Title:

Performance of *care*HPV for Detecting High-grade Cervical Intraepithelial Neoplasia among Women Living with HIV-1 in Burkina Faso and South Africa: HARP Study.

Running title:

Performance of *care*HPV in HIV infection

Authors:

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Competing interest

The authors have no conflicts of interest to disclose.

Summary

Background: The *care*HPV assay is a test for high-risk (HR) HPV detection designed to be affordable in resource-poor settings. We evaluated the performance of *care*HPV screening among 1052 women living with HIV/AIDS included in the HARP (HPV in Africa Research Partnership) study in Burkina Faso (BF) and South-Africa (SA).

Methods: Cervical samples were tested for HR-HPV by the *care*HPV and the INNO-LiPA HPV genotyping Extra assays. All women had Pap smear testing, visual inspection with acetic acid/Lugol's iodine (VIA/VILI) and colposcopy. Cervical biopsies were obtained for participants who were HR-HPV DNA positive by *care*HPV or who had abnormalities detected on cytology, VIA/VILI or colposcopy.

Results: Overall, 45.1% of women had a positive *care*HPV test (46.5% in BF, 43.8% in SA). The *care*HPV positivity rate increased with the grade of cytological lesions. Sensitivity and specificity of *care*HPV for the diagnosis of CIN2+ (n=60, both countries combined) were 93.3% (95%CI: 83.8-98.2) and 57.9% (95%CI: 54.5-61.2), respectively. Specificity increased with CD4 count. *care*HPV had a similar clinical sensitivity but higher specificity than the INNO-LiPA assay for detection of CIN2+.

Conclusion: Our results suggest that *care*HPV testing is a reliable tool for cervical cancer screening in HIV-1-infected women in sub-Saharan Africa.

Keywords: *care*HPV, cervical screening, HIV/AIDS, Africa

Introduction

Cervical cancer, which is the fourth most common cancer in women worldwide (Ferlay et al, 2015), is the most frequent cancer and the leading cause of cancer death in Sub-Saharan African women (Denny & Anorlu, 2012). Infection with human immunodeficiency virus type 1 (HIV-1) is an additional risk factor for the development of precancerous and cancerous cervical lesions (De Vuyst et al, 2008). Therefore, there is a need for developing preventive measures in this highly exposed population of African women living with HIV/AIDS (WLHA). Unfortunately, cervical cancer screening programs are lacking in most Sub-Saharan African countries and the diagnosis of cervical cancer is generally made at an advanced stage of the disease when treatment is unavailable or ineffective.

The development of precancerous cervical lesions that may evolve to invasive carcinoma is associated with persistent cervical infection with carcinogenic types of human papillomaviruses (HPV) designated as high-risk (HR) HPV. It has been shown that detection of HR-HPV in cervical samples is a highly sensitive tool for identifying women at risk of precancerous or cancerous cervical lesions (Cuzick et al, 2008; Ronco et al, 2014), but evaluation among WLHA have only rarely been conducted.

The *care*HPV assay (Qiagen Corporation, Gaithersburg, MD) is a qualitative test for HR-HPV detection targeting 14 HR-HPV types: HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV 66 and HPV68. This microplate assay, based on the hybridization of HR-HPV DNA with a cocktail of RNA probes and chemiluminescence signal amplification, was adapted from the Hybrid Capture 2 (HC2) assay (Qiagen) and designed to be simpler, more rapid to use, and more affordable than HC2 in resource-poor settings (Qiao et al, 2008; Gage et al, 2012).

HARP (HPV in Africa Research Partnership) is a research program conducted among WLHA in Burkina Faso and South Africa with the aim to prospectively evaluate several screening approaches for prevention of cervical cancer. In the first round of screening, we have shown that, compared with the INNO-LiPA HPV genotyping Extra assay, HC2 performed well in this population, with a similar sensitivity and a higher specificity for the diagnosis of high-grade cervical intraepithelial neoplasia (CIN) grade 2 or more severe (CIN2+). We have already reported an excellent agreement between HC2 and *care*HPV in a subgroup of HARP participants (Ngou et al, 2013), comparable to what was found in HIV-seronegative women in China (Chen et al, 2014; Lin et al, 2014). However, it remained to be demonstrated that *care*HPV had a good performance among African WLHA. For this reason, we used the second round of screening in HARP 18 months after enrolment to evaluate the performance of *care*HPV for the detection of CIN2+.

