Should All Women with Cervical Atypia Be Referred for Colposcopy: A HARNET* Study

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Background. Clinicians who manage women with Papanicolaou (Pap) smears showing atypical squamous cells of undetermined significance (ASCUS) may miss clinically significant cervical disease by repeating the cytology alone. We evaluated the ability of the human papillomavirus (HPV) screen and the naked-eye examination after a cervical acetic acid wash to enhance the follow-up Pap smear in predicting an abnormal colposcopic biopsy.

Methods. Pap smears were performed on all women (N = 7458) attending six family practice offices for a health maintenance examination from August 1989 through February 1991. Consenting subjects with ASCUS underwent repeat cytological testing, an HPV screen, and a cervical acetic acid wash examination immediately before colposcopy after a 4- to 6-month waiting period.

Results. Of the 122 consenting women identified with ASCUS, 67 (55%) demonstrated abnormalities on biopsy, including 26 with condyloma, 26 with cervical intraepithelial neoplasia I (CIN I), and 15 with CIN II to III. The false-negative rate, 58%, of the follow-up Pap smear alone for detecting these cases of condyloma and CIN was significantly decreased (false-negative rate, 27%) with the use of the cervical acetic acid wash as an adjunctive test. There was no additional reduction in the false-negative rate with the use of the HPV screen. Of the 15 subjects with high-grade cervical lesions (CIN II to III), 14 had either an abnormal follow-up Pap smear or an abnormal cervical acetic acid wash examination.

Conclusions. Among women with cervical atypia, a single follow-up Pap smear alone failed to detect one third of the cases of high-grade disease. Ninety-three percent of these cases were detected, however, with a follow-up Pap smear and an acetic acid wash. Our one subject with a high-grade lesion missed with this combination of tests had an unsatisfactory Pap smear. Use of both tests together may reliably guide clinical decisions regarding the management of cervical atypia.

Key words. Papanicolaou smear; cervical intraepithelial neoplasia; colposcopy; preventive health services; cervix neoplasms; acetic acid. (*J Fam Pract 1994; 38:387-392*)

The optimal management of women whose Papanicolaou (Pap) smears show atypical squamous cells of undetermined significance (ASCUS) remains controversial. Many clinicians consider cervical atypia to be of minimal concern and choose a "wait and see" approach to these patients. Other investigators report a high incidence of significant cervical disease, and therefore recommend colposcopy for all women with ASCUS.[1–5]

We previously reported a high incidence of abnormal colposcopic biopsies among women with ASCUS in the primary care setting.[5] In addition, we determined that the single follow-up Pap smear performed after 4 to 6 months did not accurately predict abnormal colposcopic findings. For this reason, additional tests to augment the Pap smear in the detection of significant cervical lesions are needed. Other potential tests include cervi-

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cography, screening colposcopy, speculoscopy, human papillomavirus (HPV) detection, and the naked-eye examination after a cervical acetic acid wash.[6–12]

Cervicography and screening colposcopy incur additional costs and may require further training or referral to a specialist. Speculoscopy consists of a low-power magnified examination of the cervix with a chemiluminescent light. Detection of significant cervical disease has been reported using this technique.[12] Speculoscopy has not, however, been assessed as a routine screening tool in the primary care setting. Improved detection of cervical disease by HPV screening has been demonstrated in the primary care setting.[13] In addition, the cervical acetic acid wash has proved to be an acceptable and effective adjunct to the Pap smear.[11]

In this study, we determined the usefulness of a human papillomavirus screen and naked-eye examination following a cervical acetic acid wash, alone or in combination, to augment the follow-up Pap smear in predicting an abnormal colposcopic examination in women with ASCUS.

