Triage of Women with ASCUS and LSIL on Pap Smear Reports: Management by Repeat Pap Smear, HPV DNA Testing, or Colposcopy?

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BACKGROUND. The purpose of this study was to determine the efficacy of a repeat Papanicolaou (Pap) smear and the Hybrid Capture tube-based (HCT) HPV DNA test for detecting cervical intraepithelial neoplasia (CIN) grade 2 or 3 in women with recent atypical squamous cells of undetermined significance (ASCUS) or low-grade squamous intraepithelial lesion (LSIL) Pap smear reports.

METHODS. Women with a recent Pap smear report of ASCUS (n=169) or LSIL (n=110) had a repeat Pap smear, sampling of the cervix for HCT HPV DNA assay and a colposcopy examination. Data were evaluated using three different triage thresholds for colposcopy examination: a repeat Pap smear of persistent ASCUS or more severe dysplasia, a finding of persistent LSIL or more severe dysplasia, and a carcinogenic HPV test result.

RESULTS. The sensitivity, specificity, and positive and negative predictive values for detecting CIN 2/3 were 70%, 45%, 7%, and 96% for a repeat Pap smear using an ASCUS-positive threshold and 20%, 86%, 8%, and 94% for a repeat Pap smear using an LSIL-positive threshold, respectively, when women with an initial ASCUS Pap smear were considered. HPV testing for carcinogenic viruses alone or in combination with a repeat Pap smear (using ASCUS as a positive threshold) yielded results of 50%, 67%, 9%, and 96%, respectively, and 70%, 37%, 7%, and 95%, respectively, for detecting CIN 2/3. In women with an initial LSIL Pap smear, respective values for detecting CIN 2/3 by a repeat Pap smear with an ASCUS threshold were 92%, 26%, 14%, and 96%, and for an LSIL threshold 23%, 64%, 8%, and 86%, respectively. Hybrid Capture HPV testing alone or in combination with a repeat Pap smear yielded 69%, 43%, 14%, and 91%, respectively, and 100%, 21%, 14%, and 100%, respectively.

CONCLUSIONS. A Pap smear triage threshold restricted to LSIL or more severe dysplasia for women with prior ASCUS or LSIL Pap smear results was clearly ineffective for detecting high-grade cervical precancerous lesions. In contrast, when the repeat Pap smear triage threshold was expanded to include persistent ASCUS as abnormal, 83% of the women with CIN 2/3 were detected. Detection of carcinogenic HPV DNA using the HCT test was almost as sensitive for detecting CIN 2/3 as a solitary repeat Pap smear using an ASCUS or more severe positive threshold. Combining the HPV test with a repeat Pap smear did not significantly improve the sensitivity of cytology for detecting high-grade CIN. This study suggests that women with ASCUS and particularly LSIL Pap smears should be referred for a colposcopy examination until better triage methods become available.

KEY WORDS. Papanicolaou; DNA probes, HPV; colposcopy; cervical dysplasia; cervical intraepithelial neoplasia; sensitivity and specificity. *(J Fam Pract 1998; 46:125-34)*

Among the approximately 50 million Papanicolaou (Pap) smears obtained annually in the United States, 5% to 10% are reported as either atypical squamous cells of undetermined significance (ASCUS) or as low-grade squamous intraepithelial lesion (LSIL). Diagnoses made on the basis of Pap smears are known to frequently underestimate cervical disease severity, as confirmed by colposcopically directed biopsies of the cervix. However, very few women with mildly abnormal Pap smears actually have invasive cervical cancer, and less than 25% have high-grade squamous intraepithelial lesions (HSIL).

Submitted, revised, June 27, 1997.
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Regardless of this low but clinically significant risk, many experts advocate performing colposcopy examinations on all women with mildly abnormal Pap smears to avoid the risk of failing to diagnose a cervical intraepithelial neoplasia (CIN) grade 2 or 3 or an early invasive cancer. Other experts advocate simply monitoring these patients with serial Pap smears and performing colposcopy only if subsequent Pap smears demonstrate persistent mild abnormalities or neoplasia. These divergent management approaches have created considerable controversy as to the safest and most effective way to further evaluate women with Pap smear reports of ASCUS and LSIL.[1]

The essential role of human papillomavirus (HPV) in the pathogenesis of cervical neoplasia is now well substantiated.[2-4] In particular, specific HPV types have been shown to convey a significant risk for the development of CIN 2/3 and cervical cancer.[5] Commercial methods are available to detect the presence of carcinogenic human papillomaviruses that infect the lower genital tract.[6] One of these tests, the Hybrid Capture tube-based (HCT) HPV DNA assay, permits quantification of the amount of virus present at the sampled site.[7]

Recently, it has been suggested that evaluating women with mildly abnormal Pap smears using an "intermediate triage" approach of HPV testing alone or in combination with a repeat Pap smear might provide the sensitivity of colposcopy and the convenience and lower cost of viral and cytologic monitoring.[8] However, the studies that have critically evaluated this intermediate triage approach have produced somewhat conflicting results.[8-10] Although all studies to date have found that combining HCT HPV testing with a repeat Pap smear increases the sensitivity of intermediate triage, the efficacy and estimated cost savings of this approach have varied greatly.[8,10] Differing patient characteristics, specimen collection modalities, HPV spectrum of disease, and HPV test sensitivity issues may explain, in part, the conflicting results with respect to efficacy and cost savings of using HPV testing for intermediate triage.

