Naked-eye Inspection of the Cervix After Acetic Acid Application May Improve the Predictive Value of Negative Cytologic Screening

Lawrence E. Frisch, MD; F. Helen Milner, NP; and Daron G. Ferris, MD
Arcata, California, and Augusta, Georgia

Background. The purpose of this study was to assess the predictive value of naked-eye inspection of the cervix (NIC) after acetic acid application as an adjunct to Papanicolaou (Pap) testing for cervical cancer screening.

Methods. Study subjects were women attending a medical college student health clinic either for cervical cytologic screening (67%) or because of a recent atypical cytologic screening result (33%). All study participants received cytologic screening, cervicography, and NIC.

Results. Of the 95 patients, 71 (75%) had abnormal findings on NIC. Fifty-one patients underwent colposcopy with biopsy, including 48 of the 71 with an abnormal finding on NIC. The results of 40 of the biopsies were abnormal: 36 showed human papillomavirus or low-grade squamous intraepithelial lesions, 3 showed high-grade squamous intraepithelial lesions, and 1 showed invasive cervical cancer. Sixty-five percent (26) of the abnormal biopsy findings occurred in women with normal cytologic test results. NIC and cervicography both were effective in identifying patients with abnormalities, but the combination of NIC followed by cervicography referred fewer women for colposcopy than did a positive result on NIC alone (52% vs 75%). The combination of a negative Pap smear and a negative NIC result had a 91% predictive value for the absence of cervical intraepithelial neoplasia. This was a significant improvement over cytologic screening alone.

Conclusions. In this study, the combination of cytologic screening (Pap smear) and NIC increased the screening yield as compared with a Pap smear alone but with some loss of positive predictive value. NIC significantly improved the predictive value of negative cytologic screening results.

Key words. Cervicography; cervix dysplasia; cervical intraepithelial neoplasia; acetic acid; Bayes' theorem.
(*J Fam Pract 1994; 39:457-460*)

There have been many attempts to increase the sensitivity of cervical cytologic screening.[1-4] One of the most common approaches has been to supplement cytologic screening with a second type of screening test. The theory is that the combined sensitivity would be greater than that of either technique used independently.[5] Proposed adjunctive tests include screening colposcopy,[6] cervicography,[7,8] repeat cervical cytologic evaluation,[2] DNA probe tests for human papillomavirus (HPV),[9] and naked-eye inspection of the cervix (NIC) after application of acetic acid.[10-13] The purpose of this study was to evaluate NIC as a supplement to cytologic screening.

Methods

Patient Population

All subjects were college students who were at least 18 years old and had signed an informed consent document approved by the Human Subjects Committee of the university where the study was conducted. Women presenting for routine cervical cytologic screening and those who

Submitted, revised, June 14, 1994.

From the Student Health Center, Humboldt State University, Arcata, California (L.E.F., F.H.M.), and the Student Health Service and Department of Family Medicine, Medical College of Georgia, Augusta, Georgia (D.E.F.). Requests for reprints should be addressed to Lawrence E. Frisch, MD, Student Health Center, Humboldt State University, Arcata, CA 95521.

© 1994 Appleton & Lange
ISSN 0094-3509

had previously had an atypical cytologic smear were eligible. Each subject received a cytologic evaluation, a cervigram, and an NIC after application of acetic acid. Subjects receiving colposcopy included those with a cytologic abnormality (atypia or cervical intraepithelial neoplasia), positive cervicography, or presumptive vulvovaginal HPV.

