

Human Papillomavirus Testing for Triage of Women With Cytologic Evidence of Low-Grade Squamous Intraepithelial Lesions: Baseline Data From a Randomized Trial

The Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesions Triage Study (ALTS) Group

Background and Objective: Human papillomavirus (HPV) infections appear to be central to the development of cervical cancer. This study addresses the question of whether testing women who have low-grade squamous intraepithelial lesions (LSILs) of the uterine cervix for HPV DNA is useful as a triage strategy. **Methods:** Four clinical centers in different areas of the United States participated in a randomized clinical trial of the use of HPV DNA testing in women with cytologic evidence of atypical squamous cells of undetermined significance (ASCUS) or LSIL. The study sample in this article consists only of women who had LSIL at enrollment. Within 6 months of an LSIL diagnosis (based on a Pap smear read by a community-based cytopathologist), women who were 18 years of age or older completed a standardized questionnaire and underwent a pelvic examination that included collection of cervical specimens for HPV DNA testing by Hybrid Capture II (HCII)[®] assay. **Results:** Among the 642 women referred with LSIL who had analyzable test results, the mean chronologic age and age at first coitus were similar among the four clinical centers, despite the centers' ethnic and geographic diversity. Overall, HPV DNA was detected in cervical samples from 532 (82.9%) of the 642 women (95% confidence interval = 79.7%–85.7%). This high frequency of HPV positivity was confirmed by polymerase chain reaction (PCR) assays in a subset of 210 paired specimens tested by HCII and PCR (81.4% were positive by both methods). **Conclusion:** Because a very high percentage of women with an LSIL diagnosis from Pap smears are positive for HPV DNA by HCII testing, there is limited potential for this assay to direct decisions about the clinical management of women with LSIL. The role of HPV testing in the management of women with ASCUS is still under study. [J Natl Cancer Inst 2000;92:397–402]

An estimated 50 million Pap smears are performed each year in the United States (1), with about 2% diagnosed as low-grade squamous intraepithelial lesions (LSILs) (2). Assuming that the cost of standard management (colposcopy and treatment) may exceed \$1000 per patient, the total annual cost is greater than one billion dollars (3). Because 70%–80% of low-grade lesions regress spontaneously (2), finding alternative, less expensive, but equally effective management strategies could yield enormous cost savings. Other important potential benefits include less invasive diagnostic testing, avoidance of unnecessary treatment, fewer medical complications, and reduced patient anxiety and inconvenience regarding referral.

Epidemiologic and virologic studies [reviewed in (4)] have clearly demonstrated that specific human papillomavirus (HPV)

types play a central role in the development of cervical cancer and its intraepithelial precursor lesions. Although the number of known genital HPV types now exceeds 40, certain types (notably, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) account for nearly 90% of the HPV types detected in high-grade squamous intraepithelial lesions (HSILs) and cancer (4). These HPV types are often described as “cancer-associated types,” with HPV16 being the most common type detected in cervical cancers in women from all geographic areas surveyed (4). Cancer-associated HPV types have been detected in a smaller percentage of LSILs. In view of these findings, it is possible that HPV DNA testing would provide a useful triage for women with LSIL. Women with cancer-associated HPV types could be referred for immediate colposcopy, and those without could be followed with annual Pap smears. Currently, only the Hybrid Capture II (HCII)[®] and the Hybrid Capture Tube (HCT)[®] assays (Digene, Silver Spring, MD) are licensed by the U.S. Food and Drug Administration, Rockville, MD, for clinical use in the United States.

The atypical squamous cells of undetermined significance (ASCUS)/LSIL Triage Study (ALTS) is a randomized clinical trial designed to determine the optimal management plan for low-grade cervical cytologic abnormalities. Sponsored by the National Cancer Institute (NCI), Bethesda, MD, the trial is based on the cytologic terminology adopted for the Bethesda System (5), which has replaced earlier Pap classification systems in the United States for reporting the results of cervical cytology. Four clinical centers located in Birmingham, AL, Oklahoma City, OK, Pittsburgh, PA, and Seattle, WA, were established to enroll and provide follow-up care to approximately 3600 women with a recent diagnosis of ASCUS and 3600 women with a recent diagnosis of LSIL.

