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Xpert human papillomavirus test is a promising cervical cancer screening test for HIV-seropositive women

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ABSTRACT

This study investigated the performance of Cepheid Xpert human papillomavirus (HPV) assay in South African human immunodeficiency virus (HIV)-infected women and compared its performance with that of hybrid capture-2 (hc2). *Methods*: Stored cervical specimens from HIV-infected women that had previously been tested using hc2 were tested using Xpert. *Results*: The overall HR-HPV prevalence was found to be 62.0% (720/1161) by Xpert and 61.2% (711/1161) by hc2. 13.6% (158/1161) were HPV16 positive, 18.8% (218/1161) were HPV18/45, 37.3% (434/1161) were HPV31/33/35/52/58, 12.7% (147/1161) were HPV51/59 and 23.3% (270/1161) were HPV39/68/56/66. Overall agreement with hc2 was 90%; Cohen's kappa was 0.78 (95% CI 0.74–0.82) indicating substantial agreement. Detection of HPV16, HPV18/45, and HPV31/33/35/52/58 were independently associated with cervical intraepithelial neoplasia (CIN)−2+ ($P < 0.0001$ for each); while HPV51/59 and HPV39/68/56/66 were not. Women infected with HPV16, HPV18/45 or HPV31/33/35/52/58 were found to have significantly higher amounts of HPV DNA detected for those with CIN2+ compared to those without CIN2+, $P < 0.0001$ for each. Xpert and hc2 were similarly sensitive (88.3% and 91.5%, respectively) and specific (48.4% and 51.0%) for CIN2+ and CIN3 (sensitivity: 95.8% and 97.9%; specificity: 41.4% and 42.8%). *Conclusions*: Xpert is a promising screening test in HIV-infected women that performs similarly to hc2.

1. Introduction

In sub-Saharan Africa, cervical cancer is the most common cancer among women [1,2]. The high burden of cervical cancer cases and death in sub-Saharan Africa is due to the lack of effective cervical cancer prevention programs. Cervical screening programmes are under-resourced and understaffed reducing women's access to these programmes. Limited human resources and clinic capacity, as well as patient costs (including travel, child care, and missed wages) are among the many challenges faced. Women in resource-limited countries often present with invasive cervical disease in its most advanced stage, reducing survival.

Visual inspection with acetic acid (VIA) has been used in many resource-limited countries as a way of expanding cervical cancer screening [3–5]. However, VIA as a screening test has lower efficacy in reducing cervical cancer than that of cervical cytology and HR-HPV molecular testing [3]. Persistent infection with high-risk (HR) human papillomavirus (HPV) is necessary for the development of cervical cancer and therefore HR-HPV testing is considered an alternative test to cytology or VIA or used in various

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combinations for cervical cancer screening [3,4,6,7]. Molecular testing for HR-HPV types has been found to be more sensitive but less specific for detection of cervical intraepithelial neoplasia-2/3 (CIN-2/3) when compared with cervical cytology [8,9].

Currently available HPV assays are batch tests, requiring skilled laboratory personnel and taking several hours to complete. The Cepheid Xpert HPV assay (Cepheid Sunnyvale, CA) is a qualitative, real time polymerase chain reaction assay detecting 14 HR-HPV DNA. A single Xpert HPV test can be completed in one hour, allowing same day screening, diagnosis and treatment [10]. Xpert HPV demonstrates good clinical performance in identifying women with CIN2/3 and it was found to have similar performance as other FDA approved HR-HPV tests among human immunodeficiency virus (HIV)-uninfected women [10–12]. Point-of-care HR-HPV testing may improve the management and control of cervical cancer.

A high proportion of people living with HIV reside in sub-Saharan Africa [13]. Multiple HPV infections and persistent HPV infection are significantly higher in HIV-positive women than HIV-negative women [14–16]. Among HIV-infected women the progression from persistent infection to CIN2/3 occurs within a shorter period of time and occurs more frequently than in HIV-negative women [15,17,18]. The association between highly active antiretroviral therapy (HAART) and HPV infection and cervical cancer is controversial with conflicting reports [15]. According to the systematic review evaluating the impact of HAART on incidence of cancers; the use of HAART does not seem to decrease the risk of developing cervical cancer [19].

