




# BMJ Open Utility of extended HPV genotyping for the triage of self-sampled HPV-positive women in a screen-and-treat strategy for cervical cancer prevention in Cameroon: a prospective study of diagnostic accuracy

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## ABSTRACT

**Objective** To explore the utility of extended Human Papillomavirus (HPV) genotyping to detect cervical intraepithelial neoplasia grade 2 or more (CIN2+) in a 'screen-and-treat' strategy for HPV-positive women in low-resource settings.

**Design** Prospective study of diagnostic accuracy.

**Setting** The study took place in West Cameroon between September 2018 and March 2020.

**Participants** 2014 women were recruited. Asymptomatic, non-pregnant women aged 30–49 years without history of CIN treatment, anogenital cancer or hysterectomy were eligible.

**Interventions** Participants performed self-sampling for HPV testing with GeneXpert followed by visual inspection with acetic acid and Lugol's iodine (VIA) triage before treatment if required.

**Main outcome measures** Liquid-based cytology, biopsies and endocervical brushing were performed in HPV-positive women as quality control. We assessed the detection rate of CIN2+ by HPV genotyping (two pools of genotypes obtained from the Xpert system, pool\_1 (HPV 16, 18, 45) and pool\_2 (HPV 16, 18, 45, 31, 33, 35, 52, 58)), VIA and cytology.

**Results** 382 (18.2%) women were HPV-positive among which 11.5% (n=44) were CIN2+. Of those 44 participants, 41 were triaged positive by extended genotyping, versus 35 by VIA and 33 by cytology. Overall, triage positivity was of 68.4% for extended genotyping, 59.3% for VIA and 14.8% for cytology, with false positive rates of 83.4%, 84.1% and 37.7%, respectively. Extended genotyping had a higher sensitivity for CIN2+ detection (93.2%, CI: 81.3 to 98.6) than VIA (79.5%, CI: 64.7 to 90.2, p=0.034) and cytology (75.0%, CI: 59.7 to 86.8, p=0.005). No significant difference was observed in the overtreatment rate in triaged women by extended genotyping or VIA (9.9%, CI: 8.6 to 11.3, and 8.8%, CI: 7.7 to 10.1), with a ratio of 6.0 and 6.3 women treated per CIN2+ diagnosed.

**Conclusion** Triage of HPV-positive women with extended HPV genotyping improves CIN2+ detection compared with

## STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ The study was conducted as would be in routine practice and with minimal exclusion criteria, therefore representing a large span of the West-Cameroonian female population for which cervical cancer screening is recommended.
- ⇒ All Human Papillomavirus (HPV)-positive women underwent cervical biopsy and endocervical brushing to minimise disease misclassification.
- ⇒ One limitation is that as a single-site study, our results cannot be generalisable to populations with different sociodemographic characteristics, HPV and HIV prevalence.
- ⇒ Further validation is therefore needed to verify if the pool of eight genotypes used in this study may be applied across other low-income and middle-income country contexts.

VIA with a minor loss of specificity and could be used to optimize the management of HPV-positive women.

**Trial registration number** NCT03757299.

## INTRODUCTION

Human Papillomavirus (HPV) testing for primary cervical cancer screening of women between 30 and 49 years old is an option recommended by the WHO for low-income countries.<sup>1</sup> Its high sensitivity and negative predictive value (NPV) in detecting cervical intraepithelial neoplasia grade 2 or worse (CIN2+) allow extending the screening intervals. Recently, the development of fully automated diagnostic devices providing rapid HPV testing of self-obtained vaginal samples have offered a great opportunity to improve

the effectiveness of cervical cancer prevention in low-resource contexts.<sup>2</sup>

However, a single-HPV test has limited specificity and can lead to unnecessary workup and overtreatment. Therefore, a triage strategy is required for HPV-positive women in order to reduce overtreatment. Cytology is generally proposed as it is an effective method for triage of HPV-positive women, but in low-resource settings, various logistic and operational reasons do not allow successful cytology implementation. Therefore, the WHO recommends visual inspection with acetic acid (VIA) for the triage of HPV-positive women, ideally integrated in a same-day ‘screen-and-treat’ strategy.<sup>1</sup> Nevertheless, VIA lacks quality control, requires expertise and its accuracy varies greatly depending on the examiner’s experience.

