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ARTICLE *in* CANCER CYTOPATHOLOGY · AUGUST 2011

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Original Article

Use of High-Risk Human Papillomavirus Testing in Patients With Low-Grade Squamous Intraepithelial Lesions

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BACKGROUND. The role of testing for high-risk human papillomavirus (HR HPV) when triaging women with a cytologic diagnosis of low-grade squamous intraepithelial lesion (LSIL) has not been well established. The objective of the current study was to correlate the status of HR HPV with the incidence of cervical intraepithelial neoplasia 2 and more severe lesions (CIN 2+) on tissue follow-up in women with LSIL. **METHODS.** A total of 1046 women with LSIL and HR HPV testing were identified in the database of a large teaching hospital within a 12-month period. HR HPV testing was performed using the Hybrid Capture 2 assay with 1 relative light unit/cutoff as the cutoff. **RESULTS.** Of the 1046 women with LSIL and concurrent HR HPV testing, 82.3% tested positive for HR HPV, 91.1% of whom were women aged $<$ 30 years and 73% of whom were women aged $\geq$ 30 years ($P < .001$). Cytologic and/or histologic follow-up was available in 979 (93.6%) women; 25.5% had negative follow-up, 62.5% were found to have CIN 1 lesions, and 12.0% had CIN 2+ lesions. The sensitivity and negative predictive value of HR HPV status as a marker of CIN 2+ lesions were 98.3% and 98.9%, respectively. The colposcopy rate was 73.3% and 96.9% for women aged $\geq$ 30 years and women aged $<$ 30 years, respectively ($P = .01$). **CONCLUSIONS.** Using 1 RLU/CO as the cutoff value, HR HPV testing was found to be highly sensitive for detecting CIN 2+ lesions in women with LSIL. The colposcopy rate was significantly lower in women aged $\geq$ 30 years compared with women aged $<$ 30 years. Triaging with HR HPV testing may be indicated in women aged $\geq$ 30 years with LSIL cytology, but not in women aged $<$ 30 years. ***Cancer (Cancer Cytopathol)* 2011;119:228–34.** © *2011 American Cancer Society.*

KEY WORDS: high-risk human papillomavirus (HPV) testing, triage of low-grade squamous intraepithelial lesion (LSIL), gynecologic cytology, Papanicolaou (Pap) test, follow-up of LSIL.

Testing for high-risk human papillomavirus (HR HPV) has been widely accepted as a triaging tool for women with a cytologic interpretation of atypical squamous cells of undetermined significance (ASC-US). HR HPV DNA testing is more sensitive and specific than repeat cytology in identifying women who harbor high-grade dysplasia. Its application in women with ASC cytology has dramatically reduced the

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Received: January 30, 2011; **Revised:** April 18, 2011; **Accepted:** May 9, 2011

Published online July 6, 2011 in Wiley Online Library (wileyonlinelibrary.com)

DOI: 10.1002/cncy.20172, wileyonlinelibrary.com

number of colposcopies performed without any significant loss in sensitivity when compared with direct referral for colposcopy.

The role of HR HPV testing in women with low-grade squamous intraepithelial lesions (LSILs) is much more complicated. According to the Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesions Triage Study (ALTS) Group, 83% of women with LSIL cytology tested positive for HR HPV.[1] Therefore, the investigators of the study concluded that reflex HR HPV testing would not be cost-effective and therefore should not be recommended for women with LSIL cytology. The mean age of women with LSIL in the ATLS study was 24.9 years. Subsequent studies have suggested that HR HPV DNA testing may be useful in triaging older women with LSIL.[2-4]

With the introduction of HR HPV cotesting, more and more women with LSIL have been tested for HR HPV. The objective of the current study was to correlate the status of HR HPV and the subsequent detection of high-grade dysplasia in women with LSIL. We also examined whether age stratification and HR HPV viral load quantification are clinically useful parameters when triaging women with LSIL.

MATERIALS AND METHODS

The Institutional Review Board of Yale University approved the current study. A computer search was performed to identify all women with LSIL cytology and HR HPV testing from the archives of the Department of Pathology at Yale between January 2007 and December 2007. Papanicolaou (Pap) tests interpreted as "LSIL, cannot exclude high-grade dysplasia" were excluded from the study. The women were divided arbitrarily into 3 age groups: Group 1: those aged $<$ 30 years; Group 2: those ages 30 to 54 years; and Group 3: those aged $\geq$ 55 years. Age 55 years was chosen as one of the dividing lines to generally reflect postmenopausal status.

