Are Papanicolaou Smears Enough? Acetic Acid Washes of the Cervix as Adjunctive Therapy: A HARNET Study

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Background. The Papanicolaou smear has a false-negative rate ranging from 10% to 50%. Adjunctive screening methods for detecting cervical disease are thus of interest. We studied an adjunctive acetic acid wash of the cervix to detect additional cases of cervical disease not found by the Papanicolaou smear.
Methods. All women attending six family practice offices for health maintenance during the period August 1989 through April 1990 were examined (N = 2827). Papanicolaou smears were obtained using a Cytobrush and wooden spatula. Each subject's cervix was also visually examined 1 minute after application of 5% acetic acid. Women with abnormal Papanicolaou smear results or abnormal acetowhite areas on visual inspection of the cervix underwent colposcopy.
Results. Ninety-three cases of biopsy-proven condyloma or cervical intraepithelial neoplasia (CIN) were found on the basis of abnormal Papanicolaou smear results alone, 33 on the basis of abnormal acetic acid wash results alone, and 14 on the basis of abnormal results from both a Papanicolaou smear and an acetic acid wash. The prevalence of CIN was 3%. The overall positive predictive value for abnormal results obtained by acetic acid wash was .55 (95% CI = .43 to .63).
Conclusions. Using a 1-minute 5% acetic acid wash improves the detection of cervical disease by 30%. Consideration should be given to augmenting the Papanicolaou smear with this safe, simple, and effective technique on premenopausal women during regular health maintenance examinations.
Key words. Acetic acids; Papanicolaou smears; cervix diseases; vaginal smear. *J Fam Pract 1992; 35:271-277.*

Routine screening for cervical disease with the Papanicolaou smear significantly reduces the incidence of invasive cervical cancer.[1,2] False-negative rates are reported, however, to range from 10% to 50%.[3–7] In addition, new cases of cervical cancer are predicted to sharply increase, especially among women who are now younger than 50 years old.[8] Concern, therefore, of failing to detect disease has increased interest in adjunctive screening methods.

Cervicography, human papillomavirus deoxyribonucleic acid (DNA) detection, and screening colposcopy have been proposed as methods to augment the detection of cervical disease.[9–11] Increased costs incurred by the use of these techniques may, however, prohibit widespread acceptance. In addition, no randomized controlled trials have demonstrated efficacy in the primary care setting.

An additional technique reported by Ottaviano and LaTorre[12] evaluated the use of an acetic acid wash in the detection of cervical disease. Findings from visual examination of the cervix following a 3% acetic acid wash were compared with those from colposcopy in 2400 women. Of the 312 women with an abnormal wash, 46% were confirmed to have abnormal cervical biopsies. Results obtained by Papanicolaou smear were not reported on any of these women.

Ficsor et al[13] found that 21% of the women reporting to their health clinic had acetowhite areas of the cervix on visual examination after application of acetic acid. Abnormal Papanicolaou smear findings were 6.6 times more likely to come from these women. Comparison between visual examination and colposcopic evaluation was not reported.

Neither study evaluated the cervical acetic acid wash as an adjunct to the Papanicolaou smear. The purpose of our study was to determine whether the use of the two procedures together would identify more cases of cervical disease than the Papanicolaou smear alone.

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The Harrisburg Area Research Network (HARNET) consists of six practices in the Harrisburg metropolitan area. See complete listing at end of article.

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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 includes persons living in urban, suburban, and semirural areas.

All women (N = 2827) having Papanicolaou smears in HARNET offices from August 1989 through April 1990 were eligible for entry into the study. Exclusion criteria included pregnancy, history of squamous intraepithelial lesions (SIL) or invasive cervical cancer, age over 45 years, and prior treatment of the cervix, including cryotherapy, laser vaporization, or cone biopsy.