HPV genotype-risk stratification is gaining importance and is also considered as an alternative method for triage.<sup>3–5</sup> The risk of CIN2+ varies with individual HPV genotypes.<sup>6,7</sup> HPV 16 and HPV 18 present the highest risk as these genotypes are associated with over 70% of all cervical cancers. Their value for triage is widely established and used in clinical practice.<sup>8,9</sup> However, the sensitivity of HPV 16/18 partial genotyping to detect CIN2+ lesions is limited and the need for extended HPV genotyping for individual risk stratification has been previously emphasized.<sup>10</sup>

A meta-analysis showed that worldwide, HPV 16, 18, 31, 33, 35, 45, 52 and 58 are the eight most common genotypes detected in about 90% of cervical cancers.<sup>11</sup> Similarly, these genotypes were the most common in paraffin-embedded samples from 10 575 cases of invasive cervical cancer, collected from 38 countries.<sup>12</sup> Therefore, several studies explored whether extended genotyping with major carcinogenic types can stratify HPV-positive women according to their risk and identify those needing treatment while reassuring those at very low risk of cervical cancer.<sup>13–17</sup> Nevertheless, there is uncertainty whether extended genotyping could enhance clinical performance in the triage of HPV-positive women in low-resource settings of sub-Saharan Africa practicing a screen-and-treat strategy.

In the present study, by retrieving data from an ongoing cervical cancer screening programme in Cameroon, we evaluated the diagnostic performance of a pool of 3 genotypes (HPV 16, 18, 45) and a pool of 8 genotypes (HPV 16, 18, 45, 31, 33, 35, 52, 58) to detect CIN2+ lesions and compared them with the performance of VIA and cytology as triage methods. Furthermore, we assessed the risk of overtreatment rate according to genotyping and VIA triage strategies in a ‘screen-and-treat’ approach.

## MATERIALS AND METHODS

### Setting, screening programme and study design

The Universities of Dschang (Cameroon) and Geneva have been working together since 2012 to evaluate innovative cervical cancer screening options adapted to the needs and means of women in Cameroon. In September

2018, a screening programme called *3T-Approach* (for Test-Triage-Treat in a 1-day visit) implementing WHO guidance on cervical cancer control was started in Dschang.<sup>18</sup>

The present study is nested in a large prospective trial started in September 2018 in West Cameroon, with the aim to recruit 6000 eligible women. Women were recruited by Dschang District Hospital through a poster campaign, through door-to-door recruitment by community health workers, and via radio announcements. All asymptomatic, non-pregnant women aged 30–49 years were eligible if they had no history of CIN treatment, of anogenital cancer or hysterectomy. Women were enrolled after providing written informed consent.

### Study procedures

Sociodemographic characteristics, eligibility and medical history of the participants (including self-reported HIV status) were obtained through a questionnaire completed with a midwife specifically trained for the study; and later electronically transcribed using the SecuTrial software (Berlin, Germany). Women were asked to provide a self-collected vaginal sample for HPV testing. Patients with a negative HPV test were discharged home without further investigations. In case of a positive result, participants were triaged by PAP smear and VIA/VILI. Endocervical brushing (ECB) and cervical biopsies were performed at the end of the procedure as the gold standard to assess the study outcome. Identified lesions were treated by thermal ablation or large loop excision of the transformation zone (LLETZ) and the patient was discharged home after 30 min surveillance. When no lesions were detected (negative VIA), HPV-positive women were followed up every 12 months until clearance of the HPV infection. VIA-positive women treated with thermal ablation or LLETZ were followed up at 6 months and 1 year by self-HPV/VIA/cytology/biopsies and ECB. More details on the *3T-study* have been previously published.<sup>18–20</sup>

### HPV testing

To perform HPV self-sampling, the FIOQSwab was used. A technician then rinsed the swab in a vial with 20 mL of saline solution (sodium chloride 0.9%) and vortexed for 30 s; 1 mL of the solution was then transferred into a single-use disposable cartridge that holds PCR reagents of the GeneXpert analyzer (Cepheid). The GeneXpert assay contains a ‘Sample Adequacy Control’ system with reagents that amplify and detect an endogenous single-copy human gene, thus determining if the sample contains sufficient patient cells for reliable performance. Moreover, a sample processing internal control in the form of an exogenous nucleic acid preloaded in the cartridge is included in order to verify adequate functioning. The Xpert system provides quality results in 60 min. Specifically, it uses five colour channels containing primers and probes for the detection of the following specific genotypes or pooled results: (i) HPV 16, (ii) HPV 18, 45 in a pooled result, (iii) HPV types 31, 33, 35 52 or 58, in a pooled result, (iv) HPV types 51 or 59, in a pooled result

