Is the Human Papillomavirus Test in Combination With the Papanicolaou Test Useful for Management of Patients With Diagnoses of Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesions?

Nak-Woo Lee, MD; Daesu Kim, MD; Jong-Tae Park, MD; Aeree Kim, MD

• ***Objective.*—To determine whether human papillomavirus (HPV) testing is useful in the evaluation of patients diagnosed with atypical squamous cells of undetermined significance (ASCUS)/low-grade squamous intraepithelial lesion (LSIL) and whether the HPV test is appropriate as an alternative screening method.**

***Design.*—The results of Papanicolaou (Pap) tests and subsequent hybrid capture tube (HCT) II tests for high-risk–type HPV were analyzed for 457 patients. Among these tests, 208 histologic diagnoses were made and correlated with the results of Pap and HPV tests. The sensitivity and specificity of the Pap test, HPV test, and the combined method of Pap and HPV tests to detect cervical intraepithelial neoplasia (CIN) 2/3 and all CIN were also measured.**

***Results.*—Sixty (63.8%) of 94 women with LSIL and 31 (26.3%) of 118 women with ASCUS tested positive for high-risk HPV. The sensitivity values for Pap tests in detecting all cases of CIN and CIN 2/3 were 91.4% and 92.9%, respectively. The sensitivity values of HCT II tests using the high-risk probe for detecting all cases of CIN and CIN 2/3 were 62.6% and 88.1%, respectively. Biopsies confirmed that 10 (22.7%) of 44 LSIL patients with high-risk HPV had CIN 2/3, but only 1 (4.5%) of 22 LSIL patients without high-risk HPV had CIN 2/3.**

***Conclusion.*—Testing for high-risk HPV with the HCT II test is useful in the detection of CIN 2/3 in LSIL groups and in the selection of patients for colposcopy in ASCUS groups, but it is not suitable for cervical cancer screening tests.**

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Although introduction of the Papanicolaou (Pap) test has reduced the incidence of cervical carcinoma, it remains the second or third most common malignancy in women worldwide.[1] Since the introduction of the Pap test, 4 reporting systems have been used.[2] The Bethesda System is the most recently adopted reporting system and is widely accepted.[3] The category *atypical cells of undetermined significance* (ASCUS) in this system means that cytologic changes are not definitively benign, but they do not satisfy the criteria of squamous intraepithelial lesion (SIL).[1,3,4] The diagnostic criteria for ASCUS are subjective and are not reproducible. Laboratory ASCUS rates have ranged from 1.6% to 9.2%.[5] Moreover, reports of the rate of SIL on biopsy follow-up after a Pap test has been classified as ASCUS have ranged from 10% to 50%.[6–10] On the other hand, benign cellular changes can be diagnosed falsely as ASCUS. Therefore, clinical management practices and the significance of ASCUS have not been uniformly agreed upon. Low-grade squamous intraepithelial lesion (LSIL) represents human papillomavirus (HPV) infection or cervical intraepithelial neoplasia (CIN) 1, known as mild dysplasia. About 2% to 3% of smears are diagnosed as LSIL in the United States.[1] However, there are also possibilities of missed or underdiagnosed high-grade squamous intraepithelial lesions (HSILs) or squamous cell carcinomas among patients diagnosed with LSIL. Therefore, in order not to miss HSIL or even carcinoma, the selection of these important patients from the LSIL and ASCUS groups is crucial.

Human papillomavirus causes precancerous SILs and carcinomas in the uterine cervix.[11] The smears showing features of HPV infection are categorized as LSIL.[3] However, latent infections without any visible histologic or cytologic abnormalities can be present.[12] The understanding of pathogenesis in cervical carcinoma and recent advances in molecular biologic techniques enable the application of molecular diagnostics in the screening program for cervical cancer. Use of HPV testing to triage low-grade cervical lesions enables the selection of patients for colposcopic examination. Although many studies based on the HPV test as a triage method for patients with mild atypical lesions or in screening programs have been reported,

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From the Departments of Obstetrics and Gynecology (Dr Lee), Pathology (Drs D. Kim and A. Kim), and Preventive Medicine (Dr Park), College of Medicine, Korea University, Seoul, Korea.

Reprints: Aeree Kim, MD, Department of Pathology, Korea University Ansan Hospital, College of Medicine, Korea University, 516 Gojan-Dong, Ansan, Kyunggi Province, 425-707, Korea (e-mail: ark@mail.korea.ac.kr).

