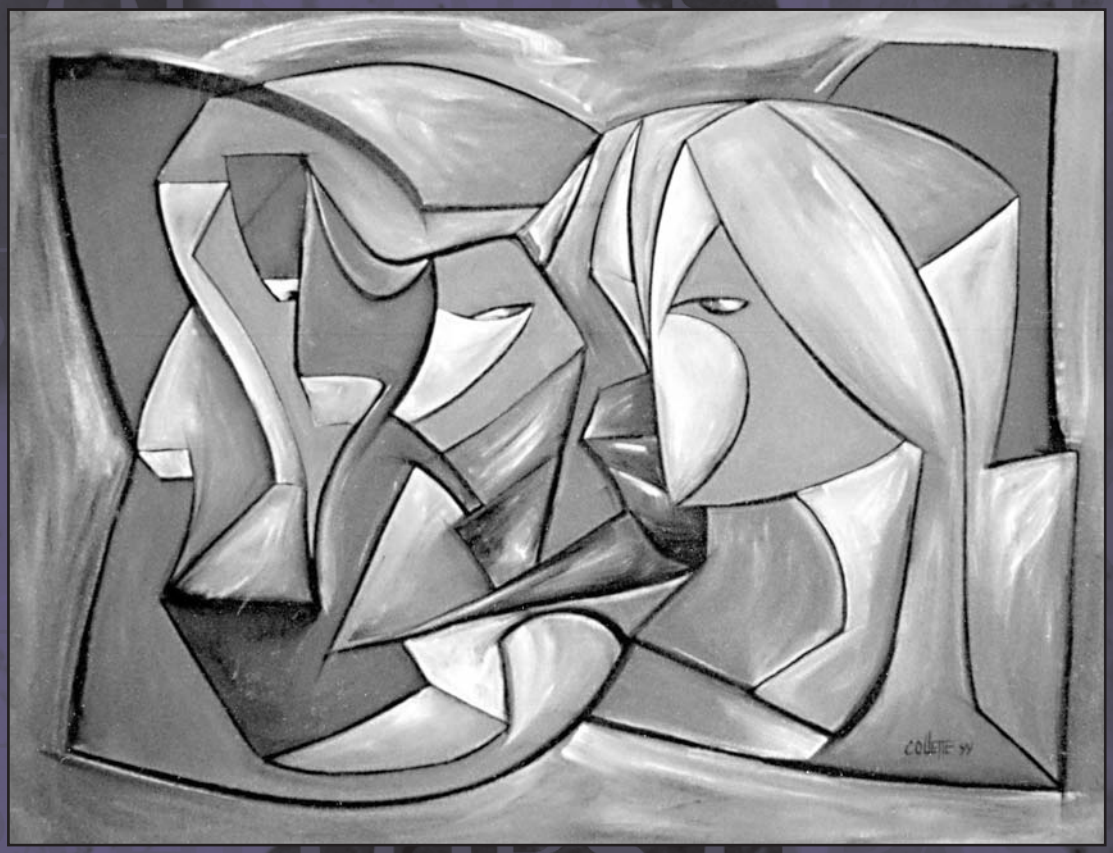
UNIT OF NON-COMMUNICABLE DISEASES

VISUAL INSPECTION OF THE UTERINE CERVIX WITH ACETIC ACID (VIA)

A CRITICAL REVIEW AND SELECTED ARTICLES



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The Noncommunicable Diseases Unit welcomes comments about this material. Please direct all comments to your local PAHO Representative or directly to cronic@paho.org.

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VISUAL INSPECTION OF THE UTERINE CERVIX WITH ACETIC ACID (VIA)

A CRITICAL REVIEW AND SELECTED ARTICLES

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Pan American Sanitary Bureau,
Regional Office of the World Health Organization

PERMISSIONS

1. Denny et al. "Evaluation of alternative methods of cervical cancer screening for resource-poor settings"
Cancer 2000; 89: 826-833
© John Wiley & Sons, Inc.
Jan. 17, 2001

2. University of Zimbabwe/JHPIEGO Cervical Cancer Project
"Visual inspection with acetic acid for cervical-cancer screening: test qualities in primary-care settings"
The Lancet 1999; 353: 869-873
© The Lancet Publishing Group
Dec. 7, 2000

3. Sankaranarayanan et al. "Visual inspection with acetic acid in the early detection of cervical cancer and precursors"
Letter, *Intl. J. of Cancer.* 1999; 80: 161-163
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Sept. 21,2001

4. Sankaranarayanan et al. "Visual inspection of the uterine cervix after the application of acetic acid in the detection of cervical carcinoma and its precursors"
Cancer 1998; 83: 2150-2156
© John Wiley & Sons, Inc
Sept. 11, 2000

5. Megevand et al. "Acetic acid visualization of the cervix: an alternative to cytologic screening"
Obstetrics and Gynecology 1996; 88: 383-386
© Elsevier Science Ltd.
Jan. 31, 2001

6. Cecchini et al. "Testing cervicography and cervicoscopy as screening tests for cervical cancer"
Tumori 1993; 79: 22-25
© Tumori
Nov. 13, 2000

Slawson et al "Are Papanicolaou smears enough? Acetic acid washes of the cervix as adjunctive therapy: a HARNET study"
J. of Family Practice 1992; 35: 271-277
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Dec. 1, 2000

Ottaviano et al. "Examination of the cervix with the naked eye using acetic acid test"
Am. J. of Obstetrics and Gynecology 1982; 143: 139-142
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CONTENTS

LIST OF TABLES ... 4
LIST OF FIGURES ... 4
PREFACE ... 5

PART 1: CRITICAL REVIEW ... 7
- Background ... 8
- Methodology followed and studies selected ... 9
  - Time period, location, purpose of the studies ... 10
  - Sample size and characteristics of study subjects ... 13
  - VIA providers and their training ... 14
  - Characteristics of VIA, other screening tests used ... 16
  - Sequence of tests used for screening and diagnosis ... 19
  - Reference standard ... 19
  - Positivity results of VIA and conventional cytology ... 19
  - Accuracy of VIA ... 21
  - Detection of moderate or worse dysplasia: VIA and conventional cytology ... 23
  - Conclusions of study authors ... 23
  - Discussion and unresolved questions ... 23
- Epilogue ... 25

REFERENCES ... 26
GLOSSARY ... 26
ANNEX: Studies not selected ... 28

PARTE 2: FULL-TEXT ARTICLES ... 29
Denny L, Kuhn L, Pollack A, Wainwright H, Wright T. Evaluation of alternative methods of cervical cancer screening for resource-poor settings. *Cancer* 2000; 89: 826-833. ... 30
University of Zimbabwe / JHPIEGO Cervical Cancer Project. Visual inspection with acetic acid for cervical-cancer screening: test qualities in a primary-care setting. *The Lancet* 1999; 353: (869-873). ... 39
Sankaranarayanan R, Shyamalakumary B, Wesley R, Sreedevi Amma N, Parkin DM, Krishnan Nair M. Visual inspection with acetic acid in the early detection of cervical cancer and precursors. (Letter) *Int J Cancer* 1999; 80: 161-163. ... 47
Sankaranarayanan R, Wesley R, Somanathan N, Dhakad N, Shyamalakumary B, Amma NS, Parkin DM, Nair MK. Visual inspection of the uterine cervix after the application of acetic acid in the detection of cervical carcinoma and its precursors. *Cancer* 1998; 83: 2150-2156. ... 51
Megevand E, Denny L, Dehaeck K, Soeters R, Bloch B. Acetic-acid visualization of the cervix: an alternative to cytologic screening . *Obstet Gynecol* 1996; 88: 383-386. ... 58
Cecchini S, Bonardi R, Mazzotta A, Grazzini G, Iossa A, Clatto S. Testing cervicography and cervicoscopy as screening tests for cervical cancer. *Tumori* 1993; 79: 22-25. ... 62
Slawson D, Bennett J, Herman, J. Are Papanicolaou smears enough? Acetic acid washes of the cervix as adjunctive therapy: a HARNET study. *J Fam Pract* 1992; 35: 271-277. ... 67
Ottaviano M, La Torre P. Examination of the cervix with the naked eye using acetic acid test . *Am J Obstet Gynecol* 1982; 143:139-142.... 75

LIST OF TABLES

1 Articles selected and time period and location of each study, by year of publication ... 10
2 Purpose of the studies included in this review ... 13
3 Sample size and selected other characteristics of the studies and the subjects ... 14
4 Providers of VIA and the training they received ... 15
5a VIA characteristics, other screening tests used ... 16
5b Criteria for positive or abnormal VIA test results ... 16
6 Sequence of actions in the conduct of screening and follow-up ... 17
7 Criteria for application of the reference standard and percent of study group tested with it ... 18
8 Comparison of positivity results, conventional cytology and VIA ... 19
9 Estimated VIA accuracy, sample size, and coverage with the reference standard ... 20
10 Cases of CIN II or worse that were positive to either VIA or the Pap test and were negative to the other ... 21
11 Conclusions of the studies, as stated by the authors ... 22

LIST OF FIGURES

1 Percent of women with abnormal conventional cytology or abnormal VIA, by study ... 20
2 Sensitivity and specificity of VIA for CIN II or higher ... 21

PREFACE

The significantly limited impact of cytology based cervical cancer screening programs in developing countries is now widely recognized. There are several reasons for these limits, ranging from the nature of participation of women in screening programs to the access and timely completion of treatment when necessary. Much of the discussion on how to improve the effectiveness of screening programs has been centered on analysis of the sensitivity and specificity of screening tests. As a result, new potential screening tests are being proposed, among them visual inspection with acetic acid, which is quite appealing for low resource settings.

In this publication we review the available evidence which speaks to the accuracy of visual inspection with acetic acid. The information and findings provided are directed to health professionals and managers of health services as an aid in the decision making process. As with any new technology, there must be a process of evaluation for this screening method. Ideally, evidence from randomized trials would provide the basis for policy changes. In such trials women are randomly assigned to be screened with either VIA or another method, and then both groups are followed up and compared. Currently, at least two studies of this nature are ongoing. In the meantime, evidence from cross-sectional studies seems to point out that VIA may be equally or more sensitive than cytology; this would signify a reduction in the rate of women with false negative results. The overarching question is how much and what level of evidence is necessary in order to incorporate this technology into public health programs.

An important factor for consideration is that the results presented in these papers were obtained under research conditions, with meticulous attention to performance of health care providers and training. In this way, sensitivity and specificity were estimated under the best of circumstances and conditions. By contrast, a 'real life' screening program entails a much more varied and complex set of circumstances. Therefore, the ultimate accuracy of the test must be evaluated under conditions, which are less than ideal; in other words, conditions which fit with the various realities of developing countries. This is important because in the end the feasibility and appropriateness of implementing VIA programs may not depend on the test itself but on the programmatic conditions surrounding the test.

No comprehensive consideration of costs is made throughout the studies contained in this report, but several of the papers assume that the cost must be relatively low since VIA relies on trained human resources and low cost materials, such as vinegar, etc. It should be noted, however, that costs may increase through high referral rates which might be required with this test, through the intensive and continuous training that a subjective technique usually requires, and most importantly, through quality control measures that a program must have.

With this report the Pan American Health Organization, as a member of the Alliance for Cervical Cancer Prevention, intends to encourage and facilitate discussion about this test in developing countries. We hope, especially, that as health professionals consider the evidence in light of their own knowledge of the situation in their respective countries, a more confident assessment of VIA may be made. This document does not constitute a formal recommendation, since important results of new studies will be forthcoming. However, it is important at this preliminary stage to pay attention to the issues of methodology and implementation that merit consideration in the design of public health programs.

PART 1.

CRITICAL REVIEW

BACKGROUND

Visual inspection with acetic acid (VIA), also called cervicoscopy, consists of naked-eye visualization of the uterine cervix (without magnification) after the application of diluted acetic acid, to screen for cervical abnormalities. A solution of 3% to 5% acetic acid is used, and the cervix is illuminated with a light source (See pictures). If low-power magnification is used, the technique is called *VIA with magnification (VIAM)*. The purpose is to identify acetowhite areas, which may indicate tissue undergoing precancerous changes. Either ablation (destroying them) or excision (cutting them out) can then eliminate these acetowhite areas.

Early studies of visual inspection involved simply looking at the cervix (unaided visual inspection, or UVI) to identify and treat pre-cancerous lesions as early as possible, a strategy referred to as downstaging. This was the main tool used before conventional cytology and, together with improved treatment and increased public and professional awareness of cervical cancer, contributed to the decline of cervical cancer deaths in Northern Europe[1]. The major drawback of this approach is that lesions are not detected early enough to prevent invasion, because a large proportion of the cancers detected are relatively advanced, requiring complex medical therapy that is difficult to provide in many settings. By contrast, visual inspection after swabbing the cervix with acetic acid causes pre-cancerous cells to turn white, enabling much earlier detection, and treatment, of pre-cancerous lesions.

Other visual inspection approaches are:

Cervicography, which entails photographing the cervix with a patented, uniquely-designed camera. The photographs, called *cervigrams*, are viewed as projected slides by colposcopists trained in their interpretation.

Speculoscopy, where acetic acid is applied to the cervix, but a chemical, or chemiluminescent, light–source and magnifying lens are used to visualize the acetowhite lesions of the cervix[2].

VILI: Visual Inspection with Lugol Iodine, which involves visualizing the cervix after applying Lugol's iodine to detect lesions. This technique is under evaluation as an independent primary screening test[3].

After conventional cytology became the standard test for cervical cancer screening, increased utilization of the colposcope ensued in order to confirm the findings. Years later, given the expense and inconvenience of colposcopy services, clinicians began to explore whether unmagnified visualization of the cervix, aided by acetic acid, could be used as an adjunct to conventional cytology to identify those patients in need of colposcopy and thereby use resources more efficiently. However, few studies were conducted which examined the value of unmagnified inspection of the cervix after the application of acetic acid for purposes of identifying a normal "transformation zone" or for detecting pre-cancerous lesions of the cervix (i.e., primary screening)[4].

In 1982, Ottaviano and La Torre published the results of a study involving 2,400 women who were examined visually and colposcopically after the application of a cervical wash with acetic acid[5]. A key result was that naked-eye (unmagnified) inspection detected an abnormality in 98.4% of the 312 patients assessed colposcopically as having an abnormal transformation zone. In addition, (unmagnified) visual inspection with acetic acid identified as normal, 98.9% of the 1,584 women diagnosed as normal by colposcopy. That landmark study, as well as others conducted since, is among those included in this document.

Several screening tests are currently available for detecting pre-invasive cervical lesions. The most commonly used method is cytology, which detects cellular changes by directly examining a sample of descamative cells taken from the cervix; this method includes conventional, as well as liquid-based, cytology. Another, more recent, option is to look for the presence of those human papillomavirus (HPV) types associated with cervical cancer in a sample of cells taken from the cervix or the vagina; several tests are available which identify HPV DNA. A third approach is to search visually for a macroscopic lesion on the cervix; VIA, VIAM, cervicography, speculoscopy and VILI are included in this category. A positive result from any of these techniques indicates an increased risk of developing cervical cancer, and a number of diagnostic tests can be used to confirm the presence of disease. Colposcopy is the most commonly used confirmatory exam, followed by histology when lesions are identified.

Most of the scientific discussion on screening for pre-cancerous lesions of the cervix has focused on identifying the technique that will provide the best balance between true and false positives. Efforts have been devoted to evaluating the effectiveness of combining screening tests in order to improve the overall sensitivity attained by the screening program. However, in addition to performing one or more screening tests, the prompt availability of results is at times critical in guaranteeing the effectiveness of a screening program. Of all the above-described screening techniques, only VIA provides instant results, allowing for immediate treatment if necessary.

METHODOLOGY FOLLOWED AND STUDIES SELECTED

Studies pertaining to visual inspection of the cervix, published from 1955 through December 2000, were retrieved by searching the PubMed database available in the U.S. National Library of Medicine. The reference lists provided in the articles retrieved were reviewed as well, and a number of researchers in the field were also contacted.

The OVID version 4.1.0 search and retrieval software was used for accessing the PubMed database. Initially, the search identified all studies with at least one of the following MeSH subject headings (both exploded and limited to a MeSH major topic):

- cervix dysplasia
- cervical intraepithelial neoplasia
- cervix neoplasms, and
- cervix uteri.

Next, the search was narrowed to those publications that also included the MeSH keyword acetic acid in the title, abstract, registry number word, or MeSH subject heading; 67 such articles were finally identified. The search through the reference lists given in the articles retrieved produced another 12 publications, and six more studies were identified at conferences or through contacts with other researchers. Thus, a total of 85 scientific articles or studies were identified. Only 20 of these met our main selection criterion that the article or study must present original data from a clinical study of the accuracy of VIA in detecting pre-invasive cervical lesions.

The following secondary criteria were then applied:

(1) The paper should have been published in a peer-reviewed journal or should be in press.
(2) The study should have included a diagnostic evaluation for all or a sample of VIA-negative, and all VIA-positive, women.
(3) The thresholds used in determining VIA- and PAP-positives should be specified in the paper.
(4) The sample size should have been large enough to have the statistical power to detect a VIA sensitivity of approximately 60%-80%. This implies that, considering various prevalences of CIN, the sample size should have been between 500 and 3,000 for confidence intervals of 80% to 90%.

Seven scientific articles met the above criteria and are included in this document; an eighth, by Ottaviano and La Torre, was selected for inclusion as well because of its landmark status and also because it was conducted in a clinical setting. This was, in fact, the first study conducted on VIA. The list of articles chosen is presented in *Table 1*.

The lack, or insufficient use, of a reference standard was common to the majority of studies identified in the search. Therefore, it proved necessary to omit a selection criterion concerning a reference standard.

It was found that some of the published studies applied a reference standard in which VIA was compared to all methods, some had no reference standard at all, and others used the Pap smear test as a reference standard screening test. In this review, the Pap smear test is considered to yield insufficiently definitive results to allow prudent use as a reference standard.

The list of papers that were considered, but not selected, is included in the *Annex.*

Authors	Reference	Period of study	Location
Denny L., Kuhn L., Pollack A., Wainwright H., Wright T.	Evaluation of alternative methods of cervical cancer screening for resource-poor settings. *Cancer* 2000; 89: 826-833.	January 1996 to September 1997, 19 months.	Khayelitsha, South Africa (squatter settlement outside Cape Town)
University of Zimbabwe / JHPIEGO Cervical Cancer Project	Visual inspection with acetic acid for cervical-cancer screening: test qualities in a primary-care setting. *The Lancet* 1999; 353: 869-873.	Phase I initiated in October 1995, Phase II in August 1997. Number of months not stated	Zimbabwe 15 primary care clinics
Sankaranarayanan R., Shyamalakumary B., Wesley R., Sreedevi Amma N., Parkin D.M., Krishnan Nair M.	Visual inspection with acetic acid in the early detection of cervical cancer and precursors. Letter, *Int J Cancer* 1999; 80: 161-163.	1995-1997, approx. 24 months.	Ernakulam, Kerala, India Early Cancer Detection Center
Sankaranarayanan R., Wesley R., Somanathan N., Dhakad N., Shyamalakumary B., Amma N.S., Parkin D.M., Nair M.K.	Visual inspection of the uterine cervix after the application of acetic acid in the detection of cervical carcinoma and its precursors. *Cancer* 1998; 83: 2150-2156.	1996-1997, approx. 12 months	Southern Kerala, India Cancer detection clinics of Trivandrum
Megevand E., Denny L., Dehaeck K., Soeters R., Bloch B.	Acetic-acid visualization of the cervix: an alternative to cytologic screening. *Obstet Gynecol* 1996; 88: 383-386.	February to September 1994, 7 months.	Cape Town, South Africa (squatter area)
Cecchini S., Bonardi R., Mazzotta A., Grazzini G., Iossa A., Clatto S.	Testing cervicography and cervicoscopy as screening tests for cervical cancer. *Tumori* 1993; 79: 22-25.	May 1991 to January 1992, 8 months.	Florence, Italy Centro per lo Studio e la Prevenzione Oncologica
Slawson D., Bennett J., Herman J.	Are Papanicolaou smears enough? Acetic acid washes of the cervix as adjunctive therapy: a HARNET study. *J Fam Pract* 1992; 35: 271-277.	August 1989 to April 1990, 8 months.	Pennsylvania, USA Harrisburg Area Research Network (HARNET)
Ottaviano M., La Torre P.	Examination of the cervix with the naked eye using acid acetic test. *Am J Obstet Gynecol* 1982; 143: 139-142.	Period not stated	Florence, Italy Florence University

TABLE 1. Articles selected and time period and location of each study, by year of publication

Time period, location, purpose of the studies

Five of the studies selected were conducted in developing countries: two in South Africa, one in Zimbabwe, two in India*. Three other studies were carried out in developed countries: two in Italy, one in the United States of America. None were conducted in Latin America. The first VIA study, by Ottaviano and La Torre, was published in 1982 and conducted in a clinical setting in Italy, while the other seven were published between 1992 and 2000 and primarily correspond to reports of community-based studies.

All the studies were cross-sectional, and aimed to evaluate VIA's ability to detect pre-cancerous lesions as compared to a reference standard. They did not investigate the overall effectiveness of a VIA-based screening program for the purpose of lowering cervical cancer incidence or mortality, which would have required a longitudinal study design.

The authors had various objectives in carrying out a study on VIA (*Table 2*). Broadly, these objectives may be classified as follows:

1. Measuring the accuracy** of VIA in screening for cervical dysp lasia.
2. Comparing the accuracy of VIA to that of other screening tests.
3. Measuring the added accuracy when using VIA as an adjunct to other screening tests.
4. Assessing the concordance of VIA with colposcopy (colposcopy being the reference standard).

* The first of the two Sankaranarayanan studies presented here began in 1995, ended in 1997, and was published in 1999; it will be referred to as "Sankaranarayanan (1995-97)." The second began in 1996, ended in 1997, and was published in 1998; it will be referred to as "Sankaranarayanan (1996-97)."

** See Box in opposite page for definitions.

Measures of data quality: accuracy and precision

Accuracy: A measure of how closely the results of the test correspond to the true state of affairs. It is measured by the calculation of sensitivity and specificity. A test with perfect accuracy will have a sensitivity of 100% (identifies every single case in the population under study) and a specificity of 100% (recognizes every normal subject as such in the population under study).

Precision: A measure of how closely replicate observation of the same thing produces the same results. Measures can be highly precise but inaccurate. Measured by the standard deviation of a series of replicate determinations. The Kappa coefficient, a measure of the degree of nonrandom agreement between observers or measurements of the same categorical variable, is also used. In the conventional cytology reading the degree of dysplasia is highly variable among pathologists,i.e. there is low agreement. However, for distinguishing between cancer and no cancer, conventional cytology is highly precise, i.e. there is a high agreement rate among pathologists.

Effectiveness of screening
Effectiveness: Reduction of incidence or mortality due to the particular disease (cancer). Most studies do not attempt to measure these long-term outcomes. Instead, they use the test sensitivity to evaluate the detection capacity of the screening and the pre-cancerous lesions treated as a proxy for invasive cancers prevented.

Biases in the interpretation of a screening program's effectiveness

Several different biases might cause overestimation of a program's benefits. Although not relevant to the studies under review, for reference purposes they are mentioned below.

1. Lead time bias: Although screening only speeds up diagnosis, it does not necessarily delay death. Upon analyzing the study group, screening appears to increase post-diagnostic lifespan. To measure lead time bias a controlled longitudinal design would be required, with one group screened and another unscreened, both followed until death.
2. Length bias: Asymptomatic cases that are detected by screening have a better prognosis, or more favorable progression of the disease, than those symptomatic cases that result in spontaneous consultation. This improved disease progression and longer life expectancy is then erroneously attributed as a benefit of the screening program. Measurement of this bias would require following asymptomatic cases without treatment, which is ethically unacceptable.
3. Self-selection bias: People who participate in the screenings are usually healthier than those who do not. This can produce an improved outcome which is erroneously attributed to the screening process itself. Measurement of this bias would also require a longitudinal controlled design.

The objectives of six of the eight studies selected fall into the first two categories. Those that aimed to assess the accuracy of VIA as a screening test, such as the University of Zimbabwe / JHPIEGO+ and Cecchini studies, often applied a reference standard (such as histology) to all subjects (i.e., regardless of whether they tested positive or negative to VIA), in order to be able to measure accuracy by means of indicators such as sensitivity, specificity, and positive and negative predictive values++. In these studies, the ultimate objective was to determine whether VIA can act as an exclusive, stand-alone screening method.

In the studies by Denny, Sankaranarayanan, and Megevand, which aimed to compare the accuracy of VIA with that of other screening tests, the reference standard was not applied to all subjects, but only to those who tested positive to any of the initial screening tests. Although in principle all study subjects should be tested with the reference standard, when several tests are used and all subjects having any positive result are then tested by means of a reference standard, the sampling fraction of people receiving the reference test will be higher, thereby decreasing the verification bias ‡.

The study by Slawson aimed to determine the increased accuracy obtained by using VIA in combination with conventional cytology. The main focus was on the number of cases that would have been missed without VIA as an adjunct, rather than on indicators of screening accuracy. For the authors of this study, the question was whether combining conventional cytology with VIA significantly improves the results of a screening approach.

+ The Zimbabwe study had two phases. In the first phase not all subjects received the reference standard, whereas in the second phase included a standard test for all study subjects. In this publication, when analyzing results only the second phase is taken into consideration.
++ See first box in next page for definitions.
‡ Verification bias: results when only those people with a positive screening test result receive the confirmatory diagnostic test. See article in page 67.

Properties of screening tests

Sensitivity is the proportion of truly diseased persons in the screened population who are identified as diseased by the screening test. Sensitivity is a measure of the probability of correctly diagnosing a case, or the probability that any given case will be identified by the test (Syn: true positive rate).

Specificity is the proportion of truly non-diseased persons who are so identified by the screening test. It is a measure of the probability of correctly identifying a non-diseased person with a screening test (Syn: true negative rate).

Positive predictive value is the proportion of people with a positive test who have the disease in question. It is a measure of the probability that a patient with a positive screening result has the disease. The prevalence of a disease in a population, as well as the sensitivity of the test being used when the disease is infrequent easily affects the positive predictive value.

Negative predictive value is the proportion of people with a negative test who do not have the disease, thereby measuring the probability that a patient with a negative screening result does not have the disease.

The above listed relationships are shown in the following table, in which the letters a, b, c, and d represent the quantities specified below the table.

