Aided visual inspection with acetic acid (VIA) and HPV detection as optional screening tools for cervical cancer and its precursor lesions

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Summary

Purpose of investigation: To assess the contribution of visual inspection with acetic acid (VIA) and Hybrid Capture II (HCII) as adjunct methods to the Pap test in detecting cervical neoplasia. *Subjects and methods:* This was a cross-sectional study with 809 women who consecutively attended gynecological consultations at Campinas University, Brazil, from January 2002 to July 2003. Pap test, HCII, VIA, and colposcopy were offered to all patients. Performance of tests (alone or in combination) in detecting histologically confirmed lesions was evaluated. *Results*: Of the 40 patients with CIN, 69% had CIN1, 26% CIN2 or CIN3 and one patient had invasive carcinoma. VIA had the best performance in detecting CIN, yielding 72% sensitivity and 91% specificity. Considering only CIN2 or worse as significant lesions, HCII had the best sensitivity (73%), while the Pap test was the most specific (93%). Combining the three exams, 92% of the CIN1 or worse were detected. When CIN1 was excluded from the analysis, Pap smear plus HCII delivered 82% sensitivity and 79% specificity. However, this combination yielded a very low (5%) PPV. *Conclusion:* VIA and HCII contributed to the screening of cervical neoplasia in a group of Brazilian women, but the cost-effectiveness of conjoint screening modalities is still debatable.

Key words: Cervical intraepithelial neoplasia; Visual inspection with acetic acid (VIA); Hybrid Capture II; Screening.

Introduction

Every year, almost half a million new cases of cervical cancer are detected worldwide, and almost 50% of these new cancers will result in death [1]. As the consequence of social, economic, sanitary and public health shortcomings, approximately 80% of the new diagnoses of cervical cancer occur in the developing world [2-4]. In Latin America, cervical cancer is the second most frequent malignancy among women and also the second cause of female deaths. It is unfortunate that, in contrast to all advances in prevention and treatment found in the developed world, the incidence of cervical cancer and its mortality rates have been stable since 1960 in the poorer regions of the southern hemisphere [4, 5].

Systematic and widespread use of cervical cytology (Pap smear or Pap test) has been key to the reduction of cervical cancer incidence in several countries. The Pap test allows the detection of precancerous abnormalities of the cervix and cervical intraepithelial neoplasia (CIN), which can be cured even with conservative treatments. Pap smear based screening programs heralded a 70% reduction in cervical cancer incidence in developed countries [6-8]. However, years of accumulated expertise disclosed important weaknesses of this test: Pap smear performance is dependent on several steps, from adequate collection to slide processing and, ultimately, availability of experienced professionals capable of interpreting cellular abnormalities. This infrastructure is not always available, resulting in remarkably discrepant figures of Pap smear sensitivity in detecting cervical cancer precursors, reported to range from 30% to 87% [9, 10].

The inherent flaws of the Pap test, associated with structural difficulties encountered in developing countries to adequately implement its usage, elicited the search for alternative screening tools for cervical cancer. Visual inspection with acetic acid (VIA) has been advocated by some investigators as an inexpensive and easy to implement screening technique, especially useful in low-resource areas as a stand-alone test or in combination with the Pap test. Consisting of naked-eye examination of the cervix, following its exposure to 5% diluted acetic acid, VIA has been shown to perform very closely to the Pap test in detecting cervical squamous lesions [11-17]. Another advantage of this test is the possibility to screen and treat the patient in a single visit, dramatically reducing costs and augmenting compliance to follow-up. However, VIA is reported to have a large number of false-positive results, leading to a number of unnecessary treatments.

