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Development of a screening algorithm for the cervical cancer prevention programme in Botswana

Thesis submitted in partial fulfilment of the requirements for the degree, PhD in Obstetrics & Gynaecology, in the Faculty of Health Sciences, University of Pretoria

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DECLARATION

I declare that this thesis, which I hereby submit for the degree Doctor of Philosophy in Obstetrics and Gynaecology at the University of Pretoria, Faculty of health Sciences, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

DEDICATION

I dedicate this work to my late grandmother, Watsie Ramosweu, who encouraged me during the analysis phase and initial write up of this thesis as she was battling her own terminal illness. I had hoped she would have lived long enough to see me triumph at the end. Spending the last months of your life listening to all the stories strengthened my faith, taught me forgiveness, and unconditional love.

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ABSTRACT

Background

Although cervical cancer is preventable, it continues to be one of the most common malignancies globally. Women living with human immunodeficiency virus (HIV) are at increased risk for cervical cancer; and the two diseases often overlap in low-resource settings where cervical cancer tends to be most prevalent.

There are four main steps in the development of cervical cancer: 1. Infection with oncogenic human papillomavirus (HPV); 2. Persistent infection with the virus; 3. Progression to pre-cancer in infected cells; 4. Progression to cancer and invasion.
Cervical cancer lends itself well to screening. The purpose of screening is to reduce incidence and mortality through detection and treatment of pre-cancer lesions. For more than half a century, cervical cytology, (Pap smear), an exfoliative cytology test, has been the mainstay of secondary prevention of cervical cancer in most industrialized settings where it has substantially impacted the incidence and mortality due to cervical cancer.

Establishing a cytology-based cervical cancer prevention programme in most low-resource settings has proven difficult. This difficulty has prompted research into evaluating alternatives such as screening using visual inspection with acetic acid (VIA), a same-visit clinical test that provides immediate results. Although VIA has operational benefits, its real-world sensitivity has significantly varied due to substantive differences in training, monitoring, and lack of standardised and objective criteria for positivity.

Testing for carcinogenic HPV in cervical specimens offers the most biologically salient and highly sensitive method of screening. The World Health Organization (WHO) has, therefore, recommended HPV testing as a primary screening tool where resources permit. While HPV testing as a primary screen is attractive, it must be done in conjunction with some other method to identify women who need further assessment or treatment for cervical pre-cancer. Both VIA and colposcopy can be used as triage methods but the evidence is limited in settings of high HIV burden, and almost non-existent for HIV-negative women. The aim of this thesis was to evaluate VIA,

colposcopy, and several partial genotyping methods as potential triage tests and propose an evidence-based screening algorithm(s) for the Botswana's national cervical cancer prevention programme.

Methods

This prospective clinical study evaluated various cervical cancer screening algorithms' performance following a positive high-risk HPV (hrHPV) screening result. A total of 1500 women living with HIV (WLWH) and HIV-negative women in the ratio of 1:1 and 25 years and older, were enrolled into the study. All women underwent primary cervical cancer screening with the AmpFire Multiplex hrHPV DNA assay using self-collected swabs. Women testing positive for the15 hrHPV (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68) and an additional 10% testing negative for hrHPV were called back for visual evaluation (VIA and colposcopy). The treatment decisions were based on colposcopy findings. Women with a visual lesion received treatment with loop electrosurgical excision procedure (LEEP), and those with no lesion or whose examination was inadequate received a biopsy or an endocervical curettage to ensure all women had a histological endpoint. The primary endpoint was cervical intraepithelial neoplasia grade 2 or worse (CIN 2+). Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) of hrHPV followed by triage with VIA, colposcopy, and three partial genotyping protocols (HPV 16/18; HPV 16/18/45; and HPV 16/18/31/33/35/45/52/58) were calculated as screening algorithms.

Results

Among 750 WLWH screened, 392 (52%) had hrHPV, and 314/750 (42%) HIV-negative women had hrHPV. CIN2+ prevalence in the screening population of WLWH was 9.6% (69/721) and it was 6% (44/728) among HIV-negative women. Among the hrHPV-positive women, CIN 2+ was detected in 19% (69/363) of WLWH, and 15% (44/292) of the HIV-negative women. Nearly a quarter of all CIN 3+ disease was detected in women 50 years and older in both WLWH and HIV-negative women who would normally be excluded by the screening target age.

The performance of the various algorithms was as follows for WLWH: Primary hrHPV in a 'screen-and-treat' setting- (sensitivity: 100%, specificity: 10%, PPV: 19%, NPV:

100%); hrHPV followed by VIA triage- (sensitivity: 61%, specificity: 73%, PPV: 33%, NPV: 89%); and hrHPV followed by colposcopy triage- (sensitivity: 68%, specificity: 70%, PPV:35%, NPV: 90%). The performance of partial genotyping was as follows: HPV 16/18- (sensitivity 35%, specificity: 79%, PPV: 26%, NPV: 85%); HPV 16/18/45- (sensitivity 42%, specificity71%, PPV24%, NPV85%); HPV 16/18/31/33/35/45/52/58- (sensitivity: 87%, specificity: 37%, PPV:23%, NPV: 93%).

The performance of the various algorithms was as follows for HIV-negative women: Primary hrHPV in a 'screen-and-treat' setting- (sensitivity:100%, specificity: 18%: PPV: 15%, NPV:100%); hrHPV followed by VIA triage- (sensitivity: 39%, specificity: 79%, PPV: 25%, NPV: 88%); and hrHPV followed by colposcopy triage- (sensitivity: 39%, specificity: 74%, PPV: 21%, NPV:87%). The performance of partial genotyping was as follows: HPV 16/18- (sensitivity: 27%, specificity: 85%, PPV: 21%, NPV:89%); HPV 16/18/45- (sensitivity: 32%, specificity: 78%, PPV: 17%, NPV: 89%); HPV 16/18/31/33/35/45/52/58- (sensitivity: 86%%, specificity:48%%, PPV:20%, NPV: 96%).

Conclusions

As Botswana prepares for the introduction of primary screening with hrHPV testing for the national cervical cancer prevention programme, utilising self-collected swabs will be a valuable strategy to increase access. Any new screening programme will need to consider risk mitigation for women in the older birth cohorts who would be missed by the screening target age of 25 to 49. These women are at high risk for invasive cancer and need special attention until the maturation of the screening programme.

Using any hrHPV test in a "screen-and treat" setting resulted in the treatment of most, if not all women with CIN 2+ in the screening population. However, this approach would lead to overtreatment of many women with no established cervical precancer. Therefore, adopting this strategy for the national screening programme can overwhelm limited resources, and result in loss of faith in the healthcare system. Triage of hrHPV-positive women by VIA lead to both over- and -undertreatment of many women. The 8-HPV-restricted genotyping provided the best triage sensitivity, and it is recommended for the Botswana cervical cancer prevention programme. However, given the relatively low specificity of the 8-hrHPV method, further research is needed

to develop other triage methods that are better at identifying women with established precancer among those with transient hrHPV infection.

Keywords

Botswana, Human papillomavirus genotyping, Human immunodeficiency virus, cervical cancer screening, visual inspection after acetic acid, colposcopy, histological verification, cervical intraepithelial neoplasia, test performance, AmpFire HPV DNA assay

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LIST OF ABBREVIATIONS

ACTG	AIDS Clinical Trials Group
ART	Anti-retroviral therapy
BLH	Bamalete Lutheran Hospital
BIDMC	Beth Israel Deaconess Medical Centre
CD4 count	T-cell count
CE	Conformité Européenne
CIN 1	Cervical intraepithelial neoplasia grade one
CIN 2+	Cervical intraepithelial neoplasia grade two or worse
CO_2	Carbon dioxide
Co-PI	Co-Principal Investigator
COVID-19	Corona virus disease 2019
CY5	Indodicarbocyanine
DNA	Deoxyribonucleic acid
FAM	6-Carboxyfluorescein
FDA	Food and drug administration
HEX	Hexachloro-6-carboxyfluorescein
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
hrHPV	High-risk human papillomavirus
IARC	International Agency for Research on Cancer
ID	Identity
IRBs	Institutional review boards
LEEP	Loop electrosurgical excision procedure

lrHPV	Low-risk human papillomavirus
LMICs	Low and middle-income countries
MOH	Ministry of Health
N_2O	Nitrous Oxide
NCD	Non-communicable disease
NCI	National Cancer Institute
NPV	Negative predictive value
PCR	Polymerase chain reaction
PI	Principal Investigator
PMH	Princess Marina Hospital
PPV	Positive predictive value
qPCR	quantitative polymerase chain reaction
RA	Research assistant
REDCap	Research Electronic Data Capture
ROX	6-Carboxy-X-rhodamine
SOP	Standard operating procedure
UB	University of Botswana
UP	University of Pretoria
USA	United States of America
USD	United States Dollar
URL	Uniform Resource Locator
VIA	Visual inspection with acetic acid
WHO	World Health Organization
WLWH	Women living with HIV
ZAR	South African Rand

CHAPTER 1. BACKGROUND AND LITERATURE REVIEW

1.1. Introduction and epidemiology

Although cervical cancer is preventable, it continues to be one of the most common malignancies globally, and its incidence is steadily increasing.[1] Global trends show that cervical cancer has dropped from being the third to the fourth most frequently occurring malignancy in women; however, it remains the leading cancer among women in some parts of the world, including sub-Saharan Africa (sSA). It was estimated that worldwide around 530,000 new cervical cancer cases and 270,000 deaths occurred in 2008[2], as compared to 570,000 new cases and 311,000 deaths in 2018.[1] Further, the proportion of cervical cancer cases occurring in low-resource countries remains relatively unchanged. In Botswana, cervical cancer is the leading cancer cause of death in women.[3]

Compared to most cancers, cervical cancer is unique in that it has a clear and well-understood aetiology. This understanding is due to various breakthroughs over the last several decades, most notably the discovery of human papillomavirus (HPV) as the causative agent of cervical cancer.[4,5] Attempts to understand cellular changes associated with, and possibly causing, cervical cancer started in the 1960's. In 1975 zur Hausen put forward the theory of HPV as a primary cause of cervical cancer.[6] An aetiologic agent was first established in an abnormal cervical koilocytic cytological finding in 1976, but was initially suspected to be a herpes virus.[7] HPV 16 was first identified in deoxyribonucleic acid (DNA) extracted from an invasive cervical cancer biopsy specimen in 1983,[8] followed closely by identification of HPV 18 in 1985.[9] Thereafter several reports confirmed HPV infections as causative agents of cervical.[5,10-12] The strong association of certain types of HPV to cancer is now widely known,[10] and the 570,000 cases of cervical cancer reported in 2018 represent 80% of all HPV attributable cancers globally.[13]

HPV is a non-enveloped, double-stranded, circular, and icosahedral DNA virus of the Papillomaviridae family.[14] More than 200 HPV subtypes have been identified, of which about 40 are known to infect the ano-genital region. These have been grouped

according to their oncogenic potential into low-risk (lr) and high-risk (hr).[15] The hrHPV group is aetiologic for invasive cervical cancer and its precursor lesions in women.[16] The following cancer related subtypes of HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 have been classified as hrHPV, or International Agency for Research on Cancer (IARC) class 1.[17] Two hrHPV types (16 and 18) account for around 70% of invasive cervical cancers and 50% of pre-cancers worldwide.[15,16,18,19] Although infection with HPV is common, most infections are transient and do not result in long term disease.[20-22] Studies have shown that up to 90% of new HPV infections will clear within 12-24 months.[23,24] Development of precancer (CIN 3) can develop within 3 years of persistent high-risk HPV infection. Most of the invasive cervical cancer cases develop within 5-10 years of CIN 3.[24]

Women living with human immunodeficiency virus (HIV) are at increased risk for HPV-associated disease, particularly cervical cancer.[25,26] This higher burden of HPV-associated disease in women living with HIV (WLWH) compared to HIV-negative women has been shown in studies of HPV prevalence and precancerous lesions, as well as those of invasive disease.[27-30] These studies have revealed prevalence rates of 30-80%, 10-40% and 1.3-1.7% for hrHPV, precancerous lesions, and invasive cervical cancer, respectively, in WLWH.

Before the widespread availability of combination antiretroviral treatment (ART), most WLWH in low-resource settings died due to other acute HIV-related conditions before cervical cancer had time to develop. Even though ART is now readily available, evidence regarding the impact of ART on prevalence and incidence of hrHPV, precancer and invasive cervical cancer has been inconsistent.[31] With increased survival due to ART,[32-34] many WLWH are now at an increased risk for developing cervical cancer, given that older age independently increases the likelihood of cancer.[35-37] Improved survival also increases potential exposure time to hrHPV leading to increased likelihood of progression to precancer and invasive cancer.[38-40] However, there are also studies that report no impact of ART on prevalence and incidence of hrHPV, precancer, and invasive cancer,[41-43] and more recently, those that support improved control of hrHPV and reduced progression to precancer following long term use of ART.[36,44] According to Bratcher and colleagues, the inconsistency of earlier studies “was likely due to different study designs,

screening and diagnostic protocols, duration and type of ART use, recruitment and referral strategies, and definitions of screening test and disease positivity",[31] challenges that more recent studies have tried to overcome.

There are four main steps in the development of cervical cancer: 1. Infection with oncogenic human papillomavirus; 2. Persistent infection with the virus; 3. Progression to precancer in infected cells; 4. Progression to cancer and invasion.[24,45] The aetiologic understanding of the natural history of cervical cancer has led to highly effective preventative approaches. Currently, the prevention of cervical cancer depends on three main strategies: 1. primary prevention of HPV infection through vaccination with an HPV vaccine; 2. secondary prevention through screening and identification of pre-cancer lesions, followed by 3. appropriate treatment of pre-cancer lesions.[46]

1.2. HPV vaccination

Primary prevention is possible through HPV vaccination. There are three types of HPV vaccines licensed by the Food and Drug Administration (FDA) of the United States of America (USA). These vaccines have been found to be safe and highly effective against vaccine-type HPV infection and precancerous lesions following large international randomised control clinical trials,[47-49] and a robust systematic review and meta-analysis.[50] These are the bivalent and quadrivalent vaccines licensed in 2006/7 and the nonavalent vaccine licensed in 2014. Both bivalent and quadrivalent vaccines are protective against the hrHPV 16/18. The quadrivalent vaccine has additional protection again low-risk HPV 6/11 which are responsible for up to 90% of the genital warts.[51] The nonavalent vaccine builds on the quadrivalent vaccine with a 20% additional protection against invasive cervical cancer through the inclusion of hrHPV 31/33/45/52/58.[52,53]

The safety of the bivalent vaccine was shown in a randomised control trial in South Africa involving 120 WLWH aged 18-25 years.[54] Similarly, the safety of the quadrivalent vaccine was shown in a randomised control trial involving 319 WLWH aged 13-45 in Brazil, South Africa and USA.[55] Several studies have subsequently confirmed the safety of both vaccines.[56-58] Although the safety of the nonavalent

vaccine has been shown in healthy populations,[59] safety data in people living with HIV is not yet available.

Both the bivalent and quadrivalent HPV vaccines have been shown to be immunogenic in people living with HIV, the antibody response has been relatively lower than in HIV-negative people.[60,61] However, the significance of the lower antibody response is unknown.

1.3. Screening

Cervical cancer lends itself well to screening, as the disease fulfils the classic 'Wilson and Jungner' screening criteria.[62] A systematic review and meta-analysis of cervical cancer screening has shown its protective benefits.[63] Cervical cancer screening takes place in an asymptomatic population at risk of developing the disease. The purpose of screening is to reduce incidence and mortality by early detection and treatment of pre-cancer lesions.[64,65]

When considering a screening strategy, the screening test's characteristics need careful consideration as there is no perfect test. Trade-offs are related to balancing the harmful effects of not detecting the people who have the disease (false negatives), especially in settings where the screening interval is long versus the identification and unnecessary treatment of those with a transient disease (false positives). Cervical cancer is a rare preventable life-threatening disease, and therefore, a highly sensitive test is preferable to detect as many people with abnormal lesions as possible. However, the high sensitivity needs to be balanced with reasonable specificity to avoid excess false-positive results which can lead to overtreatment, especially in the “screen-and-treat” setting.

1.3.1. Cytology

For more than half a century, an exfoliative cytology test of the cervix pioneered by Georgios Papanikolaou,[66] called the Pap smear has been the mainstay of secondary prevention of cervical cancer in most industrialized settings. Although the Pap smear

test has been recognised as one of the most significant advances in cancer control in the 20th century, its efficacy was never subjected to large randomised controlled trials before widespread implementation. However, it has been shown through extensive epidemiologic studies that implementation of organised cytology-based cervical screening has reduced the prevalence and mortality rate of cervical cancer in well-resourced nations.[67-69] The incidence of cervical cancer was reported to be 15 and 4.0 per 100,000 finish women in 1964 and 2003, respectively. Organised cervical cancer screening was introduced in Finland in 1963. The 60% decline in cervical cancer mortality in England and Wales observed in 1997 in women over the age of 55 was in-part credited to the national cervical cancer screening programme launched in 1988.[68] The cervical cancer rates declined by 29.1 per 100,000 women between the 1930s and 1990s in the United States of America.[69] The success of cytology-based screening in these settings is attributed to the repeated (in some cases annual) nature of screening, rather than the accuracy of a one-time cytological screening test, which can miss anywhere between 30-40% of precancerous lesions.[70] Furthermore, once women are identified as likely to have precancer, they need to be efficiently linked to diagnostic and treatment services for the screening to be effective in preventing cervical cancer.

Establishing a cytology-based cervical cancer prevention programme in most low-resource settings has proven difficult.[71,72] In these countries, availability of such screening remains inconsistent. It is also unlikely to reach the level of organisation and coverage required to make it useful.[73,74] This is one of the major reasons for the global disparity in cervical cancer incidence in low and middle-income countries (LMICs)[75] compared to high-resource countries.

1.3.2. Visual inspection with acetic acid

Limited healthcare access in developing countries necessitates maximising the yield of a singular opportunity for screening. This has prompted research evaluating alternatives to cytology-based screening, such as screening using visual inspection with acetic acid (VIA), a same-visit clinical test that provides immediate results.[72,74,76] VIA can be taught to non-physician health care providers and can be performed at low

operational costs with minimal additional investments. Women who screen positive with VIA can be given same day visit treatment, even without a confirmatory diagnostic assessment. The same day visit treatment takes away the need for additional clinic visits and expensive and logistically challenging linkages with pathology laboratories. Such 'screen-and-treat' protocols (screen with VIA and treat using simple methods like cryotherapy or thermal ablation) have an operational goal of minimising the women lost-to-follow-up. Women identified with large or inaccessible lesions from VIA need a referral for diagnostic colposcopy and cervical biopsy, followed by excisional treatment of biopsy-confirmed lesions using loop electrosurgical excision procedure (LEEP) where available.

Although VIA has operational benefits, its real-world sensitivity has significantly varied due to substantive differences in provider training, monitoring, lack of standardised and objective criteria for positivity,[77,78] and unreliably conducted (or virtually absent) quality assurance procedures.[79] On the other hand, testing for carcinogenic HPV in cervical specimens (HPV testing) offers the most biologically salient and highly sensitive method of screening.[80]

1.3.3. HPV testing

The establishment of HPV as a causative agent for cervical cancer further enabled the development of HPV-based screening tests. In 1989, Tidy and colleagues reported the first detection of HPV 16 DNA by polymerase chain reaction (PCR) from cervical scrapes of two and 12 women with normal and abnormal smears, respectively.[81] This led the authors to predict the potential clinical application of HPV testing to supplement the Pap smear in cervical cancer screening. Cox and colleagues reported their evaluation of the first FDA-approved ViraPap HPV test for clinical use in 1992.[82] Their study determined the usefulness of the HPV test as a triage method for predicting the likelihood of women referred to colposcopy to have histologically confirmed cervical intraepithelial neoplasia (CIN) disease. Four hundred and eighty-two young women each had an HPV test, a Pap smear, and colposcopy. HPV positivity was strongly associated with histologically confirmed CIN disease ($p < 0.001$).

The creation of a new abnormal cytology category called atypical squamous cells of undetermined significance (ASCUS) for Pap smears that could not be reliably designated normal or abnormal followed a National Cancer Institute (NCI)-led workshop in 1988.[83] The women designated to this category dramatically increased the number referred to colposcopy. Although many had no disease or only had low-grade disease, more high-grade lesions were diagnosed in women with ASCUS than any other category due to the sheer numbers in this category. Subsequent NCI workshops in 1991 and 2001 led to the creation of the two categories of atypical squamous cells of undetermined significance (ASC-US) and atypical squamous cells cannot rule out high-grade (ASC-H).[12] This spurred the earnest evaluation of HPV as a potential triage method. For example, Cox and colleagues reported a sensitivity of 93% for CIN 2+ and a reduction of 58% in colposcopy referral of ASCUS women if only those positive for HPV were evaluated.[84] Subsequent to this report and other reports,[85,86] the American Society for Colposcopy and Cervical Pathology (ASCCP) recommended HPV testing for triage of ASC-US lesions in their 2001 guidelines.[87]

In their review in 2003, Crum and colleagues predicted the 20-year evolution of cervical cancer screening through four stages.[88] The first stage was seen as the end of traditional screening with cytology and the beginning of the second phase of utilising HPV testing for managing cytologic abnormalities and possibly for primary screening. The third phase proposed the use of host and/or viral biomarkers to increase specificity and positive predictive value for present and future cervical epithelial neoplasia grade 2 or worse (CIN 2+) disease. The fourth predicted the use of HPV testing in the era of HPV vaccines.

Crum's third phase began to be realised with the understanding of the differential oncogenic risk of the various hrHPV types and the potential use of HPV genotyping as risk stratification. Compared to women with other hrHPV types,[11] more intensive follow-up was proposed for women with HPV 16/18/45 due to their elevated risk for cervical cancer. The elevated risk for both cervical squamous cell carcinoma and adenocarcinoma was confirmed by de Sanjose and colleagues in 2010.[19] Several researchers further established the similarity of oncogenic risk conferred by HPV 31/33/52/58 to that of HPV 18,[89-91] and the need for more nuanced management algorithms of cytology screen-positive women. Therefore, while HPV testing as a

primary screen is attractive, it must be done in conjunction with some other method to identify women who need further assessment or treatment for cervical precancer. More recently, the role of HPV-genotyping for the triage of screen-positive women has gained momentum.[92]

1.3.4. VIA and HPV screening in women living with HIV

VIA and HPV testing have been shown to perform adequately among WLWH. Studies have shown that the sensitivity of VIA among WLWH to detect cervical intraepithelial neoplasia grade two or more severe lesions (CIN2+) ranges between 65% and 86%, and specificity between 51% and 83%.[35,93-95] The sensitivity range is similar to or somewhat higher than corresponding contemporaneous estimates among HIV-negative women. There is evidence that with low T-cell (CD4) count (i.e., significant immunosuppression), the sensitivity is higher,[96] likely reflecting the larger sized aceto-white lesions that are easier detected by visualisation. On the other hand, VIA's specificity in women living with HIV is affected by the higher rates of underlying cervicitis or concurrent sexually transmitted infections,[94,97] resulting in aceto-white like false-positive lesions that may also lead to overtreatment of these women. Nonetheless, most of this topic's studies have design inadequacies and consequent limitations such as verification bias, use of inappropriate comparison groups, and lack of quality assurance of pathologic endpoints.

Studies evaluating HPV screening in HIV-positive women have reported a range of sensitivities between 84 and 94% and specificities between 51 and 56%.[35,93,96,98] These studies have differed in the choice of their HPV assay methodologies, specimen collection (clinician-collected cervical swabs vs. self-collected vaginal swabs), and processing methods (batched vs. random access). The hrHPV prevalence is high among WLWH, while the specificity of HPV testing for CIN2+ is low. However, the positive predictive value (PPV) of HPV testing is high among inadequately screened populations. Modelling estimates suggest that while the number of women who will screen positive by HPV and need triage will be high, the final number of HPV-positive women with detected cervical precancer will be ultimately low.[99,100] HPV testing will, therefore, achieve higher overall screening efficiency, provided younger women are

excluded (less than 25 years old).[101] However, this needs to be proven by empiric evidence, particularly from implementation studies. The development and evaluation of appropriate triage approaches (e.g., visual, and other low-cost molecular tests) are also critical.

1.4. Treatment of pre-cancers in low-resource settings

The focus of most cervical cancer prevention initiatives in low-resource settings has been to treat screen-detected precancerous lesions by same-visit treatment approaches ('screen-and-treat') without the need for an intermediate histological verification step.[102] These programmes have commonly utilised ablative techniques due to their relative ease of use and affordability.[103]

1.4.1. Cryotherapy

The most common ablative treatment approach is cryotherapy. This involves freezing the cervical precancerous lesions by a metal probe cooled by highly compressed refrigerant gases like nitrous oxide (N_2O) or carbon-dioxide (CO_2) to temperatures less than or equal to -50°C.[104] Freezing of the transformation zone removes the precancer through cellular necrosis. Cryotherapy does not require local anaesthesia or electricity (though it needs to rely on a supply of easily transportable tanks of the refrigerant gases). Cryotherapy can be feasibly implemented by nurses (or other non-physician health care providers) and scaled-up in low-resource settings, albeit with sustained investments and efforts.[105] However, there are several challenges related to cryotherapy, including the cumbersome nature of the gas tanks used and the difficulty in procuring high-quality refrigerant gas.[103]

1.4.2. Thermal ablation

Thermal ablation locally destroys the abnormal cervical epithelium by heating it to 100°C thus inducing tissue necrosis. The method uses electricity with a treatment time ranging between 20 and 60 seconds, shorter than cryotherapy. Similar effectiveness between the two methods, cryotherapy, and thermal ablation, has been shown in

pooled analysis of published studies (mainly observational).[106,107] The effectiveness was recently confirmed in a randomised control trial conducted in Nigeria, which also reported its cost-effectiveness.[108] Both cryotherapy and thermal ablation had similar cure rates, with a slightly higher mean patient satisfaction score and no side effects for the latter. Cryotherapy had a significantly higher mean cost per patient than thermal ablation. The first battery-operated thermal ablation was compared with cryotherapy in Zambia.[109] The added benefits of this device are its portability and its non-dependence on a continuous supply of electricity, a practical feature that makes it ideal for outreach service and use in most LMICs. The inclusion of WLWH in the Zambia study provides the reassurance that thermal ablation is safe and effective in this population, even though its effectiveness was slightly less than that observed in HIV-negative women. The less cumbersome thermal ablation has now become the preferred ablative treatment method due to its shorter treatment time, non-dependence on refrigerant gas, and cost effectiveness.

1.4.3. Loop electro-surgical excision procedure

Women who have large cervical lesions or have lesions extending into the endo-cervical canal are ineligible to be treated with ablative techniques. Excisional approaches such as loop electro-surgical excision procedure (LEEP), or conisation are necessary. The proportion of cryotherapy-ineligible lesions has been reported to be between 20-30% among WLWH in large scale implementation studies[74,110] and nested analyses within clinical trials.[33] LEEP uses wire loop electrodes to remove precancerous cells from the transformation zone and provides the advantage of allowing histopathologic verification of excised lesions. If there is evidence of residual disease from the excised margins, this can predict the likelihood of disease recurrence.[111,112] Excisional approaches require a higher level of provider skills, equipment, and infrastructure, including local anaesthesia, smoke evacuation specula, and back-up surgical services. That said, post-treatment complications are reported to be rare, and studies have not reported higher rates of complications among WLWH versus HIV-negative women.[30,113]

1.4.4. Treatment challenges of women living with HIV

There are concerns about suboptimal treatment effectiveness (as measured by rates of recurrence), particularly among HIV-positive women.[114-116] Whether this recurrence reflects procedural inefficiencies or is a reflection of the underlying higher risk of latent reactivating HPV disease, especially in the context of immunosuppression, has not been adequately addressed.[112] There are additional concerns about the increased risk of HIV shedding after cryotherapy, but its impact on increasing HIV transmission rates, particularly among women not receiving ART, has not been studied.[117,118]

From an implementation point-of-view, cryotherapy, or thermal ablation for precancer treatment are preferred due to the logistical advantage of being able to offer same-visit treatment. However, this same-visit treatment advantage may not be necessary for WLWH receiving ongoing care, as they are often more amenable to repeat visits.

The efficacy and feasibility of offering LEEP as the frontline treatment instead of cryotherapy have been evaluated in two randomised clinical trials for WLWH with confirmed CIN2+.[119,120] Although the South African trial of 166 women showed lower cumulative CIN2+ rates in the LEEP treatment group (10.8% versus 16.1%) at six months, the effectiveness of both treatments was similar at 12 months (>70%). In contrast, the LEEP treatment group in the Kenyan trial of 400 women had a lower recurrence rate of CIN2+ at 24 months (19% versus 30%). However, before considering LEEP treatment instead of cryotherapy for a national programmatic strategy in HIV-infected women, its benefits need to be weighed against the additional resources required for LEEP and the likely loss-to-follow-up of a two-step process.

1.5. Research problem

Botswana has one of the highest cervical cancer burdens worldwide,[3] driven by the high prevalence of HIV in the country.[121] The country has achieved significant HIV treatment success due to its pioneering program to provide universal government-funded access to ART.[122] Such success has led to increases in HIV prevalence and life expectancy, which both independently contribute to the high cancer incidence.

Botswana is undergoing an epidemiological transition as cancer and other non-communicable diseases (NCDs) start to replace infectious diseases as leading causes of morbidity and mortality.[123]

The available data from manual case-finding suggests that cancer accounts for 7% of all deaths in Botswana.[3] From 2005 to 2012, 11,398 cancers were diagnosed and registered in the population-based Botswana National Cancer Registry.[123] Amongst women's deaths, the top three cancers were cervical cancer (26%), breast cancer (16%), and Kaposi's sarcoma (14%).[123] The staging at diagnosis varies as a large proportion of the cancers are not reported to the national cancer registry; however, amongst the 15% of cancers reported, the majority were at advanced stage.[123]

Currently available screening methods in Botswana for cervical cancer, such as 'see and treat' using VIA,[76,123,124] and cytology have inherent limitations that further impact early detection of cancers.[125] Both methods rely heavily on sampling techniques. They also rely on health workers' ability to discriminate between healthy tissue, malignancy, or inflammation.[78,126] The cytology test has a higher sensitivity for high-grade lesions (CIN 2+) compared to VIA.[127] However, cytology requires regular follow-up systems to impact cervical cancer incidence and has remained an implementation challenge in most LMICs, including Botswana.

HPV-based testing programmes have demonstrated a 60-70% greater protection against invasive cervical cancers compared with cytology.[128] Hence, primary hrHPV testing has become first-line screening for cervical cancer in many high-income countries.[128,129] The feasibility of this approach in LMICs is less clear, as is the role of hrHPV testing among WLWH.[130,131]

Botswana has utilised a combination of cytology and VIA for screening, and cryotherapy or LEEP for treatment within a 'see and treat' setting due to its unique resource environment.[76,124] The World Health Organization (WHO) recommends using a 'screen and treat' approach using VIA for screening, treatment with cryotherapy or, where feasible, HPV testing followed by treatment.[132] The Botswana Ministry of Health (MOH) has piloted primary hrHPV testing for cervical cancer screening. Nevertheless, national guidelines for the management of positive hrHPV results are not yet

available.[133] The current practice in the southern African region, especially in research settings, has been to triage hrHPV screen-positive women with VIA.[131,134] However, preliminary data from Botswana suggest that both cytology and VIA perform poorly as triage strategies for positive hrHPV results among WLWH, with the sensitivity of 62% and 59%, respectively.[135] Primary hrHPV testing followed by colposcopy seemed the most effective screening strategy in the same study. Given these preliminary findings, a more robust study is needed to provide relevant data which can guide the development of appropriate national cervical cancer screening algorithms that are inclusive of both WLWH and HIV-negative women.

Most of the current commercially available HPV DNA assays are costly, require complex infrastructure, and some have not undergone stringent clinical and regulatory evaluation.[136] Many of the available HPV assays either depend on direct genomic detection,[137] or amplification of genome target sequences.[138,139] Reliable, accurate, affordable, quick, and easy-to-use assays are required to support screening programmes in LMICs, and new technologies are being developed to make this possible.

More recently, the new AmpFire Multiplex HPV DNA genotyping assay (Atila Biosystems, Inc, Mountain View, California) was tested on formalin-fixed, paraffin-embedded (FFPE) cervical tissue,[140] and self-collected vaginal swabs[141-143] with excellent results. The assay utilises a novel isothermal amplification with real-time fluorescent to individually detect 15-hrHPV subtypes which include the additional HPV 53/66 to the 13-hrHPV International Agency for Research on Cancer (IARC) class 1 (HPV 16/18/31/33/35/39/45/51/52/56/58/59/68) types. The AmpFire HPV assay was Conformité Européenne (CE European community) marked in 2017.

Tang and colleagues carried out an analytical validation of the AmpFire.[140] They evaluated the performance of the AmpFire assay against the Roche cobas HPV and Roche Linear Array HPV on 214 FFPE of clinical specimen from the cervix/vulva and oropharynx. These specimens were stored at Hospital Universitario Virgen de la Arrixaca in Madrid, Spain, and Memorial Sloan Kettering Cancer Centre in New York, USA. The limits of detection for HPV 16 and 18 were 2 copies/reaction and 20 copies/reaction for the other 13-hrHPV. The AmpFire detected, with positive

agreements with the other assays, 100% of the HPV 16 and 18, and 95% of the other hrHPV from the clinical samples. There was also 100% negative agreement for all the hrHPV.

Zhang and colleagues performed a clinical evaluation of the AmpFire.[143] Using a nested sample of 6000 Chinese women, they evaluated the performance of AmpFire as a primary screening tool using frozen self- and provider-collected samples stored in PreservCyt at -4°C. These samples had previously been tested using Roche cobas HPV assay. The sensitivity for detection of CIN 2+ for both collection methods was similar for the two assays. In another nested cohort of stored self-collected vaginal samples from 439 pregnant women in Pemba, Tanzania, stored in Copan eNAT® buffer (VCopan Italia, Brescia, Italy), researchers evaluated the hrHPV prevalence with the AmpFire HPV assay against the researchers' in-house assay.[141] A 100% concordance was reported. The AmpFire was also used in a large primary screening project in Mongolia.[142] Of the 3345 women screened, 18.7% (624) were hrHPV positive and were invited for colposcopy. Five hundred and fifty-two arrived for evaluation and 21% had an inadequate examination. Only those with a lesion visualised on colposcopy had a directed biopsy for histology which yielded 20 cases of CIN 2+; 10 infected with HPV 16, one with HPV 18, eight with other non 16/18-hrHPV, and one with unknown HPV type. All four AmpFire studies above were based on the general population.

Recent data from Botswana supports the accuracy, acceptability, and feasibility of self-collected swabs for hrHPV testing.[144,145] Self-collected swabs have also been shown to be acceptable to women in other settings,[146,147] with comparable accuracy to provider-collected swabs even in high HIV-burden populations.[145,146,148] Reduced discomfort related to self-collection swabs has been reported compared to other screening methods.[149] Self-collection improves the equity and coverage of cervical cancer screening programmes.[146] It provides programmatic policy makers a solution to surmount the first hurdle of the screening cascade by making the service accessible to more women and potentially those in far to reach and often underserved communities.

Our study evaluated the performance of various AmpFire-based hrHPV primary screening strategies in a population of both WLWH and HIV-negative women, utilising self-collected swabs. Additionally, we tested the performance of VIA, colposcopy, and partial genotyping as potential triage methods following a hrHPV screen-positive test. Evaluating potential triage strategies following primary hrHPV testing in high HIV prevalence settings like Botswana is essential to generate valuable data for the region and other similar sub-Saharan Africa countries.

1.6. Aim, research questions and objectives.

1.6.1. Aim

The aim of this thesis was to evaluate various triage methods for hrHPV screen-positive women and propose an evidence-based screening algorithm for Botswana's national cervical cancer prevention programme.

1.6.2. Research questions

1. What is the prevalence of hrHPV types in WLWH and HIV-negative women aged ≥25 years old in Botswana?
2. How does a two-stage algorithm of primary hrHPV followed by VIA or colposcopy perform in WLWH and HIV-negative women?
3. What new triage strategies can be suggested for the Botswana cervical cancer prevention programme?

1.6.3. Specific objectives

1. Describe the general and type-specific hrHPV epidemiology among WLWH and HIV-negative women in Botswana
2. Describe the findings of visual evaluation as triage among hrHPV-positive WLWH and HIV-negative women in Botswana

3. Describe the prevalence of histology confirmed precancer lesions among a screening population of WLWH and HIV-negative women according to a primary hrHPV screening test in Botswana
4. Calculate the test performance of the various single and dual test strategies to detect precancer lesions among a screening population in Botswana
5. Considering the unique context of Botswana and the various test performances, propose potential screening algorithm(s) for the country.

1.6.4. Thesis structure

This thesis is presented in seven chapters and the references of each chapter are presented at the end of the respective chapter.

Chapter one: Introduces the study, including a general literature review, research problem, aim and specific objectives of the study. Additionally, a review of newer literature (January 2020 through June 2021) on HPV and cervical cancer screening in southern Africa's five United Nations' sub region countries was done to set the context for the study. The review focused on hrHPV epidemiology, HPV-based secondary prevention, and the various potential triage strategies for implementation of HPV primary screening in southern Africa. The published manuscript is included as part of the appendix at the end of the thesis.

Chapter two: Details the methods used and describes the characteristics of the study population. All study-related documents are included in the appendix at the end of the thesis.

Chapter three: Provides the epidemiology of hrHPV and type-specific findings in WLWH and HIV-negative women in a semi-urban region in Botswana, meeting the **objective one** of the thesis.

Chapter four: Presents the results of concurrent VIA (by a nurse) and colposcopy (by a gynaecologist) evaluations in WLWH and HIV-negative women who screened positive for any hrHPV, meeting the **objective two** of the thesis.

Chapter five: Presents the results of histology-confirmed disease prevalence in WLWH and HIV-negative women who screened positive for hrHPV. This analysis meets the **objective three** of the thesis.

Chapter six: Presents the performance of a single hrHPV screening test compared to a two-stage triage strategy using VIA, colposcopy, or a partial hrHPV-genotype following a positive hrHPV test in WLWH and HIV-negative women. This meets the **objective four** of the thesis.

Chapter seven: Presents the general conclusions of the study and proposes potential screening algorithms for Botswana which meets objective five of the thesis. It also presents the limitations and challenges of the research, and areas for future study. This meets the **objective five** of the thesis.

1.6.5. Contribution of the candidate to the thesis

Together with the other study co-principal investigator (co-PI), the candidate conceptualised and designed the study. The two co-PIs jointly supervised the recruitment and screening of participants by research assistants, carried out colposcopy, histological sampling, and LEEP of hrHPV-positive participants. This thesis was based on a subset of a larger study and the candidate was involved in the data cleaning, coding, and analysis for the 1500 participants reported on.

The candidate was first author of a review article published in the journal Current Opinion in Infectious Disease titled “Progress and challenges in HPV and cervical cancer in southern Africa” (Appendix 1). The candidate was senior author of an e-poster abstract (number: A-AIDS-2022-02904) presented at the 24th International AIDS Conference, held in Montreal, Canada, from 29th July to 2nd August 2022, titled: “The role of expanded human papillomavirus genotyping in detecting high-grade cervical dysplasia by HIV status in Botswana” (Appendix 2). She presented another e-poster abstract (number: 930) to the 35th International Papillomavirus Conference held in Washington DC, USA April 17-21st 2023 titled “Cervical dysplasia and cancer missed among high-risk human papillomavirus primary screen-positive women with a

secondary visual triage-negative test in Botswana" (Appendix 3). Another manuscript entitled "Triage of HPV positivity in a high HIV-prevalence setting: Comparison of visual triage methods and HPV genotype restriction" has been submitted to the International Journal of Gynecology and Obstetrics. The candidate is co-first author. The preliminary results of the study were presented to the Botswana Ministry of Health on the 18th of November 2022. All research outputs are based on the data generated by the study and included in the appendix at the end.

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CHAPTER 2. METHODS AND PARTICIPANTS CHARACTERISTICS

2.1. Introduction

This prospective cohort study evaluated various algorithms for triaging women testing hrHPV-positive in Botswana. In this chapter we describe the methods used and report the characteristics of the study participants.

2.2. Research design and statistical plan

2.2.1. Study population

We enrolled 1500 women, women living with HIV (WLWH) and HIV-negative women, in the ratio of 1:1, aged 25 years and above, for cervical cancer screening with primary hrHPV testing. HPV testing is not recommended for women younger than 25 years for the following reasons: there are exceedingly low disease rates among young women, higher rates of transient HPV infection and regression of mild disease, and an increased potential for harm related to unnecessary treatment.[1,2]

2.2.2. Study sites

All participants were recruited from the Southeast District in Botswana. Recruitment took place at outpatient clinics at Bamalete Lutheran Hospital (BLH) in Ramotswa. This peri-urban village has a population of 30,000 and is 35 kilometres from the capital, Gaborone. BLH and its primary care clinics serve both village residents and neighbouring rural communities.

2.2.3. Inclusion and exclusion criteria

Inclusion criteria:

1. female
2. ≥25 years of age.

2. able to give informed consent.
3. known HIV status.

Exclusion criteria:

1 pregnancy.
2. prior hysterectomy.
3. prior diagnosis of cervical cancer.

2.2.4. Study procedures

All study processes were guided by standard operating procedures (SOPs) (Appendix 4). For participants presumed HIV-negative, evidence of a negative HIV test within one year was requested (this was based on the Botswana national HIV testing interval). If this was unavailable, participants were referred for HIV testing on-site before they could be included in the study. Participants with a new HIV diagnosis were referred for HIV-disease management as per Botswana national guidelines.

2.2.4.1 Recruitment procedures

Potential participants were recruited during routine clinic visits by study research assistants (RAs). The RAs identified age-eligible females on the morning of recruitment days with clinic staff's help, and through recruitment talks to patients waiting in line for care. This recruitment practice is an accepted strategy in Botswana. Recruitment leaflets (Appendix 5) were also available in both English and Setswana at clinic reception and in patient waiting areas. Women interested in participating were escorted to a private room available at the clinic, where study staff assessed eligibility.

One thousand five hundred out of 1530 potential participants met eligibility criteria and were invited to consent to the study (Appendix 6; note that only the English version of the consent form is included). No data was collected before the participant signed the informed consent form. The RAs collected baseline information from participants and asked demographic and risk factors questions (Appendix 7 is a composite

representation of the various data collection forms). Next, the RAs reviewed the swab collection method with the participants. A laminated and sanitised picture of how to self-collect the specimen was given to the participant (Appendix 8). Each participant was then escorted to a private room to provide a self-collected swab from the vagina and instructed to wash their hands before and after the collection. The swab, placed in a specimen transport media, was handed over to the study staff. The RAs were available to address any participant questions before and after the specimen collection. All participants were provided with an HPV take-home information leaflet (Appendix 9). Specimens were labelled with a study identity (ID) and specimen ID and put into a refrigerator or cooler box with ice packs. At the end of each day, specimens were transported by a study team member in a cooler box to the Botswana Harvard AIDS Institute Partnership Reference Laboratory in Gaborone for processing. Specimens were tracked the same day using an intake log by study ID and specimen ID and stored at -70°C or lower until tested.

2.2.4.2 HPV DNA testing procedures

HPV DNA testing was performed at the Botswana Harvard Partnership laboratory in Gaborone by virologist SM using Atila AmpFire Multiplex hrHPV detection kit (Atila BioSystems, USA).[3] The Atila AmpFire assay utilises an isothermal nucleic acid amplification to detect approximately 100-base pair targets within E1 and/or L1 regions of hrHPV types. The assay employs real-time fluorescence detection to qualitatively identify hrHPV types. It enables simultaneous detection of 15 hrHPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, and 68) in a single tube. To achieve this, specific probes labelled with indodicarbocyanine (CY5), 6-carboxy-X-rhodamine (ROX), 6-carboxyfluorescein (FAM), and hexachloro-6-carboxyfluorescein (HEX) are utilised to detect HPV16, HPV18, other specific 13 hrHPV, and the internal control, respectively.

To ensure the integrity and presence of DNA in all samples, the AmpFire assay includes a verification step through the amplification of the b-globin gene. This gene serves as the internal control and is tested in the HEX channel. The amplification process generates a sigmoid curve, and the Cycle Threshold (Ct) value is closely monitored. A sigmoid curve reaching a Ct value of 60 is considered indicative of the

presence of the target DNA. This step provides an additional layer of validation, confirming that the samples contain the necessary genetic material for accurate hrHPV detection and analysis.

The absence of an exponential amplification curve in the HEX channel is interpreted as an invalid outcome. The manufacturer specifies a threshold of 80 copies per reaction for the 13 non-HPV16/18 types and 20 copies per reaction for HPV16 or HPV18. If no exponential curve other than the internal control is present for a sample, this sample is NEGATIVE.

The assay uses a crude cell lysate, isothermal multiplex amplification in a 96 well plate format and the results are available in 90 minutes. The assay and can be used for both screening (only subtypes HPV16/18 identified) or for genotyping (all 15 subtypes identified). We used the genotying assay for this study to allow evaluation of various genotypes as triage methods.

For each dry vaginal swab specimen extraction, 1mL of 1X lysis buffer was added to each collection tube and briefly vortexed (~15 seconds) and transferred to a 1.5mL Eppendorf tube. The tubes were then incubated at room temperature for 20 minutes before being centrifuged for 30 minutes at maximum speed. The resulting supernatant was removed completely and 30µL of lysis buffer added to the cell pellet which was then thoroughly to re-suspended (sightly adjusting lysis buffer volume to ensure full resuspension). The resulting suspension from each sample tube was then transferred into a PCR tube (0.2mL) and incubated at 95 °C for 10 minutes in a thermocycler.

For HPV DNA genotyping, 2µL of sample from the lysate was then used for HPV amplification and detection. A complete AmpFire Genotype HPV kit (catalog number: GHPVF1618-100) included the following reagents: reaction mix, primer mixes one, two, three, and four, which cover the 31/51/39/16, 35/68/18/59, 33/66/IC/45, and 58/56/53/52 HPV genotypes, respectively. The specific probes for the 16/59/45/52, 39/18/66/53, 31/35/33/58, and 51/68/IC/56 HPV genotypes were labelled with CY5, ROX, FAM, and HEX, respectively. The assay was run using an isothermal quantitative polymerase chain reaction (qPCR) of 60°C, taking fluorescence reading at the FAM/HEX/CY5/ROX channels once every minute for a total of 60 minutes.

The results of the Ct values for each amplification curve in all fluorescence channels are automatically reported by the Bioer Line-gene 9600 plus real time PCR system. For each sample, genotyping results were coded and identified based on exponential amplification curves in the CY5, ROX, FAM, and HEX channels in the above layout of four reaction tubes. All samples with detectable fluorescence were considered positive. The lack of exponential amplification curve in the HEX channel from primer mix three was interpreted as an invalid result. Participants with invalid results were recalled for repeat collection of their HPV swab with closer supervision. For the AmpFire Multiplex hrHPV genotyping assay, negative and positive controls provided in the kit were included in each experiment for quality assurance.

2.2.5. Management of hrHPV-negative participants

Participants who tested HPV-negative were counselled to repeat screening in three years if they were WLWH and five years if HIV-negative, as per Botswana national guidelines recommendation. To evaluate the performance of hrHPV testing as a stand-alone screening tool in our high HIV-prevalence population and to confirm its negative predictive value, 10% of the hrHPV-negative participants from each HIV status group were called back for a second-stage triage visit and histological evaluation, as detailed below for participants positive for hrHPV.

2.2.6. Referral for VIA and colposcopy

All participants testing positive for any hrHPV were called back for a scheduled visual assessment with visual inspection after acetic acid (VIA) and colposcopy. Histological specimen were obtained for all women who attended. An additional 10% of hrHPV-negative women were also called back for evaluation including histological sampling.

2.2.6.1 Visual inspection with acetic acid procedures

VIA was performed by a trained nurse who had participated in a Botswana national training programme and was blinded to both the hrHPV type results and the baseline questionnaire responses (Appendix 7 is a composite representation of the various data

collection forms). Participants were examined in the lithotomy position. The examination began with a visual examination of the external genitalia under an intense examination light. Participants with vaginal discharge or menstrual bleeding were rescheduled. Participants with lesions suspicious of cancer were biopsied and referred for further management but included in the analysis as a high-grade abnormality. The cervix was then washed for one minute with a cotton wool ball soaked in 5% acetic acid. The nurse waited for two minutes to allow a potential lesion to develop before inspecting the cervix with the naked eye. At the end of the examination, the VIA part of the form was completed. VIA findings were categorised as normal, abnormal with a recommendation for cryotherapy, or abnormal with a recommendation for a loop electrosurgical excision procedure (LEEP). The RAs and the study co-Principal investigators (co-PIs) checked these forms daily and weekly, respectively. Missing or incongruent data was flagged and followed up for resolution or correction by the data entry clerk or RAs.

2.2.6.2 Colposcopy procedures

The candidate and the other study co-PI (RL) performed colposcopy at alternate sessions (Appendix 7 is a composite representation of all the various data collection forms). They were blinded to the questionnaire responses, the hrHPV type and the VIA results. Colposcopy examination occurred immediately after completion of VIA by the study nurse. Colposcopy impressions were recorded as normal, low-grade, or high-grade, with details about abnormalities recorded. Women with inadequate exams were not excluded from the study. All participants undergoing triage testing had a biopsy at the time of colposcopy. For visible lesions after application of acetic acid, a LEEP was performed. If no lesion was seen, a small endo-cervical excision (as proxy for a four-quadrant biopsy) was performed as per the current national guidelines for women 30 years and older, and for all women with an inadequate examination. If no lesion was seen in women under 30 years old, an endo-cervical curettage (ECC) was performed.

2.2.7. LEEP procedures

After colposcopy assessment, verbal consent was obtained from the participant for treatment as per the Botswana national guidelines. The cervix was injected with 2 to 6 ml of a local anaesthetic containing a vaso-constrictive agent (1-2% lignocaine with 1: 100,000 or 1: 200,000 of adrenaline). The loop size was selected according to the size of the lesion. The smallest loop (0.5cm diameter) was used in the case of an endo-cervical LEEP where no lesion was observed. At the end of the procedure, the cervical crater was cauterised. The participants were informed about the symptoms and signs of LEEP complications (i.e., smelly discharge, haemorrhage, and pain). The procedure was recorded in the government issued national patient-held medical record. Post-LEEP instructions and several numbers to call in case of complications were clearly written in the medical card. The participants were also informed that they could return to the clinic at any time should they need further information or visit the nearest facility with their medical record in case of an emergency. The two co-PIs phone numbers were printed on the consent forms and the patient's medical record to facilitate communication and contact during emergencies related to the study.

2.2.7.1 LEEP ineligible

If a significant aceto-white lesion was detected, but the upper limit was not seen, a "top hat" LEEP was performed (a larger LEEP followed by a second pass LEEP with a smaller loop). Where there was suspicion of micro-invasive disease the participants were referred for a cone biopsy at Princess Marina Hospital (PHM), the country's main tertiary hospital, 35 km away, and exited from the study but included in the analysis as high-grade abnormality. The cone biopsy was performed by RL, the other study co-PIs. If an apparent cancer was found on examination a biopsy was performed, and the participant was referred to PMH gynaecology department for urgent management. In both cases, the visual evaluation was recorded as high-grade lesion for the purposes of analysis. The participants' results were followed up and included in the histological analysis.

2.2.8. Histological specimen procedures and results

All histology specimens were placed in 10% formalin and saline and delivered to the Ministry of Health (MOH) National Health Laboratory (NHL) weekly, following the histopathology chain of custody SOP (Appendix 4). The specimens were processed and evaluated by the pathologists from the MOH. All women with cervical intraepithelial neoplasia grade two or worse (CIN 2+) on ECC were brought back for an excisional procedure. Women with histopathology showing micro-invasion or invasive cervical cancer were referred to gynaecology providers for further assessment and treatment, if not already referred at the time of visual assessment.

2.2.9. Tracing of defaulters

The RAs traced women who required colposcopy or treatment (all hrHPV positive, select 10% hrHPV negative, and positive ECC) on a weekly basis. If after five successive attempts a woman was not found or was found but still did not return to the clinic for examination, she was no longer contacted, and considered lost to follow up for research purposes.