Materials and Methods

Study population

Participants were confirmed HIV-1- seropositive women aged 25-50 years recruited from the HIV outpatient clinic of the University Hospital of Ouagadougou, Burkina Faso (BF), and HIV treatment centres and surrounding primary health care clinics in Hillbrow, Johannesburg, South Africa (SA). Eligible women were invited for inclusion in the study if they were resident in the recruitment city, had not had a total hysterectomy or history of cervical cancer treatment and were not pregnant or less than 8 weeks post-partum.

Ethical approval was granted from Ministry of Health in Burkina Faso (no. 2012-12-089), the Witwatersrand University in South Africa (no. 110707), and the London School of Hygiene and Tropical Medicine (no. 7400). All women provided a written informed consent at the screening visit, and they were given a reflection period of at least 7 days before enrolment in the study. A second written informed consent was obtained at the enrolment visit for enrolment and follow-up over scheduled visits at months (M) 6, 12 and 18.

*care*HPV assay

At the M18 visit, endo- and ectocervical sampling was performed using the *care*HPV sample collection device consisting of a *care*Brush and a vial containing 1 ml of *care*HPV collection medium (Qiagen). After collection, the brush was stirred into the collection medium, the cell collection was homogeneized by vortexing and divided into four 0.25-ml aliquots. One aliquot was maintained at 4°C until *care*HPV analysis, performed within less than 4 weeks, and the others were cryopreserved at -80°C. The *care*HPV test was performed using 50µl of cervical sample in collection medium according to the manufacturer's instruction. The tests were performed at the respective sites by medical scientists specifically trained by a Qiagen's scientist. The positive or negative result of the *care*HPV assay was displayed by the *care*HPV test controller without additional specification of the luminescent signal intensity.

HPV detection and genotyping

All cervical specimens were tested using the INNO-LiPA HPV genotyping Extra® assay (Fujirebio, Les Ulis, France) as previously described (Ngou et al, 2015). This assay, which is based on PCR amplification of HPV DNA using broad-spectrum SPF10 consensus primers followed by hybridization of the amplicons with type-specific oligonucleotides probes immobilized on membrane strips, allows identification of 28 HPV types, including the 14 HR-HPV types targeted by the *care*HPV assay, the possible carcinogenic types HPV26, HPV53, HPV69, HPV70, HPV73 and HPV82, and the low-risk types HPV6, HPV11, HPV40, HPV43, HPV44, HPV54, HPV71 and HPV74. Testing was performed on an aliquot preserved at -80°C. A sample was considered HPV-positive if at least one of the type-specific probes or one of the HPV control probes were detected.

Cytological and histological analysis

An additional cervical brush was collected from the ecto- and endocervix and rolled on a glass slide which was fixed with ethanol for cytological reading using the Papanicolaou method (Pap test). Conventional cytology was used as liquid-based cytology was not available at that time in the African laboratories involved in the study. All participants had visual inspection with acetic acid/Lugol's iodine (VIA/VILI) performed by trained nurses and colposcopy performed by trained colposcopists. Systematic four-quadrant biopsy and directed biopsy from any suspicious lesions were performed for participants testing positive by *care*HPV or who had abnormalities detected on cytology (≥ASC-US), VIA/VILI or colposcopy.

The Bethesda system for reporting cervical cytology (Smith, 2002) was used for cytology results and the CIN classification for histology results. Cytological and histological slides were independently examined at each site by two senior pathologists blinded to the other study results. Pathologists were trained before the start of the study in order to harmonize slide interpretation between sites. A quality assessment of over 10% of slides was organized at 6-month intervals by the reference pathology laboratory at Montpellier University Hospital for both sites, in addition to existing internal and external quality assurance schemes adhered to by the National Health Laboratory Service (NHLS) in SA.

The HARP end-point committee composed of 5 pathologists reviewed all histological slides from women with a local diagnosis of CIN2+ and approximately 10% of slides from women with normal or CIN1 histological findings; the final classification of lesions was based on a consensus of the committee.