Screening test results	Reference Standard Diseased	Not diseased	Total
Positive	a	b	a+b
Negative	c	d	c+d
Total	a+c	b+d	a+b+c+d

a = Diseased individuals detected by the test (true positives)

b = Non-diseased individuals positive by the test (false positives)

c = iseased individuals not detectable by the test (false negatives)

d = Non-diseased individuals negative by the test (true negatives)

Sensitivity = a / (a+c)
Specificity = d / (b+d)

Positive predictive value (positive test result) = a / (a+b)
Negative predictive value (negative test result) = d / (c+d)

Sources:
Last JM. A dictionary of epidemiology. Third edition, 1995. Oxford University Press. (p. 154)
Gordis L. Epidemiology 1996 W.B. Saunders Company. (pp 55-65)

Reference standard

In order to determine the accuracy of a screening test, it is usually compared with a reference or gold standard - an external source of "truth" that can determine the true disease status of an individual resulting from a more definitive and often more invasive test. Accuracy is therefore described using various indicators that explain this comparison: sensitivity, specificity, positive and negative predictive values. Currently some authors are beginning to use a score that, in one single measurement, simultaneously synthesizes the specificity and sensitivity of a test. The score indicates how close each study is to the ideal of 100% sensitivity and 100% specificity; it is called accuracy score (see Table 9).

Two other factors may be distinguished:

a) Concurrent validity: The ability of a test to detect existing cases. Its measurement requires that a reference test be applied simultaneously to the entire study group. This is the detection capacity of the test to identify those cases that the reference also identifies as cases. The studies reviewed focus on concurrent validity of VIA to detect CIN and cancer.

b) Predictive validity: The ability of a test to predict cases that will occur in the future. In some instances, even the reference standard could be negative at baseline and yet be positive at a later time. This might be the case with HPV or other molecular techniques, which are sometimes compared with morphological techniques such as cytology or histology.

The options for incorporating VIA into a screening and treatment program are:

- As an adjunct to conventional cytology. Both tests are carried out, and a positive VIA result is followed either by colposcopy or by immediate treatment.
- As a complement to conventional cytology. Identification of low grade lesions by conventional cytology is followed by VIA to decide whether to send the woman for colposcopy.
- As the sole screening technique. A positive VIA result is followed by colposcopic examination or immediate treatment.

In all studies, the outcome that determines accuracy is the actual detection of cervical lesions (squamous cell intraepithelial lesions). Detection and treatment of these precancerous lesions serve as a proxy for the reduction of cervical cancer incidence, which ultimately is the true measure of effectiveness for a cervical cancer screening program.

First author	Purpose (as stated in the paper)
General objective: To measure the accuracy of VIA in screening	
U. of Zimbabwe / JHPIEGO, Phases I & II	To assess the specificity and sensitivity of VIA carried out by non-physicians in a primary-care setting. A secondary objective was to compare the qualities of VIA with those of the Pap smear test
Cecchini S.	To evaluate the possible advantages of combining cervicography with vaginal cytology in a screened population. The study also investigated the diagnostic accuracy of direct examination of the cervix after acid lavage.
General objective: To compare the accuracy of VIA relative to other tests	
Denny L.	To evaluate the use of alternative screening methods in a resource-poor setting
Sankaranarayanan R. (1995-97)	To compare the performance of VIA and cervical cytology in detecting cervical lesions in a clinic-based study of women attending for routine examination or because of referral to rule out cervical pathology.
Sankaranarayanan R. (1995-97)	To evaluate the performance of unmagnified, naked-eye visual examination of the uterine cervix after application of 3-4% acetic acid (VIA) in detecting cervical lesions.
Megevand E.	To determine if direct visualization of the cervix after application of acetic acid would be an adequate alternative to cytology in the detection of premalignant lesions of the cervix.
General objective: To measure the added value of VIA as an adjunct to other tests	
Slawson D.	To determine whether acetic acid wash and the cytology smear, if used together, would identify more cases of cervical disease than the cytology smear alone.
General objective: To assess the concordance of VIA and colposcopy	
Ottaviano M.	To draw attention to the importance of naked-eye inspection of the cervix, employing a good light source, after preparing the cervix with a 3% acetic acid solution

TABLE 2. Purpose of the studies included in this review

It should be noted that, in order to obtain the true sensitivity or specificity of a test, it is necessary to apply a reference standard‡ test to all the study subjects, regardless of their screening results. Otherwise, only a relative comparison of the screening tests can be made. Denny and Sankaranarayanan calculated the ratio of the sensitivity of one test to that of another (ratio of the number of true cases detected by each test) and used McNemar's test of statistical significance of the difference between them. Relative comparisons of screening outputs (number of cases detected) are valid and useful in regard to cost-effectiveness analyses, since they provide information that is helpful in choosing which screening tests to implement.

Since most of the studies did not intend to measure the sensitivity and specificity of VIA, estimating accuracy indicators will incur some degree of verification bias.

Sample size and characteristics of study subjects

A combined total of 27,922 women were screened with VIA in the eight studies (*Table 3*). Sample sizes ranged between 2,000 and 3,000 subjects, except for the Sankaranarayanan (1995-97) and University of Zimbabwe / JHPIEGO Phase I studies, with 1,351 and 8,731 subjects respectively. Except for Ottaviano, whose study subjects were patients subjected to both VIA and colposcopy, all other studies were conducted among women who had come for a screening test without reason to suspect disease

‡ See second box in the opposite page for definitions.

FIRST AUTHOR	Sample size	Duration	Age range (years)	Mean age	SOURCE OF STUDY SUBJECTS	SELECTION CRITERIA
1. Denny L.:	2,944	19 months	35-65	39*(a)*	Periurban locality near the city of Cape Town, with a population of 350,000.	Not pregnant, not previously screened for cervical cancer, had not undergone hysterectomy.
2a. U. of Zimbabwe/JHPIEGO Phase I:	8,731	22 months (b)	25-55	32.2*(b)* std dev 6.6 years	Fifteen primary-care clinics in Zimbabwe.	Not pregnant, no previous history of cervical cancer or hysterectomy.
2b. U. of Zimbabwe/JHPIEGO Phase II:	2,203	22 months (b)	25-55	32,2*(b)* std dev 6.6 years	Fifteen primary-care clinics in Zimbabwe.	Not pregnant, no previous history of cervical cancer or hysterectomy.
3. Sankaranarayanan R.: (1995-97)	1,351	Aprox. 24 months	22-70	38.9 std dev 7.3 years	Women who presented themselves to the Center for a routine examination or were referred to rule out cercival pathology.	Not stated.
4. Sankaranarayanan R.: (1996-97)	3,000	Aprox.12 months	20-70+	43.4	Women recruited from among those attending open-access cancer detection clinics as part of community outreach programs.	Not stated.
5. Megevand E.:	2,426	7 months	20-83	31	Participants in a mass screening program offered as a free service in mobile clinics in squatter area.	Residents of the squatter area who had not had a smear taken within the past year.
6. Cecchini S.:	2,105	8 months	17-83	46.3	Sexually active women attending the Pap smear clinic.	Residents of the District of Florence and actively invited to undergo screening.
7. Slawson D.:	2,827	8 months	15-45	25	Six private physician practices in the Harrisburg, Pennsylvania metropolitan area.	Not pregnant, no history of SIL or invasive cervical cancer, no prior treatment of the cervix (including cryotherapy, laser vaporization or cone therapy).
8. Ottaviano M.:	2,400	Not stated	18-65	Not stated	Patients attending Department of Obstetrics and Gynecology at Florence University.	Unselected patients with normal or abnormal cervical cytology.
GRAND TOTAL: 27,922						

a) This is the median age, not the mean. Fifteen percent were 50 years or older.
b) These figures are fro phases I and II combined.

TABLE 3. Sample size and selected other characteristics of the studies and the subjects

Duration of the studies was between seven months and two years. The mean age of patients ranged from 25 to 46.3 years.

The exclusion criteria most commonly applied were:

- previous treatment of pre-cancerous lesions of the cervix;
- pregnancy;
- gross distortion of the cervical anatomy.

VIA providers and their training

In five of the studies VIA was performed by nurses, while in one (Sankaranarayanan 1996-1997) it was performed by cyto-technicians and, in two others (Slawson, Ottaviano), by physicians (*Table 4*). Not all the authors mention how many providers conducted VIA, yet those that do (Sankaranarayanan, Megevand, Cecchini, Ottaviano) indicate that only one or two providers performed all of the VIA exams. In the University of Zimbabwe / JHPIEGO study, six nurses were used (personal communication with one of the authors, LG).

The duration of the training of providers was usually three to six days, however, little or no information was given as to the number of hours of training and clinical practice, the number of patients seen during the training, or the criteria for competency of the trainees.

First author/Number, characteristics of providers of screening tests	Training received
1. Denny L.: 1 trained nurse Nurse conducted gynecological examination, VIA, Cervicography, and obtained a Pap smear and a sample for HPV DNA testing. PAP smears were read at local cytopathology laboratory; not masked. Cervigrams were evaluated in the United States of America, masked. HPV DNA test assayed at local University. Gynecological oncologist conducted colposcopy, biopsy, LEEP and endocervical curettage. All histology exams were evaluated in the United States of America, masked.	Nurses received training on all four testing methods in a four-day course involving clinical examinations and extensive review of photographs of normal and abnormal cervices.
2a. U. of Zimbabwe/JHPIEGO, Phase I: 6 trained nurse-midwives Nurse-midwives obtained a Pap smear and conducted VIA. Pap smears were analyzed by cytotechnicians in Harare, masked. All positive smears and a 10% random sample of negative smears were re-assessed by a local pathologist and also by a cytopathologist in the United States of America. Colposcopy was conducted in Harare by three faculty members, masked. Biopsies were read by the local pathologist, masked. HPV samples were analyzed in the United States of America.	Nurse-midwives received: Refresher training in speculum insertion and PAP collection. Three-day training on VIA, where trainees were familiarized with the naked-eye appearance of the cervix in various states of health and disease. Pictorial atlas was used during training and service delivery. All were assessed as competent. Over five days, the study cytotechnicians took part in a review course.
2b. U. of Zimbabwe/JHPIEGO, Phase II: 6 trained nurse-midwives Same as above, except: • All slides were reviewed, masked, by the local pathologist and by a cytopathologist in the United States of America • All patients received a masked colposcopy in a Harare clinic	Training as above, plus, before initiation of Phase II, a job refresher training of nurses in VIA was held, as well as refresher training of cytotechnicians in reading PAP smears.
3. Sankaranarayanan R. (1995-97): 1 nurse Nurse did a speculum examination, VIA exam and obtained a Pap smear. PAP smears were examined by a cytopathologist. Colposcopy was conducted by the same cytopathologist	The nurse was trained in recognizing cervical abnormalities and acetowhite lesions after acetic acid application.
4. Sankaranarayanan R. (1996-97): 2 cytotechnicians Two cytotechnicians conducted speculum examinations, VIA, and obtained cervical smears. Smears were examined by cytopathologists and cytotechnologists. Providers of colposcopy and biopsies were not mentioned.	Training for speculum examinations was provided by a gynecologist and a pathologist during a two-month period before the studies began, on the following competencies: VIA without magnification, recognition of acetowhite lesions, and identification of macroscopic abnormalities.
5. Megevand E.: 1 nurse Una enfermera adiestrada realizó IVAA y obtuvo frotis para prueba de A trained nurse conducted VIA and obtained a Pap smear. Cervical smears were screened and processed by a masked cytotechnologist. No information is given on providers of colposcopy, LEEP and biopsies conducted in mobile clinic.	Education was provided on-site, under the supervision of a qualified gynecologist trained in oncology and colposcopy, starting one week before the arrival of the mobile clinic.
6. Cecchini S.: 2 "smear-takers" Two "smear-takers," midwives without any training in colposcopy, conducted cervicoscopy (VIA). Cervicography was performed by the "smear-taker." An expert colposcopist, masked to the cytologic report, interpreted cervigrams. No information is given on providers of colposcopy nor on Pap smears.	"Smear -takers" underwent "short" training to recognize acetowhite areas.
7. Slawson D.: Clinicians; number not stated Clinicians (medical practitioners or family practice residents) obtained Pap smear and conducted VIA. A qualified cytotechnologist performed cytology, masked. Smears found to be abnormal were reviewed, masked, by a board-certified pathologist who also reviewed biopsies. Physicians performed colposcopies and biopsies. Colposcopist was unaware of which area of the cervix was abnormally acetowhite in VIA.	All clinicians received "standard instruction" on the identification of abnormal results of acetic acid washes. This included observation of photographs demonstrating normal and abnormal cervices. No specific instruction in colposcopic technique was given. Colposcopy and directed biopsies were performed by physicians with training and certification in colposcopic techniques.
8. Ottaviano M.: 1 postgraduate MD student and 1 colposcopist A postgraduate MD student conducted a naked-eye examination before and after application of acetic acid solution. An experienced colposcopist conducted colposcopic examination before and after application of acetic acid solution. Naked-eye and colposcopic findings, before and after acetic acid application, were comparedimmediately after both persons conducted the examinations.	"Adequately instructed" postgraduate MD student.

TABLE 4. Providers of VIA and the training they received

First author	VIA	Other screening tests used		
	Acetic acid lighting	Pap	Cervico-graphy	HPV
Denny L.:	5% Lighting not described	X	X	X
U. of Zimbabwe/JHPIEGO:	4% Flashlight	X		*(a)*
Sankaranarayanan R.: (1995-97)	3-4% "Adequate" light	X		
Sankaranarayanan R.: (1996-97)	3-4% "Adequate" light	X		
Megevand E.:	5% 100 W	X		
Cecchini S.:	5% 60 w halogen	X	X	
Slawson D.:	5% 100 w	X		
Ottaviano M.:	3% 100 w	*(b)*		

a. Phase II only; findings not presented.
b. The study subjects had had cervical cytology carried out prior to the study, with normal or abnormal results. The cytologic data were available for comparison to VIA and colposcopic findings.

TABLE 5a. VIA characteristics, other screening tests used

Criteria for positive or abnormal VIA test results

Denny L.*: Acetowhite lesions, cervical ulcers or cervical growths. Did not grade the severity of lesions.

U. de Zimbabwe*/JHPIEGO: *Normal*: Smooth, pink, uniform, featureless
Atypical: Cervicitis (inflammation, red spots), discharge, ectropion, polyp, nabothian cyst
Abnormal (positive): White plaques, ulcer, acetowhite epithelium
Cervical cancer (positive): Cauliflower-like growths, fungating mass

Sankaranarayanan R.*: *Positivo:* Any distinct acetowhite area(s) detected on cervix
Abnormalities: Cervicitis, cervical warts, polyps. Nabothian cysts, bleeeding, erosions, stippled cervix, irregular edematous elongated cervix, growths and ulcers
Negative: No acetowhite area, whitish appearance doubtful or faint, polyps took faint acetowhite staining, nabothian follicles (which are whitish before acetic acid application) became more prominent after acetic acid application, grape-like glands or erosions in the endocervix are slightly paler than ectocervix.

Megevand E.*: *Positivo:* Presence of acetowhite areas on the cervix

Cecchini S.*: *Negativo:* No acetowhite areas
Positive: Evidence of acetowhite areas

Slawson D.*: *Positivo (abnormal):* Acetowhite areas detected outside the transformation zone

Ottaviano M.*: *Normal:* Original columnar epithelium; physiologic transformation zone (TZ)
Abnormal or atypical transformation zone: White epithelium; other findings (metaplasia, cervicitis, atrophy, true erosions, polyps)

• Firts author

TABLE 5b. Criteria for positive or abnormal VIA test results

The information given in *Table 4* includes whether or not the health personnel examining the patient knew the results of previous tests. This is particularly important since knowledge of initial VIA results could potentially affect subsequent interpretation of Cervigrams or colposcopic evaluations. Most of the authors report that the complementary screening and diagnostic tests were conducted in a masked fashion with regard to VIA results. Unless independently assessed, serious bias may occur which could undermine the validity of the study.

Characteristics of VIA, other screening tests used

Given that VIA is still under evaluation as a cervical cancer screening tool, while conventional cytology is a widely accepted screening test, the latter was used concurrently with VIA in all the studies considered here. In the study by Denny a third screening test was also used: HPV testing. In Phase II of the University of Zimbabwe / JHPIEGO study, samples were also collected for HPV testing, but the results were not reported in the paper. Two other studies (those by Denny and Cecchini) also incorporated cervicography as a screening test.

When the reference standard screening test was not systematically applied to all study subjects, the use of additional screening tests often played a major role in determining the coverage with the reference standard diagnostic tool (colposcopy with histology in all studies).

The specifics of the application of the VIA method varied across studies, for example with respect to the acetic acid concentration used, which ranged from 3% to5%. None of the authors report how, or whether, acetic acid concentration was controlled during the intervention. Recent studies conducted under field conditions have shown that the acidity of acetic acid solution drops dramatically after exposure to air for one week.

First Author		Sequence of actions
Denny L.:	Day 0	1. Pap smear 2. HPV DNA 3. VIA and VIAM 4. Cervicography
	Days 2-6	5. If positive VIA or HPV → referred for immediate colposcopy
	Day 14	If positive Pap and not previously referred for colposcopy → tracked and referred for colposcopy
	Day 56-76	If positive cervigram and not previously referred for colposcopy → tracked and referred for colposcopy
	Day 2-76	6. If significant grade lesions at colposcopy → lesions electrosurgically excised using loop electrodes If minor grade lesions at colposcopy → biopsy If no visible lesions → endocervical curettage performed
U. of Zimbabwe/JHPIEGO Fase I:	Day 0	1. Pap smear 2. VIA
	Day not specified	3. If VIA showed abnormal result → scheduled for colposcopy and biopsy when indicated Every 10th woman with normal or atypical VIA → scheduled for colposcopy and biopsy when indicated
U. of Zimbabwe/JHPIEGO Fase II :	Day 0	1. Pap smear 2. VIA 3. HPV DNA 4. Colposcopy and biopsy when indicated
Sankaranarayanan R. (1995-97):	Day 0	1. Unaided visual inspection 2. Pap smear 3. VIA
	Day not specified	4. If positive VIA or Pap → referred for colposcopy If grossly abnormal-looking cervix → referred for colposcopy
	Day not specified	5. If abnormal colposcopic findings → performed directed biopsy
Sankaranarayanan R. (1996-97):	Day 0	1. Unaided visual inspection 2. Pap smear 3. VIA
	Day 3-80	4. If positive VIA or Pap → referred for colposcopy If grossly abnormal-looking cervix → referred for colposcopy
	Day 3-80	5. If abnormal colposcopic diagnosis → directed biopsy obtained from acetowhite and suspicious areas If no lesions or features of reparative and reactive changes found on colposcopy → no biopsy
Megevand E.:	Day 0	1. Speculum examination 2. VIA 3. Pap smear
	Day 0-3	4. If positive VIA or Pap → referred for colposcopy 5. If features consistent with HGSIL → patient treated with large loop excision of transformation zone If features consistent with LGSIL → punch biopsy of most abnormal area taken If no abnormal colposcopy → histology exam (not defined, but probably endocervical curettage)
Cecchini S.:	Day 0	1. Papsmear 2. Cervicoscopy (VIA) 3. Cervicography
	Day not specified	4. If abnormal Pap, cervicoscopy or cervicography → colposcopically-guided biopsies of all acetowhite areas
Slawson D.:	Day 0	1. Pap smear 2. VIA
	Day not specified	3. If positive Pap → immediate colposcopy
	Day 120-180	3. If abnormal VIA or atypical, inflammatory, or negative Pap → colposcopy 4. Endocervical curettage performed on all patients with colposcopy Vaginal sidewalls and vulvar areas also examined and biopsied when indicated
Ottaviano M.:.	Day not specified	1. Pap (performed prior to the study)
	Day 0	2. VIA and colposcopic exam performed on all women 3. When appropriate, punch biopsy carried out

TABLE 6. Sequence of actions in the conduct of screening and follow-up

In addition, the type of lighting used was not consistent across studies, nor was it consistently described by the authors. In some studies a halogen light was used, in others a handheld flashlight; in some, a 100-watt light source was used, while in some cases only "adequate" lighting is mentioned (*Table 5a*). Both the acetic acid concentration and the type of lighting could play a role in identifying acetowhite lesions. *Table 5b* complements *Table 5a*, and presents the criteria for a positive test as stated by the authors in their reports. All of the studies mention an acetowhite lesion as a criterion, while only the University of Zimbawe / JHPIEGO and both Sankaranarayanan studies provide additional details. All studies distinguish VIA positive from negative (normal) test results, although the acetowhite lesions are not graded

FIRST AUTHOR	Criteria for application of the reference standard test	Number of patients who received the reference test		Colposcopy coverage of the screened popuation (%)
		Colposcopy	Histology	
Denny L.:	Referred if abnormal VIA, HPV DNA test-positive (RLU>10) or abnormal Pap (LGSIL or worse). All subjects with colposcopy had histology: LEEP, biopsy or endocervical curettage.	760	760 (endocervical, curettage, punch biopsy and LEEP)	25.8
U. of Zimbabwe/ JHPIEGO	**Phase I:** Referred if abnormal VIA or Pap test (LGSIL or worse), along with every tenth woman with normal Pap or atypical VIA. Overall, in Phases I and II, 25% of HGSIL and carcinoma diagnoses were based only on colposcopy.	1,584	(biopsy)*	18.1
U. of Zimbabwe/ JHPIEGO	**Phase II:** All subjects were referred. Overall, in Phases I and II, 25% of HGSIL and carcinoma diagnoses were based only on colposcopy.	2,147	(biopsy)*	97.5
Sankaranarayanan R.:	(1995-97) Referred if positive VIA, abnormal Pap (ASCUS or LGSIL or worse), or grossly abnormal cervix.	601	201 (biopsy)	44.5
Sankaranarayanan R.:	(1996-97) Referred if positive VIA, abnormal Pap (ASCUS or LGSIL or worse), or grossly abnormal cervix.	573	277 (biopsy)	19.1
Megevand E.:	Referred if positive VIA, abnormal Pap (LGSIL or worse). All subjects with colposcopy had histology: LEEP, biopsy or endocervical curettage.	330	330 (endocervical curettage, punch biopsy and LEEP)	13.6
Cecchini S.:	Referred if abnormal cervicoscopy, cervicography or Pap (ASCUS, LGSIL or worse).	486	281 (biopsy)	23.1
Slawson D.:	Referred if positive Pap (LGSIL or worse). Referred in 4-6 months If abnormal VIA or atypical, inflammatory or negative Pap. All subjects with colposcopy had histology: either biopsy or endocervical curettage.	221	221 (endocervical curettage and biopsy)	7.8
Ottaviano M.:	All subjects had colposcopy; biopsy whenever indicated.	2,400	(biopsy) **	100.0

* Although all patients positive on colposcopy had a biopsy, the results for all patients were not available at the time of publication. Among 624 positive final diagnoses of HGSIL or cancer in Phases I and II, 74.8% were confirmed by biopsy.
** A punch biopsy was obtained when appropriate; however, the number of patients is not given.

TABLE 7. Criteria for application of the reference standard and percent of study group tested with it

Sequence of tests used for screening and diagnosis

With the single exception of the Megevand study, the Pap smear was always obtained prior to the application of acetic acid (*Table 6*).

The time interval between VIA and confirmatory tests varied greatly, ranging from zero days to 6 months. It should be noted that, when a confirmatory test is performed months after the initial screening has taken place, it is possible to obtain a result which is discordant with that obtained with the original VIA, because many lesions (particularly, those that are low-grade) may have regressed. (This would have been a false-positive VIA screening test result.) On the other hand, new lesions may also have become evident during the time interval between the tests.

Although VIA provides the opportunity for immediate diagnosis and treatment, only Ottaviano offered immediate diagnoses, and conization was provided to patients with severe dysplasia or carcinoma in situ; the other researchers offered neither immediate diagnosis nor immediate treatment.

Reference standard

Colposcopy was used as a reference standard in all of the studies. Several authors did not conduct any further tests when the colposcopy result was negative; others performed a biopsy (endocervical curettage) for histologic examination, even when the colposcopy result was negative.

Regardless of whether colposcopy was conducted alone or was accompanied by a histologic exam, in order to determine the number of true positives in the study group the reference standard test should ideally have been applied to all patients referred for evaluation. However, with the exception of Phase II of the Zimbabwe study, all the other studies only referred for application of the reference standard, the 10%-50% of patients who had abnormal results in at least one of the screening tests. Therefore, all of the studies, except Zimbabwe Phase II, carry some verification bias [6]. This bias skews the estimated sensitivity and specificity of VIA. However, a correction for this can be made by evaluating a random sample of the screen-negative subjects with the reference standard.

It is also necessary to track how many of the subjects who were intended to receive the reference standard test, actually did. In the present group of studies, between 78% and 100% of the subjects referred for it, received the reference test.

First author	Pap LGSIL and worse		Abnormal VIA	
	No.	%	No.	%
Denny L	238	8.2	534	18.1
U. of Zimbabwe/ I JHPIEGO Phase I	1,218	14.6	1,762	20.2
U. of Zimbabwe/ JHPIEGO Phase II	269	12.6	868	39.8
Sankaranarayanan R. (1995-97)	205	15.2*	509	37.7
Sankaranarayanan R. (1996-97)	307	10.2*	298	9.9
Megevand E.	315	13.0	76	3.1
Cecchini S.	21	1.0	341	25.4
Slawson D.	196	7.1*	113	4.2
Average positivity rate, weighted according to sample size		**11,1**		**18,2**

* ASCUS or worse

TABLE 8. Comparison of positivity results, conventional cytology and VIA

In summary, the proportion of all women studied who actually received the reference standard test depended not only on the criteria used for applying the standard, but also on the rate of compliance with these criteria (*Table 7*). The last column of *Table 7* shows the final figure for coverage with a reference standard test in each of the studies considered; it varied from as low as 8.1% (Slawson) to 97.5% (University of Zimbabwe / JHPIEGO Phase II).

Positivity results of VIA and conventional cytology

In five of the studies (Denny, both phases of the University of Zimbabwe / JHPIEGO study, Sankaranarayanan 1995-97, Cecchini), VIA resulted in more positives than conventional cytology, while in two studies (Megevand, Slawson), positivity was higher with conventional cytology. In Sankaranarayanan's second study (1996-1997), both techniques gave similar results (*Table 8, Figure 1*).

Positivity rates for conventional cytology varied between 1.0% and 15.2% among the eight studies, with a weighted average of 11.1 %. VIA positivity rates varied from 3.1% to 39.8%, with a weighted average of 18.2%.

