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Performance of the acetic acid test when used in field conditions as a screening test for cervical cancer

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Summary

OBJECTIVE To assess if visual inspection with acetic acid (VIA) is a useful alternative screening test for cervical cancer, when used in a resource-poor setting with an existing cytology-based screening programme.

METHODS Women living in Rivas district (Nicaragua), who attended the programme, were concurrently screened with VIA and Papanicolau (PAP) smear. Screening was performed by health providers who had received training in VIA and a refresher course in cytology. Women testing positive for either of the results were referred for colposcopy and biopsy when indicated. The performance of VIA was compared with PAP smear, calculating the relative true and false positive rate (RELTPR and RELFPR) and for a high threshold on biopsy (cervical intraepithelial neoplasia grade 2 or a higher grade). We determined the trade-off between both tests by calculating the ratio of extra false positives detected through extra true positives (EFP:ETP ratio).

RESULTS A total of 1076 patients were screened. Nearly 33% had a positive screening test. On biopsy, 7.6% had a low-grade intraepithelial lesion, 4.5% a high-grade intraepithelial lesion (HSIL) and 0.5% invasive cancer. The RELTPR (VIA to PAP) was 1.96, the RELFPR 5.02 and the EFP:ETP ratio 8.04. VIA detected twice as much HSIL and invasive cancers as the PAP smear. Yet, for every extra diagnosis, eight extra false positives had to be examined at the referral level.

CONCLUSIONS The VIA spectacularly increases the number of HSIL and invasive cancers detected. The high FPR is a concern for the organization of the referral level. There is a need to establish uniform criteria on test positivity and to further improve the performance in field conditions.

keywords cervical cancer, screening, visual inspection, cytology, developing countries

Introduction

During the last decade, the problem of cervical cancer has received renewed interest. The decrease in cervical cancer prevalence in most of the developed countries is attributed to the success of cytology-based screening programmes, hardly observed in many developing countries (Sankaranarayanan *et al.* 2001). The cost and the operational problems related to cytology-based programmes result in the lack of quality screening programmes in resource-poor settings (Parkin 1991; Sankaranarayanan *et al.* 2001; Sherris *et al.* 2001). This has attracted the attention of policy makers, health professionals and researchers, and led to the development of alternative approaches to improve the success of screening programmes. One of the new screening tools is visual inspection of the cervix with acetic acid (VIA).

This cheap technique involves the application of 3–5% acetic acid (household vinegar) on the cervix followed by inspection of the cervix 2 min later, under illumination, for the presence of acetowhite areas (Megevand *et al.* 1996; Sankaranarayanan *et al.* 1999). A number of studies report test sensitivity for high-grade squamous intraepithelial lesions (HSIL) varying between 70% and 76%, with a specificity of 64.1–79% (University of Zimbabwe/JHPIEGO Cervical Cancer Project 1999; Belinson *et al.* 2001; Denny *et al.* 2002). Yet, most of these promising results have been obtained in research settings, with specially trained research staff or health providers performing the test under adequate supervision.

The performance of VIA was desired to be assessed when used in field conditions, particularly as an adequate alternative in a setting where a screening programme based on Papanicolau (PAP) smear already exists.

Methodology

This study is part of a larger study on integration of cervical cancer screening services in primary health care in the district of Rivas, in southern Nicaragua. The study was approved by the Ethical Board of the Universidad Nacional Autónoma de Nicaragua.

Within this project, women aged 30 years or older who had never been screened, or who had not been screened for the past 3 years (the so-called target population) were invited by community health workers to attend the programme. In line with the national policy, women who attended spontaneously were also screened, irrespective of the time of their last PAP smear. Within this project, the local cytologist responsible for reading all the PAP smears taken in Rivas district within the public health system (2000–4000 annually), received a refresher-training course in July 2000.