2.2.10. Sample size

Sample size assumptions were based on data from a prior study, existing literature, and patient behaviour regarding clinical follow-up.[4] In the prior study, the most sensitive two-stage screening algorithm was hrHPV testing, followed by colposcopy, with 83% sensitivity. The prevalence of CIN2+ in the sample population of all WLWH was 10%. We assumed the prevalence of CIN2+ to be half that value in HIV-negative women, and therefore the combined prevalence was estimated to be 7.5% in an equally mixed HIV-positive/HIV-negative study sample. Assuming a two-sided alpha of 0.05, a sample size of 488 participants yielded >80% power to detect the specified difference. In the prior study, the loss to follow-up was 6%. We assumed a higher loss to follow-up rate of 20% in a more rural population from a large catchment area. Thus, we estimated that we would need data from 585 participants with positive hrHPV results.

2.2.11. Data collection, storage, and confidentiality procedures

Data was captured into REDCap (Research Electronic Data Capture, https://projectredcap.org/), a secure, Health Insurance Portability and Accountability Act (HIPAA) compliant, web-based data collection application available through the University of Botswana (UB) eHealth Research Unit. REDCap is highly adaptable for clinical care and research, and it provides user-friendly web-based case report forms, real-time data entry validation (for data types and range checks), audit trails, and the ability to set up a calendar to schedule and track critical clinic events such as specimen-collection, patient visits, and many others. REDCap allows designated users to have different levels of access. For this study, we replicated the paper forms into online forms to allow for data capture into a central online database (Appendix 7 is a composite representation of all the various data collection forms).

All the data collected on paper case forms were entered into the database by a study RA or data entry clerk, and then independently verified for accuracy of entry and completeness by a second staff member. The data collection forms that were entered into the database did not contain any personally identifying information of enrolled participants. The online database was password protected and encrypted, and data submitted to the database was secured with Secure Socket Layer certificates. The hard copy versions of data collection forms were transported in a locked trunk to be stored at the research offices in Gaborone in locked cabinets. Only authorised study personnel had access to data collection forms and any coding sheets.

The data included a study ID as an identifier that linked to the laboratory specimens. Data from the hrHPV testing and histological evaluation was retained by the laboratory until all quality checks were complete. When complete, the data was sent to the study statistician who merged the data from the laboratory and demographic files. The laboratory data included a study ID and specimen ID.

All database files were regularly and securely backed-up. Confidentiality was preserved during the use, transmission, and storage of the data. Computers were

password protected, and their access restricted to authorised study personnel. Technical and administrative stewardship responsibilities of data, documents, and specimens from the evaluation sites resided with the co-PIs. Surveys and laboratory specimens will be stored for ten years in a secured location, including paper copies of forms per University of Botswana requirements.

2.2.12. General statistical considerations

We analysed data for WLWH and HIV-negative women separately. Descriptive statistics results were presented as median with interquartile range (IQR), or proportions to describe the demographic (i.e., age, marital status, education, employment, and parity), behavioural (i.e., age of sexual debut, number of sexual partners, hormonal contraception use, condom use, smoking, and cervical cancer screen) and clinical characteristics (i.e., HIV diagnosis, length of ART, viral load, CD4 cell count for WLWH only). Medians were calculated for continuous variables (i.e., age, age of sexual debut, parity, length of HIV diagnosis, length of ART and CD4 cell count), and frequencies with the corresponding percentages for categorical variables. More comparisons between WLWH and HIV-negative women were done by: i) describing the various HPV group types (any hrHPV, individual hrHPV type (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66 and 68), HPV16/18, HPV16/18/45, HPV16/18/31/33/35/45/52/58, non-HPV16/18/45 (HPV31, 33, 35, 39, 51, 52, 53, 56, 58, 59, 66, 68), and other hrHPV (HPV 39/51/53/56/59/66/68), ii) the distribution of the histological results (≤CIN 1, CIN 2, CIN 2+, CIN 3, and CIN 3+), and iii) the proportion of the cervical cancer precancer disease (CIN 2, CIN 2+, CIN 3, and CIN 3+) both diagnosed and missed by visual evaluation (VIA and colposcopy).

The Chi-square (χ^2) or Fisher's exact tests were used to identify statistically significant differences in categorical variables including participants' characteristics, hrHPV group types, VIA, colposcopy, and histological results ($p<0.05$). After assessing for differences in histology results and HPV status by participants' characteristics using a diagnostic approach (hrHPV testing types, VIA, and colposcopy result) separately among WLWH and HIV-negative women, we then used a X^2 test to assess if those differences varied depending on the participant's HIV status (heterogeneity of effect).

Further analysis was done to determine the odds of VIA positivity, colposcopy abnormality and CIN 2+ among hrHPV-positive women with logistic regression analysis and reported both the unadjusted odds ratio (ORs) and adjusted odds ratios (aORs) with 95% confidence intervals (CIs) and corresponding p-values. The odds of CIN 2+ by HIV positivity were also calculated. We initially fitted bivariate models, then included all variables with bivariate p< 0.30 in a multiple regression model.

We further described the data by calculating the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NVP) of the two-stage screen algorithm using VIA, colposcopy and partial hrHPV tests to detect CIN 2+ in hrHPV-positive women.

All data were analysed using the Statistical Package for the Social Sciences (SPSS), version 27 (IBM, USA).

2.3. Ethical considerations and informed consent

Participation in the study was voluntary. The study's ethical approvals were granted by the Botswana MOH, the University of Botswana, and the University of Pretoria. The permit numbers were HPDME 13/18/1 (Botswana Ministry of Health, Appendix 10); URB/IRB/1543 (University of Botswana, Appendix 11); 721/2020 (Faculty of Health Sciences, University of Pretoria, Appendix 12). No recruitment was undertaken before all ethical approvals were received.

2.3.1. Informed consent procedures

Study RAs invited potential participants (see recruitment procedures section 2.1.4.1) and helped them to understand the informed consent process and document (Appendix 6). The RAs provided information about the study and answered potential participants' questions before requesting the voluntary signing of an informed to participate in the study. The process of obtaining informed consent was completed before the collection of data or specimens. The RAs gave a copy of the consent document to the participants to keep. They excluded any potential participant who was

unable to understand the informed consent or did not meet any of the study eligibility criteria. The RAs kept consent forms in a locked office in Gaborone. All study staff involved in recruiting, consenting, and examining participants completed a human research subjects certification program before the beginning of the study.

2.3.2. Minimising risks of participation.

Informed consent and strict confidentiality were rigorously enforced to minimise risks to participants and their partners. All study personnel received training in the informed consent process.

The RAs were available if participants had difficulty in any part of the specimen collection, such as pain or discomfort.

Participants with known hypertension or elevated blood pressure at the time of LEEP were injected with local anaesthetic without adrenaline. The smallest loop appropriate for the size of the lesion was used for LEEP, and participants younger than 30 years old with no lesions received an ECC to get a histological specimen for outcome verification. These measures aimed to preserve the cervical tissue and minimise bleeding and potential cervical competency problems in future pregnancies. In all participants the cervical crater was cauterised carefully to ensure good haemostasis post-LEEP, and participants injected with local anaesthesia without adrenaline received extra attention. If bleeding continued post-cautery, pressure was applied with cotton wool for several minutes until the bleeding stopped. In a few cases where bleeding persisted, an absorbable haemostat was applied to the crater, and the vagina was packed. The participant was kept in an observation room and the cervix was re-examined after 30-45 minutes with further cauterisation if necessary. The participant was then sent home with prophylactic antibiotics. No participant in the study required further intervention beyond the above measures.

Participants were provided with verbal and written post-LEEP care instructions including potential complications. The two co-PIs' phone numbers were provided, and

the participants could contact either of them in case of emergency or for any study-related questions.

2.3.3. Potential benefits of participation

Participants were tested for hrHPV, a test that was not currently available in regular care in Botswana. Participants who tested positive for hrHPV were identified as potentially at higher risk for cervical cancer earlier than they would have been under the standard care, allowing for prompt management. Education about the risks of cervical cancer and the role of regular screening were emphasised. Participants who needed care outside cervical cancer screening were linked to appropriate services.

2.4. Results of characteristics of the study participants

2.4.1. Recruitment and flow of participants

Women were recruited from clinics in the Southeast District of Botswana and the referral district hospital Bamalete Lutheran Hospital (BLH). One thousand five hundred women were consecutively enrolled out of 1530 screened between 30th April 2021 and 16th December 2021, in a ratio of 1:1 for WLWH and HIV-negative women (Figure 2.1).

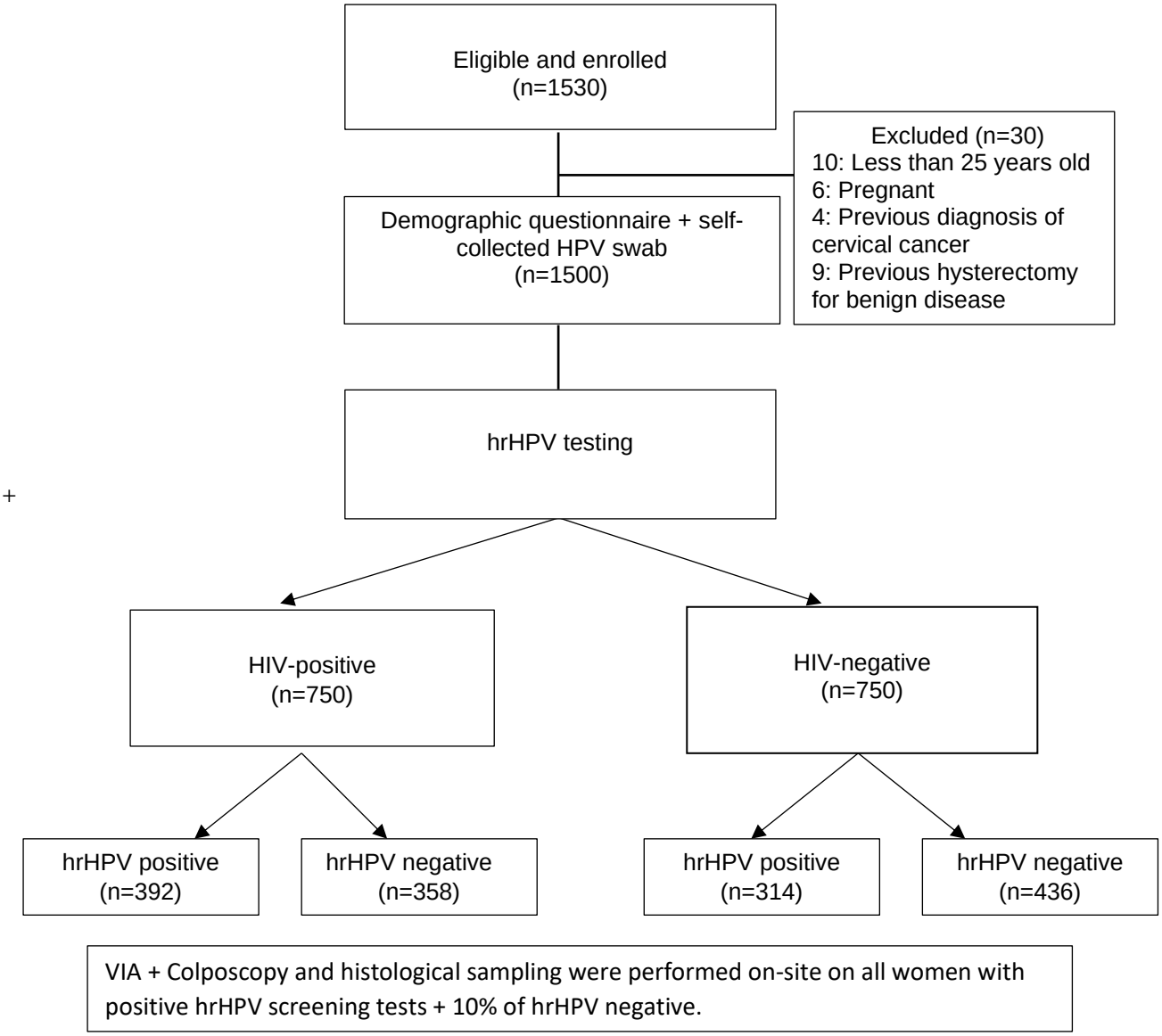

Figure 2.1 Flow of participants through study

2.4.2. Socio-demographic, behavioural and clinical characteristics of study participants

The socio-demographic characteristics, reproductive, sexual history, and cervical cancer risk factors for participants overall are presented in Table 2.1. The median age was 41 years (IQR 34-49 years) and the range was 25-76 years. Most women were in the 30–49-year age group (30-39 years n=472; 40-49 years n=505) followed by the 50–59-year age group (n=251), the 25–29-year age group (n=175), and the smallest group was in the ≥60-year age group (n=97).

Most participants were single (69.1%). Nearly a quarter was currently married (24.7%) and the remaining 6.5% had previously been married.

Almost all participants received some form of formal schooling (98.0%), with the majority having received secondary level schooling (58.2%), followed by tertiary level schooling (24.5%), and primary level schooling (15.3%). Over half were in formal employment (55.1%).

Even though the median number of children per woman was two (IQR =1-3), less than half the women had two or more children (44.1%). Nearly 80% of the women had their first sexual experience at age 18 or older (median age =19 years; IQR 18-21 years), and nearly 70% had between 1 and 5 lifetime partners. Just over 70% were currently on some form of contraception. Of those currently using contraception, 88.9% were using condoms and 24.6% were using a hormonal contraceptive. Less than 5% of the women were current smokers, and most women had previously been screened for cervical cancer (68.1%).

Table 2.1 Participant demographic, behavioural, and clinical characteristics by HIV status

	All participants N=1500 n (%)	HIV-negative N=750 n (%)	HIV-positive N=750 n (%)	P value*
Age in years, *Median (IQR)*	41 (34-49)	39 (32-48)	43 (37-49)	<0.001
25-29	175 (11.7)	131 (17.5)	44 (5.9)	<0.001
30-39	472 (31.5)	267 (35.6)	205 (27.3)	
40-49	505 (33.7)	186 (24.8)	319 (42.5)	
50-59	251 (16.7)	105 (14.0)	146 (19.5)	
≥60	97 (6.5)	61 (8.1)	36 (4.8)	
Marital status				
Single	1036 (69.1)	495 (66.0)	541 (72.1)	0.022
Married	365(24.3)	205 (27.3)	160 (21.3)	
Previously married	99 (6.6)	50 (6.7)	49 (6.5)	
Highest education				
None	28 (1.9)	13 (1.7)	15 (2.0)	<0.001
Primary/non-formal	230 (15.3)	100 (13.3)	130 (17.4)	
Secondary	873 (58.2)	387 (51.6)	486 (64.9)	
College/university	368 (24.5)	250 (33.3)	118 (15.8)	
Missing	1 (0.1)			
Employed				
Yes	826 (55.1)	419 (55.9)	407 (54.3)	0.533
No	674 (44.9)	331 (44.1)	343 (45.7)	
Parity, *Median (IQR)*	2 (1-3)	2 (1-3)	2 (2-3)	0.037
≤2	839 (55.9)	440 (58.7)	399 (53.2)	0.033
>2	661 (44.1)	310 (41.3)	351 (46.8)	
Age at first intercourse, *Median (IQR)*	19 (18-21)	19 (18-21)	19 (18-20)	0.606
<18	317 (20.9)	147 (19.7)	170 (22.8)	0.153
≥18	1175 (78.4)	598 (80.3)	577 (77.2)	
Missing	8 (0.5)	-	-	
Number of sex partners (ever)				
1-5	1043 (69.5)	549 (74.0)	494 (66.4)	0.001
>5	443 (29.5)	193 (26.0)	250 (33.6)	
Missing	14 (0.9)	-	-	
Contraception use (currently)				
Yes	1071 (71.4)	524 (70.3)	547 (73.5)	0.0171
No	418 (27.9)	221 (29.7)	197 (26.5)	
Missing	11 (0.7)	-		
Hormonal contraception use**				
Yes	263 (24.6)	150 (28.6)	113 (20.7)	0.002
No	808 (75.4)	374 (71.4)	434 (79.3)	
Condom use**				
Yes	952 (88.9)	447 (85.3)	505 (92.3)	<0.001
No	119 (11.1)	77 (14.7)	42 (7.7)	
Smoking				
Yes	73 (4.9)	20 (2.7)	53 (7.1)	<0.001
No	1427 (95.1)	730 (97.3)	697 (92.9)	
Cervical cancer screen (ever)				
Yes	1021 (68.1)	418 (55.7)	603 (80.4)	<0.001
No	479 (31.9)	332 (44.3)	147 (19.6)	

HIV: human immunodeficiency virus; IQR: interquartile range

*Pearson or fisher's exact test; **Among those using contraception

2.4.3. Socio-demographic, behavioural and clinical characteristics of study participants by HIV status

The socio-demographic characteristics, reproductive, sexual history, and cervical cancer risk factors by HIV status are presented in Table 2.1. There was no difference in age range by HIV status (range 25-76 years). HIV-negative women were slightly younger than women living with HIV (WLWH) (median age 39 years [IQR 32-48 years] compared to median age 43 years [IQR 37-49 years]), respectively. Larger differences in age by HIV status were observed in the extreme age groups. The proportion of HIV-negative women in the age group of 25-29 years was three times higher and nearly double in the 60 year and older age group compared to WLWH (17.5 vs. 5.9%; and 8.1 vs. 4.8%, respectively).

There was no significant difference in marital status, highest education, parity, employment, and age of first sexual intercourse by HIV-status categories. Lifetime partners of five or more was slightly higher in WLWH compared to HIV-negative women. Although current contraception use was similar between the two groups, HIV-negative women were more likely to use hormonal contraception compared to WLWH (28.6% versus 20.7%). However, WLHW were more likely to use condoms compared to HIV-negative women (92.3 % versus 85.3%). A greater proportion of WLWH (7.1%) were smokers compared to HIV-negative women (2.7%), and previous screening for cervical cancer among the two groups was significantly different (80.4% of WLWH compared to 55.7% of HIV-negative women had been previously screened for cervical cancer).

2.3.4 Characteristics of HIV disease in women living with HIV

The median number of years since a diagnosis of HIV and length of time on ART were 9.6 years (IQR 5.3-15.0 years) and 8.0 years (IQR 3.9-12.8 years), respectively. Seven percent of the women had a CD4 count of less than 350, and almost all had an undetectable viral load (98.7%) (Table 2.2).

Table 2.2 HIV disease and treatment characteristics

HIV diagnosis length (years)	**n**	**%/median (IQR)**
<5	158	21.1
5-9	211	28.1
≥10	381	50.8
Length of HIV diagnosis, *median years* (IQR)	750	9.6 (5.3-15.0)
CD4 count (cells/mm^3) *		
<200	15	2.0
200–349	37	4.9
350–499	115	15.3
≥500	583	77.7
CD4 count (cells/mm^3) *		
<500	167	22.3
≥500	583	77.7
CD4 T-cell count, *median cells/ mm^3 (IQR)*	750	679 (507-871)
Viral load (copies/mL) *		
≤400(undetectable)	739	98.7
>400	10	1.3
Length on ART (years)		
<2	82	10.9
≥2	668	89.1
Length on ART, *median years (IQR)*	750	8.0 (3.9-12.8)

HIV: human immunodeficiency virus; ART: antiretroviral treatment; IQR: interquartile range
*Most recent

2.5. Discussion

Although the proportion of the WLWH in the sample was set at 50% which is higher than the national female average of 26.3% in the 15-64 age group, it does come closer to the average prevalence in older age groups in the sample. In the results of the new Botswana AIDS Impact Survey 2022, the HIV prevalence by age group in women is reported as follows: 25-29 years: 15.8%; 30-34 years: 20.2%; 35-39 years: 35.6%; 40.44 years: 49.3%; 45-49 years: 52.0%; 50-54 years: 43.0%; 55-59 years: 38.7%; 60-64 years: 32.6%.[5] Most of the characteristics of the sample were similar to those reported in previous studies in Botswana.[4,6,7]

Even though one of the inclusion criteria was age 25 years and older, the majority of the women in the study population were in the age group 30-49 (65.2%) which has traditionally been the target age group for cervical cancer screening in the country, based on the older WHO recommendations.[8] There were three times more HIV-negative women in the age group 25-29 in this sample, and is closer to the national population distribution by HIV status.

Nearly 70% of women reported being single in this cohort, with WLWH marginally more likely to be single than the HIV-negative women. This finding was confirmed by two other previous studies from Botswana.[4,9] Other African settings reported prevalence of single marital status ranging from 27% in Congo-Brazzaville to 53% Namibia.[10] The reason for the predominately higher single status in Botswana is unknown.

Formal education was almost universal in this cohort, a finding previously reported by various authors from Botswana.[4,11,12] However, there was a significant difference in higher education level attained by WLWH and HIV-negative women, with the latter twice more likely to have received tertiary education. Hamda and colleagues reported a similar finding in their study involving seven health facilities around Botswana.[13] Durevall and colleagues also observed the higher HIV prevalence in those with lower education levels in Kwa-Zulu Natal, South Africa.[14]

Age at first sexual intercourse has consistently been reported to be 18 years or older in Botswana and Zimbabwe,[15-17] but other parts of Africa have reported a younger age of sexual debut.[18,19] The reasons for these differences are unknown but could be linked to sexual risk behaviour prior to the roll out of ART, differences in age of marriage, and other cultural believes and practices.

The number of lifetime partners of five or more was 30% higher in WLWH than in HIV-negative women. Multiple lifetime partners have been reported as a risk for HIV by others.[20,21] It is worth noting that reporting multiple lifetime partners has also been linked to earlier age of sexual debut and the heightened risk of HIV,[22] but this will not apply to Botswana with its older sexual debut age.

Nearly three quarters of the women reported current contraceptive usage, which may be linked to the high levels of single marital status in this cohort. The high contraception use could also in part explain the lower parity in the cohort. Among those on contraception, condom use was much more common than hormonal contraceptive use, with WLWH marginally more likely to use condoms than HIV-negative women. The higher condom use in WLWH has also been confirmed by the findings from several studies.[9,23,24] This is most likely due to the legacy of the pre-ART HIV-era messaging from the early 2000's which emphasised the use of condoms to protect against acquisition and spread of HIV. However, other studies in Africa have reported low condom use among HIV-positive couples and HIV-positive pregnant women.[25,26] The reason for these discrepancies may be due to desire by couples to conceive, assurance of being in a long-term relationship, and less fear of contracting HIV due to availability of medications.[27,28]

A small number of women reported smoking in this cohort, which is in keeping with previous reports from Botswana.[6,11] Among those few, smoking was three times more likely to be reported by WLWH. Recent studies have also evaluated the association between the HIV status and tobacco product use status and shown that HIV-infected patients showed a higher rate of current smokers.[29,30]

The study did not exclude women who had previously been screened for cervical cancer. Most of the sample (68%) reported previous cervical cancer screening, and

the rate of previous screening was 40% higher in WLWH compared to HIV-negative women. Previous studies have confirmed this finding.[31-33] Researchers have reported that WLHH were more likely to screen for cervical cancer because of the perceived benefits of screening for WLWH compared to HIV-negative women.[32] However, our finding could merely be indicative of the way the cervical cancer screening programme has evolved over the last two decades in Botswana. The development of a semi-organised screening programme was funded through the USA's President Emergency Program for AIDS Relief (PEPFAR) which prioritised WLWH. Hence the emphasis on screening for WLWH.

The HIV characteristics of this study population appear to reflect well-controlled HIV disease for most of the participants. This finding is in keeping with the report by Tawe and colleagues whose cohort of WLWH was almost all on ART (93.2%), and two thirds had a baseline CD4 count ≥350 cells/mm^3.[34] This is not surprising since Botswana has led the fight against HIV and was the first country in the world to provide free universal ART.[35] The country's drive to achieve epidemic control by reaching the 90-90-90 target (90% of people living with HIV knowing their status, 90% with HIV on ART, 90% on ART immunologically suppressed),[36] seems to be succeeding. Various reports about the state of HIV management indicate a relatively high number of WLWH with well-managed HIV disease in Botswana.[37-39] However, it is not clear if well-managed HIV disease translates into better control of hrHPV-related cervical disease.

2.5.1. Conclusions

Analysis in the next chapters should provide further information on the impact of prior cervical cancer screening and well-manged HIV disease on the prevalence of both hrHPV and cervical precancer disease in Botswana.

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CHAPTER 3. HPV EPIDEMIOLOGY IN BOTSWANA

3.1. Introduction

Given the strong body of evidence that human papillomavirus (HPV) -based screening is more effective as a screening method for cervical cancer than cytology or naked eye visual inspection after acetic acid (VIA),[1-3] increasing numbers of countries have switched or are in the phase of switching towards screening using validated HPV DNA assays. The World Health Organization (WHO) guidelines (2021) recommend primary screening with a high precision test (HPV).[4] HPV prevalence in women varies by world region, age, and the existence of immune-suppressive co-morbidities such as HIV.[5-9]

The objective of this chapter was to describe the general and type-specific high-risk human papillomavirus (hrHPV) epidemiology in women living HIV (WLWH) and HIV-negative women in a semi-urban population Botswana.

3.2. Results

3.2.1. Overall hrHPV-positivity and characteristics of hrHPV-positivity by HIV status

Forty-seven percent of the women in the cohort tested positive for any of the 15 hrHPV infections (n=706). The hrHPV types included in the assay were HPV 16/18/31/33/35/39/45/51/52/53/56/58/59/66/68. Table 3.1 shows the hrHPV positivity in relation to socio-demographic characteristics of the participants using univariate analysis. The majority (56%) of the hrHPV-positive women were WLWH. A higher proportion of women previously screened for cervical cancer was observed in the hrHPV-negative group compared to the hrHPV-positive group (71 vs. 64%). The hrHPV-positive group also had a higher proportion of women with two or less children and women of age 25 to.29 years old compared to the hrHPV-negative group (61% versus 51% and 14% versus 9%; respectively).

Among WLWH, those with lower CD4 counts were more likely to be hrHPV-Positive. There was very little difference in hrHPV-positivity as CD4 count increased above 350 cells/ mm^3. The proportion of hrHPV-positive was nearly six times higher in women with a CD4 count of less than 200 cells/ mm^3, and double in women with CD4 count 200-349 cells/ mm^3 compared to women who were hrHPV-negative. In contrast, there appeared to be no difference between the proportions of hrHPV-positive and hrHPV-negative WLWH with a CD4 count above 500 cells/ mm^3 or who had been on anti-retroviral treatment (ART) for more than two years.

Table 3.1 Participant demographic, behavioral, and clinical characteristics by hrHPV positivity

	All patients N=1500 n (%)	hrHPV-negative N=794 n (%)	hrHPV-positive N=706 n (%)	p-value*
Age (years)				
Median (IQR)	41 (43-49)	42 (35-49)	40 (34-48)	
25-29	175 (11.7)	75 (9.4)	100 (14.2)	0.026
30-39	472 (31.5)	246 (31.0)	226 (32.0)	
40-49	505 (33.7)	283 (35.6)	222 (31.4)	
50-59	251 (16.7)	142 (17.9)	109 (15.4)	
≥60	97 (6.5)	48 (6.0)	49 (6.9)	
Marital status				
Single	1036 (69.1)	526 (66.2)	510 (72.2)	0.038
Married	365 (24.3)	213 (26.8)	152 (21.5)	
Previously married	99 (6.6)	55 (6.9)	44 (6.2)	
Highest education				
None	28 (1.9)	16 (2.0)	12 (1.7)	0.740
Primary/non-formal	230 (15.3)	128 (16.1)	102 (14.5)	
Secondary	873 (58.2)	461 (58.1)	412 (58.4)	
College/university	368 (24.5)	189 (23.8)	179 (25.4)	
Employed				
Yes	826 (55.1)	444 (55.9)	382 (54.1)	0.481
No	674 (44.9)	350 (44.1)	324 (45.9)	
Parity				
Median (IQR)	2 (1-3)	2 (2-3)	2 (1-3)	
≤2	839 (55.9)	407 (51.3)	432 (61.2)	<0.001
>2	661 (44.1)	387 (48.7)	274 (38.8)	
Age at first intercourse				
Median (IQR)	19 (18-21)	19 (18-21)	19 (18-20)	
<18	317 (21.2)	160 (20.3)	157 (22.3)	0.333
≥18	1175 (78.8)	629 (79.7)	546 (77.7)	
Missing	10 (0.7)	-	-	
Number of sex partners (ever)				
1-5	1043 (70.2)	559 (70.9)	484 (69.3)	0.501
>5	443 (29.8)	229 (29.1)	214 (30.7)	
Missing	14 (0.9)	-	-	
Contraception use (currently)				
Yes	1071 (71.9)	576 (73.2)	495 (70.5)	0.251
No	418 (28.1)	211 (26.8)	207 (29.5)	
Missing	11 (0.7)	-	-	
Hormonal contraception use**				
Yes	263 (24.6)	136 (23.6)	127 (25.7)	0.438
No	808 (75.4)	440 (76.4)	368 (74.3)	
Condom use**				
Yes	952 (88.9)	506 (87.8)	446 (90.1)	0.242
No	119 (11.1)	70 (12.2)	49 (9.9)	
Smoking				
Yes	73 (4.9)	31 (3.9)	42 (5.9)	0.066
No	1427 (95.1)	763 (96.1)	664 (94.1)	
Cervical cancer screen (ever)				
Yes	1021 (68.1)	566 (71.3)	455 (64.4)	0.005
No	479 (31.9)	228 (28.7)	251 (35.6)	

HIV status				
HIV positive	750 (100)	358 (45.1)	392 (55.5)	<0.001
HIV negative	750 (100)	436 (54.9)	314 (44.5)	
	N=750	**N=358**	**N=392**	
CD4 count (cells/mm^3) ***				
Median (IQR)	679 (507-871)	730 (547-893)	650 (493-845)	
<200	15 (2.0)	2 (0.6)	13 (3.3)	0.005
200–349	37 (4.9)	11 (3.1)	26 (6.6)	
350–499	115 (15.3)	56 (15.6)	59 (15.1)	
≥500	583 (77.7)	289 (80.7)	294 (75.0)	
CD4 count (cells/mm^3) ***				
<500	167 (22.3)	69 (19.3)	98 (25.0)	0.060
≥500	583 (77.7)	289 (80.7)	294 (75.0)	
Length of ART (years) ***				
Median (IQR)	8 (4-13)	9 (5-13)	7 (3-12)	
≤2	82 (10.9)	30 (8.4)	52 (13.3)	0.032
>2	668 (89.1)	328 (91.6)	340 (86.7)	
Viral load (copies/mL) ***				
≤400(undetectable)	739 (98.7)	353 (98.6)	386 (98.7)	0.888
>400	10 (1.3)	5 (1.4)	5 (1.3)	
Missing	1 (0.1)	-	-	

HPV: human papilloma virus; hrHPV: High-risk human papilloma virus; ART: Antiretroviral therapy; IQR: Interquartile range

*Pearson or fisher's exact test**;** **Among those using birth control**;** ***Among the HIV positive

3.2.2 Characteristics associated with HrHPV-positivity among WLWH

A multivariable logistic regression model was developed using backward selection ($p < 0.3$ to enter, $p < 0.15$ to stay) to identify characteristics independently associated with having a prevalent hrHPV infection among WLWH in the study sample (Table 3.2). After adjusting for age (as a continuous variable), parity, marital status, age at first intercourse, CD4 count and length of ART, primary and secondary education were found to be protective against hrHPV infection (adjusted odds ratio [aOR] 0.51, 95%CI 0.30-0.85 and 0.59 95% CI 0.39-0.89, respectively, p=0.01). No prior screening for cervical cancer was associated with double the odds of hrHPV infection (aOR 2.06 95% CI 1.41-3.01; p=<0.001).

Table 3.2 Logistic regression analysis of factors associated with hrHPV positivity among HIV positive women

	All patients N=750 n (%)	hrHPV Negative N=358 n (%)	hrHPV Positive N=392 n (%)	Bivariate		Multivariate	
				hrHPV Positivity OR (95% CI)	p-value	hrHPV Positivity aOR (95% CI)	p-value
Age (years), *Median (IQR)*	43 (31-49)	44 (38-50)	42 (36-49)				
25-29	44 (5.9)	12 (3.4)	32 (8.2)	2.39 (0.94- 6.06)	0.067	-	-
30-39	205 (27.3)	95 (26.5)	110 (28.1)	1.04 (0.51- 2.11)	0.922		
40-49	319 (42.5)	159 (44.4)	160 (40.8)	0.90 0.45- 1.80)	0.766		
50-59	146 (19.5)	75 (20.9)	71 (18.1)	0.85 (0.41- 1.76)	0.656		
≥60	36 (4.8)	17 (4.7)	19 (4.8)	*Ref*	*Ref*		
Marital status							
Single	541 (72.1)	248 (69.3)	293 (74.7)	1.37 (0.96- 1.96	0.079	-	-
Previously married	49 (6.5)	24 (6.7)	25 (6.4)	1.21 (0.64- 2.30)	0.064		
Married	160 (21.3)	86 (24.0)	74 (18.9)	*Ref*	*Ref*		
Highest education							
None	15 (2.0)	8 (2.2)	7 (1.8)	0.50 (0.17- 1.48)	0.211	0.47 (0.16- 1.41)	0.176
Primary/non-formal	130 (17.4)	68 (19.0)	62 (15.9)	0.52 (0.31- 0.87)	0.012	0.51 (0.30- 0.85)	0.01
Secondary	486 (64.9)	239 (66.8)	247 63.2)	0.59 (0.39- 90)	0.013	0.59 (0.39- 0.89)	0.01
College/university	118 (15.8)	43 (12.0)	75 (19.2)	*Ref*	*Ref*	*Ref*	*Ref*
Employed							
Yes	407 (54.3)	198 (55.3)	209 (53.3)	*Ref*	*Ref*	-	-
No	343 (45.7)	160 (44.7)	183 (46.7	1.08 (0.81- 1.45)	0.585		
Parity, *Median (IQR)*	2 (2-3)	3 (2-4)	2 (1-3)				
≤2	399 (53.2)	176 (49.2)	223 (56.9)	*Ref*	*Ref*	-	-
>2	351 (46.8)	182 (50.8)	169 (43.1)	0.73 (0.55- 0.98)	0.34		
Number of sex partners (ever)							
1-5	494 (66.4)	237 (66.6)	257 (66.2)	*Ref*	*Ref*		
>5	250 (33.6)	119 (33.4)	131 (33.8)	1.01 (0.75- 1.38)	0.923		
Age at first intercourse, Median (IQR)	19 (18-20)	19 (18-20)	19 (18-20)				
<18	576 (77.1)	282 (79.0)	294 (75.4)	1.23 (0.87- 1.73)	0.242	-	-
≥18	171 (22.9)	75 (21.0)	96 (24.6)	*Ref*	*Ref*		

Hormonal Contraception use*							
Yes	113 (20.7)	54 (20.4)	59 (20.9)	1.03 (0.68- 1.57)	0.875	-	-
No	434 (79.3)	211 (79.3)	223 (79.1)	*Ref*	*Ref*		
Condom use*							
Yes	505 (92.3)	245 (92.5)	260 (92.2)	*Ref*	*Ref*		
No	42 (7.7)	20 (7.5)	22 (7.8)	1.04 (0.55- 1.95)	0.911		
Smoking							
Yes	53 (7.1)	22 (6.1)	31 (7.9)	1.31 (0.75- 2.31)	0.348	-	-
No	697 (92.9)	336 (93.9)	361 (92.1)	*Ref*	*Ref*		
Cervical cancer screen (ever)							
Yes	603 (80.4)	308 (86.0)	295 (75.3)	*Ref*	*Ref*		
No	147 (19.6)	50 (14.0)	97 (24.7)	2.03 (1.39- 2.95)	<0.001	2.06 (1.41- 3.01)	<0.001
CD4 count (cells/mm^3)							
Median (IQR)	679 (507-871)	730 (547-893)	650 (493-845)				
<200	15 (2.0)	2 (0.6)	13 (3.3)	6.39 (1.43-28.57)	0.015	-	-
200–349	37 (4.9)	11 (3.1)	26 (6.6)	2.32 (1.13- 4.79)	0.022		
350–499	115 (15.3)	56 (15.6)	59 (15.1)	1.04 (0.69- 1.55)	0.864		
≥500	583 (77.7)	289 (80.7)	294 (75.0)	*Ref*	*Ref*		
CD4 count (cells/mm^3)							
<500	167 (22.3)	69 (19.3)	98 (25.0)	1.40 (0.99- 1.98)	0.06	-	-
≥500	583 (77.7)	289 (80.7)	294 (75.0)	*Ref*	*Ref*		
Viral load (copies/mL)							
≤400(undetectable)	739 (98.7)	353 (98.6)	386 (98.7)	*Ref*	*Ref*	-	-
>400	10 (1.3)	5 (1.4)	5 (1.3)	0.92 (0.26- 3.19)	0.888		
Length of ART (years)							
Median (IQR)	8 (4-13)	9 (5-13)	7 (3-12)				
<2	82 (10.9)	30 (8.4)	52 (13.3)	1.67 (1.04- 2.69)	0.034		
≥2	668 (89.1)	328 (91.6)	340 (86.7)	*Ref*	*Ref*		

hrHPV: high-risk human papilloma virus; ART: Antiretroviral therapy; IQR: inter-quartile range; OR: odds ratio; aOR: adjusted odds ratio; CI: confidence interval

*****Among those using contraception

Table 3.3 Logistic regression analysis of factors associated with hrHPV positivity among HIV negative women

	All patients N=750 n (%)	hrHPV Negative N=436 n (%)	hrHPV Positive N=314 n (%)	Bivariate	Multivariate		
				hrHPV Positivity OR (95% CI)	p-value	hrHPV Positivity aOR (95% CI)	p-value
Age years							
Median (IQR)	39 (32-48)	40 (33-49)	37 (30-48)				
25-29	131 (17.5)	63 (14.4)	68 (21.7)	1.11 (0.61- 2.05)	0.725	-	-
30-39	267 (35.6)	151 (34.6)	116 (36.9)	0.79 (0.46- 1.39)	0.417		
40-49	186 (24.8)	124 (28.4)	62 (19.7)	0.52 (0.29- 0.93)	0.028		
50-59	105 (14.0)	67 (15.4)	38 (12.1)	0.59 (0.31- 1.11)	0.102		
≥60	61 (8.1)	31 (7.1)	30 (9.6)	*Ref*	*Ref*		
Marital status							
Single	495 (66.0)	278 (63.8)	217 (69.1)	1.27 (0.91- 1.77)	0.158	-	-
Previously married	50 (6.7)	31 (7.1)	19 (6.1)	1.0 (0.53- 1.89)	0.995		
Married	205 (27.3)	127 (29.10	78 (24.8)	*Ref*	*Ref*		
Highest education							
None	13 (1.7)	8 (1.8)	5 (1.6)	0.88 (0.28- 2.76)	0.823	-	-
Primary/non-formal	100 (13.3)	60 (13.8)	40 (12.7)	0.94 (0.58- 1.50)	0.783		
Secondary	387 (51.6)	222 (50.9)	165 (52.5)	1.04 (0.76- 1.44)	0.796		
Tertiary/college	250 (33.30	146 (33.5)	104 (33.1)	*Ref*	*Ref*		
Employed							
Yes	419 (55.9)	246 (56.4)	173 (55.1)	*Ref*	*Ref*	-	-
No	331 944.10	190 (43.6)	141 (44.9)	1.06 (0.79- 1.41)	0.718		
Parity							
Median (IQR)	2 (1-3)	2)1-3)	2 (1-3)				
≤2	440 (58.7)	231 (53.0)	209 (66.6)	*Ref*	*Ref*		
>2	310 (41.3)	205 (47.0)	105 (33.4)	0.57 (0.42-0.76)	<0.001	0.49 (0.34- 0.71)	<0.001
Number of sex partners(ever)							
1-5	549 (74.0)	322 (74.5)	227 (73.2)	*Ref*	*Ref*		
>5	193 (26.0)	110 (25.5)	83 (26.8)	1.07 (0.77- 1.49)	0.688		
Age at first intercourse							
Median (IQR)	19 (18-21)	19 (18-21)	19 (18-21)				
<18	147 (19.7)	85 (19.7)	62 (19.8)	1.01 (0.70- 1.45)	0.964	-	-

≥18	598 (80.3)	347 (80.3)	251 (80.2)	*Ref*	*Ref*		
Hormonal Contraception use*							
Yes	150 (28.6)	82 (26.4)	68 (31.9)	1.31 (0.89- 1.92)	0.167	-	-
No	374 (71.4)	229 (73.6)	145 (68.1)	*Ref*	*Ref*		
Condom use*							
Yes	447 (85.3)	261 (83.9)	186 (87.3)	*Ref*	*Ref*		
No	77 (14.7)	50 (16.1)	27 (12.7)	0.76 (0.46- 1.26)	0.281		
Smoking							
Yes	20 (2.7)	9 (2.1)	11 (3.5)	1.72 (0.71- 4.21)	0.233	-	-
No	730 (97.3)	427 (97.9)	303 (96.5)	*Ref*	*Ref*		
Cervical cancer screen (ever)							
Yes	418 (55.7)	258 (59.2)	160 (51.0)	*Ref*	*Ref*		
No	332 (44.3)	178 (40.8)	154 (49.0)	1.40 (1.04- 1.87)	0.026		

hrHPV: high-risk human papilloma virus; HIV: human immunodeficiency; IQR: inter-quartile range; OR: odds ratio; aOR: adjusted odds ratio; CI: confidence interval
*****Among those using contraception

3.2.2. Characteristics associated with HrHPV positivity among HIV-negative women

Having adjusted for age (as a continuous variable), prior cervical cancer screening, smoking, current use of hormonal contraception, and condom use, having more than two children was the only variable found to be associated with lower odds (aOR 0.49 95% CI 0.34-0.71; p=<0.001) of testing positive for hrHPV (Table 3.3).

3.2.3. Outcomes of primary hrHPV screening

3.2.3.1 Prevalence of any hrHPV

WLWH were more likely to have any hrHPV (52.4% versus. 41.6% in HIV-negative women, (p<0.001) (Table 3.4). The hrHPV subtypes were grouped into five categories according to their likely relative risk profile for causing cervical precancer and invasive cancer: HPV 16 and/or 18 (group 1), HPV 16 and/or 18/45 (group 2), HPV 16/18/31/33/35/45/52/58 (group 3), non-HPV 16/18/45 (group 4), and other-HPV (types 39/51/53/56/59/66/68) (group 5). WLWH were more likely to have higher proportions in all these groups compared to HIV-negative women (p <0.001), with the difference ranging from 21.0% to 49.5%.

Table 3.4 Prevalence of different hrHPV groups by HIV status

HPV types	All Patients N=1500 n (%)	HIV Negative N=750 n (%)	HIV Positive N=750 n (%)	P value
Any hrHPV	706 (47.1)	314 (41.9)	392 (52.3)	<0.001
hrHPV 16/18 (Group 1)	174 (11.6)	70 (9.3)	104 (13.9)	<0.001
hrHPV 16/18/45 (Group 2)	229 (15.3)	95 (12.7)	134 (17.9)	<0.001
hrHPV 16/18/31/33/35/45/52/58 (Group 3)	503 (33.5)	215 (28.7)	288 (38.4)	<0.001
Non-hrHPV 16/18/45 (Group 4)	482 (32.1)	218 (29.1)	264 (35.2)	<0.001
Other hrHPV (39, 51,53,56, 59, 66, 68) (Group 5)	400 (26.7)	168 (22.4)	232 (30.9)	<0.001

hrHPV: high risk human papillomavirus; HIV: human immunodeficiency virus
HPV: human papillomavirus; (HPV single types 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68)

3.2.3.2 Age distribution of hrHPV by age and HIV status

Table 3.5 shows the hrHPV prevalence by age and HIV status, and Figures 3.1 and 3.2 provide a visual representation of this data. Any hrHPV prevalence was highest in the age group of 25 to.29 years old among both WLWH and HIV-negative women. Among WLWH, the prevalence of all the five groups was highest in the age group 25 to.29 years old except for hrHPV group 3 which was highest in the age group 60 years or older. Among HIV-negative women, the prevalence for hrHPV groups 1, 2, and 5 was highest in the 25 to 29 years age group, and the prevalence of hrHPV groups 3 and 4 was highest among the 60 years and older group.

Table 3.5 HrHPV prevalence by age and HIV status

	All patients N=1500 n (%)	HIV-positive N=750 n (%)	HIV-negative N=750 n (%)
Any hrHPV			
All women	706/1500 (47.1)	392/750 (52.3)	314/750 (41.9)
25-29	100/175 (57.1)	32/44 (72.7)	68/131 (51.9)
30-39	226/472 (47.9)	110/205 (53.7)	116/267 (43.4)
40-49	222/505 (44.0)	160/319 (50.2)	62/186 (33.3)
50-59	109/251 (43.4)	71/146(48.6)	38/105 (36.2)
≥60	49/97 (50.5)	19/36 (52.8)	30/61 (49.2)
HPV 16/18 (Group 1)			
All women	174/1500/ (11.6)	104/750 (13.9)	70/750 (9.3)
25-29	18/175 (10.3)	7/44 (15.9)	11/131 (8.4)
30-39	56/472 (11.9)	28/205 (13.7)	28/267 (10.5)
40-49	58/505 (11.5)	44/319 (13.8)	14/186 (7.5)
50-59	28/251 (11.2)	21/146 (14.4)	7/105 (6.7)
≥60	14/97 (14.4)	4/36 (11.1)	10/61 (16.4)
HPV 16/18/45 (group 2)			
All women	229/1500 (15.3)	134/750 (17.9)	95/750 (12.7)
25-29	29/175 (16.6)	12/44 (27.3)	17/131 (13.0)
30-39	78/472 (16.5)	37/205 (18.0)	41/267 (15.4)
40-49	70/505 (13.9)	54/319 (16.9)	16/186 (8.6)
50-59	32/251 (12.7))	24/146 (16.4)	8/105 (7.6)
≥60	20/97 (20.6)	736 (19.4)	13/61 (21.3)
HPV 16/18/31/33/35/45/52/58 (group 3)			
All women	503/1500 (33.5)	288/750 (38.4)	215/750 (28.7)
25-29	59/175 (33.7)	18/44 (40.9)	41/131 (31.3)
30-39	164/472 (34.7)	84/205 (41.0)	80/267 (30.0)
40-49	164/505 (32.5)	121/319 (37.9)	43/186 (23.1)
50-59	78/251 (31.1)	49/146 (33.6)	29/105 (27.6)
≥60	38/97 (39.2)	16/36 (44.4)	22/61 (36.1)
Non-HPV 16/18/45 (group 4			
All women	482/1500 (32.1)	264/750 (35.2)	218/750 (29.1)
25-29	69/175 (39.4)	18/44 (40.9)	51/131 (38.9)
30-39	152/472 (32.2)	76/205 (37.0)	76/267 (28.5)
40-49	155/505 (30.7)	109/319 (34.2)	48/186 (24.7)
50-59	76/251 (30.3)	48/146 (32.9)	28/105 (26.7)
≥60	30/97 (30.9)	13/36 (36.1)	17/61 (27.9)
HPV 39/51/53/56/59/66/68 (group 5)			
All women	400/1500 (26.7)	232/750 (30.9)	168/750 (22.4)
25-29	71/175 (40.6)	28/44 (63.6)	43/131 (32.8)
30-39	121/472 (25.6)	61/205 (29.8)	60/267 (22.5)
40-49	118/505 (23.4)	84/319 (26.3)	34/186 (18.3)
50-59	60/251 (23.9)	46/146 (31.5)	14/105 (13.3)
≥60	30/97 (30.9)	13/36 (36.1)	17/61 (27.9)

hrHPV: high risk human papillomavirus; HIV: human immunodeficiency virus
HPV: human papillomavirus; (HPV single types 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68)

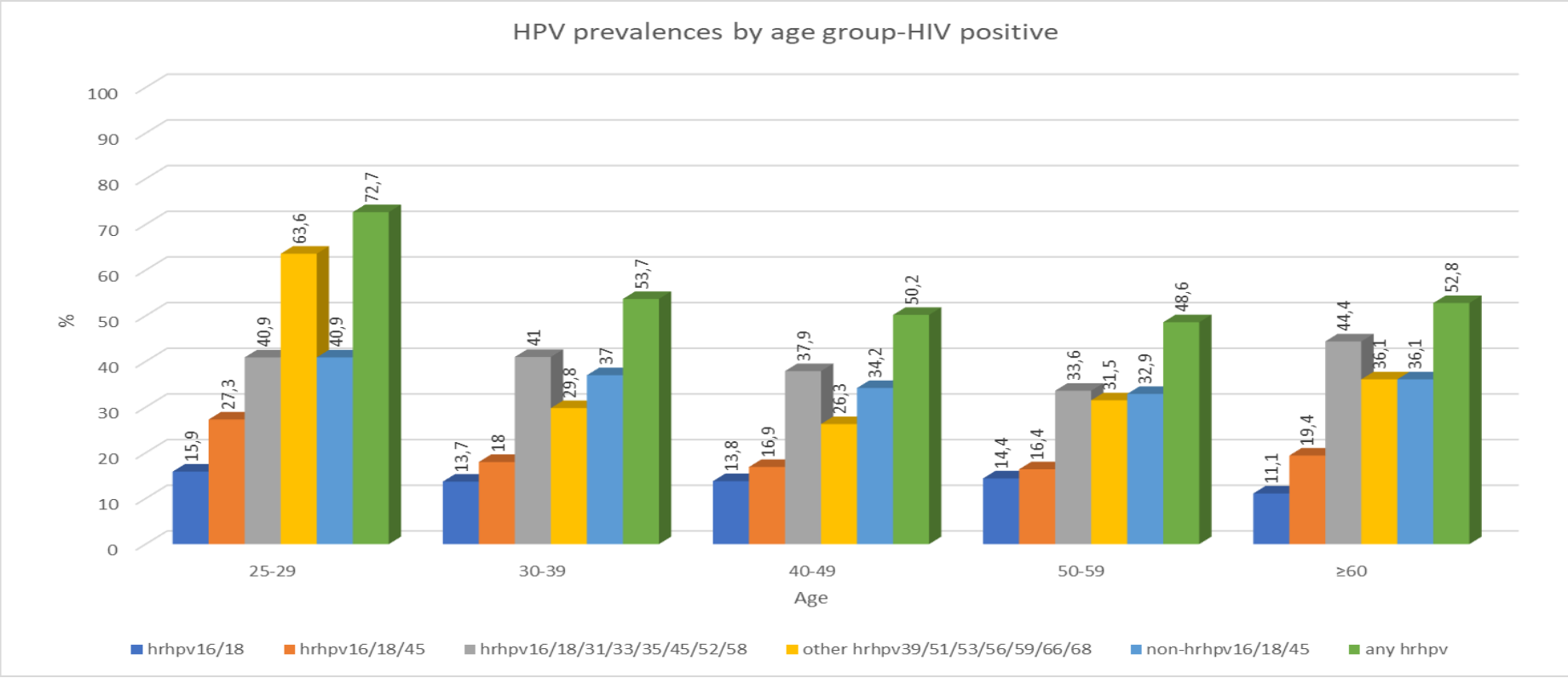

Figure 3.1 HPV prevalence by age group among HIV-positive women

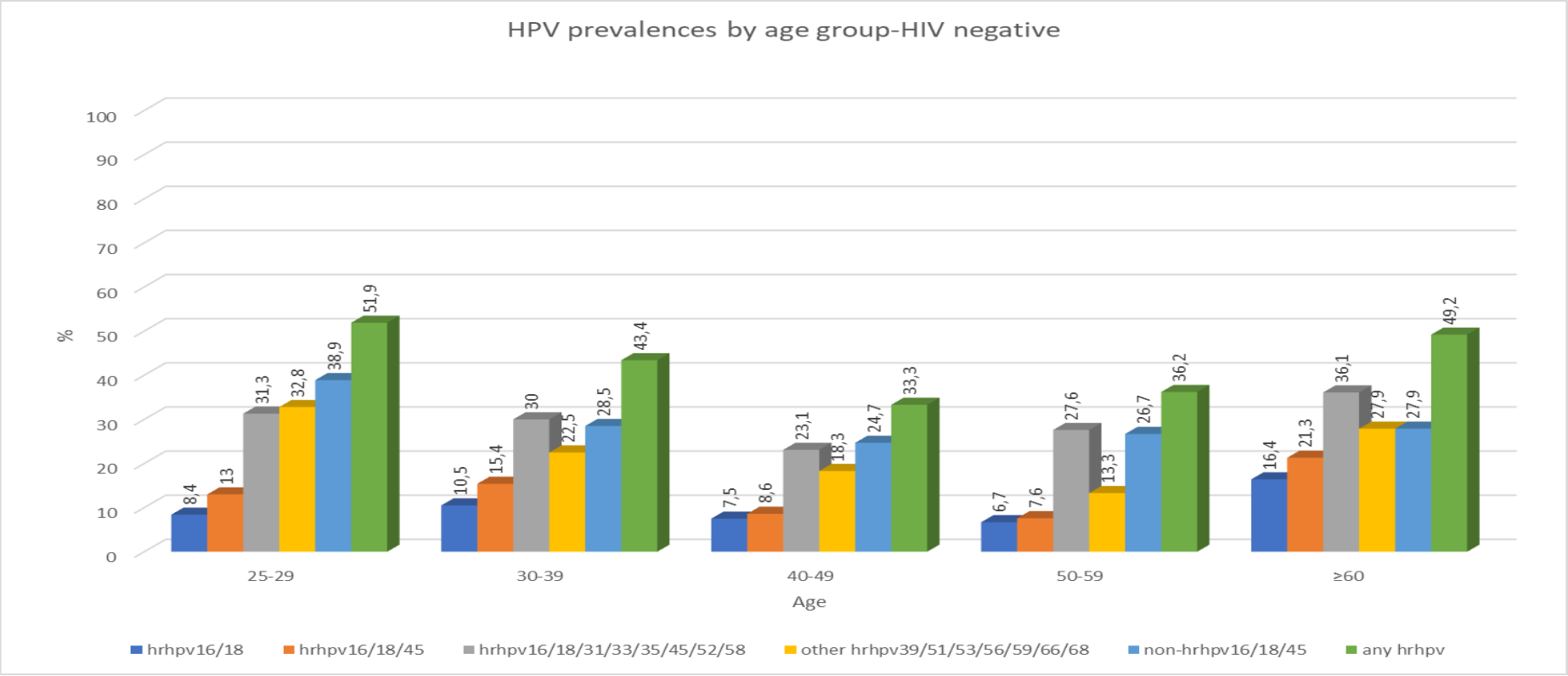

Figure 3.2 HPV prevalence by age group among HIV-negative women

Figures 3.3 and 3.4 depict linear representations of the prevalence of the various hrHPV groups for the different age groups. Across the age groups, the prevalence of group 1 stayed relatively constant, with a small increase in the proportion among older HIV-negative women. There was a substantial decrease in the proportion of women testing positive for the lower risk group (group 5) observed between the age groups 25 to 29 and 30 to 39, with this decrease more pronounced among WLWH. The proportion of group 5 remained relatively similar in WLWH with increasing age. However, there was an appreciable increase between 50 to 59 years age group and the 60 or older age group in the HIV-negative women.