There was a large difference between the two Sankaranarayanan studies as to the VIA positivity rates observed: 37.7% in the study that began in 1995 and

	CINII threshold for positive final diagnosis				
	Estimated sensitivity %	Estimated specificity %	Accuracy score (B)	N	Coverage with the reference standard (%)
Sankaranarayanan R. (1996-97):	90.2	92.2	87.5	3000	19.1
Cecchini S.:	87.5	82.3	78.3	2105	23.1
Sankaranarayanan R. (1995-97):	95.8	67.9	67.6	1351	44.5
Megevand E.:	64.5	97.7	64.4	2426	13.6
U. of Zimbabwe/JHPIEGO Phase I:	65.5	88.7	64.2	8731	18.1
Denny L.:	67.4	83.7	63.6	2944	25.8
U. of Zimbabwe/JHPIEGO Phase II	76.7	64.1	57.2	2203	97.5
Slawson D.:	29.0	97.1	28.9	2827	7.8

Studies are shown in descending accuracy score sequence

A. Sensitivity and specificity can only be estimated, given that most of the studies suffer from verification bias.

B. The accuracy score shows how close to the ideal each study is, where "ideal" is defined as 100% specificity and 100% sensitivity. The formula to obtain the score is: 100 - (SQR ([100-Sensitivity%]^2 + [100 –Specificity%]^2))

SQR: square root

TABLE 9. Estimated(A) VIA accuracy, sample size, and coverage with the reference standard

9.9% in the one that began in 1996. At the same time, the differences in positivity rates (ASCUS or worse) for conventional cytology were not as great: 15.2% and 10.2%, respectively. The lower VIA positivity found in the 1996-97 study may represent a change in the application of the positivity criteria as a result of experience gained by the provider. On the other hand, in the University of Zimbabwe / JHPIEGO study the positivity rate of VIA doubled (from 20% to 40%) between Phases I and II while the positivity of conventional cytology was stable (15% and 13%, repectively). The authors attributed this change to the application of less stringent positivity criteria in Phase II, in the interest of maximizing sensitivity.

Positivity by age

The paper by Cecchini is the only one that provides positivity results for VIA, Pap and cervicography classified by

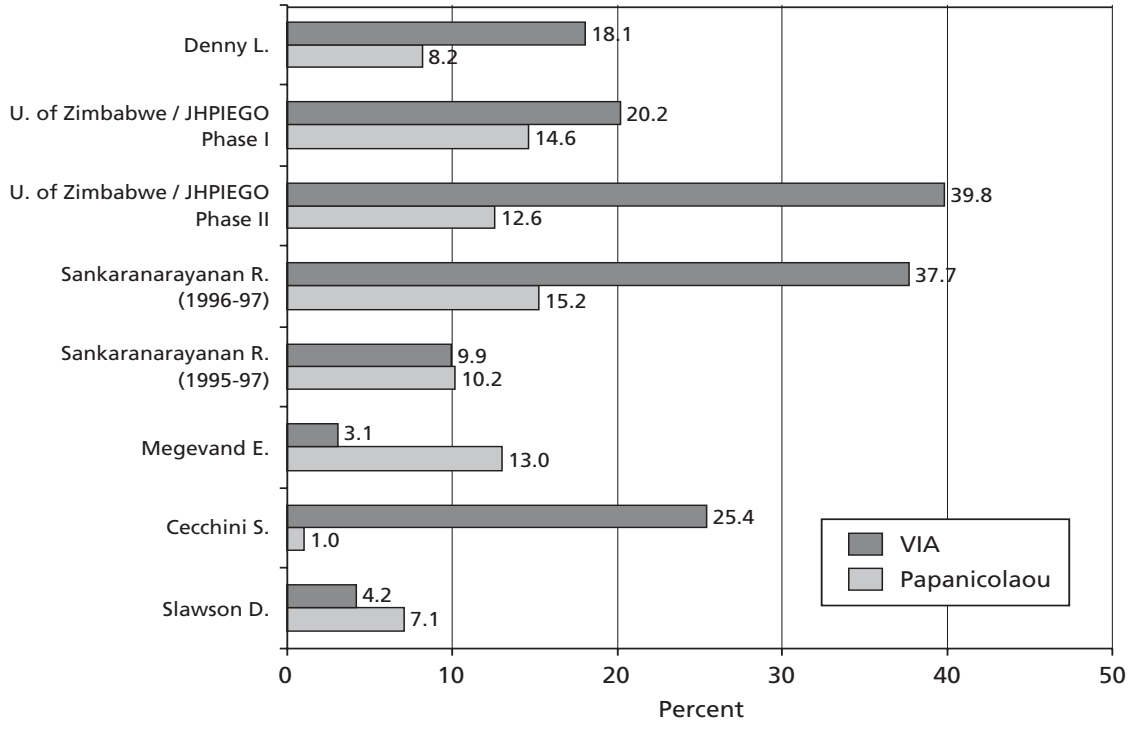

Figure 1. Percent of women with abnormal conventional cytology or abnormal VIA, by study

First author	Pap + and VIA- No.	Pap + and VIA- % of all cases	Pap - and VIA + No.	Pap - and VIA + % of all cases
Sankaranarayanan R.* (1996-97):	2	3.9	5	9.8
Sankaranarayanan R.‡ (1996-97):	3	5.9	5	9.8
Cecchini S.*‡:	1	12.5	3	37.5
Sankaranarayanan R.‡ (1995-97)	1	1.4	25	34.5
Megevand E.*:	11	35.5	0	0.0
U. of Zimbabwe/ JHPIEGO Phase I*:	99	24.9	95	23.9
U. of Zimbabwe/ JHPIEGO Phase II*:	14	7.0	80	40.0
Slawson D.‡:	22	71.0	4	12.9
Denny L.:	Not mentioned		Not mentioned	

* Positive Pap: LSIL and above
‡ Pap result: ASCUS and higher

TABLE 10. Confirmed cases of CIN II or worse that were positive to either VIA or the Pap test and were negative to the other

age. Two age groups were used: over 50 years and under 50. Statistically significant lower positivity rates were found for women in the older age group, for all the screening tests. The positivity rates found were: cytology, 5.1% among women under 50 vs. 2.5% among women over 50; cervicography, 24.3% vs. 3.2%, respectively, for those two age groups; and for VIA, 29.0% vs. 20.5%.

In that same study, approximately 50% of the women who had a positive result to any of the tests received a biopsy if colposcopic examination confirmed a lesion. The biopsy confirmation rate for high-grade lesions was similar for both age groups: 1.7% biopsy-confirmed CINII/III among women under age 50 and 1.3% among women over 50. No significant difference was found, with regard to age, in the positive predictive values of the tests.

Accuracy of VIA

Estimates of the sensitivity and specificity of VIA for detecting CINII and worse lesions were calculated for all those studies that provided the data (*Table 9, Figure 2*). Caution is advisable in comparing the results because, due to differences in study design, from 8% to 98% of the screened population was actually tested with the reference standard. When calculating estimated sensitivity and specificity it was assumed that all those women who were not tested with the reference standard were true negatives, which introduced a bias into the estimates.

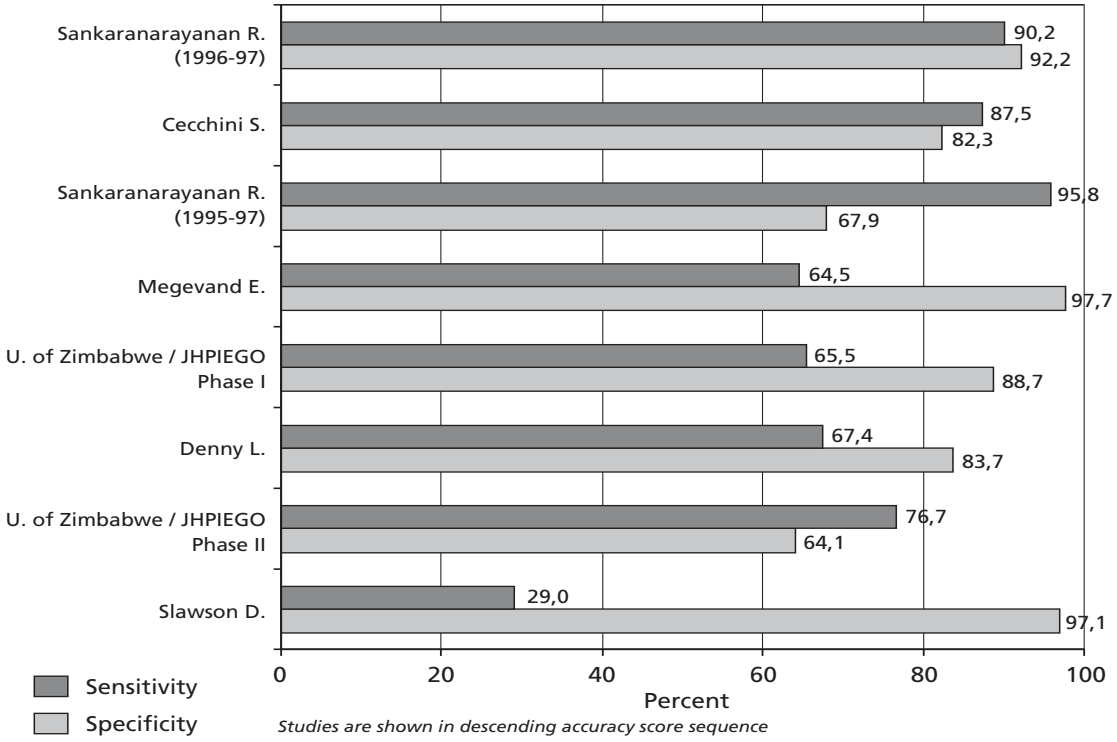

Figure 2. Sensitivity and specificity of VIA (%) for CIN II or worse, by study

First Author	Conclusions
L Denny	The sensitivity of VIA is equivalent to that of cytology for detecting high-grade SIL or invasive cervical cancers. Use of a low-magnification (2.5x) device does not enhance the sensitivity of VIA. The low specificity of VIA is due to the underlying high prevalence of STDs.
U. of Zimbabwe/ JHPIEGO	VIA can identify most true cases of cervical pre-cancer and cancer. The high number of false-positive results for VIA may lead to high rates of referrals. VIA is readily available, sustainable, and if coupled with treatment, could reduce the burden of disease. Even where cytology services are well established, VIA might be cost-effective.
Sankaranarayanan R (1996-97)	VIA demonstrated a higher detection rate of moderate dysplasia or worse lesions than cytology. VIA was much less specific than cytology and resulted in referral of more than one-third of women. VIA is a non-invasive procedure with easy applicability and immediate results that permit an immediate solution for women. Applicability of VIA in medical practice should be further evaluated.
Sankaranarayanan R. (1995-97)	Immediate colposcopy and treatment increases compliance and decreases the costs of screening. It is possible to train paramedical providers in VIA. Main problem: a high false-positive rate. Cauterization or cryotherapy may have a low risk of morbidity; treatment of false-positives may be judged as acceptable. VIA may be useful as an adjunct to improve the sensitivity of the Pap test.
Megevand E.	VIA detects two-thirds of high-grade pre-malignant cervical lesions and prevents some malignancies, at a low cost. Cytology is the best method of screening for cervical cancer. When such screening is not available, VIA warrants consideration as an alternative.
Cecchini S.	Cytology has a limited sensitivity for CIN II and III. When combined with cervicography there is limited improvement, at a very high cost. Cervicoscopy has poor specificity, but is more sensitive than cytology and cervicography, and more cost-effective than cervicography.
Slawson D.	VIA improved 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 pre-menopausal women during regular health maintenance examinations.
Ottaviano M.	VIA is an effective, inexpensive, and simple test that can be carried out routinely by all gynecologists and can be a very helpful supplement for early detection of cervical carcinoma. The colposcope is not indispensable. Colposcopic examination, on the other hand, is essential for the choice between ultraconservative or conservative treatment in every single case of CIN.

TABLE 11. Conclusions of the studies, as stated by the authors

To measure how well VIA distinguishes true negatives from true positives, an "accuracy score" was also calculated for each study (*Table 9*). This score, which combines sensitivity and specificity to produce a single figure, attempts to facilitate comparison of the results obtained in the different studies. It summarizes the trade-off between, on the one hand, the need for high sensitivity in a screening test and, on the other, the need for a sufficiently high level of specificity.

Any interpretation of the accuracy scores shown on the table must reflect the fact that those women who were not tested with the reference standard were assumed to be true negatives. This assumption resulted in inflated accuracy scores for VIA, for all the studies

except Phase II of the study by the University of Zimbabwe / JHPIEGO, which did not suffer from this bias.

In spite of the difficulties that arise when attempting to compare the results of studies that had very different design, it is evident that VIA not only has the sensitivity to detect a large number of cases but also, under certain conditions, can be fairly specific. More research is needed to explore the conditions that are necessary in order to obtain better performance in terms of the true positives / false positives balance.

Detection of moderate or worse dysplasia: VIA and conventional cytology

The results obtained in the different studies as to the ability of VIA and the Pap test to detect CIN II or worse lesions are shown in Table 10. The table shows the number of histologically confirmed cases detected by only one of the two screening tests-- that is, confirmed cases where a positive result to either VIA or conventional cytology was accompanied by a negative result to the other test. In the Sankaranarayanan 1995-97 study VIA missed only one case, whereas cytology missed 25 cases, of high-grade cellular intraepithelial lesions (CINII/III and worse). On the other hand, in the Slawson study, VIA missed 22 cases of CINII/III and worse, compared with only four missed by conventional cytology. If VIA were to be used as a complement to conventional cytology, it is likely to produce an increase of 10% to 40% in the number of cases detected. Nevertheless, in one study (Megevand), the use of VIA did not add a single case.

Conclusions of study authors

In their conclusions, all the authors agreed that VIA is sensitive and improves the detection rate of precancerous lesions over conventional cytology, but expressed concerns with the high rate of false positives (Table 11). Their recommendations ranged from using VIA as an alternative to conventional cytology screening (Denny, University of Zimbabwe / JHPIEGO, Sankaranarayanan 1996-97, Cecchini) to using VIA as an adjunct to cytology (Sankaranarayanan 1995-97, Megevand, Slawson, Ottaviano).

In general, the non-invasive nature of VIA was highlighted by the various authors, as well as the low resources required for providing it and the immediacy of the results. Despite variations in the prevalence of pre-

Key policy questions when considering VIA as a programmatic tool

- Do variations in VIA accuracy by age affect current programmatic recommendations as to age at screening?
- In settings where the conventional cytology test is already being used, when is the implementation of VIA justifiable?
- Should VIA be used to complement, or to replace, other screening methods?
- At what time interval should women be screened?
- Should VIA be incorporated alongside immediate, or deferred, treatment?

cancerous lesions among the populations studied, all the authors commented on the low specificity of the test and, consequently, its low positive predictive value. This would imply extra costs for unnecessary follow-up diagnoses or treatment. The over-referral of false positives was found to have been around 80% to 90% (University of Zimbabwe / JHPIEGO and Denny, respectively).

The authors acknowledged that the applicability of VIA in routine medical practice remains to be shown

Discussion and unresolved questions

The studies reviewed share the limitation of being cross-sectional assessments of the accuracy of VIA in detecting precancerous cervical lesions. None of them measured VIA effectiveness in preventing cervical cancer cases or deaths. Estimations of the number of cases prevented can be made on the basis of current knowledge of the natural history of the disease.

In order to directly measure the effectiveness of VIA in preventing invasive cervical cancer, a controlled longitudinal study design would be needed, ideally a randomized controlled trial. Studies of this type are long and expensive, given the amount of time required for a sufficiently large number of invasive cancers to occur, necessary for a statistically significant estimate. Another

Practical factors and questions regarding the application of VIA

Number of providers	In most reported studies, only one or two providers performed all the exams. The accuracy of VIA in a multiple-providers context remains to be evaluated
Type of providers	**In the research context, some studies have shown good VIA performance with providers who had no previous gynecological training. This performance remains to be proven under field conditions with available personnel.**
Training of providers	**Studies are needed to examine the external validity of the various training strategies that are being proposed.**
Quality control	**Studies are needed in order to design and evaluate quality control methods for VIA.**
Light used for visualization	**There is no published comparison of controlled studies using different light options, nor with regard to the best options where there is no electricity.**
Use of Lugol iodine	**There is some evidence that the use of Lugol iodine might improve the accuracy of VIA; however, its practicability and accuracy under the field conditions remains to be evaluated.**
Use of magnification devices	**Studies are required in order to clarify and resolve contradictory results pertaining to the use of magnification.**
Methodological study design issues	• Need to apply a reference standard to the study population (either in all or in a randomized sample). • Completeness of follow up for both positive and negative screened women. • Need for detailed description of the conditions under which VIA is conducted: trainers, providers, clinical practice, number of patients seen during the training, number of pictures, criteria for satisfactory completion of training, retraining requirements, quality control. • Evaluation of effectiveness: how to measure the effect on morbidity and mortality

design option is community-based intervention, where one community receives one type of screening and another type is assigned to a control community; the incidence of cervical cancer is then measured in each of the two communities. However, this is a less efficient study design than the clinical trial, and requires a larger sample size – but is possibly easier to implement.

Another limitation shared by the studies reviewed is that they were conducted in highly controlled situations which rarely, if ever, occur under field conditions. It is not possible to directly apply the results obtained, as if similar results could be expected from the use of VIA in the routine operation of the health services. Rather, these figures serve to inform as to the maximum effect that could be expected under ideal conditions.

In the research context, VIA has been shown to be at least as accurate as the currently accepted screening test, conventional cytology. However, before the evidence of VIA accuracy in a controlled setting is used for the purpose of making policy decisions, ***a determination of the effectiveness of VIA in real-life health services*** is required. Such effectiveness is determined by whether VIA can help achieve the goal of the screening program, namely, to reduce the incidence and mortality from cervical cancer through detection and treatment of precancerous lesions. Effectiveness also depends on acceptance of the technique by providers and the population, as well as the manner in which it is introduced into health services practice.

Some authors require that VIA, and indeed any new screening method, be proven effective in decreasing cervical cancer incidence and mortality, and not only in detecting cervical lesions. The argument is that a significant proportion of these lesions would have regressed without treatment, therefore the new screening techniques may be identifying lesions which could have remained undetected without severe consequences. The same requirement is not applied to conventional cytology, under the argument that its effectiveness has already been demonstrated in various countries.

Nevertheless, conventional cytology has failed to demonstrate effectiveness in low resources settings.

Any screening technique has to be sustainable. In this regard, ***quality control methods*** that will maintain acceptable accuracy are required. None of the studies reviewed here addressed this issue.

Beyond its potential usefulness for cervical cancer prevention, VIA offers the option of a ***see-and-treat approach***, based on the principle that screening and treatment of the patient might both take place during the same visit, thus improving the overall results of the program. Some studies have found that the see-and-treat approach, consisting of VIA coupled with cryotherapy, results in the most cost-effective strategy of all those evaluated, namely VIA, conventional cytology, HPV, liquid-based cytology[7]. However, the external validity of this result remains to be assessed, i.e., the reproducibility of the screening results in a large-scale primary care setting.

Prior to using ***VIA as a complement*** to other screening tests currently in use, further evaluation of its added benefit in real-life settings is required. One of these benefits might be that VIA could alert providers and patients of the potential risk of a cervical lesion before cytology results are available, and thus encourage follow-up. In low-resource settings, priority could be assigned to follow-up efforts directed at these women. In order to answer these questions, operational research and program evaluation strategies are needed.

The decision as to ***the VIA positive criteria to be applied*** will have an enormous impact on the number of women referred for evaluation or treatment, as well as on the number of women diagnosed as normal. An acceptable balance between under- and over-diagnoses must be reached. Since VIA is a highly subjective method, close monitoring will be required in order to maintain that desired balance.

The key policy questions that should be addressed when considering VIA as a programmatic tool are summarized in the Box in page 21. In addition, many other questions regarding the determinants of VIA accuracy remain unresolved, among them those related to the details concerning application of the technique itself (Box in opposite page). Additional research in this regard is needed as well.

EPILOGUE

As this publication was nearing completion, the results of a major new study by Belinson et al. were published[8]. Given the importance of this research, its main features are highlighted here.

The study was conducted in China and included 1997 women; providers were oncologic gynecologists. Each woman received a colposcopic examination followed by five biopsies, four cervical and one endocervical. The biopsies were obtained regardless of colposcopic results. Hence, in this study there was complete ascertainment of disease status.

Interestingly, the authors found that colposcopy detected only 81% of high-grade lesions and 100% of cancers, while VIA detected 71% of high-grade lesions but only 67% of cancers. VIA had 74% specificity for detection of CINII or worse lesions. The accuracy score for this study was 61.1, quite similar to the 57.2 obtained in Phase II of the University of Zimbabwe / JHPIEGO study, and may reflect the true sensitivity and specificity of VIA when performed by gynecologists. The performance results of VIA when carried out by primary care providers have yet to be confirmed.

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GLOSSARY

Acetic acid: A diluted (3-5%) vinegar solution that is applied to cervical tissue to make identification of abnormal tissue easier. The acetic acid interacts with diseased cells, causing epithelial lesions to turn white.

Bethesda classificationsystem: A system, proposed in 1988 by the US National Cancer Institute, that relies on only two grades for reporting cervical cancer precursor conditions: low-grade squamous intraepithelial lesions (LGSIL), which includes cellular atypia and CIN I, and high-grade squamous intraepithelial lesions (HGSIL), which includes CIN II, III and CIS.

Carcinoma in situ (CIS): Cellular changes in the stratified squamous epithelium associated with invasive cancer, but not extending to adjacent structures. CIS is generally a recognizable precursor of invasive squamous cell cancer.

Cervical stenosis: A narrowing of the cervical canal.

Cervicoscopy: Naked-eye visualization of the cervix after application of diluted (3-5%) acetic acid. Equivalent, in this report, to VIA.

Cervical intraepithelial neoplasia (CIN) classification system:Introduced in the 1960s, the CIN classification system for reporting cytological (Pap smear) results grades the severity of cervical lesions so that mild cervical dysplasia is categorized as CIN I; moderate cervical dysplasia as CIN II; and severe cervical dysplasia as CIN III.

Cervicography: A technique in which a photograph of the cervix is obtained after application of diluted (3-5%) acetic acid, using a specially equipped camera. Once developed, the photographs, called cervigrams, are projected as slides and interpreted by specially trained colposcopists.

Colposcopy: Examination of the vagina and cervix using an endoscopic instrument (colposcope) that provides magnification to allow direct observation and study of vaginal and cervical cells in vivo, after application of an acetic acid solution.

Cone biopsy: A surgical procedure in which a cone-shaped wedge of cervical tissue is obtained for histo-pathological analysis.

Cryotherapy: A method of outpatient treatment that uses extremely low (LIST) temperatures to freeze and destroy abnormal tissue.

Downstaging: Naked-eye visualization of the cervix without acetic acid nor magnification to identify early stages of cancer. Also known as unaided visual inspection (UVI).

Dysplasia of the uterine cervix: Epithelial abnormality involving the cervical epithelium. One of several interchangeable terms used to describe this disease process. Other terms include cervical intraepithelial neoplasia (CIN) or squamous intraepithelial lesion (SIL).

Loop electrosurgical excision procedure (LEEP): A method of outpatient excisional biopsy and treatment that is used to remove the entire transformation zone using a thin wire electrode charged with a low-voltage, high-frequency alternating current, producing a tissue specimen suitable for histologic analysis in most circumstances.

Nabothian cysts: A mucus-filled cyst that is a small, smooth, and rounded white lump commonly noted on the surface of the uterine cervix. With no clinical significance.

Negative predictive value: Proportion of women having no disease among those with a negative test result.

Positive predictive value: Proportion of women having disease among those with a positive test result.

Punch biopsy: A method by which a small sample of tissue is extracted for histological analysis.

Sensitivity: The proportion of true positives that are identified as positives.

Specificity: The proportion of true negatives that are identified as negatives.

Squamocolumnar junction: The point at which columnar cells meet ectocervical squamous cells on the cervix. This junction is located in the center of the transformation zone and is most vulnerable to abnormal changes in cervical cells.

Transformation zone: Located on the surface of the cervix, the transformation zone is composed of glandular (columnar) epithelium until the onset of puberty, when the columnar epithelium is gradually replaced by squamous epithelium, similar to the lining of the vagina. Cervical cancer generally originates at the edges of the transformation zone.

Unaided visual inspection (UVI): Naked-eye visualization without acetic acid nor magnification, to screen the cervix for gross abnormalities. Also known as downstaging.

Verification bias: Also called workup bias, this occurs when the chances of being referred for the reference test are different for those who test positive during screening and those who test negative.

Visual inspection with acetic acid (VIA): Naked-eye visualization (without magnification) of the acetic-acid-washed cervix (using diluted 3-5% acetic acid) to screen for cervical abnormalities.

ANNEX

Studies Not Selected

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PART 2

FULL-TEXT ARTICLES

Evaluation of Alternative Methods of Cervical Cancer Screening for Resource-Poor Settings

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Background: SNoncytologic methods of screening for cervical carcinoma and its precursor lesions are needed for resource-poor settings in which cervical carcinoma continues to be an important cause of morbidity and mortality.
Methods. Two thousand nine hundred forty-four women ages 36-65 years were recruited from Cape Town, South Africa, and screened using a combination of a Papanicolaou (Pap) smear, human papillomavirus (HPV) DNA testing, direct visual inspection after the application of a 5% acetic acid solution (DVI), and cervicography. Cervicography was considered primarily as a method with which to quality control the DVI examinations. Women with squamous intraepithelial lesions (SIL) or carcinoma on Pap smear, positive DVI examination (acetowhite lesion or cervical ulcer/growth), high levels of high risk HPV DNA (relative light units [RLU] > 10x positive control), or positive CervigramTM were referred for colposcopy and cervical biopsy.
Results. Pap smears were positive in 8.1% of all women screened and identified 65 (78%) of all cases of biopsy confirmed high-grade disease (high grade SIL or invasive carcinoma). DVI and cervicography were classified as positive in 18.1% and 10.5%, respectively, of women screened and identified 58 (67%) and 46 (58%) of all cases of high grade disease, respectively. The results o HPV DNA testing varied depending on the cutoff value used to define a positive result. At the standard cutoff level (RLU > 1x positive control), 16.2% of women screened were classified as high risk HPV DNA positive, as were 63 women with high-grade disease (73%).
Conclusions. DVI and HPV DNA testing identified similar numbers of high grade SIL (cervical intraepithelial neoplasia Grade 2,3) and invasive carcinoma cases as Pap smears. However, both classify considerably more women without cervical disease as being test positive. Cancer 2000; 89:826-33.

KEYWORDS: direct visual inspection (DVI), human papillomavirus, Papanicolaou (Pap) smears, squamous intraepithelial lesions (SIL), cervicography, cervical intraepithelial neoplasia (CIN).