In September 2000 and May 2001, seven medical doctors and 26 nurses from six health centres, 13 health posts, one non-governmental organization (NGO) clinic and the gynaecology consultation of the district hospital were trained. The training consisted of 1 day theoretical sessions on clinical and epidemiological aspects of cervical (pre-) cancer, the technique of VIA and a refreshment module on PAP smear sampling. A full day was spent on VIA training, using a pictorial atlas developed at the International Centre for Reproductive Health, Ghent University (not published) and a teaching set of projected 35 mm photographical slides of cervices images after application of acetic acid (cervicograms). Each participant then received 1 day of supervised practical training on women attending the clinics for cervical screening. The trainees received a 1-day refresher workshop 6 months after the initial training, using a teaching slide set and practice sessions.

A positive result on visual inspection was defined as an opaque white or grey lesion with well-defined borders, located close to the squamo-columnar junction. The PAP smears were classified according to the 1991 Bethesda classification (Kurman & Solomon 1994) and considered positive when at least atypical squamous cells of unknown origin were reported. In order to keep the reporting uniform throughout the study period, no adaptation was made to the 2001 Bethesda classification (Solomon *et al.* 2002).

The two screening tests were performed on all women of the target population attending the health facilities for screening purposes. Other women were screened by PAP smear, with or without VIA test. A PAP smear was obtained using Ayre's spatula and spray fixative for cytodiagnosis (Labofix; Labonord, Villeneuve d'Ascq, France) and after PAP staining, read by the cytologist of the district hospital in Rivas. All positive PAP smears and 10% of negative smears were revised by a pathologist from the Bertha Calderon Hospital, a referral hospital in Managua. Conventional cytology (dry slides) was used at both levels.

Immediately after the PAP smear, the health providers applied 5% acetic acid to the cervix and recorded the findings 2 min later, using a simple household torch as a light source. Women testing positive on either screening test were referred to the colposcopy clinic. Colposcopies were offered at the NGO clinic in San Juan del Sur, one of the areas of the district. Referred patients were asked to attend the clinic as soon as possible, without previous appointment. The clinic was open every Saturday and colposcopy and outpatient treatment of pre-invasive disease was free of charge.

The referral test involved colposcopy and a biopsy if indicated. Colposcopies and subsequent biopsies were performed by a trained gynaecologist. Biopsies were examined at the Bertha Calderon Hospital by a local pathologist. All biopsies were independently reviewed by a pathologist from Ghent University. This pathologist was blinded for the first result. The overall inter-observer agreement of the biopsies was 66%. Discordant biopsies were investigated by both pathologists, using a binocular training microscope with two heads. The consensus diagnosis was taken as the final result.

Statistical analysis

As only those women testing positive on either VIA or PAP smear were further investigated, hence, introducing verification bias, we used specific statistical methods (Schatzkin *et al.* 1987; Chock *et al.* 1997) to assess the accuracy of the VIA compared with the conventional PAP smear as currently used in Nicaragua.

Our data were represented using the sample scheme developed by Schatzkin. As the referral test is not applied to patients who tested negative on both screening test, sensitivity and specificity cannot be calculated. Yet, information about the relative true positive rate (RELTPR) and the relative false positive rate (RELFPR) of both the tests is available (Table 1): the RELTPR of test 2 (VIA) compared with test 1 (PAP) = $(a + b)/(a + c)$; the RELFPR of test 2 (VIA) compared with test 1 (PAP) = $(A + B)/(A + C)$.

We used Mc Nemar's test, with the usual correction for continuity, to test for a statistically significant difference in the sensitivities and specificities between the two tests, even when the sensitivity and the specificity of the tests cannot be established, as the test compares only the discordant cells within each of the diseased and non-diseased groups:

Table 1 Sample scheme used (Schatzkin *et al.* 1987)

	Diseased			Non-diseased		
	Test 1+	Test 1−	Total	Test 1+	Test 1−	Total
Test 2 +	*a*	*b*	*a* + *b*	*A*	*B*	*A* + *B*
Test 2 −	*c*	(*d*)	c + (d)	*C*	(*D*)	*C* + (*D*)
	a + *c*	*b* + (*d*)	(*n*)	*A* + *C*	*B* + (*D*)	(*N*)

Value in parentheses indicates unknown values.

$X^2 = (|b - c| - 1)^2/b + c$ and $X^2 = (|B - C| - 1)^2/B + C$, respectively (Schatzkin *et al.* 1987).

