14 | 27.5 | 15 | 14.3 | 33 |
| LGL+§ | 4 | 44.4 | 3 | 100.0 | 33 | 64.7 | 83 | 79.1 | 123 |
| Total | 9 | 100 | 3 | 100 | 51 | 100 | 105 | 100 | 168 |

Total sample includes equivocal histological disease.

* Mantel-Haenszel chi-square <0.0001 for entire table and <0.0001 for HPV16 vs other types (excluding the "negative" category).

† Both evaluators agreed to a diagnosis of normal cervix or benign changes (metaplasia).

‡ The pair of evaluators disagreed on the diagnosis (1 evaluator said normal, and the other said LGL+).

§ Both evaluators agreed that the visual diagnosis was LGL or worse.

‖ Excludes pictures considered to be inadequate by 1 of the evaluators.

¶ Mantel-Haenszel chi-square <0.01 for this entire section and 0.03 for HPV16 vs other types (excluding the "negative" category).

# Mantel-Haenszel chi-square 0.02 for this entire section and 0.38 for HPV16 vs other types (excluding the "negative" category).

** Mantel-Haenszel chi-square 0.01 for this entire section and <0.01 for HPV16 vs other types (excluding the "negative" category).

†† Mantel-Haenszel chi-square 0.06 for this entire section and 0.19 for HPV16 vs other types (excluding the "negative" category).

result: 222 women (23.6%) had a negative HPV test result; 115 women (12.3%) had noncarcinogenic HPV only, and 602 women (64.1%) were infected with at least 1 carcinogenic type of HPV. The final diagnosis at the end of the 2-year follow-up period was CIN1 or less in 684 women (72.8%, including 304 normal results), CIN2 in 83 women (8.8%), and CIN3+ in 172 women (18.3%; including 3 invasive cancers).

Table 2 shows the percentage of cases

**TABLE 3**
**Risk of acetowhite lesion that is associated with HPV-typing results***

| Variable | Pooled odds ratio (minimum and maximum values) | | |
|---|---|---|---|
| | Any acetowhite lesion† | Any LGL+ | Any metaplasia |
| Any HPV type | 2.2 (1.3-5.9) | 2.0 (0.7-4.6) | 1.1 (0.3-3.1) |
| Any carcinogenic HPV | 2.1 (0.8-6.2) | 1.8 (0.6-3.9) | 1.2 (0.4-3.2) |
| HPV16 | 3.2 (0.6-6.8) | 2.6 (1.5-7.0) | 0.9 (0.3-6.3) |
| Non-16 carcinogenic HPV | 1.6 (0.9-4.0) | 1.4 (0.6-2.5) | 1.1 (0.7-8.3) |
| Noncarcinogenic HPV | 1.4 (0.6-3.4) | 1.2 (0.4-2.8) | 1.1 (0.1-2.1) |

* Summary of the odds ratios of the 20 evaluators.
† Any acetowhite lesion includes metaplasia and LGL+.

for which pairs of evaluators agreed on a visual diagnosis of LGL+, only 1 of 2 evaluators rated the image as showing LGL+ or both agreed that no LGL+ was present. The percentage of "definite" LGL+ was higher in the group of women who were infected with HPV16 (69.1%) than in the other groups. The percentages of agreement regarding LGL+ lesions were lower and similar for the 2 groups of subjects with other HPV types (45.1% for non-16 carcinogenic types and 49.6% for noncarcinogenic types). The lowest percentage of concordant visual diagnosis of LGL+ was found in the group of women with no HPV infection, although 27.4% of the women were judged visually to have definite LGL+. The association between visual diagnosis and HPV infection status was statistically significant ($P < .0001$).

