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Performance of OncoE6™ Cervical Test in detecting cervical precancer lesions in HIV-positive women attending an HIV clinic in Bujumbura, Burundi: a cross-sectional study

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ABSTRACT

Objective New rapid and low-cost molecular tests for cervical cancer screening, such as the OncoE6 Cervical Test, are emerging and could be alternatives for low-income and middle-income countries. To this end, we evaluated the clinical performance of the OncoE6 Cervical Test in detecting cervical intraepithelial neoplasia (CIN) among HIV-infected women in Bujumbura, Burundi.

Methods From June to December 2017, a cross-sectional study was conducted in 680 HIV-positive women at the University Hospital. Women aged 25–65 years who declared having had vaginal intercourse were consecutively recruited, and cervical specimens for OncoE6, liquid-based cytology and human papillomavirus (HPV) genotyping were obtained and visual inspection with acetic acid performed. Thereafter, participants underwent a colposcopic examination. The sensitivity, specificity, and positive and negative predictive values of the different tests were calculated with reference to 'colposcopic-histological' diagnoses, and areas under the receiver operating curves of OncoE6 and cytology tests were compared.

Results The prevalence of CIN was 4.9%, and OncoE6 positivity was 3.1%. OncoE6 sensitivity varied from poor to low with increasing disease severity (42.1%, 95% CI 19.9% to 64.3% at CIN2+ threshold; and 58.3%, 95% CI 30.4% to 86.2% at CIN3+ threshold). OncoE6 had the highest specificity compared with all other tests used together. The performance of the OncoE6 test was significantly lower compared with cytology at atypical squamous cell of undetermined significance (ASCUS+) cut-off (AUC=0.68 vs 0.85, p=0.03) and low-grade squamous intraepithelial lesion (LSIL+) cut-off (AUC=0.68 vs 0.83, p=0.04) for CIN2+ diagnoses. However, the performance of the OncoE6 test was similar to that of cytology at high-grade squamous intraepithelial lesion (HSIL+) cut-off (AUC=0.68 vs 0.76; p=0.30) for CIN2+ diagnoses and was also similar to that of cytology at all cut-offs (ASCUS+, LSIL+ and HSIL+) for CIN3+ diagnoses (p1=0.76, p2=0.95 and p3=0.50, respectively).

Conclusion The current OncoE6 test proved to be a point-of-care test. However, given its poor performance for CIN2+ diagnoses, we do not recommend it for primary screening. We recommend to enrich it with more oncogenic HPV types, which may improve the performance of the test akin to that of cytology.

Strengths and limitations of this study

- ► This study is among the few studies evaluating the clinical performance of the OncoE6 Cervical Test in HIV-positive women, with a sufficiently powered sample size.
- ► Comprehensive efforts have been made to improve the precision of the parameter estimates by the use of a 'composite colposcopic-histological diagnosis', which is a reliable gold standard proxy.
- ► Women with normal-appearing cervix were not biopsied, which may have introduced a verification bias.

BACKGROUND

The causal relationship between infection with high-risk human papillomavirus (HR-HPV) and invasive cervical cancer (ICC) development is well established. Among the HR-HPV types, the 16 and 18 types cause more than 70% of all ICCs.[1] Worldwide, ICC represents the fourth most common cancer among women, accounting for an estimated incidence and mortality rates of 13.1 and 6.9 per 100 000 women, respectively.[2] In low-income and middle-income countries (LMICs), ICC is the second most common cancer among women, and this part of the world bears 87% of the ICC global burden.[2] In Burundi, cervical cancer is the most common cancer in women (age-standardised incidence and mortality rates of 57.4 and 50.3 per 100 000 women, respectively), with a high mortality to incidence ratio related to a very

low survival due to most women being diagnosed at a late stage.[3]

This high burden associated with HPV infection is more important in HIV-positive women since they bear an increased risk of HPV persistent infection,[4] a high frequency of precancerous lesions and a rapid progression from precancerous lesions to invasive cancer, compared with their HIV-negative counterparts.[5] As a corollary, HIV-infected women should be prioritised for cervical cancer control interventions.[6–8]

Currently available cervical cancer screening strategies are HPV-based, cytology-based and visual inspection-based (acetic acid (VIA) and/or Lugol's iodine (VILI)). Research has shown that HPV-DNA-based screening techniques are more effective tools for cervical cancer screening than the other two alternative techniques.[9–12] Current WHO recommendations for cervical cancer screening are HPV-based screening techniques where resources are available, whereas in low-resource settings WHO recommends VIA alone.[8 13] HPV-based and cytology-based screening techniques are hardly implementable in LMICs due to their high cost, infrastructure requirements and need for well-trained staff, all conditions that many LMICs, like Burundi, cannot satisfy.

