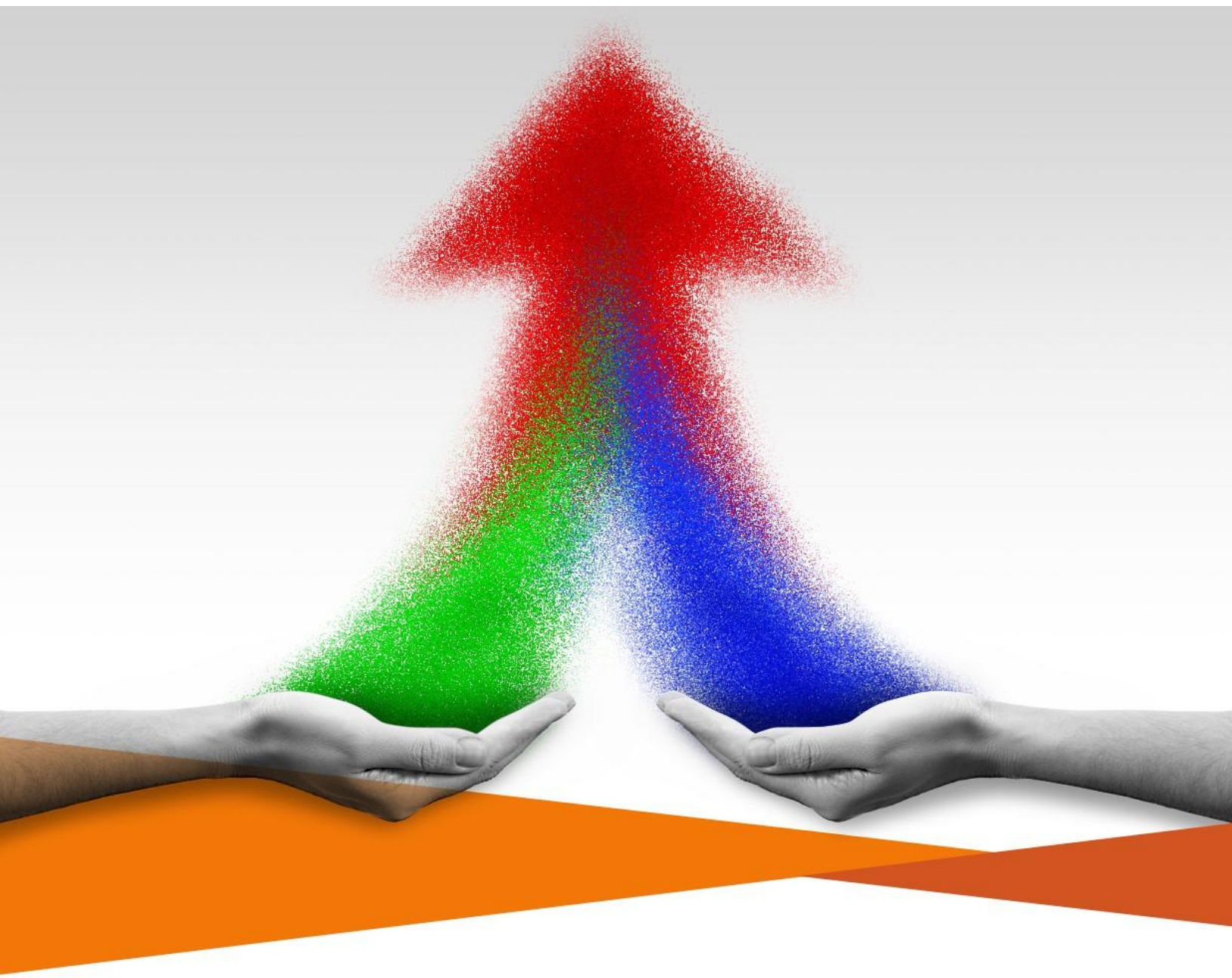

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Effectiveness of a two-stage strategy with HPV testing followed by visual inspection with acetic acid for cervical cancer screening in a low-income setting

