ORIGINAL ARTICLE

Evaluation of visual inspection with acetic acid (VIA), Lugol's iodine (VILI), cervical cytology and HPV testing as cervical screening tools in Latin America

This report refers to partial results from the LAMS (Latin AMerican Screening) study

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Objectives: To assess the performance indicators of visual inspection with acetic acid (VIA) and visual inspection with Lugol's iodine (VILI) in four Latin American centres participating in the ongoing Latin AMerican Screening (LAMS) study, in settings with moderate incidence of cervical disease and with poorly to moderately well-organized cervical cancer screening.

Setting: Three Brazilian centres (São Paulo, Campinas and Porto Alegre) and one Argentine centre (Buenos Aires) recruited a total of 11,834 healthy women to undergo VIA, VILI, conventional Pap smear and Hybrid Capture II (HCII).

Methods: Women who had a positive result from any of these tests were subjected to colposcopy and biopsies (if necessary), and women with high-grade cervical intraepithelial neoplasia (CIN) were properly treated. To control for verification bias, 5% of women with normal tests were referred for colposcopy, as were 20% of HCII-negative women.

Results: Data on VIA ($n=11,834$), VILI ($n=2994$), conventional Pap smear ($n=10,138$) and HCII ($n=4195$) were available for test comparisons, calculating sensitivity, specificity, and positive and negative predictive values. Overall test positivity was 11.6% for VIA, 23.0% for VILI, 2.2% for Pap smear (LSIL threshold), 1.1% for Pap smear (HSIL threshold) and 17.1% for HCII. VIA was positive in 61.8% of the women with CIN 1, 57.0% of those with CIN 2, 35.0% of women with CIN 3 and in 21 of 28 (75%) of women with cancer. Approximately 10% of women with no detectable disease had an abnormal VIA. Regarding VILI, 83.3% of women diagnosed with CIN 1 and 62.5% of those with CIN 3 had an abnormal test. VILI failed to detect one of three cases of cancer. Both the sensitivity, specificity and positive predictive value of VIA and VILI in detecting CIN 2 or CIN 3 could be significantly improved depending on the combination with Pap smear or HCII (sensitivity up to 100.0% and specificity up to 99.8%).

Conclusions: The LAMS study failed to reproduce the performance figures obtained with VIA and VILI (as stand-alone tests) in some other settings, where the prevalence of cervical disease was higher. However, a combined use of VIA or VILI with the Pap test or HCII allowed specific detection of cervical abnormalities.

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INTRODUCTION

Severely affected by the lack of human and material resources, many economically underprivileged geographic regions fail to provide efficient screening for cervical cancer.[1,2] The current mainstay of cervical cancer screening, cervical cytology (Pap smear), necessitates a well-organized infrastructure to achieve optimal results: health units to collect cervical material, laboratories to prepare the slides for reading, specialized personnel apt to render a diagnosis and, ultimately, physicians trained to deal with the abnormalities eventually detected.[3] This structure is not readily available, and only a few countries have managed to consistently reduce their cervical cancer incidence and prevalence rates by widespread use of Pap smear screening, most notably the Nordic Countries.[4–6]

Prompted by the need for optimal strategies for cervical cancer screening, and based upon the concepts that the majority of pre-invasive and invasive cervical lesions are visible by 'naked-eye' observation, investigators have developed novel affordable diagnostic tools suitable for large-scale screening of cervical abnormalities.[7] Visual Inspection with Acetic Acid (VIA) and Visual Inspection with Lugol's Iodine (VILI) are two modifications of a direct visual assessment of the cervix, different only in regard to the solutions applied to enhance the cervical lesions.

Several recent studies testing VIA suggest that it closely matches the Pap smear in its performance in detecting cervical cancer precursors.[7,8] In a recent report on 4444 women, VILI was also shown to perform adequately,[9] being comparable to both VIA and the Pap smear.[10,11] However, several weaknesses of VIA and VILI have been revealed, particularly the high rate of false-positive findings, which may lead to substantial number of colposcopies.[11–13] Importantly, more work is needed to evaluate the performance of these new tools under field conditions, and on implementing VIA and VILI in countries with different cancer incidence and in different screening settings.

The authors recently designed a multi-centre study testing eight different screening tools in a cohort of over 12,000 women enrolled by four clinics in regions of Brazil and Argentina with different incidence of cervical cancer, known as the Latin AMerican Screening (LAMS) study.[14] One of the two major aims of this study is to evaluate the feasibility of eight different diagnostic tests, to find out the cost-effective tools for cervical cancer screening in these low-resource settings. VIA and VILI are included in the repertoire of these eight diagnostic tests to be compared in the LAMS study. The present communication reports the performance of VIA and VILI, used as a stand-alone test and combined with other tests in detecting significant cervical pathology in our setting.