The VIA screening modality is relatively less demanding since trained nurses can perform it with simple tools. However, this strategy has also several bottlenecks, including frequent training and supervision, along with its subjective interpretation, resulting in varying accuracy in different settings. Furthermore, there is evidence that increased inflammation among HIV-positive population affects the sensitivity and specificity of VIA.[14] Despite its feasibility, these limitations are also hard to overcome in LMICs, which may explain the current delay or lack of adoption by policymakers in Burundi.

Given that HPV-based screening tests are resource-prohibitive for LMICs, there are currently a number of rapid and cheaper molecular tests that could be used in LMICs, such as the OncoE6 Cervical Test. This molecular test does not require elaborate laboratory infrastructure or highly trained staff and is reproducible, thus making HPV-based screening test accessible even in resource-poor settings.

The OncoE6 Cervical Test is designed to detect elevated levels of E6 oncoproteins, which are disease-specific biomarkers required for epithelial cell transformation to occur.[15 16] Previous findings of the OncoE6 test in the general population revealed its high specificity and positive predictive value.[7 17–19] It was then hypothesised that the test could be useful to screen high-risk populations, including HIV-positive women. Nevertheless, notwithstanding its accuracy, this hypothesis has never been investigated, and large-scale evaluation among the highest risk groups, especially among HIV-infected women, is currently lacking. In view of the lack of access to cervical cancer screening that HIV-infected Burundian women face, the OncoE6 test may offer opportunities. To this end, we evaluated the clinical performance of the OncoE6 Cervical Test in detecting cervical intraepithelial neoplasia (CIN) among HIV-infected women in Bujumbura, Burundi.

METHODS

Study design and population

A cross-sectional study was conducted in an HIV-clinic located in the University Hospital, Roi Khaled, in Bujumbura, from June to December 2017. Participants were HIV-positive women of age between 25 and 65 years, able to provide informed consent and declared having had vaginal intercourse during their lives. Women were excluded if they were pregnant, less than 6 weeks post partum, had a history of hysterectomy or treatment for cervical cancer.

Enrolment visits and study-related procedures

Participants were given an education session about cervical cancer before the start of the study. A baseline questionnaire was administered to each participant to assess risk factors for cervical dysplasia and HPV infection. All women coming to the clinic for any reason were given an opportunity to participate in the study. Women were consecutively enrolled until the target sample size was reached. All participants underwent a physical examination of the pelvis. Women who reported or presented with abnormal vaginal discharge and lower pelvic pain were given syndromic sexually transmitted infection (STI) treatments following the WHO guidelines.[13] These women on STI presumptive treatment were asked to return 2 weeks after treatment for screening and colposcopy. Women with menses were also asked to come back 2 weeks later. The initial examination was performed by three nurses and a senior medical student in the last phase of residency who followed a 7-day training on VIA/VILI screening using the International Agency for Research on Cancer (IARC) training module.[20] In addition, they were trained on specimen collection for the OncoE6 Cervical Test and liquid-based cytology (LBC).

The examination started by evaluating, in naked eye, the cervix to identify its landmarks. During this clinical examination, cervical specimens were collected in the following order: OncoE6 in dry tubes, LBC in ThinPrep vials (for both cytology and HPV-DNA testing), followed by VIA. One minute after the application of 5% acetic acid, the squamocolumnar junction (SCJ) was re-evaluated using a lamp as source of light. We considered that a woman had a positive VIA result in case of a definite acetowhite lesion touching or abutting the SCJ, or if the entire cervix or a growth on the cervix turned acetowhite, as per the IARC guidelines.[20]

After sample collection, each participant underwent a standardised non-invasive colposcopy examination by two trained medical doctors. Colposcopic diagnoses were recorded using the modified Reid Colposcopic Index.[21] Colposcopic evaluation included naked eye inspection, and reapplication of acetic acid, green filter and Lugol's iodine. An endocervical speculum was used to assist in

visualising the endocervical canal if the entire SCJ was not seen. If, despite the use of endocervical speculum, the SCJ was not entirely seen, the colposcopy was considered unsatisfactory.

