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ABCD criteria to improve visual inspection with acetic acid (VIA) triage in HPV-positive women: a prospective study of diagnostic accuracy

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ABSTRACT

Objectives A simple system for visual inspection with acetic acid assessment, named ABCD criteria, has been developed to increase accuracy for triaging of high-risk human papillomavirus (HPV)-positive women. This study aimed to determine the accuracy of ABCD criteria for the detection of histologically confirmed cervical intraepithelial neoplasia grade two or worse (CIN2+) in HPV-positive women living in a low-resource setting.

Design Prospective study of diagnostic accuracy.

Setting Cervical cancer screening programme based on a 3T-Approach (test, triage and treat) in the Health District of Dschang, West Cameroon.

Participants Asymptomatic non-pregnant women aged 30–49 years were eligible to participate. Exclusion criteria included history of CIN treatment, anogenital cancer or hysterectomy. A total of 1980 women were recruited (median age, 40 years; IQR 35–45 years), of whom 361 (18.4%) were HPV-positive and 340 (94.2%) completed the trial.

Interventions HPV-positive women underwent a pelvic examination for visual assessment of the cervix according to ABCD criteria. The criteria comprised A for acetowhiteness, B for bleeding, C for colouring and D for diameter. The ABCD criteria results were codified as positive or negative and compared with histological analysis findings (reference standards).

Primary outcome measure Diagnostic performance of ABCD criteria for CIN2+, defined as sensitivity, specificity, negative and positive predictive values.

Results ABCD criteria had a sensitivity of 77.5% (95% CI 61.3% to 88.2%), specificity of 42.0% (95% CI 36.5% to 47.7%), positive predictive value of 15.1% (95% CI 10.8% to 20.8%), and negative predictive value of 93.3% (95% CI 87.6% to 96.5%) for detection of CIN2 +lesions. Most (86.7%) of the ABCD-positive women were treated on the same day.

Conclusions ABCD criteria can be used in the context of a single-visit approach and may be the preferred triage method for management of HPV-positive women in a low-income context.

Trial registration number NCT03757299.

Strengths and limitations of this study

- Using acetowhiteness, bleeding, colouring and diameter (ABCD) criteria for visual inspection with acetic acid interpretation is a simple test with binary results (positive or negative) that are immediately available, allowing a screen-and-treat approach.
- Because all human papillomavirus-positive women underwent biopsy and endocervical brushing regardless of the ABCD criteria results, there was no risk of verification bias in the calculations of sensitivity and specificity.
- A limitation of the study was its setting in a single centre in a district hospital in West Cameroon with five clinicians administering all screening and treatment procedures.

INTRODUCTION

More than 90% of cervical cancer (CC) deaths occur in low-income and middle-income countries (LMICs), mainly due to lack of prevention.[1] Cytology-based CC screening programmes and more recent human papillomavirus (HPV)-based programmes have been successfully implemented in high-income countries and have been associated with important reductions in deaths from CC.[2] However, these strategies have not been implemented in LMICs, predominantly because of financial and logistical limitations. Alternative methods such as visual inspection of the cervix after application of acetic acid (VIA) and more recently, human papillomavirus (HPV) primary screening, are considered suitable for use in LMICs.[3 4]

A global strategy for the elimination of CC has been launched by the WHO in 2020, which relies on the screening of 70% of women using a high-performance test and the treatment of 90% of women identified with cervical disease.[5] Recommendations adopted by the WHO for screening in resource-limited settings include a strategy of HPV-screening followed by VIA triage

and treatment, or a strategy of HPV-screening followed by treatment.[3] Although no recommendations are given for the approach that should be prioritised, sub-Saharan Africa has a high HPV prevalence rate of 15%–30% and most HPV-positive women have no lesions.[3 6 7] In this context, HPV testing followed by immediate treatment can represent significant overtreatment in women with an HPV-positive test, which by itself may not confer a high risk of cervical intraepithelial neoplasia grade two or worse (CIN2+).[4 8 9] In sub-Saharan Africa, the prevalence of CIN2 +was reported to be 2%–4% in women aged 30–49 years and 7%–11% in an HPV-positive population with a low HIV prevalence rate (<10%).[6 7 10] A triage system is only a valid option if it can improve the positive predictive value (PPV) for CIN2 +and minimise the referral rate, while conserving the high sensitivity of the HPV test. The achievement of a high PPV at the cost of limited sensitivity may be considered a reasonable option when the loss to follow-up of women requiring surveillance is minimal. However, in low-resource settings, high levels of lost to follow-up constitute an important barrier to CC screening, which is why programmes having no follow-up visits or as few as possible are preferable to achieve a high degree of participation.[11] A '3T-Approach' (test, triage and treat) combining testing with a rapid HPV test, triage of HPV-positive women with VIA, and treatment by thermal ablation of VIA-positive patients within the same day, has been previously used to further reduce the risk of lost to follow-up.[12]

Triage by VIA and/or visual inspection with Lugol's iodine (VILI) requires accurate criteria to decide whether or not the findings are positive, which are generally based on the International Agency for Research against Cancer (IARC) manual.[13] However, in this setting, VIA triage in HPV-positive populations appears to be associated with an important loss of sensitivity, suggesting that triage by VIA using traditional criteria may not be of benefit.[6 7 10 14] Previous studies using histology as reference standard and having excluded verification bias had sensitivities ranging from 25.0% to 45.5%.[6 10 15]

Interpreting VIA with naked eye alone is subjective and is highly variable between healthcare providers.[16–18] This issue may be improved with continuous supervision and medical education thanks to the use of digital VIA and VILI (D-VIA/D-VILI). This includes acquisition of cervical images, native and after VIA and VILI application, through a camera or smartphone. These technologies provide an alternative to colposcopy in the context of LMICs and may constitute an important step in the improvement of VIA/VILI interpretation.[19–21] Although the image quality is probably lower than that with high-resolution colposcopy, there are significant benefits for healthcare providers, because they can move through and compare the native, VIA, and VILI images, and can also magnify suspicious lesions, before deciding whether treatment is needed.[19 20]

To improve VIA/D-VIA interpretation as a triage test in HPV-positive populations, we introduced a set of criteria, termed ABCD criteria for 'acetowhiteness', 'bleeding', 'colouring' (with Lugol's iodine) and 'diameter' of the lesion. These criteria constitute a simple structure that may contribute to preventing CC in an LMIC context. The aim of this study was to provide a rationale for the ABCD criteria and determine their performance in identifying histology-proven CIN2+.