Several small clinical studies (6–16) using the HCT assay to triage women with abnormal Pap smears have been undertaken. Results from these studies suggest that HPV DNA testing of women with LSIL for triage to colposcopy is most likely to be useful at a national level if two conditions are met: 1) HPV DNA from cancer-associated types is not detected in the great majority of women with LSIL and 2) among women with LSIL, detection of cancer-associated HPV DNA is sensitive for histologically confirmed HSIL. If condition 1 is not met—i.e., if most women with LSIL are positive for cancer-associated HPV DNA types—

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See “Notes” following “References.”

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HPV DNA testing is unlikely to be practical in the triage of women with LSIL.

To address these issues, the ALTS group planned an early analysis of the HPV DNA prevalence among women with LSIL. This article summarizes HPV findings from the enrollment visit of the first 642 women referred to ALTS with a Pap smear showing LSIL who also had an HPV test result. These data were analyzed to address the question of whether HPV DNA testing by HCII would be useful in the triage of women with LSIL.

METHODS

Overview of the ALTS Design

Although this article concerns only the enrollment data for women referred to the ALTS with a Pap smear showing LSIL, a brief description of the overall trial study design is provided for comprehensibility.

During the enrollment visit, women referred to ALTS with ASCUS or LSIL Pap smears provide cervical specimens for HPV DNA testing and repeat cytologic assessment. After the enrollment examination is complete, ALTS participants are randomly assigned to one of three arms: HPV triage, conservative management, or immediate colposcopy. Women in the HPV triage arm are sent for colposcopy at enrollment if either HSIL is detected on cytology or if the HCII HPV DNA test is positive or missing. In the conservative management arm, only cytologic evidence of HSIL triggers colposcopy. Women in the immediate colposcopy arm undergo colposcopy at enrollment, regardless of test results.

All women are examined every 6 months, for a total of 2 years. Cervical cytology showing HSIL at any time during follow-up examinations triggers colposcopy. At the 2-year exit visit, all women undergo colposcopy, and lesions suggestive of squamous intraepithelial lesions (SILs) are biopsied. Women with HSIL or persistent LSIL are offered treatment.

At colposcopy, biopsy specimens are taken of any colposcopically suspected SILs. Endocervical curettage is done at the discretion of the clinician. Histologically confirmed high-grade lesions are treated by loop electrosurgery excision procedure. All colposcopy examinations are performed by use of computerized digital imaging systems (DenVu™, Tucson, AZ). Additional safety and quality control procedures include the independent review of referral and enrollment cytology and all histology by the ALTS Pathology Quality Control Group for evidence of missed cervical intraepithelial neoplasia grade 3 (i.e., the subset of high-grade lesions that most resembles carcinoma *in situ*) and the performance of cervicography™ (National Testing Laboratories, Fenton, MO). Cervicography was performed to reduce the remote possibility of missing non-exfoliating but visible cancers among women followed in the HPV triage and conservative management arms.

Study Population

The four clinical centers participating in ALTS are diverse with respect to geographic location, racial/ethnic composition of the referral population, and proportion of insured clients. The Pittsburgh clinical center enrolls women from a health maintenance organization, a hospital-based clinic, and a number of private practice groups. The Birmingham facility accepts referrals from 19 county or state health department clinics and three hospital-based clinics. In Oklahoma City, women are recruited from a university-affiliated family medicine group, a private practice, and health department clinics. The Seattle clinical center recruits from seven Planned Parenthood clinics, 11 health department clinics, and one university student health clinic.

Women who met the following criteria were eligible for trial participation and are included in this article: current (within 6 months) referral Pap smear with a diagnosis equivalent to the Bethesda System (5) category of LSIL, 18 years of age or older, not pregnant, no previous hysterectomy, no previous ablative or excisional therapy of the cervix, and able to give written informed consent for participation in the trial for 2 years. Of the eligible women with LSIL, a similar percentage (70%) enrolled at each center. The ALTS protocol was reviewed and approved by institutional review boards located at the NCI and at each of the four clinical centers.