To determine whether the association of HPV16 with more definite visual abnormality was due to a higher frequency of intraepithelial lesions in the group of women who were infected with HPV16, we repeated the analysis, stratifying the subjects according to their worst histologic diagnosis (Table 2). Interestingly, worse visual appearance was seen in HPV16-infected women, regardless of diagnoses: normal ($P < .01$), CIN1 ($P = .02$), CIN2 ($P < .01$), and CIN3 ($P = .06$). When we excluded women whose results were HPV-negative, the trends were still consistent, although not always statistically significant.

As an alternative way of examining the relationship of visual appearance and HPV infection, we examined and summarized the results for each of the 20 individual colposcopists, comparing for each of the 112 images that were examined: HPV infection (no, no HPV; yes, infection with ≥1 HPV types) and presence of acetowhite lesion (no, no lesion; yes, presence of ≥1 lesions). These results are shown in Table 3. On average and for all individual evaluators, we found a weak association between infection with any HPV type and presence of acetowhite lesions. The pooled odds ratio was only 2.2, close to double, which means that women with an infection were only twice as likely to have a lesion than uninfected women. There was a fairly broad range of odds ratios, which shows that evaluator opinion differed considerably. The only association that was somewhat consistently strong and positive was, again, the association between HPV16 and LGL+ lesions. On average, the presence of HPV did not affect the frequency of colposcopists calling an image metaplasia.

Because a dichotomized analysis might miss important patterns, we attempted to correlate the number of HPV types that infected the cervix and the number of lesions marked by each of the evaluators. We performed the evaluation considering 3 categories for HPV infection: the number of any HPV type that infected the cervix, the number of any carcinogenic HPV type, and the number of any noncarcinogenic HPV type. The evaluation of the number of visual lesions in the cervix was performed also considering 3 possibilities: the total number of acetowhite lesions, the number of acetowhite lesions with diagnosis

**TABLE 4**
**Number of HPV types vs number of lesions**

| Variable | Median *r** (range) | | |
|---|---|---|---|
| | No. of acetowhite lesions | No. of LGL+ | No. of metaplasias |
| No. of HPV types | 0.12 (−0.07-0.38) | 0.12 (−0.08-0.38) | 0.05 (−0.19-0.20) |
| No. of carcinogenic HPV types | 0.12 (−0.01-0.34) | 0.14 (−0.05-0.34) | 0.02 (−0.16-0.23) |
| No. of noncarcinogenic HPV types | 0.08 (−0.17-0.27) | 0.08 (−0.15-0.28) | 0.01 (−0.22-0.15) |

* Spearman correlation coefficient relating HPV test results (number of HPV infections of different types) and visual appearance (number of discrete lesions) were calculated for each reviewer. The Table gives the median and range of these correlation coefficients, which shows a general lack of correlation, including some inverse correlations for individual reviewers.

TABLE 5
**Time of histologic diagnosis of patients with CIN3+ and HPV status in the immediate colposcopy randomized arm of ALTS**

| Time of diagnosis | HPV status (column %) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | HPV negative | | Noncarcinogenic + | | Non-16 carcinogenic HPV | | HPV16 | |
| | n | % | n | % | n | % | n | % |
| Enrollment | 0 | 0 | 4 | 40.0 | 22 | 41.5 | 78 | 67.2 |
| Follow-up period | 0 | 0 | 1 | 10.0 | 14 | 26.4 | 22 | 19.0 |
| Exit | 3 | 100.0 | 5 | 50.0 | 17 | 32.1 | 16 | 13.8 |

$P_{trend} < .001$ for the entire table and for HPV16 vs non-16 carcinogenic HPV.

of LGL or worse (LGL+), and the number of metaplastic lesions. Table 4 summarizes the statistical analysis (median and range of Spearman correlation coefficient) of the 9 possible combinations of the HPV categories and the acetowhite lesions categories. As can be seen in that Table, there was a poor average correlation between the number of HPV types that infected the cervix and the number of lesions detected by the evaluators. The poor correlations, which were similar to the dichotomous results in Table 3, held for all individual investigators, for all comparisons of HPV types, and either LGL+ or metaplasia. None of the *r* values exceeded 0.38, which is consistent with a fair correlation. Finally, we repeated the analysis for older vs younger women. Although the young average age of our sample (median, 24 years; range, 18-73 years) precluded full analysis of age effects, we saw no modification of the results.