**Table 1** Sociodemographic characteristics of HPV-positive and negative participants

Variable	HPV negative	HPV positive	Total	P value*
	N (%)	N (%)	N (%)	
Participants recruited	1722 (81.8)	382 (18.2)	2104	
Age (years), median (IQR)	41 (35–45)	40 (34–45)	40 (35–45)	0.066
Age groups, years				0.060
30–39	743 (43.2)	185 (48.4)	928 (44.1)	
40–50	979 (56.8)	197 (51.6)	1176 (55.9)	
Marital status				<0.001
Married/in relationship	1486 (86.4)	302 (79.3)	1788 (85.1)	
Single/divorced/widowed	234 (13.6)	79 (20.7)	313 (14.9)	
Education				0.686
Unschooling/primary education	502 (29.2)	115 (30.3)	617 (29.4)	
Secondary and tertiary education	1216 (70.8)	265 (69.7)	1481 (70.6)	
Employment status				0.133
Employed	468 (27.2)	112 (29.4)	580 (27.6)	
Independent	464 (27.0)	102 (26.8)	566 (26.9)	
Housewife	391 (22.7)	70 (18.4)	461 (21.9)	
Other (eg, student, unemployed)	58 (3.4)	21 (5.5)	79 (3.8)	
Farmer	339 (19.7)	76 (19.9)	415 (19.8)	
Age of menarche, mean±SD	14.7±1.8	14.7±1.9	14.7±1.8	0.862
≤14 years	831 (48.3)	190 (49.7)	1021 (48.5)	0.6
>14 years	891 (51.7)	192 (50.3)	1083 (51.5)	
Age at first intercourse, mean±SD	17.9±2.6	17.9±2.7	17.9±2.6	0.819
≤17 years	789 (45.8)	175 (45.8)	964 (45.8)	0.998
>17 years	933 (54.2)	207 (54.2)	1140 (54.2)	
Number of sexual partners, mean±SD	3.8±3.94	4.1±3.3	3.9±3.4	0.123
<4	1021 (59.3)	204 (53.4)	1225 (58.2)	0.035
≥4	701 (40.7)	178 (46.6)	879 (41.8)	
Age at first delivery, mean±SD	21.6±4.0	21.4±3.8	21.5±4.0	0.587
≤20 years	838 (48.7)	178 (46.6)	1016 (48.3)	0.464
>20 years	884 (51.3)	204 (53.4)	1088 (51.7)	
Parity, mean±SD	4.5±2.1	4.1±2.2	4.5±2.1	<0.001
Nulliparous	69 (4.0)	16 (4.2)	85 (4.0)	0.020
1–4	712 (41.4)	187 (48.9)	899 (42.7)	
>4	941 (54.6)	179 (46.9)	1120 (53.2)	
Contraception				0.556
None	1217 (71.0)	255 (67.3)	1472 (70.3)	
Condom	175 (10.2)	43 (11.3)	218 (10.4)	
Hormonal (IUD, implant, injectable, pill)	305 (17.8)	77 (20.3)	382 (18.2)	
Other (sterilisation, spermicide)	18 (1.0)	4 (1.1)	22 (1.1)	
Smoker	28 (1.6)	10 (2.6)	38 (1.8)	0.187
HIV status (self-reported)				<0.001
Negative	1643 (97.5)	347 (93.0)	1990 (96.7)	
Positive	42 (2.5)	26 (7.0)	68 (3.3)	

\*Calculated by  $\chi^2$  test.

HPV, human papillomavirus; IUD, intrauterine device.

**Table 2** Distribution of histopathologic outcomes stratified by triage test results among HPV-positive women

Triage test	Biopsies/ECB with valid result	Normal	CIN 1	CIN 2	CIN 3	Invasive cancer
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
HPV Pool 1						
Positive	85 (22.9)	53 (20.1)	15 (23.4)	3 (21.4)	13 (48.1)	1 (33.3)
Negative	286 (77.1)	210 (79.9)	49 (76.6)	11 (78.6)	14 (51.9)	2 (66.7)
HPV Pool 2 (extended genotyping)						
Positive	247 (68.4)	163 (64.2)	43 (68.2)	12 (85.7)	26 (96.3)	3 (100)
Negative	114 (31.6)	91 (35.8)	20 (31.8)	2 (14.3)	1 (3.7)	0 (0)
Visual assessment						
Positive	220 (59.3)	139 (52.9)	46 (71.9)	13 (92.9)	21 (77.8)	1 (33.3)
Negative	151 (40.7)	124 (47.1)	18 (28.1)	1 (7.1)	6 (22.2)	2 (66.7)
Cytology						
Positive (≥ ASC US)	53 (14.8)	15 (6.0)	5 (7.8)	8 (57.1)	22 (81.5)	3 (100)
Negative	304 (85.2)	234 (94.0)	59 (92.2)	6 (42.9)	5 (18.5)	0 (0)