Confirmation of the causative role of human papillomavirus (HPV) in the natural history of cervical cancer has led to the development of molecular techniques aimed at detecting cervical infection by this virus. Hybrid Capture II (HCII) is a commercially-available HPV detection technique, fully approved by the FDA. HCII

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has also been studied as a stand-alone screening test, or used in combination with the Pap smear. Some investigators have reported its sensitivity and negative predictive values to be better than that of the Pap test [18-22]. This paper was aimed at assessing VIA, HCII and the Pap smear as screening tests, used alone or in combination, having as the gold-standard colposcopy. Most studies that evaluated the performance of cervical cancer screening tests did not provide a colposcopic evaluation of all study subjects, preventing direct measures of performance indicators of these tests.

Material and Methods

Patient selection

For this cross-sectional study, 809 consecutive patients attended gynecological consultations at a teaching hospital from Campinas Universidade Estadual de São Paulo, Brazil. Patients were recruited through an open advertisement distributed in the University facilities. Thus, the study sample comprises University personnel and students, and their relatives or friends. No financial support has been given to women that agreed to enroll.

Women were considered eligible if they met all of the following requirements: a) age between 18 to 60 years; b) had an intact uterus (i.e., no previous surgical procedure on the cervix or *corpus uteri*); c) had no history of an abnormal Pap test in the previous year; d) were not undertaking treatments for condyloma, vaginal and cervical warts; e) had had no sexual intercourse during the three days prior to consultation; f) did not have any confirmed or clinical suspicion on immunossupression: HIV, therapy with corticosteroids, chemotherapy, chronic diseases that might affect the immune system. All patients signed an informed consent and the study protocol was reviewed and approved by the local ethics committee on medical research.

Patient examination

The consultation visit comprised an interview and pelvic examination. The interview consisted of a questionnaire regarding social, demographic and clinical issues, Samples for Pap smear and HCII were collected during pelvic examination. After sample collection, 5% acetic acid was applied to the cervix and approximately one minute later a "naked-eye" examination was performed (see description of VIA) and the examiner's impression recorded. Finally, colposcopy was performed and eventual abnormal areas were biopsied. A second appointment, to apprise the patients of their exam results, was scheduled 45 days after the first visit. Patients that had high-grade Pap results or were found to have histologically confirmed high-grade disease or cancer were treated by the investigators. Patients with normal/low-grade Pap tests or normal colposcopy/low-grade histological disease were scheduled for a follow-up visit in four months (results of these follow-up visits are not reported in this article).

Visual inspection with acetic acid (VIA)

After collection of the samples for the Pap test and HCII, 5% acetic acid was applied to the cervix through an embedded cotton at the edge of a Cherron. One minute thereafter, the cervix was illuminated with a 100W bright lamp and examined by "naked eyes". Examiners were trained to classify their visual impression according to the Atlas of Visual Inspection, which has many diagnostic possibilities (see below). For statistical purposes, these diagnoses were grouped as *negative* or *positive,* as follows:

Negative: nulliparous, multiparous, presence of cervical mucous, squamous metaplasia, ectropion, cervicitis, Naboth cysts; polyps, vaginal discharge;

Positive: suggestive of condyloma, CIN1, CIN2, CIN3 or cancer.

Pap smear

The Pap smear was taken with the Ayre spatula and endocervical brushes, then fixed in 95% ethanol and stained by the modified Papanicolaou method. Final cytological diagnoses were rendered using the Bethesda System (2002) [23] and classified as normal/inflammatory, atypical squamous cells (ASC), atypical glandular cells (AGC), low-grade squamous intraepithelial lesion (LSIL) or high-grade squamous intraepithelial lesions (HSIL). For statistical purposes, normal/inflammatory results were categorized as negative, and ASC, AGC, LSIL or HSIL as positive.