In South Africa there are no standard guidelines on so-called "screen-and-treat" methods for cervical cancer prevention in either HIV-infected or -uninfected women. Identification of optimal cervical cancer screening methods among HIV-positive women is necessary, particularly in the context of high HIV disease burden in settings like South Africa. Therefore, the aim of the study was to investigate the performance of Cepheid Xpert HPV assay in HIV-infected South African women and compare its performance with that of Digene hybrid capture 2 (hc2) (an FDA approved test).

2. Methods

2.1. Study population and design

The current study used stored samples obtained from the VICAR-1 study conducted between November 2009 and August 2011 in Johannesburg, South African. VICAR-1 study was a cohort study of HIV-infected, South African women evaluating 3 cervical cancer screening tests: hc2 test for HR-HPV, cervical cytology, and VIA [20]. Human Research Ethics Committee of University of Witwatersrand and University of Cape Town approved all aspects of the study. All study participants provided written informed consent. For the current analysis, stored cervical specimens ($n=1193$) from HIV-infected women that had previously been tested using hc2 were tested using the Xpert HPV test.

2.2. Pathology results

The cytology and histology were reviewed as part of standard of care by the National Health Laboratory Services (NHLS). The NHLS undergoes routine accreditation by the South African National Accreditation System and the pathologists undergo regular proficiency testing through the Royal College of Pathologist of Australasia Quality Assurance scheme. In addition there is a 100% second review of all negative Pap smears by a second cytotechnologist and all abnormal Pap smears are reviewed by a senior cytologist or pathologist. The cytology readings from NHLS have previously gone under an external blinded review at University of North Carolina with 80–85% concordance of results; if clinically indicated, discrepant cytological and histological results were reviewed and second procedure done [21,22].

2.3. HPV genotyping

Cervical specimens were collected with a Digene cervical sampler and stored in 1 ml Digene transport medium buffer and stored at −80°C. It is important to note that Digene transport medium buffer is not a manufacturer's approved collection medium for the Xpert HPV. The ThinPrep transport medium is one of the approved collection medium and is 20 ml while Digene transport medium is 1 ml. A total of 100 μl cervical sample was transferred to 950 μl phosphate buffered saline and 1000 μl of diluted specimen was used to run Cepheid Xpert HPV according to manufacturer's instructions. Xpert HPV gives results from 6 separate channels: (a) sample adequacy control (SAC), (b) P1-HPV16, (c) P2-HPV18/45, (d) P3-HPV31/33/35/52/58, (e) P4-HPV51/59 and (f) P5-HPV39/68/56/66.

2.4. Statistical analysis

The relationship between HPV detection in the 5 Xpert channels and CIN2/3 was determined by univariable and multivariable logistic regression. All 5 HPV channels were included in the multivariable model. We quantified HPV DNA using the cycle threshold (Ct) normalised with the SAC Ct (HPV DNA Ct/SAC Ct) as described previously [11]. The relationship of the normalized Ct value to the presence of CIN2+ was determined using the Student's *t*-test. The test performance characteristics for Xpert and hc2 are weighted to adjust for verification bias as was done for the original cohort description [20,23]. All statistical analyses were conducted using SAS 9.4 (Cary, North Carolina, USA).

3. Results

A total of 1202 women were enroled in the parent screening study between November 2009 and August 2011. These women were recruited from a HIV treatment clinic Themba Lethu Clinic located in a tertiary academic hospital in Johannesburg. Almost all of the women (98.1%) considered themselves Black African and had median age of 38 years (IQR 32–43) with a median CD4 count of 394 cells/μL. The majority of women (93.1%) were on HAART and approximately 83% had HIV plasma viral loads under 400 copies/ml.