A Papanicolaou smear was obtained from each subject by sampling the endocervix with a Cytobrush and scraping the ectocervix with a wooden spatula. Slides made from these preparations were immediately fixed with ethanol. Cytology was performed by a qualified cytotechnologist, and all smears found to be abnormal were reviewed by a board-certified pathologist at Smith-Kline Bio-Science laboratory (Philadelphia) or at Harrisburg Hospital. Cytology laboratory personnel and the pathologists were not aware of the study being conducted.

Five percent acetic acid was next applied to each subject's cervix with a large cotton swab and left for 1 minute. The cervix was then examined with a 100-watt light source. Acetowhite areas detected outside the transformation zone were considered abnormal.

All clinicians participating in the study received standard instruction on the identification of abnormal results of acetic acid washes. This training included observation of photographs demonstrating normal and abnormal cervices (Figures 1 to 3). No specific instruction in colposcopic technique was given.

Women with Papanicolaou smears showing SIL underwent immediate colposcopy. Consenting subjects with abnormal acetowhite areas detected on visual examination who had Papanicolaou smears reported as either atypical, inflammatory, or negative underwent colposcopy after a 4- to 6-month waiting period. Subjects requesting immediate colposcopy were analyzed separately. All suspected infections were appropriately treated.

After acetic acid application and immediately before colposcopy, a visual examination was repeated. The colposcopist was blind to what area of the cervix was abnormally acetowhite following the first acetic acid wash.

Colposcopy and directed biopsies were performed by physicians with training and certification in performing colposcopic techniques. Endocervical currettage was performed on all subjects. The vaginal side walls and vulvar areas were also examined and biopsied when indicated. Selected photographs were taken for documentation by means of an Olympus OM1 camera adapted for the colposcope. Colposcopic biopsies were reviewed by board-certified pathologists at Harrisburg Hospital who were not informed of the research protocol.

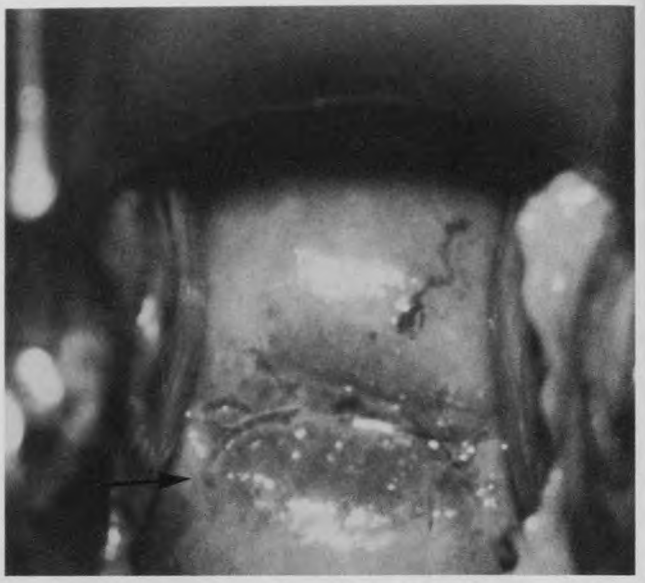

Figure 1. Normal cervix seen with the colposcope at low power (9×). Arrow indicates the thin white line between the columnar and squamous cell epithelium (squamocolumnar junction).