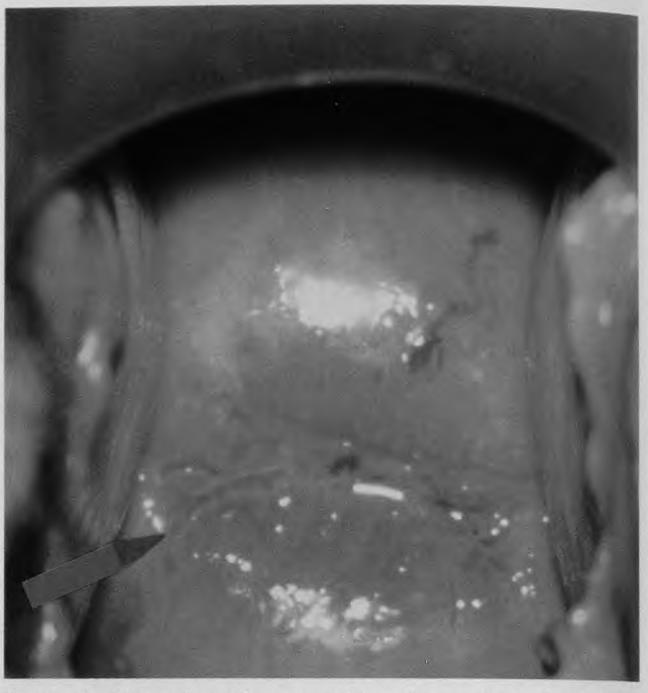

Figure 1. Normal cervix seen with colposcope at low power (×9). Arrow indicates thin white line between columnar squamous cell epithelium (squamocolumnar junction).

Methods

The Harrisburg Area Research Network (HARNET) consists of six practices in the Harrisburg, Pennsylvania, metropolitan area. Two practices are training sites for a family practice residency program. The remaining four are private practices. HARNET's patient population is drawn from urban, suburban, and semirural areas.

All women (N = 7458) who presented for a health maintenance examination in HARNET offices between August 1989 and February 1991 and underwent cervical disease screening with both a Pap smear and naked-eye examination after a cervical acetic acid wash were eligible for entry into the study. Details of patient enrollment, clinician training and certification, and sample collection and processing were reported previously.[5,11]

Women whose initial Pap smears showed squamous intraepithelial lesions (SIL) underwent immediate colposcopy. Consenting subjects who had had a Pap smear revealing ASCUS underwent colposcopy after a 4- to 6-month waiting period. Subjects with koilocytotic atypia were reclassified as having low-grade SIL in accordance with the Bethesda System for cytological reporting. All suspected infections were appropriately treated.

Before colposcopy, a repeat Pap smear, HPV screen, and cervical acetic acid wash examination were performed on all consenting women with cervical atypia. The HPV screen was obtained by sampling the endocervical canal with a Dacron applicator and placing the specimen in the transportation medium of the ViraPap kit (Life Technologies, Inc, Gaithersburg, Md). The HPV DNA hybridization test was performed at SmithKline Bio-Science Laboratory in Philadelphia. This test is based on the specific binding of complementary labeled RNA probes to target HPV DNA. DNA can then be hybridized to radiolabeled probes that are specific for HPV types 6, 11, 16, 18, 31, 33, and 35. Five percent acetic acid was then applied to each subject's cervix with a large cotton swab and left for 1 minute, after which the cervix was examined with a 100-watt light source. Acetowhite areas detected beyond the transformation zone were considered abnormal (Figures 1 and 2).

Colposcopy and directed biopsies were performed by physicians with appropriate training and certification. Endocervical curettage was performed on all subjects. The vaginal side walls and vulvar areas were also examined and biopsied when indicated. Colposcopic biopsies were reviewed by board-certified pathologists who were not aware of the research protocol.