The purpose of this study was to determine the efficacy of a repeat Pap smear and an HCT HPV DNA test for detecting the presence of cervical neoplasia in women with recent Pap smear reports of ASCUS or LSIL.

METHODS

SUBJECTS

Consenting women who were 13 years of age or older were enrolled in the study at six colposcopy clinics: the Family Medicine Center, Medical College of Georgia, Augusta, Ga; University Health Center, University of Massachusetts, Amherst, Mass; Student Health Center, Kansas University, Lawrence, Kan; Planned Parenthood of Westchester, White Plains, NY; Health Insurance Plan of New York, NY; and Columbia Presbyterian Medical Center, New York, NY. Inclusion criteria were the presence of LSIL or ASCUS on a Pap smear collected during the previous 4 months. Exclusion criteria were current pregnancy, cytologic diagnosis of HSIL or cervical cancer within the preceding 12 months, women with known causes of immunosuppression, prior colposcopy or treatment of cervical neoplasia within the past 12 months, or gynecologic conditions that precluded proper cervical evaluation. The study protocol was approved by the institutional review boards at each of the clinical sites.

EQUIPMENT AND MATERIALS

Repeat Pap smears were collected using either an Ayre's spatula and cytobrush (Medscand, Hollywood, Fla) or an Accellon sampler (Medscand, Hollywood, Fla). A Vira Pap collection kit (Dacron swab and specimen tube; Digene Corporation, Silver Spring, Md) was used to collect and transport cervical/vaginal specimens for human papillomavirus testing. All HPV testing was performed at a central site (Columbia Presbyterian Medical Center) using the HCT HPV assay (Digene Corporation, Silver Spring, Md).

STUDY DESIGN

After obtaining informed consent from women with a recent Pap smear report of ASCUS and LSIL, the cervix was visualized and a Pap smear was collected. Next, a Dacron swab was used to wipe the ectocervix, cervical os, and vagina. This swab was then placed into the Vira Pap specimen transport tube and delivered for HCT HPV testing.

Colposcopy was performed by each colposcopist as previously reported.[11] Representative cervical biopsies were obtained of abnormal cervical or vaginal lesions and an endocervical curettage performed, when indicated. Pap

smears and cervical biopsies were interpreted at a central laboratory by cytotechnologists and pathologists (Kyto, New York, NY) who were blinded with respect to the HPV status of subjects and who did not attempt to correlate the cytologic findings with the histologic findings. Pap smear results were reported using the Bethesda System[12] and histology results were reported conventionally using the CIN classification system.

Specimens for HPV DNA testing were analyzed for high-risk HPV types 16, 18, 31, 33, 35, 45, 51, 52, and 56 according to the HCT kit package instructions. Only specimens demonstrating a relative light unit (RLU) reading obtained from the luminometer equal to or greater than the mean of the positive control (10 pg/mL HPV 16 DNA) value were considered positive. The range of HPV quantification was determined by the RLU value; 1 to 4.9 times the positive control was classified as 1+ positive, 5 to 20 times the positive control was 2+ positive, and greater than 20 times the positive control was defined as 3+ positive.

Statistical Analysis

Sensitivity and specificity were calculated for each of the triage tests, using the results of colposcopy and biopsy as the criterion standard. Because controversy exists concerning whether women with CIN 1 should be aggressively evaluated and treated, two histologic levels of neoplasia were considered separately to be positive outcomes. Sensitivity, specificity, and predictive values were calculated for each level. First, CIN 1, 2, and 3 results on biopsy were considered to be positive, and results within normal limits were considered to be negative. Second, only CIN 2/3 results were considered to be positive, and results within normal limits or CIN 1 were considered to be negative. Confidence intervals for sensitivity, specificity, and predictive values of positive and of negative tests were calculated, based on the F-distribution. Comparisons of sensitivity and specificity between the different triage tests were made using McNemar's χ^2 statistic. Associations between the tests and the neoplasia categories were evaluated by Pearson's χ^2 statistic, or by Fisher's exact test if expected table cell counts were low. The Mantel-Haenszel χ^2 statistic was used to test for a linear association between the ordinal HPV viral load results and biopsy results.

RESULTS

Thirty-four women referred for the study were excluded because of nonqualifying Pap smear reports. A total of 295 women were enrolled in the study, and of these women, 279 had both a repeat Pap smear and HPV DNA test available for analysis. Of these 279 women, 169 were referred for colposcopy because of a previous Pap smear finding of ASCUS and 110 had an initial Pap smear indicating LSIL. The mean age of the women was 27.3 (SD±9.4) years, and the mean age of first sexual intercourse was 16.8 (SD±2.43) years. Fifty-five percent of women were white, 29% African American, 15% Hispanic, and 1% Asian. Although 16% of women had a prior history of genital warts, only 8% of women had previous cervical treatment, primarily cryotherapy.

