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Clinical performance validation of four point-of-care cervical cancer screening tests in HIV-infected women in Zambia

Carla J. Chibwesha, MD[1,2], **Brigitte Frett, MSW**[2], **Katundu Katundu, MSc**[2], **Allen C. Bateman, PhD**[1,2], **Aaron Shibemba, MMed**[3], **Sharon Kapambwe, MBChB**[2], **Mulindi H. Mwanahamuntu, MMed**[2,4], **Susan Banda**[2], **Chalwa Hamusimbi**[2], **Pascal Polepole, MSc**[5], and **Groesbeck P. Parham, MD**[1,4]

[1]Division of Global Women's Health, Department of Obstetrics and Gynecology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

[2]Centre for Infectious Disease Research in Zambia, Lusaka, Lusaka, Zambia

[3]Department of Pathology, Cancer Diseases Hospital, Lusaka, Lusaka, Zambia

[4]Department of Obstetrics and Gynaecology, University Teaching Hospital, Lusaka, Lusaka, Zambia

[5]Department of Department of Biomedical Sciences, School of Medicine, University of Zambia, Lusaka, Lusaka, Zambia

Abstract

Objectives—We sought to determine the clinical performance of visual inspection with acetic acid (VIA), digital cervicography (DC), Xpert HPV, and OncoE6 for cervical cancer screening in an HIV-infected population.

Methods—HIV-infected women 18 years and older were included in this cross-sectional validation study conducted in Lusaka, Zambia. The screening tests were compared to a histological gold standard. We calculated sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios, and odds ratios using cervical intra-epithelial neoplasia grade 2 or worse (CIN2+) and grade 3 or worse (CIN3+) thresholds.

Results—Between January and June 2015, 200 women were enrolled. 15% were screen positive by VIA, 20% by DC, 47% by Xpert HPV, and 6% by OncoE6. Using a CIN2+ threshold, the sensitivity and specificity of VIA was 48% (95% confidence interval [CI]: 30-67%) and 92% (95% CI: 86-95%), respectively. Similarly, the sensitivity and specificity of DC was 59% (95% CI: 41-76%) and 88% (95% CI: 82-93%). The sensitivity and specificity of Xpert HPV was 88%

Corresponding author / requests for reprints: Carla J. Chibwesha, MD, Division of Global Women's Health, Department of Obstetrics and Gynecology, University of North Carolina at Chapel Hill, 3009 Old Clinic Building, Chapel Hill, NC 27599-7577, Tel: 919-962-0760, Fax: 919-966-6049Carla_Chibwesha@med.unc.edu.

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(95% CI: 71-97%), and 60% (95% CI: 52-68%). Finally, the sensitivity and specificity of OncoE6 was 31% (95% CI: 16-50%) and 99% (95% CI: 97-100%).

Conclusions—VIA and DC displayed moderate sensitivity and high specificity. Xpert HPV performed equivalently to currently approved HPV DNA tests, with high sensitivity and moderate specificity. OncoE6 displayed excellent specificity but low sensitivity. These results confirm an important role for VIA, DC, and Xpert HPV in screen-and-treat cervical cancer prevention in low- and middle-income countries, such as Zambia.

Keywords

Cervical cancer screening; HIV; clinical performance validation; visual screening; molecular screening; low- and middle-income countries

Introduction

Invasive cervical cancer (ICC) is responsible for much of the cancer-related morbidity and mortality among women worldwide, with approximately 85% of the disease burden occurring in low- and middle-income countries (LMICs).[1,2] HIV-infected women are at increased risk of persistent high-risk HPV (hrHPV) infection and cervical cancer precursors, as well as a more rapid progression from cancer precursors to ICC.[3,4]

The vast majority of cervical cancers can be prevented by vaccination against HPV, the principal cause of cervical cancer, and by organized, population-based cervical cancer screening.[5] Comprehensive national programs to promote and provide HPV vaccination and cervical cancer screening are urgently needed in sub-Sarahan Africa, Southeast Asia, and Latin America. Evaluation of cost-effective *and* scalable cervical cancer screening and treatment modalities is also of central importance in settings of high disease burden, including in Zambia where the age-standardized incidence of cervical cancer is above 30 per 100,000[6] and the prevalence of HIV among adults aged 15-49 years is 13%.[7]

The cost-effectiveness and scalability of visual inspection with acetic acid (VIA) have previously been established in various LMIC settings.[5,8-10] However, the test's sensitivity is moderate.[11] Molecular cervical cancer screening – based on the detection of HPV DNA, RNA, or oncoproteins – has the potential to overcome some of the shortcomings of VIA. While molecular testing was once thought to be cost and resource prohibitive for LMICs, newer tests are cheaper, available for use at the point of care, and vastly simplified. These advancements enable molecular tests to be more easily integrated into screening algorithms in a wide range of settings.