Each year approximately 450,000 new cases of invasive cervical carcinoma are diagnosed worldwide.[1] The cervical cancer incidence rate is highest in countries that have little or no cytologic cervical cancer screening.[2] There are many barriers to establishing cervical cancer screening programs in resource-poor settings. These include the demands of competing health needs, such as communicable disease; poorly developed health care services that tend to be focused on curative rather preventive care; war and civil strife; and the fact that women in poor countries often are uniformed as to their needs for preventive health care.[3] The traditional method of screening, cervical cytology, presents an additional barrier. Cytologic-based screening programs require a relatively sophisticated infrastructure, including highly trained-personnel, adequately equipped laboratories, and referral systems to communicate results of the test (which usually is delayed) to women.[4]

Because of the problems intrinsic to cytologic screening, considerable attention is being given to developing alternative methods, such as direct visual inspection (DVI) and human papillomavirus (HPV) DNA testing, for cervical cancer screening in resource-poor settings. To evaluate the use of alternative screening methods in a resource-poor setting, we instituted a study in a squatter settlement outside Cape Town, South Africa. Previously unscreened women ages 35-65 years were screened using a combination of conventional cytology, DVI, HPV DNA testing, and cervicography. In this article we present a comparison of the performance of these four tests in detecting high-grade cervical squamous intraepithelial lesions (SIL) and invasive carcinoma.

Materials and Methods

Patients

From January 1996 to September 1997, 2944 women were recruited from Khayelitsha, South Africa. Women ages 35-65 years, who had not been screened previously for cervical cancer, who were not pregnant, and who had not undergone hysterectomy were eligible for the study. The study was approved by the Institutional Review Boards of Columbia University and the University of Cape Town.

Clinical Examinations

After meeting with a community health educator, written informed consent was obtained and a brief oral questionnaire was administered. A nursing sister who had been trained in DVI of the cervix, cervicography, and in obtaining a Papanicolaou (Pap) smear, performed a gynecologic examination onsite. Training consisted of a 4-day course that included hands-on clinical examinations as well as extensive review of photographs of normal and abnormal cervices. The nursing sister was trained to refer all women with acetowhite lesions as well as all women with cervical ulcers or cervical growths. No attempt was made by the nursing sister to grade the severity of the lesions.

The examinations included sequentially: 1) a Pap smear obtained using an Accellon sampler (Med Scand, Hollywood, FL); 2) a sample for HPV DNA testing obtained by breaking the tip from the Accellon into a HPV specimen collection tube (Digene Corporation, Silver Spring, MD) after the Pap smear had been prepared; 3) DVI of the cervix after application of 5% acetic acid with and without 2.5x magnification using a handheld magnification device (Edmund Scientific, Barrington, NJ); and 4) a 35-mm photograph of the cervix (CervigramTM; National Testing Laboratories, St. Louis, MO). Women were given the results of the DVI examination immediately, and all women were asked to return to the clinic within 2-6 days after the initial examination.

Laboratory Tests

HPV DNA was assayed at the University of Cape Town on a weekly basis using the first-generation Hybrid Capture I HPV DNA assay (Digene Corporation). The HPV DNA test was performed according to the manufacturer's instructions using the tube-based format and probes for "high oncogenic risk" HPV types (i.e., types 16, 18, 31, 33, 35, 45, 51, 52, and 56). HPV determinations were

read as the ratio of relative light units (RLU) of HPV DNA in the sample to that of a positive control that was set at 10pg/mL HPV DNA (corresponding to approximately 100,000 HPV genome copies per test). Because the assay is quantitative, we used two different HPV levels to define a "positive" result. The first cutoff level classifies only samples with relatively high levels of high risk HPV DNA (RLU > 10x positive control or approximately > 100pg/mL HPV DNA) as positive. This cutoff value was used to refer women for colposcopy. In addition, a second cutoff level was used in the analysis that classifies samples with lower levels of HPV DNA (RLU > 1x positive control) as being "positive." The lower cutoff level is the standard level used for clinical testing in the U.S.

Women with high levels of high risk HPV DNA (RLU > 10x positive control) or positive DVI results underwent onsite colposcopy when they returned for their results 2-6 days after their initial examination. Colposcopic lesions were graded using the Reid Colposcopic Index.[5] Lesions classified as minor grade (Reid Score < 3) were biopsied whereas lesions classified as significant (Reid Score ≥ 3) were excised electro surgically using loop electrodes. Endocervical curettage was performed if no lesions were visible. All biopsies, loop excision specimens, and endocervical curettage specimens were evaluated at Columbia University, New York, by pathologists masked to any clinical screening test information. The histologic results were reported using a two-tiered SIL terminology with low grade SIL equivalent to cervical intraepithelial neoplasia Grade 1 (CIN 1) and high grade SIL equivalent to CIN Grades 2 and 3, combined.[6]

Pap smears were evaluated at the University of Cape Town Cytopathology laboratory using the Bethesda system terminology and results were available within 2 weeks of the Pap smear being taken.[7] Women with cytologic diagnoses of low grade SIL, high grade SIL, or carcinoma who had not already undergone colposcopy because of a positive DVI or HPV test were tracked when the Pap results became available and referred for colposcopy. Women with cytologic diagnoses of atypical squamous cells of undetermined significance (ASCUS) were not referred for colposcopy if all other screening tests were negative.

Cervigrams were evaluated at National Testing Laboratories (St. Louis, MO) and were reported using the company's standard terminology. Cervigram evaluations of "warrants colposcopy," low grade SIL, high grade SIL, or carcinoma were referred for colposcopy. Cervigram results were available 8-12 weeks after being performed. It should be stressed that in this study cervicography was used primarily to quality control DVI. Therefore, no attempt was made to obtain a rapid turnaround of the Cervigrams, which were mailed to the U.S. in batches for evaluation. This approach resulted in high numbers of Cervigrams being read as technically defective because it minimized the ability of the laboratory to provide feedback to the clinical site.

Statistical Analysis

To adjust for loss to follow-up, the prevalence of cervical disease was calculated as follows: the proportions of women with histologically confirmed low grade SIL, high grade SIL, and carcinoma among those who underwent colposcopy were multiplied by the proportion of women with one or more positive screening tests. This adjustment assumes that the prevalence of disease among women with one or more positive screening test were lost to follow-up did not differ from those who underwent colposcopy.

To compare the performance of the tests, we first calculated the capacity of each test to detect all cases of disease (e.g., histologically confirmed high grade SIL or invasive carcinoma) that were detectable using all four screening tests together (i.e., the ratio of the number of test positive, disease positive women to the total number of women identified with disease). This measure is roughly equivalent to sensitivity, but does not require that the reference standard be applied to all women examined. Second, we calculated the capacity of each test to detect women free of disease correctly as the ratio of the number of test negative, disease negative women to the total number of women free of disease. This calculation is roughly equivalent to specificity but does not require the reference standard to be applied to women who are screening test negative. Women were considered to be free of disease if low grade SIL, high grade SIL, and invasive cervical carcinoma were ruled out on histollogic sampling after colposcopy among women with one or more positive screening test of if none of the four screening tests were positive. Although the two measures described may not represent true sensitivity and specificity directly, because some disease may have been missed by all four screening tests, they can be used to compare the performance of the four screening tests with each other. Specifically, a ratio of the true sensitivity (true-positive rate) of one test to another can be calculated and tested using the McNemar test.[8,9] Similarly, a ratio of the false-positive rates (1-specificity) of the two tests can be compared and tested.

The performance of the four screening tests was extrapolated to estimate the proportion of all women screened who would be test positive, disease positive. This was calculated as follows: the positive predictive value of each test multiplied by the prevalence of a positive test result among all women screened. The performance of the four screening tests also was extrapo-

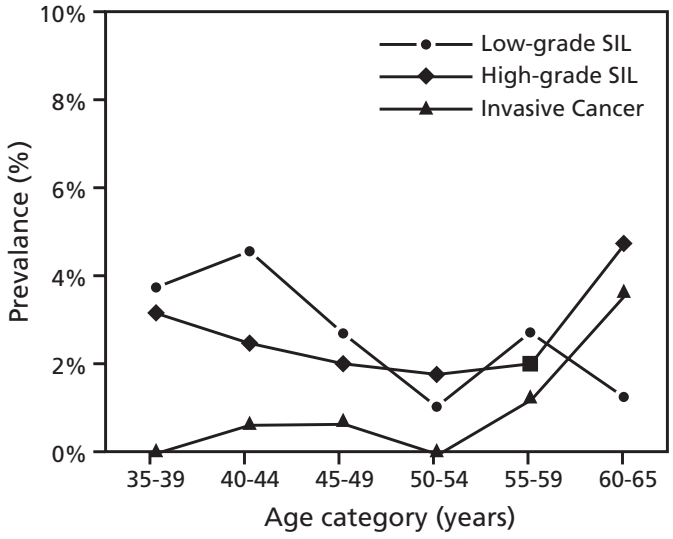

FIGURE 1. Prevalence of histologically confirmed low grade squamous intraepithelial lesion (SIL) (cervical intraepithelial neoplasia [CIN] Grade 1), high grade SIL C (CIN Grade 2.3), and carcinoma by age category adjusted for loss to follow up.

lated to estimate over treatment associated with each screening test as follows: the proportion of women test positive but free of disease on histologic sampling after colposcopy multiplied by the prevalence of a positive test result among all women screened.

Results

Demographics of the Study Population

The median age if the 2944 women enrolled into the study was 39 years and 16% were age ≥ 50 years. The majority of the women (63%) live in self-built shacks on sites with basic water and sanitation services, and 19% lived on vacant land without basic services. Few women (4%) had completed high school and a considerable proportion (14%) had received no formal schooling. The median gravidity was 3.5 and the median parity was 3.5. The median age at the time of the first pregnancy was 19.5 years. Only 17% of the women reported sexual intercourse before the age of 16 years and the median number of lifetime sexual partners was 1.5. Only 23% of the women reported having had no sexual intercourse in the month prior to their examination.

Prevalence of Cervical Disease

Abnormalities were identified, using one or more of the four screening tests, among 842 of the 2944 women screened (29%). Of these, 760 (90%) underwent colposcopy and had a cervical biopsy of any colposcopically identified abnormalities. There were 12 cases of histologically confirmed invasive cervical carcinoma, 74 cases of high grade SIL (CIN Grade 2 or 3), and 95 cases of low grade SIL (CIN Grade 1). The prevalence of biopsy confirmed invasive carcinoma was 0.5%, that of high grade SIL (CIN Grade 2,3) was 2.8%, and that of low grade SIL (CIN Grade 1) was 3.6%, after adjusting for loss to follow-up. Two of the tumors identified in the study were International Federation of Gynecology and Obstetrics (FIGO) Stage Ia, three were Stage IB, three were Stage IIB, three were Stage IIIB and one was Stage IVA. The prevalence of biopsy confirmed high grade and low grade SIL did not differ significantly by age, but the prevalence of invasive carcinoma was significantly higher in older age groups *(Fig. 1)*.

Detection of High Grade SIL (CIN, Grade 2,3) and Carcinoma

A high proportion of women (18%) were classified as having an abnormal result when screened using DVI after the application of a 5% solution of acetic acid *(Table 1)*. Of the 12 women with invasive carcinoma and 74 with biopsy confirmed high grade SIL (CIN Grade 2,3) identified in the study, DVI correctly identified 9 of 12 carcinomas (75%) and 47 of 74 high-grade SIL (CIN Grade 2,3) (64%). The addition of magnification to the DVI examination did not improve the detection of high grade SIL or carcinoma. No acetowhite lesions were detected using x2.5 magnification that had not been detected with the naked eye.

Of the 2944 Pap smears that were taken, 2922 (99%) were classified as being "satisfactory for evaluation." The overall abnormal rate, including ASCUS, SIL, and invasive carcinoma, was 17%. A diagnosis of SIL or carcinoma was made on 8.1% of all Pap smears. This was the cutoff level used to refer women for colposcopy in this study. The Pap smear was classified as SIL or carcinoma in 10 of the 12 women with invasive carcinoma (83%) and in 55 of the 74 women with biopsy confirmed high grade SIL (CIN Grade 2,3) (74%). Two women with carcinoma and one with a biopsy confirmed high grade SIL (CIN Grade 2,3) had an unsatisfactory Pap smear.

Using the Hybrid Capture I HPV DNA assay, 476 (16%) of all women screened were found to be high risk HPV DNA positive. Of these, 180 had a relatively high

Screening test	Result of screening test	No. of women (%) with each histologic diagnosis among those who underwent colposcopy					
		Number of women (%)[a]	Number of women with colposcopy (%)	No disease	Low grade SIL	High grade SIL	Invasive carcinoma
Cytology	Within normal limits	2478 (84.8)	505 (20.4)	455 (90.1)	34 (6.7)	16 (3.2)	0 (0.0)
	ASCUS	206 (7.0)	43 (20.91	34 (79.1)	7 (16.3)	2 (4.7)	0 (0.0)
	Low grade SIL	155 (5.3)	128 (82.6)	64 (50.0)	43 (33.6)	20 (15.6)	1 (0.8)
	High grade SIL	80 (2.7)	73 (91.3)	2 1 (28.8)	11 (15.1)	35 (47.9)	6 (8.2)
	Invasive carcinoma	3 (0.1)	3 (100)	0 (0.0)	0 (0.0)	0 (0.0)	3 (100)
Direct visual inspection	Negative	2410 (81.9)	250 (10.4)	174 (69.6)	48 (10.2)	25 (10.0)	3 (1.2)
	Positive	534 (18.1)	510 (95.5)	405 (79.4)	47 (9.2)	49 (9.6)	9 (1.8)
HPV DNA test	< 1	2467 (83.8)	492 (19.9)	436 (88.6)	33 (6.7)	21 (4.3)	2 (0.4)
(RLU per positive	1-10	296 (10.1)	97 (32.8)	59 (60.8)	18 (18.6)	15 (15.5)	5 (5.2)
control)	> 10	180 (6.1)	171 (95.0)	84 (49.1)	44 (25.7)	38 (22.2)	3 (2.9)
Cervicography	Negative	2279 (87.3)	427 (18.7)	346 (81.0)	49 (11.5)	30	2 (0.5)
	Atypical	56 (2.1)	28 (50)	24 (85.7)	3 (10.7)	1 (3.6)	0 (0.0)
	Warrants colposcopy	35 (1.3)	29 (82.9)	21 (72.4)	2 (6.9)	6 (20.7)	0 (0.0)
	Low grade SIL	203 (7.8)	173 (85.2)	124 (71.7)	26 (15.0)	23 (13.3)	0 (0.0)
	High grade SIL	29 (1.1)	28 (96.6)	8 (28.6)	10 (35.7)	9 (32.1)	1 (3.6)
	Invasive carcinoma	9 (0.3)	9 (100)	2 (22.2)	0 (0.0)	0 (0.0)	7 (77.8)

SIL: squamous intraepithelial lesion: atypical squamous cells of undetermined significance; HPV: human papillomavirus; RLU: relative light units.

a Columns do not add up to 2944 because 22 women had unsatisfactory Papanicolaou smears, 1 woman was missing human papillomavirus DNA rest results, and 133 women had technically defective or missing cervigrams.

TABLE 1. Results of Screening Tests among 2944 Women Screened and Histologic Diagnosis among Those Women who Underwent Colposcopy

HPV DNA viral load (RLU >10x the positive control). High risk HPV DNA was identified in 10 (83%) or 5 (42%) of the 12 women with invasive cervical carcinoma and 53 (72%) or 38 (51%) of the women with biopsy confirmed high grade SIL (CIN Grade 2,3) at the standard cutoff level (RLU > 1x positive control) and high cutoff level (RLU > 10x positive control) respectively *(Table 1)*.

Cervigram results were missing on account of difficulties with the camera or because the Cervigrams were classified as technically defective in 333 of all examinations (11%). Of the 2611 Cervigrams that were satisfactory for evaluation, 2279 (87%) were classified as negative, 56 (2%) as atypical but insufficiently abnormal to warrant referral for colposcopy. Cervicography identified 8 of the 12 carcinomas (67%) and 38 of the 74 cases of histologically confirmed high grade SIL (CIN Grade 2,3) (51%) (Table 1). Two of the 12 women with invasive cervical carcinoma had missing or technically defective Cervigrams and 2 had Cervigrams diagnosed as being within normal limits. Similarly, 5 of the 74 women with biopsy confirmed high grade SIL (CIN Grade 2,3) had technically defective Cervigrams and 30 and Cervigrams diagnosed as being within limits *(Table 1)*.

Comparison of the Overall Performance of the Four Tests

One way of comparing the performance of the four different tests is to compare the percentage of women with abnormal test result who were found at colposcopy to have biopsy confirmed with high grade SIL or invasive cervical carcinoma. A corollary of this is to compare the percentage of women with a negative test result who had no evidence of cervical disease based on screening with the other three tests and/or a colposcopic examination of one of the other three tests was abnormal. Based on these two parameters, cytology provided the best test performance. Approximately 78% of all women whose Pap smear was classified as SIL or invasive cervical carcinoma had biopsy confirmed high grade SIL (CIN Grade 2,3) or invasive cervical carcinoma. Approximately 97% of women whose Pap smear was classified as within normal limits or as ASCUS had no evidence of cervical disease based on the results of the other three screening tests and/or colposcopic examination *(Table 2).* However, despite the slightly higher point estimate, the sensitivity of DVI and of HPV DNA testing at a low cutoff level (RLU >1x positive control) to detect high grade SIL or carcinoma was within the same range as that of cytology (ratio of sensitivity of DVI to cytology, 0.85, P = 0.16; and

Screening test	Number (%) of women testing positive who have high grade disease[a]	Number (%) of women testing negative who lack disease[b]
Cytology[c]	65 (78.3)	2.755 (96.8)
Direct visual inspection	58 (67.4)	2.279 (84.91
HPV DNA (low cutoff RLU >1x positive contro)	63 (73.3)	2.354 (87.8)
HPV DNA (high cutoff RLU 10x positive control)	43 (50.0)	2.596 (96.9)
Cervicography[d]	46 (58.2)	2.210 (93.4)

HPV: human papillomavirus; RLU: relative light units.

a Histologically confirmed high grade squamous intraepithelial lesion (n=74) or invasive (n=12). Three of these women had unsatisfactory cytology results and seven had missing cervigram results and were exclude from the denominators for these tests.

b Women were considered free of disease if any grade squamous intraepithelial lesion or carcinoma was ruled out after colposcopy (n=579) or if none of the 4 screening tests were positive (n=2102). Nineteen women had unsatisfactory cytology results, 1 was missing human papillomavirus DNA test results and 316 had missing cervigram results and were excluded from the denominators for these tests,

c Cytology diagnoses of low grade squamous intraepithelial lesion (SIL) high grade, or carcinoma, were classified as positive, "atypical squamous cells of undetermined significance" and "within normal limits" were classified as negative.

d Cervicography diagnoses of low grade squamous intraepithelial lesion (SIL)m high grade SIL, carcinoma, or P0, were classified as positive; atypical and negative cervigrams were classified as negative.

TABLE 2. Detection of High-Grade Cervical Disease and Test Positivity Rate in Women with No-Evidence of Disease

ratio of sensitivity of HPV DNA testing at a low cutoff level to cytology, 0.95, P = 0.60). However, the sensitivities of HPV DNA testing at a high cutoff level (RLU > 10x positive control) and cervicography were significantly higher than cytology (ratio of sensitivity of HPV DNA testing at a high cutoff level to cytology, 0.66, P < 0.001; ratio of sensitivity of cervicography to cytology, 0.73, P = 0.005). DVI, HPV DNA testing at a low cutoff level, and cervicography all had significantly higher false-positive rates than cytology (i.e., lower specificity) (ratio of false-positive rates of DVI to cytology, 4.7; HPV DNA testing at a low cutoff level to cytology, 3.8; and cervicography to cytology, 1.9; P < 0.001 for all comparisons). The specificity of HPV DNA testing at a high cutoff level was not significantly different from cytology (ratio of false-positive rates, 0.98; P = 0.92).

DVI and HPV DNA testing using the standard cutoff level (RLU > 1x positive control) were similar to cytology in their capacity to detect high grade SIL (CIN Grade 2,3) or invasive cervical carcinoma. Cervicography and HPV DNA testing using a high cutoff level (RLU > 10x positive control) had lower detection capacities, *(Table 2)*. However, both cervicography and HPV DNA testing using the high cutoff level (RLU > 10x positive control) classified fewer women with no evidence of cervical disease as being screen negative than did the other tests *(Table 2)*.

Consequences If the Screening Tests Are Used without Colposcopy

Because of a lack of trained colposcopists and facilities for performing colposcopy, it has been suggested that in a low-resource setting treatment decisions might be based solely on the basis of a positive screening test.4 If a positive screening was followed by treatment in the absence of colposcopy, high grade SIL (CIN Grade 2,3) or carcinoma would be identified correctly and treated in a similar proportion (approximately 2%) of all women screened when any of the four screening tests were used *(Table 3)*. However, over treatment of women without cervical disease would be more widespread with DVI and with HPV DNA testing at the standard cutoff levels (RLU >1x positive control). For example, if women with positive DVI results were treated in the absence of colposcopy, 18% of the population would be treated and 79% of these would be disease free *(Table 3)*. In contrast, if a positive cytology result was used to determine who was to be treated and less than half (42%) of these women would be disease free. HPV DNA testing at the standard cutoff level (RLU > 1 x positive control) performs similarly to DVI (72% of HPV DNA positive women

have no disease), but at a high threshold levels (RLU > 10x positive control) HPV DNA testing performs similarly to cytology (only 49% of women classified as being HPV DNA positive have no disease) *(Table 3)*.

Discussion

The current study was designed to compare the effectiveness of alternatives to cytologic screening for detecting high grade SIL and invasive carcinoma in a resource-poor setting. It was conducted in a large periurban, informal settlement that is 20 kilometers from Cape Town and that is typical of many African resource-poor settings. Khayelitsha is inhabited by an estimated 350,000-500,000 people, many of whom are recent immigrants from rural areas who live in unserviced shanties and until recently, health care services were minimal.

DVI and HPV DNA testing with the Hybrid Capture 1 test (Digene Corporation) using the standard cutoff level identified similar numbers of high grade SIL (CIN Grade 2,3) and invasive cervical carcinoma cases as did conventional cytologic screening. However, both methods classified considerably more women without evidence of cervical disease as test positive that did cytology. Cervicography identified significantly lower numbers of cases of high grade ISL (CIN Grade 2,3) or invasive cervical carcinoma cases than did cytology, DIV, or HPV DNA testing at the low (RLU > 1 positive control) cutoff level.

Visual screening methods recently received considerable attention as alternatives to cytologic screening in resource-poor seetings.[10-13] They are perceived as requiring a lower level of infrastructure and as being less expensive than cytologic screening because they do not require cytology laboratories with their attendant training and quality control costs. Visual methods provide an immediate result that is preferable in settings in which transportation facilities are minimal, in which it is difficult to track women with abnormal test results, or in which women must travel for long distances to health care facilities.

Recent studies that have incorporated acetic acid washes consistently have found the sensitivity of DVI to be equivalent to that of cytology for detecting high grade SIL or invasive cervical carcinoma.[10-12, 14] For example, in a recent Indian study, 3000 women were screened using both, cytology and DVI after the application of acetic acid.[12] DVI identified 90% of the cases of high grade SIL or invasive cervical carcinoma. In another study of similar design conducted in a primary care setting in Zimbabwe, DVI had a sensitivity of 77% for high grade SIL and carcinoma compared with 44% for cervical cytology.[10] Our results from Khayelitsha confirm these findings and extend them by demonstrating that the use of a low magnification (x2.5) device does not enhance the sensitivity of DVI. To our knowledge, not a single case of high grade SIL or invasive cervical carcinoma was identified using x2.5 magnification that was not also identified by the nursing sister using the "naked eye" alone. However, it should be noted that in the current study no measures were put into place to control the biases of the nursing sister performing the visual screening. Based on the results of these studies together, it appears that the sensitivity of DVI is adequate to allow it to be used for screening in resource-poor settings.

To our knowledge, relatively limited data are available regarding the use of HPV DNA testing as an alternative screening method. In a study of 1985 women from the United Kingdom who were screened using both, cytology and a polymerase chain reaction assay for high risk PHV DNA, Cusick et al. reported that the sensitivity of HPV DNA testing for detecting high grade SIL or invasive cervical carcinoma was 78%, which was better that of cytology.[15] Similarly, in a cohort of nearly 10,000 Costa Rican women, Sherman et al, reported that 78% of women with high grade SIL or invasive cervical carcinoma identified using cytology or cervicograhy were high risk HPV DNA positive using the Hybrid Capture I test (Digene Corporation).[16] The current finding that HPV DNA TESTING for high risk HPV types identifies an equivalent number of cases of high grade SIL (CIN Grade 2,3) and invasive cervical carcinoma as cytology confirms the effectiveness of HPV DNA testing as a method with which to identify women with cervical disease.

The conventional screening protocols in widespread use throughout the U.S. and Western Europe refer women who a re screening test positive for a colposcopic examination. However, colposcopy is a relatively expensive procedure and colposcopic services often are in limited supply in resource-poor-settings. Therefore, the number of women without significant cervical disease who are classified as being screening test positive is just as important as the number of cases of high grade SIL (CIN Grade 2,3) and invasive cervical carcinoma detected by a screening method. In the current study we found that both, HPV DNA testing and DVI classify a considerable number of women without high-grade disease as being test positive. With HPV DNA testing, 1 of 6 women screened (17%) has a high risk HPV DNA detected using the standard test cutoff level and only 14% of these women who are HPV DNA positive have a high grade cervical lesion. Very similar results were found for DVI. Therefore, if either DVI or HPV DNA testing were used to screen for cervical disease in Khayelitsha, the

	Proportion of all women screened (%)			
		Correctly treated		Overtreated
	Total treated	High grade SIL or carcinoma[a]	Low grade SIL[a]	No disease[b]
Cytology	8.1	2.6	2.1	3.4
Inspección visual directa	18.1	2.1	1.7	14.4
ADN del VPH (valor límite estándar: ULR menos de 10 veces mayores que las del testigo positivo)	16.2	2.3	2.22	11.7
ADN del VPH (valor límite alto: ULR más de 10 veces mayores que las del testigo positivo)	6.1	1.5	1.5	3.0
Cervicografía	10.3	2.0	1.7	6.8

SIL:squamous intraepithelial lesion; HPV: human papillomavirus; RLU: relative light units.
A Proportion with squamous intraepithelial lesions of any grade or carcinoma among those with a positive test result who underwent colposcopy multiplied by the proportion of women who were test positive on the screening test.
B Proportion with no histologic evidence of squamous intraepithelial lesions of any grade or carcinoma among those with a positive test result who underwent colposcopy multiplied by the proportion of women who test positive on the screening test.