Clinical Procedures

A standardized interview that included demographic and behavioral questions and a brief gynecologic history was performed during the enrollment visit. After

the interview, women underwent a pelvic examination by a nurse clinician. The pelvic examination focused on the cervix, with cervical cells collected by Papette broom™ (Wallach Surgical, Orange, CT) placed directly into PreservCyt™ (Cytoc Corporation, Boxborough, MA) for liquid-based cytology (ThinPrep 2000™, Cytoc Corporation) and for HPV DNA testing. An additional cervical cell sample was collected in specimen transport medium (Digene) and stored for future HPV testing. A Cervigram™ (National Testing Laboratories) was obtained by the nurse clinician and sent to National Testing Laboratories for expert review. Finally, blood was drawn for immunologic assays.

Laboratory Assays

Cytology and HPV DNA testing were performed in a blinded fashion without knowledge of other test results or clinical data.

Cytology. Women were referred to the study with a Pap smear result equivalent to LSIL provided by a community-based cytopathologist working for a laboratory that served one of the four ALTS referral clinics. At enrollment, thin-layer cytology specimens were generated from the PreservCyt samples by use of a semiautomated processor (ThinPrep 2000; Cytoc Corporation) and were evaluated by clinical center cytologists (data not shown). The residual PreservCyt samples were then forwarded to the clinical centers' HPV laboratory for HCII testing.

HPV DNA testing by HCII. The HCII assay (Digene) for HPV DNA testing was performed with the use of 4 mL of the residual PreservCyt sample that remained after the cytology specimen was prepared. If less than 4 mL remained in the vial, the HCII test was not done. There were HCII test results for 642 (96.1%) of the first 668 women with LSIL enrolled in ALTS. Under the oversight of the ALTS HPV quality-control laboratory, technicians at each of the four clinical centers were trained to perform the HCII assay routinely.

The HCII assay for HPV DNA is a sandwich capture molecular hybridization assay that utilizes chemiluminescent detection. Specimens containing the target DNA hybridize in solution with a specific HPV RNA probe cocktail. The HCII HPV test is configured to detect in a single assay one or more of the cancer-associated HPV types, including HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. The resulting RNA:DNA hybrids are captured onto the surface of a well coated with an anti-RNA:DNA hybrid antibody. Immobilized hybrid was then reacted with an antihybrid antibody conjugated to alkaline phosphatase and detected with a chemiluminescent substrate. As the substrate is cleaved by the bound alkaline phosphatase, light is emitted that is measured in relative light units on a luminometer. The intensity of the light emitted is proportional to the amount of target DNA in the specimen. A relative light unit measurement greater than or equal to the prospectively chosen cutoff value (equivalent to 1 pg/mL HPV DNA or approximately 5000 genomic equivalents of HPV DNA per test) indicates the presence of HPV DNA sequences in the specimen. A measurement less than the cutoff value indicates the specimen is negative for the 13 HPV DNA sequences included in the test. These results indicate either the absence of the specific HPV DNA sequences tested or HPV DNA levels below the detection threshold of the assay. Although the HCII assay is similar in design to the Hybrid Capture Tube (HCT) assay (Digene), it has enhanced diagnostic sensitivity and efficiency of performance (17).

HPV DNA testing by PCR on a subset of cervical specimens. To assess the sensitivity of HCII assay, we also tested cervical specimens from the first 210 women with LSIL randomly assigned to the HPV triage arm for HPV DNA by a polymerase chain reaction (PCR)-based assay. The PCR assay was performed in the ALTS HPV quality control laboratory located at the University of New Mexico Health Sciences Center, Albuquerque. Briefly, cervical specimens collected in PreservCyt were processed by placing a 1.5-mL aliquot into a 1.5-mL Eppendorf vial and centrifuging for 15 minutes at 13 000g at room temperature. The supernatant was immediately removed and discarded. The cell pellet was dried overnight at room temperature. The pellet was then resuspended in 150 mL of digestion solution (i.e., 10 mM Tris, 1 mM EDTA, 200 µg/mL proteinase K, and 0.1% Laureth-12) and digested at 56 °C for 1 hour. The digestion was followed by a 15-minute incubation at 95 °C to inactivate the proteinase K. Crude DNA extracts were stored at -20 °C until amplification.