We included 2 such molecular tests in our validation study: Xpert HPV and OncoE6. Xpert HPV is a rapid HPV DNA test run on the Cepheid GeneXpert platform, and has a turn-around-time (TAT) of ∼ 60 minutes. The Arbor Vita OncoE6 test, which detects the presence of the HPV E6 oncoprotein, a protein upregulated in ICC and cancer precursors, has a TAT of ∼ 90 minutes. The objective of our study was to determine the clinical performance (i.e., sensitivity, specificity, positive predictive value, and negative predictive value) of VIA, digital cervicography (DC), Xpert HPV, and OncoE6 for cervical cancer

screening in HIV-infected women. The screening tests were compared to a histological gold standard using both cervical intra-epithelial neoplasia grade 2 or worse (CIN2+) and grade 3 or worse (CIN3+) thresholds.

Methods

Study design and setting

We conducted a cross-sectional clinical performance validation of 4 cervical cancer screening tests: 2 visual and 2 molecular. Our study clinic was co-located with a Cervical Cancer Prevention Program in Zambia (CCPPZ) clinic on the campus of the University Teaching Hospital in Lusaka, Zambia. Since its inception in 2006, CCPPZ has implemented cervical cancer screening based on a “see-and-treat” approach, where women undergo visual screening, followed by immediate ablation with cryotherapy or cold coagulation (thermocoagulation), or referral for biopsy. CCPPZ has screened over 300,000 women through see-and-treat services provided within the public clinics and is integrated within the PEPFAR-supported HIV care and treatment infrastructure.[12,13] Trained nurses apply acetic acid to the cervix (VIA), looking with the naked eye for acetowhite changes and vascular abnormalities. In our setting, VIA is routinely augmented with DC, which uses digital photographs captured with a commercial brand camera for magnification, patient education, telemedicine support, and quality assurance.[14-16]

Study population and procedures

Women attending the CCPPZ screening clinic were invited to participate in our study. HIV-infected women 18 years and older were eligible for inclusion. Pregnant women were excluded. After written informed consent, demographic and clinical information was collected on standardized case report forms. All participants then underwent pelvic examination with cervical samples for Xpert HPV (GeneXpert, Cepheid, 2014, Sunnyvale, CA, USA) and OncoE6 (Arbor Vita, 2013, Fremont, CA, USA) collected using a cytobroom and placed in ThinPrep solution (Hologic, 2005, Marlborough, MA, USA). Thinprep collection was followed by VIA and then DC, after which all participants underwent two-quadrant cervical biopsies to obtain tissue specimens for histology.[17] For those with acetowhite changes, cervical biopsies were obtained from areas of the transformation zone that appeared abnormal (i.e., the worst-appearing lesions). For women with no acetowhite changes, biopsies were obtained from 6 and 12 o'clock. Biopsy specimens were placed in 10% buffered formalin. Finally, blood samples were collected for HIV plasma viral load and CD4+ count testing.

VIA and DC results were communicated to participants during the initial study visit. Women returned 2-4 weeks after the initial visit to receive their histology results, HIV viral load, and CD4+ count, as well as additional treatment recommendations. (The results of the Xpert HPV and OncoE6 tests were not communicated to participants, nor were they incorporated into recommendations for clinical management, as neither test has been approved for clinical use in Zambia.) In accordance with CCPPZ clinical guidelines, those diagnosed with CIN1+ were referred to the CCPPZ clinic for further management of CIN, with either ablative or excisional treatment, or to the University Teaching Hospital or Cancer Diseases Hospital for

further management of ICC. Women with no intra-epithelial lesion or malignancy (NILM) seen on histology were counseled to return for routine screening in 3 years. With the woman's permission, HIV viral load and CD4+ cell count results were also communicated to her HIV treatment clinic.