Of these 1202 women, 1193 participants were included in the VICAR-1 analysis (9 were excluded due to incomplete test results: 6 had inadequate or no cytology and 3 had invalid hc2 HPV or VIA results). Of these, 1161 (97%) have valid Xpert HPV results (10 had no sample available for analysis, 22 had samples tested but no results were obtained because of technical issues with the Xpert machine and not enough specimen was available for a re-run). The HR-HPV prevalence was found to be 62.0% (720/1161) for Xpert and 61.2% (711/1161) for hc2. 13.6% of women (158/1161) were HPV16 positive, 18.8% (218/1161) were HPV18/45, 37.3% (434/1161) were HPV31/33/35/52/58, 12.7% (147/1161) were HPV51/59 and 23.3% (270/1161) were HPV39/68/56/66 (Table 1). Multiple HPV types were observed in 29.5% (342/1161) of specimens and a single HPV type in 32.6% (378/1161) of specimens. HR-HPV positivity on both Xpert [odds ratio (OR): 6.9, 95% confidence interval (CI): 4.7–9.6, $P<0.0001$] and hc2 (OR: 11.0, 95% CI: 7.4–16.6, $P<0.0001$) were significantly associated with CIN2+ (Table 1). HPV16 was found to be independently associated with CIN2+ (OR: 6.8, 95%

Table 1
Observed association of high-risk human papillomavirus with cervical intraepithelial lesions in HIV-seropositive women.

	Prevalence, *N* (%)	CIN2+ prevalence (test positive vs. test negative)	Univariate association with CIN2+, OR [95% CI]	Multivariate
hc2 positive	727 (61%)	42% vs. 6.2%	11 [7.4–16.6]*	n/a
Xpert HPV positive	720 (62%)	39.2% vs. 8.8%	6.9 [4.7–9.6]*	n/a
HPV16	158 (13.6%)	68.3% vs. 22.3%	7.5 [5.2–10.9]*	6.8 [4.5–10.1]*
HPV18/45	218 (18.8%)	45.1% vs. 24.2%	2.6 [1.9–3.5]*	2.5 [1.8–3.4]*
HPV31/33/35/52/58	434 (37.3%)	45.5% vs. 17.5%	3.9 [3.0–5.1]*	3.5 [2.6–4.6]*
HPV51/59	147 (12.7%)	42.1% vs. 26.1%	2.1 [1.4–2.9]*	1.3 [0.87–2.0], $P=0.19$
HPV39/68/56/66	270 (23.3%)	37.2% vs. 25.1%	1.8 [1.3–2.3]*	1.2 [0.89–1.7], $P=0.20$

* $P<0.0001$. CIN: cervical intraepithelial neoplasia; n/a: not applicable; hc2: hybrid capture-2; HPV: human papillomavirus.

Table 2
The performance characteristics for various human papillomavirus screening algorithms.

	CIN2+				CIN3			
	Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV
Digene hc2	91.5% (87.2–95.8)	51.0% (47.6–54.5)	42.1% (38.4–45.8)	93.9% (90.7–97.1)	97.9% (95.1–100)	42.8% (39.8–45.7)	14.4% (11.8–17.0)	99.5% (98.9–100)
Xpert	88.3% (83.6–93.0)	48.4% (44.9–51.9)	40.1% (36.5–43.8)	91.3% (87.6–95.0)	95.8% (91.8–99.9)	41.4% (38.4–44.4)	13.9% (11.3–16.5)	99.0% (98.0–100)
HPV16	31.5% (26.3–36.7)	93.5% (91.8–95.2)	65.5% (56.7–74.2)	77.7% (74.9–80.5)	43.0% (33.3–52.8)	89.4% (87.5–91.3)	28.8% (21.0–36.5)	94.0% (92.5–95.5)
HPV18/45	30.1% (25.0–35.3)	85.6% (83.2–88.0)	45.0% (38.1–51.8)	75.8% (72.8–78.9)	32.3% (23.1–41.5)	82.5% (80.2–84.8)	15.5% (10.5–20.4)	92.5% (90.8–94.2)
HPV31/33/35/52/58	61.1% (55.4–66.8)	71.9% (68.8–75.0)	45.9% (41.0–50.7)	82.6% (79.4–85.8)	63.9% (54.4–73.4)	65.3% (62.4–68.1)	15.4% (11.9–18.8)	94.8% (93.1–96.5)
HPV51/59	18.8% (14.5–23.2)	89.7% (87.6–91.8)	41.7% (33.5–49.9)	73.9% (70.9–76.8)	16.2% (9.00–23.5)	87.7% (85.7–89.7)	11.5% (6.2–16.9)	91.4% (89.6–93.1)
HPV39/68/56/66	33.6% (28.3–38.9)	80.7% (78.0–83.5)	40.7% (34.7–46.7)	75.6% (72.4–78.8)	36.7% (27.3–46.2)	78.0% (75.5–80.5)	14.2% (9.9–18.5)	92.6% (90.8–94.3)