Table 1. Relationship Between Cytologic Feature and Detection of High-Risk Human Papillomavirus (HPV)*

	Cytologic Finding, No. (%)					
	WNL (n = 94)	BCC (n = 116)	ASCUS (n = 118)	LSIL (n = 94)	HSIL (n = 35)	Total (n = 457)
Negative	92 (97.9)	108 (93.1)	87 (73.7)	34 (36.2)	3 (8.6)	324 (70.9)
High-risk HPV	2 (2.1)	8 (6.9)	31 (26.3)	60 (63.8)	32 (91.4)	133 (29.1)

* All values are presented as numbers (percentages). WNL indicates within normal limits; BCC, benign cellular changes; ASCUS, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; and HSIL, high-grade squamous intraepithelial lesion.

Table 2. Relationship Between Histologic Features and Detection of High-Risk Human Papillomavirus (HPV) in Each Cytologic Group*

	Histologic Feature, No. (%)		
	Normal/ Reactive	CIN 1	CIN 2/3
WNL/BCC (n = 52)	42 (80.8)	9 (17.3)	1 (1.9)
HPV+ (n = 3)	2 (66.7)	1 (33.3)	0
HPV− (n = 49)	40 (81.6)	8 (16.3)	1 (2.1)
ASCUS (n = 59)	23 (39.0)	35 (59.3)	1 (1.7)
HPV+ (n = 17)	2 (11.8)	15 (88.2)	0
HPV− (n = 42)	21 (50)	20 (47.6)	1 (2.4)
LSIL (n = 66)	4 (6.1)	51 (77.3)	11 (16.6)
HPV+ (n = 44)	0	34 (77.3)	10 (22.7)
HPV− (n = 22)	4 (18.2)	17 (77.3)	1 (4.5)
HSIL (n = 29)	0	2 (6.9)	27 (93.1)
HPV+ (n = 27)	0	0	27 (100)
HPV− (n = 2)	0	2 (100)	0

* All values are presented as numbers (percentages). CIN indicates cervical intraepithelial neoplasia; WNL, within normal limits; BCC, benign cellular changes; ASCUS, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; and HSIL, high-grade squamous intraepithelial lesion.

the advantages of the HPV test remain to be studied due to lack of histologic confirmation.[13,14] This study was designed to compare the sensitivity and specificity of the Pap test, HPV test using the hybrid capture tube (HCT) II test, and the combined method of the Pap test and HPV tests for detecting CIN and CIN 2/3. To establish clinical, cytopathologic, and microbiologic guidelines for the evaluation and management of a diagnosis of ASCUS/LSIL, we evaluated the rate of high-risk HPV infection in patients with diagnoses of within normal limits (WNL), benign cellular changes (BCC), ASCUS, LSIL, and HSIL, and compared the relationships between histologic features and the presence of high-risk HPV DNA in each cytologic group.

MATERIALS AND METHODS

A total of 2967 women were screened for cervical carcinoma and precancerous cervical lesions between March 2000 and February 2001 at Korea University Ansan Hospital. The samples for cervicovaginal cytology were taken with Cervex brushes (Boin Medical Co Ltd, Seoul, Korea). Two pathologists signed out all of the samples, without previous screening by cytotechnologists. Both pathologists used the Bethesda System[3] and adhered to relatively uniform criteria and terminology.

The subject group consisted of 457 patients who had subsequent HCT II tests performed (Digene Diagnostics, Inc, Beltsville, Md) for high-risk–type HPVs. The HCT II test for high-risk HPV was performed in patients who had diagnoses of ASCUS, LSIL, or HSIL on cytologic examination. The HCT II test for high-risk HPV was also performed in all patients with diagnoses of WNL or BCC who granted consent for HPV testing. The specimens for histologic diagnoses in these groups were obtained by hysterectomies performed to treat other benign diseases.

Specimens for the HCT II test from the uterine cervix were collected with a Digene specimen collection kit and stored at −20°C until analyzed. In the first step, the specimens were denatured with alkali. Then, 150 μL of denatured sample was hybridized to probe B (high-risk cocktail: probe for HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). The storage of specimens and reagents and the tests were conducted according to the manufacturer's instructions. Human papillomavirus status was read out as the ratio of relative light units (RLU) of HPV DNA in the sample to that of the positive control, set at 1 pg/mL HPV DNA. To be positive for HPV, the ratio of RLU of HPV DNA in the sample to that of the positive control should be more than 1.