Laboratory testing

Cervical Thinprep (Hologic, 2005, Marlborough, MA, USA) samples were transported from the study clinic to the Centre for Infectious Disease Research in Zambia (CIDRZ) Central Laboratory and stored at room temperature for ∼ 90 days before testing by Xpert HPV and OncoE6 in accordance with the manufacturers' instructions. The Xpert HPV test (GeneXpert, Cepheid, 2014, Sunnyvale, CA, USA) carries out multiplexed amplification of target DNA by real-time PCR of 13 hrHPV types and 1 possible hrHPV type (HPV66) in a single cartridge-based analysis. Xpert HPV identifies HPV16 and HPV18/45 in two distinct detection channels and reports 11 other HPV types (HPV31/33/35/39/51/52/56/58/59/66/68) as a pooled result. A human reference gene (hydroxymethylbilane synthase [HMBS]), which confirms sample adequacy, and internal probe checks are integrated within the test cartridge. The OncoE6 test (Arbor Vita, 2013, Fremont, CA, USA) is based on the capture and detection of E6 proteins from HPV16 and HPV18 using high-affinity monoclonal antibodies in a lateral-flow assay. The E6 proteins are detected by an alkaline phosphatase-conjugated monoclonal antibody, and a positive result is visualized by the addition of an enzyme substrate.

Cervical biopsies stored in formalin were transported to the Cancer Disease Hospital Pathology Department, embedded in paraffin, sectioned, stained with hemotoxylin and eosin, and read by a certified pathologist to determine the histologic diagnosis. Blood samples collected in EDTA tubes were transported to the CIDRZ Central Laboratory on the day of collection. Plasma was isolated from the blood samples and analyzed for HIV-1 viral load and CD4+ cell counts. Viral load was measured using the Cobas Ampliprep/Taqman platform (Roche Molecular Diagnostics, 2007, Pleasanton, CA, USA) and CD4+ cell count using an FC500 flow cytometer (Beckman Coulter, 2004, Pasadena, CA, USA) according to the manufacturers' recommendations.

Statistical analysis

Demographic, clinical, and laboratory data were entered into a custom-built database. Data were cleaned in MS Excel (Microsoft, 2015, Redmond, WA, USA) and exported to Stata version 12.1 (StataCorp, 2012, College Station, TX, USA) for analysis. Our primary outcome was clinical performance (sensitivity, specificity, PPV, NPV) of VIA, DC, Xpert HPV, and OncoE6 for identification of CIN2+. Our secondary outcomes included clinical performance of the tests for CIN3+, as well as likelihood ratios (LRs), odd ratios (ORs), and number needed to screen.

Descriptive analysis included generating frequencies, measures of central tendency, and measures of variability for demographic and clinical variables. Missingness was also assessed for each variable. We then calculated the sensitivity, specificity, PPV, and NPV of all 4 screening tests for identification of both CIN2+ and CIN3+. The point estimates of

these parameters are reported with corresponding 95% confidence intervals (CIs). For each of the screening tests, we also determined the positive and negative LRs and the odds of CIN2+ or CIN3+ among women who were screen positive compared to those who were screen negative. Once again, point estimates of the LRs and ORs are reported with corresponding 95% CIs.

Ethics statement

Regulatory approval was provided by the University of Zambia's Biomedical Research Ethics Committee and the University of North Carolina at Chapel Hill's Institutional Review Board. All participants provided written informed consent.

Role of the funding source

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Results

Characteristics of the study participants

Between January and June 2015, 200 eligible HIV-infected women were enrolled. The median age of women in the study was 42 years (interquartile range [IQR]: 34-47). Roughly half of the participants had completed at least some secondary school education (47%) and were married to or cohabiting with their primary partner (47%) (Table 1).

Participants were asked to provide a brief gynecologic and sexual history. The median age of sexual debut was 18 years (IQR: 16-20) and the median number of lifetime sexual partners was 3 (IQR: 2-5). Although vaginal “cleansing” with herbs, detergents, cloth, and similar items is thought to be common in settings like Zambia, only 27 (14%) women reported this practice. Encouragingly, 90 (46%) participants reported prior cervical cancer screening by VIA, 179 (90%) were receiving antiretroviral therapy (ART), and 153 (77%) had an HIV viral load < 20 copies/mL. The median CD4+ cell count was 456 cells/uL (IQR: 328-590). Despite the overwhelming majority of women receiving ART and being virologically suppressed, however, the burden of cervical disease in this population was high. Six (3%) women were diagnosed with ICC. Additionally, 14 (7%) women had CIN3, 12 (6%) had CIN2, and 35 (18%) had CIN1.