Cervical punch biopsies for histology confirmation were performed as clinically indicated on consenting participants with evidence of colposcopic cervical abnormalities, as dictated by the ethics committee. Results were reported as per the Richart CIN staging system,[22] in five categories of increasing disease severity: normal, CIN1, CIN2, CIN3 and ICC. Biopsy specimens were stored in 10% buffered formalin at room temperature until analysis. Cytology and histology reading, as well as Riatol quantitative PCR (qPCR) HPV genotyping (a clinically validated, laboratory-developed test which amplifies 18 HPV types: HPV 6E6, 11E6, 16E7, 18E7, 31E6, 33E6, 35E6, 39E7, 45E7, 51E7, 52E7, 53E6, 56E7, 58E7, 59E7, 66E6, 67L1 and 68E7),[23] were carried out at the Laboratory of Molecular Pathology, AML, Antwerp, Belgium, by a cytologist and a pathologist blinded to colposcopic findings, and OncoE6 and VIA results. Cytology results were reported according to the Bethesda 2014 guidelines. The OncoE6 test was run at the recruitment site by one trained investigator (ZN, a medical doctor).

Considerations about sample size determination

In order to have 80% power to prove that the sensitivity of the OncoE6 Cervical Test was not inferior to that of a cytology screening test at a significance level of 0.05, we needed to include at least 674 HIV-positive women. We assumed that the sensitivity of the LBC was 0.80 at atypical squamous cell of undetermined significance (ASCUS) cut-off and 0.55 for the OncoE6 Cervical Test. A non-inferiority margin of 0.05 was taken into account.

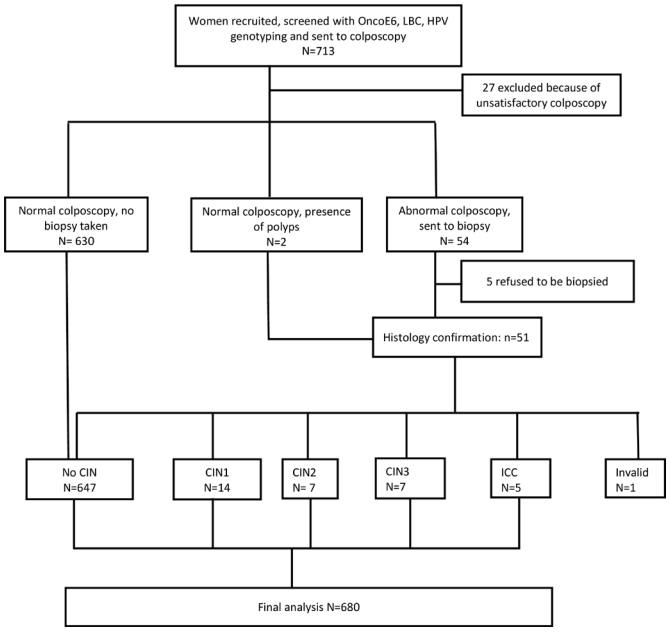

Figure 1 Flow diagram of participants. CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; ICC, invasive cervical cancer; LBC, liquid-based cytology.

Statistical analysis

The five-level categories of Richart's classification was collapsed into a four-level ordinal outcome variable as follows: no CIN, CIN1, CIN2 and CIN3+. Summary statistics were generated for basic sociodemographic and clinical information. Clinical performance of OncoE6, VIA and LBC (at ASCUS+, low-grade squamous intraepithelial lesion (LSIL+) and high-grade squamous intraepithelial lesion (HSIL+) thresholds) in predicting CIN2+ or CIN3+ diagnoses was evaluated using sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), with their respective 95% CI. Values of sensitivity, specificity, PPV and NPV between 0% and ≤40% were considered as poor, between >40% and ≤60% as low, between >60% and ≤80% as moderate, and >80% as high.

Furthermore, the performance of the OncoE6 test was compared with the performance of LBC at ASCUS+, LSIL+ and HSIL+ cut-offs, respectively, by exploring the differences in areas under their respective receiver operating curves. To this end, we used the Stata command *'roccomp status mod1 mod2, graph summary'*, with status being the gold standard, mod1 being test 1 and mod2 being test 2. The differences in areas under the curves were considered statistically significant at a p value <0.05. Our gold standard was based on (1) histopathological results for women in whom invasive procedures (punch biopsies) were performed and (2) diagnostic colposcopy results in women who had no clinical indication for undergoing invasive procedures.

Secondary analyses were performed to determine the performance of an algorithm composed of the Riatol HPV test followed by the OncoE6 Cervical Test, for women who tested positive with the Riatol HPV test, in detecting cervical lesions among HIV-positive women. Women with inconclusive colposcopic assessments (ie, unsatisfactory colposcopy), women who were eligible for histological confirmation but did not undergo biopsy procedures and one woman with invalid histopathology result were excluded from the analysis. All the analyses were carried out using the Stata V.15 software.