MATERIALS AND METHODS

Study design

LAMS is an ongoing, cross-sectional, multi-centre study sponsored by the European Commission through its INCO–DEV partnership (ICA4-CT2001-10013). In this study, consecutive women from the cities of Campinas (Brazil), São Paulo (Brazil), Porto Alegre (Brazil) and Buenos Aires (Argentina) were recruited to undergo gynaecological consultations and examination with conventional Pap smear, VIA and VILI, cervicography and screening colposcopy. Women were sampled for human papilloma virus (HPV) by Hybrid Capture II (HCII). All centres performed conventional Pap smear, HCII and VIA, but only Porto Alegre was assigned to perform VILI (Figure 1). In order to ensure homogeneous exams quality, all centres provided specialists in gynaecology and well-trained nurses to carry out the specimen collection for HCII and Pap, as well as to perform VIA and VILI. No exact numbers are available on how many exams have been collected/performed by each of these professionals. Altogether, 11,834 women were examined with VIA, 2994 with VILI, 10,138 had conventional Pap test, and 4195 with HCII at the first clinical visit. Figure 1 depicts the number of women enrolled by each centre. The study protocol has been approved by the local Ethics Committees of all participating clinics. All enrolled women gave their agreement to participate by signing the Informed Consent Forms written in their native language.

Study centres and demographics

Campinas is a city of one million inhabitants, situated in the southeast region of Brazil approximately 100 km from São Paulo city. The city is a dynamic commercial and industrial centre, with a relatively well-structured health system and some high-standard hospitals. However, a substantial proportion of the population (almost 20%) living on the outskirts of the city is composed of people who migrated from the north and northeast (the poorest) regions of the country, searching for jobs. In São Paulo state, encompassing the cities of Campinas and São Paulo, cervical cancer is the fourth major cause of cancer death among women, accounting for 3.3% of all female deaths due to cancer. In this region, breast cancer accounted for 13.3% of cancer deaths among women between 1995 and 1999.[15] Women have been enrolled in the Centro de Atenção Integral à Saúde da Mulher (CAISM), a State University of Campinas' (UNICAMP) teaching hospital, dedicated to the care of women, and in a basic health unit in the outskirts of the city.

São Paulo city is the economic powerhouse of Brazil, with 11 million inhabitants. Its population is composed of a multiple ethnic groups (European whites, Asians and African–Americans). Health care is heterogeneous, ranging from overcrowded public basic health units and hospitals to a high-quality private sector. In this city, women have been enrolled by the Hospital Leonor Mendes de Barros, a public institution which performs over 50,000 gynaecological and obstetric consultations every year.

Porto Alegre is the capital of Rio Grande do Sul state, in the south of Brazil. The state's population enjoys the best