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The World Health Organization recently advocated a two-stage strategy with human papillomavirus (HPV) testing followed by visual inspection of the cervix with acetic acid (VIA) as a suitable option for cervical cancer screening. However, its accuracy has never been directly assessed in the context of primary screening. To evaluate effectiveness of HPV testing on self-obtained specimens (self-HPV) followed by VIA (sequential testing) in a low-income setting, we recruited 540 women aged between 30 and 65 years in two Cameroonian periurban areas. Eligible women were counseled about cervical cancer and how to perform self-sampling. HPV positive and a random sample of HPV-negative women were called back for VIA and biopsy. Disease was defined by interpretation of cervical intraepithelial neoplasia Grade 2 or worse (CIN2+). Performances of VIA, self-HPV and sequential testing were determined after adjustment for verification bias. HPV prevalence was 27.0%. VIA positivity was 12.9% and disease prevalence was 5%. Sensitivity and specificity of VIA for CIN2+ were 36.4% [95% confidence interval (CI): 15.2–64.6%] and 90.4% (95% CI: 85.4–93.7%), respectively. Sensitivity of self-HPV [100.0% (95% CI: 79.6–100.0%)] was 66% higher than that of sequential testing [33.3% (95% CI: 15.2–58.3%)]. Meanwhile, specificity of self-HPV [74.5% (95% CI: 70.6–78.1%)] was 22% lower than that of sequential testing [96.7% (95% CI: 94.8–97.9%)]. A two-stage screening strategy with self-HPV followed by VIA improves specificity of cervical cancer screening, but at the cost of an important loss of sensitivity. Ways to improve VIA performance or other tools are needed to increase positive predictive value of HPV testing.

Cervical cancer is an important public health issue in low-income settings. Eighty-eight percent of yearly about 530,000 new cervical cancer cases are diagnosed in developing countries where 240,000 women die from this disease every year.[1] In Cameroon—a central African country—age-standardized cervical cancer incidence and mortality rates are estimated at 24/100,000 and 17/100,000 per year, respectively.[2] Main reason for this imbalance is the absence of cervical cancer screening programs like in most low-income settings.[3] In major cities of Cameroon, sporadic screening options exist using Papanicolaou smear (Pap test) or visual inspection of the cervix after application of acetic acid (VIA) or Lugol's iodine (VILI). However, Pap test traditionally used in developed countries for primary screening, shows sensitivity for high-grade cervical lesions of 50–60%, which necessitates regularly repeated controls.[4] The method requires highly specialized laboratories, well-trained pathologists to analyze the specimens and adequate conditions during samples transport. Therefore, Pap test is hardly feasible on a large scale in low-income settings.[5]

In contrast, VIA has the advantage of being low-cost and facile to perform. It can easily be carried out by nurses or midwifes after a short training.[6] Yet, even though VIA is easy to conduct its interpretation is challenging. A recent meta-analysis showed that VIA sensitivity for cervical intraepithelial neoplasia Grade 2 or worse (CIN2+) ranged between 41 and 92% and its specificity between 49 and 98%.[7] Thus, the informative value of a positive or negative VIA result emerged to be highly dependent of the examiner's experience.

Another option recommended by the World Health Organization (WHO) is testing of cervical or vaginal swabs for the

Key words: low-income setting, cervical cancer screening, two-stage strategy, HPV testing, VIA, sequential testing, accuracy

Abbreviations: CHUY: University Hospital Center of Yaoundé; CI: confidence interval; CIN: cervical intraepithelial neoplasia; Ct: cycle threshold; ECC: endocervical curettage; HPV: human papillomavirus; HUG: Geneva University hospitals; IQR: interquartile range; JHPIEGO: Johns Hopkins Program for International Education in Gynecology and Obstetrics; mRNA: messenger RNA; NPV: negative predictive value; PCR: polymerase chain reaction; PPV: positive predictive value; VIA: visual inspection of the cervix with acetic acid; VILI: Visual Inspection with Lugol's Iodine; WHO: World Health Organization

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What's new?

Guidelines developed by experts at the World Health Organization propose a cervical cancer screening strategy whereby human papillomavirus (HPV) testing is followed by visual inspection with acetic acid (VIA). But questions remain about the accuracy of the strategy and whether its effectiveness outweighs the resources needed for VIA. In this study, among women enrolled in a screening campaign in Cameroon who were called back for VIA after a positive HPV result, VIA was found to have low sensitivity but high specificity. The loss of sensitivity may be unacceptable, particularly for low-income settings and mass screening programs.

presence of oncogenic human papillomavirus (HPV) types (HPV testing). It is established that virtually all cervical cancers test positive for HPV and sensitivity of HPV testing for CIN2+ is usually over 90%.[8,9] Moreover, samples for HPV testing can be taken by patients themselves without markedly impairing the test performance.[10–14] A downside of HPV testing is its low positive predictive value (PPV). Since it does not directly test for cervical cancer but for HPV infection, a negative test result holds a high probability for the patient not to develop cervical cancer within 5–10 years following completion of the test, whereas a positive result only indicates the presence of an essential risk factor.[9] HPV infection is a very common condition in young women with prevalence over 25% in those aged younger than 30 years in Cameroon.[14] Studies carried out in other African countries found a PPV for HPV testing to be lower than 30%.[15–17] These findings indicate that a triage test following a positive HPV result is necessary to limit the rate of false positive and consequently the harms of overtreatment. VIA might be an affordable triage test for low resource countries, as its specificity has shown high when adequately performed.[18] Although recent WHO guidelines for cervical cancer prevention include a strategy of screening with an HPV test followed by VIA,[19] to the best of our knowledge, no study directly assessed accuracy of such a two-stage strategy. The aim of this study was to evaluate the effectiveness of HPV testing on self-obtained vaginal specimens (self-HPV) followed by VIA for cervical cancer prevention in a low resource setting.