METHODS

Study design

This prospective study was carried out between September 2018 and March 2020 in the health district of Dschang (West Cameroon) as part of a 5-year CC screening programme. The screening strategy consisted of the '3T-Approach', in which testing with HPV, Triage with VIA and treatment are provided within one visit. Asymptomatic non-pregnant women aged 30–49 years were eligible to participate in the study on a voluntary basis and were included in a consecutive manner on presentation to the screening site. Exclusion criteria included history of CIN treatment, anogenital cancer or hysterectomy. The study was conducted within a larger trial aiming to recruit 6000 women in a 5-year screening programme.[21] At the baseline visit, after obtaining written informed consent and providing guidance to participants on the procedure for vaginal self-sampling, participants undertook an HPV self-test (self-HPV) that was subsequently analysed by a point-of-care assay (GeneXpert), with most results available within an hour. HPVnegative women were reassured and advised to repeat the test in 5 years, while HPV-positive women were invited to undergo visual triage and thermal ablation or large loop excision of the transformation zone (LLETZ) if needed. Trained midwives performed gynecologic examination with VIA/VILI, assessment of ABCD criteria and transformation zone (TZ) type, and determined treatment modalities in a single visit. Two gynaecologists were available on call for a second opinion or advice.

ABCD criteria

The ABCD criteria were chosen from a synthesis of published results as well as our own experience in VIA and VILI interpretation.[3 13 22–26] We considered acetowhiteness as the most important predictor for CIN and noted that Lugol's iodine can be used to identify thin acetowhite lesions not seen on the initial VIA assessment (figure 1). Similar to the IARC criteria, the pathological area should be located within or in contact with the TZ. The ABCD criteria are codified as positive (present) or negative (absent). To be considered ABCD-positive, at least one of the following conditions needs to be fulfilled: presence of criteria A (acetowhiteness) and D (diameter) combined, or criterion B (bleeding) with or without presence of A, C (colouring) or D.

ABCD criteria were independently evaluated by one of three trained midwives and supervised by two experienced Cameroonian gynaecologists.

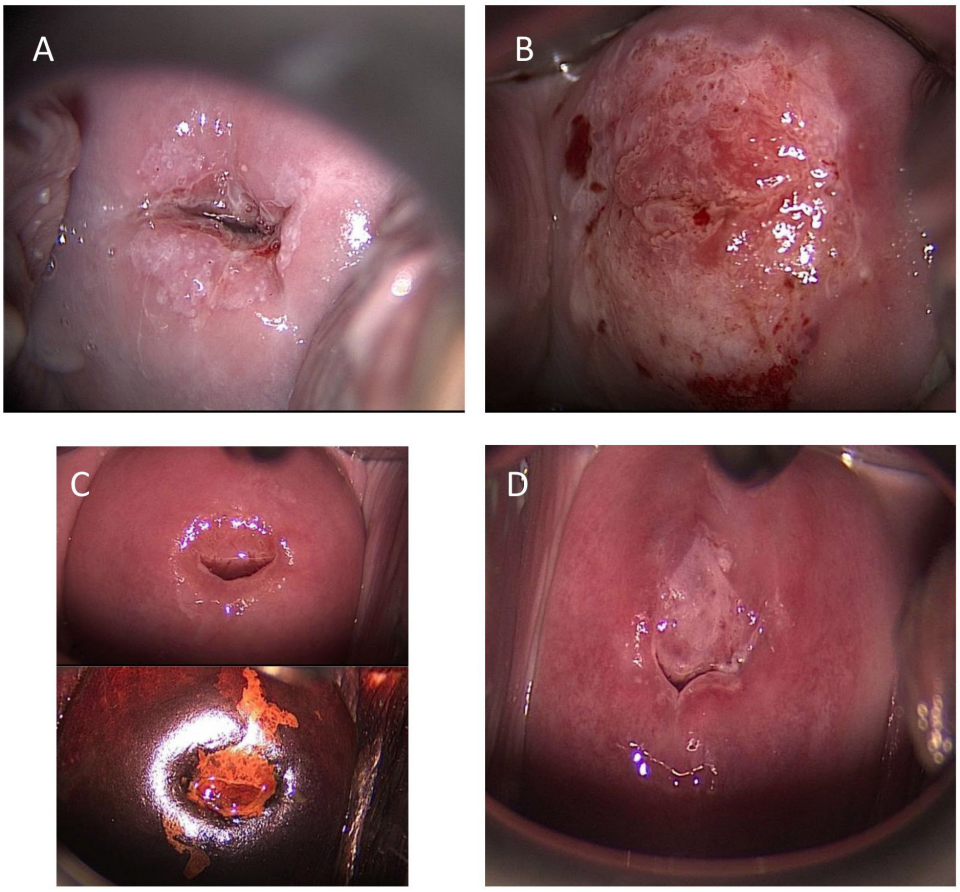

Figure 1 ABCD criteria for VIA interpretation in HPV-positive women. Criterion (A) Acetowhite area touching the transformation zone (absent on the native view and apparent after acetic acid application) is considered positive. Criterion (B) Bleeding without touching or after lightly touching (with a swab or speculum) the cervix is considered positive. Criterion (C) (optional)—colouring with VILI contributes to confirmation or identification of a faint acetowhite lesion. Criterion (D) Diameter of >5 mm (about the size of a pencil eraser) in an acetowhite area is considered positive. ABCD, acetowhiteness, bleeding, colouring and Diameter; diameter; HPV, human papillomavirus; VIA, visual inspection with acetic acid; VILI, visual inspection with Lugol's iodine.