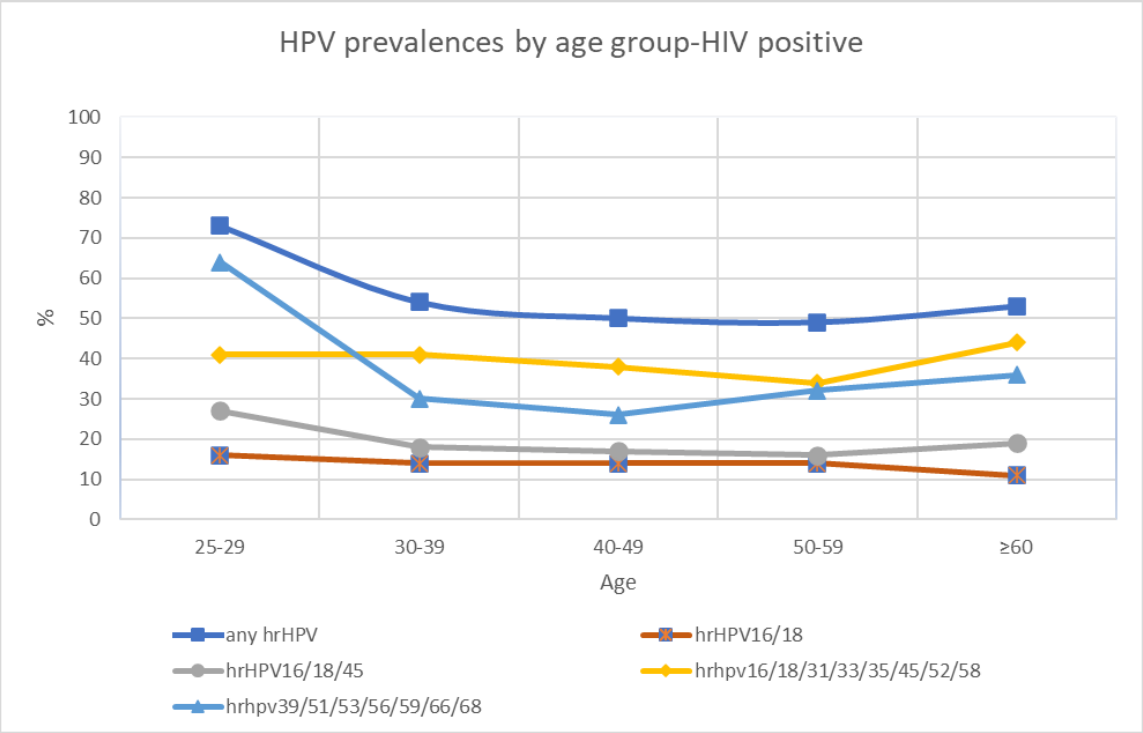

Figure 3.3 Trend of HPV prevalence by age group among HIV-positive women

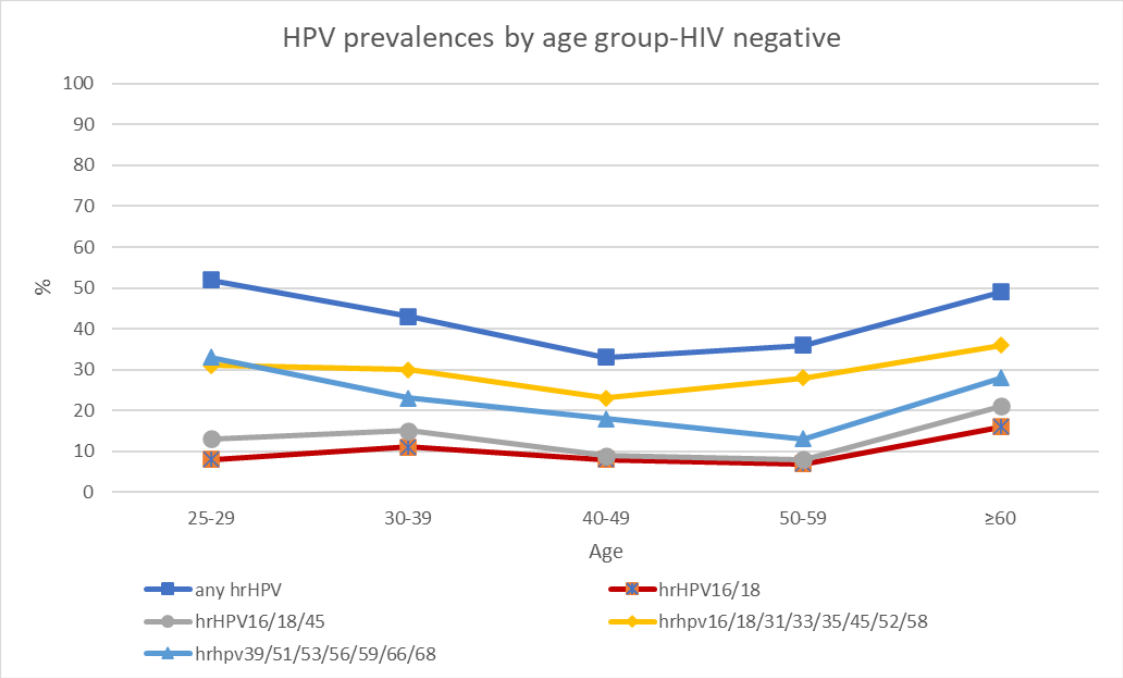

Figure 3.4 Trend of HPV prevalence by age group among HIV-negative women

3.2.3.3 Number of hrHPV types per participant

When positive for hrHPV, WLWH were more likely to be infected with two or more hrHPV types compared to HIV-negative women (45.5% vs. 35.9%, p=0.001) (Table 3.6). The prevalence of two or more types was highest in the 25 to 29 year age group (51.5%), followed by the 60 years and above age group (49.2%), the 30 to 39 year age group (43.2%), the 50 to 59 year age group (35.5%), and then the 40 to 49 year age group (33.2%). Also see Figures 3.5 and 3.6.

Table 3.6 Number of hrHPV types by age and HIV status in hrHPV positive women

	All patients N=706 n (%)	HIV-positive N=392 n (%)	HIV-negative N=314 n (%)
One single HPV type			
All women	393/706 (55.7)	194/392 (49.5)	199/314 (63.4)
25-29	49/100 (49.0)	10/32 (31.3)	39/68 (57.4)
30-39	129/226 (57.1)	61/110 (55.5)	68/116 (58.6)
40-49	125/222 (56.3)	82/160 (51.2)	43/62 (69.4)
50-59	66/109 (60.6)	37/71 (52.1)	29/38 (76.3)
≥60	24/49 (49.0)	4/19 (21.1)	20/30 (66.7)
2-4 HPV types			
All women	287/706 (40.7)	178/392 (45.4)	109/314 (34.7)
25-29	46/100 (46.0)	18/32 (56.3)	28/68 (41.2)
30-39	84/226 (37.2)	39/110 (35.5)	45/116 (38.8)
40-49	91/222 (41.0)	74/160 (46.3)	17/62 (27.4)
50-59	41/109 (37.6)	32/71 (45.1)	9/38 (23.7)
≥60	25/49 (51.0)	15/19 (78.9)	10/30 (33.3)
≥5 HPV types			
All women	26/706 (3.7)	20/392 (5.1)	6/314 (1.9)
25-29	5/100 (5.0)	4/32 (12.5)	1/68 (1.5)
30-39	13/226 (5.8)	10/110 (9.1)	3/116 (2.6)
40-49	6/222 (2.7)	4/160 (2.5)	2/62 (3.2)
50-59	2/109 (1.8)	2/71 (2.8)	0/38 (0.0)
≥60	0/49 (0.0)	0/19 (0.0)	0/30 (0.0)

hrHPV: high risk human papillomavirus; HIV: human immunodeficiency virus HPV: human papillomavirus

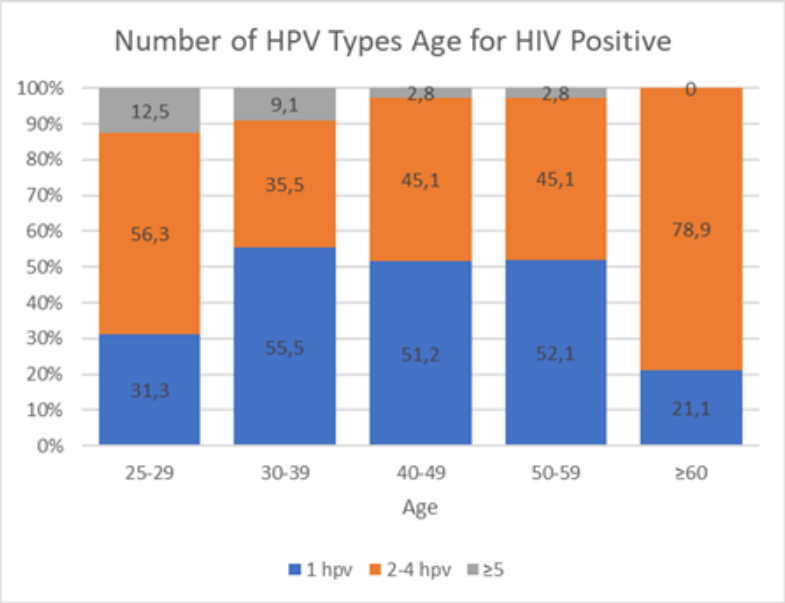

Figure 3.5 Number of hrHPV types by age in HIV positive women

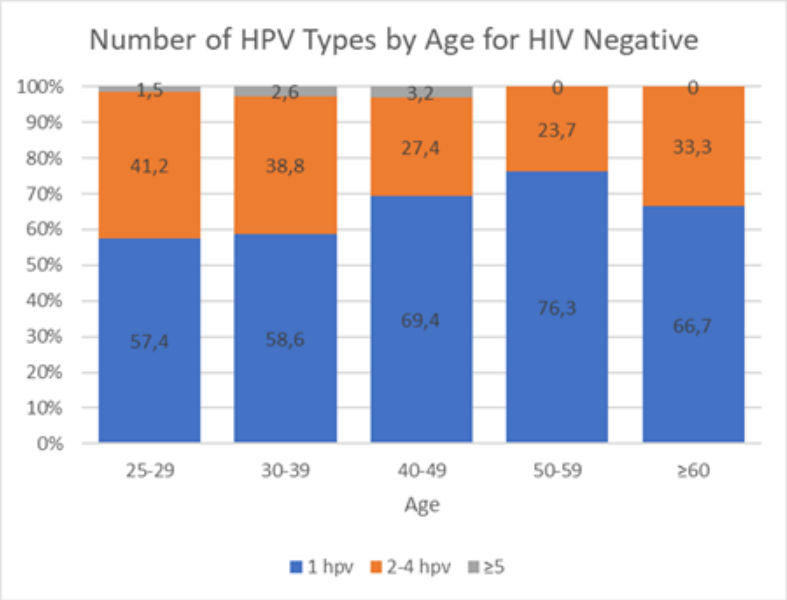

Figure 3.6 Number of hrHPV types by age in HIV negative women

3.2.3.4 Prevalence of each hrHPV type

The following hrHPV types were amongst the ten most common types regardless of HIV-status (HPV 16/33/51/52/53/58/68) (Figure 3.7). HrHPV 16 was equally common in WLWH and HIV-negative women (7.9% vs. 6.8%), whereas hrHPV 18 was 2.25 times more common in WLWH compared to HIV-negative women.

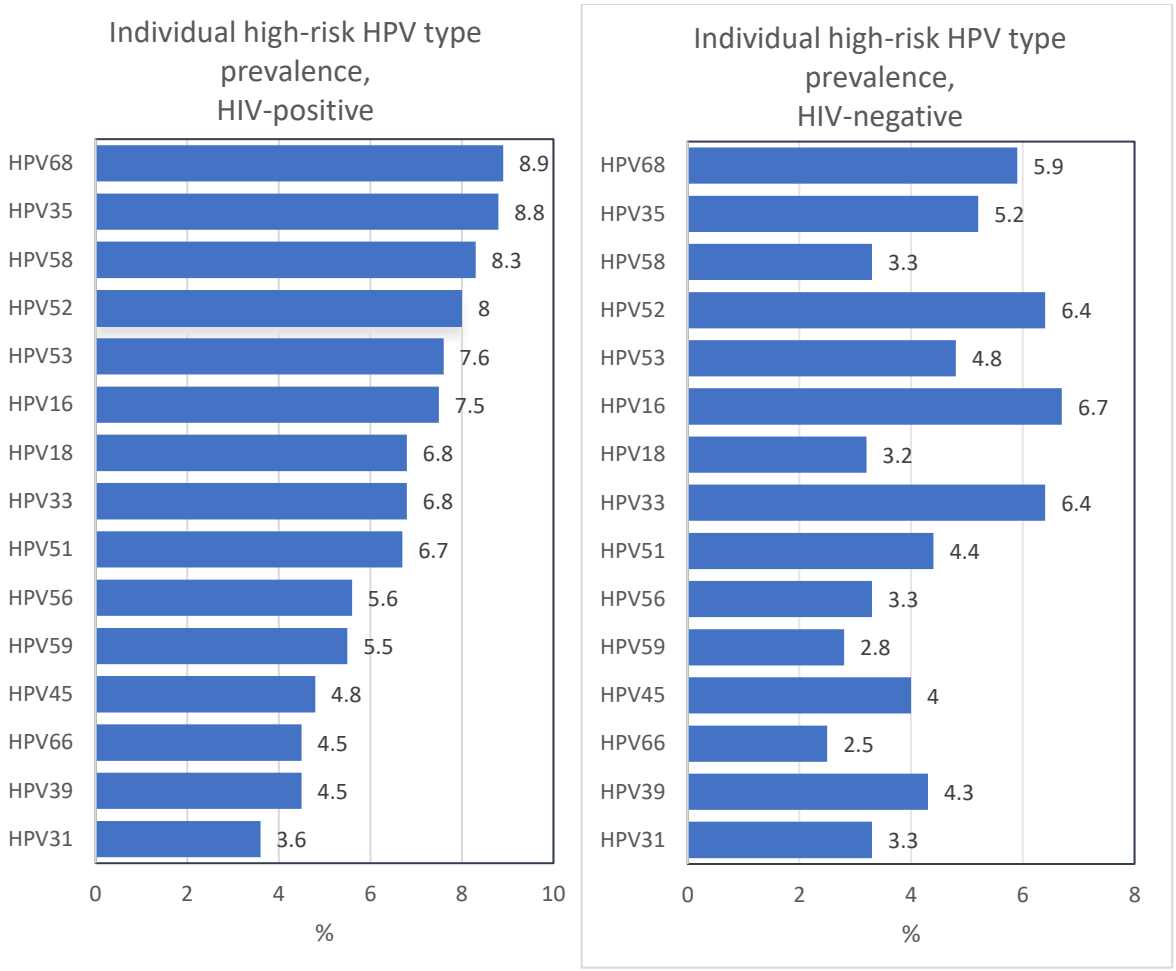

Figure 3.7 Most common hrHPV types by HIV status (as single or multiple infections). HrHPV types ordered by magnitude among women living with HIV

3.3. Discussion

Understanding the epidemiology of hrHPV in each country is critical to determining appropriate HPV vaccine policy and estimating impact and resource planning for hrHPV screening, triage and cervical precancer treatment in that country. In this chapter, we present our findings on any and type-specific hrHPV prevalence in WLWH and HIV-negative women in a semi-urban population in Botswana. Our main observations were 1) that any and type-specific hrHPV prevalence was high, varied with age and appeared to be strongly associated with HIV-status, 2) that the highest prevalence of positivity for 2-4 HPV sub-types (as opposed to a single HPV type) was in WLWH, and was more common at the extremes of age but most pronounced in the older age group, 3) that the greatest drop in hrHPV prevalence with increasing age appears to be associated with the changes in the less risky hrHPV types (39/51/53/56/58/66/68), 4) that the prevalence of the higher risk HPV 16/18 appeared to be fairly constant across the age groups, and 5) that HPV 16 was common in both WLWH and HIV-negative women.

3.3.1. Prevalence of hrHPV in women living with HIV

Numerous screening studies have reported high prevalence of hrHPV infection in WLWH in sub-Saharan Africa, and our study has provided more evidence of this from Botswana with the overall hrHPV prevalence of 52%. This prevalence was similar to that reported by Jaquet and colleagues in women of reproductive age in Cote d'Ivoire (53.9%)[10] and two other studies in women from Cape Town, South Africa (52.4% and 48.2%).[11,12] HrHPV prevalence in our study was higher than that reported by two other studies from Botswana, and one other from South Africa.[13,14] One of the Botswana studies evaluated a different age group of 30 to 49 year olds with a prevalence of 41.3%.[13] The other study was done in women 25 years and older with a prevalence of 29%, but the median ages were higher than in our study (hrHPV-positive 44 years [IQR 40-57], and hrHPV-negative 47 years [IQR 42-52]).[14] The South African study done in the Eastern Cape in 30 to 98 year olds also had a lower prevalence of 40.6% compared to our study.[15]

The higher hrHPV infection seen among women in several sub-Saharan African countries is generally linked to the higher HIV prevalence, and may be further explained by the presence of other risk factors.[15-23] These factors include early age of sexual debut,[16] the number of sexual partners of women and of their partners,[15,17] other sexually transmitted diseases,[18-22] and use of hormonal contraception.[23] It is notable that in our study population the age of sexual debut was relatively high (18 years or older), so other co-factors for hrHPV acquisition may have been more prominent in our cohort.

Our findings have shown a very high prevalence in the younger age group of 25 to 29 years of WLWH (72.7%), which was similar to that reported by Ginindza and colleagues in Swaziland (72%).[24] Historically, HPV DNA testing has been recommended for women 30 years and older; therefore, very few studies have reported on hrHPV prevalence in the 25 to 29 years age group. Two studies from Johannesburg and Cape Town, South Africa, reported hrHPV prevalence of 59.6% and 59.7% in this 25 to 29 age group in WLWH.[3,11] Other studies that have reported prevalence similar to our finding have been in younger women of less than 25 years. McClung and colleagues recently reported a hrHPV of 72.9% in a cohort of 223 sexually active 18 to 22 years old WLWH in Gaborone, Botswana.[25] Omar and colleagues reported a combined prevalence of 66.7% in HIV-infected 18 to 24 years old young adults in Mozambique, the majority of whom were females.[26]

When hrHPV genotypes were stratified by immunocompetency status based on CD4 counts, our findings showed that increased CD4 count was significantly associated with lower prevalence of HPV-infection among WLWH. This was confirmed by previous studies which showed higher CD4 count associated with lower prevalence of HPV-infection among WLWH.[27-32]

Our results also showed that ART usage was associated with decreased risk of overall hrHPV detection among WLWH, a finding that agrees with a study by Ermel et al.[33] in Kenya, and Kelly et al.[34] in South Africa and Bukina Faso. A systematic review and meta-analysis of association of ART, hrHPV, precancer, and invasive cancer, reported association of reduced hrHPV prevalence, precancer incidence (aOR, 0.59) and progression (aOR, 0.64), and incidence of invasive cervical cancer (adjusted hazard

ratio [aHR], 0.54) with effective ART use.[27] It also showed an increased regression of cervical intraepithelial abnormalities (aHR, 1.54). The positive effect of ART seems more certain when treatment duration is long (>2 years),[27,35] and started at higher CD4 count nadir,[36] and lower HIV viral loads.[37,38]

3.3.2. Prevalence of hrHPV in HIV-negative women

In most previous studies in southern Africa, the prevalence of hrHPV in HIV-negative women has generally been 50% or less than that in WLWH. In Botswana, Castle and colleagues reported a prevalence of 25.2%, and three studies from South Africa reported prevalence of 16-21.4%.[11,12,15] Our overall hrHPV prevalence in HIV-negative women was high (41.9%), especially given that more than 50% of the women reported previous screening for cervical cancer. There are two possible reasons for this finding. First, HrHPV prevalence is influenced by the circulating levels of hrHPV in the general population. The assumption of high circulating hrHPV levels in Botswana is a reasonable one given prior hrHPV prevalence reports, combined with high HIV prevalence. Second, as the AmpFire HPV platform is relatively new, it is likely that the manufacture settings have a higher sensitivity for HPV DNA than other previously used platforms leading to detection of transient infections. Nonetheless, this finding warrants further investigation.

As in WLWH in our study, we observed a higher prevalence of hrHPV in the 25 to 29 age group of HIV-negative women (51.9%) than in other HIV-negative age groups. This finding was similar to that reported by McDonald in South Africa (46.4%),[11] and Ginindza in Swaziland (44%).[24] The highest prevalence of hrHPV in younger women has also been reported in other settings in sub-Saharan Africa, Asia, Latin America, and the United States of America.[39-44]

3.3.3. The natural history of HPV over time

Over the years data has accumulated to describe the natural history of HPV. The generally accepted model is that of a linear pathway that begins with acquisition of HPV at or around the time of sexual debut through sexual exposure which explains

the highest prevalence of both low- and high-risk HPV in adolescent girls and young women.[42,45] Up to 90% of these newly acquired infections spontaneously become undetectable within 1-2 years, a process usually attributed to viral clearance.[46] In up to two thirds of women, the initial exposure to the HPV infection generates an immune response that leads to detectable specific serum antibodies to the type-specific HPV.[47] The significance of the serum antibodies in preventing future re-infection with the same HPV genotype is unclear.[48] Finally, a small percentage of infections can still be detected beyond one year, and these are thought to be due to persistent infection and represent a higher risk of progression to precancer and cancer if there is no appropriate intervention.[46]

Gravitt and colleagues proposed alternative, non-linear, non-mutually exclusive pathways that HPV infections may follow from exposure and progress over the years.[49] According to this model, new detection of HPV-infection could be from re-infection, recurrent detection of a controlled or latent infection,[50] direct auto-inoculation from other sites (e.g., anus) or indirectly from the virus carried underneath fingernails,[51,52] and finally, transient deposition of viral nucleic acid from a recent sexual exposure.[53] Conversely, loss of HPV detection that has been termed “clearance” may be due to viral eradication with or without acquired immunity against re-infection (what has been termed “clearance”) or viral control below limits of detection (viral latency).[54]

Reactivation of latent HPV may be responsible for detection of new HPV infection in older women in the absence of recent sexual exposure. Our findings indicate a drop in hrHPV detected after the age group 25 to 29 in both WLWH and HIV-negative women, with a slight uptick in the prevalence in the older age group. Most studies that show a similar pattern come from North America. Three mid-adult cohort studies using repeated HPV DNA testing linked to sexual history have shown that unlike in younger women where new detection of HPV is likely to be due to new sexual activity, new detection in older women is more likely to be due to re-activation of latent infection.[55-57] The explanation for the latter is related to age related immunosuppression.

Strickler and colleagues established the link between HPV reactivation and HIV status.[50] New detection of HPV infection in non-sexually active women increased from HIV-negative women (5%), followed by WLWH with CD4 count >500 (7%), WLWH

with CD4 count 200-500 (9%), and the highest in WLWH with CD4 count <200 (22 %). Other studies in southern Africa have reported similar findings of increased detection of hrHPV with increasing age in HIV-negative women compared with WLWH.[11,24,58,59]

Our study reported an increase in detection of hrHPV in older HIV-negative women when compared with WLWH. Other studies in southern Africa have reported similar findings of increased detection of hrHPV in older age groups in HIV-negative women compared with WLWH.[11,24,58,59] Increasing age is an independent risk factor for cervical cancer regardless of HIV status as the core tenant of cervical cancer development is persistence of hrHPV over time.[27,60] Regular screening with effective treatment of women with precancer disease has been found to reduce incidence of cervical cancer. Countries with newer screening programmes like those in LMICs have limited target age groups, commonly restricted to the 30 to 49 year olds. The exclusion of older women as programmes begin jeopardises higher risk older women who may already have high rates of persistent hrHPV or even more detrimental, those with existing precancer or cancer. The effectiveness of screening is also dependent on coverage of the target population, and most programmes are challenged in this regard. Given how the Botswana programme has disproportionally targeted WLWH, its plausible that coverage of screening in women living without HIV, especially those in the older birth cohorts, is much lower than in WLWH. Indeed, the difference in reported rates of previous cervical cancer screening in our study by HIV status suggests this.

3.3.4. Prevalence of HPV 16

It is generally agreed that not all the different specific HPV genotypes have the same potential for progression to invasive disease, with some considered higher risk than others. Indeed, in the hierarchy of risk, HPV types 16/18 are universally considered highest risk. There is, however, some debate about which types confer the lowest risk but this group seems to include some or all of HPV 39/51/53/56/59/66/68.[61] Closer analysis of the changes in detection of the various HPV types with increasing age in our study showed some interesting findings. As HPV16/18 remained relatively constant across age groups in this study, the group of seven relatively lower risk HPV genotypes appeared to account for most of the changes in the prevalence across the

age groups. These findings suggest the higher likelihood of persistence associated with infection with HPV16/18, and therefore risk of progression to precancer and cancer.[62-64] HPV 16, more so than 18, has been shown to be relatively independent of the immune status.[65] Through its evolution, HPV 16 seems to have created a better mechanism to avoid host immune surveillance relative to other HPV genotypes, and hence the relatively lower than expected rise in invasive cervical cancer due to HPV 16 in WLWH, especially in those with severely suppressed immunity compared to HIV-negative women.

Our finding of the prevalence of HPV 16 contrasted that from another study from Botswana. Even though Castle and colleagues reported prevalence of 8.3% in WLWH which is close to our finding, the prevalence in HIV-negative women was much lower than ours (2.8% vs. 6.7%). The explanation for this difference is unclear and may warrant further investigation.

3.3.5. Prevalence of multiple hrHPV genotypes

Our study has further demonstrated that when positive for hrHPV, WLWH were more likely to be infected with two or more hrHPV genotypes compared to HIV-negative women (50.5% vs. 36.6%). Previous studies have also reported a similar finding.[17,28,44,66-69] The finding of the increased risk of multiple hrHPV genotypes (both prevalent and persistent) infection in WLWH may be attributed to the difficulties in the resolution of HPV infection due to deficiencies in cell mediated immune response at the mucosal level.[28,70,71]

3.3.6. Prevalence of hrHPV and previous cervical cancer screening

In our study, history of previous cervical cancer screening was significantly associated with reduced hrHPV positivity only in WLWH. This result corroborates the findings by Kelly and colleagues.[3] Six hundred and twenty-six WLWH were enrolled from a primary and a community health clinic in Johannesburg, South Africa, and followed for 16 months. The hrHPV detection at baseline was 59.7% and 30% at 16 months in women who had been successfully treated for CIN 2+. Similarly, in a smaller sample

of 300 WLWH in Gaborone, Botswana, the baseline hrHPV detection was 29% and 20% after 12-16 months following treatment of those with abnormal colposcopy results.[72] Screening of all women regardless of HIV status is important in a national screening programme to lead to overall reduction of circulating hrHPV.

3.3.7. The effect of Botswana HIV treatment programme on hrHPV prevalence

The benefits of the successful HIV treatment programme in Botswana may not be as pronounced for the prevention of HPV acquisition and persistence as some would have anticipated. Eighty percent of WLWH in this study appeared to have systemically reconstituted CD4+ T-cells (CD4 count ≥500). However, this did not translate into lower prevalence of hrHPV, and by extension, risk of precancer and invasive cancer. Saluzzo *et al*.[71] suggests that if ART treatment is started after irreversible loss of tissue/mucosal immunity, this may not halt development of precancer/cancer despite the apparent recovery of systemic immunity. Universal ART at HIV diagnosis was started in 2016 in Botswana, and the country has been successful in reaching and exceeding the 90-90-90 HIV management targets.[73-75] However, this achievement does not appear to have positively impacted prevalence of hrHPV. Even with universal ART in Botswana, it is plausible that some women may have started treatment with advanced HIV disease resulting in poor immunologic response to treatment,[76] and lesser ability to optimally protect against cervical disease.[36] Including a measure of mucosal immunity in comparison with systemic immunity may better stratify cervical cancer risk for WLWH in future investigations.

The other reason that previous screening may not result in lower detection of hrHPV and hrHPV-related disease in future may be related to treatment failure, or delay/loss to follow-up, following a screen-positive result. Indeed, Kelly *et al*.[3] reported hrHPV detection of 66.7% in women who were previously treated for CIN 2+ and still had CIN 2+ detected at follow-up (presumed treatment failure). Two other studies in Botswana reported delays in accessing care for women presenting with invasive cervical cancer disease despite previous screening due to various reasons.[77,78] These included two specific delays: 1) a delay in results reaching the referring facility and the patient, and

2) a delay in the facility making an appointment for the patient at the gynecological oncology multi-disciplinary team clinic. Therefore, an effective prevention programme depends on the optimization of all the component in the screening pathway.

3.3.8. Conclusions

Given the above findings which seem to indicate a high level of circulating hrHPV in the general population in Botswana, diligence in cervical cancer screening and effective treatment of both WHLW and HIV-negative women in Botswana, and similar settings, is imperative.

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CHAPTER 4. VISUAL EVALUATION OF HRHPV POSITIVE WOMEN

4.1. Introduction

The use of visual inspection with acetic acid (VIA) as an alternative primary cervical cancer screening method started nearly two decades ago in low and middle income countries (LMICs).[1,2] Although this approach has made screening accessible for women in poorer countries because of its operational feasibility and affordability [3,4] its performance has been variable at best, especially in the absence of agreed quality assurance standards in most countries.[4,5] The World Health Organization (WHO) recommends primary cervical cancer screening with an hrHPV test where feasible, and reserves VIA as a "triage-and-treat" method for hrHPV screen-positive women in low-resource settings.[6]

Colposcopy is the mainstay triage method of cervical cancer screening programmes in high-income country (HIC).[7-9] It was initially used following an abnormal cytology result,[7,8] and more recently has increasingly been used for triage of hrHPV screen-positives.[9]

In this chapter, we report the outcome of visual evaluation triage of hrHPV screen-positive WLWH and HIV-negative women by VIA and colposcopy.

4.2. Results

Seven hundred and fifty WLWH and another 750 HIV-negative women were screened with hrHPV. Women who screened positive for any hrHPV (392 [52.3%] WLWH, and 314 [41.9%] HIV-negative women) were referred for visual triage with VIA and colposcopy. Forty-four women were lost to follow up (29 WLWH and 15 HIV-negative women) (Figures 4.1 and 4.2). The 662 women who attended follow-up were evaluated at the same visit, first by a nurse (VIA) followed by a gynaecologist (colposcopy). Each assessor was blinded to the type of hrHPV present and to the visual evaluation of the other.

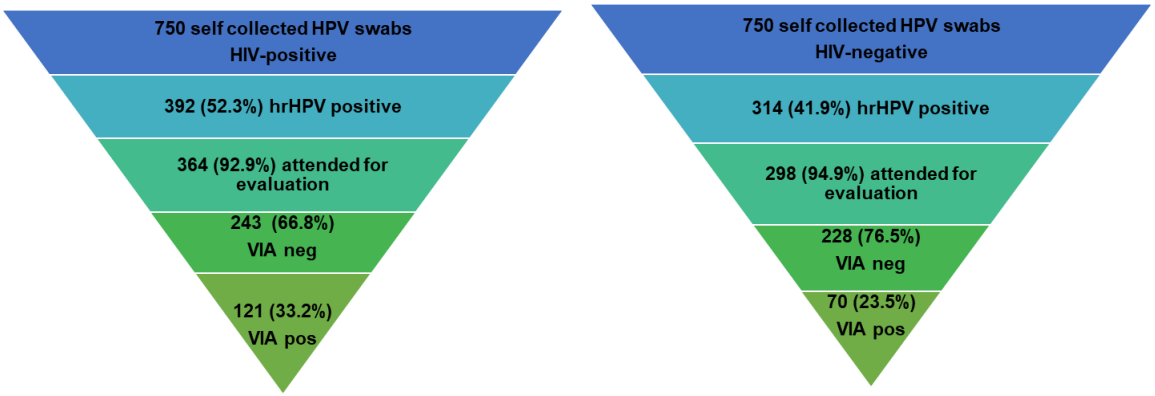

Figure 4.1 Flow of HIV-positive and HIV-negative women from high-risk HPV screening to triage with visual inspection after acetic acid (VIA)

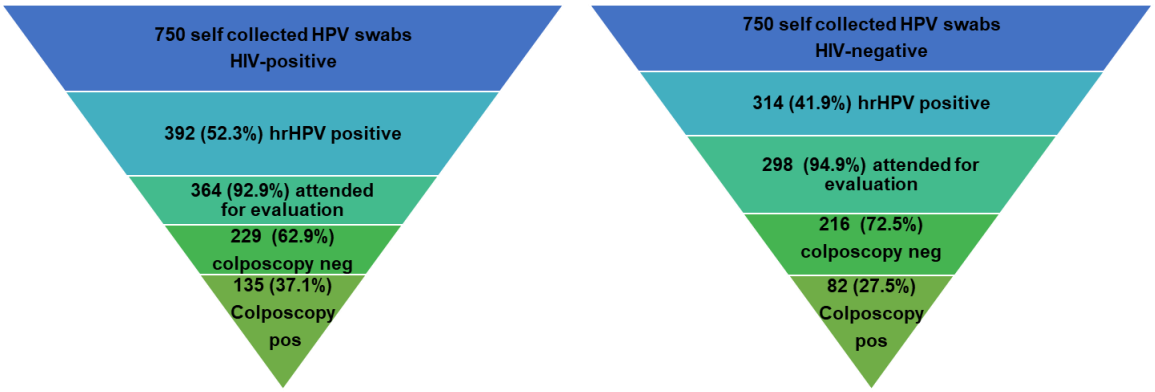

Figure 4.2 Flow of HIV-positive and HIV-negative women from high-risk HPV screening to triage with colposcopy

4.2.1. Outcome of VIA triage evaluation of HPV screen positive

The VIA positivity for WLWH was 33.2% (15.4% low-grade lesions and 17.9% high-grade lesions), and that of HIV-negative women was 23.5% (equal proportions for low- and high-grade lesions of 11.7%) (Table 4.1).

Table 4.1 VIA evaluation of hrHPV positive women

VIA	All participants N=662 n (%)	HIV positive N=364 n (%)	HIV negative N=298 n (%)	P value
Negative	471 (71.1)	243 (66.8)	228 (76.5)	0.020
Low-grade abnormality	91 (13.7)	56 (15.4)	35 (11.7)	
High-grade abnormality	100 (15.1)	65 (17.9)	35 (11.7)	

hrHPV: high-risk human papilloma virus; HIV: human immunodeficiency virus; VIA: visual inspection after acetic acid

A multivariable logistic regression model was developed using backward selection ($p < 0.3$ to enter, and $p < 0.15$ to stay) to identify the characteristics independently associated with a visualised lesion on the cervix for women positive for hrHPV (Table 4.2). After adjusting for age (as a continuous variable), number of lifetime sexual partners, age at first intercourse, hormonal contraceptive use, condom use, smoking, and length of ART, women with more than 5-lifetime partners were more likely to have a lesion visualised at VIA (aOR 2.28 (95% CI 1.32-3.95). Additionally, WLWH were more than twice likely to have a lesion if they had been on ART for less than two years.

Table 4.2 Logistic regression analysis of factors associated with VIA positivity among HPV positive women

	All patients N=662 n (%)	VIA-negative N=471 n (%)	VIA-positive N=191 n (%)	Bivariate		Multivariate	
				VIA positivity OR (95% CI)	P value	VIA positivity aOR (95% CI)	P value
Age (years)							
25-29	97 (14.7)	67 (14.2)	30 (15.7)	2.76 (1.00-6.91)	0.039	-	-
30-39	209 (31.6)	143 (30.4)	66 (34.6)	2.85 (1.14-7.03)	0.024		
40-49	208 (31.4)	142 (30.1)	66 (34.6)	2.87 (1.17-7.23)	0.023		
50-59	105 (15.9)	82 (17.4)	23 (12.0)	1.73 (0.65-4.60)	0.273		
≥60	43 (6.5)	37 (7.9)	6 (3.1)	*Ref*	*Ref*		
Marital status							
Single	472 (71.3)	329 (69.9)	143 (74.9)	1.25 (0.82-1.89)	0.301	-	-
Previously married	43 (6.5)	33 (7.0)	10 (5.2)	0.87 (0.39-1.93)	0.731		
Married	147 (22.2)	109 (23.1)	38 (19.9)	*Ref*	*Ref*		
Highest education							
None	10 (1.5)	8 (1.7)	2 (1.0)	0.51 (0.11-2.47)	0.402	-	-
Primary/non-formal	96 (14.5)	76 (16.1)	20 (10.5)	0.54 (0.30-0.96)	0.037		
Secondary	383 (57.9)	271 (57.5)	112 (58.6)	0.84 (0,57-1.24)	0.379		
College/university	173 (26.1)	116 (24.6)	57 (29.8)	*Ref*	*Ref*		
Employed							
Yes	360 (54.4)	261 (55.4)	99 (51.8)	*Ref*	*Ref*	-	-
No	302 (45.6)	210 (44.6)	92 (48.2)	1.16 (0.83-1.62)	0.402		
Parity							
≤2	414 (62.5)	295 (62.6)	119 (62.3)	*Ref*	*Ref*	-	-
>2	248 (37.5)	176 (37.4)	72 (37.7)	1.01 (0.72-1.44)	0.937		
Number of sex partners (ever)							
1-5	456 (69.5)	343 (73.8)	113 (59.2)	*Ref*	*Ref*	*Ref*	*Ref*
>5	200 (30.5)	122 (26.2)	78 (40.8)	1.94 (1.36-2.77)	<0.001	2.28 (1.32-3.95)	0.003
Age at first intercourse							
<18	148 (22.5)	111 (23.7)	37 (19.4)	0.77 (0.51-1.17)	0.226	-	-
≥18	511 (77.5)	357 (76.3)	154 (80.6)	*Ref*	*Ref*		
Hormonal Contraception use*							
Yes	118 (25.5)	76 (23.4)	42 (30.4)	1.43 (0.92-2.24)	0.112	-	-
No	345 (74.5)	249 (76.6)	96 (69.6)	*Ref*	*Ref*		

Condom use*							
Yes	417 (90.1)	297 (91.4)	120 (87.0)	*Ref*	*Ref*	*Ref*	*Ref*
No	46 (9.9)	28 (8.6)	18 (13.0)	1.59 (0.85-2.98)	0.148	2.28 (0.87--5.99)	0.095
Smoking							
Yes	38 (5.7)	24 (5.1)	14 (7.3)	1.47 (0.75-2.91)	0.265	-	-
No	624 (94.3)	447 (94.9)	177 (92.7)	*Ref*	*Ref*		
Cervical cancer screen (ever)							
Yes	431 (65.1)	303 (64.3)	128 (67.0)	1.13 (0.79-1.61)	0.512	-	-
No	231 (34.9)	168 (35.7)	63 (33.0)	*Ref*	*Ref*		
**CD4 count (cells/mm^3) ** **							
<200	12 (3.3)	8 (3.3)	4(3.3)	1.05 (0.31-3.59)	0.936	-	-
200–349	24 (6.6)	11 (4.5)	13 (10.7)	2.49 (1.07-5.77)	0.034		
350–499	55 (15.1)	39 (16.0)	16 (13.2)	0.86 (0.46-1.63)	0.648		
≥500	273 (75.0)	185 (76.1)	88 (72.7)	*Ref*	*Ref*		
**CD4 count (cells/mm^3) ** **							
<500	91 (25.0)	58 (23.9)	33 (27.3)	1.20 (0.73-1.97)	0.480	-	-
≥500	273 (75.0)	185 (76.1)	88 (72.7)	*Ref*	*Ref*		
**Viral load (copies/mL) ** **							
≤400(undetectable)	358 (98.6)	240 (99.2)	118 (97.5)	*Ref*	*Ref*	-	-
>400	5 (1.4)	2 (0.8)	3 (2.5)	3.05 (0.50-18.5)	0.225		
**Length of ART (years) ** **							
<2	48 (13.2)	26 (10.7)	22 (18.2)	1.86 (1.00-3.43)	0.049	2.41 (1.14-5.10)	0.021
≥2	316 (86.8)	217 (89.3)	99 (81.8)	*Ref*	*Ref*	*Ref*	*Ref*

VIA: visual inspection with acetic acid; HPV: human papilloma virus; ART: antiretroviral therapy; OR: odds ratio; aOR: adjusted odds ratio; CI: confidence interval

*****Among those using birth control

******Among the HIV positive

4.2.2. Outcome of colposcopy triage evaluation of HPV screen-positive Colposcopy

The colposcopy positivity for WLWH was 37.1% (20.1% low-grade lesions and 17.0% high-grade lesions), and that of HIV-negative women was 27.5% (20.5% low-grade lesions and 7.0% high-grade lesions) (Table 4.3).

Table 4.3 Colposcopy evaluation of hrHPV positive women

Colposcopy	All patients N=662 n (%)	HIV-positive N=364 n (%)	HIV-negative N=298 n (%)	P value
Normal	445 (67.2)	229 (62.9)	216 (72.5)	<0.001
Low grade abnormality	134 (20.2)	73 (20.1)	61 (20.5)	
High grade abnormality	83 (12.5)	62 (17.0)	21 (7.0)	

hrHPV: high-risk human papilloma virus; HIV: human immunodeficiency virus

In a multivariate analysis which included age (as a continuous variable), employment, number of lifetime sexual partners, hormonal contraceptive use, smoking, viral load, and length of ART, being on treatment for less than two years seemed to increase the risk of a visible lesion at colposcopy (aOR 2.11 95% CI 0.96-4.62). However, the confidence interval included one which was most likely due to the limited sample numbers (Table 4.4).

Table 4.4 Logistic regression analysis of factors associated with colposcopy grading among HPV positive women

	All patients N=662 n (%)	Colposcopy-negative N=438 n (%)	Colposcopy-positive N=217 n (%)	Bivariate		Multivariate	
				Colposcopy positivity OR (95% CI)	P-value	Colposcopy positivity aOR (95% CI)	P-value
Age (years)							
25-29	97 (14.7)	64 (13.8)	33 (16.8)	5.03 (1.65-15.3)	0.004	-	-
30-39	209 (31.7)	129 (27.7)	80 (40.6)	6.05 (2.08-17.6)	<0.001		
40-49	208 (31.4)	149 (32.0)	59 (29.9)	3.86 (1.32-11.3)	0.014		
50-59	105 (15.9)	84 (18.1)	21 (10.7)	2.44 (0.78-7.58)	0.124		
≥60	43 (6.5)	39 (8.4)	4 (2.0)	*Ref*	*Ref*		
Marital status							
Single	472 (71.3)	321 (69.0)	151 (76.6)	1.40 (0.92-2.13)	0.117	-	-
Previously married	43 (6.5)	34 (7.3)	9 (4.6)	0.79 (0.35-1.79)	0.569		
Married	147 (22.2)	110 (23.7)	37 (18.8)	*Ref*	*Ref*		
Highest education							
None	10 (1.5)	8 (1.7)	2 (1.0)	0.54 (0.11-2.61)	0.440	-	-
Primary/non-formal	96 (14.5)	74 (15.9)	22 (11.2)	0.64 (0.36-1.13)	0.124		
Secondary	383 (57.9)	265 (57.0)	118 (59.9)	0.96 (0.65-1.41)	0.817		
College/university	173 (26.1)	118 (25.4)	55 (27.9)	*Ref*	*Ref*		
Employed							
Yes	360 (54.4)	262 (56.3)	98 (49.7)	*Ref*	*Ref*	-	-
No	302 (45.6)	203 (43.7)	99 (50.3)	1.30 (0.93-1.82)	0.120		
Parity							
≤2	414 (62.5)	289 (62.2)	125 (63.5)	*Ref*	*Ref*		
>2	248 (37.5)	176 (37.8)	72 (36.5)	0.95 (0.67-1.34)	0.752		
Number of sex partners (ever)							
1-5	456 (69.5)	329 (71.7)	127 (64.5)	*Ref*	*Ref*	-	-
>5	200 (30.5)	130 (28.3)	70 (35.5)	1.40 (0.98-1.99)	0.066		
Age at first intercourse							
<18	148 (22.5)	106 (22.9)	42 (21.3)	0.91 (0.61-1.36)	0.648	-	-
≥18	511 (77.5)	356 (77.1)	155 (78.7)	*Ref*	*Ref*		
Hormonal Contraception use*							
Yes	118 (25.5)	73 (23.4)	45 (29.8)	1.39 (0.90-2.15)	0.139	-	-

No	345 (74.5)	239 (76.6)	106 (70.2)	*Ref*	*Ref*		
Condom use*							
Yes	417 (90.1)	280 (89.7)	137 (90.7)	*Ref*	*Ref*	-	-
No	46 (9.9)	32 (10.3)	14 (9.3)	0.89 (0.46-1.73)	0.740		
Smoking							
Yes	38 (5.7)	22 (4.7)	16 (8.1)	1.78 (0.91-3.47)	0.090	-	-
No	624 (94.3)	443 (95.3)	181 (91.9)	*Ref*	*Ref*		
Cervical cancer screen (ever)							
Yes	431 (65.1)	305 (65.6)	126 (64.0)	0.93 (0.66-1.32)	0.687		
No	231 (34.9)	160 (34.4)	71 (36.0)	*Ref*	*Ref*		
CD4 count (cells/mm^3) **							
<200	12 (3.3)	7 (2.9)	5(4.0)	1.43 (0.44-4.63)	0.552	-	-
200–349	24 (6.6)	11 (4.6)	13 (10.5)	2.36 (1.02-5.48)	0.045		
350–499	55 (15.1)	40 (16.7)	15 (12.1)	0.75 (0.39-1.43)	0.382		
≥500	273 (75.0)	182 (75.8)	91 (73.4)	*Ref*	*Ref*		
CD4 count (cells/mm^3) **							
<500	91 (25.0)	58 (24.2)	33 (26.6)	1.14 (0.69-1.87)	0.610	-	-
≥500	273 (75.0)	182 (75.8)	91 (73.4)	*Ref*	*Ref*		
Viral load (copies/mL) **							
≤400(undetectable)	358 (98.6)	238 (99.2)	120 (97.6)	*Ref*	*Ref*	-	-
>400	5 (1.4)	2 (0.8)	3 (2.4)	2.98 (0.49-18.0)	0.236		
Length of ART (years) **							
<2	48 (13.2)	23 (9.6)	25 (20.2)	2.38 (1.29-4.40)	0.006	2.11 (0.96-4.62)	0.063
≥2	316 (86.8)	217 (90.4)	99 (79.8)	*Ref*	*Ref*	*Ref*	*Ref*

HPV: human papilloma virus; ART: antiretroviral therapy; OR: odds ratio; aOR: adjusted odds ratio; CI: confidence interval

*****Among those using birth control

******Among the HIV positive

4.2.3. Agreement between VIA and colposcopy

Table 4.5 VIA and colposcopy visual evaluation overlap

	HIV-positive N=363 n (%)	**HIV-negative N=292 n (%)**
Agreement	**86.8% (95% CI 82.9-89.9%)**	**79.5% (95% CI 74.4-83.7%)**
Cohen's kappa	**0.711 (0.633-0787)**	**0.468 (0.353-0.582)**
VIA-positive Colposcopy-positive	104 (26.7)	46 (15.8)
VIA-positive Colposcopy-negative	17 (4.7)	24 (8.2)
VIA-negative Colposcopy-negative	211 (58.1)	186 (63.7)
VIA-negative Colposcopy-positive	31 (8.5)	36 (1.3)

HIV: human immunodeficiency virus; VIA: visual inspection with acetic acid

Assessment by VIA and colposcopy showed 86.8% and 79.5% agreement in WLWH and HIV-negative, respectively (Table 4.5). Cohen's Kappa suggests substantial agreement between VIA and colposcopy among WLWH (0.71), but only moderate agreement among HIV-negative women (0.47).

4.3. Discussion

WHO recommends VIA or colposcopy, depending on country resources to evaluate hrHPV screen-positive women to decide on who requires treatment. In this study all women screening positive for hrHPV had both visual evaluations, VIA by a nurse and colposcopy by a gynaecologist. Our findings add to the limited body of work reporting VIA as a triage test, and comparing it to colposcopy, especially in the setting of high HIV burden. Our main observations were (1) that more lesions were visualised by both methods in WLWH compared to HIV-negative women, (2) that although both evaluation methods were generally concordant, the agreement between the two was higher in WLHW compared to HIV-negative women.

Primary screening with hrHPV leads to identification of many women who either have a transient or insignificant infection.[10-13] How to best manage hrHPV-positive women is clearer in HICs as in such settings there are an array of triage tools (i.e., cytology, colposcopy, HPV-genotyping).[8,9,14] These tools are not readily available in most LMICs hence the recommendation by WHO to use VIA after HPV testing as a "triage-and-treat" method of choice in LMICs.[6]

4.3.1. VIA positivity in hrHPV positive women

Most studies that have evaluated VIA as a triage test following hrHPV screen-positive have been done in WLWH. Our VIA positivity of 33.2% in WLWH agrees with that reported by several African studies. Castle *et al*.[15] reported a VIA triage positivity of 30.5% following a 41.3% hrHPV positivity on self-collected swabs in a cohort of 570 WLWH, aged 30 to 49 years in Kweneng District, Botswana. Similarly, Kelly *et al*.[16] observed a VIA triage positivity of 28% following a 59.7 % hrHPV positivity on provider-collected swabs in a slightly younger cohort of 576 WLWH aged 25 to 50 years in Johannesburg, South Africa.

Our VIA triage positivity in HIV-negative women was a third less than that in WLWH (23.5%), a rate similar to that reported in another study from Botswana by Castle and colleagues of 23.7%.[15] Data in HIV-negative women in sub-Saharan Africa is limited.

Two studies from Cameroon observed the following VIA triage positivity rates among hrHPV positive women: Bigoni *et al*.[17] reported a VIA triage positivity of 30.9% following a 38.1% hrHPV positivity on self-collected swabs in a cohort of 846 women. These women were aged 25 to 65 years with a 12% HIV prevalence and recruited from hospital settings. Tebeu and colleagues showed a VIA triage positivity of 7.7% following a hrHPV positivity of 27.0% on self-collected swabs in a general population. Their cohort of 540 women aged 30 to 65 years were recruited from two peri-urban sites (HIV prevalence unreported).[18] The fourfold difference in VIA positivity between the two Cameroonian studies may have been related to the different populations targeted and the varying VIA skills of providers.