Patient and public involvement

Patients were not involved in the conception phase of the study. However, they were involved during the recruitment phase by explaining to other women how they experienced the sample collection processes, and this contributed to reassurance among other participants. Each participant received the results of the screening tests and colposcopy findings and was advised on the screening periodicity. The authors will disseminate the findings via conference presentations and by submitting the manuscript to journals for publication.

RESULTS

Figure 1 presents the flow chart of study participants.

Sociodemographic characteristics of study participants

Table 1 presents the sociodemographic characteristics along with screening and colposcopic results of

Table 1 Distribution of sociodemographic characteristics as per the final disease outcome (final composite colposcopic-histological diagnosis) among HIV-infected women in Bujumbura, Burundi, 2018

Variable	Overall* n (%)	Normal n (%)	CIN1 n (%)	CIN2 n (%)	CIN3/ICC n (%)
Overall	680 (100)	647 (95.1)	14 (2.1)	7 (1.0)	12 (1.8)
Age					
Median (Q1, Q3)	44 (37, 52)	44 (38, 52)	37.5 (32, 42)	41 (40, 49)	43 (37.5, 51.5)
Education					
No education	114 (16.8)	101 (16.5)	3 (21.4)	0 (0.0)	4 (33.3)
Went to primary school	361 (53.1)	346 (53.5)	7 (50.0)	3 (42.9)	5 (41.7)
Went to high school	195 (28.7)	184 (28.4)	4 (28.6)	4 (57.1)	3 (25.0)
Went to higher school	10 (1.5)	10 (1.6)	0 (0.0)	0 (0.0)	0 (0.0)
Marital status					
Married/cohabiting	286 (42.1)	271 (41.9)	7 (50.0)	5 (71.4)	3 (25.0)
Single	25 (3.7)	23 (3.6)	1 (7.1)	0 (0.0)	1 (8.3)
Divorced	122 (17.9)	115 (17.8)	3 (21.4)	1 (14.3)	3 (25.0)
Widowed	247 (36.3)	238 (36.8)	3 (21.4)	1 (14.3)	5 (41.7)
Tobacco use					
Yes	27 (4.0)	26 (4.0)	0 (0.0)	0 (0.0)	1 (8.3)
Age at first sexual intercourse (n=679)					
Median (Q1, Q3)	18 (16, 20)	18 (16, 20)	17.5 (15, 20)	18 (16, 20)	18 (15, 20)
Lifetime sexual partners (n=679)					
1	140 (20.6)	136 (21.1)	3 (21.4)	1 (14.3)	0 (O.0)
2+	539 (79.4)	510 (78.9)	11 (78.6)	6 (85.7)	12 (100.0)
Median (Q1, Q3)	3 (2, 4)	3 (2, 4)	3.5 (2, 4)	3 (2, 5)	4 (2, 5)
Sexual partners in the previous 6 months (n=679)					
0	291 (42.9)	278 (43.0)	5 (35.7)	1 (14.3)	7 (58.3)
1	351 (51.7)	333 (51.6)	8 (57.1)	6 (85.7)	4 (33.3)
2+	37 (5.4)	35 (5.4)	1 (7.1)	0 (0.0)	1 (8.3)
Median (Q1, Q3)	1 (0, 1)	1 (0, 1)	1 (0, 1)	1 (1, 1)	0 (0, 1)
Gestity					
Median (Q1, Q3)	4 (3, 6)	4 (3, 6)	3.5 (2, 5)	4 (3, 7)	5 (2.5, 6)
Parity					
Median (Q1, Q3)	4 (2, 5)	4 (2, 5)	3.5 (2, 5)	4 (2, 7)	4 (2.5, 5)
Profession					
Employed/professional	319 (46.9)	307 (47.4)	7 (50.0)	1 (14.3)	4 (33.3)
Unemployed/housewife	361 (53.1)	340 (52.6)	7 (50.0)	6 (85.7)	8 (66.7)
Menarche					
Median (Q1, Q3)	14 (13, 15)	14 (13, 15)	15 (13, 15)	16 (14, 16)	14.5 (13, 16)
Age of marriage (n=654)					
Median (Q1, Q3)	19.5 (17, 24)	19 (17, 24)	21 (20, 25)	20 (18, 25)	20.5 (18, 24)
Age at first pregnancy (n=660)					
Median (Q1, Q3)	19 (17, 23)	19 (17, 23)	20 (18, 22)	20 (18, 25)	20 (18.5, 21.5)
Contraceptive use (>5 years)					
Yes	17 (2.5)	15 (2.3)	0 (0.0)	0 (0.0)	2 (16.7)
Alcohol consumption					
Yes	264 (38.8)	255 (39.4)	6 (42.9)	1 (14.3)	2 (16.7)