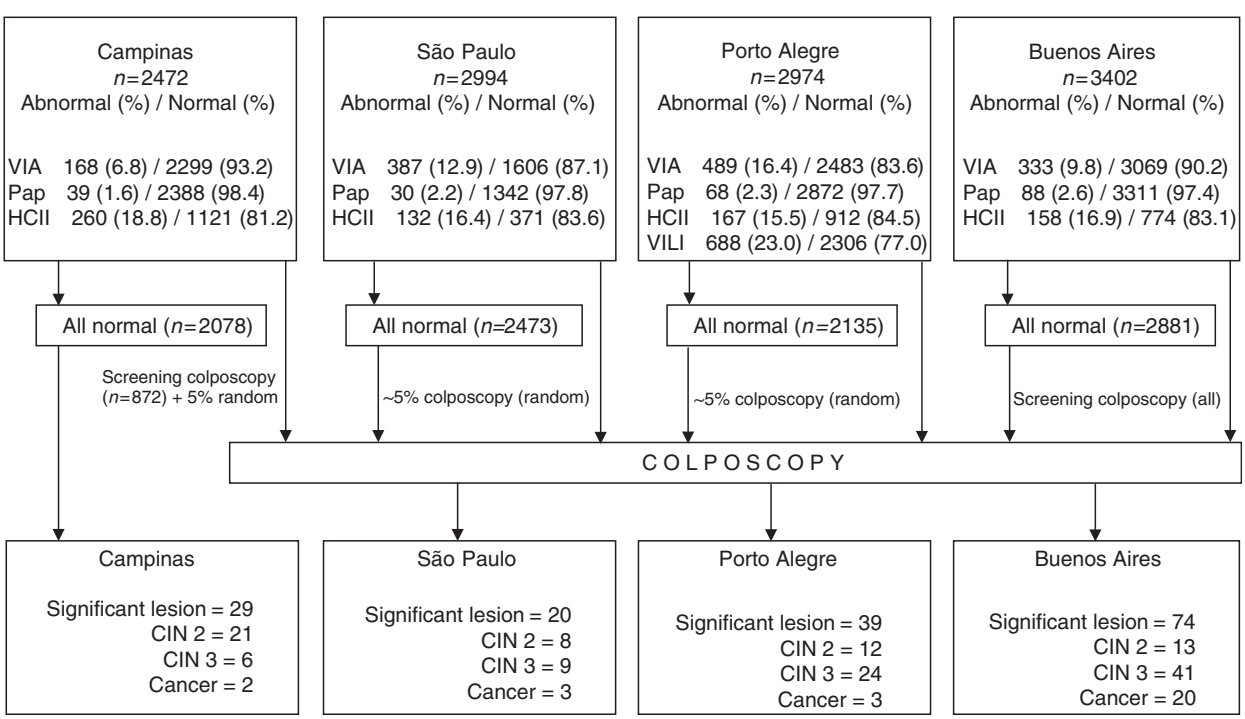

Figure 1 Screening test results for each study centre and final disease status. Significant lesion = CIN 2 or worse.

levels of quality of life in the country, although cervical cancer is the sixth major cause of cancer death among women, accounting for 6.10% of all female deaths due to cancer. In this region, breast cancer accounted for 15.1% of cancer deaths among women from 1995 to 1999.[15]. The participant centre was Hospital de Clínicas de Porto Alegre, a general hospital affiliated to Federal University of Rio Grande do Sul. The department of Obstetrics and Gynaecology of this centre is responsible for roughly 30,000 gynaecological and obstetrical consultations per year.

Buenos Aires is the capital of Argentina, and the participating centre in this country was Hospital de Clínicas José de San Martin, a general teaching hospital with large experience with gynaecology and obstetrics, and being a reference centre for colposcopy for the entire country. The country has an overall cervical cancer mortality rate of 7.6 per 100,000 women.[16]

Enrolment and eligibility

Slightly different protocols were used to recruit the women in different clinics. In São Paulo, Porto Alegre and Buenos Aires, eligible women (see below for criteria) were informed of the study protocol by their local health units, inviting them to participate. In Campinas, in addition to this approach, students and employees of the University Hospital were also informed and invited through an open advertisement, widely distributed in the university facilities.

Women were considered eligible if they met all of the following requirements:

- were aged 18–60 years;
- had an intact uterus (i.e. no previous surgical procedure of the cervix or corpus);
- had no history of abnormal Pap test in the past year;
- were not under treatment for genital condyloma (external or in the cervix);
- had no sexual intercourse during the three days prior to the consultation;
- did not have any confirmed or clinically suspected immunosuppression (HIV, corticosteroids, chemotherapy, other chronic diseases that might compromise the immune system).

Diagnostic setting

After signing the Informed Consent Form, women were subjected to a questionnaire addressing clinical and epidemiological risk factors of cervical disease (e.g. HPV). All women were subjected to a thorough pelvic examination, in this sequence comprising collection of the Pap smear, collection of the HCII sample and VIA. In Porto Alegre, most women were subjected to VILI shortly after they had been examined using VIA. Women who had one or more abnormal result were referred for colposcopic examination. In Argentina and Campinas (CAISM), women were subjected to screening colposcopy even when their exams were negative. The decision to take a histological specimen was based upon the Pap smear result and colposcopy. Abnormal colposcopy prompted punch biopsies of the cervix and women with high-grade cytological abnormalities were referred for conization. Women had their second visit scheduled after 45 days, to be informed about their exam/biopsy results and to be allotted to either the treatment or the follow-up group. Treatment was offered to all women who had high-grade lesion confirmed in the cervical biopsy. In all, 28 cases of cancer were diagnosed during the course of the recruitment phase and were treated according to each institution's protocols.

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 embedded cotton at the edge of a Cherron. After 1 min, the cervix was illuminated with a 100 W bright lamp and visually examined ('naked eye' examination). Examiners have been trained to classify their visual impression according to the Atlas of Visual Inspection,[8] which has many diagnostic possibilities. For statistical purposes, these diagnosis were grouped as negative or positive, as follows:

- negative – nulliparous, multiparous, presence of cervical mucous, squamous metaplasia, ectropium, cervicitis, Naboth cysts; polyps, vaginal discharge.
- positive – suggestive of condyloma, cervical intraepithelial neoplasia (CIN) 1, CIN 2, CIN 3 or cancer.

Visual inspection with Lugol's iodine (VILI)

Following the completion of VIA, the cervix was stained with Lugol's iodine and the visual impressions were classified into three categories: normal cervix, abnormal cervix, and cervix with suspected cancer.[9] Lugol's iodine stains glycogen-rich vaginal epithelium cells. Proliferative lesions, like CIN or cancer, are composed of cells that contain less glycogen than does the surrounding epithelium. These lesions appear as non-staining areas when Lugol is applied to the cervix, and VILI is therefore classified as 'abnormal cervix'. If ulcerated, friable lesions are found, VILI has been classified as 'suggestive of cancer'. VILI was always performed after VIA, because Lugol's iodine usually stains the cervix for 30–60 min, sometimes for many hours. As with VIA, the main purpose of VILI was not to ascertain the diagnosis, but to distinguish between a normal and an abnormal cervix.

