The epidemiology of Human Papillomavirus (HPV) infection and epigenetic factors associated with the development of cervical cancer precursor lesions in women living with HIV in Africa

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Declaration

I, Helen Kelly, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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« Comment se fait une vie? Quelle est la part des circonstances, de la nécessité, du hasard, des choix et des initiatives du sujet? »

"How is a life formed? How much of it is made up by circumstance, how much by necessity, how much by chance, and how much by the subject's own options and personal initiatives?"

Simone de Beauvoir, *Tout compte fait* (1972)

Abstract

Background: The relationships between human papillomavirus (HPV) and HIV, in an African context are limited. Given the large number of women living with HIV (WLHIV) accessing antiretroviral therapy (ART), it is important to establish associations of HIV-related factors with high-risk (HR)-HPV and cervical intraepithelial neoplasia (CIN).

Design: Prospective cohort of WLHIV attending HIV treatment centres in Ouagadougou, Burkina Faso (BF) and Johannesburg, South Africa (SA).

Methods: At baseline and endline (median 16 months), cervical samples and biopsies were analyzed for HPV genotyping (InnoLiPA) and by histology. HPV serology targeting 15 HPV types (12 HR) was by multiplexed pseudovirion-based serological assay. Methylation of a human gene *EPB41L3* and HPV16 was measured by pyrosequencing. Logistic regression was used to estimate associations of HIV-related factors with HR-HPV and CIN2+ outcomes.

Results: Among 1238 enrolled WLHIV (BF=615; SA=623) HR-HPV prevalence was 59.1% in BF and 79.1% in SA. CIN2+ prevalence was 5.8% in BF and 22.5% in SA. Prolonged ART use (>2 years) was associated with lower HR-HPV prevalence in BF and lower CIN2+ prevalence compared to short-duration ART users and ART-naïve participants in SA.

Among 963 (77.8%) women seen at endline, HR-HPV persistence was 41.1% in BF and 30.2% in SA; CIN2+ incidence over 16-months was 1.2% in BF and 5.8% in SA. HR-HPV persistence was lower among those with prolonged ART compared to ART-naïve and short-duration ART users. CIN2+ incidence was reduced among women on ART in SA.

HPV seroprevalence and seropersistence were high (93% and 95%, respectively). Seroconversion was 23.1%, and was higher among recent ART users (≤2 years) and among those with type-specific DNA persistence compared to those who cleared infection.

The human gene *EPB41L3* showed elevated methylation in CIN2+ compared to ≤CIN1 (Mann Whitney U p=<0.001) at baseline. Higher methylation levels were found among recent ART users (≤2 years) and women with CD4 ≤200 cells/mm^3.

Conclusion: WLHIV in BF and SA have high rates of HR-HPV and CIN2+, and WLHIV in SA have higher CIN2+, linked with poorer control of HIV and higher frequency of cofactors for HR-HPV and CIN2+. Prolonged and effective ART is important in controlling HR-HPV and the development of CIN2+. WLHIV are infected with multiple HR-HPV types and there is limited evidence that HPV antibodies protect against same-type reinfection. WLHIV may benefit from vaccination using a multivalent vaccine. DNA methylation of a tumour suppressor gene *EPB41L3* is elevated among women with CIN2+ and shows promise as a biomarker test for CIN2+ prediction among WLHIV.

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List of Acronyms

AFM	Age at first marriage
AFP	Age at first pregnancy
AFSI	Age at first sexual intercourse
AGUS	atypical glandular cells of underdetermined significance
AGW	Anogenital warts
AIDS	Acquired immune deficiency syndrome
AHR	Adjusted Hazard Ratio
AOR	Adjusted Odds Ratio
APR	Adjusted Prevalence Ratio
ARR	Adjusted Risk Ratio/adjusted Relative Risk
ART	Antiretroviral therapy
ARV	Antiretroviral
APC	Antigen presenting ell
ASCUS	Atypical squamous cells of undetermined significance
ASR	Age standardised rate
AUC	Area under the receiver operating characteristic
BF	Burkina Faso
BV	Bacterial vaginosis
cART	Combination antireroviral therapy
CERBA	Centre de Recherche Biomoléculaire Pietro Annigoni
CI	Confidence interval
CIN	Cervical intraepithelial neoplasia
CIN2+	High grade cervical intraepithelial neoplasia (Grade 2+)
CIS	Carcinoma *in situ*
CRF	Case Report Form
CRIS-UO	Centre de Recherches Internationales pour la Santé, Université de Ouagadougou (Burkina Faso)
CT	*Chlamydia trachomatis*
CVL	Cervicovaginal lavage
DC	Dendritic Cell
DMPA	Depot medroxyprogesterone acetate
DNA	Deoxyribonucleic acid

ELISA	Enzyme-linked immunosorbent assay
EU	European Union
FTP	Full-term pregnancy
GCP	Good Clinical Practice
GDP	Gross Domestic Product
GAVI	Global Alliance for Vaccines and Immunization
HAART	Highly active antiretroviral therapy
HARP	HPV in Africa Research Partnership
HC	Hormonal Contraception
HC2	Hybrid Capture 2
HIV	Human immunodeficiency virus
HPV	Human Papillomavirus Virus
HR	Hazard Ratio
HR-HPV	High Risk Human Papillomavirus Virus
HSIL	High-grade squamous intraepithelial lesions
HSV-2	Herpes simplex virus type-2
IARC	International Agency for Research on Cancer
IC	Injectable contraception
ICC	Invasive Cervical Cancer
ICF	Informed Consent Form
IFN	Interferon
IL	Interleukin
IQR	Interquartile range
IRR	Incidence Rate Ratio
LEEP	Loop Electrosurgical Excision Procedure
LLETZ	Large loop excision of the transformation zone
LMICs	Low and middle income countries
LR-HPV	Low risk Human Papillomavirus Virus
LSHTM	London School of Hygiene and Tropical Medicine
LSIL	Low-grade squamous intraepithelial lesions
LTSP	Lifetime number of sex partners
MC	Male Circumcision
MFI	Mean fluorescence intensity unit
MG	*Mycoplasma genitalium*

MHC	Major histocompatibility complex
mRNA	Mitochondrial ribonucleic acid
MRC	Medical Research Council
MSP	Methylation specific polymerase chain reaction
MVA	Multivariate analysis
NAAT	Nucleic acid amplification test
NET-EN	Norethisterone enanthate
NG	*Neisseria gonorrhoea*
NGO	Non-governmental organisation
NGS	Next generation DNA sequencing
NK	Natural Killer cells
OC	Oral contraception
OR	Odds Ratio
ORF	Open Reading Frame
PCR	Polymerase Chain Reaction
PLHIV	People living with HIV
POC	Point-of-care
PR	Prevalence Ratio
PSQ	Pyrosequencing
PVL	Plasma Viral Load
QC	Quality Control
qMSP	Quantitative methylation specific polymerase chain reaction
QMUL	Queen Mary University of London
RNA	Ribonucleic acid
ROC	Receiver operating characteristic
RPR	Rapid plasma reagin
RR	Risk Ratio/Relative Risk
SA	South Africa
SCJ	Squamocolumnar junction
SIL	Squamous Intraepithelial Lesions
SIR	Standardised Incidence Ratio
SOP	Standard operating procedure
SSA	Sub-Saharan Africa
STI	Sexually transmitted infection

STIRC	Sexually Transmitted Infections Reference Centre
TGF	Tumour Growth Factor
Th1	T-helper 1 immune response
Th2	T-helper 2 immune response
TLR	Toll-like receptors
TNF	Tumour necrosis factor
TPHA	*Treponema pallidum* haemagglutination assay
TSG	Tumour Suppressor Gene
TV	*Trichomonas vaginalis*
TZ	Transformation Zone
UM-1	Université of Montpellier 1
UN	United Nations
UNAIDS	The Joint United Nations Programme on HIV/AIDS
URR	Upstream regulatory region
VCT	Voluntary HIV counselling and testing
VI	Visual Inspection
VIA	Visual Inspection using Acetic Acid
VILI	Visual Inspection using Lugol's Iodine
VLP	Virus-like particle
VMMC	Voluntary Medical Male Circumcision
WITS-RHI	University of Witwatersrand, Reproductive Health & HIV Institute, (Johannesburg, South Africa)
WLHIV	Women living with HIV
WHO	World Health Organization
WHS	WHO Household Survey

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This thesis would not have been possible without the support and guidance from my supervisor Philippe Mayaud. Philippe's generosity of time and ideas over the last four years has provided motivation and inspiration for the road ahead in research.

I wish to thank my advisors on the advisory panel; Helen Weiss, Michel Segondy and Silvia de Sanjose. In particular, I would like to thank Attila Lorincz for extending an invitation to work in the lab at the Wolfson Institute of Preventive Medicine. Their combined expertise and guidance was invaluable in achieving the work in this thesis.

I would like to acknowledge colleagues with whom I worked in the HARP study; in Ouagadougou: Nicolas Meda, Bernard Sawadogo, Olga Lompo, Souleymane Zan and Aminata Pare; in Johannesburg: Sinead Delany-Moretlwe, Admire Chikandiwa, Tanvier Omar and Pam Michelow; in Montpellier: Nicolas Nagot, Jean Ngou, Marie-Noelle Didelot, Sylviane Doutre and Valerie-Costes, and in London: Clare Gilham, Lorna Gibson and Frankie Liew. The HARP study was an enormous team effort, and I feel very fortunate to have worked with such a diverse and expert team.

Charles Lacey at the University of York was as an advisor to the HARP study and provided some important insights and suggestions during the early stages of this thesis, for which I am very grateful. I would also like to thank Helena Faust and Joakim Dillner at the Karolinska Institute, and Rhian Warman, Natasa Vasiljevic and Dorota Scibior-Bentkowska for providing valuable training and support at the Wolfson Institute of Preventive Medicine.

Research in public health is guided by a principal of improving quality of life and healthcare for all, and I hope the work in this thesis does some credit to the women who dedicated their time to participate in the HARP study.

1 INTRODUCTION

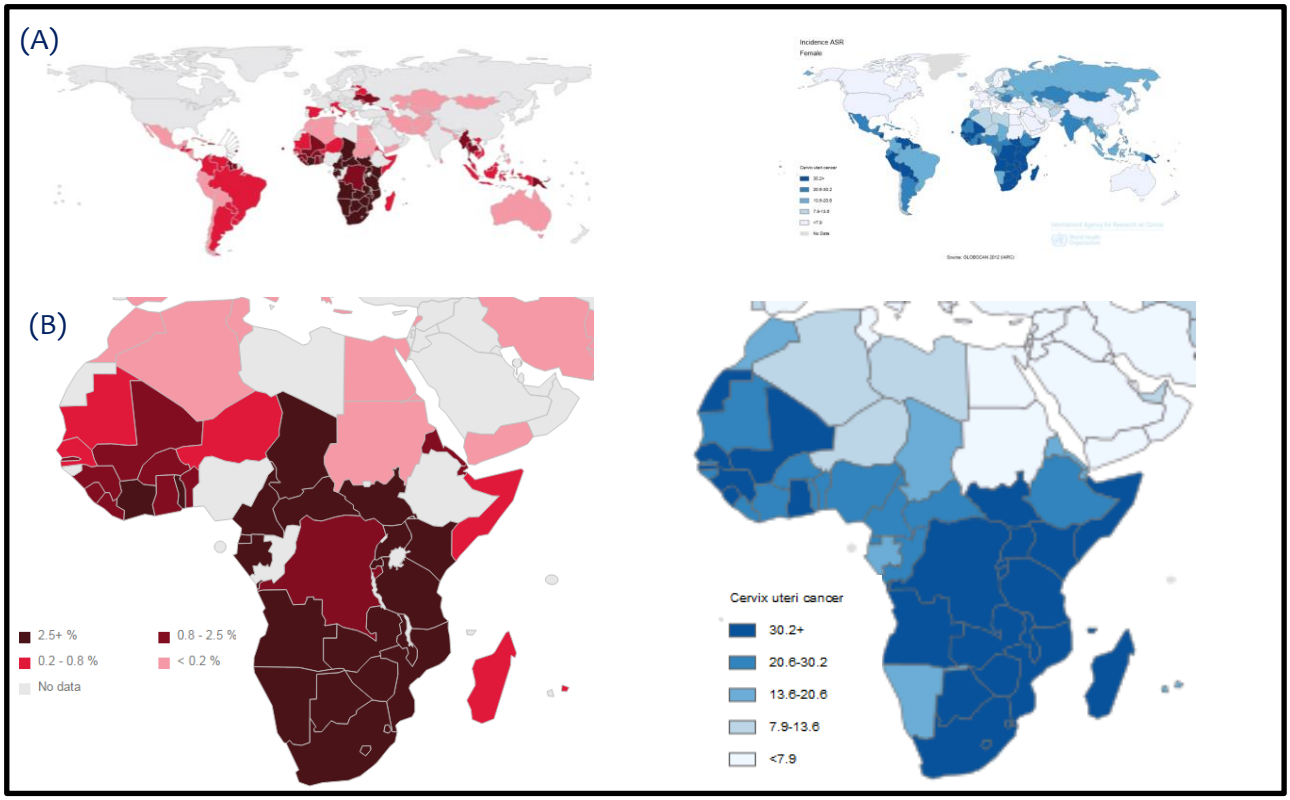
1.1 Background and Rationale for the thesis

Invasive cervical cancer (ICC) is the most common female cancer in low and middle-income countries [1], where 85% of the estimated 500,000 global annual cases occur [1, 2] and is the leading cause of cancer deaths among African women [3]. Cervical intraepithelial neoplasia (CIN) is an early stage of cancer of the cervix, which is curable if detected early. There is international consensus that persistent infection by HPV types designated as high-risk (HR), including genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68, is necessary for the development of squamous intraepithelial lesions (SIL) and cervical intraepithelial neoplasia (CIN) [4, 5]. SIL of low or high grade represent abnormal growth of squamous epithelial cells in a cytology reading, while CIN grades 1 to 3 describe the proportion and thickness of abnormal cells in a cervical biopsy section.

Cervical cancer incidence and mortality have declined in many high-income countries over the past 40 years as a result of widespread routine screening, treatment of precancerous lesions and improved socioeconomic status [6]. HPV vaccines targeting HR types associated with ICC (HPV16/18/31/33/45/52/58) and two low risk types implicated in anogenital warts (HPV 6 and 11) are licensed for use among girls from the age of nine years, and are widely implemented in high-income settings. However, ICC incidence and mortality remains high in low and middle-income countries (LIMC), where few have organised screening services and HPV vaccination programmes in place. Furthermore, the availability of services for the management of ICC is limited due to lack of skilled staff, surgical equipment and radiation facilities [3].

The Sub-Saharan African (SSA) region has both the highest incidence of ICC and the highest prevalence of HIV (**Figure 1.1**). ICC ranks as one of the most common neoplasms among women living with HIV (WLHIV) and is considered an AIDS-defining illness [7, 8]. Opportunities for control, early detection and prevention of HPV and related cervical neoplasia among WLHIV are available, although they are limited or inadequately implemented.

Figure 1.1. HIV prevalence among women aged 15-49 years (red) and age-standardised incidence of cervical cancer (blue) worldwide (panel A) and in Africa (panel B)



Cervical cancer incidence rates from IARC GLOBOCAN 2012 [9]; prevalence of HIV from UNAIDS, 2015 [10]; *ASR=Age-standardised rate

With the advent of the antiretroviral therapy (ART) era, the incidence of other AIDS-defining cancers has decreased, however the impact on cervical cancer and its precursor lesions is less clear. In a recent systematic review of observational studies evaluating the incidence of malignancies before and after the introduction of ART in people with HIV/AIDS, the risk for the development of Kaposi's sarcoma decreased by 70% (Risk Ratio [RR]=0.30, 95% Confidence Interval [CI]: 0.28-0.33) and 48% for non-Hodgkin's lymphoma (RR=0.52, 95% CI: 0.48-0.56), but a 1.5-fold increased risk was observed for invasive cervical cancer (RR=1.46, 95% CI: 1.09-1.94)[11]. As ART becomes more widely available in Africa, longer survival times may increase the rates of cervical cancer as many women remain susceptible to HR-HPV acquisition and persistence and consequently are at an increased risk for CIN or SIL incidence and progression. Given the large number of WLHIV now accessing ART in Africa, it is important to establish the impact that ART and other HIV-related factors have on the

natural history of HPV infection and CIN progression, as this may influence cervical cancer control strategies.

Current HPV vaccines offer potential for cervical cancer prevention by targeting the HR-HPV types associated with ICC. Although there are no data yet available on HPV vaccine efficacy among HIV-positive persons, HPV vaccines have been reported to be safe and immunogenic in HIV-positive children, female adolescents and adults [12]. Recent meta-analyses have shown that the HPV types associated with high-grade SIL (≥HSIL) and ICC are similar among WLHIV and HIV-negative women [13, 14]. However, data on HPV genotypes associated with CIN grade 2 and 3 (CIN2/3) are limited among WLHIV in SSA settings. It is important to assess the distribution of HPV genotypes in cervical precursor lesions of WLHIV, in order to establish the potential impact of HPV vaccination on this population.

Furthermore, the seroepidemiology of a wide range of vaccine types and evaluation of current and past exposure has seldom been estimated among WLHIV, particularly in SSA. There is also no information on antibody responses to natural infection observed longitudinally among WLHIV. An improved understanding of 1.) exposure to vaccine-related HPV types, and 2.) whether past exposure protects against same-type re-infection would be useful to understand the value of HPV vaccines in this population.

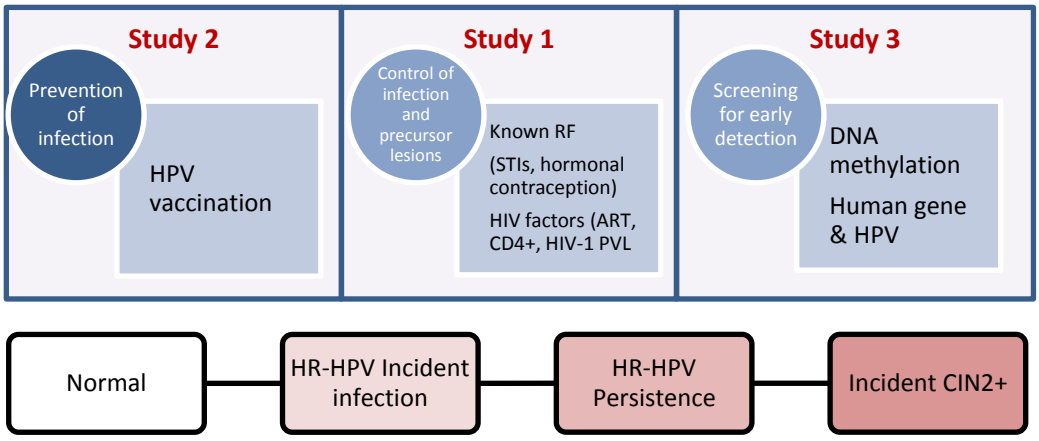
Finally, there is a need for better biomarkers for earlier detection of CIN grade 2 and higher (CIN2+), in order to avoid late stage development and subsequent expensive colposcopy/biopsy and treatment, particularly in low resource settings [1]. Epigenetic mechanisms, such as DNA methylation of human genes and the HPV virus itself have recently been shown to distinguish non-progressive HPV infections from those that progress to cancer among HIV-negative women [15, 16]. DNA methylation has rarely been studied among WLHIV [17], and its association with CIN2+, and the influence of HIV-related factors has never before been prospectively evaluated.

1.2 Aims and Objectives of the Thesis

Aims

In a cohort of women living with HIV-1 enrolled in the *HARP* (HPV in Africa Research Partnership) study in Burkina Faso and South Africa and followed-up over 18 months, I aim to evaluate the influence of HIV-related factors (ART, CD4+ cell count and HIV-1 plasma viral load [PVL]) and other known risk factors on the epidemiology of HR-HPV and high-grade cervical intraepithelial neoplasia (CIN2+) (**STUDY 1**); to evaluate the prevalence, incidence and persistence of HPV vaccines types and their association with CIN2+ (**STUDY 2**) and to investigate the association of human gene and HPV DNA methylation markers with CIN2+ (**STUDY 3**).

Each study addresses the value of different control measures that could be used in the pathway from HPV infection to CIN2+ as depicted in the box below, including: the prevention of HR-HPV infection through HPV vaccination (**STUDY 2**), control of HR-HPV infection and lesion development in part through ART use and control of other cofactors, including sexually transmitted infections (STIs) (**STUDY 1**) and detection of CIN2+ once established (**STUDY 3**).



Specific Objectives

Two systematic reviews will be performed with the following objectives (summarised in **Table 1.1**):

1.1 To evaluate the association of ART with HR-HPV prevalence, and with cervical lesion prevalence, incidence, progression and regression (**Chapter 3**)

1.2 To summarise the association of DNA methylation (of human genes and HPV16) with cervical intraepithelial lesions and invasive cervical cancer (**Chapter 4**)

Three studies will be conducted to address the following objectives (summarised in **Table 1.1**):

STUDY 1 (Chapters 6 and 7)

Using cross-sectional and prospective data collected in the HARP study, this study will describe:

[**Chapter 6**]**:**

2.1 the prevalence and persistence of HR-HPV infection;

2.2 the prevalence and incidence of CIN2+;

2.3 the associations of HR-HPV and CIN2+ prevalence with socio-demographic, behavioural and sexual behavioural factors, and other STIs;

[**Chapter 7**]:

2.4 the associations of HR-HPV prevalence and persistence, and CIN2+ prevalence and incidence with HIV-related factors (ART, CD4+ cell count, HIV-1 PVL).

STUDY 2 (Chapters 8 and 9)

Using cross-sectional and prospective data collected in the *HARP* study, this study will describe:

[**Chapter 8**]:

3.1 the prevalence, incidence and persistence of individual HPV genotypes;

3.2 their association with prevalent and incident CIN grade 2 and higher (CIN2+), and persistent and regressed CIN2+;

3.3 the associations of specific HPV types with ART, CD4+ cell count and HIV-1 PVL.

Using cross-sectional and prospective data collected in the *HARP* study (in South Africa only), this study will describe [**Chapter 9**]:

3.4 the seroprevalence of HPV types and association with socio-demographic, behavioural and sexual behavioural factors, and other STIs;

3.5 HPV type seroincidence and seroconversion and determinants, including HPV DNA status at baseline and endline, HIV-related factors and other known risk factors;

3.6 the risk of type specific reinfection according to same type seropositive at baseline.

STUDY 3 (Chapter 10)

In a nested case-control study using samples collected at baseline and endline from among the *HARP* participants, this study will:

4.1 determine the association of baseline methylation of a human gene *EPB41L3* and HPV16 with prevalent CIN2+, and *EPB41L3* baseline and endline methylation with incident CIN2+;

4.2 determine whether *EPB41L3* methylation levels at enrolment are influenced by HIV related factors (ART, CD4+ cell count, HIV-1 PVL), and other risk factors;

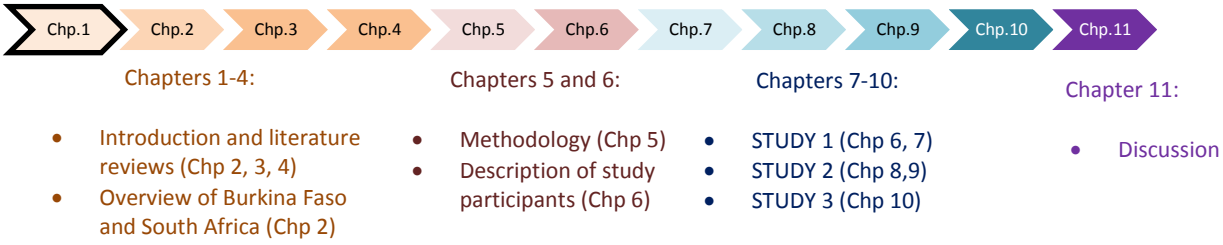
4.3 monitor the change in *EPB41L3* methylation levels over time among CIN2/3 that persist or regress.

Table 1.1 Project summary

Objective		Outcomes	Key exposure	Chapter and manuscript
SYSTEMATIC REVIEWS				
1.1	To evaluate the association of ART with HR-HPV prevalence, and with cervical lesion prevalence, incidence, progression and regression	• HR-HPV prevalence • HSIL+/CIN2+ prevalence • SIL incidence • SIL progression • SIL regression	ART	**Chapter 3** **[PAPER 1]**
1.2	To summarise the association of DNA methylation (human genes and HPV16) with cervical intraepithelial lesions and invasive cervical cancer	• CIN2+	DNA methylation of human genes and HPV16	**Chapter 4**
STUDY 1: Epidemiology of HPV infection and cervical lesions in WLHIV in Burkina Faso and South Africa over 16 months				
2.1	To describe the prevalence and persistence of HR-HPV	• HR-HPV prevalence • HR-HPV persistence	n/a	**Chapter 6** **[PAPER 2]**
2.2	To describe the prevalence and incidence of CIN2+	• CIN2+ prevalence • CIN2+ Incidence	n/a	
2.3	To evaluate socio-demographic, sex behaviour risk factors, and other STIs for HR-HPV prevalence and CIN2+ prevalence	• HR-HPV prevalence • CIN2+ prevalence	Known risk factors for HR-HPV and CIN2+	**Chapter 6**
2.4	To evaluate the association of HPV prevalence and persistence with HIV-related risk factors	• HR-HPV prevalence • HR-HPV persistence	ART CD4+ cell count HIV-1 PVL	**Chapter 7** **[PAPER 2]**
	To evaluate the association of CIN2+ prevalence and incidence with HIV-related risk factors	• CIN2+ prevalence • CIN2+ Incidence	ART CD4+ cell count HIV-1 PVL	
STUDY 2: HPV type specific infection and serodynamics among WLHIV in Burkina Faso and South Africa over 16 months				
3.1	To describe the prevalence, incidence and persistence of individual genotypes	• Prevalence, incidence and persistence of individual genotypes	n/a	**Chapter 8** **[PAPER 3]**
3.2	To assess the associations of prevalent HPV genotypes with prevalent CIN2+	• CIN2+ prevalence	Prevalent HPV genotypes	
	To assess the associations of persistent HPV genotypes with incident CIN2+.	• CIN2+ incidence	Persistent HPV genotypes	
3.3	To evaluate the association of individual HPV genotype prevalence with HIV-related risk factors	• Prevalence of individual genotypes	ART CD4+ cell count HIV-1 PVL	
(sub analysis in South Africa only)				
3.4	To describe the seroprevalence of HPV genotypes and its determinants	• HPV type-specific (and combined) seroprevalence	n/a	**Chapter 9** **[PAPER 4]**
3.5	To describe type-specific HPV seroincidence and seroconversion and its determinants	• Type-specific HPV seroconversion	ART CD4+ cell count HIV-1 PVL	
3.6	Risk of type-specific re-infection following same-type seropositivity at baseline	• Type-specific DNA incidence	Same-type seropositive at baseline	
STUDY 3: Association of DNA methylation with prevalent and incident CIN2+ among WLHIV in Burkina Faso and South Africa				
4.1	To determine the association of DNA methylation of a human gene *EPB41L3* and HPV16 with CIN2+ prevalence	• CIN2+ prevalence	*EPB41L3* methylation at baseline	**Chapter 10** **[PAPER 5]**
	To determine the association of baseline and endline *EPB41L3* DNA methylation with CIN2+ incidence	• CIN2+ incidence	*EPB41L3* methylation at baseline and endline	
4.2	To evaluate the role of HIV-related and other risk factors on the *EPB41L3* DNA methylation at enrolment	• 'High' *EPB41L3* DNA methylation	ART CD4+ cell count HIV-1 PVL	
4.3	To monitor the change in *EPB41L3* DNA methylation levels over time among CIN2+ that progress vs. regress	• CIN2+ progression • CIN2+ regression	*EPB41L3* methylation at baseline and endline	

1.3 Structure of the thesis

The thesis is organised in 4 distinct sub-sections, as depicted below.



This thesis starts in **Chapter 1** by providing the rationale and a summary of the aims and objectives of this research, as well as the role of the candidate in the research activities.

Chapters 2, 3 and 4 are review chapters setting out what is already known of HPV and related cervical disease in WLHIV. **Chapter 2** provides an overview of the epidemiology of HPV infection and related cervical disease among WLHIV in comparison to HIV-negative women. This chapter also summarises data on cervical cancer incidence globally, as well as in the Sub-Saharan Africa (SSA) region, and more specifically in Burkina Faso and South Africa, the two countries which this research is set. In order to put this research in context in the two countries, **Chapter 2** also provides a brief overview of the two countries using demographic and economic data from the World Bank, HIV related information using indicators from UNAIDS and finally details on cervical cancer screening strategies and HPV vaccination programmes in each of these two countries.

On reviewing the literature on the associations of HIV-related factors such as ART and CD4+ count on HPV infection and related cervical lesions, the data was found to be limited and often conflicting and inconsistent. Therefore, **Chapter 3** is a systematic review and meta-analysis of data for the association of ART on HPV and cervical lesions. The literature on DNA methylation makers associated with cervical lesions was similarly heterogeneous and **Chapter 4** is a systematic review on this topic. The findings of these reviews helped to identify the main research gaps which inform the objectives of this research and help put the research findings in this thesis into context.

Chapter 5 summarises the methodologies used as part of this research thesis, and provides a full description of the *HARP* study design and methods within which this thesis is nested.

Chapter 6 provides a summary of the HARP study participants at enrolment and at the follow-up. This includes a description of the socio-demographic and behavioural factors, sexual behaviour, STI screening results, cervical screening history and HIV-related factors. It also describes the HR-HPV prevalence, incidence and persistence, and CIN2+ prevalence and incidence in these populations.

Chapters 6 to 10 are dedicated to the analyses of this data. Each chapter focusses on different steps in the pathway from HPV acquistion to cervical lesion progression. **Chapter 6** investigates the association of known risk factors (socio-demographic, behavioural, sexual behaviour, STIs) on HR-HPV prevalence, incidence, persistence and clearance and CIN2+ prevalence and incidence, while **Chapter 7** investigates the association of HIV-related factors (ART, CD4+ cell count and HIV-1 PVL) on the these outcomes.

In order to understand the potential impact of HPV vaccines on high-grade cervical lesions, **Chapter 8** describes the prevalence, incidence and persistence of individual HPV genotypes and investigates their association with CIN2+ prevalence and incidence. In order to understand past exposure of WLHIV to HPV infection and the serodynamics of HPV infection among WLHIV, **Chapter 9** describes the type-specific seroprevalence of HPV genotypes, the seroconversion rates at follow-up following infection at baseline and re-infection rate among those with type-specific antibodies at follow-up. This chapter also investigates the association of HIV-related factors, such as ART, CD4+ cell count and HIV-1 PVL, with HPV seroconversion.

Finally, in order to further understand the role of epigenetic markers such as human gene DNA methylation on the natural history of lesion progression, **Chapter 10** investigates the association of human gene *EPB41L3* and HPV16 methylation with CIN2+ prevalence and incidence and explores *EPB41L3* methylation changes over time among those that develop incident lesion, those that progress and spontaneously regress.

The final section of this thesis, **Chapter 11,** summarises the key research findings and discusses the public health implications in the context of screening and vaccination.

1.4 Role of the Candidate

This PhD was undertaken part-time as a staff member at LSHTM. My salary was funded through a combination of sources –the European Union (EU) FP7 programme and the Medical Research Council (MRC) PHINDS programme, within which the individual research studies of this thesis are embedded. My role in each of these projects is detailed below, and summarised in **Table 1.2**.

HARP (HPV in Africa Research Partnership) EU FP7 Framework funding (grant No. HEALTH-2010-F2-265396*)*

As part of the HARP study, I acted as the study coordinator between 2010 and 2014, and worked closely with the Chief Investigator (Prof Philippe Mayaud) and the study statistician (Prof Helen Weiss) at LSHTM and the local study investigators in Burkina Faso (Prof Nicolas Meda), South Africa (Prof Sinead Delany-Moretlwe) and France (Dr Michel Segondy and Prof Nicolas Nagot) during the finalization of the study research protocol, Informed Consent Forms (ICFs), Case Report Forms (CRFs) and Standard Operating Procedures (SOPs). I have monitored and reported on the progress of enrolment and retention of participants throughout the study period, and monitored and reported on any Adverse Events related to the study procedures, in liaison with an External Clinical Monitor. I have undertaken site visits to ensure good research and implementation according to

Good Clinical Practice (GCP) standards. I was involved in the development of the analysis plans for the cross-sectional and longitudinal studies for the HPV and CIN related analyses.

HPV vaccination among WLHIV study - (funded by UK MRC, PHINDS scheme, Grant PH01/14-39)

This study was undertaken between March to September 2015. As part of this study, I worked with infectious disease modellers at Imperial College and provided them with HPV type-specific infection data from the HARP study for estimation of genotype attribution to CIN2+ in order to model the potential impact of HPV vaccines among WLHIV in South Africa. In addition to this, serology samples from the HARP participants were sent to the pathology laboratory of Prof Joakim Dillner at Karolinska Institute, Stockholm, Sweden, for HPV serology testing to measure the past exposure of HPV types in this population. I was involved in the study design and co-wrote the proposal with the PI Prof Philippe Mayaud and co-Investigator Prof Sinead Delany-Moretlwe.

DNA methylation study

From July to September 2015, I performed the DNA methylation assays using cervical samples from the HARP participants, at the laboratory of Prof Attila Lorincz at the Wolfson Institute of Preventive Medicine, Queen Mary University of London (QMUL).

Table 1.2. Candidate's role in the thesis

Study	Candidate's Role
Paper 1 (Chapter 4): Antiretroviral therapy, high-risk human papillomavirus and cervical intraepithelial neoplasia: a systematic review and meta-analysis	• *Study design*: HK conceptualised the study together with PM and SDS, and HK developed the research protocol • *Ethical approval*: none required • *Data extraction and cleaning*: HK extracted all data, and 25% of all data was blindly extracted by HW • *Statistical analysis and interpretation*: HK performed all analyses with input from HW and YBM • *Final manuscript*: HK wrote first draft with contributions from PM, SDS, HW and YBM • *Status of manuscript*: **Re-submitted following Reviewer comments with *Lancet HIV***
Paper 2 (Chapters 6 and 7): Epidemiology of High-risk Human Papillomavirus and Cervical Lesions in African women living with HIV/AIDS: Effect of Anti-Retroviral Therapy	• *Study design*: HARP investigators designed the HARP study: PM, SDM, NM, HW, MS, NN • *Ethical approval*: PM, SDS and NM applied and gained ethical approval from the Ministry of Health in Burkina Faso, the Witwatersrand University in South Africa, and the London School of Hygiene and Tropical Medicine (LSHTM) • *Study coordination:* HK acted as the international study coordinator and worked closely with local study coordinators BS and AC • *Data extraction and cleaning*: Data management teams in local sites performed data entry and quality checks. HK monitored data entry monthly • *Statistical analysis and interpretation*: HK performed all analyses with input from HW and CG • *Final manuscript*: HK wrote first draft with contributions from PM, HW, SDM, MS, NN • *Status of manuscript*: **Accepted and published in *AIDS* (November 2016)**
Paper 3 (Chapter 8): Associations of human papillomavirus (HPV) genotypes with high grade cervical lesions in a cohort of women living with HIV/AIDS in Africa	• *Study design*: HARP investigators designed the HARP study: PM, SD, NM, HW, MS, NN • *Ethical approval*: PM, SDS and NM applied and gained ethical approval from the Ministry of Health in Burkina Faso, the Witwatersrand University in South Africa, and the LSHTM • *Study coordination:* HK acted as the international study coordinator and worked closely with local study coordinators BS and AC • *Data extraction and cleaning*: Data management teams in local sites performed data entry and quality checks. HK monitored data entry monthly • *Statistical analysis and interpretation*: HK performed all analyses with input from HW and CG • *Final manuscript*: HK wrote first draft with contributions from PM, HW, SDM, MS, NN • *Status of manuscript*: **Accepted and published in *PLoS One (March 2017)***
Paper 4 (Chapter 9): Type-specific serologic responses to human papillomaviruses among women living with HIV in South Africa: seroprevalence, seroconversion and risk of re-infection	• *Study design*: HK, PM and SDM wrote the proposal and submitted to MRC PHINDS; use of HARP samples for HPV serology • *Ethical approval*: HK, PM, SDM gained ethical approval from the Witwatersrand University in South Africa, and the LSHTM • *Data extraction and cleaning*: HK received data from KI, extracted and cleaned data for entry in HARP database • *Laboratory testing*: HF performed all laboratory testing at KI • *Statistical analysis and interpretation*: HK performed all analyses with input from HW and HF • *Final manuscript:* HK wrote first draft with contributions from PM, HW, JD and HF • *Status of manuscript*: **Co-author review**
Paper 5 (Chapter 10): Associations of *EPB41L3* DNA methylation with cervical lesions in women living with HIV-1 in Burkina Faso and South Africa	• *Study design*: HK conceptualised the study together with PM and AL, and HK developed the research protocol • *Ethical approval*: HK, NM and SDM gained ethical approval from the Ministry of Health in Burkina Faso, the Witwatersrand University in South Africa, and the LSHTM • *Laboratory testing:* HK performed all laboratory testing with assistance from RW and NV at QMUL • *Data extraction and cleaning*: HK conducted all data entry and cleaning • *Statistical analysis and interpretation*: HK performed all analyses with input from AL • *Final manuscript*: HK wrote first draft with contributions from AL, PM, MS • *Status of manuscript*: **Co-author review**

PM=Philippe Mayaud; SDS=Silvia de Sanjose; HW=Helen Weiss; YBM=Yolanda Buenevente-Moreno; SDM=Sinead Delany-Moretlwe; NM=Nicolas Meda; MS=Michel Segondy; NN=Nicolas Nagot; CG=Clare Gilham; BS=Bernard Sawadogo; AC=Admire Chikandiwa; KI-Karolinska Institute, Sweden; JD=Joakim Dillner; HF=Helena Faust; AL=Attila Lorincz; RW=Rhian Warman; NV=Natasa Vasiljevic

1.5 Collaborating institutions and the *HARP* study

This study is nested within a larger prospective study which aimed to evaluate cervical cancer screening strategies among WLHIV. This EU-funded study called *HARP* (*HPV in Africa Research Partnership*) was undertaken in two African countries – Burkina Faso and South Africa - with different HIV epidemics, burdens of HPV infection and cervical cancer, and approaches to screening for cervical cancer. This allows the findings to be extended to a range of countries and settings in the region. The HARP study, including its methodology, is described in detail in **Chapter 5**, but is briefly described here.

The HARP study evaluated cervical cancer screening strategies against histological (CIN) and cytological (SIL) endpoints in a cohort of 1238 WLHIV (two thirds of whom were taking ART) in Burkina Faso and South Africa. The objective of HARP was to improve cervical cancer prevention programmes for WLHIV in South Africa and Burkina Faso, by evaluating the effectiveness and cost-effectiveness of alternative screening strategies, and by developing algorithms leading to earlier detection and management of cervical cancer in these high-risk populations.

The HARP study was conducted at one study site in each country, and consisted of three interlinked sub-studies:

- A cross-sectional study of HPV and cervical neoplasia screening among HIV-1 seropositive women attending HIV care centres in Burkina Faso and South Africa.
- A prospective cohort study of HIV-1 seropositive women recruited in Study 1 including women without advanced histological lesions (≤CIN1), and women with CIN2+ up to 18 months (which was reduced to 16 months because of project constraints).
- A health economics and modelling study using data from the cross sectional and cohort studies to determine cost-effectiveness of the various screening strategies in preventing cervical cancer cases.

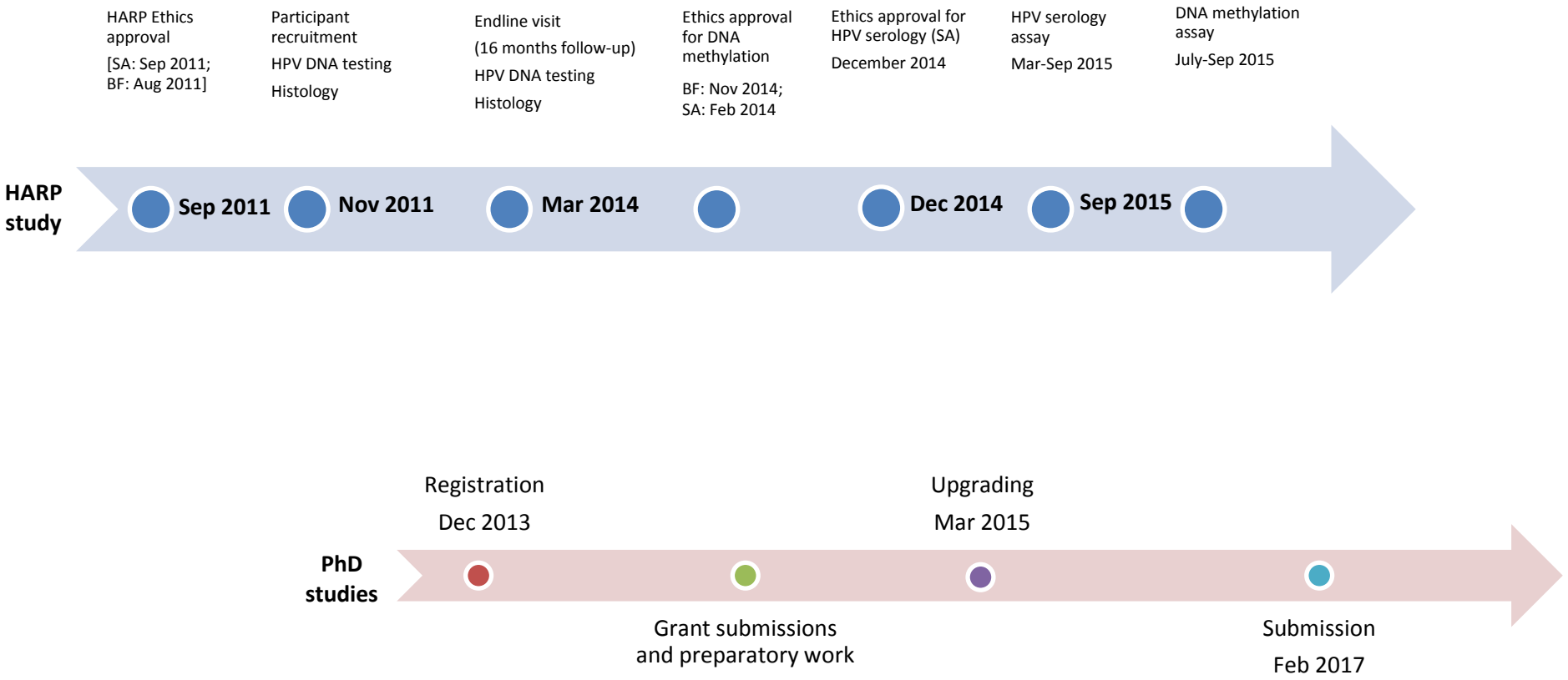
This thesis used secondary quantitative longitudinal data collected between December 2011 and August 2014 recorded during participant visits to the local clinics in Burkina Faso and South Africa as part of the HARP study and stored in the HARP database at the LSHTM. Histological measurements, in addition to data on HPV genotyping data, socio-demogepahic, behavioural, sex behaviour, STI screening results and HIV related factors, such as ART status, ART duration, CD4+ cell count and HIV-1 PVL were used during the analysis (**Chapters 6, 7 and 8**).

As part of a connected study to inform the development of an HPV vaccine study among WLHIV in South Africa and funded by the UK MRC PHINDS programme, analyses were later performed using serum collected from the HARP study participants for HPV serology performed at the Karolinska Institute in Stockholm, Sweden (**Chapter 9**).

A collaboration between LSHTM and Queen Mary University of London (QMUL) involved the use of HARP cervical specimens for DNA methylation analysis (**Chapter 10**).

The timeline of HARP activities and PhD activities is summarised in **Figure 1.2.**

Figure 1.2. Time line of PhD and HARP study



1.6 Academic Support

My supervisor is Prof Philippe Mayaud, Professor of Infectious Diseases & Reproductive Health at LSHTM and was PI of the HARP study. He has considerable research experience in the field of epidemiology and control of HIV and STIs in developing countries. He has successfully supervised over 10 PhD candidates.

Prof Helen Weiss, PhD, Professor in Epidemiology at LSHTM provided statistical overview. She has designed and analysed many epidemiological studies and trials of HIV prevention and was a Co-PI on the HARP study. She has collaborated with Prof Mayaud on many HIV/STI intervention trials. She has supervised 7 PhD students.

The Scientific Advisory Board was formed of the following people:

Dr Michel Segondy, PhD, is Associate Professor at the Faculty of Medicine and University Hospital and is the head of the Virology Laboratory at CHU Arnaud de Villeneuve, University of Montpellier (UM-1), France and was a Co-Investigator in HARP. He was responsible for overseeing the testing and quality control of HPV genotyping testing, and HPV variant testing and analysis. He advised on the virological aspects and analyses of this study.

Prof Silvia de Sanjose, MD, PhD is Chief of the Cancer Epidemiology Research Programme at Institut Català Oncologia, Barcelona, Spain. She is a leading researcher on studies of cancer-associated infectious diseases. She was a member of the International Scientific Advisory Group for the *HARP* study and has been closely involved and advised on the study progress over its duration. Prof de Sanjose inspired and was co-responsible for the conceptualisation of the meta-analysis on the association of ART with HR-HPV and cervical lesion outcomes (**PAPER 1**).

Prof Attila Lorincz, PhD, Professor of Molecular Epidemiology is a molecular biologist at Queen Mary University of London (QMUL) recognized for his research in human diagnostics and the natural history of HPV infections. He invited me to work in his laboratory at QMUL and supervised the work on DNA methylation assays.

Prof Sinead Delany-Moretlwe, MD, PhD, is Director of Research at Wits RHI, University of the Witwatersrand, Johannesburg, South Africa and was Co-PI on HARP. She provided scientific supervision in South Africa, ensured that local ethical approvals were obtained, and reviewed results and provided guidance as required. Prof Nicolas Meda, MD, PhD, is Director of the ANRS HIV research site, CRIS, University of Ouagadougou, Burkina Faso, and was a Co-PI on HARP. He provided scientific supervision in Burkina Faso, ensured that local ethical approvals were obtained, and reviewed results and provided guidance as required.

1.7 Ethical clearance

Ethical approval was granted by the Ministry of Health in Burkina Faso (no. 2012-12-089), the Witwatersrand University in South Africa (no. 110707), and the London School of Hygiene and Tropical Medicine (no. 7400) [**PAPERS 2 and 3**] with supplementary approvals for serology [**PAPER 4**] and DNA methylation analysis [**PAPER 5**].

1.8 Funding

This research was conducted as a staff member at the London School of Hygiene and Tropical Medicine, and salary costs were covered through a combination of funding from the European Commission (EC) 7th Framework Programme under grant agreement No. HEALTH-2010-F2-265396 and the UK Medical Research Council (MRC) PHINDS scheme (*PH01/14-39*).

1.9 Publications and conference presentations resulting from this PhD

PUBLICATIONS

PUBLISHED:

- PAPER 2 (**CHAPTERS 6 AND 7**)

  Kelly HA, Sawadogo B, Chikandiwa A, Segondy M, Gilham C, Lompo O, Omar T, Didelot MN, Nagot N, Meda N, Weiss HA, Delany-Moretlwe S, Mayaud P; HARP Study Group. Epidemiology of High-risk Human Papillomavirus and Cervical Lesions in African women living with HIV/AIDS: Effect of Anti-Retroviral Therapy. AIDS. 2017 Jan 14;31(2):273-285. doi: 10.1097/QAD.0000000000001301.

- PAPER 3 (**CHAPTER 8**)

  Kelly HA, Ngou J, Chikandiwa A, Sawadogo B, Gilham C, Omar T, Lompo O, Doutre S, Meda N, Weiss HA, Delany-Moretlwe S, Segondy M and Mayaud P for the HARP Study Group. Associations of human papillomavirus (HPV) genotypes with high-grade cervical lesions in a cohort of women living with HIV/AIDS in Africa PLoS One. 2017 Mar 23;12(3):e0174117. doi: 10.1371/journal.pone.0174117

UNDER REVIEW:

- PAPER 1 (**CHAPTER 3**)

  Kelly HA, Weiss HA, Benavente-Moreno Y, De Sanjose S, Mayaud P. Antiretroviral therapy, high-risk human papillomavirus and cervical intraepithelial neoplasia: a systematic review and meta-analysis [RESUBMITTED FOLLOWING PEER REVIEW WITH LANCET HIV]

- PAPER 4 (**CHAPTER 9**)

  Kelly HA, Faust H, Chikandiwa A, Ngou J, Helen Weiss, Segondy M, Dillner J, Delany-Moretlwe S, Mayaud P. Type-specific serologic responses of human papillomavirus among women living with HIV in South Africa: seroprevalence, seroconversion and risk of re-infection [CO-AUTHORS REVIEW]

- PAPER 5 (**CHAPTER 10**)

  Kelly HA, Warman R, Segondy M, Chikandiwa A, Sawadogo B, Vasiljevic N, Scibor-Bentkowska D, Meda N, Weiss HA, Delany-Moretlwe S, Mayaud P, Lorincz A. Associations of DNA methylation of *EPB41L3* and HPV16 with cervical lesions in women living with HIV in Burkina Faso and South Africa [CO-AUTHORS REVIEW]

CONFERENCE PROCEEDINGS

European Research Organisation on Genital Infection and Neoplasia (EUROGIN 2012) Prague, Czech Republic (8-11 July 2012)

- **Kelly H**, Muzah B, Sawadogo B, Didelot M, Michelow P, Lompo O, Doutre S, Gilham C, Von Knorring N, Zan S, Delany S, Omar T, Meda N, Drabo J, Weiss H, Legood R, Nagot N, Segondy M, Costes V, Mayaud P. Prospective evaluation of cervical screening methods in HIV positive women in Africa (HARP study): baseline results (oral presentation; number FC 6-2)

STI & AIDS World Congress 2013 in Vienna, Austria (14 to 17 July 2013)

- **Kelly H**, Ngou J, Sawadogo B, Muzah B, Gilham C, Nagot N, Meda N, Delany S, Segondy M, Mayaud P. HPV genotype distribution in HIV-positive African women and associations with high grade histological lesions by CD4+ count (poster)

7eme Alliance Francophone des Acteur de sante contre le VIH et les infections virales (AFRAVIH 2014) in Montpellier, France (27 to 30 April 2014)

- **Kelly H,** Ngou J, Chikandiwa A, Sawadogo B, Weiss H, Omar T, Lompo O, Nagot N, Meda, N, Delany S, Segondy M, Mayaud P. Distribution des génotypes du HPV et associations avec les lésions histologiques du col utérin chez les femmes africaines séropositives au VIH-1 (Poster; number Po M6.32)
- **Kelly H,** Ngou J, Chikandiwa A, Sawadogo B, Weiss H, Omar T, Lompo O, Nagot N, Meda, N, Delany S, Segondy M, Mayaud P. Importance du contrôle immunitaire et virologique sur le risque de lésions cervicales intraépitheliales néoplasiques de haut grade (CIN2+) et d'infection par HPV à haut-risque (hr-HPV) chez les femmes africaines infectées par le VIH-1 (Poster; number Po M6.34)

7th Association Francophone pour l'Etude des Infections á Papillomavirus et Polyomavirus (AFFIP 2014) in Sète, France (29 September to 1 October 2014) –travel bursary awarded for attendance

- **Kelly H,** Warman R, Segondy M, Chikandiwa A, Sawadogo B, Vasiljevic N, Scibor-Bentkowska D, Meda N, Weiss HA, Delany-Moretlwe S, Mayaud P, Lorincz A. Méthylation de l'ADN des cellules hôtes parmi des femmes africaines infectées par le VIH avec ou sans lésions du col utérin (oral presentation)

30th International Papillomavirus Conference 2016, in Lisbon, Portugal (17-21 September 2016) – travel bursary awarded for attendance

- **Kelly H,** Ngou J, Chikandiwa A, Sawadogo B, Gilham C, Didelot MN, Omar T, Lompo O, Weiss H, Nagot N, Meda, N, Delany S, Segondy M , Mayaud P. Persistence and incidence of high-risk HPV associated with CIN2+ among African women living with HIV-1 (oral presentation; number HPV15-0792)
- **Kelly H,** Warman R, Segondy M, Chikandiwa A, Sawadogo B, Vasiljevic N, Scibor-Bentkowska D, Meda N, Weiss HA, Delany-Moretlwe S, Mayaud P, Lorincz A. Association of DNA methylation of *EPB41L3* human gene with CIN2+ and HIV-related factors in African women living with HIV (poster; number HPV15-0794)

8eme Conference Internationale Francophone VIH/Hepatites AFRAVIH 2016, Brussels, Belgium (20-23 April 2016) –travel bursary awarded for attendance

- **Kelly H,** Ngou J, Omar T, Lompo O, Gilham C, Magooa-Mahalpe P, Djigma F, Sawadogo B, Chikandiwa A, Didelot MN, Meda N, Nagot N, Weiss HA, Delany-Moretlwe S, Segondy M, Mayaud P. Infections HPV et lésions cervicales chez les femmes VIH+ en Afrique subsaharienne : données issues des études transversale et longitudinale du projet HARP (oral presentation ; number S11.01)
- **Kelly H,** Warman R, Segondy M, Chikandiwa A, Sawadogo B, Vasiljevic N, Scibor-Bentkowska D, Meda N, Weiss HA, Delany-Moretlwe S, Mayaud P, Lorincz A. Méthylation de l'ADN des cellules hôtes chez des femmes africaines infectées par le VIH avec ou sans lésions du col utérin (poster; number PV254)

2 LITERATURE REVIEW

2.1 Anogenital HPV and associations with cervical lesions

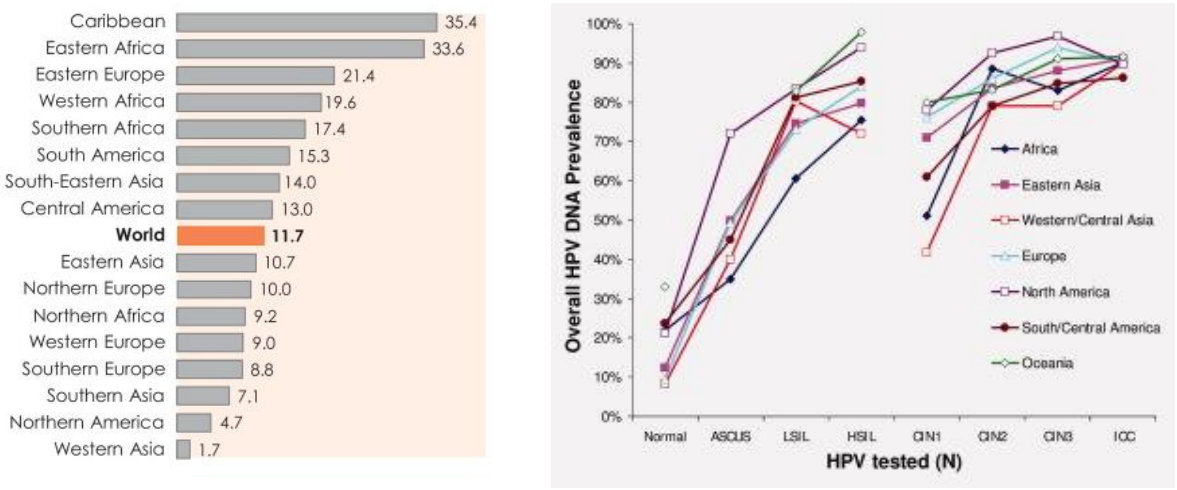
The link between a sexually transmitted agent and invasive cervical cancer (ICC) was first postulated in 1842 when an Italian physician noted a high frequency of cervical cancer among married women, widows and prostitutes, but was a rare occurrence among virgins and nuns [18, 19]. Later epidemiological studies reported the sexual behaviour of a woman and that of her male partner was associated with the risk of ICC [20-23]. ICC was often found to co-exist with other sexually transmitted infections (STIs), and studies in the 1960s reported a strong association of Herpes Simplex Virus Type 2 (HSV-2) antibodies with ICC [24-26]. However, a large prospective study among women in former Czechoslovakia found no association between HSV-2 antibodies and CIN development [27, 28]. In the meantime, the HPV virus was reported to be localised in cervical tumour tissue using a nucleic acid hybridization technique in the 1970s [29, 30] and later prospective studies demonstrated the presence of persistent HPV DNA precedes cervical lesion development [31, 32]. HPV DNA detection in cervical biopsies using polymerase chain reaction (PCR)-based tests in the 1990s confirmed that HPV was an aetiological agent of ICC [33, 34].

A recent meta-analysis of cross-sectional HR-HPV type distribution among 369,186 women and 423 studies worldwide [35] found that HPV prevalence among women with atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL) was 12.4%, 52.5%, 75.7% and 85.4%, respectively, and among women with cervical intraepithelial neoplasia grade 1 (CIN1), CIN2, CIN3 and ICC was 73.4%, 85.6%, 92.6% and 89.4%, respectively (**Figure 2.1**). Even though it is reported that HPV is a necessary cause of ICC [34, 36], it is not uncommon to find patients with ICC being HPV negative. In their review of 10,575 biopsies of invasive cervical cancer from around the world [37], De SanJose et al (2010) found that only 85% were positive for any HPV and the authors suggest this could be the result of

samples used (cervical swab vs. cervical biopsy) as it may be plausible that HPV infection may no longer be detectable from the cervical mucosa whilst having caused lesions.

Figure 2.1. Prevalence of human papillomavirus (HPV) DNA among cytological normal women (A) and by cervical disease grade and region (B)

(A) (B)

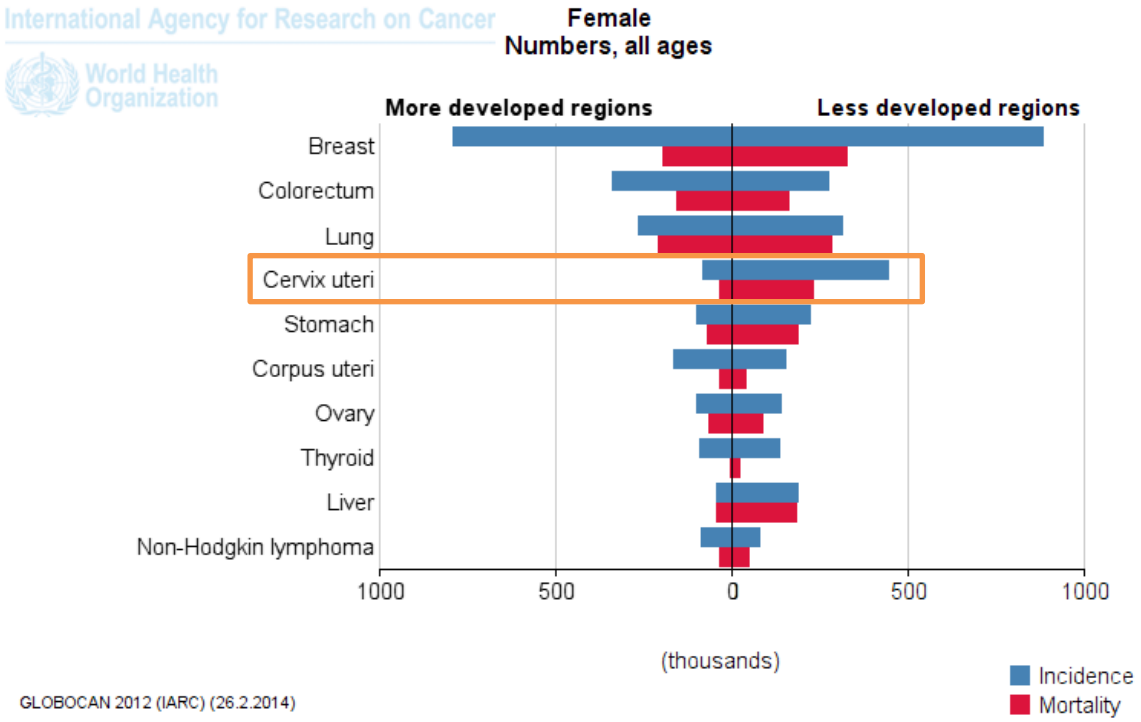


(A) from [1] and (B) from [35] Abbreviations; ASCUS: atypical squamous cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; CIN: cervical intraepithelial neoplasia; ICC: invasive cervical cancer.

2.2 Invasive cervical cancer

ICC is the most common female cancer in low and middle-income countries [1], where 85% of the estimated 500,000 global annual cases occur [1, 2] (**Figure 2.2**) and is the leading cause of cancer deaths among African women [3]. This is likely due to the lack of organised screening services for the prevention and detection of disease, and with the limited resources to manage and treat cervical disease when detected.

Figure 2.2. Incidence of and mortality from cancers (in thousands) among women in 2012



From [9]

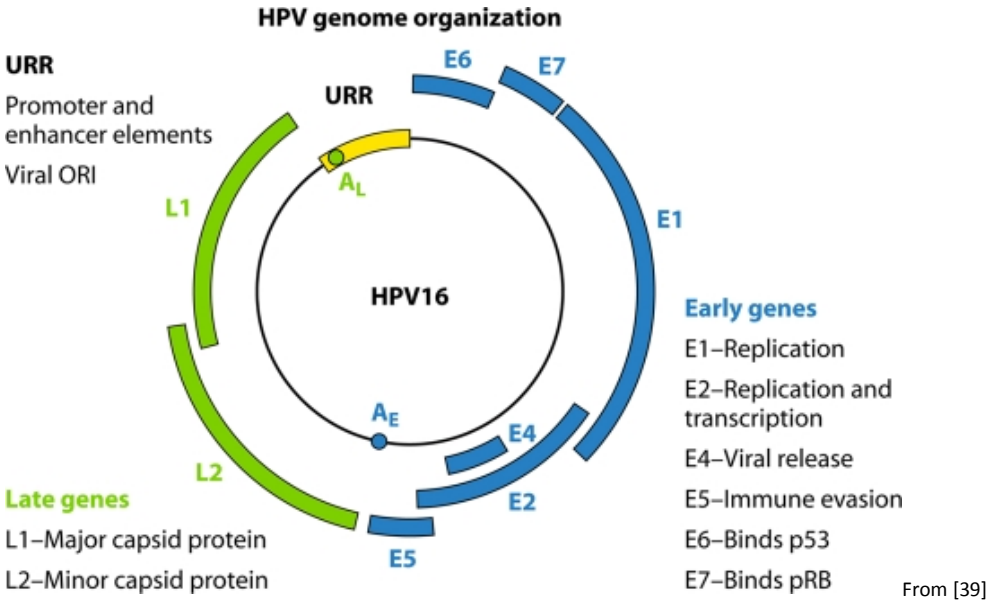
ICC is also one of the most common cancers in women living with HIV (WLHIV) [7], and ranks as one of the most common neoplasms among WLHIV and is considered an AIDS-defining illness [8]. In the African region the prevalence of both ICC and HIV are high (**Figure 1.1**). CIN is an early stage of cancer of the cervix which is curable. Persistent infection with high-risk human papillomavirus (HR-HPV) genotypes (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) is necessary for the development of squamous intraepithelial lesions (SIL) and CIN [5, 33].

2.3 HPV virus structure

HPV is a relatively small virus (8kb) containing a circular double-stranded DNA (**Figure 2.3**). The virus is organized into three regions; the non-coding upstream regulator region (URR) which controls gene expression; an early (E) region with the open reading frame (ORF) encoding genes involved in viral replication and cell transformation (E6, E7, E1, E2, E4, E5); and a late (L) region encoding the L1 and L2 capsid proteins which self-assemble to form the virion [38].

HPV is classified into genotypes based on L1 capsid protein. There are over 100 HPV genotypes identified; 40 of these are genital and 15 are oncogenic, or high-risk given their association with anogenital neoplasia. The genital HPV types are classified by the International Agency for Research on Cancer (IARC) according to the magnitude of their association with ICC. HR-HPV types include the types that are 'carcinogenic to humans' (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) and 'probable carcinogenic' (HPV68) [4]. The 'possible carcinogenic' types include HPV26, 53, 66, 69, 70, 73, and 82 and other known low-risk types include HPV6, 11, 40, 43, 44, 54, 71, and 74.

Figure 2.3. HPV16 genome organization



From [39]

2.4 The natural history of HPV and cervical lesions

The squamocolumnar junction (SCJ) is the junction between the squamous epithelium and the columnar epithelium of the cervix (**Figure 2.4**). The location of the SCJ on the cervix varies over a woman's lifetime and is dependent on age, hormonal status, oral contraceptive use, birth trauma and pregnancy [40]. Cervical lesions develop in the area of the cervix called the Transformation Zone (TZ, **Figure 2.4**), which is an area of high cellular turnover, as columnar cells change into squamous cells. It is located fully on the outer cervix in teenage girls and moves higher into the endocervical canal in older women with decreasing levels of oestrogen.

Cervical ectopy, or ectropion is defined as the presence of everted endocervical columnar epithelium on the extocervix. It is a normal occurrence in a woman's life, but when the columnar epithelium extends into the vaginal fornix, there is disruption of the mucosa which can result in squamous metaplasia and is associated with abnormal transformation in ICC.

Figure 2.4. Cervical Squamocolumnar junction (SCJ) and the Transformation Zone (TZ) in mid-later reproductive stage (30's age range)

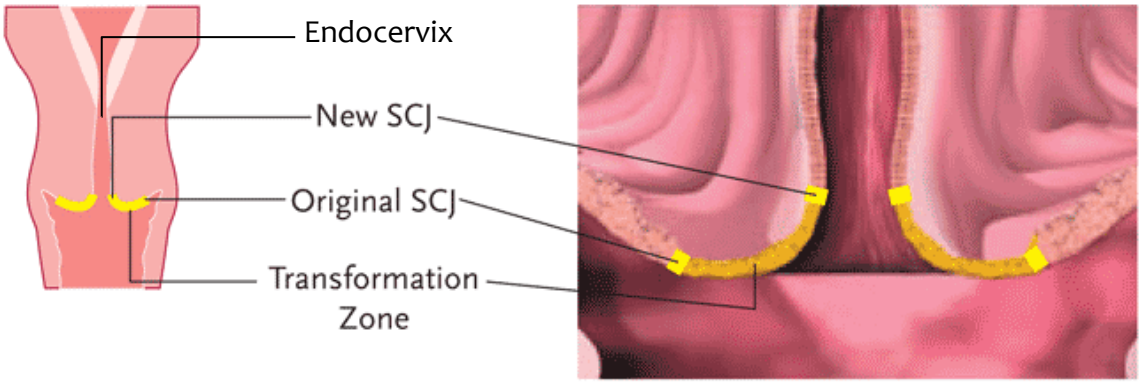


Image from http://www.prn.org/images/uploads/Palefsky-fig3-680.gif

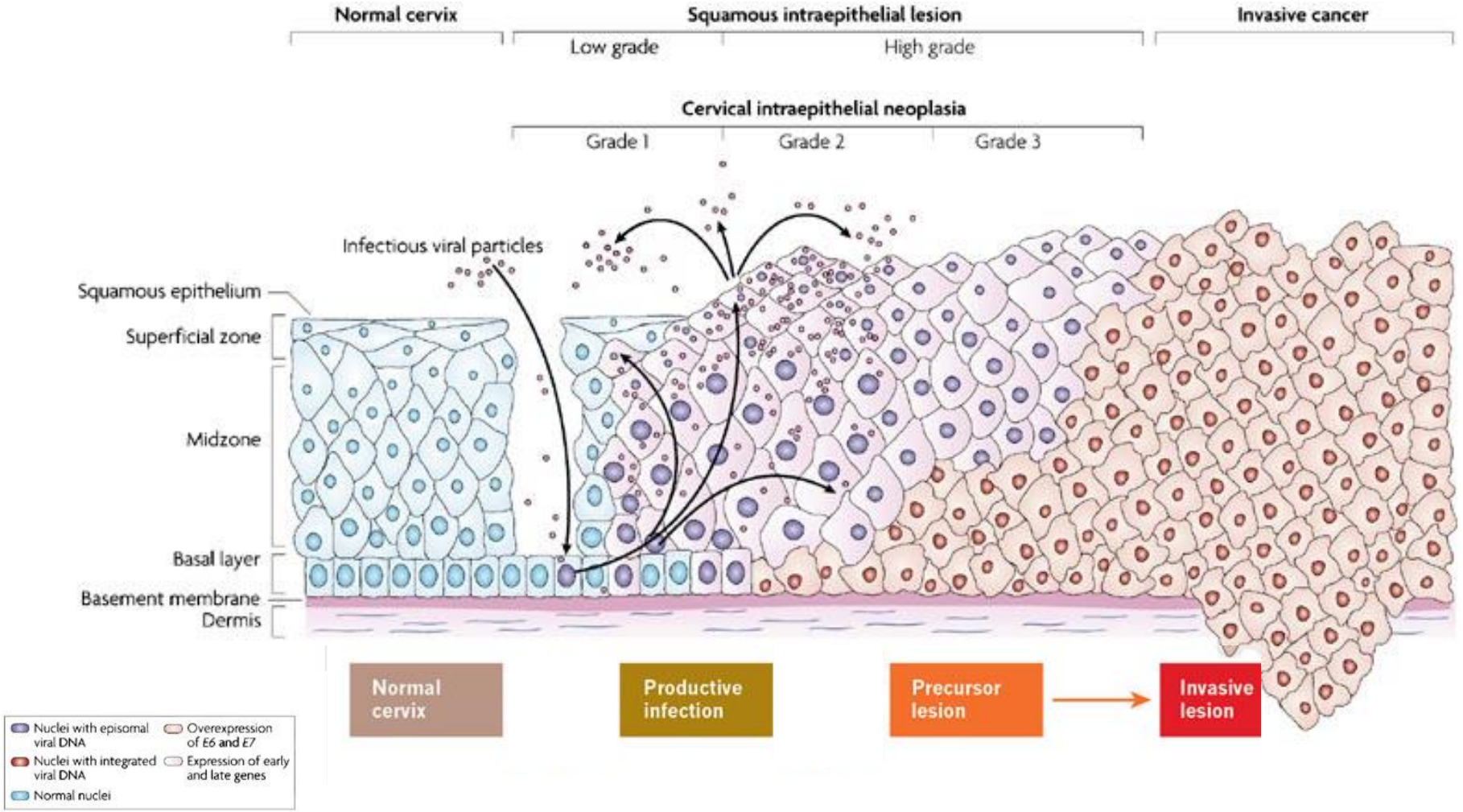
The pathway from HPV infection to ICC is depicted in **Figure 2.5** and summarised here. Two terminologies are used to determine stages of cervical neoplasia. Cytological results are reported using the Bethesda nomenclature: low-grade and high-grade squamous intraepithelial lesions (LSIL

and HSIL, respectively) [41]; histological results are reported using the 3-tier Cervical Intraepithelial Neoplasia (CIN1-3) classification system [42].

The HPV virus is dependent on a complete programme of keratinocyte differentiation [43, 44]. It infects keratinocytes, and enters the cell to attach to the basement membrane if there is a microabrasion on the surface of the cervix, which can occur through trauma during sexual intercourse, vaginal cleansing or drying and if the cervix is inflamed as a result of other sexually transmitted infections (STIs). In the normal cervix, the epithelial stem cell divides along the basement membrane and matures vertically through epithelium without further division, a process which can take 12-48 hours. The virus and cell replicate together and the viral genome remains in the basal layer at low copy number (~100 copies per cell). HPV viral replication is accompanied by the expression of 'early' proteins E1 and E2 in the basal layers. During this phase, the oncoproteins E6 and E7 are tightly controlled. The viral lifecycle goes through successive stages of genome amplification, virus assembly and virus release. As the host cell stops dividing, it begins to differentiate into a mature keratinocyte. The dividing cell population expands vertically and the virus activates all genes and the copy number increases to the thousands. This is called a productive infection, LSIL or CIN1 and can be cleared with an effective immune response. A shift in virus expression patterns to oncogene expression (E6 and E7) leads to unregulated viral DNA replication in non-cycling host cells, brought about through the suppression of normal cell cycle checkpoints. This promotes cell proliferation and delay differentiation, and this is defined as a precursor lesion (HSIL/CIN2 or 3), which can be managed if detected or can spontaneously regress. As infected cells differentiate into squamous cells, the E4 protein, and the late proteins L1 and L2 (which form the capsid), are expressed. Viral capsids are shed into the genital tract within desquamated epithelial cells. In rare events, the HPV virus integrates into the host genome, which causes deletion or disruption of E2 gene but retains E6, E7 and the URR. These cells show increasing genomic instability and a greater probability of acquiring genomic abnormalities which drives malignant transformation and invasion [39].

Figure 2.5. HPV-mediated progression to cervical cancer

Adapted from [44, 45]



2.5 HPV immune evasion mechanisms

The HPV virus has several mechanisms through which it successfully manages to evade detection from the immune response, primarily linked to the differentiation of the target cell, the keratinocyte [39, 46, 47]:

i. *Viral lifecycle occurs entirely within epithelium*

The HPV viral lifecycle occurs entirely within the stratified epithelium far from the sites of immune activity. It infects and multiplies within differentiating keratinocytes which have a short life span and are destined for death by natural causes and desquamation away from sites of immune activity. Because there is no cytolysis resulting from viral replication, the infected cell is not a target of the host immune response and there is no opportunity for the Antigen Presenting Cell (APC) to present the antigens to the immune system. The early proteins are produced in the basal layer and late proteins in the distal layer, and so are not detected by the Langerhans cells, which are the APCs of the squamous epithelium and reside in the parabasal and lower suprabasal layers of the squamous epithelium. This allows for long periods of uninterrupted viral replication.

ii. Absence of viraemia

There is no blood-borne (viraemic) phase of the HPV lifecycle and the infection is not spread systemically, and so it remains unexposed to the immune system outside the epithelium, allowing the virus to persist in the cervical tissue.

iii. Absence of inflammation

There is no virus-induced death and no inflammation, and there appears to be little or no release of pro-inflammatory cytokines that are necessary for activation and migration of the Langerhans cells and macrophages and the recruitment of effector T-lymphocyte cells. During normal turnover of cervical epithelial cells, dying cells are detected by Langerhans cells, which subsequently release

immunosuppressive cytokines (TGF-ß, IL-10 and IL-13). Dendritic cells (DCs) transiting from epithelium to lymph nodes require maturation to deliver antigen to T-cells in order to trigger a positive immune response. To do this, the DCs require co-stimulation by other co-factors (B7) which in turn requires release of inflammatory cytokines. Because the HPV virus does not need to destroy the host cell, it does not trigger inflammation or inflammatory cytokines.

iv. The viral proteins cause local immunosuppression

HPV downregulates the expression of interferon 1 genes (IFN-α and IFN-β) which have antiviral, antiproliferative and immunostimulatory properties and act as a bridge between the innate and humoral immune responses [39]. HPV oncoproteins E6 and E7 can directly inhibit these antiviral pathways in the cell. In this way, E6 and E7 maintain the keratinocyte in the cell cycle.

v. Oncoprotein evasion

E7 is located in the nucleus where Langerhans cells cannot gain access. HPV16 E7 also displays similarity to several human proteins, which might be a reason for the limited immunogenicity of HPV16 E7 [47].

2.6 Immune response to natural infection

These immune evasion mechanisms allow the HPV virus to persist. However not all infections persist and the majority of immuno-competent women clear infection [48]. Prospective studies of incident and prevalent infections among young women have shown that up to half of infections clear within 6 months and approximately 90% clear within 2 years after acquisition [49, 50].

2.6.1 Innate immune response to HPV infection

The innate immune response is the first line of recognition and defence against viral infection. The role of the innate immune response to HPV infection is still poorly understood, but current evidence is summarised here, and illustrated in **Figure 2.6**.

Detection of virus

Although keratinocytes are not classic immune cells, they can act as APCs for presentation of antigen in association with the Major Histocompatability Complex (MHC) class 1. Keratinocytes are also able to secrete pro-inflammatory cytokines and chemokines and can activate CD4+ and CD8+ T-cells [51]. The double stranded DNA and L1 and L2 capsids of the HPV virion can trigger signalling through Toll-like receptors (TLR), which are a class of proteins involved in the recognition of foreign pathogens [52] (**Figure 2.6)**. A greater expression of Toll-like receptors (TLR2, 3, 7, 8 and 9) has been reported after incident HPV16 infection compared to before infection and this expression was found to be significantly associated with subsequent clearance within 4 months [53]. Virus capsid entry is usually an activating signal for APCs of the squamous epithelia, the Langerhans cells.

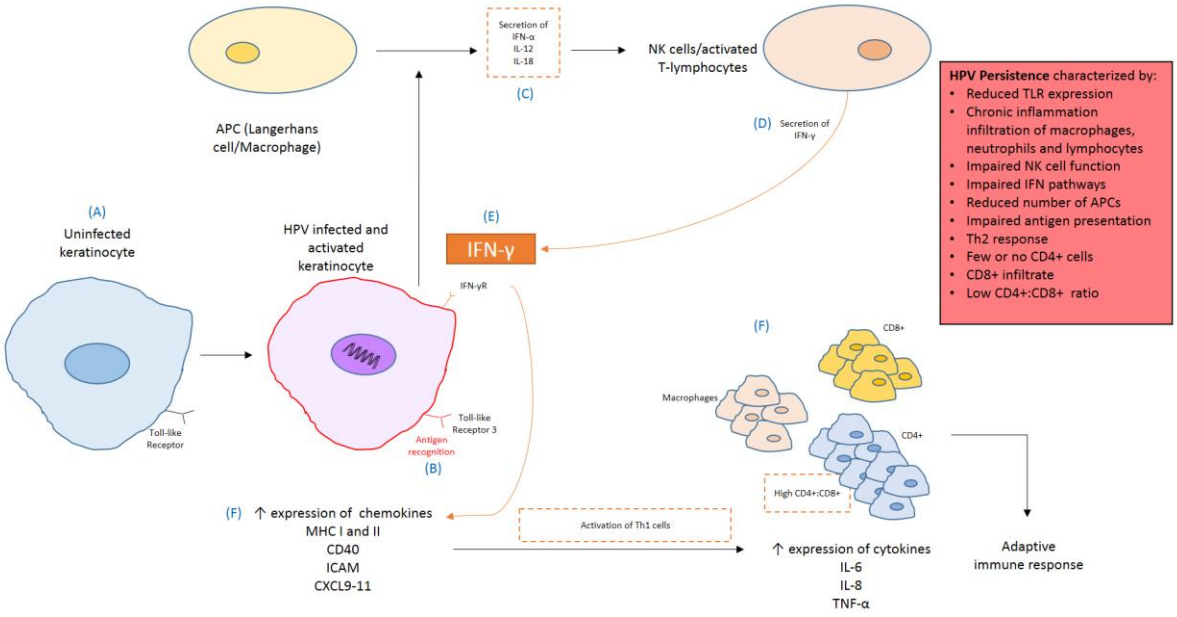
Immune response to infection

Once virus is detected, the APCs secrete cytokines to promote a T-cell response to HPV infection [54]. Two longitudinal studies have shown that IFN-γ mRNA expression in both cervical cytobrush [55] and cervical biopsy [56] specimens was associated with HPV clearance. IFN-γ is expressed by Natural Killer (NK) cells and activated T-cells which in turn are stimulated by the cytokines IFN-α, IL-12 and IL-18 produced by the infected cells or activated APCs (**Figure 2.6**).

Women who clear infection are more likely to have detectable levels of IFN-γ, indicative of a Th1 immune response compared to those whose infections persist [55]. A Th2 response (IL-4 and IL-10) is more likely among those whose infections progress to precursor lesions [57](**Table 2.1**).

Immunohistochemical studies have shown that regression of anogenital warts (AGW) is accompanied by an infiltration of mononuclear cells (CD4+, CD8+, CD56+, macrophages) into the lesion and increased expression of Th1 cytokines (IL-2, IL-12, IFN-γ, TNF-α). A 12-month prospective study of histologically confirmed CIN1 lesions found that lesion regression was strongly correlated with CD8+ and CD56+ T-cells [58].

Figure 2.6. Detection and response to HPV by host immune response



(A) Resting uninfected keratinocyte; (B) Detection of foreign DNA by infected keratinocyte and professional Antigen Presenting Cells (APCs) and signalling through Toll-like receptors; (C) Activation of Natural Killer (NK) cells and T-lymphocytes by cytokines secreted from professional APCs and infected cells; (D) IFN-γ secretion by NK cells and activated T-cells; (E) Activation of infected keratinocyte by IFN-γ ; (F) Increased surface expression of MHC I and II, CD40, ICAM and CXCL9-11 on infected keratinocyte; (F) this attracts activated Th1 cells (CD4+, CD8+, macrophages) ; IFN-γR=IFN-γ Receptor

Immune response to cervical precursor lesions

CIN2/3 lesions appear to be characterised by a reduction in the Th1 immune response, but an increase in the Th2 immune response. Previous studies have shown that in comparison to normal or CIN1 biopsy specimens, CIN2/3 biopsy specimens contained lower levels of IFN-γ mRNA [59] and contained increased numbers of immune cells from both the acquired and innate immune response in the stroma (CD4+ and CD8+ T-lymphocytes, macrophages, mast cells, B-cells,

neutrophils and NK cells) and in the dysplastic epithelium (CD4+ T-cells, macrophages and NK cells) [60-62].

Table 2.1. Th1 and Th2 cytokine responses for HPV transient infection and HPV persistence and CIN2/3

Response	Function	HPV transient infection	HPV persistence CIN2/3 lesions
Th1 immune response	Cell-mediated immunity -proinflammatory responses responsible for killing intracellular pathogens and for perpetuating autoimmune response		
IFN-γ	Activate cytotoxic T lymphocyte and NK cell mediated cytolytic functions that have been proposed to provide effective **antitumour** defence mechanisms	↑	↓
IL-2	Proliferation and activation of T- and B-cells; Induces the transformation of NK cells into lymphokine activated killer cells that have been associated with improved capacity to **destroy tumour cells**	↑	
IL-12	Activates cytotoxic T lymphocyte and NK cell mediated cytolytic functions that have been proposed to provide effective **antitumour** defence mechanisms	↑	
TNF-α	Binds 2 cell surface receptors: TNF receptor 1 and TNF receptor 2, activating multiple signalling pathways involved in antiviral activities, **growth arrest, cell proliferation, differentiation or apoptosis**, depending on cell type and GF availability	↑	↓
Th2 immune response	Humoral immunity, inhibition of inflammation		
IL-4	Inhibits Cell Mediated Immune responses		↑
IL-10	Inhibition of cytokine production; Tumour derived IL-10 suggested to modulate antitumor responses by **inhibiting tumour antigen presentation** by professional and non professional APCs; it is possible that IL-10 suppresses expression of MHC class 1 expression preventing tumour antigen presentation to CD8+ CTLS		↑

↑=increase in levels; ↓=decrease in levels; [63]

2.6.2 Adaptive immune response to HPV infection

Antigen-specific T-cell responses are weak and often transient [39] and there is still uncertainty about whether antibodies to HPV L1, elicited by natural infection, are partially protective against re-infection by the same HPV type. Detectable serum neutralising antibody responses to L1 are type specific [64]. Comparisons between sero-epidemiological studies are difficult due to the use of different serological assays, and the lack of a reference serum for establishing cut-off values [65]. The pseudovirion-based neutralization assay (PBNA) is the "gold standard" for detection of HPV antibodies and international reference sera are available for HPV16 and HPV18 (WHO LabNet)

[66, 67]. The competitive Luminex immunoassay (cLIA; Merck), the virus-like-particle (VLP)-based multiplex immunoassay (VLP-MIA; GlaxoSmithKline), and the in situ purified glutathione S-transferase L1-based MIA (GST-L1-MIA) are the most widely used assays for seroprevalence studies in several countries and trials.

Seronversion following incident infection

Seroconversion has been previously shown to be dependent on time following incident infection. Carter et al [68] enrolled 588 HIV-negative college-attending women aged 18-20 years in a prospective study over a mean 31 months follow-up with average time between visits of 4.8 months. The authors report that 60%, 54% and 69% of women seroconverted for HPV-16, -18 or -6, respectively within 18 months of incident DNA detection. HPV6 seroconversion coincided with DNA detection but HPV16 seroconversion occurred between 6 to 12 months after DNA detection, with the highest rate of seroconversion occurring 7-8 months after DNA detection.

Furthermore, HPV16 and HPV18 antibodies were more likely to persist compared to HPV6 antibodies and women with persistent HPV16 and HPV18 infections (DNA positive at more than 1 consecutive visit) were more likely to seroconvert, but there were some women with persistent DNA who never seroconverted. In contrast, HPV6 seroconversion was the same, irrespective of HPV6 DNA persistence or clearance.

Baseline seropositivity and risk of same-type re-infection

A recent meta-analysis [69] of 14 studies among >24,000 individuals (90% women) investigating whether antibodies against HPV16 and 18 were protective against type-specific re-infection found that HPV16 antibodies acquired through natural infection were associated with a 35% (pooled Risk Ratio [RR]=0.65, 95%CI: 0.50-0.80) decreased risk of subsequent cervical HPV16 infection, while HPV18 antibodies were associated with a 30% (pooled RR=0.70, 95%CI: 0.43-0.98) decreased risk of subsequent HPV18 infection.

Furthermore, Safaeian et al have shown that antibody generated in natural infections is protective against re-infection but protection was antibody titre-dependent in a study among Costa-Rican women aged 18-25 years in the control group of a community-based, randomized HPV16/18 vaccine trial [70]. The women were negative for HPV16/18 DNA at enrolment and followed up annually for a median 4 years. The study found that women who were HPV16-seropositive at enrolment with HPV16 antibody titres in the highest tertile (≥66 percentile point; ≥60 Elisa Units/mL) had a 50% decreased risk of subsequent HPV16 infection compared to HPV-16 seronegative women (aRR=0.50, 95%CI: 0.26-0.86, adjusted for age, education, marital status, lifetime number of sexual partners, and smoking) [70]. Similarly women with HPV18 antibody in the highest tertile (≥28 EU/mL) at enrolment had a 64% reduced risk of subsequent HPV18 infection (aRR=0.36, 95%CI: 0.14-0.76). These findings were not found among women whose antibodies titres were in the low tertiles for both HPV16 and 18.

2.7 Risk factors associated with the natural history of HPV: from HPV acquisition to ICC

A framework for the study of risk factors that influence the pathway from HPV acquisition to persistence and the development of CIN and ICC includes two groups of factors, corresponding to the key stages in HPV natural history:

i.) socio-demographic and behavioural factors which determine the acquisition of HR-HPV infection; and

ii.) biological factors which determine the persistence of infection and cervical precursor lesion development/carcinogenesis.

Figure 2.7 presents a conceptual framework of risk factors for HR-HPV acquisition, persistence and development of cervical lesions, based on a framework designed for the analysis and

interpretation of risk factors in demographic studies and more recently used in for studies for HIV transmission [71]. This framework links the socio-demographic factors (underlying determinants) on the left of the diagram with the health outcome on the right. The underlying determinants (socio-demographic factors) influence the proximate determinants which have both behavioural, and biological components; those, in turn, determine the health outcomes/stages (HR-HPV acquisition, persistence and cervical lesion development). In the case of HPV, the risk factors can vary according to the stage of the HPV life cycle; some risk factors are associated with only one stage of the life cycle (e.g. condom use reduces HR-HPV acquisition) and others are associated with more than one stage (e.g. hormonal contraception can influence acquisition of infection as well as promote carcinogenesis).

Published evidence has so far focused on the association of risk factors with ICC, despite the fact that some risk factors are associated with an increased risk in HR-HPV acquisition as well as persistence. Indeed, a separate group of risk factors could be considered for the progression of high-grade cervical lesions to ICC (in addition to groups i.) and ii.) above), although, some of biological risk factors considered in group ii.) above, also apply to this stage of the pathway.

Where data is available on HR-HPV outcomes, these are given (there were no meta-analyses investigating these associations on SIL or CIN outcomes). The associations reviewed here, summarized in **Table 2.2,** are based on i.) the epidemiological evidence currently available on the association of the risk factor with HR-HPV infection and/or ICC, and ii.) the biological plausibility for this association. The summarised evidence satisfied five of the nine elements of the classical epidemiological Bradford-Hill criteria for establishing causality between risk factors and health outcomes [72]. Data from meta-analyses investigating the association of the risk factors with the outcome (HR-HPV acquisition or persistence and/or ICC) were used where available as this shows *the consistency of the association*. The *strength of the association* (effect estimate) between the risk factor and the outcome is summarised in **Table 2.2.** Prospective studies *(evidence of termporality)*,

and those that report *a dose-response relationship (biological gradient)* between a risk and the outcome (e.g. smoking frequency, duration of hormonal contraceptive use) are presented where available. Finally, the *biological plausibility* of an association between the risk factor and the outcome is briefly discussed.

2.7.1 Socio-demographic factors

Age

Epidemiological evidence

The relationship between age and HPV prevalence is similar worldwide with the highest prevalence among women <25 years and a decline at older ages [73, 74]. In Europe and North America, young women <25 years have very high rates (20-30%) which decrease to very low levels over the age of 45 years (<5%). In Africa and Asia, HPV prevalence remains ≥10% among older age groups (>45 years), and in Latin America and the Caribbean, there is a decline and an increase again in middle-aged women [75]. The peak transmission of HPV infections is typically among young women following sexual debut [76] because HPV is highly prevalent in younger age groups, and is easily transmitted [77]. Several studies which examined the influence of age on persistence of infection and risk of CIN2+ have reported that for prevalently-detected infections, the risk of HPV persistence and CIN3 increases with older age [49, 78-80] but newly acquired infections were cleared as quickly by older women as by younger women [49].