Screening test positivity

Twenty-nine (15%) women were screen positive by VIA, 30 (20%) by DC, 94 (47%) by Xpert HPV, and 11 (6%) by OncoE6. The Xpert HPV test also provided results for HPV16 and HPV18/45, for which 29 (15%) and 22 (11%) women were positive, respectively. Using OncoE6, 9 (5%) women were HPV16 positive and 3 (2%) HPV18 positive. There were no

statistically significant differences in the proportion of screen positive women by ART status (Table 2).

Clinical performance using a CIN2+ threshold

Using a CIN2+ threshold, the sensitivity and specificity of VIA was 48% (95% CI: 30-67%) and 92% (95% CI: 86-95%), respectively. Similarly, the sensitivity and specificity of DC was 59% (95% CI: 41-76%) and 88% (95% CI: 82-93%). The sensitivity and specificity of Xpert HPV was 88% (95% CI: 71-97%) and 60% (95% CI: 52-68%). The sensitivity and specificity of OncoE6 was 31% (95% CI: 16-50%) and 99% (95% CI: 97-100%) (Table 3).

Compared to women who were screen negative, the odds of CIN2+ was 10 times higher among women who were VIA positive (95% CI: 4-25), and 11 times higher among those DC positive (95% CI: 4-25) and Xpert HPV positive (95% CI: 4-30). Additionally, we determined that to detect 1 case of CIN2+, 13 women would need to be screened by VIA, 11 women by DC, 7 by Xpert HPV, and 20 by OncoE6.

Clinical performance using a CIN3+ threshold

Using a CIN3+ threshold, the sensitivity and specificity of VIA was 63% (95% CI: 38-84%) and 91% (95% CI: 85-94%), respectively. Similarly, the sensitivity and specificity of DC was 75% (95% CI: 51-91%) and 87% (95% CI: 81-91%). The sensitivity and specificity of Xpert HPV was 90% (95% CI: 68-99%) and 57% (95% CI: 48-65%). Finally, the sensitivity and specificity of OncoE6 was 40% (95% CI: 19-64%) and 98% (95% CI: 95-100%).

The results of the likelihood ratio analysis for CIN3+ were similar to those obtained using a CIN2+ threshold. However, the confidence intervals were wider because we had relatively few cases of CIN3+ (Table 4). The calculation of the number needed to screen also showed a similar trend, such that to detect 1 case of CIN3+ 17 women would need to be screened by VIA, 13 women by DC, 11 by Xpert HPV, and 25 by OncoE6.

Discussion

Our study confirms a substantial risk of CIN2+ among HIV-infected Zambian women receiving combination ART (cART), nearly half of whom had previously been screened for cervical cancer. Specifically, 3% of women were diagnosed with ICC and 13% with high-grade CIN. It should be noted that the burden of cervical disease in our study was somewhat lower than in previously published Zambian studies, which have reported that ∼30-50% of HIV-infected women are VIA/DC positive,[18,19] 20% have CIN2+,[19] and 53% are hrHPV positive.[20] These studies were conducted before cART was widely available in Zambia and before the introduction of the so-called Option B+ strategy (through which the overwhelming majority of HIV-infected women of reproductive age have obtained access to cART regardless of their CD4+ cell count), possibly accounting for the observed differences.

The visual screening tests evaluated (VIA and DC) both displayed moderate sensitivity and high specificity for CIN2+ (sensitivity: 48% and 59%, respectively; specificity: 92% and 88%), which is broadly consistent with previously published validation data.[11,15,21] Not surprisingly, Xpert HPV, an HPV DNA PCR assay, demonstrated high sensitivity and

moderate specificity for CIN2+ (88% and 60%, respectively). Previous validation studies of the Xpert HPV in the U.S. have yielded similar results, with the initial evaluation (n=141) demonstrating that Xpert HPV was equally sensitive for high-grade squamous intra-epithelial lesions (HSIL) as Cobas HPV (90.8% vs. 90.8%) and more sensitive than Digene HC2 (90.8% vs. 81.6%, p=0.004). Xpert HPV was also more specific than Cobas HPV (42.6% vs. 39.6%, p=0.02) but less specific than Digene HC2 (42.6% vs. 47.7%, p<0.001). Our study adds evidence that Xpert HPV performs well in HIV-infected women and, as expected, appears to have higher PPV for CIN2+ than in HIV-uninfected women.[22] Again, not surprisingly, OncoE6, a lateral flow oncoprotein assay, demonstrated high specificity but low sensitivity (99% and 31%, respectively) for CIN2+. This finding was similar to previously published OncoE6 validation data.[23]