Laboratory Methods

Cervical cytologic samples were obtained in a standard manner using a Cytosoft cytology brush (Medical Packaging Corp, Camarillo, Calif) and spatula. NIC was performed by swabbing the cervix twice with a large cotton swab moistened with 5% acetic acid. At least 1 minute was allowed for whitening to occur. An abnormal NIC was recorded if any areas of acetowhite were observed outside the margin of the squamocolumnar junction. Cervicography was performed as previously reported[14,15] in accordance with manufacturer-recommended procedures established during on-site training. After acetic acid application, the cervix was clearly visualized through the cerviscope (camera), and two cervigrams were taken. The film was returned to the manufacturer for processing and then interpreted by a single cervicography-certified gynecological oncologist as follows: *negative* if normal; *atypical* if there was evidence of an acetowhite lesion of doubtful significance either inside or outside the transformation zone or if there was evidence of atypical immature squamous metaplasia; *positive* if there was evidence of a minor- or major-grade lesion or cancer; and *technically defective* if the film was technically uninterpretable.

Statistical Analysis and Definitions

"Disease" was defined as a squamous lesion found through biopsy. Biopsy specimens interpreted as atypia in the absence of evidence of HPV or dysplasia were treated as variants of normal. Patients were considered "free of disease" if their biopsy result was normal, *or* if they had a negative cytologic screening result, negative cervicography findings during the study, *and* (when available) a negative cervical cytologic screening result 6 to 12 months after the study concluded. The positive predictive value (PPV) and negative predictive value (NPV) were calculated for the NIC separately and for several combinations of NIC, cytologic, and cervicographic findings. A kappa statistic was calculated to assess the degree of agreement between NIC and cervicography.[6]

Results

The mean age of the 95 subjects was 24 years, with a range of 18 to 42 years. All but four were nulliparous; 63% were current users of oral contraceptives, and 33% entered the study for follow-up of a recent atypical smear. The results of NIC were abnormal in 71 study subjects, and smears detected cytologic abnormalities in 15, all but one of which were low-grade lesions. The cervigraphic findings in 28 patients were positive, and those in an additional 20 patients were atypical. All patients with positive cervigrams and all patients with a cytologic abnormality received colposcopy. Colposcopy was performed on 52 of the 95 patients, including 48 of the 71 patients with positive NIC results. In most of the cases (40), an abnormal cytologic screening result or positive finding on cervicography was the rationale for colposcopy.

Of 51 biopsies performed (one patient with a colposcopically normal transformation zone did not have a biopsy), the results of 40 were abnormal: 36 showed HPV or low-grade squamous intraepithelial lesions (SIL), 3 showed high-grade SIL, and 1 showed invasive cervical cancer. The patient in whom invasive cervical cancer was found had abnormal findings on all three screening modalities: NIC, cytology, and cervicography. Both the cytology and cervicography reports for this patient indicated a high probability of invasive disease. Twelve of the 55 patients considered free of disease were classified as such because of normal colposcopy results. Of the remaining 43, all had negative results on initial cervicography, cytologic examination, and NIC following acid wash.

The addition of either NIC or cervicography to cytologic screening significantly improved the screening yield. Of the 40 abnormal biopsy results, 26 (65%) were in women with normal cytologic findings. Although most abnormal findings were of low-grade SIL, two of the three high-grade lesions were missed by cytologic testing alone. All three high-grade lesions were detected by NIC, but one of these had negative results on a cervigram. Two patients whose low-grade SIL was detected by NIC also had negative results on cytologic screening and cervicography. Biopsies done on three of four patients with negative NIC findings showed HPV or low-grade SIL. Of these three patients, one had abnormal cytologic results, one a positive cervigram, and one had suspected vulvovaginal HPV. Although both NIC and cervicography were highly effective in increasing the yield of histologic findings of SIL, the agreement between their findings was at best only fair (κ=.23). This lack of agreement was due to the higher frequency of positive results in NIC as compared with cervicography.