Biological plausibility

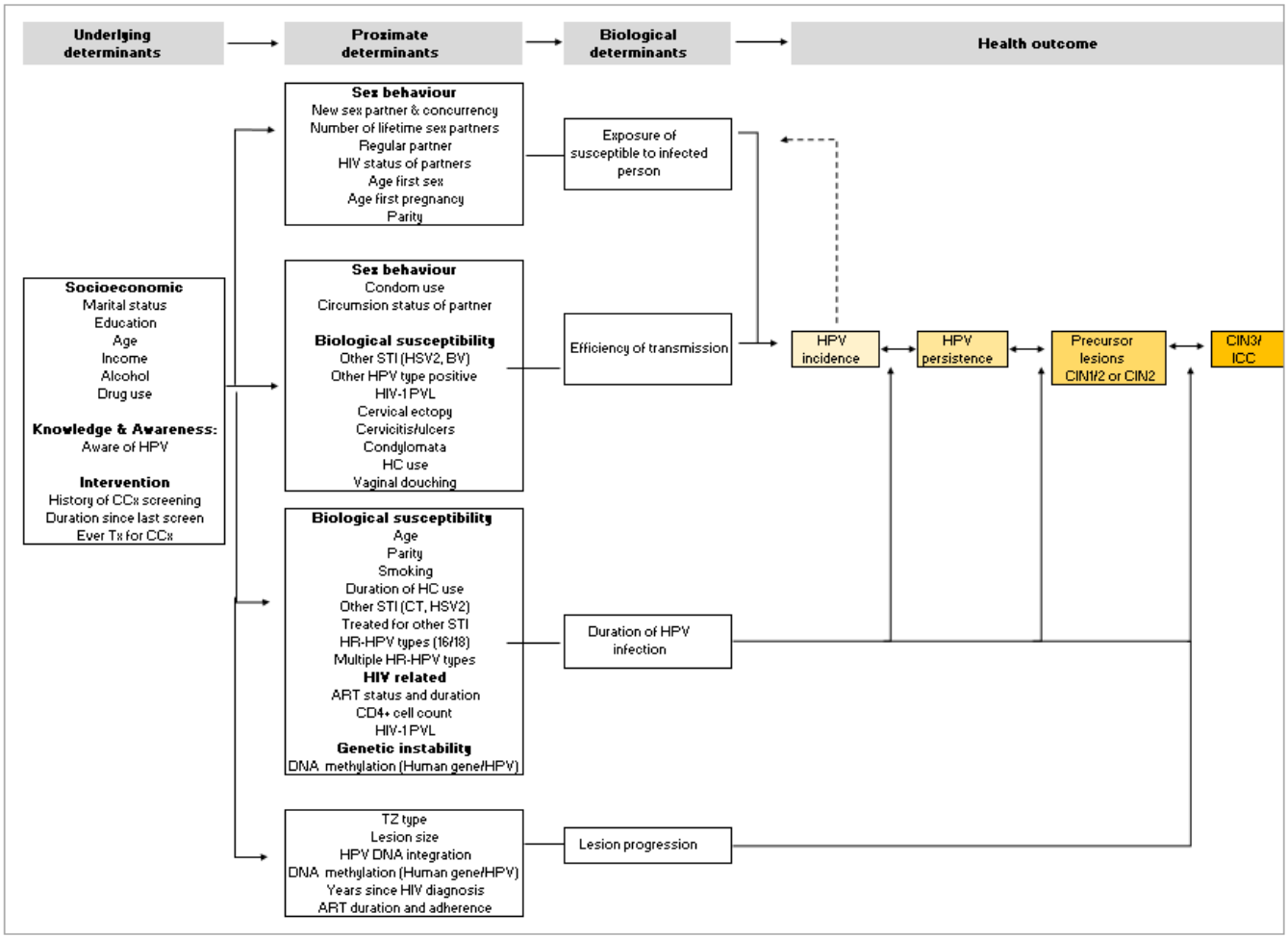
Cervical ectopy, which is more likely among young women has been shown to increase the risk of acquisition of other STIs [81], including HPV [82, 83]. The single layer columnar epithelium, indicative of ectopy, is a weak physical barrier and may facilitate entry of HPV and other STIs. Furthermore, oral contraceptive use - another risk factor for HPV infection, which is frequently used among younger women- can increase the likelihood of ectopy [84].

Table 2.2. Summary of risk factors associated with the natural history for HPV infection and cervical cancer

Outcome	Risk factor	Risk exposure	Comparison group	N studies	Effect Estimate (95%CI)	Reference
HR-HPV incidence	Circumcision status of male partner	Circumcised	Uncircumcised	1	IRR=0.77 (0.63-0.93)	[85]
HPV persistence	Age	≥30 years	17-24 years	1	aOR=5.2 (1.5-1.8)	[79]
		25-29 years	17-24 years	1	aOR=1.4 (1.4-4.8)	[79]
HPV clearance	Smoking	Ever	Never	1	HR=0.51 (0.30-0.88)	[86]
HPV Prevalence	Age	Peak prevalence ≤25 years (% prevalence variable by geographic region)		134	Not presented	[74]
HPV Prevalence	*Trichomonas vaginalis*	Positive	Negative	1	aOR=4.1 (1.7-9.8)	[87]
HPV Prevalence	Bacterial vaginosis	Positive	Negative	12	OR=1.43 (1.11-1.84)	[88]
HPV prevalence	HIV	Seropositive	Seronegative		Refer to **Table 2.3**	
ICC	Smoking	Ever	Never	7	OR=2.2 (1.5-3.2)	[89]
	Age at first sexual intercourse	≤16 years	≥21 years	9	OR=2.31 (1.85-2.87)	[90]
	Age at first pregnancy	<17 years	≥25 years	25	aRR=1.77 (1.42-2.23)	[91]
	Parity	≥7 FTP	1-2 FTP	25	aRR=1.76 (1.53-2.02)	[91]
	Oral contraception	5-9 years	Never	28	RR=1.3 (1.0-1.9)	[92]
		≥10 years	Never		RR=2.5 (1.6-3.9)	[92]
	Injectable contraception	<5 years	Never	3	RR=1.0 (0.9–1.2)	[92]
		≥5 years	Never		RR=1.2 (1.0-1.6)	[92]
	Chlamydia trachomatis	Positive	Negative	22	aOR=1.76 (1.03-3.01)	[93, 94]
	Trichomonas vaginalis	Positive	Negative	2	aRR=1.93 (1.22-2.65)	[95]
	Herpes simplex virus-2	Positive	Negative	7	aOR=1.96 (1.24-3.09)	[96]
	HIV	Seropositive	Seronegative		Refer to **Table 2.3**	

Updated from [97]; a=adjusted; IRR=Incidence Rate Ratio; OR=Odds Ratio; HR=Hazard Ratio; RR=Relative Risk; FTP=full term pregnancy

Figure 2.7 Conceptual framework for the analysis of factors affecting the epidemiology and natural history (prevalence, incidence, persistence and clearance) of HPV and development and progression of cervical lesions



Adapted from [71]

2.7.2 Behavioural factors

Smoking

Epidemiological evidence

Compared to HPV-positive women who never smoked, HPV-positive women who have ever smoked tobacco have a 4.6 (95%CI: 0.9-22.9), 2.2 (95%CI: 1.4-3.4) and 2.2 (95%CI: 1.5-3.2) increased odds of CIN2/3, CIN3 and CIS/ICC, respectively (**Table 2.2**) [86, 89, 98-103]. The amount of smoking (number of cigarettes per day) and duration of smoking history were also shown to increase the odds of CIS/ICC [89]. Ever having smoked was found to be associated with a 49% reduction in HPV clearance in a cohort study of 346 HIV-negative women aged 18-35 years in the US [86].

Biological plausibility

Tobacco smoke contains known carcinogens such as polycyclic aromatic hydrocarbons that could have a direct transformation effect on the cervix [98]. It is also known to suppress the immune response by reducing the number of Langerhans cells and other immune markers [89], thereby allowing HPV to persist and cervical lesion to develop [86, 104].

2.7.3 Sexual behaviour

Age at first pregnancy and parity

Epidemiological evidence

In a review of 25 epidemiological studies which included individual data on 11,161 women with ICC, 5,402 women with CIN3/carcinoma *in situ* (CIS) and 33,542 women without cervical carcinoma [91], multiparous women (≥7 full-term pregnancies [FTP]) had a 2-fold (aRR=1.76, 95%CI: 1.53-2.02, adjusted for age at first FTP) increased risk of ICC compared to women who had 1-2 FTP (**Table 2.2**). This association was not observed for CIN3/CIS. In a case-control study of 702 HIV-negative women

in Uganda, women with 10 or more children had a 7-fold increased risk of ICC compared to women with fewer than 4 pregnancies [105].

A younger age at FTP was also associated with a 2-fold increased risk of ICC (<17 years vs. ≥25 years: aRR=1.77, 95%CI: 1.42-2.23, adjusted for number of FTP) and CIN3/CIS (aRR=1.78, 95%CI: 1.26-2.51) in [91]. Both associations persisted even after adjustment for lifetime number of sex partners (LTSP), age at first sexual intercourse (AFSI) and history of screening, and when restricted to HPV-positive women only. The finding that number of FTP and age at FTP were not associated with HR-HPV positivity among the control group further confirms contribution of these factors to ICC development. However, this may be indicative of early HPV infection, which may have initiated cervical lesion pathway but may be undetected at time of ICC detection.

Biological plausibility

The increase in ICC risk among women with younger age at first pregnancy may be attributed to cervical trauma during delivery, which can result in cervical ectopy, thereby increasing exposure of the SCJ to HPV infection [91]. High concentrations of oestrogen and progesterone during pregnancy could facilitate cervical carcinogenesis, a mechanism similar to that reported for hormonal contraceptive users. Immunosuppression during pregnancy can also facilitate acquisition and persistence of HPV [106].

Age at first sexual intercourse and number of lifetime sex partners

Epidemiological evidence

A second review assessed the association of AFSI with ICC using a pooled analysis of case-control studies among 3,583 women from 8 developing countries [90]. The review shows that AFSI, age at first pregnancy (AFP) and age at first marriage (AFM) were highly interrelated. Compared with women with older AFSI (≥21 years), women with AFSI 17-20 years had a 1.8-fold (OR=1.80, 95%CI:

1.50-2.39) increased odds of ICC and women with AFSI ≤16 years has a 2.3-fold (OR=2.31, 95%CI: 1.85-2.87; **Table 2.2).**

Biological plausibility

Early AFSI may be a surrogate marker for early HPV infection which may have persisted. Early AFSI has shown to be associated with riskier sexual behaviour, such as unprotected sex, a higher number of lifetime sex partners, or concurrent sex partners and a woman's partner having multiple sex partners [90]. The peak transmission of HPV infections is typically among young women following sexual debut [76] and it has been suggested that the immature cervix during adolescence may be more susceptible to HPV acquisition and persistence [107].

2.7.4 Biological determinants

Male circumcision

Epidemiological evidence

Previous studies in Uganda, South Africa and Kenya have shown that male circumcision is associated with lower HPV prevalence in both HIV-negative and HIV-positive men [108-113]. Male circumcision has been shown to decrease the risk of transmission of HPV from HIV-negative men to their female partners [85], however this has not been proven among HIV-positive men [114]. In Rakai, Uganda, 1,245 HIV-negative female partners of HIV-negative men enrolled in two randomised controlled trials of male circumcision were prospectively followed for 24 months. Female partners of men in the intervention group had 23% lower HR-HPV incidence compared to women in the control group (Incidence Rate Ratio [IRR] =0.77, 95%CI: 0.63-0.93; **Table 2.2),** and increased HR-HPV clearance (RR=1.12, 95%CI: 1.02-1.22). Male circumcision was also found to decrease the genital HPV viral load among both HIV-negative men [115] and their female partners [116] and others have reported that HPV viral load is predictive of HPV transmission between heterosexual partners [117].

Furthermore, among women with male partners with high-risk behaviour, the risk of ICC is reduced if male partners have been circumcised. In a pooled analysis of 1,913 couples enrolled a case-control study of determinants of ICC in South America, Asia and Europe [118], circumcised men were less likely to be HPV infected compared to uncircumcised men (aOR=0.37, 95%CI: 0.16-085, adjusted for AFSI and number of lifetime sex partners). Monogamous women whose male partners were circumcised and who engaged in risky sexual behaviour (i.e. had 6 or more sexual partners, or sex with prostitutes) had a 58% reduction in ICC (aOR=0.42, 95%CI: 0.23-0.79, adjusted for AFSI and number of lifetime sex partners) compared to monogamous female partners of uncircumcised men with similar high risk behaviour. This association was not observed when restricting analysis to men who did not engage in risky sexual behaviour. The reduction in risk among female partners of men with multiple sex partners suggests a decrease in the risk of acquisition of HR-HPV.

Biological plausibility

The reduction in HPV acquisition and ICC among women is linked to the reduction in transmission of HPV from male partners, which result from: a.) an overall reduction in HPV prevalence among men, and b.) reduction in likelihood of transmission from infected males to their female partners. Removal of the foreskin during male circumcision decreases the surface area vulnerable to HPV (and others STIs including HIV), and decreases the likelihood of mucosal trauma during intercourse. The glans of the circumcised penis has a thicker epithelium making it more resistant to abrasions and therefore less susceptible to HPV viral entry. In these men, the distal urethra is the only mucosal epithelium, which is known to contain relatively fewer HPV-related lesions [119].

Hormonal contraception

Epidemiological evidence

Smith et al investigated the association of hormonal contraceptive use with ICC in a systematic review and meta-analysis of 28 cohort and case-control studies including a total of 12,531 women with ICC [92]. Compared with never users of oral contraceptives (OC), women taking OC for

durations of <5 years, 5-9 years and ≥10 years had increased risk of ICC with RRs of 1.1 (95%CI: 1.1-1.2, adjusted for number of sexual partners, cervical screening, smoking, or use of barrier contraceptives), 1.6 (95%CI: 1.4-1.7) and 2.2 (95%CI: 1.9-2.4), irrespective of HPV infection. Among women with HPV infection, the respective RRs were 0.9 (95%CI: 0.7-1.2), 1.3 (95%CI: 1.0-1.9), and 2.5 (95%CI: 1.6-3.9) for HPV positive women (**Table 2.2).** A more recent prospective study of 308,036 women recruited in the European Prospective Investigation into Cancer and Nutrition (EPIC) Study and followed for a median 9 years [120] reported that prolonged duration of oral contraceptives use was associated with a significantly increased risk of both CIN3/carcinoma *in situ* (CIS) and ICC (HR=1.6 and HR=1.8 respectively for long term (≥15 years) use versus never use).

The Smith et al meta-analysis [92] reported a weaker association for injectable contraception (which includes the progesterone-only norethisterone enanthate [NET-EN] and depot medroxyprogesterone acetate [DMPA]) use with ICC. Compared to never users, prolonged users (≥5 years) had 1.2- fold (RR=1.2, 95%CI: 1.0-1.6) increased risk of ICC (**Table 2.2**). No increased risk was observed among short-duration users (<5 years: RR=1.0, 95%CI: 0.9-1.2). It should be noted however that their review was performed in 2003 and there were only 3 studies which provided data on injectable contraception. Injectable contraception use is increasing in SSA [121]; the prevalence of hormonal contraceptive use among married or in-union women aged 15-49 years in 2015 was highest in Eastern Africa (31.1% in Malawi, 28.1% in Ethiopia and Rwanda), and Southern Africa (30.3% in South Africa and 27.5% in Namibia) [122]. Injectable contraceptives are highly effective, long lasting and easily reversible; a single dose is effective for months, which means fewer clinic visits and freedom from daily pills, which may be a reason for its popularity among WLHIV who may be on daily ART.

Biological plausibility

Both oral and injectable contraception have been associated with increased risk of acquisition of HPV and other STIs [123-129]. Hormonal contraception influences the differentiation and maturation of the cervical epithelium which can lead to thinning of the mucosal epithelium and microtearing [123, 126-130], acting as portal of entry for HPV. DMPA has also been shown to modify the normal genital flora reducing the levels of hydrogen peroxide-producing lactobacillus in the vagina [131], associated with an increased risk of vaginal flora changes and bacterial vaginosis (BV), which in turn is linked to increased risk of HIV, and other STIs [132, 133]. Furthermore, hormonal contraceptives can regulate cytokine and immunoglobulin expression [134] which may facilitate HPV persistence. Both oestrogen and progesterone increase the expression of the HPV oncoproteins E6 and E7 linked to ICC development [135, 136] and there is evidence of oestrogen assisting in ICC development through oestrogen response elements found in the long-chain region of HPV16 [137]. DMPA users have been reported to have higher levels of HPV16 E7 DNA compared to non-DMPA users [138].

Other Sexually Transmitted Infections (STIs)

The presence of other STIs increases the risk of acquiring HPV, possibly due to disruption of the mucosal epithelial barrier caused by inflammation and ulceration, thereby facilitating entry of the HPV virus [139]. The disruption to immune responses can facilitate HPV persistence. The link with ICC is less clear, as STI positivity is a marker of past sexual behaviour, and may act as a confounder for HR-HPV infection. However, several STIs induce inflammatory responses which are linked to HRHPV persistence and carcinogenesis, and these possibilities are reviewed here for selected pathogens, which have been better studied.

Herpes-simplex virus-2 (HSV-2)

Herpes simplex type 2 virus (HSV-2) was originally thought to be an etiological agent for ICC as early case control studies found an association of HSV-2 antibodies with ICC [140]. However, larger prospective studies failed to confirm these findings [27, 28]. As HSV-2 is found to frequently co-exist with HPV, it is thought that genital HSV-2 infection may facilitate HPV persistence and cervical lesion development [96].

Epidemiological evidence

The role of HSV-2 as a cofactor for ICC was investigated in a case-control study of 2,380 women from Africa, South America, Asia and Europe [96]. Among the HPV DNA-positive women, HSV-2 seropositivity was associated with a 2-fold (aOR=1.96, 95%CI: 1.24 to 3.09) increased risk of squamous-cell carcinoma (SCC) after adjusting for age, study site, HPV DNA type, history of pap smear, and markers of sexual or reproductive behaviour which included number of FTP, number of sexual partners, AFSI, *Chlamydia trachomatis* seropositivity, and oral contraceptive use (**Table 2.2).** As the analysis was restricted to those who were HPV DNA positive, the authors propose that genital HSV-2 may act in conjunction with HPV infection to modestly increase the risk of ICC.

Biological plausibility

As for HPV, HSV-2 can infect the cervical squamous epithelium at SCJ. Ulcerative HSV-2 lesions can facilitate HPV viral entry to the basal layer. Inflammation resulting from herpetic infections may interfere with targeted immune response to HPV infection by suppressing the T-helper cell-mediated immune response [141, 142]. Furthermore, HSV-2 can induce the production of nitric oxide which has been shown to cause cellular DNA damage in HPV-infected cells [143]. Finally, HSV-2 has been shown to facilitate HPV replication and integration of HPV DNA in infected host cells [144] and interfere with apoptosis [142], which may facilitate cervical lesion development and progression.

Chlamydia trachomatis

Epidemiological evidence

Chlamydia trachomatis infection has been shown to facilitate the entry and persistence of multiple HR-HPV types [145, 146] and has been suggested to be linked to the disruption of the immune response required to clear the virus [147]. The association of *Chlamydia trachomatis* as a cofactor for ICC has also been widely reported. A recent meta-analysis which examined the association between *Chlamydia trachomatis* and the risk of ICC [94] and included a total of 22 studies and 4,291 ICC cases found a significant association between *Chlamydia trachomatis* and ICC (aOR=1.76, 95%CI: 1.03-3.01, adjusted for HPV and age; **Table 2.2**).

Biological plausibility

Genital *Chlamydia trachomatis* may cause chronic cervicitis and pelvic inflammatory disease. The biological plausibility for an increased risk in ICC associated with *Chlamydia trachomatis* may be linked to the inflammatory cytokine responses during infection which may lead to reactive oxidative metabolite production, causing DNA damage or modification, resulting in genetic instability, particularly if infection persists [93, 147, 148]. In vitro data show that *Chlamydia trachomatis* may inhibit cell apoptosis, or programmed cell death, resulting in unregulated cell proliferation [149].

Trichomonas vaginalis

Epidemiological evidence

The link between *Trichomonas vaginalis* and ICC is less well studied. A systematic review dating from 1994 which included only 2 cohort studies investigating the association of *Trichomonas vaginalis* and ICC [95] reported a 2-fold (aRR=1.93, 95%CI: 1.22-2.65) increased risk of CIN1-3 among women positive for *Trichomonas vaginalis*, after adjustment for age, residence, past screening history and number of years since negative pap smear, number of sexual partners, HPV status and

duration of follow-up (**Table 2.2**). A longitudinal cohort study among 19,114 women attending organized mass screening in Finland in 1985-1990 evaluated whether gynaecological infections other than human papillomavirus (HPV) were related to an increased risk of cervical neoplasia. *Trichomonas vaginalis* was associated with an increased risk (Standardized incidence ratios [SIR]=6.4, 95%CI: 3.7-10) of preinvasive lesions and invasive cancer combined, however it is unclear whether the authors adjusted for HPV detection in the analyses [150].

Others have shown an increased risk of HR-HPV acquisition among 324 Tanzanian women positive for *Trichomonas vaginalis* (aOR=4.1, 95%CI: 1.7-9.8, adjusted for bacterial vaginosis and clinical pelvic inflammatory disease [87].

Biological plausibility

As the association of *Trichomonas vaginalis* with HPV and ICC has rarely been studied or reported, it remains unclear whether *Trichomonas vaginalis* generates any specific mechanism for HPV persistence or ICC development other than that reported for other STIs; that it may be a surrogate for HPV infection. However, *Trichomonas vaginalis* has shown to be associated with immunosuppression [151] and the presence of nitrosamines [152] in cervical discharge which may induce cervical epithelial cell transformation in the presence of oncogenic HPV infection [152-155]. It has also been shown to induce cervical epithelial atypia in mice [156] and to have promoter-enhancing effects [157] which facilitate cervical lesion development. However, further epidemiological evidence is required to further investigate these effects.

Neisseria gonorrhoeae (NG) and Mycoplasma genitalium (MG)

Although there is less evidence available on associations of *Neisseria gonorrhoeae* (NG) and *Mycoplasma genitalium* (MG) with HR-HPV and cervical lesions, HPV infection has been reported to be 4-fold higher among antenatal clinic attendees positive for NG compared to negative women

(aOR=4.46, 95%CI: 1.20-16.0) in Mwanza, Tanzania [158], but no such associations were reported for MG in a population of female sex workers (FSW) in Nairobi, Kenya [159].

Bacterial vaginosis (BV)

Epidemiological evidence

Two recent meta-analyses investigated the association of BV on HPV infection and cervical precursor lesions. In a meta-analysis which included 12 studies (10 cross-sectional, 2 prospective) among 6,372 women [88], BV positivity was associated with a 1.4-fold (OR=1.43, 95%CI: 1.11-1.84) increased prevalence of cervical HPV **(Table 2.2)**. A separate prospective study in the US among 1763 WLHIV and 493 HIV-negative women assessed semiannually for BV, TV and HPV found that women with BV at any time during follow-up had a higher risk of acquiring HPV (aOR=1.41, 95%CI: 1.25-1.59, adjusted for LTSP) [160].

A meta-analysis of the association of BV with cervical precursor lesions (defined as any of: LSIL, HSIL, CIN1, CIN2 or CIN3) included 17 cross-sectional and 2 incidence studies among over 10,000 women and found a 1.5-fold increase in SIL (LSIL/HSIL) or CIN (CIN1-3) prevalence (SIL and CIN combined: OR=1.51, 95%CI: 1.24-1.83) among women with BV [161]. However, the authors report significant heterogeneity in the estimate, a wide range of OR was reported across the individual studies and while only 3 studies adjusted for HPV, markers of sexual behaviour and smoking, they yielded conflicting results on the association of BV and SIL/CIN prevalence.

Biological plausibility

BV is associated with changes in the physiochemical and immunological environment of the vagina. In BV-negative women, hydrogen peroxide-producing lactobacilli dominate the vaginal microflora and have a role in defence mechanisms [88]. BV results in the loss of these protective micro-organisms and other changes in the vaginal milieu including changes in the production of cytokines such as IL-1β and IL-10 [162], which could facilitate the acquisition and persistence of other STI,

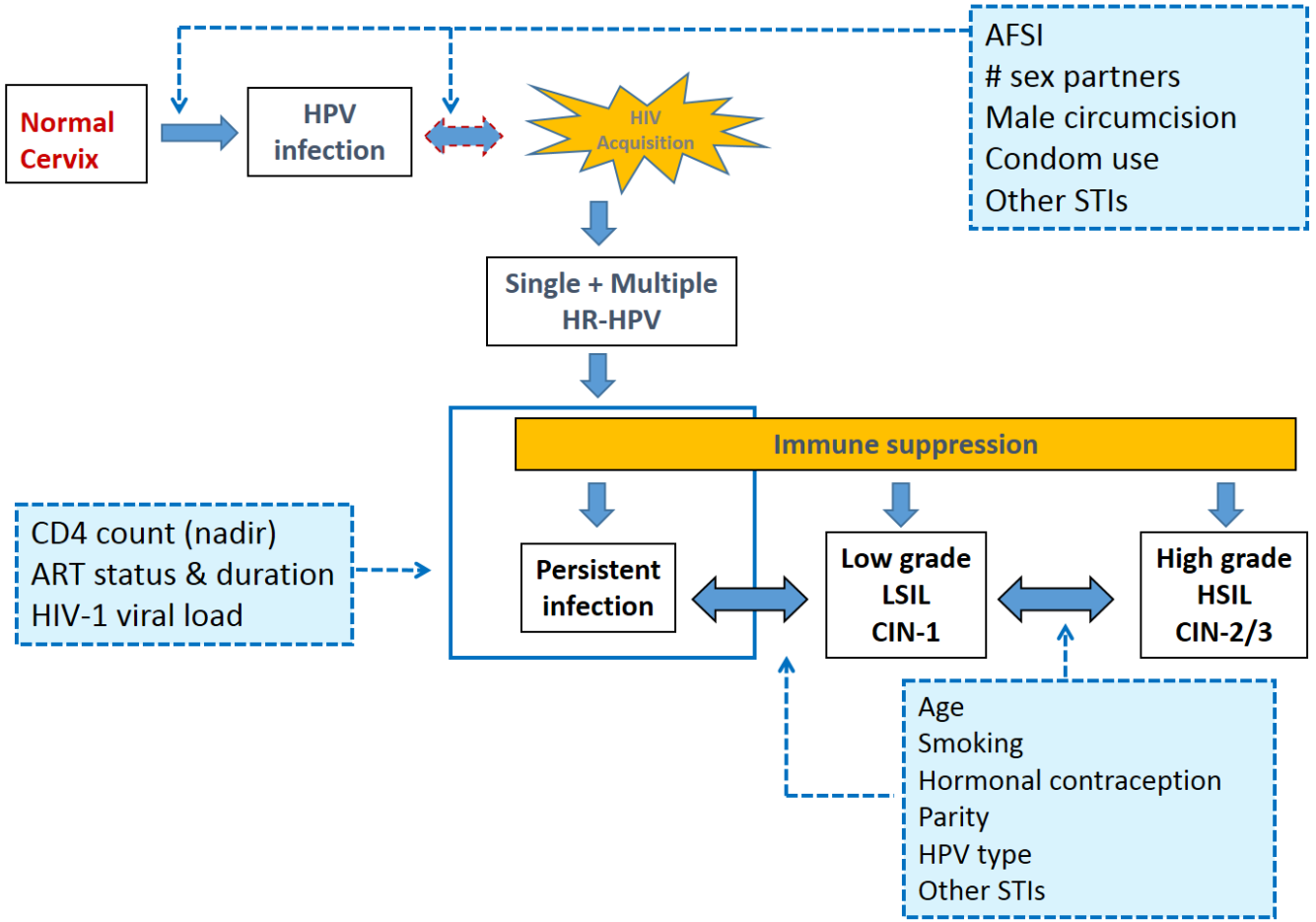
including HPV. Furthermore, BV-associated anaerobes release volatile amines which can form carcinogenic compounds such as nitrosamines in combination with nitrates, produced by nitrate reducing bacteria [163]. Others have shown that the local accumulation of nitrosamines during BV episodes may induce cervical epithelial cell transformation in the presence of oncogenic HPV infection [152-155]. Furthermore, the disrupted vaginal environment induced by BV is associated with alterations in the inflammatory cytokine profile which could promote cervical lesion development [162].

Human immunodeficiency virus (HIV)

The interrelations between HIV and HPV are bidirectional (**Figure 2.8**). Recent data has confirmed that HPV, like other STIs, facilitates the acquisition of HIV. Following on several observational studies, two systematic reviews have indicated that HPV infection confers a 2-fold increased risk of HIV acquisition in women and men [164, 165].

Likewise, HIV seropositivity increases the risk of acquiring HPV and the subsequent immunosuppression increases the risk of HPV persistence and cervical lesion progression (**Figure 2.8**). The associations of HIV-related factors (ART, CD4+ cell count and HIV-1 PVL) with HPV infection are discussed in more detail in the next **section 2.8**.

Figure 2.8. Factors associated with HPV infection and cervical lesion development among WLHIV



2.8 HPV and cervical intraepithelial neoplasia (CIN) among WLHIV

The interactions between HIV, HPV and cervical lesions have long been a subject of research but full elucidation of the mechanisms of these interactions remain a challenge. Recent systematic reviews and meta-analyses have summarized the comparative data for HPV or HR-HPV prevalence and incidence of low and high-grade cervical lesions and ICC among WLHIV and HIV-negative women, and are summarized here and in **Table 2.3.** Data on longitudinal HPV or HR-HPV outcomes (incidence, clearance and persistence) are more limited and while there have been no formal review or meta-analyses comparing these outcomes among WLHIV and HIV-negative women, recent data is summarized.

2.8.1 Effect of HIV on HPV infection epidemiology

Previous systematic reviews have shown that WLHIV have a 2-fold higher prevalence of HPV compared to HIV-negative women (pooled prevalence=62.0% vs. 29.5%; pooled PR=1.97, 95%CI: 1.81-2.15) [166], but among those with higher lesion grades (HSIL+) and ICC, HPV prevalence was similar, irrespective of HIV status [13, 14] (**Table 2.3**). However, the prevalence of multiple HPV was higher among WLHIV, irrespective of lesion grade. Four recent studies found that compared to HIV-negative women, WLHIV had between 1.8- and 2.3-fold increased risk of HR-HPV incidence [167, 168] and a 27-60% decreased likelihood of HR-HPV clearance [167, 169, 170].

2.8.2 Effect of HIV on cervical lesion incidence and progression

In a previous systematic review, WLHIV were reported to have a 4-fold increased prevalence of LSIL (pooled PR=3.63, 95%CI: 2.54-5.18) and HSIL (pooled PR=3.66, 95%CI: 2.55-5.25) compared to HIV-negative women [166]. In a large prospective study of 1,002 WLHIV and HIV-negative women in the US [171], WLHIV had a 5-fold increased risk of any SIL incidence (HR=4.50, 95%CI: 3.10-6.40) and HSIL incidence (HR=5.10, 95%CI: 1.90-14.20; [172, 173].

2.8.3 Effect of HIV on Invasive cervical cancer (ICC)

A review of 8 linkage studies using HIV/AIDS and cancer registries between 2000 and 2007 [166] reported over a 6-fold increase in ICC incidence among WLHIV compared to HIV-negative women (pooled SIR=6.64, 95%CI: 3.35-13.14). There was a single study from SSA carried out between 1989-2002 [174]; the authors report a 2.7-fold increased risk of ICC among WLHIV compared to HIV-negative women (SIR=2.7, 95%CI: 1.8-4.0). An additional case-control analysis of cancer risk among 4,399 women in Johannesburg and Soweto between 1995 to 2004, not included in the above meta-analysis, found that HIV-1 infection was associated with an increased risk of ICC (aOR=1.6, 95%CI: 1.3-2.0, adjusted for age, year of diagnosis, level of education and LTSP) [175].

2.8.4 Factors associated with HPV and related cervical disease among WLHIV

WLHIV have increased susceptibility to HPV infection and decreased ability to clear infection [176]. WLHIV have high rates of coinfection with HPV due to similar risk profiles for HIV and HPV acquisition, including early AFSI, multiple sex partners, low socioeconomic status, use of hormonal contraception and low use of barrier contraceptive methods [177]. Furthermore, both HIV and HPV infections elicit and thrive on viral and host factors that impair the immune system, thereby causing considerable co-morbidity [178]. The HIV-related factors influencing HPV infection and cervical lesion development are illustrated in **Figure 2.8.**

The role of HIV-related factors such as ART status, CD4+ cell count and HIV plasma or genital RNA levels, on HPV infection and cervical lesion development was the subject of a manuscript written as part of this thesis [179], the key points of which are summarised here.

Table 2.3. Review of epidemiology data of HPV, HR-HPV and cervical lesion outcomes among WLHIV compared to HIV seronegative women

Outcome	N studies [N women]	Percentage or No. Events		Effect Estimate (95%CI)	
		WLHIV	HIV-negative		
HPV prevalence [166]	29 [n=22,469]	62.0%	29.5%	PR[a]	1.97 (1.81-2.15)
HPV among cytology normal [13, 35]	20 [n=5578] (HIV+) 147 [n=266,671] (HIV-)	36.3%	12.0%	NR	-
HPV among HSIL+1 [13]	20 [n=5578]	84.1%	84.2%	OR	1.00 (0.70-1.40)
HPV among ICC [14]	21 [n=4616]	91.2%	89.6%	PR	1.02 (0.96-1.08)
Multiple HPV					
Multiple HR-HPV prevalence [180]	10 [n=5,436]	22.7%	14.5%	OR	2.41 (1.83-3.18)
Multiple HPV among cytology normal [13]	20 [n=5578]	11.9%			
Multiple HPV among HSIL+ [13]	20 [n=5578]	41.1%	6.7%	OR	9.30 (6.90-12.00)
Multiple HPV among ICC [14]	21 [n=4616]	27.8%	15.9%	PR	1.75 (1.18-2.58)
Longitudinal outcomes					
HR-HPV Incidence					
[167]	1 [n=1073]	NR	NR	aHR	2.28 (1.09–4.77)
[168]	1 [n=1,284]	8.1 per 100PY	4.4 per 100PY	RR	1.80 (1.30-2.70)
HR-HPV Clearance					
[167]	1 [n=1073]	NR	NR	aHR	0.41 (0.25–0.65)
[169]	1 [n=801]	37.3 per 100PY	62.0 per 100PY	aHR	0.58 (0.42-0.80)
At 6-months follow-up [170]	1 [n=334]	59.0%	75.9%	aHR	0.73 (0.52-1.01)
HPV Persistence [181]	1 [n=805]	24.1%	3.9%	aOR[4]	7.50 (3.60-16.0)
Cervical lesions					
LSIL Prevalence [166]	8 [n=5,987]	14.6%	3.9%	PR[a]	3.63 (2.54-5.18)
HSIL Prevalence [166]	7 [n=5,577]	4.5%	2.0%	PR[a]	3.66 (2.55-5.25)
SIL Incidence [171]	3 [n=3,371]	8.9-11.5 per 100YR	2.2-2.6 per 100PY	HR	4.50 (3.10-6.40)
HSIL incidence [171]	1 [n=1,002]	1.6 per 100YR	0.3 per 100PY	HR	5.10 (1.90-14.20)
ICC Incidence [166]	8 [n=102,212]	NR	NR	SIR	6.64 (3.35-13.14)

a=adjusted; HR=Hazard Ratio; RR=Relative Risk; PY=person-years; SIR=Standardised Incidence Ratio for invasive cervical cancer in women with HIV or AIDS through cancer registry linkage studies; acrude Prevalence Ratio obtained from pool extracted data from [166]; 1estimates given for any HPV; [2]Adjusted for study; [3]adjusted for age, number of lifetime sex partners and ethnicity; [4]adjusted for number of visits

CD4+ cell-count and HPV

CD4+ cell count has been shown to be one of the strongest predictors of HR-HPV infection [182, 183] and cervical lesion development [173] in multivariate analysis.

HR-HPV prevalence has been shown to increase by 1.5- and 3-fold among those with low CD4+ cell count (<200 cells/µl) compared to high CD4+ cell count (≥500 cells/µl) [184-186]. A decrease of between 11% to 18% in HR-HPV prevalence per 100 cells/µl increase was observed in a cohort of WLHIV with no evidence of cervical lesions [187] and among women with evidence of cervical lesions [188], respectively. Few studies have reported the association of CD4+ cell count with HR-HPV among ART users and ART-naïve separately.

De Vuyst et al [186] reported that ART-naïve participants with CD4+ cell count <250 cells/µl had a higher HR-HPV prevalence compared to those with CD4+ cell count ≥500 cells/µl (PR=1.51, 95%CI: 1.02-2.25) but no association was found among ART users, irrespective of duration on ART. Furthermore, Konopnicki et al [185] reported that a nadir CD4+ cell count <500 cells/µl was associated with a 3-fold increased risk of HR-HPV prevalence compared to those with CD4+ cell count ≥500 cells/µl.

Although few studies have reported the association of CD4+ cell count with longitudinal HPV outcomes, high CD4+ cell count has been shown to be associated with increased clearance of any HPV, and decreased persistence of HR-HPV. Paramsothy et al reported a 10% increased likelihood of HPV clearance per 100 cells/µl increase in CD4+ cell count among women with normal (aHR=1.1, 95%CI: 1.0-1.2) or ASCUS cytology (aHR=1.1, 95%CI: 1.0-1.3) [189]. Furthermore, Lillo et al reported a marginal 8% decreased risk of HR-HPV persistence per 50 CD4+ cells/µl increase [190].

Few studies have reported the impact of ART on specific HPV genotypes, and have tended to focus on HPV16/18 because of their greater importance in the aetiology of CIN and their supposed ability to escape immunological control [191]. Some studies, like Mane et al found that ART users had a

3.5-fold greater risk of HPV16 prevalence compared to ART-naïve individuals (aOR=3.47, 95%CI: 1.40-8.59) [188], whilst Blitz et al reported that ART users had a 2-fold greater hazard risk of clearing HR-HPV types other than HV16/18 and had a non-significant decreased risk of HPV16 clearance [192]. In contrast, Lillo et al found that ART users had a 72% decreased risk of incident HPV16 and 18 infections (aOR=0.28, 95%CI: 0.09-0.86), however the sample size was low in this study. Studies reporting the effect of CD4+ cell count on HPV16/18 infections have shown a clearer picture. Mane et al reported a 1.4-fold increased risk of prevalent HPV16 infection per 100 CD4+ cell decrease [188], while Blitz et al reported an almost 5-fold increased hazard risk of incident HPV16 among women with CD4+ cell count <200 cells/μl compared to those with CD4+ cell count >500 cells/μl (HR=4.82, 95%CI: 1.51-15.41), while no corresponding increase was observed for non-HPV16/18 types (HR=1.01, 95%CI: 0.39-2.60) [192].

CD4+ cell count and cervical lesions

Several studies suggest that CD4+ cell count is one of the strongest predictors of SIL prevalence, incidence and progression. Compared to women with baseline or current high CD4+ cell count (≥500 cells/mm^3), low CD4+ cell count (<200 cells/mm^3) resulted in a 2-fold increased risk of cervical lesion prevalence [186, 193, 194], SIL incidence [195-197] and SIL progression [195, 197, 198]. Others have reported a 10% decreased hazard of SIL progression per 100 CD4 cells/mm^3 increase [189, 199]. In contrast, no study reported an association between baseline or current CD4+ cell count with the likelihood of SIL regression [192, 195, 197] but Paramsothy et al reported a marginal 10% increased hazard risk of SIL regression per 100 CD4+ cells/mm^3 increase (aHR=1.1, 95%CI: 1.0-1.2) [189].

Longitudinal studies have shown that in populations with controlled HIV disease (i.e,, with undetectable HIV PVL and CD4+ ≥350 cells/mm^3), ART users present a similar risk of SIL incidence to ART-naïve with higher CD4+ cell counts [200] while ART use is most effective in the prevention of SIL incidence or progression at low CD4+ cell count. In South Africa, a 53% decreased risk in the rate of SIL incidence or progression was observed among ART users compared to ART-naïve

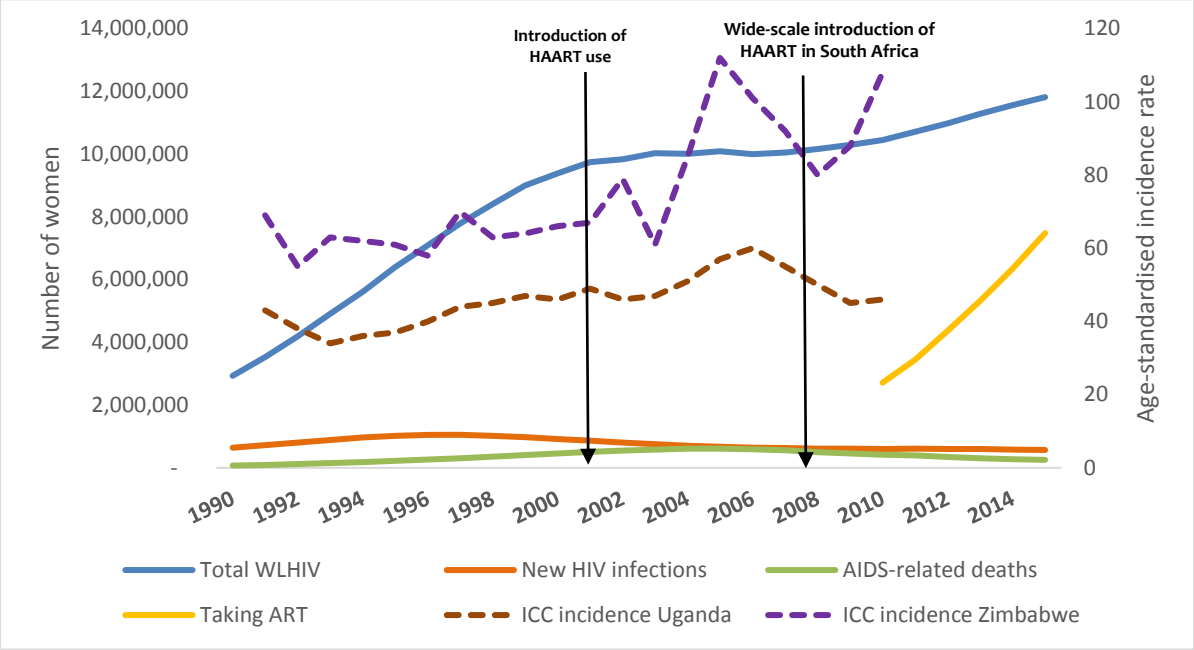
(incidence rate ratio [IRR]=0.47, 95%CI: 0.30-0.73) when the analysis was restricted to women with CD4+ <350 cells/mm^3, but no association was observed among those with CD4+ cell count ≥350 cells/mm^3 [201].

In contrast, cross-sectional studies report a significant association of low CD4+ with CIN2+ prevalence among ART-naïve but no association among ART users. De Vuyst et al have shown that ART-naïve Kenyan women with CD4+ cell count <250 cells/µl had a 4-fold increased risk of CIN2+ prevalence compared to those with CD4+ cell count ≥500 cells/µl (aOR=4.23, 95%CI: 1.27-14.07], but no association was observed among ART users, irrespective of duration on ART [186]. Similarly, Huchko et al also reported that ART-naïve women in Kenya with CD4+ count ≥500 cells/µl had a 58% decreased risk of CIN2+ prevalence compared to those with <200 cells/µl (aOR=0.42, 95%CI: 0.22-0.80) but there was no association observed among ART users [193]. A major limitation of cross-sectional studies is the difficulty to disentangle the chronology of events, e.g. the timing of CIN incidence with regard to timing of ART initiation and nadir CD4+ cell count. Several authors suggest that immune reconstitution by ART may not be established early enough after HPV infection to prevent or reverse the development of CIN2+ [186]. Huchko et al reported that nadir CD4+ count >500 cells/µl was associated with 40% reduction in CIN2+ detection compared to nadir CD4+ count <200 cells/µl (aOR=0.61, 95%CI: 0.38-0.97) [193]; while De Andrade et al showed that nadir CD4+ count ≥350 cells/µl was associated with greater than 80% decreased risk of CIN2+ compared to nadir CD4+ count <350 cells/µl (aPR=0.17, 95%CI: 0.04-0.67) [202].

Antiretroviral therapy (ART)

With the advent of the ART era, the incidence of other AIDS-defining cancers has decreased, however the impact on cervical cancer and its precursor lesions has been less clear. A recent meta-analysis of observational studies evaluating the incidence of malignancies before and after the introduction of highly-active antiretroviral therapy (HAART) found that while the risk for the development of Kaposi's sarcoma and non-Hodgkin's lymphoma decreased by 70% (RR=0.30, 95%CI: 0.28-0.33) and 48% (RR=0.52, 95%CI: 0.48-0.56), respectively, the incidence of invasive cervical cancer increased by 46% (RR=1.46, 95%CI: 1.09-1.94) after the introduction of HAART [11]. These opposing trends are depicted in a time-trend graph (**Figure 2.9**) between 1990 and 2015 which compares numbers of WLHIV aged ≥15 years, sourced from UNAIDS [10] and the age-standardized cervical cancer incidence in SSA sourced from cancer registries from the International Agency for Research on Cancer (IARC) [203]. Data on cervical cancer incidence is limited in SSA; only data from the general population of women in Uganda (Kyadondo County) between 1993 and 2007 is available. With the increased availability of ART from 2001 onwards (2004 in South Africa) and HIV prevention programmes in the region, the number of new infections is stabilizing and AIDS-related deaths are in decline and the total number of women living with HIV is increasing. Data from the general population of women in Uganda (Kyadondo County) and Harare, Zimbabwe between 1991 and 2010 however show a trend of increasing cervical cancer incidence.

Figure 2.9. Time-trends of numbers of women living with HIV (cumulative), new HIV infections (per year), AIDS-related deaths (per year) and numbers taking ART (cumulative), and age-standardised cervical cancer incidence rates among women in Sub-Saharan African region



WLHIV=women living with HIV; ASR=age-standardised incidence rate; data for variables WLHIV, new HIV infections, AIDS-related deaths and taking ART extracted from UNAIDS AIDSInfo [10]; data for Cervical cancer incidence ASR from Kyadondo County, Uganda extracted from IARC C15Plus Cancer Incidence Time Trends registry [203] and updated up to 2010 from [204]; data for Cervical cancer incidence ASR from Harare, Zimbabwe from [205] [no other country from Sub-Saharan Africa had sufficient data for set time period]

Recently studies have shown that immediate ART reduces the risk of infection-related cancer during early HIV infection. The Strategic Timing of Antiretroviral Treatment (START) trial randomized HIV positive adults with a CD4+ count over 500 cells/mm^3 to immediate combination ART initiation or deferral until CD4+ counts dropped below 350 cells/mm^3 [206]. Immediate ART reduced the risk of infection-related cancers by 64% with greatest decreases observed for Kaposi's sarcoma and non-Hodgkin's lymphoma, but there were too few cases of cervical cancer to derive any meaningful associations.

The interactions of ART with HR-HPV and CIN are complex. ART increases potential exposure time to HR-HPV by decreasing mortality and increasing life expectancy of WLHIV. However, many individuals remain susceptible to HPV acquisition, persistence and incidence and progression of CIN or SIL, increasing the probability of accumulation of cellular genetic changes that increase the

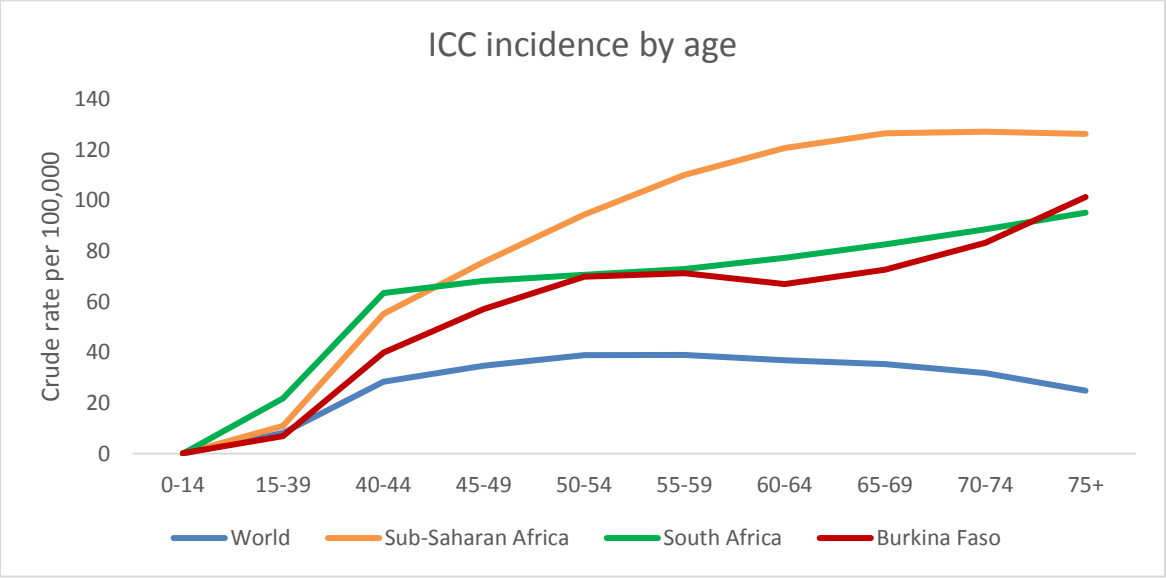
likelihood of ICC [176, 207]. Improved survival among WLHIV may thus lead to increased cervical cancer rates [208]. Conversely, by decreasing HIV PVL, ART can restore mucosal immune competence to help clear HR-HPV and reduce the incidence of precursor lesions [209, 210]. Previous systematic reviews have explored the association of ART with HR-HPV and cervical lesions [173, 211, 212] but report conflicting results. Some studies show that "effective" ART (i.e. with high adherence, HIV viral suppression and immune reconstitution) over longer durations decreases HR-HPV prevalence [184, 209, 213], by decreasing incidence [214, 215] and promoting clearance [209, 214, 216]. ART has also been shown to reduce high-grade cytological SIL incidence [197] and progression [217, 218] especially among adherent users [214] and those with sustained HIV viral suppression [219]. However, other studies have reported no such benefits of ART on HR-HPV [188, 220] or histological lesions [193, 213, 221-224], with some reporting a significant increased risk of CIN2+ among ART users [225]. Moreover, few longitudinal studies have reported the effect of ART and other HIV-related factors on the combined natural history of HR-HPV infection and histological cervical lesions. These observational studies differed with respect to study design, outcomes, timing of ART initiation and effectiveness of ART use, making it difficult to estimate the true effect of ART.

Given that no meta-analysis has quantified the risk of HR-HPV and cervical lesions among ART users compared to ART-naive women, or considered other confounding factors, such as nadir CD4+ cell count and ART duration, one of the aims of this PhD was to systematically review and summarise the literature on the association of ART with HR-HPV prevalence, and with cervical lesion prevalence, incidence, progression and regression, and to investigate the role of HIV-related co-factors that might modify these associations, such as ART duration, timing of ART initiation and immune suppression and recovery. These findings are summarised and discussed in **Chapter 3**.

2.9 Control of HPV related cervical disease –screening and management

Between 80-90% of confirmed ICC cases occur among women aged 35 years and older. In the SSA region, incidence rates rise steeply between the ages of 15-39 years and continues to rise among older age groups, and are particularly marked for the countries in which this study took place (**Figure 2.10**).

Figure 2.10. Incidence of invasive cervical cancer, by age, in 2012



Crude rate per 100,000 women. data from [9]

The World Health Organisation (WHO) 2014 Cervical cancer screening guidelines recommend that screening should be performed at least once between the ages of 39-49 years, and this may be extended to women younger than 30 years if there is evidence of high risk for CIN2+ [226]. However, cervical cancer screening coverage, and linkage to care, is low in LMIC countries where infrastructure and personnel requirements for cervical screening and management put high demands on the health systems [227].

The recommended cervical cancer screening methods include cervical cytology using the Papanicolaou method, Visual Inspection (VI) using acetic acid (VIA) or Lugol's iodine (VILI) add HPV DNA based testing, which are briefly described here.

2.9.1 Screening tests

Cytology

Cytology based programmes have improved cervical cancer control in developed countries but implementation in developing countries is constrained by cost, lack of infrastructure and trained staff, and lag time between sample collection and availability of test result [228]. In a meta-analysis of studies evaluating performance of screening in Africa and India [229], cytology, whether using positive test result cut-offs of ≥ASCUS, ≥LSIL or ≥HSIL had poor sensitivity for the detection of CIN2+ (range: 42.6 for ≥HSIL to 57.0% for ≥ASCUS) and CIN3+ (range: 51.6-63.0%) but high specificity (range: 92.8-99.3% and 92.3-99.0% for CIN2+ and CIN3+, respectively) (**Table 2.4**).

Visual Inspection

Visual inspection using acetic acid (VIA) or Lugol's iodine (VILI) is advantageous in low resource settings as it can be performed by trained midwifes and nurses, and requires simple tools to perform (speculum, acetic acetic acid, Lugol's iodine, lamp). However, frequent training and supervision is required. In a meta-analysis of 21 studies from SSA and India among over 58,000 women [228, 229], VIA showed good sensitivity for the detection of CIN2+ (range: 79.2-82.4%, **Table 2.4**) and CIN3+ (82.9%) and good specificity (84.7%-87.4% and 84.2% for CIN2+ and CIN3+, respectively). VILI performed even better with higher sensitivity (CIN2+: 91.2-95.1%; CIN3+: 93.8%) and similar specificity (CIN2+: 84.5-87.2%; CIN3+: 83.8%). However sensitivity has been reported to be as low as 50% in other studies [230]. The wide variation in its performance is attributed to the subjective nature of test interpretation. High rates of positivity have resulted in over referral to colposcopy services and overtreatment [6]. Despite this, a single round of VIA screening has been associated with a 25-35% reduction in cervical cancer incidence, cervical cancer mortality and the frequency of CIN2+ lesions, in randomised-controlled trials in Thailand, Ghana and Zambia [231-233]. In the absence of other adaptable cervical screening strategies in LMIC, VIA/VILI is a simple, inexpensive test for resource limited settings. A systematic review of 5 cross-sectional studies

which evaluated the performance of VIA and cytology for the detection of CIN2+ among WLHIV in Brazil, India, Kenya, Nigeria, South Africa, Tanzania, Thailand, Zambia and Zimbabwe [234] found that VIA sensitivity ranged from 65% to 80% and specificity ranged from 51% to 83%. Sensitivity of VIA was similar or better than that of cytology however VIA specificity was slightly lower. However, screening using VIA/VILI has also been shown increase detection and treatment rates among WLHIV in South Africa [227] as it allows for immediate treatment with cryotherapy.

HPV DNA based tests

Prior studies have shown that HPV DNA based screening allows for earlier diagnosis of high-grade CIN and is more effective in prevention of ICC [235]. A single round of the Digene Hybrid Capture 2 (HC2) PCR based test which detects 13 HR-HPV types has shown to halve the rate of ICC (HR=0.47, 95%CI: 0.32-0.69) and ICC-related mortality (HR=0.52, 95%CI: 0.33-0.83) compared to VIA among 131,746 women aged 30-59 years in India [235].

Point-of-care (POC) tests, such as the *care*HPV test (Qiagen) which is based on a simplification of the HC2 test platform, detects 14 HR-HPV types and is optimised for developing countries (shorter run time, portable batteries, longer shelf-life which does not require refrigeration) has shown good performance for the detection of CIN2+ in a large evaluation trial among 2,500 women in China [236]; *care*HPV had sensitivity of 90% for the detection of CIN2+ compared to 41% for VIA; and a specificity of 84% compared to 95% for VIA.

Table 2.4. Summary of 3 meta-analyses among the general population and 1 cross-sectional study among WLHIV assessing performance of cervical tests for CIN2+ and CIN3+ detection

			CIN2+				CIN3+			
Location and citation	**Test**	**Test strategy**	**N studies**	**Sensitivity % (95%CI)**	**Specificity % (95%CI)**	**Pooled CIN2+ Prevalence % (95%CI)**	**N studies**	**Sensitivity % (95%CI)**	**Specificity % (95%CI)**	**Pooled CIN3+ Prevalence % (%, 95%CI)**
General population										
Worldwide [237]	CareHPV	Primary screening					1	87.0 (73.2-100.0)	86.1 (84.7-87.5)	1.0 (0.6-1.4)
	HC2[a]	Primary screening	31	90.4 (88.0-92.8)	88.5 (87.0-90.0)	2.2 (1.8-2.6)	22	95.3 (93.3-97.3)	89.0 (87.2-90.8)	1.1 (0.8-1.3)
		Triage ASCUS	39	90.4 (88.1-92.3)	58.3 (53.6-62.9)	12.0 (10.2-13.7)	17	93.7 (90.4-95.9)	52.3 (45.7-58.7)	6.8 (5.0-8.6)
		Triage LSIL	24	95.4 (94.0-96.5)	27.8 (23.8-32.1)	21.3 (17.7-24.9)	14	96.4 (90.5-98.7)	23.7 (19.4-28.7)	8.9 (6.6-11.2)
Africa and India [229]	VIA	Primary screening	11	79.2 (73.3-85.0)	84.7 (80.7-88.8)	2.3	11	82.9 (77.1-88.7)	84.2 (80.0-88.3)	NR
	VILI			91.2 (87.8-94.6)	84.5 (81.3-87.8)			93.8 (90.6-97.1)	83.8 (80.5-87.1)	
	≥ASCUS			57.0 (37.6-76.3)	92.8 (88.7-96.8)			63.0 (37.9-88.2)	92.3 (88.1-96.6)	
	≥LSIL			51.2 (30.0-72.4)	94.9 (92.1-97.7)			56.1 (32.7-79.6)	94.5 (91.6-97.5)	
	≥HSIL			42.6 (26.5-58.6)	99.3 (98.8-99.7)			51.6 (32.0-71.1)	99.0 (98.4-99.5)	
	HC2			61.9 (56.2-67.7)	93.6 (92.4-94.8)			68.4 (61.5-75.4)	93.4 (92.2-94.6)	
Sub-Saharan Africa [228]	VIA	Primary screening	10	82.4 (76.3-87.3)	87.4 (77.1-93.4)	3.3 (2.1-4.7)				
	VILI		8	95.1 (90.1-97.7)	87.2 (78.1-92.8)	2.3 (1.5-3.3)				
	HC2[b]		3	88.3 (73.1-95.5)	73.9 (50.7-88.7)	6.9 (1.7-15.2)				
WLHIV										
South Africa[c] [238]	VIA	Primary screening	1	65.4 (59.7-76.1)	68.5 (65.3-71.7)	26.0	1	68.2 (59.3-77.2)	61.5 (58.6-64.4)	8.5
	≥ASCUS			94.8 (90.5-99.2)	35.6 (32.2-38.9)			99.9 (98.8-100.0)	29.6 (26.9-32.3)	
	≥HSIL			75.8 (70.8-80.8)	83.4 (80.9-85.9)			94.5 (89.8-99.7)	72.7 (70.0-75.3)	
	HC2			91.9 (88.5-95.3)	51.4 (48.0-54.8)			97.9 (95.0-100.0)	42.8 (39.8-45.7)	
	HC2[d]	Triage HSIL+		96.6 (94.4-98.9)	17.3 (11.0-23.6)			97.8 (94.9-100.0)	10.4 (6.9-13.9)	
	VIA[e]	Triage HSIL+		81.7 (77.0-86.5)	46.7 (38.4-55.1)			77.5 (69.3-85.8)	30.4 (25.1-35.6)	
	≥HSIL[f]	VIA		81.9 (77.1-86.7)	72.2 (66.8-77.6)			96.1 (91.7-100.0)	53.6 (49.0-58.3)	

[a]HC2 as a second test; [b]2 studies used HC2, 1 used PCR; [c]this study enrolled 1193 WLHIV in Johannesburg, South Africa; [d]HC2 as second test; [e]VIA as second test; [f]Cytology HSIL+ as second test

2.9.2 Novel biomarkers for CIN2+ detection

HPV testing may detect many transient infections, meaning that its specificity for high-grade CIN can be low. A recent review of HPV DNA based tests for the detection of cervical lesions reported that for a 10% increase in HR HPV prevalence, HR-HPV DNA based test specificity for CIN2+ detection decreased by 8.4% (95%CI: 8.0-8.8%) [239], which has important implications for HIV-positive populations who have a high prevalence of HR-HPV. HC2 has shown to have high sensitivity for CIN2+ and CIN3+ among WLHIV, but poor specificity (**Table 2.4**) [238].

Novel methods are therefore required to identify which HPV positive women need colposcopy referral or management, avoiding repeat tests/visits, which can result in substantial loss to follow-up [240, 241].

While infection with HR-HPV is necessary for the development of cervical lesions and cancer [35, 37], other molecular changes occur with HR-HPV infection, leading to subsequent development of alterations in the functions and abundance of gene products regulating oncogenesis, tumor suppression, DNA repair, apoptosis, metastasis and invasion, which are necessary for developing a malignant phenotype [242, 243]. Such alterations can result from DNA nucleotide mutations, structural genomic variations or epigenetic alterations, such as DNA methylation [244]. Aberrant DNA methylation is a potentially good early indicator of existing disease [245] and predictor for future development of disease [246].

Human gene and HPV viral DNA methylation are novel biomarkers that may help distinguish non-progressive HPV infections from those that progress to cancer. DNA methylation assays are now widely available for use in early detection of various cancers, including bladder, breast, colorectal, oesaphagal, gastric, head and neck, liver, lung and prostate cancers [246] and increased DNA methylation has more recently been shown to be associated with increasing persistence of HPV genotypes [247-249] and severity of CIN lesions [15].

Methylation markers have seldom been tested among WLHIV. A combined panel of *CADM1, MAL* and *MIR124-2* have previously shown good performance with an area under the receiver operating characteristic curve of 0.80 and 0.85 for the detection of CIN2 and CIN3, respectively among WLHIV in Nairobi, Kenya [17].

The human gene *EPB4L3* is a member of the band 4.1 protein superfamily, was originally identified as a tumour suppressor gene, which is down-regulated in lung adenocarcinoma [250]. *EPB41L3* has been shown to be implicated in breast cancer [251], prostate cancer [252] and meningiomas [253] as a result of loss to its tumour suppressor activity. The performance of the human *EPB41L3* has been previously shown to have reasonable sensitivity and specificity for the detection of CIN2/3 relative to ≤CIN1 in the general population [254-256]. Three recent reviews [247-249] report methylation of different regions of the HPV16 genome and there are consistent reports of increased methylation of the L1 gene and high-grade cervical lesions. However, there are as yet no reports of the DNA methylation of *EPB41L3* and HPV16 in the detection of CIN2/3 among WLHIV.

There have been a wide range of studies on DNA methylation of human genes and the HPV virus for the detection of HPV related lesions. Many of these studies have looked at different human genes and HPV types, or different CpG sites within genes and the HPV virus [257], and it is therefore to understand the role of the different DNA methylation markers for detection of CIN2+. One of the aims of this PhD was to perform a systemic review of the literature on DNA methylation markers associated with CIN2+ among HIV-positive women. The review is extended to HIV-negative women due to the relative scarcity of data among HIV-positive women. The review is summarised in **Chapter 4**.

2.10 Prevention of HPV infection: vaccination

HPV vaccine type distribution among invasive cervical cancer (ICC) cases in HIV-negative and HIV-positive women

Infection with HR-HPV types 16 and 18 account for 70% of all cervical cancers and infection with an additional five types (31/33/45/52/58) account for >90% of cancers among HIV-negative women [73]. Vaccine manufacturers have designed a range of vaccines targeting HPV16 and 18 (combined in a bivalent and a quadrivalent vaccine, which also includes HPV6 and 11). The nonvalent vaccine, more recently available, includes the five additional HR types mentioned above (targeting HPV6/11/16/18/31/33/45/52/58). These vaccines have been extensively evaluated in HIV-negative populations [258], but there are as yet no efficacy data among WLHIV.

A recent systematic review by investigators at International Agency for Research on Cancer (IARC) compared the HPV type distribution and the HPV vaccine type distribution in ICC biopsy and cervical cell specimens of 770 HIV-positive and 3846 HIV-negative women from 21 studies in 12 African countries [14]. The authors report that the fraction of ICC attributable to the HPV types included in the current bivalent (HPV16/18) and nonavalent (HPV16/18/31/33/45/52/58) vaccines was similar among HIV-positive and HIV-negative women (bivalent: 61.7% and 67.3%; nonavalent: 88.9% and 89.5%, respectively). However, a non-negligible proportion of ICC from both HIV-positive and HIV-negative women were infected with non-vaccine types in the absence of any of the vaccine types (7.0% and 7.9% of ICC from HIV-positive and HIV-negative women, respectively), and this was highest for HPV35. These findings confirm that the currently available HPV vaccines could prevent a similar proportion of ICC cases in HIV-positive as in HIV-negative women.

2.10.1 HPV vaccinations among people living with HIV

Although there is no direct data yet available on HPV vaccine efficacy among persons living with HIV (PLHIV) (**Table 2.5**), 2 recent reviews of trials of the bivalent (targeting HPV16 and -18) and quadrivalent (targeting HPV6/11/16/18) vaccines among PLHIV confirms that HPV vaccines are safe and immunogenic in children, female adolescents and adults **(Table 2.5**) [12, 259]. Seroconversion rates have been high in both ART users and ART naive vaccinees with high antibody titres sustained up to 12 months in both groups [260]. Antibody titres were comparable between HIV negative and ART users, but lower among ART-naïve patients [260, 261]. A recent meta-analysis of seropositivity studies among PLHIV following vaccination reported HPV antibody responses of 98% for HPV16 and 94% for HPV18 following 3 doses of vaccination [259]. There are no data on safety, immunogenicity or efficacy of the more recently available nonavalent vaccine (targeting 7 HR types: HPV16/18/31/33/35/52/58 and the two LR types [HPV6/11]) among PLHIV to date. The nonavalent HPV vaccine offers greater scope for protection against a greater range of HPV types, important in a population with high levels of multiple HR infection. Given the high prevalence and incidence of HPV types other than HPV16/18 and high number of multiple HR-HPV infections in WLHIV, the nonavalent HPV vaccine has greater scope for potential prevention of diseases associated with these HPV types.

Table 2.5. Summary outcomes of vaccine studies in target groups

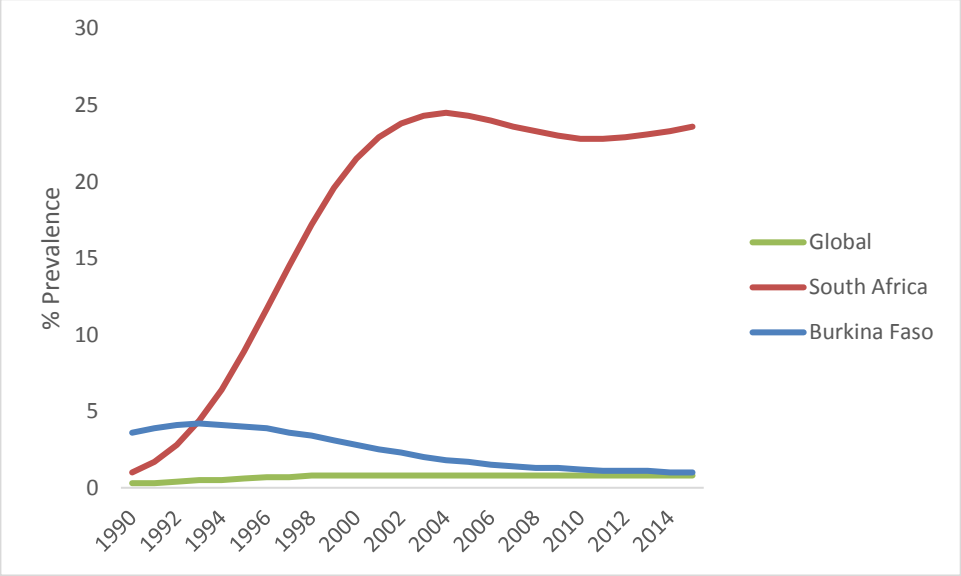
Outcome	Young women	Mid-adult women	Young men	Children	HIV+
Infection efficacy	✓	✓	✓	X	X
CIN2+ efficacy	✓	✓	-	X	X
CIN3 efficacy	✓	X	-	X	X
VIN/VIN 2/3 efficacy	✓	X	-	X	X
Genital warts efficacy	✓	X	✓	X	X
Anal infection efficacy	✓	X	✓	X	X
AIN2+ efficacy	X	X	✓	X	X
Partial cross-protection infection	✓	X	X	X	X
Partial cross-protection CIN2+	✓	X	X	X	X
Therapeutic efficacy	None	X	X	X	X
Immunogenicity	✓	✓	✓	✓	✓
Safety	No concerns	No concerns	No concerns	No concerns	No concerns

Adapted from [12, 258]

2.11 Country profiles

The research in this PhD was performed among WLHIV in Burkina Faso (BF) and South Africa (SA), two countries with different burdens of HPV infection and cervical cancer, and different approaches to screening for cervical cancer. The two countries also represent the spectrum of HIV infection (**Figure 2.11**), from relatively low prevalence in BF to high prevalence in SA, thereby providing useful case studies to determine the natural history HPV-related disease among WLHIV in different African contexts.

Figure 2.11. HIV prevalence over time among women aged ≥15 years, 1990-2015



Data extracted from UNAIDS AIDSInfo (2015) [10]

2.11.1 Burkina Faso

Burkina Faso has a total population of 18.1 million (**Table 2.6**). It is a country with a young demographic (46% are less than 15 years old). Life expectancy is 59 years, compared to 71 years worldwide. Just under half (44%) of the population live below the poverty line of less than US$1.90 a day.

Table 2.6. Demographic, sexual behavior and HIV-related factors in Burkina Faso and South Africa, 2012

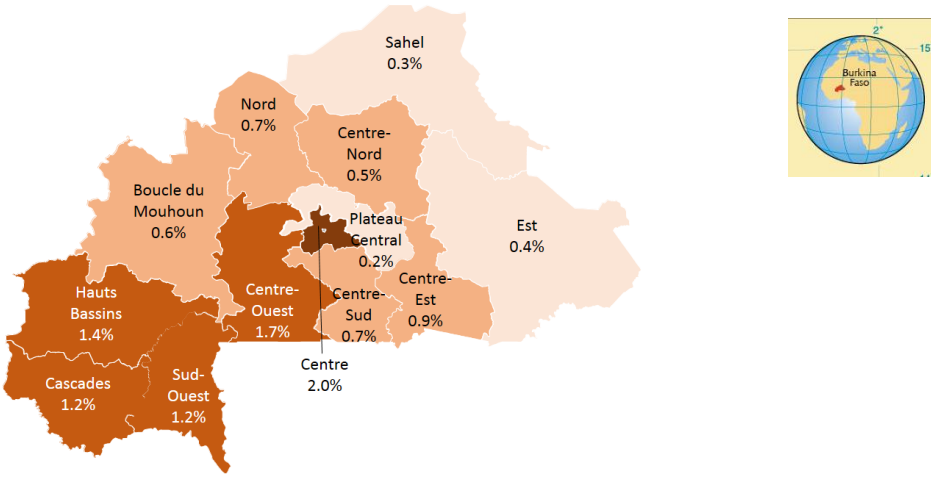
	Burkina Faso	South Africa
Demographic factors		
Population, total[a]	18,105,570	54,956,920
Population <15 years (%)[a]	45.6	29.0
Life expectancy at birth, total (years) [a]	58.6	57.2
Fertility rate (births/woman) [a]	5.7	2.41
Population, female (% of total) [a]	50.4	50.8
Population, female aged ≥15 years[a]	5, 040,000	19, 810, 000
Urban population (% of total) [a]	29.9	64.8
GDP per capita (current US$) [a]	589.8	5,724.0
Poverty headcount ratio at national poverty lines (% of population) [a,b]	40.1	53.8
Poverty headcount ratio at $1.90 A DAY (2011 PPP) (% of population) [a]	43.7	16.6
Health service		
Health expenditure (% of GDP) [a]	5.0	8.8
Number physicians/1000 population [a,c]	0.05	0.8
Nurses/midwives/1000 population [a,c]	0.57	4.7
HIV-related		
HIV prevalence among adults aged 15 to 49 years, %[d]	0.8	19.2
HIV prevalence among young women (15-24 years) , %[d]	0.4	11.6
HIV incidence rate among adults (15-49 years) [d]	0.05	1.44
Number of adults (≥15 years) living with HIV[d]	88,000	7,000,000
Number of women (≥15 years) living with HIV[d]	53,000	4,000,000
Number of deaths due to AIDS (women ≥15 yrs) [d]	1,300	81,000
Number of women (≥15 years) receiving ART[d]	35,468	2,133,020
HIV spending		
HIV spending from domestic public and international sources (US$)[e]	49,000,000	1,880,000,000
HIV spending from domestic public sources (%)[e]	7,780,061 (15.9%)	1,492,672,908 (79.4%)
Cervical cancer estimates		
Cervical cancer incidence rate per 100,000 per year (age standardised)[f]	23.3	31.7
Mortality form cervical cancer rate per 100,000 per year (age standardised)[f]	18.6	18.0
Most frequent cancer among women (ranked by total # cases) [f]	Cervix uteri	Breast (followed by cervix uteri)
Control and prevention measures		
HPV vaccination	No	Yes – school aged girls (started 2014)
Primary cervical cancer screening modality	Visual inspection (with acetic acid or Lugol's Iodine)	Cervical cytology

[a]World Bank (2015); [b]National poverty headcount ratio is the percentage of the population living below the national poverty lines. National estimates are based on population-weighted subgroup estimates from household surveys; in BF this is below CFA 82,672 (US$165); [c]data for Burkina Faso from 2010 at CIA: https://www.cia.gov/library/publications/the-world-factbook/fields/2226.html and WHO: http://apps.who.int/iris/bitstream/10665/136973/1/ccsbrief_bfa_en.pdf; [d]UNAIDS (2015); [e]UNData, from http://data.un.org/Data.aspx?d=UNAIDS&f=inID%3A49 [f]HPV information Centre (2016), Institut Català d'Oncologia at http://www.hpvcentre.net

2.11.1.1 *HIV control and Prevention*

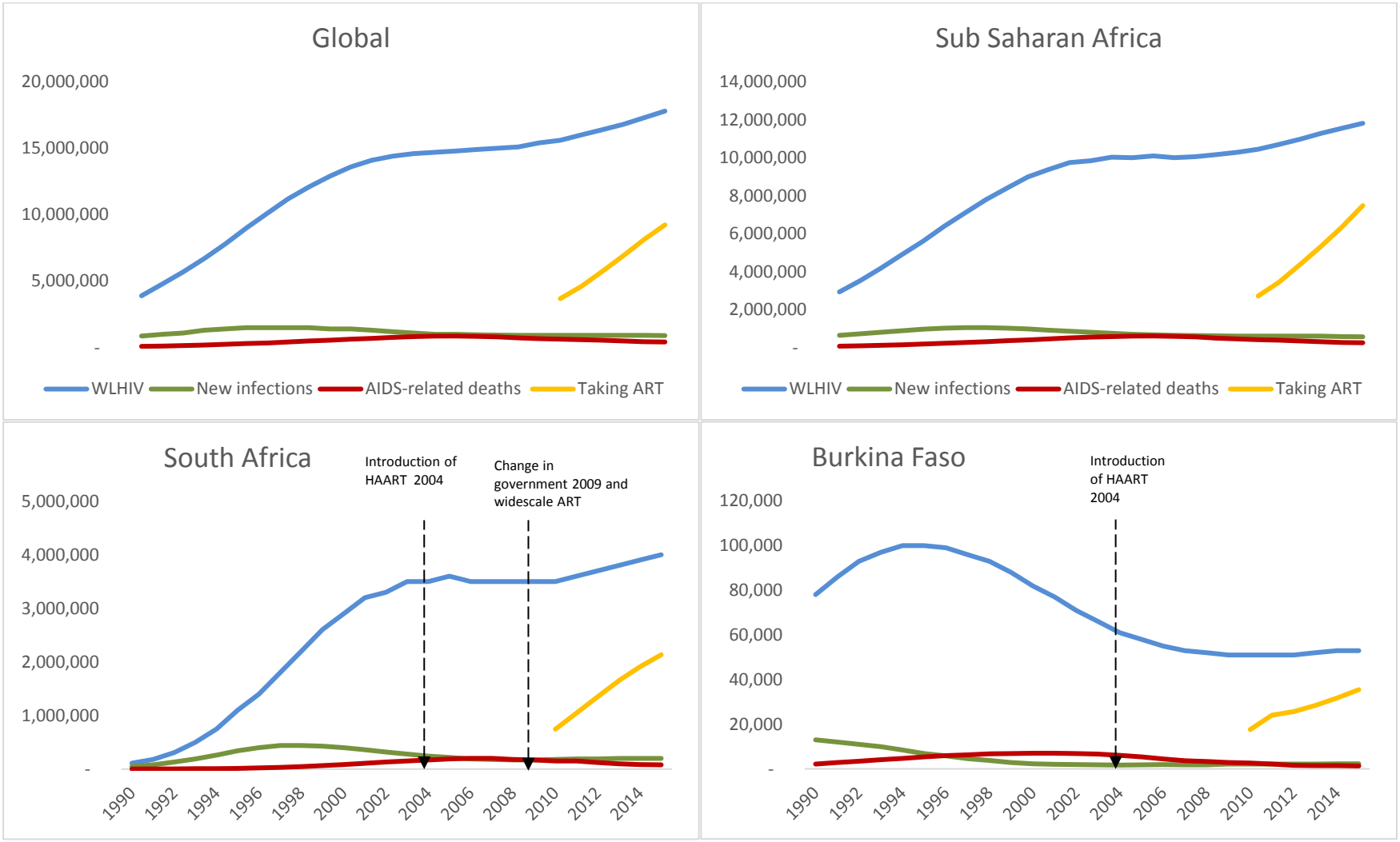
In 1990, there were 78,000 women ≥15 years with HIV (prevalence of 3.6%). At the end of 2015, 53,000 women ≥15 years were living with HIV (prevalence of 0.8%, **Table 2.6**), with the highest prevalence in the capital city of Ouagadougou (Centre Region, **Figure 2.12**). In the same year, there were 2,300 new infections and 1,300 AIDS-related deaths among women ≥15 years [10] (**Figure 2.13**). By end-2015, 35,468 women aged ≥15 years were receiving ART (coverage of 66%) [10].

Figure 2.12. HIV Prevalence by region in Burkina Faso, 2010 DHS estimates [262]



The first ARVs were made available in local private pharmacies from 1996 but their high cost was a significant barrier and this led to irrational use as most patients were able to afford only intermittent monotherapy or dual therapy [263]. Up until 2009, Burkina Faso was the exception to the trend in Africa of abolishing user fees for ART access. It had a direct out-of-pocket payment for ARVs at the point of care delivery, with subsidized payments for ARVs, laboratory tests and physician's consultations, while the poorest received these services for free [264]. In 2005, the National AIDS Council decided that each HIV-infected person could have access to ART with an individual monthly income of CFA 5,000 (US$10), which was decreased to CFA 1,500 (US$3) in 2008. Patients identified as "worst off" would receive ART for free [264].

Figure 2.13. Time-trend analysis of HIV epidemic worldwide, in Sub-Saharan Africa, South Africa and Burkina Faso*



*Data extracted from UNAIDS AIDSInfo (2015) [10]; (no data available on ART use before 2010)

In 2015, 40% of the population lived on less than US$1.90 a day. A 2010 study interviewing 499 PLHIV from 26 public health centres, non-governmental organisation (NGOs) and faith-based health facilities reported that 79% did not pay for their treatment, while among those that were charged, 95% paid the subsidized amount of US$ 10 and 5% paid only a part of the subsidized amount ranging from US$1 To US$2.50 [264]. The State procures ARVs via the Global Fund and distributes them to public services centres or NGOs based on estimated needs [265]. In 2011, the then President, Blaise Compaoré, announced ARVs would be free of charge, and in 2012, committed to reducing the country's dependence on external funding by expanding the share of domestic resources allocated to the HIV response from 15% to 25% (**Table 2.6**).

2.11.1.2 *Cervical cancer control and prevention*

Burkina Faso has a population of 5 million women aged 15 years and older who are at risk of developing cervical cancer [266] **(Table 2.6**). Cervical cancer ranks as the most frequent cancer among women in Burkina Faso. In Burkina Faso, cervical cancer incidence and mortality was 23.3 and 18.6 per 100,000 per year- in 2012 (age standardised rate) [9].

Visual inspection using VIA or VILI is the current method for cervical cancer screening. A WHS (WHO Household Survey) carried out between 2001 and 2002 found that 5.5% of all women aged 18-69 years were screened within the previous 3 years [266]. An updated WHS survey in 2008 reported cervical cancer screening coverage to be 7.8%; this was highest among women 25-34 years old (11.8%) and decreased to 6.5% among those 35-44 years old with further decreases among older women.

The quadrivalent (Gardasil®, Merck) vaccine is licensed for use in Burkina Faso, but there is currently no organised vaccination programme.

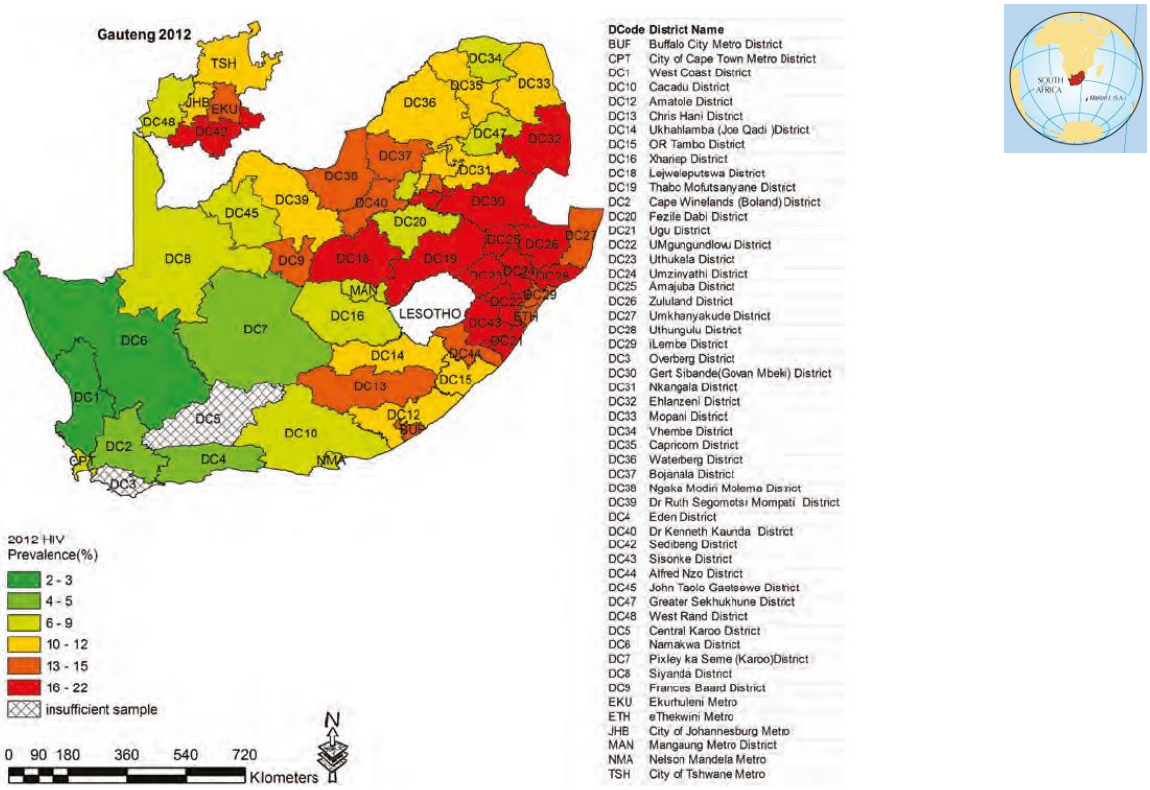
2.11.2 South Africa

South Africa has a total population of 55 million (**Table 2.6**), 29% of whom are <15 years old, with a life expectancy of 57 years. South Africa is an upper middle-income country (GDP of US$5,724), and is the second largest economy in the African region (after Nigeria), yet just under a fifth of the population live below the poverty line of less than $1.90 a day.

2.11.2.1 HIV control and prevention

South Africa has the largest number of WLHIV in the world, with the highest prevalence (4 million women ≥15 years among a total of 19.8 million women in 2015, **Figure 2.13**), and the highest number of new infections per year (200,000 in 2015) [10]. The highest HIV prevalence is in the eastern province of KwaZulu-Natal; 27.6% among girls <18 years and 52.0% among women ≥18 years [267] (**Figure 2.14**).

Figure 2.14. HIV Prevalence by district in South Africa 2012 [268]



HIV incidence among women ≥15 years peaked in 1997 and 1998 (440,000 new infections recorded in each year) and was in decline between 2000-2009, which may be due to the scaling up of combination prevention programs, such as medical male circumcision and treatment expansion at higher CD4+ cell counts [269]. However, from 2010 to 2016, it has been on the increase (from 170,000 new infections among women ≥15 years in 2009 to 200,000 in 2016) (**Figure 2.13**). Although South Africa's National Integrated Strategy on HIV, STIs and TB (2012-2016) [270] aimed to reduce HIV incidence by 50%, young people aged 15-24 years continue to have high incidence of HIV. In particular, young women aged 15-19 years have the highest incidence (4.7 per 100 woman years in KwaZulu-Natal [267]) and are 8-times more likely to become HIV-infected compared to males in the same age group [268], which may in part be attributed to having older male partners [271].

ART became available to adults and children in April 2004 in several accredited service points across the country. By September 2005, only 5% of all public healthcare facilities were providing ART and 85,000 people were enrolled on ART in the public sector. The availability of wide-scale ART was hampered during the Mbeki government between 1999 and 2008, characterised by denial of the causal link between HIV and AIDS. As a direct result of delay in wide-scale roll-out of ART, it is estimated that more than 330,000 lives were lost [272], and those that survived may have suffered more severe HIV disease progression. In 2009 with a change in government, a national counselling and testing (HCT) campaign was established, in addition to the abandonment of the accreditation of health centres for ART provision and an increase in HIV prevention measures such as medical male circumcision [269]. The national ART guidelines were revised in 2010, expanding treatment to children <1 year, all pregnant women regardless of CD4+ cell count and all TB-HIV co-infected patients with CD4+ count of <350 cells/μl, as well as changing ART for first and second-line therapy, followed by the inclusion of sex workers, men who have sex with men, truckers and adolescents in 2011 [269]. In 2012, the initiation CD4+ count threshold was increased to 350 cells/μl for all adults, as well as expanded access thresholds for children. By mid-2016 3,710,130 people living with HIV

were receiving ART; and 2,133,020 of these were women aged 15 years and over (coverage of 53%) [10] (**Figure 2.13**).

A study using demographic and HIV surveillance data in KwaZulu-Natal between 2001 and 2014 investigated changes in life expectancy before, during, and after ART was rolled out. The authors report that 5.2 and 17.1 years of life were gained for men and women, respectively, after ART introduction [273], however these estimates were dependent on lower CD4+ cell count thresholds for ART initiation and decentralised healthcare availability. Beginning in September 2016, the Department of Health announced the introduction of immediate ART initiation for those testing positive for HIV in the public sector [274] following WHO recommendations. The wide-scale availability of this "test-and-treat" strategy is still uncertain. Studies have shown that women are at decreased risk of HPV persistence [209] and CIN2+ [193] if ART is initiated at higher CD4+ counts (>500 cells/mm3), and earlier ART initiation promises a potential reduction in cervical precursor lesions and ICC if ART is made available to all. However, there remains a generation of women who started ART at lower CD4+ cell counts according to older guidelines and they remain at increased risk of ICC. Improved survival among these women may lead to increased ICC rates [208].

2.11.2.2 *Cervical cancer control and prevention*

South Africa has a population of 19.8 million women aged 15 years and older who are at risk of developing cervical cancer [275] **(Table 2.6**). Cervical cancer ranks as the second most frequent cancer among women (breast cancer being the first) and the most frequent cancer among women between 15 and 44 years of age. Cervical cancer incidence and mortality was 31.7 and 18.0 per 100,000 per year-in 2012 (age standardised rate) [9] (**Table 2.6**).

The South Africa national policy on cervical screening, established in 2002, allows for three cytological smears in a woman's lifetime taken at 10-year intervals from 30 years of age. [276]. Women with LSIL or ASCUS on cytology have repeat smears within 12 months and are referred to colposcopy if the outcome is the same or worse. Women with HSIL or atypical glandular (i.e. from

the endocervical epithelium) cells of underdetermined significance (AGUS) are immediately referred to colposcopy, and if they have abnormal findings, are managed for cervical lesions. This policy has been implemented in some areas but not throughout the country. Currently there is no population-wide screening programme in SA. In several areas partial screening does take place, and opportunistic screening is commonly practised in the private sector. Using data from the WHS in 2008, cervical cancer coverage in South Africa was reported to be 23.2% in 2008; this was highest among younger women; 27.7%, 28.5%, 13.3% and 14.0% among women aged 25-34, 35-44, 45-54 and 55-64 years, respectively [277]. And in 2014, the uptake of cervical screening was estimated to be 21.4% among women aged 30-39 [275]. The goal set in 2002 was to achieve screening of 70% of women ≥30 years within 10 years of the introduction of screening [274].

The national cancer control programme is currently being revised, to be implemented in 2017, to 1.) include HPV DNA testing where feasible; 2.) upgrade cytology-based programmes where they exist; and 3.) include a programme for WLHIV following international recommendations [274]. Current international guidelines recommend that cervical cancer screening should be started in sexually active girls and women, as soon as they have tested positive for HIV [278]. If the screening test is negative, a repeat test is done within three years. Women receiving treatment should receive follow-up at one year post-treatment [278]. WLHIV who have invasive cervical cancer should be managed in the same way as HIV-negative women [278, 279].