Continued

Table 1 Continued

Variable	Overall* n (%)	Normal n (%)	CIN1 n (%)	CIN2 n (%)	CIN3/ICC n (%)
Result of visual inspection with 5% acetic acid					
Positive	38 (5.6)	11 (1.7)	11 (78.6)	7 (100.0)	9 (75.0)
Colposcopy result					
Positive	51 (7.5)	17 (2.6)	14 (100.0)	7 (100.0)	12 (100.0)
OncoE6 results (n=679)					
Positive	21 (3.1)	11 (1.7)	2 (15.4)	1 (14.3)	7 (58.3)
Cytology results (n=670)					
Normal	543 (81.0)	538 (84.5)	2 (14.3)	0 (0.0)	3 (25.0)
ASCUS	14 (2.1)	12 (1.9)	1 (7.1)	0 (0.0)	1 (8.3)
AGC	3 (0.4)	3 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)
LSIL	55 (8.2)	40 (6.3)	11 (78.6)	2 (28.6)	2 (16.7)
HSIL	25 (3.7)	17 (2.7)	0 (0.0)	3 (42.9)	5 (41.7)
ASC-H	1 (0.2)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)
ICC	4 (0.6)	2 (0.3)	0 (0.0)	2 (28.6)	0 (0.0)
Invalid	25 (3.7)	24 (3.8)	0 (0.0)	0 (0.0)	1 (8.3)
HPV result (n=670)					
Positive	256 (38.2)	224 (35.2)	13 (92.9)	7 (100.0)	12 (100.0)

*Excluding women with unsatisfactory colposcopy (n=27), women who refused/failed to be biopsied (n=5) and a woman with invalid histology result (n=1).
AGC, atypical glandular cell; ASC-H, atypical squamous cells cannot exclude high-grade lesion; ASCUS, atypical squamous cell of undetermined significance; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; ICC, invasive cervical cancer; LSIL, low-grade squamous intraepithelial lesion; Q1, 25th percentile; Q3, 75th percentile.

the participants. A total of 713 women participated in the study, of whom 33 participants were excluded from the analyses (27 had unsatisfactory colposcopy, 5 had abnormal colposcopy and refused/failed to come for biopsy, and 1 woman had invalid histopathology result), resulting in 680 women being included in the final analysis. The median age of our participants was 44 years (IQR: 37–52). Majority of the participants (316 women, 53.1%) went to primary school. Of the women, 286 (42.1%) were married or cohabiting, 247 (36.3%) were widowed and 122 (17.9%) were divorced.

The median age at first intercourse was 18 years old (IQR: 16–20); the median number of lifetime sexual partners was 3 (IQR: 2–4), and the median number of sexual partners in the last 6 months was 1 (IQR: 0–1). Majority of the participants (361, 53.1%) were employed or had a professional activity.

Prevalence of cervical cancer lesions and screening test results

Colposcopy was performed in all 713 participants and displayed abnormal results in 56 women. Biopsies for histopathology diagnosis were taken from 51 patients. Hence, colposcopy results served as a final diagnosis in 630 women, while histopathology results served as a final diagnosis in 50 women. Among the 680 participants included in the analysis, 14 women (2.1%) had CIN1 lesions, 7 (1%) had CIN2 lesions and 12 (1.8%) had CIN3/ICC lesions. With VIA, 38 women (5.6%) had a positive result. With the OncoE6 test, 21 women (3.1%) had a positive result. Positivity rates were 15.2% and 38.2% for cytology and HPV-DNA test (Riatol qPCR), respectively.

Clinical performance of the tests used at different thresholds

The clinical performances of different screening tests are presented in table 2. At CIN2+ threshold, OncoE6 had sensitivity and specificity of 42.1% (95% CI 19.9 to 64.3) and 98% (95% CI 97 to 99.1), respectively, with a good PPV. VIA displayed both high sensitivity and high specificity, with the highest PPV. The HPV-DNA test displayed the highest sensitivity, with the lowest specificity and PPV.