We further determined the trade-off between VIA and PAP smear by calculating the ratio of extra false positives (EFPs) to extra true positives (ETPs) detected. According to Chock *et al.* (1997) this EFP:ETP ratio equals $(A + B) - (A + C)/(a + b) - (a + c)$ and the 95% confidence interval:

$$CI = \exp[\ln(B - C)/(b - c)] \pm 1.96[(b + c)/(b - c)^2 + (B + C)/(B - C)^2]^{0.5}.$$

The performance of VIA was compared with the PAP test using a high threshold for the referral test: cervical intraepithelial neoplasia (CIN) grade 2 or higher, on biopsy.

The effect of the number of tests done on the test result was assessed in univariate analysis, calculating a *P* value for the difference in the false positive rate (FPR) between providers having used the test at least 100 times and the others. The FPR is subject to verification bias as no biopsy was performed on VIA-positive patients with negative colposcopy, but we assume the bias to be equal in both the groups.

Results

Visual inspection was used as a screening tool by six of seven (85.7%) trained medical doctors and by 14 of 26 (53.8%) trained nurses. It was implemented in all health centres, in the NGO clinic, in four of 13 (30.8%) health posts and for a few months in the gynaecology consultation of the district hospital.

Between September 2000 and July 2002, 1080 patients underwent visual inspection. Of them 572 (53.0%) were seen in the health centres, 362 (33.5%) in the NGO clinic, 133 (12.3%) in the health posts and 13 (1.2%) in the hospital. Medical doctors performed 580 (53.7%) of the tests and nurses 500 (46.3%). A total of 977 (90.5%) women were aged 30 or older. Of them, 381 had never been screened and 432 had not been screened in the last 3 years, resulting in 813 (75.3%) patients belonging to the defined target population. The 1076 patients who had both a PAP test and a visual inspection were included for further analysis on the test performance. Patients had a mean age of 39.8 years (range: 16–86), a mean parity of 5.6 (range: 0–25) and started their sexual life at a mean age of 17.4 years (range: 11–35). Only 7.5% were smokers.

Overall, 352 patients (32.7%) had a positive screening test: 47 (4.4%) tested positive on both VIA and PAP smear, 275 (25.5%) had a positive visual inspection only and 30 (2.8%) only a positive cytology. Of the screen positive patients, 290 (82.4%) were assessed at the colposcopy clinic, where colposcopy and biopsy was performed. Seventeen patients with two negative screening tests underwent additional colposcopies, because they had cervical polyps. Five patients (0.5%) had a histological diagnosis of invasive cancer, 46 (4.5%) of CIN2/CIN3 and 77 (7.6%) of CIN1 and human papilloma virus (Table 2).

Table 2 Distribution of histological results by outcome of screening tests

						Biopsy			
VIA	PAP	*N*	Did not attend colposcopy	Colpo performed	Colpo normal	No dysplasia	CIN1/ HPV	CIN2/ CIN3/*in situ*	Invasive cancer
+	+	47	4 (8.5%)	43	7	4	15	15	2
+	−	275*	52 (18.9%)	223	112	33	50	25	3
−	+	30	6 (20.0%)	24	6	3	9	6	0
−	−	724†	–	17‡	6	8	3	0	0
Total		1076	62§	307	131	48	77 (7.6%)	46 (4.5%)	5 (0.5%)

VIA, visual inspection with acetic acid; CIN, cervical intraepithelial lesion; HPV, human papilloma virus.
* Including four patients with no diagnosis on PAP smear because of bad quality.
† Including two patients with no diagnosis on PAP smear because of bad quality.
‡ Patients referred to colposcopies for other reasons, mainly because of presence of polyps.
§ Patients excluded from analysis on comparison of the two tests.