Our results prompted us to hypothesize that, if HPV16 is associated with worst visual appearance, we would expect a higher influence of HPV16 on the CIN3+ lesions that were detected at first colposcopic evaluation at enrollment of ALTS (when the largest lesions were seen) than on the CIN3+ cases that were diagnosed during the 2-year follow-up period or by loop electrosurgical excision procedure at exit (when lesions were less likely to be seen and were smaller).[20] Table 5 shows CIN3+ cases that were detected in the immediate colposcopy randomization arm of ALTS, the time when they were diagnosed, and the HPV status. There we can see that HPV16-related CIN3+ cases were more likely to be diagnosed earlier in the study (at enrollment colposcopy) than were the CIN3+ cases that were related to other HPV types ($P_{trend} < .001$).

## Comment

To our knowledge, this is the first study to explore systematically the relationship between type-specific HPV infection and visual changes of the uterine cervix. HPV infection is the necessary cause of cervical cancer, and we had hypothesized that colposcopic impression at the most basic level (lesion vs no lesion) would be associated strongly with molecular evidence of infection. Previous reports suggested that there is some level of tropism of some HPV types for infecting preferentially the squamous or the glandular epithelium of the cervix.[21,22] These findings prompted us to hypothesize that the different HPV types could develop separately on the cervix and colonize different areas of the epithelium; therefore, we could expect that the more HPV types that infected the cervix, the higher the number of acetowhite lesions to be found on visual evaluation.

Based on our results, we suggest that this correlation does not exist; even when we evaluated the results as simple dichotomies (presence or absence of lesion vs presence or absence of HPV). A possible explanation for this finding is that not all the HPV infections are associated with visual changes of the epithelium. It is still not clear whether this lack of association is that some acetowhite lesions could be located out of the reach of the visual evaluation (endocervix) or that human papillomavirus produces no detectable alterations of the squamous epithelium in a subgroup of subjects. We consider that the last statement is the more probable explanation, because previous data have demonstrated that cytologic abnormalities are discernible in only the minority (approximately 25%) of HPV infections that are detected by molecular assays.[23] Another possible explanation of the lack of correlation between the numbers of infections and the numbers of lesions could be that several HPV types might infect the epithelium in the same area and result in a single lesion.

Based on these results, we additionally evaluated whether some HPV genotypes are associated with a higher risk for causing visual changes of the cervix. Our data suggest that HPV16 acts differently than the other HPV types (carcinogenic or noncarcinogenic). There is a higher risk of becoming chronically infected if HPV16 is present[11]; women chronically infected with HPV16 are more likely to produce malignant transformation of the epithelium.[11,12] Our finding that HPV16 is more likely to produce a clinically identifiable lesion than other HPV types persisted even after stratification of the subjects according to the worst histologic diagnosis; therefore, it is not an artifact of HPV16 producing more high-grade disease. Interestingly, even if the histologic result was negative, HPV16 infection led to the colposcopic impression of LGL+ being present.

There are several factors that may limit the interpretation of our data. Colposcopists with even greater expertise may have superior ability to distinguish HPV-related lesions, but our raters were selected by national leaders in colposcopy and likely represent the upper range of colposcopy skill. We used 2-dimensional images that were displayed on computer screens of varying quality, and better results might have emerged during in vivo assessment or on optimal equipment. Care should be taken when cervigram results are extrapolated to colposcopy. We currently are exploring the accuracy and interrater variability in the application of modified Reid index components to our dataset, but the use of a rating system might have improved correlations. The use of paired images that were obtained after iodine application might have improved the reliability of lesion grading. We did not perform HPV assays on biopsy tissue and presumed that HPV-related lesions shed virus, but we cannot exclude the possibility of false-negative HPV results because of the lack of exfoliation.