TABLE 3. Proportion of Women Screened Who Would Be Treated Correctly and Those Who Would Be Overtreated by Each Screening Test in the Absence of Colposcopy

available colposcopic services would be overwhelmed rapidly. The most likely explanation for the low specificity of both HPV DNA testing and DVI in the current study is the underlying high prevalence of sexually transmitted diseases in this population. Although information concerning sexually transmitted diseases was not obtained in the current study, in a subsequent survey we found a very high prevalence of sexually transmitted diseases among women in this age group in Khayelitsha with a human immunodeficiency virus (HIV) seropositivity rate of approximately 8%, Trichomonas vaginalis in 25% of the women, and *Chlamydia trachomatis* or *Neisseria gonorrhea* in 9% of the women. Severe cervicovaginal infections make DVI considerably more difficult and it is our impression that many of the cases of false-positive DVI examinations are attributable to cervical ulceration and inflammation associated with infection.

Several approaches have been suggested to handle the problem of low specificity in DVI and HPV DNA testing. One, which we recently modeled, using data from the Khayelitsha study, uses two screening tests in succession, with the second test performed only if the first test is positive (unpublished data). This two-state screening approach greatly increases the specificity of the screening process, but the increase in specificity is accompanied by a reduction in sensitivity. The other approach is to ignore the low specificity of DVI and HPV DNA testing and simply treat all women who are screening test positive.[17] Advocates of this approach point out that cytologic and colposcopic services are unlikely to become available in many resource-poor settings in the foreseeable future and that invasive cervical cancer represents a serious health problem for women that needs to be dealt with immediately. Moreover, outpatient treatment using modalities such as cryosurgery is easy to perform, relatively inexpensive, and has a low rate of complications. Although combining DIV or HPV DNA testing with the immediate treatment of women who are test positive has obvious advantages, large-scale safety and efficacy studies are needed to evaluate carefully the medical and programmatic side effects of over treating significant numbers of women without cervical disease and the efficacy of outpatient modalities when performed by midlevel clinicians in the absence of colposcopic guidance in a low-resource setting. Moreover, to determine the comparative cost-effectiveness of the different screening tests and approaches in reducing mortality from invasive cervical carcinoma in low resource settings, formal cost-effective modeling using country specific costs needs to be performed.

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Visual inspection with acetic acid for cervical-cancer screening:

test qualities in a primary-care setting

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Background: Naked eye visual inspection of the cervix with acetic-acid wash (VIA), or cervicoscopy, is an alternative to cytology in screening for cervical cancer in poorly resourced locations. We tested the sensitivity, specificity, and predictive value of VIA done by nurse-midwives in a less developed country.

Methods: Women were screened by six trained nurse-midwives in a two-phase, cross-sectional study at 15 primary-care clinics in Zimbabwe. VIA and Pap smears were done concurrently, and their sensitivity and specificity compared. Colposcopy, with biopsy as indicated, was used as the reference test to allow a direct comparison of the test unaffected by verification bias.

Findings: 10 934 women were screened. In phase II, 2148 (97.5%) of the 2203 participants for whom there was a screening result also had a reference test result. Also in phase II, VIA was more sensitive but less specific than cytology. Sensitivity (95% CI) was 76.7% (70.3-82.3) for VIA and 44.3% (37.3-51.4) for cytology. Specificity was 64.1% (61.9-66.2) for VIA and 90.6% (89.2-91.9) for cytology.

Interpretation: The high sensitivity of VIA shows that the test could be valuable in detection of pre-cancerous lesions of the cervix. However, there are costs to the patient and system costs associated with high numbers of false-positive results, so attention should be given to improving the specificity of VIA.

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Introduction

In most less-developed countries, cervical-cancer screening programmes are small-scale or non-existent.[1-3] Consequently, there are few opportunities to diagnose pre-cancerous disease, and most patients present with invasive disease at an advanced stage.[3-5] In some less-developed countries Pap-smear-based screening is available, but usually only in urban areas or in the private health sector that serves a small proportion of the female population. Screening programmes based on Pap smears require technical capabilities and systems for transportation, communication, follow-up, and training that are beyond the capacity of healthcare infrastructure in most less-developed countries.[2-6] Thus, other methods of cervical-cancer screening provision have been investigated.[1-6-5]

One such method is visual inspection with acetic acid (VIA). The cervix is washed with acetic acid and then inspected by eye for evidence of disease (also known as cervicoscopy, or direct visual inspection). This has potential advantages over traditional screening techniques in poorly resourced locations –there is immediate feedback of test results to the patient and, importantly, treatment can be provided immediately after the test.[9-11]

Given the potential significance of VIA, we undertook a field-based study in Zimbabwe to screen more than 10,000 women with both, the Pap smear and VIA. The primary endpoint was to assess the specificity and sensitivity of VIA done by non-physicians in a primary-care setting. A secondary objective was to compare these test qualities with those of the Pap smear, the current screening method in Zimbabwe.

Methods

Participants

Our study was a cross-sectional, screening test study that took place between October 1995, and August 1997. Women attending 15 primary-care clinics in Chitungwiza and the greater Harare area, Zimbabwe, were invited to attend a health-education talk on cervical cancer for the purpose of recruitment into the study. The talk was given every morning while women were waiting to be seen for other health matters. After the talk, those interested in being screened were invited to take part in our study. Women aged between 25 and 55 years, who were not pregnant and had no previous history of cervical cancer or hysterectomy, were eligible for enrollment.

Study design

The study had two phases: in both, participants were interviewed by a trained female nurse- midwife who used a standardized questionnaire. The study objectives were explained, and verbal consent was obtained after an informed-consent statement was read out. Immediately after the participant's history was taken, both screening tests were done. Each woman was placed in a modified lithotomy position on an examination table. An unlubricated bivalve speculum was inserted into the vagina by the nurse-midwife and a cervical cytology specimen was obtained with a wooden Ayres spatula. The nurse-midwives were trained to scrape the cervix around the entire transformation zone to obtain an adequate specimen. Immediately after a cytology specimen was obtained, the nurse-midwife cleansed away any excess mucus thoroughly with a saline-soaked swab, and applied a solution of 4% acetic acid to the cervix with a cotton-tipped applicator. With the aid of a handheld flashlight, the nurse-midwife then visually inspected the whole cervix. Categories of VIA findings (panel) were recorded on the study questionnaire.

In phase I of the study, if the VIA assessment showed an abnormal result, the woman was scheduled for colposcopy by a study coordinator. By use of a systematic random sampling scheme, every tenth woman with a normal or atypical VIA assessment was also scheduled for colposcopy. If the Pap smear was abnormal, attempts were made to tell the woman of the result by home visit, letter and communication through non-study clinical staff at the 15 clinics. If contacted, the woman was encouraged to make an appointment for a colposcopy. Colposcopy was done in Harare by three University of Zimbabwe faculty members (ZMC, JK, NH). If no lesion was found during colposcopy, the patient was reassured and asked to return to her local clinic every year for rou-

tine follow-up. Women assessed as abnormal on colposcopy (confirmed, if indicated, by biopsy) were referred for appropriate treatment and followed up according to standard local clinical protocol.

Phase II of the study began about 1 year after the start of phase I, and continued until the end of the study. Phase II differed from phase I primarily in that recruitment took place on a given day, and on the next day all screening and diagnostic tests were done. Thus, in phase II all test-positive and all test-negative women were sent for the reference test. Transport was provided to a Harare clinic, where colposcopy was done by two investigators (EN, ZMC). In phase II, a specimen was obtained from all women to be tested for human-papillomavirus. Human-papillomavirus data were collected to assess the specificity and sensitivity of that test as a single screening test in a less-developed country, and to assess the usefulness of human-papillomavirus tests as an adjunct to VIA screening. These findings will be presented elsewhere.

Outcome measures

The reference standard used in our study was colposcopy with biopsy (whenever the latter was clinically indicated) – a commonly used and accepted reference standard for cervical-cancer screening studies.[9,10,12-16] We defined two thresholds of disease: a low threshold of low-grade squamous intraepithelial lesion (LGSIL) or worse on colposcopy (or their equivalents on biopsy); and a high threshold of high-grade squamous intraepithelial lesion (HGSIL), or worse. LGSIL or more was taken as a positive test for the Pap smear, and, consistent with local cytology norms, atypical squamous cells of undetermined significance and atypical glandular cells of undetermined significance were taken to be test-negative. We defined a positive test for VIA as either abnormal or cancer, as shown by acetowhite lesions and other visual markers of (pre) cancerous lesions of the cervix.

Quality Control

In both phases of the study, Pap smears were analyzed by cytotechnicians in Harare, who were unaware of the VIA results. All positive smears and 10% random sample of smears assessed as negative by each cytotechnician were forwarded for a second assessment by the study cytopathologist (RM). In addition, in phase I of the study, a certified cytopathologist at the Johns Hopkins Bayview Medical Center, Baltimore, MD, USA, reviewed a sample of 10% of all negative and all positive smears. In phase II, all slides reviewed by the local cytopathologist were sent for review by the US cytopathologist. Study protocol called for colposcopy results to be recorded without knowledge of the VIA or Pap smear findings, and for biopsies to be read without knowledge of any test result. The study was approved by the ethics committee or the Zimbabwe Research Council and the Institutional Review Board of the Johns Hopkins Bayview Medical Center, USA.

Personnel were trained and assessed, and clinical standardization was ensured at the start of the study. Refresher training was provided for the nurse-midwives in speculum insertion and Pap collection, followed by practical training in VIA over 3 days. The nurse-midwives were familiarized with the naked-eye appearance of the cervix in various states of health and disease. A pictorial atlas was used for reference during training and during the study.[17]

Over 5 days, the study cytotechnicians took part in a review course designed to standardize their skills run by the study cytopathologist (RM) who continued to work with the technicians for a week. In addition, the US-based cytopathologist visited Zimbabwe for 1 week at the beginning of the study to work with the cytotechnicians and to standardize assessment categories (modified Bethesda system) with the local cytopathologist.

Statistical analysis

The initial sample size for the study was set at 24,000 patients, to allow statistical precision of 0.05. We assumed a 60% sensitivity, the presence of high-grade lesions in 5-6% of patients, and that 10% of all participants who tested negative on all screening would receive the reference test. The overall sample-size estimate was lowered markedly to 12,000 women at the start of phase II of the study, when high-grade lesions were found in around 10% of patients – protocol changes meant that 100% of all test-negative women on screening would be referred for the reference test.

Univariate analyses were done for all questionnaire study variables. Analysis of sensitivity, specificity, and predictive value were done by use of standard formulae for these test qualities.[18] The rate of disease (pre-cancer and cancer) detection was calculated as the number of true-positive results detected by the test divided by the total number of women with a screening result from that test.[19] Exact binomial 95% CIs were calculated for predictive evaluation.[18]

Reference standard results	Phase I (n = 8.731)							Phase II (n = 2.203)								
	VIA negative			VIA positive			Total	VIA result missing		VIA negative			VIA positive			Total
	Pap X	Pap -	Pap +	Pap X	Pap -	Pap +		Pap -	Pap +	Pap X	Pap -	Pap +	Pap X	Pap -	Pap +	
Missing	261	5.712	461	50	507	156	7.147	4	0	2	31	0	2	15	2	56
Negative	20	217	155	34	561	182	1.169	15	1	35	1.128	70	14	570	107	1.940
Positive	6	38	99	12	95	165	415	1	0	3	31	14	3	80	75	207
Total	287	5.967	715	96	1.163	503	8.73	20	1	40	1.190	84	19	665	184	2.203

Pap X = Pap result missing; Pap- =Pap result negative; Pap+ =Pap result positive.

Table 1: Test results by study phase

Results

Our study enrolled 10,934 women (8731 in phase I, 2203 in phase II). 22 other women were recruited but not included in the analysis because important identifying information was missing. In phase I, 1584 (18.1%) women had colposcopy (and biopsy as indicated) as a result of a positive VIA or Pap smear screen, or because they were selected as one of the test-negative women to receive the reference-standard test.

Characteristic	Phase I	Phase II	Total
Demographic	(n = 8.731)	(n = 2.203)	(n =10.934)
Mean (SD) age (years)	32.0 (6.5)	33.2 (7.1)	32.2 (6.6)
Ever married (%)	96.2	94.8	95.9
Educaction (%)			
None	3.3	6.0	3.8
1-7 years completed	44.3	47.3	44.9
8-12 years completed	51.0	46.5	50.1
Higher education	1.3	0.3	1.1
Adult literacy	0.1	0.0	0.1
Sexual			
Men gravidity (SD)	3.4 (2.1)	3.3 (2.3)	3.4 (2.2)
Ever used family planning (%)	91.1	86.2	90.1
Sexually active in past year (%)	100	100	100
STI or suspected STI (%)	45.5	59.6	48.3
Currente STI symptoms(%)	67.8	83.8	71.0
Previous Pap smear (%)	10.2	12.9	10.7

ITS = sexually transmitted infection.

Table 2: Participant characteristics

In phase II, the reference standard test was done on 2147 (97.5%0 of the 2203 women for whom there was a Pap smear or VIA result (*Table I*)

Most of the women were married, all were sexually active, most had used a method of family planning at some time, and few (<15%) had ever been screened for cervical cancer in the past (*Table 2*). Most participants were in their late 20s or early 30s, and had completed either primary or secondary school. These statistics are characteristic of women who attend primary-care clinics in Zimbabwe. There was little difference in baseline characteristics between study phases, except for variables related to sexually transmitted infections. The difference in experience of such infections reflects increased self-selection by women who came to or returned to the clinic specifically for screening in the last year of study.

Test	Numbers tested (%)		
	Phase I	Phase II	Total
VIA			
Normal	2.688 (30,8)	495 (22,7)	3.183 (29,2)
Atypical	4.281 (49,0)	819 (37,5)	5.100 (46,7)
Abnormal	1.747 (20,0)	857 (39,3)	2.604 (23,9)
Cancer	15 (0,2)	11 (0,5)	26 (0,2)
Total	**8.731 (100)**	**2.182 (100)**	**10.913 (100)**
Pap smear			
Normal	4.998 (59,9)	1.300 (60,6)	6.298 (60,0)
Inflammation	1.150 (13,8)	252 (11,8)	1.402 (13,4)
ASCUS911	(10,9) 285	(13,3) 1.196	(11,4)
AGUS	71 (0,9)	38 (1,8)	109 (1,0)
LGSIL	828 (9,9)	196 (9,1)	1.024 (9,8)
SGSIL	371 (4,4)	69 (32)	440 (4,2)
Cáncer de Squamous cancer	19 (0,2)	3 (0,1)	22 (0,2)
Adenocarcinoma	0 (0)	1 (<0,1)	1 (<0,1)
Total	**8.348 (100)**	**2.144 (100)**	**10.492 (100)**

ASCUS=atypical squamous cells of undetermined significance;
AGUS=atypical glandular cells of undetermined significance;
LGSIL=low-grade squamous intraepithelial lesion;
HGSIL=high-grade squamous intraepithelial lesion

Table 3. Results of screening tests

Test	Parameter (95% CI)		
	Phase I	Phase II	Total
VIA			
PPV	25.9% (23.3-28.7)	18.6% (16.1-21.4)	22.7% (20.6-24.6)
NPV	73.3% (69.3-77.0)	96.3% (95.1-97.2)	89.5% (88.0-90.9)
Detection rate*	31/1.000	72/1.000	39/1.000
Pap smear			
PPV	43.9% (39.9-48.0)	33.3% (27.7-39.3)	40.7% (37.4-44.0)
NPV	85.4% (82.9-87.6)	93.9% (92.7-94.9)	91.1% (89,9-92,1)
Dtection rate†	32/1.000	42/1.000	34/1.000

PPV=positive predictive value; NPV=negative predictive value.
* Among the 8731 phase I and 2182 phase II women with an informative VIA result.
† Among the 8348 phase I and 2144 phase II women with an informative Pap smear resultu.

Table 4: Detection rates and predictive values for VIA and Pap smear

VIA assessment was adequately completed for 10,913 women (8731 in phase I, 2182 in phase II). Adequate Pap smears were obtained from 8348 women in phase I and 2144 in phase II. In phase I, 20.2% of the women tested positive for VIA and 14.6% were Pap smear positive. In the second phase, test positive rates were 39.8% and 12.6% for VIA and Pap smear, respectively (*Table 3*).

Among the 8731 women in phase I with definitive Pap or VIA result there were 305 cases of LGSIL, 398 cases of HGSIL, and 17 cancers detected by the reference standard. Among the 2182 women in phase II, there were 294 cases of LGSIL, 204 cases of HGSIL, and three cancers. Of the 602 cases of HGSIL and 20 cancers, 74.8% were confirmed by biopsy.

Table 4 shows detection rates for both tests, by phase and for the study as a whole, together with their predictive values. We used HGSIL or worse on colposcopy or biopsy to define true disease status. *Table 5* shows screening test results according to reference test outcomes for phase II data for both thresholds that we used to define disease. 2130 (97.6%) of 2182 women with a VIA result, and 2092 (97.5%) of 2144 women with an adequate Pap smear had both, a screening-test result and a reference-test result in phase II. *Table 6* lists the test qualities for phase II derived from the data in *table 5*. Phase I sensitivity and specificity values are not shown because, despite the use of randomized-selection protocol in phase I to identify a proportion (10%) of test-negatives to receive the reference test, upon which statistical adjustments for verification bias were to have been made, the sample of VIA test-negative women who actually underwent colposcopy was non-random. Consequently, adjustment for verification bias by use of phase I data was inappropriate, and the unadjusted test-quality results were biased. Verification bias (also called workup bias) occurs when the chances of being referred for the reference test are different for those who test positive on screening and for those who test negative.[20] Such bias inflates estimates of sensitivity and falsely reduces specificity.[21]

Discussion

In more-developed countries, Pap smears have formed the basis of cervical cancer screening and detection programmes for many years. National cytology-based screening programmes have contributed substantially to the marked decline in deaths from cervical cancer in these countries.[22-24] Yet, in many less-developed countries, the technical complexity of cytological testing, and the infrastructure required to implement it, precludes the effectiveness of national Pap-smear programmes.[2,6,19]

Several studies have shown the potential value of VIA (cervicoscopy) as a screening approach in less-developed countries,[9-11,14,25-27] but none established precisely the quality of the VIA test under clinical conditions likely to occur in less-developed countries – screening done by non-physicians in a basic health facility.

In such a setting, our results showed that VIA can effectively identify most cases of cervical pre-cancer and cancer. In phase I, the detection rates for VIA and the Pap smear were equal. In phase II, however, the detection rate for VIA was higher than that for cytology. The detection rate for both tests increased in phase II since 98% of VIA-positive and 99% of Pap-smear-positive women received the reference standard in that phase compared with only 60% (VIA) and 40% (Pap smear) of women in phase I.

The test-positive rate for VIA doubled between phase I and phase II of the study. The priority for the study clinicians was detection of cervical cancer rather

Test	T+, D+	T+, D-	T-, D+	T-, D-
VIA (n = 2.130)				
Low threshold	316	533	182	1,099
High thresold	158	691	48	1,233
Pap smear (n = 2.092)				
Low threshold	144	123	343	1,482
High threshold	89	178	112	1,713

T+=test positive; T-=test negative; D+=disease according to reference test; D-=no disease according to reference test; Low threshold ≥ LGSIL on reference test; High threshold ≥ HGSIL on reference test

Table 5: Phase II screening-test results by reference standard outcomes

Test	Sensitivity (CI de 95%)	Specificity (CI de 95%)
VIA (n = 2.130)		
High threshold*	76,7% (70,3-82,3)	64,1% (61,9-66,2)
Low threshold†	63,5% (59,1-67,7)	67,3% (65,0-69,6)
Pap smear (n = 2.092)		
High threshold*	44,3% (37,3-51,4)	90,6% (89,2-91,9)
Low threshold†	29,6% (25,6-33,8)	92,3% (90,9-93,6)

* ≥HGSIL on the reference test.
† ≥LGSIL on the reference test.

Cuadro 6. Sensibilidad y especificidad de cada prueba en la fase II

than avoidance of over-referral, particularly in phase II, because that protocol called for all women to receive the follow-up test irrespective of their screening result. Given that the nurse-midwives knew that all of their VIA assessment would be confirmed or rejected that same day by a physician who did colposcopy, the nurse-midwives may have defined borderline cases as abnormal in phase II to ensure that they missed as few cases as possible.

The positive predictive value of VIA was lover than that of the Pap smear in both study phases, partly because the specificity of cytology was consistently higher that that of VIA. We could not obtain an accurate estimate of the prevalence of disease from phase I but, given that rates of sexually transmitted infection were different in the two study phases, the incidence of cervical lesions may have differed between phases. Differences in positive predictive value between phases may thus be due to prevalence differences, but are also in part an artifact of biases introduced in phase I that affected our estimates of sensitivity, specificity, and the prevalence of disease. As expected, definition of disease by use of lower threshold (LGSIL or worse) increased the positive predictive value of both tests.

Given that the appearance of high-grade lesions was used to dictate clinical treatment decisions, the high negative predictive value shown by both tests warrants particular mention. In places characterized by similar incidence of cervical disease, including many countries in Africa, Asia and Latin America, the use of VIA as a primary screening test means that women assessed as test-negative would be reassured that most probably they do not have HGSIL or cancer.

Symptoms of sexually transmitted infection were more common among women in phase II of the study, which suggests that the women tested in that phase were a slightly different reference population from that of phase I. Nevertheless, the presence of sexually transmitted infections did not affect screening capabilities between phases in different ways, and the full spectrum of disease was observed in both phases. Therefore, we believe that the sensitivity and specificity results of phase II show the potential for VIA screening from a group of trained nurse-midwives.

Given our study conditions, our results are likely to represent the lower end of the range for sensitivity and specificity of VIA. Higher test qualities for both tests are likely to be observed under better service-delivery conditions (e.g., better lighting, examination tables, speculae) and with more standardized VIA training than was available in our study. Additional improvements in VIA specificity could result from repeat VIA testing in women likely to return to the same site for healthcare, or by use of VIA for triage of those who should undergo further tests when such follow-up is feasible.[10,26,27]

Our results are consistent with recent studies that have shown that VIA is more sensitive but usually less specific than cytology.[19,28] The finding that a visual inspection test can identify a greater proportion of diseased cases under certain clinical circumstances than the Pap smear is not unexpected, since measurements of sensitivity and specificity shown by the Pap smear in various studies are not consistently high and have ranged from 20%-85%.[20,29]

When attempting to diagnose whether a cervix is healthy, or diseased and in need of treatment, the clinician must "find the lesion". In our study, in more than 75% of the cases in which a lesion was found on colposcopy or biopsy, the lesion was also found with simple VIA. We agree with Ottaviano and La Torre[9] that "colposcopy magnification is not essential…[to identify] the cervix at risk". Our data show that VIA could be an acceptable means of screening for cervical cancer, especially in poorly resourced locations. However, the acceptance of VIA depends on the attitudes of policymakers and opinion leaders in various countries. The observed high number of false-positive results for VIA may lead to high rates of referral, and may increase rates of treatment, with the associated potential for increased patients' discomfort and increased numbers of side-effects. Nonetheless, our study shows that VIA can identify most true cases of cervical pre-cancer and cancer. Where large-scale Pap smear screening is not now available and is not likely to be available consistently in the future, VIA could be a readily available, potentially sustainable means of testing that, when coupled effectively with treatment, could reduce the burden of disease in populations in which the incidence of cervical cancer is high. Even where cytology services are well established, VIA might be a cost-effective method of rapidly differentiating between a potentially diseased cervix and a healthy one.

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Visual inspection with acetic acid in the early detection of cervical cancer and precursors

(LETTER TO THE EDITOR. International Journal of Cancer 1999; vol.80: 161–163)

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Dear Sir,

Organized cytology screening programs are not feasible in many developing countries where four-fifths of the world burden of cervical cancer occurs. Among other possible approaches to prevention, the performance of unaided visual inspection (to detect lesions of the cervix) has been shown to be unsatisfactory, particularly in detecting re-invasive lesions (Nene et al…1997: Wesley et al… 1997: Snkaranarayanan et al… 1997). Unmagnified, naked eye inspection of the cervix, after the application of 3-1% acetic acid [termed visual inspection with acetic acid (VIA) or cervicoscopy] has been reported to detect lesions missed by cytology (Cecchini et al… 1993: Le et al… 1993).

Aceto-whitening of cervical epithelium may reflect the presence of an abnormal transformation zone as a result of increased cellular density with increased abnormal nuclei and DNA content. Acetic acid application is routinely used in colposcopic examination to visualize abnormal epithelium. Here, we report a comparison of the performance of VIA and cervical cytology in detecting cervical lesions in a clinic-based study in Kerala, India.

The study subjects were 1,351 women aged 22-70 years attending the Early Cancer Detection Centre (ECDC). Ernakulam, Kerala, India during 1995-1997. These women presented themselves for a routine examination or were referred from elsewhere to rule out cervical pathology. Their mean age was 38.9 years (SD 7.3 years). Their educational levels were as follows: nil: 66 (4.9%); primary school: 305 (22.6%); middle school 221 (16.4%); high school: 495 (36.6%); college and above: 260 (19.2%); not known: 4 (0.3%). They were subjected to speculum examination by a nurse trained in recognizing cervical abnormalites and aceto-white lesions after acetic acid application. The nurse initially recorded the findings of unaided visual inspection, and then took a cervical smear using Ayre's spatula. This was followed by application of 3-4% freshly prepared acetic acid using a cotton swab and the cervix was examined after 1-2 min under adequate light. If aceto-white areas were detected in the cervix, the test outcome was recorded as being positive.