In this report, we focus on the performance of the OncoE6 Cervical Test compared with cytology; other comparisons will be presented in further reports. The OncoE6 test had a lower performance in diagnosing CIN2+ lesions, compared with cytology at ASCUS+ and LSIL+ cut-offs (AUC=0.68 vs 0.85, p=0.03 and AUC=0.68 vs 0.83, p=0.04, respectively (Area Under the Curve, AUC)) (see online supplementary figure 1). However, the performance of OncoE6 test was as good as cytology at HSIL+ cut-off (AUC=0.68 vs 0.76, p=0.30).

At CIN3+ threshold, OncoE6 had both the highest specificity and PPV. VIA also had high specificity. The sensitivity and specificity for cytology were 45.5% (95% CI

Table 2 Clinical performance of different screening tests at CIN2+ and CIN3+ thresholds among HIV-infected women in Burundi

	n	TP	FP	TN	FN	SE, % (95% CI)	SP, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
Disease-positive threshold 'CIN2+ on Colposcopic-histopathological results'									
OncoE6 (HPV 16 and 18)	679	8	13	647	11	42.1 (19.9 to 64.3)	98.0 (97.0 to 99.1)	38.1 (17.3 to 58.9)	98.3 (97.3 to 99.3)
HPV	670	19	237	414	0	100.0 (100 to 100)	63.6 (59.9 to 67.3)	7.4 (4.2 to 10.6)	100.0 (100 to 100)
VIA	680	16	22	639	3	84.2 (67.8 to 100.0)	96.7 (95.3 to 98.0)	42.1 (26.4 to 57.8)	99.5 (99.0 to 100.0)
Cytology ASCUS+	645	15	87	540	3	83.3 (66.1 to 100.0)	86.1 (83.4 to 88.8)	14.7 (7.8 to 21.6)	99.4 (98.8 to 100.1)
Cytology LSIL+ cut-off	645	14	71	556	4	77.8 (58.6 to 97.0)	88.7 (86.2 to 91.2)	16.5 (8.6 to 24.4)	99.3 (98.6 to 100)
Cytology HSIL+ cut-off	645	10	20	607	8	55.6 (32.6 to 78.5)	96.8 (95.4 to 98.2)	33.3 (16.5 to 50.2)	98.7 (97.8 to 99.6)
Disease-positive threshold 'CIN3+ Colposcopic-histopathological results'									
OncoE6 (HPV 16 and 18)	679	7	14	653	5	58.3 (30.4 to 86.2)	97.9 (96.8 to 99.0)	33.3 (13.2 to 53.5)	99.2 (98.6 to 99.9)
HPV	670	12	244	414	0	100.0 (100 to 100)	62.9 (59.2 to 66.6)	4.7 (2.1 to 7.3)	100.0 (100 to 100)
VIA	680	9	29	639	3	75.0 (50.5 to 99.5)	95.7 (94.1 to 97.2)	23.7 (10.2 to 37.2)	99.5 (99.0 to 100.1)
Cytology ASCUS+	645	8	94	540	3	72.7 (46.4 to 99.0)	85.2 (82.4 to 87.9)	7.8 (2.6 to 13.1)	99.4 (98.8 to 100.0)
Cytology LSIL+ cut-off	645	7	78	556	4	63.6 (35.2 to 92.1)	87.7 (85.1 to 90.3)	8.2 (2.4 to 14.1)	99.3 (98.6 to 100.0)
Cytology HSIL+ cut-off	645	5	25	609	6	45.5 (16.0 to 74.9)	96.1 (94.5 to 97.6)	16.7 (3.3 to 30.0)	99.0 (98.2 to 99.8)

ASCUS+ cut-off includes ASCUS, AGC, LSIL, HSIL, ASC-H, adenocarcinoma and SCC lesions; LSIL+ cut-off includes LSIL, ASC-H, HSIL, adenocarcinoma and SCC lesions; HSIL+ cut-off includes HSIL, ASC-H, adenocarcinoma and SCC lesions.
AGC, atypical glandular cell; ASC-H, atypical squamous cells cannot exclude high-grade lesion;ASCUS, atypical squamous cell of undetermined significance;CIN, cervical intraepithelial neoplasia; FN, false negative;FP, false positive; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; NPV, negative predictive value; PPV, positive predictive value; SCC, squamous cell carcinoma; SE, sensitivity;SP, specificity; TN, true negative; TP, true positive; VIA, visual inspection with acetic acid.

16 to 74.9) and 96.1% (95% CI 94.5 to 97.6), respectively, and had the lowest PPV.

The performance of the OncoE6 test was not significantly different from that of cytology at ASCUS+, LSIL+ and HSIL+ cut-offs in diagnosing CIN3+ lesions (p1=0.76, p2=0.95 and p3=0.50, respectively).