- Criterion A for acetowhiteness—Criterion A is obtained after application of 3%–5% acetic acid. Any acetowhite area touching the TZ and having a diameter of >5 mm (criterion D) is considered positive. Compared with the IARC criteria, which require a degree of whiteness combined with the presence of a sharp, distinct, well defined, dense (opaque/dull or oyster white) acetowhite area,[13] we considered here any acetowhite lesion exceeding 5 mm to be positive.
- Criterion B for bleeding on touch—Criterion B is obtained on native examination or after acetic acid application. Presence of cervical bleeding without touching or after lightly touching the cervix in the TZ area is considered positive. This means that any bleeding from the surface of the cervix, after excluding bleeding of intrauterine origin, can be associated with CIN2 +lesions. Although bleeding can also be caused by ulceration or infection, any signs should be thoroughly investigated to rule out the possibility of early preclinical invasive cancer. This sign is easy to recognise and is considered a risk finding for precancerous lesions and CC.[25 26] Presence of bleeding in association with criteria A and C may require referral for further testing like biopsy and colposcopy.
- Criterion C for colouring with Lugol's iodine—Criterion C is optional. Lugol's iodine staining can be used as an adjunct to VIA to recognise epithelial change that would otherwise be difficult to identify by VIA only. The colour changes with VILI can be easier to appreciate than those after VIA and may contribute to identification of a missed thin acetowhite lesion. To be considered positive, an iodine-negative lesion should correspond to a VIA lesion having criteria A and D. Compared with the IARC criteria, which require the presence of a well-defined, bright yellow, iodine non-uptake area,[13] we consider any non-iodine uptake areas to be positive, providing they match an acetowhite lesion.
- Criterion D for diameter—Criterion D is evaluated after application of acetic acid (or Lugol's iodine). An acetowhite lesion measuring >5 mm in diameter (about the size of a pencil eraser) is considered positive. Defining a minimal size of 5 mm allows exclusion of benign conditions such as dot-like, line-like or streak-like areas.[24]

A set of three images (native, acetic acid, Lugol's iodine) were obtained on a Galaxy S5 smartphone (Samsung, Seoul, South Korea). Diagnosis and treatment were based on combined results of VIA/VILI and smartphone-enhanced D-VIA, using aids such as zooming in on lesions and performing comparisons between the native, VIA and VILI images. Women with positive ABCD criteria were eligible for treatment by thermal ablation, with the exception of (1) lesions extending into the endocervix which could not be covered by the probe tip and (2) suspicions of carcinoma, in-situ adenocarcinoma or invasive adenocarcinoma, which were referred to a gynaecologist to determine the need for further treatment (LLETZ or oncological management). Cervical liquid-based cytology, biopsy at the TZ and endocervical brushing (ECB) were performed on all HPV-positive women prior to treatment.

Cytology

Cervical liquid-based cytology was performed using the SurePath (September 2018 to July 2019) and ThinPrep (July 2019 to March 2020) techniques. All vials were analysed in Switzerland (CytoPath, Unilabs, Geneva and University Hospital of Geneva). The slides were independently read by qualified cytotechnologists and classified according to the 2014 Bethesda classification system: negative for intraepithelial lesion or malignancy, inflammatory atypical squamous cells of undetermined significance (ASC-US), inflammatory ASC that cannot exclude high-grade squamous intraepithelial lesion (HSIL) (ASC-H), atypical glandular cells with low-grade squamous intraepithelial lesion, HSIL, and invasive cancer. The cytotechnologists were aware of the HPV-positive status (but not of the HPV type) of participants but were blinded to the ABCD criteria interpretation.

Histology findings (reference standard)

Cervical biopsies were performed using biopsy forceps, and ECB was carried out with an endocervical brush. Cervical biopsies were performed at 6 o'clock in the TZ when ABCD criteria were negative. If ABCD criteria were positive, one or more biopsies were performed at the most suspicious areas. All samples were stored in formalin. Biopsy slides and ECB samples (processed by cellular block) were read by two experienced gynaecologic pathologists of the Geneva University Hospitals, Switzerland, who were blinded to the screening test results and ABCD criteria findings. There was no external review of histological analyses. The histological results were classified as normal, CIN1, CIN2, CIN3, adenocarcinoma in situ, invasive carcinoma or adenocarcinoma. The cut-off for a pathological result was set at CIN2+. When histological results varied within the samples of one participant, only the worst result was considered as the reference standard.

Patient and public involvement

Preferences of and experience with former patients of a preliminary research study on CC screening in Dschang, Cameroon, were considered in the design and conduction of this study. During the study, focus groups were organised with members of the community (women and men), healthcare workers and community health workers, to explore barriers to CC screening and further improve the programme and recruitment strategy. Patients were also involved at their arrival at the screening centre where they were offered a 1-hour information session on CC and sexual health by trained midwives. Furthermore, the public is kept informed about the progress of our research through the publication of biannual newsletters disseminated among health workers and the general community. Newsletters will be published until the end of the 3T study.