Colposcopy-directed biopsy was performed in patients with LSIL or HSIL, or with ASCUS and high-risk HPV. The surgical specimens for histologic confirmation were obtained through colposcopy-directed biopsy, loop electrosurgical excisional procedure, conization, or total hysterectomies in 208 patients. Two pathologists confirmed all histologic diagnoses without any knowledge of the HPV status. In the case of equivocal specimens, we made diagnoses jointly with a second pathologist. Clinical data were recorded from the patients' medical records.

For statistical analysis of the correlation of clinicopathologic findings and the results of cytologic diagnosis and HPV testing, we used the Fisher exact test. To compare the HPV DNA quantities of each diagnostic group, we used analysis of variance and a *t* test.

RESULTS

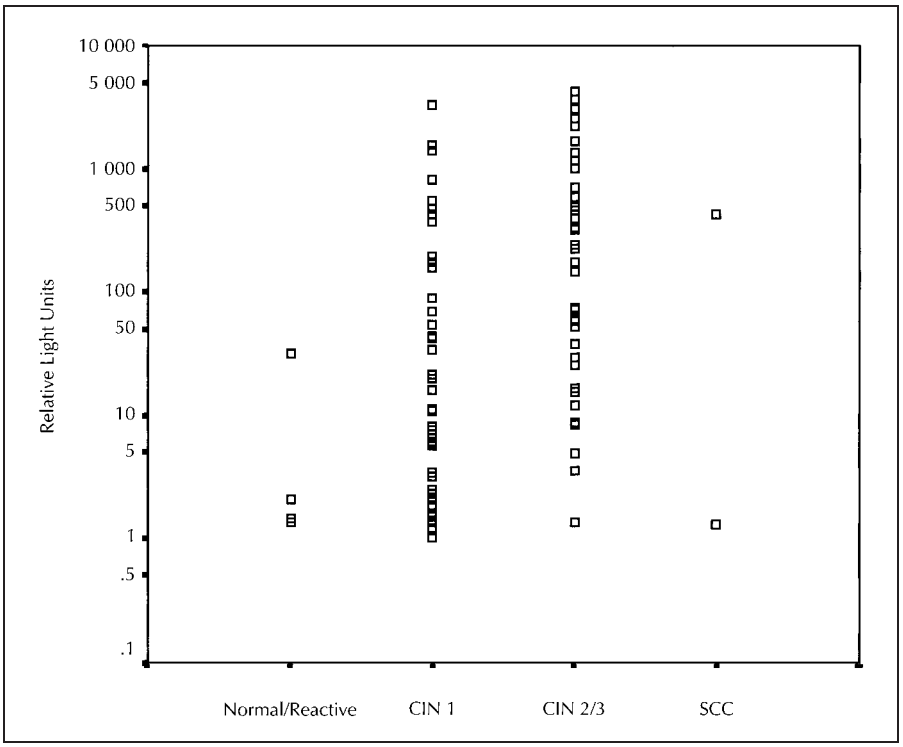
Of the 2967 patients who underwent the Pap test, 297 specimens (10.0%) were diagnosed as ASCUS, 145 (4.9%) as LSIL, 57 (1.9%) as HSIL, 7 (0.2%) as invasive squamous cell carcinoma, 23 (0.8%) as atypical glandular cells of undetermined significance, 695 (23.4%) as BCC, 1693 (57.1%) as WNL, and 50 (1.7%) as unsatisfactory. The age distribution of patients ranged from 19 to 85 years. The correlation between high-risk HPV positivity and the cytologic findings is presented in Table 1. Among 118 ASCUS cases, 69 (58.5%) were qualified as ''favor LSIL,'' 31 (25.4%) as ''favor reactive,'' and 18 (15.2%) as ''not otherwise specified.'' Human papillomavirus was detected in 22 (31.9%) of 69 ASCUS, favor LSIL; 5 (27.8%) of 18 ASCUS, not otherwise specified; and 4 (12.9%) of 31 ASCUS, favor reactive. Two patients with WNL diagnoses by cytology had positive HPV tests. Review of the Pap smears for these 2 patients revealed that one showed LSIL, but the other remained WNL. Three patients with HSIL did not test positive for high-risk HPV DNA. Two of these patients underwent colposcopy-directed biopsies, which confirmed CIN 1.