Data analysis

Women included in this analysis comprise all of the HARP participants who were not lost to follow-up at M18 visit, including women who may have been treated for CIN2+ detected at baseline, except if this had been by hysterectomy. Proportions were compared between groups using Chi-square or Fisher's exact test, as appropriate. Sensitivity, specificity, positive and negative predictive values (PPV and NPV) were calculated with exact binomial 95% confidence intervals (CI), separately for each country first and then for both countries combined (sensitivity and specificity only). Additionally, sensitivity and specificity analysis to detect CIN2+ was stratified by levels of CD4 T-cell counts ($\leq$200 cells/mm^3, 201-350 cells/mm^3, >350 cells/mm^3) at entry in the study and at the time of screening (M18), and by age (<35y and $\geq$35y) and compared across strata using Chi-squared or Fisher's exact tests as appropriate. The comparative analysis of performance of all other methods and triage combinations for the detection of CIN2+ is not reported in this paper. Agreement between the *care*HPV assay and the INNO-LiPA HPV genotyping Extra assay was assessed by percentage overall agreement and prevalence-adjusted bias-adjusted (PABA) kappa coefficient. All analyses were done using the Stata version 14 software (Stata Corp, College Station, TX, USA).

Results

A total of 1249 WLHA were enrolled in the study between November 2011 and October 2012, 625 in BF and 624 in SA. The analysis was based on the 1052 women (94% overall; BF=492; SA=560) who had an adequate *care*HPV result at the M18 visit, which actually occurred at a median 16 months [interquartile range (IQR), 15.5-16.8] (Figure 1). Among those with valid *care*HPV result, 929 (88%; BF=426, SA=503) also had valid cytology results, and 976 returned for colposcopy (93%; BF=469, SA=507). Adequate biopsies were taken from 718 (74%) women, 265 (57%) in BF and 453 (89%) in SA. A total of 225 women (BF=179; SA=46) did not require biopsy as they had no abnormal cytology, VIA/VILI or colposcopy findings and a negative *care*HPV test; they were classified as having "normal/negative" histology. Thirty three women (BF=25; SA=8) did not have biopsy taken for reasons such as pregnancy, pain, cervix atrophy or stenosis. Overall, valid histology results were available for 943/1052 (90%) women, 444 (90%) in BF and 499 (89%) in SA. These women had a median (IQR) CD4+ T-cell count of 495 (355-684) cells/mm^3, 583 (412-813) cells/mm^3 in BF and 438 (331-571) cells/mm^3 in SA. The number of women on ART was 796 (75%), 400 (81%) in BF and 396 (71%) in SA, including those who were initiated onto treatment during the study period.

Overall, 45.1% (474/1052) of women had a positive *care*HPV test, 46.5% (229/492) in BF and 43.8% (245/560) in SA (*P*=0.36). Any HPV DNAwas detected in 83.4% (877/1052) cervical samples, [80.3% (395/492) in BF and 86.1% (482/560) in SA] by the INNO-LiPA genotyping Extra assay. When considering only the 14 HR-HPV types targeted by *care*HPV, 70.0% (736/1052) of samples [67.9% (334/492) in BF and 71.8% (402/560) in SA] were positive by genotyping. The overall agreement between the two tests for HR-HPV DNA detection was 62.4%, with a PABA-Kappa value of 0.25 (95%CI: 0.19-0.31) indicating a fair agreement. However, when analysis was restricted to the 60 women with CIN2+, the agreement between tests was 93.3% with a PABA-Kappa value of 0.87 (95%CI: 0.74-0.99) indicating an excellent agreement. Compared with the INNO-LiPA HPV genotyping Extra assay, analytical sensitivity of *care*HPV for detecting HR-HPV DNA was 55.4% (95%CI: 51.8%-59.1%). The positivity rates of the *care*HPV test according to the HR-HPV types detected by genotyping in single HR-HPV infection cases are presented in Table 1. The detection rates ranged from 40.0% (HPV56) to 86.4% (HPV58).