Cervical intraepithelial neoplasia of any grade was detected in 43 (25.4%) of 169 women with an initial ASCUS Pap smear (Table 1). Thirty-three (19.5%) of these women had CIN 1 and 10 (5.9%) women had CIN 2/3. Of the 110 women with an initial Pap smear report of LSIL, 57 (51.8%) had CIN, 44 (40.0%) with CIN 1 and 13 (11.8%) with CIN 2/3. Cervical cancer was not detected in either group. HPV DNA tests detected cancer-associated HPV types in 58/169 (34.3%) women with an initial Pap smear report of ASCUS and 64/110 (58.2%) women with an initial Pap smear report of LSIL.

When only the 169 women with an initial Pap smear of ASCUS were considered, a repeat Pap smear with a positive triage threshold of ≥ASCUS (persistent ASCUS or more severe dysplasia) detected 30/33 (91%) cases of CIN 1 and 7/10 (70%) women with CIN 2/3. Overall, 56% (95/169) of women had a repeat Pap smear of persistent ASCUS or more severe dysplasia. When a positive threshold of ≥LSIL was used for the repeat Pap smear, 11/33 (33%) women with CIN 1 and 2/10 (20%) women with CIN 2/3 were identified. A positive carcinogenic HPV DNA test correctly identified 15/33 (45%) cases of CIN 1 and 5/10 (50%) women with CIN 2/3. When a repeat Pap smear finding of ≥ASCUS was combined with a positive HPV test result, 30/33 (91%) women with CIN 1 and 7/10 (70%) women with CIN 2/3 were correctly identified. When the repeat Pap smear threshold was increased to ≥LSIL, the combined tests correctly identified 18/33 (55%) women with CIN 1 and 5/10 (50%) women with CIN 2/3.

TABLE 1

Detection of Cervical Intraepithelial Neoplasia (CIN) in Women with Pap Smear Reports of ASCUS and LSIL Using Various Triage Tests (N=279)

Triage Test	Triage Threshold[a]	Initial ASCUS Pap Smear Total	CIN 1[b]	CIN 2/3[c]	Initial LSIL Pap Smear Total	CIN 1	CIN 2/3
Colposcopy	All women	169	33	10	110	44	13
		95	30	7	84	43	12
Pap smear	≥ASCUS[d]	95	30	7	84	43	12
	≥LSIL[e]	25	11	2	38	21	3
HPV DNA test							
	Positive at any level	58	15	5	64	32	9
	1+ to 2+ RLU[f]	25	4	1	22	10	4
	2+ to 3+ RLU	10	2	2	18	7	2
	3+ or greater RLU	23	9	2	24	15	3
Repeat Pap smear and HPV test combined	Pap smear ≥ASCUS or HPV positive[g]	107	30	7	90	43	13
	Pap smear ≥LSIL or HPV positive[h]	64	18	5	75	36	11

[a] Level at which test is considered positive.
[b] Number of cases of CIN 1 detected.
[c] Number of cases of CIN grade 2 or 3 detected.
[d] Repeat Pap smear considered positive if ASCUS or more severe dysplasia.
[e] Repeat Pap smear considered positive if LSIL or more severe dysplasia.
[f] HPV quantification by relative light units; 1+ = 1 to 4.9 x positive control, 2+ = 5 to 20 x positive control and 3+=20 x positive control.
[g] Considered positive if repeat Pap smear ≥ASCUS or positive carcinogenic HPV DNA test result.
[h] Considered positive if repeat Pap smear ≥LSIL or positive carcinogenic HPV DNA test result.

ASCUS denotes atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; HPV DNA test, Hybrid Capture human papilomavirus virus DNA test.

When only the 110 women with an initial Pap smear of LSIL were considered, a repeat Pap smear with a positive triage threshold of ≥ASCUS detected 43/44 (98%) cases of CIN 1 and 12/13 (92%) women with CIN 2/3. Overall, 76% (84/110) of women had a repeat Pap smear of ASCUS or more severe. When a positive test threshold of ≥LSIL was used for the repeat Pap smear, 21/44 (48%) women with CIN 1 and 3/13 (23%) cases with CIN 2/3 were identified. A positive oncogenic HPV DNA test detected 32/44 (73%) women with CIN 1 and 9/13 (69%) women with CIN 2/3. When a repeat Pap smear finding of ≥ASCUS was combined with a positive HPV test result, 43/44 (98%) women with CIN 1 and 13/13 (100%) of women with CIN 2/3 were detected. When the Pap smear threshold was increased to ≥LSIL, the combined tests detected 36/44 (82%) women with CIN 1 and 11/13 (85%) women with CIN 2/3.

The sensitivity, specificity, and positive and negative predictive values for the triage tests alone and in combination, compared with the criterion standard of colposcopically directed biopsy, are shown in Table 2 for women with initial Pap smear reports of ASCUS and in Table 3 for women with initial Pap smear reports of LSIL. When women with initial Pap smear findings of ASCUS were considered (Table 2), all of the tests appeared to have relatively low positive predictive values and reasonably high negative predictive values. When women with an initial Pap smear report of LSIL were considered (Table 3), few differences were again noted among triage tests in regard to positive and negative predictive values. The best test sensitivities were obtained by the combination of Pap smear and HPV test (one or both pos-

TABLE 2

Triage Test Efficacy for Detecting CIN 2/3 and CIN 1, 2, 3 in Women with Initial ASCUS Pap Smears (n=169)