To evaluate the ability of the cervical acetic acid wash and the HPV screen to augment the Pap smear, a series of component tests were created. The results of a component test, which is any combination of the individual tests, are considered abnormal if either one of the individual tests is abnormal, and normal if all the individual tests are normal. The sensitivity, specificity, and negative and positive predictive values were estimated for each of the component tests. Because the predictive val-

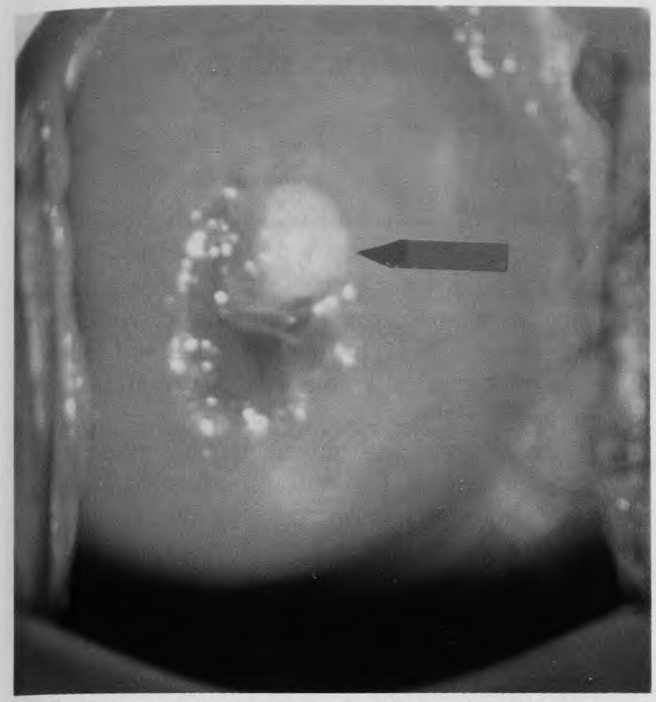

Figure 2. Cervix after 1-minute application of 5% acetic acid. Note the acetowhite area indicated by arrow. Colposcopically directed biopsies of the site revealed moderate dysplasia (CIN II).

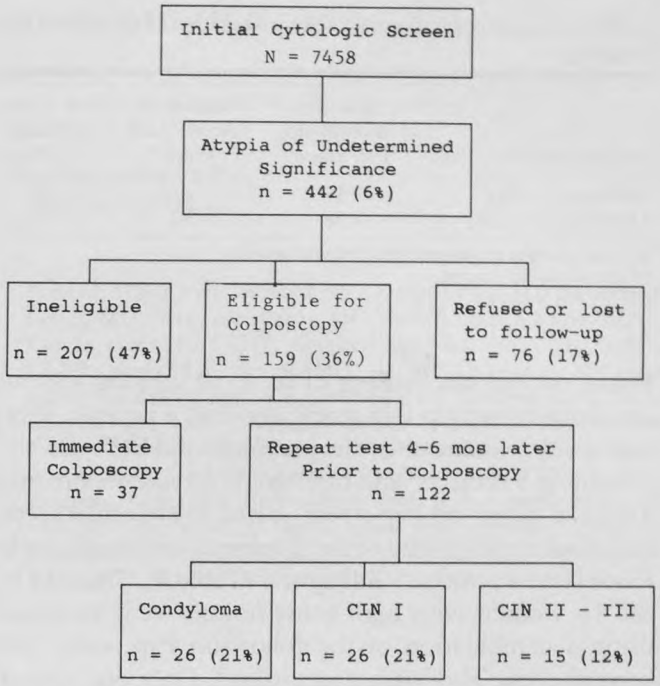

Figure 3. Diagram of research architecture for sample of 7458 women.

ues are a function of the population disease prevalence, it should be noted that the predictive values reported are based on the observed disease prevalence. The lower bounds of 95% one-sided confidence limits for sensitivity and specificity were calculated based on exact methods. The lower bounds of 95% one-sided confidence limits for the negative and positive predictive values were calculated based on large-sample variance estimates using the delta method (JR Landis. The analysis of categorical data: applications to the biomedical and health sciences. Unpublished data, 1994.).