Methods

The Faculty of Medicine and Biomedical Sciences, Yaoundé, the National Cancer Control Committee, Cameroon and the Geneva University Hospitals (HUG), Switzerland work together to evaluate innovative cervical cancer screening options in order to develop a screening approach adapted to the needs and means of Cameroon. The present cross sectional study as part of this collaborative platform was approved by the National Ethics Committee of Cameroon (number of approbation: 244/CNE/SE/09).

Study population

Between February 2010 and August 2012, we organized screening campaigns and enrolled women in two cities in Cameroon: Tiko in the South-West Region and the capital city Yaoundé in the Centre Region. Tiko is a semiurban city with an estimated population of 120,000 inhabitants in 2010. Women represent about half of the population and most of them were previously unscreened. In Yaoundé, screening as part of this campaign was limited to a neighborhood with a primary-level hospital. This popular area had an estimated population of 40.000 inhabitants in 2010, mostly of low socioeconomic status. Such campaigns offer opportunity for unscreened women to have free access to cervical screening. Target population was estimated at about 12,000 and 5,000 women in Tiko and Yaoundé, respectively. In Tiko, sensitizations through radio channels, announcements in churches, on the street with loudspeakers and through word-of-mouth advertising were organized and women were invited to attend screening at the district hospital. In Yaoundé, we recruited participants in a neighborhood hospital with informational posters and word-of-mouth advertising. Women were eligible if they were aged between 30 and 65 years and nonpregnant. Women with a history of hysterectomy, those who presented with gynecological symptoms and those who did not consent to participate were excluded. A sample of 540 women was consecutively enrolled for HPV testing (Fig. 1).

Screening procedure

Women were invited to follow a short lecture about HPV, cervical cancer and screening for cervical cancer. Lectures were done in English and French, illustrated with pictures to help get the message. Translation in local language was provided when necessary. Investigators explained how to use the self-sampling device, a sterile, flocked swab (*ESwab*®; Copan, Brescia, Italy). Women were advised to perform self-sampling and to complete a questionnaire assessing socio-demographic characteristics. Those who agreed were led to a room within the hospital where they collected vaginal samples without assistance of health care workers. Samples were analyzed in Switzerland using polymerase reaction chain (PCR) system of the Abbott RealTi*m*e High Risk HPV assay (Abbott Laboratories, Abbott Park, IL) which detects separately HPV 16, HPV 18 and 12 other high-risk types as a group (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68).

All women testing positive for HPV and a randomly chosen sample of the same size of HPV-negative women were called back for VIA examination. Histological samples were also taken after VIA without colposcopy and consisted of four-quadrant biopsies at 3, 6, 9 and 12 o'clock, and endocervical