Statistical analysis

Initially, we planned a sample of 6000 women. However, the COVID-19 pandemic and public health measures to control the virus have impacted on-site clinical activity since mid-March 2020. In this context, we decided to consider an interim analysis to the trial of the primary endpoints which included performance of the ABCD criteria. Descriptive statistics were used to analyse the baseline characteristics of the study population. Sensitivity, specificity, PPV, negative predictive value (NPV) and positivity rate plus their 95% CIs were calculated for each triaging test. Student's t-test, Mann-Whitney test or Pearson's χ^2 test were used, where appropriate, to identify sociodemographic and reproductive characteristics of the patients that could differ between ABCD criteria results. A $p<0.05$ was considered statistically significant. An exploratory analysis was performed to assess the relationships between each independent variable and the correct prediction of the ABCD criteria. This correct prediction score was equal to one when ABCD criteria were positive and there was a CIN2 +on histology or if the ABCD criteria were negative and histology was also negative. All other incorrect predictions were assigned the value 0. Univariate and multivariate logistic regression analyses were carried out to identify predictors of a correct ABCD criteria score according to histology. Participants with missing or indeterminate results for ABCD criteria or histopathology were excluded from the analysis. ORs were adjusted for potential confounders, such as age, marital status, number of lifetime sexual partners, age at first sexual intercourse, age at first delivery, parity, HIV status, and type of TZ, and 95% CIs were calculated. All data analyses were conducted using Stata Statistical software Release V.13 (StataCorp).

RESULTS

A total of 1980 women aged 30–49 years were enrolled (median age: 41 years; IQR36–50 years). Overall, 1964 women performed Self-HPV, of whom 361 (18.5%) had an HPV-positive test and underwent pelvic examination, three were excluded from the results analysis for lack of ABCD criteria assessment, and 340 (94.2%) had interpretable histology findings and constituted the study

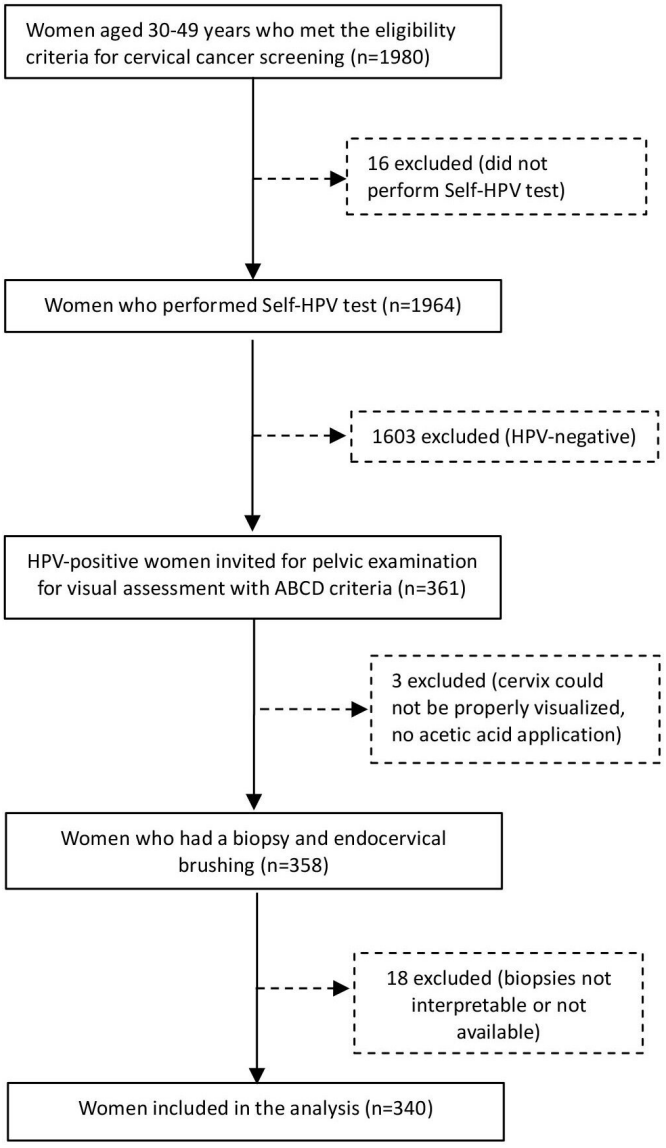

Figure 2 Flow chart of participants for the 3T-Approach in Cameroon. ABCD, acetowhiteness, bleeding, colouring and diameter; HPV, human papillomavirus.

population (figure 2). Table 1 provides details of the baseline sociodemographic, reproductive and clinical characteristics of the participants. Median age at first sexual intercourse was 18 years (IQR 16–19 years) and median number of sexual lifetime partners was 3 (IQR 2–5).

Thirty-four (9.5%) samples were positive for HPV-16, 53 (14.9%) for HPV-18/45 and 300 (84.0%) for other HPV types. Overall, 218 (60.9%) participants were classified as ABCD criteria-positive. All patients positive for ABCD were treated with thermal ablation with the exception of one patient who underwent LLETZ and one patient suspicious of cancer who was biopsied and referred for multimodal therapy. Thermal ablation was provided on the same day as HPV screening in 86.7% of cases. Reasons for delaying treatment included referral for further evaluation, technical issues, bleeding at the time of screening, or choice of the patients themselves. No serious adverse event occurred as a result of the screening procedure.

Among all 358 women with HPV-positive results, 343 samples with valid cytological results and 340 samples with valid histological results were obtained. Of the 343 valid cytological results, 21.6% had abnormal cytology (ASC-US+). Four patients had ASC-H, 25 had HSIL, and three had cytology suggesting cancer. All three cancers identified by cytology were confirmed by histology. Of the 340 valid histological results, 63 (18.5%) CIN1 were identified, 13 (3.8%) CIN2, 24 (7.1%) CIN3 and 3 (0.9%) invasive cancers. The prevalence of CIN2 +and CIN3 +was 11.8% and 7.9%, respectively. Details for the disease prevalences are also shown in table 1.