- negative – homogeneous staining of the cervix was obtained after application of Lugol's iodine.
- positive – a well-delimited non-staining area was present.

Cervical cytology (Pap smear)

Conventional Pap smears were taken using the Ayre spatula and endocervical brush, fixed in 95% ethanol and stained by the modified Papanicolaou method. Final cytological diagnoses were issued using the Bethesda System (2002)[18] and were classified as normal/inflammatory, atypical squamous cells (ASC), atypical glandular cells (AGC), low-grade squamous intraepithelial lesion (LSIL) or high-grade squamous intraepithelial cells (HSIL).

- negative – normal/inflammatory and ASC results.
- positive – LSIL, HSIL, and 'suggestive of invasive carcinoma' (two thresholds were used for positivity: LSIL or higher and HSIL or higher).

Hybrid capture II (HCII)

The specimens for HCII were tested with probe B (high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68)[19] and the tests were classified positive at the relative

light unit/positive control (RLU/CO) ratio of 1 pg/mL or greater. These RLU/CO ratios provide a semi-quantitative estimate of the amount of HPV DNA in the specimens (i.e. the viral load in the sample). Storage of specimens and reagents, as well as exams processing, were carried out in manufacturer-certified laboratories, under the responsibility of the investigators, following the manufacturer's instructions (Digene Diagnostics Inc., USA). São Paulo and Buenos Aires processed their own HCII samples in-house. Campinas and Porto Alegre had their HCII specimens processed at a Campinas University hospital laboratory.

Colposcopy

Colposcopy was performed immediately after an abnormal VIA/VILI or, in case of a positive Pap smear or HCII, at the second appointment. All women who attended consultations at the Campinas University Hospital (CAISM, 872 cases) and in Buenos Aires (2881 cases; see Figure 1) were examined with screening colposcopy. In the other study centres, 5% of the women with negative screening exams were randomly assigned for colposcopic assessment. Random assignment was performed in the interim between collection and processing of the screening tests and the first visit to be appraised of the test result, the women being informed of her assignment status at that moment. All examinations were performed by experienced and certified colposcopists. Careful examination of the cervix and transformation zone was carried out approximately 1 min after applying 5% acetic acid on the entire cervix, with up to 40 × magnification (DF Vasconcellos Inc., Brazil). Acetowhite epithelium, punctuation, mosaic, iodine negativity and atypical vessels prompted colposcopically targeted punch biopsies.[21]

Cervical biopsies

Tissue samples were fixed in formalin, embedded in paraffin, and processed into 5-μm-thick haematoxylin–eosin-stained sections for light microscopy, following the routine procedures. All biopsies were examined as part of the daily routine in the Pathology Departments of the four clinics, and diagnosed using the commonly agreed CIN nomenclature. For the study purposes, the pathologists were also asked to notify the morphological changes suggestive for the presence of HPV in cases with no CIN (i.e. HPV-non CIN [= flat condyloma]). The slides from two of these centres (Campinas, São Paulo) have been subjected to re-examination by a panel of pathologists from European Union countries (ME, KS). The consensus diagnosis of the panel was considered to be the final diagnosis, also comprising the specific diagnostic categories used in classifying cervical pathology. CIN 2 or worse was regarded as a 'clinically significant lesion', whereas all other histological subtypes of cervical disease were categorized as 'non-significant'.

Statistical analysis

Colposcopy and cervical biopsies (i.e. punch biopsies or cervical cones) were considered to be the reference investigations. Women with pathologically confirmed CIN 2 or worse were regarded as positive, whereas women with normal colposcopy, abnormal colposcopy with non-CIN, CIN 1, or other non-cancer diagnosis, or those who were not examined with colposcopy but had all screening tests negative, were considered to be negative. Differences in women's age distribution according to the study centres have been tested through the paired *t*-test. Sensitivity, specificity, positive and negative predictive values (PPV and NPV) were calculated for all screening tests, alone and in combination, by using the cervical biopsy as the gold standard, with different cut-off points (CIN 2 or CIN 3). In order to avoid distorted performance results, calculations have been carried out after excluding all women with abnormal colposcopy but no histological assessment, and women with an abnormal screening test, therefore necessitating a colposcopic assessment according to the study protocol, but that did not show up for colposcopy or that did not comply with the exam. All calculations were performed with the *R* environment for statistical computing[20] within 95% confidence intervals (95% CI).