Both the bivalent (Cervarix®, GSK) and quadrivalent (Gardasil®, Merck) vaccines are licensed for use in SA. Between March and April 2014, the SA government piloted an HPV vaccination programme using 2 doses of the Cervarix® vaccine among schoolgirls and achieved 91% school coverage, in total vaccinating over 340,000 girls [280]. The vaccine was introduced in the public sector in 2014 through the Integrated School Health Program and is offered to schoolgirls ≥9 years, annually. Pilot studies have been completed in KwaZulu-Natal and Gauteng and have been positively evaluated in terms of vaccine uptake in 2015 [281, 282].

3 ANTIRETROVIRAL THERAPY, HIGH-RISK HUMAN PAPILLOMAVIRUS AND CERVICAL INTRAEPITHELIAL NEOPLASIA: A SYSTEMATIC REVIEW AND META-ANALYSIS

As combined ART is scaled-up, longer survival times among WLHIV may increase the rates of cervical cancer. The interactions of ART and the natural history of HR-HPV and cervical lesions in WLHIV are poorly understood. Observational studies differ with respect to study design, outcomes, timing of ART initiation and effectiveness of ART use, making it difficult to estimate the true effect of ART.

Previous systematic reviews have explored the association of ART and HR-HPV and cervical lesions [173, 211, 212] but no meta-analysis has quantified the risk of HR-HPV and cervical lesions among ART users compared to ART-naive women. Given the large and increasing number of women on ART, improved understanding of the interplay of ART, immune recovery and virological control on the natural history of HR-HPV infection and CIN progression is needed to guide screening programs.

*In this chapter, I perform a systematic review of the literature on the association of ART with HR-HPV prevalence, and cytological and histological cervical lesions. This review was originally performed to inform the cross-sectional and prospective analyses as part of Study 1 (**Chapter 7**) which investigated the association of HIV-related factors, including ART, CD4+ cell count and HIV-1 PVL with HR-HPV and CIN2+ outcomes.*

Note: An updated version of this review was performed in November 2016 to update the literature before publication (currently under review with Lancet HIV) as there were additional studies published that were relevant to the review. As such, the data originating from this thesis on the association of ART with HR-HPV prevalence and CIN2+ prevalence and incidence (as part of the HARP study) are also included in the meta-analysis.

3.1 Objectives

- To evaluate the association of ART with HR-HPV prevalence, and with cervical lesion prevalence, incidence, progression and regression ***(Objective 1.1)***
- To investigate the role of HIV-related co-factors that might modify these associations, such as ART duration, timing of ART initiation and immune suppression and recovery

3.2 Methods

3.2.1 Literature search

Medline and Embase databases were searched for publications in the English language using search terms for HPV, CIN, SIL and ART (**Appendix 1**). Reference lists of review articles and all articles identified in the systematic search were checked. The search was carried out from January 1996 (when highly active ART [HAART] became utilised) up to 05 November 2016. All abstracts were screened by one author (HK). Full text copies of relevant publications were obtained and assessed for eligibility by two authors (Helen Kelly [HK] and Philippe Mayaud [PM]). Consensus was reached on potential relevance.

3.2.2 Inclusion and exclusion criteria

Exposure and outcome measures

Studies were eligible if they reported the association of combination ART (cART) or HAART use with the following outcomes: HR-HPV prevalence and cervical lesion prevalence, incidence, progression or regression among WLHIV. Studies were also considered eligible if they provided raw data to calculate an unadjusted effect estimate.

For HR-HPV outcomes, studies reporting genital HR-HPV only were included. There were no exclusions on HPV test methods. For the prevalent lesion outcomes, studies reporting low-grade

lesions and greater (LSIL+) or a combined outcome measure of "any CIN grade" which could include CIN1, were excluded as they are not considered true measures of disease, as were studies reporting cervical lesions using visual inspection using acetic acid (VIA) or Lugol's iodine (VILI) due to the poor sensitivity and specificity in detecting high-grade lesions. Studies on invasive cervical cancer (ICC) cases were excluded due to the difficulty in accounting for temporal associations in the longer duration of ICC development.

Types of studies

For prevalent outcomes, cross-sectional studies were included if they reported the association of ART use with HR-HPV and/or high-grade lesions (histological CIN grade 2 or higher [CIN2+] or cytological high-grade SIL or higher [HSIL+]). Cohort studies were included if participants initiated ART at enrolment, were followed-up and had measures of HR-HPV at baseline and in the follow-up visit.

For the longitudinal outcomes, cohort studies reporting the association of ART with the incidence, progression and regression of "any SIL", which could include atypical squamous cells of undetermined significance (ASCUS) as well as LSIL and HSIL+ lesions were included as SIL represents various incremental degrees of HR-HPV persistence and subsequent lesion development.

For publications that reported results from the same cohort, but at different follow-up visits, the publication that gave the most relevant description of the cohort and study design and the most complete set of results was included. There was no restriction on age or geographical location.

3.2.3 Data extraction

From the consensus list, data were extracted by one author (HK) and a random sample of 25% was checked by a second author (Helen Weiss [HW]). For studies reporting prevalence of HR-HPV or

cervical lesions, Odds Ratios (OR) were extracted. For studies reporting cervical lesion incidence, progression or regression, Hazard Ratios (HR) or OR were extracted.

3.2.4 Methodological quality assessment

Studies were assessed primarily on adjustment for HIV-related factors (current and nadir CD4+ cell count and ART duration). Cross-sectional studies that adjusted for either current or nadir CD4+ count or ART duration were considered separately in sensitivity analysis, as were cohort studies that adjusted for time on ART during follow-up. Study quality was also assessed on participant selection, statistical method and HPV test used, and cervical lesion classification (cytological or histological) (**Appendix 2 to Appendix 4**).

3.2.5 Statistical analysis

Meta-analyses were performed for the discrete outcomes of HR-HPV prevalence, high-grade lesion (CIN2+ or HSIL+) prevalence and incidence, progression and regression of "any SIL", respectively.

Adjusted effect estimates were reported where available. For the cross-sectional studies in which adjusted effect estimates were not reported but raw data were provided, crude ORs were calculated (HK) and independently verified (HW and PM). Authors were contacted when the paper suggested that relevant data was collected but not reported.

Random-effects meta-analysis were used to estimate pooled effects to account for between-study heterogeneity.[283] Heterogeneity was examined using the I^2 statistic and publication bias using funnel plots and Begg's test for correlation between the effect estimate and their variances.[284, 285] An influence analysis was performed to assess the robustness of the pooled summary effects by excluding each of the studies from the pooled estimate. Sub-group analyses by geographic region were performed to compare pooled effects and heterogeneity. Sensitivity analyses excluding studies unadjusted for HIV related factors were performed. Data were analysed using Stata version 14 (Stata Statistical Software, College Station. TX: Stata Corporation).

This review was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)[286] and the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines [287] and was registered on the PROSPERO database at the Centre of Reviews and Dissemination, University of York; registration number CRD42016039546.

3.3 Results

3.3.1 Antiretroviral therapy and HR-HPV Prevalence

The review identified 521 publications through Medline and Embase searches, of which 176 duplicates were removed and 312 excluded after abstract review, leaving 33 articles for full text review. Finally, 14 articles matched the inclusion criteria and four additional publications were identified through cross-referencing (**Figure 3.1 (A)**). Data were extracted from 18 publications (14 cross-sectional; four cohort) representing 19 discrete populations and providing data from 6,441 WLHIV, of whom 3,677 (57%) were taking ART (ranging from 19-85% in cross-sectional studies), 2,032 (32%) were ART-naive and 732 (11%) were ART initiators. Three ART initiation studies compared HR-HPV before and after ART initiation (i.e. women acted as their own controls) [288-290](**Table 3.1**). One publication provided data from two countries [291] and was considered as two individual studies in the analysis, resulting in a total of 19 included studies.

The pooled OR among 19 studies [184-186, 188, 192, 288-300] indicates that WLHIV on ART had a lower risk of HR-HPV prevalence compared to ART-naïve (crude Odds Ratio [OR]=0.80, 95%CI: 0.66-0.97), but there was a high degree of heterogeneity between studies (I^2=72%, p-value for heterogeneity <0.001; **Table 3.4**, **Figure 3.2**). Restricting the analysis to the 12 studies that adjusted for either current or nadir CD4+ cell count, or ART duration [185, 186, 188, 288-293, 295, 300] the OR was similar but with a moderate degree of heterogeneity (adjusted OR=0.83, 95%CI: 0.70-0.99, I^2=51%, p=0.02). The reduction in heterogeneity on adjustment for confounding was most

noticeable among the studies from Africa; among six studies[186, 288, 291-293] the aOR was 0.70 (95%CI: 0.56-0.88) with no evidence of heterogeneity (I^2=0%, p=0.97). Similarly, among studies from Europe/North America, three studies [185, 289, 290] showed a similar reduction in HR-HPV (aOR=0.74, 95%CI: 0.59-0.93, I^2=48%, p=0.14). This was in contrast with the two studies from Asia[188, 295] (aOR=1.72, 95%CI: 1.10-2.68, I^2=0%, p=0.34) and three from Latin America [298-300] (crude OR=1.08, 95%CI: 0.84-1.39, I^2=0%, p=0.99).

The pooled OR from three cohort studies that followed women before and after ART initiation [288-290] provides strong evidence of a decreased risk of HR-HPV after compared to before ART initiation (aOR=0.79, 95%CI: 0.71-0.88, I^2=48%; p=0.15, data not shown).

Nine studies reported the association of ART duration with HR-HPV prevalence.[185, 186, 291-293, 295, 296, 299] While HR-HPV prevalence was similar among the ART-naïve and short-duration users (≤2 years), the pooled OR suggests that HR-HPV was lower among prolonged ART users (>2 years) compared to short-duration users and ART-naïve combined (crude OR=0.65, 95%CI: 0.55-0.77, I^2=0.0%, p=0.92 data not shown), and among the seven adjusted studies [185, 186, 291-293, 295] the association was similar (aOR=0.65, 95%CI: 0.55-0.78, I^2=0%, p=0.91, adjusted for current and nadir CD4+ cell count, data not shown).

Sensitivity analysis

There was no evidence to suggest publication bias; i.e. smaller studies were not more likely to report a positive association (Beggs rank correlation test; p=0.12 for the crude analysis; p=0.34 for adjusted analysis; **Appendix 5)**.

3.3.2 Antiretroviral therapy and cervical cytological and histological lesions

This review identified 661 publications for ART and cervical lesion prevalence, incidence, progression or regression, of which 165 duplicates were removed and 446 excluded after abstract

review, leaving 50 articles for full review. Finally, 20 articles matched the inclusion criteria and 12 additional publications were identified through cross-referencing **(Figure 3.1 (B)).**

Thirteen studies [186, 193, 194, 202, 222-225, 291, 301-303] reported the association of ART with CIN2+/HSIL+ prevalence among 8,262 WLHIV, of whom 4,463 (54%) were taking ART (range across studies: 16-76%) and 3,799 (46%) were ART-naïve (**Table 3.2**). One publication provided data from two countries,[291] and was considered as two individual studies in the analysis.

Ten studies reported the association of ART with incident SIL or LSIL from a combined total of 3,266 women, all of which used time-to-event analyses [195-197, 200, 201, 289, 304-307], and two studies reported the association of ART with incident CIN2+ using logistic regression[291, 308](Table 3). Ten studies [189, 190, 192, 195, 197-199, 201, 218, 309] were included for SIL progression from a combined total of 6,212 women; six of these used time-to-event analysis[189, 192, 198, 199, 201, 218], and nine studies [189, 192, 195, 197, 218, 289, 309-311] for SIL regression from a combined total of 5,059 women, five of these used time-to-event analysis [189, 192, 218, 289, 310](**Table 3.3**).

Cervical lesion prevalence

The pooled OR among 13 studies [186, 193, 194, 202, 222-225, 291, 301-303] suggests no evidence of an association of ART with high-grade cervical lesion prevalence (crude OR=0.90, 95%CI: 0.68-1.20, I^2=61.0%, p=0.002; **Table 3.4**, **Figure 3.3**). The nine African studies [186, 193, 194, 222-224, 291, 301] found weak evidence of an association of ART with high-grade lesion prevalence [crude OR=0.84, 95%CI: 0.64-1.10, I^2=45.5%, p=0.07). When analyses were restricted to four studies [186, 291, 302] that adjusted for both current CD4+ cell count and ART duration there was some evidence that ART users had decreased odds of high-grade lesion prevalence compared to ART-naive women (aOR=0.65, 95%CI: 0.40-1.06, I^2 =29.5%, p=0.24).

Sensitivity analysis

There was a large variation in the study size [range: 70-3185]; the largest study [193] enrolled 3,185 WLHIV (40% of participants included in the meta-analysis). However, excluding this study did not change the overall results. The next largest study [201] enrolled 1,010 WLHIV in Johannesburg and reported an increased risk among ART users (crude OR=1.31, 95%CI: 0.92-1.86), but the estimate was not adjusted for CD4+ cell count or ART duration. When excluded, the pooled OR among eight studies in Africa showed a protective effect of borderline significance (crude OR=0.77, 95%CI: 0.59-1.01) with less between-study heterogeneity (I^2=30.4%; p=0.19). There was no evidence to suggest publication bias (Beggs rank correlation test; crude analysis: p=0.46, adjusted analysis: p=0.50; **Appendix 5**).

Cervical lesion incidence

The pooled Hazard Ratio (HR) among 10 studies [195-197, 200, 201, 289, 304-307] provides weak evidence of an association of ART with incident SIL (crude HR=0.75, 95%CI: 0.56-1.00, I^2=41%, p=0.09; **Table 3.4**, **Figure 3.4**). Among five studies that adjusted for the time-varying effects of ART [196, 197, 289, 306, 307] there was evidence of a reduction in SIL incidence among ART users (aHR=0.64, 95%CI: 0.47-0.86, I^2=19.4%, p=0.29).

When restricting analysis to five studies reporting incidence of any SIL grade [197, 201, 289, 306, 307] there was a reduction among ART users (crude HR=0.66, 95%CI: 0.52-0.83, I^2=0.0%; p=0.72), but not among five studies reporting the association of ART on incidence of ≥LSIL[195, 196, 200, 304, 305](crude HR=0.91, 95%CI:0.44-1.87, I^2=64.8%; p=0.02, data not shown).

Whilst not included in the meta-analysis due to the use of logistic regression instead of time-to-event analysis, two studies reported a reduction in incident CIN2+ among ART users. Clifford et al[308] report that those with prolonged ART use (>2 years) at baseline had a decreased odds of CIN2/3 incidence (aOR=0.64, 95%CI: 0.42-0.98, adjusted for nadir CD4+ cell count) compared to

ART-naïve in a case-control study in Switzerland (Table 3). Similarly, Kelly et al[291] reported a reduction in incident CIN2+ over 16 months among ART users compared to those who remained ART-naïve (aOR=0.39, 95%CI: 0.15-1.01, adjusted for baseline CD4+ cell count) in South Africa.

There was no evidence to suggest publication bias for SIL incidence studies (Beggs rank correlation test; crude analysis: p=0.42, adjusted analysis: p=1.00; **Appendix 5**).

Cervical lesion progression and regression

The pooled HR among six studies[189, 192, 198, 199, 201, 218] suggests a decreased hazard of SIL progression among ART users (crude HR=0.64, 95%CI: 0.56-0.74, I^2=0%, p=0.42; **Table 3.4**; **Figure 3.5**). Restricting the analysis to four studies[189, 198, 199, 218] that adjusted for time-varying ART did not vary the estimate (aHR=0.64, 95%CI: 0.54-0.75, I^2=17.8%, p=0.30). Similarly, there was no variation by region.

The pooled HR among five regression studies[189, 192, 218, 289, 310] suggests an increased likelihood of SIL regression among ART users (crude HR=1.67, 95%CI: 1.32-2.12, I^2=31.0%, p=0.22; **Table 3.4**, **Figure 3.6**). Restricting the analysis to four studies[189, 218, 289, 310] that adjusted for time-varying ART during follow-up did not vary the estimate (aHR=1.58, 95%CI: 1.28-1.94, I^2=18.2%, p=0.30).

Sensitivity analysis

While most studies reported progression of any SIL grade, one study reported progression of LSIL to a higher grade.[218] No effect in the estimate was observed when excluding that study (crude HR=0.63, 95%CI:0.51-0.78, I^2=18%, p=0.30). There was no evidence to suggest publication bias for the progression studies (Beggs rank correlation test; p=0.85, **Appendix 5**), but there was some evidence for the regression studies (p=0.01; **Appendix 5**), as more of the small studies reported positive association of ART with regression. However, even the largest study (enrolling 1048 WLHIV followed up over a median 18 months[218]) reported a significant increased likelihood of

regression among ART users compared to ART-naïve (aHR=1.71, 95%CI: 1.29-2.27, adjusted for ART duration, age and excision treatment), suggesting a real beneficial effect of ART.

3.4 Discussion

This systematic review and meta-analysis confirms that women on ART have a lower prevalence of both HR-HPV and high-grade cervical lesions, a reduction in SIL incidence and progression, and an increase in regression, after adjusting for CD4+ cell count and duration on ART. It is also the first study to quantify the association of ART with prevalent HR-HPV and high-grade cervical lesions.

Performing a meta-analysis of observational studies for HR-HPV and cervical lesion outcomes has difficulties due to inherent differences in study populations, definitions of exposure and outcomes used, and varying approaches to adjustment of effect estimates. In particular, a major limitation of cross-sectional studies is the difficulty in establishing the timing of HPV infection and development of cervical lesions with regard to ART initiation and nadir CD4+ cell count. While pooling studies with and without adjustment for nadir or current CD4+ cell count or ART duration may have shown a limited effect of ART on HR-HPV or cervical lesions, restricting analysis to those studies that did adjust for any of these factors suggests that ART is associated with a reduction in any of the outcomes, with less between-study heterogeneity. Among studies that report limited or no association, immune reconstitution by ART may not be established early enough after HPV infection to prevent or reverse the development of HR-HPV persistence or CIN2+.

Several studies reported that a high nadir CD4+ cell count was associated with between a 36-70% decreased risk of HR-HPV [185, 300] and 36-80% decreased risk of CIN2+ [193, 202, 308] compared to those with low nadir CD4+ cell count. Once on ART, others have shown that 'effective' ART (i.e. prolonged duration with sustained HIV-1 viral suppression and stable high CD4+ cell count) was associated with a reduction in HR-HPV persistence and CIN2+ [185, 291]. The protective effect of

ART may only be seen over prolonged duration, accompanied by HIV-1 viral suppression and immune reconstitution.

There has been a steadily increasing representation of studies from African settings in recent years, while many of the earlier studies were performed in the USA or Europe, leading to a geographical and period heterogeneity. The African studies provide encouraging indication that earlier initiation and effective ART over prolonged duration can prevent cervical lesion incidence and progression and promote regression.

There were fewer studies from Latin America and Asia and the majority were cross-sectional in design. These studies reported an opposite increased risk of HR-HPV and high-grade cervical lesions among ART users. It is still unclear if this is due to lower nadir CD4+ cell count before ART initiation. Thus, regular cervical cancer screening remains important especially among women on ART if they have started at a low nadir CD4+ cell count. This concerns a generation of women who may have started ART under older guidelines at specific lower CD4+ cell count thresholds and who may never have fully recovered their HPV-specific mucosal immune response.

Limitations

The majority of cross-sectional studies compared HR-HPV and CIN2+/HSIL+ using a binary category of ART users and ART-naïve. A more informative analysis would be to measure the effect of ART duration as there is a non-comparability among women initiated on ART due to decreasing CD4+ cell count compared to those with stable CD4+ cell count not yet needing treatment. Women who initiate ART are more likely to have advanced HIV disease, lower nadir CD4+ cell counts and higher HIV-1 viral loads compared to those who have not yet started ART.

Significant between-study heterogeneity was found when pooling geographical regions, which suggests differing epidemiology and related risk factors by region. Subgroup analyses by regions however reduced this heterogeneity.

There was variation in the outcome definitions for cervical lesions, in particular the use of cytological versus histological measurement and definition of progression and regression between grades varied. The majority of the prospective studies used cytological outcomes instead of a more desirable histological endpoint and there was variation in the way SIL were grouped (i.e. any grade change vs. LSIL and HSIL). This may overestimate the effect of ART as the beneficial effect of ART is less pronounced in advanced SIL. Coupled with the variation in ART exposure (e.g. varying ART regimens and duration) between populations makes interpretation of pooled data less clear. There is also the possibility of unmeasured confounding. Additional data that would have been useful to understand predictors of progression or regression of cervical lesions include the nadir CD4+ count, ART adherence and virological control, which was not always reported across studies. Although, where available, we performed sensitivity analysis that adjusted for time-varying ART.

3.5 Recommendations

This review has practical implications for the management of HIV patients and cervical cancer control worldwide. Encouraging earlier ART initiation with rapid virological control, and ensuring sustained adherence is likely to improve and maintain higher CD4+ cell counts, leading to possibly more complete immune reconstitution. It is expected that the consequence will be a reduction of HR-HPV infection and SIL incidence or progression. ART users with low or unknown nadir CD4+ cell count should be screened frequently.

3.6 Summary of findings

METHODS:

- A systematic review and meta-analysis was performed by searching Medline and Embase databases for cross-sectional or cohort studies from 1 January 1996 to 5 November 2016 that reported the association of ART with prevalence of HR-HPV or prevalence, incidence, progression or regression of histological/cytological cervical abnormalities.
- Random-effects meta-analyses were used to estimate summary statistics. Heterogeneity was examined using the I^2 statistic.

RESULTS:

- A total of 6,441 and 8,262 WLHIV were included from 29 studies evaluating the association of ART with prevalence of HR-HPV, and high-grade cervical intraepithelial neoplasia (CIN2+) or squamous intraepithelial lesions (HSIL+), respectively.
- WLHIV on ART had lower HR-HPV prevalence than those not on ART (adjusted Odds Ratio [aOR]=0.83, 95%CI: 0.70-0.99, I^2=51%, adjusted for CD4+ cell count and ART duration).
- There was some evidence of association with CIN2+/HSIL+ (aOR=0.65, 95%CI: 0.40-1.06, I^2=30%).
- Sixteen studies reported the association of ART with longitudinal cervical lesion outcomes, from a combined 6,664 WLHIV.
- ART was associated with a decreased risk of SIL incidence (adjusted Hazard Ratio [aHR]=0.64, 95%CI: 0.47-0.86, I^2=19%), and progression (aHR=0.64, 95%CI: 0.54-0.75, I^2=18%) and increased likelihood of regression (aHR=1.58, 95%CI: 1.28-1.94, I^2=18%).

CONCLUSION:

- Prolonged ART use in WLHIV can decrease the risk of HR-HPV, SIL incidence and progression and induces regression of lesions.

3.7 Findings in context

3.7.1 Evidence before this study

This is the first meta-analysis investigating the association of ART with prevalent HR-HPV and high-grade cervical lesion outcomes. Studies that adjusted for either nadir or current CD4+ cell count and time-varying effects of ART were more likely to show a protective effect of ART on these outcomes.

Studies from Africa and Europe/North America provide indication that ART is associated with lower prevalence of HR-HPV and cervical lesions, and over prolonged duration, ART can prevent cervical lesion incidence and progression and promote regression. Fewer studies exist from Asia and Latin America with the majority being cross-sectional in design, and these studies were less likely to report any protective association of ART. As some studies from Latin America have reported an increased risk of HR-HPV and CIN2+ among women with a lower nadir CD4+ count, the lack of association may reflect the timing of ART in relation to HPV infection and cervical lesion development in these populations, although more longitudinal studies from these settings are warranted.

3.7.2 Implications of the available evidence

These findings highlight the importance of early ART initiation (before reaching a low nadir CD4+ cell count) and sustained effectiveness, as evidenced by duration, high adherence, virological control and CD4+ cell recovery, in controlling HPV infection and cervical disease progression. ART users with low or unknown nadir CD4+ cell count should be screened frequently.

Table 3.1. Summary of studies investigating the association of ART with HR-HPV prevalence

First author, year	Study design	Location	Enrolment period	Sample size	% ART users	HR-HPV+ prevalence	CD4+ count, cells/mm^3 Median [IQR] or mean (SD or range)	Comparison group	Crude Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)
Africa										
Kelly, 2016 [291]	Cohort	Burkina Faso- Ouagadougou	2011-2012	570	67%	59.3%	ART-naïve: 417 (315-606) ART: 446 (309-600)	ART vs. ART-naive	0.95 (0.66-1.39)	0.76 (0.47-1.22)[a]
Kelly, 2016 [291]	Cohort	South Africa-Johannesburg	2011-2012	613	65%	78.8%	ART-naïve: 448 (353-614) ART: 420 (279-567)	ART vs. ART-naive	0.69 (0.45-1.05)	0.61 (0.37-1.01)[b]
Zeier, 2015 [288]	Cohort: ART initiation	South Africa –Western Cape	2009-2011	300	68% initiated during FU	94.3%	(Mean, SD): [188]194 [77-311]	ART vs. 'not on treatment', i.e. not yet initiated or interruption for >1 month	0.33 (0.24-0.44)	0.72 (0.39-1.32)[c]
Ezechi, 2014 [184]	Cross-sectional	Nigeria-Ogun & Lagos	NR	220	72%	24.5%	500 [347-685]	ART vs. ART-naive	0.36 (0.19-0.69)	0.40 (0.30-0.50)[d]
Reddy, 2014 [292]	Cross-sectional	Malawi-Lilongwe	2011-2012	294	85%	38.8%	337 [215,491]	ART vs. ART-naive	0.78 (0.40-1.52)	0.71 (0.30-1.67)[e]
De Vuyst, 2012 [186]	Cross-sectional	Kenya-Nairobi	2009	497	75%	52.7%	ART naïve: 407 In ART users <2y: 333 In ART users ≥2y: 483	cART vs. cART-naive	0.75 (0.49-1.13)	0.64 (0.40-1.02)[f]
Jaquet, 2012 [293]	Cross-sectional	Côte d'Ivoire-Abidjan	Jun-Oct 2010	254	75%	52.8%	471 [318-629]	ART vs. ART-naive	1.07 (0.60-1.88)	0.84 (0.46 - 1.54)[g]
Veldhuijzen, 2011 [294]	Cross-sectional	Rwanda-Kigali	2006-2009	124	40%	50.8%		ART vs. ART-naive	0.77 (0.38-1.59)	-
Asia										
Menezes, 2015 [296]	Cross-sectional	India-Chennai	July-Aug 2011	50	48%	48.0%	425 [range: 106-1229]	ART vs. ART-naive	0.61 (0.20-1.88)	-
Zhang, 2014 [295]	Cross-sectional	China-Yunnan	NR	301	64%	37.5%	571	ART vs. ART-naive	0.97 (0.60-1.58)	2.30 (1.09-4.85)[h]
Mane, 2012 [188]	Cross-sectional	India-Pune	NR	277	56%	35.3%	372 [241-556]	ART vs. ART-naive	-	1.46 (0.84-2.54)[i]
Aggarwal, 2012 [297]	Cross-sectional	India-Chandigarh	NR	130	75%	20.0%	398	HAART vs. HAART-naive	3.11 (0.87-11.13)	-
Latin America										
Rocha-Brischiliari, 2014 [298]	Cross-sectional	Brazil-Maringa city	Apr-Oct 2011	178	79%	46.6%	64% with CD4+≥200	HAART vs. HAART-naive	1.04 (0.50-2.14)	-
Dames, 2014 [299]	Cross-sectional	Bahamas-Nassau	Feb-Sep 2008	165	81%	78.2%	47% with CD4+ >200	HAART vs. HAART-naive	1.06 (0.41-2.70)	
Grinsztejn, 2009 [300]	Cross-sectional	Brazil-Rio de Janeiro	1996-2006	634	68%	45.0%	74% with CD4+≥200	HAART ≥2 months vs. HAART-naive	-	1.09 (0.82-1.44)[j]
Europe/North America										
Konopnicki, 2013 [185]	Cohort	Belgium-Brussels	2002-2011	652	79%	42.8%	426 [302-601]	cART vs. cART-naive	0.73 (0.50-1.07)	0.72 (0.41-1.27)[k]
Blitz, 2013 [192]	Cohort	Canada-11 cities	1993-2002	750	19%	46.3%	336 [180-515]	HAART vs. ART-naïve or non-HAART regimen	0.70 (0.48-1.01)	-
Minkoff, 2010 [289]	Cohort: ART initiation	USA-5 US cities	1994-2002	286	0%	22.4%	73% with CD4+ ≥200	Adherent ART users followed up to 30 months after vs. before ART initiation (within woman analysis)	-	0.60 (0.44-0.81)[l]
Fife, 2009 [290]	Cohort: ART initiation	USA/Puerto Rico	2001-2005	146	0%	62.0%	238 [121-339]	6 months after vs. before ART-initiation (within woman analysis)	0.40 (0.24-0.69)	0.83 (0.74-0.94)[m]

NR=not reported; cART=combination ART; HAART=highly active antiretroviral therapy; [a]adjusted Odds Ratio, adjusted for **CD4+ count, ART duration,** alcohol use, marital status, age at first pregnancy and cervicitis,**;** [b]adjusted Odds Ratio, adjusted for **CD4+ count, ART duration**, age, smoking, injectable contraception, genital warts, condom use, vaginal cleansing, *Chlamydia trachomatis*, Bacterial vaginosis and *Trichomonas vaginalis***;** [c]adjusted Odds Ratio, adjusted for **CD4+ count, ART duration**, age, sexual activity, months since cervical lesion excision, HIV-1 plasma and cervical viral load; [d]adjusted Odds Ratio, adjusted for age, type of community, life time sexual partner and marital status; [e]adjusted for **nadir CD4,;** months since HIV diagnosis and age; [f]adjusted Odds Ratio, adjusted for **CD4+ count, ART duration** and age; [g]adjusted for **CD4+ count**, age, marital status and age at first sex**;** [h]adjusted Odds Ratio, adjusted for **CD4+ count, ART duration** and age; [i]Adjusted Odds Ratio, adjusted for **CD4+ count**, age, marital status, education, income, parity, age at first sex, LTSP, past history of STI and tobacco**;** [j]Adjusted Prevalence Ratio, adjusted for **nadir CD4+ count,** age, marital status, drug use, age at first sex, LTSP, history of HPV and condom use; [k]Adjusted Odds Ratio, adjusted for **current and nadir CD4+ count**, **ART duration**, age, , CDC stage, HIV duration of follow-up**,** and HIV plasma VL; [l]Adjusted Odds Ratio, adjusted for **CD4+ count pre- and post-HAART** and treatment of CIN, [m]adjusted Odds Ratio, adjusted for **current CD4+ count**, age, sexual activity at baseline LSIL+ at baseline and HIV-1 PVL.

Table 3.2. Summary of studies investigating the association of ART with high-grade cervical lesion prevalence

First author, year	Study design	Location	Enrolment period	Sample size	% ART users	CD4+ count, cells/mm^3 Median [IQR] or mean (SD or range)	Lesion definition	Lesion prevalence	Comparison group	Crude Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)
Africa											
Kelly, 2016 [291]	Cohort	Burkina Faso-Ouagadougou	2011-2012	530	73%	ART-naïve: 417 (315-606) ART: 446 (309-600)	CIN2+	5.8%	ART vs. ART-naive	1.63 (0.66-4.04)	0.86 (0.26-2.83)[a]
Kelly, 2016 [291]	Cohort	South Africa-Johannesburg	2011-2012	613	65%	ART-naïve: 448 (353-614) ART: 420 (279-567)	CIN2+	22.4%	ART vs. ART-naive	0.80 (0.53-1.20)	0.54 (0.32-0.91)[b]
Memiah, 2015 [223]	Cross-sectional	Kenya-Kiambu	2009-2010	686	16%	45% had baseline CD4+ <200 cells/mm3	CIN2+	6.1%	ART vs. ART-pre-ART	0.55 (0.19-1.56)	-
Huchko, 2014 [193]	Cross-sectional	Kenya-Kisumu	2007-2010	3185	50%	356 [218-530]	CIN2+	9.0%	HAART vs. HAART-naïve	0.96 (0.75-1.23)	1.01 (0.79-1.30)[c]
De Vuyst, 2012 [186]	Cross-sectional	Kenya-Nairobi	2009	470	75%	ART naïve: 407 ART users <2 yrs: 333 ART users ≥2 yrs: 483	CIN2+	24.0%	cART vs. cART-naïve	1.12 (0.69-1.84)	0.91 (0.51-1.62)[d]
Mabeya, 2012 [222]	Cross-sectional	Kenya-Eldoret	NR	149	67%	Mean (range): 400 (10-1198)	CIN2+	30.9%	HAART vs. HAART-naïve	1.18 (0.56-2.49)	-
Ezechi, 2014 [301]	Cross-sectional	Nigeria-Ogun & Lagos	NR	490	76%	mean: 532 [263-801]	HSIL+	5.1%	ART vs. ART-naive	0.44 (0.19-1.01)	-
Firnhaber, 2010 [194]	Cross-sectional	South Africa-Johannesburg	NR	1010	65%	231 (range:1-1789)	HSIL+	18.0%	HAART vs. HAART-naïve	1.31 (0.92-1.85)	-
Mogtomo, 2009 [224]	Cross-sectional	Cameroon-Douala	NR	70	50%	Mean : 253 among ART and 165 among ART-naive	HSIL+	31.4%	HAART vs. HAART-naïve	0.44 (0.16-1.26)	-
Asia											
Feng, 2012 [302]	Cross-sectional	China-Yunnan	2009	301	64%	571	CIN2+	9.3%	HAART vs. HAART-naïve	0.45 (0.20-0.98)	0.14 (0.02-1.09)[e]
Sahasrabuddhe, 2010 [225]	Cross-sectional	India-Pune	2006-2007	271	26%	343 [244-495]	CIN2+	15.9%	ART vs. ART-naïve	2.16 (1.09-4.28)	2.24 (1.17-4.26)[f]
Latin America											
De Andrade, 2011 [202]	Cohort	Brazil-Rio de Janeiro	1996-2007	340	26%	347 [193-546]	CIN2+	6.5%	ART (≥2 months) vs. ART-naive	2.31 (1.02-5.22)	-
Europe											
Patrelli, 2013 [303]	Cohort	Italy-Parma	1993-2010	194	66%	ART-naïve: 487 [±238] ART users: 411 [±188]	HSIL+	35.1%	Receiving ART vs. ART-naïve or women refusing treatment (but in need of treatment)	0.64 (0.35-1.18)	-

NR=not reported; cART=combination ART; HAART=highly active antiretroviral therapy; [a]adjusted for **CD4+ count**, **ART duration,** age, Bacterial vaginosis and cervical ectopy; [b]adjusted for **CD4+ count**, **ART duration,** age at first pregnancy, injectable contraception and number of lifetime sex partners; [c]Adjusted for age and site; [d]Adjusted for **CD4+ count**, **ART duration** and age; [e]Adjusted for **CD4+ count**, **ART duration** and age; [f]Adjusted for **CD4+ count**, age, education, income, age at first sex, LTSP, parity, WHO stage and HR-HPV DNA.

Table 3.3. Summary of studies investigating the association of ART with cervical lesion incidence, progression and regression

First author, year	Location	Enrolment period	Sample size	% ART users	CD4+ count, cells/mm^3 Median [IQR] or mean (SD)	Median interval between smears (months)	Outcome definition	Comparison group	Effect size (ES)	Adjusted ES (95%CI)
Incidence studies										
Africa										
Adler, 2012 [197]	South Africa-Soweto	2003-2010	767	2% at baseline; 17% initiation during FU	CD4+ count <350; ART users: 56% ART naïve: 42%	14 [range: 6-77]	Normal to ASCUS+	ART ≥6 mths vs. ART-naive at baseline	HR	0.62 (0.42-0.91)[a]
Firnhaber, 2012 [201]	South Africa-Johannesburg	NR	326	71% at baseline	ART users: 248 (152,382); ART-naive: 299 (174,448)	6	Normal to ASCUS+	ART at baseline vs. ART-naive at baseline	HR	0.55 (0.34-0.90)[b]
Kelly, 2016 [291]	South Africa-Johannesburg	2011-2012	379	71% at end of FU	ART users: 439[322-604] ART-naïve: 437 [346-543]	16	<CIN2 to CIN2/3	ART >16 months vs. ART-naive	OR	0.39 (0.15-1.01)[c]
Latin America										
Kreitchmann, 2013 [304]	Brazil-Porto Alegre	1997-2007	349	38%	436 [range: 9-1571]	14	<LSIL to LSIL+	HAART vs. HAART-naive	HR	1.90 (0.90-4.01)[d]
Europe/North America										
Minkoff, 2010 [289]	USA- 5 cities	1994-2002	286	All ART initiators	73% with CD4+ ≥200	6	Normal to ASCUS+	Adherent ART period vs. pre-HAART period (within-woman analysis)	HR	0.68 (0.25-1.85)[e]
Sirera, 2008 [200]	Spain- Barcelona	1997-2006	127	71% at baseline	Mean: ART users: 646 ART naive: 681	12	Normal to LSIL+	HAART prior to baseline vs. ART-naive throughout FU	HR	1.66 (0.16-17.03)[f]
Soncini, 2007 [196]	Italy-Parma	1993-2003	101	43% through FU	50% with CD4+ count between 200-499	6	Normal to LSIL+	HAART (time-dependent) vs. ART-naïve	HR	0.30 (0.13-0.68)[g]
Lehtovirta, 2006 [305]	Finland-Helsinki	1989-2003	55	48% at baseline; 64% at FU	45% with CD4+ >500	6	Normal to LSIL+	HAART during FU vs. ART-naive	HR	0.80 (0.35-1.83)[h]
Heard, 2006 [306]	France- Paris	1993-2005	298	49% through FU	400 [250,574]	6	Normal to ASCUS+	HAART during FU vs. non-HAART and ART-naive	HR	0.70 (0.40-1.20)[i]
Schuman, 2003 [195]	USA- 4 cities	1993-1995	629	33% at baseline	16% with CD4+ <200; not stratified by ART	6	Normal to LSIL+	HAART (time-dependent) vs. ART-naive	HR	1.20 (0.49-2.94)[j]
Ellerbrock, 2000 [307]	USA –New York	1991-1996	328	54% on ≥1 ARV for at least 6mths during study period; 41% on only 1 ARV; 0.9% on 3 drugs.	429; 24% with CD4+ <200	6	Normal to ASCUS+	ARV (time-dependent) vs. ARV-naive	HR	1.00 (0.50-2.00)[k]
Clifford, 2016 [308]	Switzerland-5 cities	1988-2013	1451	54%	NR	~5 years	<CIN2 to CIN2/3	>2years vs. ART naive	OR	0.64 (0.42-0.98)[l]
Progression studies										
Africa										
Zeier, 2012 [218]	South Africa-Western Cape	2004-2009	1,048	18% started before LSIL+; 41% after LSIL+	312	Not reported	LSIL to HSIL+	HAART (time-varying) vs. naive before lesion detection	HR	0.66 (0.54-0.81)[m]
Firnhaber, 2012 [201]	South Africa - Johannesburg	NR	326	As before	As before	As before	ASCUS+ to HSIL+	ART at baseline vs. ART-naive at baseline	HR	0.52 (0.27-1.01)[n]
Omar, 2011 [198]	South Africa- Soweto	2003-2010	1,074	6% on ART at baseline; 20% were initiated during FU	356 [215,474]	5.5	Normal to LSIL+; LSIL to HSIL+/ASCH	HAART (time-varying) vs. naive	HR	0.72 (0.52-0.99)[o]
Adler, 2012 [197]	South Africa-Soweto	2003-2010	1,123	As before	As before	As before	Subsequent smear with worsening dysplasia	ART ≥6 mths vs. ART-naive at baseline	OR	0.80 (0.57-1.13)[p]
Europe/North America										
Kim, 2013 [199]	USA- New York	1991 - 2011	245	NR	Nadir: 206	12	normal->ASCUS+; ASCUS->LSIL+	HAART vs. other regimens or ART naïve	HR	0.47 (0.33-0.67)[q]
Blitz, 2013 [192]	Canada- 11 cities	1993-2002	326	19% on HAART at baseline; 64% by end of study	336 [180, 515]	8 [6,13]	ASCUS to greater	HAART during study vs. ART-naïve or non-HAART	HR	1.02 (0.40-2.59)[r]

First author, year	Location	Enrolment period	Sample size	% ART users	CD4+ count, cells/mm³ Median [IQR] or mean (SD)	Median interval between smears (months)	Outcome definition	Comparison group	Effect size (ES)	Adjusted ES (95%CI)
Paramsothy, 2009 [189]	USA- 4 cities	1996-2000	537	46.9% during study	ART-naïve: 48% CD4≥500 vs. 24% of ART users	6	Normal to ASCUS; ASCUS to LSIL; LSIL to HSIL	HAART use during study period vs. pre-HAART or never on ART	HR	0.70 (0.60-1.00)[s]
Schuman, 2003 [195]	USA- 4 cities	1993-1995	629	As before	As before	As before	Normal/ASCUS to LSIL+, LSIL to HSIL	HAART (time-dependent) vs. ART-naive	OR	1.50 (0.90-2.49)[t]
Minkoff, 2001 [309]	USA- 6 cities	1994-1995	741	0.9% at baseline	36% with CD4<200	6	subsequent pap with any grade higher than baseline	HAART vs. off-HAART (women never received HAART or among initiators, prior to HAART initiation	OR	0.68 (0.52-0.88)[u]
Lillo, 2001 [190]	Italy- Milan	1995-1997	163	46% through FU	HAART : 260 (±23); ART-naïve: 627 (±38)	6	Normal to LSIL+; LSIL to HSIL	HAART during study vs. ART-naïve or non-HAART	OR	3.50 (1.01-12.12)[v]
Regression studies										
Africa										
Zeier, 2012 [218]	South Africa-Western Cape	2004-2009	1,048	18% started before LSIL+; 41% after LSIL+	312	Not reported	≥LSIL to <LSIL (2 normal results at least 4 weeks apart)	HAART (time-varying) vs. naive before lesion detection	HR	1.71 (1.29-2.27)[m]
Adler, 2012 [197]	South Africa-Soweto	2003-2010	1,123	As before	As before	As before	Subsequent improvement in cytological results	ART ≥6 mths vs. ART-naive at baseline	OR	2.61 (1.75-3.89) [p]
Europe/North America										
Blitz, 2013 [192]	Canada- 11 cities	1993-2002	326	As before	As before	As before	≥ASCUS to <ASCUS	HAART during study vs. ART-naïve or non-HAART	HR	3.32 (1.22-9.04)[r]
Minkoff, 2010 [289]	USA-5 US cities	1994-2002	286	As before	As before	As before	SIL to lower grade	Adherent ART period vs. pre-HAART period (within-woman analysis)	HR	2.25 (1.03-4.92)[w]
Paramsothy, 2009 [189]	USA- 4 cities	1996-2000	537	As before	As before	As before	HSIL to LSIL, LSIL to ASCUS, ASCUS to normal	HAART use during study period vs. pre-HAART or never on ART	HR	1.30 (1.00-1.70)[s]
Heard, 2002 [310]	France- Paris	1993-1999	168	56% through FU	250 [139-400]	6	Reversion to normal or from HG to LG	HAART (time-dependent) vs. ART-naive	HR	1.93 (1.14-3.28)[x]
Del Mistro, 2004 [311]	Italy- Vicenza & Padova	1994-2002	201	37%	292 (range: 2-1445)	6-12	≥LSIL to <LSIL	HAART at baseline and FU vs. ART-naive at baseline and FU OR non-HAART OR change in regimen during FU	OR	1.87 (0.71-4.93)[r]
Schuman, 2003 [195]	USA- 4 cities	1993-1995	629	As before	As before	As before	≥LSIL+ to <LSIL	HAART (time-dependent) vs. ART-naive	OR	0.86 (0.50-1.47) [t]
Minkoff, 2001 [309]	USA- 6 cities	1994-1995	741	As before	As before	As before	Lower grade abnormality than baseline east one HR-HPV infection at all visits	HAART vs. off-HAART (women never received HAART or among initiators, prior to HAART initiation	OR	1.40 (1.06-1.85) [u]

NR-not reported; FU=follow-up; [a]adjusted for **time-varying ART**, current CD4+, weight, sexual activity, STI symptom; [b]adjusted for CD4+ at baseline; [c]adjusted for number of lifetime sex partners and baseline CD4+ count; [d]age, education, log viral load and CD4+ count; [e]adjusted for **time-varying ART** and adherence; [f]adjusted for baseline/nadir CD4+ count; [g]adjusted for **time-varying ART** and baseline CD4+ count; [h]adjusted for age; [i]adjusted for **time-varying ART**, age, ethnicity, smoking, LTSP, [j]adjusted for baseline CD4+, time (visit), study site, age, race/ethnicity and education contraception use, condom, inclusion period; [k]adjusted for **time-varying ART**, baseline CD4+, age, smoking, HPV persistence; [l]case-control study matched on enrolment centre, HIV-transmission category, age at enrolment, year of enrolment and adjusted for nadir CD4+; [m]adjusted for **duration on ART**, age and excision treatment; [n]adjusted for CD4+ at baseline; [o]adjusted for **time-varying ART**; CD4+ at baseline, age, baseline smear result, smoking; [p]adjusted for current CD4+, weight, sexual activity, STI symptom; [q]adjusted for **time-varying CD4+,** duration of HIV infection, menopausal, smoking; [r]unadjusted; [s]adjusted for **time-varying CD4+**; HPV positive, baseline pap result; [t]adjusted for baseline CD4+, time (visit), study site, age, race/ethnicity and education; [u]adjusted for baseline CD4+ and baseline pap result; [v]adjusted for CD4+, HIV-1 RNA and gynecological treatment; [w]adjusted for **time-varying ART**, treatment of CIN, baseline CD4+; [x]adjusted for **time-varying ART**, CIN grade, CD4+ at lesion detection.

Table 3.4. Meta-analysis of the association of ART with HR-HPV and cervical lesions among WLHIV

	Crude analysis[1]				Adjusted analysis[2]			
	N studies	OR (95%CI)	I^2	*P* for heterogeneity	N studies	OR (95%CI)	I^2	*P* for heterogeneity
HR-HPV Prevalence								
All	19	0.80 (0.66-0.97)	71.7%	<0.001	**12**	**0.83 (0.70-0.99)**	**51.0%**	**0.02**
Africa	8	0.62 (0.49-0.80)	49.4%	0.05	**6**	**0.70 (0.56-0.88)**	**0.0%**	**0.97**
Asia	4	1.60 (0.93-2.75)	38.6%	0.18	**2**	**1.72 (1.10-2.68)**	**0.0%**	**0.34**
Latin America	3	1.08 (0.84-1.39)	0.0%	0.99	-	-	-	-
Europe/North America	4	**0.75 (0.63-0.88)**	**29.9%**	**0.23**	3	**0.74 (0.59-0.93)**	**48.4%**	**0.14**
CIN2+ Prevalence								
All	13	0.90 (0.68-1.20)	61.0%	0.002	4	0.65 (0.40-1.06)	29.5%	0.24
Africa	9	0.84 (0.64-1.10)	45.5%	0.07	3	0.70 (0.48-1.01)	0%	0.40
Asia	2	0.67 (0.05-9.90)	84.3%	0.01	-	-	-	-
Latin America	1	2.31 (1.02-5.23)	-	-	-	-	-	-
Europe/North America	1	0.64 (0.35-1.18)	-	-	-	-	-	-

	Crude analysis[1]				Adjusted analysis[3]			
	N studies	HR (95%CI)[4]	I^2	*P* for heterogeneity	N studies	HR (95%CI)[4]	I^2	*P* for heterogeneity
SIL Incidence								
All	10	0.75 (0.56-1.00)	41.0%	0.09	**5**	**0.64 (0.47-0.86)**	**19.4%**	**0.29**
Africa	2	**0.59 (0.44-0.80)**	0.0%	0.71	1	0.62 (0.42-0.91)	-	-
Latin America	1	1.90 (0.90-4.01)	-	-	-	-	-	-
Europe/North America	7	0.73 (0.52-1.03)	14.0%	0.32	4	0.64 (0.40-1.02)	39.0%	0.18
SIL Progression								
All	6	**0.64 (0.56-0.74)**	0.0%	0.42	4	**0.64 (0.54-0.75)**	17.8%	0.30
Africa	3	**0.67 (0.56-0.79)**	0.0%	0.68	2	**0.68 (0.57-0.80)**	0.0%	0.65
Europe/North America	3	**0.62 (0.43-0.90)**	46.4%	0.16	2	**0.57 (0.39-0.85)**	58.0%	0.12
SIL regression								
All	5	**1.67 (1.32-2.12)**	31.0%	0.22	4	**1.58 (1.28-1.94)**	18.2%	0.30
Africa	-	-	-	-	1	**1.71 (1.29-2.27)**	-	-
Europe/North America	4	**1.76 (1.21-2.58)**	44.5%	0.14	3	**1.57 (1.13-2.19)**	32.5%	0.23

[1]includes studies with either 1.) no adjustment and 2.) studies that adjust for sociodemographic factors only and no adjustment for HIV related factors
[2]adjusted for at least one of the following: current CD4+; nadir CD4+; ART duration
[3]adjusted for at least one of the following: time-varying ART, time-varying CD4+ count
[4]only studies that reported Hazard Ratio (HR) from time-to-event analysis included in the meta-analysis

Figure 3.1.Study selection for outcomes of HR-HPV (A) and cervical lesions (B).

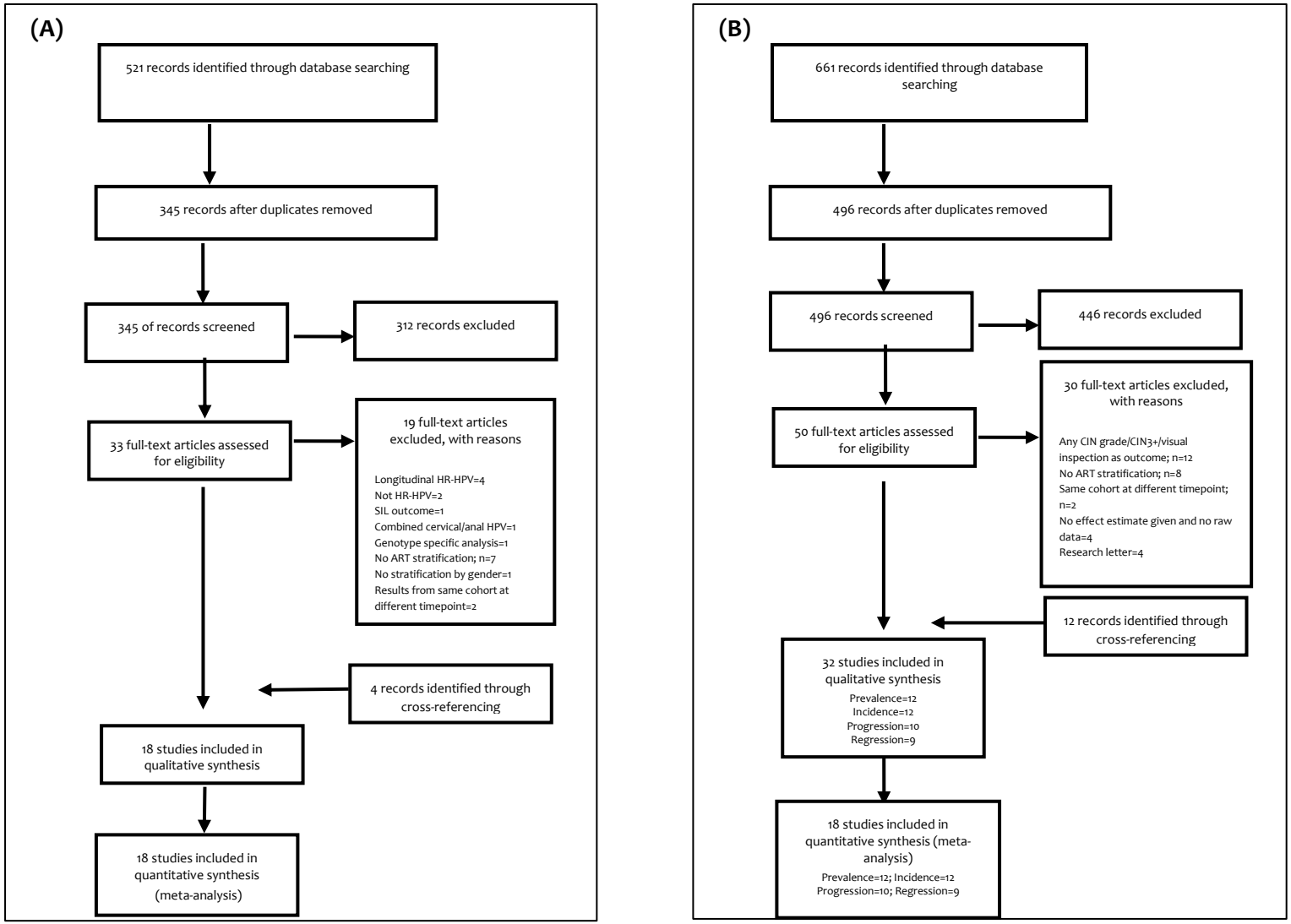
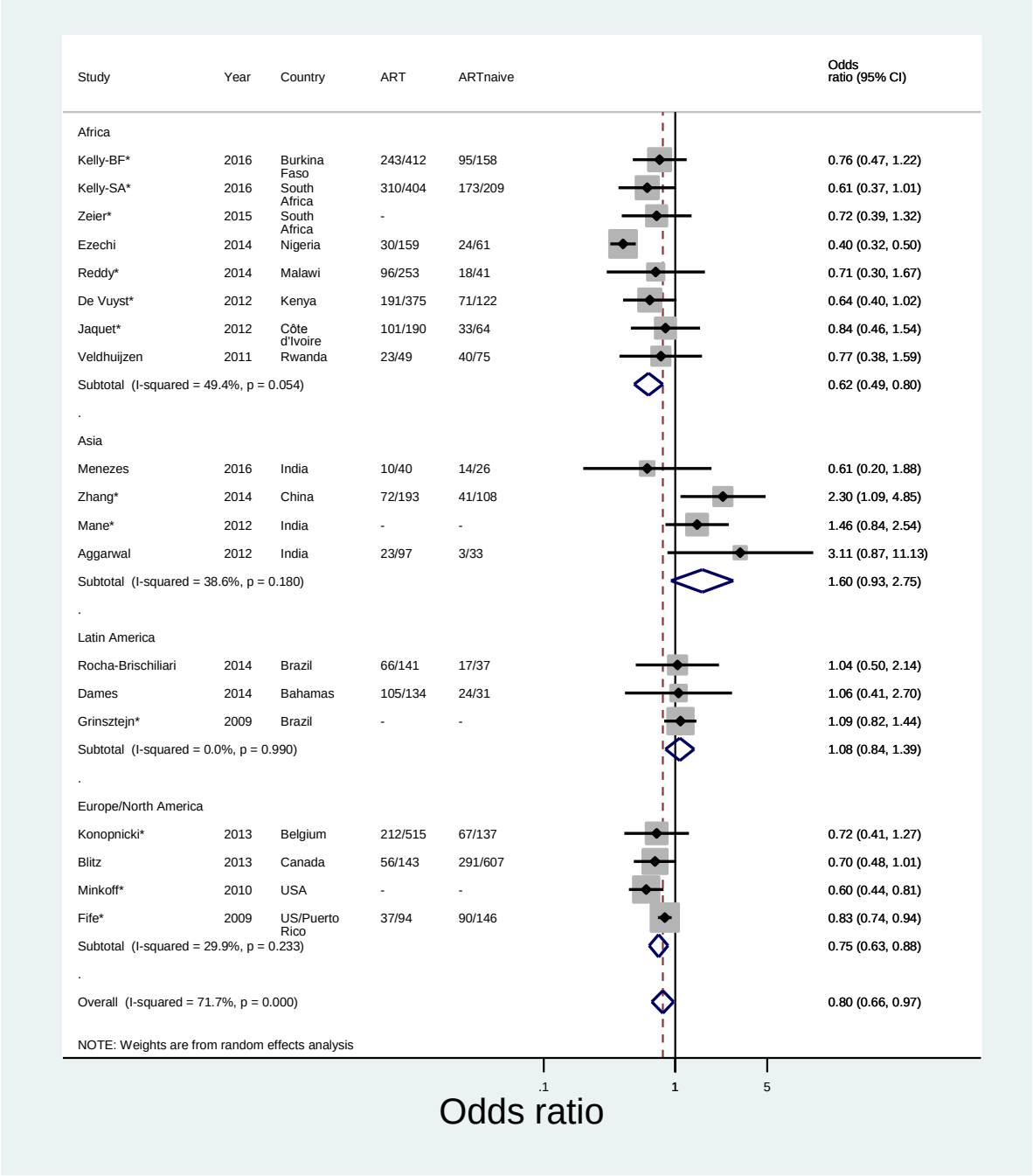
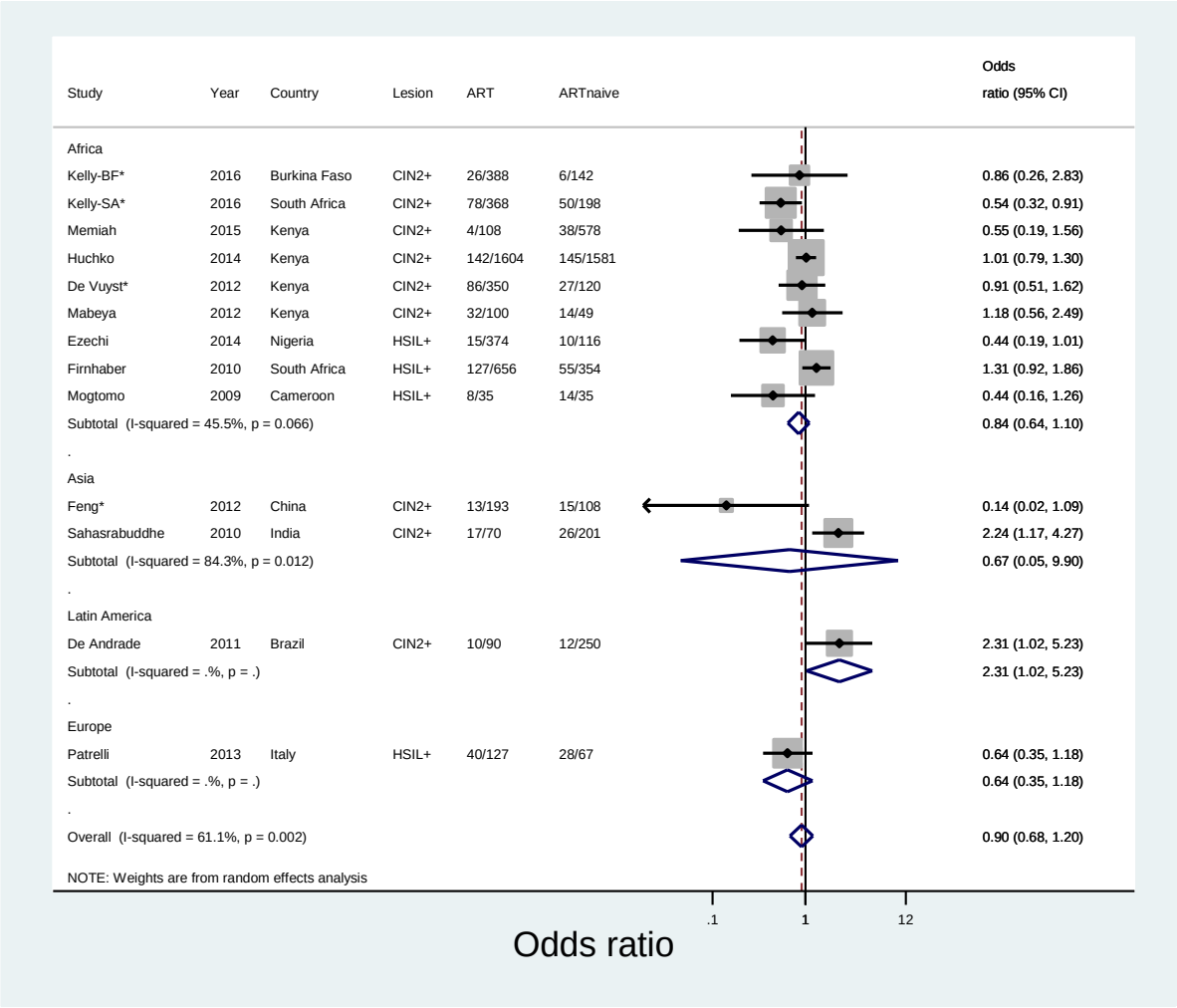


Figure 3.2. Meta-analysis of HR-HPV prevalence among ART users compared to ART-naive in 19 studies



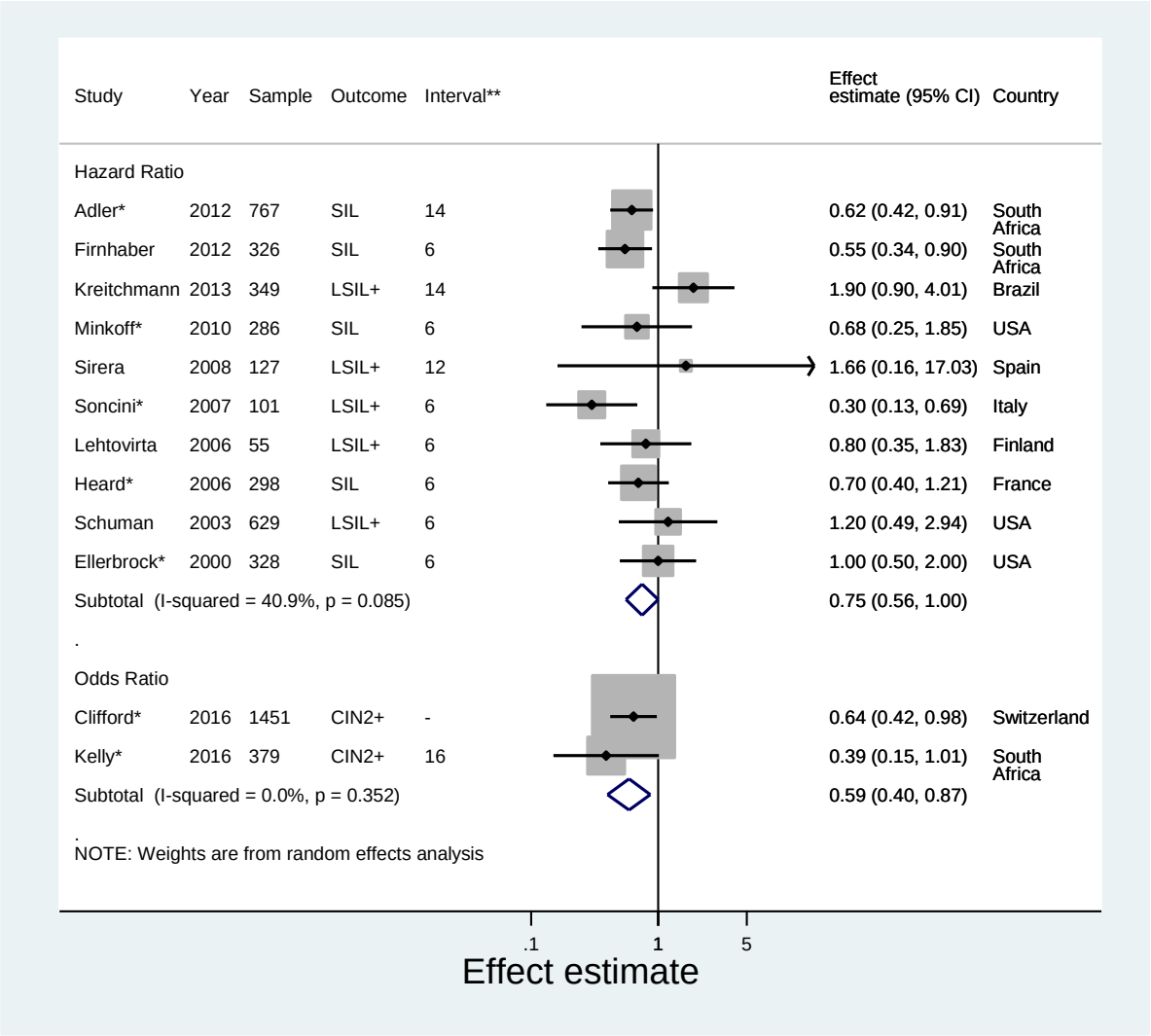
*Studies that adjusted for ART duration, current or nadir CD4+ cell count

Figure 3.3. Meta-analysis of high grade cervical prevalence among ART users compared to ART-naive in 13 studies



*Studies that adjusted for ART duration, current or nadir CD4+ cell count

Figure 3.4. Meta-analysis of high grade cervical incidence among ART users compared to ART-naïve in 10 studies [1]

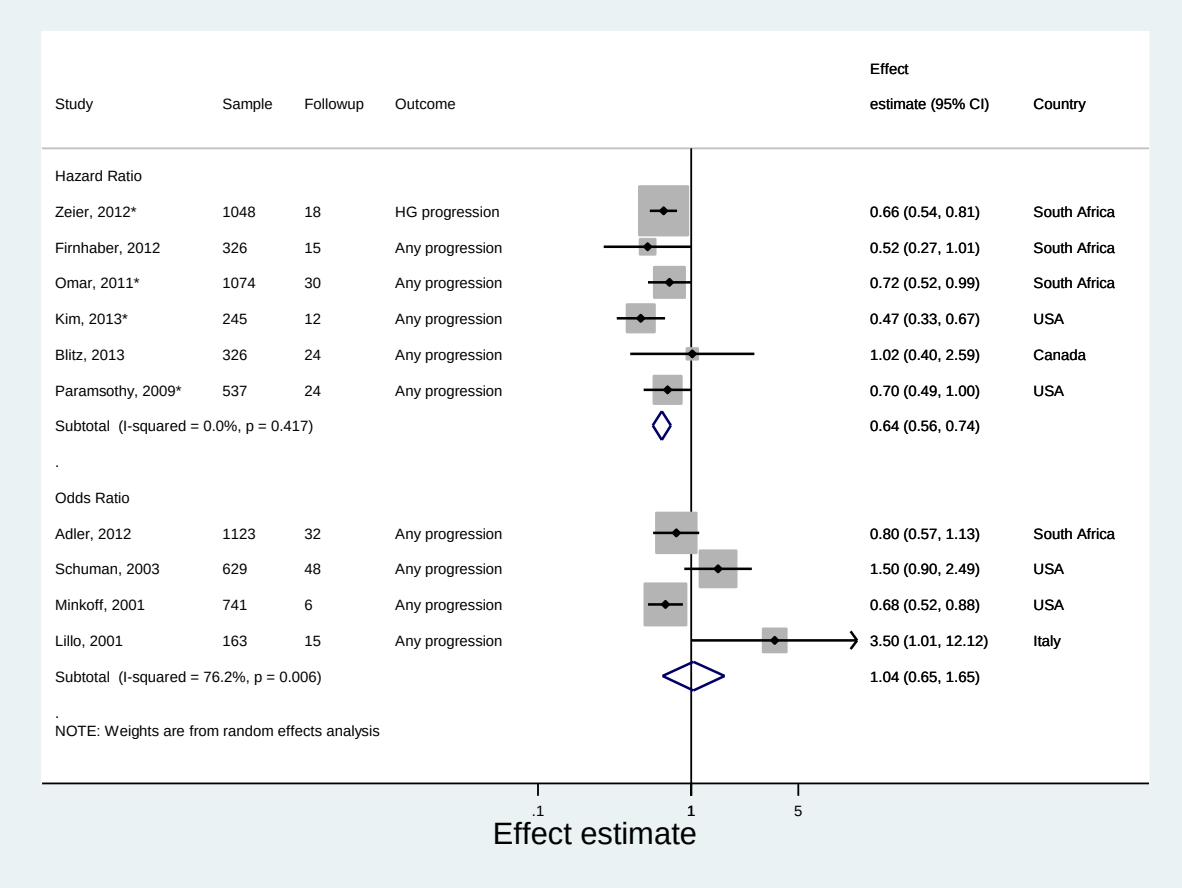


[1]only studies that reported Hazard Ratio (HR) from time-to-event analysis included in the meta-analysis in **Table 3.4.**

*****adjusted for the time-varying ART or CD4+ cell count

****** median interval (months) between pap smears, or between histological evaluations

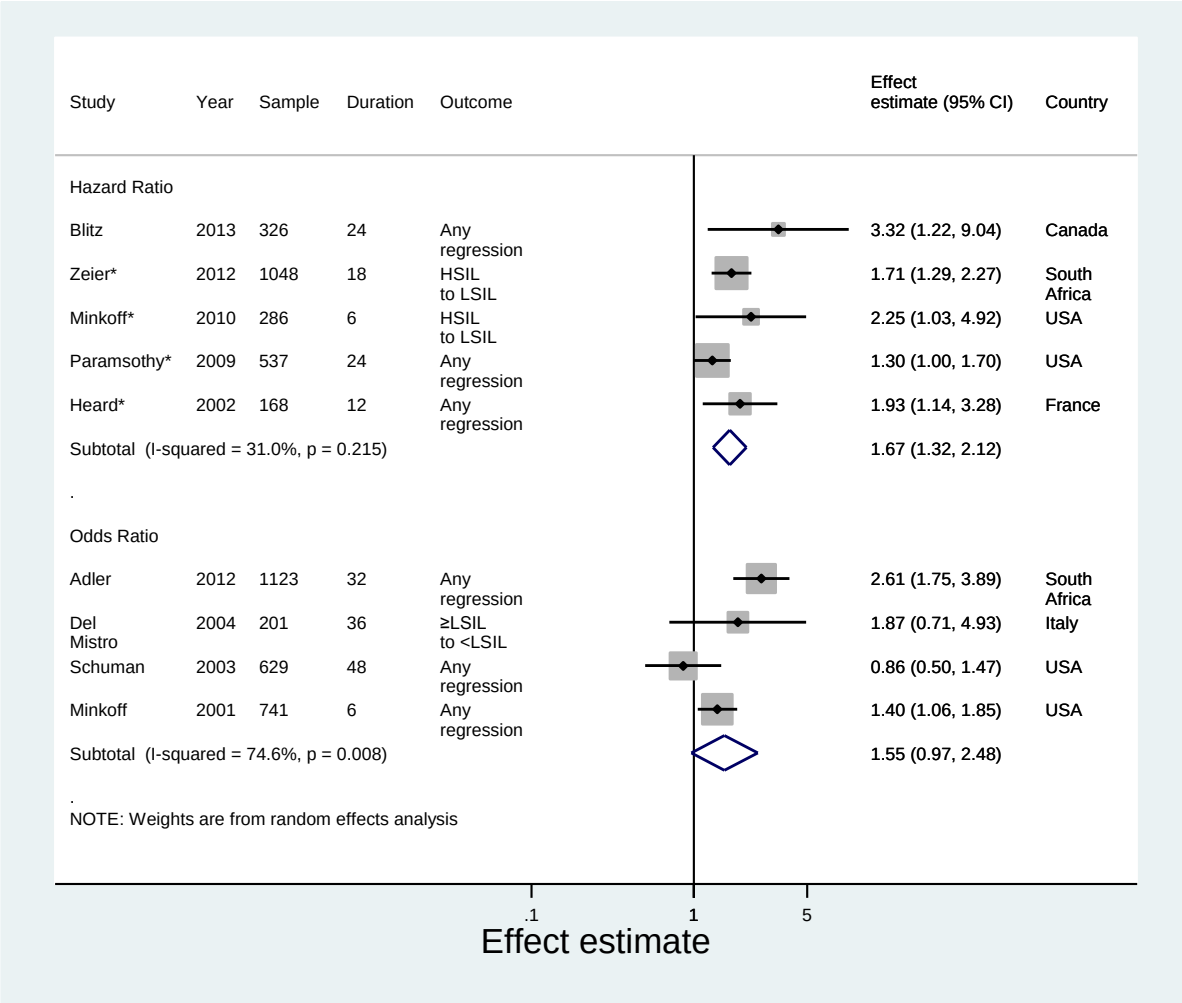
Figure 3.5. Meta-analysis of high grade cervical progression among ART users compared to ART-naïve in 6 studies [1]



[1]only studies that reported Hazard Ratio (HR) from time-to-event analysis included in the meta-analysis in **Table 3.4**.

*adjusted for the time-varying ART and CD4+ cell count

Figure 3.6. Meta-analysis of high grade cervical regression among ART users compared to ART-naïve in 5 studies [1]



[1]only studies that reported Hazard Ratio (HR) from time-to-event analysis included in the meta-analysis in **Table 3.4.**

*****adjusted for the time-varying ART and CD4+ cell count

4 THE ASSOCIATION OF DNA METHYLATION OF HUMAN GENES AND HPV16 WITH CERVICAL INTRAEPITHELIAL NEOPLASIA AND INVASIVE CERVICAL CANCER: A SYSTEMATIC REVIEW

The utility of HPV-DNA based assays for the detection of CIN2+ are limited among WLHIV because of the high prevalence of HR-HPV in these populations. Human gene and HPV viral DNA methylation are novel biomarkers that may help distinguish non-progressive HPV infections from those that progress to cancer. DNA methylation assays using human genes are now available for use in early detection of various cancers, including bladder, breast, colorectal, oesaphagal, gastric, head and neck, liver, lung and prostate cancers. Data on the validation of DNA methylation assays of various human genes and the HPV virus for CIN2+ detection are more recently available. However, a wide variety of human genes have been studied, and associations of various genes and HPV types with CIN2+ is still unclear.

In this chapter, I perform a systematic review of the literature on DNA methylation of human genes and HPV types, and their association with CIN2+, in order to summarise the evidence on the most frequently studied human genes and HPV types.

The majority of publications in the field reported this association using performance measures (receiver operating characteristic [ROC] curves, and sensitivity and specificity). I aim in this chapter to interpret this data in order to understand the role of DNA methylation with CIN2/3, CIN3 and ICC.

*This data will inform the case-control study in Study 3 (**Chapter 10**), which evaluated DNA methylation assays for human gene EPB41L3 and HPV16 for the detection of CIN2+ among WLHIV.*

4.1 Introduction

There is strong evidence that HPV DNA based screening allows for earlier diagnosis of high-grade CIN and is more effective in prevention of ICC [235]. However, HPV testing detects many transient infections, meaning that its specificity for high-grade CIN is low. Furthermore, a recent (2012) review of HPV DNA based tests for the detection of cervical lesions reported that for a 10% increase in HR HPV prevalence, HR-HPV DNA based test specificity decreased by 8.4% [95% confidence interval (CI): 8.02-8.81] [239]. This has important implications for screening WLHIV who have a high prevalence of HR-HPV. Cervical cytology and visual inspection using VIA/VILI are more frequently used in the Africa region, but also have poor accuracy and reproducibility [227]. Novel methods are thus required to identify which HPV positive women need colposcopy referral, avoiding repeat testing which can result in substantial loss to follow-up [240, 241].

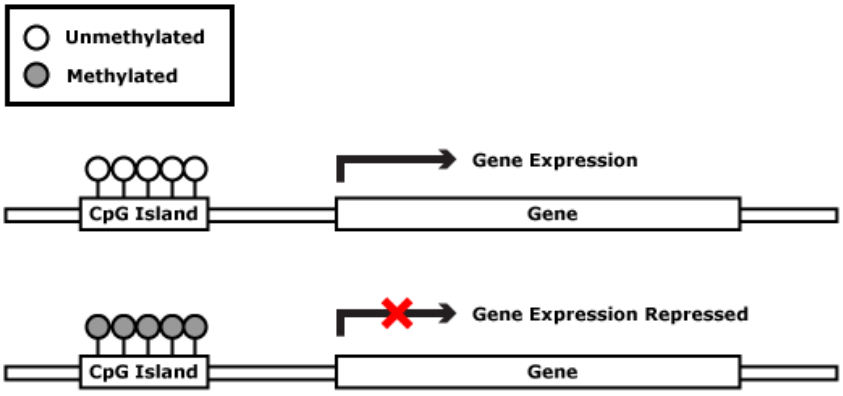
While infection with HR-HPV is necessary for the development of CIN [35, 37], other molecular changes occur with HR-HPV infection, leading to subsequent development of alterations in the functions of gene products regulating tumor suppression, DNA repair, apoptosis, metastasis and invasion [242, 243]. Such alterations can result from DNA nucleotide mutations, structural genomic variations or epigenetic alterations, such as DNA methylation [244, 312]. Aberrant DNA methylation is a potentially good early indicator of existing disease [245] and a predictor of future disease development [246].

4.1.1 DNA methylation of human genes

DNA methylation mainly occurs at CpG dinucleotide sites (cytosine and guanine separated by one phosphate), known as CpG islands. CpG islands are most commonly associated with promoter regions and are present in approximately 60% of human genes [313]. DNA methylation occurs following the addition of a methyl group to position 5 of the cytosine (C) ring immediately preceding a guanine (G) in the DNA sequence (**Figure 4.1**). The process is mediated by DNA

methyltransferases (DNMTs), which are histone modifying enzymes. This change forms methylated CpG dinucleotides which is associated with the loss of gene expression and function [314].

Figure 4.1.DNA methylation of a CpG island resulting in gene silencing



From http://missinglink.ucsf.edu/lm/genes_and_genomes/methylation.html

Controlled methylation of these CpG islands are essential for normal biological process [315, 316]. It has an important role in regulating cellular processes such as embryonic development, chromosome instability and protection from invading foreign viral DNA [317, 318]. However, aberrant DNA methylation of certain genes is a widespread phenomenon in cancer and may be among the earliest changes to occur in oncogenesis [319]. It is therefore a potentially good early indicator of existing disease [245] and predictor for future development of disease [246].

4.1.2 DNA methylation of HPV virus

DNA methylation of HPV can occur in two ways depending on the site of methylation; as a form of defence by the host, or to allow the virus to persist. As part of a host defence mechanism, the genomes of tumour viruses are subject to selective differential methylation and silencing. E6 and E7 have shown to be down-regulated through the DNA methylation of the HPV16 LCR [320]. DNA

methylation induced gene silencing on the integrated viral DNA is important for the host cell to subdue the foreign DNA and maintain genome stability [313].

In contrast, methylation of the HPV late regions (L1 and L2) has been reported to be an indicator of viral persistence and cell immortalization [321]. A number of HR-HPV types have been investigated for methylation of the L1/L2 regions, including HPV16, 18, 31, 33, 45, 58 and 58 [322-325]. These studies have shown that the methylation patterns are similar across the different HPV types. In a study using cervical specimens with a range of pathological diagnoses, HPV18 L1 sequences were found to be more strongly methylated in all cervical carcinomas, while the LCR and E6 remained unmethylated [326]. In a separate study using samples from women with normal cytology, there was low or absent methylation levels in the LCR and E6 regions, but increased methylation levels were found in E1, E2 and E7, with the highest levels in the L1 and L2 regions. Methylation levels in the L1 and L2 regions have shown to become higher with increasing disease severity [321].

Human gene and HPV viral DNA methylation are novel biomarkers that may help distinguish non-progressive HPV infections from those that progress to cancer. There have been a wide range of studies on DNA methylation of human genes and the HPV virus in earlier detection of HPV related lesions. Many of these studies have looked at different human genes and HPV types, or different CpG sites within genes and the HPV virus. [257].

4.1.3 DNA methylation methylation markers for CIN2+ detection

Host gene methylation

A previous systematic review conducted in 2009 [15] of host gene methylation markers and their association with high-grade cervical lesions (including HSIL+, CIN2/3 and ICC) reported 51 studies analysing 68 different human genes among 4,376 specimens representing all stages of cervical

disease. Many of the genes were selected for the review because they are known tumour genes (TSG) and aberrant methylation had been previously reported in other types of cancer [327]. Only 3 host genes *DAPK1, CADM1* and *RARB* showed elevated methylation in cervical cancers consistently across all studies. This review reported methylation frequencies of each gene and the authors concluded that a rigorous meta-analysis was not possible given the high degree of heterogeneity in methylation methods (methylation specific polymerase chain reaction [MSP], quantitative methylation specific polymerase chain reaction [qMSP], bisulfite sequencing, restriction enzyme-based methylation detection, pyrosequencing and next generation DNA sequencing [NGS] methods) and specimens used (fresh frozen material, exfoliated cells and paraffin-embedded tissue) combined with the low number of studies analysing the same gene.

More recently in 2014, the methylation of 26 genes which showed promise in earlier studies CIN2+ detection, including *DAPK1, CADM1* and *RARB,* were evaluated using 20 CIN3 cases and 20 ≤CIN1 controls [328]. Eight of these genes showed elevated methylation in CIN3 cases and were further validated using 205 CIN2/3 cases and 366 ≤CIN1 controls from a colposopy referral following a cytology finding of 'mild dyskaryosis or worse'. Of the 8 genes, the *EPB41L3* gene demonstrated the best separation of CIN2+ cases from ≤CIN1 controls with an area under the Receiver Operating Characteristic (AUC) of 0.73.

HPV DNA methylation

Three recent reviews performed between 2012-2013 [247-249] have reported DNA methylation of different regions of the HPV16 genome given it is the most widely studied HPV type for DNA methylation studies; while a positive association between increased methylation of L1 gene and high-grade cervical lesions has been shown consistently, there are inconclusive results about methylation of CpGs in the upstream regulatory region (URR), with some studies reporting decreasing methylation with increasing grades of CIN and others showing increasing methylation levels with increasing grades of CIN.

4.2 Rationale for an updated review

There has not been a systematic review of the literature performed since 2013. Given that there has been a growing number of publications on DNA methylation of both human genes and the HPV virus, and their association with CIN2+ and ICC detection in the intervening time period, an update of the evidence on DNA methylation of human genes and HPV virus is warranted.

4.3 Aim of review

The aim of this review is to systematically summarise the data on the levels of DNA methylation markers (human genes and HPV virus) associated with histologically confirmed high-grade cervical lesions. Given the relative sparsity of studies investigating DNA methylation of HPV types other than HPV16, this review will focus on the literature which investigated DNA methylation of HPV16.

4.4 Objectives

- To summarise the association of DNA methylation (human genes and HPV16) with cervical intraepithelial lesions and invasive cervical cancer; ***(Objective 1.2)***
- To summarise the diagnostic performance of DNA methylation assays (human genes and HPV16) for the detection of CIN2+.

4.5 Methods

4.5.1 Outcome

Studies were included if they reported the percentage of DNA methylation according to CIN grade, or sensitivity and specificity of the DNA methylation assays for the detection of the outcome. This review was restricted to all human genes which reported associations with histologically confirmed cervical lesions (CIN). Studies reporting DNA methylation of HPV16 were included, however, given the small number of studies which evaluated DNA methylation of HPV18, 31, 33 and 45, they were not included in this review.

4.5.2 Reference or comparison group

Studies were included if methylation markers were assessed against a histological endpoint of CIN grade 2 or higher (CIN2/3, CIN2+ or CIN3+ which can include carcinoma *in situ* [CIS] and ICC). Studies with cytological endpoint assessment only were excluded because of the lower sensitivity for cytology measures in detection of high-grade disease [229]. Studies among ICC cases only were excluded when authors did not include a reference group (i.e. normal cervix) as the aim of this review is to investigate the association of DNA methylation with cervical lesions compared to normal cervix.

4.5.3 Search

Medline, Embase and Cochrane databases were searched using the following search terms: DNA methylation [Title/Abstract] OR epigenetic [Title/Abstract] OR methylation [MeSH Terms] AND nucleic acids [MeSH Terms] OR CpG islands [MeSH Terms] AND HPV [Title/Abstract] OR human papillomavirus [Title/Abstract] OR carcinogenesis [MeSH Terms] OR Cervical Intraepithelial Neoplasia/ or intraepithelial neoplasia [Title/Abstract] OR Anus Neoplasms/ or anal intraepithelial lesion [Title/Abstract]. The search was carried out up to 5 November 2016. All abstracts were screened by one author (HK).

4.5.4 Inclusion and exclusion criteria

Studies that reported only crude percentage methylation estimates without a validated cut-off for CIN2+ detection were excluded as they were not verified or validated. Studies not in the English language or conference abstracts were excluded due to difficulty in assessing the quality of the methodology, as were studies with fewer than 50 participants which could result in an imprecise effect measurement. Whereby publications provided DNA methylation measures using a combination set of gene markers, the DNA methylation of the individual markers as well as the combination panel are presented separately in the results.

4.5.5 Data extraction

From the consensus list, data were extracted by one author (HK) using a standardized form. The majority of studies which investigate the association of DNA methylation of human genes and HPV virus do so through validation of DNA methylation assays for the detection of CIN2+. HK extracted performance measures, including sensitivity and specificity, to interpret the association of DNA methylation with CIN2+. For all studies, the following variables were recorded: year of study, study location, origin (country) of study population, outcomes of interest (histological confirmed lesion CIN2+/CIN3+), DNA methylation marker evaluated, DNA methylation positive among CIN2+ (true positives), and ≤CIN1 (false positives), and DNA methylation negative among ≤CIN1 (true negatives) and CIN2+ (false negatives), where given.

4.5.6 Statistical analysis

The numbers of true positives, false positives, true negatives and false negatives were extracted where available, obtained using study-specific thresholds (cut-offs) to define methylation positivity. Many of the included studies reported performance measures for the detection of CIN2+ (sensitivity or specificity, or receiver operating characteristics [ROC] curves). Where several thresholds for methylation positivity were reported or where only ROC curves were presented, data was extracted based on a threshold that obtained at minimum a 70% specificity, as 1.) this

would be considered to be the minimum requirement for an acceptable screening test for CIN2/3 [229] and 2.) this was most commonly reported minimum specificity across the publications for the various methylation markers. Results are summarised in table format, separately by individual human gene and HPV16.

This review is registered with on the PROSPERO database at the Centre of Reviews and Dissemination, University of York; registration number CRD42016052119.

4.6 Results

4.6.1 Search result

The review identified 2,106 publications through Medline, Embase and Cochrane library searches which reported the association of the methylation of human genes or of any HPV type with any of the following outcome groups: CIN2/3, CIN3, CIN2+ and CIN3+ (**Figure 4.2**), of which 802 duplicates were removed and 1,142 excluded after abstract review, leaving 162 articles for full text review. Finally, 31 articles were selected which matched the inclusion criteria, among which there were at minimum three reports for any single gene. The characteristics of these studies are summarised in **Appendix 6**. Among these, 23 reported the association of single human gene DNA methylation with CIN2+, including seven for cell adhesion molecule 1 (*CADM1*) [17, 329-333]; seven for myelin and lymphocyte (*MAL*) [17, 329-333]; five for erythrocyte membrane protein band 4.1 like (*EPB41L3*) [254, 256, 328, 334, 335]; eight for paired box 1 (*PAX1*) [329, 336-342] and two for sex-determining region Y, box 1 (*SOX1*) [339, 342]. Ten studies reported the association of HPV16 methylation with CIN2+ [256, 334, 343-350].

In addition, nine studies reported the association of combination panel markers with CIN2+ which included five for *CADM1* and *MAL* [330, 332, 351, 352]; two for *MAL* and microRNA 124-2 (*MIR*) [331, 353] and two for *CADM1, MAL* and *MIR* [17, 354].

Of the 23 human gene studies, the majority included women who were HR-HPV positive, with the exception of two studies which evaluated *CADM1, MAL* and *MIR*-124 DNA methylation [330, 354] and two studies which evaluated *EPB41L3* DNA methylation irrespective of HPV status [334, 335]. Of the eight studies which evaluated *PAX1* or *SOX1* DNA methylation, six included women irrespective of HPV positive test result and two studies did not report HPV testing result [337, 340]. All studies which evaluated HPV16 DNA methylation included women who were HPV16 positive.

4.6.2 DNA methylation of CADM1, MAL and MIR for the detection of CIN2/3, CIN3 and CIN3+

Because *CADM1, MAL* and *MIR* were tested in the same populations by the same authors, and used in multi-gene panels, result are presented here together. For *CADM1*, seven publications were identified; four of these provided data to estimate performance measures for detection of CIN2/3, three for CIN3 and two for CIN3+ (**Appendix 7**). The same seven publications also provided estimated for *MAL* (**Appendix 8**). For *MIR*, there were only 2 studies that provided performance measures as a single gene; 1 for CIN2/3 [17] and 2 for CIN3+ [17, 331]. Because there were few studies that reported performance of MIR independently, only the studies whereby *MIR* is part of a multi-gene panel with *CADM1* and *MAL* are presented. Combination panels of *CADM1, MAL* and *MIR* genes are summarised in **Appendix 9**.

Sensitivity, specificity, PPV (positive predictive value) and NPV (negative predictive value) for individual studies are summarised in **Appendix 7** (*CADM1*), **Appendix 8** (*MAL*) and **Appendix 9** (combination panels of *CADM1, MAL* and *MIR*). From the data available in the literature, it was possible to obtain a range of sensitivity estimates based on a specificity of 70% for all single and combination markers among *CADM1, MAL* and *MIR*. These summary ranges of estimates, by gene, are summarised in **Table 4.1**.

Based on a minimum specificity of 70%, sensitivity estimates for *CADM1* as a single marker ranged between 13-79% for CIN2/3, 33-87% for CIN3 and 47-79% for CIN3+ (**Table 4.1**). The sensitivity estimates for MAL as a single marker ranged between 2-64% for CIN2/3, 42-83% for CIN3 and 60-67%

for CIN3+ (**Table 4.1**). The best sensitivity estimates across all outcome group (CIN2/3, CIN3, CIN3+) appeared to be obtained when *CADM1, MAL* and *MIR* were combined in a single panel; with increasing sensitivity estimates achieved with increasing CIN grades; 48-65% for CIN2/3, 69-77% for CIN3 and 95% for CIN3+ (Table 4.1).