Triage Tests	Triage Positive Threshold[a]	Sensitivity % (95%CI)	Specificity % (95%CI)	PPV % (95%CI)	NPV % (95%CI)
Detection of CIN 2/3					
Pap smear	≥ASCUS	70.0 (34.8, 93.3)	44.7 (36.8, 52.7)	7.4 (3.0, 14.6)	96.0 (88.6, 99.2)
	≥LSIL	20.0 (2.5, 55.6)	85.5[c,d] (79.1, 90.6)	8.0 (1.0, 26.0)	94.4 (89.3, 97.6)
HPV DNA	positive, high risk[b]	50.0 (18.7, 81.3)	66.7[e] (58.8, 73.3)	8.6 (2.9, 19.0)	95.5 (89.8, 98.5)
Pap smear and HPV DNA	one or both positive	70.0 (34.8, 93.3)	37.1 (29.6, 45.1)	6.54 (2.7, 13.0)	95.2 (86.5, 99.0)
	both positive	50.0 (18.7, 81.3)	74.2 (66.7, 80.8)	10.9 (3.6, 23.6)	95.9 (90.8, 98.7)
Detection of CIN 1, 2, 3					
Pap smear	≥ASCUS	86.1*6[f] (72.1, 94.7)	54.0 (44.9, 62.9)	39.0 (29.1, 49.5)	91.9 (83.2, 97.0)
	≥LSIL	30.2 (17.2, 46.1)	90.5[f,g] (84.0, 95.0)	52.0 (31.3, 72.2)	79.2 (71.6, 85.5)
HPV DNA	positive, high risk[b]	46.5 (31.2, 62.3)	69.8[h] (61.0, 77.7)	34.5 (22.5, 48.1)	79.3 (70.5, 86.4)
Pap smear and HPV DNA	one or both positive	86.1 (72.1, 94.7)	44.4 (35.6, 53.6)	34.6 (25.6, 44.4)	90.3 (80.1, 96.4)
	both positive	46.5 (31.2, 62.3)	79.4 (71.2, 86.1)	43.5 (28.9, 58.9)	81.3 (73.3, 87.8)

[a] Level at which the test is considered positive.
[b] Positive high risk (carcinogenic) HPV 16, 18, 31, 33, 35, 45, 51, 52, and 56.
[c] Specificity Pap smear ≥LSIL vs HPV DNA, $P<.0001$.
[d] Specificity Pap smear ≥LSIL vs HPV DNA and Pap smear, $P=.002$.
[e] Specificity HPV DNA vs Pap smear ≥ASCUS, $P<.0001$.
[f] Sensitivity Pap smear ≥ASCUS vs HPV DNA, $P=.0001$.
[g] Specificity Pap smear ≥LSIL vs HPV DNA, $P<.0001$.
[h] Specificity Pap smear ≥LSIL vs HPV DNA and Pap smear ≥ASCUS, $P=.004$.

CIN denotes cervical intraepithelial neoplasia; ASCUS, atypical squamous cells of undetermined significance; PPV, positive predictive value; NPV, negative predictive value; LSIL, low-grade squamous intraepithelia lesion; HPV DNA, human papillomavirus DNA test by hybrid capture.

itive) for the detection of CIN 2/3 (100%) and for CIN 1, 2, and 3 (98.3%) in women with an initial Pap smear report of LSIL.

Several demographic variables were examined to determine their influence on triage test performance. Increasing patient age demonstrated a positive correlation with increasing triage test positive predictive values for most triage modalities (Table 4). There was no similar trend noted for negative predictive values and patient age. Although few subjects reported prior cervical treatment, all the triage test positive predictive values were significantly greater for women who had never received cervical therapy when compared with women who had prior treatment.

When HPV DNA viral load results were compared

TABLE 3

Triage Test Efficacy for Detecting CIN 2/3 and CIN 1, 2, 3 in women with initial LSIL Pap Smear Reports (n=110)

Triage Tests	Triage Positive Threshold[a]	Sensitivity % (95%CI)	Specificity % (95%CI)	PPV % (95%CI)	NPV % (95%CI)
Detection of CIN 2/3					
Pap smear	≥ASCUS	92.3 (17.4, 35.7)	25.8 (7.6, 23.6)	14.3 (80.4, 99.9)	96.2 (80.4, 99.9)
	≥LSIL	23.1 (5.0, 53.8)	63.9[c,d] (53.5, 73.4)	7.9 (1.7, 21.4)	86.1 (75.9, 93.1)
HPV DNA	positive, high risk[b]	69.2 (38.6, 90.9)	43.3[e] (33.3, 53.7)	14.3 (6.6, 25.0)	91.3 (79.2, 97.6)
Pap smear and HPV DNA	one or both positive	100 (75.3, 100)	20.6 (13.1, 30.0)	14.4 (7.9, 23.4)	100 (83.2, 100)
	both positive	61.5 (31.6, 86.1)	48.5 (38.2, 58.8)	13.8 (6.1, 25.4)	90.4 (79.0, 96.8)
Detection of CIN 1, 2, 3					
Pap smear	≥ASCUS	96.5[f] (87.9, 99.6)	45.3 (31.6, 59.6)	65.5 (54.3, 75.5)	97.3 (74.9, 99.1)
	≥LSIL	42.1 (29.1, 55.9)	73.6 (57.7, 84.7)	63.2 (46.0, 78.2)	54.2 (42.0, 66.0)
HPV DNA	positive, high risk[b]	71.9[g] (58.5, 83.0)	56.6 (42.3, 70.2)	64.1 (51.1, 75.7)	65.2 (49.8, 78.6)
Pap smear and HPV DNA	one or both positive	98.3 (90.6, 100)	35.9 (23.1, 50.2)	62.2 (51.4, 72.2)	95.0 (75.1, 99.9)
	both positive	70.2[h] (56.6, 81.6)	66.0 (51.7, 78.5)	69.0 (55.5, 80.5)	67.3 (52.9, 79.7)