All Pap tests were liquid-based preparations: 20% were ThinPrep (Hologic, Marlborough, Mass) and the remaining 80% were SurePath (BD Diagnostics, Burlington, NC). All ThinPrep slides were screened using the ThinPrep imaging system, whereas the SurePath slides were evaluated using the FocalPoint Slide Profiler (BD Diagnostics).

HR HPV testing was performed using the Hybrid Capture 2 (Qiagen, Gaithersburg, Md) method on residual fluid from liquid-based Pap tests. The probes used were directed against high-risk HPV types 16,18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. The result was expressed as the RLU/CO, the ratio of each specimen's light emission compared with that of the controls, which contained 1 pg/mL of HPV DNA. The result was considered positive when the RLU/CO ratio was $\geq$ 1. The RLU/CO ratios also provided an estimate of the amount of HPV DNA in the specimens (ie, the viral load in the sample). Viral load was classified as low if the RLU/CO ratio was between 1 and 100, moderate when the viral load was $\geq$ 100 and $<$ 1000, and high when the viral load was $\geq$ 1000. The protocol for reporting positive results and viral load classification was followed as per the manufacturer's instructions and guidelines (HC-II method; Digene Corporation, Gaithersburg, Md). The division of viral load groups appears to have been assigned arbitrarily by the manufacturer.

HR HPV testing was performed at the request of the clinicians under 1 of the 2 following options: reflex testing triggered by a cytologic interpretation of LSIL or cotesting with the Pap test regardless of the cytologic result. The latter option included women aged $\geq$ 30 years, as well as those aged $<$ 30 years. Although to our knowledge HPV-Pap cotesting is not approved by the US Food and Drug Administration (FDA) in women aged $<$ 30 years, some clinicians may cotest in a clinical setting in which the patient is considered "high risk," in an attempt "to determine whether the infection has cleared" (eg, if a patient had a prior history of an abnormal colposcopy and biopsy result or an abnormal Pap finding with positive HR HPV, or in an immunocompromised patient [personal communication with a referring clinician]).

Follow-up included repeat cytology and/or surgical pathology obtained within 24 months after the index Pap test. For women undergoing $\geq$ 2 procedures during the follow-up period, only the worst diagnosis based on cytologic or histologic examination was recorded. Because all the specimens were submitted by a dozen community-based gynecologic practices whose patient populations were largely derived from a suburban area, the methods of follow-up and evaluation were entirely at the discretion of the referring physicians and health care providers.

Table 1. Age Distribution and Methods of Follow-Up

Age Group	No Follow-Up	Follow-Up		
		Repeat Pap Only	Biopsy	Total
Age <30 y	45/530 (8.5%)	129/485 (26.6%)	356/485 (73.4%)	485/979 (49.5%)
Age ≥30 y	22/516 (4.3%)	138/494 (27.9%)	356/494 (72.1%)	494/979 (50.5%)
HPV status				
HPV positive	48/861 (5.6%)	205/813 (25.2%)	608/813 (74.8%)	813/979 (83.0%)
HPV negative	19/185 (10.3%)	62/166 (37.3%)	104/166 (62.7%)	166/979 (17.0%)
Total	67/1046 (6.4%)	267/1046 (25.5%)	712/1046 (68.1%)	979/1046 (93.6%)

Abbreviations: HPV, human papillomavirus; Pap, Papanicolaou.

Therefore, there was no uniform protocol for managing women with LSIL.

In the study institution, all gynecologic specimens are reviewed by subspecialty trained gynecologic pathologists and cytopathologists, including internationally recognized experts in the field of gynecologic pathology and cytopathology. There is a rigorous internal review process for gynecologic specimens with daily consensus review and cytology-histology correlation when possible. For the purposes of the current study, the original cytologic interpretations of LSIL were not reviewed; however, all follow-up diagnoses of cervical intraepithelial neoplasia 2 (CIN 2) and more severe lesions (CIN 2+) on biopsy, as well as high-grade squamous intraepithelial lesion interpretations by cytology were reviewed by 1 of the authors (A.W.L.) and confirmed by consensus. In the group of women aged $<$ 30 years, the majority of cervical biopsies diagnosed as CIN 2+ underwent consensus review by gynecologic pathologists before the review process in the current study. In addition, all Pap tests interpreted as "negative" and flagged as high risk (a history of SIL by cytology or biopsy, or concurrent HR HPV positivity) underwent a manual rescreen followed by cytopathologist review. Any discrepancy was resolved by a consensus opinion.