DNA methylation of *EPB41L3* for the detection of CIN2/3, CIN3 and CIN3+

Four studies were identified that reported performance of *EPB41L3* for the detection of CIN2/3, two for CIN3, two for CIN2+ and one for CIN3+ (**Appendix 10**). The available data allowed extraction of sensitivity estimates for a minimum specificity of between 57-60%. Based on this specificity, sensitivity estimates for *EPB41L3* as a single marker ranged between 50-60% for CIN2/3 and 68-84% for CIN3 (**Table 4.1**). When combined with HPV16, 18, 31 and 33, sensitivity for CIN2+ ranged between 65-74%, and combined with other human genes (*JAM3, TERT* and *C13ORF18*), sensitivity for CIN2+ ranged between 82-84%.

4.6.3 DNA methylation of PAX1 and SOX1 for the detection of CIN2/3, CIN3, CIN2+ and CIN3+

For *PAX1*, eight publications were identified; four of these provided data to estimate performance measures for detection of CIN2/3, two for CIN3, four for CIN2+ and five for CIN3+ (**Appendix 11**). For *SOX1*, two publications were identified; one of these provided data to estimate performance measures for detection of CIN2+ and both for CIN3+ (**Appendix 12).**

The data for both *PAX1* and *SOX1* suggests that for both markers the sensitivity was low for CIN2/3 (**Table 4.1**). For a minimum specificity of 90%, sensitivity estimates for *PAX1* as a single marker ranged between 15-79% for CIN2/3, 36-92% for CIN3 and 52-95% for CIN3+. *SOX1* was less frequently studied. For a minimum specificity of 80%, sensitivity estimates for *SOX1* as a single marker were 44% for CIN2+ but ranged between 81-88% for CIN3+.

4.6.4 DNA methylation of HPV16 L1/L2 and LCR regions for the detection of CIN2/3, CIN3, CIN2+ and CIN3+

Ten studies were identified that reported methylation of HPV16 L1/L2 regions associated with CIN2+ (**Appendix 13**). The studies reported a wider range of specificity estimates, making it difficult to provide a summary range of sensitivity estimates. Based on a specificity of 70% that was reported in four studies, sensitivity for CIN2/3 ranged from 21-93%, and 61-80% for CIN3+ (**Table 4.1**). As previously described, a combination panel which included *EPB41L3*, and HPV types 18, 31 and 33 had reasonable sensitivity and specificity for CIN2+ and CIN3+ detection.

4.6.5 Meta-analysis of the performance of DNA methylation markers for the detection of CIN2/3 and CIN3+

When restricting the analysis to studies that used samples from population-based screening (**Table 4.2**), there were too few studies to evaluate pooled sensitivity and specificity for individual gene markers. However, when combining the various DNA methylation markers (HPV16, EPB41L3, MAL and MIR combination, CADM1+MAL and CADM1, MAL and MIR combination), the pooled sensitivity and specificity for CIN2/3 detection were 65% (95%CI: 55-74) and 70% (95%CI: 68-72) respectively among 6 studies (**Figure 4.3**). The corresponding pooled estimates for sensitivity and specificity for CIN3+ detection were 62% (95%CI: 56-69) and 70% (95%CI: 68-73) respectively among 7 studies (**Figure 4.4**). Heterogeneity across studies was high for both CIN2/3 and CIN3+ outcomes (**Figure 4.3, Figure 4.4**), and a more formal meta-anaysis (which is on-going) would be required to take into account the different study characteristics (study design, sample types and storage, and the assays used), as well as separate analysis for the HPV16 and human gene markers.

4.7 Summary

DNA methylation assays using single genes for the detection of CIN2/3, CIN3 or CIN3+ appeared to vary in sensitivity, based on an established specificity of >60%. But there appeared to be less variation in sensitivity when gene markers were combined. Based on a set specificity of between 60-90% (depending on the DNA methylation marker evaluated), the sensitivity estimates appeared to increase with increasing grades from CIN2/3 to CIN3+ (**Table 4.1**). Furthermore, the best performance, incorporating high specificity and sensitivity, was observed among women with CIN3+ relative to ≤CIN1. These data also suggest that the association between human gene and HPV DNA methylation increases with increasing CIN grade, and the variability in this association may suggest that different tumour suppressor genes (TSG) are implicated at different stages of cervical carcinogenesis, however there is no data to confirm this. Furthermore, the differences in thresholds used to define methylation positivity in this review prevent a full assessment of this.

An increase in aberrant DNA methylation patterns of TSG with increasing CIN grade is plausible. Silencing of TSG activity through DNA methylation has been reported in cervical cancer cells for *CADM1* [355, 356] and *MAL* [357]. Methylation of the CpG regulatory regions of micro RNAs (miRNA), including that of *MIR-124-2*, can lead to their deregulation. Deregulation of *MIR-124-2* has been shown in cervical cancer, and has been linked to increased promoter methylation of its genes [358-361]. *EPB41L3* encodes the DAL-1 protein, and methylation of its promoter is associated with a loss in tumour suppressor activity. It has been frequently studied in lung adenocarcinoma [250, 362] and breast [251] and prostate cancer [252], but there are as yet no studies which have investigated its role in cervical carcinogenesis.

PAX1 is a transcription factor involved in developmental processes. It is suggested to be a TSG and is silencing by methylation in ovarian cancer [363] and ICC [364]. Its precise function is yet to be determined. In animal studies, overexpression of *SOX1* was associated with tumour suppression by

inhibiting cell proliferation and invasion in vitro, and interferes with the Wnt/β-catenin signalling pathway in cervical cancer [365]. Its silencing in DNA methylation restricts this regulation.

While aberrant methylation of HPV during disease progression is common, and most pronounced in the L1 and L2 regions [366] , it remains uncertain whether HPV DNA methylation provides the infected cell with a growth advantage [366] or whether it is indirectly involved in DNA methylation of human genes.

It was not the aim of this review to assess the accuracy of the various DNA methylation markers for the detection of CIN2/3, CIN3 and CIN3+ and a more formal pooling and meta-analysis of the data, as well as full assessment of sources of heterogeneity would be required to do this. The diversity of studies and performance is further compounded by the diversity of use in different screening/triaging settings. However, based on a crude assessment of the summary data in **Table 4.1,** it appears that the majority of DNA methylation markers detect CIN3+ with greater accuracy (i.e. high sensitivity and specificity), suggesting the DNA methylation is a process which occurs late in the pathway to ICC, and it could be used in triage after a more sensitive test such as HPV DNA testing.

Previous data has shown that if left untreated, lesions from approximately <5% of HIV-negative women with CIN1, 12-20% of women with CIN2 and 35-65% of women with CIN3 or carcinoma *in situ* (CIS) would progress to ICC during a normal life span [367], and there could be a role for a marker that could detect women who were likely to have lesion progression, similar to what has been reported for $p16^{INK4A}$ (cyclin-dependent kinase inhibitor), a tumour suppressor gene implicated in CIN2+. In a study among 1,137 HIV-negative women in Italy, among whom 40% were found to over-express $p16^{INK4A}$ at baseline, the cumulative incidence of CIN2+ over 3 years was 8.8% in $p16^{INK4A}$ -positive HR-HPV+ women and 3.7% in $p16^{INK4A}$ -negative HR-HPV+ women, giving a RR of progression to CIN2+ of 2.61 [368].

Given that WLHIV have faster progression to CIN2 and greater [171]– and the value of HPV DNA testing is limited because of the high prevalence of HPV in this population – such a marker would be valuable in distinguishing CIN2/3 lesions that persist or progress from those that spontaneously regress.

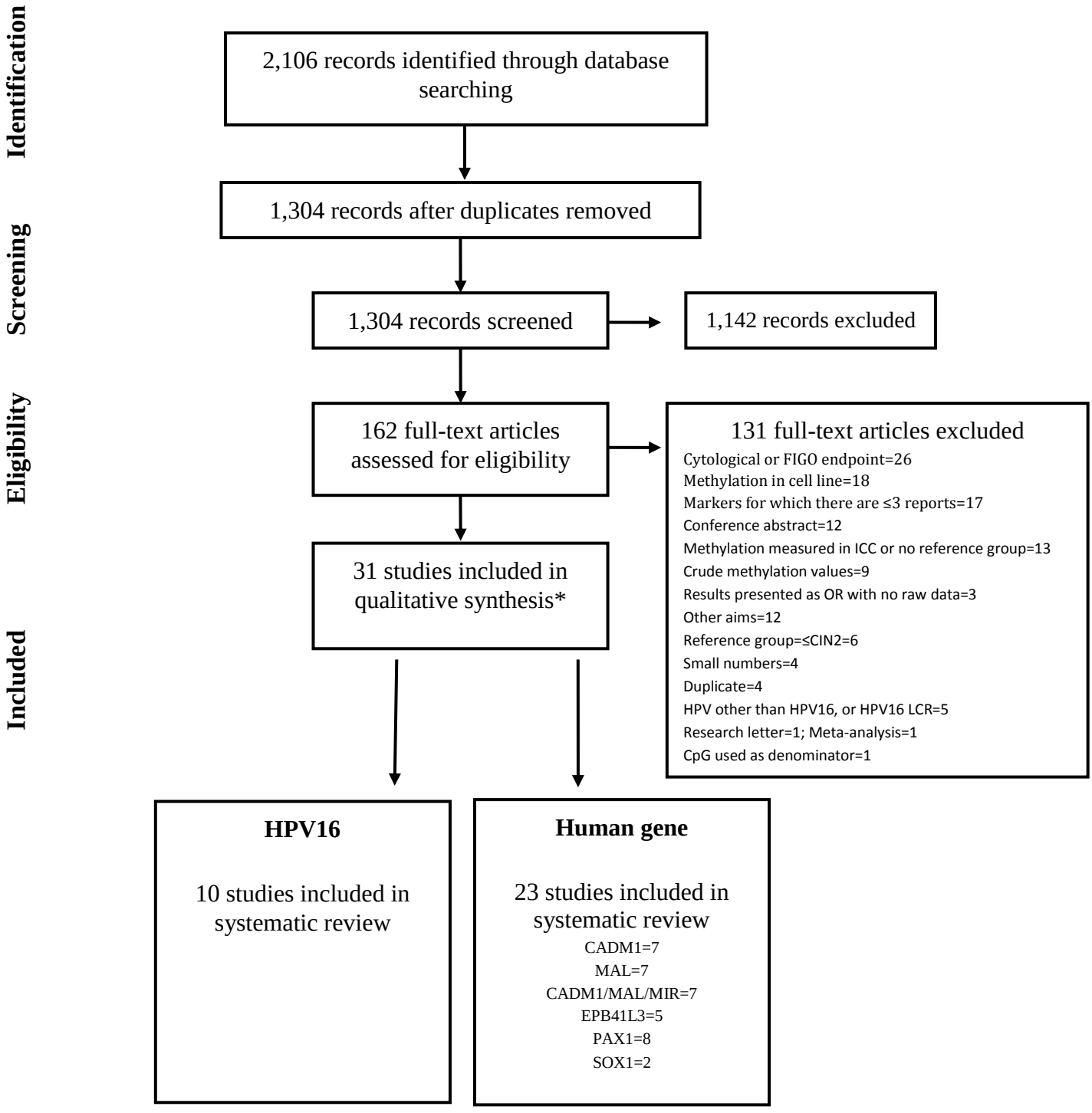
DNA methylation of human genes have rarely been studied among WLHIV and HPV16 DNA methylation has never been studied among WLHIV. In this thesis, baseline and endline samples from the HARP study were used in a case-control design to evaluate the DNA methylation of the human gene *EPB41L3* and HPV16 for the detection of CIN2+ at baseline, and incident CIN2/3 at endline. Furthermore, among the women who were not treated for prevalent CIN2+ during the 18-months follow-up period, the baseline methylation levels of EPB41L3 among women with persistent or progressive CIN2/3 were compared with those from women that had spontaneous regression of CIN2/3 lesions at endline. The finding of these analyses are summarised in **Chapter 10.**

EPB41L3 gene was selected as it generated reasonable performance among HIV-negative women, and had more consistent sensitivity and specificity estimates across studies with less variation in these estimates. DNA methylation of the HPV16 L1 region was also explored so that a comparison of its association with CIN2+ is similar to that of EPB41L3 methylation.

Summary of findings

- Databases were searched using search terms for human gene and HPV DNA methylation and cervical lesions.
- Of 2,106 records initially identified, **23 studies** were included that investigated the association of **human gene** DNA methylation with histologically confirmed CIN2+ relative to ≤CIN1, including *CADM1, MAL, MIR-124, EPB41L3, PAX1* and *SOX1*, and **12 studies** were included that investigated **HPV16** DNA methylation with CIN2+.
- There was significant variation in reporting of data across studies; studies reported various thresholds of DNA methylation to define methylation positivity and various outcome groups were used (CIN2/3, CIN3, CIN2+, CIN3+).
- ***CADM1, MAL* and *MIR-124*** genes were most frequently studied in combination panels together. For a **specificity of 70%,** a combination of the 3 genes gave a range of **sensitivity** between **48-65% for CIN2/3, 69-77% for CIN3 and 95% for CIN3+**.
- ***EPB41L3*** as a single test had a range of sensitivity between **50-60% for CIN2/3 and 68-84% for CIN3**, based on a **specificity of 57%.** In **combination** with HPV16, 18, 31, 33, or with other human genes, sensitivity was increased; **65-74% for CIN2+ and 82-84% for CIN3+.**
- ***PAX1* and *SOX1*** both had very **high specificity** when evaluated individually **(≥80%). Sensitivity** was low for **CIN2/3 (15-79%) but higher for CIN3 (37-78%) and CIN3+ (52-95%).**
- **HPV16** as a single gene had a wide range of **sensitivity values (21-93%) for CIN2/3** detection based on **specificity of 70%,** but increased **to 61-80% for CIN3+.**
- Although there was much variation in reporting of DNA methylation of human genes and HPV16, the data appears to suggest that DNA methylation markers are able to detect **CIN3+ lesions with high sensitivity** and with a reasonable specificity (70%).
- Restricting the analysis to studies using samples from population based screening, the pooled sensitivity and specificity of various DNA markers combined (HPV16, EPB41L3, CADM1, MAL and MIR) for CIN2/3 detection were were 65% (95%CI: 55-74) and 70% (95%CI: 68-72) respectively among 6 studies, and for CIN3+ were 62% (95%CI: 56-69) and 70% (95%CI: 68-73) respectively among 7 studies. However, heterogeneity across studies was high, and a more formal meta-anaysis would be required to take into account the different study characteristics.

Figure 4.2. Flowchart for study selection



*some studies looked at combination of human genes and HPV16

Table 4.1. Summary range of Sensitivity estimates for detection of CIN2/3, CIN3 and CIN3+ relative to ≤CIN1, based on different specificity values as reported in literature

			CIN2/3		CIN3		CIN2+		CIN3+	
	Marker Panel	Minimum specificity*	N studies	Range Sens.	N studies	Range Sens.	N studies	Range Sens.	N studies	Range Sens.
CADM1	Single	70%	4	13-79%	3	33-87%	-	-	2	47-79%
MAL	Single	70%	4	2-64%	3	42-83%	-		2	60-67%
CADM1/MAL	Combination	70%	3	36-62%	3	50-90%	-	-	2	68%
MAL/MIR	Combination	70%	1[a]	31%	1	41%	-	-	-	-
CADM1/MAL/MIR	Combination	70%	2	48-65%	2	69-77%	-	-	1	95%
EPB41L3	Single	57%	3	50-60%	2	68-84%	-	-	-	-
	Combination	60%	-	-			2[b]	65-74%	2[c]	82-84%
PAX1	Single	90%	4	15-79%	2	36-92%	4	37-78%	5	52-95%
SOX1	Single	80%	-	-	-	-	1	44%	2	81-88%
HPV16 L1/L2	Single	70%	4	21-93%	1	61%	-	-	1	80%
		60%	-	-	-	-	4	60-92%	-	-
		40%	2	90-92%	-	-	-	-	-	-
	Combination	60%	-	-	-	-	1	74%	1	84%

[a]based on 57% specificity; [b]based on 65% specificity and combination panel (either HPV16,18,31,33 or JAM3, TERT, C13ORF18; [c]based on 60% specificity and combination panel (either HPV16,18,31,33 or JAM3, TERT, C13ORF18);* obtained the highest possible specificity values for each marker as reported in the literature. From these estimates, a range of sensitivity values was generated

Table 4.2. Performance of DNA methylation markers for the detection of CIN2/3, CIN2+, CIN3 and CIN3+ among population based screening studies

Author, year	Methylation marker	Country	Sample size	Endpoint	N cases	Sens.	Spec.	PPV	NPV
DeVuyst, 2015	CADM1+MIR+MAL	Kenya-Nairobi	248	CIN2/3	93	72.0%	70.0%	58.8%	80.7%
Verhoef, 2015	CADM1+MAL	The Netherlands	358	CIN2/3	84	57.1%	72.3%	38.7%	84.6%
Verhoef, 2014	MAL+MIR	The Netherlands	1019	CIN2/3	212	46.7%	68.1%	28.1%	82.7%
Vasiljevic, 2014	EPB41L3	UK-London	1456	CIN2/3	531	61.0%	70.0%	54.9%	75.0%
Brentnall, 2014	HPV16	UK-London	556	CIN2/3	323	78.0%	70.0%	78.3%	69.7%
Lorincz, 2013	HPV16	UK-Wales	73	CIN2/3	25	77.0%	70.0%	57.6%	85.0%
DeStrooper, 2014	CADM1+MAL	The Netherlands	234	CIN2+	58	62.1%	78.4%	48.6%	86.3%
Hesselink, 2014	MAL+MIR	The Netherlands	355	CIN2+	94	59.6%	70.0%	41.8%	82.8%
Bryant, 2015	HPV16	UK-Cardiff	200	CIN2+	145	61.9%	66.0%	82.6%	39.6%
DeVuyst, 2015	CADM1+MIR+MAL	Kenya-Nairobi	248	CIN3	43	79.0%	70.0%	42.0%	92.4%
Verhoef, 2015	CADM1+MAL	The Netherlands	330	CIN3	56	66.1%	72.3%	32.7%	91.2%
Verhoef, 2014	MAL+MIR	The Netherlands	1019	CIN3	134	51.5%	68.1%	21.4%	89.3%
DeStrooper, 2014	CADM1+MAL	The Netherlands	234	CIN3+	38	68.4%	75.5%	35.1%	92.5%
Hesselink, 2014	MAL+MIR	The Netherlands	355	CIN3+	74	64.9%	70.0%	36.4%	88.3%
Verhoef, 2014	MAL+MIR	The Netherlands	1019	CIN3+	147	55.1%	68.1%	24.3%	89.1%
Bryant, 2015	HPV16	UK-Cardiff	200	CIN3+	145	60.5%	70.3%	84.6%	40.6%

PPV=Positive predictive value; NPV=Negative predictive value

Figure 4.3. Meta-analysis of the performance of DNA methylation markers for the detection of CIN2/3 in 6 studies.

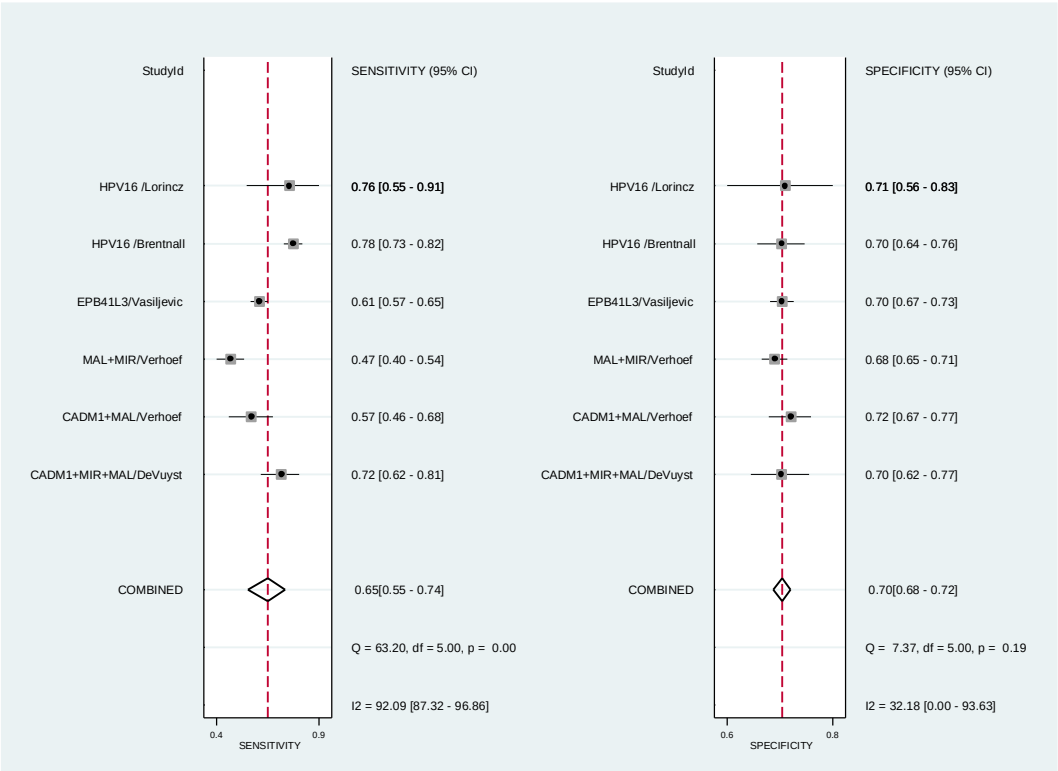
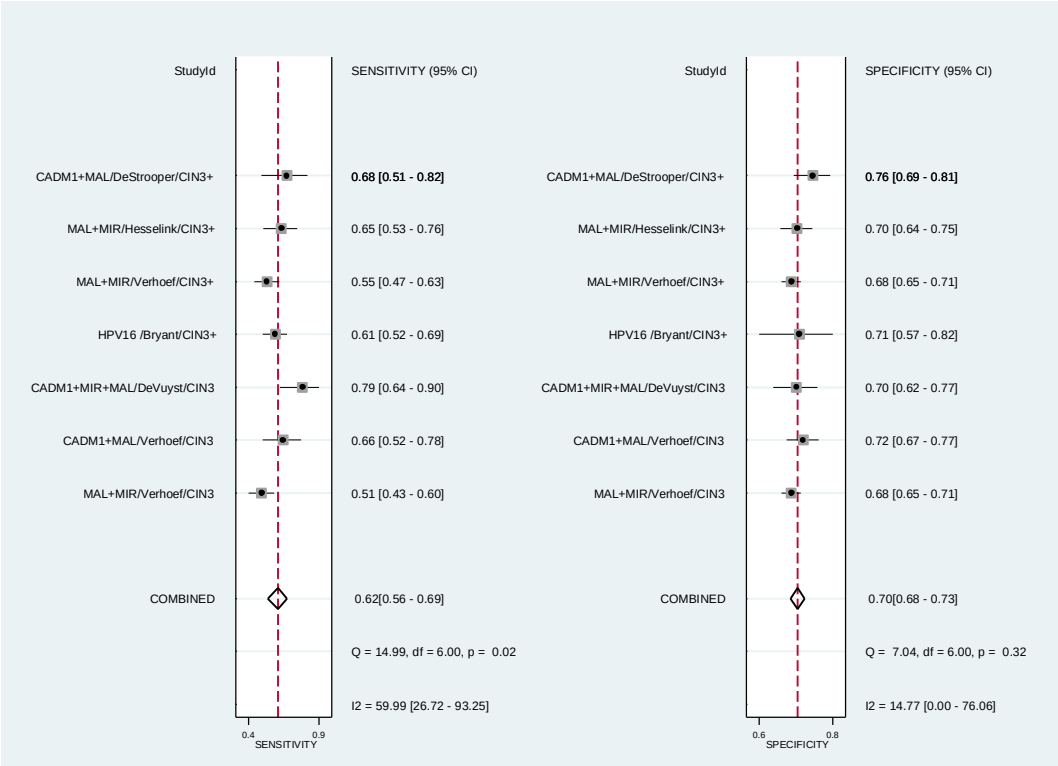


Figure 4.4. Meta-analysis of the performance of DNA methylation markers for the detection of CIN3 and CIN3+ in 7 studies.



5 OVERALL STUDY METHODOLOGY

In this chapter, I firstly describe the overall design of this research thesis. I then describe the HARP specific study design, objectives and methods within which this PhD is nested, and laboratory methods for HPV serology and DNA methylation. The statistical approaches are briefly described but specific statistical analyses for each of the research objectives are more clearly described in the respective chapter (Chapters 6 to 10 inclusive).

5.1 PhD overall study design

As described in **Chapter 1 (Table 1.1),** this PhD is comprised of three interrelated studies, each with their specific design and methods, although some of the laboratory methods and analytical approaches are shared. **Figure 5.1** illustrates how the thesis is organised into the individual studies. The thesis begins with a series of systematic reviews. In terms of research study designs, the PhD comprises a combination of cross-sectional, cohort and case-control studies.

A systematic review and meta-analysis exploring the association of ART with HR-HPV and cervical lesions (**Objective 1.1**) was needed to inform the analyses for **STUDY 1** and **STUDY 2**, in order to understand whether women on ART had a different risk profile for HPV infection and cervical lesion incidence and progression compared to women not on ART.

A second systematic review of the literature on DNA methylation of human genes and HPV types, and their association with CIN2+ (**Objective 1.2**), was performed in order to summarise the evidence on the most frequently studied human genes and HPV types, to justify the choice of DNA methylation targets in this thesis and to inform the analysis of **STUDY 3**.

The different study designs used to address the objectives in **STUDIES 1, 2 and 3** are illustrated in **Figure 5.2. STUDY 1** used a combination of cross-sectional and prospective data from the *HARP* study to describe the epidemiology of HR-HPV (prevalence, incidence, persistence and clearance) and CIN2+ (prevalence, incidence, persistence and spontaneous regression) at baseline and over 18-months follow-up, and to explore socio-demographic, behavioural and HIV-related factors associated with these outcomes. **STUDY 1** used a broader definition for HR-HPV and was defined as positive for any HR-HPV types.

STUDY 2 also used a combination of cross-sectional and prospective data from the *HARP* study, but focussed on HPV genotype specific analyses. **STUDY 2** uses the cross-sectional data to describe the HPV genotype specific prevalence and their association with prevalent CIN2+, as well as describing HPV type seroprevalence. The prospective data is used to explore the association of specific HPV genotype persistence with CIN2/3 incidence, as well as HPV type specific seroincidence and seroconversion and associations with same-type HPV re-infection.

The prospective study data was used from the *HARP* study as: i.) it allows for a precise definition of HPV incidence and HPV persistence; and CIN2+ incidence over the follow-up period, and ii.) it addresses the temporality between the risk exposure and the outcome, thereby allowing for exploration of the association of baseline risk factors with early disease outcomes, such as HR-HPV persistence and CIN2+ incidence. The prospective study excluded prevalent CIN2+ in the risk factor analysis so as to extend the findings to a wider population of women without CIN2+.

Finally, **STUDY 3** used a case-control design to investigate associations between human gene *EPB41L3* and HPV16 DNA methylation and CIN2+ prevalence and incidence, and this is detailed in **section 5.5**.

Figure 5.1. Organization of the thesis in 4 sections and 11 Chapters.

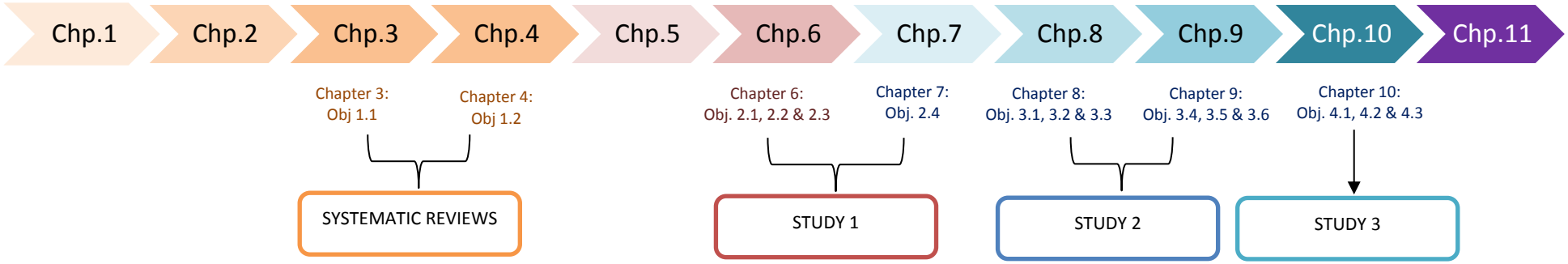
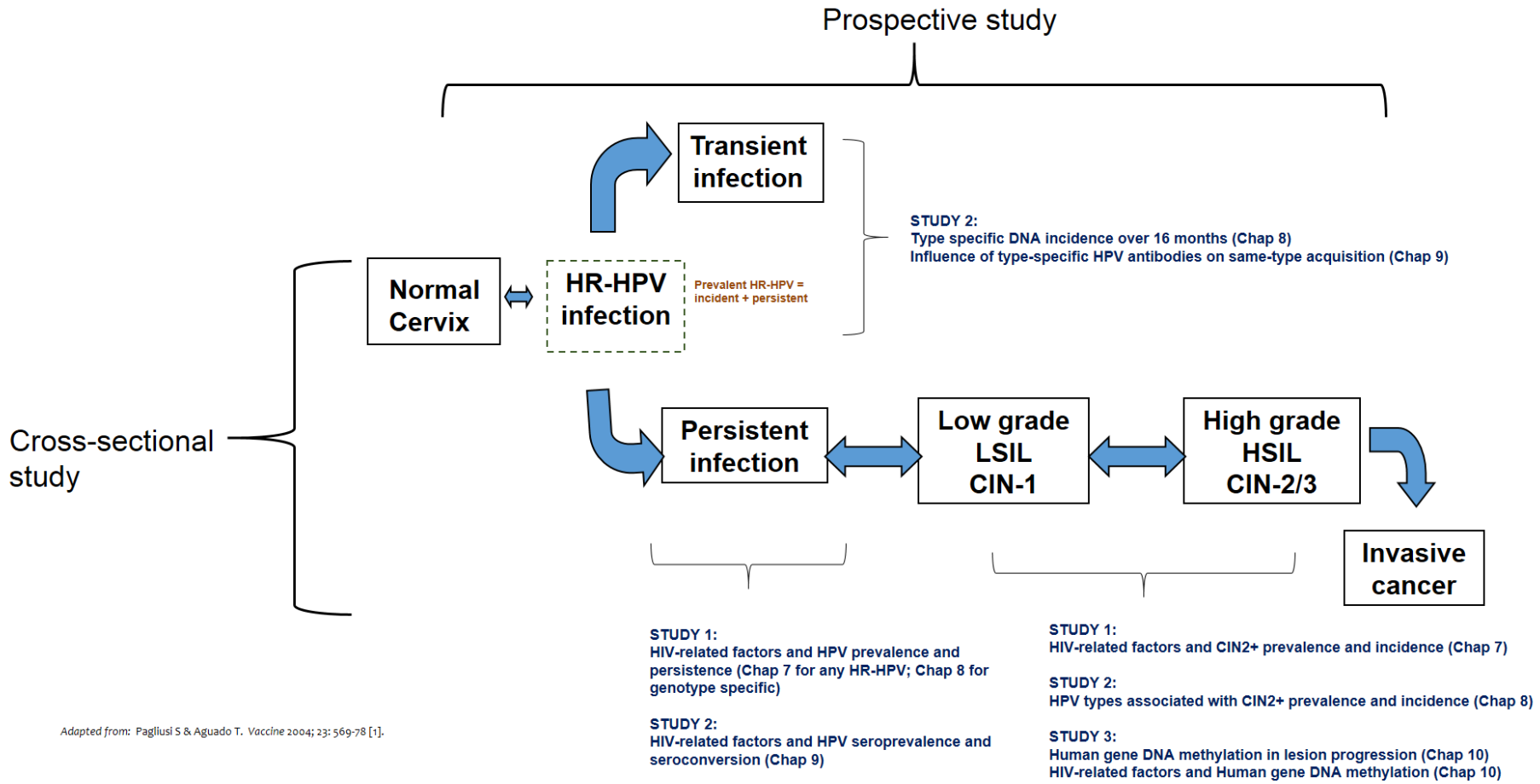


Figure 5.2. Elements of the pathway from HPV infection to invasive cervical cancer addressed in this thesis



Adapted from: Pagliusi S & Aguado T. *Vaccine* 2004; 23: 569-78 [1].

5.2 The *HARP* study

The EU-funded *HARP* (*HPV in Africa Research Partnership*) study was established to evaluate cervical cancer screening strategies against histological (CIN) and cytological (SIL) endpoints among WLHIV recruited according to ART status in Burkina Faso and South Africa. The overall aim of HARP was to improve cervical cancer prevention programmes for WLHIV in South Africa and Burkina Faso, by evaluating the effectiveness and cost-effectiveness of alternative screening strategies, and by developing algorithms leading to earlier detection and management of cervical cancer in these high-risk populations.

5.2.1 Study design

The HARP study was conducted in two countries, with one study site in each country, and consisted of three interlinked substudies:

- A cross-sectional study of HPV and cervical neoplasia screening among HIV-1 seropositive women attending HIV care centres in Burkina Faso and South Africa.
- A prospective cohort study of HIV-1 seropositive women recruited in Study 1 including women without advanced histological lesions (≤CIN1), and women with CIN2+ up to 18 months (which was reduced to 16 months because of project constraints).
- A health economics and modelling study using data from the cross sectional and cohort studies to determine cost-effectiveness of the various screening strategies in preventing cervical cancer cases.

5.2.2 Sample size

Sample size calculations for the HARP study were based on the comparison of the rate of progression to CIN2+ between HIV-1 positive patients taking ART or having low CD4+ T-lymphocytes cell count at study entry versus those not on ART at study entry. Prevalence and

progression rates were based on the only available estimates of CIN2+ prevalence and incidence in WLHIV in UK and South African populations at the time of study design [369]. The sample size calculations took into account the approximate presentation of patients at HIV clinics in Africa with a 2:1 ratio of women enrolled ART-users:naïve. With total of 1200 women (400 not on ART, 800 on ART) over both sites, the study had over 80% power to detect a difference of 10% vs. 5% CIN2+ prevalence at enrolment. With total of 1200 women (400 not on ART, 800 on ART) enrolled in both sites, the study had 95% power to detect a difference of 15% vs. 7.5% in cumulative CIN2+ incidence over 18 months (the initially intended duration of the study). Calculations also allowed for 15% loss to follow-up, including any change of ART/CD4 group status.

5.2.3 Visit schedule

At the screening visit (Visit 0), participants were invited to enrol for the HARP study following assessment of eligibility criteria and were given at least 7 days reflection period before enrolment (Visit 1) in the study. Four to six weeks following enrolment (Visit 2), allowing for results guiding biopsy indication to be returned, all participants were examined by digital colposcopy; and four-quadrant biopsy was taken if indicated.

Participants were followed up every 6 months for 18 months, reflecting routine schedules for HIV follow-up and care in both countries, to evaluate the performance of screening methods for the detection of incident CIN2+. For that purpose, a colposcopy and biopsy visit was organised at Month 18 (M18) follow-up visit. In addition, a venous blood sample was collected at month 6 (M6), month 12 (M12) and M16 for CD4+ T-lymphocyte counts. The HARP study flow of procedures is shown in **Figure 5.6.**

5.2.4 Study population

Participants were recruited from the Hôpital de Jour, the HIV outpatient clinic of the University Teaching Hospital of Ouagadougou, Burkina Faso (BF), and HIV treatment centres (Esselen Street Clinic and Ward 21, Hillbrow Community Health Centre) in inner city Johannesburg, South Africa (SA) from December 2011 to October 2012. Inclusion criteria were being HIV-1 seropositive, aged 25-50 years and resident in the city. Exclusion criteria were history of prior treatment for cervical cancer, previous hysterectomy, and being pregnant or less than 8 weeks postpartum. To enable analysis of the effects of ART on cervical disease progression, enrolment was stratified in a 2:1 ratio of ART-users:naïve, similar to proportion of WLHIV using ART in other SSA countries [10]. At enrolment, eligibility for ART initiation in both countries followed the 2010 WHO guidelines with a CD4+ count threshold of 350 cells/mm^3 [370]. Written informed consent was obtained at the screening visit when eligibility for the study was assessed and at enrolment. Data on clinical, socio-demographic and behavioural characteristics were collected by interviewer-administered questionnaire. Participants were followed-up every 6 months for CD4+ cell count up to Month 18 visit (endline) when procedures similar to baseline (Visit 1) were repeated.

5.2.5 Specimen collection

At enrolment, a venous blood sample was collected to confirm HIV-1 serostatus if needed, to perform HSV-2 and syphilis serologies and to obtain baseline HIV-1 RNA PVL and CD4+ cell counts. Urine pregnancy testing was performed. Cervical samples were collected by a nurse-midwife using a Digene cervical sampler (Qiagen, Courtaboeuf, France) at enrolment and using the *care*HPV cervical sampler (Qiagen, Gaithesburg, MD) at month 12 and at month 18 follow-up visits for HPV-DNA testing and genotyping. A cytobrush for Papanicolaou smear cytology and a swab from the ecto/endocervix to detect cervical STIs by molecular methods were also collected. A vaginal smear was collected to diagnose bacterial vaginosis and *Candida albicans* by Gram stain. Participants were

assessed clinically using visual inspection with acetic acid or Lugol's iodine (VIA/VILI). All participants were referred for colposcopy performed by trained colposcopists. Systematic 4-quadrant cervical biopsy, including directed biopsy of any suspicious lesions, was performed for participants who had abnormalities detected by cytology (≥LSIL or atypical glandular cell of undetermined significant or greater [≥AGUS]), VIA/VILI or colposcopy, or who were HR-HPV DNA positive (by Digene HC-II). The same genital sampling and examination procedures were repeated at the endline visit at Month 18 (HR-HPV DNA testing was done then by *care*HPV).

5.2.6 HIV and STI laboratory testing

HIV-1 serostatus was diagnosed according to national guidelines for each country [371, 372]. HSV-2 serology was performed using the Kalon® IgG2 ELISA (Kalon Diagnostics, UK) and syphilis serology by a combination of a *Treponema pallidum* haemagglutination assay (TPHA) and rapid plasma reagin (RPR; BioMérieux, Lyon, France in BF; Immutrep carbon antigen RPR, Omega Diagnostics in SA). Plasma HIV-1 RNA was assessed using real-time PCR (Abbott RT HIV-1) in BF and COBAS Taqman (Roche Diagnostics) in SA, with a lower limit of detection of 40 copies/ml. Testing for CD4+ T-lymphocytes was performed using FACScount (Becton-Dickinson, NJ). Laboratories subscribed to international external quality assessment schemes; the UK-National External Quality Assessment Service for CD4+ cell counts [373] and Quality Control for Molecular Diagnostics for HIV-1 PVL testing [374].

Neisseria gonorrhoeae, Chlamydia trachomatis, Mycoplasma genitalium and *Trichomonas vaginalis* were detected using nucleic acid amplification tests [NAATs], the Sacace simplex assays (Sacace, Como, Italy) in BF and the APTIMA Combo (Gen-Probe, San Diego, CA) in SA. The Nugent's score [375] was used for vaginal flora reading of Gram-stained vaginal smears, with diagnosis of bacterial vaginosis made for scores ≥7, and examined for the presence of *Candida spp*.

5.2.7 HPV DNA assays

HPV-DNA genotyping of all samples was performed at the virology laboratory of the Gui de Chauliac Teaching Hospital, University of Montpellier, France, using the qualitative Digene HC-II (Qiagen, Gaithersburg, MD), and genotyping with the INNO-LiPA HPV genotyping Extra® assay (Innogenetics, Courtaboeuf, France)[376, 377]. The Digene HC-II assay is based on hybridization of HPV DNA with a RNA probe cocktail with chemiluminescence signal amplification to detect the 13 HR types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68).

The *care*HPV assay (Qiagen, Gaithersburg, MD) is a new signal-amplification assay adapted from HC-II assay and targets the same 13 HR types as Digene, in addition to HPV66. Modifications were made to deploy this assay in low-resource environments. In *HARP* the careHPV assays were performed at the local laboratories, the Centre de Recherche Biomoléculaire Pietro Annigoni (CERBA) in Ouagadougou and the Sexually Transmitted Infections Reference Centre (STIRC) of the National Health Laboratory Service (NHLS) in Johannesburg.

The INNO-LiPA HPV genotyping Extra® assay is based on the amplification of a 65-bp fragment in the L1 gene using the SFP10 broad-spectrum primers that enables the identification of 32 HPV types [376]. HR-HPV types were defined using the current International Agency for Research on Cancer (IARC) classification [4] to include: 'carcinogenic to humans' (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) and 'probable carcinogenic' (HPV68). The 'possible carcinogenic' types (HPV26, 53, 66, 69, 70, 73, and 82) and other known low-risk types (HPV6, 11, 40, 43, 44, 54, 71, and 74) were considered as low-risk (LR-HPV), as summarised in **Table 5.1.** HPV types are further classified into sub-groups according to the nucleotide sequence of the L1 protein; the HR types are part of the alpha-7 and alpha-9 sub-group of HPV types.

Table 5.1. Definition of HPV categories using INNO-LiPA genotyping assay

Outcome Group	HPV types
HPV positive[1]	6, 11, 16, 18, 25, 26, 30, 31, 33, 35, 39, 40, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 69, 70, 71, 73, 74, 81, 82, 84
HR-HPV ('carcinogenic' & 'probable carcinogenic'[2]	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68
HR-HPV 'possible carcinogenic'[2]	26, 53, 66, 69/71 (without distinction between 69 and 71), 70, 73, 82
LR-HPV[2]	6, 11, 40, 43, 44, 54, 62, 74
Alpha-9 HR-HPV[3]	16, 31, 33, 35, 52, 58
Alpha-7 HR-HPV[3]	18, 39, 45, 59, 68
Alpha-5 HR-HPV[3]	51
Alpha-6 HR-HPV[3]	56
Vaccine groups	
Bivalent vaccine types (2vHPV)	16, 18
Quadrivalent vaccine types (4vHPV)	6,11,16,18
Nonavalent vaccine types (9vHPV)	6, 11, 16, 18, 31, 33, 45, 52, 58

[1]types detected by INNO-LiPA; [2]Classification by [4]; [3]classification in [378]

5.2.8 Histology

Cervical biopsies were processed at the local pathology laboratories in Ouagaoudou and Johannesburg and read using the three-tier CIN classification system [42]. Histology was classified as 'negative' (≤CIN1) or 'positive' (CIN2+) based on the highest reading across all findings from the 4-quadrant biopsies and endocervical curettage, if indicated. All histological slides from women with a local diagnosis of CIN2+, all slides with an undecided diagnosis of CIN1/2 and approximately 10% of slides from women with ≤CIN1 histological findings were reviewed by the HARP Endpoint Committee of five pathologists (one from each site plus 3 international from France and Spain) after each major endpoint visits were completed (i.e. Visit 2 and Visit 6, see HARP Flowchart**, Figure 5.6**), for consensus classification (and if necessary adjustment of management of the patients).

5.2.9 Management of CIN2+

Participants were recalled for CIN2+ management, if found to have CIN2+ lesions by histology at enrolment. This visit was scheduled at the earliest convenient date once the result was known. Due to demands on local health services, especially in SA, this often meant that CIN2+ management was scheduled up to 14 months later. CIN2+ status was therefore defined according to whether the participant had received CIN2+ management between enrolment and follow-up (**Table 5.3**).

Management of CIN 2+ was according to local guidelines at each site, and included the following options:

- Immediate treatment with cold coagulation or laser ablation
- Use of loop excision techniques (Large loop excision of the transformation zone [LLETZ]/Loop Electrosurgical Excision Procedure [LEEP]) techniques according to IARC guidelines (http://screening.iarc.fr/colpo.php).
- Referral for surgery for cone excision, cervix amputation or hysterectomy for higher grade or invasive lesions and according to the child-bearing intention of the women.

Women who underwent hysterectomy were withdrawn from the follow-up study.

5.3 Study 1: Epidemiology of HPV infection and cervical lesions

Study 1 used secondary quantitative cross-sectional and longitudinal data collected as part of the HARP study and stored in the HARP database at the LSHTM. Histological measurements, in addition to data on HPV genotyping data and HIV related factors, such as ART status, ART duration, CD4+ cell count and HIV-1 PVL, socio-demographic and behavioural factors were used during the analysis (**Chapters 6, 7 and 8**).

5.3.1 Definitions of HPV infection states

HR-HPV type-specific persistence was defined as being positive for the same type at baseline and endline by genotyping (**Table 5.2**). Type-specific clearance was defined as being positive for a specific HR type at baseline and negative for the same type at endline, while complete clearance was defined as being positive for at least one HR type at baseline and negative for all HR types at endline. Results are presented in **Chapter 6 (Objective 2.1)** for HR-HPV prevalence, incidence, persistence and clearance, and in **Chapter 8 (Objectives 3.1 & 3.2)** for individual HPV genotype prevalence, incidence and persistence.

Table 5.2 Definition of HPV infection status at baseline and endline

Outcome Group		Definition	Baseline result (Denominator)	Endline result (Numerator)	Analysis approach
Incidence	**1**	Type specific incidence	Type specific negative	Same type positive	Per infection analysis
	2	Type swap	Positive for X HPV Negative for Y HPV	Negative for X HPV Positive for Y HPV	Per woman analysis
	3	Any HR-HPV incidence[1]	Negative for any HR-HPV	Positive for HR-HPV type not detected at baseline	Per woman analysis
Persistence	**4**	Type specific persistence	Type specific positive	Same type positive	Per infection analysis
	5	Any HR-HPV persistence	Positive for any HR-HPV	Any HR-HPV type-persistence	Per woman analysis
Clearance	**6**	Type specific clearance	Type specific positive	Same type negative	Per infection analysis
	7	HR-HPV complete clearance	Positive for any HR-HPV	Negative for all HR-HPV	Per woman analysis

[1]For the definition of ANY HR-HPV incidence : given that no woman was infected by all 13 HR types, all women were at risk of acquiring at least one HR-HPV infection and all women were included in the risk factor analysis for HR-HPV incidence; the numerator include women with incident infection of any HR type

5.3.2 Definitions of CIN2+ status

CIN2+ status at baseline and endline was defined, as described in **Table 5.3**. Prevalent CIN2+ was defined as the number of women with CIN2+ detection at baseline among all women enrolled in the HARP study. There were no ICC detected at endline, therefore CIN2/3 is used at in the endline definitions of CIN status. Incident CIN2/3 at endline was defined as newly detected CIN2/3 at the endline visit among those without CIN2/3 at baseline. Recurrent CIN2/3 was defined as CIN2/3 detection at endline among women who received management for prevalent CIN2+ diagnosis before the second biopsy visit at endline.

Persistent CIN2, persistent CIN3 and progression from CIN2 to CIN3 were calculated among those participants that did not receive management for prevalent CIN2/3 diagnosis before the second biopsy visit at endline. Participants with CIN3 regression to CIN2 and CIN2/3 spontaneous regression to ≤CIN1 were similarly described.

These descriptive results are presented in **Chapter 6 (Objective 2.2)**.

Table 5.3. Definition of CIN2+ status at baseline and endline

Management status	Outcome Group	Denominator	Baseline	Endline
Not required	CIN2+ prevalence	All women at baseline	CIN2+	
Not required	Twice ≤CIN1	Women ≤CIN1 at baseline	≤CIN1	≤CIN1
	Incident CIN2/3		≤CIN1	CIN2/3
Treated for CIN2+	Successfully treated	Women with CIN2+ at enrolment and treated before M18	CIN2+	≤CIN1
	Recurrence		CIN2+	CIN2/3
Not treated for CIN2+	Persistent CIN2	Women with CIN2+ at enrolment and not treated before M18	CIN2	CIN2
	Persistent CIN3		CIN3	CIN3
	Progression CIN2 to CIN3		CIN2	CIN3
	Regression CIN3 to CIN2		CIN3	CIN2
	Regression CIN2/3 to≤CIN1		CIN2/3	≤CIN1

5.3.3 Statistical analysis

Individual research chapters provided thorough details of statistical analyses used, specific to each chapter, although some statistical approaches commonly used in the research chapters are described here.

As HR-HPV prevalence was common, associations with exposure variables were estimated with prevalence ratios (PRs) obtained from logistic regression using marginal standardization to estimate PRs, and the delta method to estimate 95% confidence intervals (CI) [379]. Associations between HR-HPV persistence and exposure variables were estimated with generalised estimating equation to account for multiple HR-HPV infection and multiple infection states (persistence and clearance) [380]. For associations with CIN2+ prevalence and incidence over follow-up (measured once at endline), logistic regression was used to estimate odds ratios (ORs) and 95% CI.

Multivariable analyses were adjusted for site and socio-demographic and behavioural factors which were independently associated in univariate analyses (with p values <0.10) with HR-HPV or CIN2+ for each country (Model 1). Given the wide range of risk factors included in the initial model,

a p-value of <0.10 was used to identify factors to carry forward in multivariate analysis. Results are presented in **Chapter 6 (Objective 2.3)**.

To explore associations of HR-HPV and CIN2+ outcomes with HIV-related factors, pre-specified analyses included stratification by site, ART use and duration (≤ or >2 years), HIV-1 viral suppression (< or ≥1000 RNA copies/ml) and CD4+ cell counts. Stable high CD4+ cell count was defined as having CD4+ counts >500 cells/mm^3 at baseline, Month 12 and endline (M18) visits. A second logistic regression model (Model 2) incorporated baseline CD4+ cell count to Model 1 to explore associations with ART. Results are presented in **Chapter 7 (Objective 2.4)**.

All data in this thesis were analysed using Stata version 14 (Stata Statistical Software, College Station. TX: Stata Corporation).

5.4 Study 2: HPV type specific infection and serodynamics among WLHIV

5.4.1 Definitions of HPV type-specific DNA

HR-HPV type-specific persistence and clearance are defined, as described previously (**Table 5.2**). Vaccine-type HPV groups are defined in **Table 5.1.** Results are presented in **Chapter 8 (Objectives 3.1 & 3.2)** for individual HPV genotype prevalence, incidence and persistence.

5.4.2 HPV serology assay

HPV serology was tested using a multiplexed binding assay, which uses pseudovirions as antigen and detects HPV type specific IgG antibodies [PsV-Luminex]). Serology was performed for 'carcinogenic/probable carcinogenic' types HPV16/18/31/33/35/39/45/52/56/58/59/68 (but not HR-HPV type 51) and the 'possible carcinogenic' or low-risk types HPV6/11/73 at Karolinska Institute, Stockholm, Sweden [381]. Serum samples were analysed in a 1:50 and 1:150 dilutions. Cut-off values to define seropositivity were calculated independently for each HPV type by analysing the mean fluorescence intensity unit (MFI) values obtained from 100 Swedish children's sera (≤12 years old).

The cut-off algorithm was as recommended by the global HPV LabNet (mean MFI value of a negative control serum panel plus 3 standard deviations) [382]. If this cut-off value was unreasonably low (less than 400 MFI), 400 MFI was used as cut-off to have sensitivity and specificity similar to classical ELISA [383].

5.4.3 HPV serology definitions

HPV serology results are presented as binary results (positive and negative for a given type) based on the cut-off estimated using negative panel (minimum 400 MFI). HPV seroprevalence rates are presented in **Chapter 9 (Objective 3.4)**.

HPV type-specific seroincidence was defined as being HPV-type seronegative at baseline and becoming same type seropositive at endline, irrespective of the DNA status at either baseline or endline.

A more conservative definition was applied considering the HPV DNA status at baseline as evidence of past infection. HPV type-specific seroconversion was defined as being HPV DNA positive and same type seronegative at baseline, and becoming same type seropositive at endline, irrespective of the DNA status at endline. Results are presented in **Chapter 9 (Objective 3.5)**.

5.4.4 Statistical analyses

HPV genotype-specific DNA (Objectives 3.1-3.3)

For associations of prevalent HPV genotypes with CIN2+ prevalence at enrolment, and persistent HPV genotypes with CIN2+ incidence over follow-up, logistic regression was used to estimate odds ratios (ORs) and 95% CI. Results are presented in **Chapter 8 (Objective 3.2)**.

To explore associations of HR-HPV vaccine types (HPV16/18, nonavalent types, and non-vaccine types) with HIV-related factors, associations were estimated with PRs obtained from logistic regression using marginal standardization to estimate PRs, and the delta method to estimate 95% CI. Results are presented in **Chapter 8 (Objective 3.3)**.

HPV Serology (Objectives 3.4-3.6)

Associations between HPV seroconversion and exposure variables were estimated with generalised estimating equation to account for seropositivity by multiple HPV types [380]. To explore associations of HPV seroconversion with HIV-related factors, pre-specified analyses included stratification by ART duration (≤ or >2 years), HIV-1 viral suppression (≤ or >40 copies/ml) and CD4+ cell counts (Model 1). A second logistic regression model (Model 2) incorporated endline CD4+ cell count to Model 1 to explore associations with ART. Results are presented in **Chapter 9 (Objective 3.5)**. For associations of baseline seropositivity with HPV DNA incidence over follow-up, logistic regression was used to estimate ORs and 95% CI. Results are presented in **Chapter 9; Objective 3.6)**.

5.5 Study 3: Association of DNA methylation with CIN2+

Study 3 used cervical samples collected using a Digene cervical sampler (Qiagen, Courtaboeuf, France) at enrolment and using the *care*HPV cervical sampler (Qiagen, Gaithesburg, MD) at month 18 follow-up visits, as previously described (**section 5.2.5 Specimen collection**).

5.5.1 Study design

Study 3 is a set of two case-control studies nested within the HARP study using cervical samples with matched genotyping and histological endpoints. Case-control study 1 was retrospective (**Figure 5.3**) and used samples collected at baseline to compare DNA methylation between CIN2+ cases and ≤CIN1 controls at baseline. Case-control study 2 was prospective (**Figure 5.4 and Figure 5.5**) and compared i.) the DNA methylation between incident CIN2/3 and ≤CIN1 controls at endline, and ii.) the change in methylation levels between baseline and endline among women with incident CIN2/3 and women who remained ≤CIN1 .

Cases and control were primarily selected for testing by the *EPB41L3* human gene methylation assay (i.e. irrespective of whether they were HR-HPV positive), and a subsample was tested by the HPV16 methylation assay among those that were HPV16 positive at baseline.

Case-control Study 1 (Retrospective design) ***(Figure 5.3)***

Definition of cases

All cases of histologically confirmed CIN2+ detected at baseline (prevalent) were included in the case-control study 1, matched 1:1 with ≤CIN1 controls, as detailed in **Figure 5.3**.

Definition of controls

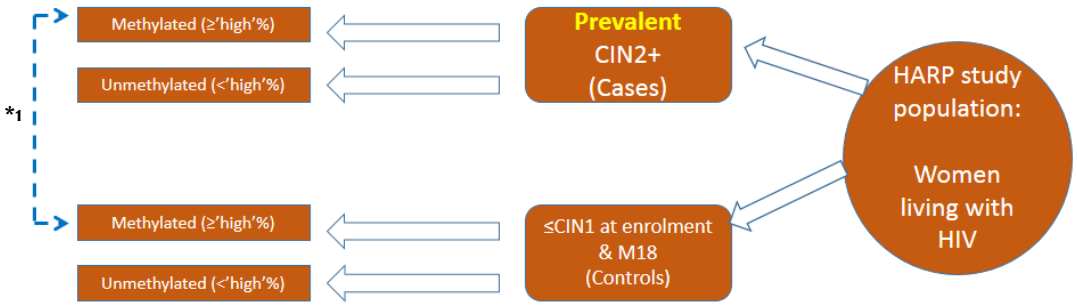
Controls were defined as ≤CIN1 at baseline with a corresponding histological result at endline, irrespective of whether they developed CIN2/3 or remained ≤CIN1 at endline.

Controls were to be matched 1:1 to cases, according to:

1) Age group (<35 years and ≥35 years), as methylation changes can occur during healthy aging as well as during disease development, and aberrant methylation changes during aging could underlie the development of cancer [384];
2) Country of recruitment due to potential genetic differences and different environmental exposures in the two populations.

Controls were randomly selected from among HARP participants who matched age and site requirements and had histological diagnoses at both time points.

Figure 5.3. Retrospective Case-control Study 1 design



*1:Comparison of 'High' methylation (exposure) between prevalent CIN2+ cases and ≤CIN1 controls

Case-control Study 2 (Prospective design) **(Figure 5.4 and Figure 5.5)**

Definition of cases

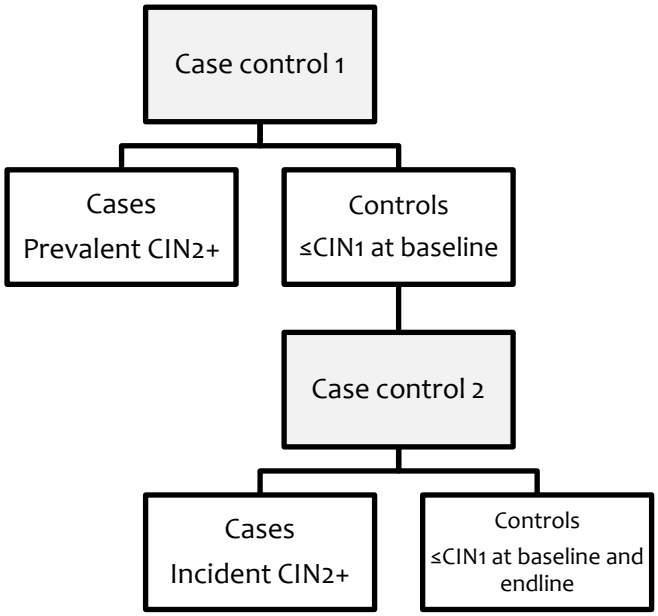
All participants with ≤CIN1 in Case-control Study 1 were followed up at endline visit. Cases for Case-control Study 2 were defined as all histologically-confirmed incident CIN2/3 detected at endline.

Definition of controls

Controls were sampled from the population still at risk at the end of the study and were defined as ≤CIN1 at both baseline and endline, confirmed histologically at both time points.

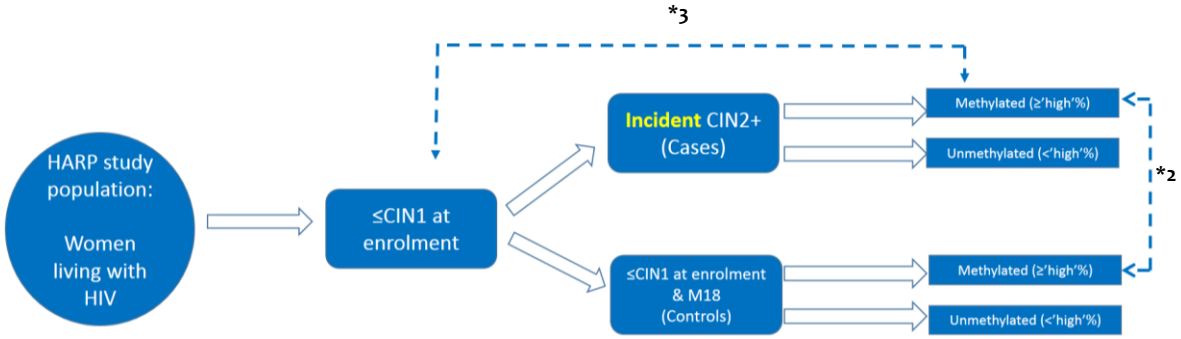
Controls were to be matched 1:1 to cases according to age and country of recruitment, as described for Case-control Study 1.

Figure 5.4. Case-control study 1 and 2 flowchart



Case-control study 2 compared i.) the DNA methylation between incident CIN2/3 and ≤CIN1 controls at endline, and ii.) the change in methylation levels between baseline and endline among women with incident CIN2/3 and women who remained ≤CIN1 .

Figure 5.5. Prospective Case-control Study 2 design†



†Figure depicts cases of incident CIN2/3, but same applies for CIN2/3 persistence, progression and regression *2:Comparison of 'High' methylation (exposure) between incident CIN2+ cases and ≤CIN1 controls at endline; *3: Comparison of baseline and endline methylation levels of incident CIN2+ cases to observe change over time

EPB41L3 DNA methylation was also performed among women with CIN2+ included in Case-Control 1 and with an histological outcome at endline, and included the following outcomes: persistent

CIN2/3 and spontaneous regression to ≤CIN1 among those not treated. However, given the small number of cases, these analyses were considered to be exploratory.

HPV16 DNA methylation analysis was not performed on longitudinal outcomes given the small number of incident HPV16-positive CIN2/3 outcomes.

5.5.2 Sample size

Sample size estimates were established for Case-control Study 1 using prevalent cases, given the higher number of cases. Methylation levels for EPB41L3 were dichotomized into 'high' and 'low' methylation levels using the second tertile (66.7 percentile point) based on the distribution in the control as a cut-point to define 'high' methylation, as previously used in other studies [385, 386]. To answer objective 3.1 (To determine the association of DNA methylation of a human gene *EPB41L3* with CIN2+ prevalence), the study would have 95% power to observe 'high' methylation in at least 67% of prevalent CIN2+ cases (n=159) compared to 33% in ≤CIN1 controls (n=159), using the second tertile as a cut-point and a 1:1 ratio of cases: controls, as summarised in **Table 5.4.**

Table 5.4 Power calculation to detect difference in 'high' methylation levels (exposure) between ≤CIN1 controls (n=159) and prevalent CIN2+ cases (n=159)

Cut-point (percentile point)	% exposed ≤CIN1	% exposed prevalent CIN2+	Power
66.7	33.3%	50%	>90%
		60%	>95%
		70%	>95%

5.5.3 DNA isolation, bisulfite conversion and methylation assay

The methylation assays for *EPB41L3* (CpG sites: 438, 427 and 425) and HPV16 L1 region (CpG sites: 6389 and 6367) were performed using DNA isolation, bisulfite conversion and quantification of methylation using pyrosequencing (PSQ) assays in single separate reactions [387].

Sodium bisulfite deaminates cytosine residues on single stranded DNA molecules and converts them to uracils, whereas 5-methyl cytosines (C) remain protected from conversion. When bisulfite modified DNA is subjected to PCR, the uracil residues are converted to thymidines (T) by DNA polymerase in the amplified products. The ratio of C/C+T indicates the proportion of methylated cytosines at each C in the assayed sample. Pyrosequencing provides an average estimate of methylation levels at a specific site by providing % incorporation of C (methylated) vs. T (unmethylated) in bisulfite treated DNA [388].

Cervical samples collected at the baseline using the Digene sampling brush and collection medium, and endline visit using the *care*HPV sampling brush and collection medium were used for the DNA methylation assays. After collection, the brush was stirred into the collection medium, the cell collection was homogenised by vortexing and divided into four 0.25-ml aliquots. DNA was extracted from one of the aliquots with the QIAamp DNA Mini Kit (Qiagen Inc, Hilden, Germany). Two hundred and fifty nanograms of DNA was used in the bisulfite conversion reactions, where unmethylated cytosines were converted to uracil with the EZ DNA methylation kit (Zymo research, Irvine, USA). Converted DNA was amplified by methylation-independent PCR primers and the amplicons were tested by pyrosequencing for DNA methylation of *EPB41L3* and the late (L1) region of HPV16.

Briefly, 12.5 µl PCR master mix, 2.5 µl Coral red, 1 µl primer-mix, 2 µl DNA and optimised amount of $MgCl_2$ were adjusted with water to give a final 25 µl reaction. The final concentration of each primer for *EPB41L3* and HPV16 assays was 0.2 mM. All the assays were run with thermal cycling conditions: 95°C for 15 min, optimised number of cycles: 30 s at 94°C; 30 s at the optimised annealing temperature; 30 s at 72°C and a final extension varied between 5 and 10 min at 72°C. In each run, a non-template negative control was run in addition to a standard curve consisting of 1 pg/ml of 0, 50 and 100% methylated HPV plasmid in a background of 10 ng/ml human DNA. The amplified DNA was confirmed on QIAxel capillary electrophoresis instrument (Qiagen). 10 µl of

PCR product was pyrosequenced using a PyroMark™Q96 ID (Qiagen) instrument [387]. Percentage methylation was taken from the singlicate result.

5.5.4 Statistical analyses

A description of the statistical analyses used for Study 3 are provided in **Chapter 10, section 10.2.3**.

5.6 Data Management

Type of data:

This study will involve the following types of data:

1) The use of secondary quantitative longitudinal data collected and recorded during participant visits to the local clinics in Burkina Faso and South Africa as part of the *HARP* study and stored in the *HARP* database using Stata version 14 at the LSHTM. Endpoint data (histological measurements), in addition to data on HPV genotyping data, socio-demographic, behavioural and HIV related factors, such as ART status, ART duration, CD4+ count and HIV-1 PVL were used during analysis.
2) The generation of new quantitative data using results of HPV serology assays and DNA methylation assays on samples previously collected as part of the HARP study.

Format of data:

As part of the HARP study, socio-demographic and behavioural, HPV genotyping, HIV-related and histological endpoint data have been collected using Case Report Forms (CRFs) at the local sites in Burkina Faso and South Africa and double entered using Stata version 14 and validated locally before being sent to the central database at LSHTM. The Stata file was stored on a shared, password-protected folder on the LSHTM main server, which was backed up on a daily basis. The HPV serology and DNA methylation data were received in Excel format and added to the HARP database by HK.

Data quality and standards:

Standard operating procedures (SOPs) for data management procedures were prepared in English and French for the HARP study for use at the local sites. All CRFs were checked for missing data and other errors and quality control of 10% of all source documents and records are performed on a weekly basis at local sites for HARP study documents. A senior data manager managed central coordination at LSHTM. Overall monitoring of the quality of the data collection processes across the sites was co-ordinated by the LSHTM statistician and myself (HK), as the *HARP* international study coordinator. Similar methods were used during the collection and validation of the serology and DNA methylation data.

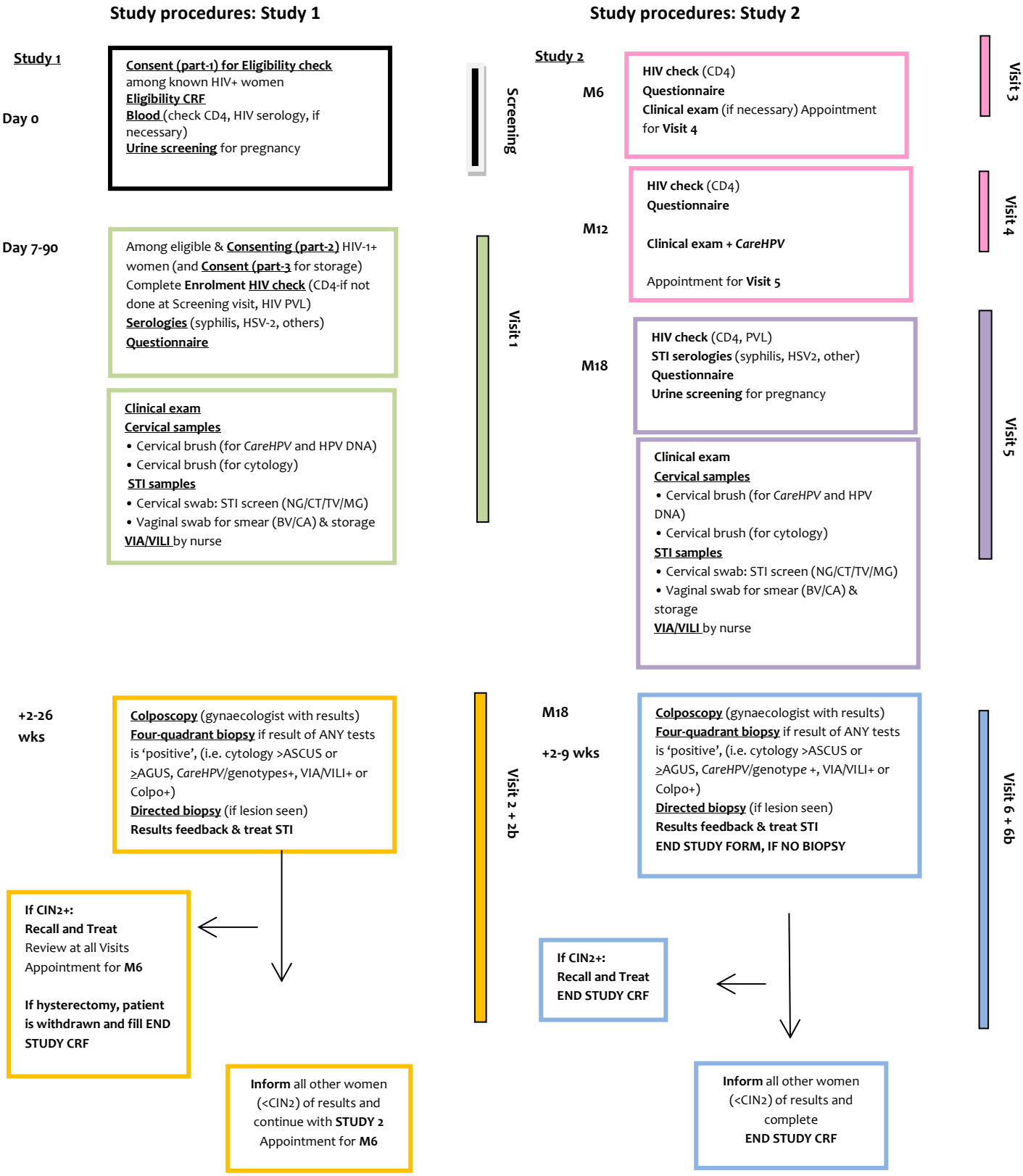
Metadata standards and data documentation:

The HARP study had an associated predefined Data Management Plan, including a series of SOPs describing procedures for data entry, CRF completion and Quality Control (QC), and a statistical analysis plan. All HARP related data was coded and annotated CRFs and a data dictionary provides traceability to all codes and variables. The data dictionary was password protected. HPV serology and methylation data were added as continuous variables to the database.

Formal information/data security standards:

Data and specimens collected from participants were labelled with the corresponding unique pre-printed bar-coded numbers and were linked to the research data but not to names or other individually-identifying information. All study data were kept in a secure location in accordance with the terms of the Data Protection Act 1998. The laboratory testing was conducted under GLP (Good Laboratory Practice), including security of data and specimens, in a masked fashion. All test results were treated as confidential medical records according to legal requirements. Access to the data was only available to the principal investigators and the study coordinators. In both countries, signed consent forms, bearing identifiers of the participants, were kept separately from the other study data to preserve anonymity and confidentiality. HARP data was double-entered locally and saved daily on password protected computers.

Figure 5.6. HARP study visits and procedures



6 STUDY PARTICIPANTS DESCRIPTION AND RISK FACTORS FOR HR-HPV AND CIN2+

In this Chapter, I will firstly provide a summary of the study population characteristics at baseline including HR-HPV and CIN2+ prevalence, by country. I will summarise the socio-demographic, behavioural and HIV-related factors known to be associated with HR-HPV infection and CIN2+, as summarised in Chapter 2, section 2.7 (according to Conceptual framework in ***Figure 2.7****). I will also provide a summary of HIV-related factors (ART status and CD4+ cell count) at endline to reflect changes in immune reconstitution or immunosuppression over time.*

Finally, I will investigate the association of socio-demographic and behavioural factors with the prevalence of HR-HPV and CIN2+ at baseline in multivariate risk factor analyses. I will then extend the multivariate risk factor analyses to explore the association of baseline risk factors for longitudinal HR-HPV outcomes (HR-HPV incidence, persistence and clearance) and CIN2+ incidence.

I will then discuss these findings in the context of what is already known of these risk factors, before evaluating the effect of HIV-related factors on HR-HPV and CIN2+ outcomes in ***Chapter 7.***

6.1 Objectives

In a cohort of WLHIV in Burkina Faso and South Africa, at enrolment:

- To describe the prevalence of HR-HPV infection and CIN2+; ***(Objectives 2.1 & 2.2)***
- To investigate the associations of HR-HPV and CIN2+ prevalence with socio-demographic and sexual behaviour factors, and other STIs. ***(Objective 2.3)***

In a cohort of WLHIV with ≤CIN1 at enrolment and followed over 18 months:

- To describe the incidence and persistence of HR-HPV, and incidence of CIN2+; ***(Objectives 2.1 & 2.2)***
- To investigate the associations of HR-HPV incidence and persistence, and CIN2+ incidence with socio- demographic and sexual behaviour factors, and other STIs. ***(Objective 2.3)***

In a cohort of WLHIV with CIN2+ at enrolment and followed over 18 months:

- To describe the recurrence of CIN2+ among those who were treated for CIN2+ at baseline, and persistence and regression to ≤CIN1 among those not treated for CIN2+.

6.2 Statistical Methods

A full description of the HARP enrolment procedures is provided in **Chapter 5, section 5.2**. Specific statistical methods for univariate and multivariate risk factor analysis are detailed here.

Univariate and multivariate risk factor analyses were performed for HR-HPV and CIN2+ prevalence, using all known risk factors for HR-HPV and CIN2+, as presented in (**Figure 2.7, Chapter 2**). The frequencies of these risk factors are summarised, by country, in **Table 6.2** of this chapter**.**

As prevalence measures do not allow assessment of the temporal associations between a risk factor and an outcome, I extended the risk factor analyses to explore the baseline risk factors (and the endline status of ART and CD4+ cell count as a measure of immune reconstitution or immunosuppression over time) associated with longitudinal HR-HPV and CIN2+ outcomes. The definitions used for each of the HR-HPV outcomes according to the baseline and endline status are summarised in **Table 5.2,** and include:

- Incidence of any HR-HPV type among all women at risk of acquiring a HR-HPV infection (outcome 3 of **Table 5.2**).
- Type-specific HR-HPV persistence among women who were HR-HPV positive at baseline (outcome 5 of **Table 5.2**).
- Clearance of all HR-HPV types among women who were HR-HPV positive at baseline (complete clearance; outcome 7 of **Table 5.2**).
- Incidence of CIN2+ among women with ≤CIN1 at baseline (defined **Chapter 5, Table 5.3**)

Risk factor analysis was not performed for type-specific clearance as this is the opposite of type-specific persistence (**Table 5.2**). Instead, risk factor analysis was performance for complete clearance of all HR-HPV types. Because known risk factors are specific to each woman, risk factor analyses were performed using a 'per woman analysis', rather than a per infection analysis.

6.2.1 Univariate risk factor analysis

For the univariate analysis of risk factors, risk factors were chosen *a priori* as part of a conceptual framework (**Figure 2.7**) of known risk factors for HPV acquisition, persistence and cervical lesion development and progression. Risk factors were organised into the following categories: socio-demographic, sexual behaviour-related, STI-related, clinical signs and symptoms, hormonal or carcinogenic determinants and HIV-related factors (**Table 6.1**). Socio-demographic and sexual behaviour-related factors were considered important for understanding determinants of HR-HPV incidence, as illustrated in **Figure 6.1.** The presence of other STIs and clinical signs and symptoms were considered to understand the effects of inflammation on HR-HPV persistence and clearance, and the development of precursor lesions, as reviewed in **Chapter 2 (section 2.7)**. The biological determinants included factors linked to carcinogenesis, including smoking, pregnancy and hormonal contraceptive use. HIV-related factors (ART, CD4+ cell count and HIV-1 PVL) were included in the univariate and multivariate risk factor analyses in order to evaluate the independent

association of socio-demographic, behavioural and biological determinants of HR-HPV infection and CIN2+ among WLHIV. An in-depth investigation of the independent associations of HIV-related factors on HR-HPV infection and CIN2+ is provided in **Chapter 7**.

Unadjusted Prevalence Ratios (PR) for each risk factor with each HR-HPV outcome (HR-HPV prevalence, incidence, persistence and clearance) and unadjusted Odds Ratios (OR) for CIN2+ outcomes (CIN2+ prevalence and incidence) were obtained using Stata version 14. PRs or ORs were considered significant if p<0.10 for HR-HPV prevalence and CIN2+ prevalence. Given the wide range of risk factors included in the univariate model and larger number of prevalent outcomes, a p-value of <0.10 was used to identify factors to carry forward in multivariate analysis (MVA) for prevalent outcomes. A higher p-value (p<0.20) was considered for the longitudinal outcomes (HR-HPV prevalence, incidence, persistence and clearance and CIN+ incidence) given the smaller number of cases for each outcome group.

For the longitudinal outcomes, analyses were restricted to women with ≤CIN1 at baseline in order to understand the natural history of HPV infection among women without cervical disease and remove the potential bias among women with prevalent CIN2+ as the majority will be HR-HPV infected, and could have a higher persistence of infection.

The total number of women was used as denominator (as opposed to number of infections). Denominators for each of the individual analyses are summarised in **Appendix 14**.

6.2.2 Multivariate risk factor analysis

Factors considered significant in univariate analysis were adjusted for each other in multivariate analysis, using PR for HR-HPV outcomes and OR for CIN2+ outcomes. Factors that persisted in MVA (with *p<0.10*) were selected for the final model. Final models were specific for each outcome group, and were done separately for each country.

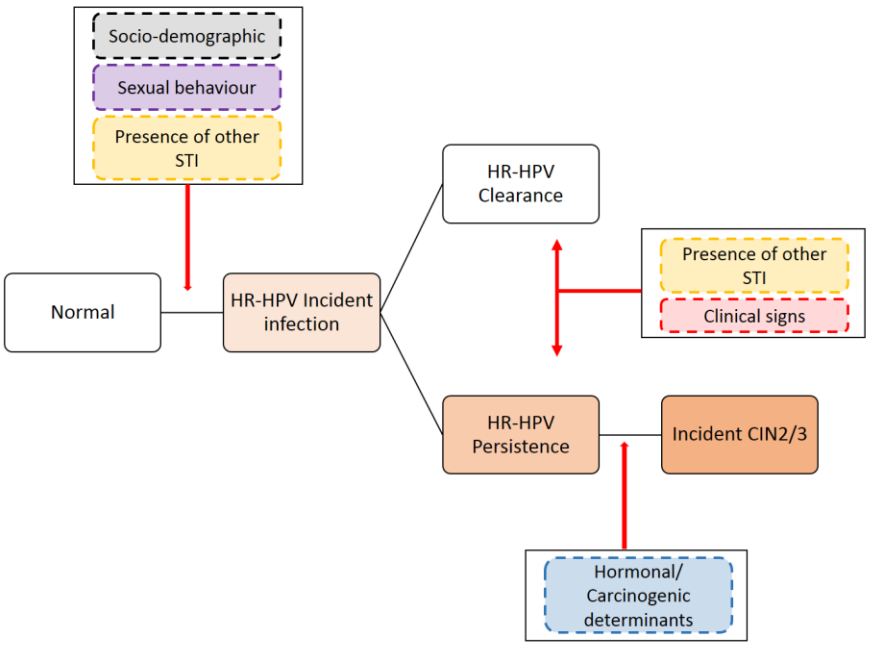
Table 6.1. Known risk factors* for HR-HPV infection and CIN2+

Socio-demographic	Sexual behaviour-related	STI-related	Clinical signs and symptoms	Hormonal/ carcinogenic factors	HIV-related
Age	AFSI	*Neisseria gonorrhoeae*	Genital warts	Smoking	Years since HIV diagnosis
Education	AFP (proxy for above)	*Chlamydia trachomatis*	Cervicitis	AFP	
Marital status	Condom use	*Trichomonas vaginalis*	Cervical ectopy[1]	Parity	ART status
Alcohol use	Regular male sex partner	*Mycoplasma genitalium*	Vulvar lesions	Hormonal contraception	ART duration
	Age of current male sex partner	Bacterial vaginosis	Genital blisters, ulcers or sores		ART adherence
	Number of LTSP	*Candida albicans*	Past history of genital blisters, ulcers or sores		HIV-1 PVL detection
	Number of recent male sex partners	HSV-2 serology			HIV-1 PVL suppression
	HIV status of current male sex partners	Active syphilis serology			CD4+ count at baseline
	Circumcision status of male partner				CD4+ count at endline
	Vaginal cleansing				CD4+ count change during follow-up

*Some of the risk factors included in this table have been shown to associated with HR-HPV and CIN/SIL lesions and ICC, as reviewed in Chapter 2, section 2.7; and 2.8; although not all risk factors included in the table were reviewed as they were less commonly studied (and Chapter 2 focussed on evidence from meta-analyses), these risk factors have been shown to increase acquisition of other STIs, and a priori decision was made to include them in univariate analysis.

AFSI=age at first sexual intercourse; AFP=age at first pregnancy; LTSP=lifetime sex partners; defined as normal, [1]<20% and ≥20% of columnar epithelium on the ectocervix

Figure 6.1. The association of risk factors types with HR-HPV infection and cervical lesion development



6.3 Results 1: Study population description

6.3.1 Study population at baseline

Of 1,473 women screened, 1,238 were enrolled (BF: 615; SA: 623; **Figure 6.2**). In SA, 775 women were screened. Among those screened, 128 failed to present for enrolment within the specified window; and 24 women were ineligible for the study; of whom: 7 were HIV negative; 6 had previous cervical abnormality; 6 were pregnant or planning to become pregnant; 3 were outside the age criteria; 1 was planning to relocate and 1 was not well enough to be enrolled. In BF, 698 women were screened. Among those screened, 62 failed to present for enrolment within the specified window (phone contacts not working, not answering phone call, moved to another town or death); and 21 women were ineligible for the study; of whom: 3 were HIV-2 positive; 11 had previous cervical abnormality; 2 were pregnant or planning to become pregnant and 5 were outside the age criteria.

The median age of participants was 36 (interquartile range [IQR], 31-41) years in BF and 34 (IQR, 30-40) years in SA (**Table 6.2**). Participants in BF had lower levels of education and were less likely to be employed than those in SA. Classical risk factors for HR-HPV and CIN, including smoking, hormonal contraception use and higher number of sexual partners, were more prevalent in SA than BF, as were all STIs, except *Candida* infection. About half (49.6%) of SA participants had ever had a Pap smear, and a fifth (20.8%) of BF participants had ever had a VIA/VILI examination, the primary cervical cancer screening modality in each country, respectively.

At enrolment, 422 (68.6%) participants were on ART in BF and 406 (65.2%) in SA, reflecting the 2:1 stratification ratio. There were 126 women in BF and 22 in SA who initiated ART in the month prior to enrolment. The median duration on ART was 17 (IQR, 0-63) months in BF and 28 (IQR, 10-50) months in SA. The median CD4+ count among ART-naive and ART users was 417 (IQR, 315-606) cells/mm^3 and 446 (IQR, 309-600) cells/mm^3, respectively, in BF; and 448 (IQR, 353-614) cells/mm^3 and 420 (IQR, 279-567) cells/mm^3, respectively, in SA. HIV-1 RNA was undetectable ($\leq$40 copies/ml)

in 69.9% and 33.7% of ART users in BF and SA, respectively, while HIV-1 viral suppression (<1000 copies/ml) was noted for 79.9% and 80.3% among ART users in BF and SA, respectively. Overall, 32 participants (BF: 24; SA: 8; overall 2.6%) denied being on ART at enrolment despite undetectable HIV-1 PVL. Of these, 23 (72%) had CD4+ count >500 cells/mm^3. Given the uncertainty of their status, these women were excluded from the risk factors analyses.

6.3.2 Prevalence of HR-HPV and CIN2+ at baseline

Of the 1,238 participants enrolled, 1215 (98.1%) had valid HPV genotyping results (BF: 96.6%; SA: 99.7%) (**Figure 6.2)**. The prevalence of any HPV type was 75.3% in BF and 88.7% in SA (p<0.001). HR-HPV prevalence was lower in BF than SA (BF: 59.1% vs. SA: 79.1%; p<0.001) (**Table 6.2**).

Overall, 1,128 (91.1%) participants (BF: 90.1%; SA: 92.1%) had valid histology. CIN2+ prevalence was 5.8% (32/554) in BF and 22.5% (129/574) in SA (p<0.001) (**Table 6.2)**.

6.3.3 Study population at endline

Of the 1,077 women without CIN2+ at baseline, 963 (89.4%) were seen at endline visit (median follow-up 16 months, IQR, 15.6-16.8; **Figure 6.2)**. Fifty-three women (10.4%) in BF and 25 (5.5%) in SA initiated ART during follow-up (**Table 6.3**). The median CD4+ count changes among ART users at baseline, ART initiators and those who remained ART-naive were +105, +123 and +65 cells/mm^3 per year respectively in BF, and +5, +83 and -53 cells/mm^3 per year respectively in SA.

Genotyping data at both baseline and endline was available for 922 (95.7%) women (BF: 476; SA: 446) and histology results were available for 809 (84.0%) women (BF: 430; SA: 379)**.**

6.3.4 Cumulative HR-HPV incidence over 16 months

Among 922 women without CIN2+ at baseline and with HPV-genotyping results for baseline and endline (BF: 476; SA: 446), 448 (48.6%) had incident HR-HPV of any type during follow-up: this was similar by site (BF: 47.9%; SA: 49.3%). When restricted to women who were negative for all HR-HPV types at baseline, 169/312 (54.2%) had an incident infection at endline, which was also similar by site

(BF: 55.3%; SA: 51.9%). The total number of incident HR-HPV infections was 350 in BF and 328 in SA (**Table 6.3**).

6.3.5 HR-HPV persistence and complete clearance at endline

Among 610 women without CIN2+ but HR-HPV positive at baseline, the total number of baseline infections was 1028 (BF: 416; SA: 612; **Table 6.3**). Persistent infection of an HR-HPV type at endline was slightly higher in BF (BF: 41.1% vs. SA: 30.2%; p<0.001). Complete clearance of all HR-HPV types was similar in both sites (BF: 24.4% of women; SA: 26.5% of women).

6.3.6 CIN2/3 incidence over 16 months

The incidence of CIN2+ over 16 months was higher in SA (BF: 1.2% [5/430] vs. SA: 5.8% [22/379]; p<0.001; **Table 6.3**).

6.3.7 Outcomes among prevalent CIN2+ detected at baseline

In BF, of 32 CIN2/3 detected at baseline, 28 returned for management of their CIN2/3 lesions before the colposcopy/biopsy visit at endline. In SA, among 97 prevalent CIN2+ cases who returned for endline visit, only 61 had obtained treatment before the endline colposcopy/biopsy visit. Of the 36 participants who did not undergo treatment before the endline colposcopy/biopsy visit, 20 (55.6%) had CIN2/3 detected again at endline (persistent CIN2/3), and 16 (44.4%) had ≤CIN1 (spontaneous regression to ≤CIN1 ; **Figure 6.3**).

6.4 Results 2: Risk factor analysis

The socio-demographic, behavioural and biological determinants found to be associated with each of the HR-HPV and CIN2+ outcomes are summarised in **Table 6.4 and Figure 6.4**. HIV-related factors found to be associated with HR-HPV and CIN2+ are discussed in **Chapter 7**.

6.4.1 Socio-demographic and behavioural factors associated with HR-HPV at baseline

In BF, HR-HPV prevalence was higher among those with occurrence of anogenital warts (AGW) compared to those without (80.0% vs. 57.4%; adjusted Prevalence Ratio [aPR]=1.47, 95%CI: 1.18-1.83, adjusted for all factors that persisted in MVA; **Table 6.5**). Women in BF with a HIV-positive partner were less likely to have HR-HPV compared to women with a HIV-negative partner (53.2% vs. 71.2%; aPR=0.75, 95%CI: 0.58-0.97).

In SA, HR-HPV prevalence was higher among younger age groups (25-29 years vs. 45-50 years: 88.1% vs. 65.3%; aPR=1.28, 95%CI: 1.04-1.58), and among smokers (ever smoked vs. never smoked: 92.1% vs. 76.9%; aPR=1.18, 95%CI: 1.09-1.29; **Table 6.5**).

6.4.2 Socio-demographic and behavioural factors and STIs associated with HR-HPV incidence

Women who ever used condoms were less likely to have a new HR-HPV infection detected at endline compared to women who never used condoms, in both countries (in BF: sometimes vs. never: 40.7% vs. 59.6%; aPR=0.66, 95%CI: 0.45-0.97, in SA: always vs. never: 46.8% vs. 72.0%; aPR=0.63, 95%CI: 0.46-0.86; **Table 6.6**).

In SA, women were more likely to have HR-HPV incidence if any of the following were detected at baseline; bacterial vaginosis (presence vs. absence: 54.1% vs. 45.4%; aPR=1.26, 95%CI: 1.02-1.56) and *Chlamydia trachomatis* (positive vs. negative: 73.7% vs. 48.2%; aPR=1.63, 95%CI: 1.22-2.19) and was marginally associated with *Trichomonas vaginalis* (positive vs. negative: 57.8% vs. 47.7%; aPR=1.30, 95%CI: 1.00-1.68). But women who ever performed vaginal cleansing were less likely to have HR-HPV incidence (ever vs. never: 45.5% vs. 52.2%; aPR=0.76, 95%CI: 0.61-0.97).

6.4.3 Socio-demographic and behavioural factors associated with HR-HPV persistence

In BF, HR-HPV persistence was associated with frequent alcohol use compared to women who never drink alcohol (80.0% vs. 52.2%, aPR=1.45, 95%CI: 1.11-1.91), and marginally associated with cervicitis at baseline (62.4% vs. 48.0% without cervicitis; aPR=1.25, 95%CI: 1.00-1.57; **Table 6.7**).

In SA, HR-HPV persistence was associated with evidence of genital blisters, ulcers or sores at baseline (60.9% vs. 43.4%; aPR=1.43, 95%CI: 1.01-2.03) and with AGW occurrence at baseline (68.4% vs. 43.2%, aPR=1.61, 95%CI: 1.16-2.24).

6.4.4 Socio-demographic and behavioural factors associated with HR-HPV complete clearance

In BF, clearance of all HR-HPV infection was less likely among women with cervicitis at baseline (9.4% vs. 30.9% without cervicitis; aPR=0.06, 95%CI: 0.03-0.15; **Table 6.8**Error! Reference source not found.**)**. Women with cervical ectopy at baseline were also less likely to have clearance of all HR-HPV types (<20% ectopy vs. normal: 11.1% vs. 29.0%; aPR=0.30, 95%CI: 0.23-0.40; ≥20% ectopy vs. normal: 9.7% vs. 29.0%; aPR=0.31, 95%CI: 0.24-0.41). While women who sometimes used condoms were more likely to have complete HR-HPV clearance compared to women who never used condoms (36.7% vs. 15.2%; aPR=2.76, 95%CI: 1.11-6.86).