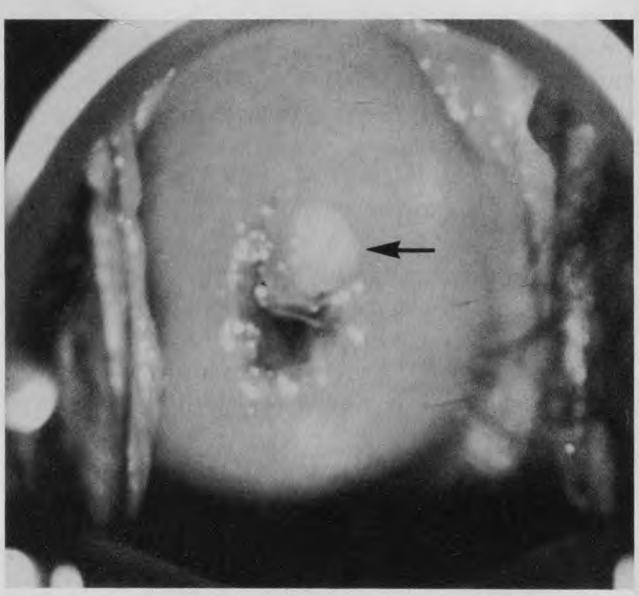

Figure 2. Cervix after 1-minute application of 5% acetic acid. Note the acetowhite area indicated by arrow. The Papanicolaou smear was normal, but colposcopically directed biopsies of the site revealed moderate dysplasia (CIN II).

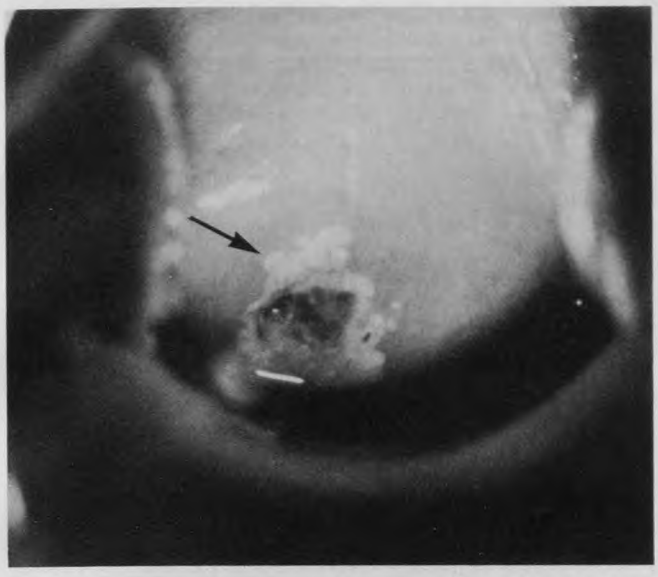

Figure 3. Cervix after 1-minute application of 5% acetic acid. Arrow indicates one of several areas of acetowhite staining around the cervical os. The Papanicolaou smear was normal, but colposcopically directed biopsies of the site revealed mild dysplasia/condyloma.

Predictive values and their associated confidence intervals were calculated using standard techniques.

Results

The mean age of the women was 25 years (range 15 to 45 years). Of the 2827 women screened, 358 (13%) were found to have an abnormal result on the acetic acid wash or the Papanicolaou smear or both (Figure 4). Of these, 74 were ineligible and did not undergo colposcopy. Forty-seven of the ineligible women were over 45 years of age, 20 had a history of cryotherapy, and 7 were pregnant. Sixty-three eligible subjects refused colposcopy. Of these, 25 had abnormal results only on acetic acid wash, 3 had abnormal results on both acetic acid wash and Papanicolaou smear, and 35 had abnormal Papanicolaou smear results only. Subjects accepting and refusing colposcopy were compared. There were no statistically significant differences between these groups with respect to age, ethnicity, or history of cervical disease.

The remaining subjects were eligible and participated in the study. Results of colposcopy for the three groups of these subjects are reported below and summarized in Table 1.

Group I: Abnormal Acetic Acid Wash Only

Sixty-three eligible women with an abnormal acetic acid wash and either an inflammatory or negative Papanicolaou smear result agreed to undergo colposcopy. Abnormalities were found on biopsy in 33 (52%) of the women, including 15 with condyloma, 14 with cervical intraepithelial neoplasia (CIN) I, and 4 with CIN II to III. Eleven of the 63 subjects requested immediate colposcopy after abnormal results were obtained on the initial acetic acid wash. Of these, seven (64%) had abnormal colposcopic findings (Group Ia).