Statistical Analysis

Statistical analysis of the data was performed and included the 2-tailed chi-square test for comparison of follow-up results and HR HPV status. We computed the sensitivity and specificity of HPV testing in identifying patients with CIN 2+ lesions on follow-up among patients in different age groups and with different levels of viral load. The statistics were calculated using SPSS software (version 16.0; SPSS Inc. Chicago IL). Statistical significance was set at a level of $\leq .05$.

RESULTS

During the 12-month study period, 4004 women received a diagnosis of LSIL, accounting for 4.7% of the total number of Pap tests. Among these women, 1046 (26.1%) also had concomitant HR HPV testing; the mean and median ages were 33 years and 30 years, respectively. Overall, 82.3% of women with LSIL tested positive for HR HPV, 91.1% of whom were women aged $<$ 30 years, 73.8% of whom were women ages 30 to 54 years, and 70.3% of whom were women aged $\geq$ 55 years. The difference in the rate of HR HPV positivity was statistically significant between women aged $<$ 30 years and those aged $\geq$ 30 years ($P < .001$). There was no statistically significant difference noted with regard to the rate of HR HPV positivity between women ages 30 to 54 years and those aged $\geq$ 55 years ($P > .05$).

Overall, 67 (6.4%) women were lost to follow-up. Women aged$<$ 30 years with LSIL were more likely to be lost to follow-up than women aged $\geq$ 30 years. (8.5% vs 4.2%; chi-square test, $P = .02$). Furthermore, women with negative HR HPV status were more likely to be lost to follow-up than women with positive HR HPV status among all age groups (7.3% vs 3.2%; chi-square test, $P < .01$).

The remaining 979 (93.6%) women with follow-up comprised the study population. Their ages ranged from 15 years to 85 years with a mean age of 33 years and a median age of 30 years; 485 (49.5%) women were aged $<$ 30 years, 424 (43.3%) were ages 30 years to 54 years, and 70 (7.2%) were aged $\geq$ 55 years. Because of the small number of women aged $\geq$ 55 years, group 2 (women ages

Table 2. Follow-Up Outcomes and HR HPV Status Among Various Age Groups

Age Groups	HR HPV Status	Follow-Up Result			Total
		Negative	CIN 1/HPV	CIN 2+	
<30 y	Negative	19 (50.0%)	18 (47.4%)	1 (2.6%)	38 (7.8%)
	Positive	103 (23.0%)	278 (62.2%)	66 (14.8%)	447 (92.3%)
	Total	122 (25.2%)	296 (61.0%)	67 (13.8%)	485
≥30 y	Negative	61 (47.7%)	66 (51.6%)	1 (0.0%)	128 (25.9%)
	Positive	67 (18.3%)	250 (68.3%)	49 (13.4%)	366 (74.1%)
	Total	127 (25.7%)	316 (64.0%)	50 (10.1%)	494
Overall	Negative	80 (48.2%)	84 (50.6%)	2 (1.2%)	166 (17.0%)
	Positive	170 (20.9%)	528 (64.9%)	115 (14.2%)	813 (83.0%)
	Total	250 (25.5%)	612 (62.5%)	117 (12.0%)	979

Abbreviations: CIN, cervical intraepithelial neoplasia; HR HPV, high-risk human papillomavirus.

Table 3. Operating Characteristic of Positive HR HPV Status as a Marker for CIN 2+ Lesions on Follow-Up

Age Groups	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
<30 y	98.5%	8.9%	14.8%	97.4%
≥30 y	98.0%	28.6%	13.4%	99.2%
Overall	98.3%	19.0%	14.1%	98.9%

Abbreviations: CIN, cervical intraepithelial neoplasia; HR HPV, high-risk human papillomavirus.

30 years to 54 years) and group 3 (women aged ≥ 55 years) were combined to form 1 group. Table 1 summarizes the mode of follow-up between the 2 age groups. The percentage of women who underwent colposcopy and cervical biopsy was similar between those aged ≥ 30 years and those aged < 30 years (73.4% vs 72.1%; chi-square test, $P > .05$). However, not unexpectedly, women with positive HR HPV status were more likely to undergo colposcopy and cervical biopsy than women with negative HR HPV status among all age groups (74.8/% vs 62.7%; chi-square test, $P < .01$).