Cytology results showed that the overall prevalence of high-grade squamous intraepithelial lesions (HSIL) was 10.5% (98/929) with a prevalence of 2.1% (9/426) in BF and of 17.7% (89/503) in SA. As shown in Table 2, the prevalence of HR-HPV detected by *care*HPV or the INNO-LiPA genotyping Extra assay increased with the lesion grade (*P*<0.0001), and the sensitivity and specificity values, combined for both countries, for the detection of HSIL were respectively, 88.8% (87/98) and 61.8% (514/831) for *care*HPV, and 91.8% (90/98) and 33.7% (280/831) for INNO-LIPA genotyping Extra (14 types). The sensitivity of *care*HPV for detecting HSIL was lower in SA than in BF (87.6% vs 100%) but specificity was higher (64.7% vs 56.3%). Negative predictive values for HSIL were 100% (95%CI: 98.4-100.0) and 96.1% (95%CI: 93.1-98.0) in BF and SA, respectively.

A total of 60 (6.4%) women had high-grade (CIN2+) histological lesions, 9 (2.0%) in BF and 51 (10.2%) in SA (*P*<0.0001). Overall, the *care*HPV test was positive in 56/60 (93.3%) women with CIN2+, 9 (100%) in BF and 47 (92.2%) in SA, respectively (*P*>0.5). The performance characteristics of the *care*HPV and INNO-LiPA genotyping Extra assays for the diagnosis of CIN2+ in each country and overall are presented in Table 3. The negative predictive values of both tests were very similar (98.6% to 100%), and when combining the results for the two countries, the *care*HPV test was slightly less sensitive (93.3% vs 96.7%) but more specific (57.9% vs 32.9%) than the INNO-LiPA HPV genotyping Extra assay.

Stratification of results by CD4+ T-cell count showed that there was no significant difference in sensitivities by CD4+ T-cell count levels at study entry or contemporary to CIN biopsy at M18. However, specificities decreased with decreasing CD4+ T-cell counts both at study enrolment (*P*=0.01) and at M18 (*P*=0.0008), which corresponded to increasing HR-HPV prevalences (Table 4). Participants on ART at the time of enrolment had lower HR-HPV prevalence at M18. *care*HPV was slightly more specific in these women (*P*=0.05). There were no significant difference by age (Table 4).

Discussion

Considering the high prevalence of HIV infection in sub-Saharan Africa and the fact that WLHA have an increased risk of development of cervical precancerous and cancerous lesions, it is important to target this population in cervical cancer prevention programs. It is also important to verify that the performance of screening tests is not modified by HIV serostatus. Several studies conducted in China (Qiao et al, 2008), India (Labani et al, 2014), Thailand (Trope et al, 2013), Brazil (Lorenzi et al, 2013), Nigeria (Gage et al, 2012) on clinician- or patient-collected cervical samples have shown that cervical screening based on *care*HPV testing was a feasible and performant strategy in low-resource settings. However, none of these studies had been conducted among WLHA. Thus, the possible impact of HIV infection on the performance of *care*HPV for cervical screening deserved further investigation.

As expected, the positivity rate of *care*HPV increased with the grade of cytological lesions, ranging from 87.6% (SA) to 100% (BF) in women with HSIL. The endpoint in this study was CIN2+ and our results indicate that *care*HPV detected CIN2+ with a high sensitivity (93.3%) and a high negative predictive value (≥99%) in this population of WLHA. The sensitivity of *care*HPV for detecting CIN2+ observed in the present study was very similar to that (94.3%) recently reported in the only other study among WLHA, although specificity was not reported in that Ugandan study (Bansil et al, 2015). The overall specificity of *care*HPV was relatively low (58%) and varied by CD4 T cell strata (between 43% and 62%). A similar finding, albeit with even lower specificity, was reported in a study of a similar group of WLHA in Johannesburg tested with HC2 (Firnharber et al, 2013). The high prevalence of HR-HPV in this highly exposed population, which increases by level of immune-suppression, may lead to low test specificity, as not all HPV infections will progress to CIN2+ lesions, or only much later.