Of the remaining 52 subjects who had a second wash after 4 to 6 months, 30 had persistently abnormal

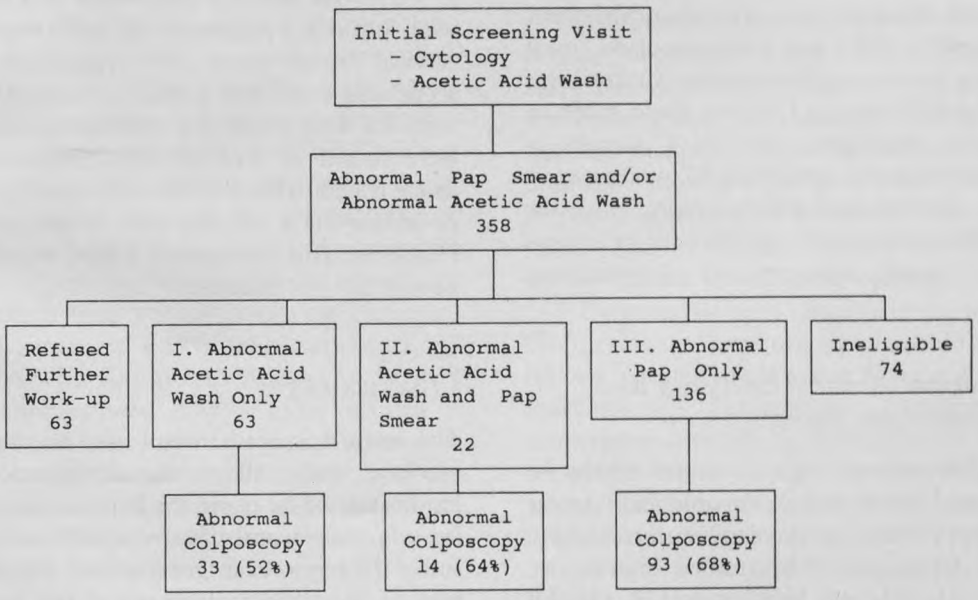

Figure 4. Diagram of group assignment within the sample of 2827 women.

Table 1. Women with Abnormal Colposcopic Biopsy, Grouped by Screening Test

Screening Test Group	Women with Abnormal Biopsy* No. (%)
Group I. Abnormal acetic acid wash only	
a. Immediate colposcopy (n = 11)	7 (64)
b. Colposcopy in 4–6 months, second wash abnormal (n = 30)	19 (63)
c. Colposcopy in 4–6 months, second wash normal (n = 22)	7 (32)
Total (n = 63)	33 (52)
Group II. Abnormal acetic acid wash and abnormal Pap (n = 22)	14 (64)
Group III. Abnormal Pap only (n = 136)	93 (68)
Total (I, II, III) (n = 221)	140 (63)

**Abnormal indicates the presence of condylomatous changes or cervical intraepithelial neoplasia.*

wash results. Nineteen (63%) of these 30 had abnormal colposcopic findings (Group Ib). Twenty-two women had a normal second acetic acid wash result after a 4- to 6-month waiting period. Of these, seven (32%) had abnormal colposcopic findings (Group Ic).

We wished to examine whether clinicians improved in their ability to detect abnormal areas as the study progressed. Forty-five women were evaluated in the first 6 months of the study. Of these, 21 (47%) had abnormal colposcopic findings. This was compared with the remaining 18 subjects who were evaluated 6 months after introduction of this technique in the study setting. Of these, 12 (67%) had abnormal colposcopic findings.

Of the 63 consenting subjects with abnormal acetic acid wash findings, 6 (10%) had a Papanicolaou smear showing moderate to severe inflammation. Colposcopic findings were abnormal in 2 (33%) of these subjects. Both demonstrated condyloma or CIN I. Results of Papanicolaou smears on the remaining 57 women were either normal or showed mild inflammation. Thus, the rest of the abnormal colposcopic results were in women with normal or mildly inflammatory Papanicolaou smears.