Before amplification, the crude digests were allowed to reach room temperature and briefly centrifuged for 5 minutes at 13 000g. PCR and reverse-line blot hybridization for genotyping were performed essentially as described previously (18). Briefly, a biotinylated version of the MY09-MY11-HMB01 L1 consensus primer system was used to amplify HPV DNA (MYB09, MYB11, and HMBB01). (Note: L1 is an HPV capsid protein.) To determine specimen adequacy, biotinylated GH20 and PC04 (BGH20 and BPC04) primers were used to

coamplify human β -globin DNA. The reverse-line blot hybridization assay for HPV genotyping contained probes to detect the following HPV types: 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 66, 68, MM4 (W138), MM7 (P291), MM8 (P155), and MM9 (P238A), as well as β -globin DNA.

Statistical Analysis

Proportions of study subjects testing positive for HPV DNA and exact 95% confidence intervals (CIs) were calculated for each of the four centers and for all centers combined. Mean values and standard deviations (SDs) were calculated for continuous variables. Differences in proportions were first analyzed by the χ^2 test. Univariate and multivariate logistic regression analyses were performed to estimate odds ratios (ORs) and 95% CIs (Statistical Package for the Social Sciences, Inc., Chicago, IL). Variables examined in these analyses included age, race/ethnicity, age at first coitus, lifetime number of sex partners, use of hormonal contraception in the last 2 years, and parity. Interassay variation for both HPV assays (HCII and the PCR-based test) was assessed with the unweighted kappa statistic (SAS version 6.12; SAS Institute Inc., Cary, NC).

RESULTS

Study Population

From January through October 1997, 668 women with LSIL were enrolled in ALTS; 642 (96.1%) had adequate HPV results by HCII assay. The overall mean age of these women was 24.9 years (SD = 6.6 years), and it was similar for all four centers (range, from 24.1 years [SD = 5.9 years] for Seattle to 25.7 years [SD = 7.3 years] for Oklahoma City). The racial/ethnic

composition of the four center populations was quite variable, with African-Americans accounting for 69.0% of the women enrolled in Birmingham and only 8.0%–30.0% of the women enrolled in the other three centers (Table 1). More Asian women were enrolled in Seattle (12.4%) than were enrolled in the other three centers (0.5%–4.6%). All women reported at least one male sex partner. Women enrolled in Birmingham reported fewer sex partners and more live births than women enrolled in the other three centers. Age at first intercourse and current use of hormonal contraception were similar for women enrolled in all centers.

Overall, HPV DNA was detected by HCII assay in cervical specimens of 532 (82.9%) of the 642 women with LSIL (Table 2). Center-specific prevalence estimates were similar and ranged from 79.1% for Oklahoma City to 86.1% for Seattle.

In this population of women with LSIL, only age and lifetime number of sex partners were associated with detection of HPV DNA in the multivariate analysis (Table 3). Those who were 30 years of age or older were less likely to have HPV DNA detected than those who were younger (adjusted OR = 0.5; 95% CI = 0.3–0.9). Although there was no upper age limit for participation in this study, only 113 (17.6%) of the women with LSIL were 30 years of age or older, and only 3.3% were more than 40 years of age. Compared with women who reported one to two sex partners, women who reported three or more were more likely to be positive for HPV DNA (adjusted OR = 1.9; 95% CI = 1.1–

Table 1. Demographic characteristics of women with low-grade squamous intraepithelial lesions (LSILs): center-specific* and combined findings