The Table reports the PPV and NPV for cytologic testing, NIC, and the combination of cytologic, NIC, and

Table. Positive and Negative Predictive Values for Naked-Eye Inspection of the Cervix, Cytologic Evaluation, and Both Screening Methods for the Detection of Cervical Cancer

Screening Method	Positive Predictive Value, % (95% CI)	Negative Predictive Value, % (95% CI)
Cytology	82 (64–100)	67 (57–77)
Naked-eye inspection of the cervix	52 (40–64)*	88 (75–100)†
Cytology + naked-eye inspection of the cervix (either positive or both negative)	57 (45–69)	91 (80–100)
Cytology + naked-eye inspection of the cervix with cervigram if naked-eye inspection is positive	68 (55–81)	91 (83–99)

NOTE: Disease endpoint is taken to be histologic cervical intraepithelial neoplasia (CIN) or human papillomavirus (HPV).
** If disease endpoint is CIN only (excluding HPV), then the positive predictive value is 23% (95% CI, 13–33).*
† If disease endpoint is CIN only (excluding HPV), then negative predictive value is 96% (95% CI, 88–100).
CI denotes confidence interval.

(only when NIC results were positive) cervicographic findings. Fewer women would have been referred for colposcopy if referral had required positive cervicographic findings in addition to a cytologic evaluation to confirm a positive outcome of NIC (52% vs 75%, $P<.01$, nonindependent samples).

Discussion

The study population had a high prevalence of low-grade squamous lesions, and most enrolled subjects were nulliparous, young, and taking oral contraceptives. Consequently, the findings of this study may not be directly applicable to populations with a differing age distribution or underlying prevalence of abnormality. With this caveat, the study's major finding is a statistically significant difference in NPV between cytologic evaluation alone and cytologic evaluation augmented by NIC (Table). If confirmed by other studies in an unselected population, a high NPV could have importance in screening. By Sackett's rule of "SnNout"[16,17] (when a test has high sensitivity, a negative test rules out disease), a negative NIC result makes CIN much less likely. Although case-finding is the central goal in screening programs, the ability to identify normal subjects is also of great importance in determining the frequency and intensity of screening efforts. The combination of NIC and cytologic screening might be used to essentially rule out SIL in women who have negative results on both tests.

This study offers estimates of the PPV and NPV for NIC alone, and for a combination of cytologic evaluation, NIC, and NIC-contingent cervicography. These estimates must be regarded with some caution because 43 patients in the study did not receive colposcopy and, hence, may have harbored undetected SIL. We would like to have performed colposcopy on all study subjects but were constrained by ethical and financial considerations.

Because we were concerned about bias deriving from undetected SIL in patients who did not undergo colposcopy, we performed a sensitivity analysis on the combination of cytologic, NIC, and (for positive NIC results) cervicographic evaluation, as reported in the Table. To do this, we first looked at the 20 patients with atypical cervigrams. Of these, 12 had biopsies, with a positivity rate of 83% (all HPV). We consequently postulated that 7 of the 8 patients who did not have biopsies were SIL-positive. We then looked at the 19 women with negative results on NIC, cytologic screening, and cervicography who did not receive colposcopies. Only if a minimum of 9 of these had undetected SIL would the 95% confidence limits of the NPV for the combination of cytologic tests, NIC, and NIC-contingent cervicography begin to overlap those of unaugmented cytologic screening. Since it might seem unlikely that such a high proportion of women with multiple negative studies would harbor undetected SIL, this sensitivity analysis argues that the observed high predictive value for negative cytologic screening results and NIC is not solely an artifact of incomplete colposcopic ascertainment. In contrast, only 6 additional positive findings (missed SIL) occurring among the 16 women without colposcopies but with positive NIC would raise the PPV to its maximum of 92% (95% confidence interval, 84 to 100). Hence, our findings for the PPV might appear to be more sensitive to ascertainment bias than are those for NPV.

It is important to recognize a number of additional qualifications to the study's conclusions. First, while the study's main finding is a high NPV for the combination of cytologic screening and NIC, high NPVs are always to be expected when outcome events are rare, as is a positive result on biopsy in most screened populations. Indeed, by Bayes' theorem, the NPV of NIC would be *higher* in a conventional screening population than in this study, in which more than 40% of participants had biopsies showing HPV or SIL. Second, since most study participants (75%) had positive NIC findings, the advantage of high NPV would apply only to the minority of women whose NIC result was negative. Third, all but two cases detected by NIC-augmented screening were low-grade lesions (typically HPV infection), the malignant potential of which may be limited. This study did not have the statis-

tical power to evaluate the performance of NIC in detecting higher grade SIL.