Table 3 Performance of visual inspection in relation to experience*

Number VIA	VIA result		Final result		False positive rate		
	Negative	Positive	Negative	≥HSIL	Overall	Doctors	Nurses
≥100	282 (74.0%)		280	2			
		99 (26.0%)	76	23	76 of 99 (76.8%)	64 of 83 (77.1%)	12 of 16 (75.0%)
5–99	446 (74.7%)		442	4			
		151 (25.3%)	131	20	131 of 151 (86.8%) $P = 0.04$	78 of 89 (87.6%)	53 of 62 (85.5%)

VIA, visual inspection with acetic acid; HSIL, high-grade intraepithelial lesion.
* The cases where final results were not available are excluded from the analysis: in groups ≥100: four (14.8%) VIA negative and 23 (84.6%) VIA positive; in groups 5–99: six (15.4%) VIA negative and 33 (84.6%) VIA positive; and 32 VIA carried out by various health providers during training sessions.

Of 51 patients diagnosed as CIN2 and more on biopsy, 17 were positive on both screening tests, whereas 28 had a positive VIA test only and six a positive PAP smear only. Of 256 patients with a negative referral test, 26 were both PAP and VIA positive, 195 had a positive VIA test only and 18 a positive PAP smear only. The RELTPR of VIA compared with PAP smear was 1.96 (45 : 23), $P < 0.001$. The RELFPR was 5.02 (221 : 44), $P < 0.001$. The EFP:ETP ratio was 8.05 (95% CI: 4.68–13.86).

The FPR of VIA was 82.8%. The FPR decreased with experience: it was 86.8% for health providers who used the test <100 times, compared with 76.8% when used at least 100 times ($P = 0.04$). These rates were similar for nurses and for doctors (Table 3).

Time between screening and diagnosis was significantly shorter for visual inspection than for PAP smear. For 206 patients with a positive VIA and a negative PAP smear, mean time to colposcopy was 17.5 days (95% CI: 14.3–20.8, median 10) compared with 68.9 days (95% CI: 47.5–90.3, median 54) for 23 patients who had a positive PAP but a negative VIA and 36.2 days (95% CI: 24.8–47.5, median 30) for 42 patients with both tests positive ($P < 0.001$).

Discussion

Despite the fact that cytology-based screening programmes for cervical cancer have been introduced in most of the countries in South and Central America since the 1970s, they have had very limited success (Sankaranarayanan *et al.* 2001). Low screening coverage and inappropriate collection and reading of PAP smears and limitations in the accuracy of this test have been shown to be important reasons for the observed ineffectiveness of these programmes (Eluf-Neto & Nascimento 2001).

Our study shows that, despite additional training in correct sampling and reading of PAP smears, the detection rate for dysplasia was only 4.7% in a high-risk population. Earlier data, whereby PAP smears were taken by one single gynaecologist in a general population in Nicaragua, showed a detection rate for abnormal smears of 7.7% (Claeys *et al.* 2002b). Quality control data revealed that sampling (including lack of endocervical cells and poor fixation) rather than misclassification was the main problem. Yet, compared with the centres where personnel was not trained, the detection rate was three times higher and the number of inadequate samples halved, indicating that the training had an effect on the quality of the PAP smears (data not shown in this paper). Although the performance was improved, the PAP test only detected 47 of 138 lesions, missing nearly half of the HSIL and more than half of the invasive cancers. Conversely, through visual inspection, twice as many pre-malignant lesions of the cervix were detected. This result was obtained after a very short training, without further supervision and by a variety of health providers using the test. This confirms VIA to be a cheap test, easy to perform and with a high sensitivity (Kitchener & Symonds 1999). Our study further showed that the performance increases with experience, as reflected by a decrease in the FPR. The study design does not allow an assessment of the false negative rate, as no gold standard was applied to all people with a negative test. Yet, this might be less important as the main problem of VIA is the low specificity (Wright *et al.* 2002). Other advantages of visual inspection include the shorter delay in referral and final diagnosis, which is crucial for compliance and timely treatment.

Unfortunately, this does not mean that the ideal screening test for cervical cancer screening has been found. Comparing the test performance of VIA with PAP smear reflects a common situation where one test has a higher true positive rate than the other at the expense of having a higher FPR (Chock *et al.* 1997). Whereas VIA is known to have a higher sensitivity than PAP smear, its specificity is

substantially lower. This results in a high number of women needing unnecessary confirmatory investigation. Normal diagnostic procedures consist in colposcopy and biopsy, which are performed at the referral level. The practical impact of this trade-off is shown by the EFP:ETP ratio for VIA compared with PAP smear. This ratio was 8.05, indicating that for every extra case of at least a CIN2 on histology, eight extra false positives had to be attended at the referral level.