However, we believe that this evaluation is clinically relevant. Moreover, the performance of colposcopy and digital colposcopy images in the ALTS population yielded similar conclusions, specifically that colposcopy has modest reproducibility and accuracy.[8,24]

The clinical implications of our results are that, if a woman has an intraepithelial lesion that is associated with HPV16 rather than other types, it may more likely to be detected and accurately targeted for biopsy during the colposcopic evaluation. But at the same time, it may be easier to miss an intraepithelial lesion that is associated with carcinogenic types other than HPV16. These data raise the possibility that the performance of colposcopy might be altered in vaccinated populations if the relative prevalence of HPV 16 as a proportion of all oncogenic HPV infections is reduced. ■

**ACKNOWLEDGMENT**

Affiliations of the NIH-ASCCP Research Group: Lori Boardman, Obstetrician Gynecologist, Department of Obstetrics and Gynecology, Women and Infants' Hospital, Providence, RI; Peter Cartwright, Obstetrician Gynecologist, Department of Obstetrics and Gynecology Duke University, NC; Philip Castle, Investigator, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD; Charles Dunton, Gynecologist Oncologist, Division of Gynecologic Oncology, Lankenau Hospital, Philadelphia, PA; Julia Gage, Investigator, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD; Richard Guido, Obstetrician Gynecologist, Magee-Women's Hospital of the University of Pittsburgh Health Care System, Pittsburgh, PA; Fernando Guijon, Obstetrician Gynecologist, Department of Obstetrics, Gynecology and Reproductive Sciences, University of Manitoba, Canada; Thomas Herzog, Gynecologist Oncologist; Columbia University, New York, NY; Warner Huh, Gynecologist Oncologist, University of Alabama at Birmingham, Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, Birmingham, AL; Jose Jeronimo, Gynecologist Oncologist, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD; Abner Korn, Obstetrician Gynecologist, Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, CA; Edward Kost, Gynecologist Oncologist, Division of Gynecologic Oncology, Brooke Army Medical Center, Fort Sam Houston, TX; Ramey D. Littell, Gynecologist Oncologist, Kaiser Permanente, San Francisco Medical Center, CA; Rodney Long, Engineer visual data management, Communications Engineering Branch, National Library of Medicine, Bethesda, MD; Stewart Massad, Gynecologist Oncologist, Department of Obstetrics and Gynecology, the Southern Illinois University School of Medicine, Springfield, IL; Jorge Morales, Obstetrician Gynecologist, Proyecto Epidemiologico Guanacaste, Costa Rica; Leif Neve, visual data management, Communications Engineering Branch, National Library of Medicine, Bethesda, MD; Dennis O'Connor, Gynecologic Pathologist, CPA Lab, Louisville, KY; Janet S. Rader, Gynecologist Oncologist, Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, Washington University School of Medicine, St Louis, MO; George Sawaya, Obstetrician Gynecologist, Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, CA; Mark Schiffman, M.D., Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD; Mario Sideri, Gynecologist Oncologist, Division of Gynecology, European Institute of Oncology, Milan, Italy; Karen Smith-McCune, Obstetrician Gynecologist, Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, CA; Mark Spitzer, Obstetrician Gynecologist, Department of Obstetrics and Gynecology, Brookdale University Hospital, Brooklyn, NY; Alan Waxman, Obstetrician Gynecologist, Departments of Obstetrics and Gynecology, University of New Mexico Health Sciences Center, Albuquerque, NM; Claudia Werner, Obstetrician Gynecologist, Department of Obstetrics and Gynecology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX.

**REFERENCES**

**1.** Bosch FX, Lorincz A, Munoz N, Meijer CJ, Shah KV. The causal relation between human papillomavirus and cervical cancer. J Clin Pathol 2002;55:244-65.