Biopsies showed that 2 patients had invasive squamous cell carcinoma. Table 2 shows the relationship between histologic features and the detection rate of high-risk HPV in patients within each cytologic group. In the ASCUS group, the percentage of CIN on biopsy was higher in the

Table 3. Sensitivities and Specificities of Cytology, Human Papillomavirus (HPV) Test, and Combined Triage for Detecting Cervical Intraepithelial Neoplasia (CIN) and CIN 2/3

		Histology			CIN		CIN 2/3	
	Total	p11Negative	CIN	CIN 2/3	Sensitivity, %	Specificity, %	Sensitivity, %	Specificity, %
Cytology*								
ASCUS/SIL	154	27	127	39	91.4	60.9	92.9	30.7
WNL/BCC	52	42	10	1				
HPV								
Positive	91	4	87	37	62.6	94.2	88.1	67.4
Negative	115	65	50	3				
Combined triage								
Positive	157	29	128	39	92.1	58.0	92.9	28.9
Negative	49	40	9	1				
Total	**206**	**69**	**137**	**40**				

* ASCUS indicates atypical squamous cells of undetermined significance; SIL, squamous intraepithelial lesion; WNL, within normal limits; and BCC, benign cellular changes.



Amounts of human papillomavirus DNA measured according to each diagnostic group. CIN indicates cervical intraepithelial neoplasia; SCC, squamous cell carcinoma.

patients with high-risk HPV than in those without high-risk HPV. The percentage of CIN 2/3 in the patients with high-risk HPV was higher than in those without high-risk HPV among the patients with LSIL. Biopsies confirmed CIN 2/3 in all of the patients with high-risk HPV in the HSIL group. In the WNL/BCC group, 3 patients had high-risk HPV DNA. One showed only chronic cervicitis in the colposcopy-directed biopsy, but had a history of conization resulting from CIN 3, 3 years earlier. The other patient showed CIN 1 on the biopsy.

Table 3 shows sensitivities and specificities of cytology, the HPV test, and combined triage for detecting CIN and CIN 2/3. In comparison with the Pap test, the sensitivity for detecting all cases of CIN and high-grade CIN was not improved with combined triage.

The mean RLU levels (±SD) were 9.0 ± 14.8 in women with benign reactive lesions, 214.6 ± 551.6 in women with CIN 1, 742.9 ± 1092.9 in women with CIN 2/3, and 216 ± 303.8 in women with invasive squamous cell carcinoma. The mean RLU levels were not correlated according to disease severity (Figure). The mean RLU level of women with high-grade or higher lesions was 716.1 ± 1071.9, and that of women with low-grade or lower lesions was 199.1 ± 532.8. The difference in the RLU levels of these 2 groups was statistically significant ($P = .007$). The minimum RLU levels in each diagnostic category were lower than 2.

Only 77 (16.8%) of the participants in this study were in menopause. Thirty-eight of these 77 women were classified in the ASCUS/LSIL group. The rate of high-risk HPV positivity in the ASCUS/LSIL group overall was higher in premenopausal than in menopausal women (Table 4).

Table 4. Relationship Between Detection of High-Risk Human Papillomavirus (HPV) and Menopause in Atypical Squamous Cells of Undetermined Significance/Low-Grade Intraepithelial Lesion Group

	High-Risk HPV, No. (%)	
	Positive	Negative
Menopause (n = 38)	13 (34.4)	25 (65.8)
Premenopause (n = 174)	78 (44.8)	96 (55.2)

* All values are presented as numbers (percentages).

COMMENT

Although the Pap test is the most reliable screening method for the detection of high-grade intraepithelial lesions and invasive cervical carcinoma, it has not eradicated carcinoma in the uterine cervix, even in populations with a high screening rate.[15] It is also not always effective in the detection of potential premalignant lesions. Accuracy of Pap tests is poor, especially in the low-grade cervical lesions.[16] The category of ASCUS constitutes a heterogenous group, encompassing minimal cellular changes of benign reactive lesions to invasive squamous cell carcinoma. This wide range results in unnecessary colposcopies and biopsies.

Persistent presence of high-risk–type HPV is related to the development of cervical carcinoma. Therefore, the detection of high-risk HPV can be an additional tool in the management of LSIL and ASCUS lesions and in the screening program for cervical carcinoma.