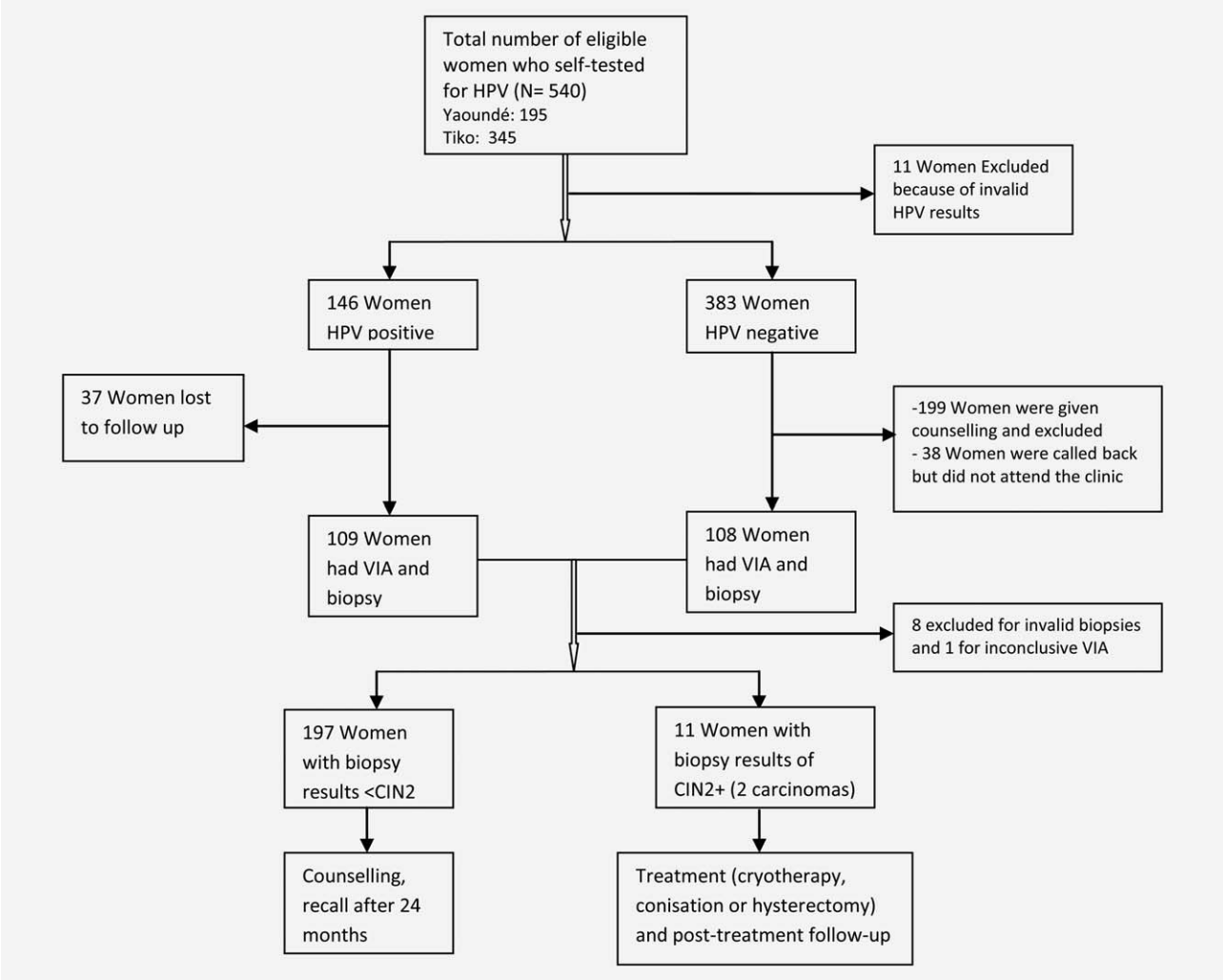

Figure 1. Flow chart of the study.

curettage (ECC). In the case where any visible cervical lesion was suspected, punch biopsy in the corresponding quadrant focused on the suspect area. In the study protocol, the minimum number of biopsies required was four per women. However, in some women whose cervix was elusive or tended to bleed profusely, less than four biopsies could be taken.

VIA was performed by two experienced Cameroonian physicians. Both screeners had followed a two weeks theory-based didactic education and a hands-on competency-based skill acquisition at the gynecologic Unit of HUG. The theoretical part (5 days) included learning how to prepare a 5% diluted solution of acetic acid, how to perform VIA and how to recognize normal and pathologic cervical pictures before and after application of acetic acid. Training was based on the teaching manual developed by IARC.[20] The practical part (10 days) consisted of performing VIA and interpreting results in at least 25 women consulting at the colposcopy clinic of HUG under supervision of well-trained specialists. They are currently conducting routine VIA-based cervical cancer screening at the University Hospital Center of Yaoundé (CHUY), Cameroon. Before participating in this study, they had performed more than 1,000 VIA examinations each, as part of cervical cancer screening activities in Cameroon. A daylight-like light source was used to perform VIA during the study. The 5% acetic acid solution applied on the cervix was obtained following a well-standardized dilution protocol of a 100% solution supplied by the CHUY pharmacy. Biopsies were sent to Geneva where histological analyses were performed by two experienced pathologists who were blinded to both HPV and VIA results and discrepant cases were discussed between the two pathologists to reach a final decision. Histological results were classified into five categories: negative or inflammatory, CIN1, CIN2, CIN3 and carcinoma. When results were available, women were informed and managed accordingly (Fig. 1).