Clinical performance of the algorithm HPV+, followed by OncoE6 or VIA test

A screening algorithm consisting of a first screening test using the Riatol qPCR HPV test and a second test (OncoE6 test or VIA) only for women who tested HPV-positive on the first test, to identify CIN2+ or CIN3+ lesions, suggests that the clinical performance of OncoE6 or VIA did not improve (table 3).

DISCUSSION

Our report shows a proportion of cervical intraepithelial lesions of 4.9% in this never-screened, HIV-positive Burundian women population. To our knowledge, this study is the first report from Burundi attempting to provide accurate data on the prevalence of cervical precancer lesions confirmed by colposcopy and/or histopathology among HIV-positive women. All study participants received standardised colposcopy evaluation, and biopsies for histopathology confirmation were taken when clinically recommended.[24–26]

Clinical implications of the results

In this study, the performance of OncoE6 in predicting CIN2+ and CIN3+ diagnoses was evaluated. The sensitivity of OncoE6 in detecting CIN lesions increased with increasing disease severity—42.1% and 58.3% for CIN2+ and CIN3+, respectively—and corroborates previous findings.[7 17] A major drawback of this test's low sensitivity is its inability to detect a high proportion of cervical precancerous lesions that, if treated, would have resulted in reduced incidence and mortality related to ICC. Adhering to the WHO recommendations[13] to treat HIV-positive women with CIN2+ lesions, the performance of the OncoE6 test was significantly lower compared with cytology at ASCUS+ cut-off.

This low sensitivity may be due to the fact that a substantial proportion of cervical lesions among these HIV-infected women were caused by HR-HPV types other than HPV 16/18 included in the current version of the test. In fact, only 12 women out of 19 with histologically confirmed CIN2+ lesions (63.2%) were HPV 16-positive or HPV 18-positive (online supplementary table 1). This implies that at least 37% of all CIN2+ were associated with HR-HPV types other than the HPV 16 and 18 types.

Another hypothesis of the low sensitivity of the OncoE6 Cervical Test may be the possibility of HPV-related lesions with a lower progressive potential being missed. This may be due to the ability of the E6 oncoprotein to biologically differentiate between HPV-related lesions with a high progressive potential (thus, testing OncoE6-positive) and

Table 3 Clinical performance of the algorithm HPV+ test followed by OncoE6 or VIA test

	n	TP	FP	TN	FN	SE, % (95% CI)	SP, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
Disease-positive 'CIN2+' threshold									
OncoE6	255	8	13	223	11	42.1 (19.9 to 64.3)	94.5 (91.6 to 97.4)	38.1 (17.3 to 58.9)	95.3 (92.6 to 98.0)
VIA	256	16	13	224	3	84.2 (67.8 to 100.0)	94.5 (91.6 to 97.4)	55.2 (37.1 to 73.3)	98.7 (97.2 to 100.0)
Disease-positive 'CIN3+' threshold									
OncoE6	255	7	14	229	5	58.3 (30.4 to 86.2)	94.2 (91.3 to 97.2)	33.3 (13.2 to 53.5)	97.9 (96.0 to 99.7)
VIA	256	9	20	224	3	75.0 (50.5 to 99.5)	91.8 (88.4 to 95.2)	31.0 (14.2 to 47.9)	98.7 (97.2 to 100.0)

OncoE6 Cervical Test includes the HPV 16 and 18 types.
CIN, cervical intraepithelial neoplasia; FN, false negative; FP, false positive;HPV, human papillomavirus; NPV, negative predictive value; PPV, positive predictive value; SE, sensitivity; SP, specificity; TN, true negative;TP, true positive; VIA, visual inspection with acetic acid.

lesions with a lower progressive potential (resulting in OncoE6-negative), as has been reported by Valdez *et al*.[18] Nevertheless, our cross-sectional study design precludes us from exploring this hypothesis. An alternative explanation of the low sensitivity of the OncoE6 test could be the potential mutations in the HPV 16 and 18 E6 DNA sequence, which alter the E6 oncoprotein binding to the anti-E6 specific monoclonal antibodies applied in the OncoE6 test, as documented by Krings *et al*.[27] This could explain why the Riatol HPV test was in some samples positive for HPV 16 and 18, and the OncoE6 test being negative for the same samples. However, this hypothesis cannot be verified with our data.