Among the 979 women with follow-up, 250 (25.5%) had a negative follow-up, 612 (62.5%) had CIN 1 lesions and/or HPV cytopathic changes, and 117 (12.0%) had CIN 2+ lesions. The latter included 90 cases of CIN 2 lesions, 24 cases of CIN 3 lesions, and 3 squamous cell carcinomas. All CIN 2+ lesions were biopsy proven. Table 2 summarizes the results of follow-up and HR HPV status among the 2 age groups. Only 2 women who were negative for HR HPV were found to have CIN 2+ lesions on follow-up. The incidence of CIN 2+ lesions in women who were positive for HR HPV was significantly higher than the incidence of CIN 2+ lesions in women who were negative for HR HPV. This was true for the entire study population, as well as for each age group ($P < .001$)

Table 3 summarizes the operating characteristic of HR HPV status as a marker for CIN 2+ lesions on subsequent follow-up. As a marker for CIN 2+ lesions, HR HPV demonstrated excellent sensitivity and negative predictive value among all age groups. However, the specificity was quite poor in women aged ≥ 30 years and even worse in women aged < 30 years. Overall, HR HPV-negative women with LSIL were found to be at only at a 1.2% risk of harboring CIN 2+ lesions on follow-up, whereas the risk of harboring CIN 2+ lesions on follow-up was 2.6% and 0.8% among HR HPV-negative women who were aged < 30 years and those aged ≥ 30 years, respectively.

Among the women with positive HR HPV status, 30.8%, 29.2%, and 40.0% had a low, moderate, and high viral load, respectively. Overall, 91 (16.2%) women with moderate and high viral loads were found to have CIN 2+ lesions on follow-up whereas only 24 (9.6%) women with a low viral load were found to have CIN 2+ lesions on follow-up; the difference was statistically significant

($P = .01$). Using 100 RLU/CO as a cutoff value (ie, moderate and high viral loads), the sensitivity, specificity, and negative predictive value of HR HPV as a marker for CIN 2+ lesions were 78%, 45%, and 94%, respectively, for all women.

DISCUSSION

In the current study, the detection rate for HR HPV among all women with LSIL regardless of age was 82.3%, which was comparable to rates reported in the literature.[1,4,5] A recent meta-analysis reported that the pooled estimate of HR HPV positivity among women with LSIL was 76.6%.[5] In the ALTS study, only 18% of women were aged $\geq$ 30 years and $<$ 5% were aged $\geq$ 40 years. Conversely, in the current study, 51% of women were aged $\geq$ 30 years, 31% of women were aged $\geq$ 40 years, and 7% were aged $\geq$ 55 years.

Our observations demonstrated that the HR HPV detection rate was significantly lower in women aged $\geq$ 30 years with LSIL when compared with those aged $<$ 30 years (73% vs 91%). Other studies have also reported that fewer older women with LSIL tested positive for HR HPV when compared with younger women.[2,4,6,7] Moss et al reported that positive HR HPV status was noted in 90% of women aged $<$ 35 years with LSIL (n = 1335), 70% of those ages 35 years to 49 years (n = 373), and 51% in those aged $\geq$ 50 years (n = 117).[4] Sherman et al also reported that 75% of women with LSIL who were aged $\geq$ 30 years were positive for HR HPV (n = 166) whereas nearly 90% of women with LSIL who were aged $<$ 30 years were positive (n = 682).[6] The lowest reported HR HPV detection rate was 43% in a cohort of 291 women aged $\geq$ 35 years.[8]

Overall, approximately 75% of the women in the current study were found to have CIN 1+ lesions on subsequent follow-up. Another 12% were found to have CIN 2+ lesions on follow-up. The latter observation was consistent with the literature, which reported the incidence of CIN 2+ lesions after a diagnosis of LSIL to be in the range of 12% to 16%.[9-11] In addition, women aged $<$ 30 years had a significantly higher incidence of CIN 2+ lesions than women who were aged $\geq$ 30 years.

There are issues to be examined before HR HPV testing can be considered a clinically useful triage tool. One is whether the test would identify a significant percentage of women who would benefit from immediate colposcopy and biopsy, and could that result justify the cost of the triage test. For example, if the results of HR HPV testing are almost always positive in a given subset of women, the cost savings from a minimally reduced number of colposcopies would be unlikely to cover the costs of HR HPV testing. Another important consideration is whether HR HPV-negative women can be followed safely with conservative management.