All of the tests we selected for our validation study can be performed at (or near) the point-of-care, a characteristic of great importance when considering which testing modalities to scale in screen-and-treat cervical cancer prevention programs. Same-day screening and treatment services are highly efficient,[24] and offer women and providers several advantages. First, they reduce attrition from care, a near-ubiquitous problem in both non-communicable disease care and HIV treatment programs across sub-Saharan Africa.[25,26] Second, same-day services have the potential to decrease health care costs, as well as the financial burden that women and their families face when multiple return visits are required to obtain test results, referral, and treatment.

Although well established, it bears repeating that limited access to cervical cancer screening, prevention, and treatment services is among the most important reasons for the disparities in cervical cancer incidence and mortality observed globally between high and low- and middle-income countries, between urban and rural women, and between rich and poor women.[27] Screen-and-treat programs can help to address these disparities. We have previously shown that in the CCPPZ VIA/DC see-and-treat program, 1 cancer death is prevented for every 46 HIV-infected women screened (range: 28–68).[28] A landmark randomized trial conducted by Denny and colleagues in South Africa also showed that for every 100 women screened, a VIA see-and-treat strategy can prevent 7 cases of CIN2+ among HIV-infected women and 1 case among HIV-uninfected women. Among 100 women screened using an HPV test-and-treat strategy, 12 cases of CIN2+ would be prevented among HIV-infected women and 3 among HIV-uninfected women.[29] Encouragingly, the cost-effectiveness of both screen-and-treat approaches has also been confirmed.[5,8,24] With the efficacy and cost-effectiveness of screen-and-treat cervical cancer prevention proven, what remains to be seen is how rapidly and how fully global guidance[30] can be implemented and scaled to reduce the burden of this preventable cancer, including among HIV-infected individuals who are at the highest risk of cervical disease.

We acknowledge several limitations of our work. As noted above, the burden of cervical disease in our study population was somewhat lower than anticipated, affecting measures of variability, such as 95% confidence intervals. We were also unable to evaluate the other available near-patient HPV DNA test, *care*HPV, or other FDA-approved HPV tests (e.g., Digene HC2 and Roche Cobas HPV) due to limited commercial availability of these tests in Zambia. Despite these limitations, we anticipate that our work will help to expand the

nascent literature on point-of-care molecular diagnostics for cervical cancer screening and to create a foundation for future clinical and implementation science research exploring optimal strategies for HPV-based screening in LMICs.

Conclusions

Among HIV-infected women, VIA and DC displayed moderate sensitivity and high specificity, Xpert HPV high sensitivity and moderate specificity, and OncoE6 excellent specificity but low sensitivity. Based on these results, we recommend that future evaluations of screen-and-treat cervical cancer prevention modalities include HPV DNA testing with the GeneXpert platform, as well as with VIA/DC to determine the comparative effectiveness of these strategies for reducing CIN2+. Due to its poor sensitivity, OncoE6 is not recommended for primary screening, but may be investigated as a triage test. Importantly, we also show that the burden of hrHPV and CIN2+ among HIV-infected Zambian women remains substantial despite rapidly increasing access to cART and measurable improvements in immune function.