Statistical analysis

The results of histological examination were used as reference standard and cervical intra-epithelial neoplasia Grade 2 or worse (CIN2+) was considered as disease threshold. HPV

test was considered positive if the real-time PCR detected at least one of the 14 high-risk types. The HPV target cut-off]32.00 cycle threshold (Ct)] and the internal control target cutoff (35.00 Ct) were established by the manufacturer, and samples with insufficient content of cervicovaginal cells were automatically invalidated. VIA was deemed positive when a well-defined acetowhite area on the external os, the transformation zone or close to the squamo-columnar junction or an acetowhite growth on the cervix was seen 1 min after application of 5% acetic acid. Accuracy of VIA in HPV-positive women and in all those who underwent VIA was measured. We also calculated performances of self-HPV alone and sequential testing (self-HPV followed by VIA) in the study population. Sensitivity, specificity, PPVs and negative predictive values (NPVs) were calculated with their 95% confidence interval (CI). For sequential testing, net sensitivity and specificity were calculated as the joint probability of disease or nondisease accurately detected when using both self-HPV and VIA. The tests were considered significant when p-value was found to be below 0.05. Statistical analyses were conducted using OpenEpi and Stata version 11 (StataCorp, TX).

Method for correcting for verification bias

Since all women who underwent VIA had biopsy and ECC, no risk of verification bias was expected in the calculation of sensitivity and specificity for VIA. Moreover, estimates of sensitivity and specificity for VIA were transposable to the study population because the selection of women who underwent biopsy was not conditional on VIA results. However, PPV and NPV of VIA in this subset could not be generalized to the study population as the prevalence of cervical lesions might be different.

The fact that the disease outcome was determined in a random sample of women who tested negative for HPV allowed us to derive more valid estimates of sensitivity and specificity for self-HPV alone and sequential testing.[21] To prevent the effect of verification bias, an adjustment procedure was necessary. This bias is caused by the fact that only a fraction of the women testing negative by self-HPV (~38%) and a much larger proportion of women HPV positive (100% as specified in the protocol) were recalled for histological verification, which decreases the relative proportions of negative subjects within the disease case and noncase groups. We adjusted for verification bias by correcting the frequencies of test results by adding to them the proportion of unverified subjects with the same test result who had the same lesion status. This was done as follows. Denote the frequency of subjects in each combination of test results and lesion status by F_{HVL}, where the subscripts H, V and L indicate the results for HPV and VIA and the lesion status, respectively. Let U_{HV} and C_{HV} be the frequency of unverified and verified subjects in each combination of H and V, respectively. To compensate for the verification bias the adjusted frequency for each combination was calculated as follows: $A_{HVL} = F_{HVL} + U_{HV} \times [F_{HVL}/C_{HV}]$.

To use this formula, one has to assume that for each subset of women with a given HPV results or combination of HPV and VIA results, the distribution of lesions among those with unverified lesion status is the same as for those with lesion status ascertained by histology. This is a plausible assumption because HPV test results was the only criteria which determined performing biopsy, and thus women without histological results are likely to have the same prevalence of lesion as those in the same category represented by the HPV results. The same assumption is tenable for sequential testing and histological verification because the decision to biopsy was not conditional on the clinician's impression during the VIA examination, as all women who underwent VIA also had biopsy.

Results

Patient's characteristics

A total number of 540 women were enrolled. Socio-demographic and reproductive information of the study population and comparison with HPV-positive women and both HPV and VIA positive women are presented in Table 1. Median age was 41 years [interquartile range (IQR), 36–50]. Most women were married (58.2%) or widowed (17.5%) and well educated (secondary education or more, 66%). Median age at first sexual intercourse was 17 years (IQR, 16–19) and median number of sexual lifetime partners was 3 (IQR, 2–5). Socio-demographic characteristics of HPV-positive women and of those who tested positive for both HPV and VIA were comparable to those of the study population (Table 1).

HPV prevalence

HPV prevalence in the study population was 27.0% (95% CI: 23.5–30.9%). In 383 women, HPV testing results were negative and in 11 cases, HPV analysis was inconclusive due to poor quality of samples. HPV prevalence was higher in women younger than 35 years, decreasing in women aged 35–49 years and reascending in women aged older than 49 years, but none of these trends was statistically significant (Fig. 2).

VIA positivity and biopsy results

All 146 HPV-positive women and a randomly selected sample of 146 HPV-negative women were called back for VIA examination and biopsy. Although we asked women with invalid HPV test results to repeat self-sampling, they were not included in the analyses. Among HPV-positive women, 37 (25.3%) did not attend VIA examination and among HPV-negative women who were recalled for further evaluation, 38 (26%) did not show up. VIA and biopsies were therefore obtained from 217 women. Biopsy results were invalid in 8 women and revealed 9 CIN1 (4.1%), 9 CIN2 or CIN3 (4.1%) and 2 carcinomas (0.9%). VIA was positive in 28 (12.9%) women and not conclusive in one woman. Among the 208 women whose biopsy results and HPV were interpretable, 11 (5.3%) were CIN2+ (Table 2). All 11