4.3.2. Comparison of VIA and colposcopy positivity in hrHPV positive women

Our study findings showed similar triage positivity rates between VIA and colposcopy by HIV status, with marginally higher rates for colposcopy. In WLWH, 33.2% women were positive by VIA, and 37.1% by colposcopy; and in HIV-negative women the positivity rates were 23.5% and 27.5% for VIA and colposcopy, respectively. Very few studies report on colposcopy as a visual triage test. A study from India of a cohort of 4947 women from the general population living in urban slums, aged 30 to 49 years, with a hrHPV positivity of 7.6%, reported VIA triage positivity of 19% compared to 26% of colposcopy.[19] The HIV prevalence in this cohort was not reported. A study from Botswana of WLWH aged 25 years and older from an urban centre, reported hrHPV prevalence of 29%, VIA, and colposcopy triage positivity rates of 53.7, and 62.2%, respectively.[20] Although the hrHPV prevalence was nearly half of that in our study, the triage positivity rates were surprisingly higher than those observed in our study (more than one and half times higher). The reason for these differences is unclear, however, we believe that the HPV DNA assay used by Luckett and colleagues may have been better at excluding women with transient hrHPV-infection compared to the newer assay used in our study. As a result, a higher proportion of women with a likelihood of established CIN 2+ disease would have increased VIA/colposcopy positivity compared to our study, as visualisation of a lesion improves with increasing severity of a cervical abnormality.[21]

We also reported a high concordance of visualised acetowhite lesion between VIA performed by a nurse and colposcopy performed by a gynaecologist in WLWH. This is in keeping with the findings of other researchers and is likely due to the dependency of both methods on visual cues.[22,23] However, there was only moderate agreement in HIV-negative women. Although the reason for this in unclear, it could be related to the difficulty of visualising lesions in HIV-negative women which tend to be smaller compared to WLWH.[24]

4.3.3. Factors associated with visual positivity in hrHPV-positive women

One of the two factors associated with VIA triage positivity in hrHPV positive women in our logistic regression analysis was more than 5-lifetime sexual partners (p=0.003). In addition to increasing risk of hrHPV, number of lifetime sexual partners may also increase risk of other sexually transmitted diseases,[25-29] which may manifest as cervicitis on histology.[30] Inflammation caused by high rates of cervicitis can increase proportions of visualised acetowhite lesions.[31,32] Although we did not report on the rate of cervicitis in our study, a sub-group analysis of the study by Luckett and colleagues reported a 27% cervicitis rate in women who had a histology result.[30]

The number of years on ART was associated with both VIA and colposcopy triage positivity in hrHPV-positive women. In WLWH, large acetowhite lesions which are easier to visualise have been associated with no or short duration of ART treatment.[33-35] In a cohort of 200 WLWH in Zambia, Chibwesha and colleagues also showed that hrHPV-positive women were twice more likely to be VIA-positive if they were not on ART compared with those on ART (25% VS. 14%).[36]

Although age was not associated with VIA positivity in our study, other studies have observed decreasing positivity with increasing age in both screening and triage cohorts. A screening cohort of 25 to 49 year old of mainly WLWH (90%) in Botswana reported higher VIA positivity was higher in the age group 25-29 regardless of HIV status.[37] Additionally, in their screening and triage cohort of 18-55 year old WLWH in Kenya, VIA positivity was higher in women under 40 compared with women 40 years

and older ($p<0.001$).[24] There are two possible explanations for this finding. Younger women are more likely than older women to have a transient hrHPV-infection which may manifest as mild acetowhite lesions during VIA. Furthermore, the transformation zone in younger women is generally easier to visualise than it is in older women, hence, reducing the likelihood of a clinician missing an acetowhite lesion on visualisation.

4.3.4. Conclusions

The data presented in this chapter provides evidence in support of the possible equivalence of VIA to colposcopy evaluation where the VIA providers are skilled and there is robust quality assurance. This, therefore, lends support to the argument for using VIA in a “triage-and-treat” setting, especially where there are limited colposcopy providers and limited ability to have biopsy-guided treatment. It is, however, worth mentioning that both methods may miss lesions in the endocervix, especially in older women as the squamo-columnar junction recedes into the endocervical canal. Detection rates of histologically confirmed CIN 2+ by triage method will be discussed in the next chapter.

4.4. References chapter 4

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CHAPTER 5. HISTOLOGICAL RESULTS OF HRHPV POSITIVE WOMEN

5.1. Introduction

Detection of hrHPV is associated with diagnosis of cervical precancer disease in only a few of the screen-positive women.[1-3] The prevalence of both hrHPV and precancer depends on geographical region, age, previous screening rates, and prevalence of immunosuppressive disease.[4-8] Most reports of precancer prevalence are usually based on cohorts with colposcopy directed biopsy of visualised lesions.[9,10] Therefore, lack of histological verification of all hrHPV screen-positive women brings to question the accuracy of the reported prevalence rates of precancer in the literature.[11] Furthermore, studies from sub-Saharan Africa often focus on women living with HIV (WLWH) leaving out HIV-negative women, which makes planning of comprehensive cervical cancer prevention programmes challenging.[8] The limited histological verification end-point of screen-positive women in both WLWH and HIV-negative has implications for determining the optimal and effective national cervical cancer prevention programming for the general population.

In this chapter, we report the histological end-point for all women who screened hrHPV-positive, attended for visual evaluation, and had a valid histology result. We also calculated the proportion of CIN 2+ precancer disease in both WLWH and HIV-negative women.

5.2. Results

5.2.1. Outcome of histological evaluation of hrHPV-positive women

A total of 655 hrHPV-positive women (363/364 WLWH, and 292/298 HIV-negative women) had a valid histology result. Among WLWH, 69/363 (19%) had CIN 2 or worse, and CIN3+ accounted for 51/363 (14.1%). The overall CIN 2+ in the WLWH screening cohort was 69/721 (9.6%)). Among HIV-negative women, 44/292 (15.0%) had CIN 2 or worse, with CIN 3+ accounting for 30/292 (10.3%). The overall CIN 2+ in the HIV-negative screening cohort was 44/728 (6.0%). (Table 5.1 and Figure 5.1)

Table 5.1 Outcome of histological evaluation of hrHPV-positive women

Histology results	All patients N=662 n (%)	HIV-positive N=364 n (%)	HIV-negative N=298 n (%)	P value*
≤CIN1	542 (81.9)	294 (80.8)	248 (83.2)	0.477
CIN2	32 (4.8)	18 (4.9)	14 (4.7)	
CIN3	75 (11.3)	46 (12.6)	29 (9.7)	
Adenocarcinoma-in-situ	3 (0.5)	2 (0.5)	1 (0.3)	
Cervical Cancer	3 (0.5)	3 (0.8)	0 (0.0)	
Inadequate sample (no histological endpoint)	7 (1.1)	1 (0.3)	6 (2.0)	

hrHPV: high-risk human papilloma virus; HIV: human immunodeficiency virus; CIN: cervical intraepithelial neoplasia

***Pearson or Fisher Exact test**

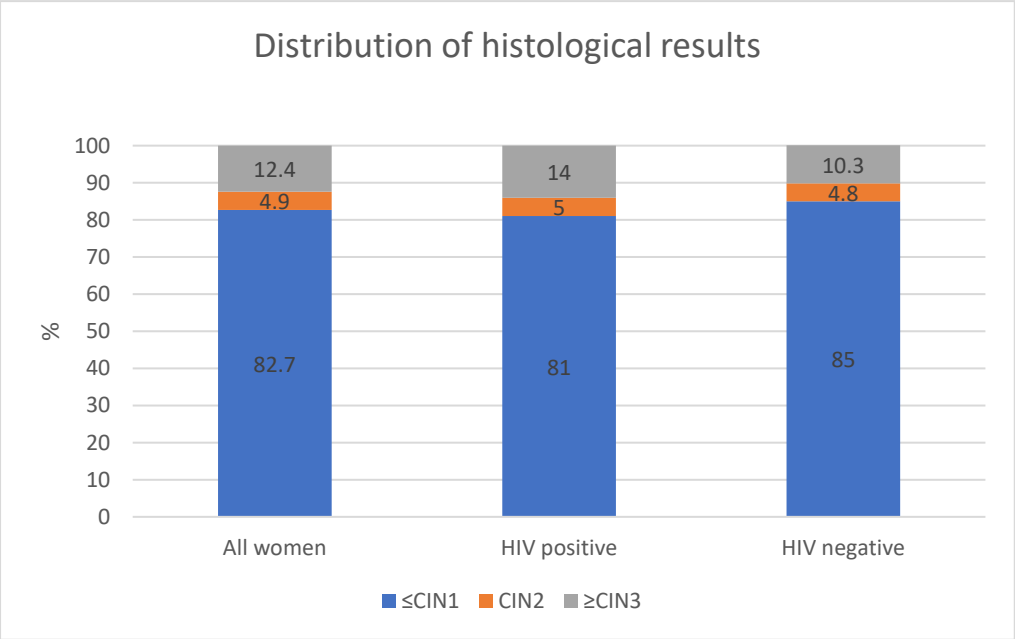


Figure 5.1 Percentage distribution of histological diagnosis in women with valid histology results

A multivariable logistic regression model was developed using backward selection ($p < 0.3$ to enter, and $p < 0.15$ to stay) to identify characteristics independently associated with having CIN 2+ disease among hrHPV screen-positive women in the study (Table 5.2). We adjusted for age (as a continuous variable), marital status, employment, condom use, prior cervical cancer screening, CD4 count, viral load, and length of ART.

Being unemployed was associated with histologically confirmed CIN 2+ (aOR 2.31 95% CI 1.18-4.54, p=0.014). A detectable viral load among WLWH appeared to increase the risk of CIN 2+ (aOR 7.16 95% CI 1.12-45.8, p=0.038), however, the interpretation of this finding should be done with caution given the wide confidence intervals reflecting the limited number of women in this category (5/363).

Table 5.2 Logistic regression analysis of factors associated with CIN2+ among hrHPV positive women

	All patients N=655 n (%)	≤ CIN1 N=542 n (%)	≥ CIN2 N=113 n (%)	Bivariate		Multivariate	
				CIN2+ OR (95% CI)	P value	CIN2+ aOR (95% CI)	P value
Age (years)							
25-29	92 (14.0)	84 (15.5)	8 (7.1)	0.42 (0.15-1.20)	0.104	-	-
30-39	208 (31.8)	168 (31.0)	40 (35.4)	1.04 (0.45-2.42)	0.924		
40-49	207 (31.6)	170 (31.4)	37 (32.7)	0.95 (0.41-2.22)	0.910		
50-59	105 (16.0)	85 (15.7)	20 (17.7)	1.03 (0.42-2.56)	0.950		
≥60	43 (6.6)	35 (6.5)	8 (7.1)	*Ref*	*Ref*		
Marital status							
Single	466 (71.1)	388 (71.6)	78 (69.0)	0.75 (0.47-1.20)	0.230	-	-
Previously married	42 (6.4)	38 (7.0)	4 (3.5)	0.39 (0.13-1.19)	0.098		
Married	147 (22.4)	116 (21.4)	31 (27.4)	*Ref*	*Ref*		
Highest education							
None	10 (1.5)	7 (1.3)	3 (2.7)	2.57 (0.62-10.6)	0.192	-	-
Primary/non-formal	96 (14.7)	80 (14.8)	16 (14.2)	1.20 (0.60-2.39)	0.604		
Secondary	381 (58.2)	311 (57.4)	70 (61.9)	1.35 (0.82-2.24)	0.816		
College/university	168 (25.6)	144 (26.6)	24 (21.2)	*Ref*	*Ref*		
Employed							
Yes	357 (54.5)	304 (56.1)	53 (46.9)	*Ref*	*Ref*	*Ref*	*Ref*
No	298 (45.5)	238 (43.9)	60 (53.1)	1.45 (0.96-2.17)	0.075	2.31 (1.18-4.53)	0.014
Parity							
≤2	407 (62.1)	341 (62.9)	66 (58.4)	*Ref*	*Ref*	-	-
>2	248 (37.9)	201 (37.1)	47 (41.6)	1.21 (0.80-1.83)	0.369		
Number of sex partners (ever)							
1-5	452 (69.4)	375 (69.7)	77 (68.1)	*Ref*	*Ref*	-	-
>5	199 (30.6)	163 (30.3)	36 (31.9)	1.08 (0.70-1.66)	0.743		
Age at first intercourse							
<18	148 (22.7)	121 (22.4)	27 (23.9)	1.09 (0.68-1.75)	0.731		
≥18	505 (77.3)	419 (77.6)	86 (76.1)	*Ref*	*Ref*		
Hormonal Contraception use*							
Yes	117 (25.5)	99 (25.9)	18 (23.7)	0.89 (0.50-1.58)	0.684	-	-
No	341 (74.5)	283 (74.1)	58 (76.3)	*Ref*	*Ref*		

Condom use*							
Yes	413 (90.2)	347 (90.8)	66 (86.8)	*Ref*	*Ref*	-	-
No	45 (9.8)	35 (9.2)	10 (13.2)	1.50 (0.71-3.18)	0.288		
Smoking							
Yes	38 (5.8)	30 (5.5)	8 (7.1)	1.30 (0.58-2.92)	0.524	-	-
No	617 (94.2)	512 (94.5)	105 (92.9)	*Ref*	*Ref*		
Cervical cancer screen (ever)							
Yes	429 (65.5)	363 (67.0)	66 (58.4)	0.69 (0.46-1.05)	0.082	-	-
No	226 (34.5)	179 (33.0)	47 (41.6)	*Ref*	*Ref*		
**CD4 count (cells/mm^3) ** **							
<200	12 (3.3)	9 (3.1)	3 (4.3)	1.56 (0.41-5.99)	0.515	-	-
200–349	24 (6.6)	17 (5.8)	7 (10.1)	1.93 (0.76-4.91)	0.167		
350–499	54 (14.9)	43 (14.6)	11 (15.9)	1.20 (0.58-2.49)	0.627		
≥500	273 (75.2)	225 (76.5)	48 (69.6)	*Ref*	*Ref*		
**CD4 count (cells/mm^3) ** **							
<500	90 (24.8)	69 (23.5)	21 (30.4)	1.43 (0.80-2.55)	0.229	-	-
≥500	273 (75.2)	225 (76.5)	48 (69.6)	*Ref*	*Ref*		
**Viral load (copies/mL) ** **							
≤400(undetectable)	357 (98.6)	292 (99.3)	65 (95.6)	*Ref*	*Ref*	*Ref*	*Ref*
>400	5 (1.4)	2 (0.7)	3 (4.4)	6.74 (1.10-41.1)	0.039	7.16 (1.12-45.8)	0.038
**Length of ART (years) ** **							
<2	47 (12.9)	35 (11.9)	12 (17.4)	1.56 (0.76-3.19)	0.225	-	-
≥2	316 (87.1)	259 (88.1)	57 (82.6)	*Ref*	*Ref*		

CIN: cervical intraepithelial neoplasia; hrHPV: high-risk human papilloma virus; ART: antiretroviral therapy; CI: confidence interval; OR: odds ratio; aOR adjusted odds ratio

*****Among those using birth control

******Among the HIV positive

5.2.2. Outcome of CIN 2+ of hrHPV-positive women stratified by age

Table 5.3 shows the proportions of CIN 2+ disease in each age group, among WLWH and HIV-negative women. The proportion of CIN 2+ among WLWH ranged from 16.7% to 33.3%, with the lowest proportion in the 25 to 29 age group and the highest proportion in the age group of 60 years and above. Among HIV-negative women, the proportions of CIN 2+ ranged from 4.8% (25 to 29 age group) to 19.3% (30to 39 age group). The age group 25 to 29 years for both WLWH and HIV-negative women had the lowest prevalence of CIN 3+ disease (3.3 and 3.2%, respectively).

Table 5.3 Age distribution of histology in hrHPV-positive women by HIV status

	CIN 2+ n (%)	**CIN 2 n (%)**	**CIN 3+ n (%)**
HIV-positive			
All women	69/363 (19.0)	18/363) (5.0)	51/363 (14.0)
25-29	5/30 (16.7	4/30 (13.3)	1/30 (3.3)
30-39	19/99 (19.2)	4/99 (4.0)	15/99 (15.2
40-49	26/148 (17.6)	3/148 (2.0)	23/148 (15.5)
50-59	13/68 (19.1)	6/68 (8.8)	7/68 (10.3)
≥60	6/18 (33.3)	1 /18 (5.6)	5/18 (27.8)
HIV-negative			
All women	44/292 (15.1)	14/292 (4.8)	30/292 10.3)
25-29	3/62 (4.8)	1 /62 (1.6)	2/62 (3.2)
30-39	21/109 (19.3)	8 /109 (7.3)	13/109 (11.9)
40-49	11/59 (18.6)	3/59 (5.1)	8/59 (13.6)
50-59	7/37 (18.9)	2/37 (5.4)	5/37 (13.5)
≥60	2/25 (8.0)	0 (0.0)	2/25 (8.0)

CIN: cervical intraepithelial neoplasia; hrHPV: high-risk human papilloma virus; HIV: human immunodeficiency virus

5.2.3. Histological outcome by number of hrHPV genotypes and HIV status

Table 5.4 shows a breakdown of the proportion of the CIN 2 and CIN3+ disease in among WLWH and HIV-negative women with single versus multiple infections. Roughly, one third of the CIN 2 lesions were identified in WLWH with a single hrHPV genotype, and half of CIN 3+ lesions were identified in those with 2-4 genotypes. Few

WLWH had five or more hrHPV genotypes, and the proportion with CIN 2 was double that in those with CIN 3+ (16.7% versus 7.8%).

The lesions in HIV-negative women were associated with a single or 2-4 hrHPV genotype(s) only. The proportions of single or 2-4 hrHPV genotypes were equal in women with CIN 2. Among women with CIN 3+, the proportion with 2-4 genotypes was slightly higher than those with a single hrHPV genotype (60% versus 40%).

Table 5.4 Proportions of number of hrHPV subtypes in CIN 2 and CIN 3+ lesions, by HIV status

	HIV-positive N=69			**HIV-negative N=44**		
Number of hrHPV types	**Number evaluated N=363 n (%)**	**CIN2 N=18 n (%)**	**≥CIN3 N=51 n (%)**	**Number evaluated N=292 n (%)**	**CIN2 N=14 n (%)**	**≥CIN3 N=30 n (%)**
1	178 (49.0)	6(33.3)	19 (37.3)	189 (64.7)	7 (50.0)	12 (40.0)
2-4	166 (45.7)	9(50.0)	28 (54.9)	98 (33.6)	7 (50.0)	18 (60.0)
≥5	19 (5.2)	3 (16.7)	4 (7.8)	5 (1.7)	0 (0.0)	0 (0.0)

CIN: cervical intraepithelial neoplasia; HIV: human immunodeficiency virus; hrHPV: high-risk human papillomavirus

Regardless of HIV status, the odds of CIN 2+ disease was higher in women with two or more hrHPV genotypes (Table 5.5).

Table 5.5 Logistic regression analysis of the association between HIV status and histology results

	All patients n (%)	≤ CIN1 n (%)	≥ CIN2 n (%)	Bivariate	
				CIN2+ OR (95% CI)	p-value
HIV-positive	**363 (100)**	**294 (81.0)**	**69 (19.0)**		
Number of hrHPV genotypes detected					
1	178 (49.0)	153 (52.0)	25 (36.2)	*Ref*	*Ref*
2-4	166 (45.7)	129 (43.9)	37 (53.6)	1.76 (1.00-3.07)	0.045
≥5	19 (5.2)	12 (4.1)	7 (10.1)	3.57 (1.28-9.94)	0.015
HIV-negative	**292 (100)**	**248 (84.9)**	**44 (15.1)**		
Number of hrHPV genotypes detected					
1	189 (64.7)	170 (58.2)	19 (43.2)	*Ref*	*Ref*
2-4	98 (35.6)	73 (25.0)	25 (56.8)	3.06 (1.59-5.91)	<0.001
≥5	5 (1.7)	5 (1.7)	0 (0.0)	-	-

CIN: cervical intraepithelial neoplasia; HIV: human immunodeficiency virus; hrHPV: high-risk human papillomavirus; CI: confidence interval; OR: odds ratio

5.2.4. Single hrHPV infection in CIN 2+ disease

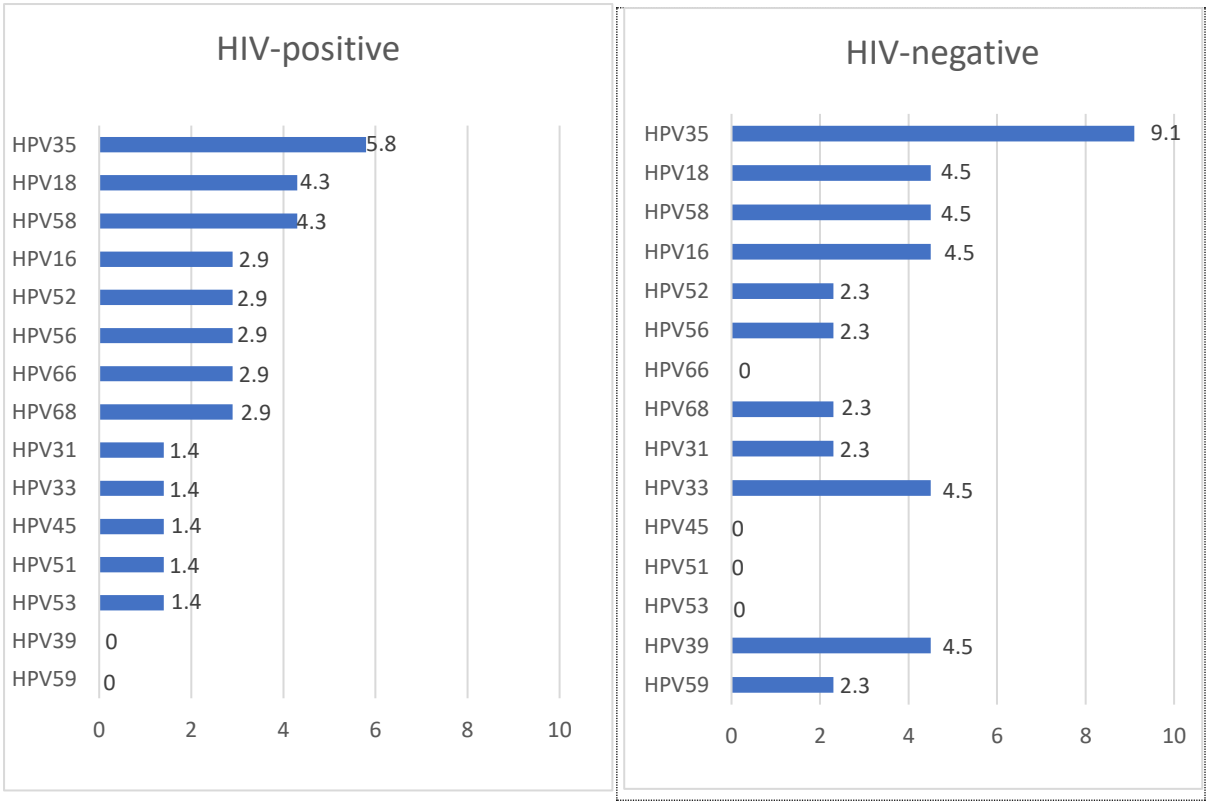


Figure 5.2 Participants with a specific single HPV genotype, by CIN2+ disease

Figures 5.2 and 5.3 show proportions of single hrHPV genotype in women with histologically confirmed CIN 2 and CIN 3+, respectively. One woman living with HIV infected with a single HPV 18 was diagnosed with adenocarcinoma. The following were the type-specific hrHPV detected in WLWH diagnosed with CIN 3: HPV 35 and 58 were each responsible for four cases of CIN 3. HPV 16, 52, and 66 were each responsible for two cases of CIN 3. HPV 18, 31,33, 45, 51, 56 were each responsible for one case of CIN 3. In HIV-negative women, HPV 18, 33, 35, 58 were each responsible for two cases of CIN 3. HPV 16, 31, 39, and 52 were each responsible for one case of CIN 3. HPV 53, 59, and 68 were not responsible for any CIN 3 in either WLWH or HIV-negative women as single infections.

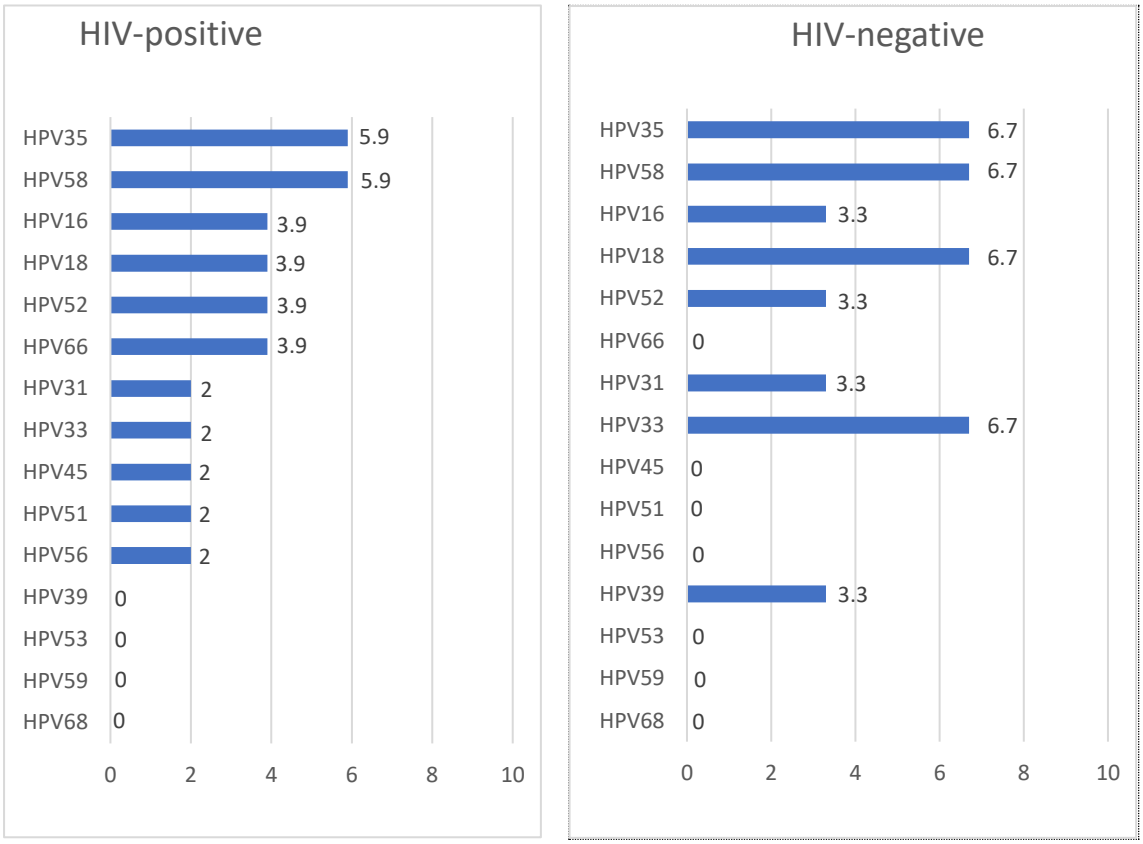


Figure 5.3 Participants with a specific single HPV genotype, by CIN3+ disease

5.2.5. Histological outcome by hrHPV genotype

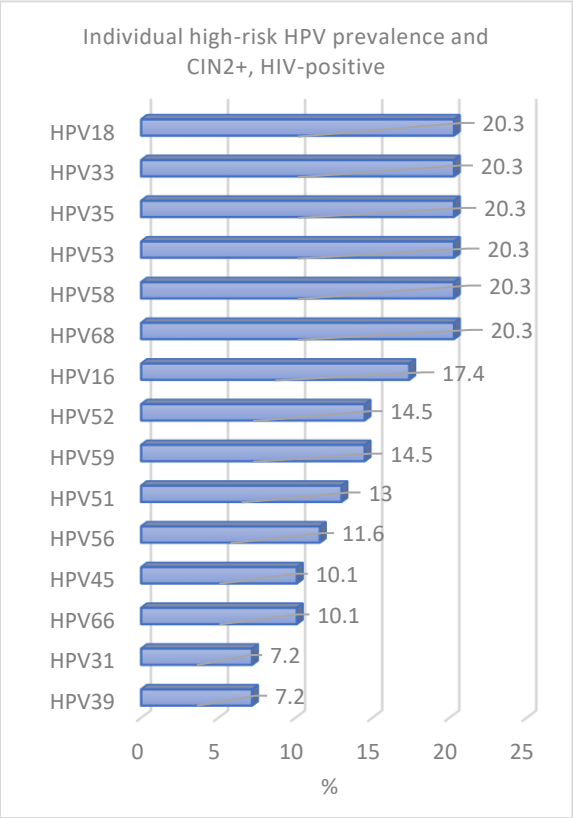
Table 5.6 shows the proportions of CIN 2 and CIN 3+ identified by the various genotype groupings. In WLWH, 3-hrHPV (hrHPV 16/18/45) and 8-hrHPV (hrHPV 16/18/31/33/35/45/52/58) were associated with half and 90% of CIN 3+ disease, respectively. The 3-hrHPV and 8-hrHPV accounted nearly 40% and over 90% of CIN 3+, respectively in HIV-negative women.

Table 5.6 Proportions of CIN 2 and CIN 3+ identified by various hrHPV genotypes, by HIV status

	HIV-positive N=69			**HIV-negative N=44**		
HrHPV types	**Number evaluated N=363 n (%)**	**CIN2 N=18 n (%)**	**≥CIN3 N=51 n (%)**	**Number evaluated N=292 n (%)**	**CIN2 N=14 n (%)**	**≥CIN3 N=30 n (%)**
Any hrHPV	363 (100)	18 (5.0)	51 (14.1)	292 (100)	14 (4.8)	30 (10.3)
hrHPV 16/18 (group 1)	93 (25.6)	3 (16.7)	21 (41.2)	59 (20.2)	3 (21.4)	9 (30)
hrHPV 16/18/45 (group 2)	122 (33.6)	4 (22.2)	25 (49.0)	83 (28.4)	3 (21.4)	11(36.7)
hrHPV 16/18/31/33/35/45/52/58 (group 3)	267 (73.5)	14 (77.8)	46 (90.2)	201 (68.8)	10 (71.4)	28 (93.3)
Non-hrHPV 16/18/45 (group 4)	242 (66.6)	14 (77.8)	26 (51.0)	215 (73.6)	11 (78.6)	19 (63.3)

CIN: cervical intraepithelial neoplasia; HIV: human immunodeficiency virus; hrHPV: high-risk human papillomavirus

Figure 5.4 shows the CIN 2+ proportions associated with each individual HPV genotype, either as a single infection or in combination with others. Six genotypes (HPV 18/33/35/53/58/68) were equally represented at 20.3%, followed by HPV 16 (17.4%), HPV 52/59 (14.5%), HPV 51 (13.0%), and HPV 56 (11.6%) in WLWH. and the top five hrHPV genotypes in HIV-negative women. The top five genotypes in HIV-negative women were HPV 52 (29.5%), HPV 35 (22.7%), HPV 33 (20.5%), HPV 58 (15.9%), and HPV 18/52 (13.6%).



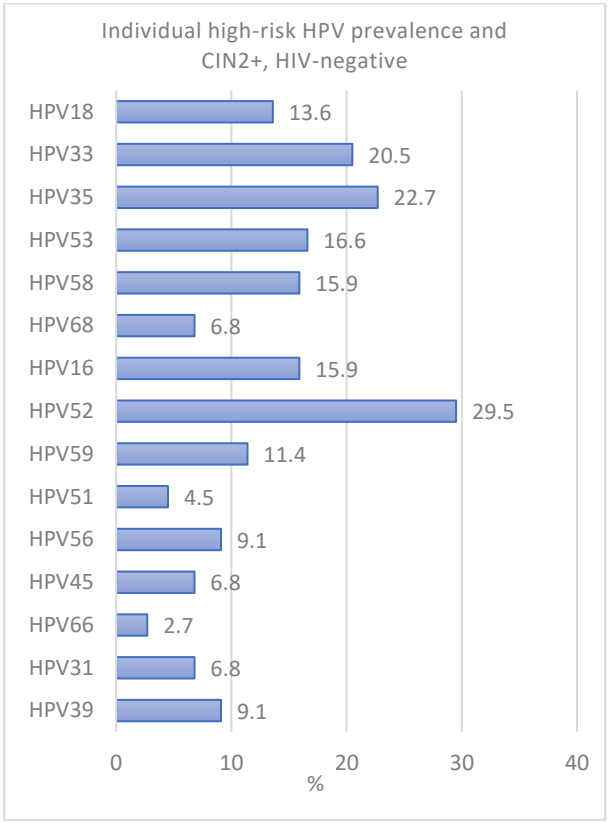


Figure 5.4 Prevalence of the 15 hrHPV genotypes in HIV-positive and -negative women with CIN 2 disease

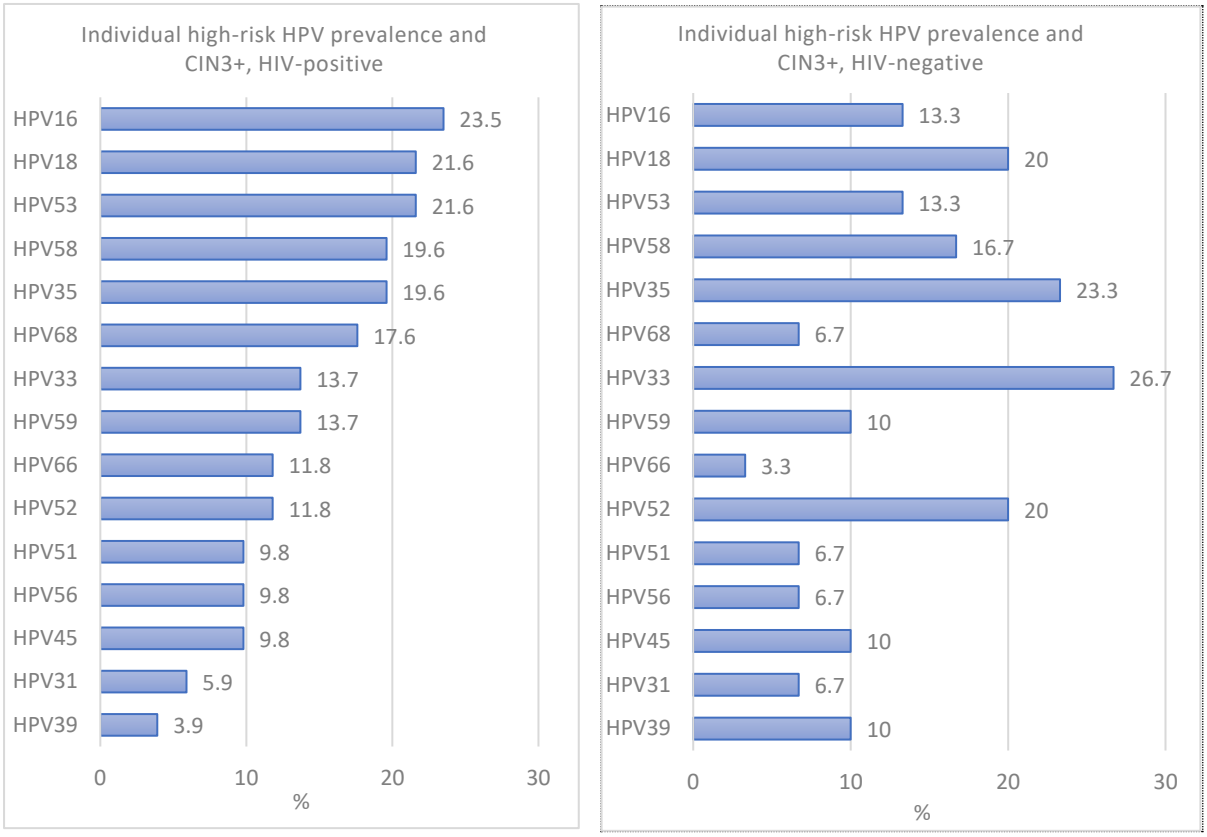


Figure 5.5 Prevalence of the 15 hrHPV genotypes in HIV-positive and -negative women with CIN 3+ disease

Figure 5.5 shows the CIN 3+ proportions associated with each individual hrHPV genotype, either as a single infection or in combination with others. The top five hrHPV genotypes in WLWH were HPV 16 (23.5%), HPV 18/53 (21.6%), HPV 58 (19.6%), HPV 68 (17.6%), and HPV 33/59 (13.7%). In HIV-negative women, the top five hrHPV genotypes were HPV 33 (26.7%), HPV 35 (23.3%), HPV 18/52 (20.0%), and HPV 58 (16.7%).

5.2.6. Outcome of CIN 2+ of hrHPV-positive women stratified by VIA result

Table 5.7 shows the proportions of hrHPV women with valid histology results by VIA positivity. Thirty-five percent (42/121) of WLWH with VIA lesions had CIN 2+, and 29%

(35/121) had CIN 3+. In HIV-negative women, 25% (17/69) with VIA lesions had CIN 2+, and 22% (14/69) had CIN 3+.

Table 5.7 Results by VIA triage, histological finding, and HIV-status (percentage)

	HIV-positive		HIV-negative		Overall		p-value*
	%	(a/n)	%	(a/n)	%	(a/n)	
% who tested positive for hrHPV	52.3%	(392/750)	41.9%	(314/750)	47.1%	(706/1500)	
% hrHPV+ histologically evaluated	92.6%	(363/392)	93.0%	(292/314)	92.8%	(655/706)	
Among histologically evaluated…							
% positive by VIA	33.3%	(121/363)	23.6%	(69/292)	29.0%	(190/655)	0.007
% negative by VIA	66.7%	(242/363)	76.4%	(223/292)	71.0%	(465/655)	
% VIA-positive with CIN2+	34.7%	(42/121)	24.6%	(17/69)	31.1%	(59/190)	
% VIA-negative with CIN2+	11.2%	(27/242)	12.1%	(27/223)	11.6%	(54/465)	0.75
% VIA-positive with CIN3+	28.9%	(35/121)	21.7%	(14/69)	25.8%	(49/190)	
% VIA-negative with CIN3+	6.6%	(16/242)	7.2%	(16/223)	6.9%	(32/465)	0.81

CIN: cervical intra-epithelial neoplasia; HIV: human immunodeficiency virus; hrHPV: high-risk human papillomavirus; VIA: visual inspection after acetic acid
*Chi-square test

5.2.7. Outcome of CIN 2+ of hrHPV-positive women stratified by colposcopy result

Table 5.8 shows the proportions of hrHPV women with valid histology results by colpocopy positivity. Thirty-five percent (47/135) of WLWH with colposcopy lesions had CIN 2+, and 21% (17/81) had CIN 3+. In HIV-negative women, 21% (17/82) with colposcopy lesions had CIN 2+, and 16% (13/82) had CIN 3+.

Table 5.8 Results by colposcopy triage, histological finding, and HIV-status (percentage)

	HIV-positive		HIV-negative		Overall		p-value*
	%	(a/n)	%	(a/n)	%	(a/n)	
% who tested positive for hrHPV	52.3%	(392/750)	41.9%	(314/750)	47.1%	(706/1500)	<0.001
% hrHPV+ histologically evaluated	92.6%	(363/392)	93.0%	(292/314)	92.8%	(655/706)	0.84
Among histologically evaluated…							
% positive by colposcopy	37.2%	135/363	28.1%	82/292	33.1%	217/655	0.01
% negative by colposcopy	62.8%	228/363	71.9%	210/292	66.9%	438/655	0.01
% colposcopy-positive with CIN2+	34.8%	47/135	20.7%	17/82	29.5%	64/217	0.03
% colposcopy-negative with CIN2+	9.6%	22/228	12.9%	27/210	11.2%	49/438	0.29
% colposcopy-positive with CIN3+	28.9%	39/135	15.9%	13/82	24.0%	52/217	0.03
% colposcopy-negative with CIN3+	5.3%	12/228	8.1%	17/210	6.6%	29/438	0.23

CIN: cervical intra-epithelial neoplasia; HIV: human immunodeficiency virus; hrHPV: high-risk human papillomavirus
*Chi-square test

5.2.8. Assessment of individuals with adenocarcinoma-in-situ, adenocarcinoma, and squamous cell carcinoma

A total of five women had a diagnosis of adenocarcinoma-in-situ (AIS) or adenocarcinoma (ADC). All these occurred in WLWH except for one HIV-negative woman had an adenocarcinoma-in-situ. The rest of the abnormalities were in HIV-infected women (two adenocarcinoma-in-situ; two adenocarcinomas; one squamous cell carcinoma) (Table 5.9).

Table 5.9 Individual demographic, behavioral and clinical characteristics of women diagnosed with glandular disease and cervical cancer

Variable	AIS	AIS	AIS	ADC	ADC	SCC
Age (years)	37	60	35	49	44	40
Marital Status	Single	Married	Single	Single	Single	Single
Education	Tertiary	Secondary	Secondary	Secondary	Secondary	Secondary
Employed	Yes	Yes	No	Yes	Yes	Yes
Parity	0	3	1	2	2	3
Age at first sex (years)	20	20	20	24	21	15
Number of sexual partners	1-5	>5	>5	1-5	1-5	>5
Birth Control	Yes	No	Yes	No	Yes	No
Hormonal use	No	-	Yes	-	No	-
Condom use	Yes	-	Yes	-	Yes	-
Ever screened	No	Yes	Yes	No	Yes	Yes
HrHPV type	hrHPV 16/66	hrHPV 39/52/53/68	hrHPV 58/68	hrHPV18	hrHPV 18/53	hrHPV 45/51
VIA outcome	Normal	High-grade	Low-grade	High-grade	Normal	Visible lesion
Colposcopy outcome	Normal	Low-grade	High-grade	High-grade	Normal	Visible lesion
HIV status	Negative	Positive	Positive	Positive	Positive	Positive
Cd4 cell count	-	1032	974	650	489	500
Viral load	-	<400	<400	<400	<400	<400
Length of ART (years)	-	3	<1	2	4	6

AIS: adenocarcinoma-in-situ; ADC: adenocarcinoma; SCC: squamous cell carcinoma; ART: antiretroviral treatment; hrHPV: high-risk human papillomavirus; VIA: visual inspection after acetic acid

5.2.9. Outcome of histological evaluation of hrHPV-negative women

A random sample of roughly 10% of hrHPV-negative women was also brought back for histological evaluation. None of the 85 women (32 WLWH; 53 HIV-negative) had CIN 2+ (results not shown).

5.3. Discussion

The value of screening in cervical cancer prevention programmes is to identify women with precancer and adequately treat them.[12,13] In this cohort of women from a semi-urban area in Botswana 93% of all hrHPV screen-positive, and 10% of hrHPV screen-negative women had a valid histological endpoint. Our main observations were 1) that CIN 2+ disease was detected in a small proportion of women with hrHPV screen-positive results, 2) that infection with multiple hrHPV genotypes was associated with higher odds of detection of CIN 2+ disease, 3) that most of the disease was detected in women 30-59 years old, 4) that hrHPV screening identified women with glandular disease, but a fraction was missed by visual triage, 5) that evaluation by VIA and colposcopy was better at detecting CIN 2+ in WLWH compared to HIV-negative women, with significant disease missed in the screening target age group.

5.3.1. Prevalence of CIN 2+ disease in women living with HIV

We detected 9.6% and 19.0% cases of CIN 2+ in the screening population and hrHPV screen-positive of WLWH, respectively. Luckett *et al.*[14] reported a similar prevalence of CIN 2+ (9,7%) in the screening population of 300 WLWH in Gaborone, Botswana, but a much higher prevalence of 35% in hrHPV screen-positive women. There are two potential explanations for this vast difference. It is possible that the HPV platform (Gene Xpert) used in the earlier study was better at excluding women with hrHPV transient infection than the newer one used in the current study (Antila Amp Fire), thus identifying a screen-positive group with a high specificity for CIN 2+ disease. Self-collected swabs were used in the current study compared with the earlier study that used provider-collected swabs. It has been reported that self-collected swabs are more likely to test HPV-positive resulting in lower specificity for CIN 2+ disease.[15]

In their systematic review and meta-analysis of WLWH mainly from sub-Saharan Africa (sSA) and India, Kelly *et al*.[8] reported a pooled hrHPV screen-positive prevalence range of 20-64%, and pooled prevalence of 12.0% (95% CI: 9.8-14.1), and 6.7% (95% CI: 5.0-8.4%) for CIN 2+ and CIN 3+, respectively. The studies included

were restricted to those with a histological endpoint in the whole screening population or in those with hrHPV screen-positive results.

In Zambia a screening study of 200 WLHW 18 years and older who all had a histological endpoint, the hrHPV screen-positive was 47%, and the CIN 2+ prevalence was 16.0% in the screening population.[16] In another study of 18 to 55 years old WLWH in Kenya, Chung *et al*.[17] screened 498 women with a hrHPV screen-positive rate of 53.0%. Like the Zambia study, they also reported a histological end point for all the women. The overall CIN 2+ prevalence was 22% in the whole cohort.

The observed histologically verified CIN 2+ prevalence in the screening populations from Kenya (22.7%) and Zambia (16%) were both higher than the overall prevalence in our screening population of WLWH (9.6%). Seventy-five percent of the Kenyan cohort was on ART with a median CD4 count of 371 (IQR 245-533), whereas almost all the Zambia cohort was on ART with a median CD4 count of 456 (IQR 328-590). In comparison, our median CD4 count was much higher (679 [IQR 507-871]). Furthermore, just under 50% of the Zambia cohort reported prior cervical cancer screening compared with our reported 80.4% prior screening. Prior screening was not reported in the Kenyan cohort. Both the level of CD4 count and proportion of prior screening may explain the differences in the overall prevalence of CIN 2+ in our study compared with these two studies.

Two other studies that reported CIN 2+ in WLWH observed slightly lower CIN 2+ prevalence in the screening populations compared with our study.[18 19,20] In their cohort of 30 to 59 year olds both the hrHPV prevalence and the CIN 2+ proportion in WLWH were around 70% of that in our study (37.5 and 6.4%), even though the HIV disease severity seemed worse than in our study.[18,19] Just over 70% of the WLWH in this India study were on ART, and the mean CD4 count at HIV diagnosis and at recruitment were low (303 [range 0-1388] and 305 [39-1493] cells/mm3) compared with our study. A large study from Cape Town, South Africa, in 17 to 65 years old women reported a similar hrHPV positivity of 52.4%, but a slightly lower CIN 2+ proportion of 7.2% in the screening cohort of WLWH compared with our study.[20] However, it is difficult to make any further comparisons to this study since the researchers did not report on the severity of HIV disease in their cohort.

High viral load in WLWH has also been shown to be associated with risk of CIN 2+ disease in WLWH ,[21-23] a finding we confirmed in our small number of women with a detectable viral load. Low CD4 count and high HIV viral load are independently associated with risk of hrHPV persistence,[24] and in turn risk of CIN 2+ disease .[22-25]

5.3.2. Prevalence of CIN 2+ disease in HIV-negative women and the general population

One of the major challenges is that few of the screening studies in LMICs especially sub-Saharan Africa (sSA) include HIV-negative women. The prevalence of CIN 2+ in our study was lower for HIV-negative women at 6.0% and 15.1% for the screening population and hrHPV screen-positive women, respectively. This occurred on a background of a higher hrHPV positivity of 42%. McDonald and colleagues reported a lower hrHPV positivity of 20.8%, and a CIN 2+ proportion of 11.3% in screen-positive women compared to our study.[20] The overall CIN 2+ proportion in the screening population of HIV-negative women was much lower than in our study at 1.6%. Boddu and colleagues reported a much lower hrHPV positivity of 5.9% in HIV-negative women with CIN 2+ proportion of 0.5% in the screening population in their Indian cohort.[18] The CIN 2+ proportion in this study was lower compared with our study and the McDonald study. The reason for the differences in CIN 2+ positivity between these screening studies from Botswana, India, and South Africa is unclear but may be related to the different characteristics of the study populations including age and cervical cancer risk.

In a large Indian screening study in a general population of 5000+ women aged 30 to 49 years, 377 women were hrHPV positive and 273 had a histologically verified endpoint.[19] Poli and colleagues reported a surprisingly high prevalence of 22.0% and 15.0% of CIN 2+ and CIN 3+ disease in screen-positive women, respectively, even though the hrHPV prevalence was low (7.6%). The extrapolated CIN 2+ of the screening population was 1.2%. However, the HIV prevalence of this cohort was not stated and no further comparison with our study could be made.

Two studies from Cameroon reported relatively similar prevalence for CIN 2+ in the general screening population. Bigoni et al.[26] evaluated 846 women aged 25 to 65 years old with a 12% HIV prevalence. The hrHPV prevalence was 38.5% and CIN 2+ was identified in 8.2% of all screen-positive women, which translated to 3.0% in the screening population. The other study by Tebeu et al.[27] screened 540 women aged 30 to 65 years old, with hrHPV positivity of 27%. CIN 2+ prevalence of 10.4% in hrHPV-positive women, or 2.2% in the screening population.

5.3.3. Association of CIN 2+ disease and multiple hrHPV infections

In this thesis we showed that detection of multiple hrHPV genotypes was slightly higher in WLWH compared with HIV-negative women (64 vs. 57%). When present, multiple hrHPV infections were significantly associated with detection of CIN 2+, more so in HIV-negative women than WLWH ($p<0.001$ in HIV-negative vs. $p= 0.015$ in WLWH). In another report from Botswana of 100 WLWH with confirmed CIN2/3, a slightly lower proportion of 56% had two or more hrHPV detected.[28] The finding of higher proportions of multiple hrHPV infections in WLWH compared with HIV-negative women was further confirmed by other reports. The finding from a South African study showed a more pronounced difference in coinfections between WLWH and HIV-negative women (40.9 vs 24.3%, $p<0.001$), and this difference persisted in women with CIN 2+ disease.[20] In contrast, another study from South Africa of 225 WLWH and 45 HIV-negative women with confirmed CIN2/3, reported much higher rates of multiple hrHPV infections.[29] There were 73% WLWH and 49% HIV-negative women with multiple hrHPV infections. Although Menon *et al*.[30] reported an association between multiple hrHPV infections with CIN 2+ in their earlier systematic review of studies from Kenya, this finding was not supported by their subsequent cross-sectional study of 74 women.[31] They observed that HPV 16 and 31 were the only independent predictors of CIN 2+ in the latter study.[31]

5.3.4. Prevalent hrHPV genotypes in women with CIN 2+ disease

In our study the most prevalent hrHPV genotypes in descending order in WLWH with CIN 2+ were HPV (18, 33, 35, 58, 68 tied),16, and 52. In HIV-negative women the

most prevalent genotypes were HPV 52, 35 33, and (53, 16 tied). In comparison to the study from Pretoria, South Africa, the most prevalent HPV in WLWH were HPV 16, 58, 35, 51, and 52. In HIV-negative women these were HPV 16, 52, 31, 35, and 58.[29] More recently, a study from the Eastern Cape reported an association of infection with two or more HrHPV in women with CIN 2/3 with rates independent of HIV status. The most detected hrHPV in multiple infections were HPV 16, 35, and 45 in WLHW, and hrHPV 16, 35 and 66 in HIV-negative women.[32]

5.3.5. Prevalent hrHPV genotypes in CIN 3+ disease

Unlike CIN 2 which may still regress, CIN 3 is seen as a true precursor for invasive cancer. The most prevalent hrHPV genotypes in our study in women with CIN 3 were HPV 16, (18, 53 tied), (58,35 tied), and 68 for WLWH, and HPV 33, 35, (18, 52 tied), and 58 for HIV-negative women. McDonald and colleagues reported HrHPV 16, (33,35 tied), and 58 as the most prevalent in WLWH, and hrHPV 16, 35, 45 in HIV-negative women with CIN 3.[20] A more recent study from another region of South Africa reported prevalent genotypes observed in women with CIN 3 as HPV 16, 35, and 45 in WLHW, and hrHPV 16, 35 and 66 in HIV-negative women.[32] In their cohort of WLWH from Kenya, Menon and colleagues reported the most prevalent genotypes in women with CIN 3 were HPV 16, 56, 33, and 53,[33] Whilst Clifford *et al*.[34] reported HPV 16, 18, 45, 35 and 58 as the most prevalent in WLWH in Africa with CIN 3. Meanwhile, a combined cohort of 1240 women with high-grade precancerous cervical lesions from four developed countries with extremely low HIV rates (Denmark, Iceland, Norway, and Sweden), reported the most prevalent hrHPV in women with CIN 3 as HPV 16, 31, and 33.[35] Analysis of most studies regardless of continent shows that HPV 16 remains relatively important in severe precancer disease regardless of region.