Results

The mean age of the subjects who underwent colposcopy was 25 years (range 15 to 45). Of the 7458 women who had initial cytological screening, 442 (6%) had a Pap smear demonstrating atypia of undetermined significance (Figure 3). Of these, 207 were ineligible for participation in the research protocol. One hundred four of the ineligible women were over 45 years of age, 54 had a history of cryotherapy, 41 had a history of either atypia or SIL, and 8 were pregnant. Seventy-six women refused colposcopy or were lost to follow-up. Subjects accepting and refusing colposcopy were compared. There were no statistically significant differences between these groups with respect to age or race.

The remaining 159 subjects agreed to colposcopy. Of these, 37 women requested colposcopy without further delay. Results on these women are reported elsewhere.[5] The 122 remaining women agreed to wait 4 to 6 months for a follow-up Pap smear, ViraPap screen, and repeat naked-eye examination after a cervical acetic acid wash. Abnormalities were detected on colposcopic biopsy in 67 (55%) of these women, including 26 with condyloma, 26 with CIN I, and 15 with CIN II to III. Complete data were available for 121 subjects and are reported in Table 1. Sensitivity, specificity and positive and negative predictive values with their associated lower bounds of 95% one-sided confidence limits for each test alone and in combination are reported in Table 2.

Prediction of abnormal findings on colposcopy was improved significantly by augmenting the follow-up Pap smear with the cervical acetic acid wash. While the Pap smear alone identified 42% of the abnormalities found on colposcopy, both tests together found 73%. In contrast, the Pap smear was minimally augmented by the addition of the ViraPap (42% vs 44%). The use of all three tests together was no better at predicting an abnormal colposcopic biopsy than was a combination of only the follow-up Pap smear and cervical acetic acid wash.

Of 15 women with high-grade lesions (CIN II to III) found by colposcopic biopsy, 10 had an abnormal

Table 1. Colposcopic Results Categorized by Diagnostic Test Findings

Colposcopy (N = 121)	Abnormal Follow-up Pap Smear*	Abnormal Acetic Acid Wash†	Positive ViraPap‡
Normal (n = 55)	17	17	1
Abnormal (n = 66)	28	32	7

**Presence of atypia or squamous intraepithelial lesions.*
†Acetowhite lesions detected.
‡HPV DNA detected.

follow-up Pap smear. Four of these 10 subjects with an abnormal follow-up Pap smear also had a positive ViraPap result, confirming the presence of HPV. Of the remaining 5 subjects with high-grade cervical lesions and a normal follow-up Pap smear, 4 had an abnormal acetic acid wash result. None of the 5 subjects with high-grade lesions had a positive ViraPap test (Table 3). Thus, 14 of the 15 women with high-grade lesions were identified using a combination of the follow-up Pap smear and cervical acetic acid wash examination. Only one subject with a high-grade lesion had negative results on all three tests. The follow-up Pap smear obtained on this subject was unsatisfactory, limited by hyperkeratosis and a heavy candidal infestation.

Forty-three (35%) of the 122 women with cervical atypia had both a normal follow-up Pap smear and a normal acetic acid wash examination. Abnormalities were detected on colposcopic biopsy in 18 (42%) of these women, including 17 with condyloma or CIN I and one with CIN II to III. The negative predictive value of this combination of tests for a high-grade lesion (CIN II to III) among 122 women with ASCUS was 0.98.

We further analyzed the ability of the initial cervical acetic acid wash examination performed at the time of the original health maintenance visit to predict an abnormal colposcopically directed biopsy result for women found to have cervical atypia. This analysis showed no additional improvement in the ability to detect a subsequently abnormal colposcopic examination.