RESULTS

Women's age did not differ significantly between study centres ($P = 0.89$). Mean age was 37.9 years, with 90% central range of 26–56 years (data not shown). Overall test positivity was 11.6% for VIA, 23.0% for VILI, 2.2% for Pap smear (LSIL threshold), 1.1% for Pap smear (HSIL threshold) and 17.1% for HCII. The highest proportions of positive VIA (17.3%) and VILI (33.4%) were found among women aged 21–30 years, whereas women aged ⩾41 years were less likely to have a cervical abnormality with VIA (7.8%) or VILI (19.5%). The highest proportions of women with abnormal Pap tests (LSIL) were encountered among those 21–30 years old (2.4%) and 31–40 years (2.9%), whereas for HSIL, the majority of abnormal tests concentrated in the group of women aged 31–40 years (1.7%). No woman ⩽20 years old presented with an HSIL Pap test. In contrast, HCII positivity rates decreased with increasing age, as 38.1% of women aged ⩽20 years had a positive HPV test and only 10.9 of those aged ⩾41 years had a positive HCII (Table 1).

Table 1 Results of the screening tests in different age groups

	Positive/total (% positive)				
Age (years)	VIA	VILI	Pap smear (LSIL)	Pap smear (HSIL)	HPV*
⩽20	51/355 (14.4)	14/57 (24.6)	5/307 (1.6)	0/307 (0.0)	53/139 (38.1)
21–30	531/3076 (17.3)	155/464 (33.4)	63/2648 (2.4)	15/2648 (0.6)	264/1070 (24.7)
31–40	397/3290 (12.1)	192/794 (24.2)	81/2773 (2.9)	47/2773 (1.7)	205/1204 (17.0)
⩾41	398/5113 (7.8)	327/1679 (19.5)	76/4410 (1.7)	52/4410 (1.2)	195/1782 (10.9)
Total	1377/11834 (11.6)	688/2994 (23.0)	225/10138 (2.2)	114/10138 (1.1)	717/4195 (17.1)

*Detected with Hybrid Capture 2®

VIA, visual inspection with acetic acid; VILI, visual inspection with Lugol's iodine; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; HPV, human papillomavirus

Table 2 Screening test results as related to the final diagnosis

	Positive/total (% positive)				
Final diagnosis	VIA	VILI	Pap smear (LSIL)	Pap smear (HSIL)	HPV
Negative*	1213/11536 (10.5)	637/2919 (21.8)	123/9878 (1.2)	34/9878 (0.3)	631/4067 (15.5)
CIN 1	84/136 (61.8)	30/36 (83.3)	16/113 (14.2)	4/113 (3.5)	34/65 (52.3)
CIN 2†	31/54 (57.4)	4/12 (33.3)	22/47 (46.8)	16/47 (34.0)	21/31 (67.7)
CIN 3†	28/80 (35.0)	15/24 (62.5)	44/74 (59.5)	40/74 (54.1)	28/29 (96.6)
Invasive†	21/28 (75.0)	2/3 (66.6)	20/26 (76.9)	20/26 (76.9)	3/3 (100.0)
Total	1377/11834 (11.6)	688/2994 (23.0)	225/10138 (2.2)	114/10138 (1.1)	717/4195 (17.1)

*All tests negative; normal colposcopy; abnormal colposcopy with histological diagnosis of: cervicitis, acute colpitis, condyloma, HPV-non CIN
†'Significant' lesions
VIA, visual inspection with acetic acid; VILI, visual inspection with Lugol's iodine; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; HPV, human papillomavirus

Table 3 VIA and VILI used alone or combined with Pap test and HCII in detecting significant cervical pathology