The rate of HPV positivity in our LSIL group was lower than that reported in the ASCUS-LSIL Triage Study (79.1%–86.1%), but higher than reported in other investigations (59.4%).[17,18] In the ASCUS-LSIL Triage Study, because a high proportion of patients with LSIL on Pap test was positive for high-risk HPV, the investigators concluded that the HCT II test has limited potential in management.[17] In our study, the high-risk HPV test in the LSIL group was useful for the detection of CIN 2/3. Most patients with HSIL (91.4%) were positive for high-risk HPV in this study. Therefore, the HPV DNA test is unlikely to be practical in the triage of patients with HSIL. Because only 26.3% of the patients with ASCUS had high-risk HPV, and 88.2% of them showed CIN 1, the HPV test proved useful for women with ASCUS in the selection of patients for colposcopic examinations. Recently, the ASCUS-LSIL Triage Study group reported that the sensitivity to detect CIN 3 or above using the HCT II test for high-risk HPV was very high (96.3%).[19] The treatment of patients with high-risk HPV and CIN 1 is controversial, because low-grade lesions may progress toward high-grade lesions in only a small number of patients. In our study, a considerable proportion of the cases with ASCUS and without high-risk HPV were pathologically confirmed as CIN 1. The patients could have had low-risk HPV. It is well known that low-risk HPV types are found more often in condyloma acuminatum and CIN 1.[20]

Three patients with confirmed CIN 3 by histologic examination did not have high-risk HPV. Recent studies have demonstrated that almost all cervical carcinomas in humans are associated with high-risk HPV.[20] Rare cases in which HPV is not detected can be explained by (1) improper sampling, (2) disruption of HPV by integration events, (3) the existence of still unidentified HPVs, (4) sensitivity of the method, and (5) the mechanism of transformation.[21]

In our study, the differences between mean RLU levels of patients with low-grade lesions and serious lesions (eg, CIN 2, CIN 3, and invasive squamous cell carcinoma) were statistically significant. The cutoff level to define a positive result of HPV HCT II testing can be adjusted according to the specificity and sensitivity.[22] Because the lowest RLU levels of each diagnostic category were just above 1, the low RLU level should not be overlooked; for those patients whose cervical lesions have been misdiagnosed, that leads to clinically, emotionally, and economically serious consequences.

In the ASCUS/LSIL group, menopausal women were less likely to have high-risk type–HPV DNA than premenopausal women. This discrepancy results from cytologic overdiagnosis in the menopausal group due to atrophic change.

Our findings revealed no differences between the sensitivities of the Pap test only and the combined triage for the detection of CIN or CIN 2/3. Combined triage is not superior to the Pap test as a screening test in terms of cost-effectiveness. The HPV HCT II test is also not suitable as a screening test because it has low sensitivity in the detection of CIN, and it is expensive. There should be several prerequisite factors for the success of a screening program for cervical carcinoma: (1) adequate sampling, (2) subjectivity in the detection and interpretation of abnormal cells, and (3) a well-organized screening program with legislative mandates by the government.[23] In low-resource settings, such as South Africa, the HPV test was suggested for primary cervical cancer screening.[22] Although an organized screening program and legislative mandates by the government (eg, Clinical Laboratory Improvement Amendments of 1988 in the United States) are lacking, high-risk HPV testing was not superior to the Pap test in sensitivity for detecting CIN in this study.

The studies of false-negative Pap tests revealed that laboratory errors in screening and interpretation might cause misclassification.[24–26] Recently, several new methods beyond well-organized quality assurance programs, such as computer-assisted screening, thin prep method, and Autopap, have been developed and used in several large institutions. The HCT II test is simple and is a highly sensitive method for the detection of HPV DNA. In our study, a few women with BCC/WNL diagnoses had high-risk HPV. This result suggests that the HPV test in this group can be another method of quality assurance for countries where there is a lack of organization in the screening program and no legislative mandates by the government.

In summary, we conclude that testing for high-risk HPVs with the HCT II test is useful in the detection of CIN 2/3 in LSIL groups and is useful in the selection of patients for colposcopic examination in ASCUS groups, but it is not suitable as a cervical cancer screening test.

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References

1. Schiffman M, Adrianza ME. ASCUS-LSIL triage study. *Acta Cytol.* 2000;44:726–742.

2. Morin C, Bairati I, Bouchard C, et al. Cytologic predictors of cervical intraepithelial neoplasia in women with an ASCUS Pap smear. *Acta Cytol.* 2000;44:576–586.

3. NCI Workshop. The 1988 Bethesda System for reporting cervical/vaginal cytological diagnosis. *JAMA.* 1989;262:931–934.

4. Bonfiglio TA. The cytopathology of squamous epithelial lesions of the cervix

and vagina. In: Bonfiglio TA, Erozan YS, eds. *Gynecologic Cytopathology.* Philadelphia, Pa: Lippincott-Raven; 1997:73–105.