ASC-US, atypical squamous cells of undetermined significance; CIN1/2/3, cervical intraepithelial neoplasia grade 1/2/3; ECB, endocervical brushing; HPV, Human Papillomavirus; HPV Pool 1, HPV 16, HPV 18/45; HPV Pool 2 (extended genotyping), HPV 16, HPV 18/45, HPV 31/33/35/52/58.

and (v) HPV types 39, 56, 66 or 68 in a pooled result. In case of a negative HPV result, women were reassured and advised to repeat the test in 5 years. If positive for at least one of the channels, a pelvic examination with visual assessment of the cervix was completed.

#### Visual assessment and treatment

To carry out VIA, the nurse applied a cotton swab soaked with acetic acid on the cervix and waited 1 min to evaluate the result, followed by Lugol iodine application. Midwives were trained before the beginning of the study to perform VIA and received ongoing training from a gynaecologist on a bimonthly basis. In order to optimize the sensitivity of the test, we introduced point-of-care digital imaging of the cervix using a smartphone application named 'Exam' developed by the Swiss Federal Institute of technology, Lausanne (EPFL), to obtain high-quality digital pictures as an adjunct to naked-eye inspection.<sup>21–23</sup> All pictures were double-checked by a gynaecologist.

Furthermore, we introduced an ABCD mnemonic method to alert healthcare providers for positivity of the visual assessment according to the following simple criteria: A for 'Acetowhiteness', B for 'Bleeding of a lesion in the transformation zone' (TZ), C for 'Colouring confirmation with Lugol's Iodine' and D for 'Diameter of the acetowhite area' (≥0.5 cm).<sup>24 25</sup> To be considered ABCD positive, at least one of the following conditions needed to be fulfilled: presence of criteria A and D combined or criterion B (with or without presence of A, C or D).<sup>24</sup> All participants positive on VIA (reported hereafter as VIA) were treated by thermal ablation or LLETZ, depending on eligibility criteria. If an invasive cancer was suspected, women got a full assessment, and the treatment was completely covered by the programme.

A quality assurance plan was put in place for the 3T study and included, among other indicators, cytology, biopsies and ECB, performed for all HPV-positive cases.<sup>18</sup>

#### Cytology

Cervical liquid-based cytology was performed using the SurePath (September 2018 to July 2019) and ThinPrep (July 2019 to March 2020) techniques. A spatula was used to collect cells from the TZ of the cervix, and the cell-covered end of the spatula was introduced into a vial containing a preservative solution. All vials were analysed in Switzerland (CytoPath, Unilabs, Geneva and University Hospital of Geneva). The cells in the vials were separated from excessive blood and mucus, and representative samples of the cells were placed onto slides. The slides were independently read by qualified cytotechnologists and classified according to the Bethesda classification system.<sup>26</sup>

#### Histopathology

Adjudicated histopathology diagnosis of cervical tissue obtained from biopsies and ECB served as the reference standard for diagnostic accuracy in this study. Guided biopsies were performed on all visible lesions or at 6 o'clock within the TZ and near the squamocolumnar junction when no lesion was seen. ECB was performed on all women, with or without visible lesions, at the end of visual inspection. Hematoxylin-eosin-stained slides of the tissue were prepared in the Division of Clinical Pathology (Geneva University Hospitals). Two pathologists specialized in gynaecopathology provided diagnosis according to the CIN classification system.