5.3.6. The effect of hrHPV 53 and 66

In settings where both HPV 53 and 66 are included in the screening test, they tend to increase the hrHPV test positivity, often with reduced specificity. The IARC class 1 hrHPV list excludes HPV 53 and 66,[36] and most HPV assays exclude HPV 53. The oncogenic potential of HPV 53 and 66 was initially deemed extremely low.[37-39] In their

systematic review Clifford and colleagues reported a ratio of cervical cancer to low-grade disease of 0.05 and 0.02 for HPV 53 and 66 respectively, compared with ratios of 2.0 and 1.5 in women with HPV 16 and 18, respectively.[37] However, the studies above were carried out in women outside sSA. In their Kenyan study of WLWH Menon and colleagues reported HPV 53 as the fourth most prevalent hrHPV genotype in and HPV 66 as the least prevalent in women with CIN 3+ disease. HPV 53 was also the stand-alone infection in the only cervical cancer case in their cohort.[33] In our study, HPV 53 was the second most prevalent in WLWH with CIN 3+, and two (4%) WLWH with a diagnosis of CIN 3 had HPV 66 as a stand-alone HPV infection. In their recent review, Kombe and colleagues reported the prevalence of HPV 66 in invasive cervical cancer as 0.4% in Africa compared with Europe (<0.01%) and was not detected in North America.[40] In contrast, HPV 53 was not recorded in Africa but was reported as 0.3 and 0.6% in Europe and North America, respectively.[40] Although both HPV 53 and 66 have been shown to be of extremely low cancer potential in some settings, we do not have enough data to confidently ascribe oncogenic risk potential to these genotypes in sSA, and more research is needed to evaluate this further.

5.3.7. Prevalence of HPV 35

The new nonavalent HPV vaccine excludes HPV 35. This may have resulted in part from the reliance on evidence of earlier epidemiological studies that tended to have little sSA representation and had no or limited numbers of WLWH.[1,37,41-44] In their more recent systematic review and meta-analysis, Clifford and colleagues included a substantial representative of studies from sSA.[34,45] Outside of HPV 16, 18, and 45, they reported HPV 35 as more frequent in WLWH in sSA diagnosed with CIN 3+ disease. The epidemiology of HPV from studies done in sSA, including our study, report high prevalence of HPV 35. In an earlier study from Botswana of a 100 WLWH diagnosed with CIN 2 and 3, the prevalence of HPV 35 was 40%, third after HPV 16 and 18.[28] In their Cape Town study, McDonald and colleagues reported the prevalence of HPV 35 associated with CIN 3 as second after HPV 16 regardless of HIV.[20] A cross-sectional study involving three sSA countries (Ghana, Nigeria, and South Africa) reported HPV16, 18, 45 and 35 as the most common HPV types detected from DNA extracted from paraffin-embedded cervical cancer specimen, and HPV 35 as the third

highest stand-alone infection.[46] A study carried out in the Eastern Cape, South Africa Taku and colleagues reported HPV 35 as the most common infection in women with CIN 3 regardless of HIV status, and second and third most common as a stand-alone in HIV-negative women and WLWH, respectively.[32] More recently, a cross-sectional study from the same region using HPV data of DNA extracted from cervical biopsy specimen instead of vaginal/cervical swabs, confirmed the importance of HPV 35, which was the second and fourth most common subtype in women with CIN 3 and ICC, respectively.[47] WLWH made up more than two thirds of this cohort. Exclusion of HPV 35 from the nonavalent vaccine which has been shown to prevent 90% of ICC outside sSA is a missed opportunity for improved protection in regions with a high burden of HIV and HPV 35 like sSA.

5.3.8. Association of CIN 2+ and age

Another finding in our study was the increasing severity of histological lesions with increasing age. This was more pronounced in WLWH compared to HIV-negative women where CIN 2 was four times higher in the 25 to 29 years old age group compared to the 60 and older group, and the reverse was observed in the proportion of CIN 3+ in the latter group compared to the younger women. Increasing age is one of the independent factors associated with severe precancer and invasive cancer regardless of HIV status.[22,32,34] This has a critical importance in settings like ours where the formal screening target age excludes women 50 years and older. As these screening programmes are relatively new it means that the older birth cohorts would never have had an opportunity to be screened and therefore, carry a significant risk for cervical cancer. Education of healthcare providers is needed to ensure that these women are offered screening whenever they present to health facilities.

5.3.9. Glandular disease

Even though squamous cell carcinoma rates have come down especially in countries with organised screening programmes the rates of adenocarcinoma have not changed much, and in some settings their relative proportion has gone up.[48-50] One of the reasons for missed adenocarcinomas in screening programmes utilising cytology has

been attributed to the site of this type of cancer (endocervical), making it difficult to sample and more likely to be diagnosed at an advanced stage.[51,52] Adenocarcinomas are usually diagnosed in women in their mid-40's. HPV 16/18/31/33/45 has been associated with more than 90 % of the adenocarcinomas.[52,53] Since the disease occurs in the endocervix, it is likely to be missed by visual evaluation methods like VIA and colposcopy, especially in women with atrophy of the cervix which impairs examination inside the endocervical canal. Two women in our study were missed by both VIA and colposcopy. The first one was a 37-year-old HIV-negative woman who had never been screened, positive for hrHPV 16 and 66 and diagnosed with adenocarcinoma-in-situ. The second one was a 44-year-old woman with well controlled HIV disease who had prior cervical screening, was positive for hrHPV 18/53, and diagnosed with microscopic adenocarcinoma. Compared to visual evaluation of the cervix, hrHPV is more likely to identify glandular lesions but only when all hrHPV women get a histological verification regardless of visual evaluation outcome.

5.3.10. HrHPV-negative precancer

Between 7 and11% of global cervical cancer cases have been reported to be hrHPV-screen negative.[41,54,55] A recent cancer genome study reported that approximately 5% of the primary cervical cancer in the USA were hrHPV-negative. Failure to detect hrHPV in known cases of cervical cancer could be due to sampling, testing and storage errors.[56] Other hrHPV negative cases may be those of glandular origin with a different pathway other than hrHPV-associated carcinogenesis.[57,58] As most screening programmes have partial histological verification it is difficult to ascertain the true hrHPV negative disease. In our study we brought back 10% of hrHPV for histological verification. No CIN 2+ was detected in any of the women. This finding was similar to that of Tebeu and colleagues in Cameroon who brought back 27% of their hrHPV negative women for histological verification and found no CIN 2+ disease.[27] This is in contrast to two other studies in which all screened women underwent histological verification. Four and 19 CIN 2+ cases were detected in hrHPV negative women in Zambia and Kenya, respectively, a proportion of 2 and 4% in the screening population.[16,17]

In their pooled analysis comparing hrHPV-positive and negative precancers, Castle *et al*.[59] reported a 9.0 % prevalence of hrHPV-negative CIN 2+. They put forward three explanations for this relatively high prevalence. They believed 1) that most of the apparent hrHPV-negative CIN 2+ cases were due to a false negative test, 2) that some of the CIN 2+ were caused by low carcinogenic HPV types, 3) that some of the lesions that appeared to be CIN 2+ were benign lesions which were misclassified. The misclassification occurred due to conditions on the cervix that mimicked CIN 2+ such as immature squamous metaplasia, atrophy, and reparative epithelial changes.[59]

The importance of true hrHPV-negative cervical cancer is two-fold. Primary hrHPV screening will miss these women at precancer stage, and they will be given a false sense of security by a negative test. Once they have developed cancer, these women tend to present late leading to poor prognosis and survival.[60,61] Better ways of identifying women with glandular disease remain a challenge.

5.3.11. Visual evaluation and histological outcome

Providing histological verification for all hrHPV screen-positive women who presented for visual evaluation allowed us to quantify the magnitude of both the over- and under-treatment if VIA is utilised as a “triage-and-treat” method. In our study, for women reported as VIA triage-negative, similar proportions of CIN 2+ cases were missed in both WLWH and HIV-negative women (11% [27/243] and 12% [27/248], respectively). Slightly more HIV-negative women reported as VIA triage-positive were overtreated compared with WLWH (76% [53/60] and 65% [79/121]). In a general population cohort of 5000 Indian women 12% of the VIA triage-negative women would have had a missed CIN 2+, and 49% of the VIA triage-positive women would have been overtreated.[19] Other studies have not included this level of detail to allow comparison.

The reason that VIA is recommended for women younger than 50 is because those are likely to still have a relatively normal cervix not affected by atrophy. However, reports have indicated that WLWH reach menopause relatively early,[62] and this can affect the performance of visual evaluation even in younger WLWH. Our study included women 25 years and older and did not exclude women with cervical atrophy.

Both VIA and colposcopy missed similar proportions of CIN 2+ lesions in women under and over 50 years old in WLWH. However, in HIV-negative women, both methods missed more CIN 2+ lesions in younger women (the order of 2.4 and 2.9 times higher for colposcopy and VIA, respectively, compared to women over 50 years old). All women with atrophy of the cervix or inadequate squamous columnar junction received a small central LEEP for histological evaluation. These women are the ones most likely to be referred for colposcopy (if available) by VIA providers. Women with atrophy or inadequate examination were more likely to be recorded under the category of “no lesion” if no obvious lesion was visualised in our study and this may partially explain the high proportion of missed CIN 2+. However, given the small number of WLWH and HIV-negative women in the younger age group of 25 to 29, and the older age group of over 50, we cannot draw any further inference from these results.

5.3.12. Conclusions

Despite the higher hrHPV prevalence in both WLHW and HIV-negative women, the CIN 2+ detection in this study was similar to previous reports from Botswana among WLWH. In this study, more WLWH had prior cervical cancer screening compared to HIV-negative women, reflecting the bias of international donor funding towards people living with HIV. Although our findings showed that HIV-negative women had slightly lower proportions of CIN 2+ than WLWH, the overall prevalence was significant enough to warrant their inclusion in a comprehensive national programme. Deliberate inclusion of older women in the study has provided a valuable insight into the potential burden of cervical disease in the birth cohorts older than the screening target age group of 25 to 49 years old in new programmes.

The other important finding from the study was the high proportion of hrHPV-positive women identified with an acetowhite lesion by visual evaluation who had no CIN 2+ disease, regardless of HIV status. This indicates that VIA as a triage method would result in overtreatment of many women.

We have also confirmed the importance of HPV 35 in precancer in keeping with other sub-Saharan African studies. This has an implication for the future HPV vaccination

programmes even if the current nonavalent vaccine is used as it does not protect against HPV 35. Additionally, emerging evidence from this study seems to indicate a stronger oncogenic role of HPV 53 and 66 in the development of severe precancer than previously reported. Finally, our data contribute evidence on the use of the new AmpFire HPV DNA platform in a clinical setting with a high HIV burden.

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CHAPTER 6. PERFORMANCE OF DIFFERENT TRIAGE ALGORITHMS

6.1. Introduction

Using self-collected hrHPV as a primary test tool has made screening accessible to more women.[1-5] HrHPV prevalence is high in most countries in sub-Saharan Africa (sSA), Botswana included.[6] Only a small fraction of hrHPV positive women have verifiable CIN 2+ disease.[7-9] A screening tool needs to identify as many of the women with precancer disease as possible so that they can be treated to prevent development of invasive disease.[10] However, if most women screened positive turn out to have no disease, this can lead to anxiety and harm related to unnecessary treatment, especially in a "screen-and-treat" setting.[11,12]

In this chapter, we present the test performance of the various algorithms to detect cervical precancer lesions.

6.2. Results

6.2.1. Histological outcome of hrHPV positive women

Overall, 655 women who tested positive for hrHPV had valid histological results (Table 6.1). Among WLWH, 18/363 (5.0%) had CIN 2, 46/363 (12.7%) had CIN 3, 2/363 (0.6%) had adenocarcinoma-in-situ, 2 (0.6%) had adenocarcinoma, and 1/363 (0.3%) had squamous cell carcinoma. Among HIV-negative women, 14/292 (4.8%) had CIN 2, 29/292 (9.9%) had CIN 3, and 1/292 (0.3%) had adenocarcinoma-in-situ.

Table 6.1 Histological outcome of hrHPV positive women with valid results

Histology diagnosis	All patients N=655 n (%)	HIV positive N=363 n (%)	HIV negative N=292 n (%)
≤CIN1	542 (87.5)	294 (81.0)	248 (84.9)
CIN2	32(4.8)	18 (5.0)	14 (4.8)

CIN3	75 (11.3)	46 (12.7)	29 (9.9)
Adenocarcinoma-in-situ	3 (0.5)	2 (0.6)	1 (0.3)
Adenocarcinoma	2 (0.3)	2 (0.6)	0 (0.0)
Squamous cell carcinoma	1 (0.2)	1 (0.3)	0 (0.0)

hrHPV: high-risk human papilloma virus; HIV: human immunodeficiency virus; CIN: cervical intraepithelial neoplasia

6.2.2. VIA triage

VIA positivity is defined as all women with an acetowhite lesion (both low- and high-grade). Of the 655 hrHPV positive women with a histological end-point, 121/363 (33.3%) WLWH were VIA positive compared to 69/292 (23.6%) HIV-negative women (Table 6.2).

Table 6.2 Results by VIA triage, histological finding, and HIV-status (percentage)

	HIV-positive		HIV-negative		Overall		p-value*
	%	(a/n)	%	(a/n)	%	(a/n)	
% who tested positive for hrHPV	52.3%	(392/750)	41.9%	(314/750)	47.1%	(706/1500)	
% hrHPV+ histologically evaluated	92.6%	(363/392)	93.0%	(292/314)	92.8%	(655/706)	
Among histologically evaluated…							
% positive by VIA	33.3%	(121/363)	23.6%	(69/292)	29.0%	(190/655)	0.007
% negative by VIA	66.7%	(242/363)	76.4%	(223/292)	71.0%	(465/655)	
% VIA-positive with CIN2+	34.7%	(42/121)	24.6%	(17/69)	31.1%	(59/190)	
% VIA-negative with CIN2+	11.2%	(27/242)	12.1%	(27/223)	11.6%	(54/465)	0.75
% VIA-positive with CIN3+	28.9%	(35/121)	21.7%	(14/69)	25.8%	(49/190)	
% VIA-negative with CIN3+	6.6%	(16/242)	7.2%	(16/223)	6.9%	(32/465)	0.81

CIN: cervical intra-epithelial neoplasia; HIV: human immunodeficiency virus; hrHPV: high-risk human papillomavirus; VIA: visual inspection with acetic acid
*Chi-square test

VIA missed 27/69 (39.1%) CIN2+ and 16/51 (31.4%) CIN 3+ in WLWH compared with 27/44 (61.4%) CIN 2+ and 16/30 (53.3%) CIN 3+ in HIV-negative women. (Figures 6.1 and 6.2, and Table 6.3)

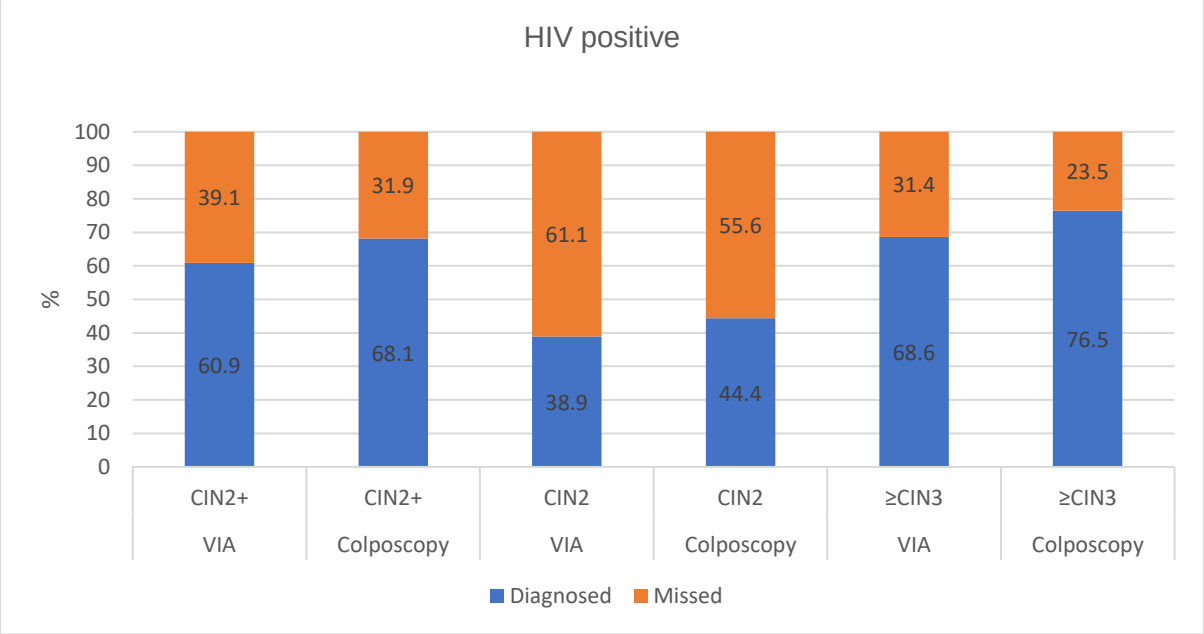

Figure 6.1 Disease diagnosed and missed by visual evaluation

Table 6.3 Proportion of CIN cases missed during VIA, by HIV-status

	HIV-positive		**HIV-negative**		**Overall**		**p-value***
CIN2+ cases missed by VIA result	39.1%	(27/69)	61.4%	(27/44)	47.8%	(54/113)	0.02
CIN3+ cases missed by VIA result	31.4%	(16/51)	53.3%	(16/30)	39.5%	(32/81)	0.07

CIN: cervical intra-epithelial neoplasia; HIV: human immunodeficiency virus: VIA: visual inspection with acetic acid
*Chi-square test

A higher proportion of CIN2+ cases were missed among HIV-negative women, compared with WLWH (p-value=0.02) (Table 6.3).

6.2.3. Colposcopy triage

Colposcopy positivity is defined as all women with an acetowhite lesion (both low- and high-grade). Of the 363 hrHPV positive WLWH women evaluated, 135 (37.2%) were positive compared with 82/292 (28.1%) of HIV-negative women (Table 6.4).

Table 6.4 Results by colposcopy triage, histological finding, and HIV-status (percentage)

	HIV-positive		HIV-negative		Overall		p-value*
	%	(a/n)	%	(a/n)	%	(a/n)	
% who tested positive for hrHPV	52.3%	(392/750)	41.9%	(314/750)	47.1%	(706/1500)	<0.001
% hrHPV+ histologically evaluated	92.6%	(363/392)	93.0%	(292/314)	92.8%	(655/706)	0.84
Among histologically evaluated…							
% positive by colposcopy	37.2%	135/363	28.1%	82/292	33.1%	217/655	0.01
% negative by colposcopy	62.8%	228/363	71.9%	210/292	66.9%	438/655	0.01
% colposcopy-positive with CIN2+	34.8%	47/135	20.7%	17/82	29.5%	64/217	0.03
% colposcopy-negative with CIN2+	9.6%	22/228	12.9%	27/210	11.2%	49/438	0.29
% colposcopy-positive with CIN3+	28.9%	39/135	15.9%	13/82	24.0%	52/217	0.03
% colposcopy-negative with CIN3+	5.3%	12/228	8.1%	17/210	6.6%	29/438	0.23

CIN: cervical intra-epithelial neoplasia; HIV: human immunodeficiency virus; hrHPV: high-risk human papillomavirus
*Chi-square test

Colposcopy missed 22/69 (31.9%) CIN 2+ and 12/51 (23.5%) CIN 3+ in WLWH, compared with 27/44 (61.4%) CIN 2+ and 17/30 (46.7%) CIN 3+ in HIV-negative women (Figures 6.1 and 6.2).

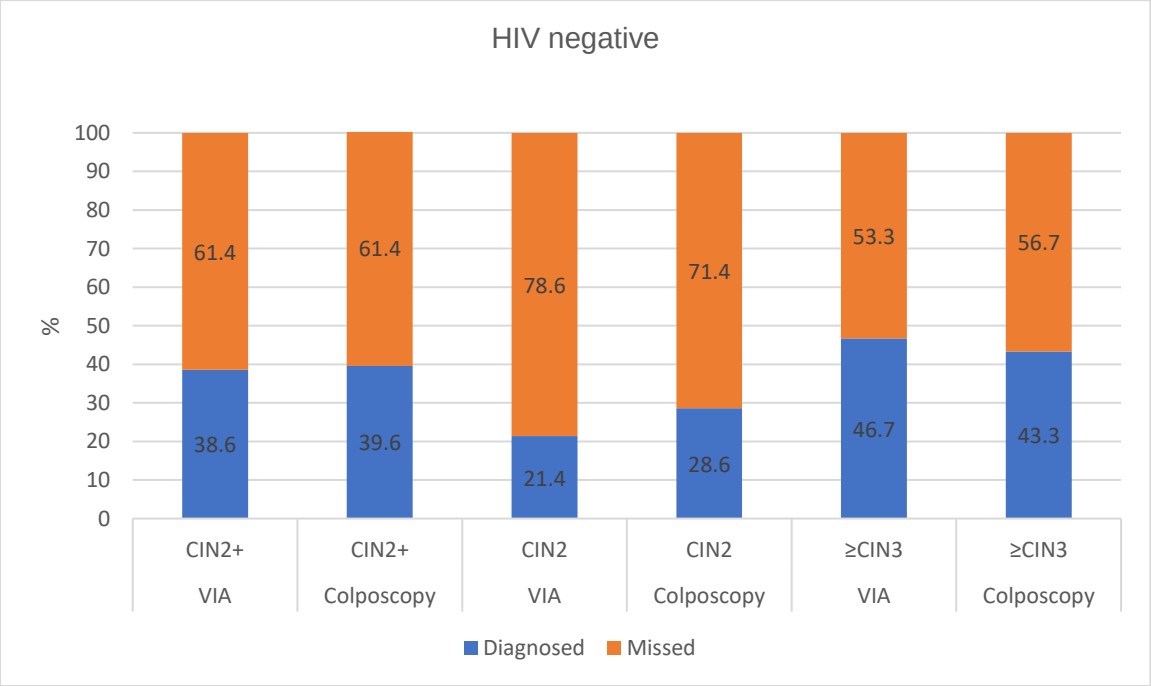

Figure 6.2 Disease diagnosed and missed by visual evaluation

Table 6.5 Proportion of CIN cases missed during colposcopy, by HIV status

	HIV-positive		HIV-negative		Overall		p-value*
CIN2+ cases missed by colposcopy	31.9%	(22/69)	61.4%	(27/44)	43.4%	(49/113)	0.002
CIN3+ cases missed by colposcopy	23.5%	(12/51)	56.7%	(17/30)	16.0%	(13/81)	0.003

CIN: cervical intra-epithelial neoplasia; HIV: human immunodeficiency virus
*Chi-square test

A higher proportion of both CIN2+ and CIN 3+ cases were missed among HIV-negative women, compared with WLWH (p-value 0.02 and 0.003, respectively) (Table 6.5).

Table 6.6 Missed disease by VIA and colposcopy by HIV status and age

	CIN 2+ n (%)	CIN 2 n (%)	CIN 3+ n (%)
VIA			
HIV-positive			
All women	27/69 (39.1)	11/18 (61.1)	16/51 (31.4)
<50	15/69 (21.7)	4/18 (22.2)	11/51 (21.6)
≥50	12/69 (17.4)	7/18 (38.9)	5/51 (9.8)
HIV-negative			
All women	27/44 (61.4)	11/14 (78.6)	16/30 (53.3)
<50	20/44 (45.4)	9/14 (64.3)	11/30 (36.7)
≥50	7/44 (15.9)	2/14 (16.3)	5/30 (16.7)
Colposcopy			
HIV-positive			
All women	22/69 (31.9)	10/18 (55.6)	12/51 (23.5)
<50	9/69 (13.0)	3/18 (16.7)	6/51 (11.8)
≥50	13/69 (18.8)	7/18 (38.9)	6/51 (11.8)
HIV-negative			
All women	27/44 (61.4)	10/14 (71.4)	17/30 (56.7)
<50	19/44 (43.2)	8/14 (57.1)	11/30 (36.7)
≥50	8/44 (18.2)	2/14 (14.3)	6/30 (20.0)

CIN: cervical intra-epithelial neoplasia; HIV: human immunodeficiency virus. VIA: visual inspection with acetic acid

Table 6.6 represents the missed lesions by age group. Both visualisation methods missed similar proportions of CIN 2+ lesions in WLWH younger than 50 years old and those 50 years and older. However, both methods missed more CIN 2+ lesions in younger women compared with older women (by the order of 2.4 and 2.9 times by colposcopy and VIA, respectively) in HIV-negative women.

6.2.4. Histological outcome for overlap of VIA and colposcopy evaluation for hrHPV-positive women

Table 6.7 Histological outcome of VIA and colposcopy overlap for hrHPV-positive women

	HIV-positive N=69			HIV-negative N=44		
Variable	**CIN 2+ N=69**	**CIN 2 N=18**	**CIN 3+ N=51**	**CIN 2+ N=44**	**CIN 2 N=14**	**CIN 3 N=30**
VIA-positive Colposcopy-positive	40 (58.0%)	7 (38.9%)	33 (60.8%)	12 (27.3%)	3 (21.4%)	9 (30.0%)
VIA-positive Colposcopy-negative	2 (2.9%)	0 (0.0%)	2 (3.9%)	5 (11.4%)	0 (0.0%)	5 (16.7%)
VIA-negative Colposcopy-negative	20 (29.0%)	10 (55.6%)	10 (17.6%)	22 (50.0%)	10 (71.4%)	12 (40.0%)
VIA-negative Colposcopy-positive	7 (10.1%)	1 (5.6%)	6 (11.8%)	5 (11.4%)	1 (7.1%)	4 (13.3%)

CIN: cervical intraepithelial neoplasia; HIV: human immunodeficiency virus; hrHPV: high-risk human papilloma virus; VIA: visual inspection after acetic acid

Table 6.7 shows the overlap of VIA and colposcopy. Fifty-eight percent of all CIN 2+ lesions among WLWH were detected by both VIA and colposcopy. The detection by both methods was 27.3% in HIV-negative women. Both visualisation methods missed 29.0% in WLWH, and 50.0% in HIV-negative women. VIA identified 2.9% and 11.4% of CIN 2+ in WLWH and HIV-negative women that colposcopy missed. Conversely, colposcopy identified 10.1% and 11.4% of CIN 2+ in WLWH and HIV-negative women that VIA missed.

Table 6.8 Prevalence of CIN2/CIN3+ and missed disease by hrHPV genotyping

HrHPV genotypes	No undergoing evaluation n (%)	No with CIN2 n (%)	No with ≥CIN3 n (%)	No ≥CIN3* missed n (%)*
HIV-positive				
Any hrHPV	363 (100)	18 (5.0)	51 (14.1)	0 (0.0)
hrHPV 16/18 (group 1)	93 (25.6)	3 (0.8)	21 (5.8)	30 (58.8)
hrHPV 16/18/45 (group 2)	122 (33.6)	4 (1.1)	25 (6.9)	26 (51.0)
hrHPV 16/18/31/33/35/45/52/58 (group 3)	267 (73.5)	14 (3.9)	46 (12.7)	5(9.8)
Non-hrHPV 16/18/45 (group 4)	242 (66.6)	14 (3.9)	26 (7.2)	25 (49.0)
HIV-negative				
Any hrHPV	292 (100)	14(4.8)	30 (10.3)	0 (0.0)
hrHPV 16/18 (group 1)	59 (20.2)	3 (1.0)	9 (3.1)	21 (70.0)
hrHPV 16/18/45 (group 2)	83 (28.4)	3 (1.0)	11(3.8)	19 (63.3)
hrHPV 16/18/31/33/35/45/52/58 (group 3)	201(68.8)	10 (3.4)	28 (9.6)	2 (6.7)
Non-hrHPV 16/18/45 (group 4)	215 (73.6)	11 (3.8)	19 (6.5)	11 (36.6)

CIN: cervical intraepithelial neoplasia; hrHPV: high risk human papilloma virus; HIV: human immunodeficiency virus
*Denotes percentage of n/number of CIN 3+ missed by restricted hrHPV genotyping

6.2.5. Triage of hrHPV-positive women by HPV16/18

If any hrHPV-positive women were triaged with 2-hrHPV (group 1) this would miss 45/69 (65.2%) CIN 2+ and 30/51 (58.8%) CIN 3+ in WLWH, and 32/44 (72.8%) CIN2+ and 21/30 (70%) CIN3+ in HIV-negative women (Table 6.8).

6.2.6. Triage of hrHPV-positive women by HPV16/18/45

If any hrHPV-positive women were triaged with 3-hrHPV (group 2) this would miss this would miss 40/69 (58.0%) CIN 2+ and 26/51 (51.0%) CIN 3+ in WLWH, and 30/44 (68.2%) CIN 2+ and 19/30 (63.3%) CIN3+ in HIV-negative women (Table 6.8).

6.2.7. Triage of hrHPV-positive women by HPV16/18/31/33/35/45/52/58

If any hrHPV-positive women were triaged with 8-hrHPV (group 3) this would miss this would miss 9/69 (13.0%) CIN 2+ and 5/51 (9.8%) CIN3+ in WLWH, and 6/44 (28.6%) CIN 2+ and 2/30 (6.7%) CIN3+ in HIV-negative women (Table 6.8).

6.2.8. Performance of any hrHPV in detection of CIN 2+

The sensitivity of any hrHPV test was 100% (95% CI 95-100%), specificity 10% (95% CI 7-14%) positive predictive value 19% (95% CI 15-23%), and negative predictive value 100% (95% CI 89-100%), in WLWH. In HIV-negative women the sensitivity was 100% (95% CI 92-100%), specificity 18% (95% CI 14-22%), positive predictive value 15% (95% CI 11-20%), and negative predictive value 100% (95% CI 93-100%)

Table 6.9 Performance of hrHPV screen in detecting CIN 2 or higher among all women

	HIV-positive				
	Histology results	**HPV screen characteristic**			
hrHPV screen using different cutoffs	**≥ CIN2 Prop (a/n)**	**Sensitivity % (95% CI)**	**Specificity % (95% CI)**	**PPV % (95% CI)**	**NVP % (95% CI)**
HPV Negative*	0/32	-	-	-	-
Any hrHPV	69/363	100 (95-100)	10 (7-14)	19 (15-23)	100 (89-100)
hrHPV16/18 (group 1)	24/93	35 (25-47)	79 (74-83)	26 (18-36)	85 (81-89)
hrHPV16/18/45 (group 2)	29/122	42 (31-54)	71 (66-76)	24 (17-32)	85 (81-89)
hrHPV16/18/31/33/35/45/52/58 (group 3)	60/266	87 (77-93)	37 (32-42)	23 (18-28)	93 (87-96)
Non-hrHPV16/18/45 (group 4)	40/273	58 (46-69)	29 (24-34)	15 (11-19)	76 (68-83)
	HIV-Negative				
	Histology results	**HPV screen characteristic**			
hrHPV screen using different cutoffs	**≥ CIN2 Prop (a/n)**	**Sensitivity % (95% CI)**	**Specificity % (95% CI)**	**PPV % (95% CI)**	**NVP % (95% CI)**
hrHPV-negative*	0/53	-	-	-	-
Any hrHPV	44/292	100 (92-100)	18 (14-22)	15 (11-20)	100 (93-100)
hrHPV16/18 (group 1)	12/57	27 (16-42)	85 (81-89)	21 (13-33)	89 (85-92)
hrHPV16/18/45 (group 2)	14/81	32 (20-47)	78 (73-82)	17 (11-27)	89 (84-92)
hrHPV16/18/31/33/35/45/52/58 (group 3)	38/195	86 (73-94)	48 (42-53)	20 (15-26)	96 (92-98)
Non-hrHPV16/18/45 (group 4)	30/264	68 (53-80)	22 (18-27)	11 (8-16)	83 (73-89)

*Among subset of random selection of 10% of HPV negative women (both HIV positive and HIV negative).
CIN: cervical intraepithelial neoplasia; hrHPV: high-risk human papilloma virus; HIV: human immunodeficiency virus; PPV: positive predictive value; NPV: negative predictive value; CI: confidence interval

6.2.9. Performance of HPV 16/18 in detection of CIN 2+

The sensitivity of the 2-hrHPV (group 1) test was 35% (95% CI 25-47%), specificity 79% (95% CI 74-83%) positive predictive value 26% (95% CI 18-36%), and negative predictive value 85% (95% CI 81-89%), in WLWH. In HIV-negative women the

sensitivity was 27% (95% CI 16-42%), specificity 85% (95% CI 81-89%), positive predictive value 21% (95% CI 13-33%), and negative predictive value 89% (95% CI 85-92%) (Table 6.9).

6.2.10. Performance of HPV 16/18/45 in detection of CIN 2+

The sensitivity of the 3-hrHPV (group 2) test was 42% (95% CI 31-54%), specificity 71% (95% CI 66-76%) positive predictive value 24% (95% CI 17-34%), and negative predictive value 85% (95% CI 81-89%), in WLWH. In HIV-negative women the sensitivity was 32% (95% CI 20-47%), specificity 78% (95% CI 73-82%), positive predictive value 17% (95% CI 11-27%), and negative predictive value 89% (95% CI 84-92%) (Table 6.9).

6.2.11. Performance of HPV 16/18/31/33/35/45/52/58 in detection of CIN 2+

The sensitivity of 8-hrHPV (group 3) test was 87% (95% CI 77-93%), specificity 37% (95% CI 32-42%), positive predictive value 23% (95% CI 18-28%), and negative predictive value 93% (95% CI 89-100%), in WLWH. In HIV-negative women the sensitivity was 86% (95% CI 73-94%), specificity 48% (95% CI 42-53%), positive predictive value 20% (95% CI 15-26%), and negative predictive value 96% (95% CI 92-98%) (Table 6.9).

6.2.12. Performance of VIA in detection of CIN 2+ in hrHPV-positive women

Using low-grade acetowhite lesion as a cut-off, the sensitivity of VIA triage was 61% (95% CI 49-72%); specificity 73% (68-78%); positive predictive value 33% (95% CI 24-42%); and negative predictive value 89% (95% CI 84-92%) for WLWH. In HIV negative women the sensitivity was 39% (95% CI 26-53%); specificity 79% (74-84%); positive predictive value 24% (95% CI 16-36%); and negative predictive value 88% (83-92%) (Table 6.10).

6.2.13. Performance of colposcopy in detection of CIN 2+ in hrHPV-positive women

Table 6.10 Performance of VIA/colposcopy triage in detecting CIN 2 or higher among all women who tested positive for hrHPV

	HIV-positive				
	Histology results	Triage characteristic			
Triage using different cutoffs	**≥ CIN2 Prop (a/n)**	**Sensitivity % (95% CI)**	**Specificity % (95% CI)**	**PPV % (95% CI)**	**NVP % (95% CI)**
VIA					
Normal	27/242	-	-	-	-
Low-grade impression	15/56	22 (14-33)	86 (82-90)	27 (17-40)	82 (78-86)
High-grade impression	27/65	39 (28-51)	87 (83-90)	42 (30-54)	86 (82-89)
Low-grade impression or worse	42/121	61 (49-72)	73 (68-78)	33 (24-42)	89 (84-92)
Colposcopy					
Normal	22/228	-	-	-	-
Low-grade impression	18/73	26 (17-36)	81 (76-85)	26 (16-36)	82 (78-86)
High-grade impression	29/62	42 (31-54)	89 (85-92)	47 (35-59)	87 (82-90)
Low-grade impression or worse	47/135	68 (56-78)	70 (65-75)	35 (27-43)	90 (86-94)
	HIV-negative				
	Histology results	Triage characteristic			
Triage using different cutoffs	**≥ CIN2 Prop (a/n)**	**Sensitivity % (95% CI)**	**Specificity % (95% CI)**	**PPV % (95% CI)**	**NVP % (95% CI)**
VIA					
Normal	27/223	-	-	-	-
Low-grade impression	9/34	20 (11-35)	90 (86-93)	26(15-32)	86 (82-90)
High-grade impression	8/35	18 (10-32)	89 (85-92)	23(12-39)	86 (81-90)
Low-grade impression or worse	17/69	39 (26-53)	79 (74-84)	25 (16-36)	88 (83-92)
Colposcopy					
Normal	27/210	-	-	-	-
Low-grade impression	10/61	23 (13-37)	79 (74-84)	16 (9-28)	85 (80-89)
High-grade impression	7/21	16 (8-29)	94 (91-97)	33 (17-55)	86 (82-90)
Low-grade impression or worse	17/82	39 (26-53)	74 (68-79)	21 (13-31)	87 (82-91)

CIN: cervical intraepithelial neoplasia; hrHPV: high-risk human papilloma virus; HIV: human immunodeficiency virus; VIA: visual inspection after acetic acid; PPV: positive predictive value; NPV: negative predictive value; CI: confidence interval

Using low-grade acetowhite lesion as a cut-off, the sensitivity of colposcopy triage of any hrHPV positive was 68% (95% CI 56-78%); specificity 70% (95% CI 65-75%); positive predictive value 35% (95% CI 27-43%); and negative predictive value 90% (95% CI 86-94%) for WLWH. In HIV negative women the sensitivity was 39% (95% CI 26-53%); specificity 74% (68-79%); positive predictive value 21% (95% CI 13-31%); and negative predictive value 87% (82-91%) (Table 6.10).

6.3. Discussion

The optimal management of hrHPV screen-positive women that ensures the balance of benefits and harm remains a challenge, especially in high HIV prevalent countries.[13] In this chapter we evaluated several strategies for management of hrHPV screen positive women. We observed and will discuss below, that 1)although nearly half of the women screened positive with hrHPV, a relatively small percentage had histologically verified CIN 2+ regardless of HIV status, 2) visual triage methods (VIA and colposcopy) had similar performance, 3) “triage and treat” with VIA led to both overtreatment and undertreatment, 4) triage with a partial panel of 2-hrHPV and 3-hrHPV missed significant disease, and 4) triage with 8-hrHPV provided reasonable sensitivity to detect most of the CIN 2+ compared to 2-hrHPV and 3-hrHPV.

6.3.1. Performance of primary hrHPV screening in a ‘screen-and treat’ setting

As no CIN 2+ was confirmed in the random 10% sample of hrHPV-negative women in our cohort we assume no CIN 2+ disease would have been missed during screening. By extrapolation, using hrHPV screening in a “screen-and treat” strategy identified all women with CIN 2+ disease in this study as all screen-positive women had histological verification. This included one adenocarcinoma-in-situ in an HIV-negative woman, two adenocarcinoma-in-situ, two microscopic adenocarcinomas, and one macroscopic squamous carcinoma in WLWH. However, approximately four in five and six in seven hrHPV screen-positive WLWH and HIV-negative women, respectively, without established CIN 2+ disease would have been overtreated.

The sensitivity of the hrHPV “screen-and-treat” strategy in WLWH was 100% (95% CI 95-100); positive predictive value (PPV) of 19% (95% CI 15-23%); negative predictive value (NPV) of 100% (95% CI 89-100%), with a low specificity of 10% (95% CI 7-14%) for confirmed CIN 2+. The performance of hrHPV primary screening in HIV-negative women was as follows: sensitivity of 100% (95% CI 92-100%); specificity of 18% (95% CI 14-22%); PPV of 15% (95% CI 11-20%); NPV of 100% (95% CI 93-100%) for confirmed CIN 2+. The specificity was marginally better than in WLWH.

In their meta-analysis Kelly and colleagues reported a pooled hrHPV screen-positivity of 20 to 64% and sensitivity of 91.6% (88-94.1%) for CIN 2+ disease for various hrHPV DNA tests.[13] From a few studies with histological verification, the sensitivity of hrHPV ranged from 84 to 100%, and specificity was between 55 and 70% in WLWH in Bukina Faso, Kenya, India, South Africa and, and Zambia.[14-18] Limited data exists for HIV-negative women. A study from South Africa that included an HIV-negative cohort reported sensitivity of 89% (95% CI 83-94%), and specificity of 87% (95% CI 87-90%).[18] One study from Cameroon reported performance in the general population with a 12% HIV prevalence. The sensitivity was 100% (95% CI 80-100%) and specificity of 75% (95%CI 71-78%).[19]

Although the sensitivities reported in these studies were similar to our study finding, we reported much lower specificities. There are possible explanations for these: 1) the high sensitivity of the new Atila AmpFire assay that identifies HPV DNA regardless of established disease,[20] 2) the inclusion of younger women (<30), who are known to have high rates of HPV infections not associated with CIN 2+ disease, 3) unprotected sexual intercourse within the last five days prior to HPV testing can detect deposited sexual partners' HPV DNA, and this was not ascertained in our study.[21]

6.3.2. Performance of VIA and colposcopy as independent triage methods

This study is one of the few studies that assessed visual evaluation as a triage method comparing VIA and colposcopy. Colposcopy is the standard for evaluation of screen-positive women in many HICs screening guidelines,[22-25] but this is usually to guide biopsy where there is a visualised lesion. In this study, visual evaluation performance by VIA and colposcopy were similar, making a case for VIA as a reasonable "triage-and-treat" strategy for hrHPV screen-positive women. Providing histological verification for all hrHPV screen-positive women who presented for visual evaluation allowed us to quantify the magnitude of over- and undertreatment if utilising VIA as a "triage-and-treat" method. Of the women reported as VIA negative, CIN 2+ disease would have been missed in 11% (27/243) and 12% (27/228) of WLWH and HIV-

negative women, respectively. In the VIA positive group, 65% (79/121) WLWH, and 76% (53/60) HIV-negative women would have been overtreated.

VIA and colposcopy missed significant disease, more so in HIV-negative women compared with WLWH. Furthermore, VIA missed a third of the CIN 3+ compared with nearly a quarter by colposcopy in WLWH. Most concerning was that both methods missed more than half of the CIN 3+ disease in HIV-negative women. VIA is recommended for women younger than 50 because those are likely to have a relatively normal cervix not affected by atrophy.

The established criteria for VIA include referral to colposcopy for further evaluation for women with inadequate examination.[26-29] It is worth noting that our study included older women outside the 25 to 49 screening target age and did not exclude women with atrophy or inadequate examinations. Instead, most of the women with inadequate examination were more likely to be recorded under the category of “no lesion”. This classification may partially explain the high proportion of missed CIN 2+ disease. The women with atrophy and/or inadequate examination recorded as no lesion, received a small central excisional procedure leading to attainment of a histological endpoint. VIA and colposcopy missed similar proportions of CIN 2+ lesions in women under 50 years old and in those 50 years old and above in WLWH. However, in HIV-negative women, both methods missed more CIN 2+ lesions in younger women (the order of 2.4 and 2.9 times by colposcopy and VIA, respectively).

There is almost no data reporting histological verification for cases missed and overtreated when using VIA and colposcopy as triage of hrHPV screen positive women. In a general population cohort of 5000 women in India of evaluation of VIA as triage for hrHPV screen-positive women with histological verification, 12% of VIA negative women would have had a missed CIN 2+. Of the VIA positive women, 49% had no CIN 2+ and would have been over-treated.[16]

Overtreatment has been shown to be inevitable in a “screen-and-treat” programme, and it has been accepted as a necessary evil.[26,30] The reason for its acceptance is because it has been shown to be relatively safe with its benefits outweighing the harms, especially in limited-resource settings and where there is high likelihood for

loss-to-follow up.[3,26,28,30] However, in settings where close to half the women are hrHPV screen-positive and less than 20% of them have established disease, a "screen-and-treat" is likely to overwhelm resources in most screening programmes. This may even jeopardise the ability to treat the few with disease as waiting periods for assessment for all screen-positive for treatment would be inevitably long. This can further overwhelm the pathological services if excisional treatment is carried out and place a significant strain on health care systems that are already overburdened by many other diseases such as HIV, TB, and malaria.

Furthermore, a screen-positive result can lead to stress, anxiety, feelings of stigma, shame, and concerns about the possibility of a cancer diagnosis.[12] Loss of confidence in the service can also result from high screen-positive results with many false positive results. Therefore, as we design screening programmes, the need to treat those with precancer whilst minimising loss-to-follow-up, should be balanced against overtreatment with its potential negative consequences.

6.3.3. Performance of partial hrHPV genotyping as triage methods

Full genotyping of all hrHPV-positive women allowed us to assess the various restricted genotype triage strategies. Both 2-hrHPV (HPV 16/18) and 3-hrHPV (HPV 16/18/45) strategies had good specificities but poor sensitivities. They missed more than half the CIN 3+ in WLWH and even higher in HIV-negative women, making them inferior to VIA as triage strategies. Out of all the tested triage strategies, 8-hrHPV (HPV 16/18/31/33/35/45/52/58) had a better sensitivity (87% [77-93%] vs. 86% [73-94%] for WLWH and HIV-negative women, respectively) of detecting CIN 2+ disease. 8-hrHPV missed less than 10% of CIN 3+ in both WLWH and HIV-negative women. However, this triage strategy had a sub-optimal specificity as many women still needed evaluation for treatment [207/267 (77.5%) for WLWH, and 163/201 (81.1%) for HIV-negative women]. The specificity was as follows: WLWH - 37% (32-42); HIV-negative women – 48% (42-53%).

Other researchers have reported a better balance of sensitivity and specificity of 8-hrHPV than our study. Johnson and colleagues reported from their Cape Town study

hrHPV prevalence of 48.2% in WLWH and 16.2% in HIV-negative women.[18] The performance of the 8-hrHPV genotype triage was as follows: 1) WLWH – sensitivity 90.7% (85.3-94.6%); specificity 67.8% (61.9-73.3%). 2) HIV-negative women – sensitivity 87.1% (79.9-92.4%); specificity 89.7% (86.0-92.6%).[18]

Kelly and colleagues in their combined cohort of 1130 WLWH from Bukina-Faso (hrHPV prevalence 41.8%) and South Africa (hrHPV prevalence 59.7%), the performance of 8-hrHPV was as follows: sensitivity 76.9% (69.6-83.2%); specificity 73.5% (70.6-76.2%).[14] The reason for this variance between our study and the other two studies remains unclear and warrants further investigation.

Kuhn and colleagues in the same cohort reported by Johnson *et al*.[18] evaluated the performance of the GeneXpert HPV DNA platform by varying the manufacture-set cut-off levels to get a sensitivity of 80%.[31] By so doing, they were able to improve the specificity of the 8-hrHPV triage from 67.5 to 83.2% in WLWH, and from 89.7 to 94.1% in HIV-negative women.

6.3.4. Comparison of the new Atila AmpFire DNA assay to GeneXpert assay in the literature

The AAF HPV DNA assay is relatively new. Its attraction is its simplicity short turn-round time (no need for DNA extraction), and cheaper than most available platforms in sSA.[32] Adding to the available performance data for this platform is important, as it stands to be a viable option for countries that have a reasonable laboratory infrastructure to handle centralised specimen processing such as Botswana and South Africa.

New data from Rwanda has compared the performance of AmpFire to the GeneXpert in WLWH.[20] This study was nested within a large cervical cancer screening study of 5000. Nearly 300 women who had tested hrHPV positive by GeneXpert or were VIA positive were invited for colposcopy. At colposcopy, new specimens were collected and tested with both GeneXpert assay and AmpFire genotyping assay. The agreement for detection of any hrHPV was 89%, Cohen kappa 0.77 (95% CI 0.70-0.85). AmpFire

was highly likely to test positive for HPV 16 (p< 0.001), and marginally likely to test positive for 13-hrHPV which excludes HPV 66 (p= 0.05). Of note, GeneXpert detected 54% of HPV 51 and 43% of HPV 39 detected by AmpFire. The channel that tested for HPV 31, 33, 35, 52, and 58 in GeneXpert detected more cases than AmpFire. Even though the study did not report a histological endpoint, it does add to the AmpFire body of evidence as a primary screening tool.

6.3.5. The effect of inclusion of HPV 53 and 66 in HPV DNA screening assays in the literature

In their recent publication Schiffman and colleagues discuss the reasons and the potential harms of the “false positive” results in cervical cancer screening tests. They explained false positive as “detection of HPV infections that are not destined to cause cervical cancer”.[8] The reasons include use of ultra-sensitive screening tests that detect borderline HPV types with low likelihood of causing cervical cancer such as HPV 53 and 66, and the harm caused by treatment of all screen-positive women.

The IARC class 1 excludes HPV 53 and 66 and classifies them as rarely carcinogenic to humans.[33] HPV 53 and 66 are relatively common in the general population, do cause some high-grade precancerous lesions, but rarely cause invasive cancer according to several reports.[8,34-40] With this in mind, Desai and colleagues have recently collaborated with the Atila Biosystems Scientists responsible for the AmpFire assay to redesign the assay for public use and only included the IARC class 1 13-hrHPV types.[41] Additionally, they grouped the HPV types into four channels similar to the Xpert test (HPV 16, HPV 18,45, HPV 31, 33, 35, 52, 58, and HPV 39, 51, 56, 59, 68) designating the differential cervical cancer risk.

The new assay has been named ScreenFire, it is portable, and the reagents are stable at room temperature for two weeks, at 4C for up to one month, and -20C for a year. The assay was independently validated against MY09-My11-based PCR assay with genotyping in a screening population in Nigeria of 16–88-year-old women. They reported a rapid result within 20 to 60 minutes and the hierarchical agreement of 97% (95% CI 96-98%) weighted kappa of 0.90 (95% CI 0.86-0.93) showed no major

difference when the analysis was restricted to the 25-49 age group. The risk-based hierarchical HPV groups were considered as HPV 16 positive, HPV 18 or 45 positive (If HPV 16 was not present), HPV 31, 33, 35, 52, or 58 (if HPV 16, 18, and 45 were not present), HPV 39, 51, 56, or 68 (if other 8-hrHPV not present). The extended genotyping in a single tube is accurate, fast, affordable ($5/sample), and portable.

The original Ampfire assay individually genotypes 15 types. Removing HPV 53 and 66 will increase the specificity for CIN2+ as these types are frequent in infection but very rarely result in dysplasia, at least in low-risk populations. However, in WLWH this may be different, and in such populations more severe dysplasia than in the normal population might occur in conjunction with HPV 53 and 66. The data from our study hints at this possibility with each genotype detected as the only infection in a few cases of cervical disease in WLWH. HPV 53 was detected in one woman diagnosed with CIN 2, and HPV 66 was diagnosed in another woman with CIN 3. As the studies reporting low carcinogenesis of these two genotypes are skewed towards low-risk populations, more data from different settings such sSA with a high HIV burden is needed.

6.3.6. Conclusions

This study has shown that where good quality assurance for VIA exists, the triage test performs as well as that of experienced colposcopists. Although the specificity of both methods was better than that of hrHPV in a screen and treat setting, the accompanying loss in sensitivity negates the benefits of hrHPV primary screening test. However, these results probably give a better indication of the burden of disease in the screened population compared to studies that exclude women with inadequate visual examination of the cervix from the analysis. Of all the triage methods, the 8-HPV partial genotyping had the best sensitivity for the detection of CIN 2+ even though the specificity was relatively low. The specificity of this method was most likely linked to the overall specificity of the primary screening test and would probably be improved by a more restricted cutoff threshold for hrHPV positivity.

6.4. References chapter 6

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CHAPTER 7. CONCLUSIONS AND RECOMMENDATIONS FOR THE BOTSWANA CERVICAL CANCER SCREENING PROGRAMME

7.1. Introduction

The World Health Organization (WHO) guidelines (2021) recommend primary screening with a high precision human papillomavirus (HPV) test, followed by various triage methods depending on resources.[1] The triage recommendations relied heavily on modelling data with limited primary evidence, thus reducing their broad applicability, particularly to sub-Saharan Africa.[2] Botswana is moving from screening with visual inspection after acetic acid (VIA) towards high-risk HPV (hrHPV) testing.[3] It is mandatory that introduction of healthcare programmes such as population-level primary hrHPV screening are based on evidence.[4] Risk-stratification of hrHPV screen-positive women is critical to ensure better management of those at highest risk of cancer, sparing unnecessary effort and treatment for those with transient abnormalities, thereby rationalising scarce resources.[4]

The aim of this study was to evaluate some of the triage methods for hrHPV-positive women and recommend possible screening algorithms for the Botswana cervical cancer screening programme for both women living with HIV (WLWH) and HIV-negative women. The study utilised self-collected swabs for hrHPV testing. The prevalence calculation of CIN 2+ was based on the histological outcome for women who screened positive for hrHPV and attended the visual evaluation follow-up (93% of the total screening cohort). This histological verification allowed a more accurate evaluation of the undertreatment and overtreatment proportions of the various triage methods, compared to studies that only used biopsy-driven outcomes of women with visualised lesions.