Table 1. Sociodemographic characteristics, with respect to HPV and VIA status

Characteristics	Total, $N = 540$ (100%)	HPV+, $n = 146$ (27.0%)	HPV+ and VIA+, $n = 18$ (3.3%)
Age (years)			
Median (IQR)	41 (36–50)	41 (34–51)	34.5 (31–44)
30–39	230 (42.6)	67 (45.9)	41 (38.0)
40–49	173 (32.0)	41 (28.1)	40 (37.0)
50–65	137 (25.4)	38 (26.0)	27 (25.0)
Marital status			
Married	326 (60.4)	80 (54.8)	9 (50.0)
Single	95 (17.6)	29 (19.9)	6 (33.3)
Divorced	19 (3.5)	2 (1.4)	0 (0.0)
Widowed	92 (17.0)	32 (21.9)	2 (11.1)
Separated	8 (1.5)	3 (2.1)	1 (5.6)
Education			
Unschooled	32 (5.9)	13 (8.9)	0 (0.0)
Primary	163 (30.2)	41 (28.1)	2 (11.1)
High school	263 (48.7)	66 (45.2)	10 (55.6)
University or other degree	82 (15.2)	26 (17.8)	6 (33.3)
Number of different sexual partners (median, IQR)	3 (2–5)	4 (2.5–5)	4.5 (3.5–5)
Age(years) at first sexual intercourse (median, IQR)	17 (16–19)	17 (16–19)	17 (16–19)
History of previous screening			
Yes	86 (15.9)	19 (13.0)	4 (22.2)
No	454 (84.1)	127 (87.0)	14 (77.8)

Abbreviation: IQR: interquartile range.

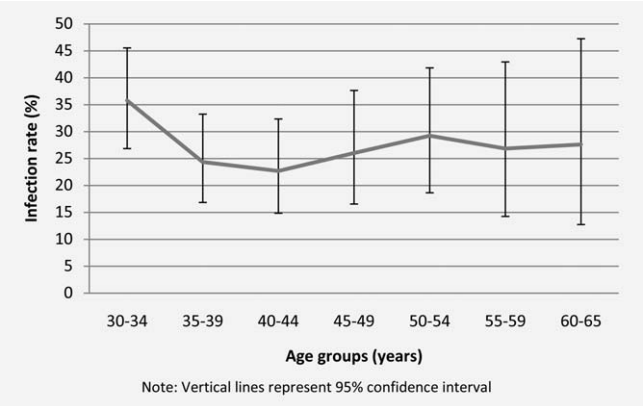

Figure 2. HPV prevalence in the study sample by age group ($N = 2{,}529$). Note: Vertical lines represent 95% confidence interval.

CIN2+ lesions were HPV positive and only 4 of them were VIA positive. Both carcinomas were classified as VIA negative.

Performances of VIA, self-HPV and sequential testing (self-HPV followed by VIA)

In HPV-positive women, VIA showed a sensitivity of 36.4% (95% CI: 12.9–65.4%) for CIN2+ and detected no case of carcinoma (Table 3). Specificity of VIA was 87.4% (95% CI: 84.7–90.7%) and its PPV was 25.0% (95% CI: 8.9–45.0%) in this subgroup. In the study population, accuracy measures of VIA were not different from those obtained among HPV-positive women: sensitivity and specificity of VIA were 36.4% (95% CI: 15.2–64.6%) and 90.4% (95% CI: 85.4–93.7%), respectively (Table 3).

Sensitivity of self-HPV [100.0% (95% CI: 79.6–100.0%)] to detect CIN2+ was 66% higher than that of sequential testing [33.3% (95% CI: 15.2–58.3%); Table 4]. Meanwhile, specificity of self-HPV [74.5% (95% CI: 70.6–78.1%)] was 22% lower than that of sequential testing [96.7% (95% CI: 94.8–97.9%)]. However, PPV was two times higher for sequential testing [22.7% (95% CI: 10.1–43.4%)] than for self-HPV as a stand-alone screening tool [10.3% (95% CI: 6.3–16.3%); Table 4].

Discussion

To date, this is the first study that evaluates accuracy of a two-stage strategy with self-HPV followed by VIA for primary screening for cervical cancer. We found that the gain in specificity when adding VIA to HPV testing was obtained at the expenses of a loss of sensitivity that is unacceptable in the context of a mass screening program.