5. Davey DD, Nielsen ML, Naryshkin S, Robb JA, Cohen T, Kline TS. Atypical squamous cells of undetermined significance: current laboratory practice of participants in the College of American Pathologists interlaboratory comparison program in cervicovaginal cytology. *Arch Pathol Lab Med.* 1996;120:440–444.

6. Davey DD, Naryshkin S, Nielsen ML, Robb JA, Kline TS. Atypical squamous cells of undetermined significance: interlaboratory comparison and quality assurance monitors. *Diagn Cytopathol.* 1994;11:390–396.

7. Genest DR, Dean B, Lee KR, Sheets E, Crum CP, Cibas ES. Qualifying a cytologic diagnosis of ''atypical squamous cells of undetermined significance'' affects the predictive value of a squamous intraepithelial lesion on subsequent biopsy. *Arch Pathol Lab Med.* 1998;122:338–341.

8. Selvaggi SM. Cytologic features of squamous cell carcinoma in situ involving endocervical glands in endocervical cytobrush specimens. *Acta Cytol.* 1994; 38:687–692.

9. Selvaggi SM, Haefner HK. Reporting of atypical squamous cells of undetermined significance on cervical smears: is it significant? *Diagn Cytopathol.* 1995;13:352–356.

10. Williams ML, Rimm DL, Pedigo MA, Frable WJ. Atypical squamous cells of undetermined significance: correlative histologic and follow-up studies from an academic medical center. *Diagn Cytopathol.* 1997;15:288–291.

11. Koutsky LA, Holmes KK, Critchlow CW, et al. A cohort study of the risk of cervical intraepithelial neoplasia grade 2 or 3 in relation to papillomavirus infection. *N Engl J Med.* 1992;327:1272–1278.

12. Oriel JD. Natural history of genital warts. *Br J Vener Dis.* 1971;47:1–13.

13. Kaufman HR, Adam E. Is human papillomavirus testing of value in clinical practice? *Am J Obstet Gynecol.* 1999;180:1049–1053.

14. Adam E, Kaufman HR, Berkova Z, Icenogle J, Reeves WC. Is human papillomavirus testing an effective triage method for detection of high grade cervical intraepithelial neoplasia? *Am J Obstet Gynecol.* 1998;178:1235–1244.

15. Koss LG. The Papanicolaou test for cervical cancer detection: a triumph and a tragedy. *JAMA.* 1989;261:737–743.

16. Duggan MA, Brasher PMA. Accuracy of Pap tests reported as CIN I. *Diagn Cytopathol.* 1999;21:129–136.

17. ALTS Group. Human papillomavirus testing for triage of women with cytologic evidence of low-grade squamous intraepithelial lesions: baseline data from a randomized trial. *J Natl Cancer Inst.* 2000;92:397–402.

18. Bergeron C, Jeannel D, Poveda JD, Cassonnet P, Orth G. Human papillomavirus testing in women with mild cytologic atypia. *Obstet Gynecol.* 2000;95: 821–827.

19. Solomon D, Schiffman M, Tarone R. Comparison of three management strategies for patients with atypical squamous cells of undetermined significance: baseline results from a randomized trial. *J Natl Cancer Inst.* 2001;93:293–299.

20. Brown DR, Bryan JT, Cramer H, Fife KH. Analysis of human papillomavirus typed in exophytic condylomata acuminata by hybrid capture and Southern blot techniques. *J Clin Microbiol.* 1993;31:2667–2673.

21. Walboomers JMM, Meijer CJLM. Do HPV-negative carcinomas exist? *J Pathol.* 1997;181:253–254.

22. Kuhn L, Denny L, Pollack A, Lorincz A, Richart RM, Wright T. Human papillomavirus DNA testing for cervical cancer screening in low-resource settings. *J Natl Cancer Inst.* 2000;92:818–825.

23. Infantolino C, Fabris P, Infantolino D, et al. Usefulness of human papilloma virus testing in the screening of cervical cancer precursor lesions: a retrospective study in 314 cases. *Eur J Obstet Gynecol Rep Biol.* 2000;93:71–75.

24. Robertson JH, Woodend B. Negative cytology preceding cervical cancer: causes and prevention. *J Clin Pathol.* 1993;43:509–512.

25. Bosch MMC, Rietweld-Scheffers PEM, Boon M. Characteristics of false negative smears tested in the normal screening situation. *Acta Cytol.* 1992;36: 711–716.

26. Howell S, Theodor M, Pacey NF, Patwardhan JR, Ayer B. Quality assurance in cytology: rescreening of previously negative smears from high grade squamous intraepithelial lesions. *Acta Cytol.* 1997;41:1085–1090.