Despite these further limitations, the present study concurs with others in suggesting that NIC has some potential for enhancing the effectiveness of cytologic screening. Adding either cervicography or NIC to cytologic screening increased the SIL yield nearly threefold. Although other authors have reported improved screening sensitivity with the use of NIC, our data in this population suggest that NIC may also significantly augment the ability of screening to identify *normal* women. In a cost-conscious era, this may be a particularly worthy goal. Cytologic screening that is not augmented with adjunctive testing (such as NIC, with cervicography for patients in whom the NIC result is positive) relies heavily on repeat screening. In a transient population, such as that of a college health clinic, the required follow-up may be difficult to achieve[18] and, in our experience, nearly impossible to document.

References

1. Koss LG. The Papanicolaou test for cervical cancer detection, a triumph and a tragedy. JAMA 1989; 261:737–43.
2. Frisch LE, Parmar H, Buckley LD, Chalem SA. Improving the sensitivity of cervical cytology: a comparison of duplicate smears and colposcopic examination of patients with cytological inflammatory epithelial changes. Acta Cytol 1990; 34:136–9.
3. Frisch LE. Inflammatory atypia and the false negative smear in CIN. Acta Cytol 1987; 31:873–7.
4. Ferris DG. Cervicography—an adjunct to Papanicolaou screening. Am Fam Physician 1994; 50:363–70.
5. Fleiss JL. Statistical methods for rates and proportions. 2nd ed. New York, NY: John Wiley & Sons, 1981.
6. Hocutt JE, Clark RR, Pfenninger JL, Queripel P. Papanicolaou testing and colposcopic screening. J Fam Pract 1992; 34:38–40.
7. Ferris DG, Willner WA, Ho JJ. Colpophotography systems: a review. J Fam Pract 1991; 33:633–9.
8. Greenberg MD, Rutledge LH, Weinberg JH, Campion MJ, Reid R. Cervicography: a useful adjunct for screening. Female Patient 1991; 16:57–68.
9. Reid R, Greenberg MD, Lorincz A, et al. Should cervical cytology be augmented by cervicography or HPV DNA detection? Am J Obstet Gynecol 1991; 164:1461–71.
10. Ottaviano M, La Torre P. Examination of the cervix with the naked eye using acetic acid test. Am J Obstet Gynecol 1982; 143:139–42.
11. Ficsor G, Fuller SK, Jeromin JL, et al. Enhancing cervical cancer detection using nucleic acid hybridization and acetic acid tests. Nurs Pract 1990; 15:26–8.
12. Slawson DC, Bennett JH, Herman JM. Are Papanicolaou smears enough? Acetic acid washes of the cervix as adjunctive therapy: a HARNET study. J Fam Pract 1992; 35:271–7.
13. Van Le L, Broekhuizen FF, Janzer-Steel R, et al. Acetic acid visualization of the cervix to detect cervical dysplasia. Obstet Gynecol 1993; 81:293–5.
14. Ferris DG, Payne P, Frisch LE, Milner FH, diPaola FM, Petry LJ. Cervicography: adjunctive cervical cancer screening by primary care clinicians. J Fam Pract 1993; 37:158–64.
15. Ferris DG, Payne P, Frisch LE. Cervicography: an intermediate triage test for the evaluation of cervical atypia. J Fam Pract 1993; 37:463–8.
16. Sackett DL, Haynes RB, Guyatt GH, Tubwell P. Clinical epidemiology: a basic science for clinical medicine. Boston, Mass: Little, Brown, 1991.
17. Boyko EJ. Ruling out or ruling in disease with the most sensitive or specific diagnostic test: short cut or wrong turn? Med Decis Making 1994; 14:175–9.
18. Frisch L. Effectiveness of a case management protocol in improving follow-up and referral of Papanicolaou smears indicating cervical intraepithelial neoplasia. J Am Coll Health 1986; 35:112–5.