Hybrid Capture II (HCII)

The specimens for HCII were tested with probe B (high-risk HPVs: types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) [24] and the tests were classified positive at the relative light unit/positive control (RLU/PC) ratio of 1 pg/ml or greater. These RLU/PC ratios provide a semi-quantitative estimate of the amount of HPV DNA in the specimens, i.e., the viral load in the sample. The storage of the specimens and all reagents as well as conduction of the tests took place at the Medical School Hospital Laboratory, following the manufacturer's instructions *(Digene Diagnostics Inc., USA).*

Colposcopy

Colposcopy was performed immediately after an abormal VIA or, in case of a positive Pap smear or HCII, at the second appointment. The examination was always performed by an experienced and certified colposcopist. Careful examination of the cervix and transformation zone (TZ) was carried out, approximately one minute after applying 5% acetic acid to the entire cervix, with up to 40 times magnification (DF Vasconcellos Inc, Brazil). Acetowhite epithelium, punctuation, mosaic, iodine negativity and atypical vessels prompted colposcopically targeted punch biopsies.

Cervical biopsies

Directed punch biopsies (and cone biopsies) were fixed in formalin, embedded in paraffin, and processed into 5-mm-thick hematoxylin-eosin (HE) stained sections for light microscopy, following the routine procedures. All biopsies were examined among the daily routine in the Pathology Departments of the four clinics, and diagnosed using the commonly agreed CIN nomenclature. For study purposes, the pathologists were also asked to note any morphological changes suggestive of the presence of HPV in cases with no CIN, i.e., HPV-NCIN (= flat condyloma). The slides from two of these centers were subjected to re-examination by a panel of pathologists from EC countries. The consensus diagnosis of the panel was considered as the final diagnosis, comprising also the specific diagnostic categories used in classifying cervical pathology.

Statistical analysis

Sensitivity, specificity, positive and negative predictive values (PPV and NPV) were calculated for the Pap test, VIA and HCII,

alone or in combination, in detecting squamous cervical lesions. Two settings of histological results were considered: patients were regarded as *positive* when they had histologically-confirmed CIN1 or worse or CIN2 or worse. All calculations were performed with the R environment for statistical computing [25], within 95% confidence intervals (95%CI).

Results

Patients' age ranged from 18 to 60 years old (mean 37 years). The cervix was considered abnormal with VIA in 99 (12%) women and these abnormalities were described as suggestive of either condyloma (28%), CIN1 (57%), CIN2/3 (13%) or invasive carcinoma (1%). Ninety-three percent (751 women) of the Pap tests were rendered as normal, whereas 22 (38% of the abnormal Pap tests) were classified as ASCUS, 20 (35%) as LSIL and 14 (25%) as HSIL, one as AGC and one was considered inadequate for analysis. Regarding HPV detection with HCII, 151 (19%) women had positive results. Almost 96% (773/809) of the patients were deemed to have a normal cervix, i.e., normal colposcopy or cervical biopsy rendered as normal epithelium/cervicitis. Of the 40 patients with histologically confirmed CIN, 72% presented with low-grade disease (CIN1) and the remainder with high-grade lesions (CIN2, CIN3 or cancer). One patient was found to have macroinvasive carcinoma (Table 1).

VIA was the test that performed best in detecting CIN, yielding 72% (95% CI: 69% to 75%) sensitivity and 91% (95%CI: 89% to 93%) specificity. Conversely, the Pap test detected only 45% of the CIN cases, and HCII also detected only 62% of the patients with histologically confirmed disease. Nevertheless, while considering only high-grade lesions (CIN2 or worse), HCII had the best sensitivity 73% (95% CI: 69% to 76%), while the Pap test was still the most specific screening tool (93%; 95% CI: 91% to 95%) (Table 2).

Table 1. — *Visual inspection with acetic acid (VIA), Pap test, Hybrid Capture II (HCII) and final diagnosis.*

Test	n	%
VIA		
Negative	710	(88)
Positive	99	(12)
Condyloma	28	(28)
CIN 1	57	(57)
CIN 2	11	(11)
CIN 3	2	(2)
Invasive	1	(1)
Pap test		
Normal	751	(93)
Abnormal	57	(7)
ASCUS	22	(38)
LSIL	20	(35)
HSIL	14	(25)
AGC	1	(2)
Inadequate	1	(0.2)
HCII		
Negative	635	(81)
Positive	151	(19)
Final diagnosis		
Negative	773	(95)
Positive	40	(5)
CIN 1	29	(72)
CIN 2	7	(18)
CIN 3	3	(7)
Invasive	1	(3)