[a] Level at which the test is considered positive.
[b] Positive high risk (carcinogenic) HPV 16, 18, 31, 33, 35, 45, 51, 52, and 56.
[c] Specificity Pap smear ≥LSIL vs HPV DNA, P=.002.
[d] Specificity Pap smear ≥LSIL vs HPV DNA and Pap smear, P=.01.
[e] Specificity HPV DNA vs Pap smear ≥ASCUS, P=.002.
[f] Sensitivity Pap smear ≥ASCUS vs HPV DNA, P=.001.
[g] Sensitivity Pap smear ≥LSIL vs HPV DNA, P=.003.
[h] Sensitivity Pap smear ≥ASCUS and HPV vs Pap smear ≥LSIL, P=.005.

CIN 2/3 denotes cervical intraepithelial neoplasia grade 2 or 3; LSIL, low-grade squamous intraepithelial lesion; PPV, positive predictive value; NPV, negative predictive value; ASCUS, atypical squamous cells of undetermined significance; HPV DNA, human papillomavirus DNA test by hybrid capture.

with the frequency of CIN, a distinct linear association was demonstrated for women with a report of ASCUS (P=.005), of LSIL (P=.003), and of ASCUS or LSIL on initial Pap smear (P=.001) (Figure 1). As the reported HPV DNA viral load results increased in number, so did the frequency of CIN detected. A significant linear association between HPV DNA viral load and cervical biopsy results or severity of cervical neoplasia was also observed for women with initial Pap smear report of ASCUS (P=.009) (Figure 2).

DISCUSSION

Cervical intraepithelial neoplasia grade 2 or 3 or cervical cancer are reasonably well detected by interval screening cervical cytology. Women with Pap smear

TABLE 4

Effect of Patient Age on Triage Test Performance for Detecting CIN 2/3

Triage Tests	Triage Positive Threshold[a]	Age (years)	PPV, %	*P* Value[b]	NPV, %	*P*
Pap smear	≥ASCUS	<20	4.6		100	NS
		20-30	9.0	.046	95.4	
		>30	20.0		95.4	
	≥LSIL	<20	0		96.3	
		20-30	2.3	.001	90.1	NS
		>30	36.4		92.5	
HPV DNA	positive[c]	<20	6.3		100	
		20-30	9.6	NS[e]	93.9	NS
		>30	22.7		92.9	
Pap smear and HPV DNA	one or both positive[d]	<20	3.9		100	
		20-30	8.6	.030	94.6	NS
		>30	19.1		97.2	

[a] Level triage test considered positive.
[b] *P* value from χ^2 test for trend.
[c] Carcinogenic HPV DNA (16, 18, 31, 33, 35, 45, 51, 52 and 56) present.
[d] Pap smear ≥ASCUS or carcinogenic HPV DNA present.
[e] NS, not significant, *P*>.05

CIN 2/3 denotes cervical intraepithelial neoplasia grade 2 or 3; PPV, positive predictive value; NPV, negative predictive value; ASCUS, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; HPV DNA, human papillovirus virus DNA test by hybrid capture.