The increase in both ETPR and EFPR has a serious impact on the organization of the referral level. In our setting, the detection of more than twice as many lesions through the use of VIA meant quadruplicating the number of patients referred to colposcopy and a doubling of the number of patients needing treatment for high-grade lesions or invasive cancer. Using the PAP smear as a primary screening tool, only 77 women would have been referred, 24 low-grade intraepithelial lesion (LSIL) followed-up, 21 HSIL and two cancers treated. In absolute terms, VIA meant a surplus of 245 (of whom 199 attended) referrals to the colposcopy clinic, of 41 extra LSIL needing close follow-up, of 19 extra HSIL and three extra invasive cancers needing specialized treatment and this for 1080 women screened over a period of nearly 2 years.

If visual inspection were to be used as a common screening test, an easily accessible colposcopy clinic would have to be set up at the level of district hospital. Gynaecologists would have to be trained in colposcopy and outpatient treatment modalities, and accept to examine many false positive patients. However, the increase in workload could be countered by focusing the screening programme on older women and increasing the screening interval to 3 years. This would reduce the total number of tests provided and increase the cost-effectiveness of the programme.

Recently, it was shown using a population-based simulation model that VIA, with immediate treatment when abnormalities were found, would be the most cost-effective approach in Thailand if the test was applied at 5-year intervals in women aged 35–55. In the model, treatment consisted of cryotherapy provided at community site and referral for hospital evaluation when a suspected invasive cancer is revealed by the test. However, the authors comment that, depending on resources, test performance and compliance with screening and follow-up, several other options are viable alternatives (Mandelblatt *et al.* 2002). In Nicaragua, as in most of the Latin American countries, where screening and referral systems, as well as large number of professionals exist, a see and treat approach can hardly be defended. In our study, only 45 of 266 (16.9%) women with a positive VIA test had a lesion that needed immediate treatment (high-grade dysplasia or more). The others would have been unnecessarily overtreated. Moreover, compliance with referral was very good: 82% of referred women attended the colposcopy clinic, which is much higher than the estimated 50% in the previous study. This high compliance rate might have been influenced by the organization of the programme, including the invitation of women of the target population and the provision of diagnosis and treatment free of charge. Yet, health promotion to increase the uptake of a screening programme and the (geographical and financial) accessibility of diagnostic and treatment services should be taken into account in the design of all screening programmes. From an operational point of view, it is also easier and more feasible to provide treatment at the referral level, than it would be to make cryotherapy available in all primary health centres where screening is currently provided.

It might be too early to advocate widespread use of visual inspection as a screening test. Our study, as most of the studies on VIA, focuses on one single test and no information is available on test performance when the test is repeated. It cannot be excluded that the results are positively influenced by the motivation of the health workers who used the test, as half of the nurses did not use it and no information is available on their performance. Yet, these nurses neither performed PAP smears, so most probably they were assigned to other programmes during the study period.

There is an urgent need to establish uniform criteria on test positivity and on definitions to evaluate test performance (Denny *et al.* 2002). Our criteria resulted in nearly 30% tests reported as positive. Other authors report 24–25% positive tests (University of Zimbabwe/JHPIEGO Cervical Cancer Project 1999; Belinson *et al.* 2001; Denny *et al.* 2002). Using the same criteria in a research setting in Kenya, more than 27% of the tests were positive (H. De Vuyst, personal communication). VIA is a promising test and further field testing to increase its performance is surely needed. Now that more results are available on its effectiveness, standardization and methods for quality control are highly required. Meanwhile, efforts should be targeted into further improving the existing cytology-based programme. Measures to increase coverage (Claeys *et al.* 2002a) need to be complemented by additional in-depth training of health professionals for correct sampling of PAP smears and further exploring VIA as an alternative screening test.

In conclusion, this study shows that VIA when applied on a larger scale spectacularly increases the number of CIN and invasive cancer detected in a general, but inadequately screened, population. However, the relatively high FPR remains an important concern for the organization of the referral level.

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