VIA in our study showed an unexpectedly low sensitivity (36.4% (95% CI: 15.2–64.6%)) as compared with that reported by other studies carried out in sub-Saharan African countries, and a rather high specificity [90.4% (95% CI: 85.4–93.7%)].[18,22–25] To reduce possible sources of bias in this study, VIA was performed by well-trained physicians and two experienced pathologists blinded to HPV and VIA results performed histological interpretation of biopsies. A major strength of this work is the use of histology alone as reference standard, unlike most reports evaluating accuracy of VIA for cervical cancer. In previous studies conducted worldwide under screening conditions, the gold standard was colposcopy and colposcopy-guided biopsy of abnormal areas although this method has proven to yield errors in disease recognition.[26,27] It is established that the high correlation between VIA and colposcopy results may result in an overestimation of test accuracy,[28] supporting that sensitivity measures of VIA reported in many studies might have been overestimated. Furthermore, since interpretation of cervical biopsies is dependent on skills and experience of pathologists,[29] and as immature squamous metaplasia can be mistakenly interpreted as high-grade precancerous cervical lesions,[30] misclassification errors might have resulted in unrealistically high estimates of VIA sensitivity as proven in some African settings.[31,32]

VIA is a subjective test and many reports have highlighted the need to properly train and adequately monitor

Table 2. Distribution of biopsy results according to the HPV and VIA status of women

	HPV+, $N = 106$ (50.3%)		HPV−, $N = 102$ (49.7%)	
Biopsy results[1]	VIA+, $n = 16$ (7.7%)	VIA−, $n = 90$ (43.3%)	VIA+, $n = 7$ (3.4%)	VIA−, $n = 95$ (45.7%)
Negative ($n = 188$)	9 (4.8)	79 (42.0)	7 (3.7)	93 (49.5)
CIN 1 ($n = 9$)	3 (33.3)	4 (44.4)	0 (0.0)	2 (22.2)
CIN 2 ($n = 5$)	3 (60.0)	2 (40.0)	0 (0.0)	0 (0.0)
CIN 3 ($n = 4$)	1 (25.0)	3 (75.0)	0 (0.0)	0 (0.0)
Cancers ($n = 2$)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)
CIN2+ ($n = 11$)	4 (36.4)	7 (63.6)	0 (0.0)	0 (0.0)

[1]A total of 208 women were included, since VIA or biopsies results were not interpretable in 9 women.

Table 3. VIA performance for CIN2+ detection with respect to the HPV status of women

HPV status	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
HPV+ ($N = 106$)	36.4 (15.2–64.2)	87.4 (79.2–92.6)	25.0 (10.2–49.5)	92.2 (84.8–96.2)
HPV− ($N = 102$)	Not calculable[1]	93.1 (86.5–96.6)	00.0 (00.0–35.4)	100.0 (96.1–100.0)
Total[2] ($N = 208$)	36.4 (15.2–64.6)	90.4 (85.4–93.7)	NA	NA

[1]All CIN2+ were HPV positive.
[2]All women with interpretable biopsy and VIA results.
Abbreviation: NA: not applicable.

Table 4. Estimated performance for CIN2+ detection of HPV testing as standalone tools and sequential screening (HPV testing and VIA)[1]

	HPV testing alone	HPV testing followed by VIA
Sensitivity % (95% CI)	100.0 (79.6–100.0)	33.3 (15.2–58.3)
Specificity % (95% CI)	74.5 (70.6–78.1)	96.7 (94.8–97.9)
PPV % (95% CI)	10.3 (6.3–16.3)	22.7 (10.1–43.4)
NPV % (95% CI)	100.0 (99.0–100.0)	98.0 (96.4–99.9)

[1]After adjustment for verification bias.

screeners.[33,34] Even though the low sensitivity of VIA could question training of providers and effectiveness of quality assurance in this study, this may not be a sufficient explanation. A population-based study conducted in rural India where VIA providers were gynecologists with renowned experience who rigorously followed the JHPIEGO Cervical Cancer Prevention Guidelines for Low-Resource Settings competency-based training tool[35] and who were quality assessed on a yearly basis during the study period, showed a sensitivity of VIA for detecting CIN2+ of 26.3%.[36] In a more recent study of 4,656 women in India where screening was performed by well-trained nurses with regular retraining and quality control, sensitivity of VIA to detect CIN2 + was 21.9%.[37] Another study conducted in United States some years ago where training and monitoring of test providers should have been optimal, found sensitivity of VIA for CIN2+ to be 29%.[38] In a meta-analysis of 26 cross-sectional studies on the accuracy of VIA in low- and middle-income countries, VIA sensitivity for CIN2+ varied from 41 to 92%.[7] Authors of that review found that region, capacity of VIA provider, study period and size of the study population had no effect on VIA performance. These results are consistent with the multi-factorial nature of the variability of VIA's accuracy and suggest that the low reproducibility of VIA is mainly due to factors other than background and experience of screeners. More importantly, the conditions under which we carried out this work are close to the clinical practice where it is not always feasible to provide regular on-site supervision and refresher training to VIA providers, especially in remote areas in limited resource settings. Therefore, accuracy measures obtained here are more likely to reflect field conditions than those reported in most demonstration studies where screening was performed under ideal circumstances.