Table 2 shows demographic and pathological characteristics associated with a correct prediction of the ABCD criteria.

ABCD criteria were more likely to be correct in the presence of TZ type 3 (adjusted OR, aOR 6.47; 95% CI 2.59 to 16.21; p<0.001) and high-grade lesions on cytology (aOR 3.37; 95% CI 1.35 to 8.44; p<0.009). Overall, a correct prediction of the ABCD criteria was not impacted by the multiple sociodemographic characteristics of the population in the multivariate analysis, apart from women working as farmers who were less likely to have a correct prediction of ABCD criteria than employed women (OR 0.41, 95% CI 0.18 to 0.95).

Performance of ABCD and cytology for detection of high-grade cervical lesions (CIN2 +and CIN3+) is shown in table 3.

ABCD criteria for CIN2 +detection showed a sensitivity of 77.5% (95% CI 61.3% to 88.2%), specificity of 42.0% (95% CI 36.5% to 47.7%), PPV of 15.1% (95% CI 10.8% to 20.8%) and NPV of 93.3% (95% CI 87.6% to 96.5%). Cytology-classified HSIL +for CIN2 +detection showed lower sensitivity of 62.5% (95% CI 46.1% to 76.5%), but higher specificity of 98.6% (95% CI 96.3% to 99.5%), PPV of 86.2% (95% CI 67.0% to 95.1%) and NPV of 95.0% (95% CI 91.8% to 97.0%). Meanwhile, cytology-classified ASC-US +showed improved sensitivity of 80.0% (95% CI 64.0% to 89.9%) and specificity of 87.5% (95% CI 83.1% to 90.7%). Screening by HPV 16/18/45 genotyping alone had a much lower sensitivity of 37.5% (95% CI 23.5% to 53.9%) and a specificity of 79.9% (95% CI 74.9% to 84.1%). When combining HPV 16/18/45 partial genotyping with VIA triage of other HPV types, sensitivity rose to 85.0% (95% CI 70.2% to 94.3%) and NPV to 94.4% (95% CI 88.2% to 97.9%), while specificity decreased to 33.7% (95% CI 28.3% to 39.3%) and PPV to 14.6% (95% CI 10.3% to 19.8%). ABCD criteria for CIN3 +lesion identification showed a sensitivity of 70.4% (95% CI 49.6% to 85.2%), specificity of 40.6% (95% CI 35.2% to 46.1%), PPV of 9.3% (95% CI 6.0% to 14.1%), and NPV of 94.1% (95% CI 88.5% to 97.0%).

DISCUSSION

The ABCD criteria were established to improve the performance of visual-based approaches for triage of HPV-positive women. Previous studies conducted in LMICs

Table 1 Baseline sociodemographic, reproductive health, and clinical characteristics according to ABCD criteria (N=358)*

Variable	ABCD criteria-negative	ABCD criteria-positive	Total	P value
Participants recruited, n (%)	140 (39.1)	218 (60.9)	358	
Age (years), median (IQR)	41 (35–45)	40 (34–45)	40 (34–45)	0.4464
Marital status, n (%)				0.8910
Single	15 (10.7)	20 (9.2)	35 (9.8)	
With partner	109 (77.9)	173 (79.3)	282 (78.8)	
Divorced/widowed	16 (11.4)	25 (11.5)	41 (11.4)	
Education, n (%)				0.3900
Unschooled	1 (0.7)	5 (2.3)	6 (1.7)	
Primary education	37 (26.4)	66 (30.3)	103 (28.8)	
Secondary education	67 (47.9)	105 (48.2)	172 (48.0)	
Tertiary education	35 (25.0)	42 (19.2)	77 (21.5)	
Employment status, n (%)				0.1750
Employed	50 (35.7)	57 (26.2)	107 (29.9)	
Independent	39 (27.9)	56 (25.7)	95 (26.5)	
Housewife	23 (16.4)	41 (18.8)	64 (17.9)	
Unemployed	7 (5.0)	12 (5.5)	19 (5.3)	
Farmer	21 (15.0)	52 (23.8)	73 (20.4)	
Age at menarche (years), mean±SD	14.7±1.8	14.7±1.9	14.7±1.8	0.8914
Age at first intercourse, median (IQR)	17 (16–19)	18 (16–20)	18 (16–19)	0.2390
No of sexual partners, median (IQR)	4 (3–6)	3 (2–5)	3 (2–5)	0.0008
Contraception, n (%)				0.5950
None	93 (66.9)	142 (65.5)	235 (66.0)	
Condom	18 (13.0)	25 (11.5)	43 (12.1)	
Hormonal pill	1 (0.7)	7 (3.2)	8 (2.3)	
IUD/implant/injection	25 (18.0)	41 (18.9)	66 (18.5)	
Other	2 (1.4)	2 (0.9)	4 (1.1)	
HIV status, n (%)				0.9420
Negative	128 (92.7)	198 (93.0)	326 (92.9)	
Positive	10 (7.3)	15 (7.0)	25 (7.1)	
Age at first delivery (years), mean±SD	21.4±3.7	21.4±2.5	21.4±3.8	0.9137
Parity, n (%)				0.0080
Nulliparous	11 (7.9)	3 (1.4)	14 (3.9)	
1–4	66 (47.1)	108 (49.5)	174 (48.6)	
>4	63 (45.0)	107 (49.1)	170 (47.5)	
Transformation zone, n (%)				<0.0001
TZ1	76 (57.1)	150 (73.5)	226 (67.1)	
TZ2	26 (19.6)	45 (22.1)	71 (21.1)	
TZ3	31 (23.3)	9 (4.4)	40 (11.8)	
HPV testing results, n (%)				
HPV-16	11 (7.9)	23 (10.6)	34 (9.5)	0.3890
HPV-18/45	22 (15.8)	31 (14.2)	53 (14.9)	0.6770
Other HPV	114 (82.0)	186 (85.3)	300 (84.0)	0.4060
Cytology, n (%) (Total=343)				0.0990
Normal	108 (82.5)	161 (75.9)	269 (78.4)	
ASC-US	7 (5.3)	10 (4.7)	17 (5.0)	