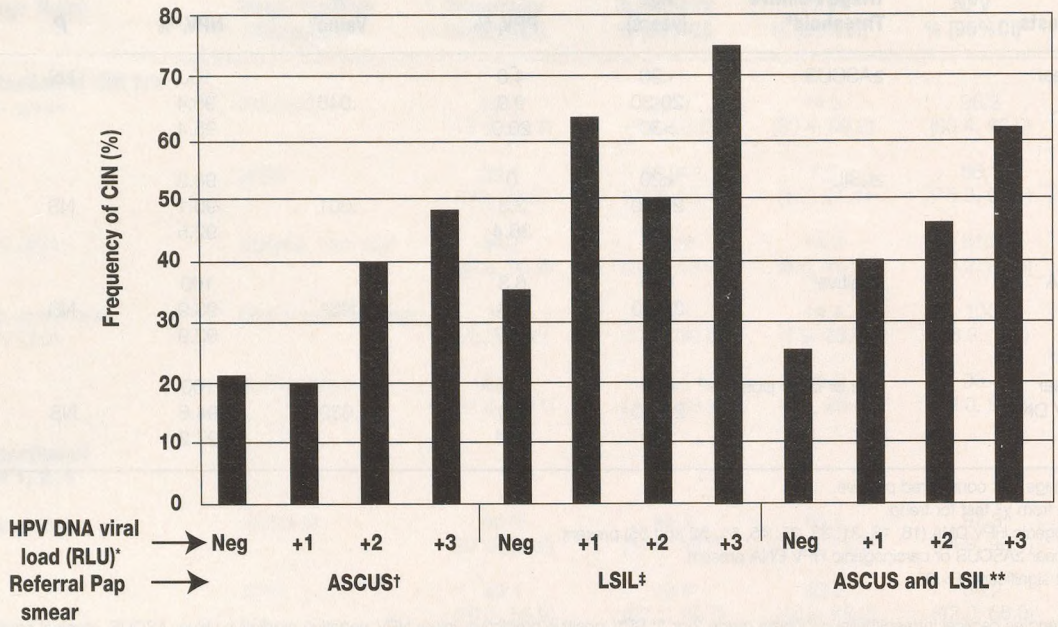
reports of HSIL should have definitive evaluation by colposcopy, and treatment if the examination and biopsy confirm the severity of the Pap smear findings. This approach is taken because many women with CIN 2 or 3 are likely to eventually develop a cervical malignancy. The management of women with reports of ASCUS or LSIL on Pap smear and less severe cervical disease confirmed by biopsy is controversial and complex.[1,13] In contrast to high-grade lesions that tend to persist or progress, low-grade cervical lesions, particularly those associated with low-risk HPV types, frequently spontaneously regress. Therefore, observation using repeat Pap smears may be appropriate, provided the initial Pap smear was accurate and the repeat Pap smears are sensitive enough to detect the majority of women with CIN 2/3. However, a small percentage of women with a report of ASCUS or LSIL on Pap smear (6% and 12%, respectively) will be found to actually have a CIN 2/3 when colposcopy is performed. A triage protocol that could detect women with occult CIN 2/3 or cancer and refer those women for colposcopy and management while allowing women with less severe lesions to be monitored by cytology or viral testing would be an improvement over universal colposcopy.

Based on our data, a repeat Pap smear can detect 70% and 92% of women with CIN 2/3 who have an initial Pap smear report of ASCUS and of LSIL, provided that women with any degree of cytologic abnormality (ASCUS or more severe dysplasia) on repeat Pap smears are referred for colposcopy. However, this triage strategy still requires 64% of women to be examined by colposcopy. If only women with LSIL or more severe dysplasia on repeat Pap smears were directed to colposcopy, merely 22% of our cases of CIN 2/3 would have been detected. Such a high threshold for a screening test, relegated to providing triage or diagnostic test capabilities, assumes intolerable risk. The sensitivity of HPV testing for detecting CIN 2/3 was less than a repeat Pap smear when a triage threshold of ASCUS was used and was better than a repeat Pap smear when a threshold of LSIL was used.

Other researchers have found that HPV DNA tests are more sensitive for detecting CIN 2/3 than those

FIGURE 1

Human papillomavirus DNA viral load by hybrid capture compared with presence of cervical intraepithelial neoplasia (CIN 1, 2, 3)



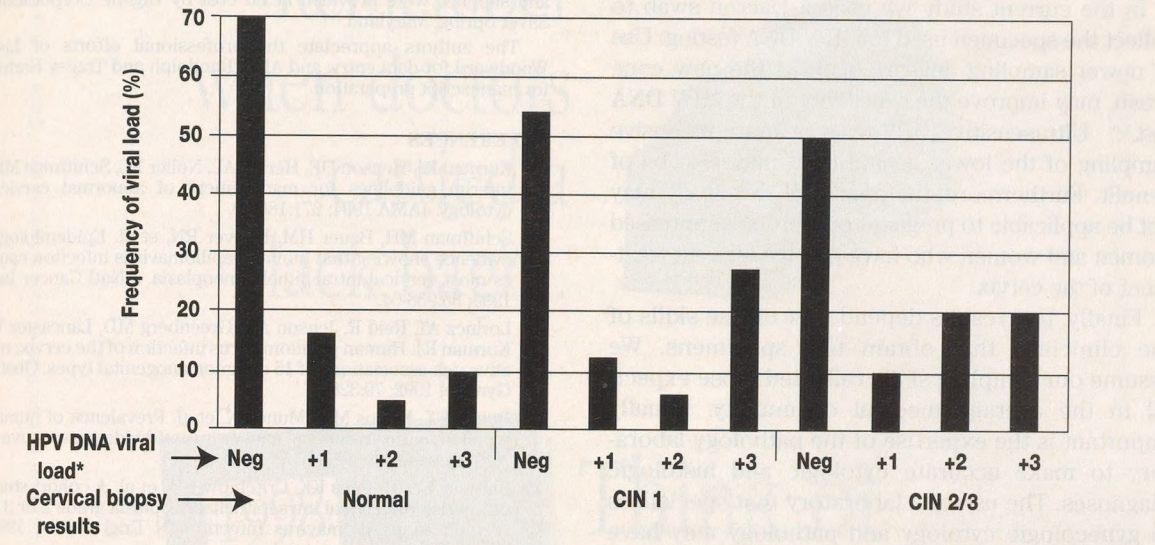
*Range of relative light units, +1 = 1.0 to 4.9 times positive control, +2 = 5 to 20 times positive control, and +3 = greater than 20 times positive control.
†ASCUS, atypical squamous cells of undetermined significance, linear association between viral load and frequency of CIN (Mantel-Haenszel χ^2=7.75, *P*=.005).
‡LSIL, low-grade squamous intraepithelial lesion, linear association between viral load and frequency of CIN (Mantel-Haenszel χ^2=8.82, *P*=.003).
**Linear association between viral load and frequency of CIN Mantel-Haenszel χ^2=23.33, *P*=.001).