| Characteristic | Birmingham, AL (n = 197) | Oklahoma City, OK (n = 153) | Pittsburgh, PA (n = 155) | Seattle, WA (n = 137) | All (n = 642) |
|--|-----------------------------|--------------------------------|-----------------------------|--------------------------|------------------|
| Mean age, y (standard deviation) | 24.3 (5.6) | 25.7 (7.3) | 25.4 (7.7) | 24.1 (5.9) | 24.9 (6.6) |
| Mean age at first coitus, y (standard deviation) | 16.0 (2.1) | 16.2 (3.0) | 16.4 (2.4) | 16.0 (2.7) | 16.1 (2.6) |
| Race/ethnicity, No. (%)† | | | | | |
| White | 59 (29.9) | 106 (69.3) | 99 (66.0) | 94 (68.6) | 358 (56.2) |
| African-American | 136 (69.0) | 21 (13.7) | 45 (30.0) | 11 (8.0) | 213 (33.4) |
| American Indian | 0 (0) | 10 (6.5) | 4 (2.7) | 3 (2.2) | 17 (2.7) |
| Asian | 1 (0.5) | 7 (4.6) | 1 (0.7) | 17 (12.4) | 26 (4.1) |
| Hispanic | 1 (0.5) | 9 (5.9) | 1 (0.7) | 12 (8.8) | 23 (3.6) |
| Lifetime No. of sex partners, No. (%) | | | | | |
| 1 | 10 (5.1) | 7 (4.7) | 6 (3.9) | 6 (4.4) | 29 (4.6) |
| 2 | 19 (9.7) | 16 (10.8) | 18 (11.7) | 3 (2.2) | 56 (8.8) |
| 3–5 | 96 (49.0) | 52 (35.1) | 70 (45.5) | 37 (27.2) | 255 (40.2) |
| 6–9 | 45 (23.0) | 26 (17.6) | 18 (11.7) | 34 (25.0) | 123 (19.4) |
| ≥ 10 | 26 (13.3) | 47 (31.8) | 42 (27.3) | 56 (41.2) | 171 (27.0) |
| Hormonal contraception use in last 2 y, No. (%) | 108 (54.8) | 87 (56.9) | 91 (58.7) | 94 (68.6) | 380 (59.2) |
| Parity† | | | | | |
| 0 | 46 (23.4) | 54 (35.3) | 80 (51.6) | 84 (61.3) | 264 (41.1) |
| 1 | 75 (38.1) | 54 (35.3) | 39 (25.2) | 32 (23.4) | 200 (31.2) |
| 2 | 42 (21.3) | 30 (19.6) | 14 (9.0) | 12 (8.8) | 98 (15.3) |
| ≥ 3 | 34 (17.3) | 15 (9.8) | 22 (14.2) | 9 (6.6) | 80 (12.5) |

*Missing data for one or more variables were recorded for one woman enrolled in Birmingham, five enrolled in Oklahoma City, eight enrolled in Pittsburgh, and one enrolled in Seattle.

† χ^2 test indicates center-specific differences ($P < .05$).

Table 2. Percent human papillomavirus (HPV) DNA positive by use of Hybrid Capture II assay by clinical center

| | Birmingham, AL (n = 197) | Oklahoma City, OK (n = 153) | Pittsburgh, PA (n = 155) | Seattle, WA (n = 137) | All (n = 642) |
|---|------------------------------|--------------------------------|------------------------------|------------------------------|------------------------------|
| HPV positive using the 1-pg/mL HCII cutoff value (exact binomial 95% confidence interval) | 167 (84.8%) (79.0%–89.5%) | 121 (79.1%) (79.0%–85.2%) | 126 (81.3%) (74.2%–87.1%) | 118 (86.1%) (79.2%–91.4%) | 532 (82.9%) (79.7%–85.7%) |

Table 3. Relationship between detection of human papillomavirus (HPV) DNA by Hybrid Capture II and demographic and behavioral characteristics among 627 study participants with cytologic evidence of low-grade squamous intraepithelial lesion*

| Characteristic | No. of HPV positive | No. of HPV negative | Odds ratio (95% confidence interval) | |
|--|---------------------|---------------------|--------------------------------------|----------------|
| | | | Unadjusted | Adjusted† |
| Location | | | | |
| Oklahoma City, OK | 117 | 31 | 1.0 (referent) | 1.0 (referent) |
| Pittsburgh, PA | 120 | 27 | 1.2 (0.7–2.1) | 1.1 (0.6–2.1) |
| Seattle, WA | 117 | 19 | 1.6 (0.9–3.1) | 1.5 (0.8–2.9) |
| Birmingham, AL | 167 | 29 | 1.5 (0.9–2.7) | 1.3 (0.7–2.4) |
| Age, y | | | | |
| 18–29 | 439 | 75 | 1.0 (referent) | 1.0 (referent) |
| ≥30 | 82 | 31 | 0.5 (0.3–0.7) | 0.5 (0.3–0.9) |
| Race/ethnicity | | | | |
| White | 288 | 62 | 1.0 (referent) | 1.0 (referent) |
| African-American | 181 | 31 | 1.3 (0.8–2.0) | 1.2 (0.7–2.2) |
| Other | 52 | 13 | 0.9 (0.4–1.7) | 0.9 (0.4–1.8) |
| Age at first coitus, per year (as continuous variable) | | | 0.9 (0.9–1.0) | 1.0 (0.9–1.1) |
| Lifetime No. of sex partners | | | | |
| 1–2 | 61 | 23 | 1.0 (referent) | 1.0 (referent) |
| ≥3 | 460 | 83 | 2.1 (1.2–3.6) | 1.9 (1.1–3.4) |
| Hormonal contraception use in the last 2 y | | | | |
| No | 207 | 47 | 1.0 (referent) | 1.0 (referent) |
| Yes | 314 | 59 | 1.2 (0.8–1.8) | 1.0 (0.6–1.6) |
| Parity | | | | |
| 0 | 216 | 44 | 1.0 (referent) | 1.0 (referent) |
| 1–2 | 246 | 44 | 1.1 (0.7–1.8) | 1.2 (0.7–2.0) |
| ≥3 | 59 | 18 | 0.7 (0.4–1.2) | 0.9 (0.4–2.0) |