Table 2. — *Performance of VIA, Pap test and HCII in detecting CIN 1 or worse or/CIN 2 or worse.*

Screening test	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV %	NPV %
VIA (CIN1 or worse)	72 (69 to 75)	91 (89 to 93)	28	98
VIA (CIN2 or worse)	54 (51 to 58)	88 (86 to 91)	6	99
Pap test (CIN1 or worse)	45 (41 to 48)	94 (93 to 96)	31	97
Pap test (CIN2 or worse)	54 (51 to 57)	93 (91 to 95)	10	99
HCII (CIN1 or worse)	62 (59 to 66)	83 (80 to 86)	16	98
HCII (CIN2 or worse)	73 (69 to 76)	81 (79 to 84)	5	99

PPV = positive predictive value; NPV = negative predictive value.

Combining the Pap smear with either VIA, HCII or both, very different figures for performance arise. The addition of VIA raised Pap smear performance in detecting CIN1 or worse to 85% (95% CI: 82% to 87%), being also the most specific two-by-two combination (86%; 95% CI: 83% to 88%). Assessing the three exams altogether, 92% of the CIN1 or worse lesions were detected. When CIN1 lesions were excluded from the analysis, the most sensitive two-by-two combination of screening exams was the Pap smear plus HCII, delivering 82% (95% CI: 79% to 84%) sensitivity and 79% (95% CI: 76% to 81%) specificity, even higher than that provided by the association of the three exams (70%; 95% CI: 67% to 74%). However, this combination yielded a very low (5%) PPV (Table 3).

Table 3. — *Association of screening methods in detecting CIN 1 or worse/CIN 2 or worse.*

Test association	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV %	NPV %
Pap and/or VIA (CIN1 or worse)	85 (82 to 87)	86 (83 to 88)	24	99
Pap and/or VIA (CIN2 or worse)	73 (70 to 76)	83 (81 to 86)	6	99
Pap and/or HCII (CIN1 or worse)	72 (69 to 76)	80 (78 to 83)	17	98
Pap and/or HCII (CIN2 or worse)	82 (79 to 84)	79 (76 to 81)	5	100
Pap and/or VIA and/or HCII (CIN 1 or worse)	92 (91 to 94)	73 (70 to 76)	15	99
Pap and/or VIA and/or HCII (CIN 2 or worse)	82 (79 to 84)	70 (67 to 74)	4	99

PPV = positive predictive value; NPV = negative predictive value.

Discussion

Still investigational, alternate and adjunctive methods of screening are currently considered a necessity to further decrease mortality associated with cervical cancer. Even in developed countries, the sensitivity of the screening, based on Pap tests exclusively, should be augmented, and in economically disadvantaged areas, cost-effective prevention is yet to be implemented. In the present study, VIA and HCII contributed significantly to

the Pap test in detecting cervical abnormalities, and the main improvement was found in the sensitivity of the screening. Importantly, because colposcopy was available for all patients, direct measures of the performances of VIA, HCII and Pap smear in detecting cervical abnormalities could be obtained. The homogeneous set of patient social and demographic characteristics (e.g., age, family income, years of study) also permitted selection bias to be reduced ensuring a more reliable comparison of test performances.

In this study, performance figures for the Pap test are very close to those that have been reported previously: the JPIEGO Cervical Cancer Project [26], reporting on 10,934 women, found the Pap test to be 30% sensitive in the detection of CIN 1 or worse and 44% sensitive for high-grade disease. Cronjé *et al.* [27] reported a sensitivity of 23% for CIN1 or worse and 53% for high-grade disease, while stating that performance improved with patient age. Almost ten years ago, Fahey *et al.* [28] anticipated these figures with a 50% sensitivity for cervical disease in their case series. These allowed us to conclude that Pap tests in the present study performed very similarly as in previous reports.