we observed in this study. Hatch et al[9] reported that the sensitivity of HPV testing for detecting CIN 2/3 in women referred to colposcopy for a variety of reasons was 74% compared with our sensitivity of 61%. When used in combination with the Pap smear, the sensitivity improved to 91% compared with our observed 87%. When women with either ASCUS and LSIL Pap smear reports were evaluated separately, the HCT HPV test identified a greater percentage of women with CIN 2/3, 60% and 76%, respectively, compared with our 50% and 69%, respectively. The specificity of the HCT test in the Hatch series of women with initial reports of ASCUS or LSIL on Pap smears was 68% and 43%, respectively, which is nearly identical to our reported specificity of 67% and 43%, respectively. However, the positive predictive values for the HCT test for identifying women with CIN 2/3 who had Pap smear reports of ASCUS or LSIL was 35% and 47%, respectively, which is significantly greater than the 9% and 14%, respectively, identified in our series. In a study of women referred for colposcopy with an ASCUS Pap smear report, Cox et al[8] reported that the combination of a repeat Pap smear and the HCT HPV test had a greater sensitivity (90% vs 86%), specificity (58% vs 44%), positive (39% vs 35%) and negative predictive (95% vs 90%) values for detecting CIN than found in our study. Wright and colleagues[10] previously demonstrated that a positive HCT test had a sensitivity and specificity of 73% and 66%, respectively, for detecting CIN, 59% and 65%, respectively, for women with initial ASCUS Pap smears, and 80% and 68%, respectively, for women with initial LSIL Pap smear reports. These results contrasted with those reported in this trial, 61% sensitivity and 66% specificity for detecting CIN, 47% and 70% for women with ASCUS Pap smears, and 72% and 57% for women with initial LSIL Pap smear results.

In the current study, increasing age correlated with increasing positive predictive values for triage by Pap smear alone or for Pap smear combined with HPV DNA testing. As such, intermediate triage may

FIGURE 2

Human papillomavirus DNA viral load by hybrid capture compared with cervical biopsy results for women with an initial Pap Smear report of ASCUS



*Range of relative light units, +1 =1.0 to 4.9 times positive control, +2 = 5 to 20 times positive control, and +3 = greater than 20 times positive control.
Note: Linear association between HPV DNA viral load and cervical biopsy result (Mantel-Haenszel χ^2=6.9, *P*=.009).
ASCUS denotes atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia, grade 1, 2, or 3; HPV, human papillomavirus.

be less useful for younger women. Our study also demonstrated that prior treatment has a detrimental effect on the accuracy of intermediate triage tests. Because of the relatively diminished accuracy of triage tests in women who have undergone previous therapy for CIN, evaluation by colposcopy would be prudent for these women following a mildly abnormal Pap smear result.

Although our study, as others,[8,14] demonstrated a significant association between increased carcinogenic HPV DNA viral load levels and increased severity of CIN, the strength of this association was weak. In the study of Cox et al,[8] 92% of CIN 2 lesions positive by Hybrid Capture HPV DNA testing had RLUs >5 (+2). In our trial only 40% of women with CIN 2/3 and an initial ASCUS Pap smear and 38.5% of women with CIN 2/3 and an initial LSIL Pap smear had positive carcinogenic HPV DNA above the same level (>5 RLU). In addition, 33% of women with CIN 1 and an ASCUS Pap smear and 50% of women referred with an LSIL Pap smear had comparable levels of carcinogenic HPV DNA. Therefore in our population, carcinogenic HPV DNA levels were unable to reliably discriminate the severity of cervical neoplasia. Nevertheless, women without CIN who were HPV DNA positive had the lowest levels of HPV DNA on average.

Since women with CIN 2/3 were identified during colposcopy and referred for treatment, one limitation of our study is that it is unknown whether these women with CIN 2/3 missed by either expert cytologic examination or HPV DNA testing would have been identified subsequently at a follow-up visit if colposcopy had not been performed. Prospective serial monitoring with these moderately accurate, but less expensive tests, may be as effective over time as is more comprehensive initial evaluation with colposcopy.

An additional limitation of this study is that the results do not consider the ability of triage tests to detect rare and occult cancers, which occasionally are detected in women with LSIL and, particularly, in those with ASCUS. Hatch and colleagues found that the HCT HPV test failed to detect 4 of 9 women with histologically confirmed cervical cancer.[9] In a study of 30 women with histologically confirmed invasive cervical cancer, we found that 19 (63%) were positive for high-oncogenic-risk HPV using the HCT

test.[15] Larger studies are needed to accurately define the ability of HPV DNA testing to detect carcinogenic HPV in women with invasive cervical cancer.

In the current study we used a Dacron swab to collect the specimen used for HPV DNA testing. Use of newer sampling devices, such as the new cone brush, may improve the sensitivity of the HPV DNA test.[8,16] Ultrasensitive HPV tests or more extensive sampling of the lower genital tract may also be of benefit. Furthermore, the results of this study may not be applicable to pregnant or immunosuppressed women and women who have received recent treatment of the cervix.