In this chapter, we summarise the important study findings and propose options for the screening algorithms for Botswana based on these results, with appropriate motivation. We also consider the study strengths, limitations, and identify some future research areas.

7.2. The main study findings

7.2.1. Participant characteristics

The inclusion age criteria of this study was 25 years old and above. The reason to include women beyond the current Botswana screening exit criterium of 49 years old was to quantify the burden of disease in the older birth cohorts that would generally not have been screened. This data is important when reconsidering appropriate age parameters for recommendations towards the revised screening policy. Twenty two percent of WLWH and 24% of HIV negative women were 50 years and older in the study.

The two study groups of WLWH and HIV-negative women were analysed separately. As our sample was not randomly selected, the median age of the HIV-negative group was slightly younger than that of WLWH. A significant difference was observed in the age group 25 to 29 years which was three times higher in HIV-negative compared to WLWH. This difference was, however, closer to the distribution in the national population.

Two thirds of HIV-negative women and nearly three quarters of WLWH were single. A third of WLWH reported five or more lifetime partners compared to a quarter of the HIV negative women. Contraception use, including condom use, was high in both groups. WLWH were three times more likely to smoke compared to HIV-negative women in the small sub-group that smoked in the cohort. All these characteristics were in keeping with previous reports from Botswana.

Eighty percent of WLWH reported previous cervical cancer screening compared to just over half of the HIV negative women. Cervical cancer screening for WLWH has been prioritised by the Botswana Ministry of Health and supported by many international organisations including the federal government of the United States of America through the President's Emergency Plan for AIDS Relief. While this is commendable, equitable health care provision needs to be inclusive of all women. Although WLWH are at a higher risk for cervical cancer, HIV-negative women's risk increases with age

and if these women are not included in national programmes their risk can potentially surpass that of screened WLWH. Furthermore, the coverage of a target screening age group needs to be closer to 80% to have impact on the prevention of cervical cancer. This is an area that the Botswana Ministry of Health needs to pay attention to in its national screening programme to ensure no woman of screening age is left behind.

WLWH in this cohort had stable HIV disease. Ninety nine percent of the women had undetectable viral load, 93% had CD4 count 350 cells/mm^3 or higher, and 90 % had been on long term anti-retroviral treatment. These important parameters indicate the key milestones in Botswana's management of HIV, and the progress made towards the global targets of 95, 95, 95.

7.2.2. HPV epidemiology in Southeast Botswana

The prevalence of hrHPV was high in both WLWH and HIV-negative women in Southeast Botswana (52 and 42%, respectively). In a multivariate regression analysis, the only variable that was associated with hrHPV-positivity in WLWH was no previous cervical screening (adjusted odds ratio 2.06, 95% CI 1.41-3.01, p value = <0.001). In HIV-negative women parity of more than two seemed protective (adjusted odds ratio 0.49, 95% CI 0.34-0.71, p value = <0.001).

The highest prevalence of any hrHPV was observed in the younger age groups regardless of HIV status. The prevalence of any hrHPV decreased from age group 30 to 39 and remained generally constant for WLWH. After dropping to the lowest nadir at age group 40 to 49 the prevalence rose again to reach the original highest level in the 60 years and older age group in HIV-negative women. In contrast to the prevalence of any hrHPV, the highest risk HPV genotypes 16, 18, remained relatively constant across the age groups in WLWH, whereas the prevalence generally increased with age in HIV-negative women with the highest prevalence in the 60 years and above.

Fifty percent of WLWH were more likely to have multiple hrHPV genotypes compared to a third of HIV-negative women. The prevalence by HIV status when the hrHPV genotypes were stratified into three potential risk groups (HPV 16, 18 =group 1; HPV

16,18,45 = group 2; HPV 16,18, 31,33,35,45,52,58 = group 3) were as follows: In WLWH group 1 was 14%; group 2 was 18%; group 3 was 38%. In HIV-negative women group 1 was 9%, group 2 was 13%, and group 3 was 34%.

There was a lot of overlap for the 10 commonest hrHPV genotypes amongst WLWH and HIV-negative women. The prevalence of each type (as single or multiple infection) in descending order were HPV 68, 35, 58, 52, 53, 16, 18, 33, 51, 56 for WLWH, and HPV 16, 33, 52, 68, 35, 53, 51, 39, 45, and joint 10th 31, 56, and 58 for HIV negative women.

7.2.3. Visual triage of hrHPV-positive women

Although assessment of the acceptability of the use of self-collection swabs was not formally evaluated, women often mentioned their appreciation of the method, especially when they presented for visual examination which required insertion of a speculum.

Visual evaluation by VIA providers was comparable to that of experienced colposcopists in this setting where there was good quality assurance for VIA. The Cohen's Kappa suggested substantial agreement between the two methods among WLWH (0.71) and moderate agreement in HIV-negative women (0.47).

VIA-positivity was associated with more than five lifetime partners (adjusted odds ratio 2.28 (95% CI 1.32-3.95, p value =0.003). Being on anti-retroviral treatment for less than two years more than doubled the odds of a visualised lesion at VIA (adjusted odds ratio 2.41, 95% CI 1.14-5.10, p value =0.021) in WLWH.

7.2.4. Histology results of hrHPV-positive women

Although the prevalence of hrHPV was high in the Southeast District of Botswana in both WLWH (52%) and HIV-negative women (42%), the prevalence of CIN 2+ disease in the screening population of WLWH (9.6%) was similar to that reported by earlier studies from other parts of country. The prevalence of CIN 2+ in the HIV-negative screening population was 6%, the first reported in Botswana.

Even though the numbers were small in each age group, the severity of cervical disease seemed to increase with age. CIN 2 or less disease was detected in 97% of women in the age group 25 to 29 years for both WLWH and HIV-negative women, and 86% and 89% in the age group 50 years and above for WLWH and HIV-negative women, respectively. CIN 3+ was more prevalent in the age group 50 years and above (14% and 11% of WLWH and HIV-negative women, respectively). In contrast, CIN 3+ was detected in only 3% of the 25 to 29 years old age group for both WLWH and HIV-negative women**.**

Multiple hrHPV infections were associated with the presence of CIN 2+, more so in HIV-negative women compared to WLWH (p value = <0.001, and 0.015, respectively). The most prevalent hrHPV genotypes in descending order in WLWH with CIN 2+ were HPV (18, 33, 35, 58, 68, tied), 16, and 52. The most prevalent genotypes in HIV-negative women were HPV 52, 35, 33, and (53, 16, tied) in HIV-negative women. There was some overlap between the two groups.

Although not as common as the other hrHPV genotypes in CIN 2+ in this cohort, both HPV 53 and 66 were identified in some severe cervical disease cases in WLWH. HPV 66 was identified as the only infection in 4% of the WLWH who had CIN 3 disease. HPV 53, in combination with other infections, was the second commonest infection in WLWH who had CIN 3+.

Screening with hrHPV identified all women with cervical disease including glandular abnormalities. VIA identified 60% of CIN 2+ disease and colposcopy identified nearly

70% of the disease. Both VIA and colposcopy missed one of the two adenocarcinomas, and one of the three adenocarcinoma-in-situ cases.

Despite the well-manged HIV disease in almost all WLWH, this did not seem to translate into better control of hrHPV prevalence and cervical disease. Botswana implemented universal ART in 2016, and it is likely that many WLWH included in this study started treatment following the older guidelines that were based on level of CD4 count. Therefore, the current study findings may not be representative of the potential benefit of universal ART on cervical precancer risk, especially when started at higher CD4 counts.

7.2.5. Performance of various triage methods

7.2.5.1 Any hrHPV

Using any hrHPV test in a “screen-and treat” setting resulted in treatment of all women with CIN 2+ in the screening population in this cohort. In WLWH, the sensitivity was 100% (95-100%), specificity 10% (7-14%), positive predictive value 19% (15-23%), and negative predictive value 100 (89-100%). In HIV-negative women, the sensitivity was 100% (92-100%), specificity 18% (14-22%), positive predictive value (15 (11-20%), and negative predictive value 100% (93-100%). Although this strategy would result in treatment of women with precancer in the screening population, the financial and social cost of overtreatment would overwhelm the already burdened healthcare system due to its poor specificity.

7.2.5.2 Triage with visual assessment

Visual evaluation by VIA providers was comparable to that of experienced colposcopists in this setting where there was good quality assurance for VIA. However, use of either VIA and colposcopy as triage methods in this study would have led to undertreatment of a significant number of women with glandular precancer disease regardless of HIV status, and HIV-negative women with CIN 2+ disease. VIA identified 40% of WLWH with CIN 2 disease and 69% with CIN 3+ disease. In HIV-negative

women, VIA identified 21% and 47% with CIN 2 and CIN 3+ disease, respectively. Colposcopy identified 33% of WLWH with CIN 2 disease and 76% with CIN 3+ disease. In HIV-negative women colposcopy identified 29% with CIN 2 and 43% with CIN 3+ disease.

The performance of VIA triage to detect CIN 2+ disease was as follows: for WLWH, the sensitivity was 61% (49-72%), specificity 73% (68-78%), positive predictive value 33% (24-42%), negative predictive value 89% (84-92%). In HIV-negative women, the sensitivity was 39% (26-56%), specificity 79% (74-84%), positive predictive value 25% (16-36%), and negative predictive value 88% (83-92%).

The performance of colposcopy triage to detect CIN 2+ disease was as follows: for WLWH, the sensitivity was 68% (56-78%), specificity 70 (65-75%), positive predictive value 35% (27-43%), negative predictive value 90% (86-94%). In HIV-negative women, the sensitivity was 39% (26-53%), specificity 74% (68-79%), positive predictive value 21% (13-31%), and negative predictive value 87% (82-91%).

7.2.5.3 Triage with partial hrHPV genotyping

Both 2-hrHPV (HPV 16/18) and 3-hrHPV (HPV 16/18/45) strategies had good specificities but poor sensitivities. They missed more than half the CIN 3+ in WLWH and even higher in HIV-negative women, making them inferior to VIA as triage strategies.

The performance of the 2-hrHPV triage to detect CIN 2+ disease was as follows: for WLWH, the sensitivity was 35% (25-47%), specificity was 79% (74-83%), positive predictive value 26% (18-36%), negative predictive value 85% (81-89%). In HIV-negative women, the sensitivity was 27% (16-42%), specificity 85% (81-89%), positive predictive value 21% (13-33%), and negative predictive value 89% (84-92%).

The performance of the 3-hrHPV triage to detect CIN 2+ was as follows: for WLWH, the sensitivity was 42% (31-54%), specificity 71% (66-76%), positive predictive value 24% (17-32%), negative predictive value 85% (81-89%). In HIV-negative women, the

sensitivity was 32% (20-47%), specificity 78% (73-82%), positive predictive value 17% (11-27%), and negative predictive value 89% (84-92%).

The 8-hrHPV (HPV 16/18/31/33/35/45/52/58) genotyping had the best sensitivity of all the triage methods. For WLWH, the sensitivity was 87% (77-93%), specificity 37% (32-42%), positive predictive value 23% (18-28%), negative predictive value 93% (87-96%). In HIV-negative women, the sensitivity was 86% (73-94%), specificity 48% (42-53%), positive predictive value 20% (15-26%), and negative predictive value 96% (92-98%).

7.3. Recommendations for the Botswana cervical cancer screening programme

As Botswana prepares for the introduction of primary screening with hrHPV testing for the national cervical cancer prevention programme, we are making the following recommendations based on our study results.

7.3.1. Screen participants

7.3.1.1 HIV status

Although the screening of women in Botswana has prioritised WLWH as can also be seen from the questionnaire data with more WLWH reporting previous screening, a holistic and comprehensive programme needs to include all women within the screening target age group. The prevalence of CIN 3+ disease observed in our overall screening cohort was 7% for WLWH and 4% for HIV-negative women. Although this prevalence was lower in HIV-negative women compared with WLWH, this level of severe precancer is not insignificant. We, therefore, recommend a deliberate effort to ensure an inclusive screening programme for Botswana, regardless of HIV status based of these findings.

7.3.1.2 Entry criteria

The recommended target screening age by WHO is 25 to 49 years for WLWH and 30 to 49 years for the general population. In our study, the prevalence of CIN 3+ in the screening population for the age group 25 to 29 years old was 2.4% and 1.6% for WLWH and HIV-negative women, respectively. Given these results, we recommend a uniform target screening age of 25 to 49 for all women regardless of HIV status. This will also simplify the implementation of the new national screening programme.

7.3.1.3 Exit criteria

Countries which have mature screening programmes usually exit women from routine screening when they are 65 years and older, provided they have had several screen-negative tests. These women are generally at low risk of developing cervical cancer. WHO recommends exiting women from the screening programme when they are 50 years old. This is a pragmatic public health decision that ensures the most cost-effective use of limited resources, especially in low-resource settings. However, with a relatively new screening programme women older than the target exit age would be screen naive. Although the primary goal of screening is to focus on those who are likely to have precancer and treat them appropriately to reduce their risk of developing invasive cancer, new screening programmes need to carefully consider care of these older age cohorts that have an elevated risk of severe precancer and invasive disease.

7.3.1.4 Screening of women older than 49 years old

During the introduction of primary hrHPV screening, we recommend an initial campaign that would include all women 25 years even those beyond the exit age of 49 years to cover older women with no prior screening. The programme can then revert to the recommended 25 to 49 years old age group once a coverage of the 50 to 65 years old category has reached 80%. This is based on our finding of nearly a quarter of all CIN 3+ disease detected in women 50 years and older in both WLWH and HIV-negative women. Although not evaluated in our study, the cost of screening these additional women would most likely be much lower than the cost of treating

invasive cervical cancer in this older age group, and the preservation of life for women in this key demographic bodes well for any society.

7.3.1.5 Screening interval

WHO recommends the screening interval of three to five years for WLWH, and five to 10 years for HIV-negative women. Our study does not provide data to support or refute this recommendation, but due to the high sensitivity we demonstrated, we suggest that a longer interval may be safe. Therefore, we recommend a screening interval of five years for WLWH and 10 years for HIV-negative women as we implement the new screening programme in Botswana. Spreading out the interval will allow more women to be screened whilst having enough time to follow up those that screen positive for treatment and post treatment care.

7.3.2. Sampling methods

As Botswana prepares for the introduction of primary screening with hrHPV testing for the national cervical cancer prevention programme, using self-collected swabs will be a valuable strategy to increase access. The acceptability of self-collected swabs for hrHPV testing in Botswana has been confirmed in previous studies. We successfully used this method in this study and hrHPV testing was adequately done on self-collected swabs. We, therefore, recommend this collection method which will make speculum examination only necessary at the time of treatment, unless a women requests for a provider-collected swab, or where there is need to do a speculum examination for other reasons.

7.3.3. Screening and triage tests

The AmpFire HPV Multiplex DNA assay used in this study detects 15 hrHPV genotypes, giving this platform a built-in triage ability. Our study confirms that hrHPV DNA testing is an appropriate screening test for Botswana because we demonstrated a very high sensitivity to detect disease (see chapter 6, page 158). We calculated a 100% sensitivity across both study groups, but this was influenced by the low number

of HPV negative women who underwent biopsy (chapter 5, page 131). In this study, we also showed the effectiveness of a variety of reflex genotyping options (chapter 6, pages 158-159). Additionally, VIA and colposcopy were also evaluated as triage methods (chapter 6, pages 159-161). Both methods reduced sensitivity to 61%-68% in WLWH and 39% in HIV-negative women. Triage with 2-hrHPV reduced sensitivity to 35% in WLWH and 27% in HIV-negative women. Triage with 3-hrHPV reduced sensitivity to 42% in WLWH and 32% in HIV-negative women. Amongst the various visual and partial genotyping triage tests, the 8-hrHPV (HPV 16, 18, 31, 33, 35, 45, 52, 58) demonstrated the best sensitivity of 87% for WLWH and 86% for HIV-negative women (chapter 6, page 159).

The AmpFire HPV DNA assay used in this study is currently being considered for the national primary hrHPV screening programme in Botswana because of its simplicity, ease of use, short run time, and relative affordability. Furthermore, the AmpFire assay can also be used on most DNA platforms. To implement primary hrHPV screening, the Botswana cervical cancer prevention programme should leverage on the many DNA platforms across the country that were set up for the management of HIV and Covid-19, and the accompanying robust specimen transport and tracking system. Other suitable tests that can conform or partially conform to the recommendations made here, include the BD Onclarity test and the Roche test. However, the use of these assays requires the use of their own proprietary DNA platforms, which can add to the outlay costs of setting up a primary hrHPV screening programme.

Use of VIA and cytology as primary screening tests may need to be continued until hrHPV screening becomes widely available as per the WHO guidelines. However, all efforts must be made to ensure accessibility to primary hrHPV screening to all screen eligible women.

7.3.4. Management by risk category

We recommend that women who are triage-positive with partial genotype of 8-hrHPV (16, 18, 31, 33, 35, 45, 52, 58) should be referred for visual assessment for treatment decision. Based on our study, in comparison to referral of all hrHPV-positive women,

this triage will reduce the referred proportion from 52% to 38% for WLWH, and 42% to 29% for HIV-negative women. Furthermore, 90% of WLWH and 93% of HIV-negative women with CIN 3+ occurred in this category of women in our study. Prioritising this group for referral would ensure most of the highest risk women will receive treatment during the first round of screening. Women screening positive for other hrHPV should be re-screened in one year regardless of HIV status, and referred for treatment if they still test positive. The reason for this recommendation will be discussed further below.

7.3.5. Treatment options

7.3.5.1 WHO treatment guidelines

WHO recommends the following treatment guidelines after primary hrHPV testing:1. In a “screen-and-treat” approach, all women who screen hrHPV-positive should be treated, and 2. In a screen, triage, and treat approach, all women who screen-positive by whatever triage method should be treated. Using partial genotyping, WHO recommends treatment of all those who are triage-positive for HPV 16 and 18, and referral for VIA for all women who are triage-positive for other hrHPV genotypes and treating only those with acetowhite lesions.

Given the high hrHPV-positivity in our cohort but with CIN 2+ disease detected in only 19% of WLWH and 12% of HIV-negative women who were hrHPV-positive, we do not recommend the “screen-and-treat” approach for Botswana. This option would result in high numbers of women referred for treatment, a decision that can overwhelm the healthcare system, and lead to overtreatment of many women without disease.

7.3.5.2 Treatment of women with partial 8-hrHPV genotyping

Instead, we recommend a screen with hrHPV, triage with partial genotyping, and treat all women who triage-positive for the 8-hrHPV genotypes (HPV 16, 18, 31, 33, 35, 45, 52, and 58). As per the WHO guidelines, treatment method should be guided by visual inspection after acetic acid. All women with no lesion or those with mild acetowhite

lesions should receive thermal ablation, and all with moderate to severe acetowhite lesions, or with an inadequate examination, should receive a loop electrosurgical excision procedure. The reason for this recommendation is that this group of women had a high risk of CIN 3+ in our study. Ninety percent of all CIN 3+ disease in WLWH and 93% of HIV-negative women occurred in women who were triage-positive for the 8-hrHPV genotypes. Women suspected of invasive cervical cancer should be referred urgently to the appropriate service for further management.

7.3.5.3 Treatment of women with other hrHPV

In contrast to the WHO guidelines, we recommend repeat hrHPV testing after one year in women who are triage-positive for other 7-hrHPV genotypes (39, 51, 53, 56, 59, 66, 68) instead of referring them for VIA and treatment after the first round of screening. Those who still test positive for the 7-hrHPV genotypes after one year should then be referred for VIA and treatment as per the WHO treatment guideline for this group of women. This is based on the low risk of harbouring CIN 3+ in our study for this category of women (10% of CIN 3+ occurred in WLWH, and 7% in HIV-negative women). Referring both triage groups of women (8-hrHPV and 7-hrHPV) with different management algorithms after the first round of screening may lead to confusion of both healthcare workers and patients in a new screening programme. Furthermore, only referring one group of women for treatment will avoid total collapse of the health care system with the influx of all screen-positive women in the first year of the programme. This recommendation can be evaluated two to three years into the programme as data to guide decision-making becomes available.

7.3.5.4 Follow up of hrHPV-positive women

All women who screened positive for any hrHPV regardless of triage results and treatment should have a repeat hrHPV test after one year. Women with histology results confirming micro-invasive disease should be referred to the multi-disciplinary gynaecological-oncology clinic four months post treatment for further management as per the current Botswana national guidelines.

7.3.5.5 Management of women screened with VIA or cytology

All women still receiving primary screening with VIA or cytology should be managed according to the current WHO guidelines.

7.4. Strengths of the study

This is the first study in Botswana to evaluate hrHPV primary screening with full genotyping and histological verification in both WLWH and HIV-negative women. The study provided a histological outcome for all hrHPV screen-positive women, allowing for evaluation of undertreatment and overtreatment of various triage-methods. The study is also the first one to report CIN 2+ prevalence in HIV-negative women in Botswana. This study further adds to the body of knowledge in sub-Saharan Africa which makes robust primary data available to guide recommendations from bodies like the WHO.

This study also adds valuable information about the true prevalence of CIN 2+ disease in the screening target age group of 25 to 49 years, and further provides data about prevalence of underlying disease in the older birth cohorts outside the screening population.

The study utilised a relatively low cadre of healthcare workers (health care auxiliary) as research assistants to do the recruitment and facilitate the self-collection of HPV swabs. Several women from outside the study areas presented for the study due to word of mouth about the ease of screening without the need for a speculum, which suggested that the service was desired. The VIA evaluation was provided by the regular MOH VIA nurse-providers. The two gynaecologists who did all the colposcopies and provided treatment on-site worked with two medical officers from the district hospital and provided colposcopy and LEEP training for them during the study period.

Histological verification was done as part of the Ministry of Health routine laboratory work. This provided additional histopathological material used in the training of

pathology registrars. Apart from the HPV testing, the study team strove to work within the healthcare system rather than create a parallel system because when research is done this way, it makes adoption of the study findings and recommendations relatively easy.

The use of the AmpFire assay adds more evidence about its clinical use, especially in WLWH.

7.5. Limitations of the study

Our study had several limitations. As histological verification was done as part of routine laboratory work, there were delays in getting results back within the six weeks that participants had been told to expect their results. This was due to the increased workload created by the additional histology specimen generated by the study outside routine practice. To mitigate patient anxiety, research assistants still called participants at the six-week mark to explain the delay, and called every two weeks thereafter until the results were available. The longest wait was three months. Due to the global Covid 19 related personnel shortage it was not possible to carry out additional quality assurance on the histology slides as originally planned.

As the study participants were not randomly selected, and the limited number in some important subgroups (such as age), the results need to be interpreted with caution. Furthermore, data collection of some variables such as number of lifetime sexual partners, was set as binary, making it impossible to calculate measures such as mean and standard deviations for more precise outcomes.

Trying to ascertain the outcome of previous cervical cancer screening was found difficult in our earlier research studies due to poor recall and missing records. We did not include this information in our current study. Therefore, analysis of the effect of the screening results and treatment decision on the current hrHPV and CIN2+prevalence was not possible.

The study did not exclude women with inadequate examination of the cervix during visual assessment. Furthermore, documentation of the visibility of the squamocolumnar junction, and adjustment for lack of its visibility may have impacted the observed effectiveness of visual triage methods.

The messaging about HPV testing is complicated given the sexually transmitted nature of the infection. An important limitation of this study is the lack of qualitative evaluation of the women's views about the impact of being diagnosed with a sexually transmitted infection in the context of cervical cancer prevention.

7.6. Future research

Increasing the options for easier and affordable assays to utilise in hrHPV primary screening programmes, especially in resource-limited settings is importance. Evaluating newer assays against established ones provides confidence when recommending them. We intend to use our stored samples from this study to evaluate the performance of the AmpFire platform against the known GeneXpert, and the newer ScreenFire test. The ScreenFire platform is a modification of the AmpFire platform using the GeneXpert risk-grouping of the various hrHPV genotypes (which excludes HPV 53).

Additional data to guide decisions of who to refer for immediate treatment and who to adopt a watchful wait approach on in a hrHPV primary screening setting is urgently needed. The key thing is to find screening and/or triage tests that are easily accessible due to affordability and ease of use for the resource-limited settings, which often bear the greatest burden of cervical cancer disease. Evaluation of the various cycle threshold cutoffs for hrHPV positivity that is associated with CIN 2+ disease for the AmpFire assay to improve its specificity without compromising the sensitivity will add value.

Potential triage strategies for women who screen positive for hrHPV such as reflex biomarkers at the point of primary screening are needed to identify women most likely to develop invasive cervical cancer. We will further evaluate different cycle cutoff

thresholds for hrHPV-positivity to try and improve the specificity of the AmpFire platform with minimal effect on the sensitivity. Such triage methods will avoid unnecessary visits and treatment of women with transient hrHPV infections and streamline the referral for women most likely to harbour severe precancer disease.

The significance of hrHPV 53/66 needs to be evaluated further in the sub-Saharan context as these types are currently considered of rare oncogenic risk, and often excluded from HPV DNA assays.

Public health and clinical evaluations are not complete without the inclusion of the cost-effective analysis and implementation science to guide policymakers when faced with various recommendations, particularly in the setting of limited resources and many competing needs often facing nations.

Finally, there is need to understand women's lived experience of undergoing HPV testing, particularly the potential fear, stigma, shame, and the impact of women's relationships with regarding the fidelity and the source of infection. A qualitative aspect of HPV screening research regarding the impact of HPV infections on women and their quality of life is urgently needed to ensure we develop appropriate messaging for HPV testing.

7.7. Conclusions

We have evaluated various options for triage of primary screened hrHPV-positive women in the context of a high HIV burden, including both WLWH and HIV-negative women. We utilised self-collected swabs for hrHPV testing, which were well received by the participants. Both VIA and colposcopy performed comparably as triage methods by HIV status. However, they both reduced the sensitivity of the primary hrHPV screening test by about a third in WLWH and by 60% in HIV-negative women, leading to an overall sub-optimal performance that eroded the benefit of primary hrHPV screening. The 8-HPV restricted genotyping provided the best triage sensitivity and is recommended as the triage method when the primary hrHPV screening is implemented in the country.

The importance of HPV 35 as a serious oncogenic risk was confirmed in association with CIN 2+ disease in both WLWH and HIV-negative women. This finding has implication for future vaccine programmes and the global goal of cervical elimination since it is excluded from the current nonavalent vaccine.

7.8. References chapter 7

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Appendix 1. Current opinions in infectious disease manuscript

Progress and challenges in human papillomavirus (HPV) and cervical cancer in southern Africa

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ABSTRACT

Purpose of review

Although cervical cancer is preventable, it is the leading cancer among women in southern Africa. The association of high-risk human papillomavirus (HR-HPV) with almost all invasive cervical cancers has led to the development of effective primary and secondary prevention measures. This review focuses on updated and new evidence of the epidemiology of HPV and HPV-based secondary prevention in southern Africa.

Recent findings

HR-HPV prevalence in southern Africa differs between regions, and varies most by HIV prevalence and age. HR-HPV prevalence among women living with HIV (WLWH) is reported between 29% and 59.7%, and between 16.2% and 25.2% among women without HIV. HPV16 is the most common HR-HPV type present in invasive cervical cancers in the region; and vaccination may potentially prevent approximately 80% of these cancers. Concerning preliminary data suggests faster development of new cervical precancer within a short follow-up period.

Summary

We need tools that identify the small number of women with precancer from the many with transient HR-HPV infection in southern Africa. The high-volume of test-positive women leads to challenges in managing triage in a HR-HPV-based screening program. Longitudinal data from the entire region is urgently needed to guide effective implemention of HPV-based screening programs.

Keywords

Human papillomavirus, cervical cancer, southern Africa, epidemiology, screening

INTRODUCTION

Persistent infection with high-risk (oncogenic) human papillomavirus (HR-HPV) is associated with most cervical precancer and invasive cancer [1]. In 2018, 690,000 new cases of infection-attributed cancer worldwide were due to HPV, with HPV16/18 (72%) and HPV31/33/45/52/58 (17%) sub-types responsible for the majority of cancer cases [2]. Cervical cancer accounted for 570,000 of the new cases [3]. Southern Africa has one of the world's highest age-standardized incidence rates for cervical cancer of 43.1 per 100,000, with respective rates: Eswatini 75.3, Lesotho 52.1, South Africa 43.5, Botswana 31.6, and Namibia 24.2 [4]. The reasons for these high rates are multifactorial; the critical ones are the inter-play of human immunodeficiency virus (HIV) and HPV [5], and the challenges of effective implementation of preventative strategies in low-middle-income countries (LMICs) [6]. The most recent global meta-analysis of cervical cancer by Stelzle *et al.* [7] reported a pooled risk estimate of 6.07, 95% CI: 4.40-8.37 for women living with HIV (WLWH) compared to the general population. In 2018, 63.8% of new cervical cancer cases in southern Africa occurred in WLWH [7].

Highly effective primary and secondary prevention measures have made cervical cancer an almost entirely preventable disease [8], leading the World Health Organization (WHO) to call for the "elimination of cervical cancer"(hereafter referred to as elimination) [9*]. Their strategy includes three specific recommendations, namely: vaccination of 90% of eligible girls, screening 70% of eligible women with a high-performance test, and effective treatment of 90% of screen-positive women [9*].

To support elimination, WHO recently released updated guidelines for screening and treatment of precancer lesions [10**]. These guidelines call for screening with HPV testing where possible, explicitly providing guidance for screening WLWH. However, recommendations rely heavily on modeling instead of primary data [11], which constrain their broad applicability, particularly to sub-Saharan Africa.

This report summarizes the latest evidence on HPV and cervical cancer screening in southern Africa's five UN subregion countries. It will only focus on the epidemiology of HPV and HPV-based secondary prevention since no updated data exists about HPV vaccination in the region for the review period (January 2020 to June 2021). We will also review evidence to guide the implementation of HPV primary screening in southern Africa, considering the WHO guidelines.

EPIDEMIOLOGY OF HPV

The prevalence and genotype distribution of HPV varies by geographic region, age, and the burden of HIV. Understanding the epidemiology of HPV in southern Africa is critical in determining HPV vaccine policy and estimating impact and resource planning for HPV screening, triage and precancer treatment.

HPV prevalence in the general population

The burden of HPV infection in young women in southern Africa is high. According to 2018 estimates, southern Africa had one of the highest HPV prevalences in all females (42.2%), and women under the age of 25 accounted for most of the infections [4]. In the rural Eastern Cape Province, South Africa, overall HPV prevalence in 15-22 year old sexually experienced girls was 76%, and prevalence of HR-HPV was 54.5% [12*]. Most girls were HIV-negative (66.9%) or had an unknown HIV status (29.6%). There was relatively low prevalence of HPV genotypes targeted by the three commercially available vaccines: 14.5% for Cervarix [HPV 16,18], 20.7% for Gardasil®4 (HPV 6,11,16, 18), and 30.1% for Gardasil®9 (HPV 6,11,16,18,31,33,45,52,58). [12*]

Another study in the Eastern Cape Province evaluated HR-HPV prevalence among a rural screening population of 417 women aged 30 to 98 years (median age 46 [IQR: 38-55]) [13*]. Thirty-seven percent were WLWH, almost all on antiretroviral treatment (ART). The prevalence among WLWH was 40.6% compared to 21.4% in women without HIV (OR 1.94 [95% CI 1.17-3.22]). HPV16, 35, and the HPV33/52/58 group were the most commonly detected HR-HPV subtypes [13*].

In an urban community screening clinic in Cape Town, South Africa, Johnson *et al*. [14*] evaluated HR-HPV prevalence among 714 women aged 30 to 65. Forty-seven percent were living with HIV, and 80% were on ART. WLWH had a higher prevalence of HR-HPV than women without HIV (48.2% versus 16.2%) and were more likely to have multiple subtypes (35.6% versus 25.8%) [14*].

Castle and colleagues determined HR-HPV prevalence in 1022 women aged 30 to 49 from a semi-rural region of Kweneng District, Botswana [15*]. Fifty-six percent were WLWH. Higher HR-HPV prevalence was observed in WLWH (40.2%) compared to women without

HIV (25.2%) (age-adjusted OR 2.3 [95% CI 1.7-3.0]). Infection with multiple HR-HPV subtypes in WLWH was double that in women without HIV (30% versus 15.9%, p=0.005) [15*]. The HR-HPV prevalence in WLWH was higher than 29%, previously reported by Luckett's study of 300 WLWH attending an urban HIV clinic at a tertiary hospital in Gaborone, Botswana [16]. Notably, all women in this urban cohort were on ART, and 95% had been previously screened [16].

The new WHO guidelines have reduced the age of screening initiation from 30 to 25 years in WLWH [10**]. However, the only study reporting HR-HPV prevalence for WLWH aged 25 to 29 was Kelly's urban community cohort of 576 WLWH in Johannesburg, South Africa) [17**]. The HR-HPV prevalence in women younger than 30 years versus 30 years and older was 59.7% and 48.8%, respectively. Of note, only a third of the WLWH were on long-term ART [17**].

HPV in women with cervical precancer

Taku and Mbulawa reported HR-HPV prevalence in 193 women with biopsy-confirmed cervical intraepithelial neoplasia grade 2 and 3 (CIN2/3) at a referral clinic in the Eastern Cape [18*]. The overall median age was 40 (IRQ: 33-48), and most were WLWH (76.2%). The prevalence of HR-HPV was high regardless of HIV status (87.1% for WLWH versus 82.6% for women without HIV-negative women). The most common HPV genotypes observed as single or co-infection were HPV 35, 16, 58, 45, and 52 (prevalences of 22.8%, 20.7%, 16.6%, 15%, 13.5%, respectively). However, WLWH were more likely to be infected with multiple subtypes than women without HIV (65.4 versus 47.5%, p=0.03) [18*].

HPV in women with invasive cancer

Chambuso *et al*. [19*] characterized HPV genotypes within cervical biopsies of 181 women over 18 with histologically confirmed cervical premalignant and malignant disease. These women were recruited from three hospitals in Cape Town, South Africa. Forty-eight percent were WLWH, and all were on ART. Genomic DNA was extracted from 85% of the cervical biopsies, and HPV 16 (single or co-infection) was detected in 72.5% and 21.1% of cancer biopsies of WLWH and women without HIV, respectively [19*].

Tawe *et al*. [20*] assessed HR-HPV prevalence and genotypes in 126 invasive cervical cancer specimens in Botswana. Seventy percent were from WLWH, almost all of whom were on long-

term ART. The mean age at cervical cancer diagnosis in WLWH was 45 versus 61 in women without HIV. HR-HPV DNA was detected in all specimens. HPV 16 (single or co-infection) was detected in 80.7% and 63.2% (p=0.04) of specimens of WLWH versus women without HIV, respectively. Multiple HR-HPV infections were present in 32.5% of specimens [20*].

In summary, HR-HPV prevalence in southern Africa differs between settings, but it varies most by HIV prevalence and age within studies. HR-HPV prevalence among WLWH is reported between 29% and 59.7%. WLWH on ART and who had prior cervical cancer screening had the lowest prevalence; the youngest WLWH not on ART had the highest among the highest prevalence. Prevalence between 16.2% and 25.2% was reported among women without HIV. HPV16, HPV35, and the HPV33/52/58 group were the most commonly detected HR-HPV subtypes. HPV16 as a single or co-infection was one of the most common HR-HPV types in cervical pathology, present in around 20% of precancer lesions and above 70% of invasive cancers.

HPV-BASED PREVENTION

Botswana and South Africa are the only two southern African countries with national HPV vaccination programs but have recently struggled with adequate coverage due to the global vaccine shortage [21]. This shortage has been exacerbated by expanding HPV vaccination across broader age groups and boys in high-income countries. While HPV vaccination may eliminate 70% of cervical cancers, secondary prevention with screening and treatment of identified precancers will remain the cornerstone of cervical cancer prevention in women already exposed to oncogenic HPV in southern Africa.

Primary HPV screening

All five southern African countries have national cervical cancer screening programs using a combination of cytology and VIA [4], but with sub-optimal coverage (≤50%) [22, 23]. Although the utility of HPV testing as a primary screening tool is well-understood, none of these countries have introduced HPV testing into their screening programs as of 2021. The main barriers are the lack of evidence to guide the transition to HPV testing and the limited resources for the new infrastructure requirements [24, 25]. Multiple WHO pre-qualified HPV testing platforms have demonstrated comparable performance [14*,17**,26*]. Although the majority are strictly laboratory-based, Xpert HPV and careHPV [14*,17**] have demonstrated the possibility of point-of-care (POC) testing in research settings.

Accuracy and acceptability of self-sampling

In combination with self-collected HPV swabs, POC testing has the potential to increase the accessibility of cervical cancer screening dramatically. Furthermore, eliminating transport medium by using DNA sample preservation cards (commonly known as FTA cards), dry swabs, or brushes that can be easily stored at room temperature makes self-sampling even more accessible [26*]. HPV self-sampling is acceptable to women, even though most women prefer to have a provider-collected swab [26*].

The accuracy of self- compared to provider-collected swabs is reassuring, even in populations with high HIV prevalence [26*,27,28*]. Taku and Meiring *et al*. [26*] compared the HR-HPV prevalence in self-and provider-collected swabs in a cohort described above[13*]. Similar prevalences for HR-HPV were reported for both collection methods (27.8% versus 26.4% self- versus provider-collection) in their community cohort. They reported an agreement of 91.4% (k= 0.711; 95% CI: 0.610-0.811) in their referral cohort and 86.9% (k=0.699; 95% CI:0.588-0.750) in their community cohort [26*]. In a Cape Town study by Saidu *et al.* [28*], the agreement of the two methods was equally high: 89.3% (k=0622; 95% CI: 0.471-0.726) for their referral clinic cohort and 86.8% (k=0.720; 95% CI: 0.699-0.771) for their community-based cohort. The agreement was maintained when stratified by HIV status [28*].

Triage of HR-HPV

The WHO screening guidelines recommend triage testing in a primary HPV screening program when possible and allow for "test-and-treat" if triage is not possible [10**]. A "test-and-treat" policy is challenging given the high prevalence of HR-HPV in southern Africa, especially in WLWH. WHO recommends triage by HPV genotyping, cytology, VIA, or colposcopy [10**], although all have challenges in the southern African setting. Strategies are needed to improve the triage of HPV-positive women to increase specificity without losing sensitivity.

Reducing HR-HPV types and DNA cut-off levels

Two studies from South Africa considered the impact of HPV triage by genotyping using HR-HPV 16,18,31,33,35,45,52,58 on the detection of CIN2+ [14*, 17**]. Both studies showed that HR-HPV type restriction improved specificity, more so in WLWH, with minimal loss in sensitivity (sensitivity 93.6%→ 90.7%, specificity 60.1% → 67.8% in Johnson *et al.*; sensitivity 86.8% → 80.6%, specificity 48.2% → 56.5% in Kelly *et al.*) [14*,17**].

Kuhn and colleagues further optimized the specificity of restricted HR-HPV types by adjusting individual channel cycle threshold cut-offs [29*]. They found that restricting HR-HPV sensitivity to 80% for each of the eight HR-HPV types increased specificity for detection of CIN2+ regardless of HIV status (specificity: in WLWH 59.9% →83.2%; in women without HIV 86.9% →94.1%) [29*].

Cytology and visual inspection methods

Kelly *et al*. [17**] reported the triage performance of visual inspection after acetic acid (VIA) and cytology in their cohort. VIA had the lowest sensitivity of 45.5%, and a cytology reading of low-grade intraepithelial lesion (LSIL) or less had the lowest specificity of 10.4%. The authors felt that the optimal balance of sensitivity and specificity was from triage with a cytology reading of high-grade intraepithelial lesion (HSIL) or worse (sensitivity of 74.8% [65.6-82.5%] and specificity of 69.8% [63.3-75.8%]) [17**]. In an earlier study from Botswana, VIA and cytology of atypical squamous cells of unknown significance (ASCUS) had comparable sensitivity (59% and 62%), but VIA had the worst specificity (49%) [16]. HSIL+ had the highest specificity (92%) but lowest sensitivity (31%), while colposcopy had the highest sensitivity (83%), but the specificity was similar to VIA [16]. Although colposcopy and cytology performed reasonably well, their use is limited to tertiary centers in South Africa.

Screening intervals in an HPV-based screening program in WLWH

WHO recommends a screening interval of three to five years for HR-HPV negative WLWH and one year for HR-HPV positive WLWH with or without treatment. Kelly's study [17**] looked at the incidence of HR-HPV/CIN2+ in both baseline HPV-negative and HPV-positive WLWH at 16 months. Of those HR-HPV positive without CIN2+ at baseline, incident CIN2+ was 5.8%, while only 0.5% baseline HR-HPV negative women developed incident CIN2+ at 16 months [17**]. Luckett *et al.* [30**] reported higher rates of incident CIN2+ at 12 to 16 months among women who were baseline HR-HPV negative [30**]. Among 237 WLWH who returned for follow-up, the prevalence of CIN2+ was 9%, similar to the baseline prevalence of 8%. Incident CIN2+ among WLWH who were HR-HPV negative at baseline was 5.3%, ten times that in Kelly's study [17**,30**]. Although the numbers were small, there was a 40% (8/20) progression from no cervical disease to CIN2+ among WLWH who were HR-HPV positive at baseline [30**]. These studies show relatively high incident CIN2+ within a

relatively short screening interval. It is promising that neither study observed progression to invasive cancer.

In summary, HPV-based screening (either laboratory or POC) can be performed on self-collected dry specimens with high acceptance and accuracy. The challenge remains the high HR-HPV prevalence which necessitates triage. Partial genotyping can be used for triage and in screen-and-treat strategies due to its higher specificity to detect CIN2+. Triage with VIA or cytology improves specificity (cytology>VIA) but at the expense of sensitivity (which is ultimately the benefit of HPV testing). HR-HPV positive WLWH may require shorter interval follow-up irrespective of their initial triage results or treatment status, given likely rapid progression to CIN2+.

CONCLUSION

Even though the possibility of eliminating cervical cancer is an exciting prospect, it is still out of reach for southern Africa. Understanding the local epidemiology of HPV and how HIV influences it, is critical to developing appropriate prevention strategies. Longitudinal data from the entire region is urgently needed to implement HPV-based prevention. Therefore, support for research and dissemination of findings from the region is essential. Despite all these challenges, southern Africa needs better solutions, and accelerating HPV vaccination and introducing primary HPV screening followed by effective therapy will move the region closer to elimination.

KEY POINTS

- HR-HPV prevalence among southern African women is high, especially in WLWH and women < 30 years.
- Cervical disease in the region is caused by known oncogenic types, suggesting that HPV-based prevention can be effective.
- National screening programmes in the region have low coverage, HPV testing has not been introduced, and evidence to guide transition to HPV-based strategies is limited.
- Primary HPV screening is complicated by high prevalence of HR-HPV and a paucity of satisfactory scalable triage strategies; type restriction and more stringent cycle threshold are promising.

- Among WLWH, short screening intervals are needed because the incidence of CIN2+ among triage negative HR-HPV positive women and incident HR-HPV infection risk is high.

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Conflicts of interest

None.

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** of outstanding interest.

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Appendix 2. IAS poster

THE ROLE OF EXPANDED HUMAN PAPILLOMAVIRUS GENOTYPING IN DETECTING HIGH-GRADE CERVICAL DYSPLASIA BY HIV STATUS IN BOTSWANA

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BACKGROUND

- The World Health Organization's elimination strategy calls for high performance cervical cancer screening with human papillomavirus (HPV) testing
- HPV primary screening and triage strategies in low- and middle-income countries (LMICs) with high HIV prevalence are not clear
- Sub-Saharan Africa carries 85% of the burden of HIV-associated cervical cancer morbidity and mortality, even in Botswana, which led the region in implementing a test-and-treat HIV policy
- Triage by HPV genotypes most associated with cervical cancer (HPV 16/18/31/33/35/45/52/58) may provide more sensitive detection of high-grade cervical dysplasia in both women with and without HIV in LMICs
- This study evaluated HPV genotypes associated with high-grade cervical dysplasia in a population of both women living with and without HIV in Botswana

METHODS

- Recruitment is on-going for a cross-sectional study of 3000 women (with and without HIV) in South East District, Botswana
- This study provides HPV primary screening at 6 government health facilities using Atila AmpFire HPV (Atila Biosystems, USA)
  - Tests for 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68
- Participants testing positive for any HPV genotype undergo visual evaluation
  - Visual inspection with acetic acid, colposcopy and image capture for automated visual evaluation by National Cancer Institute
- Biopsies are collected from all participants for histopathologic evaluation; the decision of where and how to biopsy (punch biopsy, endocervical curettage or loop electrosurgical excision procedure) is based on colposcopy impression
- High-grade cervical dysplasia is defined as histopathology of cervical intraepithelial neoplasia grade 2 or worse (CIN2+)

RESULTS

- In a preliminary analysis of 1905 participants
  - 781 (41%) were women living with HIV (WLHIV)
    - 99.9% on antiretroviral therapy
    - 98.7% virologically suppressed
    - Median CD4 count was 686 cells/μL
  - 897 (47%) participants were HPV positive
    - 431 (48%) of the HPV positive were living with HIV
  - 366 (41%) had complete histopathology data at time of analysis
    - 161 (41%) with complete histopathology were WLHIV
- Overall prevalence of CIN2+ was 18% in HPV-positive participants: 20.5% in WLHIV, 15.3% in women without HIV
- HPV 18 had the highest positive predictive value for CIN2+ both in WLHIV (47%) and women without HIV (38%)
- Sensitivity of triage of all HPV-positive participants with pooled HPV genotypes 16/18/31/33/35/45/52/58 to detect CIN2+ was 85% in WLHIV and 84% in women without HIV (p=1.00)
- Alternatively: HPV positive triage by HPV16/18 only had sensitivity of 39% in WLHIV and 29% in women without HIV, and co-infection with any 2 genotypes had a sensitivity of only 64% in both WLHIV and women without HIV

CONCLUSION

- Nearly half of women in Botswana screened HPV positive
- Prevalence of HPV-associated CIN2+ was similar in women with and without HIV
- Triage of HPV positive results with pooled HPV genotypes 16/18/31/33/35/45/52/58 maintained high sensitivity in detecting CIN2+ in all participants
- In the modern ART era, streamlining of high-performance cervical cancer screening with HPV primary testing and genotype triage in both women with and without HIV is a promising strategy
- We have now recruited 2900 women in this cohort and final recruitment and data analysis will continue to provide essential data to guide regional HPV screening policy

Table 1. Positive predictive value (PPV) of individual and pooled HPV genotypes in predicting high-grade dysplasia (CIN2+) by HIV status

HPV type	Total cohort		Women living with HIV		Women without HIV		
	HPV Infection (n)†	CIN2+ n (%)	HPV Infection (n)	CIN2+ n (%)*	Infection (n)	CIN2+ n (%)$	p
Any high-risk HPV	363	64 (18)	161	33 (21)	202	31 (15)	0.20
>1 high-risk HPV	157	41 (26)	86	22 (26)	71	19 (27)	0.87
18	25	11 (44)	17	8 (47)	8	3 (38)	1.00
59	26	10 (39)	13	6 (46)	13	4 (31)	0.42
56	34	10 (29)	22	6 (27)	12	4 (33)	0.71
58	41	11 (27)	26	7 (27)	15	4 (27)	1.00
66	23	6 (26)	14	5 (36)	9	1 (11)	0.34
52	51	13 (26)	20	3 (15)	31	10 (32)	0.17
35	57	13 (23)	30	6 (20)	27	7 (26)	0.59
53	54	12 (22)	28	7 (25)	26	5 (19)	0.61
16	60	13 (22)	26	6 (23)	34	7 (21)	0.82
33	59	12 (20)	25	6 (24)	34	6 (18)	0.55
51	41	8 (20)	26	6 (23)	15	2 (13)	0.69
45	38	6 (16)	15	4 (27)	23	2 (9)	0.19
68	63	8 (13)	33	6 (18)	30	2 (7)	0.26
31	29	4 (14)	12	2 (17)	17	2 (12)	1.00
39	31	2 (7)	12	0 (0)	19	2 (11)	0.51
Any of: 16/18/31/33/35/45/52/58	260	54 (21)	116	28 (24)	144	26 (18)	0.23

† 3 women with unknown HIV status are excluded from this table
* Calculated as proportion of WLHIV with specific HPV type who have CIN2+
$ Calculated as proportion of women without HIV with specific HPV type who have CIN2+

Table 2. HPV genotype prevalence and correlation to cervical intraepithelial neoplasia grade 2 or worse (CIN2+) by HIV status (n=177)*

HPV type	Total cohort		Women living with HIV		Women without HIV		
	HPV Infection (n)†	CIN2+ n (%)	HPV Infection (n)	CIN2+ n (%)*	Infection (n)	CIN2+ n (%)$	p
16	60	13 (22)	26	6 (23)	34	7 (21)	0.82
18	25	11 (44)	17	8 (47)	8	3 (38)	1.00
31	29	4 (14)	12	2 (17)	17	2 (12)	1.00
33	59	12 (20)	25	6 (24)	34	6 (18)	0.55
35	57	13 (23)	30	6 (20)	27	7 (26)	0.59
45	38	6 (16)	15	4 (27)	23	2 (9)	0.19
52	51	13 (26)	20	3 (15)	31	10 (32)	0.17
58	41	11 (27)	26	7 (27)	15	4 (27)	1.00

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Appendix 3. IPVC poster

VIRTUAL-024 / #930
Poster Viewing
VIRTUAL POSTERS
04-16-2023 1:00 AM - 11:00 PM
CERVICAL DYSPLASIA AND CANCER MISSED AMONG HIGH-RISK HUMAN PAPILLOMAVIRUS PRIMARY SCREEN-POSITIVE WOMEN WITH A SECONDARY VISUAL TRIAGE-NEGATIVE TEST IN BOTSWANA

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Introduction: To prevent cervical cancer, the World Health Organization (WHO) calls for highperformance
screening with high-risk human papillomavirus (hrHPV) testing followed by a second triage test (indicated for HIV patients, optional for general populations). Data about the performance of secondary triage tests is limited in Low-and-Middle-Income-Countries (LMICs).
Methods: We employed primary hrHPV screening among self-collected swabs of women matched by HIV status who presented to a peri-urban clinic in southeast Botswana using Atila AmpFire HPV (AAF)
(Atila Biosystems, USA) which detects individual hrHPV subtypes 16/18/31/33/35/39/45/51/52/53/56/58/59/66/68). All who screened positive for any hrHPV were visually
assessed and a biopsy taken for histological evaluation.
Results: Among 1,500 consenting women, 47.1% (706/1500: HIV+: 52.3% (392/750); HIV-: 41.9% (314/750) screened positive for hrHPV. We completed visual assessment for treatment (VAT) and histological evaluation on 92.8%. Of the 190/655 (29.0%) who screened positive by VAT, histological evaluation revealed 59 (31.1%) had CIN2+ [10 (5.3%) CIN 2; 49 (25.8%) CIN3+). The CIN3+ category included the diagnosis of two adenocarcinoma-in-situ, one adenocarcinoma, and one squamous cell carcinoma. However, among the 71.0% (465/655) of women who screened negative by VAT and routinely would have screened again in three or five years depending on HIV status, the histological evaluation revealed 54 (11.6%) had CIN2+. Thirty-two (6.9%) had CIN3+, including one adenocarcinomain-situ, and one adenocarcinoma). Nearly 70% (22/32) of CIN2 and 40% (32/81) of CIN3+ cases, including two-fifths (2/5) of glandular abnormalities, detected in the study would have been missed under routine screening conditions. A higher proportion of CIN2+ cases were missed among HIV-negative women, compared with HIV-positive ones (p value=0.02).