**2.** Walboomers JM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999;189:12-9.

**3.** Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. N Engl J Med 1998;338:423-8.

**4.** Ferris D, Cox JT, O'Connor DM, Wright VC. Modern colposcopy. Dubuque (IA): Kendall/Hunt; 2004.

**5.** Jeronimo J, Schiffman M. Colposcopy at a crossroads. Am J Obstet Gynecol 2006;195:349-53.

**6.** Sideri M, Schettino F, Spinaci L, Spolti N, Crosignani P. Operator variability in disease detection and grading by colposcopy in patients with mild dysplastic smears. Cancer 1995;76:1601-5.

**7.** Massad LS, Collins YC. Strength of correlations between colposcopic impression and biopsy histology. Gynecol Oncol 2003;89:424-8.

**8.** Ferris DG, Litaker MS. Prediction of cervical histologic results using an abbreviated Reid colposcopic index during ALTS. Am J Obstet Gynecol 2006;194:704-10.

**9.** Gage JC, Hanson VW, Abbey K, et al. Number of cervical biopsies and sensitivity of colposcopy. Obstet Gynecol 2006;108:264-72.

**10.** Munoz N, Bosch FX, de Sanjose S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003;348:518-27.

**11.** Schiffman M, Herrero R, Desalle R, et al. The carcinogenicity of human papillomavirus types reflects viral evolution. Virology 2005;337:76-84.

**12.** Khan MJ, Castle PE, Lorincz AT, et al. The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. J Natl Cancer Inst 2005;97:1072-9.

**13.** Jeronimo J, Long LR, Neve L, Michael B, Antani S, Schiffman M. Digital tools for collecting data from cervigrams for research and training in colposcopy. J Low Genit Tract Dis 2006;10:16-25.

**14.** Schiffman M, Adrianza ME. ASCUS-LSIL Triage study: design, methods and characteristics of trial participants. Acta Cytol 2000;44:726-42.

**15.** Results of a randomized trial on the management of cytology interpretations of atypical squamous cells of undetermined significance. Am J Obstet Gynecol 2003;188:1383-92.
**16.** Jeronimo J, Long R, Neve L, et al. Preparing digitized cervigrams for colposcopy research and education: determination of optimal resolution and compression parameters. J Low Genit Tract Dis 2006;10:39-44.
**17.** Reid R, Scalzi P. Genital warts and cervical cancer: VII, an improved colposcopic index for differentiating benign papillomaviral infections from high-grade cervical intraepithelial neoplasia. Am J Obstet Gynecol 1985;153:611-8.
**18.** Stoler MH, Schiffman M. Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL Triage study. JAMA 2001;285:1500-5.
**19.** Cox JT, Schiffman M, Solomon D. Prospective follow-up suggests similar risk of subsequent cervical intraepithelial neoplasia grade 2 or 3 among women with cervical intraepithelial neoplasia grade 1 or negative colposcopy and directed biopsy. Am J Obstet Gynecol 2003;188:1406-12.
**20.** Sherman ME, Wang SS, Tarone R, Rich L, Schiffman M. Histopathologic extent of cervical intraepithelial neoplasia 3 lesions in the atypical squamous cells of undetermined significance low-grade squamous intraepithelial lesion triage study: implications for subject safety and lead-time bias. Cancer Epidemiol Biomarkers Prev 2003;12:372-9.
**21.** Castle PE, Schiffman M, Bratti MC, et al. A population-based study of vaginal human papillomavirus infection in hysterectomized women. J Infect Dis 2004;190:458-67.
**22.** Castle PE, Jeronimo J, Schiffman M, et al. Age-related changes of the cervix influence human papillomavirus type distribution. Cancer Res 2006;66:1218-24.
**23.** Kovacic MB, Castle PE, Herrero R, et al. Relationships of human papillomavirus type, qualitative viral load, and age with cytologic abnormality. Cancer Res 2006;66:10112-9.
**24.** Ferris DG, Litaker M. Interobserver agreement for colposcopy quality control using digitized colposcopic images during the ALTS trial. J Low Genit Tract Dis 2005;9:29-35.