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References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. Mar-Apr;2011 61(2):69–90. [PubMed: 21296855]
2. Bray F, Ren JS, Masuyer E, Ferlay J. Global estimates of cancer prevalence for 27 sites in the adult population in 2008. Int J Cancer. Mar 1; 2013 132(5):1133–1145. [PubMed: 22752881]
3. Clifford GM, Goncalves MA, Franceschi S. Human papillomavirus types among women infected with HIV: a meta-analysis. AIDS. Nov 28; 2006 20(18):2337–2344. [PubMed: 17117020]
4. Denny L, Boa R, Williamson AL, et al. Human papillomavirus infection and cervical disease in human immunodeficiency virus-1-infected women. Obstet Gynecol. Jun; 2008 111(6):1380–1387. [PubMed: 18515522]
5. Campos NG, Kim JJ, Castle PE, et al. Health and economic impact of HPV 16/18 vaccination and cervical cancer screening in Eastern Africa. Int J Cancer. Jun 1; 2012 130(11):2672–2684. [PubMed: 21717458]
6. Ferlay, J.; Soerjomataram, I.; Ervik, M., et al. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No 11. Lyon, France: International Agency for Research on Cancer; 2013. [Internet]Available from: http://globocan.iarc.fr [12 January 2015]
7. UNAIDS. [January 2014] Global Report on the AIDS Epidemic 2013. http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2013/gr2013/UNAIDS_Global_Report_2013_en.pdf
8. Goldie SJ, Gaffikin L, Goldhaber-Fiebert JD, et al. Cost-effectiveness of cervical-cancer screening in five developing countries. N Engl J Med. Nov 17; 2005 353(20):2158–2168. [PubMed: 16291985]
9. Sankaranarayanan R. Screening for cancer in low- and middle-income countries. Annals of global health. Sep-Oct;2014 80(5):412–417. [PubMed: 25512156]
10. Parham GP, Mwanahamuntu MH, Kapambwe S, et al. Population-level scale-up of cervical cancer prevention services in a low-resource setting: development, implementation, and evaluation of the

cervical cancer prevention program in Zambia. PLoS One. 2015; 10(4):e0122169. [PubMed: 25885821]

11. Chibwesha CJ, Cu-Uvin S. See-and-Treat Approaches to Cervical Cancer Prevention for HIV-Infected Women. Curr HIV/AIDS Rep. Jun 25.2011
12. Mwanahamuntu MH, Sahasrabuddhe VV, Pfaendler KS, et al. Implementation of 'see-and-treat' cervical cancer prevention services linked to HIV care in Zambia. AIDS. Mar 27; 2009 23(6):N1–5. [PubMed: 19279439]
13. Mwanahamuntu MH, Sahasrabuddhe VV, Kapambwe S, et al. Advancing cervical cancer prevention initiatives in resource-constrained settings: insights from the cervical cancer prevention program in zambia. PLoS Med. May.2011 8(5):e1001032. [PubMed: 21610859]
14. Parham GP, Mwanahamuntu MH, Pfaendler KS, et al. eC3--a modern telecommunications matrix for cervical cancer prevention in Zambia. J Low Genit Tract Dis. Jul; 2010 14(3):167–173. [PubMed: 20592550]
15. Bateman AC, Parham GP, Sahasrabuddhe VV, et al. Clinical performance of digital cervicography and cytology for cervical cancer screening in HIV-infected women in Lusaka, Zambia. J Acquir Immune Defic Syndr. Oct 1; 2014 67(2):212–215. [PubMed: 24977474]
16. Firnhaber C, Mao L, Levin S, et al. Evaluation of a Cervicography-Based Program to Ensure Quality of Visual Inspection of the Cervix in HIV-Infected Women in Johannesburg, South Africa. J Low Genit Tract Dis. Jan; 2015 19(1):7–11. [PubMed: 24914887]
17. Bateman AC, Chibwesha CJ, Parham GP. Minimizing verification bias in cervical cancer screening of HIV-infected women. Int J Gynaecol Obstet. Mar; 2015 128(3):269–270. [PubMed: 25467910]
18. Mwanahamuntu MH, Sahasrabuddhe VV, Blevins M, et al. Utilization of cervical cancer screening services and trends in screening positivity rates in a 'screen-and-treat' program integrated with HIV/AIDS care in Zambia. PLoS One. 2013; 8(9):e74607. [PubMed: 24058599]
19. Bateman AC, Katundu K, Mwanahamuntu MH, et al. The burden of cervical pre-cancer and cancer in HIV positive women in Zambia: a modeling study. BMC Cancer. 2015; 15:541. [PubMed: 26205980]
20. Sahasrabuddhe VV, Mwanahamuntu MH, Vermund SH, et al. Prevalence and distribution of HPV genotypes among HIV-infected women in Zambia. Br J Cancer. May 7; 2007 96(9):1480–1483. [PubMed: 17437020]
21. Firnhaber C, Mayisela N, Mao L, et al. Validation of cervical cancer screening methods in HIV positive women from Johannesburg South Africa. PLoS One. 2013; 8(1):e53494. [PubMed: 23326441]
22. Einstein MH, Smith KM, Davis TE, et al. Clinical evaluation of the cartridge-based GeneXpert human papillomavirus assay in women referred for colposcopy. J Clin Microbiol. Jun; 2014 52(6): 2089–2095. [PubMed: 24719440]
23. Zhao FH, Jeronimo J, Qiao YL, et al. An evaluation of novel, lower-cost molecular screening tests for human papillomavirus in rural China. Cancer prevention research. Sep; 2013 6(9):938–948. [PubMed: 23878179]
24. Campos NG, Castle PE, Wright TC Jr, Kim JJ. Cervical cancer screening in low-resource settings: A cost-effectiveness framework for valuing tradeoffs between test performance and program coverage. Int J Cancer. Nov 1; 2015 137(9):2208–2219. [PubMed: 25943074]
25. Petersen M, Yiannoutsos CT, Justice A, Egger M. Observational research on NCDs in HIV-positive populations: conceptual and methodological considerations. J Acquir Immune Defic Syndr. Sep 1; 2014 67(Suppl 1):S8–16. [PubMed: 25117964]
26. Fox MP, Rosen S. Retention of Adult Patients on Antiretroviral Therapy in Low- and Middle-Income Countries: Systematic Review and Meta-analysis 2008-2013. J Acquir Immune Defic Syndr. May 1; 2015 69(1):98–108. [PubMed: 25942461]
27. Denny L, Anorlu R. Cervical cancer in Africa. Cancer Epidemiol Biomarkers Prev. Sep; 2012 21(9):1434–1438. [PubMed: 22806169]
28. Parham GPMM, Sahasrabuddhe VV, Westfall AO, King KE, Chibwesha C, Pfaendler KS, Mkumba G, Mudenda V, Kapambwe S, Vermund SH, Hicks ML, Stringer JSA, Chi BH. Implementation of cervical cancer prevention services for HIV-infected women in Zambia: measuring program effectiveness. HIV Therapy. 2010; 4(6):713–722.