Besides, the fact that both carcinomas were classified as VIA negative is a real concern. A possible explanation is the inability for screeners to properly identify the squamocolumnar junction in some women and to inadvertently classify as negative, VIA results that should be considered inconclusive. Although unlikely in this study given the training and experience of VIA providers, this situation might not be uncommon in real practice. Another explanation is endocervical localization of theses early stage cancers. Performing ECC in all women probably allowed us to catch up cancers that could hardly be visualized by VIA. This result suggests the importance of performing ECC in all women in studies on VIA accuracy, regardless of VIA results.

Self-HPV allowed adequate detection of CIN2+ in our analyses, resulting in a sensitivity of 100% (95% CI: 79.6–100%) and a specificity of 74.5% (95% CI: 70.6–78.1%). These estimates are consistent with accuracy of HPV testing by PCR assay for CIN2+ detection in other studies carried out in Africa.[16,39] This result suggests that self-HPV may be a suitable tool for primary cervical cancer screening in low-income settings. Although sensitivity of self-HPV is consistently higher than that of VIA, specificity however is lower. When the specificity of a test is low, its clinical use can result in increased numbers of follow-up assessments, psychological concerns and unnecessary treatment.[40–42] Therefore, reducing the number of false positive cases while ensuring adequate identification of true cases is essential for the effectiveness of a screening program. A strategy usually employed to improve a screening test's utility is sequential testing, the second test being performed in women screened positive on the first test. Typically, the first test is more sensitive than the second test, and the second test is more specific than the first. This is why we sought to assess performance of a two-stage screening strategy with self-HPV followed by VIA, which is currently recommended by WHO for cervical cancer prevention.[19]

Our results were disappointing, as adding VIA to HPV testing led to a loss of sensitivity too drastic to compensate for gain in specificity. In fact, sensitivity of sequential testing (33.3%) to detect CIN2+ was about 66% lower than that of HPV testing as a standalone method (100.0%) although it resulted in an average increase in specificity of 22% (96.7% for sequential testing *vs.* 74.5% for HPV testing). Besides, accuracy of sequential testing was comparable to that of VIA as a standalone screening tool in our study. Therefore, not only sequential testing did not improve accuracy of HPV testing, but also performing VIA after HPV testing canceled effect of the latter in our population. These results contradict conclusions drawn from modeling studies, where HPV testing followed by VIA was presumed to offer a better balance between sensitivity and specificity than HPV testing as a single test. In a study of 2,199 women attending a cervical cancer screening program in Zimbabwe, a computerized simulation showed that sensitivity of sequential testing with HPV testing and VIA was 16.5% lower than that of HPV testing as a standalone tool (63.6% *vs.* 80.1%, respectively) whereas specificity of sequential testing was 20.8% greater than that of HPV used as single test (81.9% *vs.* 61.1%, respectively).[43] The better results for sequential testing obtained in that report could probably be explained by the greater accuracy of VIA alone (76.7% and 64.1% for sensitivity and specificity, respectively) as compared to that in the present study. Furthermore, sequential testing was modeled to result in a 53.5% reduction in the false positive rate compared to HPV testing alone. This corroborates our findings where PPV was two times higher for sequential testing [22.7% (95% CI: 10.1–43.4%)] than for HPV testing as a standalone screening tool [10.3% (95% CI: 6.3–16.3%)]. Therefore, VIA might be useful to reduce the rate of needless treatment in the context of mass screening provided its sensitivity is improved.

Our study was limited by the small size of the study population. Accuracy estimates based on a larger number of women would have provided more accurate results. In addition, the reference standard was not performed in all women, which could yield a certain degree of verification bias. However, we minimized that bias while calculating test performances by extrapolating the results obtained among women who underwent reference test to the study population. Moreover, none of the women with negative HPV test who returned for biopsy had CIN2+, suggesting that recalling of women based on HPV-positive results identified most women with CIN2+.

Efforts must therefore emphasize on either finding ways to improve VIA accuracy like cervicography or visual inspection with acetic acid and magnification,[44,45] by reviewing criteria for VIA positivity or looking for other triage tools that might be more reliable like VILI,[18,45] or molecular tests such as E6/E7 messenger RNA (mRNA) detection.[46–48]

In conclusion, adding VIA to HPV testing improves PPV of cervical cancer screening, but at the cost of an important loss of sensitivity. Further studies are warranted to explore this strategy.

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