*Data from 15 women were excluded because of missing values on the demographic or behavioral questionnaire.

†Adjusted for all other factors in the table.

3.4). However, only 84 (13.4%) of the women with LSIL reported a lifetime total of fewer than three sex partners. Among women with three or more partners, an increasing number of sex partners was not associated with an increasing percentage of women positive for HPV DNA.

Center location, race/ethnicity, age at first coitus, use of hormonal contraception, and parity showed no clear association with detection of HPV DNA.

HCII Versus MY09/MY11/HMB01 PCR-Based Assay

Of the 210 specimens that were tested by both HCII and PCR, 180 (85.7%) were positive by HCII and 180 (85.7%) were positive by PCR, with 171 (81.4%) positive by both methods (total agreement of 91.4%; kappa: 0.65). One hundred six (58.9%) of the 180 women who had HPV DNA detected by PCR had more than one HPV type detected (24.3% had two types, 12.4% had three types, 9.5% had four types, 3.3% had five types, and 1% had six types). The HPV types detected among the 210 specimens tested by PCR are shown in Fig. 1. HPV16 was the type most frequently detected (24.8%), with several other genital HPV types detected at lower frequencies (1% for HPV26 to 12.4% for HPV52). A total of 21 (11.7%) of the 180 LSIL specimens that were positive by HCII were only positive for “low-risk” HPV types by PCR (11 were positive for DNA from HPV type 6, 40, or 42, and 10 were positive for DNA from HPV type 53 or 66). These data, in addition to more detailed type-specific analyses (data not shown), suggest that the HCII assay detects HPV types (i.e., HPV6, HPV11, HPV40, HPV42, HPV53, HPV55, HPV66, HPV70, MM4, MM7, MM8, and MM9) that are not included in the HCII combination of probes.

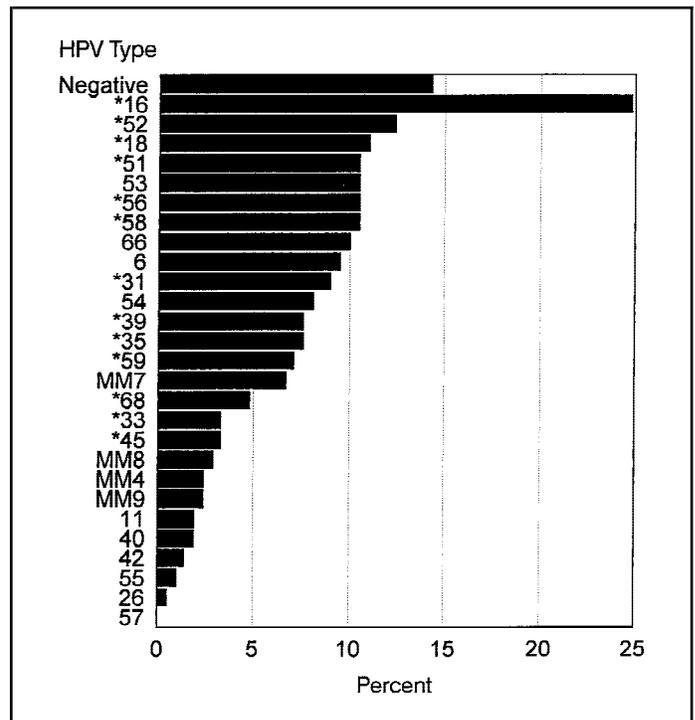


Fig. 1. Percentage of different human papillomavirus (HPV) types detected by polymerase chain reaction-based analysis of cervical specimens obtained from 210 women enrolled with low-grade squamous intraepithelial lesions. * = HPV types included in the Hybrid Capture II® probe mix.