Finally, test results depend first on the skills of the clinicians that obtain test specimens. We assume our sampling skills reflected those expected in the average medical community. Equally important is the expertise of the pathology laboratory to make accurate cytologic and histologic diagnoses. The use of a laboratory that specializes in gynecologic cytology and pathology may have biased the results in favor of greater sensitivity for the Pap smear. Further research is necessary to define the influence of cytology laboratory expertise on triage of women with Pap smear reports of ASCUS and LSIL.

The intention of intermediate triage of women with Pap smear reports of ASCUS or LSIL by HPV DNA testing or repeat Pap smears is to identify the maximum number of women with occult CIN 2/3 and cancer while simultaneously minimizing cost, limiting clinician/patient inconvenience, and avoiding patient discomfort. This study has shown that a HCT test for carcinogenic HPV alone, or in combination with a repeat Pap smear, was as effective as using a repeat Pap smear with an ASCUS threshold in the triage of women with a Pap smear report of ASCUS or LSIL. Because of the demonstrated equivalency to Pap smears, the HCT HPV tests may demonstrate particular value where cytologic resources and expertise are limited. However, both the HCT HPV test and repeat Pap smear had relatively low specificity and positive predictive values for detecting CIN 2/3 and, surprisingly, the combination of the two tests offered relatively little improvement. Therefore, the optimal triage approach to managing women with ASCUS and LSIL Pap smears remains unclear. Until better triage methods become available, examination by colposcopy remains a viable option.

ACKNOWLEDGMENTS

Financial support for this study was provided in part by a grant (22317) from the Robert Wood Johnson Foundation Generalist Physician Faculty Scholars Program and some HPV DNA tests and supplies were provided at no cost by Digene Corporation, Silver Spring, Maryland.

The authors appreciate the professional efforts of Lisa Woodward for data entry, and Alice Randolph and Tracey Barton for manuscript preparation.

REFERENCES

1. Kurman RJ, Henson DE, Herbst AL, Noller KL, Schiffman MH. Interim guidelines for management of abnormal cervical cytology. JAMA 1994; 271:1866-9.
2. Schiffman MH, Bauer HM, Hoover RN, et al. Epidemiologic evidence showing that human papillomavirus infection causes most cervical intraepithelial neoplasia. J Natl Cancer Inst 1993; 85:958-64.
3. Lorincz AT, Reid R, Jenson AB, Greenberg MD, Lancaster W, Kurman RJ. Human papillomavirus infection of the cervix: relative risk associations of 15 common anogenital types. Obstet Gynecol 1992; 79:328-37.
4. Bosch FX, Manos MM, Munoz N, et al. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. J Natl Cancer Inst 1995; 87:796-802.
5. 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; 327:1272-8.
6. Schiffman MH, Kiviat NB, Burk RD, et al. Accuracy and interlaboratory reliability of human papillomavirus DNA testing by hybrid capture. J Clin Microbiol 1995; 33:545-50.
7. Ferenczy A. Viral testing for genital human papillomavirus infections: recent progress and clinical potential. Int J Gynecol Cancer 1995; 5:321-8.
8. Cox JT, Lorincz AT, Schiffman MH, Sherman ME, Cullen A, Kurman RJ. Human papillomavirus testing by hybrid capture appears to be useful in triaging women with a cytologic diagnosis of atypical squamous cells of undetermined significance. Am J Obstet Gynecol 1995; 172:946-54.
9. Hatch KD, Schneider A, Abdel-Nour MW. An evaluation of human papillomavirus testing for intermediate- and high-risk types as triage before colposcopy. Am J Obstet Gynecol 1995; 172:1150-7.
10. Wright TC, Sun XW, Koulos J. Comparison of management algorithms for the evaluation of women with low-grade cytologic abnormalities. Obstet Gynecol 1995; 85:202-10.
11. Ferris DG: Colposcopy. In: Ferris DG, ed. Primary care: clinics in office practice. Office procedures. Philadelphia, Pa: WB Saunders, 1997.
12. Broder S. The Bethesda System for reporting cervical/vaginal cytologic diagnoses—report of the 1991 Bethesda Workshop. JAMA 1992; 267:1892.
13. ASCCP Practice Guideline. Management guidelines for follow-up of atypical squamous cells of undetermined significance (ASCUS). The Colposcopist 1996; 27:1-9.
14. Ferenczy A, Franco E, Arseneau J, Wright TC, Richart RM. Diagnostic performance of hybrid capture human papillomavirus deoxyribonucleic acid assay combined with liquid-based cytologic study. Am J Obstet Gynecol 1996; 175:651-6.
15. Sun X-W, Koulos JP, Felix JC, et al. Human papillomavirus testing in primary cervical screening. Lancet 1995; 346:636
16. Hall S, Lorincz A, Shah F, Sherman ME, Abbas F, Paull G, Kurman RJ, Shah KV. Human papillomavirus DNA detection in cervical specimens by Hybrid Capture: correlation with cytologic and histologic diagnoses of squamous intraepithelial lesions of the cervix. Gynecol Oncol 1996; 62:353-9.