Continued

Table 1 Continued

Variable	ABCD criteria-negative	ABCD criteria-positive	Total	P value
LSIL	10 (7.6)	15 (7.1)	25 (7.3)	
HSIL	4 (3.1)	21 (9.9)	25 (7.3)	
ASC-H	0	4 (1.9)	4 (1.2)	
Cancer	2 (1.5)	1 (0.5)	3 (0.8)	
Histology, n (%) (Total=340)				0.0040
Normal	108 (80.0)	129 (62.9)	237 (69.7)	
CIN1	18 (13.3)	45 (21.9)	63 (18.5)	
CIN2	1 (0.7)	12 (5.9)	13 (3.8)	
CIN3	6 (4.4)	18 (8.8)	24 (7.1)	
Invasive cancer	2 (1.5)	1 (0.5)	3 (0.9)	

*Data from the 358 participants may be missing for some variables.
ABCD, acetowhiteness, bleeding, colouring and diameter; ASC-H, atypical squamous cells that cannot exclude HSIL; ASC-US, ACS-undetermined significance; CIN1, cervical intraepithelial neoplasia grade 1; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; IUD, intrauterine device; LSIL, low-grade squamous intraepithelial lesion; TZ, transformation zone.

indicated that triage using traditional VIA criteria is not satisfactory for the detection of CIN2 +lesions, as the gain in specificity when adding VIA to HPV testing is obtained at the expense of an important loss in sensitivity.[6 7 10] The challenge for VIA screeners lies in interpreting the wide variability of cervical presentations, in populations where obstetric trauma to the cervix and history of infection are frequent, and in which CIN2 +may be difficult to identify.

The most important finding of this study is that the ABCD criteria appeared to be highly sensitive for detection of high-grade lesions in an HPV-positive population. We used both (1) a magnification technique with smartphone digital imaging that allows more detailed examination compared with naked eye alone and (2) a lower VIA/D-VIA threshold positivity to optimise identification of lesions. The ABCD criteria provided improved VIA sensitivity for triage of HPV-positive women compared with most previous studies using a comparable methodology (histology as reference standard)[6 10 15 26 27] This can be explained by the fact that the IARC criteria require dense VIA changes before being considered positive, thus limiting their sensitivity, while a reduced positivity threshold can contribute to improved sensitivity for CIN2 +detection.[13 24]

The low specificity and PPV, leading to higher overtreatment rates, arise because we considered any whitening to be positive, meaning many benign conditions (metaplasia, inflammation or other benign cervical changes) could produce false-positive results for the ABCD criteria. Criterion C (VILI/D-VILI), though dependent on criteria A and D, may contribute to the high false positive rate by categorising benign conditions as ABCD-positive through the identification of iodine-negative areas compatible with thin, transparent or patchy acetowhite lesions. Overall, 54.4% of normal histology results and 71.4% of CIN1 were considered ABCD criteria positive and consequently underwent unnecessary treatment. Thus, 85% (174 of 205) of women who screened positive were treated without CIN2+. However, when considering all women screened for CC, including HPV-negative, 174 were treated unnecessarily out of 1964 screened by self-HPV, corresponding to an overall 8.9% overtreatment rate in the total population screened. Despite the low specificity, our 3T-Approach in a single visit may be acceptable in an LMIC context because it reduces cost and lost to follow-up, which are recognised barriers to effective CC screening.[11 28] Indeed, studies in Uganda[29] and South Africa[28] have shown lost to follow-up rates between 21% and 25% after the first visit, up to 50% at 24 months. Furthermore, treatment by thermal ablation is associated with very low risks of side effects and morbidity.[30] Therefore, treatment of a significant number of false-positive cases in this context may be considered an acceptable strategy for effective control of CC in an LMIC setting and may contribute to reaching the target of the WHO's elimination initiative.[3 5] However, the use and integration of the ABCD criteria in the CC screening process warrants multidisciplinary discussion with involved stakeholders, taking into account the local context and resources, as well as regional HPV prevalence, prevalence of CIN2 +in HPV-positive participants, level of risk including HIV prevalence, availability of treatment modalities on site, and the possibility to offer further investigation when required. According to the context, the decision to refer has consequences for the patients and the healthcare system, requiring additional time and resources, and increasing the risk of loss to follow-up. Recognising the limitations of the ABCD criteria with regard to PPV and overtreatment rates, other triaging strategies merit further investigation. The use of extended HPV genotyping (HPV 16, 18, 45, 31, 33, 35, 52 and/or 58) for the triaging of HPV-positive women is one alternative that should also be explored.