Performance	Sensitivity (95% CI)	Specificity (95% CI)	PPV	NPV
CIN 2 cut-off point				
VIA	50.0 (42.2–57.7)	89.7 (89.1–90.3)	6.6	99.2
Pap (HSIL)	52.7 (44.0–60.2)	99.7 (99.5–99.7)	72.8	99.3
Pap (LSIL)	57.9 (49.9–66.0)	98.7 (98.5–98.9)	41.2	99.4
HCII	82.8 (73.6–92.1)	86.4 (85.3–87.5)	8.9	99.7
VIA or Pap*	82.4 (76.3–88.4)	87.2 (86.5–88.0)	9.2	99.7
VIA and Pap*	25.5 (18.6–32.4)	99.8 (99.7–99.9)	63.3	99.0
VIA or HCII	95.4 (91.4–99.4)	66.3 (64.7–68.0)	6.1	99.8
VIA and HCII	25.2 (17.3–33.2)	99.0 (98.8–99.2)	21.8	99.2
VILI	56.7 (40.8–72.7)	77.9 (76.2–79.7)	3.1	99.3
VILI or Pap	96.8 (93.4–100.0)	74.0 (72.1–75.7)	10.9	99.9
VILI and Pap*	14.9 (8.3–29.4)	99.8 (99.8–99.9)	48.3	99.3
VILI or HCII	100.0 (100.0–100.0)	39.3 (35.7–42.8)	5.7	100.0
VILI and HCII	51.3 (45.3–57.3)	80.9 (76.1–85.6)	31.7	90.5
CIN 3 cut-off point				
VIA	45.4 (35.9–54.6)	89.5 (88.9–90.1)	4.0	99.4
Pap (HSIL)	59.0 (49.8–68.6)	99.5 (99.3–99.6)	57.3	99.6
Pap (LSIL)	64.0 (54.6–73.4)	98.4 (98.2–98.7)	31.4	99.6
HCII	97.0 (91.1–100.0)	86.0 (84.8–87.2)	5.3	100.0
VIA or Pap*	81.4 (73.8–89.0)	86.9 (86.1–87.5)	6.1	99.8
VIA and Pap*	28.3 (19.7–36.9)	99.7 (99.6–99.8)	50.0	99.3
VIA or HCII	98.5 (95.7–100.0)	65.8 (64.2–67.5)	4.0	99.9
VIA and HCII	18.5 (9.1–26.9)	98.8 (98.6–99.0)	9.8	99.5
VILI	65.3 (47.1–83.7)	77.9 (76.2–79.6)	2.5	99.6
VILI or Pap	97.3 (93.6–100.0)	74.3 (72.5–76.2)	8.4	99.9
VILI and Pap*	18.9 (8.3–29.4)	99.8 (99.7–99.9)	35.7	99.6
VILI or HCII	100.0 (100.0–100.0)	38.7 (35.2–42.3)	3.7	100.0
VILI and HCII	59.3 (53.3–65.2)	80.2 (75.4–85.0)	25.4	94.5

*With LSIL cut-off point
VIA, visual inspection with acetic acid; VILI, visual inspection with Lugol's iodine; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; HCII, Hybrid Capture II; PPV, positive predictive value; NPV, negative predictive value

Overall, histological specimens obtained with punch biopsies or cervical conization disclosed 136 CIN 1, 54 CIN 2, 80 CIN 3 and 28 cases of cancer, totalling 162 cases of significant (CIN 2 or worse) lesions. Normal cervical tissue, or lesions rendered as acute colpitis, condyloma or HPV-non CIN, were sampled from another 1312 women. Table 2 displays screening test results as related to the final diagnosis. VIA was positive in 61.8% of the women with CIN 1, 57.0% of those with CIN 2, 35.0% of those with CIN 3 and in 21 of 28 (75%) women with cancer. Approximately 10% of women with no detectable disease had an abnormal VIA. Regarding VILI, 83.3% of women diagnosed with CIN 1 and 62.5% of those with CIN 3 had an abnormal test. VILI failed to detect one of three cases of cancer. The rate of abnormalities in Pap smears (regarded as positive with an LSIL threshold) increased in parallel with the increasing grade of the histological lesions, ranging from 14.2% among women with CIN 1 to 76.9% among those diagnosed with cancer. The same occurred with an HSIL threshold, ranging from 3.5% positivity rate in women with CIN 1 to 76.9% in women with squamous cancer. HPV tests were positive in 15.5% of women with no detectable disease, but were positive in 52.3% of women with CIN 1, reaching 100.0% (three of three cases) in women diagnosed with cancer. Interestingly, 96.6% of women with CIN 3 had a positive HCII, whereas only 67.7% of those with CIN 2, also considered high-grade disease, had a positive HPV test (Table 2).

Using the CIN 2 cut-off point, VIA and VILI as stand-alone tests performed very similarly in terms of sensitivity, detecting roughly 50% of the lesions (Table 3). However, VIA was more specific (89%) than VILI (77%). Importantly, both tests showed very low PPV: 6.6% for VIA and, even more impressive, close to 3% for VILI. The NPV of VIA (99.2 %) matched that of VILI (99.3%). Pap smear, with LSIL and HSIL cut-off points, matched the sensitivity, but were more specific than VILI and VIA. Pap smear detected 57.9% (95% CI 49.9–66.6%) of CIN 2 or worse lesions with an LSIL cut-off point, and 52.1% (95% CI 44.1–60.2%) of those with an HSIL cut-off point. Contrasting to the visual

tests, Pap smear reached 99.7% and 98.7% specificity rates at HSIL and LSIL cut-off points, also outperforming VIA and VILI in regards to PPV and NPV. HCII was the most sensitive stand-alone test (82.8%; 95% CI 73.6–92.1%), but by far the less specific (86.4%; 95% CI 85.3–87.5%).