According to the most current guidelines, the recommended management for women with a cytologic interpretation of LSIL is colposcopy. One exception to this recommendation is the adolescent population, in whom the recommendation is that they are followed conservatively with repeat cytology in 12 months' time.[12] The guidelines do not recommend reflex HR HPV testing in women with LSIL, with the exception of postmenopausal women. The rationale for this recommendation stemmed from the ATLS trial data, which reported that 83% of women with LSIL tested positive for HR HPV.[1] The authors concluded that such a high HR HPV positivity rate would result in avoidance of colposcopy in a very limited number of women with LSIL, rendering HR HPV testing not cost-effective as a triage method for women with LSIL. As mentioned earlier, the average age of the women who were enrolled in the ALTS study was 24.9 years, with $>$ 80% of women aged $<$ 30 years.[1]

In the current study, 83% of women with LSIL were found to be positive for HR HPV. Using a similar rationale as the ALTS study, HR HPV would not be a cost-effective triage tool for women with LSIL in general. However, when we only focused on the subset of women who were aged $\geq$ 30 years, only 73% of women were found to be HR HPV positive. This might suggest that approximately 1 in 4 women could avoid colposcopy if HR HPV testing was used as a triage tool in women aged $\geq$ 30 years with LSIL cytology. Other studies have also suggested that HR HPV testing of older women with LSIL may be rational because of the relatively high percentage of women who were negative for HR HPV.[3,7,13] Given the ACOG guidelines for HR HPV cotesting in women aged $\geq$ 30 years, in many cases HR HPV status may already be known. In this scenario, a known prior positive HR HPV cotesting result would avoid the need for and the additional cost of reflex HR HPV testing of a woman with LSIL cytology. In the current study, $>$ 90%

of women aged < 30 years were positive for HR HPV. Using HR HPV as a triage tool in this younger age group suggests that at least 9 of 10 women with LSIL cytology would be referred to colposcopy based on their HR HPV status. In this scenario, HR HPV would not be a cost-effective triage tool.

We observed that nearly all women who were found to have CIN 2+ lesions on follow-up were positive for HR HPV. Only 2 women who were negative for HR HPV were found to have CIN 2 lesions; none of the HR HPV-negative women were found to have CIN 3 lesions or invasive carcinoma on follow-up. Not unexpectedly, the sensitivity and negative predictive value of HR HPV as a marker for CIN 2+ lesions on follow-up were excellent; both approached 100% for all age groups. In other words, if HR HPV testing was used as a triaging tool in women with LSIL, there would be a < 1% chance that a woman who was aged ≥ 30 years with LSIL would be found to harbor CIN 2+ lesions on follow-up. Although there was a relatively low risk (2.6%) of harboring a CIN 2+ lesion on follow-up in women with LSIL who were aged < 30 years, the percentage of HR HPV-negative women in this age group was < 10%. Therefore, HR HPV testing would not be a cost-effective triaging tool for women with LSIL in this age group.

To our knowledge the relation between viral load and the degree of dysplasia on subsequent follow-up has not been well established. The results of the current study suggest that women with moderate and high viral loads were more likely to have CIN 2+ lesions than those with a low viral load. Sun et al also reported that an increasing viral load of HR HPV was associated with the presence of CIN 2+ lesions.[14,15] However, Sherman et al did not report any direct correlation between viral load and the grade of CIN lesions.[6] In the current study, if 100 RLU/CO was used as a cutoff value (ie, moderate and high viral load groups), 42.5% of women would be referred to colposcopy. Compared with a cutoff value of 1.0 RLU/CO, the specificity was higher (45% vs 19%), but at the expense of a substantial decrease in sensitivity from 98% to 78%. Using the higher threshold result as triage tool for colposcopy may not be acceptable if the goal is the maximal detection of CIN 2+ lesions.

One of the major limitations in the current study is the lack of uniformity in terms of follow-up. Overall, 6% of women were lost to follow-up. In addition, approximately 25% of women were followed with repeat cytology only. Women with negative HR HPV status were more likely to be lost to follow-up compared with HR HPV-positive women. As a result, the prevalence of CIN 1 and 2+ lesions in the current study may not be accurate. This also implies that some of our gynecologists were using HR HPV testing to triage women with LSIL. This might explain why HR HPV-positive women were more likely to be referred for colposcopy and biopsy than those who were HR HPV negative. According to a 2006 survey, 28% of cytology laboratories in the United States have been performing HR HPV testing to triage women with LSIL.[16]

Although the results of the current study suggest that using HR HPV testing as a triage tool or using known HR HPV cotesting results for colposcopy may be an option in women aged ≥ 30 years with LSIL cytology, more rigorous follow-up of the HR HPV-negative women is needed. Because the women in the current study who were HR HPV negative did not receive the same close follow-up as the HR HPV-positive group, the number of CIN2+ lesions in the HR HPV-negative women may be underestimated. Additional studies are needed before it can be suggested that using HR HPV testing in women aged ≥ 30 years with LSIL cytology could reduce the colposcopy referral rate without compromising the sensitivity of detecting CIN 2+ lesions.

FUNDING SOURCES

No specific funding was disclosed.

CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

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