**Table 3** Diagnostic accuracy of triage tests for CIN2 and CIN3 thresholds among HPV-positive women

Performance metrics and histology thresholds	HPV Pool 1 (95% CI)	P value*	HPV Pool 2 (extended genotyping) (95% CI)	Visual assessment (95% CI)	P value*	Cytology (95% CI)	P value*
<b>≥CIN2</b>							
Sensitivity	38.6% (24.4 to 54.5)	<0.001	93.2% (81.3 to 98.6)	79.5% (64.7 to 90.2)	0.034	75.0% (59.7 to 86.8)	0.005
Specificity	79.2% (74.4 to 83.5)	<0.001	35.0% (29.8 to 40.5)	43.4% (38.0 to 49.0)	0.031	93.6% (90.3 to 96.1)	<0.001
PPV	20.0% (12.1 to 30.1)	0.651	16.6% (12.2 to 21.8)	15.9% (11.3 to 21.4)	0.44	62.3% (47.9 to 75.2)	<0.001
NPV	90.6% (86.6 to 93.7)	0.052	97.4% (92.5 to 99.5)	94.0% (89.0 to 97.2)	0.07	96.4% (93.6 to 98.2)	0.137
PLR	1.86 (1.21 to 2.85)		1.43 (1.28 to 1.61)	1.41 (1.18 to 1.68)		11.74 (7.43 to 18.54)	
NLR	0.77 (0.61 to 0.99)		0.19 (0.06 to 0.59)	0.52 (0.30 to 0.92)		0.27 (0.16 to 0.45)	
<b>≥CIN3</b>							
Sensitivity	46.7% (28.3 to 65.7)	0.0001	96.7% (82.8 to 99.9)	73.3% (54.1 to 87.7)	0.008	83.3% (65.3 to 94.4)	0.046
Specificity	79.2% (74.5 to 83.4)	<0.001	34.1% (29.0 to 39.5)	41.9% (36.6 to 47.4)	0.039	91.4% (87.9 to 94.2)	<0.001
PPV	16.5% (9.3 to 26.1)	0.243	11.7% (8.0 to 16.4)	10.0% (6.4 to 14.7)	0.15	47.2% (33.3 to 61.4)	<0.001
NPV	94.4% (91.1 to 96.8)	0.865	99.1% (95.2 to 100)	94.7% (89.8 to 97.7)	0.55	98.4% (96.2 to 99.5)	0.438
PLR	2.24 (1.45 to 3.46)		1.47 (1.33 to 1.63)	1.26 (1.00 to 1.60)		9.73 (6.60 to 14.35)	
NLR	0.67 (0.48 to 0.95)		0.10 (0.01 to 0.67)	0.64 (0.35 to 1.17)		0.18 (0.08 to 0.41)	

\*P values were calculated by McNemar's  $\chi^2$  test in comparison with HPV Pool 2 (extended genotyping).

CIN2, cervical intraepithelial neoplasia grade 2; CIN3, cervical intraepithelial neoplasia grade 3; HPV, Human Papillomavirus; HPV Pool 1, HPV 16, HPV 18/45; HPV Pool 2, HPV 16, HPV 18/45, HPV 31/33/35/52/58; NLR, negative likelihood ratio; NPV, negative predictive value ; PLR, positive likelihood ratio ; PPV, predictive positive value .

### Patient and public involvement

We did not involve patients or the public in the design, or conduct, or reporting or dissemination plans of the research. However, our study design and procedures were based on a previous pilot study conducted in the same setting which was well accepted by patients and health-care providers.<sup>27</sup>

### Assessment of test accuracy and impact on referral for treatment

The primary outcome measure of the study was the diagnostic accuracy for the detection of CIN2+ and CIN3+ disease by two pools of genotypes obtained from three channels of the Xpert system: pool\_1 (HPV 16, 18, 45) and pool\_2 (extended genotyping: HPV 16, 18, 45, 31, 33, 35, 52, 58). In addition, we compared the accuracies of the genotypes pools with those of VIA and cytology. The evaluation of the proportion of women eligible for treatment by using either the extended HPV genotyping

or VIA for the triage of HPV-positive women in a 'screen-and-treat' approach was a secondary outcome.

### Statistical analysis

The initial sample size planned for this study was of 6000 women. Considering an HPV prevalence of approximately 20% in the target population among which approximately 10% have CIN2+, it was expected to obtain a total of 120 women with CIN2+. Anticipating a sensitivity to detect CIN2+ of 75% for VIA and 90% for extended genotyping, based on previous experience and preliminary analyses, calculations showed that 100 women with CIN2+ would be necessary to detect a difference in sensitivity with a confidence level of 95% and 80% power; thus, a sample size of 6000 women. However, the study had to be temporarily stopped due to the COVID-19 pandemic in March 2020, which is why this intermediate analysis was conducted with the data collected up to that time.