Comparison of the *care*HPV test with the INNO-LiPA HPV genotyping Extra assay showed that *care*HPV is less sensitive than the genotyping assay for the detection of HR-HPV DNA, and that the rate of detection varies according to the different HR-HPV types. However, despite the higher analytical sensitivity of the INNO-LiPA assay for HR-HPV DNA detection, the clinical sensitivity of the two tests for the diagnosis of CIN2+ was very comparable and the *care*HPV test had superior specificity. We may infer that the observed differences in analytical sensitivity of *care*HPV may not affect its usefulness as a screening test. We have previously reported that the HC2 assay, from which the *care*HPV test is derived, had an overall sensitivity and specificity of 88.8% and 55.2%, respectively, to detect CIN2+ lesions in this study population at their first round of screening (Ngou et al, 2015), which would make that HPV assay suitable for screening. The overall sensitivity and specificity of 93.3% and 57.9% observed in the present study indicate that *care*HPV would perform equally well for cervical cancer screening of WLHA in sub-Saharan Africa. Given the relative low specificity of HPV testing, a triage test such as cytology might be required to determine which women should be referred to colposcopy. The cost-effectiveness of this approach should be evaluated. The high prevalence of HR-HPV infection and CIN2+ observed in this study are in agreement with data obtained among similar populations in sub-Saharan Africa (Hawes et al, 2003; Didelot-Rousseau et al, 2006; Singh et al, 2009; Firnhaber et al, 2010; De Vuyst et al, 2012). Interestingly, whilst HR-HPV prevalence as determined by *care*HPV was not significantly different between BF and SA, prevalence of cytological and histological high-grade lesions were markedly higher in SA compared to BF. A similarly high prevalence of lesions among HIV-1-infected women in SA has been already reported (Firnhaber et al, 2013) and factors that may explain the differences observed between these two countries will be further investigated. At least in the HARP study, we can rule out issues of histological misclassification as final histological diagnosis of CIN2+ lesions was established by a consensus Expert Committee reviewing slides from both countries simultaneously.

In conclusion, our results indicate that the *care*HPV test would be a reliable tool for cervical cancer screening in WLHA. Such a cost-affordable test should be considered for implementation in cervical cancer prevention programs in sub-Saharan Africa targeting women living with HIV/AIDS.

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Legend Figure 1:

Study flowchart. BF, Burkina Faso; SA, South Africa.