Cervical smears were examined by the cytopathologists (B.S.) of the ECDC, who is also an experienced colposcopist. Cytology was considered to be positive if it revealed any of the following lesions: atypia, mild dysplasia, severe dysplasia, carcinoma in situ *and invasive cancer.*

Subjects with positive VIA or with positive cervical smear were referred for colposcopy. In addition, women with grossly abnormal looking cervix (bleeding on touch, hypertrophied elongated irregular edematous cervix, stippled cervix, suspected growth/ulcer) but with negative VIA and cytology were also referred for colposcopy/biopsy in order to increase the number of participants subjected to verification of their true diseases status and thereby to reduce "verification bias". Those with normal looking cervix, features of inflammation and squamous metaplasia on colposcopy were assumed to be false positives and were not subjected to biopsy. Directed biopsies were performed for those with colposcopic diagnosis of atypia or worse lesions. Those revealing moderate dysplasia or severe dysplasia or carcinoma in situ *or invasive cancer on histology were considered to be true positives. Thus, negative colposcopy and the results of histological diagnosis of colposcopically guided biopsy were considered the gold standard to evaluate the performance of the tests.*

Sensitivity and specificity measure the efficacy of a screening for diagnostic test and the positive and negative predictive values measure its accuracy. Sensitivity and specificity of the screening tests could not be calculated directly as the reference test (colposcopy with or without biopsy) was not applied to all women who were negative on screening. Estimated sensitivity and specificity may be biased ("verification bias") if all study participants are not subjected to the reference test to determine their true disease status. The performances of two tests (cervicoscopy and cytology) can, nevertheless, be compared by means of the following parameters (Schatzkin et al... 1987):

1. *The detection rate of moderate dysplasia or worse lesions (on biopsy): this is calculated by dividing the number of screened women with these lesions by the total number of screened women.*
2. *The ratio of sensitivities between the two tests: this is calculated by dividing the detection rate of VIA by the detection rate of cervical cytology: a ratio of more than unity indicates that the first test is more sensitive. McNemar's test applied to the discordant women was used for significance testing of the difference in sensitivities between VIA and cytology.*
3. *The approximated specificity of each screening test: this is calculated by dividing the number of women with negative screening tests by the total number of screened subjects less the number of true positive cases detected by the test. McNemar's statistics applied to the discordant women was used to assess the statistical significance of difference in specificities. The index (100-specificity) indicates the proportion of screen positive women recalled unnecessarily for follow-up investigations.*
4. *The positive predictive value (PPV): this is obtained by dividing the number of women with true positive screening tests by the total number of subjects with positive screening tests.*

The results of screening tests are given in Table I. *There were 509 (37.7%) women (with aceto-white cervix) positive for VIA: 205 (15.2%) were positive on Pap smear. One hundred seven women had an abnormal looking cervix on direct visual inspection but with negative VIA and cytology. The 83 subjects who did not comply with referral for colposcopy were excluded, yielding a total of 1,268 women for further analysis.*

On colposcopy, 293 women did not reveal abnormality: 162 had inflammation: 44 had features of squamous metaplasia: the remaining 102 revealed colposcopic abnormalities and were subjected to biopsy: of these, 86 (84.3%) women were positive for VIA. On histology, 4 were normal: 2 had inflammation: 9 had squamous metaplasia: 3 had squamous metaplasia with atypia: and 13 had mild dysplasia. The remaining 71 had moderate dysplasia or worse lesions. The distribution of these by results of screening tests is given in Table I*: 68/71 (95.8%) with these lesions were detected by VIA as opposed to 44/71 (62.0%) by cytology. VIA resulted in the detection of a higher proportion of mild and moderate dysplasia compared with Pap smear: both tests detected almost all cases of severe dysplasia or worse lesions among those subjects who had biopsy. VIA detected 25 lesions missed by cytology and cytology I lesion missed by VIA. One of the concerns may be the extent of true positives missed among subjects who were negative for both screening tests. We feel that it may be negligible. In view of the fact that both tests missed only two true positive cases among 107 subjects with negative screening tests but clinically abnormal cervix, who were subjected for reference investigations.*

VIA	Pap smear	Number	Lost to follow-up	Subjected to colposcopy	Subjected to biopsy	Mild dysplasia	Moderate dysplasia	Severe dysplasia	Carcinoma in situ	Invasive cancer
—	—	137	6	131	50	2	5	17	12	9
—	—	372	51	321	41	6	23	2	0	0
—	—	68	26	42	4	3	1	0	0	0
—	—	774	—	107	7	2	2	—	—	—
Total	1,351	83[1]	601	102	13	31	19	12	9	

1 Excluded from final analysis.

Table 1. Distribution of histologically diagnosed moderate dysplasia or worse lesions by outcome of screening tests.

The detection rate of moderate dysplasia or worse lesions by VIA was 53.6/1.000 women as opposed to 34.7/1.000 by cytology, yielding a ratio of sensitivities of 1.54 (χ^2 = 20.3. p>0.001). The approximated specificity of cervicoscopy was 68% and that of cytology was 89.5% ((χ^2 =7.6. p<0.01). The PPV of VIA was 14.8% (68/452) and that of cytology was 25.4% (44/173). In this clinic-based study, with a relatively high prevalence of cervical abnormalities. VIA demonstrated a higher detection rate of moderate dysplasia or worse lesions than cytology. However, it was significantly less specific than cytology and resulted in referral of more than one-third of women for colposcopy. However, its ability to detect preinvasive lesions is of interest.

Any alternative screening test considered in the developing country setting should be sensitive enough to detect pre-invasive lesions. It is easier to treat these lesions and they results in prevention of invasive cancer. Many developing countries do not have sufficient facilities to treat invasive cervical cancer (e.g. radiotherapy) and the outcome from advanced cervical cancer in these settings is not satisfactory (Nandakamar et al.. 1995: Javant et al…1996: Sankaranarayanan et al… 1996,1998). Facilities to manage pre-invasive lesions (colposcopy, LEEP, cryotherapy) can be established at much lower costs than investments for invasive cancer management.

The ability of VIA to detect lesions missed by cytology has been reported by others (Cecchini et al… 1993: Le et al… 1993). In one study, cervicoscopy was found to be more sensitive than cytology in detecting lesions, but resulted in a recall of 25.4% of 2,105 subjects for further investigations, as opposed to 3.8% with cytology (Cecchini et al… 1993). In another study, 85 subjects with aceto-white lesions on the cervix and normal Pap smear were subjected to colposcopy: 34 of them had normal colposcopic appearance and the rest were subjected for biopsy and 13 cervical intraepithelial neoplasia (CIN) lesions were detected among those (Le et al… 1993).

In a study involving 2,426 women in a suburb of Capetown, those positive on VIA or those with squamous intraepithelial lesion (SIL) on cytology were referred for colposcopy and biopsy (Megevand et al… 1996a). Of these, 61 were positive on VIA plus cytology: (15 were positive on VIA only: 254 were positive for cytology only and 2,096 were negative for both VIA and cytology. Of the total 31 histologically detected high-grade SIL lesions in this study, 20 were found positive for both tests: 11 were found positive on cytology only. It was concluded that since VIA detected more than 60% of the high-grade SIL, it warrants consideration as an alternative to cytology in low resource settings.

In a workshop: a review of preliminary or final results from several studies investigating the performance of VIA, with or without magnification, in detecting cervical neoplasia in low resource settings in Asia (India, Indonesia) and sub-Saharan Africa (Kenya, Zimbabwe, South Africa) suggested that VIA performs comparably to the Pap smear and/or other screening tests being investigated in those settings (Gaffikin et al… 1997). Sensitivity for VIA has consistently been measured at between 60 and 70% and specificity at approximately 70%.

Although the high sensitivity of VIA is offset by lower specificity, the increased costs associated with more false positive referrals can be reduced if follow-up colposcopy is performed immediately (during the same visit), even by trained para-medical staff. The feasibility of offering colposcopy and large loop excision of the transformation zone under local anesthesia during the same visit, following a positive screening test, has been well demonstrated in South Africa (Megevand et al… 1996a.b.). Furthermore, improved techniques (recognizing artifacts due to glare from the light source, wiping away extraneous mucus and secretions), and referral of those with dull aceto-white areas that cannot be wiped away and not those with faint and suspicious aceto-white lesions, may reduce false positives without compromising sensitivity. Rigorous training of providers in correctly recognizing aceto-white lesions may further

improve specificity and reduce false positive referrals. These improvements may open up new opportunities for disease control such as one-stage testing with VIA and treatment with options such as cryotherapy/LEEP, keeping the proportion (false positives) treated unnecessarily as low as possible. In one of our recently concluded studies in another location in India, 10% of the participants were scored as having positive VIA (data not shown).

The non-invasive nature and the easy applicability of the test coupled with the immediate availability of results facilitating colposcopy and treatment of pre-invasive lesions at the time of examination make VIA an attractive screening test. The usefulness of VIA, both in screening for cervical cancer in developing countries and as a case-finding tool in actual clinical practice settings, certainly merits further evaluation.

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Visual Inspection of the Uterine Cervix after the application of Acetic Acid in the Detection of Cervical Carcinoma and its Precursors

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Background: Organized cervical cytology screening programs are not feasible in many developing countries where cervical carcinoma is an important cause of mortality among adult women. This study compared visual inspection of the cervix after application of
3-4% acetic acid (VIA, or cervicoscopy) with cytology as methods for the detection of cervical carcinoma and its precursors.

Methods: Three thousand women were examined by both VIA and cytology. Those positive on one or both of the screening tests (n = 423) or those who had clinically suspicious lesions even if tests were negative (n = 215) were invited for colposcopy. Directed biopsies were obtained from 277 of 573 women at colposcopy. Those with moderate dysplasia or worse lesions diagnosed by histology were considered true-positives. Those with no lesions or with reactive or reparative changes at colposcopy and those for whom histology revealed no pathology, reactive or reparative changes, atypia, or mild dysplasia were considered false-positives. The detection rate of true-positive cases and the approximate specificity of the two tests were compared.

Results: VIA was positive in 298 women (9.8%), and cytology was positive (for atypia or worse lesions) in 307 women (10.2%). Of the 51 true-positive cases (20 cases of moderate dysplasia, 7 of severe dysplasia, 12 of carcinoma *in situ*, and 12 of invasive carcinoma), VIA detected 46 (90.1%) and cytology 44 (86.2%), yielding a sensitivity ratio of 1.05. VIA detected five lesions missed by cytology, and cytology detected three missed by VIA; both missed two lesions. The approximate specificities were 92.2% for VIA and 91.3% for cytology. The positive predictive value of VIA was 17.0% and that of cytology was 17.2%.

Conclusions: These results indicate that VIA and cytology had very similar performance in detecting moderate dysplasia or more severe lesions in this study. VIA merits further evaluation as a primary screening test in low-resource settings.
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KEYWORDS: cervical carcinoma, screening, visual inspection, control, prevention, cervicoscopy, visual inspection after acetic acid application (VIA), cytology, developing countries.

It has been well established that organized cytology screening programs can substantially reduce the incidence of and mortality from cervical carcinoma in developed countries.[1-3]. Such models of screening, based on call, recall and repeat cytology at regular intervals over a long period of time, are difficult to organize in most developing countries due to a variety of fiscal and technical constraints. On the other hand, cervical carcinoma continues to be a major public health problem in these countries, and cervical carcinoma control measures are a major priority in reducing premature death and disability during the most productive period in a woman's life.

Low-intensity cytology (a smear once in a lifetime after age 35 years or at 10-year intervals) and simple visual inspection of the cervix have been suggested as possible alternatives to organized cytology for the control of cervical carcinoma in developing countries.[4-5]

In a previous study, we investigated the performance of direct visual inspection of the cervix by health workers, without acetic acid application, and found that this test was very non-specific and not capable of detecting a large proportion of preinvasive lesions.[6-7] The study reported here was carried out to evaluate the performance of unmagnified naked-eye visual examination of the uterine cervix after application of 3-4% acetic acid, termed "VIA" or "cervicoscopy", in detecting cervical lesions.

Acetic acid application is routinely used in colposcopic examination to visualize abnormal epithelium. Acetic acid causes dehydration of the cells and some surface coagulation of cellular proteins, thereby reducing the transparency of the epithelium. These changes are more pronounced in abnormal epithelium due to a higher nuclear density and consequent high concentration of protein. It is possible to recognize acetowhitening of the cervical epithelium with the naked eye, and this constitutes a positive VIA test. We report in this article the results of a comparison of the performance of VIA and cervical cytology in detecting cervical carcinoma and its precursors.

Material and methods

The subjects studied were 3000 women (mean age, 43.4 years) residing in Kerala, India, during the years 1996-1997. They were recruited from among women attending open access cancer detection clinics conducted as part of the community outreach programs in different regions of southern Kerala, by the Community Oncology division of the Regional Cancer Centre, Trivandrum. The study protocol was reviewed and approved by the institutional ethical committee, and informed consent was obtained from each of the participating subjects. Their characteristics are given in *Table 1*: the mean age at marriage of the participants was 20.8 years, and the mean age at first childbirth, 22.4 years; one fifth of subjects had had 5 or more pregnancies, and fewer that 1 in 5 were illiterate.

Speculum examination was performed by one of two trained female cytotechnicians. Their training was provided by a gynecologist and a pathologist during a 2-month period before the onset of the study. It included direct visual inspection of the cervix (without any magnification), recognition of acetowhite lesions after the application of acetic acid, and identification of macroscopic abnormalities such as cervicitis, cervical warts, polyps, erosions, nabothian cysts, bleeding erosions, stippled cervix, irregular edematous elongated cervix, hypertrophied hard indurated cervix, growths and ulcers. Their training also included the observation of colposcopy in the women with and without lesions, so that the cytotechnicians could appreciate the pathologic significance of the acetowhite lesions.

The cytotechnicians initially recorded the findings of direct visual inspection, and then took a cervical smear using Ayre's spatula for cytologic examination. The smears were immediately fixed in a mixture of 50% ethyl alcohol and 50% ether. This was followed by application of 3.4% acetic acid on the cervix using a thick cotton swab; the cervix was then examined after 1-2 minutes under adequate light directed from a halogen lamp or torch for the determination of any dull or bright white (acetowhite) areas. No magnification was used to visualize the cervix. The test outcome was considered positive if any distinct acetowhite area was detected on the cervix. If no acetowhite areas were detected or if the whitish appearance was doubtful or faint, the test was scored as negative. All polyps with faint acetowhite staining were scored as negative.

Nabothian follicles (which are whitish even before acetic acid application) become more prominent after acetic acid application, and this was also scored as negative. The grapelike glands of the endocervix with their

Characteristic		Findings (%)
Age in years	20-29	185 (6.2%)
	30-39	954 (31.8%)
	40-49	1.124 (37.5%)
	50-59	478 (16.0%)
	60-69	201 (6.6%)
	70-	58 (1.9%)
Mean age at menarche		14.6 (DE 2.4 años)
Mean age at marriage		20.8 (DE 4.1 años)
Mean age at first childbirth		22.4 (DE 4.1 años)
Education	None	434 (14.5%)
	Elementary school	499 (16.6%)
	Secondary school	1.543 (51.5%)
	University	524 (17.5%)
Marital Status	Married	2.525 (84.2%)
	Widowed	311 (10.4%)
	Separated	164 (5.4%)
No. of pregnancies	1-2	996 (33.2%)
	3-4	1.361 (45.4%)
	5-6	421 (14.0)
	7-	222 (7.4%)

SD: Standard deviation

Table 1. Subject Characteristics

lining of columnar epithelium are slightly paler than the ectocervix after the application of acetic acid. In erosions, the endocervix is even paler than the ectocervix after acetic acid application. These were not scored as acetopositive.

Cervical smears were examined at the Cytopathology Department of the Regional Cancer Centre, Trivandrum, which is one of the accredited laboratories of the Indian Academy of Cytologists. The cytotechnologists and cytopathologists were unaware of the results of the VIA. Cytology was considered positive if it revealed any of the following lesions: atypia, mild dysplasia, moderate dysplasia, severe dysplasia, carcinoma *in situ*, or invasive carcinoma. Negative smears included those with normal cytology, reactive or reparative changes, inflammation, and infections not related to human papillomavirus (HPV).

Subjects with a positive VIA or with a positive cervical smear were invited for diagnostic investigation by colposcopy = biopsy at the Regional Cancer Centre. In addition, those with a macroscopically abnormal-looking cervix (bleeding on touch, bleeding erosion, suspected growth/ulcer, hypertrophied edematous elongated cervix, irregular hard indurated cervix, or stippled cervix) but with negative VIA and Papanicolaou (Pap) smear were also referred for colposcopy and biopsy.

Colposcopy and biopsy were not carried out if there was not abnormality on visual inspection or cytology.

The delay between the screening tests and colposcopy ranged from 3 to 80 days. At colposcopy, biopsies were obtained from acetowhite areas as well as other suspicious areas. Those with no lesions, or with features of reparative and reactive changes (e.g., inflammation or squamous metaplasia) on colposcopy were not subjected to biopsy and were assumed to be false-positive cases. Those women with no lesions, inflammation, squamous metaplasia, atypia, condyloma due to HPV infection (koilocytosis), or mild dysplasia on histologic examination of biopsy specimens were also considered false-positive cases. Those revealing moderate dysplasia or worse lesions on hystopathologic examination of biopsy specimens were considered true-positive cases.

Sensitivity and specificity of the screening tests could not be calculated directly, as the reference test was not applied to all women who were negative on screening. The performances of two tests (VIA and cytology) can nevertheless be compared by means of the following parameters:[8]

1. The detection rate of moderate dysplasia or worse lesions (on biopsy): This was calculated by dividing the number of screened women with these lesions by the total number of screened women.
2. The ratio of sensitivities between the two tests: This was calculated by dividing the detection rate of VIA by the detection rate of cervical cytology; a ration of more than unity indicated that the first test was more sensitive. McNemar's test applied to the discordant women was used for significance testing of the difference in sensitivities between VIA and cytology.
3. The approximate specificity of each screening test was calculated by dividing the number of women with negative screening tests by the total number of screened subjects minus the number of true-positive cases detected by the test. McNemar's statistics applied to the discordant women was used to assess the statistical significance of the difference in specificities. The index (100-specificity) indicates the proportion of women who were positive at screening and were recalled unnecessarily for follow-up investigation.
4. he positive predictive value: This was determined by the number of women with true-positive screening tests divided by the total number of subjects with positive screening tests.

Results

Of the 3000 women, 182 (6.1%) were positive on both VIA and cytology; 116 (3.9%) were positive on VIA only, and 125 (4.2%) were positive on cytology only (*Table 2*). Thus, 298 (9.9%) had acetowhite lesions on naked-eye inspection, and 307 (10.2%) had atypia or worse lesions on Pap smears. In addition, 215 (7.2%) women had an abnormal cervix on speculum examination but negative VIA and Pap smears, and they were also referred for colposcopy. Of the 638 women who were referred for colposcopy, 573 (89.8%) underwent this procedure. Directed biopsy was performed on 277 women (43.4%) who had abnormal findings on colposcopy (*Table 2*). The 65 women who were referred but did not undergo colposcopy were excluded from further analysis, leaving a total of 2935 subjects.

The histopathology results of the biopsies from 277 subjects are shown in *Table 3*. Of the 51 women with moderate dysplasia or worse (true-positive cases), 46 were detected by VIA, yielding a detection rate of 15.7 per 1000; cytology detected 44 lesions, corresponding to a detection rate of 15.0 per 1000. The ratio of sensitivities was 1.05, and there was non-significant difference (P 0.25). Both tests were positive for 80% of true-positive lesions. VIA detected 5 lesions (4 moderate dysplasia, 1 carcinoma *in situ*) missed by cytology, whereas the latter detected 3 lesions (2 moderate dysplasia and 1 invasive carcinoma) missed by the former.

Among the 215 cases for whom both tests were negative but who had colposcopy performed 2 cases of moderate dysplasia and 5 cases of mild dysplasia were detected. Whereas cytology resulted in the detection of 79% (61 of 77) of mildly dysplasic lesions, VIA detected 70% (54 of 77) of these lesions, 6.5% of these had been missed by both tests. The approximate specificity of VIA was 92.2% and that of cytology was 92.7%, and the difference was not statistically significant (P > 0.25). The positive predictive value of VIA was 17.0% (46 of 270) and that of cytology was 17.2% (44 of 256).

Of the 20 cases of moderate dysplasia, 15 subjects underwent a loop electrosurgical excision procedure (LEEP). Of the seven cases of severe dysplasia, five had LEEP, one had conization, and one was treated with hysterectomy. Nine cases of carcinoma *in situ* had hysterectomy, two had LEEP, and 1 had conization. The International Federation of Gynecology and Obstetrics stage[9] distribution of 12 biopsy-proven invasive carcinomas was as follows: IA, 2 cases; IB, 3 cases; IIA, 1 case; IIB, 5 cases and IIIB, 1 case. Eight of the invasive carcinomas were treated with a combination of external radiotherapy and intracavitary radiation; two with intracavitary radiation alone, and two with Wertheim's hysterectomy.

VIA	Pap smear	Number	Subjected to colposcopy	Subjected to biopsy
+	+	182	168	108
+	-	116	102	60
-	+	125	88	36
-	-	2,577	215	73
Total		3,000	573	277

VIA: Visual inspection of the uterine cervix after the application of acetic acid.

Table 2. Results of Screening Tests

VIA	Pap smear	Inflammation and infection	Squamous metaplasia	Squamous metaplasia with atypia	Mild dysplasia	Moderate dysplasia	Severe dysplasia	Carcinoma *in situ*	Invasive carcinoma	Others	Total
-	-	9	2	8	43	12	7	11	11	5	108
-	-	23	13	2	11	4	0	1	0	6	60
-	-	10	0	3	18	2	0	0	1	2	36
-	-	50	2	4	5	2	0	0	0	10	73
Total		92	17	17	77	20	7	12	12	23	277

VIA: Visual inspection of the uterine cervix after the application of acetic acid.

Table 3. Distribution of Histologic Findings by Results of Screening Tests (Positive Cytology Defined as Atypia or Worse Lesions)

VIA	Pap smear	Number	Subjected to colposcopy
+	+	158	146
+	-	140	124
-	+	83	46
-	-	2,619	257
Total		3,000	573

VIA: Visual inspection of the uterine cervix after the application of acetic acid.

Table 4. Results of Screening Tests (Positive Cytology Defined as Mild Dysplasia or Worse Lesions)

Discussion

This study was conducted as part of our effort to evaluate the performance of visual inspection-based screening approaches in the detection of cervical lesions. Our previous studies[6,7] and a review of other studies [10] in India indicated that a simple visual approach involving direct unmagnified inspection of the uterine cervix without acetic acid application ("down staging") was not satisfactory in the early detection of cervical carcinoma and precursor lesions. It has both, poor sensitivity and poor specificity in the detection of lesions, particularly preinvasive ones. This is not surprising, given the wide variability in the appearance of the cervix in a population in which obstetric trauma to the cervix is frequent, and in which cervical and vaginal infections are common.

Several reports suggest that visual inspection of the uterine cervix after the application of 3-5% freshly prepared acetic acid can lead to the satisfactory detection of cervical lesions[11-17] and lesions missed by cervical cytology,[12,13] and we wished to evaluate this technique provided by paramedical workers in India. The results of the current investigation indicate that VIA and cytology have almost the same performance in detecting cervical lesions. The proportion of women referred by each test for colposcopy was similar.

We used a low threshold to define a positive cytology (by including atypia) in order to ensure as many true-positive lesions as possible. If we had used a higher threshold to define positive Pap smears as those with mild dysplasia or worse lesions (excluding atypia), then cytology would have resulted in a referral rate of 8.0% (n = 241) (*Table 4*). However, cytology (with a detection rate of 14.7 per 1000 women) had almost the same sensitivity as VIA (with a detection rate of 15.7 per 1000 women) in the detection of lesions with this new definition (*Table 5*), but with a slightly improved specificity (95.4% for cytology as opposed to 92.2% for VIA), which was statistically significant ($P < 0.001$).

In an Italian study involving 2400 women, colposcopy identified an atypical transformation zone (ATZ) in 312 women; 307 (98.4%) of whom were identified as having distinct white cervical epithelium in naked-eye examination, after acetic acid application by medical student[11]. Histologic examination biopsies from the 312 ATZ revealed benign lesions in 169 (54.2%) and cervical intraepithelial neoplasia (CIN) 1 or worse lesions in 143 (45.8%). This is one of the earliest reports indicating that a cervix at risk can be identified by recognizing acetowhite areas with the naked eye.

Another Italian study involving 2105 women compared VIA (provided by two smear takers) cervicography (projected magnified inspection of diapositive pictures of acetic acid impregnated cervix), and cytology, and reported positivity rates of 25.4%, 15.3% and 3.8%, respectively, with these procedures*12*. Among 486 women with at least one positive test reporting for colposcopy, directed biopsies were performed in 281. Cytology, cervicography and VIA detected 5, 5, and 7 of the 8 CIN lesions detected from among them. VIA was found to be less specific but more sensitive than a Pap smear. In another study, 85 subjects with suspicious acetowhite lesions and normal Pap smears were subjected to colposcopy; 34 (40%) had normal colposcopic examinations, and the rest were subjected to biopsy, among which 13 CIN lesions were detected[13].

VIA	Pap smear	Inflammation and infection	Squamous metaplasia	Squamous metaplasia with atypia	Mild dysplasia	Moderate dysplasia	Severe dysplasia	Carcinoma *in situ*	Invasive carcinoma	Others
+	+	7	1	3	39	12	7	11	11	5
-	-	25	14	7	15	4	0	1	0	6
-	+	2	0	1	12	1	0	0	1	1
-	-	58	2	6	11	3	0	0	0	11
Total		92	17	17	77	20	7	12	12	23

VIA: Visual inspection of the uterine cervix after the application of acetic acid.

Table 5. Distribution of Histologic Findings (Positive Cytology Defined as Mild Dysplasia or Worse Lesions)

In a study involving 2426 women, conducted in a suburb of Capetown, South Africa, those positive on VIA or those with squamous intraepithelial lesions (SILs) on cytology were referred for colposcopy and biopsy[14]. Of the participants in this study, 61 were positive on VIA by trained nurses plus cytology, 15 were positive on VIA only, 254 were positive for cytology only, and 2096 were negative for both, VIA and cytology. Of the total of 31 histologically detected, high-grade SIL lesions in this study, 20 were detected by both tests and the remaining 11 by cytology. The authors concluded that because VIA detected more than 60% of the high grade SILs, it merited consideration as an alternative to cytology in low-resource settings.

A recent study involving 5692 women aged 16-60 years, conducted in southern California, utilized "speculoscopy" (4-6 times magnified examination of 5% acetic acid-impregnated cervix by a hand-held monocular optic to detect acetowhite areas, with illumination provided by a chemiluminescent light source in the upper blade of the vaginal speculum) performed by 186 trained practitioners[15]. On speculoscopy, 688 women (12.1%) were positive, and 151 (2.7%) were positive on cytology (low grade SILs or worse lesions). Of the 799 women positive for 1 or both tests, 410 attended for colposcopy, and biopsies were obtained from all of them. A total of 32 high grade SILs and 191 low grade SILs were diagnosed histologically. Speculoscopy identified 18 (56.3%) of the high grade SILs and 167 (87.4%) of the low-grade lesions; cytology identified 21 (65.6%) and 37 (19.4%) of the high and low grade lesions, respectively. Thus, the addition of magnified visual inspection of the acetic acid-treated cervix greatly improved the sensitivity of cytology.

In a workshop, a review of preliminary or final results from several studies investigating the performance of VIA, with or without magnification, in detecting cervical neoplasia in low-resource settings in Asia (India and Indonesia) and sub-Saharan Africa (Kenya, Zimbabwe, and South Africa) suggested that VIA performs comparably to the Pap smear and/or other screening tests being investigated in those settings[16]. Sensitivity for VIA was consistently 60-70% and the specificity approximately 70% in the studies reviewed.