Group II: Abnormal Acetic Acid Wash and Abnormal Papanicolaou Smear

Twenty-two eligible subjects with abnormal results on both an acetic acid wash and a Papanicolaou smear agreed to colposcopy. The Papanicolaou smears of these women showed atypia of undetermined significance (12), low-grade SIL (7), and high-grade SIL (3). Of these, findings in 14 (64%) were abnormal on biopsy

Table 2. Colposcopically Directed Biopsies for All Women with a Positive Acetic Acid Wash (n = 85)

Biopsy Results	Negative Papanicolaou Smear*	Abnormal Papanicolaou Smear†
Normal	30	8
Condyloma, CIN I	29	9
CIN II–III	4	5
Total	63	22

**Negative Papanicolaou smear indicates that there were no abnormal findings.*
†Abnormal Papanicolaou smear indicates that atypical cellular changes or squamous intraepithelial lesions were detected.
The positive predictive value of acetic acid wash, .55 (95% CI = .43 to .63).

including 3 women with condyloma, 6 with CIN I, and 5 with CIN II to III.

Group III: Abnormal Papanicolaou Smear Only

One hundred thirty-six women with normal results on acetic acid wash and abnormal Papanicolaou smear findings agreed to colposcopy. Papanicolaou smears on these subjects demonstrated atypia of undetermined significance (70), low-grade SIL (44), and high-grade SIL (22). Biopsies on these subjects were abnormal in 93 (68%), including 38 with condyloma, 33 with CIN I, and 22 with CIN II to III.

Overall, 47 of 85 eligible and consenting subjects with abnormal results on acetic acid wash had biopsy-proven abnormalities seen on colposcopy. The positive predictive value of abnormal results on acetic acid wash was therefore .55 (95% CI = .43 to .63) (Table 2). The acetic acid wash was well tolerated by all patients participating in the study.

Fifty-six cases of condyloma and 84 cases of CIN were found in a population of 2827 women screened for cervical disease using the Papanicolaou smear and an acetic acid wash. The prevalence rate of CIN was, therefore, 3%. One hundred seven cases of condyloma or CIN were found in women with abnormal Papanicolaou smear results. Thirty-three additional cases were detected by adding the acetic acid wash to our screening protocol (Table 3). This represented a 30% increase in the detection of cervical disease.

Discussion

The acetic acid wash, when used to augment the Papanicolaou smear, allows the identification of significant lesions missed by using the Papanicolaou smear alone. It is a safe, simple, and effective adjunct to the Papanicolaou smear for cervical cancer screening. Although augmentation of the Papanicolaou smear has been documented with cervicography and DNA probe testing for human

Table 3. Prevalence of Condyloma or Cervical Intraepithelial Neoplasm (CIN) and Method of Detection

	No. of Cases of Condyloma or CIN
Abnormal Pap smear alone	93
Positive acetic acid wash alone	33
Both tests positive	14
Total	140

papillomavirus, increased costs incurred may limit acceptance.[9–11] Widespread use of colposcopy as a screening tool is also expensive and impractical for many clinicians.

Of the women in this study in whom abnormal acetowhite areas of the cervix were found and negative Papanicolaou smear results were obtained, more than 50% had cervical disease. The detection rate of cervical disease was increased among women undergoing colposcopy immediately or after abnormal results were obtained on two consecutive acetic acid washes. These subgroups might represent higher risk populations for two reasons. First, women who considered themselves to be at an increased risk of cervical disease may have refused further delay in management and therefore self-selected inclusion in a high-risk group. Second, waiting the 4 to 6 months may have identified more women with truly abnormal findings. Some truly benign lesions detected on initial examination may have resolved during this time interval. We chose a 4- to 6-month waiting period because of previous studies showing maximal efficacy for the reevaluation of atypical Papanicolaou smear results.[14] As noted in the Results section, subsequent observers more accurately identified truly abnormal areas, indicating an improved expertise with time.