29. Kuhn L, Wang C, Tsai WY, Wright TC, Denny L. Efficacy of human papillomavirus-based screen-and-treat for cervical cancer prevention among HIV-infected women. AIDS. Oct 23; 2010 24(16): 2553–2561. [PubMed: 20706107]

30. WHO. Guidelines for screening and treatment of precancerous lesions for cervical cancer prevention. WHO; 2013. http://apps.who.int/iris/bitstream/10665/94830/1/9789241548694_eng.pdf [December 2015]

List of all abbreviations and acronyms

ART	Antiretroviral therapy
cART	Combination antiretroviral therapy
CCPPZ	Cervical cancer prevention program in Zambia
CI	Confidence interval
CIDRZ	Centre for Infectious Disease Research in Zambia
CIN	Cervical intra-epithelial neoplasia
DC	Digital cervicography
DNA	Deoxyribonucleic acid
FDA	Food and Drug Administration
HC2	Hybrid Capture 2
HIV	Human immunodeficiency virus
HMBS	Hydroxymethylbilane synthase
HPV	Human papillomavirus
hrHPV	High-risk human papillomavirus
ICC	Invasive cervical cancer
IQR	Interquartile range
LMICs	Low- and middle-income counties
LR	Likelihood ratio
mL	Milliliter
NILM	No intra-epithelial lesion or malignancy
NPV	Negative predictive value
OR	Odds ratio
PEPFAR	President's Emergency Plan for AIDS Relief
PPV	Positive predictive value

RNA	Ribonucleic acid
SIL	Squamous intra-epithelial lesion
uL	Microliter
VIA	Visual inspection with acetic acid

Table 1
Descriptive characteristics of the study participants

	Total (N, %)
Age (n=200), median years (IQR)	42 (34-47)
Education (n=200)	
No education	1 (1%)
Primary education	48 (24%)
Secondary education	93 (47%)
Tertiary education	58 (29%)
Marital status (n=200)	
Never married	21 (11%)
Married/cohabiting	93 (47%)
Divorced/separated	30 (15%)
Widowed	56 (28%)
Tobacco use (n=199)	
Yes	10 (5%)
Age at sexual debut (n=186), median (IQR)	18 (16-20)
Lifetime sexual partners (n=188), median (IQR)	3 (2-5)
Number of pregnancies (n=197), median (IQR)	3 (2-5)
Vaginal cleansing (n=196)	
Yes	27 (14%)
Prior cervical screening (n=194)	
Yes	90 (46%)
On antiretroviral therapy (n=199)	
Yes	179 (90%)
CD4+ cell count (n=), median cells/uL (IQR)	456 (328-590)
HIV plasma viral load (n=200)	
<20 copies/mL	153 (77%)