In an early-detection clinic-based study conducted by the authors in Ernakulam, India, involving 1351 women, 37.7% were positive on VIA carried out by trained nurses and 15.2% on cytology; 494 were subjected to colposcopy and 95 to biopsy (unpublished data). VIA detected 95.8% of 71 biopsy-proven moderate dysplasia or worse lesions as opposed to 62.0% by cytology, yielding a ratio of sensitivity of 1.54; however, it had a lower specificity (67.8%, as opposed to 98.6% for Pap smear).

The results of the current study and other reported studies indicate that VIA is a simple, objective test. The result of this procedure (positive or negative) is available immediately, allowing an algorithm of further investigations to be carried out for the identification of cervical lesions. It has been shown that follow-up colposcopy and treatment of preinvasive lesions can be performed immediately (during the same visit), which not only avoids recalls but also increases compliance to diagnostic investigation and treatment. The feasibility of offering colposcopy and large-loop excision of the transformation zone under local anesthesia during the same visit, following a positive screening test, has been well demonstrated in South Africa[14-17].

The test is not expensive, and it is possible to train providers (both, medical and paramedical) to detect acetowhite lesions with the naked eye. Whether magnification will improve the results of the procedure over and above naked-eye examination is currently not clear. However, a magnified examination can be carried out routinely without additional costs.

Notwithstanding the above advantages, the major concern is low specificity (a high false-positive rate), which means that many subjects must be recalled for colposcopy. The frequency of referral after VIA ranged from 3.1% to 38.7% in reported studies[11-16] . It seems that the objectivity of the test can further be improved with adequate training of the providers and possibly by magnification. We believe that the low proportion of acetowhite cases in our study was due to *1)* the prolonged training of our workers, *2)* including colposcopy sessions in the training, and *3)* scoring only those with distinct acetowhite areas on the cervix as positive and not including those with faint and suspicious whitish appearances. However, another explanation for the low proportion of acetopositivity is that the subjects in this study more or less resembled the general population as opposed to selected diseased populations attending the clinic-based studies. The proportion of acetopositive subjects was approximately 12-13% in 2 other studies[11-14] indicating that the proportion recalled for further investigations and false-positives may be considerably reduced by technical refreshments and in general populations. Despite this, it does seem likely that a higher proportion of subjects will require follow-up after VIA than with good quality cervical cytology. However, because the result of VIA is immediately available, colposcopy and treatment of preinvasive lesions could be performed during the same visit, which will certainly have favorable implications for the costs of screening. Alternatively, because treatment (cauterization or cryotherapy) may have a low risk of morbidity, treatment of a large number of false-positives may be judged as an acceptable price to pay for the effective control of cervical carcinoma.

The inability to identify the false-negative rate in this study may be of some concern. However, the fact that only 2 cases of moderate dysplasia and 5 cases of mild dysplasia were detected among 215 women who were negative according to the two tests (and these were women with some visual abnormality present) provides an indication that the false-negative rate is unlikely to be high in the studied population. Future studies on performance evaluation of VIA may consider colposcopy and biopsy of all subjects to establish true-positive and false-negative rates precisely. We have now initiated such a study.

It is important for this technique to be evaluated further in different settings by different providers. In the event of consistent satisfactory performance, it is likely to find applications in two settings: First, in developing countries, where it is no feasible to introduce cytology screening of acceptable quality for many years to come, VIA may find a place as an alternative low-technology and low-cost method of screening and case-finding. Second, in developed countries, it may be useful as an adjunct to improve the sensitivity of cervical cytology in detecting lesions, as it is well documented that cervical cytology is associated with substantial false-negative rates even in the best laboratories because of sampling and interpretation errors[18]. In both, the settings, cost considerations are important in view of the additional colposcopy sessions required as a result of somewhat high recall rates.

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Acetic Acid Visualization of the Cervix: An Alternative to Cytologic Screening

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Objective: To investigate the value of acetic acid visualization of the cervix as an alternative to cytologic screening.

Methods: A prospective study was conducted in a squatter area in Cape Town, South Africa, on 2426 women who underwent speculum examination, naked-eye inspection of the cervix after application of acetic acid, and cytologic smear. The smears were stained and processed at the screening site. Patients with a positive reading after acetic acid or a smear indicating a high-grade squamous intraepithelial lesion (SIL) were referred for immediate colposcopy, biopsy, and when indicated, treatment by large loop excision of the transformation zone. Therefore, histology was obtained on all patients with a positive acetic acid test or a positive cytology.

Results: Seventy-six women with positive reactions to acetic acid. Among the 2350 women with negative reactions, 254 had positive cervical smears; only 11 of these had histologic high-grade SIL. In contrast, 20 of the 61 women with positive cytology and positive acetic acid test had high-grade SIL on histology. Therefore, the acetic acid reaction enabled the observer to detect 20 of the 31 women (64%) who exhibited a high-grade SIL, both on cytology and histology.

Conclusion: In locations where access to cytopathology is limited, naked eye visualization of the cervix after application of diluted acetic acid warrants consideration as an alternative in the detection of cervical premalignant lesions. *(Obstet Gynecol 1996;88:383-6)*.

Cervical cancer is the most common cancer among women in developing countries.[1] In Africa, it is estimated that there are 36,900 new cases each year.[2] In South Africa, cervical cancer accounts for 32.7% of all cancers seen in black women. At least one in 23 black women will develop cancer of the cervix in her lifetime. Most patients still present at an advanced stage of disease.[3]

Cytologic screening of the cervix with appropriate treatment and follow-up is the only proven strategy for the prevention of cervical cancer. Developing countries lack the human, technical and financial resources to maintain and develop cytologic screening programs. Alternative methods for the detection of premalignant disease of the cervix are being investigated.

We performed this prospective study to determine if direct visualization of the cervix after application of acetic acid would be an adequate alternative to cytology in the detection of premalignant lesions of the cervix.

Materials and Methods

Under the auspices of the Cancer Association of South Africa, a pilot project was initiated to assess the feasibility of cervical screening coupled with immediate on-site treatment of detected abnormalities.[4] A fully equipped mobile clinic was taken to a squatter area in Cape Town, and the patients were drawn from the resi-

dent population. Education was part of the project, which aimed to create cancer awareness among suburban black communities in Cape Town. The educational team consisted of a professional nurse acting as a coordinator, a nurse specially trained to take cervical smears, and community health workers. Education under the supervision of a qualified gynecologist trained in oncology and colposcopy (EM) was provided on-site starting 1 week before the arrival of the clinic. We used existing facilities, mainly primary health care centers, family planning clinics, schools and churches. The focus was on cancer awareness and prevention. For the purpose of the study, a mass screening program, offered as a free service, was undertaken on all sexually active women who had not had smears taken during the past year, irrespective of age.

Each woman had a speculum examination, after which a 5% acetic acid solution was applied to the cervix. After 1 minute, the cervix was illuminated by a 100-watt lamp and examined with the naked eye by a trained nursing sister. Acetowhite areas were noted and, immediately thereafter, a cervical smear was taken with a wooden spatula. The cervical smears were processed and screened in the mobile clinic by a cytotechnologist who was aware of the study.

All women with a positive acetic acid test (i.e., the presence of acetowhite area[s] on the cervix) or a positive cytology (i.e., cervical smears showing the presence of squamous intraepithelial lesions [SIL]) were referred for colposcopy, which was performed in the mobile clinic immediately or within 3 days. If the features were consistent with high-grade SIL and the colposcopy fulfilled the criteria for local treatment of pre-invasive disease,[5] the patient was treated in the mobile clinic with large loop excision of the transformation zone using local anesthetics. If the colposcopic diagnosis was low-grade SIL, a punch biopsy of the most abnormal area was taken; if the histology showed features of low-grade SIL, the patients were followed-up. Thus, histology was obtained on all women with a positive acetic acid reaction or abnormal cytology. We compared the management results of two clinical situations, abnormal cervical smear and abnormal acetic acid test.

Results

From February to September 1994, 2426 women attended the mobile clinic. The mean age was 31 years (range 20-83), mean age at first sexual intercourse was 16 years (range 12-36), and mean parity was three (range 0-12). One hundred two patients (4%) had had a previous cer-

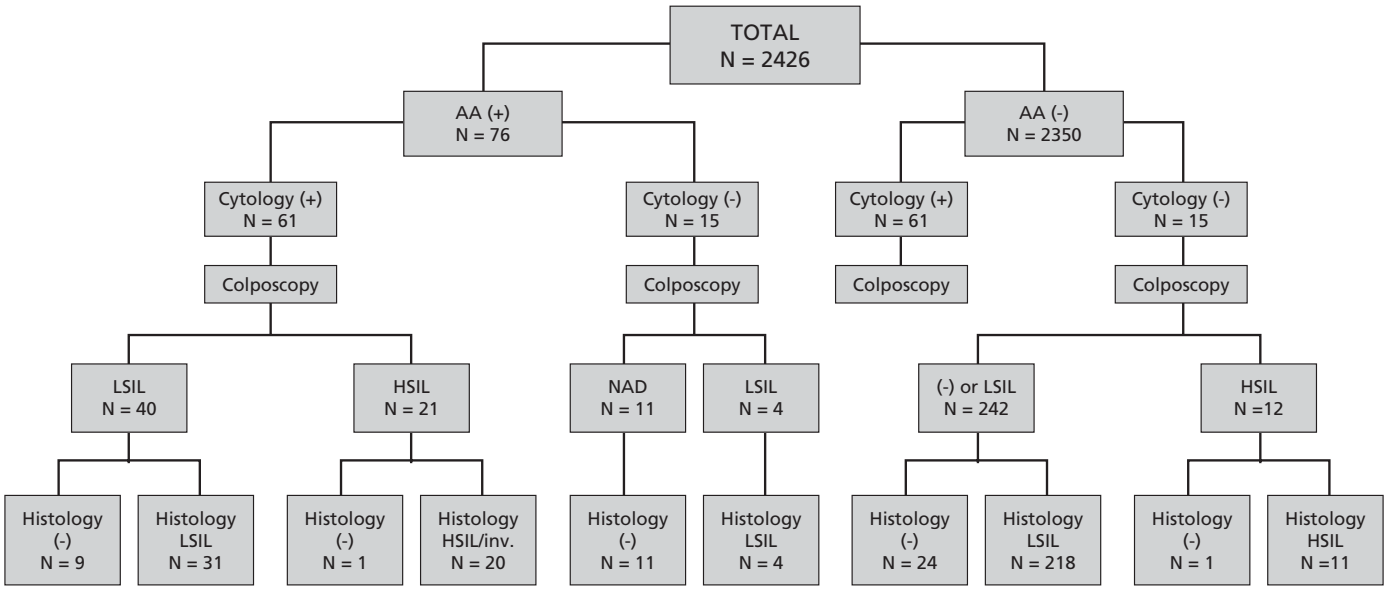

Figure 1. Acetic acid test results compared with cytology and, when indicated, histology.
AA+ = positive reaction after application of acetic acid (ie, acetowhite area on naked eye); AA- = negative reaction after application of acetic acid (ie, no acetowhite area visualized); Cytology+ = cytologic evidence of squamous intraepithelial lesion or human papillomavirus changes; Cytology- = no cytologic abnormality detected; LSIL = low-grade squamous intraepithelial lesion; HSIL = high-grade squamous intraepithelial lesion; HSIL = high-grade squamous intraepithelial lesion; INV = histologic signs of invasion; NAD = no abnormality detected.

vical smear. The clinical findings included three cases of invasive carcinoma (two stage Ib and one stage IIb), two prolapsed myomas, and one vagina metastasis of choriocarcinoma; all six patients were referred to Groote Schuur Hospital for further management.

Seventy-six women had positive reactions to acetic acid. Subsequent cervical smears revealed SIL in 6` and no evidence of SIL in 15. Among 2350 women with negative reactions to acetic acid, 254 had positive cervical smears; only 11 of these had high-grade SIL on cytology, confirmed on histology. In contrast, 20 of the 61 patients who had both, positive acetic acid tests and positive cervical smears had high-grade SIL or signs of microinvasion on histology. Thus, 20 of the 31 patients (64%) who had high-grade SIL on both, cytology and histology had a positive reaction to acetic acid. Of note, 15 women with a positive reaction to acetic acid had negative cytology; four of these had histologically proven low-grade SIL, indicating false-negative cytology. (*Figure 1*).

Patients with negative tests did not undergo colposcopy or large loop excision of the transformation zone to confirm the negative test results. Therefore, the accuracy of the tests in the screening for cervical abnormality is not known. However, because 55 of the 76 women with positive acetic acid tests had lesions proven on histology, the positive predictive value can be calculated as 72.4% for acetic acid testing. Similarly, 280 of the 315 women with positive cytology had a positive histology. Therefore, the positive predictive value for cervical cytology is 88.9%.

Discussion

Despite the prevalence of cervical cancer in developing countries, few effective screening programs are available. The reasons for this include the lack of trained personnel, laboratory facilities, and equipment; the high cost of services; and the difficulty in following-up patients. It has become necessary to find affordable alternatives that are sufficiently sensitive and specific to substitute for cytologic screening.

Some clinicians use acetic acid alone to evaluate the uterine cervix. Indeed, some continuing medical education courses have suggested that acetic acid is a good form of screening. Our aim in this study was to investigate the value of acetic acid visualization of the cervix as an alternative to cytologic screening. For this purpose, our clinical approach used cytology and the acetic acid reaction as screening methods and we compared the outcomes of these two screening tests.

Considering the 254 patients with a negative acetic acid test but a positive cytology for SIL, one would assume the sensitivity of the test to be low. However, it cannot be calculated because patients with negative tests did not undergo colposcopy, biopsy, or excision of the transformation zone to confirm the negative test results. The positive predictive value of the tests, 72% for the acetic acid and 89% for cytology, are still accurate and comparable. The higher number of false-negative tests with the acetic acid reaction than with cytology precludes its use as a screening method for premalignant cervical lesions. The cytotechnologist, aware that a study was being conducted, may have been more vigilant in reviewing smears, which would improve the sensitivity of cytology.

The understanding of the natural history of cervical cancer is that mild, moderate, and severe dysplasia represent successive stages in the pathogenesis of this disease. Considering the low risk of progression to invasive cancer in patients with cytologic low-grade SIL,[6] it seems reasonable to focus on screening for high-grade lesions in countries where cytologic cervical screening opportunities are limited. Compared with cytology, the acetic acid test enabled the observer to detect 64% of histologic high-grade SIL. In other words, in a high-risk population and when no other screening program is available, the use of this test could detect two-thirds of high-grade premalignant cervical lesions and prevent a number of malignancies at a low cost. Additionally, in this study, direct visualization of the cervix identified three cases of invasive carcinomas at early stages.

Cervical cytology remains the best method available for the detection of premalignant cervical lesions, with a sensitivity ranging between 42 and 89%.[7,8] Other sophisticated approaches, such as the expensive automated cytologic screening or the use of human papillomavirus typing, remain experimental and are not available in developing countries. Some researchers have explored various alternatives to cytologic screening. In India, direct visualization of the cervix was tested on 11,760 patients.[9,10] Visualization detected 52% of the lesions (early cancers), whereas 71% were detectable on cytology. It is not yet clear whether this approach is practical or whether it can contribute to the control of cervical cancer in developing countries; its major disadvantage is that it detects early invasive cervical cancer rather than preinvasive disease. In a study of 2400 patients,[11] naked-eye inspection of the cervix after application of acetic acid was compared with colposcopy. Only 46% of the lesions detected were proved histologically. The report did not comment on the correlation with cytology. In a series of 65 patients,[6] screen-

ing for SIL using cervicography was found to be more sensitive (83%) than the Papanicalaou smear (42%), although the tests appeared to be equally specific. By combining the two tests, the authors obtained a 100% sensitivity in the detection of cervical dysplasia.

In our study, we regard the lack of information regarding the negative tests as a major limitation. Because the false-negative rates of the two screening methods are not known, we cannot comment on sensitivity, specificity, and negative predictive values of the tests.

Cytology remains the best method available in screening for cervical cancer. However, in countries with limited resources or in situations when such screening is not available, an inexpensive and easy alternative that can be performed by nonmedical health workers is needed. Naked-eye visualization of the cervix after application of diluted acetic acid can detect more than 60% of high-grade SIL, and therefore warrants consideration as an alternative to cytologic screening.

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Testing Cervicography and Cervicoscopy as Screening Tests for Cervical Cancer

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Aims and Background: Suboptimal sensitivity is currently reported for Pap test in screening for cervical cancer. Colposcopy is known to be more sensitive than cytology but its use as a screening test is not possible due to costs and complexity. Screening by cervicography has been suggested as a compromise being less costly and feasible. The present study evaluates the feasibility of screening by cervicography and cervicoscopy (naked eye examination of the cervix after acetic lavage) on a consecutive screening series.

Methods: Cervicography and cervicoscopy were performed by the smear taker in subjects consecutively attending a screening clinic. Women with abnormal cytology (atypia or more severe lesion) and/or abnormal cervicography or cervicoscopy (acetowhite lesion) underwent colposcopic assessment. The three screening methods were compared according to positivity rate, CIN 2-3 detection rate and positive predictive value.

Results: 2105 consecutive subjects were screened. Positivity rate was 3.8%, 15,3% or 25.4% for cytology, cervicography or cervicoscopy, respectively, 486 of 555 women attended the assessment phase, 281 directed biopsies were performed and 8 CIN 2-3 lesions were detected. Cytology, cervicography and cervicoscopy detected 5, 5, or 7 of 8 CIN 2-3 lesions, respectively. The positive predictive value was 0% for cytologic atypia, 25% for cytologic SIL, 1.75% for cervicography and 2.05% for cervicoscopy. Detecting one CIN 2-3 lesion detected at cytology cost $5,543. The cost per each additional cytologically negative CIN 2-3 lesion at cervicography or cervicoscopy was $12,947 or $3,916, respectively.
CONCLUSIONS. The study confirms the limited sensitivity of cytology for CIN 2-3. The association of cervicography was not cost effective. Cervicoscopy was poorly specific but increased the detection rate of CIN 2-3 at relatively low costs. Cervicoscopy is worth further evaluation as a screening test.

KEYWORDS: Cervical cancer screening, cervical cytology, cervicography.

Although cervical screening is currently recommended to reduce cancer incidence and mortality, [10,22] suboptimal sensitivity rates for cervical intraepithelial neoplasia (CIN) and cancer have been reported for vaginal cytology. [4,5,7,11,14,18,19] Colposcopy has been found to improve CIN and cervical cancer detection rates [8,13,16] when combined with cytology but colposcopic screening is unpractical due to costs and lack of adequate trained colposcopists. Cervicography is much cheaper, has almost the same diagnostic accuracy as colposcopy [1,21] and has been proposed as a screening test in combination with cytology. Several studies [1,3,9,12,23] reported a higher sensitivity of cervicography with respect to cytology and encouraged its routine use in screening.

The present study was aimed at evaluating the possible advantages of combining cervicography with vaginal cytology in a screened population. The study investigated also the diagnostic accuracy of direct examination of the cervix by the smear taker after acetic acid lavage (cervicoscopy).

Materials and Methods

Organized screening has been performed at the Centro per lo Studio e la Prevenzione Oncologica (CSPO) of Florence since 1969. [17] All women between 25 and 60 years of age resident in the District of Florence are actively invited to undergo screening. Pap smear sampling is performed by Ayre spatula and Cytobrush. All subjects with cytologic evidence of squamous or glandular cell atypia or more severe lesions [15] are requested to attend the CSPO colposcopic clinic for a colposcopy. About 75,000 smears are examined and 5,000 are performed every year.

In the present study 2105 consecutive women attending the Pap smear clinic at the CSPO from May 1991 to January 1992 were also examined by cervicography and cervicoscopy. Age ranged from 17 to 83 years, average 46.3.

Cervicography was performed according to the method described by Stafl [21] immediately after the smear was obtained. A color slide of the cervix was taken by the smear taker after 5% acetic acid lavage, using a 35 mm camera with a 50 mm extension ring and 1 100 mm macro lens. Cervigrams were interpreted separately by one expert colposcopist (S.Ce), unaware of the cytologic report. Cervigrams were reported as technically defective, negative (no abnormal lesion present, including subjects with no visible squamocolumnar junction), or suspicious (abnormal lesion present: acetowhite epithelium, punctuation, mosaic, atypical vessels).

Cervicoscopy consisted of naked eye examination of the cervix under a 60W halogen lamp, after acetic lavage and before performing cervicography. Two smear takers performed cervicoscopy in the present study. They had no previous knowledge of colcoscopy and had undergone a short training to recognize (no acetowhite areas).

All women with *a)* abnormal cytology (squamous or glandular cell atypia or more severe lesion), *b)* suspicious cervicography, or *c)* positive cervicoscopy were invited to the assessment clinic to undergo colposcopy. Colposcopically guided biopsies of all acetowhite areas were performed. Acetowhite lesions observed at cervicography or cervicoscopy but not confirmed at colposcopy were assumed to be false positives, due to light reflections at cervicography or to error of the non-medical operator reporting at cervicoscopy.

Cytology, cervicography and cervicoscopy were compared according to their positivity rate and diagnostic accuracy. Subjects who were invited to undergo colcoscopy and did not attend were excluded from the evaluation of diagnostic accuracy. Negative colposcopy or histologic diagnosis at colposcopically guided biopsy were considered as the gold standard for the determination of sensitivity, specificity and positive predictive value for CIN II-III. Cases showing histologic evidence of papillomavirus (HPV) infection or CIN I were grouped with negative cases for the purposes of the study. Two estimates of diagnostic accuracy were performed according to cytologic report, that is assuming squamous or glandular cell atypia alternatively as a negative or a positive report. Technically defective cervigrams were presumed to be negative.

Differences in observed rates were compared by the chi-square test and statistical significance was set the 0.05 p level.

Cost analysis was based on the actual costs of the present study. Costs of the general screening organization and invitation were not considered as they were independent of the type of screening test. The average cost of a cytologic examination ($33) was inclusive of the assessment costs in cases with abnormal cytologic findings (colposcopy = $15.5; histology = $15.5). The cost of cervicography ($6) or cervicoscopy ($5.5) was also inclusive of assessment costs, but with the exclusion of cases for which assessment was already indicated by abnormal cytology (atypia or more severe lesion). The cost per each CIN II-III detected was determined for cytology and was compared to the cost per cytologically negative CIN II-III identified by cervicography or cervicoscopy.

Results

Table 1 shows the distribution of the cases studied by report at cytology, cervicography or cervicoscopy. The rate of positive tests for cytology was 3.8% or 0.99% according to whether squamous or glandular cell atypia was considered as positive or not. The positivity rate for cervicography and cervicoscopy was 15.3% and 25.4%, respectively. A significant difference in positivity rates was observed according to age. Positivity rates in women aged < 50 and > 50 were 5.1% and 2.5% for cytology (chi-square = 8.22, df = 1, $p < 0.01$), 24.3% and 3.2% for cervicography (chi-square = 169.24, df = 1, $p < 0.0001$), and 29.0% and 20.5% for cervicoscopy (chi-square = 19.82, df = 1, $p < 0.001$).

Informe	Menor de 50 años	Mayor de 50 años	Total
Estudios citológicos:			
inadecuados	15	2	17
resultado negativo	1136	871	2007
atipia de las células escamosas o glandulares	41	19	60
LIE 1	12	1	13
LIE 2	6	2	8
Cervicografía: fallas técnicas	59	70	129
resultado negativo	855	768	1623
resultado sospechoso	294	29	323
Cervicoscopia:			
resultado negativo	857	713	1570
resultado positivo	351	184	535

Cuadro 1. Distribución de 2.105 mujeres según el informe de los estudios citológicos, de la cervicografía y la cervicoscopia

The distribution of the cases by report at combined screening tests is given in *Table 2*. No abnormality was detected at cytology, cervicography and cervicoscopy in 1550 subjects who were invited to repeat a Pap smear after 3 years. Five hundred and fifty-five subjects with at least 1 positive test were invited to undergo colposcopic examination and 486 (87.6%) attended. The attendance rate was 86.9% (411 of 473) and 91.5% (75 of 82) in women aged < 50 and > 50 years, respectively.

Colposcopy was negative in 205 cases whereas colposcopic abnormalities were observed and biopsies performed in 281 cases. The histologic diagnosis was negative in 180, HPV-CIN I in 93, CIN II in 4, and CIN III in 4 cases. The CIN II-III detection rate was similar in women aged < 50 (7 of 411) and > 50 (1 of 75).

Table 3 reports the distribution of cases by final diagnosis and according to the finding at each screening test. Cytology was abnormal in 5 (low grade squamous intraepithelial lesion (SIL) in 1, high grade SIL in 4) and negative in 3 of 8 CIN II-III. Cervicography and cervicoscopy detected 5 and 7 of CIN II-III, respectively. The review of the 3 false negative cervigrams showed that the acetowhite lesion seen at colposcopy had not been identified at cervicography because of bad framing of the cervix (2 cases), or bad focusing (1 case). The positive predictive value for CIN II-III was 0% for cytologic squamous or glandular cell atypia, 25% (5 of 20) for cytologic SIL, 1.75% (5 of 285) for cervicography and 2.05% (7 of 341) for cervicoscopy. No significant differences in the positive predictive value of different tests were observed according to age.

Cervicografía	Cervicoscopia	Número de casos	%
Estudios citológicos con resultado negativo			
negativo	negativo	1550	73.6
positivo	negativo	106	5.0
negativo	positivo	176	8.4
positivo	positivo	192	17.5
Indicios citológicos de atipia de las células escamosas o glandulares			
negativo	negativo	42	2.0
positivo	negativo	5	0.2
negativo	positivo	5	0.2
positivo	positivo	8	0.4
Indicios citológicos de lesión intraepitelial escamosa			
negativo	negativo	8	0.4
positivo	negativo	1	0.0
negativo	positivo	1	0.0
positivo	positivo	11	0.5

Cuadro 2. Distribución de los casos estudiados según el informe de distintas pruebas de detección

The cost for each CIN II-III detected at cytology was $5,543 and that for each cytologically negative CIN II-III detected at respectively, cervicography and cervicoscopy, was $12,947 and $3,916.

Discussion

This study compared the accuracy of cytology, cervicography and cervicoscopy in detecting CIN II-III lesions only. We did not consider HPV lesions and CIN I as the cost-effectiveness of their detection and systematic treatment is still controversial. The observed low prevalence of CIN II-III, which was expected in a consecutive unbiased cohort at repeat screening, did not allow the detection of statistically significant differences in sensitivity between the studied tests, but does not allow some considerations on the possible role of cervicography and cervicoscopy in a screening setting.