In SA, complete clearance of HR-HPV was higher among women who were divorced, separated or widowed compared to women who were never married (42.1% vs. 23.4%; aPR=2.12, 95%CI: 1.23-3.66) but was lower among women with a higher number of lifetime sex partners (≥2 vs. 1 LTSP: 25.8% vs. 55.6%; aPR=0.41, 95%CI: 0.24-0.72). Complete clearance was also lower among women with older age at first pregnancy (≥25 years vs. <17 years: 13.2% vs. 40.5%; aPR=0.27, 95%CI: 0.12-0.63).

6.4.5 Socio-demographic and behavioural factors associated with CIN2+ prevalence and incidence over 16 months

In BF, CIN2+ prevalence was higher among older age groups (<35 years vs. ≥35 years: 2.8% vs. 8.3%; aOR=3.47, 95%CI: 1.34-9.01), among women with bacterial vaginosis (presence vs. absence: 9.6% vs. 3.9%; aOR=2.78, 95%CI: 1.25-6.20**; Table 6.9**), AGW occurrence (12.5% vs. 5.5%; aOR=3.61, 95%CI: 1.17-11.18) and cervical ectopy (≥20% ectopy vs. normal: 16.7% vs. 3.8%; aOR=7.42, 95%CI: 2.90-18.95). CIN2+ incidence was also higher among women with cervical ectopy at baseline (≥20% ectopy vs. normal: 4.9% vs. 0.7%; aOR=16.18, 95%CI: 1.59-164.12**; Table 6.10**).

In SA, CIN2+ prevalence was associated with current injectable contraceptive use compared to never users (38.2% vs. 18.6%; aOR=2.21, 95%CI: 1.19-4.10), and with cervicitis (35.6% vs. 19.7% without cervicitis; aOR=2.00, 95%CI: 1.19-3.35; **Table 6.9**). Women who reported having a partner who was circumcised had lower incidence of CIN2+ compared to women whose partner was uncircumcised (4.0% vs. 10.6%; aOR=0.36, 95%CI: 0.13-0.99; **Table 6.10**).

6.5 Discussion

This study found a high prevalence and persistence of HR-HPV among women living with HIV-1 in Burkina Faso and South Africa. However, the prevalence and incidence of CIN2+ were higher in South Africa. This was despite similar distribution of ART use, similar median duration on ART and median CD4+ cell counts at study enrolment in both countries, and similar HR-HPV persistence rates over 16 months. The higher CIN2+ prevalence and incidence in South Africa may be explained by the higher prevalence of HR-HPV in SA, and by other cofactors for HR-HPV infection and CIN2+, as well as HIV-related factors, which differed in both countries. Because women in the two countries differed with respect to socio-demographic and behavioural factors, this justified separate risk factor analyses for each country.

Co-infection with other STI, and associated inflammation, appeared to be consistently associated with HR-HPV and CIN2+ outcomes in both countries. Women in SA had a higher prevalence of other STIs which were found to be associated with HR-HPV incidence. By contrast, women in BF were more likely to have cervicitis detected during the clinical exam, and this was found to associated with HR-HPV persistence and CIN2+ prevalence. Both countries had a high prevalence of bacterial vaginosis, which was found to increase acquisition of HR-HPV among women in SA, and with CIN2+ prevalence in BF. Other co-factors known to be associated with cervical lesion progression, such as smoking and injectable contraception, were more frequently reported among women in SA, and were found to be associated with HR-HPV and CIN2+ prevalence, respectively. The risk factors

found to be associated with each of the HR-HPV and CIN2+ outcomes are illustrated in **Figure 6.4**, and summarised in **Table 6.4**, and further discussed here.

Co-infection with other sexually transmitted infections (STIs)

In BF, the factors found to be associated with HR-HPV infection and CIN2+ were related to the occurrence of AGW, cervicitis and cervical ectopy, all of which are either a consequence of HPV infection, or a co-infection with other STIs.

Women with AGW occurrence had a higher prevalence of both HR-HPV and CIN2+ in BF. A higher risk of anogenital cancers has been reported in studies among both women and men with a history of AGW. A prospective study among 10,971 patients diagnosed with AGW in Sweden and followed for a median of 13 years found that AGW occurrence was associated with an increased incidence of vulvar and vaginal cancers and cervical carcinoma among women, and penile cancer among men [389]. Furthermore, in a large prospective study which followed 50,000 Danish women and men who were diagnosed with AGW from 1978 to 2008 [390], an increased risk of ICC was reported among women with AGW diagnosis compared to the general population (SIR=1.5, 95%CI: 1.3-1.8). However, many of these studies have not directly measured LR-HPV types 6 and 11 in the biopsy tissue, and among those that did, the prevalence of single HPV6/11 infections was rare, or co-existed with a HR-HPV type [391]. It has been suggested that, rather than LR-HPV types having a direct role on cervical lesion development, this association may be due to i.) a shared exposure of LR and HR-HPV types; and ii.) impaired immune response which facilitates AGW, HR-HPV persistence and cervical lesion development [391]. The findings in this chapter show that AGW occurrence was also associated with HR-HPV persistence in SA, and these associations were independent of ART, CD4+ cell count and HIV-1 PVL. However, the association of AGW with CIN2+ was not adjusted for HR-HPV infection, and could be a function of co-infection. In both countries, all prevalent CIN2+ cases with AGW occurrence were also infected with HR-HPV types, making it

unlikely that AGW occurrence, or HPV6/11 which cause AGW, have a direct role in CIN2+ development.

Women with cervicitis were at increased risk of HR-HPV persistence, and a decreased likelihood of full clearance of HR-HPV in BF. The association of cervicitis with cytological high-grade cervical lesions has been previously reported among HR-HPV infected women [392]. Genital *Chlamydia trachomatis* (CT) can cause chronic cervicitis and pelvic inflammatory disease (PID). CT infection has been shown to facilitate the entry and persistence of multiple HR-HPV types [145, 146] and has been linked to the disruption of the immune response required to clear the virus [147]. *Chlamydia trachomatis* may be linked to the inflammatory cytokine responses during infection which may lead to reactive oxidative metabolite production, causing DNA damage or modification, resulting in genetic instability, particularly if infection persists [93, 147, 148]. Furthermore, in vitro data show that CT may inhibit cell apoptosis, linked to carcinogenesis [149].

Cervical ectopy, or the presence of columnar epithelium on the ectovervix, was associated with a decreased likelihood of complete HR-HPV clearance, and with an increased risk of CIN2+ prevalence and incidence in BF. Associations between cervical ectopy and CT, HPV and HIV have been reported [393] but not with ICC [394], although ectopy represents a region of cell transformation associated with squamous metaplasia, which could lead to abnormal transformation in the presence of HR-HPV types [40]. While cervical ectopy was not observed in as many women in SA, this may be observer-dependent during clinical exam.

The presence of STIs such as CT and *Trichomonas vaginalis* (TV), and vaginal flora changes, such as bacterial vaginosis (BV), was associated with increased risk of HR-HPV incidence among WLHIV in SA, while condom use (which is meant to be protective against STIs) was associated with a decreased risk. The presence of genital infections may disrupt the mucosal epithelial barrier through inflammation and micro-ulcerations, thereby facilitating entry of the HPV virus [139]. Researchers in Tanzania have shown an increased risk of HR-HPV acquisition among 324 women

positive for TV (OR=4.1, 95%CI: 1.7-9.8, adjusted for presence of BV and clinical PID) [87]. In a meta-analysis which included 12 studies among 6,372 women [88], BV was associated with a 1.4-fold (OR=1.43, 95%CI: 1.11-1.84) increased prevalence of cervical HPV. Bacterial vaginosis is associated with changes in the physiochemical and immunological environment of the vagina; resulting in the loss of hydrogen peroxide-producing lactobacilli which have a role in defence mechanisms, and changes in the production of cytokines such as IL-1β and IL-10 [162], which could facilitate the acquisition and persistence of other STIs, including HPV [88].

Bacterial vaginosis was also associated with an increase in CIN2+ prevalence among women in BF. This is similar to the findings of a meta-analysis which evaluated the association of bacterial vaginosis with cervical precursor lesions (cytological [LSIL, HSIL], or histological [CIN1, CIN2 or CIN3]) including over 10,000 women: the study found a 1.5-fold increase in SIL (LSIL/HSIL) or CIN (CIN1-3) prevalence (combined cytology/histology outcome: OR=1.51, 95%CI: 1.24-1.83) among women positive for bacterial vaginosis [161]. Biologically, this is plausible: as well as facilitating acquisition of other STIs, anaerobes found in the vaginal flora of women with BV can release volatile amines which may form carcinogenic compounds such as nitrosamines in combination with nitrates, produced by nitrate reducing bacteria [163]. The local accumulation of nitrosamines during BV episodes may induce cervical epithelial cell transformation in the presence of oncogenic HPV infection [152-155]. Furthermore, the disrupted vaginal environment induced by bacterial vaginosis is associated with alterations in the inflammatory cytokine profile which could promote cervical lesion development [162].

Infection with herpesvirus (HSV-2) has been reported to be strongly associated with ICC [96]. HSV-2 associated clinical and subclinical recurrences are likely to facilitate HR-HPV acquisition via the production of (mini)ulcerative lesions, and to enhance HR-HPV persistence because of increased inflammation [141, 142]. WLHIV in this study had a strikingly high prevalence of HSV-2 at baseline: nearly all women in SA (95.2%) and three-quarters of women in BF (74.9%) were HSV-2 seropositive,

and this may have precluded finding any associations, due to the small number of women who were HSV-2 negative. Despite this limitation, the high co-infection rate of HR-HPV and HSV-2 suggests there may be some common mechanisms that facilitate this co-existence. Furthermore, the increased risk of HR-HPV persistence among women with genital ulcer, blisters or sores in SA might suggest these women had frequent clinical HSV-2 recurrences, which could facilitate HR-HPV persistence. Genital HSV-2 measures (such as shedding) may have been more appropriate to evaluate associations with HR-HPV infection at the mucosal level. Further studies addressing these interactions are warranted.

The differences in the prevalence of the various STIs among women in BF and SA may in part explain the differences in associations observed; the prevalence was higher for all STIs among women in SA, although the prevalence of BV was similar in both sites.

Interestingly, vaginal cleansing was found to be associated with a decreased risk of HR-HPV incidence, and weakly associated with increased likelihood of complete HR-HPV clearance among WLHIV in SA. Just under half (43%) of women in SA reported vaginal cleansing, while >99% of women in BF reported doing so. Among those who report vaginal cleansing in SA, 93% do so daily, using water (68%) and soap (34%); 76% reported using their fingers. Others have shown that a high frequency of vaginal cleansing, and cleansing using water and soap were significantly associated with bacterial vaginosis. However no direct association between vaginal cleansing and acquisition of HIV or other STIs was found [395]. A prospective study among 503 girls aged 15-16 years seen every 3 months in Mwanza, Tanzania found weak evidence of a lower HPV incidence among girls who reported vaginal cleansing (adjusted Rate Ratio=0.69, 95%CI: 0.43-1.10), similar to this finding among WLHIV in SA [396]. On the one hand, it would be expected that vaginal cleansing may increase the risk of acquisition of other STIs, including HPV, depending on cleansing products used. Furthermore, its association with BV would suggest an inflammatory environment that would facilitate HR-HPV persistence. On the other hand, it could be possible that daily cleansing may have

removed the superficial layer of cells with HPV virus; leading to a reduction in detectable HPV on the day of cervical sampling, although this has never been previously reported in other studies.

Age, HR-HPV and CIN2+

The relationship between age and HR-HPV prevalence among WLHIV in SA is similar to what has been shown worldwide [73]; younger women have higher prevalence of HR-HPV. The peak transmission of HPV infections is typically among young women following sexual debut [76] because HPV is highly prevalent in younger age groups, and is easily transmitted [77]. In BF, older women (≥35 years) had a higher risk of CIN2+ prevalence, and this relationship between age and CIN2+ is similar to what has been shown worldwide. Several studies which have examined the influence of age on the risk of incident CIN2+ have reported that among women with prevalent HPV infections detected at baseline, the risk of HPV persistence and CIN3 incidence during follow-up increases with older age [49, 78-80]. However, the same association was not observed among women in SA and younger women (<35 years) in SA had higher CIN2+ prevalence compared to older women, although this association was not significant in MVA. This finding is similar to a study among 1,371 previously unscreened WLHIV in Cape Town, South Africa, which similarly reported high CIN2+ prevalence among this age group [397]. In South Africa, the incidence of HIV infection is highest among young women aged 15-19 years. It is possible that, depending on the number of years living with HIV, the CD4+ cell count decline in this period and access to ART, that young WLHIV could have a similarly high, or higher, risk of CIN2+ as WLHIV in older age groups. Furthermore, it is unclear whether the CIN2/3 lesions detected at baseline among young women would be more likely to spontaneously regress compared to those among older women, similar to the trend observed between HR-HPV persistence and age. The small number of non-treated CIN2/3 in this study does not allow an accurate assessment of this association, however of the 16 cases of CIN2/3 that remained untreated during the follow-up period and who had spontaneous regression to ≤CIN1 at endline, less than half of them (41%) were <35 years.

Marital status and sexual behaviour

Women who were divorced/separated or widowed in SA were more likely to clear infection compared to women who were never married, but those with a higher number of LTSP were least likely to clear all types, as well as having a higher risk of CIN2/3 incidence. Previous studies have reported that women with fewer (≤5) LTSP had increased likelihood of clearing an HPV infection compared to women with higher (>5) LTSP (aOR=4.1, 95%CI: 1.4-11.5) [398]. The decrease in risk among women with fewer partners may also signify a decreased likelihood of past STI infections which could have facilitated HR-HPV persistence and cervical lesion development.

As well as a lower number of LTSP, women with a male partner who was circumcised in SA had a reduced risk of CIN2/3 incidence, suggesting these women were possibly at lower risk of acquiring a HR-HPV type that could persist and allow cervical lesions to progress. Male circumcision has been shown to decrease the risk of ICC in female partners of circumcised men. Monogamous women whose male partners were circumcised, and who engaged in risky sexual behaviour (i.e. had 6 or more sexual partners, or sex with prostitutes) had a 58% reduction in ICC (aOR=0.42, 95%CI: 0.23-0.79, adjusted for AFSI and number of LTSP) compared to monogamous female partners of uncircumcised men. This association between male partner circumcision and a reduction in CIN2+ incidence is likely to result from an overall reduction in HPV prevalence among men as they are less likely to acquire HPV, and therefore a reduction in transmission of HR-HPV types between partners. Male circumcision has shown to decrease transmission of HPV from HIV-negative men to their female partners [85]. In Rakai, Uganda, 1,245 HIV-negative female partners of HIV-negative men enrolled in two randomised controlled trials of male circumcision had 23% lower HR-HPV incidence over 24 months compared to women in the control group (Incidence Rate Ratio [IRR]=0.77, 95%CI: 0.63-0.93), and increased HR-HPV clearance (Risk Ratio=1.12, 95%CI: 1.02-1.22).

A curious finding in this study is that women in BF who had a HIV-positive male partner had a lower prevalence of HR-HPV at baseline compared to women who had a HIV-negative male partner (53.2%

vs. 71.2%; aPR=0.75, 95%CI: 0.58-0.97), and this persisted even after adjustment for condom use with that partner (aPR=0.74, 95% CI: 0.57-0.96). This finding is contrary to the evidence that HIV seroconcordance is one of the strongest risk factors for HPV concordance between heterosexual couples [399]. Studies of heterosexual couple from Cape Town have shown that HPV concordance (penile-cervical) between couples was significantly higher among HIV-infected couples than among HIV-seronegative couples [400], and women with a high HPV viral load frequently shared HPV types with their male partners [117].

In this study, condom use and HIV status of current partner are all based on self-report, and the possibility of recall bias (in particular for condom use), or social desirability bias (higher reported condom use may seem preferable) cannot be excluded. Another limitation is that we do not know the frequency of sex acts between couples, nor the behaviour of the male partners that might explain why women in HIV-concordant partnership have lower HR-HPV prevalence.

Another unexpected finding was that women with a younger age at first pregnancy in SA were more likely to have complete clearance of HR-HPV. Young age at first pregnancy is a known risk factor for ICC [91], related to cervical ectopy and hormonal changes that occur during pregnancy. Age at first pregnancy (AFP), and age at first sexual intercourse (AFSI) are highly interrelated measures [90] and AFP is often a proxy measure for AFSI. The finding in this study could be linked with age at first exposure to HPV infection. It is possible that some women who reported older AFP may have acquired a HR-HPV infection at an older age which then persisted, and HR-HPV persistent infections become more dominant with older age [78]. However, while a young AFP is suggestive of a young AFSI, an older AFP does not suggest an older AFSI, especially given the high proportion of women who used hormonal contraception in SA. A second explanation relates to the fact that complete clearance was defined as clearance of all HR-HPV types at endline, and did not consider the total number of infections that cleared. The likelihood of clearing a single infection between baseline and endline is different to the likelihood of clearing multiple types. Women who reported a young AFP were less likely to have multiple HR-HPV infections at baseline compared to

women with older AFP (<17 years vs. >25 years: 33.8% vs. 66.0%; p=0.05), and this could explain why women with young AFP had a higher clearance of all types compared to women with older AFP.

It remains uncertain whether AFP is associated with complete clearance of HR-HPV, or whether the association is confounded by some other unmeasured factor. Furthermore, AFSI was included in the univariate analysis and did not persist in MVA. Both AFP and AFSI measures are crude as they are rely on both recall bias, and potentially social desirability bias.

Smoking and Hormonal contraception

Carcinogenic factors, such as smoking and hormonal factors, such as injectable contraception were associated with HR-HPV and CIN2+, respectively. Smoking is a well-established risk factor for HPV infection through modulating effects on local and systemic immunity [401]. It is known to suppress the immune response by reducing the number of Langerhans cells and other immune markers [89], thereby allowing HPV to persist and cervical lesion to develop [86, 104]. Compared to HPV-positive women who never smoked, HPV-positive women who have ever smoked tobacco have a 4.6 (95%CI: 0.9-22.9), 2.2 (95%CI: 1.4-3.4) and 2.2 (95%CI: 1.5-3.2) increased odds of CIN2/3, CIN3 and CIS/ICC, respectively [86, 89, 98-103]. The amount of smoking (number of cigarettes per day) and duration of smoking history were also shown to increase the odds of CIS/ICC [89].

Both oral and injectable contraception have been associated with increased risk of acquisition of HPV and other STI [123-129] by influencing the differentiation and maturation of the cervical epithelium, which can lead to thinning of the mucosal epithelium and microtearing [123, 126-130]. A systematic review and meta-analysis investigating the association of hormonal contraceptive use with ICC included 28 cohort and case-control studies, half of which were from developing countries and included 12,531 women with ICC [92]. Compared with never users of oral contraceptives (OC), women taking OC for durations of <5 years, 5-9 years and ≥10 years had Relative Risk of 0.9 (95%CI: 0.7-1.2), 1.3 (95%CI: 1.0-1.9), and 2.5 (95%CI: 1.6-3.9) among HPV positive women. However a weaker association was observed for injectable contraception (which includes the progesterone-only NET-EN and DMPA) use with ICC.

The results presented in this chapter confirm that known determinants for HR-HPV infection and CIN2+ are similar in WLHIV as in HIV-negative populations, and remain significant after adjustment for HIV-related factors, including ART status, CD4+ cell count and HIV-1 PVL.

6.6 Study limitations

This study was constrained by the limited number of intermediate visits and overall follow-up duration. The definition of cumulative HR-HPV incidence over 16 months is at the same time crude and of limited duration to assess the associations of risk factors with HR-HPV incidence. Other studies have used shorter time intervals for defining HR-HPV incidence [396] because of the transient nature of HR-HPV infection. Furthermore, the study could not rule out type-specific clearance and reinfection when estimating persistence during the 16-months interval between HPV testing. The evaluation of HPV at two time points only did not allow precise estimation of the duration of infections. Longer duration of follow-up would have allowed to accrue a larger number of incident CIN2+ cases, and more robustly assess the association of risk factors on CIN2/3 incidence.

The inclusion of a wide array of risk factors in the univariate analysis, in addition to the separate risk factor analyses for each of the HR-HPV outcomes (prevalence, incidence, persistence and clearance) and CIN2+ outcomes (prevalence and incidence), by country, did not always result in consistent findings. It is possible that the multiple comparisons may have resulted in spurious findings that have arisen by chance, such as the finding that women with a HIV-positive partner in BF were less likely to have HR-HPV at baseline compared to women with a HIV-negative partner, or that younger age at first pregnancy was associated with increased likelihood of HR-HPV clearance in SA.

The use of the baseline status of individual risk factors to predict endline outcomes was considered for all factors, except for CD4+ cell count for which baseline and endline values were used. This strategy was considered appropriate for the majority of factors, such as injectable contraception

use or smoking for which there is a residual risk, even in absence of its use (i.e. termination of use). However, contemporary measures for STIs and HIV-1 PVL may have been more informative to evaluate associations with HR-HPV incidence and persistence and CIN2/3 incidence. The presence of other STIs may present a short-term risk, at the time of infection, which is reduced if treatment is received. Unfortunately, the HARP study did not include repeat STI or HIV-PVL measure at endline. Therefore, it cannot be ruled out that HR-HPV itself may have been the causative agent of cervicitis, which was found to be associated with its persistence and with CIN2+ prevalence in BF.

The differences in the prevalence of some risk factors, such as STIs, hormonal contraception and smoking between the two countries may have led to some inconsistencies in associations, but they reflect the different risk profiles for HR-HPV and CIN2+ in the two populations. However, there was consistency in the strength and direction of associations for STIs and cervicitis with increased risk of HR-HPV and CIN2+ outcomes in both countries, even at different prevalence levels, which underscore the importance of these cofactors in the natural history of HR-HPV.

The small number of CIN2+ cases in BF at baseline, and similarly small number of incident CIN2/3 in BF and SA at endline, did not allow for a meaningful analysis of risk factors associated with CIN2+ prevalence in BF or incidence in either country.

Women who were not biopsied were considered as ≤CIN1. Biopsy decision was based on screen-positive for any test, which included VIA/VILI, cytology, colposcopy or HPV DNA test. There is a potentially greater risk of ascertainment bias in BF as fewer women were biopsied and biopsy decision was largely based on HC-II test positivity. However, given the high sensitivity and NPV of HC-II [377, 402] it is unlikely that many cases would have been missed. In addition, the *HARP* study built a strong review of histological results by consensus of 5 pathologists, which included all CIN1/2 and CIN2+ cases and 10% of ≤CIN1 cases (among women who had been biopsied for at least one positive screening tests).

6.7 Summary of findings

- In the HARP study, the prevalence of HR-HPV at baseline, was 59.1% (351/594) in BF and 79.1% in (491/621) in SA. CIN2+ prevalence was 5.8% in BF and 22.5% in SA.
- At endline, HR-HPV incidence, persistence and complete complete clearance was 47.9%, 51.5% and 24.4% respectively among women in BF and 49.3%, 44.7% and 26.5%, respectively in SA. CIN2+ incidence over 16 months was 1.2% in 405 women in BF and 5.9% in 375 women in SA.
- Condom use was associated with a decreased risk of HR-HPV incidence in both countries
- Among women in SA, the presence of other STIs (*Chlamydia trachomatis, Trichomonas vaginalis*) and BV were associated with a 1.6, 1.3 and 1.3-fold increased risk, respectively of HR-HPV incidence.
- HR-HPV persistence was marginally higher among women with cervicitis in BF (1.3-fold increased risk), and with genital ulcers (1.4 fold increased risk) in SA.
- CIN2+ prevalence was higher among women with bacterial vaginosis in BF (3.5-fold increase), and cervicitis in SA (2-fold increase).
- Older women (≥35 years) and women with large cervical ectopy (≥20%) had higher prevalence and incidence of CIN2+ in BF.
- Injectable contraception was associated with 2.2-fold increased odds of CIN2+ prevalence among WLHIV in SA.

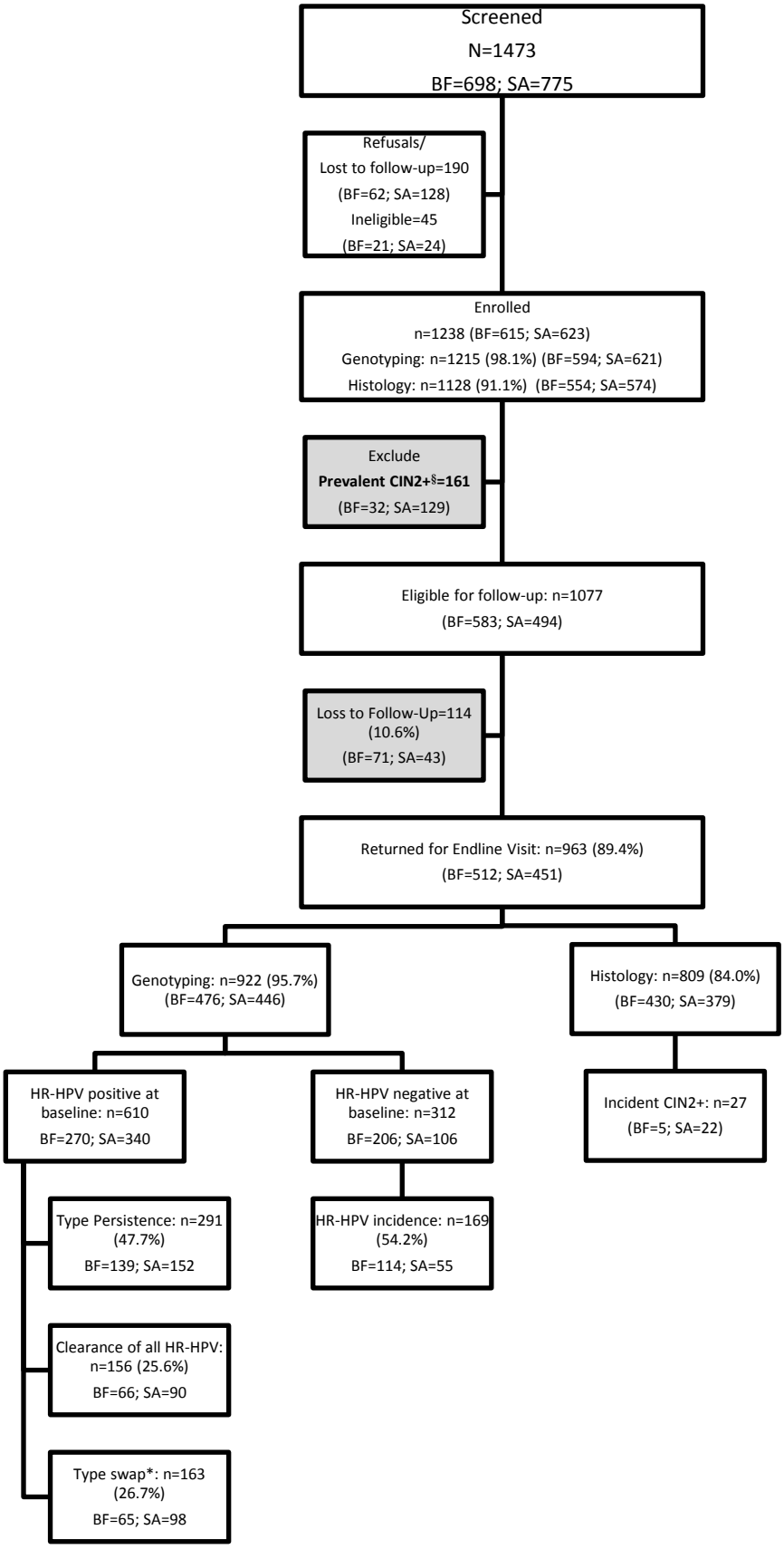
Conclusion: The presence of other STIs increases the risk of HR-HPV incidence and persistence, and may increase the risk of CIN2+ prevalence. Injectable contraception use is common among WLHIV in SA, and increases the risk of CIN2+.

6.8 Findings in context

Studies among HIV-negative women have shown that the presence of *Chlamydia trachomatis, Trichomonas vaginalis* and bacterial vaginosis were associated with an increase in prevalence, incidence and persistence of HR-HPV [87, 88, 145-147], and with ICC [94, 95, 161]. The findings in this study show that these associations are similar among WLHIV. While there are no systematic reviews that shown a similar effect on CIN2/3 lesions, the inflammatory environment associated with STI is likely to facilitate HR-HPV persistence and cervical lesion development.

A systematic review and meta-analysis investigating the association of hormonal contraceptive use with ICC [92] reported that women taking oral contraceptives (OC) had up to 2.2-fold increased risk of ICC, depending on duration of use, but a similar association was not observed for injectable contraception. This is the first study to report an increased risk of CIN2+ among women taking injectable contraception in South Africa. Others have shown no significant association of injectable or oral contraception use with cytology-determined LSIL and HSIL [403]. Given the high frequency of use among WLHIV in SA, the convenience of its use in this population, and the benefits associated with its use (unwanted pregnancy avoidance), further studies are warranted to confirm these findings.

Figure 6.2. HARP study flowchart



*Type swap is defined as clearance of one genotype and acquisition of a different genotype; §Figure 2 for prevalent CIN2+ outcomes at endline

Figure 6.3. Study flowchart among prevalent CIN2+

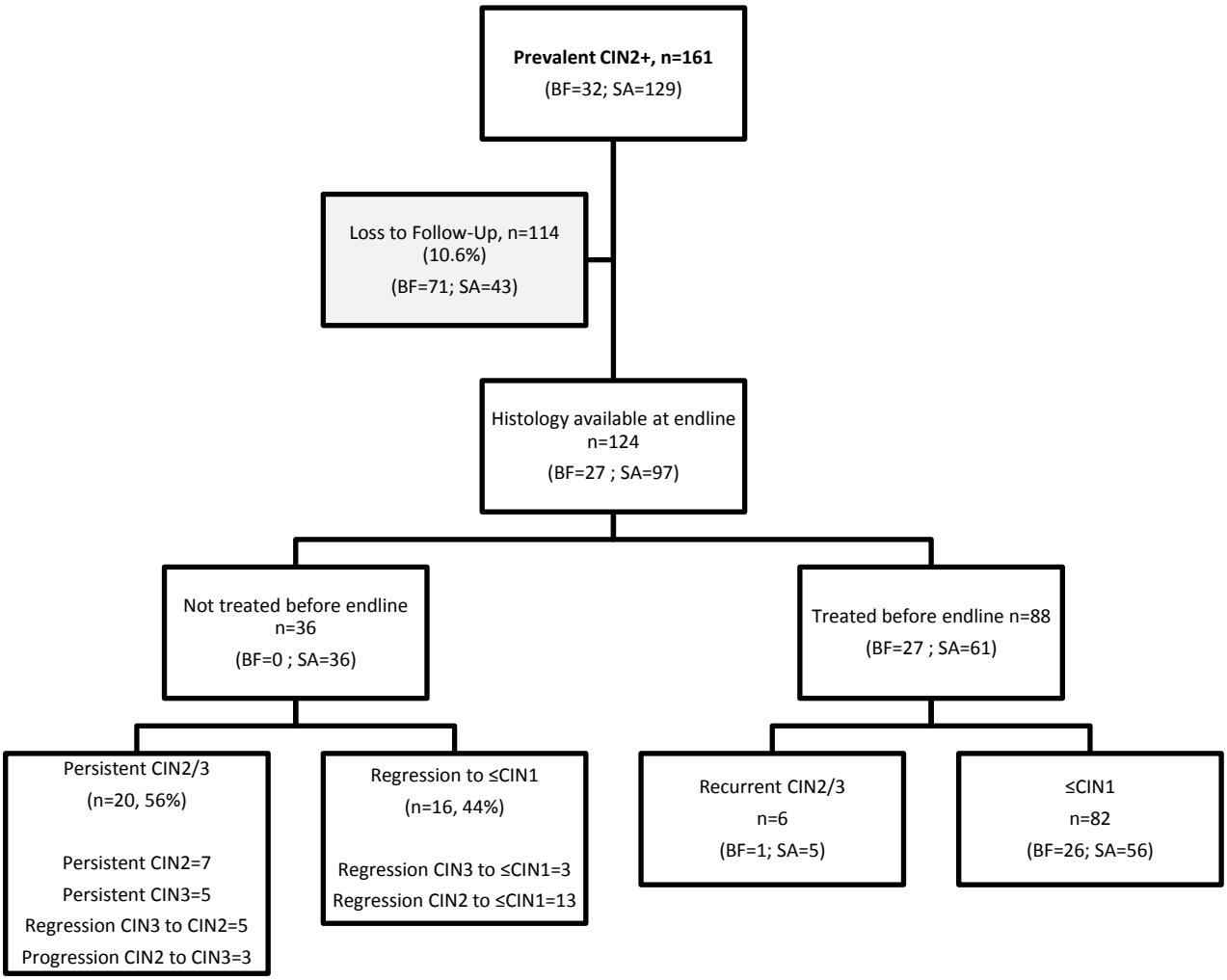


Table 6.2. Study population characteristics at baseline visit

	Burkina Faso N=615		South Africa N=623		p-value
	n or median	(%) or [IQR]	n or median	(%) or [IQR]	
Socio-demographic factors					
Age-years	36	(31, 41)	34	(30, 40)	<0.001
Place of birth					
Burkina Faso	517	(84.1)	-		
Ghana	4	(0.7)	-		
Ivory Coast	81	(13.2)	-		
Mali	4	(0.7)	-		
Niger	0	(0.0)	-		
South Africa	-		450	(72.2)	
Lesotho	-		2	(0.3)	
Mozambique	-		2	(0.3)	
Swaziland	-		3	(0.5)	
Zimbabwe	-		158	(25.4)	
Other	9	(1.5)	8	(1.3)	
Ethnicity					0.32
Black	615	(100.0)	622	(99.8)	
Coloured (Mixed-race)	0	(0.0)	1	(0.2)	
Education					<0.001
No schooling	268	(43.6)	7	(1.1)	
Primary/Incomplete secondary	324	(52.7)	349	(56.0)	
Completed secondary	15	(2.4)	249	(40.0)	
Graduate/postgraduate	7	(1.1)	18	(2.9)	
Not known	1	(0.2)	0	(0.0)	
Currently employed					<0.001
No	538	(87.5)	281	(45.1)	
Yes	77	(12.5)	342	(54.9)	
Marital status					<0.001
Never married	74	(12.0)	318	(51.0)	
Divorced/separated	95	(15.4)	23	(3.7)	
Widowed	143	(23.3)	18	(2.9)	
Married/cohabiting	302	(49.1)	264	(42.4)	
Not known	1	(0.2)	0	(0.0)	
Ever Smoke					<0.001
Never	610	(99.2)	545	(87.5)	
Ever	5	(0.8)	78	(12.5)	
Alcohol use					0.12
Never	409	(66.5)	432	(69.3)	
Sometimes but <Monthly	141	(22.9)	146	(23.4)	
At least monthly	65	(10.6)	45	(7.2)	
Age at first pregnancy-years	20	(18-22)	20	(18-22)	0.12
Number of pregnancies	3	(2-5)	2	(2-3)	<0.001
Number of live births	2	(1-4)	2	(1-3)	<0.001

	Burkina Faso N=615		South Africa N=623		p-value
	n or median	(%) or [IQR]	n or median	(%) or [IQR]	
Ever use contraception	508	(82.9)	600	(96.3)	<0.001
Ever used hormonal contraception					<0.001
No	105	(17.1)	23	(3.7)	
Yes	313	(50.9)	535	(85.9)	
Not known	197	(32.0)	65	(10.4)	
Injectable contraception					<0.001
No	487	(79.2)	173	(27.8)	
Past or current	123	(20.0)	450	(72.2)	
Not known	5	(0.8)	0	(0.0)	
Oral contraception					0.23
No	446	(72.5)	428	(68.7)	
Past or current	165	(26.8)	195	(31.3)	
Not known	4	(0.7)	0	(0.0)	
Condom use					<0.001
Never	62	(10.1)	53	(8.5)	
Ever	448	(72.9)	547	(87.8)	
Not known	105	(17.1)	23	(3.7)	
Sexual behaviour factors					
Age at first sex-years	18	(17, 19)	18	(16, 19)	0.01
Lifetime sex partners					<0.001
1	143	(23.3)	17	(2.7)	
2-4	406	(66.0)	284	(45.6)	
5+	66	(10.7)	228	(36.6)	
Not known	0	(0.0)	94	(15.1)	
Number of male partners in last 3 months					<0.001
0	224	(36.4)	111	(17.8)	
1	374	(60.8)	475	(76.2)	
≥2	17	(2.8)	35	(5.6)	
Not known	0	(0.0)	2	(0.3)	
Current male sex partner					<0.001
No	76	(12.4)	11	(1.8)	
Yes	315	(51.2)	501	(80.4)	
Did not respond	224	(36.4)	111	(17.8)	
Current male sex partner circumcised					
No	0	(0.0)	222	(44.3)	0.01
Yes	10	(3.2)	243	(48.5)	
Not known/No response	305	(96.8)	36	(7.2)	
Current male sex partner HIV seropositive					
No	64	(20.3)	113	(22.6)	0.04
Yes	100	(31.7)	196	(39.1)	
Not known	151	(47.9)	192	(38.3)	

	Burkina Faso N=615		South Africa N=623		p-value
	n or median	(%) or [IQR]	n or median	(%) or [IQR]	
Condom use with current partner					<0.001
Never	72	(22.9)	29	(4.7)	
Less than half the time	66	(21.0)	60	(9.6)	
More than half the time	7	(2.2)	112	(18.0)	
Always	168	(53.3)	299	(48.0)	
Not known	2	(0.6)	1	(0.2)	
Ever cleanse vagina					<0.001
No	3	(0.5)	353	(56.7)	
Yes	242	(39.4)	253	(40.6)	
Sometimes	370	(60.2)	17	(2.7)	
Cervical cancer screening					
Ever had Pap smear					<0.001
No	541	(88.0)	313	(50.2)	
Yes	72	(11.7)	309	(49.6)	
Not known	2	(0.3)	1	(0.2)	
Number of pap smears (IQR)	1	(1-1)	1	(1-2)	0.14
Ever had visual inspection (VIA/VILI)					<0.001
No	487	(79.2)	588	(94.4)	
Yes	128	(20.8)	16	(2.6)	
Don't know	0	(0.0)	19	(3.1)	
Number of visual inspection exams (IQR)	2	(1-3)	1	(1-2)	0.02
HIV-related factors					
Median duration since HIV diagnosis (years)	5	(2-8)	4	(2-7)	0.03
Median age at HIV diagnosis	31	(27-36)	30	(26-35)	
ART status at enrolment*					0.01
ART >2 years	196	(31.9)	227	(36.4)	
ART ≤2 years	226	(36.8)	179	(28.7)	
ART-naïve	193	(31.4)	217	(34.8)	
Median duration on ART (months)	16.6	(0.0-63.4)	28.1	(10.3-49.9)	0.002
Self-reported ART adherence (among ART users)					<0.001
>90%	0	(0.0)	0	(0.0)	
60-90%	387	(91.7)	339	(83.5)	
<60%	10	(2.4)	63	(15.5)	
Not known	25	(5.9)	4	(1.0)	
HIV-1 PVL suppression (<1000 copies/ml) among ART users					<0.001
No	56	(13.3)	76	(18.7)	
Yes	337	(79.9)	326	(80.3)	
Failed result	29	(6.9)	4	(1.0)	
HIV-1 PVL undetectable (≤40 copies/ml) among ART users					<0.001
No	98	(23.2)	265	(65.3)	
Yes	295	(69.9)	137	(33.7)	
Failed result	29	(6.9)	4	(1.0)	

	Burkina Faso N=615		South Africa N=623		p-value
	n or median	(%) or [IQR]	n or median	(%) or [IQR]	
HIV-1 PVL among ART-naïve, RNA copies/ml	48,800	(10870-206557)	19,300	(4680-55400)	<0.001
Baseline CD4+ count among ART users, cells/mm³					0.18
>500	170	(40.3)	139	(34.2)	
351-500	121	(28.7)	114	(28.1)	
200-350	82	(19.4)	102	(25.1)	
<200	48	(11.4)	51	(12.6)	
Missing	1	(0.2)	0	(0.0)	
Baseline CD4+ count among ART-naïve, cells/mm³					0.01
>500	74	(38.3)	92	(42.4)	
351-500	51	(26.4)	72	(33.2)	
200-350	49	(25.4)	47	(21.7)	
<200	19	(9.8)	6	(2.8)	
Clinical signs and symptoms					
Cervical ectopy					<0.001
Normal	419	(68.1)	597	(95.8)	
<20% cervical surface	114	(18.5)	19	(3.1)	
≥20% cervical surface	79	(12.9)	7	(1.1)	
Not known	3	(0.5)	0	(0.0)	
Cervicitis					<0.001
No	409	(66.5)	508	(81.5)	
Yes	200	(32.5)	112	(18.0)	
Not known	6	(1.0)	3	(0.5)	
Anogenital warts					0.19
No	566	(92.3)	587	(94.2)	
Yes	47	(7.7)	36	(5.8)	
Laboratory STIs					
Neisseria gonorrhoeae	4	(0.7)	14	(2.3)	0.02
Chlamydia trachomatis	13	(2.1)	31	(5.0)	0.01
Trichomonas vaginalis	-	-	101	(16.2)	
Mycoplasma genitalium	4	(0.7)	46	(7.4)	<0.001
Bacterial vaginosis	205	(34.6)	254	(41.6)	0.01
Candida albicans	87	(14.7)	52	(8.4)	<0.001
HSV-2 serology	459	(74.9)	590	(95.2)	<0.001
Active syphilis serology	1	(0.2)	7	(1.1)	0.02
HR-HPV					
HR-HPV positive	351	(59.1)	491	(79.1)	<0.001
CIN status					
Normal	373	(67.3)	261	(45.5)	<0.001
CIN1	149	(26.9)	184	(32.1)	
CIN2	19	(3.4)	76	(13.2)	
CIN3+	13	(2.4)	53	(9.2)	

*****The study was designed to include two-thirds of participants on ART in each site

Table 6.3. Study population characteristics at endline visit, among ≤CIN1 at baseline

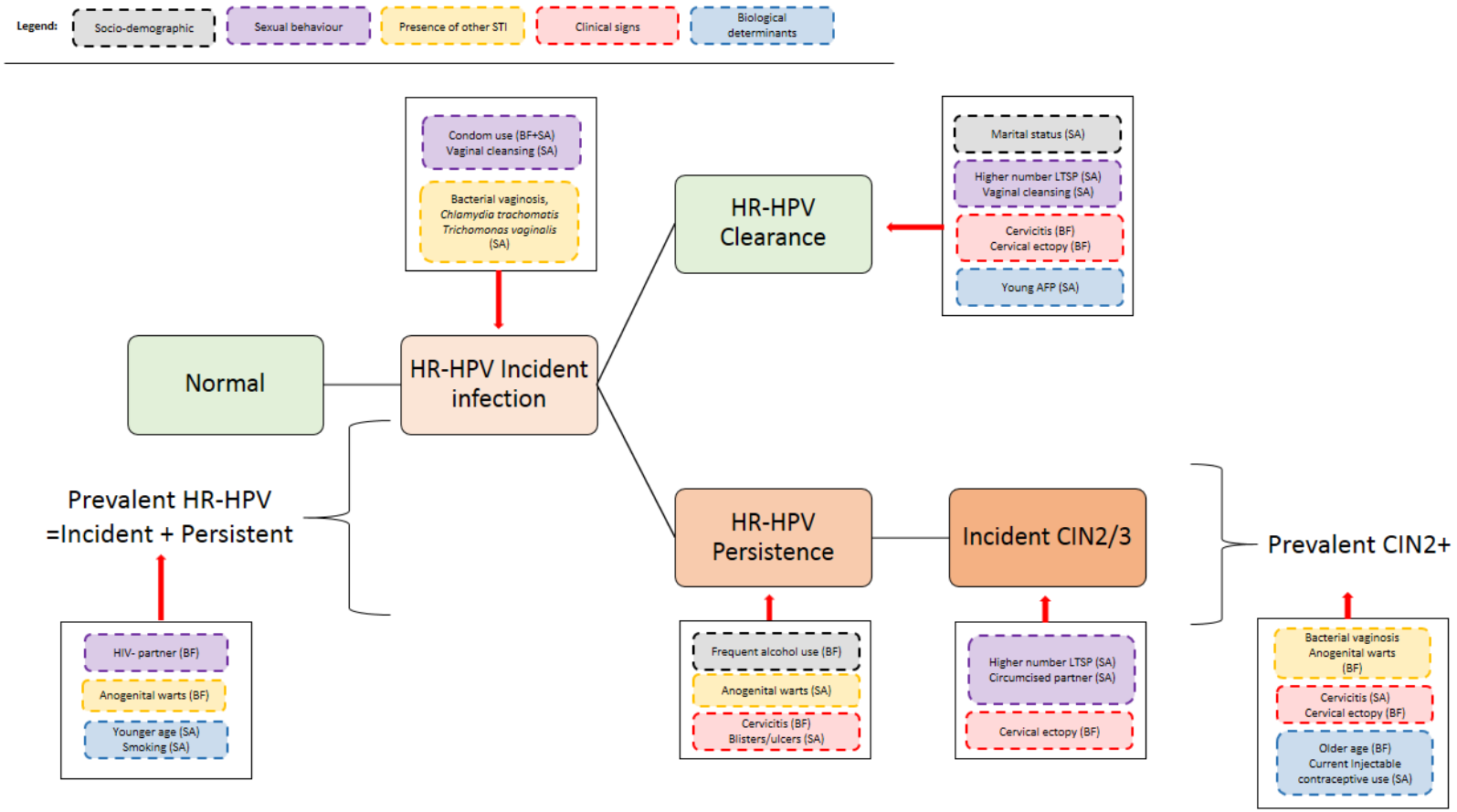
	Burkina Faso N=512		South Africa N=451		p-value
	n or median	(%) or [IQR]	n or median	(%) or [IQR]	
Duration of follow-up (FU)	16.4	[16.1-17.0]	15.9	[14.4-16.8]	
ART status at FU					0.002
ART users before enrolment	350	(68.4)	296	(65.6)	
ART initiators during FU	53	(10.4)	25	(5.5)	
ART naive at FU	109	(21.3)	130	(28.8)	
Median CD4+ count (cells/mm³) at M18					
ART users before enrolment	614	[434-819]	439	[322-604]	<0.001
ART initiators during FU	461	[312-613]	442	[366-601]	0.92
ART naive at FU	582	[439-868]	437	[346-543]	<0.001
Median CD4+ count (cells/mm³) change per year					
ART users before enrolment	105	[18-207]	5	[-56 to 88]	<0.001
ART initiators during FU	123	[35, 258]	83	[-50 to 211]	0.17
ART naive at FU	65	[-24 to 185]	-53	[-117 to 21]	<0.001
Patients with stable high CD4+ count (>500 cells/mm³)					
ART users before enrolment	129	(36.9)	62	(21.0)	<0.001
ART initiators during FU	3	(5.7)	1	(4.0)	0.76
ART naive at FU	47	(43.1)	27	(20.8)	<0.001
HR-HPV status (number of women)	**N**	**n (%)**	**N**	**n (%)**	
HR-HPV persistence	270	139 (51.5)	340	152 (44.7)	0.10
HR-HPV complete clearance	270	66 (24.4)	340	90 (26.5)	0.57
HR-HPV clearance of any type	270	180 (66.7)	340	268 (78.8)	0.001
HR-HPV incidence of any type	476	228 (47.9)	446	220 (49.3)	0.67
HR-HPV incidence among HR-HPV negative at baseline	206	114 (55.3)	106	55 (51.9)	0.56
HR-HPV status (number of infections)	**N**	**n (%)**	**N**	**n (%)**	
HR-HPV persistence	416	171 (41.1)	612	185 (30.2)	<0.001
HR-HPV clearance	416	245 (58.9)	612	427 (69.8)	<0.001
HR-HPV clearance, in absence of persistence	416	189 (45.4)	612	295 (48.2)	
HR-HPV incidence		350		328	
CIN 2+ status					
Incidence	430	5 (1.2)	379	22 (5.8)	<0.001

Table 6.4. Summary of risk factors observed among WLHIV in Burkina Faso (BF) and South Africa (SA)

Risk factor	Outcome	Effect	Effect estimate (95% CI)		Country observed
Socio-demographic					
Age	HR-HPV Prevalence	Compared to women aged 45-50 years, women aged 25-29 years had higher prevalence of HR-HPV	aPR	1.28 (1.04-1.58)	SA
	CIN2+ Prevalence	Compared to younger women (25-29 years), older women had higher prevalence of CIN2+	aOR	35-39yrs: 5.58 (1.12-27.67) 40-44 yrs: 6.79 (1.30-35.51)	BF
Marital status	HR-HPV complete Clearance	Compared to never married women, divorced/separated or widowed had increased likelihood of clearance of all HR-HPV	aPR	2.12 (1.23-3.66)	SA
Alcohol use	HR-HPV Persistence	Compared to women who never drink alcohol, women who drink alcohol at least once per month had increased risk of HR-HPV persistence	aPR	1.45 (1.11-1.91)	BF
Sex behaviour					
HIV+ partner	HR-HPV Prevalence	Compared to women with a HIV-negative partner, women with a HIV-positive partner had a decreased risk of HR-HPV prevalence	aPR	0.75 (0.58-0.97)	BF
Condom use	HR-HPV Incidence	Compared to women who never used condoms, women who **sometimes** used condoms had a decreased risk of HR-HPV incidence	aPR	0.66 (0.45-0.97)	BF
		Compared to women who never used condoms, women who **always** used condoms had a decreased risk of HR-HPV incidence	aPR	0.63 (0.46-0.86)	SA
	HR-HPV complete Clearance	Compared to women who never used condoms, women who **sometimes** used condoms had an increased likelihood of HR-HPV clearance of all HR-HPV	aPR	2.76 (1.11-6.86)	BF
Number of lifetime sex partners (LTSP)	HR-HPV complete Clearance	Compared to women with <2 LTSP, women with ≥2 LTSP had a decreased likelihood of HR-HPV clearance of all HR-HPV	aPR	0.41 (0.24-0.72)	SA
Circumcision status of male partner	CIN2+ Incidence	Compared to women whose male partner was uncircumcised, women with a partner who was circumcised had lower incidence of CIN2/3	aOR	0.36 (0.13-0.99)	SA
Vaginal cleansing	HR-HPV Incidence	Compared to women who said they never cleanse the vagina, women who said they did had a lower risk of HR-HPV incidence	aPR	0.76 (0.61-0.97)	SA
Presence of other STIs					
Bacterial vaginosis (BV)	HR-HPV Incidence	Compared to women without BV women, women with BV had an increased risk of HR-HPV incidence	aPR	1.26 (1.02-1.56)	SA
	CIN2+ Prevalence	Compared to women without BV women, women with BV had an increased risk of CIN2+ Prevalence	aOR	2.78 (1.25-6.20)	BF

Risk factor	Outcome	Effect	Effect estimate (95% CI)		Country observed
Chlamydia trachomatis (CT)	HR-HPV Incidence	Compared to CT negative women, CT positive women had an increased risk of HR-HPV incidence	aPR	1.63 (1.22-2.19)	SA
Trichomonas vaginalis (TV)	HR-HPV Incidence	Compared to TV negative women, TV positive women had an increased risk of HR-HPV incidence	aPR	1.30 (1.00-1.68)	SA
Anogenital warts (AGW)	HR-HPV Prevalence	Compared to women without AGW, women with AGW occurrence had an increased risk of HR-HPV prevalence	aPR	1.47 (1.18-1.83)	BF
	CIN2+ Prevalence	Compared to women without AGW, women with AGW occurrence had an increased risk of CIN2+ Prevalence	aOR	3.61 (1.17-11.18)	BF
	HR-HPV Persistence	Compared to women without AGW, women with AGW occurrence had an increased risk of HR-HPV persistence	aPR	1.61 (1.16-2.24)	SA
Clinical signs					
Cervicitis	HR-HPV persistence	Compared to women without cervicitis, women with cervicitis had an increased risk of HR-HPV persistence	aPR	1.25 (1.00-1.57)	BF
	HR-HPV complete Clearance	Compared to women without cervicitis, women with cervicitis had a decreased likelihood of HR-HPV clearance	aPR	0.06 (0.03-0.15)	BF
	CIN2+ Prevalence	Compared to women without cervicitis, women with cervicitis had an increased risk of CIN2+ prevalence	aOR	2.00 (1.19-3.35)	SA
Blisters, sores or ulcers on genitals	HR-HPV Persistence	Compared to women without, women with blisters, sores or ulcers on genitals had an increased risk of HR-HPV persistence	aPR	1.43 (1.01-2.03)	SA
Cervical ectopy	HR-HPV complete Clearance	Compared to women with normal cervical ectopy, women with <20% and ≥20% ectopy had a decreased risk of HR-HPV clearance	aPR	<20%: 0.30 (0.23-0.40) ≥20%: 0.31 (0.24-0.41)	BF
	CIN2+ Prevalence	Compared to women with normal cervical ectopy, women with ≥20% ectopy had an increased risk of CIN2+ prevalence	aOR	7.42 (2.90-18.95)	BF
	CIN2/3 Incidence	Compared to women with normal cervical ectopy, women with ≥20% ectopy had an increased risk of CIN2+ incidence	aOR	16.18 (1.59-164.12)	BF
Carcinogenic/Hormonal factors					
Smoking	HR-HPV Prevalence	Compared to never smokers, women who ever smoked had an increased risk of HR-HPV prevalence	aPR	1.18 (1.09-1.29)	SA
Age at first pregnancy	HR-HPV complete Clearance	Compared to young age at first pregnancy (<17 years), women with older age at first pregnancy (≥25 years) had a decreased likelihood of HR-HPV clearance	aPR	0.27 (0.12-0.63)	SA
Injectable contraceptive use	CIN2+ Prevalence	Compared to never users, current injectable contraception users had an increased risk of CIN2+ prevalence	aOR	2.21 (1.19-4.10)	SA

Figure 6.4. Risk factors associated with HR-HPV infection and CIN2+ among WLHIV in Burkina Faso and South Africa



LTSP=lifetime sex partners; AFP=age at first pregnancy

Table 6.5. Multivariate analysis of HR-HPV prevalence: associations with baseline sociodemographic factors, behavioural factors, HIV-related factors, clinical symptoms/signs and STIs

	Burkina Faso N=570					South Africa N=613			
	n (%)	aPR*	95% CI	P-value		n (%)	aPR*	95% CI	P-value
HIV status of current male partner					**Age group (years)**				
Negative	37 (71.2)	1.00			25-29	118 (88.1)	**1.28**	**1.04-1.58**	**0.02**
Positive	50 (53.2)	**0.75**	**0.58-0.97**	**0.03**	30-34	145 (81.9)	1.20	0.97-1.47	0.09
					35-39	111 (76.0)	1.13	0.92-1.40	0.24
Anogenital warts					40-44	77 (72.0)	1.07	0.86-1.33	0.56
No	300 (57.4)	1.00			45-50	32 (65.3)	1.00		
Yes	36 (80.0)	**1.47**	**1.18-1.83**	**0.001**					
					Marital status				
					Never married	251 (80.5)	1.00		
					Divorced/separated/widowed	25 (62.5)	0.83	0.67-1.04	0.10
					Married/cohabiting	207 (79.3)	0.98	0.90-1.06	0.56
					Smoker				
					Never	413 (76.9)	1.00		
					Ever	**70 (92.1)**	**1.18**	**1.09-1.29**	**<0.001**
					CD4+ count (cells/mm^3)				
					>350	313 (76.7)	1.00		
					≤350	167 (82.7)	1.07	0.99-1.17	0.10
ART status					**ART status**				
ART >2 years	101 (52.1)	1.00			ART >2 years	163 (72.4)	1.00		
ART ≤2 years	142 (65.1)	**1.41**	**1.10-1.80**	**0.01**	ART ≤2 years	147 (82.1)	1.09	0.98-1.21	0.12
ART-naive	95 (60.1)	1.21	0.94-1.56	0.14	ART-naive	173 (82.8)	1.09	0.98-1.20	0.10

*All factors in table adjusted for each other

Table 6.6. Multivariate analysis of HR-HPV incidence: associations with sociodemographic and HIV-related factors, clinical symptoms/signs and STIs[1]

	Burkina Faso (N=460)				South Africa (N=440)			
	n (%)	aPR*	95% CI	P-value	n (%)	aPR*	95% CI	P-value
Education					**Condom**			
Primary or less	154 (51.0)	1.00			Never	18 (72.0)	1.00	
More than primary	65 (42.8)	0.96	0.73-1.26	0.76	Sometimes	60 (51.7)	0.77	0.56-1.06	0.11
					Always	101 (46.8)	**0.63**	**0.46-0.86**	**0.003**
Age at first pregnancy					**Current male sex partner**			
<20 years	103 (52.0)	1.00			Yes, cohabiting	86 (45.5)	1.00	
≥20 years	112 (45.9)	0.93	0.71-1.22	0.62	Yes, non cohabiting	91 (55.2)	1.22	0.98-1.52	0.07
					No	4 (50.0)	-	-	-
Condom									
Never	31 (59.6)	1.00			**Years since HIV diagnosis**			
Sometimes	22 (40.7)	**0.66**	**0.45-0.97**	**0.03**	<5yrs	130 (54.2)	1.00	
Always	65 (50.4)	0.82	0.62-1.08	0.16	5-9 years	67 (45.0)	0.80	0.63-1.03	0.09
					≥10 years	20 (39.2)	0.71	0.44-1.15	0.17
Vulvar lesions									
No	204 (47.4)	1.00			**Bacterial vaginosis**			
Yes	18 (62.1)	1.17	0.73-1.86	0.52	Absence	114 (45.4)	1		
					Presence	99 (54.1)	**1.26**	**1.02-1.56**	**0.03**
ART status									
ART >2 years	95 (45.5)	1			***Chlamydia trachomatis***			
ART ≤2 years	69 (53.9)	1.21	0.88-1.67	0.24	Negative	203 (48.2)	1		
ART-naive	58 (47.2)	1.01	0.74-1.39	0.94	Positive	14 (73.7)	**1.63**	**1.22-2.19**	**0.001**
					Trichomonas vaginalis				
					Negative	176 (47.7)	1		
					Positive	41 (57.8)	**1.30**	**1.00-1.68**	**0.05**
					Ever cleanse vagina				
					Never	131 (52.2)	**1.00**		
					Ever	86 (45.5)	**0.76**	**0.61-0.97**	**0.02**
					CD4+ count at baseline (cells/mm³)				
					>350	149 (48.1)	1.00		
					201-350	46 (46.5)	0.88	0.64-1.19	0.40
					≤200	21 (72.4)	**1.48**	**1.06-2.05**	**0.02**
					CD4+ count at endline (cells/mm³)				
					>350	124 (45.1)	1		
					201-350	51 (55.4)	1.24	0.98-1.58	0.08
					≤200	13 (65.0)	-	-	-

*All factors in table adjusted for each other;
[1]all factors measured at baseline unless otherwise indicated

Table 6.7. Multivariate analysis of HR-HPV persistence: associations with sociodemographic and HIV-related factors, clinical symptoms/signs and STIs[1]

	Burkina Faso N=263 n (%)	aPR*	95% CI	P-value		South Africa N=334 n (%)	aPR*	95% CI	P-value
Alcohol use					**Age at first sexual intercourse**				
Never	85 (52.2)	1.00	-		<18 years	65 (38.5)	1.00		
Less than monthly	33 (44.0)	0.89	0.67-1.18	0.402	≥18 years	84 (50.9)	1.26	0.97-1.63	0.08
At least once per month	**20 (80.0)**	**1.45**	**1.11-1.91**	**0.01**					
					Number of lifetime sex partners				
Cervicitis					1	2 (22.2)	1.00		
No	84 (48.0)	1.00	-		2-4	70 (51.9)	2.42	0.74-7.86	0.14
Yes	53 (62.4)	**1.25**	**1.00-1.57**	**0.05**	≥5	53 (39.9)	2.05	0.63-6.71	0.24
					Number of male sexual partners in last 3 months (at baseline)				
ART status									
ART >2 years	51 (46.4)	1.00			0	26 (46.4)	1.00		
ART ≤2 years	35 (44.3)	0.93	0.68-1.27	0.64	1	118 (46.6)	1.05	0.75-1.45	0.78
ART-naive	52 (70.3)	**1.36**	**1.05-1.76**	**0.02**	≥2	5 (21.7)	0.28	0.08-1.02	0.05
					Anogenital warts				
CD4+ count change during follow-up					No	136 (43.2)	1.00	-	
Stable >350 cells/mm^3	70 (46.1)	1.00			Yes	13 (68.4)	**1.61**	**1.16-2.24**	**0.005**
Increasing ≤350 to >350 cells/mm^3	24 (54.6)	1.08	0.78-1.49	0.64					
Decreasing >350 to ≤350 cells/mm^3	10 (83.3)	**1.56**	**1.04-2.33**	**0.03**	**Genital blisters, ulcers or sores**				
Stable ≤350 cells/mm^3	28 (65.1)	1.30	0.99-1.72	0.06	No	135 (43.4)	1.00	-	
					Yes	14 (60.9)	**1.43**	**1.01-2.03**	**0.05**
					HIV viral suppression (<1000 copies/ml)				
					No	77 (55.0)	**1.45**	**1.13-1.85**	**0.003**
					Yes	72 (37.1)	1.00		

*All factors in table adjusted for each other; [1]all factors measured at baseline unless otherwise indicated

Table 6.8. Multivariate analysis of HR-HPV complete clearance: associations with sociodemographic factors, HIV-related factors, clinical symptoms/signs and STIs[1]

	Burkina Faso N=263				South Africa N=334			
	n (%)	aPR*	95% CI	P-value	n (%)	aPR*	95% CI	P-value
Alcohol use								
Never	38 (23.3)	1.00						
Less than monthly	22 (29.3)	0.95	0.56-1.63	0.86				
At least once per month	2 (8.0)	0.22	0.04-1.18	0.11				
Ever used injectable contraceptive								
No	43 (20.3)	1.00						
Past	16 (44.4)	1.67	0.97-2.87	0.07				
Current	3 (23.1)	1.06	0.34-3.34	0.92				
Condom								
Never	5 (15.2)	1.00						
Sometimes	11 (36.7)	**2.76**	**1.11-6.86**	**0.03**				
Always	18 (26.1)	1.76	0.72-4.30	0.22				
HSV-2								
Negative	18 (32.7)	1.00						
Positive	44 (21.2)	0.66	0.39-1.12	0.13				
Cervicitis								
No	54 (30.9)	1.00						
Yes	8 (9.4)	**0.06**	**0.03-0.15**	**<0.001**				
Cervical ectopy								
Normal	54 (29.0)	1.00						
<20%	5 (11.1)	**0.30**	**0.23-0.40**	**<0.001**				
≥20	3 (9.7)	**0.31**	**0.24-0.41**	**<0.001**				
CD4+ count at baseline (cells/mm³)								
>350	48 (27.6)	1.00						
201-350	8 (14.3)	**0.36**	**0.13-0.97**	**0.04**				
≤200	6 (18.2)	0.67	0.22-2.09	0.49				
Marital status								
Never married					40 (23.4)	1.00		
Divorced/separated/widowed					8 (42.1)	**2.12**	**1.23-3.66**	**0.01**
Married/Cohabiting					41 (28.5)	1.11	0.73-1.71	0.62
Age at first pregnancy (years)								
<17					15 (40.5)	1.00		
17-19					29 (25.0)	**0.57**	**0.34-0.93**	**0.03**
20-24					34 (31.2)	0.70	0.43-1.13	0.14
≥25					**7 (13.2)**	**0.27**	**0.12-0.63**	**0.002**
Number of lifetime sex partners								
1					5 (55.6)	1.00		
2-4					135 (20.0)	**0.32**	**0.17-0.61**	**<0.001**
≥5					42 (31.6)	**0.51**	**0.28-0.91**	**0.02**
Bacterial vaginosis								
Negative					56 (30.6)	1.00		
Positive					33 (22.6)	0.70	0.46-1.06	0.09
Ever cleanse vagina								
Never					45 (23.7)	1.00		
Ever					44 (30.6)	1.29	0.87-1.92	0.20

*All factors in table adjusted for each other; [1]all factors measured at baseline unless otherwise indicated

Table 6.9. Multivariate analysis of CIN2+ prevalence: associations with sociodemographic factors and HIV-related factors, clinical symptoms/signs and STIs[1]

	Burkina Faso N=530				South Africa N=566			
	n (%)	aOR*	95% CI	P-value	n (%)	aOR*	95% CI	P-value
Age group (years)								
25-29	2 (2.2)	1.00			35 (28.2)	1		
30-34	4 (3.2)	2.37	0.40-14.08	0.34	41 (24.0)	0.91	0.51-1.63	0.75
35-39	13 (9.9)	**5.58**	**1.12-27.67**	**0.04**	32 (23.9)	0.88	0.48-1.62	0.69
40-44	9 (8.2)	**6.79**	**1.30-35.51**	**0.02**	14 (14.1)	0.54	0.26-1.15	0.11
45-50	4 (5.6)	4.59	0.75-28.26	0.10	6 (15.8)	0.68	0.24-1.90	0.46
Bacterial vaginosis					**Age at first pregnancy (years)**			
Absence	13 (3.9)	1			<17	9 (15.3)	1	
Presence	17 (9.6)	**2.78**	**1.25-6.20**	**0.01**	17-19	56 (27.2)	1.87	0.82-4.27	0.14
Anogenital warts					20-24	46 (24.2)	1.73	0.76-3.94	0.19
No	27 (5.5)	1.00			≥25	13 (15.9)	1.00	0.38-2.64	1.00
Yes	5 (12.5)	**3.61**	**1.17-11.18**	**0.03**	**Ever used injectable contraceptive**			
Cervical ectopy					No	29 (18.6)	1		
Normal	14 (3.8)	1			Past	57 (19.0)	0.94	0.54-1.61	0.81
<20%	6 (6.7)	2.19	0.77-6.23	0.14	Current	42 (38.2)	**2.21**	**1.19-4.10**	**0.01**
≥20	12 (16.7)	**7.42**	**2.90-18.95**	**<0.001**	**Cervicitis**			
CD4+ count (cells/mm³)					No	91 (19.7)	1		
>350	19 (5.3)	1			**Yes**	36 (35.6)	**2.00**	**1.19-3.35**	**0.01**
201-350	5 (4.4)	0.84	0.29-2.44	0.75	**ART status**			
≤200	8 (13.8)	**3.59**	**1.33-9.68**	**0.01**	ART >2 years	31 (15.0)	1		
					ART ≤2 years	47 (29.2)	**1.93**	**1.09-3.42**	**0.02**
					ART-naive	50 (25.3)	1.61	0.94-2.77	0.08
					CD4+ count (cells/mm³)				
					>350	69 (18.3)	1.00		
					201-350	38 (27.5)	1.62	0.98-2.67	0.06
					≤200	**20 (41.7)**	**2.80**	**1.33-5.87**	**0.01**

*****All factors in table adjusted for each other; [1]all factors measured at baseline unless otherwise indicated

Table 6.10. Multivariate analysis of CIN2/3 incidence: associations with sociodemographic and HIV-related factors, clinical symptoms/signs and STIs[1]

	Burkina Faso N=412					South Africa N=373			
	n (%)	aOR*	95% CI	P-value		n (%)	aOR**	95% CI	P-value
Hormonal contraception					**Lifetime sex partners**				
Never	1 (0.5)	1.00			1	0 (0.0)	**1.00**		
Ever	4 (1.8)	10.69	0.79-144.79	0.08	2-4	12 (7.4)	-	-	-
					≥5	2 (1.4)	-	-	-
Cervical ectopy					**Current male partner circumcised**				
Normal	2 (0.7)	1.00			No	13 (10.6)	1.00		
<20%	1 (1.3)	1.66	0.14-19.49	0.69	Yes	6 (4.0)	**0.36**	**0.13-0.99**	**0.05**
≥20	2 (4.9)	**16.18**	**1.59-164.12**	**0.02**					
					Mycoplasma genitalium				
CD4+ count at endline (cells/mm³)					No	18 (5.3)	1.00		
>350	2 (0.6)	1.00			Yes	4 (12.9)	2.69	0.80-9.37	0.12
201-350	2 (3.8)	7.41	0.89-61.86	0.06					
≤200	1 (4.8)	**14.85**	**1.05-209.02**	**0.05**	**Anogenital warts**				
					No	19 (5.4)	1.00		
					Yes	3 (14.3)	3.48	0.84-14.35	0.08

***All factors in table adjusted for each other; ****In SA, there was no adjustment for LTSP because reference group has zero values; [1]all factors measured at baseline unless otherwise indicated

7 HIV-RELATED RISK FACTORS FOR HR-HPV AND CIN2+

*The meta-analysis in **Chapter 3** has shown that ART is associated with a decrease in HR-HPV prevalence, CIN2//HSIL+ prevalence and SIL incidence and progression, and is associated with an increased likelihood of SIL regression. These associations were strongest when ART was considered "effective" (i.e. with high adherence, HIV viral suppression and immune reconstitution) and taken over longer durations. However, few longitudinal studies have reported the effect of ART, CD4+ cell count and HIV-1 PVL on the simultaneous natural history of HR-HPV infection and histological cervical lesions. The aims of this Chapter, therefore, are to evaluate the associations of ART, CD4+ cell count and HIV-1 PVL prospectively on HR-HPV and CIN2+ outcomes in the HARP cohort.*

7.1 Objectives

In a cohort of WLHIV in Burkina Faso and South Africa at enrolment:

- To evaluate the association of HR-HPV and CIN2+ prevalence with ART status, CD4+ cell count and HIV-1 PVL. ***(Objective 2.4)***

In a cohort of WLHIV with ≤CIN1 at enrolment and followed over 18 months:

- To evaluate the association of HR-HPV incidence, persistence and complete clearance, and CIN2+ incidence with ART status, CD4+ cell count and HIV-1 PVL at baseline and (where available) at endline. ***(Objective 2.4)***

7.2 Methods

A full description of the HARP enrolment procedures is provided in **Chapter 5, section 5.2**. Specific statistical methods for univariate and multivariate risk factor analysis are detailed here.

7.2.1 Statistical methods

The HR-HPV and CIN2+ outcomes in this Chapter, are as defined previously**:** HR-HPV prevalence, incidence, persistence and complete clearance (**Table 5.2)**; and CIN2+ prevalence and incidence at endline (**Table 5.3)**.

As HR-HPV prevalence, incidence and clearance were common, associations with HIV-related variables were estimated with prevalence ratios (PRs) obtained from logistic regression using marginal standardization to estimate PRs, and the delta method to estimate 95% CI [379]. Associations between HR-HPV persistence and HIV-related variables were estimated with generalised estimating equation (GEE) to account for multiple HR-HPV infection and coexisting/concomitant infection states (e.g. persistence and clearance of different types in the same woman) [380].

For associations with CIN2+ prevalence and incidence over follow-up (much rarer outcomes), logistic regression was used to estimate odds ratios (ORs) and their 95% CI.

7.2.2 Adjustment factors

Multivariable analyses (MVA) were adjusted for site and socio-demographic and behavioural factors which were independently associated in multivariate analyses with HR-HPV or CIN2+ for each country, as described in **Chapter 6** (**Model 1**). Adjustments were made separately for each country, given the differences observed in the study population characteristics and risk factor associations with HR-HPV and CIN2+ outcomes, and are summarised in **Table 7.1.**

Adjustment factors were considered at two levels; a.) factors found to be associated with acquisition of HR-HPV infection in **Chapter 6** (condom use, age, vaginal cleansing, presence of

other STIs including bacterial vaginosis (BV), *Chlamydia trachomatis* and *Trichomonas vaginalis* and high-risk behaviours associated with HR-HPV acquisition in **Chapter 6** (such as alcohol use and number of lifetime sex partners); and b.) factors linked to inflammation and carcinogenesis and found to be associated with HR-HPV persistence (cervicitis, cervical ectopy and smoking) and CIN2+ prevalence and incidence in **Chapter 6** (age, cervical ectopy, BV, cervicitis and injectable contraception).

Table 7.1. Summary of adjustment factors found to be associated with HR-HPV and CIN2+ in multivariate analysis (Chapter 6) and used as adjustment factors in this Chapter (Model 1)

	Burkina Faso (Model 1)	**South Africa (Model 1)**
HR-HPV outcomes	Condom use Alcohol use Cervicitis Cervical ectopy	Condom use Age Vaginal cleansing Bacterial vaginosis *Chlamydia trachomatis* *Trichomonas vaginalis* Number LTSP Smoking
CIN2+ Prevalence	Age Cervical ectopy Bacterial vaginosis	Cervicitis Injectable contraception
CIN2+ Incidence	Cervical ectopy	Cervicitis Injectable contraception

Given that WLHIV in the *HARP* study were infected with multiple HR-HPV types, and had multiple coexisting infection states (i.e. an individual woman could have incidence, persistence or clearance of different types at the same time point), the same adjustment factors were used for all HR-HPV outcomes (prevalence incidence, persistence, clearance), as summarised in **Table 7.1.**

Similarly, due to possible imprecision in MVA because of the small number of incident CIN2/3 cases, I limited the range of risk factors used for adjustment and only used those found to be associated with CIN2+ prevalence at baseline for the CIN2+ incidence analyses. For BF, because there were only 5 incident CIN2+ cases, I restricted adjustment to cervical ectopy, as it was found to be associated with both CIN2+ prevalence and incidence in MVA in Chapter 6.

Other factors found to persist in MVA in **Chapter 6** (such as marital status, AGW occurrence, presence of genital blisters or sores, male partner's circumcision status, HIV-positive male partner and age at first pregnancy) were not considered as additional adjustment factors in this chapter, for the following reasons:

Marital status

In **Chapter 6,** the MVA showed that women who were divorced/separated/widowed were more likely to clear HR-HPV infection compared to never married women in SA. It could be speculated that unmarried women were younger and engaged in more risky sexual behaviours compared to other women leading to a higher risk of persistent infection, but the *HARP* data does not support this. Unmarried women, divorced/separated/widowed women and married/cohabiting women all had a similar number of LTSP and a similar number of recent sex partners (in last 3 months). It may because formerly married women were less likely to be current users of injectable contraception (9.8% vs. 19.1% of married or cohabiting women). Injectable contraception is a known immunomodulatory factor and was found to be associated with CIN2/3 prevalence in SA. It is possible that these associations are linked given that HR-HPV persistence is a precursor for CIN2/3.

Number of lifetime sex partners

Although incident CIN2+ appeared higher among those with a higher number of LTSP (in women with more than 1 LTSP) in SA, there were no cases of incident CIN2+ in the reference group (1 LTSP), and it is not clear if there an association of LTSP with incident CIN2+, as an OR could not be calculated. For this reason, LTSP was not included in the Model 1 for incident CIN2/3.

Male circumcision

Circumcision of current male sex partner was associated with a decreased risk of incident CIN2/3 in SA, but not with HR-HPV outcomes. HR-HPV incidence was similar among women with

circumcised partners and among women with uncircumcised partners (49.2% vs. 53.4%, p=0.45). Although HR-HPV prevalence was marginally higher among women with uncircumcised partner compared to those with a circumcised partner (81.9% vs. 76.0%; unadjusted PR=1.08, 95%CI: 0.98-1.18) this association was weakly statistically significant in univariate analysis at the established high cut-off (p=0.12), and it did not persist in MVA. Given the longer time period for CIN2+ development, it is possible that circumcision status is linked to a HR-HPV type acquired in the past prior to enrolment in the study, which may have been detected as a prevalent (and which may have been truly persistent) infection at baseline which developed into incident CIN2+ during the follow-up period. However, just under three-quarters of women in SA reported the circumcision status of their male partner, and given the small number of incident CIN2+ cases in SA, adjusting for this variable resulted in a less precise measure, with some missing values.

HIV-positive male partner

As discussed in **Chapter 6**, it was unclear why women with a HIV-positive male partner had lower prevalence of HR-HPV compared to those with a HIV-negative male partner, but it could be linked to the reliability of condom use reporting, as well as unmeasured confounding for sexual behaviour (male partner sexual behaviour patterns or frequency of sex).

Age at first pregnancy (AFP)

Women with a young AFP were more likely to have complete clearance of HR-HPV compared to those with older AFP. As discussed in Chapter 6, AFP can be used as a proxy measure for AFSI, but this association was not similarly observed for AFSI. Given the lack of correlation in these findings, it remains unclear if there is unmeasured confounding. Furthermore, the definition of complete clearance may be limited as it does not account for the number of infections that were cleared.

Anogenital warts (AGW)

The occurrence of AGW was shown to be associated with HR-HPV and CIN2+ prevalence in BF, and HR-HPV persistence in SA in MVA in **Chapter 6**. However, there is limited evidence in the literature that AGW occurrence results in HR-HPV persistence and CIN2+ development. AGW occurrence is not known to be a causative agent of CIN2+ and is considered to be a reflection of infection with LR-HPV types, which in turn frequently co-exist with HR-HPV types.

Presence of genital blisters, ulcers or sores

The presence of genital blisters, ulcers or sores which were associated with increased risk of HR-HPV persistence in SA, were detected during clinical exam by nurse/midwife. Their presence could be correlated with other STI, particularly HSV-2 given the high prevalence among women in SA (95%). However, HSV-2 was not found to be associated with HR-HPV or CIN2+ outcomes, probably because of its high prevalence (and there were no measures of HSV-2 shedding in this study). Given that adjustments are made for bacterial vaginosis, *Chlamydia trachomatis, Trichomonas vaginalis* and cervicitis in SA, and to avoid possible over adjustment of factors that are correlated with each other, there will be no further adjustment for genital blisters/ulcers/sores.

7.2.3 HIV-related factor definitions

To explore associations of HR-HPV and CIN2+ outcomes with HIV-related factors, pre-specified analyses included stratification by site, ART, HIV-1 viral suppression, HIV-1 viral detection and CD4+ cell counts. ART was stratified by user status (ART use:naïve) and duration of use at baseline (prolonged ART >2 years; short duration ART ≤2 years and ART-naïve), as other studies have done [209, 213], allowing for comparison with these other studies. Associations of ART initiation with HR-HPV and CIN2+ outcomes were not explored because of the small number of women that initiated ART during follow-up (BF: n=53; SA:, n=25). However, cumulative duration of ART use was

assessed from both baseline and endline (duration at baseline+follow-up duration) timepoints was assessed for the longitudinal outcomes.

Among ART users, HIV-1 viral suppression was stratified as having < or ≥1000 HIV-1 RNA copies/ml of plasma, according to WHO definitions [404], and HIV-1 viral detection (≤40 and >40 HIV-1 RNA copies/ml) was defined by the lowest limit of detection of the HIV-1 RNA assays used in each country (Abbott RT HIV-1 in BF and COBAS Taqman in SA).

A variable of "stable high CD4+ cell count" was created as a measure of immune stability and defined as having CD4+ counts >500 cells/mm^3 at all follow-up visits, i.e. baseline, Month 12 and endline visits. Stable high CD4+ cell count was assessed separately among ART users and ART-naïve.

CD4+ cell count has been shown to be an independent determinant of HR-HPV and CIN2+ [183]. Because ART users can have wide ranging CD4+ cell counts depending on how recently they initiated ART, or whether they have good treatment adherence, a second logistic regression model (**Model 2**) incorporated baseline CD4+ cell count to **Model 1** to explore associations with ART and HIV-1 PVL. **Table 7.1** summarises adjustment factors used in **Model 1**.

7.3 Results

7.3.1 Association of HR-HPV prevalence with ART and HIV-related factors

Table 7.2 reports on these findings, by country. HR-HPV prevalence was higher among those with low CD4+ count in both countries (CD4+ count <200 vs. >500 cells/mm^3: adjusted Prevalence Ratio [aPR]=1.38, 95%CI: 1.15-1.66 in BF and aPR=1.14, 95%CI: 1.01-1.30 in SA). This association was strongest among ART users (<200 vs. >500 cells/mm^3: aPR=1.43, 95%CI: 1.15-1.77 in BF and aPR=1.16, 95%CI: 1.00-1.35 in SA) but weaker evidence was observed among the ART-naive (<200 vs. >500 cells/mm^3, aPR=1.19, 95%CI: 10.84-1.69 in BF and aPR=0.98, 95%CI: 0.64-1.50 in SA).

Compared to long-duration ART users (>2 years), HR-HPV prevalence was higher among those on short-duration ART in both countries (≤2 years), however when adjusted for CD4+ cell count, this association was observed in BF only (65.1% vs. 52.1% for ≤2 years compared to >2 years; aPR=1.21, 95%CI: 1.03-1.44). There was some evidence of an increase in HR-HPV prevalence among ART-naïve compared to long-term ART users in both countries, but this was not significant.

7.3.2 Association of HR-HPV incidence with ART and HIV-related factors

Table 7.3 reports on these findings. HR-HPV incidence was higher among those with low CD4+ cell count in SA only (CD4+ count <200 vs. >500 cells/mm^3: 71.0% vs. 46.5%; aPR=1.61, 95%CI: 1.24-2.09), and this association was strongest among ART users (<200 vs. >500 cells/mm^3: 71.4% vs. 43.4%; aPR=1.77, 95%CI: 1.31-2.39). Among ART users in SA, HR-HPV incidence was marginally associated with lack of HIV-1 viral suppression at baseline (women with PVL ≥1000 vs. <1000 copies/ml: 60.0% vs. 48.3%; aPR=1.27, 95%CI: 0.98-1.64).

In BF, there was some evidence that HR-HPV incidence was higher among short-duration ART users compared to long-duration ART users, after adjustment for CD4+ cell count (53.9% vs. 45.5%; aPR=1.20, 95%CI: 0.96-1.50, **Model 2**).

7.3.3 Association of HR-HPV persistence with ART and HIV-related factors

Table 7.4 reports on these findings, by country. HR-HPV persistence was associated with low baseline CD4+ cell count in BF only (<200 vs. >500 cells/mm^3: 54.6% vs. 36.8%; aOR=1.81, 95%CI: 1.10-2.99, Model 1). HR-HPV persistence was higher among ART-naive compared to long-duration ART users in BF only (Model 1) and this association persisted when adjusted for baseline CD4+ cell count (Model 2; ART-naïve vs. ART >2 years: 58.6% vs. 37.7%; aOR=1.80, 95%CI: 1.21-2.66). HR-HPV persistence was similar among long-duration and short-duration users in BF (37.7% vs. 33.3%).

By contrast, in SA, HR-HPV persistence was higher among short-duration compared to long-duration ART users even after adjustment for CD4+ cell count (ART ≤2 years vs. ART >2 years: 35.2%

vs. 24.5%; aOR=1.75, 95%CI: 1.17-2.64; Model 2). HR-HPV persistence was also higher among ART-naïve compared to long-duration users, but there was weak evidence of an association (ART >2 years vs. ART naive: 31.8% vs. 24.5%; aOR=1.50, 95%CI: 0.94-2.40). Among ART users in SA, HR-HPV persistence was associated with lack of HIV-1 viral suppression at baseline (women with PVL ≥1000 vs. <1000 copies/ml: 49.3% vs. 24.8%; aOR=3.35, 95%CI: 1.85-6.04).

Among participants who remained ART-naive throughout the follow-up period, women with unstable CD4+ count or stable low CD4+ count (≤500 cells/mm^3) had increased persistence compared to those with stable high CD4+ count but the association was not statistically significant in BF, and borderline significant in SA (>500 cells/mm^3; in BF: 64.2% vs. 45.0%; aOR=1.50, 95%CI: 0.33-6.76 and in SA: 33.1% vs, 16.7%; aOR=2.20, 95%CI: 0.99-4.91).

7.3.4 Association of HR-HPV complete clearance with ART and HIV-related factors

Table 7.5 reports on these findings, by country. Among those with HR-HPV infection at baseline, complete clearance was lower among those with low CD4+ cell count in both countries, but the association was significant in SA only (CD4+ cell count <200 vs. >500 cells/mm^3: 12.0% vs. 33.6%; aPR=0.33, 95%CI: 0.11-0.96), and this association was strongest among ART users (<200 vs. >500 cells/mm^3: 13.6% vs.38.5%; aPR=0.31, 95%CI: 0.10-0.94).

Complete HR-HPV clearance was lower among ART-naïve women compared to long-duration ART users in both countries, but the association was significant in SA only (ART naive vs. ART >2 years: 21.1% vs. 33.3%; aPR=0.65, 95%CI: 0.42-0.99, adjusted for CD4+ cell cell count; **Model 2**).

7.3.5 Association of CIN2+ prevalence and incidence with ART and HIV-related factors

Table 7.6 and Table 7.7 report on these findings, by country. CIN2+ prevalence was higher among participants with low baseline CD4+ cell count in both countries (CD4+ count <200 vs. >500 cells/mm^3: in BF: 14.0% vs. 6.3%; aOR=2.99, 95% CI: 1.05-8.58, and in SA: 42.0% vs. 19.1%; aOR=3.39,

95%CI: 1.70-6.75; **Table 7.6**) and this association was strongest among ART users (BF: aOR=4.34, 95%CI: 1.25-15.10. SA: aOR=3.78, 95%CI: 1.73-8.24).

Among ART-naive, CD4+ cell count below the threshold for ART initiation according to the 2010 WHO guidelines was associated with higher risk of CIN2+ in SA only (≤350 vs. >350 cells/mm^3: 38.0% vs. 20.4%; aOR=2.57, 95%CI: 1.21-5.45, data not shown).

CIN2+ prevalence was higher among participants on short-duration ART (≤2 years) and among ART-naïve compared to long-duration ART users in SA and these associations persisted after adjustment for baseline CD4+ cell count (ART ≤2 years vs. >2 years: 29.2% vs. 15.0%; aOR=1.81, 95%CI: 1.03-3.17; ART-naïve vs. ART >2 years: 25.% vs. 15.0%; aOR=1.78, 95%CI: 1.06-2.99, **Table 7.6**).

In SA, CIN2+ incidence was similar among short-duration and long-duration ART users at endline (4.5% each, **Table 7.7**), but there was some evidence that CIN2+ incidence was higher among those who remained ART-naïve at endline compared to all ART users, irrespective of duration (9.6% vs. 4.5%; aOR=2.00, 95%CI: 0.80-5.04, adjusted for injectable contraception, cervicitis and baseline CD4+ count, data not shown). No association was observed in BF due to the small number of incident CIN2+ cases. No associations were observed with CD4+ cell count at either baseline or endline in either country.

7.4 Discussion

It was reported in **Chapter 6** that WLHIV in Burkina Faso and South Africa had high prevalence and persistence of HR-HPV, but CIN2+ prevalence and incidence were higher in South Africa despite balanced distribution of ART use, similar median duration on ART and median CD4+ cell counts at study enrolment in both countries, and similar HR-HPV persistence rates over 16 months.

In this Chapter we report that low baseline CD4+ cell count (<200 cells/mm^3) was associated with a higher prevalence of HR-HPV and CIN2+ in both countries, with HR-HPV incidence in SA, and with

HR-HPV persistence in BF, similar to other studies that have shown CD4+ count to be one of the strongest predictors of HR-HPV infection [183] and cervical lesion development [173]. The lack of association of CD4+ count with CIN2/3 incidence is surprising, but may be a consequence of the short duration of follow-up resulting in a small number of cases for meaningful analysis.

Prolonged duration of ART was also associated with lower CIN2+ prevalence and HR-HPV persistence, similar to other reports [209, 213, 288], and this association was independent of baseline CD4+ count. Although HR-HPV persistence was similar in both countries, cervical lesion progression was higher in South Africa and this may have been influenced to some extent by the role of HIV-related factors and other contributing cofactors such as injectable contraception and co-infection with other STIs, the frequency of which differed between the two countries.

In Burkina Faso, prolonged and short-duration ART users had a similar risk of HR-HPV persistence when CD4+ counts were >500 cells/mm^3. However the risk was higher among short-duration compared to long-duration ART users when CD4+ count was low (<200 cells/mm^3). This suggests that once the recent ART initiators begin to recover CD4+ T-lymphocytes, they can control HR-HPV persistence, leading to the lower prevalence of CIN2+. The short duration of follow-up coupled with the small number of incident CIN2/3 and ART initiators over follow-up in BF may have influenced the lack of association between CD4+ count reconstitution and CIN2/3 incidence.

By contrast, in South Africa, short-duration ART users had higher persistence of HR-HPV and higher prevalence of CIN2+, irrespective of baseline CD4+ cell count. This may be a consequence of several factors. Firstly, ART users in South Africa were not as well HIV-controlled as their counterparts in Burkina Faso. Effective ART use, as measured by HIV-1 RNA suppression (PVL <1000 copies/ml), was associated with a decrease in HR-HPV persistence over 16 months in South Africa, independent of baseline CD4+ cell count. Others have shown that HR-HPV persistence was increased 2-3 fold among women (6% ART users) with detectable genital tract HIV RNA levels, after adjusting for CD4+ cell count but not when adjusted for plasma HIV [405], thereby suggesting a possible direct role of HIV on HR-HPV through both local and systemic mechanisms. The greater proportion of

ART users in South Africa with detectable HIV-1 PVL (65% in South Africa vs. 23% in Burkina Faso) may be a consequence of their poor self-reported adherence, which was observed among both short and long-duration ART users. Women with suboptimal adherence may have developed, or initially acquired, some antiretroviral-resistant HIV strain, although we cannot verify this in the absence of HIV genotyping. Secondly, ART use in the short-term may be ineffective in clearing HR-HPV if the infection is already well established. Women on short-duration ART in SA were less likely to clear all HR-HPV infections at endline compared to women on long-duration ART. Others have shown that ART users are at a higher risk of cervical disease if they have initiated ART at a lower nadir CD4+ count [193, 406, 407]. In such cases, the CD4+ T-lymphocyte reconstitution accompanying ART may be partial or functionally impaired and the beneficial effects of ART may only become apparent after long treatment periods [176].