VIA results are also aligned with those reported by Sankaranayanan *et al.* [16, 29]. This author detected abnormalities in 9% to 10% of the visual exams, very close to the 12% of altered VIA found by us. In Africa, however, more than 20% of the patients were considered to have an abnormal cervix when examined with VIA [26]. In the present study, colposcopic examination confirmed most of the abnormalities found with VIA. For instance, 82% of the patients with VIA, classified as " suggestive of condiloma/CIN1", also had an abnormal colposcopy. Among patients with VIA "suggestive of CIN2/3", more than 92% also had colposcopic abnormalities. Nevertheless, colposcopy was performed by the same physician that had previously examined the cervix with VIA. In theory, VIA usage is supposed to be restricted to areas where resources are scarce, and performed by non-medical professionals, therefore preventing one from overtly extrapolating the hereby reported results to actual field conditions. As a stand-alone test, VIA outperformed HCII and the Pap smear, but study limitations, such as an experienced colposcopist performing visual inspection, should be carefully taken into account.

HCII, as a stand-alone test, detected 73% of CIN2 or worse cases, whereas its sensitivity was decreased to 63% when all lesions (i.e., CIN1 or worse) were considered altogether. This finding is not unexpected: HCII was performed only with probe B, dedicated to the detection of high-oncogenic risk HPV types [30]. Robust evidence has been furnished by the ASCUS-Low SIL Triage Study, in that detection of high-risk HPV is only useful in the management of patients with CIN1 for discriminating women at increased risk of progression to high-grade disease, not for the detection of CIN1 itself [31]. In alignment with this large report, in our study HCII was found to be negative in 41% of the women with CIN1, unequivocally disclosing the low sensitivity of this test for the detection of low-grade disease.

Many ongoing studies are paving the way for new cervical cancer screening strategies. These reports are almost universally consonant in that the association of screening techniques may improve overall sensitivity and, in some instances, also specificity and predictive values. Nevertheless, strategies on how to deal with increasing costs and the larger number of women to be referred to colposcopy are still pending. An overall gain in sensitivity was also found when the Pap test was used in combination with either VIA, HCII or both, at some expense to sensitivity and positive predictive values. On the other hand, the association of the Pap test and VIA performed very well in the detection of CIN1 or worse, and the association of HCII had an excellent summative effect for the detection of high-grade disease. The techinical features of VIA and HCII explain these focal advantages: whereas VIA is suitable for visually apparent disease, as the large aceto-white areas characteristic of CIN1, HCII detects viral types most associated with severe disease. Moreover, VIA can not detect lesions that are restricted to the cervical canal, whereas Pap tests and and HCII are able to find these abnormalities. With increasing age, prevalence of high- and low-grade reverses, with CIN1 prevailing in younger women and CIN2/3 being more common among older patients. Thus, the screening strategy can be tailored according to the age distribution of the screened populations, aiming at specific lesions, maximizing detection and reducing costs and false results.

Conclusion

As stand-alone tests, HCII, the Pap test and VIA were unable to detect all low- or high-grade lesions. The combination of tests advanced the overall performance of the screening, but even the three tests altogether proved to be unable to refer all patients with significant histological lesions to colposcopy. In the recent past, some authors have discussed the application of colposcopy as a screening tool for cervical cancer [32], but this discussion has not gained momentum because the introduction of colposcopy in the screening fields is not economically plausible. It is also important to consider that screening for cervical cancer takes advantage of the slow progression of cervical cancer precursors: over the time necessary for a mild lesion to become an invasive neoplasia, women may be screened several times, therefore reducing the probability of the lesion remaining undetected.

The ongoing efforts aimed at improving cervical cancer screening are vigorous. A large body of knowledge is being created and it has become possible to forecast that different and creative screening strategies will address peculiarities in disease and HPV prevalence and fit the economical and social characteristics of target populations.

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