Discussion

The majority of women identified with ASCUS were found on colposcopic biopsy to have relatively benign lesions. Although controversy exists regarding the management of women with low-grade cervical lesions (condyloma or CIN I), further treatment and follow-up for high-grade cervical lesions (CIN II to III) is widely recommended.[14–19]

When analyzed with respect to high-grade lesions, the single follow-up Pap smear performed at 4 to 6 months failed to detect one third of the lesions found after colposcopic biopsy. Ninety-three percent were detected, however, with a follow-up Pap smear and an acetic acid wash. The one subject with a high-grade lesion missed by this combination of tests had an unsatisfactory Pap smear.

Fifty-five percent of the women in this study for whom screening Papanicolaou smear showed atypia of undetermined significance were found after colposcopy to have condyloma or CIN. The false-negative rate of the single follow-up Pap smear was 58%. Adding a 1-minute 5% acetic acid wash followed by a naked-eye examination of the cervix decreased the false-negative rate to 27%. Therefore, 73% of all lesions were identified by this combination of tests. The addition of the ViraPap as an adjunctive test had no additional effect.

The negative predictive value of the follow-up Pap smear and cervical acetic wash together is disappointingly low when identifying all grades of lesions (58%). The combination of the two tests may be more useful for correctly predicting the absence of disease when screening populations with a lower prevalence of disease.

The inability of the ViraPap to accurately detect the

Table 2. Single or Combination Test Reliability for Detecting CIN or Condyloma in a Population of Women with Cervical Atypia

Screening Test	Sensitivity LB (95% CI)	Specificity LB (95% CI)	Negative Predictive Value LB (95% CI)	Positive Predictive Value LB (95% CI)
Pap smear*	.42 (.32)	.69 (.57)	.50 (.38)	.63 (.53)
Acetic acid wash†	.48 (.38)	.69 (.57)	.52 (.42)	.66 (.57)
ViraPap	.11 (.05)	.98 (.92)	.47 (.17)	.88 (.69)
Pap smear or acetic acid wash	.73 (.62)	.45 (.34)	.58 (.53)	.62 (.56)
Pap smear or ViraPap	.44 (.34)	.67 (.55)	.50 (.39)	.62 (.53)
Pap smear or ViraPap or acetic acid wash	.73 (.62)	.45 (.34)	.58 (.53)	.62 (.56)

**Follow-up Pap smear performed at 4- to 6-month follow-up visit.*
†Cervical acetic acid wash examination performed at 4- to 6-month follow-up visit.
CIN denotes cervical intraepithelial neoplasia; LB denotes lower bounds of 95% one-sided confidence limit.

Table 3. Results of Diagnostic Tests for 15 Women Found to Have High-Grade Cervical Lesions (CIN II–III)

Single test	Follow-up Pap smear abnormal in 10 of 15	Acetic acid wash abnormal in 9 of 15	ViraPap positive in 4 of 15
Combination	Pap smear and/or acetic acid wash abnormal in 14 of 15	Pap smear and/or ViraPap abnormal in 10 of 15	

majority of disease may be related to our method of obtaining the sample. Other investigators studying the primary care population have reported a higher sensitivity.[13]

Evaluation of additional screening methods such as speculoscopy and cervicography may improve reliability for identifying CIN in women with cervical atypia. Randomized, controlled trials that compare these different techniques for both cost-effectiveness and prevention of cervical cancer are necessary.

Clinicians choosing not to recommend colposcopy for their patients with ASCUS should consider follow-up examinations that include both the Pap smear and cervical acetic wash to avoid missing high-grade lesions. By limiting colposcopy to patients with either an abnormal follow-up Pap smear or an abnormal acetic acid wash, over one third of the women in our study would not have required a colposcopic examination.

We currently offer a colposcopic examination to all women identified with ASCUS. For those choosing to defer colposcopy, we repeat both the Pap smear and acetic acid wash examination after a 4- to 6-month interval. Colposcopy is recommended after an abnormal result of either test. If both tests remain normal for 1 to 2 years, annual follow-up is recommended. This management scheme minimizes unnecessary colposcopy while ensuring identification of high-grade lesions.

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