**Table 4** Performance of screening tests stratified by referral rates to treatment, histology results, overtreatment and number of treatments per  $\geq$ CIN2

Triage test	HPV-positive women* (trriage)	Referred for treatment	Histology <CIN2	Histology $\geq$ CIN2	Proportion of false positive triage†	Overtreatment rate (%; 95% CI)‡	Women treated per $\geq$ CIN2 diagnosed
HPV Pool 1	371	85/371 (22.9%)	68	17	68/85 (80.0%)	68/2093 (3.2%, 2.5 to 4.1)	5.0
HPV Pool 2 (extended genotyping)	361§*	247/361 (68.4%)	206	41	206/247 (83.4%)	206/2083 (9.9%, 8.6 to 11.3)	6.0
Visual assessment	371	220/371 (59.3%)	185	35	185/220 (84.1%)	185/2093 (8.8%, 7.7 to 10.1)	6.3
Cytology	357**¶	53/357 (14.8%)	20	33	20/53 (37.7%)	20/2079 (1.0%, 0.6 to 1.5)	1.6

\*Only cases with valid histological results are shown.

†Calculated as the proportion of participants triaged positively with no confirmed CIN2+ upon histopathological analysis.

‡Calculated as the proportion of women overtreated among all screened women, after excluding HPV-positive participants with no valid results for either the triage method or the histological diagnosis.

§10 results missing for HPV Pool 2.

¶14 missing or invalid cytological results.

CIN2, cervical intraepithelial neoplasia grade 2; HPV Pool 2 (extended genotyping), HPV 16, HPV 18/45, HPV 31/33/35/52/58.

Sensitivity, specificity, positive predictive value (PPV) and NPV were calculated as proportions with their associated 95% CIs. Student's t-test, Mann-Whitney test or Pearson's  $\chi^2$  test (as appropriate) were used to compare sociodemographic characteristics between HPV positive and negative women. McNemar's test for paired samples was used to compare sensitivity, specificity, PPV and NPV of different tests. All analyses were two-sided and p values less than 0.05 were considered as statistically significant. All analyses were carried out using the STATA software, V.16 (StataCorp, College Station, Texas, USA).

## RESULTS

### Study population

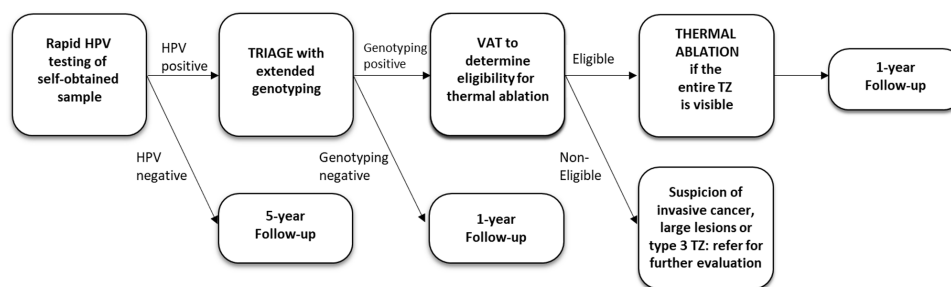
Overall, 2104 eligible women accepted to participate in the 3T-study between September 2018 and March 2020 and performed self-sampling for HPV testing. Three hundred and eighty-two (18.2%) were HPV-positive, among which 380 (99.5%) underwent VIA triage. All HPV-positive women had a Pap-test, cervical biopsies

and endocervical brushing (table 1) as quality control. Median age of screened women was 40 (IQR, 35–45) years old. Compared with HPV-negative women, HPV-positive participants were more frequently single than HPV-negative women (20.7% vs 13.6%,  $p < 0.001$ ). A majority of all women (70.3%) did not use contraception. A higher proportion of women reported living with HIV disease in the HPV-positive group (7.0%) than in the negative (2.5%,  $p < 0.001$ ).

### Accuracy of triage tests to detect CIN2+ and CIN3+

Histopathologic outcomes stratified by triage test results are described in table 2; 247 women (68.4%) were triaged as positive by extended genotyping (HPV pool\_2), 220 (59.3%) with VIA and 53 (14.8%) by cytology.