Compared with screening by HPV-16/18/45 genotyping without triage, the sensitivity of the ABCD criteria

Table 2 Demographic and pathological characteristics associated with a correct prediction of the ABCD criteria (N=340)*

Variable	Total	Unadjusted OR (95% CI)	P value	Adjusted OR (95% CI)†	P value
Age (years), n (%)					
30–40	186 (54.7)	1.00 (Reference)		1.00 (Reference)	
41–50	154 (45.3)	1.39 (0.90 to 2.14)	0.133	1.51 (0.87 to 2.60)	0.140
Marital status, n (%)					
Single	34 (10.0)	1.00 (Reference)		1.00 (Reference)	
With partner	265 (77.9)	1.15 (0.56 to 2.36)	0.706	1.07 (0.43 to 2.63)	0.887
Divorced/widowed	41 (12.1)	0.81 (0.32 to 2.04)	0.656	0.63 (0.19 to 2.04)	0.442
Education, n (%)					
Unschooled/primary education	101 (29.7)	1.00 (Reference)		1.00 (Reference)	
Secondary/tertiary education	239 (70.3)	1.04 (0.65 to 1.65)	0.879	0.92 (0.47 to 1.82)	0.818
Employment status, n (%)					
Employed	104 (30.6)	1.00 (Reference)		1.00 (Reference)	
Independent	93 (27.3)	0.90 (0.51 to 1.57)	0.706	0.73 (0.38 to 1.43)	0.363
Housewife	58 (17.1)	0.81 (0.43 to 1.55)	0.528	0.74 (0.34 to 1.63)	0.461
Unemployed	19 (5.6)	0.72 (0.27 to 1.95)	0.528	0.89 (0.27 to 2.91)	0.852
Farmer	66 (19.4)	0.69 (0.37 to 1.29)	0.248	**0.41 (0.18 to 0.95)**	**0.037**
Age at first intercourse (years), n (%)					
≤17	154 (45.6)	1.00 (Reference)		1.00 (Reference)	
≥18	184 (54.4)	0.70 (0.46 to 1.08)	0.106	0.75 (0.43 to 1.31)	0.315
No of sexual partners‡, median (IQR)	**3 (2–5)**	**1.08 (1.01 to 1.16)**	**0.031**	1.06 (0.97 to 1.1.7)	0.176
1–2, n (%)	98 (28.8)	1.00 (Reference)		1.00 (Reference)	
3–5, n (%)	177 (52.1)	1.39 (0.84 to 2.30)	0.195	1.22 (0.67 to 2.22)	0.506
>5, n (%)	**65 (19.1)**	**1.96 (1.04 to 3.70)**	**0.038**	1.53 (0.70 to 3.38)	0.284
Contraception, n (%)					
No	225 (66.6)	1.00 (Reference)		1.00 (Reference)	
Yes	113 (33.4)	0.84 (0.54 to 1.33)	0.466	0.92 (0.54 to 1.85)	0.769
HIV status, n (%)					
Negative	309 (92.8)	1.00 (Reference)		1.00 (Reference)	
Positive	24 (7.2)	1.21 (0.53 to 2.77)	0.657	0.95 (0.36 to 2.53)	0.589
Age at first delivery (years), n (%)					
≤20	157 (47.7)	1.00 (Reference)		1.00 (Reference)	
≥21	172 (52.3)	0.70 (0.45 to 1.08)	0.102	0.60 (0.34 to 1.07)	0.085
Parity, n (%)					
Nulliparous	14 (4.1)	1.00 (Reference)		1.00 (Reference)	
1–4	**165 (48.5)**	**0.21 (0.06 to 0.79)**	**0.020**	0.26 (0.02 to 2.91)	0.274
>4	**161 (47.4)**	**0.23 (0.06 to 0.86)**	**0.029**	0.28 (0.02 to 3.22)	0.307
Transformation zone, n (%)					
TZ1	210 (65.8)	1.00 (Reference)		1.00 (Reference)	
TZ2	70 (22.0)	1.17 (0.68 to 2.02)	0.575	1.24 (0.67 to 2.26)	0.492
TZ3	**39 (12.2)**	**6.72 (2.84 to 15.93)**	**<0.0001**	6.47 (2.59 to 16.21)	<0.0001
HPV testing results, n (%)					
Other HPV (without co-infection)	264 (77.9)	1.00 (Reference)		1.00 (Reference)	
HPV-16/18/45	75 (22.1)	1.19 (0.70 to 1.98)	0.514	1.18 (0.64 to 2.17)	0.605
Cytology, n (%)					
High-grade+§	**29 (8.9)**	**2.47 (1.11 to 5.49)**	**0.027**	3.37 (1.35 to 8.44)	0.009

Bold values are statistically significant.
*Data from the 340 participants may be missing for some variables.
†Adjusted for age, marital status, age at first intercourse, number of lifetime sexual partners, age at first delivery, parity, HIV status and type of transformation zone.
‡ORs for continuous variables indicate the change in odds for an increase of 1 SD.
§High-grade lesions include ASC-H, HSIL, AIS and cancer.
ABCD, acetowhiteness, bleeding, colouring and diameter; AIS, adenocarcinoma in situ; ASC-H, atypical squamous cells that cannot exclude HSIL; CIN2+, cervical intraepithelial neoplasia grade 2 or worse; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; TZ, transformation zone.