Completely different performance figures are obtained when VIA and VILI are combined with Pap smear and HCII. When VIA and Pap were both positive, only 25.5% of the women had CIN 2 or worse, but there is a significant gain in specificity from 89.7% to 99.8%, as well as in PPV (6.6–63.3%). The same was also true with VILI. Combining with HCII increased the specificity and PPV of both VIA and VILI and also the NPV of VILI, compromising their sensitivity. Nevertheless, when the combination of exams was considered as positive when at least one of them was positive, the combination VILI or HCII was the most sensitive (100%), but VIA and HCII combination and VIA or Pap were also highly sensitive. Of course, specificity suffers with this approach, dropping to 87.2% in the case of VIA or Pap, 66.3% with VIA or HCII and 74.0% with VILI or Pap, and only 39.3% with VILI or HCII. The same has occurred with the PPV.

With the CIN 3 cut-off point, performance indicators of VIA and VILI differed only slightly from those obtained with CIN 2. HCII was still the most sensitive stand-alone test, reaching 97.0% (95% CI 91.1–100.0%) sensitivity versus 45.4 (95% CI 35.9–54.6%) of VIA, but was slightly exceeded by VIA in specificity: 89.5% (95% CI 88.9–90.1%). The effects of combining with Pap test and HCII were similar as those described with the CIN 2 cut-off point (Table 3).

DISCUSSION

Most of the previous reports addressing the test performance indicators of VIA and VILI have been carried out in developing countries. Investigators from India and some sub-Saharan countries have significantly contributed to our current knowledge regarding the potential of direct visual assessment of the cervix, by examining large cohorts to assess these tests in large-scale screening settings.[3,8,12] These previous studies have been conducted in a multitude of health services and different economic backgrounds, ranging from extremely poor and unassisted regions to areas with moderately developed health care structures.[8,9,12] Although Brazil and Argentina are still considered developing countries and still have relatively elevated overall mortality rates due to cervical cancer, the national regions where the LAMS study is being run[14] certainly do not equate with regard to cervical disease burden with those countries where the most extensive testing of VIA and VILI have been made.[1,2,14,15] While comparing the results of the present study with those previous reports, therefore, we should take into account the differences in these screening settings, which in our case represent a mixture of regions with moderately- to well-developed preventive health care. For instance, in the state of São Paulo, including our centres in Campinas and São Paulo, cervical cancer accounted for 3.5% of all female cancer deaths in 1995–99.[14] In Porto Alegre, cervical cancer is only the sixth most common cause of cancer deaths among women, totalling 6.1% of all female deaths due to cancer. In Argentina, estimated cervical cancer mortality was 7.6 per 100.000 women in the year 2000.[15]

The fact that a substantial proportion of the subjects have been tested for HPV using HCII assay is noteworthy: this testing provides information that most of the prior VIA/VILI studies lack, but which must be considered critical for the understanding of the disease dynamics in the studied population.[21–25]

In this cohort of almost 11,500 women, abnormal patterns in both VIA and VILI were more common among those who presented with LSIL and HSIL in their cervical Pap smears compared with those women with normal cytology (data not shown). This significant association of abnormal VIA and VILI with cytological abnormalities suggests that both tests have a potential to detect cervical disease. Unfortunately, however, the high numbers of women with an abnormal pattern in VIA/VILI raised our concern about the misleading false-positive images found by the naked-eye examiners. In a series of 2754 African women, Denny *et al.*[11] found that 29.4% of the women aged 35–39 years and 23.4% of those aged 50–65 years had a positive VIA, which are significantly higher figures as compared with the 11.6% overall positivity rate in the present study. A part of this difference is probably explained by the different incidence of cervical disease among the African and Brazilian populations, but, more importantly, may reflect the difficulties in reproducing the same criteria of categorizing the findings in the visual inspection. Sankaranarayanan *et al.*,[7] who examined 3000 Indian women, trained paramedical personnel to grade the aceto-white lesions as positive only when a distinct pattern was noted, considering faint and doubtful aceto-whitening as a negative VIA result. We tried to adopt the same policy, but even then an unacceptable proportion of women were classified as VIA-positive in whom no cervical lesions were detected on colposcopy, Pap smear or biopsy. With even graver consequences to the performance of the exam, VILI was considered positive in 23.0% of all study subjects and 33.4% of those aged 21–30 years. This high number of false-positive results yielded a specificity of less than 80% and PPV that, for high-grade disease, did not surpass 2.5%. Even mild abnormalities of the uterine cervix, highly prevalent in young women, may possibly confound VILI examiners. In the present study, nurses and doctors performed the visual tests after being trained to render a diagnosis based upon the Atlas of Visual Inspection.[8] However, the exact numbers of VIA/VILI performed by each of these professionals (doctors versus nurses) have not been recorded, thus precluding the investigators to assess whether nurses and doctors differed in their performances as naked-eye examiners of VIA and VILI.