PPV: positive predictive value; NPV: negative predictive value; CIN: cervical intraepithelial neoplasia; hc2: hybrid capture-2; Xpert: considered positive if any channel is positive; HPV: human papillomavirus.

CI: 4.5–10.1, $P<0.0001$), this finding was also observed for HPV18/45 (OR: 2.5, 95% CI: 1.8–3.4, $P<0.0001$) and HPV31/33/35/52/58 (OR: 3.5, 95% CI: 2.6–4.6, $P<0.0001$); but not for HPV51/59 (OR: 1.3, 95% CI: 0.87–2.0, $P=0.19$) and HPV39/68/56/66 (OR: 1.2, 95% CI: 0.89–1.7, $P=0.20$).

Overall agreement was 90% where 385 (33%) were negative by both tests and 655 (56%) were positive by both tests. The agreement beyond chance (Cohen's kappa) was 0.78 (95% CI: 0.74–0.82), indicating excellent agreement. Samples with discordant results had similar cellularity when compared with those with concordant results as measured by the Xpert reporter gene cycle threshold (Ct; 31.6 cycles for discordant samples compared to 31.5 for concordant samples, not statistically significant). Discordant samples had lower amounts of HPV DNA detected. The 56 samples that were positive by hc2 and negative by Xpert, had lower relative light units (RLU, an indication of lower HPV viral load) as compared to samples where both tests were positive (2.3 log 10 RLU vs. 5.0 log 10 RLU, $P<0.0001$). Similarly, the 65 samples that were Xpert positive and hc2 negative had lower numbers of HPV channels positive by Xpert (HR-HPV was detected in an average of 1.8 out of 5 channels for Xpert positive/hc2 positive vs. 1.1 channels for Xpert positive/hc2 negative, $P<0.0001$). In addition, lower levels of HPV DNA as measured by higher Ct values were seen for all 5 HPV channels.

The relationship between the amount of HR-HPV DNA detected and the prevalence of CIN2+ was investigated. Among women infected with HPV16, HPV18/45 or HPV31/33/35/52/58, women with CIN2+ detected were found to have significantly higher amounts of HPV DNA detected compared to those without CIN2+, $P<0.0001$ for each (Supplementary 1). The performance of Xpert and hc2 for CIN2+ and CIN3 are presented in Table 2. Xpert and hc2 were similarly sensitive (88.3% and 91.5%, respectively) and specific (48.4% and 51.0%, respectively) for CIN2+ and CIN3 (sensitivity: 95.8% and 97.9%, respectively; specificity: 41.4% and 42.8%, respectively). The presence of HPV16 by Xpert increased the specificity for CIN2+ from 48.4% to 93.5% and for CIN3 from 41.4% to 89.4%.