Extended genotyping (HPV pool\_2) had a statistically significantly higher sensitivity for CIN2+ (93.2%, CI: 81.3 to 98.6) and CIN3+ (96.7%, 95% CI: 82.8 to 99.9) detection as compared with the other triage tests, but came at the cost of a lower specificity for CIN2+ (35.0%, 95% CI:



**Figure 1** Integration of extended genotyping as triage test in the 3T strategy (same day Test-Triage-and-Treatment). VAT is used exclusively to assess eligibility for thermal ablation or referral for further evaluation. HPV, Human Papillomavirus; VAT, visual assessment for treatment; TZ, transformation zone.

29.8 to 40.5) and CIN3+ (34.1%, 95% CI: 29.0 to 39.5) (table 3).

The PPV of CIN 2+ and CIN3+ were not statistically different between extended genotyping (16.6%, 95% CI: 12.2 to 21.8; 11.7%–95% CI: 8 to 16.4) and visual assessment (15.9%, 95% CI: 11.3 to 21.4; 10.0%–95% CI: 6.4 to 14.7). Cytology had a higher specificity (93.6%, 95% CI: 90.3 to 96.1) and PPV (62.3%, 95% CI: 47.9 to 75.2) compared with extended genotyping ( $p < 0.001$ ).

### Comparison of performance between extended genotyping and visual assessment for immediate referral to treatment

We compared the overtreatment rate of extended genotyping and visual assessment as triage susceptible to be used in a test-triage-and-treat approach in a single visit. The results show that there is hardly no difference in the proportions of women overtreated after triage by extended genotyping and visual assessment (9.9%, CI: 8.6 to 11.3, and 8.8%, CI: 7.7 to 10.1, respectively) and in the ratio of women treated per CIN2+ diagnosed (6.0 and 6.3, respectively) (table 4).

### Study power

Using McNemar's test for paired samples, the final sample size (including 44 CIN2+) was powered at 57% to detect a difference at the  $\alpha = 0.05$  level between the obtained sensitivities for VIA and extended genotyping, and at 83% for the difference in sensitivity between cytology and extended genotyping. For the difference in specificity, the power of our sample size was of 57% for the comparison of VIA to extended genotyping, and of 100% for the comparison of cytology to extended genotyping.

## DISCUSSION

Using data from a population-based sample of women aged 30–49 years old, our study found that extended genotyping as a triage method of HPV-positive women offered higher sensitivity for CIN2+ detection than traditional the triage methods by VIA and cytology, at the cost of lower specificity.

As compared with VIA and cytology, genotyping has the advantage to be an objective method for triage obtained simultaneously with HPV testing without additional analysis, as genotyping is part of the standard output from the Xpert analysis and other commercial tests. Risk stratification of CIN2+ is related to individual HPV genotypes, and the subdivision of 14 high-risk genotypes used for primary screening in groups according to their risk assessment for precancer and cancer could be useful for triage and relevant clinical management of HPV-positive women.<sup>28</sup>

Using a pool of eight genotypes from the major oncogenic risk groups, our study demonstrated a sensitivity of 93.2% and 96.7% for the detection of CIN2+ and CIN3+ lesions, respectively. These values were significantly higher than those of visual assessment (79.5% for CIN2+ and 73.3% for CIN3+) and those of cytology (75.0% for CIN2+ and 83.3% for CIN3+). Triage by

extended genotyping failed to detect 3 CIN2+ lesions, whereas visual assessment and cytology failed to detect 9 and 11 CIN2+ lesions, respectively. However, the higher sensitivity of extended genotyping came at a cost in terms of specificity (35.0% for CIN2+ and 34.1% for CIN3+).

These results are consistent with previously published studies.<sup>13 14</sup> However, other studies carried out in various geographic regions using either the same PCR system and extended genotyping<sup>14</sup> or different PCR systems and different pools of genotypes<sup>16 17</sup> have shown higher specificities, but lower sensitivities. These findings suggest that the performance obtained by extended genotyping may vary across geographic regions. Furthermore, disease (CIN2+) prevalence,<sup>17</sup> genotype-specific prevalence,<sup>16</sup> HIV infection and prevalence<sup>29</sup> and probably other factors may affect specificity of triage testing. Among HIV-positive women, factors such as age, duration of antiretroviral therapy and CD4+ cell count could be associated with a decrease of HPV specificity.<sup>29</sup> HIV status and CD4+ cell count have also been shown to change the relative proportion of HPV genotypes compared with the general population.<sup>30</sup> Further research is needed in different geographic regions and populations to find combinations of genotypes that optimally provide information to stratify risk of CIN2+ disease, and to understand the causes that can affect diagnostic accuracy.