IQR: interquartile range

Table 2

Screening test positivity for VIA, DC, Xpert HPV, and OncoE6 by antiretroviral status

	Total	On antiretroviral therapy	Not on antiretroviral therapy
VIA (n=199)			
Positive	29 (15%)	24 (14%)	5 (25%)
DC (n=200)			
Positive	39 (20%)	33 (18%)	6 (30%)
Xpert HPV (n=199)			
Any hrHPV positive	94 (47%)	82 (46%)	12 (60%)
HPV16 positive	29 (15%)	26 (15%)	3 (15%)
HPV18/45 positive	22 (11%)	20 (11%)	2 (10%)
HPV31/33/35/52/58 positive	64 (32%)	54 (30%)	10 (50%)
HPV51/59 positive	14 (7%)	13 (7%)	1 (5%)
HPV39/68/56/66 positive	18 (9%)	15 (8%)	3 (15%)
OncoE6 (n=200)			
Any hrHPV positive	11 (6%)	11 (6%)	0
HPV16 positive	9 (5%)	9 (5%)	0
HPV18 positive	3 (2%)	3 (2%)	0

VIA: visual inspection with acetic acid; DC: digital cervicography;

* $p<0.05$;

** $p<0.01$;

*** $p<0.001$

Table 3
Clinical performance of the cervical cancer screening tests using a CIN2+ threshold

	VIA	DC	Xpert HPV	OncoE6
True positive	15	19	28	10
False positive	14	20	66	1
True negative	153	147	100	166
False negative	16	13	4	22
Sensitivity (95% CI)	48% (30-67%)	59% (41-76%)	88% (71-97%)	31% (16-50%)
Specificity (95% CI)	92% (86-95%)	88% (82-93%)	60% (52-68%)	99% (97-100%)
PPV (95% CI)	52% (33-71%)	49% (32-65%)	30% (21-40%)	91% (59-100%)
NPV (95% CI)	91% (85-95%)	92% (87-96%)	96% (90-99%)	88% (83-93%)
PLR (95% CI)	10 (4-25)	5 (3-8)	2 (2-3)	52 (7-394)
NLR (95% CI)	0.6 (0.4-0.8)	0.5 (0.3-0.7)	0.2 (0.1-0.5)	0.7 (0.5-0.9)
OR (95% CI)	10 (4-25)	11 (4-25)	11 (4-30)	76 (12-.)

VIA: visual inspection with acetic acid; DC: digital cervicography; 95% CI: 95% confidence interval; PPV: positive predictive value; NPV: negative predictive value; PLR: positive likelihood ratio; NLR: negative likelihood ratio; OR: odds ratio

Table 4
Clinical performance of the cervical cancer screening tests using a CIN3+ threshold

	VIA	DC	Xpert HPV	OncoE6
True positive	12	15	18	8
False positive	17	24	76	3
True negative	162	155	102	176
False negative	7	5	2	12
Sensitivity (95% CI)	63% (38-84%)	75% (51-91%)	90% (68-99%)	40% (19-64%)
Specificity (95% CI)	91% (85-94%)	87% (81-91%)	57% (48-65%)	98% (95-100%)
PPV (95% CI)	41% (24-61%)	39% (23-55%)	19% (12-29%)	73% (39-94%)
NPV (95% CI)	96% (92-98%)	97% (93-99%)	98% (93-100%)	94% (89-97%)
PLR (95% CI)	7 (4-12)	6 (4-9)	2 (2-3)	24 (7-83)
NLR (95% CI)	0.4 (0.2-0.7)	0.3 (0.1-0.6)	0.2 (0.1-0.6)	0.6 (0.4-0.9)
OR (95% CI)	16 (6-46)	19 (7-56)	12 (3-.)	39 (10-154)

VIA: visual inspection with acetic acid; DC: digital cervicography; 95% CI: 95% confidence interval; PPV: positive predictive value; NPV: negative predictive value; PLR: positive likelihood ratio; NLR: negative likelihood ratio; OR: odds ratio