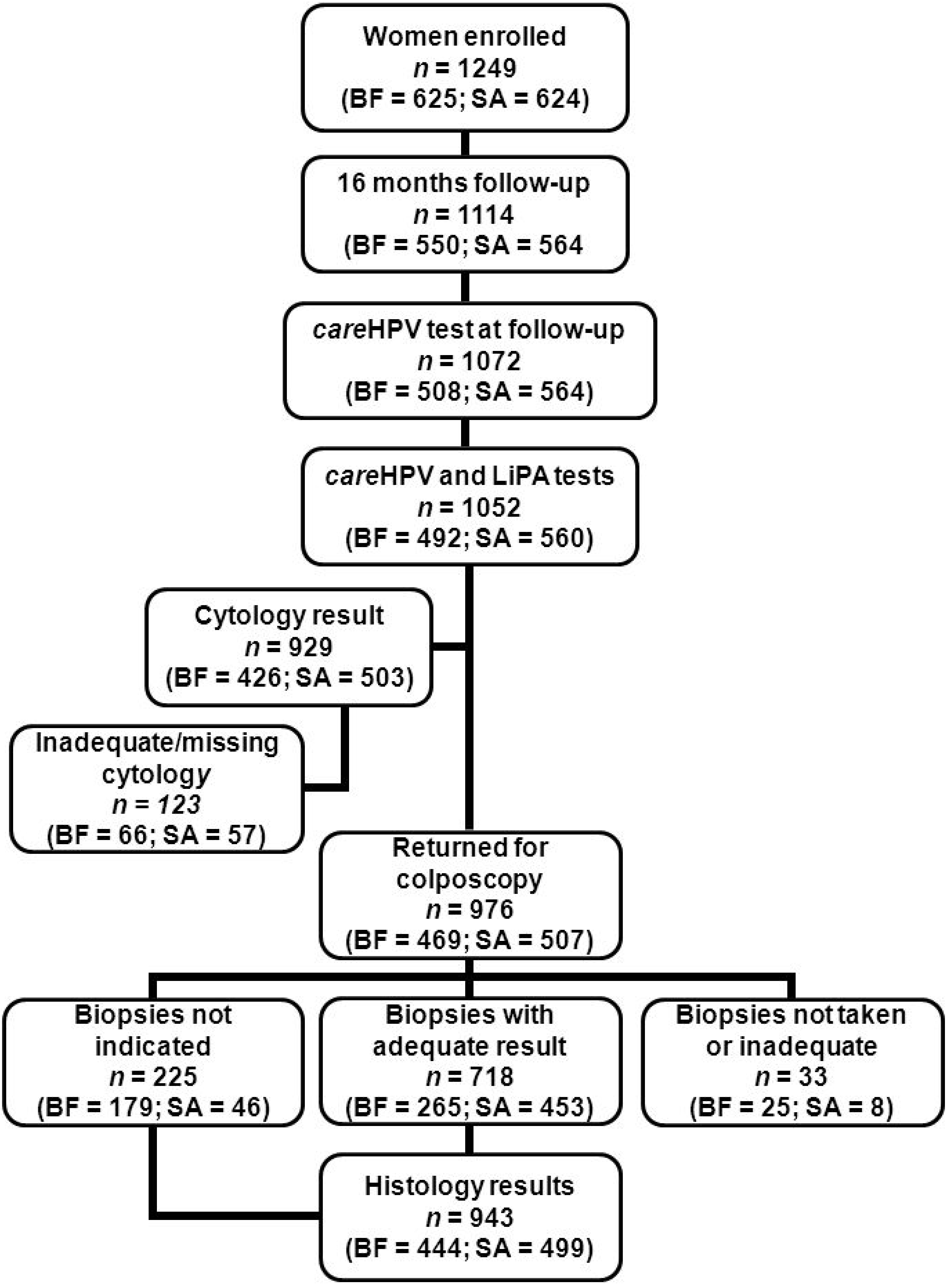
Women enrolled
n = 1249
(BF = 625; SA = 624)
16 months follow-up
n = 1114
(BF = 550; SA = 564)
careHPV test at follow-up
n = 1072
(BF = 508; SA = 564)
careHPV and LiPA tests
n = 1052
(BF = 492; SA = 560)
Cytology result
n = 929
(BF = 426; SA = 503)
Inadequate/missing cytology
n = 123
(BF = 66; SA = 57)
Returned for colposcopy
n = 976
(BF = 469; SA = 507)
Biopsies not indicated
n = 225
(BF = 179; SA = 46)
Biopsies with adequate result
n = 718
(BF = 265; SA = 453)
Biopsies not taken or inadequate
n = 33
(BF = 25; SA = 8)
Histology results
n = 943
(BF = 444; SA = 499)

Table 1. Detection rate of the 14 high-risk HPV types by the *care* HPV test among samples with single-type HPV infection as identified by the INNO-LiPA HPV genotyping Extra assay

	INNO-LiPA	*care* HPV	
HPV types	**No.**	**No.**	**%**
HPV 16	41	19	46.3
HPV 18	33	16	48.5
HPV 31	49	22	44.9
HPV 33	16	10	62.5
HPV 35	36	24	66.7
HPV 39	21	9	42.9
HPV 45	20	16	80.0
HPV 51	23	12	52.2
HPV 52	83	43	51.8
HPV 56	25	10	40.0
HPV 58	22	19	86.4
HPV 59	3	2	66.7
HPV66	27	15	55.6
HPV 68	23	10	43.5
Overall	736	408	55.4

Table 2. Prevalence of HR-HPV detection by *care* HPV and INNO-LiPA genotyping Extra assay according to cytological results

	Burkina Faso (n=426)			South Africa (n=503)		
Cytology	**No.**	***care* HPV-positive (%)**	**INNO-LiPA-positive (%)**	**No.**	***care* HPV-positive (%)**	**INNO-LiPA-positive (%)**
No anomalies	368	148 (40.2)	240 (65.2)	14	4 (28.6)	6 (42.9)
Atypical cells[a]	16	9 (56.2)	12 (75.0)	84	28 (33.3)	58 (69.0)
LSIL	33	25 (75.8)	28 (84.8)	316	114 (36.1)	215 (68.0)
HSIL+	9	9 (100)	9 (100)	89	78 (87.6)	81 (91.0)
P [b]		<0.0001	0.0016		<0.0001	0.0011