The study confirms the limited sensitivity of cytology for CIN II-III [4,20,23] and the need for alternative screening tests. The null detection rate of CIN II-III observed for cytologic atypia is probably explained by the limited size of the series studied, and by the expected low positive predictive value of cytologic atypia for CIN II-III [6].

Estudios citológicos	Cervicografía	Cervicoscopia	Diagnóstico definitivo		
			Negativo para VPH-NIC 1	NIC 2	NIC 3
negativo	positivo	negativo	93	—	—
negativo	negativo	positivo	148	1	1
negativo	positivo	positivo	168	—	1
atipia	negativo	negativo	38	—	—
atipia	positivo	negativo	5	—	—
atipia	negativo	positivo	4	—	—
atipia	positivo	positivo	7	—	—
LIE	negativo	negativo	7	—	1
LIE	positivo	negativo	1	—	—
LIE	negativo	positivo	1	—	—
LIE	positivo	positivo	6	3	1

Cuadro 3. Distribución de los casos estudiados según el informe de pruebas combinadas y el diagnóstico definitivo. Se excluyeron 68 casos que se negaron a someterse a una colposcopia.

Cervicography identified 1 cytologically negative and missed 1 cytologically positive CIN III. The overall CIN II-III detection rate of cervicography was similar to that of cytology. As previously reported, [2,23] the specificity of cervicography was poor, causing an excess of recalls for colposcopic assessment. These results were not as satisfactory as those reported by Tawa et al. 23 in a similar screening study, and may be explained by a difference in the accuracy of either cytology or cervicography between the two studies. In our study false negative cervigrams were evaluated as technically defective at review, especially as far as framing of the whole cervix is concerned. An acetowhite lesion was seen a cervicoscopy in 2 of 3 false negative cases at cervicography. This finding suggests that longer training, more accurate performance and increased confidence with the method are likely to improve the accuracy of cervicography, but this would probably increase the costs and the difficulty of making cervicography available at any cytologic clinic.

Cervicoscopy has not previously been described or employed, at least to our knowledge, as a screening method. The results observed in the present study are encouraging. The detection rate of CIN II-III was higher than those of cytology and cervicography. The latter finding might be surprising as both techniques are based on examination of the cervix after acetic acid lavage. Taking a good photograph of the cervix is not always easy, and bleeding due to previous endocervical brushing or reflections of the flash light may mask or simulate acetowhite lesions. On the contrary, cervicoscopy is not subject to such inconveniences as the cervix may be examined from different angles and bleeding may be controlled by cotton swab pressure. On the other hand, cervicoscopy was poorly specific and this caused a high recall rate for colposcopic assessment.

Cost analysis is important to evaluate alternative screening methods. We considered cytology as the basic screening method and estimated the costs of the systematic combination of cervicography or cervicoscopy with cytology. Absolute costs show considerable variations between different centers and countries and cost analysis would be better based on evaluation of the relative cost of different methods in a single setting. The specificity of cervicography and cervicoscopy was poor and their final cost was heavily influenced by the cost of unnecessary colposcopies and biopsies in false positive cases. Cervicography had also a limited sensitivity and the final cost per cytologically negative CIN II-III detected was unacceptable. Cervicoscopy was even less specific and the cost due to unnecessary assessment was higher, but this was balanced by a higher sensitivity and the final cost per cytologically negative CIN II-III detected was more favorable compared to cervicography.

In conclusion, the present study confirms that cytology has a limited sensitivity for CIN II-III. Combination with cervicography yields a limited improvement of the detection rate of CIN II-III at a very high cost. Cervicoscopy was less specific but it was also more sensitive and finally it turned out to be more cost effective than cervicography. These results need to be confirmed by a further study of cervicoscopy in a larger screening series.

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

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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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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 19909 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.*

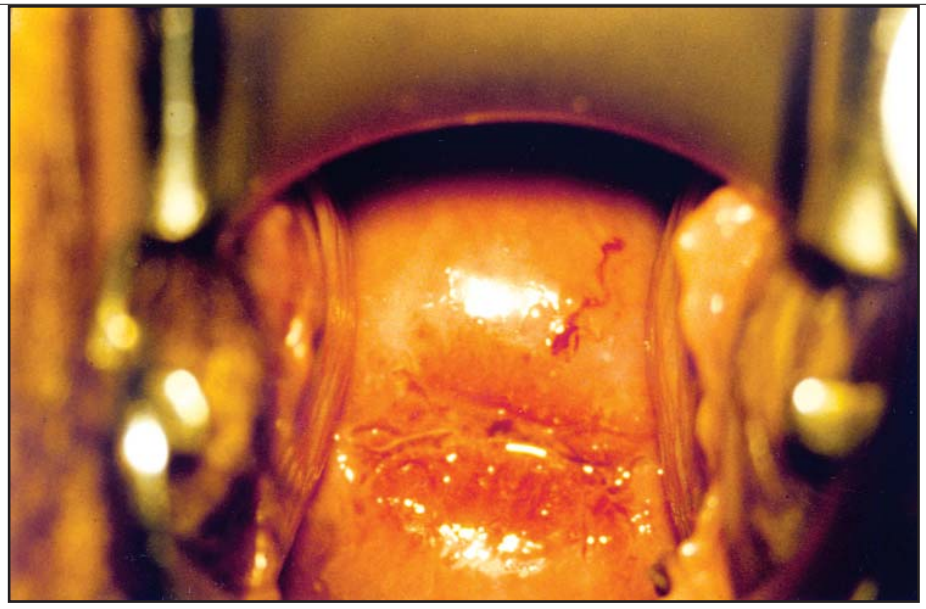

Figure 1: Normal cervix seen with the colposcope at low power (9X). Arrow indicates the thin white linen between the columnar and squamous cell epithelium (squamocolumnar junction).

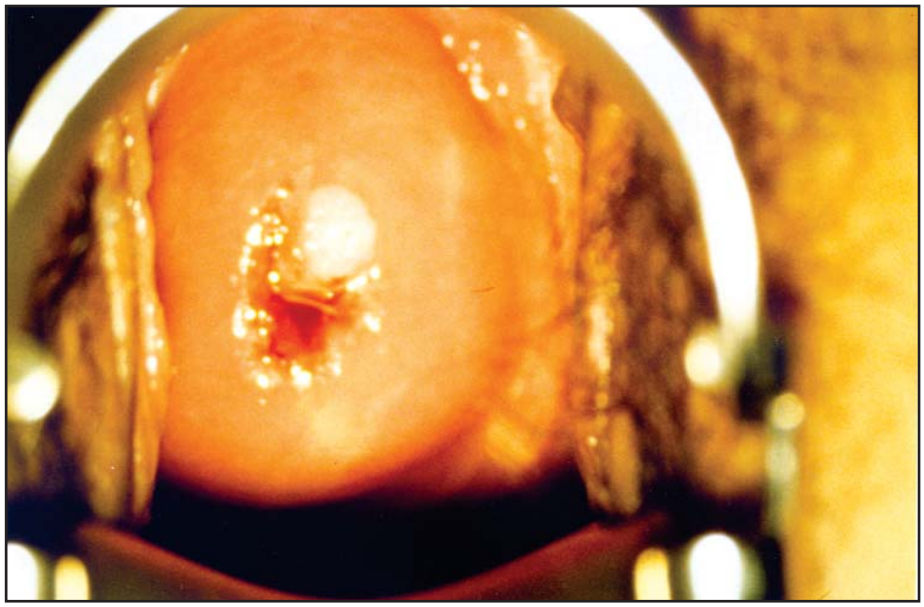

Figure 2: Cervix after 1-minute application of 5% acetic acid. Note the acetowhite area indicated by arrow. The Papanicolaou smear was normal, but colposcopically directed biopsies of the site revealed moderate dysplasia (CIN II).

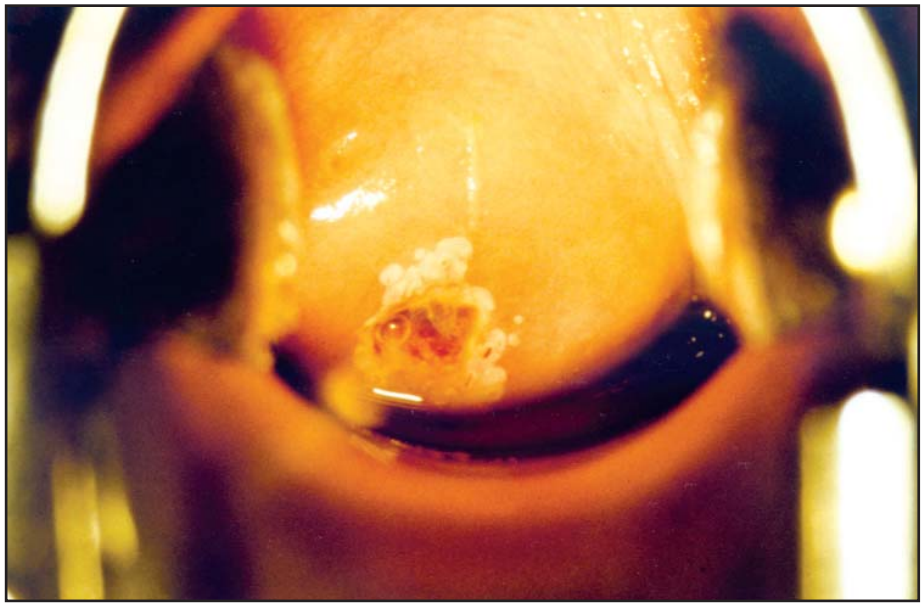

Figure 3. Cervix after 1-minute application of 5% acetic acid. Arrow indicates one of several areas of acetowhite strining around the cervical os. The Papanicolaou smear was normal, but colposcopically directed biopsies of the site revealed mild dysplasia/condyloma.

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%tp 50%[3,7]. In addition, new cases of cervical cancer are predicted to sharply increase, especially among women who are now younger that 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 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 form these women. Comparison between visual examination and colposcopic evaluation was not reported.

Neither study evaluated the cervical acetic 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 Papanicolau smear alone.

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 office 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 -in opposite page*). 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 curettage was performed on all subjects. The vaginal side walls and vulvar areas were also examined and biopsied with indicated. Selected photographs were taken for documentation by means of an Olympus OMI camera adapted for

the colposcope. Coloposcopic biopsies were reviewed by board-certified pathologists at Harrisburg Hospital, who were not informed of the research protocol.

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 o 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 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 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 to III.

Overall, 47 of 85 eligible and consenting subjects with abnormal results on acetic acid wash had biopsy-proven abnormalities seen on colposcopy. Th 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. Thirthy-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 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 that 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].

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)

* Un resultado anormal indica la presencia de cambios condilomatosos o neoplasia intraepitelial cervical.

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

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 non-detection 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 col-

Biopsy Results	Negative Papanicolaou Smear*	l Abormal Papanicolaou†
Normal	30	8
Condyloma, CIN I	29	9
CIN II-111	4	5
Total	63	22

* Negative Papanicolaou smear indicates that there were no abnormal finding

† Abnormal Papanicolaou smear indicates that atypical cellular changes or squamosas intraepithelial lesions were detected.

The positive predictive value of acetic acid wash, .55 (95% CI - .43 to .63)

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

poscopy[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%.

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

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

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.

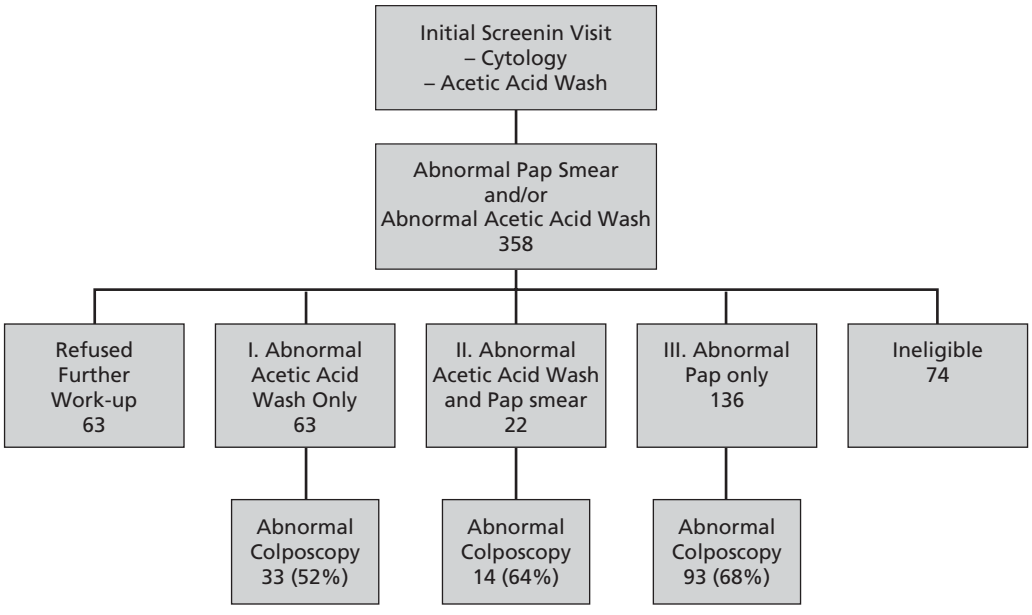

Figure 4. Diagram of group assignment within the sample of 2827 women

Harrisburg Area Research Network

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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 way improve the accuracy of identifying acetowhite areas on the cervix. No controlled trials have been published comparing 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, which 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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Examination of the cervix with the naked eye using acetic acid test

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Examination of the cervix was carried out on 2,400 patients, by use of acetic acid test with the naked eye and the colposcope. The physiologic transformation zone was clearly identified both with the naked eye and the colposcope in 1,568 of 1,594 (99%) cases. Colposcopic examination was unsatisfactory in 108 of the 264 (41%) patients in whom the cervix was completely covered by normal squamous epithelium. An atypical transformation zone (ATZ) was identified with the naked eye as the white epithelium in 98.4% and as "suspicious" in 1.6% of 312 colposcopically controlled cases. An unsatisfactory colposcopic examination occurred in 39 of the 312 (12.5%) patients with an ATZ. Final histologic diagnosis for 312 ATZs was benign lesion in 169 of 312 (54.2%), cervical intraepithelial neoplasia (CIN) grades 1 and 2 in 81 of 312 (26%), grade 3 CIN in 56 of 312 (17.9%), and preclinical invasive carcinoma in 6 of 312 (1.9%). The detection of intraepithelial or preclinical invasive cervical neoplasias should not depend on the possession of a colposcope. On the other hand, the use of a colposcope is essential for the selection of CIN that can be treated with ultraconservative therapy or with colposcopically directed conization. (AM.J.OBSTET.GYNECOL.143:139,1982).

It is difficult to understand why it should be so hard to convince American gynecologists of the value of examining the cervix under lens magnification. A physician who omits such examination is much the same as an astronaut who insists that he can see the stars without the use of a telescope[1].

The detection of in situ or invasive cervical cancer does not depend on possession of a colposcope[2].

Colposcopy has been emphasized as an "astronomic revolution" in the twentieth-century gynecology, and colposcopic magnification has been opposed to the poorness of the naked eye's vision. Two consequences derived from this: the development of progressively sophisticated and expensive apparatuses and the frustration of gynecologists when examining the cervix without a colposcope.

We would like to draw attention to the importance of the naked-eye inspection of the cervix, employing a good light source and preparing the cervix with a 3% acetic acid solution.

Patients and methods

A total of 2,400 unselected patients, with normal or abnormal cervical cytology, ranging from 18 to 65 years of age, were examined in Florence, Italy, at the G.N. Papanicolaou Laboratory and at the Department of Obstetrics and Gynecology of the Florence University.

The cervix of each patient was inspected by an experienced colposcopist (M.O.) and by an adequately instructed postgraduate student of the department (P.L.T.)

The inspection was carried out by P.L.T. with the naked eye and by M.O. with the colposcope, before and after the application of 3% acetic acid solution. For the naked-eye inspection a 100 W lamp or the colposcope light source was used.

Naked-eye and colposcopic findings, before and after acetic acid application, were compared as soon as both observers had completed their examinations.

Immediately afterward the cytologic data of the patient were checked and correlated to the cervical picture observed. When appropriate, a punch biopsy was carried out. Photographs were taken for the documentation of exemplar cases, by means of an Olympus OM-1 camera with standard lens, plus a focal length duplicator (Panagor automatic macro converter) and a ring-flash unit (Sunpack).

The colposcopic findings in this study, were classified as follows *(1)* original columnar epithelium, *(2)* physiologic transformation zone (TZ), *(3)* squamocolumnar junction within the endocervix, *(4)* atypical transformation zone (ATZ), and *(5)* other findings (vaginocervicitis, atrophic epithelium, true erosions, polyps).

We have adopted Richart's terminology in the classification of the significant entraepithelial changes: cervical intraepithelial neoplasia (CIN) or grade 1 CIN (mild dysplasia), grade 2 CIN (moderate dysplasia), and grade 3 CIN (severe dysplasia and carcinoma *in-situ*). All grade 3 CIN patients were treated with diagnostic conization: When there was no sign of invasion in the cone (cut into a minimum of eight blocks) and when the margins of the cone were free of disease, conization was considered as therapeutic.

Cases of mild to moderate dysplasia, selected on the basis of substantial biopsies and in which ATZ did not penetrate into the cervical canal were treated with diathermic electrocautery (DTC). When ATZ penetrated into the cervical canal even the patient with minor dysplasia underwent diagnostic conization.

Results

Before acetic acid application only keratosis was clearly identifiable, but no clinical diagnosis was possible with the colposcope or with the naked eye, excepting one case of overt carcinoma.

It must be remarked that before acetic application, the "innocent" cervical appearance, both to the naked eye and via colposcope, was always considered uncertain; furthermore, in most cases the colposcopic magnification only magnified the confusion.

After acetic acid application the comparison was made between findings seen with the naked eye and the colposcope (*Table I*)

The grape-like structure of original columnar epithelium was clearly visible with the naked eye and colposcopically confirmed in 132 of 2,400 (5.5% cases).

The physiologic TZ was identified in 1,584 of 2,400 (66%) cases with the colposcope and in 1,568 with the naked eye. TZ is the clinical aspect of the normal columnar squamous metaplasia and can be seen in its early or fully developed stage. Early metaplasia was diagnosed when fusion of the columnar villi (in central areas and/or in the peripheral rim of the original junction) or extending pale-red "tongues" of new squamous epithelium appeared. As fusion and flattening of the villi progresses, the grape-like original tissue becomes diffusely smooth and of a whitish or reddish color.

Fully developed metaplasia was diagnosed when the gland openings and/or nabothian cysts were included in the new, well-differentiated (iodine-stained) squamous epithelium.

In our series 16 of 1,584 (1%) cases of TZ were classified as "doubtful" with the naked eye and colposcopically clarified as early metaplasia.

The cervix was completely covered by normal squamous epithelium in 264 of 2,400 (11%) patients. In some of these cases, it was possible to distinguish, both with the naked eye and the colposcope, the original squamous from the metaplastic epithelium; in others, this distinction was not possible with either method. In all these patients, however, the real problem was the identification of the squamocolumnar junction. By use of the Kogan-Martin endocervical speculum and by wetting with acetic acid solution an adequately lighted endocervical epithelium, in 156 of 264 cases the squamocolumnar junction was visible with the naked eye up to a height of approximately 1 cm. inside of the cervical canal.

In 108 of 264 (41%) patients in whom the cervix was completely covered by normal squamous epithelium, the endocervical inspection was unsatisfactory, both with the naked eye and with the colposcope because of cervical stenosis or because the junction was too high into the cervix to be visible. In two of these cases of abnormal cervical cytology, cone biopsy showed a carcinoma

	Colposcope	Naked Eye
Original columnar epithelium (ectopy)	132 (5.5%)	132
Physiologic TZ	1,584 (66%)	1,568*
Squamocolumnar junction within the endocervix		
ATZ	312 (13%)	307‡
Other findings	108 (4.5%)	108
Total	2,400	2,379

* Sixteen cases (1%) of TZ, corresponding to the colposcopic appearance of early metaplasia, were classified as "doubtful" with the naked eye.

† In two cases of abnormal cytology and unsatisfactory inspection of the cervical canal, cone biopsies revealed a carcinoma *in-situ* in one case and severe dysplasia in the other.

‡ Five cases of ATZ (1.6%_ with very flat, white epithelium, histologically insignificant, were classified as "suspicious" with the naked eye.

Table I. Comparison between colposcopic and naked-eye findings with acetic acid test in 2,400 examinations of the cervix.

	Número	%
Benign lesions	169	54.2
Cervical intraepithelial neoplasia		
Grades l and 2	81*	26.0
Grade 3	56†	17.9
Preclinical invasive carcinoma	6‡	1.9

* Consistent biopsy before DTC revealed mild to moderate dysplasia in three cases with abnormal cytology and insignificant histologic appearance in the first punch biopsy.

† One carcinoma *in-situ* was found in the biopsy, whereas moderate dysplasia was diagnosed with punch biopsy of an ATZ penetrating out of sight within the endocervix.

‡ In one case the diagnosis of microinvasive carcinoma derived from cone biopsy in contrast with severe dysplasia diagnosed with punch biopsy of an ATZ penetrating out of sight within the endocervix.

Table 2. Final histologic diagnoses in 312 cases of ATZ

in-situ in a 57 year-old patient and a severe dysplasia in the other 30 year-old patient.

An ATZ was identified with the colposcope in 312 of 2,400 cases (13%); with the naked eye 307 (98.4%) of these were clearly seen as white epithelium and five (1.6%) were classified as "suspicious" because of a very flat, white epithelium that needed the colposcope to be clearly identifiable. In all these five cases an insignificant histologic appearance was found. Obviously, only the colposcopic magnification after the acetic acid test demonstrated the abnormal intraepithelial capillaries within the white areas of ATZ.

The principal aim, however, of the colposcopic examination in our present practice is to ascertain whether the upper limits of the lesion can be seen, in order to plan the diagnostic and therapeutic strategy.

Multiple punch biopsies demonstrating benign lesions were considered sufficient for the diagnosis without further histologic investigations in 169 of 312 patients whose lesions were treated with DTC.

Forty-eight patients with the whole grade _ CIN area visible on the ectocervix were treated with DTC after substantial biopsies; 54 patients with grades 2 and 3 CIN lesions wholly visible on the endo or ectocervix were treated with colposcopically directed conization. In 39 of 312 (12.5%) patients with ATZ, the colposcopic examination was unsatisfactory because the ATZ penetrated into the cervical canal, beyond visual detection. In two of these cases the final histologic diagnosis on the cone biopsy was more severe than the first one on the punch biopsies. Four of the six cases of preclinical invasive carcinoma belong to the group "unsatisfactory examination".

Final histologic diagnoses of the 312 ATZs are detailed in Table II.

Comment

White epithelium is the basic clinical appearance of the ATZ and is due to an increased cellular density [number of nuclei and deoxyribonucleic acid (DNA)].

Townsend[3] writes: "Although each pattern of the atypical transformation zone is a separate entity, they are all primarily white epithelium which, when having certain vascular structures, take on specific appearances (i.e., punctuation, mosaic structure or atypical vessels)".

On the other hand, punctuation and mosaic structures without an associated white epithelium can be seen in normal, atrophic, or inflammatory conditions and are not fields of neoplastic potential.

The horizontal atypical vessels are an infrequent finding in the site of early invasive carcinoma.

White epithelium was visible with the naked eye, after the acetic acid test, in 98.4% and was suspected in 1.6% of our colposcopically controlled cases. We believe, therefore, that colposcopic magnification is not essential in clinical practice for the identification of the cervix "at risk".

The Papanicolaou smear is the fundamental approach to a screening program; however, an effective, inexpensive, and simple clinical test can be carried out routinely by all gynecologists can be a very helpful supplement for early detection of cervical carcinoma.

In our opinion, once the ATZ has been identified with the naked eye and acetic acid solution, a colposcopically directed biopsy can be useful; however, the colposcope is not indispensable.

Colposcopic examination, on the other hand, is essential for the choice between ultraconservative or conservative treatment in every single case of CIN.

Interesting researches have been undertaken on the colposcopic-histologic correlations. Kolstad and Staft[4] authors of an atlas with splendid colposcopic magnifications, have achieved elaborate photographic methods accurately predicting the histologic results, based on the measurements of the intercapillary distance in atypical colposcopic lesions. "In preinvasive and invasive carcinoma on the cervix", they write, "the intercapillary distance usually increases as the stage of the disease advances". However, since the blood vessels move toward cancer when the preinvasive carcinoma becomes invasive, Kolstad and Staft specify that: "Initially the intercapillary distance may be reduced, but as the proliferation of the cancer cell continues, relatively large vascular areas are formed".

Unfortunately, these sophisticated methods are not available in clinical practice.

Australian authors[5] have suggested a three-grade classification of ATZ with reference to the whiteness of the epithelium, the aspect of the surface, and the form

of the Capillaries. The same authors admit that their scheme is subjective and can help to recognize an invasive preclinical carcinoma when spectacular atypical changes are present (grade 3). A biopsy is obviously required for definitive diagnosis.

Coppleson, Pixley and Reid[5] write: "Although the atypical transformation zone identifies the site and extent of major dysplastic and cancerous epithelia, there are numerous exceptions. It is a matter of disappointment, even disillusion to many clinicians, that so many atypical transformation zones show on histological examination only the most minor disturbances, even normal appearances in the cervical epithelium and have no apparent clinical significance". In our series a benign histologic appearance was found in 54.2% of ATZ.

The explanation of this paradox is that immature metaplasia can also take on clinical appearance of ATZ. The Australian authors, moreover, have drawn attention to this very important point: It can be difficult to distinguish histologically the undifferentiated ("full thickness") metaplastic epithelium from dysplasia or from carcinoma *in-situ*.

Recently diagnostic investigations have reached the point of correlating histopathology with DNA analysis[6].

Singer and Jordan[7] have published a photomicrograph of an immature metaplastic squamous epithelium that, as they comment, "can easily be confused with invasive squamous carcinoma".

The explanation of this paradox could lie in the physiologic phenomena of columnar-squamous metaplasia which, according to the Australian authors[8] hypothesis begins with the "crossing" of two processes: *(1)* death of the columnar epithelium and degeneration of its basement membrane and *(2)* origin in the stroma of the new squamous epithelium and formation of a new basement membrane. Fortunately, the immature metaplastic epithelium in most cases can be recognized, after acetic acid test, both with the colposcope and with the naked eye, and this must be emphasized.

When immature metaplasia has the appearance of ATZ, a close collaboration between the clinician and the pathologist is necessary to be able to exclude that some intraepithelial neoplasias or some early invasive carcinomas will contribute to the good results of conservative or radical treatment – because they never existed!

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