In the present series, the proportion of women with positive HPV tests was far higher than that of women with abnormal Pap smears. Despite this fact, however, HCII results did not concur with those of VIA/VILI any better than did the Pap test. In their study on African women, Denny *et al.*[11] found a 20% HPV prevalence, ranging from 22.4% among women aged 35–39 years to 17.1% among those aged 50–65 years, which are in perfect alignment with the figures of the present study. Similarly, HCII prevalence was also significantly higher among VILI-positive women in our study, but there are no published data reporting the associations of HPV and VILI. In the study by Denny and colleagues, the sensitivity of VIA in detecting high-risk HPV was 13%, specificity 88%, PPV 29% and NPV 84%, the corresponding figures for VILI being 45%, 69%, 24% and 85%.

Owing to their subjective visual nature, VIA and VILI should, at least in theory, correlate well with the colposcopic findings. This could not be confirmed in the present study, however. Colposcopy did not confirm almost 50% of the abnormalities in VIA or almost 65% of those in VILI. More importantly, close to 25% of the women considered as

having a normal cervix on VIA actually had a significant abnormality detected on colposcopy, as did 12% of the women categorized as normal with VILI. Like all studies where less than 100% of the women are examined by the test used as the gold standard, the present study suffers from verification bias. Only those women who are referred to colposcopy on the basis of a positive screening test (Pap, VIA, VILI, HCII) and almost 3500 women subjected to screening colposcopy or randomly assigned to this exam had the chance to become examined by the agreed gold standard (i.e. cervical biopsy). Thus, some women with true cervical abnormalities who tested negative with VIA, VILI, HCII and Pap smear might still remain undisclosed, resulting in over-optimistic performance indicators of the screening tests used.

In a cohort of 4444 women examined with Pap smear, VIA and VILI in Kerala, India, Sankaranarayanan *et al*.[9] recently achieved more than 80% sensitivity and specificity with VIA, associated with 17.5% PPV. In our hands, VIA was far less sensitive, but showed comparable specificity and lower PPV. The results were equal with VILI, which reached 87.2% sensitivity, 84.7% specificity and 16.6% PPV,[7] clearly superior to our present results. While comparing these different performance indicators, however, it is essential to remember that these are dramatically influenced by the prevalence of the cervical disease in the study populations, and also on the use of the gold standard. One plausible explanation for these discrepancies might be the shorter experience of our investigators on the use of VIA and VILI, contributing to the failure of reproducing the high-performance indicators, especially sensitivity, of VIA and VILI in the African and Indian series. It is sensible to presume, however, that trained doctors experienced at performing colposcopy should render visual assessments of the cervix better than nurses or technicians. The PPV figures are probably representative of the lower incidences of cervical lesions in the present series compared with Indian and African populations.

Combining VIA and VILI with Pap smear and HPV testing markedly improved their performance as screening tools. Many ongoing studies are paving the way for new screening strategies for cervical cancer.[4,8,9,12] These reports are almost universally consonant in that the combination of screening techniques may improve the overall sensitivity and, in some instances, specificity and predictive values. However, strategies to deal with the increasing costs and the larger number of women to be referred for colposcopy need to be developed further.

For screening purposes, investigators should devise strategies that provide reasonable detection rates and avoid false positives. This obvious and simple assertion represents the most important challenge in regard to cervical cancer, because either detection rates or specificity of the screening tests currently available for pre-invasive cervical neoplasia demand improvements. In the present study, for instance, Pap smear failed to detect almost 24% of the cancer cases. In recent years, investigators have been able to clearly demonstrate these inherent problems of screening, and the results of the present report are in close alignment with these previous findings. VIA, VILI, Pap smear and HCII showed their flaws as stand-alone tests: combinations of tests provided some improvement in terms of specific performance indicators, but always at the expense of the other indicators. Specifically addressing VIA and VILI, despite the fact that we failed to reproduce the previously reported performance figures with these unaided visual methods as stand-alone tests in our screening settings, the present data clearly demonstrate an improvement of both VIA and VILI as screening tools when these visual methods were used together with conventional Pap smears and HCII assays for HPV detection. This is in alignment with the current efforts made in several ongoing studies to develop and test new innovative screening strategies, tailored according to the local demands and by taking into account the economical and social characteristics of each individual setting. The results of the present investigation suggest that VIA and VILI do not – in settings with prevalence of cervical disease similar to that encountered were the LAMS study is being run – deserve investment as major screening strategies, or as adjunctive screening tools.

This is also one of the key aims of the ongoing LAMS study, where eight different diagnostic tests are compared as potential screening tools in Latin American settings. It is to be anticipated that the optimal results are most probably achievable by an innovative combination of two or more of these tests, but highly sensitive and specific screening strategies have not yet been devised.

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