[a] Atypical squamous cells of undetermined significance (ASC-US), n=13 (BF) and n=52 (SA); Atypical squamous cells, cannot exclude HSIL (ASC-H), n=2 (BF) and n=32 (SA); Atypical glandular cells (AGC), n=1 (BF)

[b] Test for trend

Table 3. Performance of the *care* HPV and INNO-LiPA assays for the diagnosis of CIN2+ lesions (n=943)

	Burkina Faso (CIN2+, n=9)		South Africa (CIN2+, n=51)		Overall (CIN2+, n=60)	
	***care* HPV**	**INNO-LiPA**	***care* HPV**	**INNO-LiPA**	***care* HPV**	**INNO-LiPA**
Performance indicators						
No. of positive tests	206	300	222	358	428	658
No. of CIN2+ positive by test	9	8	47	50	56	58
Sensitivity %	100 (66.4-100)	88.9 (51.7-99.7)	92.2 (81.1-97.8)	98.0 (89.6-99.6)	93.3 (83.8-98.2)	96.7 (88.5-99.6)
Specificity %	54.7 (49.9-59.5)	32.9 (28.5-37.5)	60.9 (56.3-65.5)	31.3 (27.0-35.8)	57.9 (54.5-61.2)	32.0 (29.0-35.2)
PPV %	4.4 (2.0-8.1)	2.7 (1.2-5.2)	21.2 (16.0-27.1)	14.0 (10.6-18.0)	-[a]	-
NPV %	100 (98.5-100)	99.3 (96.2-100)	98.6 (96.3-99.6)	99.3 (96.1-100)	-	-

[a] PPV (positive predictive value) and NPV (negative predictive value) have not been combined for the two countries as they depend on disease prevalence/incidence which are different for each country.

Table 4. Effect of baseline or contemporary CD4 count, age and antiretroviral therapy (ART) on the performance of *care* HPV

	Burkina Faso n (%) *care* HPV+	South Africa n (%) *care* HPV+	CIN2+ n	Sensitivity % (95%CI)	P value (Fisher's Exact)	Specificity % (95%CI)	P value (chi-squared for trend/chi-squared)
Baseline CD4 count, cells/mm^3							
<200	35 (64.1)	23 (50.0)	10	80.0 (44.4-97.5)	0.2	45.7 (34.6-57.1)	0.01 (for trend)
201-350	49 (48.5)	62 (47.7)	12	100.0 (73.5-100)[a]		55.9 (48.6-63.0)	
>350	145 (43.0)	158 (41.5)	37	94.6 (81.8-99.3)		60.2 (56.1-64.1)	
Contemporary CD4 count, cells/mm^3							
<200	20 (69.0)	14 (41.2)	6	100.0 (0.54-100)[a]	>0.9	50.9 (36.8-64.9)	0.0008 (for trend)
201-350	40 (69.0)	68 (56.7)	15	93.3 (68.1-99.8)		42.6 (34.4-51.0)	
>350	157 (41.0)	159 (40.2)	38	92.1 (78.6-98.3)		61.8 (57.9-65.5)	
Age, years							
<35	100 (50.3)	140 (48.8)	36	91.7 (77.5-98.2)	0.6	54.8 (49.8-59.8)	0.1
≥35	129 (44.0)	105 (38.5)	24	95.8 (78.9-99.9)		60.3 (55.8-64.6)	
ART status							
On ART before enrolment	156 (43.9)	147 (40.7)	38	89.5 (75.2-97.1)	0.4	60.6 (56.5-64.5)	0.05
ART initiated after enrolment	24 (53.3)	17 (48.6)	3	100 (29.2-100)[a]		49.3 (37.2-61.4)	
Not on ART	49 (53.3)	81 (49.4)	19	100 (82.4-100)[a]		52.9 (45.9-59.9)	

[a] one-sided 97.5% CI