DISCUSSION

Most women enrolled in ALTS with a Pap smear showing LSIL tested positive for the HPV types included in the HCII probe combination. Moreover, PCR analysis of the first 210 women with LSIL who were randomly assigned to the HPV arm showed good agreement between HCII and PCR for detection of HPV DNA. A small percentage of women with positive HCII test results were positive for HPV types not included as probes in the HCII assay. The potential impact of HCII cross-reactivity with phylogenetically related and unrelated HPV types, including types currently considered to be "low risk," has not yet been determined. Finally, the designation "low-risk" versus "high-risk" HPV types remains controversial because data on risk of cancer for most HPV types are extremely limited.

The percentage of women positive for HPV DNA did not vary substantially by center, despite the fact that centers were located in different parts of the country, received referrals from community-based cytopathologists with different levels of training in the Bethesda Cytologic Classification System, and served women of different racial/ethnic backgrounds. This finding suggests that the community-based cytopathologists who participated in ALTS were consistent in their diagnoses of LSIL. HPV DNA detection rates did vary somewhat by age and reported number of sex partners. The patterns were similar when PCR data were used. Compared with women who were younger than 30 years of age, those who were 30 years of age or older were less likely to have a positive HPV DNA sample. This lower rate of detection may be due to a greater frequency of false-positive LSIL Pap smears or to decreased detection of HPV DNA among women 30 years of age or older. Compared with younger women, older women are more likely to have false-positive cytology (19,20) and less likely to be positive for HPV DNA (4). Similarly, compared with women with a lifetime total of three or more sex partners, those with fewer partners may have had false-positive LSIL Pap smears or lower levels of HPV. While statistically significant, the differences in the percentage of women who were positive for HPV DNA by age and by number of male sex partners were not sufficiently large to be of clinical importance in this study population. Although the vast majority of low-grade lesions are detected among women less than 40 years of age, additional studies are needed to clarify whether HPV DNA testing is useful for triage of older women with LSIL.

Earlier studies of HPV DNA testing as a triage for LSIL (6–10,14,15) used earlier generation, less-sensitive HCT assays, included fewer than 100 women with LSIL, or did not report results separately for LSIL and ASCUS. HPV DNA was detected in only 40%–61% of LSIL case subjects in these studies. In the only published study (21) of HCII for detection of HPV DNA in specimens from women with LSIL (6–10,14,15), HCII was positive in 43 (76.8%) of 56 women with LSIL on smear. In another, larger unpublished study of more than 200 women with LSIL (Manos M: personal communication), about 85% were positive for HPV DNA by HCII. When a sensitive assay (such as HCII) is used, current evidence suggests that at least 75% of cervical LSIL will be positive for HPV DNA.

The high percentage of HPV DNA positivity in LSIL limits the usefulness of currently available HPV DNA assays to direct decisions about the clinical management of these lesions. It is estimated that the cost of HPV testing of all women with a cytologic diagnosis of LSIL would outweigh savings gained from avoiding colposcopy for only 20%–27% of women. Thus,

in October 1997, the ALTS Steering Committee, together with the NCI and the ALTS Data Safety and Monitoring Committee, decided that women with LSIL would no longer be randomly assigned to a follow-up protocol that used HPV DNA results to triage women. Women with LSIL enrolled in ALTS after October 31, 1997, have been randomly assigned either to immediate colposcopy or to follow-up with cervical cytology every 6 months. Women with ASCUS Pap smears continue to be randomly assigned to all three arms of ALTS. It remains quite possible that, as others have reported (8,16), HPV DNA testing will be useful for triage of women with ASCUS Pap smears.

In summary, increased specificity in the diagnosis of LSIL and improvements in HPV DNA testing have led to a convergence of the cytologic and molecular diagnoses. It appears that, given adequate HPV testing and adherence to current U.S. cytologic diagnostic criteria, the great majority of those diagnosed with LSIL are HPV DNA positive. Paradoxically, these diagnostic refinements have limited the usefulness of HPV DNA testing for the triage of LSIL.

APPENDIX: AFFILIATIONS OF THE ALTS GROUP

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