Table 1: Results by screening type, histological finding, and HIV-status (percentage)

	HIV-positive		HIV-negative		Overall		p-value*
	%	(a/n)	%	(a/n)	%	(a/n)	
% who tested positive for HPV	52.3%	(392/750)	41.9%	(314/750)	47.1%	(706/1500)	
% HPV+ histologically evaluated	92.6%	(363/392)	93.0%	(292/314)	92.8%	(655/706)	
Among histologically evaluated…							
% positive by VAT	33.3%	(121/363)	23.6%	(69/292)	29.0%	(190/655)	0.007
% negative by VAT	66.7%	(242/363)	76.4%	(223/292)	71.0%	(465/655)	
% VAT-positive with CIN2+	34.7%	(42/121)	24.6%	(17/69)	31.1%	(59/190)	
% VAT-negative with CIN2+	11.2%	(27/242)	12.1%	(27/223)	11.6%	(54/465)	0.75
% VAT-positive with CIN3+	28.9%	(35/121)	21.7%	(14/69)	25.8%	(49/190)	
% VAT-negative with CIN3+	6.6%	(16/242)	7.2%	(16/223)	6.9%	(32/465)	0.81

CIN: cervical intra-epithelial neoplasia; HIV: human immunodeficiency virus; VAT: visual assessment for treatment
*Chi-square test

Table 2: Proportion of cases missed, by HIV-status

	HIV-positive		HIV-negative		Overall		p-value*
CIN2+ cases missed by VAT result	39.1%	(27/69)	61.4%	(27/44)	47.8%	(54/113)	0.02
CIN3+ cases missed by VAT result	31.4%	(16/51)	53.3%	(16/30)	39.5%	(32/81)	0.07

CIN: cervical intra-epithelial neoplasia; HIV: human immunodeficiency virus; VAT: Visual assessment for treatment
*Chi-square test

Conclusions: Although hrHPV DNA testing offers a highly sensitive primary screening solution, currently recommended secondary visual triage testing may miss severe disease in those who screen negative. Better approaches to secondary triage testing for LMICs remain urgently needed.

Appendix 4. SOPs

SOP 1: Recruitment and Screening of Participants

Recruitment and Screening of Patients

Purpose: To recruit patients from Bamalete Lutheran Hospital (BLH) and surrounding health facilities and screen them for eligibility for participation in the HPV self-collection study.

Procedure:

Responsible party: The study research assistants (RA) are responsible for recruitment and screening of patients.

Timing: Participants will be recruited throughout the study until study closure.

Process:

- Participants will be recruited from BLH and surrounding health facilities Monday-Friday starting at 8am.
- On a usual recruitment day, one research assistant will be assigned to BLH and another assigned to surrounding facilities. Each RA will be responsible for recruitment at their respective location. If no surrounding facilities are available for recruitment, both RAs will be assigned to BLH.

At the beginning of each recruitment day, make sure:

- *1 room is available, with supplies:*
    - Hand sanitizer, surface disinfectant, disposable masks
    - One room with two chairs for consenting and survey (RA)
    - Pens, ink pad, markers for specimen labeling
    - HPV kits, gloves and cooler box
    - Restroom or screen in the same room available for self-collections
- *Documents printed and available:*
    - Recruitment leaflet (several copies), RA
    - HPV & cervical cancer info handout (15+ copies), RA
    - Eligibility flowchart, RA
    - Intake form, RA
    - ID link form, RA
    - English consent forms (30+ copies, 2 copies per patient), RA
    - Setswana consent forms (30+ copies, 2 copies per patient), RA
    - Questionnaires (1 copy per patient), RA
    - Self-swab instructions (laminated), RA
- *We are aware of which Study ID's will be given to newly recruited participants:*
    - Study IDs will be created using the following code: **SN-SC-NNNN**, where SN is the BHP study number [135], SC is the site code, and NNNN is a unique 4-digit number that is generated sequentially, starting with 0001.

Site	Site code
Bamalete Lutheran Hospital	01
Otse clinic	02
Mogobane Clinic	03
Lesetlhana Clinic	04
Siga Clinic	05
Taung Clinic	06

- For example, the study ID given to the 2nd patient recruited in the study, who was recruited at BLH would be 135-01-0002.
- The RA should come with prepopulated ID link sheets with NNNN prepopulated to assign study IDs to the patients recruited that day at each site.

In the hospital and health facility reception area:

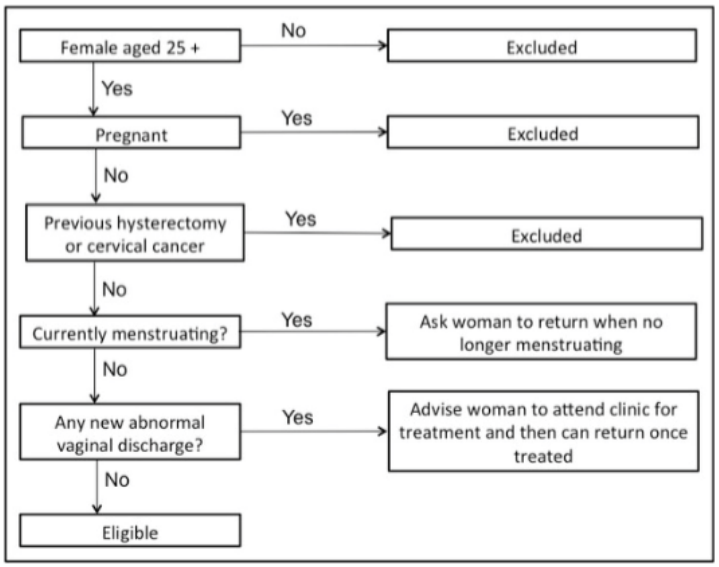
- Patients will arrive at the reception every morning. They will go to have their vitals taken before returning to reception and then to join the queue for their clinic room. We may have access to the vitals room for recruitment.
- Patients waiting in the reception area, or in the queue are given a general announcement, delivered by the RA. For example:

  "My name is _____. I am a research assistant in the clinic. We are conducting a study to find the best way to screen for cervical cancer in Botswana, and we are looking for people to participate while they are waiting to see the doctor. If you participate, we will first administer a short survey, and then you will receive a free HPV screening test. Once you are done, you can go back in line and resume your original place in the queue. Please let me know if you are interested."

- The announcement should provide assurance that women will not lose their place in the queue, and that they will resume their place following the survey and HPV collection. This is a common concern.
- **Recruitment leaflets** can also be distributed at this time.
- With eligibility criteria in mind, the RA's then approach women individually and, if interested, are taken into a consultation room.

In the consultation room:

- In the consultation room, women are given more information about the study and have the opportunity to ask questions.
- The RA will go through eligibility criteria (see "***Eligibility flowchart***") and document criteria on the **Intake form (see "*Intake form*").**
  - Inclusion criteria for the study are: 1) women ≥25 years of age and 2) competent to understand study procedures and give informed consent. Exclusion criteria are: 1) currently pregnant, 2) currently menstruating or having persistent vaginal discharge, 3) previous hysterectomy, 4) previous diagnosis of cervical cancer 5) males.
  -



- All women taken into the consultation room, regardless of whether they ultimately consent, will be recorded on the **Intake form**.
- If they do not consent, please **note the reason for refusal**.
- If a woman is currently ineligible because she is **menstruating**, or she has a **new/abnormal vaginal discharge** then she can **return on another day and still take part in the study**.
- For patients who wish to participate, they must **sign the informed consent** prior to any other study activity.

SOP 2: Consent and Questionnaire

Consent and Questionnaire

Purpose: To obtain consent and administer the questionnaire for participants recruited to the HPV self-collection study.

Procedure:

Responsible party: The research assistants are responsible for obtaining consent and administering the questionnaire.

Timing: Patients will be consented during recruitment, after verification of eligibility. Questionnaire administration occurs immediately after consent has been obtained.

Process:

Obtain consent:

- For patients who wish to participate, they must **sign the informed consent** prior to any other study activity.
- The study interviewer says: “We would like to read through this consent form with you because we want to make sure that you understand everything in it and that we answer any questions you may have.”
- The study interviewer reviews the consent form, paragraph by paragraph, and stops as needed for any questions or clarifications.
- The interviewer asks the potential participant short questions about the consent to make sure that she fully understands the study. This process gives the interviewer time to assess the potential participant to make sure there are no “gross Impairments” that would invalidate the informed consent (e.g. participant does not fully understand the study, is intoxicated, etc.) and make study participation unethical and prohibited.
- After reading the consent, study staff needs to provide adequate time for the patient to think about it, ask questions, discuss with family members or others if they choose to before they sign the consent form.
- The study staff should explain to the patient that their HPV specimen collected will be labeled without any identifying information. Any biopsies or LEEP specimens will be processed per standard national protocol at the National Health Laboratory.
- If they are eligible and consenting, they will need to be **given a study ID** (see ‘**Recruitment and Screening’ SOP** for details on how to generate a study ID).
- The study interviewer then prints and signs their name on the consent form on the line for ‘Signature of Study Staff’ and writes the date.
- The participant’s study ID is then written on the consent form
- Please ensure that the **participant’s study ID** and **date** are written on each page (header)
- Participants should read and sign the consent form in their language of preference (English or Setswana). If the patient is illiterate, a witness must be present for the consenting process and the participant must verbalize understanding of the consent; the participant can place their thumb print on the signature line, while the witness signs on the witness line. No witness needs to be present or sign a consent form for a literate participant.

- The participant needs to **sign each page of the consent (header)**, as well as the section at the end of the consent and the portions about sample storage and future participation, including phone numbers if interested.
    - If a participant is illiterate, the witness should sign each page of the consent (header) and the participant should put their thumb print. The witness should also sign the sample storage and future participation, and the participant place their thumb print.
- Study staff consenting the participant also needs to sign the consent at this point.
- Give the participant their **copy of the consent form (signed by study staff)** but can be unsigned by patient).
- You will then need to fill out the **Study ID link form** (see "*Study ID link form*") which links the study ID to the participant's details and contact information.

Administer the questionnaire

- The questionnaire consists of questions about the participant's medical history, lifestyle, risk factors, knowledge, and preferences regarding cervical cancer screening and prevention.
- All questions, in both English and Setswana, are printed out in the Questionnaire form.
- The RA should reference the paper Questionnaire form while verbally directing questions to the patient, in English or Setswana, depending on the participant's preferred language.
- Prior to administering the questionnaire, and again prior to the Sexual History section of the questionnaire, remind the participant that she is free to skip any questions that she does not wish to answer.
- Responses are documented on paper by the RA for entry into REDCap at a later time.
- Following administration of the questionnaire, thank the participant and orientate on self-sample collection.

SOP 3: HPV Sample Collection

HPV Sample Collection

Purpose: This is the SOP to describe the procedures for collecting HPV DNA samples for patients recruited to the Ramotswa HPV AVE study.

Procedure:

Responsible party: Research assistant is responsible for conducting HPV DNA self-collection orientation.

Timing: HPV sample collection occurs on the day of recruitment, after completion of the consent form and questionnaire with the RA.

Process:

- Preparation
    - Ensure all necessary supplies neatly arranged and on hand:
        - HPV DNA test kits: collection container, vaginal swab
    - Label the HPV sample collection container with the study ID, date, time, and your initials.
    - Complete HPV requisition form
- Provide instructions for the self-collection
    - Show participant the packaging and demonstrate which end of the swab can be held
    - Participant Instructions:
        - Advise participant to go to bathroom or behind screen in same room
        - Prior to collection, clean hands, make sure you are in a comfortable position and then lower your underwear
        - Remove the swab from the packaging
        - Do not allow the swab to touch any other surfaces. If the swab is contaminated by coming into contact with a wet surface, return to the consent room to request a new swab.
        - Place the swab inside the vagina and swab gently for 15 seconds. RA should use photos to demonstrate (see images at end of document).
        - Remove the swab from the vagina and place into the provided collection container. Break the swab stick at the perforation and leave the swab in the specimen container. The remaining end can be thrown away. Screw the cap on and return to RA.
- RA receives the self-collected HPV DNA specimen
- Store specimens
    - Double check that the collection tube is capped tightly and bears the Study ID, date, time on the label. Place the tube in the specimen rack in the cooler box.
- At the end of each recruitment day, store the requisition forms and take the HPV samples in the cooler box to local refrigerator (see HPV sample Chain of Custody SOP).
- All specimens received from participants should be recorded in the study specimen logbook.

SOP 4: Instructions on the Vaginal Self Swab

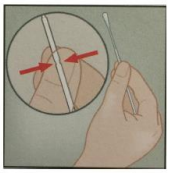
1

A = Transport reagent pot
B = Swab

2

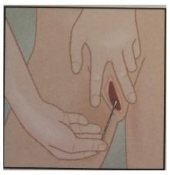
Open the swab wrapper (B). Remove the swab being careful not to touch the tip or lay it down.

3



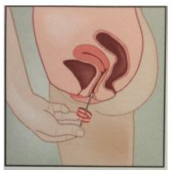
Hold the swab in your hand placing your thumb and forefinger in the middle of the swab shaft along the score line.

4



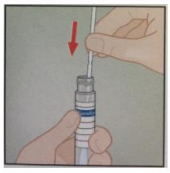
Carefully insert the swab into your vagina about two inches inside the opening of the vagina.

5



Gently rotate the swab for 10-30 seconds. Make sure the swab touches the walls of the vagina. Withdraw the swab and continue to hold in your hand.

6

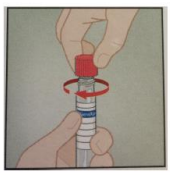


Unscrew the cap from the transport pot (A). Immediately place the collection swab into the transport pot.

7

Find the scoreline and break the swab shaft at this point against the side of the tube. Discard the top part of the swab shaft.

8



Re-cap the transport pot and tighten securely.
Return the pot to study staff. They will then label the pot and send for analysis.

SOP 5: HPV Swab Specimen Chain of Custody

PURPOSE

To describe the procedures for documenting collection, transport, receipt, processing and result reporting of **HPV swab** specimens collected from subjects of the HPV study.

SCOPE

This procedure applies to all study clinic and laboratory staff involved with handling human biological materials collected for the HPV study.

RESPONSIBILITIES

All staff involved with handling human biological materials for the HPV study are responsible for understanding and following this SOP.

PROCEDURE

Specimen Collection

Specimens will be collected at time points described in the HPV protocol.

The requisition forms will be completed for each participant at the clinic to ensure that all specimen requirements (number; type and volume of specimens, processing instructions) for that visit have been met.

HPV swabs will be collected per procedure described in the "***HPV Sample Collection" SOP***

Specimen transport and storage

When specimens have been collected from the participants they should be stored under the correct temperatures as stated in table 1 while awaiting transportation to the laboratory.

All swabs should be clearly labeled with the Study ID, collection date and time.

At the end of each recruitment day, research assistants will transport the samples to a study fridge at BLH. Approximately twice per week, study staff or a courier service will transport samples to BHHRL.

When being transported ensure that samples are neatly and securely arranged on a specimen rack and then placed in a break resistant cooler box labeled biohazard.

Ice packs should be placed in the cooler boxes to ensure low transportation temperatures.

Cooler boxes should be secured to avoid opening them unnecessarily and exposing people to potentially harmful agents.

At all times safety precautions should be followed as prescribed and specimens should be handled in the same manner that does not compromise specimen integrity.

All specimens should be transported with a requisition [electronic or paper based, or details contained in the barcode].

A list of all the specimens to be transported will be written in the **specimen transport book**. This list should contain the study IDs of the samples and their date and time of collection. Study staff will also maintain their own records of all HPV samples sent over to the BHP lab (in the **electronic study journal**), which will include the study ID of the sample, and the date and time of collection.

The order of the specimens in the specimen transport book should be the same as the order of the requisition forms and the arrangement of the specimens on the holding rack.

At the Lab, the study staff will enter the specimens into LIS. The specimen reception staff member should log in the time the transporter delivered the specimens using the specimen delivery log. Both the staff member and transporter should sign the form acknowledging the time specimens were delivered.

Specimen reception staff should cross-check the specimens sent versus the ones received using the delivery checklist. Confirm if all specimens were transported appropriately, by checking the packaging and time from collection to delivery.

Table 1: Specifications for specimens being sent to Referral Laboratory

Test	**Specimen type and container**	**Maximum delivery time/temperature**
HPV	Vaginal swab in plastic collection tube	7 days / 2-8 ^{0}C

Specimen Reception and Processing

Specimens will be received and processed as per *Specimen Reception and Processing* SOP **BHHRL/002PR05.**

Laboratory and specimen issues, including missing, unlabeled, mislabeled, broken or leaking specimens will be communicated as per *Specimen Reception and Processing* SOP **BHHRL/002PR05.**

After the specimens have been received, processed, and separated in the Specimen Receiving area, they are processed and stored appropriately until tested or transported to the appropriate laboratory.

Results Reporting

For tests done at BHHRL Lab, the results will be available on LIS within established *Test Results Turnaround Times* **Document ID: FR74.** Study staff can access results on LIS.

Critical values / urgent lab results will be reported as per Release of Results SOP **Document ID: PR19**

Health and Safety

All specimens must be treated as infectious, hence all staff members handling specimens during reception, processing and transport must observe universal safety precautions and operate in accordance with BHHRL safety procedures at all times.

The person carrying out this task should ensure that he/she is wearing personal protective clothing (including gloves); one hand should be gloved and the other ungloved to open doors. The laboratory coat (must be buttoned up completely and not permitted to flap open). The specimens should be arranged in a specimen rack and transported in a break-resistant container, labeled "biohazard".

Spills should be wiped up immediately following the procedure on Decontamination of Biohazardous Spills. In case of contamination on the outside of the container, 0.5% hypochlorite solution should be used to clean the container followed with 70% Alcohol.

References

BHHRL Safety Manual

Specimen Reception and Processing SOP **BHHRL/002PR05.**

SOP 6: Histopathology Specimen Chain of Custody

PURPOSE

To describe the procedures for documenting collection, transport, receipt, processing and result reporting of ***histopathology*** specimens collected from subjects of the HPV Study.

SCOPE

This procedure applies to all study clinic and laboratory staff involved with handling human biological materials collected for the HPV Study.

RESPONSIBILITIES

All staff involved with handling human biological materials for the HPV Study are responsible for understanding and following this SOP.

PROCEDURE

Specimen Collection

Specimens will be collected at time points described in the HPV Study protocol.

The NHL requisition forms will be completed for each participant at the clinic to ensure that all specimen requirements (number; type and volume of specimens, processing instructions) for that visit have been met.

Histopathology specimens will be collected per procedure described in the **VIA and Colposcopy SOP**

Specimen transport and storage

When histopathology specimens have been collected from the participants they should be stored in formalin at room temperature awaiting transportation to the national health laboratory.

All collection bottles should be clearly labeled with the patient's name, hospital encounter (ie PA) number, specimen type and date of collection.

At the end of each colposcopy day, research assistants or study physicians will transport the samples to the NHL.

When being transported ensure that samples are neatly and securely arranged on a specimen rack and then placed in a break resistant cooler box labeled biohazard.

Cooler boxes should be secured to avoid opening unnecessarily and exposing people to potentially harmful agents.

At all times safety precautions should be followed as prescribed and specimens should be handled in the same manner that does not compromise specimen integrity.

All specimens should be transported with a requisition [electronic or paper based or details contained in the barcode].

A list and details of all the specimens to be transported will be written in the **specimen logbook.** This list should contain the names of the samples and their date and time of collection. Study staff will also maintain their own records of all histopathology samples sent over to the NHL (in the electronic **HPV study journal**), which will include the name of the participant, study ID of the sample, and the date of collection.

The order of the specimens in the histopathology specimen transport book should be the same as the order of the requisition forms and the arrangement of the specimens on the holding rack.

At the NHL, specimen reception staff member should log in the specimens that were brought.

Specimen reception staff should cross check the specimens sent versus the ones received.

Health and Safety

All specimens must be treated as though infectious, hence all staff members handling specimens during reception, processing and transport must observe universal safety precautions and operate in accordance with NHL safety procedures at all times.

The person carrying out this task should ensure that he/she is wearing personal protective clothing (including gloves); one hand should be gloved and the other ungloved to open doors. The laboratory coat (must be buttoned up completely and not permitted to flap open). The specimens should be arranged in a specimen rack and transported in a break-resistant container, labeled "biohazard".

Spills should be wiped up immediately following the procedure on Decontamination of Biohazardous Spills. In case of contamination on the outside of the

container, 0.5% hypochlorite solution should be used to clean the container followed with 70% Alcohol.

SOP 7: Communicating and Documenting Results and Follow-ups

Communicating and Documenting Results and Follow-ups

Purpose: This is the SOP for communicating new test results (HPV, biopsy, LEEP) to participants, documenting the results in study records, and scheduling follow-up visits for patients based on results.

Procedure:

Responsible party: The research assistants are responsible for communicating and documenting participant results and scheduling follow-up visits.

Timing: New results and follow-up visits will be documented and communicated with participants as soon as results become available from BHP Laboratory and National Health Laboratory. This will occur as long as the study is recruiting participants and awaiting new results. The expected turnaround time for HPV results from the BHP Lab is approximately **30** days or less. The expected turnaround time for pathology specimens from the NHL is approximately 4-6 weeks.

Process:

Important documents used for patient tracking and documentation:

- **'HPV study journal':** An electronic spreadsheet that summarizes the status of each participant recruited in the study. This includes data on those who have missed colposcopy appointments and needs rescheduling.
- '**Colposcopy clinic schedule' –** The list of scheduled colpo appointments for a given date.
- **'Missed Colpo follow-up list'** – The list of participants who have missed colpo visits and have not yet been rescheduled

Folder descriptions (This folder will be locked in a file cabinet at BLH)

Pending REDCap Entry	Paper forms of participants who have been recruited for the study and completed the questionnaire, but questionnaire data has not been entered into REDCap yet
Results Pending	Participants awaiting HPV or histopathology results
Communication Pending	New results have been received that must be communicated to the participant, and/or the participant still needs to be scheduled for a colposcopy based on her results
Scheduled for Colpo	Results have been communicated to the participant and the participant has a scheduled appointment for a colposcopy.
Missed Colpo	Participant missed the colposcopy appointment. The participant needs to be contacted in order to reschedule.

REDCap form status descriptions:

Form	Gray	Red (Incomplete)	Green (Complete)
Identifier; Questionnaire; Risk factors	No data entered.	Data entry is incomplete.	Data entry is complete.
Past results	No data entered.	Data entry is incomplete.	Data from IPMS search for past results is complete.
HPV results	Results not received. No data entered.	Results received, but patient has not been contacted about the results **or** a necessary colpo appointment has not been scheduled.	Results received and the patient has been notified of the results, **and** she has been scheduled for colpo appointment, if she needs one.
VIA/Colposcopy	No data entered yet.	Patient missed the VIA/Colpo appointment. Patient currently is in the process of being rescheduled.	Patient arrived at the VIA/Colpo appointment. VIA and Colpo findings have been documented in REDCap.

Pending REDCap Entry

- After screening and recruitment, each participant will have a completed questionnaire. The questionnaires are stored in the folder **'Pending REDCap entry'** until data is entered into REDCap.
- **REDCap data entry**:
  - A new record is created in REDCap for each participant and data is entered for the following forms from the **Questionnaire** and the **ID Link Form**:
    - **Identifiers/Socio demographics**
    - **Patient questionnaire**
    - **Risk factors, Screening History, Knowledge**
  - A member of the study staff should also look up the participants' record in IPMS to see if there have been previous Pap smear or biopsy results. This should be documented in the following form:
    - **Past results**
  - Once all of the above sections are input into REDCap, file the questionnaire in the participant file
- Note: HPV and histopathology results can both be placed in this folder, until results are entered into REDCap

Results pending

- Put participants' **HPV lab requisition** form in the **"Results Pending"** folder after specimen has been dropped at the BHP lab.
- Put the participants' **'MOHW Colposcopy/LEEP form'** in the "**Results Pending**" folder after it has been copied in Gaborone and the original given to the clinic.
- HPV results availability should be checked once per week in LIS (usually Friday). Results are usually available within 30 days.

- Histopathology results availability should be checked once per week in IPMS. Results are usually available in 4-6 weeks.
- **Receipt of HPV or Histopathology results**
  - **Document results**
    - Copy the original HPV results and/or Histopathology result form (IPMS printout). The original is to be filed in the participant's file and the copy to be given to participant.
    - Retrieve the participant's **Questionnaire** from the participant file and fill out the "**HPV Results**" (p.17) or the "**Histopathology Results**" (p.21) section.
    - Enter results in **REDCap:**
      - Fill out the relevant **HPV results** or **Histopathology results** form.
      - At this point, the status of the **HPV Results** or **Histopathology Results** form should be set to **'Incomplete' (Red)**
    - Update the **'HPV study journal'** electronic spreadsheet.

Communication Pending

- If participant cannot be contacted immediately with HPV or histopathology results, place the HPV results form or the Histopathology results form (IPMS printout) in the **'Communication Pending'** folder.
- **Communicating results and colpo scheduling**:
  - Prior to calling a participant with HPV results:
    - **Have the ORIGINAL HPV result form from the laboratory or the Histopathology results (IPMS printout) in hand in order to confirm the result *(do not call participants based on data entered in REDCap)***
  - Call the participant, **verify their identity**, and advise them of their result.
    - **If giving HPV results**
      - **If giving positive HPV results:** have a list of available colposcopy dates and times
        - Schedule them for a colposcopy appointment.
        - Record this appointment in the '**VIA/Colposcopy schedule'**
        - Document this appointment on the **'HPV study journal'** electronic spreadsheet
      - **If they are negative for high-risk HPV**, tell the participant that they should repeat cervical cancer screening in 2 years if HIV positive or 5 years if HIV negative.
    - **If giving Histopathology results:**
      - Confirm the recommendation for follow-up with a study physician
      - If recommending referral, have details to facilitate linkage to care
      - Arrange a time for them to come in to pick up paper copies of results
  - **Document the call:**
    - **Questionnaire ('HPV Results'** [p.17] or **'Histopathology Results'** [p.21]**)**.
    - **REDCap** (**'HPV Results'** form of **'Histopathology Results'** form)
      - The status of the form should be set to **'Incomplete' (Red)** as long as we are still in the process of calling the participant about her results or scheduling a colposcopy/LEEP (if necessary).
      - The status of the form should be set to **'Complete' (Green)** when the participant has been notified of her results ***and*** scheduled for any necessary follow-up visit
    - Note: If participant cannot be reached, we must still document the call attempt. At least five attempts should be made with each call occurring one week apart and at different times of the day. Do not make more than one attempt a day, unless the participant has specified that we call them back later that day. Once five attempts are documented, the participant's file can be closed.

Scheduled for Colpo

- If the participant was successfully notified and does not require colposcopy (HPV negative), file all participant's study documents in the corresponding participant file and consider their participation complete.
- If the participant was scheduled for colposcopy, write the participant information on the **'VIA/Colposcopy schedule'** in the '**Scheduled for Colpo'** folder.
- Prepare the **MOHW colposcopy/LEEP form** and study **Visual assessments visit data collection form** for each participant who is scheduled for colposcopy.
- If an attempt was made, but participant still needs to be called back (for example, if she could not be reached, or if she was unable to schedule a colposcopy appointment at the time), keep the copy of the results in the '**Communication Pending'** folder.

Colpo appointments

- The day before Colpo clinic, participants should be called and reminded about their Colpo appointments. Ensure the **MOHW colposcopy/LEEP forms** and study **Visual assessments visit data collection forms** are prepared
- After the appointment, do paper, electronic and REDCap data entry:
  - Complete the participant's **Questionnaire** with the data entered onto **'VIA/Colposcopy'** form (p.19)
  - **REDCap** – update the **'VIA/Colposcopy'** form, and change form status to 'Complete' (green)
  - Update the **'HPV Study Journal'** electronic spreadsheet
- If biopsy/LEEP was performed:
    - Ensure specimen data is entered fully into the **"Specimen logbook"**
    - Put the **MOHW colposcopy/LEEP form** in the **'Results Pending'** folder after it has been copied and the original given back to the clinic.
    - If no biopsy/LEEP was required, the participant file can be closed and sent to Gaborone.
- File the **Visual assessments visit data collection forms** in the participants file after data entry

Missed Colpo

- Participants who miss their scheduled Colpo appointments should be called to reschedule as soon as possible (ideally, the afternoon of their original appointment).
- While awaiting rescheduling:
  - Their information should be entered onto the **'Missed Colposcopy follow-up list'**
  - Their prepared **MOHW colposcopy/LEEP form** and study **Visual assessments visit data collection forms** should be moved to **'Missed Colpo'** folder.
  - The **'HPV study journal'** should be updated with the missed appointment
- If the appointment is successfully rescheduled, document this in:
    - '**Missed Colpo follow-up list'** – write in the reschedule date
    - '**Colposcopy clinic schedule'** – for rescheduled date
    - **'HPV study journal'** electronic spreadsheet
    - Move the **MOHW colposcopy/LEEP form** and study **Visual assessments visit data collection forms** to the '**Scheduled for Colpo'** folder.

- If an attempt was made but the patient could not be reached, document this attempt in:
    - **Questionnaire ('VIA/Colposcopy',** p.19**)**
    - **REDCap** – keep the '**VIA/Colposcopy'** form status as 'Incomplete' (red)
    - Keep the **MOHW colposcopy/LEEP form** and study **Visual assessments visit data collection forms** in the **'Missed Colpo'** folder.

At the beginning of every day:

Check the following follow-up items

Follow-up item	**Where to check**
Questionnaires to be entered into REDCap	'Pending REDCap entry' folder
Pending HPV results	LIS 'Awaiting Results' folder REDCap 'HPV Results': gray dot
Participants who still need to be notified of HPV results	'Awaiting communication' folder REDCap 'HPV Results': red dot
Participants who still need to be scheduled for colposcopy	'Awaiting communication' folder REDCap 'HPV Results': red dot
Missed colpo appointments that need to be rescheduled	'Missed Colpo' folder 'Missed Colpo follow-up list'
Pending histopathology results	IPMS 'Specimen logbook' REDCap 'Histopathology Results': gray dot
Participants who still need to be notified of Histopath results	'Awaiting communication' folder REDCap 'Histopathology results': red dot

In preparation for colpo clinic:

Follow-up item	**Where to check**
Participants scheduled for colpo this week	'**VIA/Colposcopy schedule'** * Call participants 1-2 days prior to their scheduled visit to be reminded. * If they do not show up, they must be added to the '**Missed Colpo follow-up list**', and their **MOHW colposcopy/LEEP form** and study **Visual assessments visit data collection forms** should be filed into the '**Missed Colpo'** folder.

SOP 8: VIA and Colposcopy

VIA and Colposcopy

Purpose: This is the SOP for VIA and Colposcopy follow-up visits.

Procedure:

Responsible party: The research assistants are responsible for coordinating follow-up visits and collecting data from the VIA nurse and the colposcopy provider. VIA will be conducted by a trained VIA nurse. Colposcopy will be conducted by a study physician.

Timing: Once recruited women receive HPV results indicating a second visit for VIA and colposcopy is necessary.

Process:

Prior to the day of the visit:

- All participants who test HPV-positive are asked to return for colposcopy
- The RA should verify the HPV laboratory results prior to calling participants for colposcopy
- All participants should be called the day before their appointment to be reminded to come to the appointment. VIA and Colposcopy follow-up visits are generally scheduled on Thursdays, and reminder phone calls should be made on the Wednesday prior.

On the day of the visit:

- Both the VIA nurse and study physician must be blinded to the participants' prior cervical cancer screening history, and therefore **must not** be given a copy of the participants' questionnaire responses nor HPV results.
- When participants present to register for their colposcopy visit, they will be given a copy of the printed HPV results and have the opportunity to have any questions answered.
- The RAs should organize the participants to queue in the order in which they arrived.

The examination:

- The VIA nurse or study physician will call the participants in one-by-one
- The participant will be counseled on the procedures that will be performed
- The study VIA nurse will set the participant up in lithotomy position for examination
- The study VIA nurse will place the speculum and apply acetic acid to the cervix

VIA

- The VIA nurse will then conduct VIA using the Botswana standard protocol.
- The VIA nurse will then record visual results on the '**VIA data collection form'.**
    - If VIA is positive, based on assessment of the lesion(s), the nurse will record a recommendation for either cryotherapy or LEEP.
    - The nurse should not inform the participant or the study physician of their findings

Colposcopy

- The study physician will then perform colposcopy according to standard practice
- A biopsy or LEEP specimen will be collected if indicated
- Findings should be documented on the **'Colposcopy data collection form.'** Complications associated with colposcopy should also be documented on the '**Colposcopy data collection form**'.
- Colposcopy impression should be recorded on the standard **'MOHW Colposcopy/LEEP Form'**
- Specimen container should be labeled appropriately with participant name, specimen type, doctor, location, date, specimen requisition number
- A standard pathology requisition form will be completed

After all procedures, the participant is thanked and sent home. Study staff should advise her that she will be contacted again in 4-6 weeks with the results of her pathology specimen, and appropriate follow-up.

After each study participant completes their examination

- Study staff are responsible for collecting the '**VIA data collection form',** '**Colposcopy data collection form', 'MOHW Colposcopy/LEEP Form' and NHL histopathology requisition form.**

At the end of the day

- Images captured using the MobileODT device will be uploaded to the EVA Research system and identified by the patient study ID only
- The biopsies for participants will be ordered on IPMS to comply with National Health Laboratory ordering procedure
- The study team will keep a record of biopsies ordered in the **Specimen logbook** and delivered to the NHL according to the "**Chain of Custody Histopathology**" SOP
- Participant **'MOHW Colposcopy/LEEP Forms'** for those who had biopsies collected should be placed in the **'Results Pending'** folder.
- All participants who miss their scheduled VIA/Colposcopy appointment, should be added to the 'Missed Colpo Follow-up List'. Study staff will then follow-up with her to reschedule the appointment per the procedures described in the **'Communicating and Documenting Results' SOP.**
- Update **'HPV study journal'** electronic spreadsheet
- A copy of the **''MOHW Colposcopy/LEEP Form'** should be made and maintained in BLH Colposcopy/LEEP records
- All reportable incidents must be documented per the **'Incident Reporting'** SOP.

Communication of results

- Results of biopsy/LEEP specimens are provided in the same manner of HPV results described in **'Communicating and Documenting Results'** SOP**.**

SOP 9: Incident Reporting

Incident Reporting

Purpose: This is the SOP for documenting and reporting incidents.

Procedure:

Responsible party: The research assistant (RA) is responsible for completing incident reports. The RA is responsible for bringing any questionable events to the attention of the Principal Investigator (PI). The PI will then assess incidents and determine immediate course of actions. The Principal Investigator is responsible for reviewing all incidents and overseeing appropriate follow-up actions.

Timing: Timely reporting of incidents should occur throughout the length of the study.

Process:

- Unexpected events involving study staff or participants that may be caused by study procedures can be considered reportable incidents.
    - For example, if a participant returns to clinic complaining of pain or bleeding following a study-related biopsy, this is considered a reportable incident.
    - If a participant becomes agitated during the questionnaire process and assaults study staff, this is also considered a reportable incident.
- Incidents must be documented with the following details:
    - Date and time of incident
    - Study ID of participant involved
    - Description of incident
    - Action taken
- In cases of severe or life-threatening injuries, study staff should assist the affected individual(s) to receive emergency care at a government facility.
- All incidents which are considered serious adverse events related of the study must be documented and reported to the Principal Investigator *immediately*. Serious adverse events include events resulting in participant death, participant illness or condition requiring hospitalization, or participant condition that is life threatening or requires an emergency response.
- Other Incidents, such as protocol deviations and other adverse events, must be documented and reported to the Principal Investigator within 24 hours of occurrence.
- All incidents must be reviewed and discussed in a weekly study report.
- The Principal Investigator is responsible for reviewing the incident and ensuring that appropriate action was taken.
- **Any study protocol deviation or serious adverse event must be reported to the IRBs immediately.**

SOP 10: Storage of Documents

Storage of Documents

Purpose: To describe how critical documents used in the study are to be stored.

Procedure:

Responsible party: All members of the research team are responsible for ensuring proper storage of documents. Research assistants are primary handlers of study documents, and thus have primary responsibility in overseeing document storage.

Timing: Documents must be properly stored during the length of the study.

Process:

- The study binder must contain the following documents
    - Regulatory binder: IRB approvals amendments, and communications with both the BIDMC IRB and the local HRDC and University of Botswana
    - Study SOP's and MOP
    - IRB Protocol
    - Current IRB approved consent forms, both English and Setswana (stamped)
    - Information sheet and recruitment flyer
    - Participant questionnaire and contact log (ID link form)
    - Support letters
    - Personnel credentials: CV's and CITI training certificates
    - Delegation of responsibility log
    - Lab certifications
    - Lab SOPs
    - Specimen log forms
    - Training logs
    - Training materials

- Blank forms (consent forms, questionnaires) are stored in a separate folder.
- Questionnaires for enrolled participants are stored in designated folders depending on their current stage in the study. See the SOP of **'Communicating and Documenting Results'** for more detail.
- All active participant forms are to be stored in the locked file cabinet in a study office, which is also locked. For participants recruited from surrounding facilities, participant forms will be transported back to a study or site office on a daily basis.
- Participant forms in the **'Awaiting REDCap entry'** folder should be entered into REDCap every afternoon.
- Once results are received, copied and documented in REDCap, they remain in the locked file cabinet at BLH for storage and handing out to participants.
- Any completed participant files are transported back to the BHP office in Gaborone for storage in the locked file cabinet until the close of the study.

- Members of the study staff have keys to both the BLH site office and BHP HPV study office (by IDCC at PMH) and to the locked file cabinets.
- At the conclusion of the study, custody of all essential documents and records, including those containing patient information, will be transferred over to the Principal Investigator.

Transportation of study documents

- All completed study documents should either be stored in the locked BLH site office or the locked BHP study office
- Completed study documents from a study site outside of BLH (ie surrounding health facility) should be transported in a secure manner in a locked bag
- Completed study documents from BLH should be transported to Gaborone in a secure manner using a locked briefcase
- Transportation of all documents from the BLH site office to the BHP study office should be documented in the **Document Transfer Logbook**
- Ideally study staff transport documents. In a rare case, a courier service may be utilized if necessary.
- Transport of documents should be directly from one secure site to another site without making any stops on the way.

SOP 11: Data entry

Purpose: To describe how study data entry is to be verified

Procedure: Study data entry verification

Responsible party: Research assistants (RAs) are primarily responsible for ensuring proper data entry and verification of correct data entry. The PI and program coordinator are ultimately responsible for ensuring that data is verified in a systematic manner.

Timing: Data entry should occur within one week of collection and cross-checked for accuracy by a different study team member. PI data verification should occur on a bi-monthly basis.

Process

Data entry

- RAs will collect data on the paper questionnaires from Monday through Friday.
- At the end of each day, the RA will ensure that the data collected from the clinics is checked for quality and completeness.
- All questionnaires completed will be entered into the electronic database as soon as possible by the RA.

Data Verification

- All electronic data entry needs to be double checked for accuracy. After one RA or other study staff has entered data into the electronic database, a second staff member must cross check with the original source documentation that all data has been entered accurately by going through every field of entry. This includes questionnaire data, prior results and all study related results.
- Correct HPV result data entry into the electronic database must be confirmed again (with original source documentation) at the time of calling participants back with results and scheduling of colposcopy. When phoning participants with results, the study staff should have the original results document in their hands to verify accurate reporting of results.
- The study PI or a designated study staff will verify the accuracy of data entry by randomly selecting 10% of study files and cross-checking all results fields. A smaller subset of 5% of study files will undergo cross-checking of all data entry fields.
- Participants with incomplete data will be flagged and referred to the responsible RA for correction within a week

- Corrections will be made in the database where necessary and comments added where modification/changes are made

Data Cleaning

- The PI and program coordinator will run data reports once a month to identify any fields requiring data cleaning.
- Where data cleaning is required, the program coordinator will place the identified queries from data base as a spreadsheet in a created folder that will be shared with the other study team members.
- The list of questionnaires that require data cleaning/corrections will be communicated with the concerned RAs.
- Once the cleaning process is complete, the RA will send an email to notify all that the batch is now clean.

SOP 12: Script for counselling a participant before colposcopy/LEEP

Welcome the patient and introduce yourself and whoever is in the room

Ask Patient's name and age

Check for understanding of why she is at the clinic (Do you know why you are here today?)

Quickly explain to the patient that she was tested for HPV and her test came back positive.

Follow the following script:

HPV is very common in both men and women. Most commonly we get the virus when we first become sexually active

In most people it may disappear by itself or remain in our bodies without causing any problems. Just like right now you have been found to have the virus but you were not aware of it.

However, if we do find that one is carrying the virus, we need to examine them more carefully to ensure that the virus hasn't started to cause problems. It does that by creating a "seed" on the cervix. This seed doesn't have symptoms and the danger with it is that if left on the cervix, it can turn into cancer many years later.

If we find that you have a seed during today's examination, we are going to remove it after numbing the cervix.

If we don't see any seed, we are going to numb the cervix and remove the top of the cervix and send it for for tests to ensure that the seed is not hiding.

At the end of today's procedure you will have a small painless wound on the cervix. This takes 4-6 weeks to heal and during this time we need to protect the wound and make sure we don't interfer with it. There are three ways we can interfer with the wound: 1. By putting the finger inside the private parts when washing; 2. Using a tampoon during your period; having sexual intercourse. Before we can proceed we need to ensure that you will be able to abstain from sexual intercourse for 6 weeks without getting into trouble with your partner.

Setswana Version:

A o waitse gore ke eng o tsile go bona ngaka gompieno?

Maloba o itlhatlhobetse mogare wa HPV mme o itlthetswe mo go wena.

Mogare o o montsi mo sechabeng; mo go bo Mme le mo go bo Rre.

Bontsi jwa rona mo tsenwa ke mogare o ka nako ya fa re tlhakanela dikobo lwa ntlha.

Mogare o kgona go inyeletsa ka bo one, kana wa nna mo mmeleng go sena mathata jaaka ona le mogare mme o ntse o sa itse gore oteng ka one o sa go lwatse.

Le fa gontse jalo, fa re thwerwe mogare mo go wena jaaka gompiono jaana, re a tlamega go go tlhatlhoba ka tlhatlhobo e e tseneletseng go rurifasta gore mogare ga wa ijwalawa wa itira peo. Peo e fa e sa ntshiwe e kgona go jwelelela e itira kankere ya molomo wa popelomo dingageng tse ditlang.

Fa re itlhela peo gompiono fa re go tlhatlhoba, re ya go bolaya bogatsu mo molomung wa popelo re bo re e kgobola.

Fa re sa bone peo, re a go nna re bolaya bogatsu re bo rekgobola mo molomung wa popelo re ya go tlhatlhoba gape go bona gore a peo ga e a iphitlha.

Fa re fetsa tlhatlho ya gompiono, go tlaa bo go na le nthonyana mo molomung wa popelo e e seng botlhoko. Nthonyana e e tsaya sebaka sa beke tse nne go ya go tse thataro go fola. Mo nakong ya fa ntho e fola ga e a tshwnela go ronkgelwa. O ka e ronkgela ka makgetho a le mararo: 1. Go tsenya monwana mo bosading fa o tlhapa; 2. Go dirisa lilet/tampoon fa o le mo setswalong; 3. Go kopanela dikobo le Rre.

Pele ga re ka tswelela re tshwanela go rurifatsa gore go sa kopanela dikobo le Rre dibeke tse thataro ga gona go tsenya botshelo jwa gago mo diphatseng.

Fa go sena mathata le go tswelela, ke kopa gore o apole o tlo o palame bolao re go tlhatlhobe.

SOP 13: Script for scheduling HPV negative participants for colposcopy

Hello and thank you for taking part in the HPV cervical cancer screening study.

As you know, you tested negative for the virus that causes cervical cancer and so you have a very low risk of developing cervical cancer in the next few years.

We are offering some women who tested HPV negative, the opportunity to come back in for an examination by the doctor to ensure the test is accurate.

This is completely optional and if you decline, will not affect the care you receive in the future.

You would be compensated 30 BWP for your participation in the examination.

Would you be interested in coming to BLH for an examination?

SOP 14: Script for giving histopathology results for HPV positive participants

Benign/CIN1: You do not have cancer nor the seed of cervical cancer. You are still at risk for developing the seed of cervical cancer in the future because you have the HPV virus.

Maloba ha ngaka a go tlhatlhoba, o ne a kgobola molomo wa popelo go ya go tlhatlhoba gore a ona le peo. Maduo a gago a supa ha o ne o se na peo ya kankere ya molomo wa popelo. Se o re a se itumelela.

If they had a LEEP done, counsel that LEEP helps to clear the HPV infection, but some women will still have HPV even after LEEP.

Re ne re setse re kgobotse molomo wa popelo. Se se thusa go ka hokotse mogare wa HPV, mme re a itse gore bo mme bangwe bat la sala ba ntse bana le mogare

1. **HIV +:** We still have to be vigilant for cervical cancer. You should repeat screening in 2 years
    a. Mogare o ka nna wa jwala peo mo bo ka mosong. Ka jalo, o tla tlhoka go tlhatlhobelwa mogare gape morago ga ngwaga tse pedi

    HIV -: We still have to be vigilant for cervical cancer. You should repeat screening in 3 years

    a. Mogare o ka nna wa jwala peo mo bo ka mosong. Ka jalo, o tla tlhoka go tlhatlhobelwa mogare gape morago ga ngwaga tse tharo

CIN2/CIN3 (*regardless of whether margins are involved, regardless of HIV status*):

Your results show that you had a seed of cervical cancer.

Maloba ha ngaka a go tlhatlhoba, o ne a kgobola molomo wa popelo go ya go tlhatlhoba gore a ona le peo. Maduo a gago a supa ha o ne o na le peo ya kankere ya molomo wa popelo, mme e ntshitswe yotlhe. Peo e supa ha e ne eise e jwelelele go nna kankere. Se o re a selebogela. A kere wa tlhaloganya gore mogare o ne o setse o jwetse peo, mme peo e ntshitswe?

Women who had LEEP: We hope we have removed it with the LEEP procedure. We need to be vigilant because you still have a risk of developing cervical cancer. You need to repeat colposcopy/LEEP in 1 year (this is for both HIV positive and HIV negative women).

Re solofela gore re re kgonne go kgobola peo yotlhe. Re a itse gore mogare o katswa o ntse o setse mo go wena. Re batla go rurifatsa gore mogare ga o jwale peo gape mo bo ka mosong. Ka jalo, re tla batla go go tlhatlhoba gape morago ga ngwaga

Women who had only ECC: We need to schedule you for a LEEP procedure which is intended to remove the seed of cervical cancer.

Maloba jale ha ngaka a go tlhatlhoba one a hala a ntsha leswe mo teng ga molomo wa popelo le le ne la tlhatlhobiwa go bona go re a peo ga ea iphitlha. Go supagala ha o na le peo. Ka jalo o tshwanelwa ke gore o bonwe ke ngaka gore a tle a kgobole peo eo

Cancer or CIN3 *with* microinvasion: results should be given by Dr. Luckett or Dr. Masire

HPV negative: Script for giving histopathology results

CIN2 or worst pathology should be discussed with Dr. Luckett or Masire before giving results.

CIN1 or less: We are happy to let you know that you did not have the seed of cervical cancer, and because you tested negative for HPV, you have a low risk of developing cervical cancer in the next few years.

Maloba jale o ne wa bonwa ke ngaka a batla go rurifatse go re le ha o sena mogare, a o katswa o na le peo ya kankere ya molomo wa popelo. Re itumelela go go bolelela go re ga o na yone.

HIV neg: You can repeat cervical cancer screening in 5 years: O tla tlhoka go tlhatlhobelwa mogare wa kankere ya molomo wa popelo morago ga di ngwaga tse tse tlhano

HIV positive: You can repeat cervical cancer screening in 3 years: O tla tlhoka go tlhatlhobelwa mogare wa kankere ya molomo wa popelo morago ga di ngwaga tse tse tharo

Appendix 5. Recruitment leaflets

Taletso go Tsenelela Tlhatlhobo ya Kankere ya Molomo wa Popelo

Dumela Mma

O ka tswa o setse o kile wa utlwa ka kankere ya molomo wa popelo e di goga kwa pele mo go bakeng dintsho tsa bomme, lefa go ntse jalo se se itumedisang ke gore e ka thibelwa ka go e itlhatlhobela.

Tsatsi jeno re tlaa bo gape re lekeletsa mothale mongwe oo dirisiwang go tlhatlhobela kankere ya molomo wa popelo. Re eletsa gape go botsa dipotso dingwe go bona fa le ka tswa le na le kgonagalo ya go ka tsenwa ke kankere, re tlaa bo gape re le botsa ka maikutlo a lona ka tlhatlhobo e.

Fa o na le keletso ya go itse go feta se kgotsa ya go tsenelela tlhatlhobo e re tlaa leka go ikopanya nao fa o sa ntse o letetse go bona ngaka.Fa e le gore ga go kgonagale gore o tsenelele tlhatlhobo e gompieno mme e le nako ya gore o tlhatlhobele kanakere ya molomo wa popelo kgotsa o ise o ko o tlhatlhobiwe mo botshelong o tshwanetse go ya tlhatlhobong kwa kokelong nngwe le nngwe ya puso . Bomme ba ba tshelang le mogare wa HIV ba tshwanetse go tlhatlhobelwa kankere ya ya molomo wa popelo Morago ga ngwaga tse tharo, bomme ba ba senang mogare wa HIV bone ba ka tlhatlhoba mo ngwageng tse tlhano.

Re a leboga Mma

Invitation to Enroll for Cervical Cancer Screening

Good Morning.

As you might have heard, cervical cancer is a leading cause of cancer deaths among women, but the good thing is it can be prevented through screening!

Today we are offering women an extra test to assess one of the methods used to screen for cervical cancer. We would also like to ask you some questions to see if you are at risk of cervical cancer, and ask you what you think about the testing process.

If you are interested to know more about this study and/or to participate, please approach a member of our study team. We will try to see you while you wait for consultation with the doctor.

If you are unable to take part in this study today but are due for cervical screening or have never been screened, you should go for screening at selected clinics. Women with HIV should be screened every 3 years and women without HIV should be screened every 5 years.

Thank you

Appendix 6. Consent form

INFORMED CONSENT FORM

PROJECT TITLE*:* Performance of two-stage cervical cancer screening algorithms using primary high-risk human papillomavirus testing in Botswana

PROJECT ETHICS NUMBERS:
HPDME 13/18/1(Botswana Ministry of Health); **URB/IRB/1543** (University of Botswana); **721/2020** (Faculty of Health Sciences, University of Pretoria)

Principal Investigators
Rebecca Luckett MD MPH, Botswana Harvard Partnership, Gaborone, and Faculty of Medicine, University of Botswana. Phone: (267) 7433 3773

Doreen Ramogola-Masire, MD MSc(MPH), Faculty of Medicine, University of Botswana. Phone: (267) 355-5558/4564 or (267) 72687480

WHAT YOU SHOULD KNOW ABOUT THIS RESEARCH STUDY:

- Written permission to carry out this research study has been given by the office of Health Research Committee at the Ministry of Health and Wellness, Botswana; the Office of Research and Development, University of Botswana; and the Faculty of Health Sciences Research Ethics Committee, University of Pretoria
- We give you this informed consent document so that you may read about the reasons, risks, and benefits of this research study.
- You have the right to refuse to take part or agree to take part now and change your mind later.
- Please review this consent form carefully. Ask any questions before you make a decision.
- Your participation is voluntary. If you choose to participate, you do not have to answer questions that make you feel uncomfortable
- The questionnaire will take 20 to 30 minutes to complete

WHY ARE WE DOING THIS STUDY?

The purpose of the study is to test women in Botswana for a virus called Human Papillomavirus, or HPV, from a sample taken from the private parts. We want to know how to care for women who test positive for this. HPV is a common infection that is spread through sexual contact. You may have it without having any symptoms. Sometimes it goes away on its own. If it does not go away on its own, it can cause genital warts and cervical cancer. The tests available if you have the virus are visual inspection with acetic acid (vinegar test) or colposcopy (looking at the cervix with a special microscope). In this study we will also find out

more about a new test called automated visual evaluation. This involves taking pictures of the cervix (mouth of the womb) after applying acetic acid (vinegar). This picture will then be tested by a machine to tell whether there is cervical pre-cancer (seed of cancer) and cancer. Our aim is to see how well all of these tests work, and come up with the best way to tell if someone has cervical pre-cancer (seed of cancer) in an effort to prevent cervical cancer in women in Botswana.

You are being asked to participate in this study because you are attending a Southeast District clinic and you are 25 years of age or older. Before you sign this form, please ask any questions on any aspect of this study that is unclear to you. You may take as much time as necessary to think it over.

STUDY PROCEDURES

If you decide to take part in the study the research staff will give you some questions about yourself, like your age and marital status, as well as a few questions about your sexual health. Answering the questions will take around 20 to 30 minutes to complete. You do not have to answer questions that make you feel uncomfortable. We will also ask you to collect a swab from your private parts in a private room and give it to the study staff to be tested for HPV. The researchers will also read through your medical records. We are only collecting information about testing for cervical cancer in the past. You will receive the results of the test by phone in about two weeks, and the results will be explained to you. We can make an appointment for you to come in to talk about them if you want to. If you test positive for HPV will ask you to come back for more tests. This testing will take about 15 minutes and will include a pelvic examination (internal examination through the private parts) with more tests to check for cervical cancer. These tests include the application of acetic acid (vinegar) to the cervix (mouth of the womb). You will also undergo a colposcopy (looking at the cervix with a special microscope) and biopsy (taking a small piece) of the cervix to see if you have precancer (seed of cancer) or cancer. These two tests, are the usual tests we do in women whom we suspect have precancer (seed of cancer). 10 out of every 100 women who test negative for HPV will be called back for more testing to make sure HPV testing is accurate.