Table 3 Diagnostic accuracy of ABCD criteria, cytology and HPV for detection of CIN2 +and CIN3+

Variable	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Positivity rate % (95% CI)
	CIN2+ (N=40, 11.8%)				HPV+ (N=358)
ABCD criteria-positive	77.5 (61.3 to 88.2)	42.0 (36.5 to 47.7)	15.1 (10.8 to 20.8)	93.3 (87.6 to 96.5)	60.9 (55.6 to 65.9)
Cytology ASC-US+	80.0 (64.0 to 89.9)	87.5 (83.1 to 90.7)	47.1 (35.3 to 59.2)	96.9 (93.9 to 98.5)	21.6 (17.4 to 26.4
Cytology LSIL+	70.0 (53.5 to 82.6)	91.3 (87.4 to 94.1)	52.8 (39.1 to 66.2)	95.6 (92.4 to 97.5)	16.6 (12.9 to 21.1)
Cytology HSIL+	62.5 (46.1 to 76.5)	98.6 (96.3 to 99.5)	86.2 (67.0 to 95.1)	95.0 (91.8 to 97.0)	9.3 (6.6 to 13.0)
HPV-16/18/45+	37.5 (23.5 to 53.9)	79.9 (74.9 to 84.1)	20.9 (12.3 to 30.8)	90.5 (86.3 to 93.5)	23.3 (19.1 to 28.1)
	CIN3+ (n=27, 7.9%)				
ABCD criteria-positive	70.4 (49.6 to 85.2)	40.6 (35.2 to 46.1)	9.3 (6.0 to 14.1)	94.1 (88.5 to 97.0)	
Cytology ASC-US+	88.9 (68.9 to 96.7)	85.4 (80.9 to 89.0)	35.3 (24.7 to 47.6)	98.8 (96.4 to 99.7)	
Cytology LSIL+	81.5 (60.9 to 92.5)	89.7 (85.7 to 92.7)	41.5 (28.7 to 55.5)	98.2 (95.7 to 99.2)	
Cytology HSIL+	74.1 (53.2 to 87.8)	97.0 (94.3 to 98.4)	68.9 (49.0 to 83.7)	97.7 (95.2 to 98.9)	
HPV-16/18/45+	44.4 (26.2 to 64.3)	79.8 (75.0 to 83.9)	16.0 (9.2 to 26.4)	94.3 (90.8 to 96.6)	

Cytology ASC-US+=ASC US, LSIL, ASC-H, HSIL, AIS and cancer; cytology LSIL+=LSIL, ASC-H, HSIL, AIS and cancer; cytology HSIL+=ASC hour, HSIL, AIS and cancer; HPV-16/18/45+=HPV DNA test positive for HPV-16, HPV-18 and HPV-45; 95% CI.
ABCD, acetowhiteness, bleeding, colouring and diameter; AIS, adenocarcinoma in situ; ASC-US, atypical squamous cells of undetermined significance; CIN2, cervical intraepithelial neoplasia grade 2; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; NPV, negative predictive value; PPV, positive predictive value.

was much higher, at the cost of a lower specificity. PPV was also slightly lower with triage by ABCD criteria (15.1%) than with HPV partial genotyping (20.9%). One of the screening strategies currently recommended by the WHO is combined HPV 16/18/45 genotyping (treated immediately) and VIA triage of non-16/18/45 HPV genotypes.[3] In our study population, this combined strategy resulted in an increased sensitivity of 85.0%, but even further decreased the specificity and PPV, which would therefore even further increase overtreatment rates. On the contrary, triage by cytology (using a threshold of ASC-US for a positive triage) improved both sensitivity (80.0%, 95% CI 64.0% to 89.9%) and specificity (87.5, 95% CI 83.1% to 90.7%) compared with the ABCD criteria. However, although this strategy may be adapted to higher-middle and high-income countries, the lack of trained cytotechnicians and well-equipped laboratories in low-income countries, the higher cost, and the inability to provide same-day treatment to patients positively triaged with cytology, render this triaging strategy unsuitable for low-resource settings. In comparison, the ABCD criteria require only basic equipment at a low cost, and allow initiation of therapy without delay. In our series, 86.7% of participants underwent the 3T-Approach in 1 day. ABCD criteria comprise a simple tool with binary results (positive or negative) that can alert healthcare professionals to the clinical features of CIN2+, and the use of 'relaxed IARC criteria' may greatly decrease the risk of missing CIN2 +lesions. While digital imaging by smartphone may facilitate ABCD interpretation and enhance diagnostic performance, it may result in slightly prolonged examination time and may not be accessible in all settings.

Having a TZ3 was associated with a better prediction of ABCD criteria compared with TZ1 (table 2), which is unexpected as VIA is generally considered inadequate for the evaluation of TZ3 cervixes. This may be due to the use of B, C and D criteria in addition to acetowhiteness, enabling the detection of lesions extending to the ectocervix and bleeding in the absence of visible lesions. However, as A, B, C and D criteria were not assessed separately within this study sample, it is currently not possible to determine which criterion contributes most to a correct interpretation of VIA. A study is currently underway to assess each criterion individually for the detection of CIN2+. The lack of association between multiple sociodemographic variables and a correct prediction of the ACBD criteria (table 2) supports the generalisability of these criteria to the overall population of women aged 30–49 years in West Cameroon. However, the limited sample size and the fact that the study was conducted in a single centre, do not allow to extend these results to the overall female population, especially considering the differences in HPV prevalence in other regions.

A further limitation is that the study was conducted in a single centre in a district hospital in West Cameroon with five healthcare providers administering all screening and treatment procedures.

It should be noted that two out of three CC were assessed as ABCD-negative on site by the front-line healthcare providers and did not receive immediate treatment. After reviewing the digital images of these two cases offsite, it was determined that criterion B (bleeding) was present in both cases, which should have led to a positive ABCD result (online supplemental figure S1).

Strengths of our study included the application of ABCD criteria on VIA examination in real-life conditions with immediate treatment when necessary, therefore, supporting the feasibility of a 'screen-and-treat' strategy. Furthermore, because all HPV-positive women underwent biopsy and cervical brushing regardless of the ABCD criteria results, there was no risk of verification bias in the calculations of sensitivity and specificity for all diagnostic strategies assessed.

In conclusion, ABCD criteria can improve CIN2 +diagnosis in HPV-positive women and may provide a unique opportunity to improve CC screening programmes in LMICs using a one-visit approach. This strategy may be particularly beneficial because the criteria are easily remembered and to use for healthcare providers.

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