It is possible in our study that immune reconstitution achieved through recent ART initiation at the previous WHO cut-off of 350 cells/mm^3 may have been insufficient to prevent HR-HPV persistence and/or CIN2+ development, in particular when coupled with detectable HIV, lower ART adherence and lower CD4+ T-lymphocyte recovery over time, as was the case among women in South Africa. Early ART initiation, coupled with rapid virological control, is likely to rapidly improve and maintain CD4+ count at a higher level [408], leading to possibly more complete and sustained immune reconstitution, thereby reducing the risk of persistent HR-HPV and cervical lesion development. Finally, the higher CIN2+ prevalence and incidence in South Africa could additionally be explained by other cofactors for such as greater frequency of contraceptive use and smoking and higher prevalence of mucosal STIs [89, 409, 410], as also described in **Chapter 6**. The presence of STIs can trigger genital HIV replication, a cascade of mucosal immunological synergies and vaginal biome changes [142, 411], which may interact with HR-HPV [412]. Addressing such cofactors should also be a priority of cervical cancer prevention programmes.

Importantly, this study has found that ART use was associated with increased likelihood of clearing HR-HPV infections, and with a reduction in incident CIN2+ over 16 months in South Africa.

Furthermore, by endline, ART duration had increased to a median 44 months which may have played a role in promoting HR-HPV clearance and averting CIN development.

ART-naïve women in South Africa had similar HR-HPV persistence, complete HR-HPV clearance and CIN2+ prevalence as prolonged ART users at high baseline CD4+ (>500 cells/mm^3), but these outcomes worsened among the ART-naïve at low baseline CD4+ cell counts. This finding highlights the role of early ART initiation to favourably influence the natural history of HR-HPV and CIN.

7.5 Study limitations

As stated in previous chapters, this study is limited by the duration of follow-up and HR-HPV testing intervals. The definition of cumulative HR-HPV incidence over 16 months is of limited duration to assess the actual roles of HR-HPV incidence and persistence on CIN2+ incidence. The study could not rule out type-specific clearance and reinfection when estimating persistence during the 16-months interval between HPV testing. A longer duration of follow-up would have allowed to accrue a larger number of incident CIN2+ cases and more robustly assess the role of HIV-related factors on CIN development over sufficient follow-up time.

This analysis was constrained by the absence of consistent data on nadir CD4+ cell count which would have allowed a better understanding of the higher CIN2+ rates among women in SA compared to women in BF, and may have helped to explain the higher CIN2+ prevalence among the short-duration ART users in comparison with the long-duration ART users. A low nadir CD4+ cell count has previously been shown to a strong predictor for HR-HPV infection among WLHIV in Belgium and Brazil [209, 220] and CIN2+ prevalence among WLHIV in Kenya and Brazil [193, 406].

The absence of a HIV-1 PVL measure at the endline visit prohibited classification of ART users into and; i.e. according to whether they had sustained HIV-1 viral suppression (“effective” ART) or not (“ineffective” ART). Others have previously shown that sustained HIV-1 PVL suppression over 40

months was associated with a 20% reduction in HR-HPV detection during follow-up [209]. In this study, duration of ART was a proxy measure for "effective" ART use. This assumes that women were adherent, yet no woman reported >90% adherence in either country. ART adherence was crudely measured using self-reported use rather than pill count, pharmacy records or ARV drug compound testing which may be more robust methods. However, the *HARP* findings are similar to what has been reported elsewhere in SSA based on self-reporting [413]. In South Africa, Gauteng province where HARP was conducted, adherence was previously reported as 77% [414].

7.6 Summary of findings (Table 7.8 and Figure 7.1)

- WLHIV with CD4+ <200 cells/mm3 had up to a 1.4-fold increased risk of HR-HPV prevalence, 1.6-fold increased risk of HR-HPV-incidence, 1.8-fold increase in HR-HPV persistence and over a 3-fold increase in CIN2+ prevalence.
- In BF, compared to long-duration ART users (>2 years), short-duration ART users (≤2 years) had higher HR-HPV prevalence (aPR=1.24, 95%CI: 1.04-1.47), while ART-naïve women had higher persistence of HR-HPV (aOR=1.80, 95%CI: 1.21-2.66).
- In SA, compared to long-duration ART users (>2 years), short-duration ART users had higher persistence of HR-HPV (aPR=1.75, 95%CI: 1.17-2.64) and prevalence of CIN2+ (aOR=1.81, 95%CI: 1.03-3.17).
- HR-HPV persistence was associated with lack of viral suppression in SA (HIV-1 PVL ≥1000 vs, <1000 copies/ml; aPR=2.87, 95%CI: 1.63-5.05)
- ART-naïve women in SA had a similarly high risk of CIN2+ prevalence compared to long-duration ART users (aOR=1.78, 95%CI: 1.06-2.99) at baseline.
- By endline, CIN2/3 incidence was similar among short and long-duration ART users in SA, but was higher among women who remained ART-naive compared to all ART users (9.6% vs. 4.5%; aOR=2.00, 95%CI: 0.80-5.04)

Conclusion: Prolonged and effective ART, with high CD4+ cell count, is important in controlling HR-HPV and the development of CIN2+.

7.7 Findings in context

The meta-analysis in **Chapter 3** which evaluated the association of ART use with HR-HPV and cervical lesion outcomes found that ART use over prolonged duration, and with sustained HIV-1 viral suppression, was associated with a reduction in the prevalence of HR-HPV and HSIL+/CIN2+, a reduction in SIL incidence and progression and an increase in SIL regression.

The results of this study confirm the findings in the meta-analysis that prolonged ART use is associated with a reduction in HR-HPV prevalence and persistence and CIN2+ prevalence and incidence over 16 months. ART users with suppressed HIV-1 PVL at baseline in SA were less likely to have HR-HPV persistence at endline, similar to findings among other WLHIV [209].

Table 7.2. Effect of HIV related factors on HR-HPV prevalence among 570 WLHIV in Burkina Faso and 613 in South Africa

	HR-HPV Prevalence							
	Burkina Faso (N=570)				South Africa (N=613)			
	N	n (%)	Model 1[1] aPR (95% CI)	Model 2[2] aPR (95% CI)	N	n (%)	Model 1[1] aPR (95% CI)	Model 2[2] aPR (95% CI)
Baseline CD4+ count (cells/mm3)[§]								
<200	**66**	50 (75.8)	**1.38 (1.15-1.66)**	-	57	50 (87.7)	**1.14 (1.01-1.30)**	-
200-350	**125**	76 (60.8)	1.10 (0.91-1.33)	-	148	120 (81.1)	1.07 (0.96-1.19)	-
351-500	**161**	92 (57.1)	1.04 (0.87-1.25)	-	183	141 (77.1)	1.02 (0.92-1.14)	-
>500	**217**	119 (54.8)	1.00	-	225	172 (76.4)	1.00	-
ART status at baseline								
ART >2 years	**194**	101 (52.1)	1.00	1.00	**225**	163 (72.4)	1.00	1.00
ART ≤2 years	**218**	142 (65.1)	**1.26 (1.07-1.50)**	**1.21 (1.03-1.44)**	**179**	147 (82.1)	**1.12 (1.01-1.24)**	1.09 (0.98-1.22)
ART-naive	**158**	95 (60.1)	1.16 (0.96-1.41)	1.12 (0.93-1.36)	209	173 (82.8)	1.11 (1.00-1.23)	1.10 (1.00-1.22)
Among ART users:								
HIV-1 viral suppression[†]								
<1000 copies/ml	**328**	196 (59.8)	1.00	1.00	**324**	246 (75.9)	1.00	1.00
≥1000 copies/ml	**55**	32 (58.2)	0.97 (0.76-1.24)	0.80 (0.59-1.10)	**76**	62 (81.6)	1.00 (0.87-1.16)	0.98 (0.84-1.14)
HIV-1 viral detection[†]								
≤40 copies/ml	**286**	169 (59.1)	1.00	1.00	**135**	110 (81.5)	1.00	1.00
>40 copies/ml	**97**	59 (60.8)	1.02 (0.84-1.23)	0.92 (0.74-1.15)	**265**	198 (74.7)	0.90 (0.81-1.00)	0.90 (0.81-1.00)
ART adherence[‡]								
Moderate adherence (60-90%)	**378**	223 (59.0)	1.00	1.00	**337**	253 (75.1)	1.00	1.00
Low Adherence (<60%)	**10**	7 (70.0)	1.17 (0.77-1.80)	1.11 (0.68-1.79)	**63**	54 (85.7)	1.11 (0.97-1.26)	1.09 (0.96-1.25)
Baseline CD4+ count (cells/mm^3)[§]								
<200	**47**	36 (76.6)	**1.43 (1.15-1.77)**	-	**51**	45 (88.2)	1.16 (1.00-1.35)	-
200-350	**80**	50 (62.5)	1.16 (0.93-1.45)	-	**102**	83 (81.4)	1.09 (0.95-1.25)	-
351-500	**119**	68 (57.1)	1.06 (0.86-1.31)	-	**113**	81 (71.7)	0.99 (0.86-1.15)	-
>500	**165**	88 (58.3)	1.00	-	**138**	101 (73.2)	1.00	-
Among ART-naïve:								
Baseline CD4+ count (cells/mm^3)								
<200	**19**	14 (73.7)	1.19 (0.84-1.69)	-	**6**	5 (83.3)	0.98 (0.64-1.50)	-
200-350	**45**	26 (57.8)	0.95 (0.68-1.32)	-	**46**	37 (80.4)	0.98 (0.81-1.18)	-
351-500	**42**	24 (57.1)	0.89 (0.62-1.27)	-	**70**	60 (85.7)	1.06 (0.93-1.21)	-
>500	**52**	31 (59.6)	1.00	-	**87**	71 (81.6)	1.00	-

Adjusted Prevalence Ratio (aPR): [1]Model1: In BF, associations with HR-HPV were adjusted for condom use, alcohol use, cervicitis, and cervical ectopy; and in SA, associations with HR-HPV were adjusted for age, smoking, condom use, vaginal cleansing, bacterial vaginosis (BV), infection with *Chlamydia trachomatis* and *Trichomonas vaginalis*, and number of lifetime sex partners ; [2]Model 2: same as Model 1 with additional adjustment for baseline CD4+ count; [§]CD4+ count was unavailable for 1 participant on ART in BF; [†]HIV-1 PVL data was unavailable for 29 ART users in BF and 4 in SA; [‡]Data on self-reported adherence was unavailable for 24 participants in BF and 4 in SA; there was no participants in either country in the high adherence category.

Table 7.3. Effect of HIV related factors on HR-HPV incidence among 460 WLHIV in Burkina Faso and 440 in South Africa

	Burkina Faso (N=460)				South Africa (N=440)			
	N	n (%)	Model 1[1] aPR (95% CI)	Model 2[2] aPR (95% CI)	N	n (%)	Model 1[1] aPR (95% CI)	Model 2[2] aPR (95% CI)
Baseline CD4+ count (cells/mm³)								
<200	**46**	24 (52.2)	1.14 (0.83-1.57)	-	**31**	22 (71.0)	**1.61 (1.24-2.09)**	-
200-350	**98**	51 (52.0)	1.15 (0.90-1.48)	-	**99**	46 (46.5)	1.03 (0.79-1.34)	-
351-500	**136**	64 (47.1)	1.01 (0.80-1.29)	-	**140**	70 (50.0)	1.10 (0.87-1.38)	-
>500	**180**	83 (46.1)	1.00	-	**170**	79 (46.5)	1.00	-
ART status at baseline								
ART >2 years	**209**	95 (45.5)	1.00	1.00	**175**	85 (48.6)	1.00	1.00
ART ≤2 years	**128**	69 (53.9)	1.22 (0.98-1.52)	1.20 (0.96-1.50)	**122**	64 (52.5)	1.08 (0.87-1.36)	1.01 (0.79-1.28)
ART-naive	**123**	58 (47.2)	1.05 (0.82-1.35)	1.03 (0.80-1.32)	**143**	68 (47.6)	0.95 (0.75-1.20)	0.95 (0.76-1.20)
Among ART users[§] :								
HIV-1 viral suppression*								
<1000 copies/ml	**274**	130 (47.5)	1.00	1.00	**240**	116 (48.3)	1.00	1.00
≥1000 copies/ml	**39**	19 (48.7)	1.02 (0.72-1.44)	1.00 (0.67-1.48)	**55**	33 (60.0)	1.27 (0.98-1.64)	1.24 (0.96-1.61)
HIV-1 viral detection*								
≤40 copies/ml	**240**	113 (47.1)	1.00	1.00	**96**	51 (53.1)	1.00	1.00
>40 copies/ml	**73**	36 (49.3)	1.01 (0.77-1.33)	0.98 (0.72-1.32)	**199**	98 (49.3)	0.95 (0.75-1.20)	0.94 (0.74-1.19)
Baseline CD4+ count (cells/mm³)								
<200	**31**	16 (51.6)	1.09 (0.74-1.61)	-	**28**	20 (71.4)	**1.77 (1.31-2.39)**	-
200-350	**63**	37 (58.7)	1.30 (0.99-1.70)	-	**75**	36 (48.0)	1.16 (0.85-1.60)	-
351-500	**99**	45 (45.5)	0.97 (0.73-1.29)	-	**88**	47 (53.4)	1.24 (0.92-1.66)	-
>500	**144**	66 (45.8)	1.00	-	**106**	46 (43.4)	1.00	-
Stable high CD4+ (≥500 cells/mm³)								
No	**213**	106 (49.8)	1.06 (0.84-1.33)	-	**235**	120 (51.1)	1.08 (0.81-1.44)	-
Yes	**124**	58 (46.8)	1.00	-	**62**	29 (46.8)	1.00	-
Among ART-naïve:								
Baseline CD4+ count (cells/mm³) [†]								
<200	**1**	1 (100.0)	-	-	**1**	1 (100.0)	-	-
200-350	**21**	7 (33.3)	0.64 (0.30-1.34)	-	**14**	7 (50.0)	1.24 (0.76-2.03)	-
351-500	**26**	15 (57.7)	1.13 (0.70-1.84)	-	**46**	18 (39.1)	0.84 (0.54-1.31)	-
>500	**33**	15 (45.5)	1.00	-	**62**	31 (50.0)	1.00	-
Stable high CD4+ (≥500 cells/mm³)[‡]								
No	**54**	27 (50.0)	1.10 (0.63-1.93)	-	**99**	44 (44.4)	0.78 (0.52-1.18)	-
Yes	**27**	11 (40.7)	1.00	-	**24**	13 (54.2)	1.00	-

Adjusted Prevalence Ratio (aPR): [1]Model1: In BF, associations with HR-HPV were adjusted for condom use, alcohol use, cervicitis, and cervical ectopy; and in SA, associations with HR-HPV were adjusted for age, smoking, condom use, vaginal cleansing, bacterial vaginosis (BV), infection with *Chlamydia trachomatis* and *Trichomonas vaginalis*, and number of lifetime sex partners ; [2]Model 2: same as Model 1 with additional adjustment for baseline CD4+ count; [§]ART use was defined as being on ART at both baseline and endline; *HIV-1 PVL data was unavailable for 24 ART users in BF and 2 in SA; [†]Baseline CD4+ among participants who were ART-naïve at baseline [‡]ART-naïve participants were defined as being ART-naive at both baseline and endline.

Table 7.4. Effect of HIV-related factors on HR-HPV persistence, using infections as unit of measure, among 404 WLHIV in Burkina Faso and 598 in South Africa

	Burkina Faso (N=404)				South Africa (N=598)			
	N	n (%)	Model 1[1] aOR (95% CI)	Model 2[2] aOR (95% CI)	N	n (%)	Model 1[1] aOR (95% CI)	Model 2[2] aOR (95% CI)
Baseline CD4+ count (cells/mm³)								
<200	**55**	30 (54.6)	**1.81 (1.10-2.99)**	-	**39**	11 (28.2)	1.10 (0.39-3.12)	-
200-350	**86**	39 (45.4)	1.12 (0.64-1.95)	-	**148**	47 (31.8)	1.27 (0.97-1.66)	-
351-500	**127**	51 (40.2)	1.05 (0.72-1.52)	-	**189**	63 (33.3)	1.36 (0.89-2.08)	-
>500	**136**	50 (36.8)	1.00	-	**222**	60 (27.0)	1.00	-
ART status at baseline								
ART >2 years	**170**	64 (37.7)	1.00	1.00	**200**	49 (24.5)	1.00	1.00
ART ≤2 years	**123**	41 (33.3)	0.79 (0.51-1.22)	0.73 (0.45-1.21)	**162**	57 (35.2)	**1.78 (1.24-2.57)**	**1.75 (1.17-2.64)**
ART-naive	**111**	65 (58.6)	**1.93 (1.28-2.91)**	**1.80 (1.21-2.66)**	**236**	75 (31.8)	1.55 (0.97-2.46)	1.50 (0.94-2.40)
Among ART users[§] :								
HIV-1 viral suppression*								
<1000 copies/ml	**244**	82 (33.6)	1.00	1.00	**294**	73 (24.8)	1.00	1.00
≥1000 copies/ml	**32**	13 (40.6)	1.21 (0.67-2.18)	0.99 (0.49-1.99)	**67**	33 (49.3)	**3.25 (1.76-6.02)**	**3.35 (1.85-6.04)**
HIV-1 viral detection*								
≤40 copies/ml	**205**	63 (30.7)	1.00	1.00	**124**	32 (25.8)	1.00	1.00
>40 copies/ml	**71**	32 (45.1)	**1.64 (1.06-2.54)**	**1.56 (1.01-2.42)**	**237**	74 (31.2)	1.24 (0.57-2.70)	1.22 (0.57-2.60)
Baseline CD4+ count (cells/mm³)								
<200	**34**	16 (47.1)	1.57 (0.93-2.65)	-	**34**	9 (26.5)	1.04 (0.34-3.21)	-
200-350	**56**	23 (41.1)	1.24 (0.72-2.14)	-	**103**	28 (27.2)	1.11 (0.75-1.64)	-
351-500	**93**	30 (32.3)	0.92 (0.64-1.32)	-	**103**	35 (34.0)	1.47 (0.79-2.74)	-
>500	**110**	36 (32.7)	1.00	-	**122**	34 (27.9)	1.00	-
Stable high CD4+ (≥500 cells/mm³)								
No	**196**	75 (38.3)	1.28 (0.83-1.98)	-	**298**	88 (29.5)	1.10 (0.54-2.23)	-
Yes	**97**	30 (30.9)	1.00	-	**64**	18 (28.1)	1.00	-
Among ART-naïve:								
Baseline CD4+ count (cells/mm³) †								
<200	**21**	14 (66.7)	1.37 (0.48-3.89)	-	**5**	2 (40.0)	2.54 (0.45-14.32)	-
200-350	**30**	16 (53.3)	0.62 (0.21-1.87)	-	**45**	19 (42.2)	1.41 (0.73-2.74)	-
351-500	**34**	21 (61.8)	1.23 (0.49-3.09)	-	**86**	28 (32.6)	1.30 (0.59-2.89)	-
>500	**26**	14 (53.9)	1.00	-	**100**	26 (26.0)	1.00	-
Stable high CD4+ (≥500 cells/mm³)‡								
No	**53**	34 (64.2)	1.50 (0.33-6.76)	-	**160**	53 (33.1)	2.20 (0.99-4.91)	-
Yes	**20**	9 (45.0)	1.00	-	**42**	7 (16.7)	1.00	-

Adjusted Odds Ratio (aOR) using generalised estimating equation: [1]Model 1: In BF, associations with HR-HPV were adjusted for alcohol use , condom, cervicitis, and cervical ectopy; In SA, associations with HR-HPV were adjusted for age, smoking, condom, vaginal cleansing, bacterial vaginosis, *Chlamydia trachomatis*, *Trichomonas vaginalis* and LTSP; [2]Model 2: same as Model 1 with additional adjustment for baseline CD4+ count; [§]ART use was defined as being on ART at both baseline and endline; *Baseline HIV-1 PVL data was unavailable for 12 participants in BF (representing 17 infections) and 1 in SA (representing 1 infection); †Baseline CD4+ among participants who were ART-naïve at baseline ‡ART-naïve participants were defined as being ART-naive at both baseline and endline.

Table 7.5. Effect of HIV-related factors on HR-HPV complete clearance among 263 WLHIV in Burkina Faso and 334 in South Africa

	Burkina Faso (N=263)				South Africa (N=334)			
	N	n (%)	Model 1[1] aPR (95% CI)	Model 2[2] aPR (95% CI)	N	n (%)	Model 1[1] aPR (95% CI)	Model 2[2] aPR (95% CI)
Baseline CD4+ count (cells/mm³)								
<200	**32**	6 (18.8)	0.83 (0.40-1.76)	-	**25**	3 (12.0)	**0.33 (0.11-0.96)**	-
200-350	**57**	8 (14.0)	0.57 (0.28-1.15)	-	**78**	20 (25.6)	0.77 (0.50-1.19)	-
351-500	**80**	23 (28.8)	1.16 (0.73-1.83)	-	**103**	23 (22.3)	0.77 (0.51-1.15)	-
>500	**94**	25 (26.6)	1.00	-	**128**	43 (33.6)	1.00	-
ART status at baseline								
ART >2 years	**110**	31 (28.2)	1.00	1.00	**126**	42 (33.3)	1.00	1.00
ART ≤2 years	**79**	18 (22.8)	0.79 (0.48-1.29)	0.81 (0.50-1.33)	**94**	23 (24.5)	0.70 (0.46-1.06)	0.82 (0.53-1.26)
ART-naive	**74**	13 (17.6)	0.69 (0.40-1.20)	0.70 (0.41-1.22)	**114**	24 (21.1)	**0.65 (0.42-0.99)**	**0.65 (0.42-0.99)**
Among ART users[§] :								
HIV-1 viral suppression[*]								
<1000 copies/ml	**157**	41 (26.1)	1.00	1.00	**177**	57 (32.2)	1.00	1.00
≥1000 copies/ml	**20**	7 (35.0)	1.42 (0.80-2.52)	1.49 (0.82-2.70)	**42**	7 (16.7)	0.49 (0.23-1.02)	0.52 (0.25-1.06)
HIV-1 viral detection[*]								
≤40 copies/ml	**136**	38 (27.9)	1.00	1.00	**78**	24 (30.8)	1.00	1.00
>40 copies/ml	**41**	10 (24.4)	1.06 (0.62-1.80)	1.14 (0.64-2.04)	**141**	40 (28.4)	1.00 (0.65-1.54)	1.00 (0.65-1.52)
Baseline CD4+ count (cells/mm³)								
<200	**21**	6 (28.6)	1.17 (0.58-2.38)	-	**22**	3 (13.6)	**0.31 (0.10-0.94)**	-
200-350	**38**	5 (13.2)	0.55 (0.23-1.30)	-	**59**	18 (30.5)	0.74 (0.45-1.21)	-
351-500	**56**	18 (32.1)	1.35 (0.82-2.22)	-	**61**	14 (23.0)	0.72 (0.43-1.22)	-
>500	**74**	20 (27.0)	1.00	-	**78**	30 (38.5)	1.00	-
Stable high CD4+ (≥500 cells/mm³)								
No	**125**	33 (26.4)	1.11 (0.68-1.80)	-	**177**	50 (28.3)	0.92 (0.56-1.51)	-
Yes	**64**	16 (25.0)	1.00	-	**43**	15 (34.9)	1.00	-
Among ART-naïve:								
Baseline CD4+ count (cells/mm³) [†]								
<200	**1**	0 (0.0)	-	-	**1**	0 (0.0)	-	-
200-350	**12**	1 (8.3)	0.33 (0.07-1.50)	-	**12**	2 (16.7)	0.46 (0.11-2.02)	-
351-500	**18**	2 (11.1)	0.61 (0.19-1.98)	-	**37**	8 (21.6)	0.76 (0.37-1.57)	-
>500	**19**	5 (26.3)	1.00	-	**48**	13 (27.1)	1.00	-
Stable high CD4+ (≥500 cells/mm³)[‡]								
No	**35**	3 (8.6)	0.33 (0.10-1.06)	-	**78**	17 (21.8)	0.83 (0.38-1.82)	-
Yes	**15**	5 (33.3)	1.00	-	**20**	6 (30.0)	1.00	-

Adjusted Prevalence Ratio (aPR): [1]Model1: In BF, associations with HR-HPV were adjusted for condom use, alcohol use, cervicitis, and cervical ectopy; and in SA, associations with HR-HPV were adjusted for age, smoking, condom use, vaginal cleansing, bacterial vaginosis (BV), infection with *Chlamydia trachomatis* and *Trichomonas vaginalis*, and number of lifetime sex partners ; [2]Model 2: same as Model 1 with additional adjustment for baseline CD4+ count; [§]ART use was defined as being on ART at both baseline and endline; [*]HIV-1 PVL data was unavailable for 12 ART users in BF and 1 in SA; associations of HIV-PVL in SA not adjusted for *Chlamydia trachomatis* due to small numbers; [†]Baseline CD4+ among participants who were ART-naïve at baseline [‡]ART-naïve participants were defined as being ART-naive at both baseline and endline.

Table 7.6. Effect of HIV-related factors on CIN2+ prevalence among 530 WLHIV in Burkina Faso and 566 in South Africa

	CIN2+ Prevalence							
	Burkina Faso (N=530)				South Africa (N=566)			
	N	n (%)	Model 1[1] aOR (95% CI)	Model 2[2] aOR (95% CI)	N	n (%)	Model 1[1] aOR (95% CI)	Model 2[2] aOR (95% CI)
Baseline CD4+ count (cells/mm3)[§]								
<200	**57**	8 (14.0)	**2.99 (1.05-8.58)**	-	**50**	21 (42.0)	**3.39 (1.70-6.75)**	-
200-350	**116**	5 (4.3)	0.72 (0.23-2.21)	-	**138**	38 (27.5)	1.61 (0.95-2.74)	-
351-500	**151**	6 (4.0)	0.69 (0.24-2.01)	-	**174**	30 (17.2)	0.90 (0.52-1.54)	-
>500	**205**	13 (6.3)	1.00	-	**204**	39 (19.1)	1.00	-
ART status at baseline								
ART >2 years	**182**	10 (5.5)	1.00	1.00	**207**	31 (15.0)	1.00	1.00
ART ≤2 years	**206**	16 (7.8)	1.44 (0.59-3.54)	1.22 (0.48-3.08)	**161**	47 (29.2)	**2.35 (1.39-3.97)**	**1.81 (1.03-3.17)**
ART-naive	**142**	6 (4.2)	1.33 (0.42-4.25)	1.20 (0.37-3.94)	**198**	50 (25.3)	**1.76 (1.05-2.94)**	**1.78 (1.06-2.99)**
Among ART users:								
HIV-1 viral suppression[†]								
<1000 copies/ml	**309**	22 (7.1)	1.00	1.00	**294**	62 (21.1)	1.00	1.00
≥1000 copies/ml	**52**	4 (7.7)	1.45 (0.43-4.89)	0.55 (0.12-2.45)	**71**	15 (21.1)	0.94 (0.49-1.80)	0.72 (0.36-1.44)
ART adherence[‡]								
Moderate adherence (60-90%)	**358**	26 (7.3)	1.00	1.00	**311**	64 (20.6)	1.00	1.00
Low Adherence (<60%)	**7**	0 (0.0)	-	-	**53**	13 (24.5)	1.31 (0.65-2.65)	1.27 (0.62-2.63)
Baseline CD4+ count (cells/mm^3)[§]								
<200	**42**	7 (16.7)	**4.34 (1.25-15.10)**	-	**45**	19 (42.2)	**3.78 (1.73-8.24)**	-
200-350	**76**	4 (5.3)	1.10 (0.29-4.13)	-	**92**	20 (21.7)	1.22 (0.61-2.44)	-
351-500	**113**	6 (5.3)	1.03 (0.31-3.39)	-	**107**	16 (15.0)	0.78 (0.38-1.61)	-
>500	**156**	9 (5.8)	1	-	**124**	23 (18.6)	1.00	-
Among ART-naïve:								
Baseline CD4+ count (cells/mm^3)								
<200	**15**	1 (6.7)	0.60 (0.05-7.56)	-	**5**	2 (40.0)	2.27 (0.27-19.47)	-
200-350	**40**	1 (2.5)	0.30 (0.03-3.16)	-	**46**	18 (39.1)	**2.97 (1.25-7.07)**	-
351-500	**38**	0 (0.0)	-	-	**67**	14 (20.9)	1.21 (0.52-2.81)	-
>500	**49**	4 (8.2)	1.00	-	**80**	16 (20.0)	1.00	-

Adjusted Odds Ratio (aOR): [1]Model 1 : In BF, associations with CIN2+ were adjusted for age, BV and cervical ectopy; and in SA, associations with CIN2+ were adjusted for injectable contraception and cervicitis; [2]Model 2: same as Model 1 with additional adjustment for baseline CD4+ ; [§]CD4+ count was unavailable for 1 participant on ART in BF; [†]HIV-1 PVL data was unavailable for 29 ART users in BF and 4 in SA; [‡]Data on self-reported adherence was unavailable for 24 participants in BF and 4 in SA

Table 7.7. Effect of HIV-related factors on CIN2/3 incidence among 412 WLHIV in Burkina Faso and 373 in South Africa

	Combined sites (N=785)				South Africa (N=373)			
			Model 1[1]	Model 2[2]			Model 1[1]	Model 2[2]
	N	n (%)	aOR (95% CI)	aOR (95% CI)	N	n (%)	aOR (95% CI)	aOR (95% CI)
Baseline CD4+ count (cells/mm³)[3]								
<200	**62**	2 (3.2)	0.93 (0.20-4.41)	-	**24**	1 (4.2)	0.58 (0.07-4.87)	-
200-350	**177**	5 (2.8)	0.72 (0.24-2.14)		**88**	4 (4.6)	0.68 (0.20-2.29)	
351-500	**239**	9 (3.8)	0.85 (0.33-2.21)		**120**	8 (6.7)	0.99 (0.36-2.69)	
>500	**307**	11 (3.6)	1.00	-	**141**	9 (6.4)	1.00	-
Endline CD4+ count (cells/mm³)								
<200	**44**	1 (2.3)	0.75 (0.46-3.16)		**23**	0 (0.0)	-	
200-350	**132**	6 (4.6)	1.19 (0.42-3.36)		**79**	4 (5.1)	0.73 (0.22-2.43)	
351-500	**180**	8 (4.4)	1.21 (0.46-3.16)		**107**	8 (7.5)	1.09 (0.41-2.92)	-
>500	**429**	12 (2.8)			**164**	10 (6.1)	1.00	-
ART status at baseline								
ART >2 years	**337**	7 (2.1)	1.00	1.00	**153**	6 (3.9)	1.00	1.00
ART ≤2 years	**214**	9 (4.2)	1.80 (0.63-5.10)	1.95 (0.66-5.74)	**99**	5 (5.1)	1.33 (0.39-4.52)	1.53 (0.43-5.46)
ART-naive	**234**	11 (4.7)	2.01 (0.75-5.41)	2.04 (0.76-5.49)	**121**	11 (9.1)	2.26 (0.80-6.39)	2.26 (0.80-6.41)
ART status at endline								
ART >2 years	**422**	13 (3.1)	1.00	1.00	**202**	9 (4.5)	1.00	1.00
ART ≤2 years	**171**	4 (2.3)	0.90 (0.28-2.88)	0.91 (0.30-3.04)	**67**	3 (4.5)	0.95 (0.25-3.64)	1.03 (0.25-4.22)
ART-naive	**192**	10 (5.2)	1.63 (0.67-3.96)	1.65 (0.67-4.04)	**104**	10 (9.6)	2.08 (0.80-5.39)	2.02 (0.77-5.29)
Among ART users[§] :								
HIV-1 viral suppression*								
<1000 copies/ml	**84**	3 (3.6)	1.00		**46**	2 (4.4)	1.00	1.00
≥1000 copies/ml	**448**	13 (2.9)	1.29 (0.35-4.76)	1.30 (0.34-4.97)	**204**	9 (4.4)	0.98 (0.19-4.90)	1.01 (0.20-5.14)
HIV viral detection*								
≤40 copies/ml	**301**	6 (2.0)	1.00	1.00	**83**	3 (3.6)	1.00	1.00
>40 copies/ml	**231**	10 (4.3)	2.05 (0.62-6.79)	2.17 (0.65-7.27)	**167**	8 (4.8)	2.03 (0.58-7.08)	1.96 (0.56-6.87)
Baseline CD4+ count (cells/mm³)								
<200	**47**	2 (4.3)	1.16 (0.23-5.92)	-	**22**	1 (4.6)	0.84 (0.09-7.81)	-
200-350	**122**	3 (2.5)	0.64 (0.16-2.57)	-	**65**	2 (3.1)	0.52 (0.09-2.84)	-
351-500	**163**	4 (2.5)	0.52 (0.13-2.09)	-	**77**	3 (3.9)	0.66 (0.15-2.97)	-
>500	**219**	7 (3.2)	1.00	-	**88**	5 (5.7)	1.00	-
Stable high CD4+ (≥500 cells/mm³)								
No	**388**	13 (3.4)	1.19 (0.32-4.45)	-	**202**	9 (4.5)	0.92 (0.19-4.53)	-
Yes	**163**	3 (1.8)	1.00	-	**50**	2 (4.0)	1.00	-
Among ART-naïve:								
Baseline CD4+ count (cells/mm³) [†]								
<200	**15**	0 (0.0)	-	-	**2**	0 (0.0)	-	-
200-350	**55**	2 (3.6)	1.18 (0.19-7.30)	-	**23**	2 (8.7)	1.12 (0.18-7.00)	-
351-500	**76**	5 (6.6)	1.54 (0.37-6.40)	-	**43**	5 (11.6)	1.55 (0.37-6.43)	-
>500	**88**	4 (4.6)	1.00	-	**53**	4 (7.6)	1.00	-
Stable high CD4+ (≥500 cells/mm³)[‡]*								
No	**124**	8 (6.5)	1.23 (0.24-6.37)	-	**78**	8 (10.3)	1.23 (0.24-6.37)	-
Yes	**54**	2 (3.7)	1.00	-	**23**	2 (8.7)	1.00	-

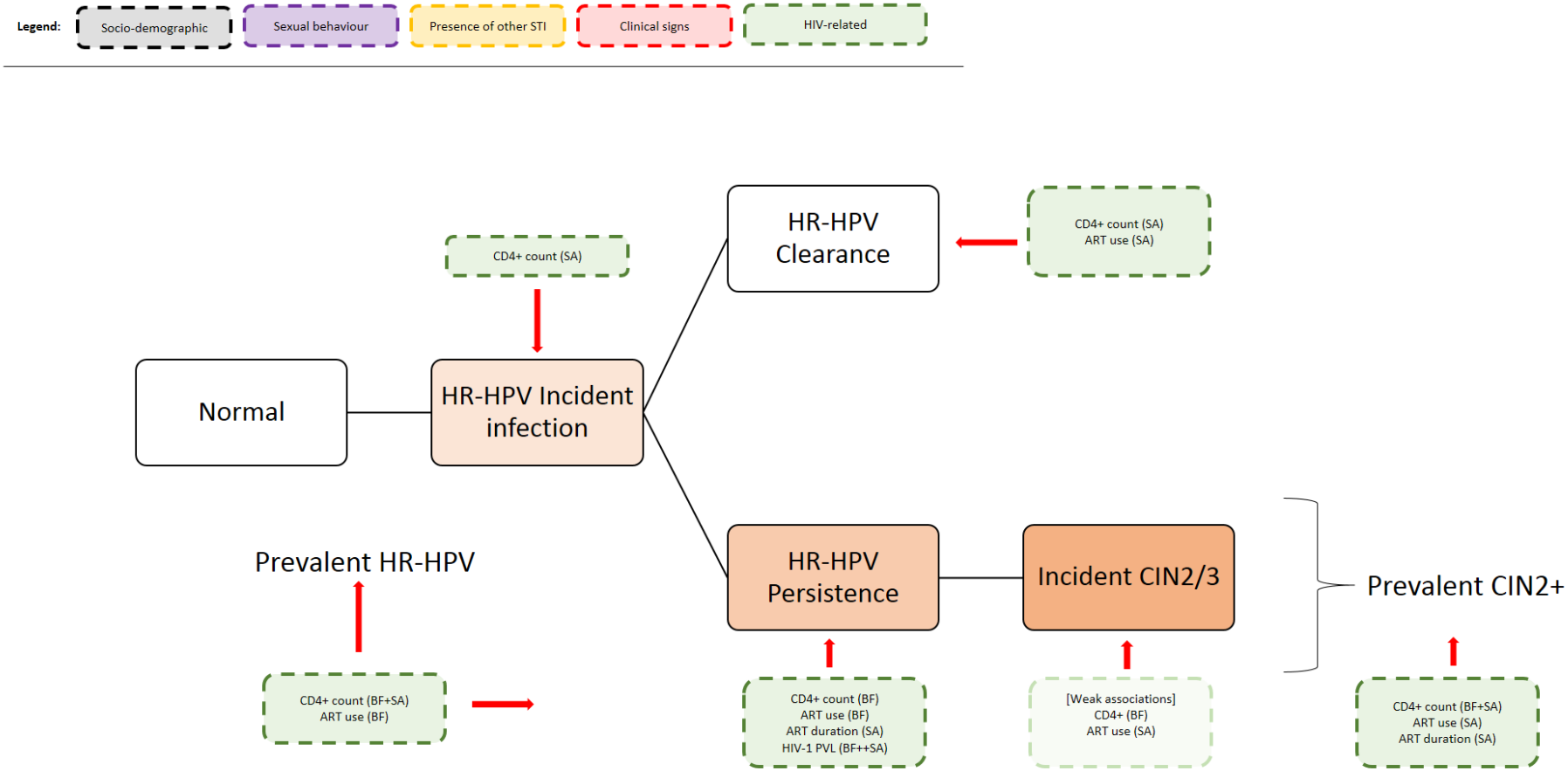
Adjusted Odds Ratio (aOR): [1]Model 1 : In BF, associations with CIN2+ incidence were adjusted for cervical ectopy; and in SA, associations were adjusted for injectable contraception and cervicitis; [2]Model 2: same as Model 1 with additional adjustment for baseline CD4+ ; [3]too few cases to allow for greater number of CD4+ groups as per previous tables; [§]CD4+ count was unavailable for 1 participant on ART in BF; [†]HIV-1 PVL data was unavailable for 29 ART users in BF and 4 in SA;* There were no incidence CIN2/3 among ART-naive in BF, hence the association when combining sites is the same as that in SA.

Table 7.8. Summary of HIV-related risk factors observed among WLHIV in Burkina Faso (BF) and South Africa (SA)

Risk factor	Outcome	Effect	Effect estimate (95% CI)		Country observed
HIV-related factors					
CD4+ count	HR-HPV Prevalence	Compared to women with CD4+ >500 cells/mm^3, women with CD4+ <200 cells/mm^3 had higher prevalence of HR-HPV	aPR aPR	1.38 (1.15-1.66) 1.14 (1.01-1.30)	BF SA
	HR-HPV Incidence	Compared to women with CD4+ >500 cells/mm^3, women with CD4+ <200 cells/mm^3 had higher incidence of HR-HPV	aPR	1.61 (1.24-2.09)	SA
	HR-HPV Persistence	Compared to women with CD4+ >500 cells/mm^3, women with CD4+ <200 cells/mm^3 had higher persistence of HR-HPV	aOR	1.81 (1.10-2.99)	BF
	HR-HPV complete clearance	Compared to women with CD4+ >500 cells/mm^3, women with CD4+ <200 cells/mm^3 had decreased likelihood of complete clearance of HR-HPV	aPR	0.33 (0.11-1.96)	SA
	CIN2+ Prevalence	Compared to women with CD4+ >500 cells/mm^3, women with CD4+ <200 cells/mm^3 had higher prevalence of CIN2+ Compared to women with CD4+ >500 cells/mm^3, women with CD4+ <200 cells/mm^3 had higher prevalence of CIN2+	aOR aOR	2.99 (1.05-8.58) 3.39 (1.70-6.75)	BF SA
	CIN2+ Incidence*	Compared to women with CD4+ >350 cells/mm^3, women with CD4+ ≤350 cells/mm^3 had higher incidence of CIN2+	aOR	5.80 (0.93-36.19)	BF
ART	HR-HPV Prevalence	Compared to long-duration ART users (>2 years), women with short-duration ART use (≤2 years) had higher prevalence of HR-HPV	aPR	1.21 (1.03-1.44)	BF
	HR-HPV Persistence	Compared to long-duration ART users (>2 years), women with short-duration ART use (≤2 years) had higher persistence of HR-HPV Compared to long-duration ART users (>2 years), women not taking ART had higher persistence of HR-HPV	aOR aOR	1.75 (1.17-2.64) 1.80 (1.21-2.66)	SA BF
	HR-HPV complete clearance	Compared to long-duration ART users (>2 years), women not taking ART had decreased likelihood of complete clearance of HR-HPV	aPR	0.65 (0.42-0.99)	SA
	CIN2+ Prevalence	Compared to long-duration ART users (>2 years), women with short-duration ART use (≤2 years) had higher persistence of HR-HPV Compared to long-duration ART users (>2 years), women not taking ART had higher persistence of HR-HPV	aOR aOR	1.81 (1.03-3.17) 1.78 (1.06-2.99)	SA SA
	CIN2+ Incidence*	Compared to women taking ART, women not taking ART at endline had higher CIN2+ incidence	aOR	2.00 (0.80-5.04)	SA
HIV-1 suppression	HR-HPV Persistence	Compared to women with HIV-1 viral suppression (<1000 copies/ml), women with lack of HIV-1 viral suppression had higher persistence of HR-HPV	aOR	3.35 (1.85-6.04)	SA
HIV-1 viral detection	HR-HPV Persistence	Compared to women with undetectable HIV-1 viral load (≤40 copies/ml), women with detectable HIV-1 had higher persistence of HR-HPV	aOR	1.56 (1.01-2.42)	BF

***** signifies associations that were not significant

Figure 7.1. HIV-related risk factors associated with HR-HPV and CIN2+ outcomes in Burkina Faso and South Africa



8 ASSOCIATIONS OF HPV GENOTYPES WITH CIN2+

WLHIV have been shown to be more commonly infected with types other than HPV16 or 18 [415] and their high-grade cytological lesions are frequently attributed to types other than HPV16/18 [13]. Primary prevention of HPV infection through vaccination could reduce the burden of infection and disease and on screening and treatment services. Current bivalent and quadrivalent HPV vaccines target two HR types (HPV16 and 18) responsible for about 70% of cervical cancers [37], whereas the nonavalent vaccine which protects against a wider range of HR-HPV types (HPV16/18/31/33/45/52/58), is estimated to prevent up to 90% of cervical cancers in women from the general population [37, 416]. These potential benefits have seldom been estimated among WLHIV, particularly in sub-Saharan Africa. An improved understanding of HPV type distribution associated with histological lesions in this population is needed to guide HPV vaccine programme decisions. In this chapter, I describe (i) the prevalence, persistence, incidence and genotype distribution of HPV, and (ii) their association with prevalent and incident CIN2+.

8.1 Objectives

In a cohort of WLHIV in Burkina Faso and South Africa at enrolment:

- To describe the prevalence of individual genotypes; ***(Objective 3.1)***
- To assess the associations of prevalent HPV genotypes with prevalent CIN2+. ***(Objective 3.2)***

In a cohort of WLHIV with ≤CIN1 at enrolment and followed over 18 months;

- To describe the incidence and persistence of individual genotypes; ***(Objective 3.1)***
- To assess the associations of persistent HPV genotypes with incident CIN2+. ***(Objective 3.2)***

- To evaluate the association of individual HPV genotype prevalence with HIV-related risk factors. ***(Objective 3.3)***

8.2 Methods

8.2.1 Statistical analysis

A full description of the HARP procedures is provided in **Chapter 5, section 5.2**. Specific statistical methods for univariate and multivariate risk factor analysis are detailed here.

Definition of HPV infection status at baseline and endline

Definitions for HR-HPV prevalence, incidence, persistence and clearance are provided in Chapter 5, section 5.3. HPV groupings (alpha-9 and alpha-7 types, and HPV vaccine types) are defined in **Table 5.1** and definition of HPV infection status are defined in **Table 5.2**, but in brief:

- Cumulative incidence of any HR-HPV was defined as the proportion of women who were negative for a specific type at baseline and positive for that type at endline visit (outcome group 1 of **Table 5.2**).
- HR-HPV genotype-specific persistence was defined as being positive for the same HR-HPV type at baseline and endline visits (outcome group 4**)**.
- Any type clearance was defined as being positive for a specific HR type at baseline and negative for that type at endline visit (outcome group 6).

Association of prevalent CIN2+ with prevalent HPV gentypes, and incident CIN2+ with persistent HPV genotypes.

Logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CI) for associations of prevalent and incident CIN2+ with individual HR-HPV types. These associations were

adjused for other co-factors found to be associated with CIN2+ in this study, as described in **Chapter 7, Table 7.1.**

Association of ART and CD4+ count with prevalent HPV genotypes at baseline

As HR-HPV prevalence was common, associations with HIV-related variables were estimated with PRs obtained from logistic regression using marginal standardization to estimate PRs, and the delta method to estimate 95% CI [379].

8.3 Results

8.3.1 Prevalence of HPV genotypes and association with CIN2+ at baseline

Of the 1238 participants enrolled, 1215 (98.1%) had valid HPV genotyping results (BF: 96.6%; SA: 99.7%) and 998 (82.1%) were positive for any HPV (BF: 75.3%; SA: 88.7%). As described in **Chapter 6**, HR-HPV prevalence was lower in BF (BF: 59.1%, 95%CI: 55.0-63.1 vs. SA: 79.1%, 95%CI: 75.7-82.2; p<0.001; result provided again here in **Table 8.1),** as was the proportion of women with multiple HR-HPV genotypes (BF: 41.9% vs. SA: 55.2%; p<0.001). HPV52 was the most prevalent type in both countries (BF: 20.4%; SA: 24.2%). HPV16/18 were detected in 15.0% of participants in BF vs. 29.0% in SA (p<0.001). Among those without infection with either HPV16 or HPV18, the proportion infected by any of the additional HR types included in the nonavalent vaccine (HPV31/33/45/52/58) was 30.1% in BF and 36.7% in SA (p<0.001). The prevalence of any of the non-vaccine HR types (HPV35/39/51/56/59/68) was 35.5% in BF and 49.1% in SA (p<0.001) irrespective of co-infection with any of the vaccine types, and was 14.0% in BF and 13.4% in SA among those not co-infected by any vaccine types.

Overall, 1128 (91.1%) participants had valid histology results at enrolment. CIN2+ prevalence was 5.8% (32/554) in BF (95%CI: 4.0-8.1%) and 22.5% (129/574) in SA (95%CI: 19.1-26.1%). Among women with both histology and HPV results (BF: 546; SA: 573), the prevalence of HR-HPV was higher

among those with higher CIN grades in both settings (BF: 48.4% in CIN normal, 71.8% in CIN1, 100% in CIN2 and 100% in CIN3+, p<0.001; SA: 72.4% in CIN normal, 81.4% in CIN1, 89.5% in CIN2 and 92.5% in CIN3+, p<0.001;) (**Figure 8.1 and Figure 8.2**). The prevalence of any HR-HPV genotypes included in the bi/quadrivalent and nonavalent HPV vaccines was 45.2% and 90.3% among women with CIN2+ in BF, respectively, and 37.2% and 79.8% in SA, respectively. In the absence of any vaccine types, the prevalence of any non-vaccine types was 9.7% (3/31) in BF and 10.9% (14/129) in SA.

In both countries, HPV58 was the genotype most strongly associated with CIN2 compared to ≤CIN1 cases (adjusted Odds Ratio [aOR]=5.40, 95%CI: 2.77-10.53), while types most strongly associated with CIN3+ were HPV16 in BF (aOR=34.56, 95%CI: 5.70-209.49) and HPV58 in SA (aOR=3.65, 95%CI: 1.42-9.37) (**Figure 8.3; Figure 8.4 and Table 8.2**). The prevalence of HPV58 was 22.1% and 21.1% among women with CIN2 in BF and SA respectively, and 23.1% and 15.1% among women with CIN3+.

The prevalence of CIN2+ was significantly higher among those positive for HPV16/18 compared to those who were negative for both types in BF (17.7% vs. 3.6%; aOR=7.90, 95%CI: 3.23-19.33) and in SA (29.1% vs. 19.9%, aOR=1.49, 95%CI: 0.95-2.34), although the latter was not statistically significant (**Table 8.2**). Among 875 women without co-infection of either HPV16 or 18, the prevalence of CIN2+ was higher among women positive for any of the additional HR-HPV types targeted by the nonavalent vaccine (31/33/45/52/58) compared to women negative for all of these types in both countries (BF: 8.8% vs. 1.0%; SA: 25.2% vs. 13.7%; aOR=2.78, 95%CI: 1.67-4.64 for both countries combined). The prevalence of CIN2+ was similar among women who were positive or negative for any of the non-vaccine HR-HPV types. However, among 498 women without infection with vaccine types (BF: 308, SA: 190), the prevalence of CIN2+ was higher among women positive for any of the non-vaccine types compared to women who were HR-HPV negative (11.1% vs. 3.4%; aOR=3.35, 95%CI: 1.36-8.26).

8.3.2 Incidence and persistence of HR-HPV genotypes at endline

Of the 963 women without CIN2+ at enrolment who returned at follow-up M18 visit (median of actual follow-up 16 months [IQR, 15.6-16.8]), genotyping data at both visits was available for 922 (95.7%) women. The proportion of women with an incident HR-HPV infection was similar in both sites (BF: 47.9% [228/476] vs. SA: 49.3% [220/446]; p=0.67). Type-specific incident infection was highest for HPV52 in both countries (BF: 12.7%; SA: 16.6%). Incident infection of HPV16/18 was observed in 14.6% of women in BF and 14.7% in SA. In the absence of HPV16/18 co-infection, incident infection of any of the additional HR-HPV types of the nonavalent vaccine was observed in 19.7% of women in BF and 23.9% in SA; incident infection of non-vaccine HR-HPV types in the absence of vaccine type co-infection was observed in 14.0% in BF and 13.5% in SA (**Table 8.1**).

Persistence of any HR-HPV was more frequent in BF than SA (BF: 51.5% [139/270] vs. SA: 44.7% [152/340]; p=0.10) (**Table 8.1**). Persistence was highest for HPV58 (72.2%) in BF and HPV35 (42.6%) in SA. Forty-nine women (18.2%) in BF and 80 (23.5%) in SA had at least one persistent and one cleared infection during the 16 months follow-up period.

8.3.3 Association of incident CIN2/3 with HR-HPV persistence at endline

Among 922 women with genotyping data at both visits, concomitant histology results were available for 780 (84.6%) women (BF: 405; SA: 375). The cumulative incidence of CIN2+ over 16 months was 1.2% (5/405) in BF and 5.9% (22/375) in SA (p=0.001). Cumulative incidence of CIN2+ was higher among those with HR-HPV persistence compared to those who cleared or who were HR-HPV negative at baseline in both settings (BF: 3.5% vs. 0.3%; SA: 13.4% vs. 2.0%; both countries combined aOR=7.90, 95%CI: 3.11-20.07, **Table 8.3)**.

CIN2+ incidence was found to be significantly associated with the persistence of any members of the alpha-9 HPV (HPV16-related) and any alpha-7 HPV (HPV-18 related) genotypes in both countries **(Table 8.3).** In particular, CIN2+ incidence was higher among women with persistent HPV16/18

compared to those who cleared either of those types, or who were negative for both at baseline (BF: 7.7% vs. 0.8%; SA: 18.2% vs. 4.7%; both sites combined: aOR=5.25, 95%CI: 2.14-12.91).

Among 721 women without persistent HPV16 or 18, CIN2+ incidence was higher among those with persistence of any of the additional 5 HR-HPV included in the nonavalent (both sites combined: 6.1% vs. 2.0% aOR=3.23, 95%CI: 1.23-8.54). In the absence of co-persistence by any of the vaccine types (606 women), CIN2+ incidence was higher among women with persistent non-vaccine types compared to those who cleared or who were HR-HPV negative at baseline in both settings (8.8% vs. 1.1%; aOR=7.87, 95%CI: 2.40-25.81).

8.3.4 HR-HPV persistence and CIN2/3 persistence at endline

Among the 36 women in SA with prevalent CIN2/3 at baseline and who remained untreated before endline visit, any type HR-HPV persistence was highest among those with persistent CIN2/3 at endline (79.0%; 15/19) but lower among those that had spontaneous regression to ≤CIN1 (42.9%; 6/14). The small number of cases percluded genotype-specific analysis.

8.3.5 Association of HIV-related factors with HR-HPV prevalence at baseline

The prevalence of HR-HPV types was similar among ART users and ART-naive participants at baseline in BF, whilst in SA, the prevalence of HPV16/18 was higher among ART-naïve participants (**Table 8.4, Table 8.5**). Among ART users in BF, the prevalence of any nonavalent HR-HPV in the absence of HPV16/18 was higher among women with low CD4+ count (<200 cells/mm^3) compared to those with high CD4+ count (>500 cells/mm^3) and this association was significant among ART users only, however no association was observed for HPV16/18 or the non-vaccine types. In SA, only the prevalence of non-vaccine HR-HPV was higher among ART-taking women with low CD4+ cell count (**Table 8.5**).

8.4 Discussion

Women living with HIV (WLHIV) in Burkina Faso and South Africa have a high prevalence, incidence and persistence of HR-HPV, and a high prevalence and persistence of multiple genotypes. While HR-HPV prevalence was higher in South Africa at baseline, HR-HPV persistence was higher among women in Burkina Faso. This may be due to the fact that estimates of HR-HPV persistence were restricted to women without evidence of CIN2+ at baseline, who underwent treatment. As there was a higher prevalence of CIN2+ at baseline in South Africa, their exclusion from the follow-up analysis potentially reduced the overall proportion of women with persistent infection at endline. By contrast, because of the lower prevalence of CIN2+ in Burkina Faso at baseline, fewer women were excluded in the follow-up analysis, which potentially allowed a greater number of persistent HR-HPV cases to accrue during the follow-up period. In addition, we cannot exclude the possibility that the 4-quadrant biopsy may have helped clear HR-HPV from the cervical epithelium, as a greater proportion of women in South Africa underwent a 4-quadrant biopsy (97% in South Africa vs. 60% in Burkina Faso).

HPV52 was the type most frequently detected overall in both countries, but HPV58 was the type most strongly associated with cervical lesions, and one of the most likely to persist in both countries. The difference in the distribution profiles between BF and SA were further explored by assessing the prevalence of HPV types among HR-HPV positive women, thereby comparing the two HPV profiles adjusting for the overall higher prevalence in SA. There were four HPV types which appeared more prevalent in SA: HPV16, 18, 33 and 56. The higher prevalence of CIN2 in SA may be partially explained by the higher prevalence of HPV33 in SA given the strength of association of this type with CIN2 (**Table 8.2**); and the higher prevalence of CIN3 in SA may be partially explained by the higher prevalence of HPV16 in SA. However, there are other cofactors that explain the higher CIN2 and CIN3 in SA, including the higher frequency of other STI, injectable contraception, and HIV-related factors, which have been further discussed in **Chapters 6 and 7.**

In a meta-analysis describing HPV type distribution among 5578 WLHIV from 20 studies including 5 from African countries, HPV16, 18 and 58 were the most prevalent among those with cytological HSIL+ [417]. Additionally, WLHIV with HSIL+ were less likely to harbour HPV16 compared to those with HSIL in the general population, and more likely to be infected with HPV18/33/51/52/58 [418]. The high prevalence of types other than HPV16 and HPV18 among WLHIV in this study is similar to what has been reported in a study among WLHIV in Belgium, 80% of whom were of African origin [415].

This study found that up to 45% of CIN2/3 in BF and 37% of CIN2/3 in SA could be prevented by the HPV16/18-targeting vaccines, and that a further 45% of CIN2/3 in BF and 43% of CIN2/3 in SA could be prevented by the additional 5 types (HPV31/33/45/51/52) contained in the nonavalent vaccine, similar to studies in the general population [416, 419]. However, not all CIN2/3 develop into ICC, and not all HPV types found in CIN2 have the same propensity to evolve towards ICC. Clifford et al, in their systematic review comparing the HPV type distribution in ICC biopsy and cervical specimens of 770 HIV-positive women from 21 studies in 12 African countries [14], report a higher pooled prevalence of HPV16/18 (61.7%) than found in this study, whilst the prevalence of the other nonavalent vaccine types was 11.2%, lower than what we report in our study. These findings indicate that the relative contribution of HPV16/18 increases with increasing lesion grade, whilst the contribution of the additional nonavalent vaccine types still represents a significant preventable fraction of ICC.

This study also shows that the persistence of any of the bi/quadrivalent and nonavalent HR-HPV types is strongly associated with the incidence of cervical lesions among WLHIV, as is the case for HIV uninfected women [5]. Moreover, this study found a significant proportion of women had an incident infection with any of the nonavalent vaccine types (35%) as well as incident infection of multiple HR-HPV types over the 16-month follow-up period. This emphasises the potential impact of vaccination in this population using an HPV vaccine with a broader-range protection potential, given that these types are also likely to persist in this population [167].

A smaller but non-negligible proportion (11%) of women with CIN2+ were exclusively infected by non-vaccine types at enrolment. In addition, the persistence of non-vaccine types in the absence of current vaccine types, was also strongly associated with incident CIN2+, and this was largely driven by persistence of HPV35 and 56. It cannot be ruled out that the CIN2+ lesions in this study could be attributed to other HPV genotypes which were undetected in the cervical samples, but which could be localised in the biopsy tissue. However, a large retrospective cross-sectional study using histologically conformed ICC collected from 38 countries worldwide reported that 7% of WLHIV with ICC were infected with non-vaccine types in the absence of any of the vaccine types, and this was highest for HPV35 [37]. Until further generation vaccines incorporate a wider range of HPV types, cervical screening will remain important among WLHIV where non-vaccine types are prevalent.

ART-naïve women were at increased risk of HPV16 or 18 infection in South Africa at baseline. Among ART users, a decrease in CD4+ cell count was also associated with an increased risk of any of the nonavalent other than HPV16/18 and the non-vaccine type prevalence in Burkina Faso, and of the non-vaccine types in South Africa. Given that women on ART in South Africa were less likely to control HIV (measured by level of detectable HIV-1 PVL and ART adherence), this may have accounted for the persistence of non-vaccine types, such as HPV35, and their association with CIN2/3 among women in South Africa.

However, there was no reduction in HPV16 or 18 prevalence with increasing CD4+ cell counts in either country. This is consistent with previous reports suggesting that HPV16 might be more weakly associated with immune suppression than other HR types due to its potential to evade the host immune response, thus explaining its greater contribution to high-grade lesions and cancer in immunocompetent women [420]. However, these associations may also be reflective of the overall increased risk of any HR-HPV prevalence among those with low CD4+ cell count, as reported **in Chapter 7** [291].

Given that the bi/quadrivalent and nonavalent types are the most important contributors to cervical lesions in this population, maintaining a stable high CD4+ cell count is necessary to reduce the risks of HR-HPV persistence and cervical lesion development. This should be achieved by early initiation of ART before CD4+ cell counts decline and maintaining good HIV-1 virological control thereafter. In addition, HPV vaccination could be offered to these women before CD4+ cell counts decline.

In conclusion, this study confirms that HR-HPV infection and cervical lesions are very common among WLHIV in Africa, and that a broader range of genotypes are potentially associated with CIN2+ development. While currently available bi/quadrivalent vaccines could prevent up to 45% (95%CI: 27.3-64.0) of treatable precursor lesions in BF and 37% (95%CI: 28.8-46.2) in SA, the nonavalent vaccine has the potential to prevent up to 90% (95%CI: 74.2-98.0) and 80% (95%CI: 71.9-86.4) of cases in WLHIV in BF and SA, respectively. HPV vaccination could reduce the incidence of HR-HPV related disease among WLHIV in addition to contributing cost saving to current screening and treatment programmes, but this would require a formal health economic evaluation.

8.5 Study limitations

As stated in previous chapters, the definition of cumulative HR-HPV incidence over 16 months is of limited duration to assess the actual roles of HR-HPV incidence and persistence on CIN2+ incidence.

The study could not rule out type-specific clearance and reinfection when estimating persistence during the 16-months interval between HPV testing. Longer duration of follow-up would have allowed to accrue a larger number of incident CIN2+ cases and more robustly assess the role of incident HR-HPV on CIN development over sufficient follow-up time.

HPV genotyping was measured in cervical swabs and not from the biopsies. This can preclude accurate attribution of CIN to individual genotypes, particularly when the co-existence of multiple

HR-HPV types was common. Other studies have accurately attributed individual HPV genotypes to specific CIN lesions using laser capture microdissection technique from biopsy tissue [421].

While HPV18 was found to be marginally associated with CIN2+ in BF (aOR=2.81, 95%CI: 0.83-9.44), the opposite was observed in SA (aOR=0.53, 95%CI: 0.27-1.05), however for both sites, those associations were not found to be significant, in part because of the small number of cases involved. HPV18 is a well-known contributor to CIN2 or CIN3 in many other studies [35]and it can only be speculated this was a spurious finding. In the longitudinal analyses, persistent HPV18 was found to be associated with incident CIN2/3, which supports its role in CIN2/3 development among WLHIV.

Approximately 10% of prevalent CIN2+ cases in SA were HR-HPV negative by INNO-LiPA at baseline. It is not uncommon to find patients with CIN2+ being HR-HPV negative, and in most studies among CIN2+ and ICC cases, the prevalence of HR-HPV does not reach 100%. In their systematic review comparing the HPV type distribution in ICC biopsy and cervical cell specimens of 770 HIV-positive women from 21 studies in 12 African countries [14], Clifford et al report that prevalence of any HPV was 89% in biopsy samples and 95% in cervical samples. Moreover, in their review of 10,575 biopsies of invasive cervical cancer from around the world, De SanJose et al [37] found that only 85% were positive for ANY HPV. Similarly, in a subanalysis of a large cervical cancer screening study (ATHENA), among 497 cases of CIN2+, 55 (11%) tested negative by Cobas HPV test and 12 (2.4%) were negative by all HPV tests (Cobas, Amplicor and Linear Array) [422]. In this analysis, 4.7% (6/129) participants with CIN2+ in SA were negative for any HPV and 10.2% (12/129) were negative for any HR-HPV. Thus this finding of 5% of cervical samples being negative for any HPV is not dissimilar to these large international studies. It is not expected that this finding has obliterated the ability to find associations with CIN2/3 in SA compared to BF, given in part to the large number of CIN2/3 cases in SA. Furthermore, the Study Endpoint Committee undertook an extensive review of all

cases of CIN2 and final classification was based on consensus of a minimum of 5 experts in order to minimize (but never can eliminate) the risk of misclassification.

This study could not explore the association of HPV genotypes with ICC, as the majority of cases were CIN2/3. The contribution of individual HPV types to precursor lesions (CIN2 in particular) may not translate into a role in cervical cancer. However, it is still important to know the contribution of certain types to treatable lesions (CIN2 and CIN3), as vaccination against such types could have an impact on colposcopy referrals and management services.

There was a higher number of multiple biopsies taken among women in SA because they were more frequently positive for screening tests, particularly HPV DNA, and it is possible that this may have led to clearance of undetected lesions between baseline and endline, which may harbour HR-HPV. While there are no known studies which have reported clearance of HR-HPV following cervical biopsy, other have reported HPV clearance following excision of cervical lesions [423]. However, the biopsies taken in the HARP study were small (2mm) and a direct comparison of biopsy tissue with excisional treatment which involves a larger and deeper tissue section may not be appropriate– although this hypothesis cannot be ruled out.

8.6 Summary of findings

- At baseline, the prevalence of HR-HPV was 59.1% in BF and 79.1% in in SA; and the prevalence of multiple genotypes was 41.9% in BF and 55.2% in SA.
- HPV52 was the most prevalent genotype in both countries, but HPV58 was most strongly associated with CIN2+ (aOR=5.40, 95%CI: 2.77-10.53).
- Among women with CIN2+, the prevalence of any HR-HPV genotypes included in the bi/quadrivalent (HPV16/18) or nonavalent (HPV16/18/31/35/45/52/58) HPV vaccines ranged from 37% to 90%, while approximately 10% of CIN2+ cases were infected with non-vaccine types only.
- At endline, HR-HPV persistence was 51.5% in BF and 44.7% in SA; CIN2+ incidence was 1.2% in 405 women in BF and 5.9% in 375 women in SA.
- Persistence was highest for HPV58 (72.2%) in BF and HPV35 (42.6%) in SA.
- Persistence of any HR-HPV was strongly associated with incident CIN2+ (aOR=7.90, 95%CI: 3.11-20.07), as was persistence of HPV16/18 (aOR=5.25, 95%CI: 2.14-12.91) and the additional HR types in the nonavalent vaccine (aOR=3.23, 95%CI: 1.23-8.54).

Conclusion: HR-HPV persistence is very common among African WLHIV and is linked to incident CIN2+. HPV vaccines could prevent between 37-90% of CIN2+ among African WLHIV. Cervical cancer screening will continue to remain important as there remains a proportion of CIN2/3 cases that may not be preventable by currently available vaccines.

8.7 Findings in context

A recent systematic review (Clifford et al, [14]) compared the HPV type distribution and the HPV vaccine type distribution in ICC biopsy and cervical cell specimens of 770 HIV-positive and 3846 HIV-negative women from 21 studies in 12 African countries. The authors report that the fraction of ICC attributable to the HPV types included in the current bivalent (HPV16/18) and nonavalent (HPV16/18/31/33/45/52/58) vaccines was similar among HIV-positive and HIV-negative women (bivalent: 61.7% and 67.3%; nonavalent: 88.9% and 89.5%, respectively). However, a non-negligible proportion of ICC from both HIV-positive and HIV-negative women were infected with non-vaccine types in the absence of any of the vaccine types (7.0% and 7.9% of ICC from HIV-positive and HIV-negative women, respectively), and this was highest for HPV35.

The findings in this study also show that HPV16/18 are highly prevalent in CIN2/3 and there is proportion of CIN2/3 infected with nonvalent types, in the absence of HPV16/18, and a proportion of CIN2/3 infected exclusively with non-vaccine types, especially with HPV35.

Primary prevention of HPV infection through vaccination could reduce HPV infection and HPV-related disease in Africa among WLHIV. However, cervical cancer screening will continue to remain important as there remains a proportion of ICC cases that may not be preventable by currently available vaccines.

Figure 8.1. HR-HPV prevalence by CIN grade among 546 women living with HIV in Burkina Faso

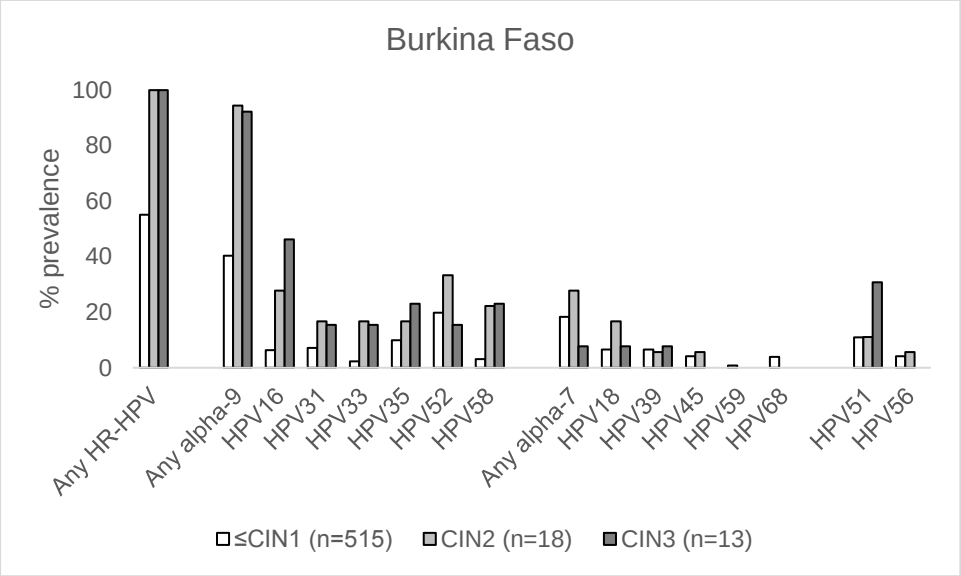


Figure 8.2. HR-HPV prevalence by CIN grade among 573 women living with HIV in South Africa

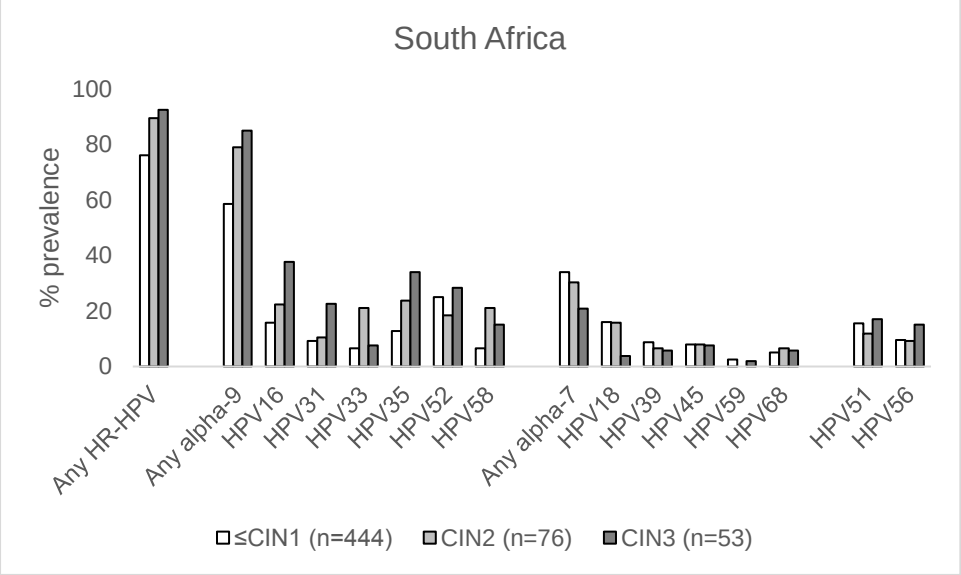
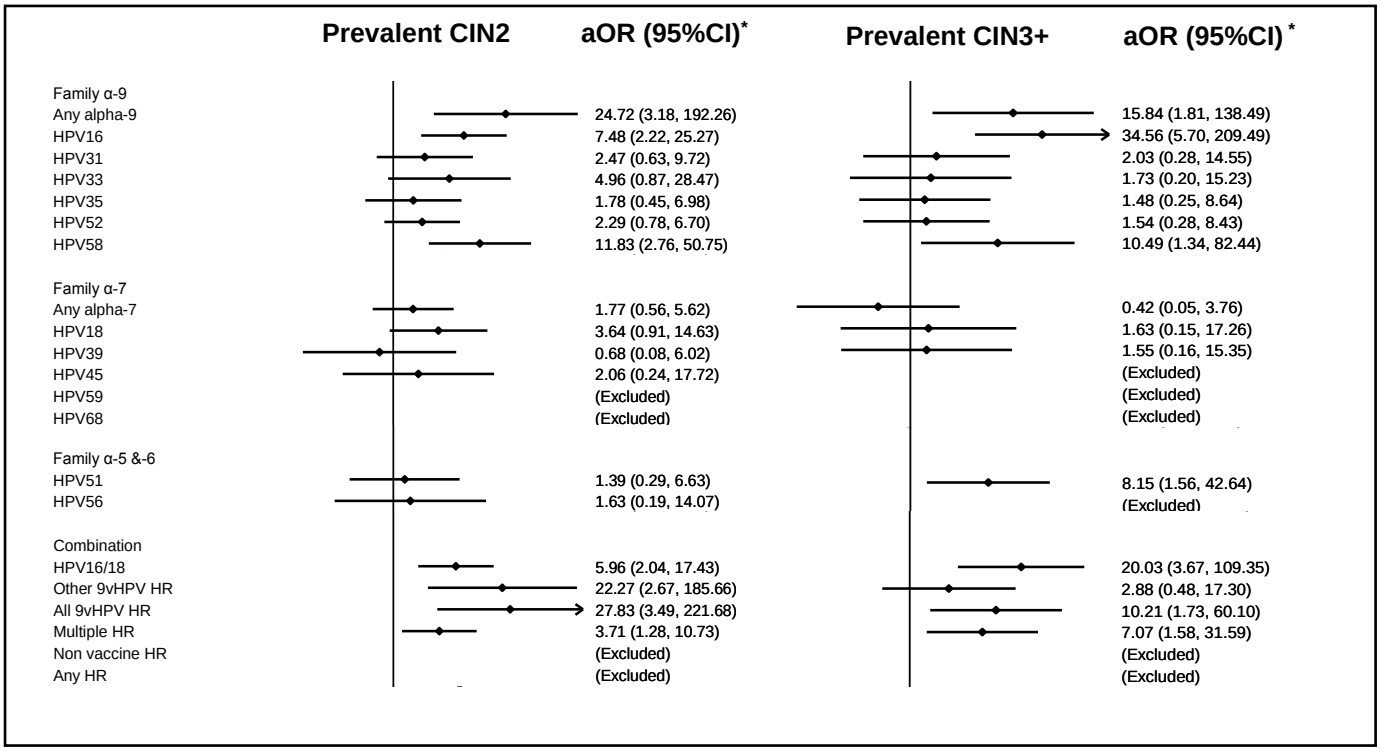
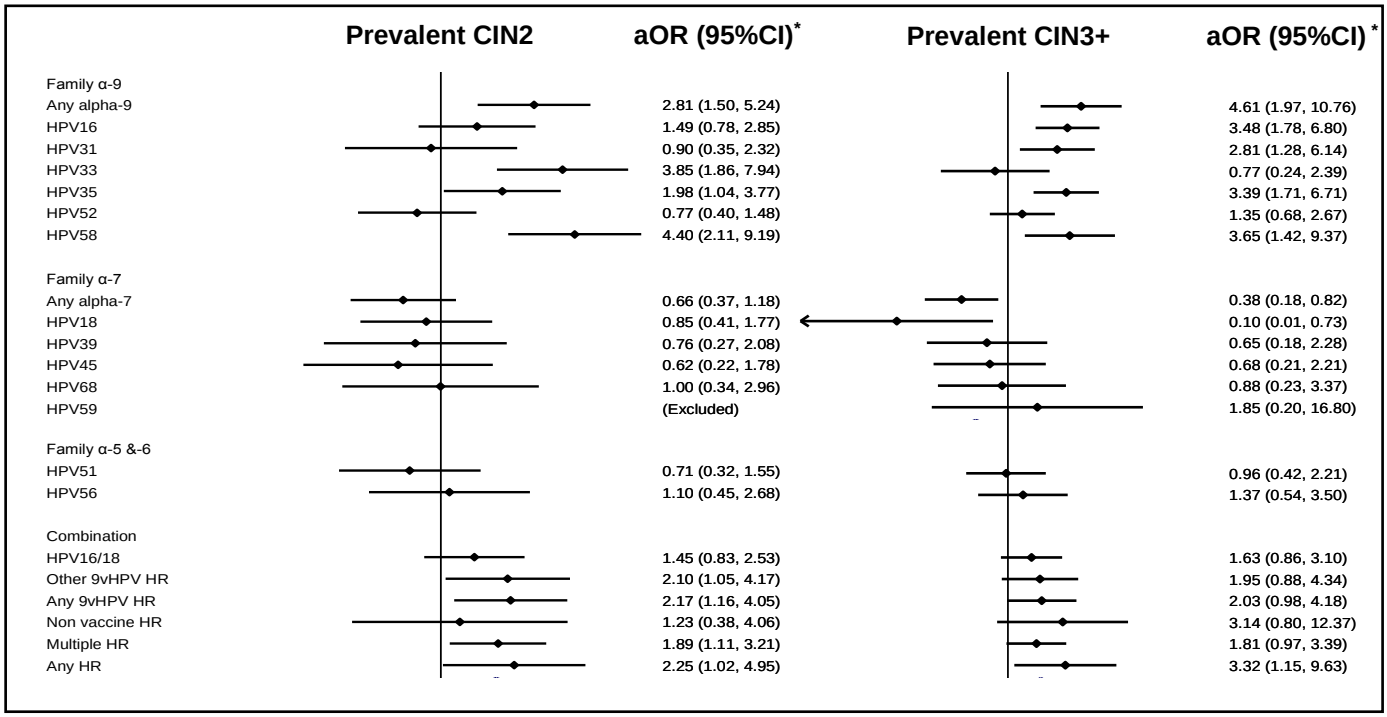


Figure 8.3. Association of HR-HPV prevalence with prevalent CIN2 and CIN3+ among 546 women living with HIV in Burkina Faso



*Adjusted for age, cervical ectopy, bacterial vaginosis, CD4+ count and ART duration.

Figure 8.4. Association of HR-HPV prevalence with prevalent CIN2 and CIN3+ among 573 women living with HIV in South Africa



*Adjusted for injectable contraception, cervicitis, CD4+ count and ART duration

Table 8.1. HR-HPV infection at baseline and endline follow-up among women living with HIV (WLHIV) in Burkina Faso (BF) and South Africa (SA)

	HPV Prevalence		HPV Incidence				HPV Persistence			
	BF	SA	BF		SA		BF		SA	
	N=594 n (%)	N=621 n (%)	At risk[a] N	Incidence n (%)	At risk[a] N	Incidence n (%)	Positive at M0 N	Persistence n (%)	Positive at M0 N	Persistence n (%)
Any HR types[b]	351 (59.1)	491 (79.1)	476	228 (47.9)	446	220 (49.3)	**270**	139 (51.5)	340	152 (44.7)
Any alpha-9	264 (44.4)	392 (63.1)	476	152 (31.9)	446	154 (34.5)	**198**	98 (49.5)	**260**	99 (38.1)
HPV16	51 (8.6)	119 (19.2)	445	43 (9.7)	377	49 (13.0)	**31**	17 (54.8)	**69**	23 (33.3)
HPV31	47 (7.9)	65 (10.5)	445	44 (9.9)	404	22 (5.5)	**31**	19 (61.3)	**42**	6 (14.3)
HPV33	19 (3.2)	51 (8.2)	464	8 (1.7)	419	17 (4.1)	**12**	3 (25.0)	**27**	4 (14.8)
HPV35	62 (10.4)	103 (16.6)	429	24 (5.6)	385	34 (8.8)	**47**	18 (38.3)	**61**	26 (42.6)
HPV52	121 (20.4)	150 (24.2)	378	48 (12.7)	337	56 (16.6)	**98**	37 (37.8)	**109**	39 (35.8)
HPV58	27 (4.6)	55 (8.9)	458	15 (3.3)	417	14 (3.4)	**18**	13 (72.2)	**29**	11 (37.9)
Any alpha-7	117 (19.7)	198 (31.9)	476	117 (24.6)	446	122 (27.4)	**89**	37 (41.6)	**145**	46 (31.7)
HPV18	42 (7.1)	90 (14.5)	445	29 (6.5)	378	18 (4.8)	**31**	17 (54.8)	**68**	24 (35.3)
HPV39	43 (7.2)	50 (8.1)	442	24 (5.4)	408	25 (6.1)	**34**	7 (20.6)	**38**	8 (21.1)
HPV45	26 (4.4)	48 (7.7)	457	12 (2.6)	415	22 (5.3)	**19**	11 (57.9)	**31**	11 (35.5)
HPV59	6 (1.0)	12 (1.9)	473	4 (0.9)	436	5 (1.2)	**3**	0 (0.0)	**10**	2 (20.0)
HPV68	23 (3.9)	35 (5.6)	457	24 (5.3)	424	28 (6.6)	**19**	3 (15.8)	**22**	4 (18.2)
Alpha-5 and -6										
HPV51	70 (11.8)	97 (15.6)	423	30 (7.1)	378	22 (5.8)	**53**	15 (28.3)	**68**	18 (26.5)
HPV56	25 (4.2)	60 (9.7)	456	45 (9.9)	408	16 (3.9)	**20**	11 (55.0)	**38**	9 (23.7)
Combinations										
HPV16/18	89 (15.0)	180 (29.0)	473	69 (14.6)	422	62 (14.7)	**59**	33 (55.9)	**113**	45 (39.8)
Other 9vHPV[c]	179 (30.1)	228 (36.7)	473	93 (19.7)	422	101 (23.9)	**155**	70 (42.5)	**198**	61 (30.8)
Any 9vHPV HR[d]	268 (45.1)	408 (65.7)	476	162 (34.0)	446	163 (36.6)	**200**	103 (51.5)	**275**	106 (38.6)
Non-Vaccine[e]	83 (14.0)	83 (13.4)	473	66 (14.0)	422	57 (13.5)	**112**	36 (32.1)	**141**	46 (32.6)
Multiple HR[f]	147/351 (41.9)	271/491 (55.2)	228	81 (35.5)	220	79 (35.9)	**270**	27/139 (19.4)	340	27/152 (17.8)
Low risk types										
HPV6	34 (5.7)	33 (5.3)	450	22 (4.9)	422	13 (3.1)	**26**	6 (23.1)	**24**	2 (8.3)
HPV11	9 (1.5)	33 (5.3)	470	3 (0.6)	420	15 (3.6)	**6**	1 (16.7)	**26**	4 (15.4)

[a]negative for that HPV type at enrolment (3 participants in BF with positive 16 *AND* 18 at enrolment, and 24 in SA; no participant was infected by ALL nonavalent HR types or ALL non vaccine types; [b]Any HR-HPV type prevalence defined as positive for at least one HR type at baseline; any HR incidence defined as incident infection from at least one HR type among those at risk for any HR infection (no participant was infected by all HR types at baseline); persistence defined as persistence of at least one HR type among those positive for any HR type at baseline; [c]Other 9vHPV=Positive for any of HPV31/33/45/52/ 58 in absence of HPV16/18; [d]9vHPV HR=Positive for any of HPV16/18/31/33/45/52/58; [e]Non Vaccine=Positive for any of HPV35/39/51/56/59/68 in absence of any vaccine type.; [f]Calculated among those positive for any HR type.