Colposcopy was not performed on all 2827 subjects having Papanicolaou smears for several reasons. First, performing 2827 colposcopies in the private practice setting would be overly time-consuming and prohibitive in cost. Second, referral bias would likely be introduced by including women with normal results on Papanicolaou smears and acetic acid washes who consent to colposcopy. Women who consider themselves to be at a higher risk of cervical disease may be more inclined to participate. Third, and most important, such a large-scale intervention on normal women is not justified, given the goal of studying the acetic acid wash as an adjunct to the Papanicolaou smear. Nevertheless, some women not undergoing colposcopy may have had undetected CIN. It is unlikely, however, that a significant number of cases of CIN were missed. The 84 cases of CIN identified represent a prevalence rate of 3% in our study. This agrees closely with other reported prevalence rates for CIN.[15–20]

A significant percentage of women (20% to 35%) with atypical Papanicolaou smear results have been shown to have undetected CIN.[15,16,21–24] Eighty-three of our subjects with atypia did not undergo colposcopy because of exclusion or refusal. The majority of studies reporting nondetection rates of CIN, however, included all cases of atypia, including koilocytotic atypia. Under the Bethesda System,[25] women with koilocytotic atypia would be reclassified as having SIL. In our study, subjects refusing colposcopy included only those with atypia of undetermined significance. It is therefore unlikely that a significant number of cases of CIN were missed in this manner.

Similarly, 35% of women with Papanicolaou smears showing only moderate to severe inflammation have recently been reported to have undetected CIN on colposcopy.[26] Less than 10% of the subjects in our study with abnormal results obtained on acetic acid washes had moderate to severe inflammation detected by Papanicolaou smear. Thus, the finding of moderate to severe inflammation by Papanicolaou smear would not have predicted the presence of undetected CIN for the majority of the subjects in this study.

It is conceivable that the discovery of additional cases of CIN was related only to performing additional colposcopies, and not to the acetic acid wash results. If this were true, however, the prevalence rate of condyloma or CIN in our population would have exceeded 50%. A prevalence rate this high has never been reported. In addition, the false-negative rate of the Papanicolaou smear under these circumstances would have been over 90%.

A significant number of women with biopsy-proven cervical lesions had negative results on acetic acid wash. Most abnormal cervical lesions are detected in the transformation zone. This area is less visible because of location near or inside the endocervical canal. Additional case findings with the acetic acid wash may be due to the increased detection of abnormal lesions on the cervical "face." This area is more visible to the examiner and may be less suitable for adequate cytologic sampling.

Concern has been raised over the use of colposcopic biopsies as the reference standard for detecting cervical disease, particularly in those women with mildly abnormal findings.[27] Future studies correlating histologic abnormalities with in situ hybridization for human papillomavirus DNA detection in biopsies from abnormal acetowhite epithelium seen on visual examination will be important in delineating the answer to this question.

It is possible that a longer acetic acid wash may improve the accuracy of identifying acetowhite areas on the cervix. No controlled trials have been published com-

paring the yield of cervical disease with varying lengths of acetic acid washes. We believe, however, that a 1-minute interval represents a practical compromise for the busy clinician who is also concerned with patient comfort.

We chose not to perform this study on patients of menopausal age for several reasons. First, colposcopy is more difficult in this population because of migration of the transformation zone into the endocervical canal.[28] Second, menopausal patients with atrophic vaginal changes are more likely to complain of a burning sensation after application of the acetic acid wash.

Most women in whom cervical disease was identified by acetic acid wash had benign lesions (condyloma or CIN I). Controversy exists regarding the management of these patients. Some clinicians elect to treat them immediately, whereas others choose to follow them closely and treat them only if the lesions progress. The recognition of women with these abnormalities is, however, important in both management scenarios.

Although one subject underwent unnecessary colposcopy for each case of condyloma or CIN discovered, only 8% of women in our entire study population underwent the procedure. False-positive results could be further decreased by reserving colposcopy for women with other risk factors for cervical disease or a history of previously abnormal results obtained on acetic acid wash.

In summary, we have shown that using a 1-minute 5% acetic acid wash improves the detection of cervical disease by 30%. Consideration should be given to using this safe, simple, and effective technique along with the Papanicolaou smear on premenopausal women during regular health maintenance examinations. Further studies are necessary to compare the cost and effectiveness of adjunctive screening between the acetic acid wash, cervicography, human papillomavirus testing, and routine colposcopy.

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