RISKS AND DISCOMFORTS

Undergoing cervical cancer screening can cause worry or concern, regardless of the test results. For this reason, both before and after you have these tests done, we will give you counseling about the meaning of the results. If you have HPV, we will provide you with appropriate follow-up testing and discuss ways to take care of yourself and avoid cervical cancer.

Cervical cancer screening might cause mild discomfort. Other less common side effects of cervical cancer screening include: 1) heavy bleeding; 2) fever or chills; and 3) yellow-colored or bad-smelling discharge from your vagina. You should contact study staff if you feel you have any of these symptoms.

BENEFITS AND/OR COMPENSATION

You will know whether you have HPV, which can cause cervical cancer. You will undergo follow-up testing if you have a positive result. The benefit to you of cervical cancer screening is that we can diagnose and treat pre-cancer in the cervix and prevent cervical cancer. You will be referred for further evaluation if you need it. We hope this information will help improve cervical cancer prevention in Botswana.

There is no cost to you for participation in this study. Ten out of every 100 women who test negative for HPV will be asked to come for follow-up. This is not needed for your health but will help us understand how the HPV test works. As this is not part of normal health care in Botswana you will be given 30 Pula for this to help with your transport costs.

CONFIDENTIALITY

All information about you will remain confidential. Your name will not be placed on the HPV sample or the pictures of the cervix we collect from you; they will be identified only by a code and date of collection. Written documents for the study will be kept in locked study offices. Any images or data stored on computers will have a security code and stored on secure servers. Only the research team and your provider will have access to your name and the study results.

The research team and your provider have access to the research information to monitor the study. We will not give your name or results to other people. We will do everything possible to ensure that your results and your participation in the study remain confidential. Publications and/or presentations that result from this study will not identify you by name.

VOLUNTARY PARTICIPATION

Participation in this study is voluntary. If you decide not to participate in this study, your decision will not affect your care, and you can still seek cervical cancer screening in a government facility**.** If you decide to participate, you are free to withdraw your consent and to discontinue participation at any time without penalty. Withdrawal from the study will not affect your care. Any refusal to observe and meet appointments agreed upon with the study team will be considered withdrawal from the study. Remember that you do not have to answer any questions that make you feel uncomfortable.

If you believe you are injured as a direct result of your participation in this study or should you have questions about the study and your rights as a research participant, contact one of the Investigators at the top of this consent. You will be offered the necessary care to treat your injury at a government facility.

AUTHORIZATION

You are making a decision whether or not to participate in this study. Your signature indicates that you have read and understood the information provided above, have had all your questions answered, and have decided to participate.

Name of Research Participant (please print)	Date
Signature of Research Participant	Date
Signature of Study Staff who consented the participant	Date

SAMPLE STORAGE

Do you agree to allow us to store your samples for future studies? If your samples are used for future studies, they may be transported to collaborating laboratories, but your identity will always remain confidential. Future studies will be limited to cancer, and will require ethical approval.

☐ Yes Signature_____

☐ No

FUTURE CONTACT

Do you agree to be contacted in the future regarding participation in future research related to cervical cancer screening and treatment?

☐ Yes Signature_____Phone 1_____Phone number 2_____

☐ No

YOU WILL BE GIVEN A COPY OF THIS CONSENT FORM TO KEEP.

If you have any questions concerning this study or consent form beyond those answered by the investigator, including questions about the research, your rights as a research participant; or if you feel that you have been treated unfairly and would like to talk to someone other than a member of the research team, please feel free to contact the Health Research Committee at the Ministry of Health and Wellness, Botswana, telephone 3632028;

the Faculty of Health Science Research Ethics Committee of the University of Pretoria telephone numbers 0027 12 356 3084 /3085

Appendix 7. Data collection forms

Fill out this page of the questionnaire PRIOR to HPV sampling

SOCIODEMOGRAPHICS ("Identifier" Form):

1	Patient signed consent? **If this answer is no, nothing further should be available for this participant in REDCap*	☐ 1. Yes ☐ 2. No
2	Patient agreed to future contact?	☐ 1. Yes ☐ 2. No
3	Which clinic site is patient recruited from?	_____
7	Date of Birth Letsatsi la matsalo	_____ (Yrs) (Dingwaga)
8	What is your physical address? *O nna ko kae (lefelo)?*	
9	Phone *Mogala* **Please include 2 numbers, even if 2nd is next of kin (NOK), given patient permits us to contact them through NOK*	Phone 1: Mogala 1_____ Phone 2 Mogala 2:_____ ☐ Phone 2 is my number. ☐ Phone 2 is my next of kin's number. ☐ I give permission to contact my next of kin. Whatsapp Number: _____ ☐ OK to text?
10	Education *Dithutego* (Tick only one option)	☐ 1. None / *Ga ke a tsena sekolo* ☐ 2. Non – formal / *Ke tsene thuto ga e go lelwe* ☐ 3. Primary / *Sekolo se se botlana* ☐ 4. Junior secondary / *Sekolo sa juniara* ☐ 5. Senior secondary / *Sekolo sa sekondary* ☐ 6. Tertiary / *Mma dikolo*
11	Are you employed?	☐ 1. Yes ☐ 2. No
11a	*Appears if Q10 "yes"	_____[Free text]

	Occupation / *Tiro*	
12	Marital status *Seemo sa nyalo* (Tick only one option)	☐ 1. Single / *Ga ke a nyalwa* ☐ 2. Married / *Ke nyetswe* ☐ 3. Divorced / *Ke tlhadilwe* ☐ 4. Separated / *Ke kgaoganye le monna* ☐ 5. Widowed / *Moswagadi*

PATIENT INTERVIEW PRE-SAMPLING("Questionnaire" Form):

13	Are you postmenopausal A o emisitse go bona setswalo?	☐ 1. Yes / *Ee* ☐ 2. No / *Nnya*
14	**appears if Q12 "No"* When was the first day of your last normal menstrual period *O bonye setswalo sag ago sa bofelo leng (tsatsi la ntlha)* (DD/MM/YY, if Date or Month unknown, default both fields to "01")	__ __ / __ __ / __ __
15	How many times have you been pregnant? *O imile ga kae?*	_____ [List 1-9 or >=10]
16	**Q15-19 appear if Q14 > 0* How many times have you given birth? *O belege ga kae?*	_____ [List 1-9 or >=10]
17	How many preterm births have you had? *Ke makgetho a a kae a o kileng wa belega pele ga nako e e tshwanetseng ya pelegi?*	_____ [List 1-9 or >=10]
18	How many miscarriages have you had? *O senyegetswe ga kae?*	_____ [List 1-9 or >=10]
19	How many children do you have? *O na le bana ba le kae?*	_____ [List 1-9 or >=10]
20	How many stillbirths have you had? *Ke ga kae o belega ngwana a tlhokafetse?*	_____ [List 1-9 or >=10]
21	Do you want (more) children? A o batla go nna le bana ba bangwe?	☐ 1. Yes / *Ee* ☐ 2. No / *Nnya* ☐ 3. Unsure / *Ga ke itse*

22	** appears if Q20 "yes"* Do you plan to get pregnant in the next 2 years? *A o batla go ima mo dingwageng tse pedi tse di tlang?*	☐ 1. Yes / *Ee* ☐ 2. No / *Nnya* ☐ 3. Unsure / *Ga ke itse*
23	What is your HIV status? *Seemo sa gago sa mogare se ntse jang?* *If negative skip to Section C, 'risk factors'*	☐ 1. Positive / *Ke na le mogare wa HIV* ☐ 2. Negative / *Ga ke na mogare wa HIV* ☐ 3. Unsure / *Ga ke itse*
24	When did you first test positive for HIV? *O tshwerwe leng ka mogare wa HIV?* (MM/YY, if Month unknown, default to "01")	__ __ / __ __
25	Do you know your CD4 nadir (lowest CD4 count)? A o itse selekanyo sa ga go sa bokete jwa masole a mmele (se se ko tlase tlase)	☐ 1. Yes / *Ee* ☐ 2. No / *Nnya* ☐ 3. Unsure / *Ga ke itse*
26	** appears if Q25 "yes"* What is your CD4 nadir? Bokete jwa masole a mmele a gago ke bokae?	_____ [Range 0-1500]
27	Do you know your most recent CD4 count? *A o itse seemo sa bokete jwa masole a gago a mmele sa bošeng?*	☐ 1. Yes / *Ee* ☐ 2. No / *Nnya* ☐ 3. Unsure / *Ga ke itse*
28	** appears if Q27 "yes"* What is your most recent CD4 count? *Masole a gago a bofelo a CD4 ke bo kae?*	_____ [Range 0-1500]
29	** appears if Q27 "yes"* Most recent CD4 count test date *Masole a gago a bofelo a CD4 a dirilwe leng?* (DD/MM/YY, if Date or Month unknown, default both fields to "01")	__ __ / __ __ / __ __

30	Do you know your most recent viral load? *A o itse bokete jwa mogare mo mmeleng wa gago jwa bošeng?*	☐ 1. Yes / *Ee* ☐ 2. No / *Nnya* ☐ 3. Unsure / *Ga ke itse*	
31	** appears if Q30 "yes"* What is your most recent viral load? *Bokete jwa mogare mo mmeleng wa gago jwa bošeng ke bokae?*	_____ [Range 0 to > 1,000,000]	
32	** appears if Q30 "yes"* Most recent viral load count test date *Tlhatlhobo ya bofelo ya bokete jwa mogare mo mmeleng wa gago e ne e le leng?* (DD/MM/YY, if Date or Month unknown, default both fields to "01")	__ __ / __ __ / __ __	
33	Are you on ARV treatment? *A o nwa diritibatsi tsa mogare wa HIV?*	☐ 1. Yes / *Ee* ☐ 2. No / *Nnya*	
34	** appears if Q33 "yes"* When did you start ARV treatment? *O simolotse diritibatsi tsa mogare wa HIV leng?* (MM/YY, if Month unknown, default to "01")	__ __ / __ __	
35	** appears if Q33 "yes"* What is your current regimen? *O tsaya kalfi efe ya diritibatsi?* (Tick best option or options. If none of these combinations in the first column applies, tick all individual drugs in the regimen in the second column)	☐ 1. Atripla ☐ 2. Truvada/Dalutegravir ☐ 3. Truvada/Nevirapine ☐ 4. Truvada/Alluvia ☐ 5. Combivir/Nevirapine ☐ 6. Combivir/Alluvia ☐ 7. Combivir/Efavirenz ☐ 8. TLD	☐ 8. Truvada ☐ 9. Combivir ☐ 10. Abacavir ☐ 11. Lamivudine ☐ 12. Zidovudine ☐ 13. Efavirenz ☐ 14. Nevirapine ☐ 15. Dolutegravir ☐ 16. Raltegravir ☐ 17. Alluvia ☐ 18. Atazanavir/r ☐ 19. Darunavir/r ☐ 20. Other _____ _____
TLD = tenofovir TDF, lamivudine 3TC, Dolutegravir DTG			

r = ritonavir	Lamivudine = 3TC	Truvada = Emtricitabine/Tenofovir
Abacavir = ABC	Zidovudine = AZT	Combivir - Lamivudine/zidovudine
		Alluvia = Lopinavir

Risk Factors (Continue "Questionnaire" Form):

35	Do you smoke or have you ever smoked cigarettes? *A o a goga kgotsa o kile wa goga motsokwe wa sekerese?*	☐ 1. Yes / *Ee* ☐ 2. No / *Nnya*
36	** Appears if Q35 "yes"* Are you a current or ex-smoker? *A mo nakong eno o goga motsokwe wa sekerese ?*	☐ 1. Current smoker / *Ke a goga* ☐ 2. Ex-smoker / *Ke tlogetse go goga*
37	** Appears if Q35 "yes"* What age did you start smoking? *O simolotse go goga o le ngwaga tse kae?*	_____[Range 0-100]
38	** Appears if Q36 "Ex-smoker"* What age did you quit smoking? *O emisitse go goga motsokwe o le dingwaga tse kae?*	_____[Range 0-100]

I am going to ask you some questions about your sexual history. If you do not want to answer them you do not have to. If you do not know the answer you can say so.
Dipotso tse di latelang di botsa ka tsa tlhakanelo dikobo. O gololesgile go seke o di araba fa o bona go sa tlhokafale. Fa o sa itse dikarabo dingwe o ka nkitsese.

39	Have you had sexual intercourse? *A o kile wa tlhakanela dikobo?*	☐ 1. Yes / *Ee* ☐ 2. No / *Nnya*
40	**Appears if Q39 "yes"* How old were you when you had sexual intercourse for the first time? *O tlhakanetse dikobo lwa ntlha mo botshelong o le dingwaga di kae?*	_____[Range 0-100]
41	**Appears if Q39 "yes"* How many partners have you had sexual intercourse with in your lifetime?	_____[List 0, 1-5, 6-10, 11-20, 21-50, >50]

	Mo botshelong jotlhe jwa gago o ka tswa o tlhakanetse dikobo le borre ba le kae?	
42	**Appears if Q39 "yes"* Do you have a current sexual partner? *A go na le yoo tlhakanelang dikobo nae mo nakong eno?*	☐ 1. Yes / *Ee* ☐ 2. No / *Nnya*
43	** Appears if Q 42 "yes"* What is your relationship to your (primary) sexual partner? *O amana jang le motho yo o tlhakanelang dikobo nae mo nakong eno?* (Tick only one option)	☐ 1. Married / *Nyalo* ☐ 2. Boyfriend / *Tsala/Nyatsi* ☐ 3. Fiancée / *Peelelo* ☐ 4. Live-in partner / *Re nna mmogo* ☐ 5. Casual relationship / *Re kopana nako nngwe* ☐ 6. Other / *Tse dingwe* _____
44	** Appears if Q 42 "yes"* Do you have more than one current sexual partner? *A o na le batho ba ba fetang bongwe ba o tlhakanelang dikobo nabo?*	☐ 1. Yes / *Ee* ☐ 2. No / *Nnya*
45	** Appears if Q 44 "yes"* How many sexual partners do you currently have? *O na le batho ba le kae ba o tlhakanelang dikobo nabo mo bogompienong?*	_____[List 0, 1-5, 6-10, 11-20, 21-50, >50]
46	Are you using anything for pregnancy prevention at the moment (including tubal ligation)? *A o dirisa sengwe go thibela boimana (go akaretsa loaro la go emisa tsholo)?*	☐ 1. Yes / *Ee* ☐ 2. No / *Nnya*
47	** Appears if Q 46 "yes"* What do you currently use for pregnancy prevention? *O dirisa eng go thibela boimana?* (Tick all that apply)	☐ 1. Condoms only / *Sekausu fela* ☐ 2. Pill / *Pilisi* ☐ 3. Injection / *Mokento* ☐ 4. Implant / *Implant* ☐ 5. Loop / *Loop* ☐ 6. Tubal ligation / *Loaro la go emisa tsholo*

		☐ 7. Other / *Tse dingwe* _____ ☐ 8. Condom with other method / *Sekausu le mofuta o mongwe*
48	** Appears if Q 46 "yes"* With your current or most recent partner, how often do you use condoms? *Le dirisa sekausu ga kae le motho yo o tlhakanelang dikobo nae mo nakong eno kgotsa motho wa bofelo o kopanna dikobo nae?* (Tick only one option)	☐ 1. Every time / *Nako tsotlhe* ☐ 2. Most times / *Nako e ntsi* ☐ 3. Sometimes / *Nako nngwe* ☐ 4. Rarely / *Ka selebalo* ☐ 5. Never / *Ga re se dirise sekauso nako epe*

History of cervical cancer screening (Continue "Questionnaire" Form):

This section of the questionnaire is based on participant recall. / Karolo e e botsa se motsaa karolo a se gakologelwang

49	Have you been screened for cervical cancer in the past? *A o kile wa tlhatlhobelwa kankere ya molomo wa popelo?* *Instructions: Help the patient go through their cards to see if there are any prior screenings documented and if not, answer the following based on recall*	☐ 1. Yes / *Ee* ☐ 2. No / *Nnya* ☐ 3. Not sure / *Ga ke itse*
50	**appears if Q49 "No"* Why have you never screened for cervical cancer? *Ka goreng o ise o itlhatlhobele kankere ya molomo wa popelo?*	_____[Free text]
51	**appears if Q49 "Yes"* At the time of your last screening for cervical cancer, why did you decide to get tested? *Lwa bofelo fa o itlhatlhoba, ke eng se seneng se go gwetlhile gore o itlhatlhobele kankere ya molomo wa popelo?*	_____[Free text]
51	**appears if Q49 "Yes"* Have you been screened more than once? *A o kile wa tlhatlhobiwa go feta gangwe?*	☐ 1. Yes / *Ee* ☐ 2. No / *Nnya* ☐ 3. Not sure / *Ga ke itse*

52	*appears if Q52 "Yes"* How many times have you been screened? *O itlhatlhobile ga kae?*	_____[drop down list 2-10]
54	*appears if Q49 "Yes" How were you screened for cervical cancer? O ne o dirisa mothale ofe wa tlhatlhobo ya kankere ya molomo wa popelo? (Tick all that apply)	☐ 1. VIA / *VIA (Tlhatlhobo ka vinegar)* ☐ 2. Pap smear / *Pap smear* ☐ 3. Not sure / *Ga ke itse*
55	**appears if Q49 "Yes"* In what year were you last screened? *O itlhatlhobetse kankere ya molomo wa popelo ka ngwaga ofe?* *(YY)*	— —
56	**appears if Q49 "Yes"* In which health facility were you last screened? *O no o tlhatllhobela kwa kokelong efe?*	_____[Free text]
57	**appears if Q49 "Yes"* What was the outcome of screening? *Maduo a tlhatlhobo a ne a ntse jang?*	☐ 1. Normal / *A ne a siame* ☐ 2. Abnormal / *A ne a sa siama* ☐ 3. Not sure / *Ga ke itse/gakologelwe*
58	**appears if Q57 "Abnormal"* Please specify how the outcome was abnormal *Tlhalosa ka botlalo gore maduo a ne a sa siama jang*	_____[Free text]
59	**appears if Q57 "Abnormal"* Did you have to have a procedure? *A o ne wa tshwanelwa ke go dirwa loaro?* (Tick only one option)	☐ 0. Yes / *Ee* ☐ 1. No / *Nnya* ☐ 2. Not sure / *Ga ke itse*
59a	**appears if Q59 "Yes"* Which procedure? Loaro lofe?	☐ 1. Colposcopy ☐ 2. LEEP ☐ 3. Cone biopsy ☐ 4. Other _____

Knowledge (Continue "Questionnaire" Form):

60	Do you know what causes cervical cancer? *A o itse gore kankere ya molomo wa popelo e bakiwa ke eng?*	☐ 1. Yes / *Ee* ☐ 2. No / *Nnya* ☐ 3. Not sure / *Ga ke itse*
61	What do you think causes cervical cancer? *O akanya gore kankere ya molomo wa popelo e bakiwa ke eng?*	_____[Free text]
62	Before today had you heard of HPV? *A o ne o kile wa utlwalela ka ga mogare wa HPV pele ga gompieno?*	☐ 1. Yes / *Ee* ☐ 2. No / *Nnya*
63	**Appears if Q62 "Yes"* How and where did you hear about HPV? *O utlwaletse ka mokento wa HPV jang kgotsa kae ?*	_____[Free text]
64	Have you heard of the HPV vaccine? *A o kile wa utlwalela ka mokento wa HPV*	☐ 1. Yes / *Ee* ☐ 2. No / *Nnya*
65	**Appears if Q64 "Yes"* Do you think people should get the HPV vaccine? *A o akanya gore batho ba tshwanetse go tsaya melemo ya Thibelo HPV?*	☐ 1. Yes / *Ee* ☐ 2. No / *Nnya* ☐ 3. Not sure / *Ga ke itse*
66	**Appears if Q64 "Yes"* What do you think the HPV vaccine does? *O akanya gore melemo ya thibelo HPV e dira eng?*	_____[Free text]
67	**Appears if Q64 "Yes"* Have you received a vaccination for HPV? *A o,klie wa fiwa mokento wa HPV?*	☐ 1. Yes / *Ee* ☐ 2. No / *Nnya* ☐ 3. Not sure / *Ga ke itse*
68	For the purposes of this study we are giving you your results over the phone. In the future you may have other options. How would you like to receive your test results? *Mo tlhatlhobong ya gompieno re tlaa go fa maduo ka mogala. Mo isagoong go ka nna le tsela tse dingwe go go fa maduo. O ka eletsa*	☐ 1. Over the phone / *Ka mogala* ☐ 2. Return to the same clinic for the results in person / *Go boela kwa kokelong e o tlhatlhobetsweng teng* ☐ 3. Present to your local clinic for the results in person / *Go ya kwa kokelong ee gaufi go utwa maduo* ☐ 4. Text message / *Go romelelwa molaetsa wa cell phone*

	go fiwa maduo a gago jang mo isagong? (Tick all that apply)	☐ 5. Other / *Tse dingwe* _____

Thank you for your participation in the study today. That is the end of the questionnaire. We will now do the exam and cervical cancer screening tests.

Re lebogela nako ya gago go nna karolo ya patlisiso e gompieno. Re tlile ko bokhutlong jwa potsolotso e. Jaanong re tla dira ditlhatlhobo di akaretsa tlhatlhobo ya kankere ya molomo wa popelo.

PRIOR HISTOPATHOLOGY REPORTS ("Past results" Form):

Look in IPMS

69	Is there a prior Pap Smear documented?	☐ 1. Yes ☐ 2. No
70	**Appears if Q69 "Yes"* Date of most recent Pap smear (MM/YY)	__ __/__ __/__ __
71	**Appears if Q69 "Yes"* What were the results of the most recent Pap smear (Tick only one option)	☐ 0. NILM ☐ 1. ASCUS ☐ 2. LSIL ☐ 3. HSIL ☐ 4. ASC-HI ☐ 5. Atypical glandular cells ☐ 6. Adenocarcinoma in-situ ☐ 7. Squamous cell carcinoma ☐ 8. Adenocarcinoma ☐ Other _____
72	**Appears if Q69 "Yes"* Is there another Pap smear documented?	☐ 1. Yes ☐ 2. No
73	**Appears if Q72 "Yes"* Date of next Pap smear (MM/YY)	__ __/__ __/__ __
74	**Appears if Q72 "Yes"* What were the results of this Pap smear	☐ 0. NILM ☐ 1. ASCUS

	(Tick only one option)	☐ 2. LSIL ☐ 3. HSIL ☐ 4. ASC-HI ☐ 5. Atypical glandular cells ☐ 6. Adenocarcinoma in-situ ☐ 7. Squamous cell carcinoma ☐ 8. Adenocarcinoma ☐ Other _____
75	**Appears if Q72 "Yes"* Is there another Pap smear documented?	☐ 1. Yes ☐ 2. No
76	**Appears if Q74 "Yes"* Date of next Pap smear (MM/YY)	__ __/__ __/__ __
77	**Appears if Q75 "Yes"* What were the results of this Pap smear (Tick only one option)	☐ 0. NILM ☐ 1. ASCUS ☐ 2. LSIL ☐ 3. HSIL ☐ 4. ASC-HI ☐ 5. Atypical glandular cells ☐ 6. Adenocarcinoma in-situ ☐ 7. Squamous cell carcinoma ☐ 8. Adenocarcinoma ☐ Other _____
78	Is there a prior cervical pathology specimen documented?	☐ 1. Yes ☐ 2. No
79	**Appears if Q78 "Yes"* Date of most recent cervical pathology (MM/YY)	__ __/__ __/__ __
80	**Appears if Q78 "Yes"* What were the results of the most recent cervical pathology? (Tick all applicable options)	☐ 0. Benign / no dysplasia ☐ 1. CIN1 ☐ 2. CIN2 ☐ 3. CIN3 ☐ 4. CIN3 with invasive features

		☐ 5. Glandular hyperplasia ☐ 6. Adenocarcinoma in-situ ☐ 7. Squamous cell carcinoma ☐ 8. Adenocarcinoma ☐ Other / Details: _____
81	**Appears if Q78 "Yes"* Is there another cervical pathology specimen documented?	☐ 1. Yes ☐ 2. No
82	**Appears if Q81 "Yes"* Date of next cervical pathology: (MM/YY)	__ __/__ __/__ __
83	**Appears if Q81 "Yes"* What were the results of this cervical pathology specimen? (Tick all applicable options)	☐ 0. Benign / no dysplasia ☐ 1. CIN1 ☐ 2. CIN2 ☐ 3. CIN3 ☐ 4. CIN3 with invasive features ☐ 5. Glandular hyperplasia ☐ 6. Adenocarcinoma in-situ ☐ 7. Squamous cell carcinoma ☐ 8. Adenocarcinoma ☐ Other / Details: _____
84	**Appears if Q81 "Yes"* Is there another cervical pathology specimen?	☐ 1. Yes ☐ 2. No
85	**Appears if Q84 "Yes"* Date of next cervical pathology: (MM/YY)	__ __/__ __/__ __
86	**Appears if Q84 "Yes"* What were the results of this cervical pathology specimen? (Tick all applicable options)	☐ 0. Benign / no dysplasia ☐ 1. CIN1 ☐ 2. CIN2 ☐ 3. CIN3

		☐ 4. CIN3 with invasive features ☐ 5. Glandular hyperplasia ☐ 6. Adenocarcinoma in-situ ☐ 7. Squamous cell carcinoma ☐ 8. Adenocarcinoma ☐ Other / Details: _____

HPV RESULTS ("HPV Results" Form):

HPV Results		
87	What were the results from the HPV swab? (Tick only one option)	☐ 1. Negative ☐ 2. HPV 16 ☐ 3. HPV 18/45 ☐ 4. Other pooled HR-HPV ☐ 5. Indeterminate
88	Date results obtained (DD/MM/YY)	__ __ / __ __ / __ __
If the patient has a positive HPV test, please schedule them for colposcopy at the time of phone call		
89	Was the participant given the results?	☐ 1. Yes ☐ 2. No
90	**Appears if Q89 "No"* Please specify why the patient was not given their results	_____
91	**Appears if Q89 "Yes"* Date the results were given to the participant (DD/MM/YY)	__ __ / __ __ / __
92	**Appears if Q89 "Yes"* How were the results given to the participant?	☐ 1. By phone ☐ 2. In person
93	**Appears if Q89 "Yes"* If a VIA/Colposcopy appointment is necessary, has it been made?	☐ 1. Yes ☐ 2. No ☐ 3. N/A

94	**Appears if Q93 "Yes"* Date of the appointment (DD/MM/YY)	__ __ / __ __ / __ __
95	**Appears if Q093 "No"* If an appointment was not made, why not?	_____[Free text]
96	**Appears if Q92 "By phone"* When you successfully got through, how many minutes were you on the phone for?	_____ mins [Free text, Range 0-60]
97	How many attempts were made to contact the participant?	_____[List 1-5]

Call log:

* *Number of documentation options that appear corresponds to answer in Q85*

Date of call 1: __ __ / __ __ / __ __ Time of call 1: _____ Results of call: _____

Date of call 2: __ __ / __ __ / __ __ Time of call 2: _____ Results of call: _____

Date of call 3: __ __ / __ __ / __ __ Time of call 3: _____ Results of call: _____

Date of call 4: __ __ / __ __ / __ __ Time of call 4: _____ Results of call: _____

Date of call 5: __ __ / __ __ / __ __ Time of call 5: _____ Results of call: _____

Make "Results of call" a drop down menu with the following options: No answer, participant not available, network down, phone out of service, wrong number, other (specify)

VIA / COLPOSCOPY ("VIA/Colposcopy" Form):

98	Did the patient miss their VIA / Colposcopy visit?	☐ 1. Yes ☐ 2. No
99	** Appears if Q109 "Yes"* Was the participant contacted to reschedule?	☐ 1. Yes ☐ 2. No
100	** Appears if Q110 "Yes"* Date of the new appointment (DD/MM/YY)	__ __ / __ __ / __ __
101	**Appears if Q110 "No"*	_____[Free text]

	If an appointment was not made, why not?	
102	** Appears if Q109 "Yes"* How many attempts were made to contact the participant?	[List 1-5]

Call log:

** Number of documentation options that appear corresponds to answer in Q85*

Date of call 1: __ __ / __ __ / __ __ Time of call 1: _____ Results of call: _____

Date of call 2: __ __ / __ __ / __ __ Time of call 2: _____ Results of call: _____

Date of call 3: __ __ / __ __ / __ __ Time of call 3: _____ Results of call: _____

Date of call 4: __ __ / __ __ / __ __ Time of call 4: _____ Results of call: _____

Date of call 5: __ __ / __ __ / __ __ Time of call 5: _____ Results of call: _____

Make "Results of call" a drop down menu with the following options: No answer, participant not available, network down, phone out of service, wrong number, other (specify)

103	What was the assessment by the VIA nurse? (Tick only one option)	☐ 1. Negative ☐ 2. Positive, for cryotherapy ☐ 3. Positive, referral for LEEP Specify reason for LEEP_____
104	What was the colposcopic impression? (Tick all applicable options)	☐ 0. Normal – SCJ fully visualized ☐ 1. Abnormal – SCJ not fully visualized ☐ 2. Acetowhite lesion ☐ 3. Mosaicism ☐ 4. Punctations ☐ 5. Atypical vessels Other: _____
104a.	What was the grade of the colposcopic impression? (tick only one option)	☐ 0. Normal ☐ 1. Low grade abnormality ☐ 2. High grade abnormality
105	Was an image of the cervix taken for mobile image capture, mobile ODT and AVE	ODT ☐ 1. Yes NCI ☐ 1. Yes

		☐ 2. No ☐ 2. No
106	What diagnostic procedure was performed? (Tick only one option)	☐ 0. None ☐ 1. Biopsy ☐ 2. Biopsy + ECC ☐ 3. ECC ☐ 4. Central LEEP ☐ 5. LEEP of transformation zone Any additional notes from provider: _____
107	Were there any complications?	☐ 1. Yes ☐ 2. No
117a	**Appears if Q117 "Yes"* What were the complications?	_____[Free text]
108	What is the date of the results/follow-up visit?	__ __ / __ __ / __ __

RESULTS OF COLPOSCOPY ("Colposcopy Results" Form):

110	What was the histopathology result of the diagnostic procedure? (Tick all that apply)	☐ 0. Benign / no dysplasia ☐ 1. CIN1 ☐ 2. CIN2 ☐ 3. CIN3 ☐ 4. CIN3 with invasive features ☐ 5. Glandular hyperplasia ☐ 6. Adenocarcinoma in-situ ☐ 7. Squamous cell carcinoma ☐ 8. Adenocarcinoma ☐ Other / Details: _____
111	Date results obtained (DD/MM/YY)	__ __ / __ __ / __ __
112	What is the follow-up plan for the patient? (Tick only one option)	☐ 0. No dysplasia – Repeat screening in 1 year ☐ 1. CIN1 – Repeat screening in 1 year ☐ 2. CIN2 on biopsy - return for LEEP ☐ 3. CIN2 on LEEP - Repeat colposcopy in 1 year

		☐ 4. CIN3 on biopsy – return for LEEP ☐ 5. CIN3 on LEEP – Repeat colposcopy in 1 year ☐ 6. CIN3 with invasive features – referral to gynaecologist ☐ 7. Glandular hyperplasia – Repeat screening in 1 year ☐ 8. Adenocarcinoma in-situ – referral to gynaecologist ☐ 9. Squamous cell carcinoma – referral to gynaecologist ☐ 10. Adenocarcinoma – referral to gynaecologist ☐ 11. Other in consultation with Dr. Ramogola-Masire or Dr. Lucke _____
113	Was the participant given the results?	☐ 1. Yes ☐ 2. No
114	**Appears if Q122 "No"* Please explain why the patient did not receive their results	_____[Free text]
115	**Appears if Q122 "Yes"* Date the results were given to the participant (DD/MM/YY)	__ __ / __ __ / __ __
116	**Appears if Q122 "Yes"* How were the results given to the patient?	☐ 1. By phone ☐ 2. In person
117	**Appears if Q112 response "0", "3", "5", or "7"* If the patient is for follow-up in 1 year were they asked to come in to have results written in their card?	☐ 1. Yes ☐ 2. No
118	**Appears if Q112 response "2" or "4"* If the patient requires LEEP, was this communicated to the patient?	☐ 1. Yes ☐ 2. No
119	**Appears if Q112 response "2" or "4"* What is the date of their LEEP appointment (DD/MM/YY)	__ __ / __ __ / __ __
120	**Appears if Q112 response "6", "8", "9", or "10"* If result required referral to gynaecologist, was this communicated to the patient?	☐ 1. Yes ☐ 2. No
121	**Appears if Q112 response "6", "8", "9", or "10"*	__ __ / __ __ / __ __

	What is the date of their gynaecology appointment?	
122	* *Appears if Q113 response "Yes"* When you successfully spoke with the patient, how long did it take to give the results?	_____ mins [Free text, Range 0-60]
123	How many attempts were made to contact the participant?	_____[List 1-5]

Call log:

* *Number of documentation options that appear corresponds to answer in Q85*

Date of call 1: __ __ / __ __ / __ __ Time of call 1: _____ Results of call: _____
Date of call 2: __ __ / __ __ / __ __ Time of call 2: _____ Results of call: _____
Date of call 3: __ __ / __ __ / __ __ Time of call 3: _____ Results of call: _____
Date of call 4: __ __ / __ __ / __ __ Time of call 4: _____ Results of call: _____
Date of call 5: __ __ / __ __ / __ __ Time of call 5: _____ Results of call: _____

Make "Results of call" a drop down menu with the following options: No answer, participant not available, network dow
out of service, wrong number, other (specify)

FOLLOW-UP / TREATMENT VISIT ("Follow-up/Treatment Visit" Form):

124	**Appears if Q93 "N/A" AND Q101 "N/A"* If the patient did not require colposcopy, did the patient present to have the results written in their card?	☐ 1. Yes ☐ 2. No ☐ 3. N/A
125	**Appears if Q121 response "0", "3", "5", or "7"* If the patient is for review in 1 year, did they present to have results written in card?	☐ 1. Yes ☐ 2. No
126	**Appears if Q121 response "1", "2" OR "4"* If the patient required LEEP did they present for their visit?	☐ 1. Yes ☐ 2. No

127	**Appears if Q137 response "Yes"* Were there any complications?	☐ 1. Yes ☐ 2. No
128	**Appears if Q139 "Yes"* What were the complications?	_____[Free text]
129	**Appears if Q137 response "LEEP"* What is the date of the results/follow-up visit? (DD/MM/YY)	__ __ / __ __ / __ __

RESULTS OF LEEP ("LEEP Results" Form):

What was the result of LEEP? (Tick all that apply)	☐ 0. No dysplasia – Repeat screening in 1 year ☐ 1. CIN1 – Repeat screening in 1 year ☐ 2. CIN2 - Repeat colposcopy in 1 year ☐ 3. CIN3– Repeat colposcopy in 1 year ☐ 4. CIN3 with invasive features – referral to gynaecologist ☐ 5. Glandular hyperplasia – Repeat screening in 1 year ☐ 6. Adenocarcinoma in-situ – referral to gynaecologist ☐ 7. Squamous cell carcinoma – referral to gynaecologist ☐ 8. Adenocarcinoma – referral to gynaecologist ☐ 9. Other in consultation with Dr. Ramogola-Masire or Dr. Luckett: _____
Was the participant given the results?	☐ 1. Yes ☐ 2. No
**Appears if Q143 "No"* Why did the patient not receive their results	_____[Free text]
**Appears if Q143 "Yes"* Date the results were given to the participant (DD/MM/YY)	__ __ / __ __ / __ __
**Appears if Q143 "Yes"* How was the participant given their results?	☐ 1. By phone ☐ 2. In person

	If by phone, please ask participant to come in to have results written in card
**Appears if Q142 "4", "6", "7", OR "8"* If result required referral to gynaecologist, what is the date of appointment?	__ __ / __ __ / __ __
**Appears if Q142 "Yes"* When you successfully got through, how long were you on the phone for?	_____ mins [Free text, Range 0-60]
How many attempts were made to contact the participant?	_____[List 1-5]

er of documentation options that appear corresponds to answer in Q85

call 1: __ __ / __ __ / __ __ Time of call 1: _____ Results of call: _____
call 2: __ __ / __ __ / __ __ Time of call 2: _____ Results of call: _____
call 3: __ __ / __ __ / __ __ Time of call 3: _____ Results of call: _____
call 4: __ __ / __ __ / __ __ Time of call 4: _____ Results of call: _____
call 5: __ __ / __ __ / __ __ Time of call 5: _____ Results of call: _____

Results of call" a drop down menu with the following options: No answer, participant not available, network down, ut of service, wrong number, other (specify)

Appendix 8. HPV information leaflet Self-collection swab instructions

English

HPV and cervical cancer information leaflet

What is human papillomavirus (HPV)?

HPV is a group of viruses that can infect both men and women. It can be transmitted sexually and is very common – most women will have an infection with HPV at some point in their lives. There are several types of HPV, many of which will cause no problems at all. Some types of HPV can cause skin warts and others can predispose to cervical cancer. There is no treatment for HPV and mostly the infection goes away with time.

HPV and cervical cancer

Cervical cancer is one of the most common types of cancer in women. Some types of HPV are involved in the development of cancer of the cervix (high-risk HPV). The HPV infection can cause cells in the cervix to change over time leading to pre-cancer. If these changes are not treated they can develop into cancer.

Cervical screening

Cervical screening is important for preventing cervical cancer. This is commonly done by a pap smear which involves examination of the cervix by a health care professional and a sample being sent to the lab for analysis. This service is offered at all government clinics in Botswana. Some clinics also offer visual inspection with acetic acid (VIA), also referred to as the vinegar test, which allows the health care professional to directly look for any changes in the cervix which can then be treated immediately. Testing for HPV is another screening method that may not require a pelvic examination. Currently HPV testing is being introduced in Botswana. Testing for HPV should easily identify women who are at high risk for cervical cancer, and these women can then be offered further management.

What are we doing today?

We are looking at the best way to screen for cervical cancer in Botswana using HPV testing. This will help develop the cervical cancer screening programme for Botswana.

If you test positive for HPV, you will be called back to have another examination and testing to see if you are at risk for having a pre-cancer or cancer of the cervix.

Setswana

Mogare wa HPV le Kankere ya Molomo wa Popelo

Human papillomavirus (HPV) ke eng?

HPV ke setlhopha sa megare e e bidiwang diviruse e e kgonang go ama borre le bomme. Mogare o o kgona go tsena ka tlhakanelo dikobo, e bile o nna teng gantsi. Bontsi jwa bomme ba nna le mogare o nako nngwe mo matshelong a bone.Go na le mefuta e mentsi ya mogare wa HPV. Bontsi jwa yone ga bo bake mathata ape. Le fa go le jalo go na le mefuta mengwe e e ka bakang dikakana fa mengwe e ka baka kankere ya molomo wa popelo. Mogare wa HPV ga o na kalafi mme eblie nako e ntsi o inyeletsa fela ka bo one fa nako e ntse e tsamaya.

Mogare wa HPV le kankere ya molomo wa popelo

Kankere ya molomo wa popelo ke nngwe ya tse di tlwaetsweng thata mo go bomme. Mefuta mengwe ya HPV (high risk) e ka baka kankere ya molomo wa popelo . Fa nako e ntse e tsamaya mofuta o wa HPV o ka dira gore dikarolo dingwe tsa molomo wa popelo di fetoge di be di nne le peo ee ka bakang kankere fa diphetogo tseo di sa alafiwe.

Tlhatlhobo ya kankere ya molomo wa popelo

Tlhatlhobo ya kankere ya molomo wa popelo e botlhokwa thata mo thibelong ya kankere. Tlhatlhobo e e tlwaetswe go dirwa ka pap smear e mo go yone ba botsogo ba tsayang matute a bosadi le go a romela kwa lebong (laboratory) go tlhatlhobiwa.Tlhatlhobo e e dirwa mo dikokelong tsotlhe tsa puso mo Botswana. Dikokelo dingwe di dira tlhatlhobo ya molomo wa popelo ka viniger ,mo go tlwaelesegileng ka Visual Inspection after acetic acid (VIA) e yone e fang ba botsogo tshono ya go lebelela diphetogo tse di ka tswang di le teng mo molomomg wa popelo, le go di alafa gone foo. Mothale o mongwe oo ka dirisiwang go tlhatlhobela kankere ya molomo wa popelo o sa dire gore go tsenelelwe kwa teng ke wa HPV. Lefa gone go na le thulaganyo ya go dira tlhatlhobo ya HPV mo bogautshwaneng, ea similodisiwa mo Botswana . Tlhatlhobo ya HPV e botlhofo go ka tlhaola bomme ba ba mo diphatseng tsa go ka tsenwa ke kankere ya molomo wa popelo le go ba romela go bona thuso/kalafi ee lebaneng.

Re dira eng gompieno?

Re batlisisa tsela e e gaisang ya go tlhatlhobela kankere ya molomo wa popelo mo Botswana ka go tlhatlhobela mogare wa HPV . Re tla dira le tlhatlhobo ya Pap smear kay one nako eo gape. Se se tlaa thusa mo go direng lenaneo la thibelo kankere ya molomo wa popelo ka mothale o mo Botswana.

Fa o tshwarwa ka mogare wa HPV, o tlaa bidiwa gape go tla go dira ditlhatlhobo tse dingwe go bona fa o le mo diphatseng tsa go tsenwa ke kankere kgotsa o na le kankere ya molomo wa popelo.

Appendix 9. University of Botswana ethics approval

UNIVERSITY of BOTSWANA

Office of the Deputy Vice Chancellor (Academic Affairs)

Office of Research and Development

Corner of Notwane and Mobuto Road
Gaborone, Botswana

Pvt Bag 00708
Gaborone
Botswana

Tel : (267) 355 2900
Fax : (267) 395 7573
Email: ord@ub.ac.bw

12th April 2023

CURB/IRB/1543

Permanent Secretary
Ministry of Health and Wellness
Private Bag 0038
Gaborone, Botswana

Study Title: "Assessment of the Acceptability and Feasibility of Self-Collected Samples for High-Risk Human Papillomavirus (HPV) Screening at two Women's Health Clinics in Gaborone, Botswana"

Researcher (s): Dr Rebecca Luckett, Dr Doreen Ramogola-Masire & Dr Chelsea Morroni

Review Type: Expedited Review by UB Institutional Review Board Committee

APPROVAL DATE : 12th April, 2023
EXPIRATION DATE : 11th April, 2024

This certifies that continuing review request for the above protocol was reviewed and approved for a period of one year.

☐ The study is still ongoing
☒ **(Closed to Enrolment) Open for analysis only. Expected end date: 06/2027**
☐ The study has not started recruitment
☐ Includes only collection of data from voice, video, digital, or image recordings made for research purpose
☐ Research on individual or group characteristics or behaviour (including, but not limited to, research on perception, cognition, motivate ion, identity, language, communication, cultural beliefs or practices, and social behaviour)
☐ Research employing survey, interview, oral history, focus group, program evaluation, human factors evaluation, or quality assurance methodology

Continuing Review
In order to continue work on this study (including data analysis) beyond the expiration date, the UB Institutional Review Board (IRB) must reapprove the protocol after conducting a substantive, meaningful, continuing review. This means that you must submit a Continuing Report form as a request for continuing review. To best avoid a lapse, you should submit your request three months before the lapse date. Reporting of serious problems or other events in writing to the UB IRB include:

- Modifications on the study protocol
- Suspension or Termination of study
- Unexpected problems involving risk to subject or others
- Adverse events, including unanticipated or anticipated but severe physical harm to subjects

QUESTIONS: Please contact ORD ext 2900 or e-mail on ORD@ub.ac.bw.

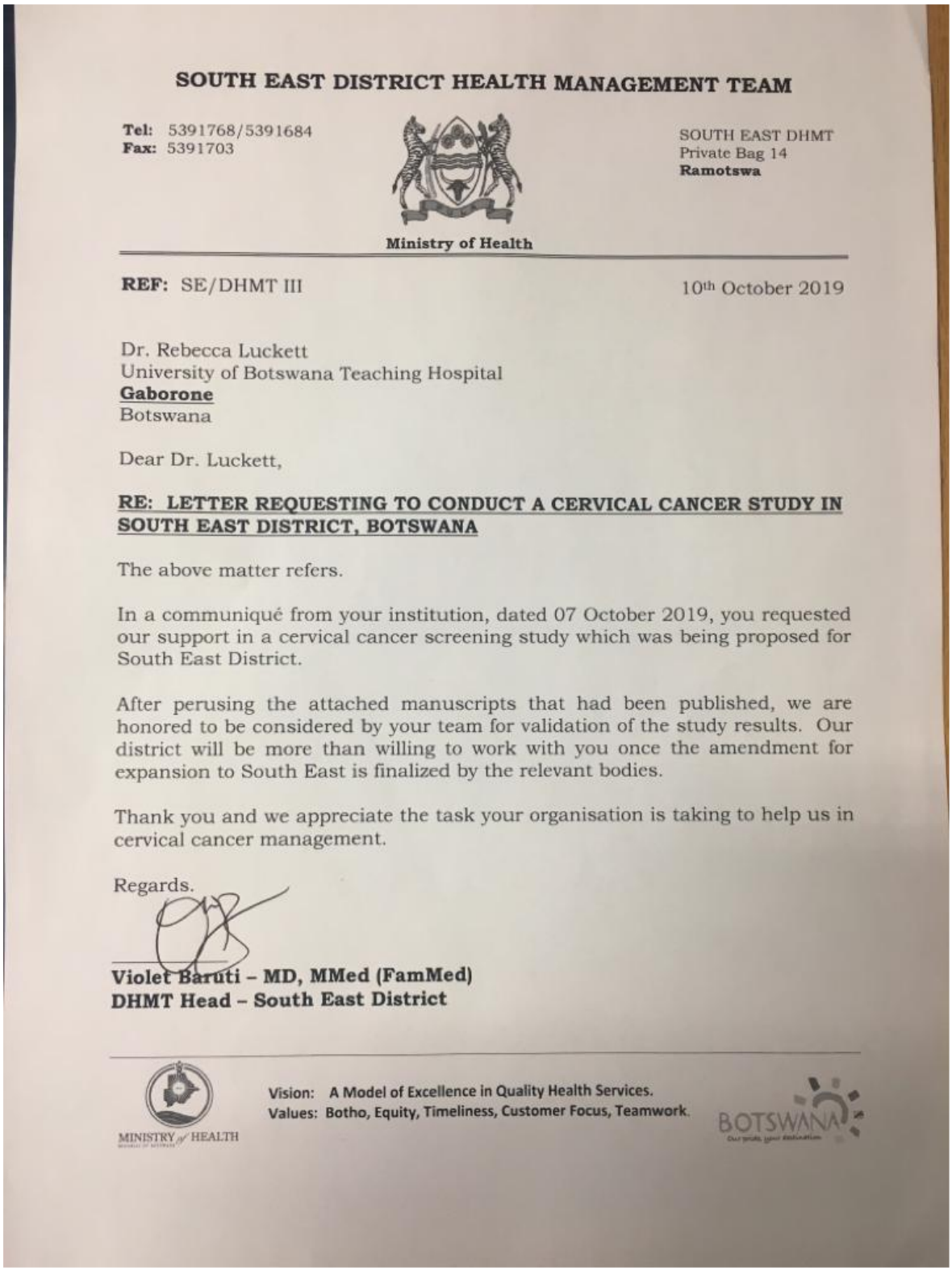
The Office of Research and Development supports continuation of the study.

We will appreciate your assistance and consideration of this application.

Sincerely,

For The Secretariat, University of Botswana Institutional Review Board
Office of Research and Development

Appendix 10. Southeast District letter of support



SOUTH EAST DISTRICT HEALTH MANAGEMENT TEAM

Tel: 5391768/5391684
Fax: 5391703

Ministry of Health

SOUTH EAST DHMT
Private Bag 14
Ramotswa

REF: SE/DHMT III

10th October 2019

Dr. Rebecca Luckett
University of Botswana Teaching Hospital
Gaborone
Botswana

Dear Dr. Luckett,

RE: LETTER REQUESTING TO CONDUCT A CERVICAL CANCER STUDY IN SOUTH EAST DISTRICT, BOTSWANA

The above matter refers.

In a communiqué from your institution, dated 07 October 2019, you requested our support in a cervical cancer screening study which was being proposed for South East District.

After perusing the attached manuscripts that had been published, we are honored to be considered by your team for validation of the study results. Our district will be more than willing to work with you once the amendment for expansion to South East is finalized by the relevant bodies.

Thank you and we appreciate the task your organisation is taking to help us in cervical cancer management.

Regards.

Violet Baruti – MD, MMed (FamMed)
DHMT Head – South East District

MINISTRY of HEALTH

Vision: A Model of Excellence in Quality Health Services.
Values: Botho, Equity, Timeliness, Customer Focus, Teamwork.

BOTSWANA

Appendix 11. Botswana Ministry of Health ethics approval

TELEPHONE: 363 2500
FAX: 317 0155
TELEGRAMS: RABONGAKA
TELEX: 2818 CARE BD

Republic of Botswana

MINISTRY OF HEALTH
PRIVATE BAG 0038
GABORONE

REFERENCE NO: HPRD: 6/14/1 **19th May 2023**

Health Research Development Division

Principal Investigator: Rebecca Luckett

Notification of IRB Review: **Continuing Review**

PROTOCOL TITLE: ASSESSMENT OF THE ACCEPTABILITY AND FEASIBILITY OF SELF-COLLECTED SAMPLES FOR HIGH-RISK HUMAN PAPILLOMAVIRUS (HPV) SCREENING AT TWO WOMEN'S HEALTH CLINICS IN GABORONE, BOTSWANA

Review Type: Expedited/Health Research Development Division
Review Date: 17th May 2023
Approval Date: 19th May 2023
Effective Date: 11th June 2023
Expiration Date: 10th June 2024

This certifies that the continuing review request for the protocol above was reviewed under review procedures. Approval is valid for a period of 1 year.

__X__ Study is continuing.
_____ Open for enrollment
_____ Accrual complete with treatment intervention and /or participant interviews/ surveys continuing.
_____ Subject interventions/data collection ended on (date): _____
_____ Open for analysis only. Expected end date:_____
_____ Competed (including all analysis). Dated completed: _____
_____ Cooperative Review
_____ Other, Please describe: _____

Attachment(s);

- HRDC CR application form
- Protocol
- CVs for Lorato and Thabo
- Current approvals

If you have any questions please do not hesitate to contact Mr Abia Sebaka at asebaka@gov.bw, Tel +267-3632754 or Mr. Kgomotso Motlhanka at kgmmotlhanka@gov.bw, Tel +267-3632751.

Thank you for your cooperation and your commitment to the protection of human subjects in research.

Yours Sincerely

Mr Abia Sebaka
for /PERMANENT SECRETARY

MINISTRY OF HEALTH
RESEARCH DIVISION
19 MAY 2023
P/BAG 0038, GABORONE
HEALTH POLICY RESEARCH & DEVELOPMENT

Vision: *A Healthy Nation by 2023.*
Values: *Botho, Equity, Timeliness, Customer Focus, Teamwork, Accountability.*

BOTSWANA
our pride, your destination

Appendix 12. University of Pretoria ethics approval

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

Institution: The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 18 March 2022 and Expires 18 March 2027.
- IORG #: IORG0001762 OMB No. 0990-0278 Approved for use through August 31, 2023.

Faculty of Health Sciences **Research Ethics Committee**

18 May 2023

Approval Certificate
Annual Renewal

Dear Dr DR Ramogola-Masire,

Ethics Reference No.: 721/2020 – Line 2
Title: Performance of two-stage cervical cancer screening algorithms using primary high-risk human papillomavirus testing in Botswana

The **Annual Renewal** as supported by documents received between 2023-05-02 and 2023-05-17 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on 2023-05-17 as resolved by its quorate meeting.

Please note the following about your ethics approval:

- Renewal of ethics approval is valid for 1 year, subsequent annual renewal will become due on 2024-05-18.
- Please remember to use your protocol number (721/2020) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

On behalf of the FHS REC, Dr R Sommers
MBChB, MMed (Int), MPharmMed, PhD
Deputy Chairperson *of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria*

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health)