Table 8.2. Association of HPV type prevalence with prevalent CIN2 and CIN3+ among 546 women living with HIV in Burkina Faso and 573 in South Africa

	Burkina Faso							South Africa						
	CIN2 (n=18)			CIN3+ (n=13)			CIN2+ (n-31)	CIN2 (n=76)			CIN3+ (n=53)			CIN2+ (n=129)
	HPV negative n (%)	HPV positive n (%)	aOR (95% CI)[a]	HPV negative n (%)	HPV positive n (%)	aOR (95% CI)[a]	aOR (95% CI)[a]	HPV negative n (%)	HPV positive n (%)	aOR (95% CI)[b]	HPV negative n (%)	HPV positive n (%)	aOR (95% CI)[b]	aOR (95% CI)[b]
Any HR-HPV	**0 (0.0)**	**18 (6.0)**	**(-)**	**0 (0.0)**	**13 (4.4)**	**(-)**	**(-)**	**8 (7.0)**	**68 (16.8)**	**2.25 (1.02-4.95)**	**4 (3.6)**	**49 (12.7)**	**3.32 (1.15-9.63)**	**2.64 (1.37-5.07)**
Any alpha-9	1 (0.3)	17 (7.6)	**24.72 (3.18-192.26)**	1 (0.3)	12 (5.5)	**15.84 (1.81-138.49)**	**19.89 (4.51-87.84)**	16 (8.0)	60 (18.8)	**2.81 (1.50-5.24))**	8 (4.2)	45 (14.8)	**4.61 (1.97-10.76)**	**3.36 (1.99-5.66)**
HPV16	13 (2.6)	5 (13.2)	**7.48 (2.22-25.27)**	7 (1.4)	6 (15.4)	**34.56 (5.70-209.49)**	**10.82 (4.03-29.06)**	59 (13.6)	17 (19.5)	1.49 (0.78-2.85)	33 (8.1)	20 (22.2)	**3.48 (1.78-6.80)**	**2.06 (1.25-3.39)**
HPV31	15 (3.0)	3 (7.5)	2.47 (0.63-9.72)	11 (2.3)	2 (5.1)	2.03 (0.28-14.55)	2.27 (0.71-7.26)	68 (14.4)	8 (16.3)	0.90 (0.35-2.32)	41 (9.2)	12 (22.6)	**2.81 (1.28-6.14)**	1.63 (0.85-3.11)
HPV33	15 (2.9)	3 (20.0)	4.96 (0.87-28.47)	11 (2.1)	2 (14.3)	1.73 (0.20-15.23)	3.15 (0.76-13.06)	60 (12.6)	16 (35.6)	**3.85 (1.86-7.94)**	49 (10.6)	4 (12.1)	0.77 (0.24-2.39)	**2.46 (1.29-4.70)**
HPV35	15 (3.1)	3 (5.6)	1.78 (0.45-6.98)	10 (2.1)	3 (5.6)	1.48 (0.25-8.64)	1.55 (0.51-4.70)	58 (13.0)	18 (24.0)	**1.98 (1.04-3.77)**	35 (8.3)	18 (24.0)	**3.39 (1.71-6.71)**	**2.60 (1.56-4.34)**
HPV52	12 (2.8)	6 (5.6)	2.29 (0.78-6.70)	11 (2.6)	2 (1.9)	1.54 (0.28-8.43)	1.91 (0.76-4.83)	62 (15.7)	14 (11.2)	0.77 (0.40-1.48)	38 (10.2)	15 (11.9)	1.35 (0.68-2.67)	0.97 (0.59-1.59)
HPV58	14 (2.7)	4 (20.0)	**11.83 (2.76-50.75)**	10 (2.0)	3 (15.8)	**10.49 (1.34-82.44)**	**10.74 (3.18-36.33)**	60 (12.6)	16 (35.6)	**4.40 (2.11-9.19)**	45 (9.8)	8 (21.6)	**3.65 (1.42-9.37)**	**4.13 (2.16-7.88)**
Any alpha-7	13 (3.0)	5 (5.1)	1.77 (0.56-5.62)	12 (2.8)	1 (1.1)	0.42 (0.05-3.76)	1.19 (0.43-3.27)	53 (15.3)	23 (13.2)	0.66 (0.37-1.18)	42 (12.5)	11 (6.8)	**0.38 (0.18-0.82)**	**0.55 (0.34-0.89)**
HPV18	15 (3.0)	3 (8.1)	3.64 (0.91-14.63)	12 (2.4)	1 (2.9)	1.63 (0.15-17.26)	2.81 (0.83-9.44)	64 (14.7)	12 (14.5)	0.85 (0.41-1.77)	51 (12.0)	2 (2.7)	**0.10 (0.01-0.73)**	0.53 (0.27-1.05)
HPV39	17 (3.4)	1 (2.9)	0.68 (0.08-6.02)	12 (2.4)	1 (2.9)	1.55 (0.16-15.35)	0.90 (0.18-4.45)	71 (14.9)	5 (11.4)	0.76 (0.27-2.08)	50 (11.0)	3 (7.1)	0.65 (0.18-2.28)	0.74 (0.33-1.70)
HPV45	17 (3.3)	1 (4.6)	2.06 (0.24-17.72)	13 (2.6)	0 (0.0)	(-)	1.22 (0.15-10.04)	70 (14.6)	6 (14.6)	0.62 (0.22-1.78)	49 (10.7)	4 (10.3)	0.68 (0.21-2.21)	0.65 (0.28-1.49)
HPV59	18 (3.4)	0 (0.0)	(-)	13 (2.5)	0 (0.0)	(-)	(-)	76 (14.9)	0 (0.0)	(-)	52 (10.7)	1 (8.3)	1.85 (0.20-16.80)	0.50 (0.06-4.16)
HPV68	18 (3.5)	0 (0.0)	(-)	13 (2.6)	0 (0.0)	(-)	(-)	71 (14.4)	5 (18.5)	1.00 (0.34-2.96)	50 (10.6)	3 (12.0)	0.88 (0.23-3.37)	1.00 (0.40-2.50)
HPV51	16 (3.4)	2 (3.5)	1.39 (0.29-6.63)	9 (1.9)	4 (6.7)	**8.15 (1.56-42.64)**	2.54 (0.87-7.37)	67 (15.2)	9 (11.5)	0.71 (0.32-1.55)	44 (10.5)	9 (11.5)	0.96 (0.42-2.21)	0.78 (0.43-1.44)
HPV56	17 (3.3)	1 (4.6)	1.63 (0.19-14.07)	13 (2.6)	0 (0.0)	(-)	0.90 (0.10-7.85)	69 (14.7)	7 (14.3)	1.10 (0.45-2.68)	45 (10.1)	8 (16.0)	1.37 (0.54-3.50)	1.20 (0.60-2.42)
Combinations														
HPV16/18	11 (2.4)	7 (9.7)	**5.96 (2.04-17.43)**	6 (1.3)	7 (9.7)	**20.03 (3.67-109.35)**	**7.90 (3.23-19.33)**	49 (13.0)	27 (18.8)	1.45 (0.83-2.53)	32 (8.9)	21 (15.2)	1.63 (0.86-3.10)	1.49 (0.95-2.34)
9vHPV 5 HR	1 (0.3)	10(6.5)	**22.27 (2.67-185.66)**	2 (0.7)	4 (2.7)	2.88 (0.48-17.30)	**12.30 (2.88-52.46)**	15 (8.4)	34 (17.3)	**2.10 (1.05-4.17)**	11 (6.3)	21 (11.4)	1.95 (0.88-4.34)	**1.98 (1.15-3.42)**
Any 9vHPV 7 HR	1 (0.3)	17 (7.5)	**27.83 (3.49-221.68)**	2 (0.7)	11 (5.0)	**10.21 (1.73-60.10)**	**16.38 (4.43-60.58)**	15 (8.4)	61 (17.9)	**2.17 (1.16-4.05)**	11 (6.3)	42 (13.0)	2.03 (0.98-4.18)	**2.07 (1.26-3.41)**
Non Vaccine	0 (0.0)	1 (1.3)	**(-)**	0 (0.0)	2 (2.6)	(-)	(-)	8 (7.0)	7 (10.8)	1.23 (0.38-4.06)	4 (3.6)	7 (10.8)	3.14 (0.80-12.37)	1.93 (0.80-4.67)
Multiple HR[c]	10 (2.4)	8 (6.7)	**3.71 (1.28-10.73)**	6 (1.5)	7 (5.9)	**7.07 (1.58-31.59)**	**4.26 (1.79-10.13)**	34 (11.3)	42 (19.1)	**1.89 (1.11-3.21)**	22 (7.6)	31 (14.8)	1.81 (0.97-3.39)	**1.85 (1.20-2.85)**
Low risk types														
HPV6	17 (3.4)	1 (3.3)	1.07 (0.13-9.07)	12 (2.4)	1 (3.3)	2.13 (0.19-23.91)	1.52 (0.30-7.59)	71 (14.5)	5 (17.2)	1.08 (0.35-3.34)	52 (11.0)	1 (4.0)	0.36 (0.05-2.84)	0.78 (0.28-2.18)
HPV11	18 (3.4)	0 (0.0)	(-)	13 (2.5)	0 (0.0)	(-)	(-)	73 (14.8)	3 (10.7)	0.62 (0.17-2.21)	50 (10.7)	3 (10.7)	0.59 (0.12-2.80)	0.62 (0.22-1.76)

Adjusted Odds Ratio; [a]adjusted for age, cervical ectopy, bacterial vaginosis, CD4+ count and ART duration in BF; [b]adjusted for injectable contraception, cervicitis, CD4+ count and ART duration in SA; [c]Reference group for any multiple HR-HPV is negative for any HR-HPV *OR* single HR-HPV infection

Table 8.3. Risk of incident CIN2+ according to HR-HPV infection status over 16 months among 405 women living with HIV (WLHIV) without CIN2+ at enrolment in Burkina Faso and 375 WLHIV in South Africa

	Burkina Faso						South Africa						Sites combined						
	Always negative or incident infection		Cleared infection or type swap*		Persistent infection		Always negative or incident infection		Cleared infection or type swap*		Persistent infection		Always negative or incident infection		Cleared infection or type swap*		Persistent infection		aOR (95%CI)[d,e]
	N[a]	n (%)	N[b]	n (%)	N[c]	n (%)	N[a]	n (%)	N[b]	n (%)	N[c]	n (%)	N[a]	n (%)	N[b]	n (%)	N[c]	n (%)	
Any HR type[f]	**177**	**0 (0.0)**	**113**	**1 (0.9)**	**115**	**4 (3.5)**	**91**	**1 (1.1)**	**157**	**4 (2.6)**	**127**	**17 (13.4)**	**268**	**1 (0.4)**	**270**	**5 (1.9)**	**242**	**21 (8.7)**	**7.90 (3.11-20.07)**
Any alpha-9	236	1 (0.4)	85	1 (1.2)	84	3 (3.6)	158	3 (1.9)	135	5 (3.7)	82	14 (17.1)	394	4 (1.0)	220	6 (2.7)	166	17 (10.2)	**6.91 (3.05-15.63)**
HPV16	380	4 (1.1)	10	0 (0.0)	15	1 (6.7)	321	16 (5.0)	39	3 (7.7)	15	3 (20.0)	701	20 (2.9)	49	3 (6.1)	30	4 (13.3)	**4.75 (1.47-15.36)**
HPV31	379	5 (1.3)	11	0 (0.0)	15	0 (0.0)	343	21 (6.1)	28	1 (3.6)	4	0 (0.0)	722	26 (3.6)	39	1 (2.6)	19	0 (0.0)	-
HPV33	395	4 (1.0)	7	1 (14.3)	3	0 (0.0)	354	21 (5.9)	19	1 (5.3)	2	0 (0.0)	749	25 (3.3)	26	2 (7.7)	5	0 (0.0)	-
HPV35	360	4 (1.1)	28	0 (0.0)	17	1 (5.9)	327	18 (5.5)	28	0 (0.0)	20	4 (20.0)	687	22 (3.2)	56	0 (0.0)	37	5 (13.5)	**4.72 (1.62-13.71)**
HPV52	320	3 (0.9)	52	1 (1.9)	33	1 (3.0)	278	17 (6.1)	60	2 (3.3)	37	3 (8.1)	598	20 (3.3)	112	3 (2.7)	70	4 (5.7)	1.67 (0.55-5.05)
HPV58	392	5 (1.3)	3	0 (0.0)	10	0 (0.0)	349	18 (5.2)	17	0 (0.0)	9	4 (44.4)	741	23 (3.1)	20	0 (0.0)	19	4 (21.1)	**9.56 (2.77-32.98)**
Any alpha-7	333	4 (1.2)	42	0 (0.0)	30	1 (3.3)	249	11 (4.4)	86	5 (5.8)	40	6 (15.0)	582	15 (2.6)	128	5 (3.9)	70	7 (10.0)	**3.34 (1.33-8.37)**
HPV18	381	4 (1.1)	12	0 (0.0)	12	1 (8.3)	318	15 (4.7)	38	3 (7.9)	19	4 (21.1)	699	19 (2.7)	50	3 (6.0)	31	5 (16.1)	**5.39 (1.83-15.82)**
HPV39	378	5 (1.3)	21	0 (0.0)	6	0 (0.0)	340	20 (5.9)	28	2 (7.1)	7	0 (0.0)	718	25 (3.5)	49	2 (4.1)	13	0 (0.0)	-
HPV45	388	5 (1.3)	7	0 (0.0)	10	0 (0.0)	348	20 (5.8)	16	1 (6.3)	11	1 (9.1)	736	25 (3.4)	23	1 (4.4)	21	1 (4.8)	1.28 (0.16-10.13)
HPV59	404	5 (1.2)	1	0 (0.0)	0	0 (0.0)	365	21 (5.8)	8	0 (0.0)	2	1 (50.0)	769	26 (3.4)	9	0 (0.0)	2	1 (50.0)	16.03 (0.96-268.33)
HPV68	388	5 (1.3)	14	0 (0.0)	3	0 (0.0)	357	21 (5.9)	14	0 (0.0)	4	1 (25.0)	745	26 (3.5)	28	0 (0.0)	7	1 (14.3)	4.15 (0.45-38.02)
HPV51	364	5 (1.4)	33	0 (0.0)	8	0 (0.0)	319	19 (6.0)	43	2 (4.7)	13	1 (7.7)	683	24 (3.5)	76	2 (2.6)	21	1 (4.8)	1.21 (0.15-9.53)
HPV56	387	4 (1.0)	9	0 (0.0)	9	1 (11.1)	340	20 (5.9)	27	0 (0.0)	8	2 (25.0)	727	24 (3.3)	36	0 (0.0)	17	3 (17.7)	**6.77 (1.69-27.04)**
Combination																			
HPV16/18	358	3 (0.8)	21	0 (0.0)	26	2 (7.7)	284	12 (4.2)	58	4 (6.9)	33	6 (18.2)	642	15 (2.3)	79	4 (5.1)	59	8 (13.6)	**5.25 (2.14-12.91)**
9vHPV 5 HR[g]	250	0 (0.0)	70	2 (2.9)	59	1 (1.7)	173	8 (4.6)	113	2 (1.8)	56	6 (10.7)	423	8 (1.9)	183	4 (2.2)	115	7 (6.1)	**3.23 (1.23-8.54)**
Any 9vHPV	240	0 (0.0)	80	2 (2.5)	85	3 (3.5)	145	5 (3.5)	141	5 (3.6)	89	12 (13.5)	385	5 (1.3)	221	7 (3.2)	174	15 (8.6)	**4.46 (2.02-9.86)**
Non-Vaccine[h]	224	1 (0.5)	66	0 (0.0)	30	1 (3.3)	164	3 (1.8)	84	2 (2.4)	38	5 (13.2)	388	4 (1.0)	150	2 (1.3)	68	6 (8.8)	**7.87 2.40-25.81)**
Selected low risk types																			
HPV6	382	5 (1.3)	19	0 (0.0)	4	0 (0.0)	354	22 (6.2)	19	0 (0.0)	2	0 (0.0)	736	27 (3.7)	38	0 (0.0)	6	0 (0.0)	-
HPV11	401	5 (1.3)	3	0 (0.0)	1	0 (0.0)	354	21 (5.9)	18	1 (5.6)	3	0 (0.0)	755	26 (3.4)	21	1 (4.8)	4	0 (0.0)	-

[a]Total number of women who were negative throughout follow-up or who had incident type specific HPV infection; [b]total number of women who had a cleared type specific infection or acquired a new type (type swap); [c]total number of women who had type specific persistent infection; [d]OR for incident CIN2+ among those with HR-HPV persistence compared to all other participants at endline (includes those that were negative for that type or those that developed incident infection with that type during follow-up; [e]adjusted for site and ART status at enrolment [f]Any HR type persistence is defined as those that had at least one HR type persistence; clearance or incidence among those who did not persist; and negative at baseline includes those who were negative for all HR types at baseline; [g]Persistence of any of HPV31/33/45/52/58 among those without persistent HPV16/18; [h]Persistence of any of HPV35/39/51/56/59/68 among those without persistent HPV16/18/31/33/45/52/58; *Type swap is defined as clearance of one genotype and acquisition of a different genotype

Table 8.4. Association of HR-HPV type prevalence with ART and CD4+ count at enrolment among 570 women living with HIV in Burkina Faso

		Burkina Faso[a]					
		HPV16/18		9vHPV HR[b]		Non vaccine types[c]	
	N	n (%)	aPR (95% CI)	n (%)	aPR (95% CI)	n (%)	aPR (95% CI)
On ART	412	63 (15.3)	1.00	123 (32.2)	1.00	69 (30.5)	1.00
ART-naive	158	22 (13.9)	0.94 (0.59-1.51)	51 (37.5)	1.03 (0.78-1.36)	25 (29.4)	0.91 (0.59-1.41)
CD4+ count among ALL, cells/mm^3							
<200	66	14 (21.2)	1.44 (0.80-2.60)	24 (46.2)	**1.52 (1.06-2.18)**	12 (42.9)	1.75 (1.00-3.07)
201-350	125	17 (13.6)	0.92 (0.53-1.58)	42 (38.9)	1.16 (0.84-1.61)	21 (31.8)	1.23 (0.76-2.01)
351-500	161	23 (14.3)	0.95 (0.57-1.59)	44 (31.9)	1.00 (0.72-1.38)	31 (33.0)	1.27 (0.82-1.96)
>500	217	31 (14.3)	1.00	63 (33.9)	1.00	30 (24.4)	1.00
CD4+ count among ART users, cells/mm^3							
<200	47	9 (19.2)	0.98 (0.47-2.06)	16 (42.1)	**1.66 (1.07-2.58)**	11 (50.0)	**2.05 (1.14-3.65)**
201-350	80	11 (13.8)	0.75 (0.39-1.43)	30 (43.5)	1.42 (0.96-2.10)	12 (30.8)	1.32 (0.74-2.35)
351-500	119	17 (14.3)	0.75 (0.42-1.35)	33 (32.4)	1.16 (0.79-1.72)	23 (33.3)	1.23 (0.74-2.05)
>500	165	26 (15.8)	1.00	43 (30.9)	1.00	23 (24.0)	1.00
CD4+count among ART-naïve, cells/mm^3							
<200	19	5 (26.3)	2.02 (0.63-6.45)	8 (57.1)	1.15 (0.59-2.24)	1 (16.7)	0.88 (0.13-5.76)
201-350	45	6 (13.3)	1.25 (0.43-3.60)	12 (30.8)	0.66 (0.37-1.20)	9 (33.3)	1.25 (0.48-3.22)
351-500	42	6 (14.3)	1.22 (0.41-3.63)	11 (30.6)	0.71 (0.39-1.27)	8 (32.0)	1.44 (0.58-3.57)
>500	52	5 (9.6)	1.00	20 (42.6)	1.00	7 (25.9)	1.00

aPR=adjusted Prevalence Ratio; [a]adjusted for condom use, alcohol, cervicitis and cervical ectopy in BF; [b]9vHPV HR includes HPV31/33/45/52/58 in absence of HPV16/18 compared to being negative for all of HPV31/33/45/52/58; [c]Non vaccine types includes HPV35/39/51/56/59/68 in absence of any nonavalent vaccine type compared to being HR-HPV negative.

Table 8.5. Association of HR-HPV type prevalence with ART and CD4+ count at enrolment among 613 women living with HIV in South Africa

		South Africa[a]					
		HPV16/18		9vHPV HR[b]		Non vaccine types[c]	
	N	n (%)	aPR (95%CI)	n (%)	aPR (95%CI)	n (%)	aPR (95%CI)
On ART	404	100 (24.8)	1.00	148 (48.7)	1.00	69 (44.2)	1.00
ART-naive	209	79 (37.8)	**1.42 (1.11-1.83)**	76 (58.5)	1.16 (0.96-1.40)	20 (37.0)	0.92 (0.62-1.37)
CD4+ count among ALL, cells/mm^3							
<200	57	17 (29.8)	1.22 (0.77-1.93)	23 (57.5)	1.13 (0.85-1.50)	10 (58.8)	**1.71 (1.06-2.75)**
201-350	148	53 (35.8)	1.47 (1.07-2.01)	48 (50.5)	0.90 (0.70-1.17)	21 (44.7)	1.27 (0.82-1.95)
351-500	183	54 (29.5)	1.18 (0.86-1.63)	64 (49.6)	0.97 (0.78-1.20)	26 (40.0)	1.12 (0.73-1.70)
>500	225	55 (24.4)	1.00	89 (52.4)	1.00	32 (39.5)	1.00
CD4+ count among ART users, cells/mm^3							
<200	51	15 (29.4)	1.57 (0.91-2.73)	20 (55.6)	1.05 (0.76-1.46)	10 (62.5)	**1.82 (1.08-3.05)**
201-350	102	34 (33.3)	**1.72 (1.09-2.71)**	34 (50.0)	0.89 (0.65-1.20)	16 (47.1)	1.33 (0.79-2.24)
351-500	113	26 (23.0)	1.20 (0.73-1.96)	35 (40.2)	0.79 (0.59-1.07)	23 (44.2)	1.21 (0.74-1.97)
>500	138	25 (18.1)	1.00	59 (52.2)	1.00	20 (37.0)	1.00
CD4+count among ART-naïve, cells/mm^3							
<200	6	2 (33.3)	1.06 (0.34-3.29)	3 (75.0)	1.53 (0.83-2.83)	0 (0.0)	-
201-350	46	19 (41.3)	1.26 (0.80-1.98)	14 (51.9)	1.03 (0.62-1.70)	5 (38.5)	0.86 (0.37-2.00)
351-500	70	28 (40.0)	1.26 (0.80-1.98)	29 (69.1)	**1.45 (1.04-2.01)**	3 (23.1)	0.57 (0.19-1.69)
>500	87	30 (34.5)	1.00	30 (52.6)	1.00	12 (44.4)	1.00

aPR=adjusted Prevalence Ratio; [a]adjusted for age, condom use, smoking, LTSP, vaginal cleansing, *Chlamydia trachomatis, Trichomonas vaginalis* and bacterial vaginosis in SA; [b]9vHPV HR includes HPV31/33/45/52/58 in absence of HPV16/18 compared to being negative for all of HPV31/33/45/52/58; [c]Non vaccine types includes HPV35/39/51/56/59/68 in absence of any nonavalent vaccine type compared to being HR-HPV negative.

9 TYPE-SPECIFIC SEROLOGIC RESPONSES TO HPV AMONG WLHIV IN SOUTH AFRICA: SEROPREVALENCE, SEROCONVERSION AND RISK OF RE-INFECTION

The seropositivity for a range of HPV types and possible sero-protection following natural infection have never been evaluated prospectively among WLHIV and the evidence regarding protection against re-infection for the same HPV type induced after natural infection remains uncertain in both HIV-negative women and WLHIV. An improved understanding of HPV type-specific serological responses in such high-risk populations and their risk of both reinfection of the same type or a newly acquired type is needed to guide HPV control efforts, including possible use of HPV vaccination.

This Chapter incorporates data that was collected as part of a nested study which aimed to estimate the impact of HPV vaccination among WLHIV in South Africa. This Chapter will describe the HPV serodynamics among WLHIV in South Africa; including the type-specific seroprevalence of 15 HPV genotypes, the seroconversion rates at endline following natural infection at baseline and the re-infection rate among those with type-specific antibodies endline. Given the uncertainty regarding immune responses following natural HPV infection, this chapter will also explore the association of CD4+ cell count and ART at baseline and endline in mediating HPV serological response.

9.1 Objectives

In a cohort of WLHIV in South Africa at baseline, to describe:

- To determine the seroprevalence of HPV genotypes and its determinants; ***(Objective 3.4)***

- To describe the correlations of type-specific HPV serology and DNA.

In a cohort of WLHIV with ≤CIN1 at baseline and followed over 18 months:

- To determine type-specific HPV seroconversion rates and their determinants; ***(Objective 3.5)***
- To evaluate the risk of type-specific re-infection and DNA persistence following same-type seropositivity at baseline ***(Objective 3.6)***

9.2 Methods

A full description of the HARP procedures including HPV serology testing is provided in **Chapter 5, sections 5.2 and 5.4**. Epidemiological definitions and specific statistical methods for univariate and multivariate risk factor analysis related to the use of HPV serology data are detailed below.

9.2.1 HPV DNA and serology definitions

The HPV serology assay detects only 15 HPV types while the HPV genotyping assay detects 28 HPV types. To ensure comparability between the two tests, I have modified the definition of HPV DNA positive in this chapter, as follows:

HPV DNA positive

- Women was considered "HPV DNA positive" if positive, by INNO-LiPA, for any of the HPV types included in the serology assay (HR types 16/18/31/33/35/39/45/52/56/58/59/68 and LR types 6/11/73), and "HPV DNA negative" if negative for any of these types.
- Women who were positive for any of the other HPV DNA types detected by INNO-LiPA were considered "HPV DNA negative".
- All HR-HPV types briefly described in Chapters 6, 7 and 8 are included in this definition, except for HPV51.

HPV serology positive

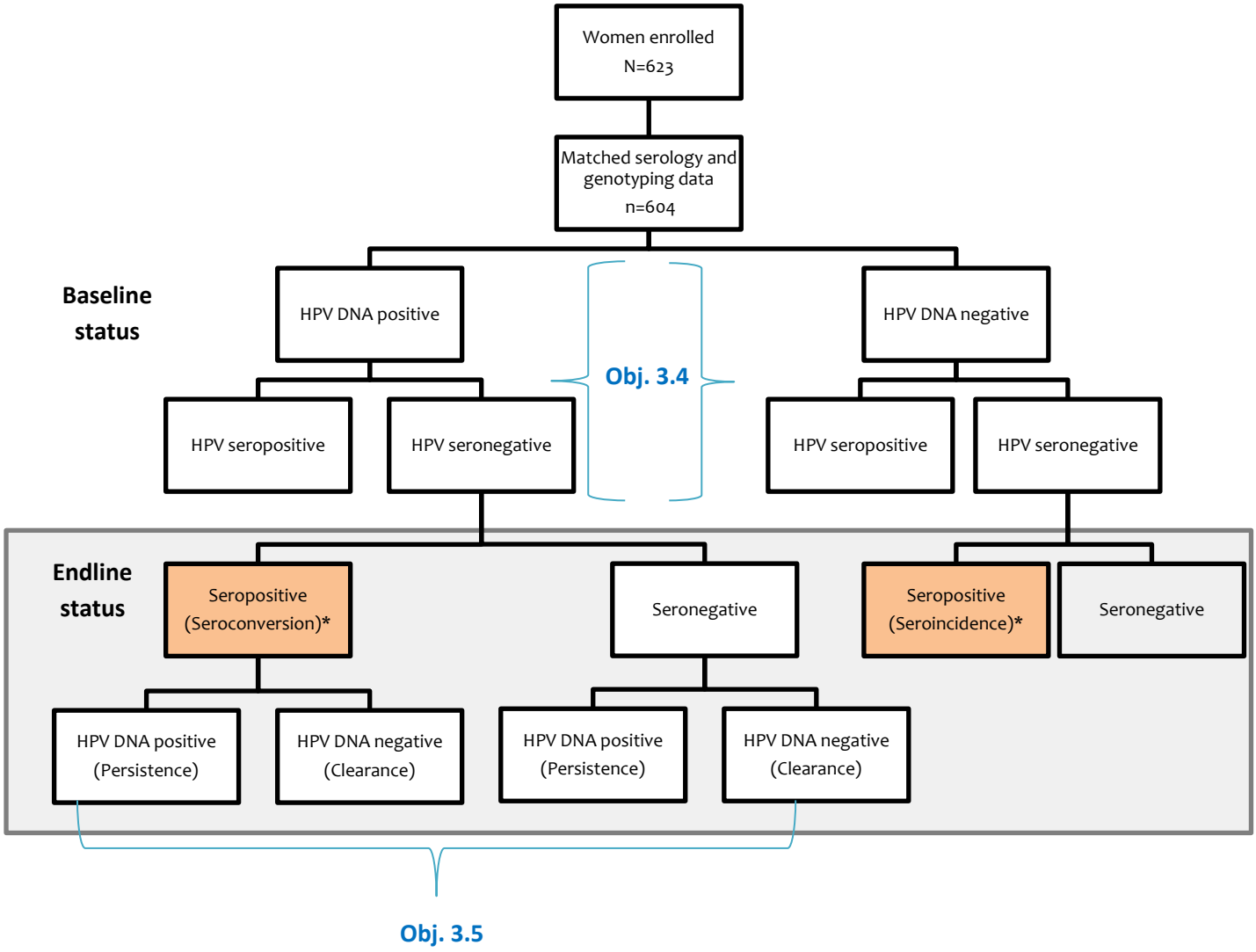
HPV serology results are presented as binary (positive or negative for a given type) based on the cut-off estimated using the negative panel (minimum 400 MFI), as described in **Chapter 5, section 5.4.2**. Serology outcomes groups are defined as positive for any of the 15 types (12 HR types 16/18/31/33/35/39/45/52/56/58/59/68 and 3 LR types 6/11/73) included in the serology assay.

Each of the HPV serology outcome groups were defined as follows:

- HPV seroprevalence was defined as being seropositive for any type at baseline among women with serology data at baseline.
- HPV type-specific seroincidence was defined as being type seronegative at baseline and becoming same-type seropositive at endline, irrespective of the DNA status at either baseline or endline (**Figure 9.1)**. The denominator was all women with serology data at both time points, and who were not seropositive for all HPV types at baseline.
- A more conservative definition was applied considering the HPV DNA status at baseline as evidence of detectable infection. HPV type-specific seroconversion was defined as being HPV DNA positive and same type seronegative at baseline, and becoming same type seropositive at endline, irrespective of the DNA status at endline (**Figure 9.1**).
- Type-specific seropersistence was defined as having same-type detectable antibody at baseline and endline, among all women who were seropositive for any HPV type at baseline.
- Type-specific seroreversion was defined as being seropositive for a specific HPV type at baseline and seronegative for the same type at endline, among all women who were seropositive for any HPV type at baseline.

The longitudinal analyses were restricted to women without prevalent CIN2+ at baseline, as the natural history of HPV infection and serology dynamics in these women, many of whom would have received ablative therapy, may be less easy to interpret.

Figure 9.1. Flowchart describing the seroincidence and seroconversion groups, according to serology and DNA status at baseline and endline



*Seroincidence is defined as being type seronegative at baseline and becoming same-type seropositive at endline, irrespective of the DNA status; represented as boxes shaded in orange

9.2.2 Statistical methods

Univariate and multivariate risk factor analyses were performed for HPV seroprevalence and seroincidence, as described for HPV DNA in **Chapter 6, section 6.2,** but in brief: unadjusted PR for each risk factor (as described in **Chapter 6, Table 6.1)** with HPV seroprevalence and HPV seroincidence were obtained using Stata version 14. PRs were considered significant if p<0.10.

A separate risk factor analysis was performed for women with seroconversion, even though they were a subset of women within those with seroincidence, to confirm whether the same associations still applied. While MVA for seroconversion was performed using women as denominator, MVA were re-performed using infections as denominator, to confirm if the same risk factors persisted in both analyses.

For analyses investigating the association of type-specific DNA prevalence (exposure) with same-type seroprevalence (outcome), associations were adjusted for factors found to be independently significantly associated with HPV seroprevalence in MVA (**Objective 3.4**). As HR-HPV seroprevalence and seroincidence were common, associations with risk factors were estimated with PRs obtained from logistic regression using marginal standardization to estimate PRs, and the delta method to estimate 95% CI, as previously described for HR-HPV DNA outcomes in **Chapter 7, section 7.2.1** [379].

Associations between HPV seroconversion and HIV-related factors were estimated with generalised estimating equation to account for multiple baseline HPV infections per woman and seropositivity by multiple HPV types [380].

To explore associations of HPV seroconversion with HIV-related factors, pre-specified analyses included stratification by ART duration (≤ or >2 years), HIV-1 viral suppression (≤ or >40 copies/ml) and CD4+ cell counts. Multivariable analyses were adjusted for socio-demographic and behavioural factors which were independently associated in multivariate analyses with HPV seroconversion

(**Model 1**). A second logistic regression model (**Model 2**) incorporated endline CD4+ cell count to **Model 1** to explore associations with ART.

For associations of baseline seropositivity with HPV DNA incidence and persistence over follow-up, logistic regression was used to estimate ORs and 95% CI, adjusted for socio-demographic and behavioural factors found to be associated with HR-HPV infection in South Africa, as described in **Chapter 7, Table 7.1.** Although the serology panel includes 12 of the HR types used in previous definitions for HR-HPV infection (HPV51 is not part of the serology assay), it is assumed that its absence should not contribute to a difference in findings.

For associations of baseline seropositivity with CIN2/3 incidence over follow-up, logistic regression was used to estimate ORs and 95% CI, adjusted for socio-demographic and behavioural factors found to be associated with HR-HPV infection, as described in **Chapter 7, Table 7.1.**

9.3 Results

9.3.1 Study population

Of the 623 WLHIV enrolled in South Africa, 604 (97.0%) had valid results for both HPV serology and genotyping at baseline. Participant characteristics were as described in **Chapter 6, section 6.3.1**; median age was 34 years (IQR: 30-40), 396 (65.6%) participants were taking ART and their median duration on ART was 28 (IQR, 10-50) months. Prevalence by INNO-LiPA of HPV DNA for the 15 HPV types, and 12 HR-HPV types, tested by serology was 78.2% and 75.5%, respectively (compared to 88.7% for all 28 HPV types and 79.1% for 13 HR-HPV types, as reported in **Chapter 6, section 6.3.2**). CIN2+ prevalence in this substudy was 22.3% (124/557) compared to 22.5% (129/574) in the full *HARP* population.

9.3.2 HPV seroprevalence at baseline

Prevalence of any HPV type by serology was 93.2% (95%CI: 90.9-95.1%), of whom 89.9% (506/563) were seropositive for multiple types. The prevalence of any HR-HPV type by serology was 90.7% (95%CI: 88.1-92.9%). Overall, 591 (97.8%) women were positive by either serology or DNA for any HPV and 583 (96.5%) for any HR-HPV types.

Seroprevalence was highest for HPV31 (59.6%), followed by HPV58 (54.8%) and HPV16 (43.1%) (**Figure 9.2**). The seroprevalence of any HPV genotypes included in the bivalent (HPV16/18), quadrivalent (HPV6/11/16/18) or nonavalent (HPV16/18/31/35/45/52/58) HPV vaccines were 59.3%, 73.5% and 87.6%, respectively (**Figure 9.2**). Simultaneous seropositivity for all HPV types included in the nonavalent vaccine was 2.8%, while 21.4% and 10.9% were seropositive for all of the bivalent and quadrivalent HPV types, respectively.

There was no difference in HPV or HR-HPV seroprevalence across age groups (**Figure 9.3**).

9.3.3 Risk factors associated with HPV seroprevalence at baseline

HPV seroprevalence was higher among past or current users of injectable contraception compared to never users (ever vs. never use: 95.2% vs. 88.5%; aPR=1.07, 95%CI: 1.01-1.14; **Appendix 15**), but was lower among women with lack of HIV-1 viral suppression (88.8% vs. 96.8% for women with PVL ≥1000 copies/mL vs. <1000 copies/ml; aPR=0.93, 95%CI: 0.87-0.99). However, this association was marginally significant when the analysis was restricted to ART users (aPR=0.94, 95%CI: 0.88-1.01, data not shown).

9.3.4 Correlation of HPV DNA and HPV antibody-specific prevalence

Of 472 women DNA positive for any of the 15 HPV types by INNO-LiPA, 59.1% were seropositive for the same HPV type (range by type: 25.5% for HPV45 to 68.9% for HPV31), while among 456 women positive for any HR-HPV DNA, 58.6% were seropositive for the same type (**Table 9.1**). Of 132 women who were HPV DNA negative for all 15 types, 90.2% (119/132) had detectable antibody for at least

one HPV type, while among 148 HR-HPV negative women, 85.8% (127/148) had detectable antibody for at least one HR type.

HPV seroprevalence was significantly higher among those with same-type DNA positive compared to same-type DNA negative for HPV35, HPV39, HPV52, HPV58 and low-risk type HPV11 (**Table 9.1**).

9.3.5 HPV seroincidence and associated risk factors

Of the 451 women without CIN2+ at enrolment who returned at follow-up visit (median follow-up 16 months [IQR, 15.6-16.8]), genotyping and serology data at baseline and endline were available for 433 (96.0%) women. Seroincidence of any HPV type was 52.2% (224/429) and any HR-HPV type was 46.0% (196/426). Four women were seropositive for all 15 HPV types at baseline, and 7 for all 12 HR-HPV types.

9.3.6 HPV type-seroconversion and associations with same-type DNA persistence

There were 219 women who were type-specific HPV DNA positive and same type seronegative at baseline and same-type seroconversion was 23.3% (51/219) for any HPV and 23.1% (48/208) for any HR-HPV. Among the 219 women, there was a total of 326 infections detected that were same-type seronegative at baseline. When considering the total number of baseline infections as denominator, there were 56 (17.2%) HPV seroconversion events (**Table 9.2**). There were differences in seroconversion rates according to HPV type. The type-specific seroconversion rate was highest for HPV31 (53.9%), HPV33 (33.3%) and jointly for HPV58 and HPV59 (25.0%), and lowest for HPV16 and HPV18 (2.4% and 17.1%, respectively).

Overall, the seroconversion rate was higher among women with same-type persistent infection compared to those who cleared infection (any HPV type seroconversion: 23.0% vs. 15.1%; crude OR=1.68. 95%CI: 0.91-3.11). A similar association was observed for seroconversion of any HR-HPV (23.2% vs. 14.8%; crude OR=1.73, 95%CI: 0.92-3.27). The numbers for individual HPV types were too small to calculate associations with DNA persistence.

9.3.7 Factors associated with HPV type-seroconversion

HPV seroconversion rates were similar among WLHIV with baseline CD4+ count ≤350 and >350 cells/mm^3 (19.8% vs. 16.7%, p=0.51) but seroconversion was lower among those with endline CD4+ ≤350 cells/mm^3 compared CD4+ >350 cells/mm^3 (10.8% vs. 19.4%; aOR=0.61, 95%CI:0.29-1.30, adjusted for injectable contraceptive use), and this association was significant among the ART users only (≤350 vs. >350 cells/mm^3: 8.0% vs. 26.3%; aOR=0.25, 95%CI: 0.08-0.75, adjusted for injectable contraceptive use**; Table 9.3**).

Seroconversion rates were similar among women who remained ART-naïve throughout the follow-up period and long-term (>2 years) ART users (ART naïve vs. long-term ART at endline: 14.2% vs. 17.7%, p=0.43). However, the highest seroconversion was observed among short-duration ART users (≤2 years ART vs. ART-naïve at endline: 24.6% vs. 14.2%; aOR=2.39, 95%CI: 1.02-5.62, adjusted for injectable contraceptive use and endline CD4+ cell count). HPV seroconversion was also more likely among women who reported high adherence to ART at baseline (60-90% vs. <60% adherence: 22.4% vs. 7.1%; aOR=8.93, 95%CI: 1.13-70.36, adjusted for endline CD4+ cell count).

9.3.8 Newly detected HPV DNA over 16 months according to type-specific seropositivity at baseline

Among the 433 women with genotyping and serology data at both visits, 221 (51.0%) had newly detected HPV DNA during follow-up, including a total of 327 incident infections. The risk of incident infection was lower among women who were same-type seropositive compared to seronegative at baseline for HPV18 (1.5% vs. 6.4%; aOR=0.14, 95%CI: 0.02-0.80, adjusted for baseline CD4+ cell count, ART status and seropositivity for any type from same HPV phylogenetic family, **Table 9.4)**; HPV35 (4.1% vs 11.9%; aOR=0.26, 95%CI: 0.10-0.68) and HPV58 (1.8% vs. 5.4%; aOR=0.19, 95%CI: 0.04-0.89;). Conversely, the risk was higher among those with same-type seropositivity at baseline for HPV45 (10.5% vs. 4.0%; association did not persist following adjustment for seropositivity from same

HPV family; aOR=2.81, 95%CI: 0.87-9.04) and HPV68 (11.5% vs. 4.4%; aOR=4.07, 95%CI: 1.52-10.90) (**Table 9.4**).

9.3.9 HPV DNA persistence over 16 months according to type-specific seropositivity at baseline

Persistent DNA infections were marginally higher among women who were seropositive for the same type at baseline (seropositive vs. seronegative: 32.3% vs. 26.1%, **Appendix 16**). Persistent HPV DNA was highest for HPV16, 33, 39, 45 and 56 although the association was significant only for HPV56 (seropositive vs. negative: 45.6% vs. 15.4%; aOR=12.79, 95%CI: 1.14-143.23).

9.3.10 Persistence of type-specific antibody at endline

The persistence of any HPV antibody was high over 16 months; among 403 women with detectable antibody at baseline, 384 (95.3%) had the same type detected again at endline (seropersistence). Despite this, DNA incidence remained high, particularly for HPV16, HPV56 and HPV68 compared to women who had seroreversion for the same type (**Table 9.4**).

DNA persistence was similar among women with seropersistence and seroreverson (**Appendix 16**). Among 577 infections detected at baseline, 166 (28.8%) persisted at endline; 31.6% (67/212) among women with seropersistence, and 35.9% (14/39) among women with seroreversion.

Seropersistence was high, irrespective of CD4+ cell count (96.1% and 96.6% among CD4+ <500 and <200 cells/mm^3, respectively, data not shown). Compared to ART users, ART-naïve women had marginally higher seropersistence (97.6%, 95.5% and 92.1% among ART-naïve, short-duration ART and long-duration ART users, respectively).

9.3.11 Incident CIN2/3 and seropositivity at baseline

Among 365 women with histology data at endline, 22 (6.0%) had incident CIN2/3 at 16 months. All 22 incident CIN2/3 cases were seropositive at baseline for any HPV, and all had type-specific seropersistence at endline.

Seropositivity at baseline was associated with incident CIN2/3 (**Appendix 17**), even after adjustment for same-type HPV DNA positivity at baseline. The associations were strongest for HPV types -31, -56, -58, -59 and 68. This independent association of seropositivity at baseline with incident CIN2/3 may be due to serum antibodies generated from a prior infection which may have persisted but either cleared, or was undetectable at the time of enrolment, but which nonetheless may be have initiated the pathway to cervical lesion incidence during follow-up in this study.

CIN2/3 incidence was also significantly higher among women with seropersistence for HPV31, 58 and 56 compared to women who had same-type seroreversion, after adjustment for same-type HPV DNA positivity at baseline (**Appendix 17**).

9.4 Discussion

This prospective study of serological dynamics for 15 HPV types among South African WLHIV found a very high seroprevalence (93%) at baseline and evidence of seroconversion (26%) and high seropersistence (95%) at endline. However, this population had high incidence (49%) and persistence of HPV DNA (45%) and high incidence of CIN2/3 (6%) over 16 months. Despite the high prevalence and persistence of detectable antibodies, there is therefore limited evidence to suggest that HPV antibodies can prevent same-type HPV DNA incidence and persistence.

Seroprevalence is very high among WLHIV

Although we found high prevalence of HPV vaccine types (59-88% depending on vaccine types), the simultaneous seropositivity for all HPV types included in the current HPV vaccines was low (21% for bivalent vaccine and 3% for nonavalent vaccine). We report a similar seroprevalence for types HPV6, 11 and 16 (42.2%, 35.8% and 43.1%, respectively) as that reported elsewhere among WLHIV in South Africa (38.2%, 33.8% and 29.3% for HPV-6, -11 and -16, respectively) [424] but higher seroprevalence for HPV18 (37.6% in this study vs. 15.9%). These findings are also similar to that

reported by Viscidi et al [425] in a study enrolling 2815 WLHIV and 963 HIV-negative women in the US that found 54% of WLHIV were seropositive for HPV16, compared to 47% of HIV-negative women.

A lower seroprevalence was reported among 54 HIV-positive pregnant women and 1103 HIV-negative pregnant women in Uganda [426] for HR-HPV (HPV16/18/31/33/35/45/52/58); 43% among WLHIV compared to 36% HIV-negative women. The lower seroprevalence of all HR types could be due to differences in serology assay methods across studies, but also to the immunosuppression associated with pregnancy [427].

Seroprevalence correlates with DNA detection at baseline among WLHIV

We found that HPV seropositivity correlated with DNA detection at baseline; 59.1% of those with HPV infection, and 58.6% of those with HR-HPV infection had seropositivity for the same type at baseline. These findings are also similar to what was reported by Viscidi et al for HPV16 [425] whereby 56% of WLHIV with HPV DNA were seropositive for HPV16, compared to 63% of HIV-negative women. Furthermore, among those who were DNA negative for all types in this study, 10% had detectable antibody for any HPV type and 14% for HR-HPV at baseline. This does not necessarily suggest false positives, but may reflect the high past exposure in this population.

As reported in HIV-negative women [428-430], a proportion of women were DNA positive but concurrently seronegative for the same type. This may reflect the timing of sampling in relation to HPV acquisition, as sampling early in course of infection may not yield detectable antibody; low titer or waning antibody, or failure to seroconvert [68, 69].

These data suggests that WLHIV mount an antibody response, given the high seroprevalence, and they can maintain detectable antibody over 16 months, given the high rates of seropersistence. But these high rates of seroprevalence and seropersistence may simply be markers of past

infection, and it is necessary to ascertain the rate of seroconversion following infection, and whether HPV antibodies prevent establishment and persistence of infection.

Seroconversion is associated with type-specific persistent infection among WLHIV

At 16 months follow-up, we found that among those that were DNA positive and same type seronegative at baseline, 26% of women seroconverted for the same type, and this was higher among those with persistent compared to cleared infection at endline. To our knowledge, this is the first report to confirm seroconversion following natural infection among WLHIV.

Seroconversion rates reported here are lower to what has been reported among HIV-negative unvaccinated women. Carter et al [68] in a study among 588 college women aged 18-20 years reported that 59.5%, 54.1% and 68.8% seroconverted for HPV-16, -18 and -6, respectively within 18 months of detection of same-type incident infection, and seroconversion was more likely among those with same-type persistent infection. Lower seroconversion estimates for HPV16 were reported in a population of 6528 women from the general population in Guancaste, Costa Rica followed for a longer time period. Wang et al [431] reported that 21.7% of women with detectable DNA at baseline had seroconversion for HPV16 over a median of 6.4 years, and, similar to Carter et al, seroconversion was higher among those with persistent compared to cleared infection (37.9% vs. 16.3%, respectively). Castro et al [432] reported much lower seroconversion rates over a long follow-up period, and among women with a wider age range. Among 600 women from the general population in Chile aged 15-85 years with a HR-HPV seroprevalence of 43.2% at baseline and followed over 5 years, 21.6% of women who were initially seronegative seroconverted for any HR-HPV type, and 7.4% for HPV16, although HPV DNA detection at baseline was not considered in these estimates. Antibodies titres are known to peak and then wane over time, even in the presence of HPV DNA [68] and this may explain the lower seroconversion for HPV16 reported by the studies in Costa Rica and Chile.

While there are no studies among WLHIV to make any comparison, seroconversion among HIV-infected MSM is similar to what is reported in this study. Among 245 HIV-infected MSM recruited from infectious disease and STI clinics in Amsterdam [433], seroconversion against any of 7 HR-HPV types (HPV16/18/31/33/45/52/58) was 23% over 12 months following anal or penile HPV infection. Among 281 HIV-infected MSM initiating ART in the Swiss HIV Cohort Study (SHCS), seroconversion for any of 8 HR-HPV types (HPV16/18/31/33/35/45/52/58) was 42.1% over 24 months.

It is however difficult to compare seroconversion estimates between women and men as HIV-negative men are known to have lower seroprevalence compared to HIV-negative women, and this has been suggested to be linked to the site of infection; as infection at the mucosal cervical epithelium of the cervix and anal canal may induce stronger immune responses compared to infection at keratinised epithelia such as the genital skin [434, 435].

Seroconversion in this study varied by HPV type, and was highest for HPV31 (53.9%) but the lowest seroconversion was observed for HPV16 (2.4%). The type seroconversion rates reflected the type-specific infection states at follow-up. The overall incident and persistent DNA was higher for HPV16 than for other types (13.1% and 33.3% for HPV16 incidence and persistence respectively, compared to 5.1% and 14.3% for HPV31). The lower seroconversion and subsequent increased risk of HPV16 incidence and persistence could be a result of its immune evasion mechansims. HPV16 prevalence amomg WLHIV has been reported to be less affected by CD4+ count decreases compared to other HR-HPV types [420].

Seroconversion is associated with CD4+ count and ART

While seroprevalence was not associated with CD4+ count at baseline, consistent with what has been reported elsewhere [424, 436], seroconversion was higher among women with higher contemporary (endline) CD4+ cell count (>350 cells/mm^3) and this association was only observed in the ART users. Seroconversion was also higher among short-duration (≤2 years) ART users at

endline compared to women who remained ART-naïve throughout follow-up (24.6% vs. 14.2%, respectively). While no other studies among WLHIV are available for comparison, the SHCS study among 281 MSM initiating ART and followed for a median of 2 years [437] reported that those with lower nadir CD4+ cell count (<200 cells/mm^3) at the time of ART initiation had the highest seroconversion rates for HR-HPV at endline, compared to MSM who initiated ART at ≥350 cells/mm^3. The authors suggest that ART-related immune reconstitution during the 2 year follow-up may have promoted seroconversion, and this was highest among those with the lowest nadir CD4+ cell count. Although the authors did not report HPV DNA detection, it is possible that MSM with low nadir CD4+ cell count at ART initiation also had higher persistent infection compared to those with higher nadir CD4+ cell count, which may have resulted in higher seroconversion rates.

While nadir CD4+ cell count is not available in this study, women on short-duration ART at endline (≤2 years) had a lower baseline CD4+ cell count (median baseline CD4+: 324 cells/mm^3, IQR: 236-403) compared to both the long-duration ART users (461, IQR: 335-593), or ART-naïve (497, IQR: 386-622). Although baseline CD4+ cell count was not associated with seroconversion, the subsequent increase in CD4+ cell count at endline through ART-initiated immune reconstitution may have promoted seroconversion among the recent ART users in response to the high rate of HPV DNA persistence.

A second explanation for higher seroconversion among short-duration ART users is that these women had high DNA prevalence at baseline and higher persistence of infection at endline compared to prolonged ART users. This study, and others, have shown that seroconversion rates were higher among women with persistent compared to cleared infection. But while seroconversion may have increased, immune reconstitution achieved through recent ART initiation at the previous WHO-recommended cut-off of CD4+ 350 cells/mm^3 may have been ineffective to prevent persistence in the short-term, as reported in **Chapter 7** . Seroconversion has been previously shown to be dependent on time following incident infection; among HIV-negative

women aged 18-20 years, HPV6 seroconversion coincided with DNA detection but HPV16 seroconversion occurred between 6 to 12 months after DNA detection [68]. The association of seroconversion with endline CD4+ count in this study, and not baseline CD4+ cell count, may also reflect the longer duration required for seroconversion to occur after infection. The frequency of HPV testing in this study however does not allow an estimation of duration of the infection detected at baseline.

Thirdly, short-duration ART users and ART-naïve women had the highest CIN2+ prevalence at baseline. However, at endline, ART-naïve women had the highest CIN2/3 incidence (9.2%) while short-duration ART users had similar CIN2/3 incidence as the prolonged ART users (4.5%). While the numbers were too small to investigate associations of seroconversion with CIN2/3 incidence, the short-term ART users appeared to have gained sufficient immune responses to reduce the risk of cervical lesion incidence over 16 months.

Of note, women taking ART for a prolonged duration (>2 years) had similarly low seroconversion (17.7%) as ART-naïve women (14.2%). The low seroconversion among prolonged ART users may be reflective of the lower rates of prevalent (72%) and persistent infection (25%; **Chapter 7**). However, women who were ART-naïve had equally high rates of persistent HR-HPV infection as short-duration ART users (31.8% and 35.2%, respectively; **Chapter 7**), and in contrast to ART users, CD4+ cell count at either time point was not associated with seroconversion among the ART-naïve women. These data suggest there may be some beneficial impact of ART in promoting seroconversion.

ART-related increases in HPV seropositivity have shown to be independent of CD4+ count in other studies among among MSM [437] and among WLHIV [425]. There are no studies that have compared seroconversion among ART users and ART-naïve women, but vaccination studies among WLHIV have shown that, while seroconversion rates are similar among ART users and ART-naive

women in the US and Puerto Rico [261], HPV16 and HPV18 antibody titres were lower among ART-naïve but comparable between HIV-negative and ART users [261].

HPV seropositivity at baseline provides limited protection against HPV re-infection and persistence among WLHIV

HPV-16 and -18 antibodies have been shown to confer partial protection against reinfection by the same type in HIV-negative women [69]. This study found limited evidence that antibodies detected at baseline provided protection against reinfection against same-type infection. However, those with baseline seropositivity for HPV18, 35 and 58 were between 74-86% less likely to have newly detected DNA for the same type 16 months later, after adjustment for covariates of HPV infection and HPV antibody from the same HPV phylogenetic family at baseline. A recent meta-analysis which assessed whether naturally acquired immunity conferred protection against reinfection by the same type [69] reported that HIV-negative women with antibodies against HPV16 and HPV18 had 35% and 30% lower risk of re-infection, respectively against the corresponding type. While this study saw no protection conferred by HPV16 antibodies against HPV16 re-infection, others have reported that HPV16 is better able to evade the host immune surveillance relative to other HPV types [438], which may explain its predominance in high-grade cervical lesions in HIV-positive and HIV-negative women [14] .

In a prospective study among 829 WLHIV and risk matched with 413 HIV-negative women in the US, Viscidi et al [436] reported no statistically significant difference among WLHIV in the risk of reinfection of any HR-HPV (HPV16/18/31/35/45) among women with same-type seropositivity compared to same-type seronegative at baseline over a median 4.5 years, with the exception of a reduced risk of infection with HPV45 (Hazard Ratio=0.21, 95%CI: 0.05-0.89). Similarly, among HIV-negative women in that study, HPV seropositivity at baseline was not associated with a reduced risk of any HPV infection. For both WLHIV and HIV-negative women, there was no evidence of protection for either transient or persistent same-type infection.

Other studies have evaluated whether HPV antibodies detected at two or more time points were associated with same-type re-infection among HIV-negative women. A prospective study with longitudinal serology measurements among 608 HIV-negative women (mean age of 20 years) seen at 6-month intervals for 3 years in the US [439] reported a 50% reduction in risk of HPV16 re-infection among women with a sustained high level of HPV16 antibody (seropositivity at two or more time points) and its genetically related types (HPV31/33/58). No study has investigated whether persistent seropositivity is a marker of protection against re-infection among WLHIV.

HPV seropersistence is high among WLHIV but is not associated with HPV DNA incidence or persistence

HPV seropersistence among WLHIV in SA over 16 months was very high (95% for any HPV type), and was high for all individual HPV types (>80%), with the exception of HPV18, 39 and 45 (65.0%, 57.1% and 46.9%, respectively). Seropersistence has been reported to be similarly high in HIV-negative women; 82% of women in Chile had seropersistence for any HR-HPV type over 5 years [432] and 58% of women from the general population in Costa Rica had seropersistence for HPV16 over 7 years [431].

In this study, WLHIV who had type-specific seropersistence had lower HPV DNA incidence compared to women with seroreversion at endline for all HPV types, with the exception of the HR types HPV16, 18, 45, 56 and 68. By contrast, women with seropersistence had higher, or similar rates of persistent infections as women with seroreversion. Although there are no other data among WLHIV for comparison purposes, persistent infection has been shown to be higher among HIV-negative women with seropersistence. Among 79 HIV-negative women in Guanacaste, Costa Rica, persistent HPV16 infection over a median of 6.4 years was 37.9% among those with seropersistence compared to 28.6% among women with seroreversion [431].

While there is no strong evidence to suggest that baseline serum antibodies protect against re-infection and persistence, the higher rates of persistent infection among those with seropersistence may simply reflect the higher exposure to past infections in these populations. Women with baseline seropositivity and seroperistence of any HPV type were also more likely to have CIN2/3 incidence over 16 months compared to women with seroreversion or women without detectable antibody at baseline. This finding suggests that, rather than being markers of protection among WLHIV, seroprevalence and seropersistence may be markers of past exposure to HPV infection and persistence. In **Chapter 7**, it was reported that women with DNA persistence over 16 months had an increased risk of CIN2/3 compared to women with HPV clearance, and this was highest for types (in order of decreasing magnitude) HPV-58, -56 and -18. Similarly, women with seropersistence for HPV-58 and -56 had increased risk of CIN2/3 incidence, possibly reflecting the level of HPV DNA persistence.

Implications for vaccination of WLHIV in South Africa

This study shows that WLHIV have high seroprevalence and high rate of seropersistence of type-specific antibodies, and there is evidence of seroconversion over 16 months. Despite this, WLHIV have high prevalence, incidence and persistence of HPV DNA, including multiple type infection. Given the limited evidence that natural immunity can protect against reinfection and persistence of infection, WLHA could benefit from HPV vaccination.

Seroconversion following vacination with the bi- and quadri-valent vaccines has been reported to be high; between 92-100% [261] for the quadrivalent vaccine among 99 WLHIV aged 16-23 years in the US, and 100% for the bivalent vaccine among and 125 WLHIV aged 18-25 years in South Africa [440]. Antibody titres following vaccination were similar among WLHIV and HIV-negative women at 12-months post-vaccination, although ART-naïve women had lower antibody titres for HPV-16 and -18 [261, 440]. However, there is no data yet available on HPV vaccine efficacy against HPV persistence and CIN outcomes. A prophylactic HPV vaccination could be beneficial for WLHIV at

risk for exposure to new infections, given that current antibodies provide limited protection against re-infection, and clearance of infection. Furthermore, a multivalent vaccine, such as the nonavalent vaccine targeting 7 HR types (HPV16/18/31/33/45/52/58) and 2 LR types (HPV6/11) would be beneficial among WLHIV given that 55% of WLHIV have multiple HR-HPV infection at baseline and 18% had multiple persistent types at endline, and relatively few women had simultaneous seropositivity or DNA positivity for all vaccine types.

9.5 Study limitations

As stated in previous chapter, this study was constrained by the limited number of intermediate visits and overall follow-up duration. We cannot establish whether the seroconversion event occurred in response to either: i.) an infection which persisted during follow-up, or ii.) a baseline infection which cleared, but the woman was re-infected again with the same type during the 16-months follow-up.

Similarly, when evaluating the risk of re-infection according to same-type seropositivity at baseline, we cannot exclude the possibility that the newly detected infection at 16-months may truly be a reinfection or whether it is a recurrence of an existing infection that was latent or had persisted at an undetectable level. If it is the latter case, then we would not expect to see a decrease in risk of new DNA detection among women who were seropositive for that type at baseline, as antibodies are not known to clear existing infections.

The reference panel used for generation of cut-off values for the serology assay was based on sera from children <12 years from Sweden. We cannot rule out the possibility that the assay was less specific for an African population, as others have reported higher prevalence of HPV among children <15 years SSA [441]. It cannot be ruled out that the serology assay had different specificities for individual HPV types and there may have been cross-reaction with types from the

same phylogenetic family, particularly given the very high rate of multiple HPV infections in this population (55%).

There is a possibility that HPV infection could have occurred at another anatomical site (anal or oral) rather than cervix, which could have affected the estimates for seroconversion in response to cervical DNA infection.

The type specific analyses were limited by the small numbers, in particular when investigating type-specific seroconversion among those with DNA at baseline.

The risk factor analysis for HPV seroprevalence at baseline should be treated with caution, given the very high seroprevalence (93%). A separate risk factor analysis was carried out for both seroincidence and seroconversion outcomes. Given that seroconversion is a sub-set of women from the seroincidence group, this may have been unnecessary. However, the MVA analyses further confirmed that injectable contraception use was associated with a decreased risk of seroconversion, as observed for seroincidence.

9.6 Summary of findings

- Seroprevalence for any of 15 HPV types was very high among WLHIV (**93%**), reflecting high exposure through past and current infection
- Among women positive for any of the 15 HPV DNA types, 60% were also seropositive for the same type at baseline, while among the those who were DNA negative for all 15 types tested, 10% had detectable antibodies for any of those types
- Seroconversion for any of the 15 HPV types over median 16 months was 23%, and was 1.7 times higher among those with the same-type persistent HPV infection compared to those who cleared the same infection
- Seroconversion was highest among women who recently (<2 years) initiated ART, and among those with higher CD4+ count at endline
- There was some evidence that women with detectable HPV antibodies at baseline had a decreased risk of reinfection by the same type, but only for HPV18, 35 and 58
- Although over 95% of women had type-specific antibodies detected at both baseline and endline, DNA incidence and persistence of infection was high
- Incident CIN2/3 was higher among women who were seropositive at baseline, reflecting past infection, but little evidence of protection

Conclusion:

WLHIV have high levels of HPV antibodies at baseline and endline, they can mount an antibody response in response to detectable DNA infection at baseline, which is dependent on CD4+ cell count. However the presence of antibodies does not appear to be effective at clearing existing infection or preventing DNA re-infection

9.7 Findings in context

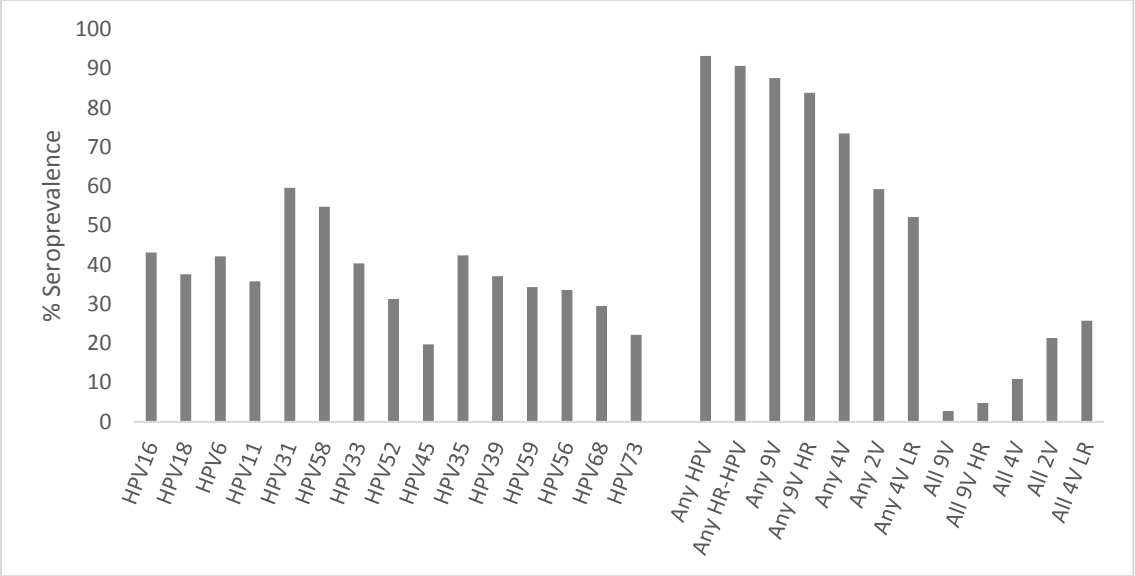
There are few studies which have reported seroprevalence for a wide range of HPV types among WLHIV. Others have shown that WLHIV in Johannesburg have similarly high seroprevalence of HPV types as shown in this study (38.2%, 33.8% and 29.3% for HPV-6, -11 and -16, respectively).

One other study in the US reported limited evidence that WLHIV with type-specific HPV antibodies had a decreased risk of re-infection, similar to this study. That study also reported similar findings among HIV-negative women in the same cohort.

No studies have reported seroconversion among unvaccinated WLHIV. However, WLHIV have been shown to have similar seroconversion rates following vaccination with bi- or quadrivalent vaccine as HIV-negative and antibody titres were sustained up to 12 months post vaccination.

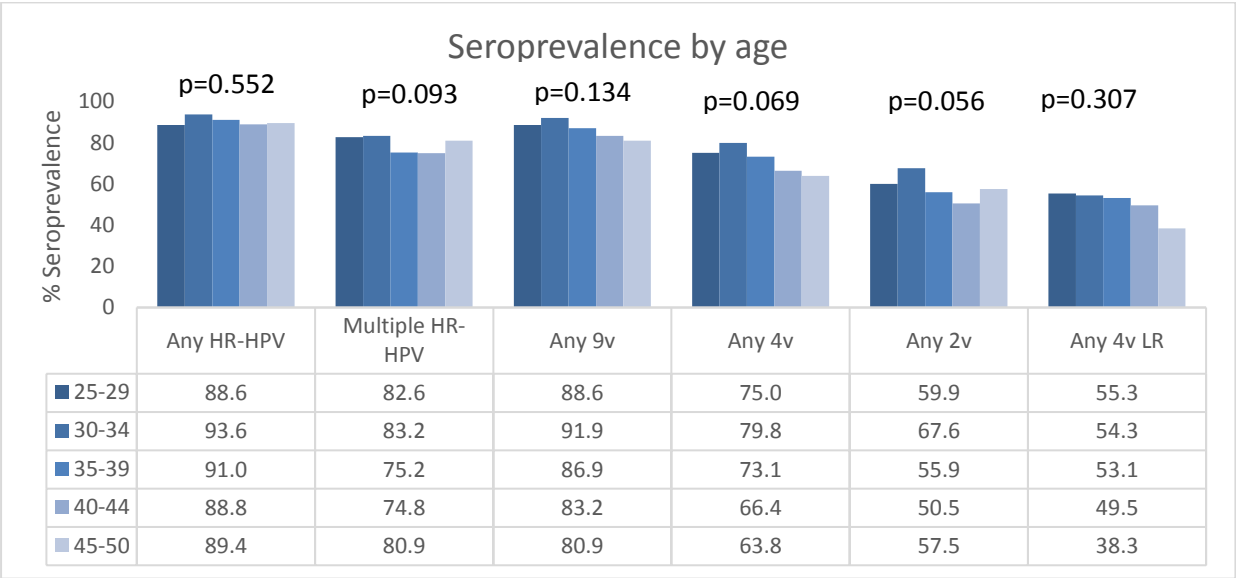
Seropositivity appears to be marker of past infection, rather than conferring protection against re-infection over 16 months. Given the limited evidence that natural immunity can protect against reinfection and persistence of infection, WLHA would benefit from HPV vaccination.

Figure 9.2. HPV type seropositivity among 604 WLHIV in South Africa at baseline



* Any HPV type prevalence defined as positive for at least one HPV type (6/11/16/18/31/33/35/39/45/52/56/58/59/68/73) at baseline; Any HR-HPV type prevalence defined as positive for at least one HR type (16/18/31/33/35/39/45/52/56/58/59/68) at baseline; Any 9V=Positive for any of HPV6/11/16/18/31/33/45/52/58; Any 9V-HR=Positive for any of HPV16/18/31/33/45/52/58; Any 4V= Positive for any of HPV6/11/16/18; Any 4V-LR= Positive for any of HPV6/11; Any 2V= Positive for any of HPV16/18; All 9V=Positive for ALL of HPV6,11,16,18,31,33,45,52 and 58; All 9V-HR=Positive for ALL of HPV16,18,31,33,45,52 and 58; All 4V=Positive for ALL of HPV6,11,16 and 18; All 2V=Positive for BOTH HPV16 and 18; All 4V-LR=Positive for BOTH HPV6 and 11.

Figure 9.3. HPV seroprevalence by age among 604 WLHIV in South Africa at baseline



	Any HR-HPV	Multiple HR-HPV	Any 9v	Any 4v	Any 2v	Any 4v LR
25-29	88.6	82.6	88.6	75.0	59.9	55.3
30-34	93.6	83.2	91.9	79.8	67.6	54.3
35-39	91.0	75.2	86.9	73.1	55.9	53.1
40-44	88.8	74.8	83.2	66.4	50.5	49.5
45-50	89.4	80.9	80.9	63.8	57.5	38.3

Table 9.1. Comparison of HPV seropositivity among type-specific HPV DNA positive and DNA negative WLHIV (N=604) at baseline

	DNA positive (N)	Antibody positive (n)	Sero+/DNA+ (%)	DNA negative (N)	Antibody positive (n)	Sero+/DNA- (%)	aPR (95%CI)[1]
Bi-/Quadri-valent types							
HPV6	33	14	**42.4**	571	241	**42.2**	0.98 (0.65-1.49)
HPV11	33	19	**57.6**	571	197	**34.5**	**1.68 (1.23-2.29)**
HPV16	115	50	**43.5**	489	210	**42.9**	1.02 (0.81-1.29)
HPV18	89	34	**38.2**	515	193	**37.5**	1.03 (0.78-1.37)
Additional nonavalent types							
HPV31	61	42	**68.9**	543	318	**58.6**	1.19 (0.99-1.42)
HPV33	48	25	**52.1**	556	219	**39.4**	1.29 (0.96-1.74)
HPV45	47	12	**25.5**	557	107	**19.2**	1.47 (0.88-2.43)
HPV52	146	60	**41.1**	458	129	**28.2**	**1.47 (1.15-1.88)**
HPV58	54	36	**66.7**	550	295	**53.6**	**1.26 (1.03-1.53)**
Non-vaccine types							
HPV35	99	59	**59.6**	505	197	**39.0**	**1.53 (1.26-1.86)**
HPV39	48	25	**52.1**	556	199	**35.8**	**1.48 (1.11-1.98)**
HPV56	58	20	**34.5**	546	183	**33.5**	1.06 (0.73-1.53)
HPV59	11	6	**54.5**	593	201	**33.9**	1.59 (0.91-2.78)
HPV68	33	12	**36.4**	571	166	**29.1**	1.26 (0.79-2.01)
HPV73	10	4	**40.0**	594	130	**21.9**	1.97 (0.94-4.10)
Any HPV	472	279	**59.1**	132	13	**9.8**	
Any HR-HPV	456	267	**58.6**	148	21	**14.2**	

[a]HPV type seropositive among DNA positive for the same type; [b] All HPV type seronegative among DNA negative for all types; [1]adjusted Prevalence Ratio [PR] for type-specific seroprevalence if same-type DNA positive compared to DNA negative, adjusted for injectable contraception and HIV-1 viral suppression

Table 9.2. Type-specific seroconversion at 16 months among 219 WLHIV with ≤CIN1 at baseline

	All participants[a]		Participants with HPV DNA persistence[b]		Participants with HPV DNA clearance[c]	
	DNA+ & sero- at baseline	Seroconversion events	DNA+ & sero- at baseline	Seroconversion events	DNA+ & sero- at baseline	Seroconversion events
	N infections	n (%)[a]	N infections	n (%)[b]	N infections	n (%)[c]
HPV16	**41**	1 (2.4)	11	1 (9.1)	30	0 (0.0)
HPV18	**41**	7 (17.1)	14	3 (21.4)	27	4 (14.8)
HPV6	**13**	3 (23.1)	0	0 (0.0)	13	3 (23.1)
HPV11	**13**	2 (15.4)	3	1 (33.3)	10	1 (10.0)
HPV31	**13**	7 (53.9)	2	2 (100.0)	11	5 (45.5)
HPV33	**15**	5 (33.3)	1	1 (100.0)	14	4 (28.6)
HPV45	**25**	4 (16.0)	8	1 (12.5)	17	3 (17.6)
HPV52	**63**	12 (19.1)	21	6 (28.6)	42	6 (14.3)
HPV58	**12**	3 (25.0)	4	1 (25.0)	8	2 (25.0)
HPV35	**26**	6 (23.1)	11	3 (27.3)	15	3 (20.0)
HPV39	**19**	0 (0.0)	2	0 (0.0)	17	0 (0.0)
HPV56	**26**	4 (15.4)	4	1 (25.0)	22	3 (13.6)
HPV59	**4**	1 (25.0)	1	0 (0.0)	3	1 (33.3)
HPV68	**13**	1 (7.7)	3	0 (0.0)	10	1 (10.0)
HPV73	**2**	0 (0.0)	2	0 (0.0)	0	0 (0.0)
Any HPV	**326**	56 (17.2)	**87**	20 (23.0)	**239**	36 (15.1)
Any HR-HPV	**298**	51 (17.1)	**82**	19 (23.2)	**216**	32 (14.8)

[a]Seroconversion calculated among 219 women with DNA+/sero- status at baseline representing 326 infections; [b]Seroconversion calculated among DNA+/sero- at baseline and with type-specific persistence at endline; [c]Seroconversion calculated among DNA+/sero- at baseline and type-specific clearance at endline

Table 9.3. HIV-related factors associated with HPV seroconversion at 16 months using generalised estimating equation (GEE) using 319 events of DNA positive/same type seronegative at baseline (among 219 WLHIV)

		HPV Seroconversion		
	N	**n (%)**	**aOR (95% CI) [1]**	**aOR (95% CI) [2]**
All participants				
Baseline CD4+ count (cells/mm³)*				
<200	**14**	2 (14.3)	0.88 (0.19-4.14)	-
201-350	**82**	17 (20.7)	1.24 (0.65-2.37)	-
>350	**221**	37 (16.7)	1.00	-
Endline CD4+ count (cells/mm³)*				
<200	**15**	0 (0.0)	-	-
201-350	**78**	10 (12.8)	0.61 (0.29-1.30)	-
>350	**191**	37 (19.4)	1.00	-
ART status at baseline				
ART >2 years	**91**	15 (16.5)	1.00	1.00
ART ≤2 years	**81**	19 (23.5)	1.53 (0.72-3.26)	1.56 (0.72-3.40)
ART-naive	**147**	22 (15.0)	0.85 (0.41-1.75)	0.85 (0.41-1.75)
ART status at endline				
ART >2 years	**124**	22 (17.7)	1.00	1.00
ART ≤2 years	**61**	15 (24.6)	1.48 (0.70-3.12)	1.35 (0.59-3.07)
ART-naive	**134**	19 (14.2)	0.74 (0.38-1.45)	0.56 (0.27-1.18)
Baseline ART users				
HIV-1 viral suppression at baseline				
<1000 copies/ml	146	27 (18.5)	1.00	1.00
≥1000 copies/ml	26	7 (26.9)	1.58 (0.60-4.17)	1.91 (0.67-5.38)
ART adherence at baseline **				
Low Adherence (<60%)	**28**	2 (7.1)	1.00	1.00
Moderate adherence (60-90%)	**143**	32 (22.4)	3.54 (0.79-15.83)	**8.93 (1.13-70.36)**
Baseline CD4+ count (cells/mm³)				
<200	**12**	2 (16.7)	1.00 (0.20-5.02)	
201-350	**52**	13 (25.0)	1.46 (0.65-3.28)	-
>350	**106**	19 (17.9)	1.00	-
Endline CD4+ count (cells/mm³) [§]				
<200	**11**	0 (0.0)	-	-
201-350	**39**	4 (10.3)	**0.32 (0.10-0.99)**	-
>350	**99**	26 (26.3)	1.00	-
ART-naïve [†‡]				
Baseline CD4+ count (cells/mm³) [†]				
<200	**2**	0 (0.0)	-	-
201-350	**30**	4 (13.3)	0.77 (0.23-2.50)	-
>350	**115**	18 (15.7)	1.00	-
Endline CD4+ count (cells/mm³) [‡]				
<200	**2**	0 (0.0)	-	
201-350	**38**	6 (15.8)	1.47 (0.48-4.51)	-
>350	**81**	9 (11.1)	1.00	-

Adjusted Odds Ratio (aOR) using generalised estimating equation ; [1]In Model 1, associations with HPV seroconversion were adjusted for injectable contraception use found to be associated with seroprevalence in **Appendix 15**; [2]In Model 2, additional adjustment for CD4+ count at endline (except for associations with ART at baseline when baseline CD4+ was used for adjustment; *CD4+ count at baseline available for 317; CD4+ count at endline available for 284; **ART adherence + measure available for 171 of 172 ART at baseline; [†]Baseline CD4+ among participants who were ART-naïve at baseline [‡] ART-naïve participants were defined as being ART-naive at both baseline and endline.

Table 9.4. Newly detected HPV DNA among 433 WLHIV, measured over 16 months follow-up, stratified by same type seropositivity at baseline

	All participants		Seronegative at baseline		Seropositive at baseline		aOR (95%CI)[3]	aOR (95%CI)[4]	Among seropositive at baseline				aOR (95%CI)[5]
									Seropersistence at endline		Seroreversion at endline		
	N[1]	n (%)[2]	N[1]	n (%)[2]	N[1]	n (%)[2]	Model 1	Model 2	N[1]	n (%)[2]	N[1]	n (%)[2]	
Any Alpha-9 HR-HPV types													
HPV16	366	48 (13.1)	203	25 (12.3)	163	23 (14.1)	1.27 (0.66-2.44)	1.48 (0.69-3.15)	135	20 (14.8)	28	3 (10.7)	1.50 (0.39-5.72)
HPV31	394	20 (5.1)	169	8 (4.7)	225	12 (5.3)	1.12 (0.45-3.11)	1.12 (0.36-3.45)	204	10 (4.9)	21	2 (9.5)	0.57 (0.11-2.92)
HPV33	408	17 (4.2)	248	11 (4.4)	160	6 (3.8)	0.81 (0.28-2.35)	0.70 (0.21-2.33)	143	5 (3.5)	17	1 (5.9)	0.59 (0.06-5.60)
HPV35	374	33 (8.8)	227	27 (11.9)	147	6 (4.1)	**0.26 (0.10-0.68)**	**0.26 (0.10-0.68)**	110	4 (3.6)	37	2 (5.4)	0.67 (0.11-4.13)
HPV52	327	55 (16.8)	233	41 (17.6)	94	14 (14.9)	0.76 (0.37-1.55)	0.77 (0.36-1.65)	82	12 (14.6)	12	2 (16.7)	0.78 (0.14-4.37)
HPV58	404	14 (3.5)	187	10 (5.4)	217	4 (1.8)	**0.22 (0.05-0.88)**	**0.19 (0.04-0.89)**	191	3 (1.5)	26	1 (3.9)	0.29 (0.02-3.48)
Any Alpha-7 HR-HPV types													
HPV18	367	17 (4.6)	235	15 (6.4)	132	2 (1.5)	**0.20 (0.04-0.91)**	**0.14 (0.02-0.80)**	84	2 (2.4)	48	0 (0.0)	
HPV39	397	24 (6.1)	251	14 (5.6)	146	10 (6.9)	1.48 (0.60-3.62)	1.69 (0.65-4.42)	83	5 (6.0)	63	5 (7.9)	0.78 (0.21-2.85)
HPV45	403	21 (5.2)	327	13 (4.0)	76	8 (10.5)	**3.30 (1.17-9.31)**	2.81 (0.87-9.04)	37	4 (10.8)	39	4 (10.3)	1.42 (0.27-7.66)
HPV59	424	5 (1.2)	280	4 (1.4)	144	1 (0.7)	0.48 (0.04-5.77)	0.41 (0.02-8.74)	107	1 (0.9)	37	0 (0.0)	
HPV68	412	26 (6.3)	299	13 (4.4)	113	13 (11.5)	**2.85 (1.20-6.78)**	**4.07 (1.52-10.90)**	89	11 (12.4)	24	2 (8.3)	1.30 (0.26-6.60)
Other HPV types													
HPV56	396	16 (4.0)	264	10 (3.8)	132	6 (4.6)	1.56 (0.51-4.76)	-	98	5 (5.1)	34	1 (2.9)	1.51 (0.17-13.76)
LR-HPV types													
HPV6	410	13 (3.2)	241	7 (2.9)	169	6 (3.6)	1.28 (0.41-4.03)	-	142	5 (3.5)	27	1 (3.7)	1.09 (0.12-10.26)
HPV11	408	15 (3.7)	269	10 (3.7)	139	5 (3.6)	1.16 (0.37-3.67)	-	119	4 (3.4)	20	1 (5.0)	0.73 (0.08-7.07)
HPV73	428	3 (0.7)	331	1 (0.3)	97	2 (2.1)	28.03 (0.98-798.1)	-	72	1 (1.4)	25	1 (4.0)	0.40 (0.02-6.73)
Any HPV	**433[a]**	**327**	**427[b]**	**209 (48.9)**	**400[c]**	**118 (29.5)**							

[1]Number of women negative for that type at baseline; [2]number of incident infections among women negative or that type at baseline; [3]Model 1, adjusted Odds Ratio (OR) for DNA incidence among same-type seropositive vs. seronegative at baseline, adjusted for age, smoking, condom, vaginal washing, chlamydia, BV, CT, TV, CD4 count and ART status at baseline; [4]Model 2, additional adjustment for seropositivity for HPV type from same family group, i.e. HPV16 DNA incidence adjusted seropositivity for types HPV31/33/35/52/58; [5]adjusted OR for DNA incidence among seropersistent vs. seroreverted at endline, adjusted for CD4+ at baseline only due to small numbers; [a]All women at risk of acquiring a HPV infection (no woman infected by all types at baseline); [b]all women with any HPV type seronegative and same type DNA negative at baseline; [c]All women with any HPV type seropositive and same type DNA negative at baseline.

10 ASSOCIATIONS OF DNA METHYLATION OF HUMAN GENE *EPB41L3* AND HPV16 WITH CIN2+

It was shown in ***Chapter 4*** *that the DNA methylation of the human gene EPB41L3 and HPV16 was associated with CIN2+, and had reasonable sensitivity and specificity for the detection of CIN2+ relative to low or absent disease (≤CIN1) in the general population. However, there are as yet, no reports of the DNA methylation of EPB41L3 or HPV16 in the detection of CIN2+ among WLHIV.*

This Chapter describes the associations of DNA methylation of a human gene EPB41L3 and HPV16 with prevalent CIN2+ at baseline, and incident CIN2/3 at endline. The change in EPB41L3 methylation levels over time among CIN2/3 that persisted or regressed will also be described. Given that aberrant DNA methylation is a possible marker for high-grade disease, this chapter will also explore whether DNA methylation is associated with HIV-related factors, such as CD4+ cell count, HIV-1 PVL and ART.

While the aim of this Chapter is to understand the associations of DNA methylation with CIN2+, its performance as a tool for detection of CIN2/3 among WLHIV will also be discussed.

10.1 Objectives

In a cohort of WLHIV in Burkina Faso and South Africa at baseline:

- To determine the association of DNA methylation of a human gene *EPB41L3* and HPV16 with CIN2+ prevalence; ***(Objective 4.1)***
- To evaluate the role of socio-demographic, behavioural and HIV-related factors on the *EPB41L3* DNA methylation at enrolment. ***(Objective 4.2)***

Among WLHIV with ≤CIN1 at baseline and followed over 18 months:

- To determine the association of baseline and endline DNA methylation of *EPB41L3* with CIN2+ incidence. ***(Objective 4.1)***

Among WLHIV with CIN2+ at baseline and followed over 18 months without receiving management for CIN2+ lesions:

- To monitor the change in *EPB41L3* methylation levels over time among CIN2/3 that persisted or spontaneously regressed to ≤CIN1. ***(Objective 4.3)***

10.2 Methods

A full description of the HARP enrolment procedures is provided in **Chapter 5, section 5.2**. A full description of Study 3 design and sample selection is provided in **Chapter 5, section 5.5**. Specific statistical methods for Study 3 are detailed here.

10.2.1 Participant samples for *EPB41L3* DNA methylation assay

The case-control study design was described in **Chapter 5, section 5.5.1**. The participant samples included in the study are described here.

All cases of histologically confirmed CIN2+ detected at baseline (prevalent) with sufficient cervical material (BF: 28; SA: 124) were included in the study and matched with at least one control without CIN (≤CIN1) by (i) age group (<35 years and ≥35 years) and (ii) country of recruitment (BF: 66; SA: 144; **Table 10.1**).

Table 10.1. Sample selection for Case Control Study 1 matched for age and country

	BF*		SA		Total BF	Total SA	Total samples
	< 35 yrs	≥ 35 yrs	< 35 yrs	≥ 35 yrs			
Cases	5	23	72	52	28	124	152
Controls	21	45	84	60	66	144	210

***** additional controls in BF to compensate for low number of cases

Among the 210 women with ≤CIN1 at baseline (both countries combined), 185 (88.1%) had histology results at endline. Methylation assays were performed for 26 of the 27 incident CIN2/3 detected in the HARP study (BF: 4; SA: 22) at endline. All women with ≤CIN1 in the baseline study and who remained ≤CIN1 at endline (n=159; BF: 53; SA: 106) were included as controls.

Among 36 women with prevalent CIN2+ included in Case Control Study 1 in SA and who did not receive ablative treatment before the endline visit, *EPB41L3* DNA methylation was measured at baseline and endline to monitor methylation levels over time. The number of cases included in Case Control Study 2 are summarised in **Table 10.2**.

Table 10.2. Control Selection decisions for Case Control Study 2*

	BF		Total BF	SA		Total SA	Total samples
	< 35 yrs	≥ 35 yrs		< 35 yrs*	≥ 35 yrs		
Total Cases:	2	3	**5**	40	23	**63**	**68**
Incident	2	2	**4**	15	7	**22**	**26**
Recurrent	0	1	**1**	2	3	**5**	**6**
Persistent	-	-	**-**	13	7	**20**	**20**
Regressed	-	-	**-**	10	6	**16**	**16**
Controls	14	39	**53**	59	47	**106**	**159**

*Cases and controls will come from same pool as for Case-control Study 1, detailed in **Table 10.1 Error! Reference source not found.**

10.2.2 Participant samples for HPV16 DNA methylation assay

HPV16 L1 DNA methylation was performed for all HPV16 positive sample at baseline only. In BF, 33.3% (9/27) of CIN2+ cases and 27.3% (18/66) of ≤CIN1 controls were HPV16 positive at baseline. In SA, 28.2% (35/124) of CIN2+ cases and 33.3% (48/144) of ≤CIN1 controls were HPV16 positive at baseline.

The HPV16-positive CIN2+ cases available for testing (BF: 8; SA: 30) were tested using HPV16 DNA methylation assays, and matched 1:1 with all HPV16-positive ≤CIN1 controls (BF: 15; SA: 28) selected from the controls in **Table 10.1**.

HR-HPV status of selected cases and controls

Whilst HR-HPV DNA positivity was not part of the selection criteria, it is a potential confounder for *EPB41L3* methylation. For the CIN2+ cases at enrolment, HR-HPV positivity was 100% in BF and 90.7% in SA. Among the controls at baseline, HR-HPV positivity was 75.8% in BF and 84.0% in SA (**Table 10.3).** At endline, HR-HPV was 100% among the cases in both countries, and among the controls at endline was 83.0% in BF and 82.1% in SA which was slightly higher than that observed in the overall cohort (59% in BF and 79% in SA, refer **Chapter 6, Table 6.2**).

Table 10.3. HR-HPV DNA positivity among selected cases and controls

		Outcome	BF	SA
Case-control Study 1	Case	Prevalent CIN2+	100.0 (31/31)	90.7 (117/129)
	Control	≤CIN1 at baseline	75.8 (50/66)	84.0 (121/144)
Case-Control Study 2	Case	Incident CIN2/3	100.0 (5/5)	100.0 (22/22)
		Recurrent CIN2/3	0.0 (0/1)	100.0 (5/5)
		Persistent CIN2/3	-	95.0 (19/20)
		Regression CIN2/3	-	87.5 (14/16)
	Control	≤CIN1 at baseline and endline	83.0 (44/53)	82.1 (87/106)

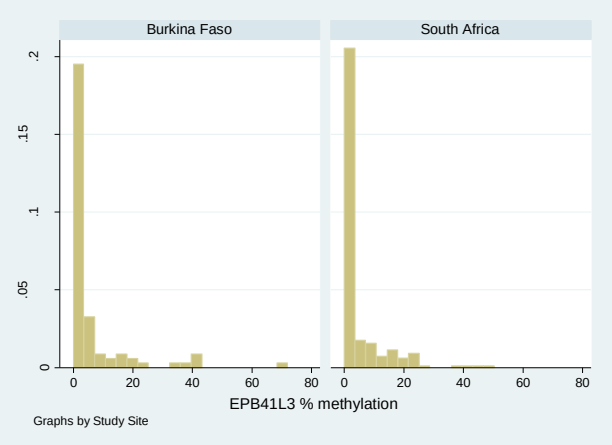
10.2.3 Statistical analysis

Comparison of methylation levels between cases and controls

Given the *EPB41L3* and HPV16 methylation values were not normally distributed (**Figure 10.1**), the median methylation of the average of the CpG sites for each gene in CIN2/3 cases and ≤CIN1 controls were compared using the non-parametric Mann-Whitney U test [442]. All women who were ≤CIN1 at baseline were included as controls in the baseline study, irrespective of whether they went on to develop incident CIN2/3 or not (i.e. inclusive sampling) because there was no difference in the percentage baseline *EPB413* median and IQR between women that remained ≤CIN1 at endline and those who developed incident CIN2/3 at endline.

The Cuzick test for trend was used to evaluate trend in methylation levels by CIN grade [443]. The one-sided Wilcoxon matched pairs test was used to evaluate changes in median methylation levels between baseline and endline among the CIN2/3 outcome groups, and a p-value of 0.05 was considered significant [444].

Figure 10.1. Histogram of *EPB41L3* methylation percentage distribution, by country



Measures of performance of EPB41L3 methylation assay for CIN2/3 detection

Receiver operating characteristic (ROC) curves were plotted for *EPB41L3* and HPV16 for the outcomes of CIN2/3 and CIN3, separately, as *EPB41L3* methylation has shown to be higher among CIN3 and greater compared to CIN2 in previous studies reviewed in **Chapter 4**. The area under the ROC curve (AUC) was used to evaluate the performance of the methylation assay for the detection of CIN2/3 and CIN3, separately.

Definition of 'high' methylation and associated risk factors

Baseline methylation levels for *EPB41L3* were further dichotomized into 'high' and 'low' methylation levels; whereby values were considered 'high' if above the second tertile (66.7 percentile point) of the distribution in the control (≤CIN1) samples at baseline, and the cut-off was country-specific given the variation in percentage methylation values in each country. All women who were ≤CIN1 at baseline were included in estimation of the cut-off, irrespective of whether they

went on to develop incident CIN2/3 or not (i.e. inclusive sampling). This also maximised the number of samples on which the cut-off was based.

Given that case-control studies do not directly estimate prevalence among the exposed and non-exposed, prevalence ratios are not appropriate and logistic regression was used to obtain ORs and 95% CI for 'high' methylation associated with socio-demographic, behavioural and HIV-related factors [445]. Multivariable analyses, as described for HPV DNA outcomes in **Chapter 6, section 6.2,** were adjusted for factors which were independently associated with 'high' methylation in univariate analyses for each country and a p-value of <0.10 was considered significant, given the smaller sample size for this study. *A priori* adjustments for HR-HPV and CIN status at baseline were included, as both HR-HPV and CIN2+ are confounders of *EPB41L3* methylation, but it is not yet clear if they are necessary for increased EPB41L3 methylation levels.

10.3 Results

10.3.1 Study population

A full description of the participants is provided in **Chapter 6.** Among the 161 CIN2/3 cases detected in the HARP study at baseline, *EPB41L3* methylation was performed for 152 CIN2/3 (CIN2: BF=17; SA=73; CIN3+: BF=11; SA=51) and 210 ≤CIN1- age and site matched controls (BF: 66; SA: 144) (**Figure 10.2**). A single invasive cancer case was identified in BF. Among the 362 women selected for Case Control Study 1, HR-HPV positivity was slightly higher in cases (BF: 100.0%, SA: 90.7%) compared to controls (BF: 75.8%; SA: 84.0%), and HPV16 was detected in 29% (27/93) of all selected participants in BF and 31.0% (83/268) in SA.

A description of the HARP study population characteristics is provided in **Chapter 6, Section 6.3.1**. In brief, for this study, the median age of 94 participants in BF was 39 (IQR, 35-43) years and among 268 participants in SA was 33 (IQR: 30-38), similar to the overall HARP cohort. The median CD4+ T cell count at enrolment was 453 cell/mm^3 (IQR: 303-594) in BF and 403 cell/mm^3 (IQR: 283-551) in

SA. At enrolment, 80 (85.1%) participants were taking ART in BF and 162 (60.5%) in SA. While the proportion of ART users in BF was higher in that sub-sample than that reported in the main HARP cohort (68.6%), the median duration of ART use was similar: 16.9 (IQR: 0.0-79.1) months in BF and 23.8 (IQR: 8.1-42.9) in SA.

Among the 210 women with ≤CIN1 at baseline, 185 (88.1%) had histology results at endline, a median 16 months later (IQR: 14.5-16.7). In the *HARP* study, there were 27 incident CIN2/3 detected over 16 months and *EPB41L3* methylation testing was performed for 26 incident CIN2/3 cases (BF: 4; SA: 22, **Figure 10.2**); 22 CIN2 (BF: 3; SA: 19) and 4 CIN3 (BF: 1; SA: 3) included in Case Control Study 2. All participants with ≤CIN1 in the baseline study who remained ≤CIN1 at endline (n=159; BF: 53; SA: 106) were included as controls for Case Control Study 2.

Among the 124 SA women with prevalent CIN2/3 at baseline, 36 could not receive management for their lesions before the endline repeat colposcopy/biopsy visit, and thus the study was able to ascertain that 20 (55.6%) had persistent CIN2/3 and 16 (44.4%) had spontaneously regressed to ≤CIN1 at endline (**Table 10.4**). When presenting results by CIN grade, among 23 women with baseline CIN2, 30.4% (7/23) had persistent CIN2, 13.0% (3/23) progressed to CIN3 and 56.5% (13/23) had spontaneous regression to ≤CIN1 at endline. Among 13 women with CIN3 at baseline, 38.5% (5/13) had persistent CIN3, 38.5% (5/13) regressed to CIN2 and 23.1% (3/13) had spontaneous regression to ≤CIN1 at endline.

Table 10.4. CIN2+ status at endline among women with prevalent CIN2+ and untreated at endline

	Baseline status		Total
Endline status	**CIN2**	**CIN3**	
Persistent	7	5	12
Progression CIN2 to CIN3	3	0	3
Regression CIN3 to CIN2	0	5	5
Regression CIN3 to≤CIN1	0	3	3
Regression CIN2 to≤CIN1	13	0	13
Total	23	13	36

10.3.2 Methylation of *EPB41L3* at baseline for detection of prevalent CIN2/3

The median methylation percentage of the average of three CpG sites for *EBP41L3* increased with increasing CIN grade (**Figure 10.3, Table 10.5**). The Mann-Whitney U test indicated that methylation levels were elevated in CIN2/3 cases compared to controls in both countries (in BF: CIN2/3 median=7.05, IQR: 1.72-17.68 vs. ≤CIN1 median=1.15, IQR: 0.0-2.93, p<0.001; in SA: CIN2/3 median=1.77, IQR: 0.0-10.62 vs. ≤CIN1 median=0.0, IQR: 0.0-1.53, p<0.001, data not shown). The Cuzick test for trend indicated increasing methylation levels by CIN grade (BF and SA combined: p<0.001, **Table 10.5**).

The ROC curve analysis for CIN2/3 detection relative to ≤CIN1 (**Table 10.6**) generates an area under the curve (AUC) of 0.77 (95%CI: 0.65-0.89) in BF and 0.68 (95%CI: 0.62-0.74) in SA. For a specificity set at 70%, this would give a sensitivity of 60.7% in BF and 58.9% in SA for CIN2/3 detection (**Table 10.6**). The AUC did not vary when considering CIN3 alone as an outcome measure relative to ≤CIN1 (**Table 10.6**). A set specificity of 70% would give a sensitivity of 63.6% in BF and 60.8% in SA for CIN3 detection.

10.3.3 Methylation of *EPB41L3* at baseline and endline for the detection of incident CIN2/3

The median methylation level for *EBP41L3* at baseline was not significantly higher among women who developed incident CIN2/3 over 16 months compared to those who remained ≤CIN1 in either country (incident CIN2/3 vs. ≤CIN1: Mann-Whitney p=0.42 for BF, p=0.68 in SA; **Table 10.7**). However, the endline methylation was significantly higher in both countries among those with CIN2/3 compared to ≤CIN1 (BF: Mann-Whitney p=0.05; SA: p=0.07), and in SA for CIN3 compared to ≤CIN1 (p=0.001; **Table 10.7**). Furthermore, *EPB41L3* methylation increased at endline among those who developed incident CIN2/3 in BF (Wilcoxon matched pairs p=0.06), and among those that developed incident CIN3 in SA, although not significant due to small numbers of incident CIN3.

The ROC curve analysis of endline methylation for incident CIN2/3 detection relative to ≤CIN1 generated an AUC of 0.80 (95%CI: 0.63-0.97) in BF and 0.60 (95%CI: 0.47-0.73) in SA, and an AUC of 0.95 (95%CI :0.86-1.00) for CIN3 detection in SA (**Table 10.6**).