Undeniably, by offering an objective and practical tool using molecular technology for primary screening and triage in a single visit, it may be possible to extend high-quality prevention of cervical cancer to more Cameroonian women. In our study, triage with extended genotyping compared favourably to triage with visual assessment. Unlike VIA, extended genotyping is an effective method that does not depend on the examiner's expertise. Of note, in our study, VIA missed the diagnosis of two cancers out of three, whereas all three cancers were identified through extended genotyping.

In this context, decision-makers should weight the risk of unnecessary treatment and harm against the benefits of early detection and prompt treatment. Triage by extended genotyping has a low specificity but high sensitivity, which may be an appropriate and effective strategy in low-resource settings. Our study was performed in a resource-constrained region with underserved rural communities, where loss to follow-up is of high concern. The study showed that in the whole population screened, the referral rate to treatment was 12.1% (255/2104) and the overtreatment rate 9.9% (206/2083). These rates may be tolerated for settings with a reduced budget and risk of loss to follow-up, where women are screened once or twice in a lifetime.

Therefore, high sensitivity and low specificity of a triage test among HPV-positive women is suitable for low-resource settings as it maintains the sensitivity of primary HPV-based screening, thus reducing missed opportunities for diagnosis and allowing immediate treatment of CIN2+ disease. In our study, 93% (41/44) of CIN2+

lesions diagnosed would have received treatment if a triage strategy by extended genotyping had been adopted.

Of note, thermal ablation therapy used in our programme has proven to be affordable with a good cure performance<sup>31</sup> and adherence (>90%).<sup>18</sup> Women expressed well-tolerated temporary side effects such as minimal discomfort, lower abdominal cramping during or immediately after the procedure and watery vaginal discharge. No complications requiring hospitalisation occurred.<sup>32</sup>

Applied to our setting in Cameroon, a screening and triage algorithm with an acceptable risk level for CIN2+ could be designed as follows: (1) self-collected rapid HPV testing, (2) triage by extended genotyping, (3) treatment of all positive women on triage and (4) follow-up of all HPV-positive women at 1 year (figure 1). VIA would be used exclusively to assess eligibility for thermal ablation or referral for further evaluation. For the use of thermal ablation, the TZ should be entirely visible (TZ type 1 or 2) and there should be no suspicion of invasive cancer. Presently, an analysis is underway in Cameroon to assess feasibility and cost-effectiveness of this algorithm.

Strengths of this study are that it was conducted as would be in routine practice and with minimal exclusion criteria, therefore representing a large span of the West-Cameroonian female population for which cervical cancer screening is recommended. Furthermore, all HPV-positive women underwent cervical biopsy and ECB to minimise disease misclassification. One limitation is that as a single-site study, our results cannot be generalisable to populations with different sociodemographic characteristics, HPV and HIV prevalence. Further validation is therefore needed to verify if the pool of eight genotypes used in this study may be applied across other LMIC contexts. Another limitation is related to the small sample size, which warrants for these results to be confirmed on a larger scale. A randomised controlled study is currently underway with the objective of confirming these initial results (ClinicalTrials.gov ID NCT05385406). However, most of the results obtained in the study are statistically significant despite a smaller sample size than initially expected (p value<0.05 for every result except PPV and NPV of different triage tests for CIN2 and CIN3, table 3). As all women between 30 and 49 years of the general population of the health district were eligible for the study, no random sampling was performed. Therefore, the study sample is likely not completely representative of the general population in terms of sociodemographic characteristics such as educational level and professional status. However, efforts were made to recruit patients living in outlying areas with less access to care by deploying recruitment through community health workers and covering travel costs to the screening centre. Finally, our gold standard is based on cervical biopsies which could be performed in the wrong place, thus leading to missed diagnosis of CIN2+. Multiple biopsies could be more representative of true disease; however, the decision to sample only one biopsy at 6 o'clock in VIA-negative

women was made based on previous experience where multiple biopsies were not well tolerated by patients.

## CONCLUSION

Triage of HPV-positive women with extended genotyping obtained through self-sampling significantly improves the detection rate of CIN2+ lesions compared with VIA without a major loss of specificity. Genotyping offers the benefit of high sensitivity which may outweigh harm of overtreatment in a resource-constrained setting. A large-scale comparison of visual assessment and genotyping conducted in a low-resource setting is needed to provide more guidance on the optimal triage strategy.

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