4. Discussion

To our knowledge this is the first published report on performance of Xpert HPV assay among HIV-positive women conducted in a resource-limited country. Xpert HPV results were found to be comparable to the results by hc2 and substantial agreement between the two assays was demonstrated. Discordant results between the assays were related to lower HPV DNA amounts as indicated by lower RLU in hc2 and high Ct values in Xpert. It is important to note that the samples that were HPV negative by Xpert (including those that were hc2 positive) were adequate samples according to the internal SAC control. A number of specimens were Xpert or hc2 negative but CIN2+ positive. These observations may indicate false negative HR-HPV results, histology false positive or the observed CIN2+ maybe due to HPV types that are not detected by Xpert or hc2 [24,25].

HPV16 positivity by Xpert was strongly associated with CIN2+. Einstein et al. also reported strong association of HPV16 detected by Xpert with CIN2+ and this association was comparable with HPV16 detected by cobas. HPV18/45 and HPV31/33/35/52/58 were also associated with CIN2+ however the positive predictive value was not as high [10]. These observations confirm the strong association between HPV16 with high grade lesions and cervical

cancers [25] in HIV-infected women, and suggest that HPV16 detection should be a strong indication for a test and treat approach in resource limited settings. HPV viral load was higher in HPV infected women with CIN2+ compared to CIN2+ negative. High HPV viral load is reported to be the predictor of abnormal cervical cytology [26,27]. Future studies should investigate with HPV quantitation can be used to increase the specificity for CIN2+.

Both Xpert and hc2 were found to be similarly sensitivity and specificity in contrast to the study reported by Einstein et al. where hc2 was found to be more specific than Xpert while Xpert was more sensitive than hc2. The HIV status was not specified in Einstein et al. study. The negative predictive value (NPV) of Xpert and hc2 for CIN2+ is similar to the one reported by Einstein et al. however the current report presents higher PPV by both Xpert (40.1% vs. 29%) and hc2 (42.1% vs. 23%). This is likely due to the greater likelihood of HR-HPV infection causing CIN2+ in HIV-infected women as compared to HIV-uninfected women. The differences between this report and Einstein et al. could also be due to different type of samples used; as they used ThinPrep specimen while the current report used the specimen stored in Digene transport media [10].

Limitations of the study: Specimens used were cervical specimens stored in Digene Specimen Transport Medium that had been frozen and thawed several times. The type of specimen used in this study is not the type approved for use with the Xpert HPV assay; ThinPrep (Hologic, Marlborough, MA) is the approved transport medium. This may have led to biased results as compared to processing samples according to the manufacturer's instructions. Despite these limitations the Xpert HPV assay was still found to perform similarly to hc2. Additionally, we used stored specimens and are therefore unable to comment on Xpert HPV as a point-of-care test. Additional studies are needed to evaluate Xpert HPV as it will be used in clinical practice.

The GeneXpert technology is been widely used in South Africa for *Mycobacterium tuberculosis* screening [28–30]. Therefore cervical cancer screening using Xpert HPV technology may be modelled after programmes and systems developed for Xpert TB testing. Point-of-care HPV screening and treatment is expected to increase the screening coverage and reduce attrition from care. However it should be noted that screening needs to be linked to treatment programmes at the clinics which comes with additional challenges. In conclusion, we demonstrate that Xpert is sensitive and specific for detecting CIN2+ and CIN3; and its performance is similar to hc2. Xpert is a promising cervical cancer screening test for HIV-infected women. Additional studies incorporating DNA quantification and detection of specific HR-HPV types into screening modalities for HIV-infected women are needed, as are real-world evaluations of point-of-care HPV assays that may be able to be incorporated into screen-and-treat cervical screening programmes.

Transparency declaration

ZM: has received financial support to attend the 7th South African AIDS conference 2015 and 30th International Papillomavirus Conference & Clinical and Public Health Workshop.

TW, BG, AS, SW, SL, MF, and JS: have no relevant disclosures.

CJC: reports no conflicts of interests, financial or otherwise.

ALW: laboratory was loaned instrumentation to perform Xpert tests and donation of 25% of the kits used in the study.

CSF: laboratory was loaned instrumentation to perform Xpert tests and reduced pricing for Xpert cartridges for other HPV Xpert studies.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.pvr.2016.02.004.

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