Among the 12 women with 16/18-related CIN2+ lesions, 8 (66.7%) were OncoE6-positive. Our analyses show there were 17 cases of CIN2+ lesions related to HR-HPV types included in the nine-avalent vaccine (ie, 16/18/31/33/45/52/58) were 17 cases. An improved OncoE6 test, including all these HR-HPV strains, with the same detection rate as the currently available test would have detected at least 12 cases. As a corollary, an improved OncoE6 test would have a sensitivity of 63.2% (12 of 19) in identifying CIN2+ lesions, which is higher than that of the current OncoE6 test.

On the other hand, we noted that the OncoE6 test had the highest specificity and PPV, compared with the HPV-DNA and cytology at all cut-offs in detecting CIN2+ and CIN3+ lesions. This finding was similar to other OncoE6 validation data published in other settings[7 17] and suggests that OncoE6 may be more useful than HPV and cytology in detecting CIN2+ lesions. Given this attribute, Zhao *et al*[17] have suggested that OncoE6 could be useful for screening high-risk populations, including HIV-infected women among others. However, our analyses highlight the limitations of the test when used as a primary screening test for cervical precancer lesions in this high-risk population of HIV-infected women. Moreover, the use of OncoE6 as a triage test for HPV-positive women did not result in an improved sensitivity of the test.

At CIN3+ threshold, nearly 60% of clinically important disease (CIN3+) could be identified with OncoE6. The study by Qiao *et al*[19] also found similar values of specificity and sensitivity of OncoE6 in identifying CIN3+ cervical lesions. At different cytology cut-offs, OncoE6 was performing at least as well as cytology. Among the 12 CIN3+ cases, 75% were HPV 16/18-related, of which the OncoE6 test was positive in 66.7% (6 of 9), as it was also for CIN2+ diagnosis. Of all CIN3+ cases, 91.7% (11 of 12) were related to HR-HPV types included in the nine-valent vaccine, which implies that 8 CIN3+ lesions would have been detected, resulting in a sensitivity of 66.7%.

This implies that given the barriers to implementing a cytology-based screening programme, an improved OncoE6 Cervical Test with more HPV strains may be an option to be considered by test developers due to it being amenable to a see-and-treat approach.

The current version of OncoE6 test includes 16/18 HPV types and has a lower clinical performance, compared with cytology at ASCUS+ cut-off, for CIN2+ diagnoses.

In our analysis, VIA proved to have both high sensitivity and high specificity for CIN2+ diagnoses, along with the highest PPV. This corroborates the WHO recommendations[8 13] to use VIA in 'a screen and treat' approach in resource-constrained settings, for cervical cancer screening, even just once in a woman's lifetime.

In the context of the absence of a screening strategy, as it is currently the case for Burundi, any investment to be made for cervical cancer screening should first consider visual inspection-based strategy as the most feasible. The OncoE6 test might be justifiable as a triage test for screened positive women given its highest specificity and ease of use. However, cost-effectiveness analyses are also needed to further elucidate the suitability of this strategy.

Strengths and limitations

Our study was conducted in a pragmatic setting, where OncoE6 Cervical Test proved to be a point-of-care test. Another strength is that all women underwent colposcopic evaluation, although colposcopy is not univocally considered as gold standard and has limitations in identifying all CIN2+ lesions.[28] However, we acknowledge an important limitation of this study. Women who had no visible lesions at colposcopy could not be biopsied, and therefore we may have overestimated the clinical estimated parameters of the test evaluated, as has been previously documented.[19 28] However, some authors have also raised concerns related to biopsies on normal-appearing cervix to ascertain the status of the cervix.[29] While balancing ethical concerns and conforming to the best clinical practices, our approach represented a comprehensive effort for improving the precision of our parameter estimates, and thus our 'composite colposcopic-histological diagnosis' was a reliable proxy as gold standard. The lower performance of the test in identifying CIN2+ lesions makes it inappropriate for primary screening of cervical precancerous lesions.

CONCLUSIONS

This study focused on the field use of the currently available OncoE6 Cervical Test, and displayed low sensitivity, high specificity and high PPV for CIN2+ diagnoses. Given its low sensitivity and poor performance in identifying CIN2+ lesions, we do not recommend the OncoE6 Cervical Test for primary screening. We highlighted the need for an OncoE6™ test to incorporate a wide range of HR-HPV strains, which would result in a good test performance for primary cervical cancer screening with less stringent equipment and personnel requirements. In the meantime, and in the absence of other alternatives for cervical cancer screening, low-income countries should first consider implementing a visual inspection-based strategy as recommended by WHO. Taking into account the Burundian context, appropriate screening strategies

for Burundi and other LMICs will undoubtedly differ from strategies appropriate for a high-income country.

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