10.3.4 Methylation *EPB41L3* among women with CIN2/3 at baseline and followed up over 16 months in SA

An analysis of the baseline methylation values of women with prevalent CIN2/3 and followed up over 16 months, show that women with persistent CIN3, or CIN2 which progressed to CIN3 (CIN2/3 persistence) had higher baseline methylation compared to women who regressed (CIN2/3 persistence, median methylation=15.67, IQR: 3.13-24.70; CIN2/3 regression to ≤CIN1, median methylation=0.0, IQR: 0.0-3.83; Mann-Whitney p=0.016; **Table 10.8**). Women with persistent CIN2 had low methylation values at both baseline and endline (Wilcoxon p=1.00).

10.3.5 Socio-demographic and behavioural factors associated with high *EPB41L3* methylation

The proportion of women with 'high' methylation at baseline (defined as greater than the 66.7 percentile point of the distribution in the ≤CIN1 samples) was 41.5% (39/94) in BF and 45.9% (123/268) in SA.

In BF, a higher education level was associated with 'high' methylation in univariate analysis, which persisted in MVA (≤primary school attendance vs. >primary: 26.7% vs. 66.7%; aOR=3.89, 95%CI: 1.20-12.62**; Appendix 18).** However, in SA, a lower education level was associated with 'high' methylation in univariate analysis, but this did not persist in MVA (**Appendix 19**).

In BF, women with *Candida albicans* detected at baseline were less likely to have 'high' methylation compared (positive vs. negative: 15.4% vs. 42.5%; aOR=0.08, 95%CI: 0.01-0.99).

Due to uncertainty of the role of education in levels of *EPB41L3* methylation, further analyses will not be adjusted for education. A priori adjustment factors include HR-HPV and CIN status at baseline in both countries, in addition to *Candida albicans* in BF.

10.3.6 HIV-related factors associated with *EPB41L3* methylation

Low CD4+ cell count was significantly associated with 'high' methylation in both countries (BF: 66.7% among women with CD4+ count ≤200 cells/mm^3 vs. 34.9% among women with CD4+ count ≥350 cells/mm^3: aOR=7.45, 95%CI: 1.53-36.22, adjusted for HR-HPV, CIN status and *Candida albicans*; **Table 10.9 ;** in SA: 68.8% vs. 39.4%; aOR=2.74, 95%CI: 1.16-6.47, adjusted for HR-HPV and CIN status). This was highest among ART users in both countries, although the associations were not significant (**Table 10.9**).

In both countries, 'high' methylation was less frequent among ART-naïve women compared to long-term (>2 years) ART users, but this association was significant in SA only (35.6% vs. 54.4%; aOR=0.38, 95%CI: 0.20-0.74, adjusted for CD4+ cell count; **Table 10.9**). There was also some evidence in SA that short-duration (≤2 years) ART users were less likely to have 'high' methylation compared to long-duration ART users (aOR=0.48, 95%CI: 0.23-0.99, adjusted for CD4+ cell count). This finding was not observed in BF.

10.3.7 HPV16 DNA methylation associated with prevalent CIN2/3 at baseline, and persistent infection and incident CIN2/3 at endline

Among the 362 cases and controls selected for this study, there were 81 samples that were positive for HPV16 positive at baseline and available for testing for HPV16 L1 methylation (BF: 23; SA: 58). In the HARP cohort, the overall HPV16 prevalence was 14.0% (170/1215) and was higher in SA (BF: 8.6%; SA: 19.2%, p<0.001, **Chapter 8, Table 8.1**).

HPV16 L1 methylation was higher among the prevalent CIN2/3 cases compared to ≤CIN1 in BF (CIN2/3 median methylation: 11.23, IQR: 4.18-43.38 vs. ≤CIN1 median methylation: 7.20, IQR: 2.80-15.20) but the difference was not significant (Mann-Whitney p=0.61). There was no difference in HPV16 L1 methylation levels between participants in CIN2/3 and ≤CIN1 in SA (p=0.49**; Appendix 20**).

10.4 Discussion

EPB41L3 is associated with CIN2+ among WLHIV

This study reports that methylation of the host gene biomarker *EPB41L3* increased with increasing grades of prevalent CIN among women living with HIV in Sub-Saharan Africa. Furthermore, *EPB41L3* methylation increased with increasing progression of CIN lesions over 16 months. These data suggest that *EPB41L3* methylation may be useful in identifying women at risk of persistent or progressive high-grade CIN.

While this is the first report of *EPB41L3* methylation among WLHIV, the performance of *EPB41L3* to detect CIN2/3 compared to ≤CIN1 is similar to reports among HIV-negative women in Canada [334], the Netherlands [254, 335] and the UK [256, 328].

EPB41L3 as a single marker has reasonable performance for detection of CIN2+

In Burkina Faso, *EPB41L3* methylation values increased across all grades of CIN, and EPB41L3 methylation assay had similar sensitivity estimates (61% for CIN2+ and 64% for CIN3+) for a set specificity of 70%, comparable to the studies conducted among HIV-negative women (also reported in **Chapter 4**). Such performance would not be superior to the current cervical cancer screening method of VIA/VILI in BF, which had a sensitivity of 56% and specificity of 78% for detection of CIN2/3 in the HARP study [446].

In South Africa, the greatest increase in methylation values was among those with CIN3+, and with an AUC of 0.68 (95% CI 0.62-0.74) and for a set specificity of 70%, *EPB41L3* methylation had a sensitivity of 59% for CIN2/3 detection. The current cervical cancer screening method in South Africa is cytology high-grade SIL and greater (HSIL+), which had a sensitivity of 69% and specificity of 82% in this study, whilst cytology of low-grade SIL and greater (LSIL+) had a sensitivity of 98% and specificity of 13% for LSIL+ [446], findings which also have been reported among WLHIV in

Johannesburg [238]. There were small increases in sensitivity for CIN3+ detection in both countries (64% in Burkina Faso and 61% in South Africa, at set 70% specificity). These findings suggest that *EPB41L3* on its own would not be any better than the current screening method, but in combination with other existing human gene markers and possibly HR-HPV methylation may increase performance as shown by others [256, 335], and reviewed in **Chapter 4**.

An increase in performance was observed in the AUC of endline *EPB41L3* methylation for the detection of incident CIN2/3 in Burkina Faso, and incident CIN3 in South Africa. A set specificity of 70% gave a sensitivity of 75% for CIN2/3 detection in Burkina Faso, and 100% sensitivity for CIN3 detection in South Africa. This suggests that *EPB41L3*-based methylation assay may be useful in a regularly screened population for the detection of incident CIN.

EPB41L3 DNA methylation distinguishes persistent CIN2/3 from CIN2/3 that spontaneously regresses

This study found that *EPB41L3* methylation levels increased with increasing duration and severity of CIN lesion over 16 months, similar to what has been reported for other host gene markers *CADM1, MAL* [447] and *FAM19A4* [448] in the general population in the Netherlands. CIN2 and CIN3 are transforming stages, and a marker which could predict progression or regression would be useful. This study found that an AUC of 0.84 (95%CI: 0.66-1.00) gave a sensitivity of 86% and a specificity of 81% for the detection of persistent CIN3 or CIN2 progressing to CIN3 relative to those who remained ≤CIN1 or who regressed to ≤CIN1 over 16 months. Although the number of outcomes were small in this study, it suggests that *EPB41L3* methylation assay may be useful in predicting CIN2+ that progress from those that regress. CIN2 reflects heterogeneous disease, of which a substantial portion represents productive HR-HPV infections [449] that will regress spontaneously [450]. Given that 56% of the women in this study with CIN2/3 at baseline had spontaneous

regression to ≤CIN1 over 16 months, such a diagnostic tool would be useful to reduce over-referral with unnecessary colposcopies and overtreatment of women with clinical disease, particularly in settings with limited resources.

While this is the first report on *EPB41L3* methylation among WLHIV, others have recently reported on the performance of a CADM1/MAL/MIR human genes combined methylation assay among 248 WLHIV in Kenya [17] and found that the tri-marker panel had an AUC of 0.80 for detection of prevalent CIN2/3 with comparable sensitivity and specificity as cytology (using cut-off of ASCUS); 89% and 50%, respectively. The Kenya study also included women who were treated for CIN2+ lesions and followed-up 6-months later, but reported that the methylation levels for MAL and MIR remained unchanged 6-months later despite treatment and suggest a possible role of HR-HPV persistence in maintaining these methylation levels.

HPV16 L1 DNA methylation does not distinguish CIN2+ from ≤CIN1

DNA methylation of HPV16 L1 did not appear to discriminate between CIN2+ and ≤CIN1 in this study. While aberrant methylation of HPV during disease progression is common, and most pronounced in the L1 and L2 regions [366] , data are still largely inconsistent, as reviewed in **Chapter 4**, and by others [247, 248], and it remains uncertain whether HPV DNA methylation provides the infected cell with a growth advantage [366], or whether HPV methylation coincides or facilitates other host gene changes. HPV16 is known to evade the host immune response, and is likely to persist in WLHIV, and does not change according to CD4+ cell count, as has been reported for other HPV types [420]. A larger study with a HIV-negative comparator group may shed light on HPV16 methylation profiles and the influence of HIV-related factors.

CD4+ cell count and ART status is associated with higher *EPB41L3* methylation

We found higher levels of *EPB41L3* methylation among women with low CD4+ cell count (≤200 cells/mm^3). There have been no previous reports of cervical cancer-specific methylation markers and associations with immune status.

It is unclear from the literature whether CD4+ T-lymphocytes are directly involved in regulating tumour suppressor genes such as *EPB41L3*, but CD4+ T-lymphocytes can regulate tumour cells indirectly through the action of cytokines. CD4+ T-cell mediated immune responses against tumours involve the secretion of cytokines IFN-γ and TNF-α (refer **Figure 2.6, Chapter 2).** Recent studies have shown that interferon (IFN)-γ and tumour necrosis factor (TNF)-α induce senescence of tumour cells in various cancers in mice and humans [451] and growth arrest and vulvar intraepithelial lesions (VIN) 3 are associated with IFN-γ producing CD4+ T-helper 1 cells [452, 453].

The inverse correlation of methylation levels with CD4+ cell count may also reflect the association of HR-HPV infection with CD4+ cell count. HPV oncogenes E6 and E7 can activate DNA methyltransferases (DNMT1, DNMT3A and DNMT3B), which are responsible for initiating and maintaining tumour suppressor gene methylation and are overexpressed in several cancers [44]. HPV16 E7 has also been shown to bind DNMT1 and to stimulate its enzymatic activity [454]. While the association of CD4+ cell count with *EPB41L3* methylation was independent of HR-HPV status, unmeasured HR-HPV, particularly in integrated form (E6/E7 integrated in host DNA) cannot be ruled out. Finally, chronic inflammation caused by past infection with other STIs, also linked with lower CD4+ cell count, can promote the initiation or progression of cancer (as reviewed **in section 2.7.4, Chapter 2**).

Higher methylation of *EPB41L3* was found among ART users at baseline, in particular among women with long-duration ART use in SA and this was independent of CD4+ cell count and HR-HPV and CIN status but this association was not observed in BF. This is a curious finding because long-

duration ART users in SA had higher median CD4+ cell count and lower CIN2+ prevalence at baseline compared to ART-naïve women. Further examination of the data among the ≤CIN1 controls showed that both long-duration and short-duration ART users with ≤CIN1 had slightly higher median methylation levels at baseline compared to ART-naïve women with ≤CIN1 (median of 0.0 (IQR: 0-2.9) for all ART users combined vs. 0.0 (IQR: 0.0-0.0) among ART naïve. It is possible that women who initiated ART at lower nadir CD4+ cell count may have experienced associated deregulation of normal TSG activity, resulting in their methylation and silencing. Over time, with increasing duration on ART, the corresponding CD4+ cell count recovery may be unable to completely reverse the DNA methylation and they could remain at low levels even among ≤CIN1. It is also possible that these women may have developed CIN2/3 lesions which were undetected but which resolved through CD4+ count recovery. Because the ART-naïve women had a median CD4+ count >350 cells/mm^3, they never experienced severe immunosuppression which may have been a catalyst for past DNA methylation among the ART users. This may also explain why the *EPB41L3* methylation assay generated poor performance data among women in SA, as the women without CIN2+ may have residual methylation levels from when they were severely immunosuppressed. Nadir CD4+ cell count data would be required to accurately assess this hypothesis. A second possible explanation is the potential for ART genetoxcity and the long term toxic effects of ART drugs are not yet known. Animal studies have shown that antiretroviral nucleoside analog drugs, such as zidovudine are incorporated into host DNA, where it causes mutations of genes associated with gene expression, cell cycle arrest and chromosomal abberations [455].

10.5 Study limitations

This study was constrained by its small sample size and its post-hoc design nested into the *HARP* parent study. While 94% of prevalent cases and 96% of incident cases from the parent HARP study were included, only a proportion of the controls (BF: 66/583 (11%), and in SA: 144/494 (29.1%)) were sampled because of limited resources and available samples. Therefore, the data might not be entirely generalizable to all women with ≤CIN1 in the *HARP* cohort. Furthermore, as the definition

for 'high' methylation was determined by the distribution of methylation values among the selected ≤CIN1 controls, this cut-off may not be reflective of the entire population without disease. The study was also limited in its ability to evaluate associations of HPV16 DNA methylation with CIN2+, due to the relatively small number of HPV16-positive samples included in the study. This study design favoured samples selected for evaluation of *EPB41L3*, and associations of HPV16 DNA methylation with HPV16 persistence could not be adequately assessed without the full set of HPV16 persistent cases existing in the *HARP* study. The high co-infection rate of other HR types (35% in BF and 72% in SA) among the HPV16 positive samples and the immune evasion capabilities of HPV16 [420] may have impacted the association of HPV16 methylation and CIN. Larger studies are required among both HIV positive and negative women using a variety of HPV methylation markers (which are available for HPV16, 18, 31 and 33) to investigate these associations.

Other studies have restricted measurement of DNA methylation markers to HR-HPV positive women to evaluate their performance as triage tests. In this study, the *EPB41L3* DNA methylation assay was performed irrespective of HR-HPV DNA status to describe the full range of epidemiological associations. A secondary sensitivity analysis restricted to HR-HPV positive women would help evaluate the performance of the assay as a possible triage test among WLHIV but would likely lack power.

This study had only one prevalent ICC case, hence the study cannot verify the findings of studies that have shown even increased methylation *EPB41L3* levels in ICC cases [256, 334].

There was a higher proportion of particpants in SA with zero percentage methylation for *EPB41L3* compared to BF. It remains uncertain why this may be the case, but there are two possible reasons. The proportion of ART users in BF in this study was higher than that reported in the main HARP cohort (85% vs. 68.6%), and also higher than the proportion of ART users in SA. Given that ART users had higher levels of EPB41L3 methylation, this may partly explain why median percentage levels were higher overall among women in BF. Secondly, there may be epigenetic differences between the two populations linked to different environmental exposures that cannot be fully investigated

in this study. Others have reported that alcohol and dietary factors, such as folate, vitamin B6 and vitamin B12 which supply the methyl units for DNA methylation can modify the effects of DNA methylation, especially in combination with environmental carcinogens and infectious agents [456]. Although the study did not collect information on diet, WLHIV in BF reported similar alcohol intake as their counterparts in SA, and alcohol was found to be associated with an increase in HR-HPV prevalence among women in BF. Others have shown that naturally occurring seasonal variations in food-consumption patterns, which vary by geographic region, have an effect on DNA methylation markers [457]. A larger study enrolling both HIV-negative women and WLHIV would be required to test whether there are epigenetic differences between the two populations.

The availability of an histological verification at both baseline and endline may have introduced a bias towards positive methylation (even in the absence of true disease) as women needed to have evidence of some abnormality in order to have been biopsied. In BF, 88% (58/66) of ≤CIN1 and 99% (143/144) of ≤CIN1 in SA were biopsied as they were positive for any one of the screening tests (the remainder were negative on all screening test and were considered CIN negative). This may had reduced the chances of discriminatory power of women with total absence of abnormalities.

Despite these limitations, this study had several strengths, including its longitudinal design, the availability of a rigorously validated histological endpoint for the majority of women thereby minimizing disease ascertainment bias; and the availability of methylation data at both time points. Furthermore, the inclusion of women with prevalent CIN2/3 at baseline and followed, untreated, up to the endline visit allowed monitoring of methylation levels over time among women with varying severity and duration of lesions.

10.6 Summary of findings

- Median methylation levels for *EBP41L3* were significantly higher among prevalent CIN2/3 vs. ≤CIN1 in BF and SA (Mann-Whitney p<0.001 and Cuzick p-trend by CIN grade p<0.001 in both countries).
- *EPB41L3* DNA methylation distinguished women with CIN2/3 with an area under the curve (AUC) of 0.77 (95% CI 0.65-0.89) in BF and 0.68 (95%CI 0.62-0.74) in SA.
- *EPB41L3 m*ethylation levels increased over time among the incident CIN2/3 at endline (Wilcoxon matched pairs test p=0.06 in BF, and p=0.11 in SA).
- *EPB41L3* methylation at endline generated an AUC of 0.80 (95%CI 0.63-0.97) in BF and 0.60 (95%CI 0.47-0.73) in SA for incident CIN2/3 detection and 0.95 (95%CI 0.86-1.00) in SA for incident CIN3.
- Among women with prevalent CIN2+ at baseline in SA, *EPB41L3* median methylation levels at baseline were higher among women with persistent CIN3, or CIN2 which progressed to CIN3 compared to women who spontaneously regressed to ≤CIN1 (Mann-Whitney p=0.016)
- In both countries 'high' *EPB41L3* methylation levels at baseline (>66.7 percentile point of distribution among ≤CIN1 controls) was associated with low CD4+ count ≤200 cells/mm^3 compared to CD4+ count >350 cells/mm^3 (BF: adjusted Odds Ratio [aOR]=7.45, 95%CI 1.53-36.22; SA: aOR=2.74, 95%CI 1.16-6.47; adjusted for HR-HPV and CIN status).
- HPV16 L1 DNA methylation was similar between CIN2+ and ≤CIN1 in both countries (Mann Whitney p=0.61 in BF and 0.49 in SA; Cuzick p-trend=0.24 in BF and 0.72 in SA).

Conclusion: Methylation of human biomarker gene *EPB41L3* DNA is elevated in prevalent CIN2/3, and incident and persistent CIN3 cases, and independently associated with lower CD4 count.

10.7 Findings in context

DNA methylation assays show promise as an earlier indicator of precancer and cervical lesion progression in high risk populations, such as WLHIV, alongside existing screening tools with lower specificity, such as HPV DNA tests. *EPB41L3* showed reasonable performance for the detection of CIN2/3. Given that DNA methylation markers in combination panels generate higher sensitivity and specificity compared to individual genes on their own (refer **Chapter 4**), multiplex DNA methylation assays including a combination of human genes may have a potential as primary screening of CIN2+ among WLHIV.

An ideal bio-marker for progressive CIN2+ among WLHIV would therefore be sensitive enough to detect clinically relevant HPV (i.e. associated with CIN2+) but specific enough to rule out HPV-positive women without evidence of disease (Test A). Furthermore, the ideal test (or a second test in triage; Test B) would be able to distinguish CIN2/3 that would persist from CIN2/3 that may spontaneously regress, thus prompting management for those most in need, but avoiding unnecessary management, use of resources and associated costs. Test B would be particularly useful for settings with limited resources for management, by prioritising women who are most likely to persist or progress to ICC for early management.

The current screening modalities for cervical cancer screening in BF (visual inspection) and SA (≥HSIL on cytology) already have shown reasonable performance for Test A, but still result in over-referral for colposcopy [446]. HPV DNA based testing would not entirely satisfy criteria for Test A given its low specificity (though high sensitivity) in this population [446]. Such triage strategies do not currently exist in either country. Human gene DNA methylation assays may have the potential to satisfy criteria for Test B, as they can be performed using the same clinician-collected or self-collected sample used for cytology or HPV testing [458] and commercially-available kits are being developed but current platforms and the requirement for highly trained laboratory staff preclude their use for lower resource settings currently.

Figure 10.2. Study flowchart

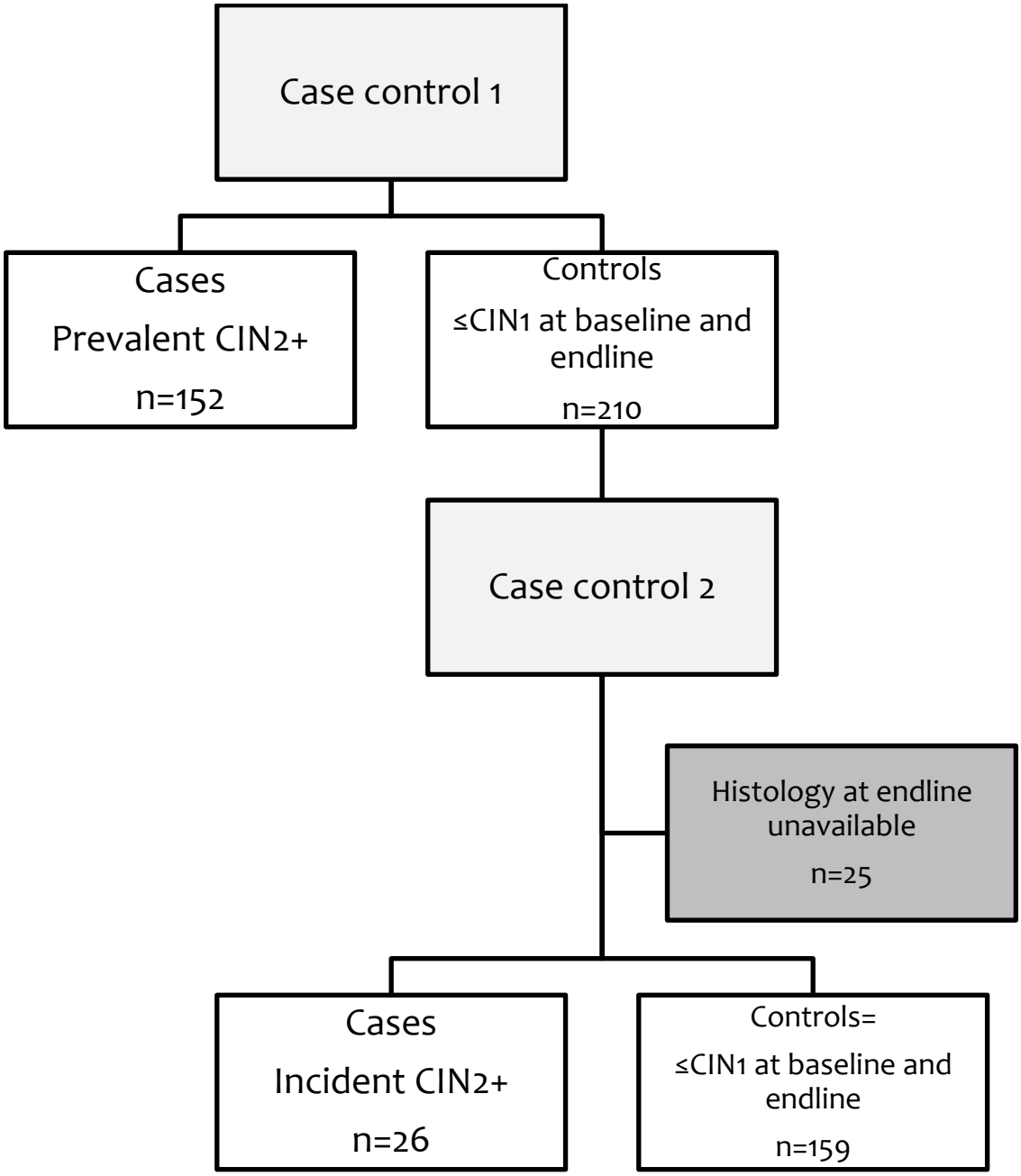


Figure 10.3. *EPB41L3* methylation levels (percentages) by CIN status among 94 WLHIV in Burkina Faso (BF) and 268 in South Africa (SA)

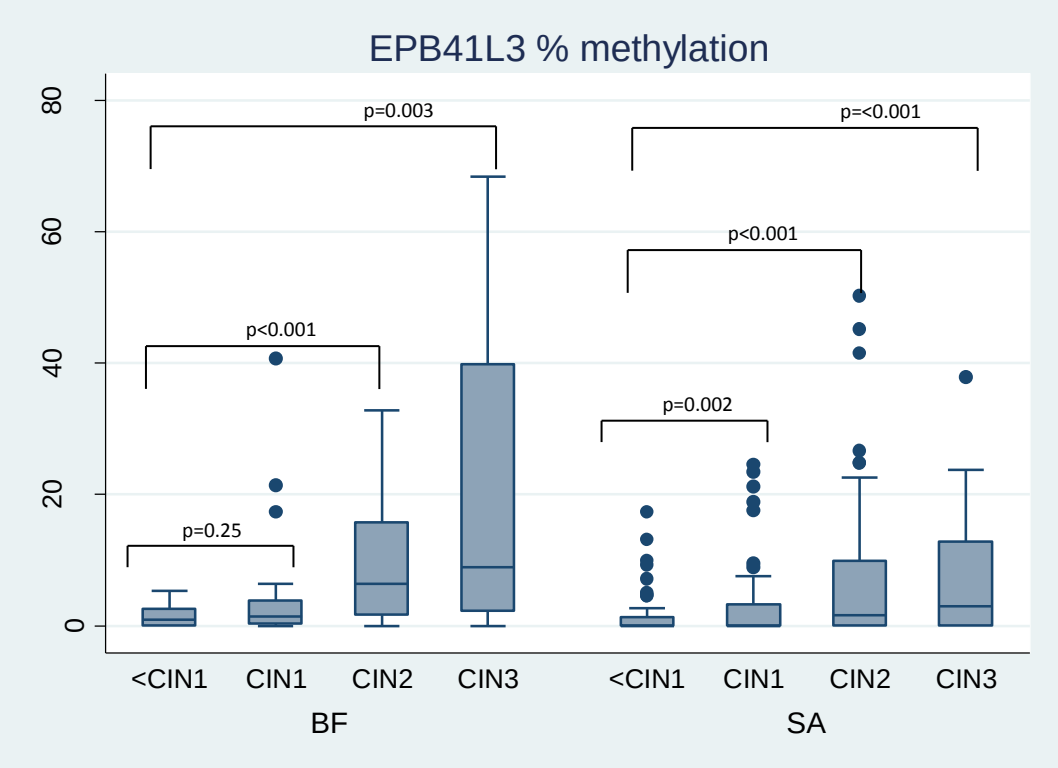


Table 10.5. *EPB41L3* median methylation levels (%) among 94 WLHIV in Burkina Faso and 268 in South Africa by CIN grade

	Burkina Faso			South Africa			Sites combined		
	N	**Median % (IQR)**	***p*[1]**	**N**	**Median % (IQR)**	***p*[1]**	**N**	**Median % (IQR)**	***p*[1]**
<CIN1	34	0.97 (0.0-2.60)	Ref	85	0.0 (0.0-1.30)	Ref	119	0.0 (0.0-1.50)	Ref
CIN1	32	1.42 (0.32-3.83)	0.25	59	0.0 (0.0-3.30)	0.05	91	0.87 (0.0-3.47)	0.02
CIN2	17	6.40 (1.63-15.73)	<0.001	73	1.60 (0.0-9.87)	<0.001	90	1.80 (0.0-11.17)	<0.001
CIN3	11	8.93 (2.20-39.80)	0.003	51	2.97 (0.0-12.80)	<0.001	62	3.85 (0.0-13.13)	<0.001
p-trend[2]			<0.001						<0.001

[1]Mann-Whitney U test, p-value for difference in median values of CIN2/3 relative to ≤CIN1;
[2]Cuzick test for trend; p-value for trend in methylation levels by CIN grade

Table 10.6. Sensitivity, specificity and area under the curve (AUC) for the detection of prevalent CIN2/3 relative to ≤CIN1 among 94 WLHIV in Burkina Faso and 268 in South Africa; and incident CIN2/3 relative to ≤CIN1 at endline among 57 WLHIV in Burkina Faso and 128 in South Africa

	Burkina Faso			South Africa		
	AUC (95% CI)	Sensitivity[1] (%)	Specificity[1] (%)	AUC (95% CI)	Sensitivity[1] (%)	Specificity[1] (%)
Baseline *EPB41L3* methylation						
Prevalent CIN2/3	0.77 (0.65-0.89)	60.7	72.7	0.68 (0.62-0.74)	58.9	70.8
Prevalent CIN3	0.77 (0.57-0.97)	63.6	72.7	0.70 (0.62-0.78)	60.8	70.8
Endline *EPB41L3* methylation						
Incident CIN2/3	0.80 (0.63-0.97)	75.0	71.7	0.60 (0.47-0.73)	40.9	71.7
Incident CIN3	0.62 (-)*			0.95 (0.86-1.00)	100.0	71.7
Baseline HPV16 methylation						
Prevalent CIN2/3	0.57 (0.30-0.83)	50.0	73.3	0.45 (0.29-0.60)	16.7	71.4
Prevalent CIN3	0.77 (0.56-0.99)	80.0	73.3	0.47 (0.30-0.65)	18.8	71.4

[1]Sensitivity obtained from reading ROC curve for a set 70% specificity; *only 1 case of incident CIN3 in BF

Table 10.7. *EPB41L3* median methylation levels (%) at baseline and endline for incident CIN2/3 over 16 months among 57 WLHIV in Burkina Faso and 128 in South Africa

	Burkina Faso				South Africa				Sites combined			
	N	Baseline values % (IQR)	Endline values % (IQR)	*P*[2]	N	Baseline values % (IQR)	Endline values % (IQR)	*P*[2]	N	Baseline values % (IQR)	Endline values % (IQR)	*P*[2]
≤CIN1*	53	1.43 (0.73-2.63)	1.20 (0.63-3.63)	*0.20*	106	0.0 (0.0-1.43)	0.0 (0.0-1.10)	*0.68*	159	0.0 (0.0-1.9)	0.0 (0.0-2.37)	*0.34*
Incident CIN2/3	4	2.45 (0.85-12.62)	5.35 (2.98-18.45)	*0.06*	22	0.0 (0.0-2.70)	0.0 (0.0-9.80)	*0.11*	26	0.0 (0.0-3.83)	0.85 (0.0-9.80)	*0.02*
p-value[1]		*0.42*	*0.05*			*0.68*	*0.07*			*0.85*	*0.10*	
≤CIN1*	53	1.43 (0.73-2.63)	1.20 (0.63-3.63)	*0.20*	106	0.0 (0.0-1.43)	0.0 (0.0-1.10)	*0.68*	159	0.0 (0.0-1.9)	0.0 (0.0-2.37)	*0.34*
Incident CIN2	3	3.83 (0.63-21.4)	7.10 (3.60-29.80)	*0.13*	19	0.0 (0.0-2.70)	0.0 (0.0-3.77)	*0.15*	22	0.0 (0.0-3.83)	0.0 (0.0-7.10)	*0.03*
p-value[1]		*0.27*	*0.04*			*0.76*	*0.44*			*0.90*	*0.50*	
Incident CIN3	1	1.07 (-)	2.35 (-)	*0.50*	3	0.0 (0.0-21.20)	14.2 (2.8-24.5)	*0.50*	4	0.53 (0.0-11.13)	8.50 (2.58-19.35)	*0.31*
p-value[1]		*0.77*	*0.67*			*0.71*	*0.001*			*0.85*	*0.005*	

*≤CIN1 at both baseline and endline; [1]Mann Whitney U p-value for difference in median values of incident CIN2/3 vs. ≤CIN1; [2]one-sided Wilcoxon matched pairs test, where the alternative hypothesis is that the median of the difference between baseline and endline values is greater than zero

Table 10.8. *EPB41L3* median methylation (%) at baseline and endline for CIN2/3 persistence, progression or regression over 16 months among 36 WLHIV not treated before final biopsy in South Africa

	N	Baseline values % (IQR)	Endline values % (IQR)	*P***
≤CIN1*	106	0.0 (0.0-1.43)	0.0 (0.0-1.10)	*0.68*
Persistent CIN2	7	2.20 (0.0-3.10)	0.93 (0.0-5.5)	1.00
Persistent CIN3[a]	5	11.35 (3.52-16.47)	13.67 (11.67-47.2)	0.31
Progression CIN2 to CIN3	3	24.70 (3.13-24.83)	14.0 (0.0-18.53)	0.88
CIN2/3 persistence/progression	8	15.67 (3.13-24.70)	13.82 (8.30-18.75)	0.50
Regression CIN3 to CIN2	5	3.70 (0.0-5.40)	7.60 (2.70-9.50)	0.06
Regression CIN2 to ≤CIN1[b]	13	0.43 (0.0-7.50)	0.0 (0.0-0.0)	0.997
Regression CIN3 to ≤CIN1	3	0.0 (0.0-1.37)	4.57 (0.0-17.03)	0.50
Any regression CIN2/3 to ≤CIN1	16	0.0 (0.0-3.83)	0.0 (0.0-0.0)	0.97

*≤CIN1 at both baseline and endline; **one-sided Wilcoxon matched pairs test, where the alternative hypothesis is that the median of the difference between baseline and endline values is greater than zero; [a]1 case of persistent CIN3 with missing value at baseline; [b]1 case of CIN2 regression to ≤CIN1 with missing values at baseline.

Table 10.9. Associations of HIV-related factors on 'high' *EPB41L3* methylation among 94 WLHIV in Burkina Faso and 266 in South Africa

	Burkina Faso				South Africa			
	N	**n (%)**	**aOR (95% CI) [1]**	**aOR (95% CI) [2]**	**N**	**n (%)**	**aOR (95% CI) [1]**	**aOR (95% CI) [2]**
All participants								
CD4+ count (cells /mm³)								
<200	15	10 (66.7)	**7.45 (1.53-36.22)**	-	32	22 (68.8)	**2.74 (1.16-6.47)**	-
201-350	16	7 (43.8)	1.79 (0.50-6.39)	-	73	38 (52.1)	1.52 (0.84-2.75)	-
>350	63	22 (34.9)	1.00	-	160	63 (39.4)	1.00	-
ART status								
>2 years	38	15 (39.5)	1.00	1.00	79	43 (54.4)	1.00	1.00
≤2 years	42	20 (47.6)	1.13 (0.38-3.33)	0.70 (0.22-2.29)	83	43 (51.8)	0.64 (0.33-1.27)	**0.48 (0.23-0.99)**
ART-naive	14	4 (28.6)	0.40 (0.09-1.79)	0.32 (0.06-1.58)	104	37 (35.6)	**0.37 (0.19-0.71)**	**0.38 (0.20-0.74)**
ART users								
HIV-1 viral suppression								
<1000 copies/ml	64	27 (42.2)	1.00		129	68 (52.7)	1.00	1.00
≥1000 copies/ml	13	7 (53.9)	2.34 (0.56-9.90)	1.29 (0.25-6.72)	32	18 (56.3)	1.19 (0.51-2.77)	0.94 (0.38-2.29)
HIV-1 viral detection								
≤40 copies/ml	55	22 (40.0)	1.00	1.00	46	25 (54.4)	1.00	1.00
>40 copies/ml	22	12 (54.6)	2.40 (0.75-7.73)	1.49 (0.37-5.90)	115	61 (53.0)	1.10 (0.51-2.37)	1.12 (0.52-2.43)
ART adherence **								
Low Adherence (<60%)	1	0 (0.0)	1.00	1.00	22	15 (68.2)	1.00	1.00
Moderate adherence (60-90%)	74	34 (46.0)	-	-	138	69 (50.0)	1.76 (0.63-4.92)	1.65 (0.58-4.66)
CD4+ count (cells/mm³)								
<200	13	8 (61.5)	4.88 (0.87-27.29)	-	30	21 (70.0)	2.42 (0.93-6.28)	-
201-350	13	7 (53.9)	2.15 (0.54-8.59)	-	45	26 (57.8)	1.80 (0.82-3.94)	-
>350	54	20 (37.0)	1.00	-	87	39 (44.8)	1.00	-
ART-naïve								
CD4+ count (cells/mm³)								
<200	2	2 (100.0)	-	-	2	1 (50.0)	2.35 (0.12-45.64)	-
201-350	3	0 (0.0)	-	-	28	12 (42.9)	1.15 (0.44-3.02)	-
>350	9	2 (22.2)	-	-	73	24 (32.9)	1.00	-

Adjusted Odds Ratio (aOR) [1]Model 1 adjusted for HR-HPV and CIN status in both countries, in addition to *Candida albicans* in BF; [2]Model 2 is same as Model with additional adjustment for CD4+ cell count; 3 ART users with missing HIV-1 PVL in BF and 1 in SA; 5 participants with no data on adherence in BF and 2 in SA; 1 ART-naïve particiants with no CD4+ cell count in SA

11 DISCUSSION AND CONCLUSIONS

In this chapter, I will present a summary of the key findings of the thesis, including a discussion of the factors found to be associated with HPV incidence and persistence and CIN2+ prevalence and incidence among WLHIV. I will illustrate how the potential interactions of cofactors with HR-HPV and with each other might facilitate the persistence of HR-HPV and the eventual incidence and progression of CIN2+. The role of ART on HPV and CIN2+ outcomes will be summarised and discussed. I will discuss the generalisability of these study findings to a wider population of WLHIV, and finally suggest recommendations for the control and prevention of HR-HPV infection and cervical lesions.

11.1 Summary of findings in this thesis

11.1.1 High levels of HR-HPV prevalence, incidence and persistence

This study found a high prevalence of HR-HPV, of multiple HR-HPV types and high rates of persistence and incidence over a median 16-months follow-up, in both countries. The women included in this study did not report recent high-risk sexual behaviours, such as a high number of recent sex partners that might have explained the high incidence in these populations. Yet, while 50% of all women in BF and 80% in SA had a single male sex partner at baseline, approximately half of these women in both countries self-reported using a condom all of the time and condom use all of the time was associated with a decrease in HR-HPV incidence. However, around one-third of women in both countries had a HIV-positive male partner, and under half of male partners in SA were uncircumcised (limited data from BF). These parameters are indicative of an increased risk of HPV acquisition.

HIV seroconcordance has been shown to be one of the strongest risk factors for HPV concordance between heterosexual couples [399]. Although this study found an inverse association in the cross-

sectional study (women with HIV-positive partners had lower HR-HPV prevalence compared to women with HIV-negative partners), this was not verified in the prospective for HR-HPV incidence, and there may have been some misclassification bias or unmeasured confounding at baseline. Studies of heterosexual couples from Cape Town have shown that HPV concordance (penile-cervical) between couples was significantly higher among HIV-infected couples than among HIV-seronegative couples [400], and women with a high HPV viral load frequently shared HPV types with their male partners [117]. Because PLHIV are less likely to clear HPV infections, frequent re-infection between partners may occur. It cannot be ruled out, that in this study, newly detected HPV DNA at endline could be re-activations of a latent infection, coinciding with periods of lack of HIV virological suppression and attendant immunosuppression in one of the partners. There is a possibility of non-synchronous reactivation of infections over time within HIV-seroconcordant couples as viral loads of certain genotypes at one point may increase during HIV disease progression, thereby increasing transmission potential. The risk may further increase if condom use is low or inconsistent, and if male partners are uncircumcised.

Male circumcision decreases HPV prevalence in both HIV-negative and HIV-positive men [108-113], and decreases transmission of HPV to female partners among HIV-negative men [85]. Although this study did not find a protective association of male circumcision with HR-HPV incidence, it was associated with lower CIN2+ prevalence among women in South Africa, and this may have indicated a HR-HPV infection which persisted. An association between male circumcision and HR-HPV incidence could not be demonstrated in BF, due to the low number of responders to the question at enrolment (only 10 women in BF responded to the question), which may indicate the question was not well understood by the interviewer or by the woman. The prevalence of male circumcision in Burkina Faso is known to be high (83-97%), based on DHS surveys [459] and it is therefore less likely that we would find an association of MC with HR-HPV and CIN2+ outcomes.

While data on HPV prevalence among men in Burkina Faso is unavailable, studies among men in Johannesburg report equally high prevalence of HPV and multiple HPV genotypes; among 304 HIV-

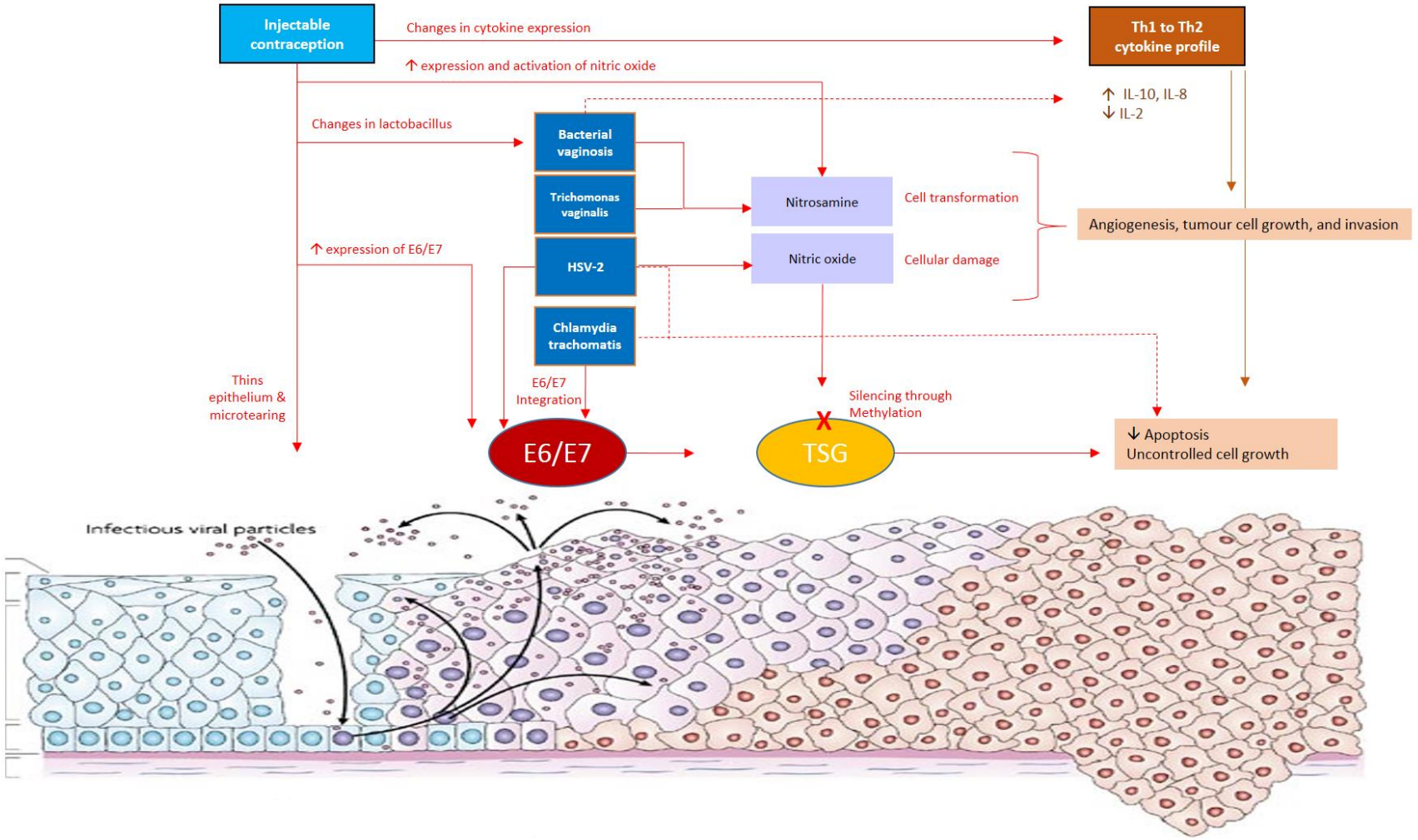
positive men enrolled in a study in Johannesburg, 95% of whom identifying as heterosexual, the prevalence of penile HPV was 79%, of HR-HPV was 52% and of multiple HR-HPV was 27%. [460].

11.1.2 Poor immunological response to HPV infection

The women in this study had a very high exposure to HPV, which was not effectively cleared resulting in high rates of DNA persistence and CIN2+ prevalence and CIN2/3 incidence over 16 months. The study has also shown that WLHIV in South Africa had very high HPV seroprevalence and seropersistence, with evidence of seroconversion over 16 months, however this did not protect against HPV DNA incidence and there is a possibility that seroconversion was a response to HR-HPV persistence. A decreased likelihood of seroconversion and DNA clearance and increased risk of HR-HPV persistence and CIN2+ prevalence were strongly associated with HIV-related factors, such as CD4+ cell count and ART. In addition to these HIV-related factors which were discussed in detail in **Chapter 7,** the persistence of HR-HPV infection and CIN2+ prevalence were further influenced by inflammation linked to co-infection with other STIs and BV, as well as being facilitated by the potential carcinogenic effects of injectable contraceptive use.

The possible interactions of the various cofactors in the pathway from HPV infection to CIN2+ are summarised in **Figure 11.1**.

Figure 11.1.Possible interactions of cofactors (injectable contraception and STIs) on the pathway from HPV acquisition to CIN2+



A full description of the pathway from HR-HPV acquisition to ICC is reviewed in **Chapter 2; section 1.4**; Th1=T-helper 1 immune response; Th2=T helper 2 immune response; IL=interleukin; TSG=Tumour Suppressor Gene; cross-sectional view of cervix on bottom of diagram from [44]

11.1.3 The role of immunity and inflammation

In this study, the presence of other STIs, cervicitis and BV was associated with an increase in HR-HPV incidence, HR-HPV persistence and CIN2+ prevalence.

The inflammatory response to infection is part of the normal host response to eliminate foreign pathogens, however chronic inflammation caused by bacterial and viral infections can promote cancer development and progression. While HPV can subvert host immunity and establish persistent infection, the co-existence of other STIs can create an inflammatory environment in the cervical mucosa, resulting in 1.) inflammatory cytokine responses and 2.) increasing levels of nitric oxide that can facilitate the persistence of HR-HPV and allow for carcinogenic changes to occur. **Figure 11.1** illustrates the interactions of the various cofactors for HPV persistence and cervical lesion development and progression which may occur among WLHIV.

1. *Specific cytokine profile involved in inflammation and tumour initiation*

Available evidence suggests that CIN2/3 lesions appear to be characterised by a reduction in the Th1 immune response, which stimulates cytokines in response to intracellular parasites such as bacteria and parasites, but an increase in the Th2 immune response which modulates antitumor responses. Despite the inconsistency and variation in cytokine panels tested across a number of studies among women with CIN2+, increased levels of IL-8 and IL-10 have consistently been shown to be associated with CIN2+, while IL-2 and IFN-γ appear to be reduced. There are however difficulties associated with interpretation of cytokine profiles for infection, particularly in blood and plasma samples, as HPV does not elicit a systemic response, and no cytokine profile has been identified that is specific to HPV infection.

Higher levels of IL-2 in peripheral blood lymphocyte culture supernatants have been reported in women with normal pap smears compared to women with LSIL, HSIL and ICC [461], whilst lower concentrations of IL-2 and IFN-γ are been reported in some studies in blood samples of HIV-negative women with CIN3+ [462, 463] compared to women without cervical disease.

By contrast, concentrations of IL-10 have repeatedly been shown to be increased in cervicovaginal lavage (CVL) [464, 465], blood [462], serum [466] and plasma [467] of HIV-negative women with CIN3 or ICC compared to women with no disease. However, IL-10 levels were similar among HR-HPV positive and HR-HPV negative without cervical disease [466], suggesting that involvement of IL-10 is in the later stages of carcinogenesis rather than infection.

Levels of IL-8 have also been shown to be elevated in biopsy specimens [468] and CVL samples [465, 469] of HIV-negative women with ICC, and were also higher in plasma of women with persistent HR-HPV compared to women who cleared infection [470]. Two recent studies among 93 HIV-positive and 72 HIV-negative women in Cape Town, South Africa [471], and 45 HIV-positive and 27 HIV-negative women in Miami, Florida [472], have shown that IL-8 levels were higher among HIV-positive women compared to HIV-negative women. Furthermore, women who had presence of BV had higher levels of IL-8, after adjustment for HIV status and other confounders [472].

A similar switch from the Th1 to Th2 immune response is also observed with HIV-disease progression. A small study among 13 HIV-positive women and 20 HIV-positive men initiating ART in Kigali, Rwanda [473] reported a negative correlation between CD4+ cell count and IL-10, but IL-10 was positively correlated with HIV-1 plasma viral load ($p<0.05$). This is consistent with the observation that advanced HIV disease is associated with an increased risk of CIN2+.

Both oral and injectable hormonal contraceptives can regulate cytokine and immunoglobulin expression [134] which may facilitate HPV persistence. The injectable hormonal contraceptive DMPA (used by 56% WLHIV in South Africa in this study) has been shown to facilitate mucosal inflammation, resulting in increases in the concentration of genital cytokines among HIV-negative women [474]. DMPA was also found to be associated with a suppressed Th1 response with lower levels of IFN-γ, IL-2, IL-4, IL-6, IL-12 and TNFα among HIV-negative women in the USA [475], and with increased levels of IL-8 in a cross-sectional analysis of 376 Kenyan and South African HIV-negative women.

2. *Elevated nitric oxide is associated with carcinogenesis*

Infections with *Trichomonas vaginalis* (TV), HSV-2 and BV have been associated with the production of nitric oxide and nitrosamine in cervical cells. Nitric oxide is a short-lived, endogenously produced free-radical gas that acts as a signalling molecule, induced by immunological stimuli, and is regulated by the Tumour Suppressor Gene p53 which inhibits production at elevated concentrations through negative feedback loop mechanism [476]. Tannenbaum et al first showed that infection resulted in increased levels of nitrite [477]. Nitric oxide is required to kill tumour cells, but in high levels, it can also form carcinogenic nitrosamines which can modify DNA or DNA repair proteins (**Figure 11.1**) [478]. Higher levels of nitric oxide have been reported in the serum of women with cervical cancer compared to healthy control [479, 480], in vaginal and endocervical swabs of women with CIN2+ [465] and increased expression of nitric oxide synthase (nitric oxide producing enzyme) was reported in HPV-infected epithelial and inflammatory cells from CIN lesions [481]. Nitric oxide has also been shown to act directly on the HPV virus causing earlier mRNA expression, silencing of Tumour Suppressor Genes (pRb and p53), resulting in increased survival of mutant cells leading to carcinogenesis [476].

Oestrogen and progesterone can upregulate the nitric oxide production; studies have shown that oestrogen can stimulate release of nitric oxide synthase in breast tissue [482] and progresterone has been found to activate nitric oxide synthase expression [483].

It is therefore possible that in this study, women with uncontrolled HIV disease, i.e. low CD4+ cell count and high HIV-1 plasma viral load may have higher levels of Th2 cytokines, further exacerbated by the presence of BV and injectable contraceptive use, which may have facilitated HR-HPV persistence and cervical lesion progression (**Figure 11.1**). Furthermore, elevated levels of nitric oxide associated with infection by other STIs including TV, HSV-2 and BV result in DNA damage, silencing of Tumour suppressor Genes and cell transformation. While there is no evidence that this

is the case for human gene *EPB41L3*, selected to be examined in this study, given that it is a TSG implicated in other cancers, it is plausible that similar mechanisms may be targeting *EPB41L3*.

11.1.4 Role of antiretroviral therapy

In this study, WLHIV taking ART for prolonged duration (>2 years) had the best outcomes in terms of burden of HR-HPV infection and CIN2+ prevalence and incidence. They had the lowest HPV DNA prevalence, incidence and persistence and CIN2+ prevalence and incidence when compared to short-duration (≤2 years) users or ART-naïve women in both countries. They also had the highest likelihood of clearing all HR-HPV infections (**Table 11.1 and Table 11.2**).

By contrast, short duration ART users at baseline had the highest HR-HPV prevalence and incidence, and the highest CIN2+ prevalence possibly due to the cumulative effect of starting at a lower nadir CD4+ cell count. However, by endline, short-duration ART users had high HPV seroconversion rates and low CIN2/3 incidence in SA (equivalent to long-duration ART users). A longer duration on ART by endline with a corresponding increase in CD4+ cell count may have played a part in reducing the risk of CIN2/3 incidence over 16 months.

ART-naïve participants appeared to have the worst outcomes over time. Although these women had a similar risk of HR-HPV prevalence and incidence, and CIN2+ prevalence as short-duration ART users (despite higher median CD4+ cell counts), they had the highest CIN2/3 incidence of all the groups in South Africa, and the highest persistence of HR-HPV in BF. While short-duration ART users appeared to recover their CD4+ T-cells over time and reduce their risk of HR-HPV persistence and CIN2/3 incidence, the risk remained elevated for ART-naive women (**Table 11.1 and Table 11.2**). This may be because ART-naïve WLHIV experience a continual decrease in mucosal immunological competence over time, despite maintaining a reasonable number of T-lymphocytes. By contrast, short-duration users, depending on the timing of ART initiation, may take a longer path to recovery, but will eventually restore some level of competence. In this study, the short-duration ART users appear to have similar risk of CIN2/3 incidence as long-duration ART users after 16 months duration.

These results confirm that there is a beneficial effect of ART initiation on HR-HPV and CIN lesion development, preferably early at a high nadir CD4+ cell count when a degree of competence is retained, but that positive effects may only appear over prolonged duration. It is unclear whether ART has a direct effect on the HPV virus; the most common classes of ARVs, nucleoside and non-nucleoside reverse transcriptase inhibitors (NRTIs and NNRTIs) inhibit the transcription of HIV RNA into double-stranded HIV DNA [484] and it is not yet clear whether there may be some similar inihibition of the HPV virus. However the indirect effects of ART use include increases in CD4+ cell count with a certain degree of competence (perhaps not complete) needed to clear infection and prevent lesion progression, in addition to a decrease in other cofactors associated with HR-HPV persistence and CIN2+. Indeed, prolonged ART use is associated with a reduction in cervicovaginal HSV-2 shedding [142, 485] and regulation of the inflammatory environment [411]. ART use, accompanied by an increase in CD4+ cell count was associated with a decrease in IL-10 and an increase in IL-2 and IFN-γ among ART initiators in Kigali, Rwanda [473] and among ART initiators in in Brazil, Haiti, India, Malawi, Peru, South Africa, Thailand, the United States, and Zimbabwe as part of randomized controlled trial of ART regimens [486].

A recent Brazilian study has shown that ART users have decreased nitric oxide compared to ART-naïve participants. Soccal et al reported [487] among 18 HIV-positive ART-naïve participants, 49 HIV-positive participants on ART (for at least 6 months) and 46 HIV-negative participants, that there was a statistically significant increase in nitric oxide levels in the ART-naïve group (164.0 ± 166.6 μmol/L), compared with both the ART users (71.7 ± 53.3 μmol/L) and the HIV-negative group (98.9 ± 59.4 μmol/L), and the differences in nitric oxide levels were independent of gender and other covariates [487]. The similarities in nitric oxide levels between the ART users and the HIV-negative group suggest that ART somehow inhibits nitric oxide production among PLHIV, but whether this is a direct or indirect effect remains to be understood.

Table 11.1. Summary of HPV and CIN2+ outcomes and their association with ART among WLHIV in Burkina Faso

		ART-naïve Throughout FU	Short-duration ART	Long-duration ART	Is association independent of CD4+ cell count?
	Median CD4+ count at baseline (IQR)	414 (314, 604)	411 (276, 574)	478 (368, 639)	
	Median CD4+ count at endline (IQR)	586 (432, 888)	519 (368, 710)	627 (420, 829)	
HPV DNA	HR-HPV Prevalence	60.1%	65.1%	52.1%	
	aPR	1.12 (0.93-1.36)	**1.21 (1.03-1.44)**	1.00	Yes
	HR-HPV Incidence	47.2%	53.9%	45.5%	
	aPR	1.03 (0.80-1.32)	1.20 (0.96-1.50)		No
	HR-HPV persistence	58.6%	33.3%	37.7%	
	aOR	1.80 (1.21-2.66)	**0.73 (0.45-1.21)**		Yes
	HR-HPV complete clearance	17.6%	22.8%	28.2%	
	aPR	0.70 (0.41-1.22)	0.81 (0.50-1.33)		No
CIN2+	CIN2+ Prevalence	4.2%	7.8%	5.5%	
	aOR	1.20 (0.37-3.94)	1.22 (0.48-3.08)	1.00	No
	CIN2/3 Incidence	0.0%	1.0%	1.8%	

Table 11.2. Summary of HPV and CIN2+ outcomes and their associations with ART among WLHIV in South Africa

		ART-naïve throughout FU	Short-duration ART	Long-duration ART	Is association independent of CD4+ cell count?
	Median CD4+ count at baseline (IQR)	448 (353, 614)	325 (207, 460)	478 (368, 639)	
	Median CD4+ count at endline (IQR)	437 (346, 543)	427 (311, 509)	457 (326, 628)	
HPV DNA	HR-HPV Prevalence	82.2%	82.1%	72.4%	
	aPR	**1.10 (1.00-1.22)**	1.09 (0.98-1.22)	1.00	No (marginal)
	HR-HPV Incidence	47.6%	52.5%	48.6%	
	aPR	0.95 (0.76-1.20)	1.01 (0.79-1.28)	1.00	No
	HR-HPV persistence	31.8%	35.2%	24.5%	
	aOR	1.50 (0.94-2.40)	**1.75 (1.17-2.64)**	1.00	Yes
	HR-HPV complete clearance	21.1%	24.5%	33.3%	
	aPR	**0.65 (0.42-0.99)**	0.82 (0.53-1.26)	1.00	Yes (naïve only)
HPV serology	HPV Seroprevalence	89.1%	94.3	94.6%	
	aPR	0.98 (0.92-1.03)	0.98 (0.92-1.04)	1.00	No
	HPV Seroincidence	41.7%	61.3%	43.5%	
	aPR	1.03 (0.83-1.29)	**1.26 (1.01-1.56)**	1.00	Yes (short vs. long)
	HPV Seroconversion	14.2%	24.6%	17.2%	
	aOR	0.85 (0.41-1.75)	1.56 (0.72-3.40)	1.00	No
		1.00	**2.39 (1.02-5.62)**		Yes (short vs. naïve)
CIN2+	CIN2+ Prevalence	25.3%	29.2%	15.0%	
	aOR	**1.78 (1.06-2.99)**	**1.81 (1.03-3.17)**	1.00	Yes
	CIN2/3 Incidence	9.6%	4.5%	4.5%	
	aOR	2.26 (0.80-6.41)	1.53 (0.43-5.46)	1.00	No (marginal)

11.1.5 Why were disease rates higher in South Africa?

The rates of CIN2+ at baseline and endline were strikingly higher among WLHIV in South Africa compared to Burkina Faso, despite similar incidence and persistence rates of HR-HPV, and similar HPV genotype distribution and type-specific persistence. The difference in CIN2+ rates is possibly due to: a.) the difference in prevalence of co-factors associated with HR-HPV infection which may facilitate its persistence, and b.) poorer control of HIV disease among WLHIV in South Africa.

The prevalence of STIs (*Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma genitalium* and HSV-2) was consistently higher among women in South Africa at baseline, except for *Candida albicans* which was higher among women in Burkina Faso. In particular, nearly all the women (95.2%) enrolled in South Africa were HSV-2 seropositive compared to 74.9% in Burkina Faso. The resulting inflammation response associated with clinical recurrences and shedding [142, 411] and associated cytokine profile that accompany HSV-2 [141, 142, 488] may have facilitated cervical lesion progression.

The prevalence of injectable contraceptive use was also far greater among WLHIV in South Africa compared to in Burkina Faso (72.2% vs. 20.0%, $p<0.001$), even though the proportion of oral contraceptive users was largely similar (31.3% vs. 26.8%, $p=0.23$).

Women in South Africa were less likely to control HIV disease. Despite similar median CD4+ cell counts among ART users in Burkina Faso and South Africa at baseline, a greater proportion of women in South Africa had detectable HIV-1 PVL and also lower reported adherence to ART. Short duration ART users in South Africa had lower median CD4+ cell counts than their counterparts in Burkina Faso at baseline, and possibly started ART at a lower nadir CD4+ cell count, although this data was unavailable in this study.

11.2 Generalisability of study findings

Women enrolled in the *HARP* study in Burkina Faso and South Africa represent the broad spectrum of WLHIV attending primary healthcare services in the cities of Ouagadougou and Johannesburg, however there may be differences that could affect the generalisability of these findings to the wider population of WLHIV. **Table 11.3** summarises some of the key characteristics of the *HARP* study population, which were associated with HR-HPV or CIN2+ outcomes, and the known prevalence of these factors among a wider population of WLHIV in the respective countries, using national estimates, or individual studies when national estimates were unavailable. For some parameters, estimates are only available from the general population. The major differences between the HARP study participants and the wider population of WLHIV are age, stratification by ART and the exclusion of women who have already been treated for CIN.

Age range

The median age of women in the HARP study was 36 years (IQR: 31-41) in Burkina Faso and 34 years (IQR: 30-40) in South Africa. Women younger than 25 years were excluded from the study, as the aim was to evaluate cervical cancer screening strategies, including HPV DNA tests, which are not usually recommended for young women, and the exclusion of women <25 years in the *HARP* study may have influenced the overall prevalence of HR-HPV, which could be even higher had we included younger age groups. Mostly, persistence of HR-HPV in younger women is known to occur less frequently than in older women. Hence our lower age limit of 25 may have resulted in **selection bias** towards lower prevalence and higher persistence of HR-HPV. A population—based prospective case-control study in the UK reported that cervical screening in HIV-negative women <25 years (20-24 years) had no impact on ICC up to the age of 30 years [489].

However, others have shown a high rate of CIN2+ among WLHIV aged 20-25 years in Cape Town [397], suggesting that cervical lesions occur earlier among WLHIV compared to their HIV-negative counterparts, linked to increased HR-HPV persistence. Women aged >50 years were excluded from

the *HARP* study due to the difficulty in interpretation of visual inspection in older age groups [490-492]. Women are now living longer with HIV but increases in AIDS-defining illnesses, including ICC in older age are expected. This age exclusion in the *HARP* study limits the ability to extend findings to an older population with higher rates of CIN3 and ICC.

Antiretroviral viral therapy use

The proportion of ART users in Burkina Faso and South Africa in the *HARP* study is representative of the national percentage of WLHIV on ART in both countries, according to UNAIDS estimates from 2015 [10] although the proportion of ART users in SA is slightly higher than the national estimate (63% vs. 53%, respectively). However, the coming years may see an increase in ART use among WLHIV if the UNAIDS "90-90-90" strategy launched in 2014 is successful in its aims of ensuring that, by 2020, "90% of all people living with HIV will know their HIV status, 90% of all people with diagnosed HIV infection will receive sustained antiretroviral therapy and 90% of all people receiving antiretroviral therapy will have viral suppression" [493]. The feasibility of achieving these aims by 2020 remains uncertain; by the end of 2015, the greatest challenge to meeting the "90-90-90" targets was in the provision of ART, with large disparities identified across countries [494].

It would be expected that the increasing availability of ART, with earlier initiation, should contribute to lower rates of HR-HPV persistence and CIN2+ incidence among WLHIV, compared to what was observed in this study.

Hormonal contraceptive use

In both Burkina Faso and South Africa, a higher proportion of HARP participants reported ever having used injectable and oral contraception compared to other cohorts of WLHIV in the respective countries, which may have resulted in **selection bias**, making the study findings less generalizable to the wider population of WLHIV. This is especially relevant to women in South Africa, as this study found an increased risk of CIN2+ prevalence among injectable contraceptive

users. It is not clear why the proportion of WLHIV reporting hormonal contraceptive use in *HARP* was higher than other cohorts of WLHIV in South Africa. In this study both past and current users were considered as a single group of 'ever users', whereas other studies may have restricted analyses to current users only, though it is not clear from the reports. In this study, current users had the highest risk of CIN2+ prevalence. The residual risk among past users justified their inclusion in the 'ever users' risk group. It is also surprising that other cohorts of WLHIV report such low use of hormonal contraception, when current prevalence of use in the general population of married and in-union women in SA in 2015 was 30.3% [122].

Other potential sources of bias

Few women in Burkina Faso provided a response to the question on whether their male partner was circumcised or not. The prevalence of male circumcision in Burkina Faso is high (83-97%), based on DHS surveys (**Table 11.3**), and it is therefore less likely that we would find an association of MC with HR-HPV and CIN2+ outcomes.

The HARP study maximised the chances of obtaining histological results by basing biopsy decision on positivity of ANY of FOUR screening tests (Hybrid Capture 2 [HC2], cytology with any reading ≥ASCUS, and abnormal findings on visual inspection [VIA/VILI] or colposcopy) to which ALL participants were subjected. This approach and the threshold to trigger biopsy for histology are in excess of usual recommendations, to minimise ascertainment bias in this study. Women negative by all tests were considered to be at extremely low risk of CIN2 since in particular HPV DNA and cytology have very high negative predictive values for CIN2 diagnosis [402]. Based on this biopsy algorithm, there is a potentially greater risk of ascertainment bias in BF as fewer women were biopsied and biopsy decision was largely based on HC-II test positivity. The CIN2+ prevalence and incidence estimates could be underestimated in BF. However, given the high sensitivity and NPV of HC-II [377, 402] it is unlikely that we would have missed many cases. In addition, the study built a strong review of histological results by consensus of 5 pathologists, which included all CIN1/2 and

CIN2+ cases and 10% of ≤CIN1 cases (among women who had been biopsied for at least one positive screening tests).

As discussed in the limitations of the research **Chapters 6 to 10**, this study was constrained by the limited number of intermediate visits and overall follow-up duration. The definition of cumulative HR-HPV incidence over 16 months is of limited duration to assess the associations of risk factors with HR-HPV incidence. Furthermore, the study could not rule out type-specific clearance and reinfection when estimating persistence during the 16-months interval between HPV testing. The evaluation of HPV at two time points only did not allow precise estimation of the duration of infections. Potential misclassifications of HR-HPV infection states could have led to inaccuracies in the description of the natural history of HPV and the associations of the risk factors with the different HR-HPV infection states. However in using a highly sensitivity genotyping assay (INNO-LiPA), it is unlikely that persistent HR-HPV infections would have been missed, but HR-HPV clearance could have been missed in during the 16-months follow-up period.

The lack of repeat testing for HIV-1 PVL may have resulted in misclassification of the effectiveness of ART use. ART duration was used as a proxy measure for effective ART use in this study, but others have reported that sustained HIV-1 viral suppression over 40 months among ART users was associated with a decreased risk of HR-HPV persistence [209].

Similarly, the lack of repeat testing for STIs at endline prevented further investigation of known risk factors for HR-HPV persistence at the time of detection. The use of baseline STI result have resulted in inaccuracies in the risk factor analyses for HR-HPV persistence and CIN2/3 incidence.

Much of the behavioural and sexual behaviour factors were self reported (smoking, hormonal contraception, condom use, male partner circumcision and HIV status of male partner) , which can result in recall and social desirability bias,and this may have influenced the accuracy of the risk factor analyses.

Table 11.3. Generalizability of study findings to wider population of WLHIV, based on study population characteristics

	Burkina Faso			South Africa		
	Prevalence in HARP	**National estimates**	**Generalizability to WLHIV population**	**Prevalence in HARP**	**National estimates**	**Generalizability to WLHIV population?**
Proportion on ART	68.6%	66% [10]	Comparable	65.2%	53% [10]	Unclear, HARP slightly higher **Possible selection bias**
HIV-1 viral suppression among ART users	79.9%	81.8% among WLHIV (FSW) & 100% among WLHIV (non-FSW) [495][1]	Comparable	80.3%	87% among WLHIV* [496]	Lower proportion with HIV-1 PVL suppression in HARP **Measurement bias** when using different PCR methods and cut-offs
Smokers	0.8%	5% [497] *among general population*	HARP is lower than general population **Possible selection or information bias**	12.5%	7.3% [498] *among general population*	Comparable to general population
Male partner circumcision	No precise estimate	83-97% *among general population* [459]	Difficult to ascertain as HARP data was mostly missing **Possible Information bias**	48.5%	46 *among general population* [268]	Comparable to general population
Oral contraception use	26.8%	13.3% any HC among WLHIV [499]	Higher than previous reports, but limited data **Possible selection bias**	31.3%	9% [500] to 14% [501]	HARP is higher than other reports among WLHIV. **Possible selection bias** in HARP or in other reports
Injectable contraception use	20.0%	3.3% to 7.3% *among general population* [502, 503]	Not Comparable to generable population, but no available estimates among WLHIV	72.2%	Various from 8% [403], 40% [501] to 45% [500]	
HSV-2 serology	74.9%	59.6% among WLHIV [502]	HARP data Is higher, but only 1 study	95.2%	77% (mix of HIV positive and negative)[504]	HARP data Is higher, but only 1 study
Bacterial vaginosis	34.6%	35.7% among WLHIV [499] [502]	Comparable	41.6%	62.5% WLHIV aged 17-21 years [505]	HARP is lower but difference in age range with [52] **Possible selection bias, or measurement bias for BV detection**

[1]HIV-1 PVL undetectable, defined as <300 copies/ml; FSW=female sex workers; *study combined WLHIV from South Africa and Uganda for a single estimate

11.3 Recommendations

In considering the findings of this study, and taking into account the generalizability of the study population to a wider population of WLHIV in Africa, the following recommendations are discussed in four key areas: 1.) prevention of CIN through screening; 2.) monitoring and control of HIV disease for control of HPV-related disease; 3.) control of co-factors for HPV infection and cervical disease progression, including STI prevention and 5.) prevention of HPV infection through vaccination.

11.3.1 Prevention of CIN through screening

Cervical cancer screening tests for WLHIV

Current international guidelines from WHO recommend that cervical cancer screening should be started in sexually active girls and women, as soon as they have tested positive for HIV, and if the screening test is negative, a repeat test is done within three years [278].

Repeated screening of WLHIV is extremely important. This study has shown that even among previously screened CIN negative women, the incidence of CIN2/3 over a 16-month duration was 1.2% among WLHIV in Burkina Faso and 5.8% among women in South Africa. It is unclear how many of the incident CIN2/3 may have persisted or progressed to higher grades beyond the end of this study, however 44% of women with CIN2+ at baseline had spontaneous regression to ≤CIN1 at endline.

The suitability of screening tests for WLHIV is dependent upon: i.) resource requirements; and ii.) performance of the test for CIN2+ detection in this population.

- Visual inspection by VIA/VILI has shown to be the most useful test in resource limited setting, requiring minimal equipment and training, and can be performed by trained nurse or midwife, however it has variable sensitivity and specificity for CIN2+ [229, 238]. Its potential benefit, however, is that frequent screening should be possible if integrated into HIV services. A potential limitation is that it may result in a high number of unnecessary

colposcopy referrals; but VIA/VILI can also be coupled with immediate treatment by cryotherapy with good overall impact on the early management of CIN2+, as demonstrated in Cape Town, South Africa [227].

- HPV testing is an increasingly recommended primary screening strategy. HPV DNA tests are now available in a format that can be easily adapted to resource-limited settings. The *care*HPV test (Qiagen) has shown good performance among HIV-negative women for the detection of CIN2+ [236] and in the *HARP* study [446], although it loses specificity when used among WLHIV given the high prevalence of HR-HPV in these populations. HPV testing may detect many transient infections, but as a repeat test over the duration of the HARP study, its specificity and PPV for detection of CIN2+ increased [446].
- There is a potential role for novel screening biomarkers, such as DNA methylation in this population, although DNA methylation based tests are not yet ready, nor would they be cost-effective as a primary screen for resource limited settings. Their utility as a triage test should be evaluated as it could reduce the number of colposcopy referrals, which would be valuable in resource-limited settings.

Increase availability and uptake of cervical cancer screening

Uptake of cervical cancer screening is still low in both countries; in Burkina Faso, 7.8% of all women aged 18-69 years in 2008 were screened within the previous 3 years [266], while in SA the uptake of cervical screening in 2014 was estimated to be 21.4% among women aged 30-39 [275]. Furthermore, the level of awareness of cervical cancer and HPV varies according to population. In this study, there was a high level of awareness of cervical cancer among women in Burkina Faso and South Africa (93% and 76%, respectively), despite the fact that a very low number of women were aware of HPV (6% and 20%, respectively). A lower proportion of women in Burkina Faso were worried about cervical cancer (28% vs. 42% in BF and SA, respectively). However, a recent survey among 87 WLHIV aged 30-69 attending the Kisenyi Health Unit in Kampala, Uganda, found that

99% of WLHIV did not think it necessary to be screened for cervical cancer and 96% had never heard of HPV, despite the fact that 45% had tested positive for HR-HPV in that study [506].

More efforts may be needed in emphasising the importance of cervical cancer screening in this population. Integration of cervical cancer screening within HIV care services could lead to continuity in primary prevention, favoring early detection and management of HPV-related cervical lesions with minimal loss to follow-up [507].

11.3.2 Control of HIV disease to enhance the control HPV-related cervical disease

Early initiation of antiretroviral therapy (ART)

CD4+ cell count is one of the strongest predictors of HR-HPV infection, persistence and SIL/CIN incidence and progression. But it has a limited effect on regression once lesions have established. Encouraging early ART initiation could have a significant impact on reducing CIN2+ incidence. The South African Department of Health announced the introduction of immediate ART initiation for those testing positive for HIV in the public sector in 2016, however the feasibility of wide-scale access with respect to financing, burden on healthcare facilities and access to ART, adherence forgiveness and potential risk of drug resistance strains in the community is uncertain.

Cost-effectiveness studies weighing costs of drugs for all compared to the cost of lives lost or co-morbidities requiring treatment including AIDS-related, non-AIDS-related cancers and opportunistic infection (cardiovascular, kidney and liver disease) are warranted [508].

Routine screening for women starting ART at low or unknown CD4+ cell count

ART users with low or unknown nadir CD4+ cell count should be screened frequently, although the optimal screening intervals remain unclear. Some authors have suggested that a screening interval triaged by CD4+ cell count may be of value considering the dose relationship observed in some

lesion progression studies [198]. However, such recommendation may become obsolete as more women become treated at higher CD4+ cell count. Of importance, maintaining a sustained viral suppression and stable high CD4+ cell count is required to reduce the risks of HR-HPV persistence and CIN/SIL development. This requires strong counseling on ART adherence and patience for the ART effects to take place.

High levels of ART adherence

Treatment success needs strict lifelong drug adherence. Reasons for non-adherence and interruption of treatment include the inconvenience of a daily regimen, and side effects associated with ART, including lipoatrophy (peripheral fat wasting), lipohypertrophy (central fat accumulation), insulin resistance, renal and bone dysfunction and increased risk of cardiovascular disease and hepatoxicity [509-513]. Suboptimal adherence allows HIV replication to continue in the presence of insufficient drug concentrations leading to drug resistance, which can be monitored using HIV genotyping. Encouraging high adherence, especially among women who started ART at low nadir CD4+ cell count is necessary to reduce the risk of cervical lesion incidence.

Regular monitoring of CD4+ cell count and HIV plasma viral load

ART-naïve women are at higher risk of HR-HPV infection and cervical lesion development when CD4+ cell count decreases. CD4+ cell count should be monitored closely and ART initiated at high CD4+ count. Starting ART early, coupled with rapid virological control, is likely to rapidly improve and maintain CD4+ at a higher level [408], leading to possibly more complete immune reconstitution at the systemic and mucosal levels, thereby reducing the risk of persistent HR-HPV infection and CIN incidence or progression.

Continued access to ART treatment

Urbans areas, such as Ouagadougou and Johannesburg attract a substantial migrant population, but migrants may not have equal access to healthcare. A recent study among attendees at a public-

sector HIV care centre in Johannesburg reported that unconfirmed South Africa citizens (without national South Africa ID number) were more likely to die or become lost to follow-up after 1-year post-ART initiation compared to confirmed SA citizens (with national ID number) [514].

Both Burkina Faso and South Africa depend on external donor funding for financing of HIV and sexual and reproductive health (SRH) services (refer **Chapter 2, Table 2.6**). Recent changes in political will may threaten continued financing of international programmes to provide these services to LMIC [515, 516].

11.3.3 Influence of other co-factors for HPV infection and ICC

Prevention of sexually transmitted infections (STIs) acquisition

Condom promotion and male circumcision

There was a high proportion of WLHIV in this study who had a regular male partner who was also HIV-positive (~40%), and there was a relatively higher proportion of injectable contraception users compared to condom users. While there are no data available to further understand low condom use, these women may be less concerned about HIV transmission risk if their partner is also HIV-seropositive, but they remain at increased risk of acquiring HR-HPV types and other STIs from their male partners. Further efforts are required at the levels of service provision; a national evaluation to assess STI services in 50 public sector clinical sentinel surveillance facilities in nine provinces in South Africa reported using patient actors reported that 31% were offered condoms, 67% were offered HIV test, 61% were offered recommended syndromic treatment, and only 6% of providers discussed male circumcision with male patient actors [517]. Furthermore, men were more likely than women to be offered all services.

The South African government introduced a Voluntary Medical Male Circumcision (VMMC) policy and programme in 2010 with a target of reaching 80% of HIV-negative men aged 15-49 years by 2015, according to WHO targets [518]. The most recently available data on male circumcision

prevalence from 2012 in a survey by the Health Sciences Research Council (HSRC), reported that 46.4% of men self-reported being circumcised, and this was highest among black men (52.4%) [268]. A recent household study has shown that male circumcision was highly acceptable among men aged 18 to 49 years in Orange Farm, South Africa and was further enhanced through motivational interviews with a male circumcision adviser [519].

Screening for, and treatment of, sexually transmitted infections (STIs)

The 2006 WHO guidelines for sexual and reproductive health care, treatment and support of WLHIV [520] recommend screening for STIs as part of the initial clinical evaluation of WLHIV, including genital examination to detect clinical signs of STIs and laboratory tests, and at each subsequent visit, careful attention should be paid to symptoms or examination findings suggesting a new or recurrent STIs. Early and effective treatment is recommended, in addition to counselling on safe sex practices and notifying and managing partners. The syndromic approach for STI case management has shown to be feasible and cost-effective in LMIC [521, 522].

The prevalence of both bacterial vaginosis (BV) and HSV-2 are very high among WLHIV in this study. The underlying causes of BV are not well understood but hormonal changes during menstruation or due to hormonal contraception use, and hygiene practices (vaginal cleansing during menstruation and after sex) have been shown to cause alterations in vaginal flora. In addition, vaginal flora changes are known to occur in response to HIV [142, 523] and HSV-2 infection [524-526]. Treatment of BV, combined with encouraging safe sex practices (condom use) and discouraging some harmful vaginal cleansing practices may contribute to a reduction in recurrences.

Suppressive HSV-2 therapy using antiviral agents, such as acyclovir, reduces HIV-1 genital and systemic immune activation, which would lead to a reduction in the inflammatory mucosal environment, and balance in vaginal flora. HSV-2 is only partially reduced by ART use, but women with low CD4+ were reported to have higher genital HSV-2 DNA [499]. It is unclear whether

recurrent HSV-2 virus coinciding with high viral load is required to facilitate inflammatory responses and nitric oxide production associated with HR-HPV persistence and cervical lesion development, or whether these events also occur even when the virus is in a latent stage. ART initiation at a higher CD4+ cell count and maintaining a stable high CD4+ cell count could help reduce HSV-2 recurrences among WLHIV [485, 527], but further studies are needed to fully investigate associations between HSV-2 and HPV virus among WLHIV.

Hormonal contraception

Levels of HC use among women in South Africa were very high in this study. A nationally representative survey of 6217 South African aged 15-24 year olds found that 52% reported using any form of contraception, among whom 67% reported hormonal contraception [528]. Depot medroxyprogesterone acetate (DMPA) was reported among 49% of women and has been reported to be as high as 90% in some areas [529]. A study among 349 sexually active, non-pregnant HIV-positive women (18–44 years) matched with 214 HIV-negative women in Soweto, South Africa [500] reported that WLHIV were more likely than HIV-negative to report contraception use of any kind, which included hormonal, barrier and permanent methods (84% vs. 69%), but similar numbers used hormonal contraception (53% vs. 60%). Among the WLHIV, ART users were more likely than ART-naïve women to use injectable contraception (56% vs. 34%), possibly because a single dose is effective for months, which means fewer clinic visits and freedom from daily pills.

As part of a technical statement on the use of hormonal contraception among WLHIV [530], the WHO reviewed the literature on its association with HIV disease progression and found no strong evidence that suggested that hormonal contraception was associated with negative HIV outcomes, although the included studies were was largely limited by high rates of contraception switching and loss to follow-up. In the technical statement, the WHO recommends that "*Women living with HIV can continue to use all existing hormonal contraceptive methods without restriction*". It was not the purpose of this statement or review to consider the effects of hormonal

contraception on HPV infection and cervical lesion development and progression. However, the role of hormonal contraception in cervical lesion development among high-risk women such as WLHIV should be considered. Given the high prevalence of injectable contraceptive use among WLHIV, the convenience and the benefits associated with its use (unwanted pregnancy avoidance) and the fact there is limited data available among WLHIV suggesting an unclear association with cervical lesions, a systematic review of the data is would complement this work.

Smoking

While smoking was not associated with cervical lesion progression in this study, it was associated with HR-HPV prevalence among women in South Africa. Smoking prevalence was low among women in Burkina Faso, but in South Africa, it was slightly higher than the national estimate for women in South Africa. The overall smoking prevalence is higher among PLHIV compared to the general population [531] and the consequences of smoking are greater among PLHIV due to the increase in risk of cancers, cardiovascular disease, inflammation and lung infections. Reported reasons for smoking among PLHIV include stress relief, coping with depression and anxiety, weight control, or habit [531]. Smoking cessation interventions for PLHIV are not widespread. Despite that fact that PLHIV were reported to engage in quitting smoking if an intervention is provided by a healthcare worker, healthcare workers report patient resistance to smoking cessation interventions, in addition to systemic barriers (access to pharmacotherapy, or lack of time) [531]. It is unclear whether smoking cessation remains a priority among South African women, however WLHIV could be counselled on the added risk of smoking for HIV related diseases.

11.3.4 Primary prevention of HPV through vaccination among WLHIV

HPV vaccination for PLHIV is recommended in very few countries (includes the US [532], Brazil [533] and the UK [534]), possibly due to the belief that PLHIV can no longer be protected because of multiple exposure to HPV types.

This study has shown that, while WLHIV mount an antibody response to infection, HPV antibodies did not protect against re-infection by the same type. This may be because immune responses are weak with low antibody titres that are insufficient to clear incident infections. But recent studies have shown that WLHIV seroconvert for HPV vaccine types following vaccination, and antibody titres are equivalent to those among HIV-negative women and are stable up to 12-months post-vaccination [261, 440]. However there are yet no efficacy data against HPV persistence and CIN2+ outcomes.

In this study, few women were infected by all of the bivalent (HPV16/18) and quadrivalent (HPV6/11/16/18) HPV types, and no woman was infected by all the nonavalent (HPV6/11/11/18/31/33/45/52/58) vaccine types. WLHIV could potentially benefit from protection offered by a multivalent vaccine, such as the nonavalent vaccine, against types that have not yet acquired. This study found that up to 45% of CIN2/3 in Burkina Faso and 37% of CIN2/3 in South Africa could be prevented by the HPV16/18-targeting vaccines, and that a further 45% of CIN2/3 in Burkina Faso and 43% of CIN2/3 in South Africa could be prevented by the additional 5 types (HPV31/33/45/51/52) contained in the nonavalent vaccine. However, not all CIN2/3 develop into ICC, and not all HPV types found in CIN2+ have the same propensity to evolve towards ICC. At the same time, this data shows that vaccination could contribute to a reduction in screen-test positive women and subsequent colposcopy referral. Therefore, vaccination could also have an impact on screening and colposcopy services. Modelling studies would be required to more accurately quantify this impact.

In 2014, South Africa introduced vaccination for school girls aged 9 years and older using the bivalent vaccine. HPV vaccination is expected to have some impact on HIV acquisition in this population, due to the fact that HPV infection is associated with increased HIV acquisition [165]. There could be a consequent reduction in HIV incidence among young women in the coming years as the first cohort of vaccinated girls reach adulthood and sexual debut.

Summary recommendations

- Screening
  - ⇨ Current methods of visual inspection in Burkina Faso and cervical cytology in South Africa appear to be feasible and have adequate performance but result in over-referral to colposcopy.
  - ⇨ HPV DNA tests would not be suitable for a single round screening, but could be used as repeated screening.
  - ⇨ There is scope for molecular based tests such as DNA methylation, in the future.
- HIV care
  - ⇨ Early ART initiation may provide strong benefits in terms of impact on HR-HPV and CIN, and these effects should be monitored by research.
  - ⇨ All newly discovered infected women should be screened for cervical cancer, a rapid proactive approach is warranted for those women starting treatment late (at low nadir CD4+ cell count).
  - ⇨ Encourage ART adherence
  - ⇨ Gynaecologists should be aware of the importance of close monitoring of CD4+ T-lymphocyte count among ART naive and sustained HIV virological suppression among ART-treated women.
  - ⇨ HIV treatment services should integrate cervical cancer screening and raise awareness among their staff to the needs of WLHIV.
- Reduction in HPV transmission is of paramount importance
  - ⇨ Condom promotion
  - ⇨ Voluntary male circumcision services
- Screening and treatment of STIs are essential services for WLHIV
- HPV vaccination should be evaluated in these high-risk populations

11.4 Conclusions

This thesis has described the natural history of HR-HPV infection and CIN2+ in a prospective cohort of WLHIV from two countries with different HIV epidemics, burdens of HPV infection and cervical cancer, and approaches to screening for cervical cancer. This allows the findings to be extended to a range of countries and settings in the region.

The findings in this thesis confirm that women living with HIV have very high prevalence and persistence of HR-HPV, and high rates of CIN2+ prevalence and incidence over 16 months. Despite the similar rates of incident and persistent HR-HPV infection, women in South Africa had higher rates of CIN2+, which is linked with the poorer control of HIV disease and higher frequency of cofactors for CIN2+ in this population, but immune recovery over time corresponded with a reduction in risk of CIN2/3 incidence over 16 months. These findings are consistent with those of the meta-analysis which investigated the association of ART use on HR-HPV and CIN/SIL outcomes. The implications of these findings are that early ART initiation, prolonged use with sustained viral suppression and CD4+ cell count recovery is important in controlling HR-HPV and the development of CIN2+.

Although WLHIV have high background rates of HPV infection, there is still scope for further prevention of infection. Women living with HIV are infected with a broad range of HPV genotypes, were multiply infected and had high HPV type seroprevalence, with limited evidence that HPV antibodies protect against same-type reinfection. WLHIV may benefit from vaccination using a multivalent vaccine.

The control of HPV and CIN is important in WLHIV and this thesis explored the role of possible biomarkers to be evaluated in this context. DNA methylation of a tumour suppressor gene *EPB41L3* shows promise as a biomarker test for CIN2+ prediction among women living with HIV, although larger studies are required to accurately demonstrate this.

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13 APPENDIX

Appendix 1. The search strategy for systematic review of the association of ART on HR-HPV and cervical lesion outcomes

Antiretroviral therapy, high-risk human papillomavirus and cervical intraepithelial neoplasia: a systematic review and meta-analysis

Search performed using MEDLINE and EMBASE on 12 July 2016

HPV STUDIES:

HPV [Title/Abstract] OR Human papillomavirus [Title/Abstract] AND Antiretroviral therapy [Title/Abstract] OR ART [Title/Abstract] OR Antiretroviral therapy, highly active [MeSH Terms] AND Cervix Uteri [MeSH Terms] OR Uterine cervix [MeSH Terms] OR cervical

CERVICAL LESION STUDIES:

Intraepithelial neoplasia [Title/Abstract] OR Squamous intraepithelial neoplasia [Title/Abstract] OR Neoplasms [MeSH Terms] OR Precancer [MeSH Terms] OR Intraepithelial lesion [Title/Abstract] OR Carcinoma, Squamous Cell [MeSH Terms] OR Precursor lesions [Title/Abstract] OR Cervical intraepithelial lesion [MeSH Terms] AND Cervical [Title/Abstract] OR Cervix uteri [MeSH Terms] OR Uterine cervix [MeSH Terms] AND Antiretroviral therapy [Title/Abstract] OR ART [Title/Abstract] OR Antiretroviral therapy, highly active [MeSH Terms]

Appendix 2. Quality assessment of studies reporting the effect of ART on HR-HPV prevalence, and other significant findings reported

First author, year	Participant selection	HR-HPV test method	Adjustment for confounding	Other significant published findings related to HIV	
				Comparison groups	Effect estimate, adjustment
Africa					
Kelly, 2016	WLHIV attending HIV outpatient and treatment centres in Ouagadougou and Johannesburg invited to participate in a study comparing cervical cancer screening methods. **Low risk**	PCR based (INNO-LiPA). **Low risk**	In BF: alcohol, marital status, age at first pregnancy, cervicitis, CD4+ and ART duration. In SA: age, smoking, injectable contraception, genital warts, condom use, vaginal cleansing, *Chlamydia trachomatis*, BV, *Trichomonas vaginalis*, CD4+ and ART duration. **Low risk**	Short duration ART (≤2yrs) vs. Long duration ART (≥2yrs)	BF: aPR=1.24 (95%CI:1.04-1.47), adjusted for CD4+
				CD4<200 vs. >500 cells/µl	BF: aPR=1.43 (95%CI:1.18-1.72), SA: aPR=1.15 (95%CI:1.01-1.30)
Zeier, 2015	Known HIV-positive women were approached for enrolment provided they were ART-naive. Decision to start ART based on South African guidelines. LTFU unclear. **Selection bias of ART initiators.**	PCR based. **Low risk**	Time dependent covariates: time on ART, HIV-1 PVL, age, sexual activity, months since excision. **Low risk**	Months since ART initiation (per months analysis)	aOR=0.95 (95%CI: 0.93-0.97), adjusted for time on ART, HIV-1 PVL, age, sexual activity, months since excision
Ezechi, 2014	80% recruited from cervical cancer screening clinic; 20% from community cervical cancer screening outreach in urban and rural locations.	HPV4A ACE PC. **Unclear risk**	age, type of community, LTSP, marital status; **no adjustment for ART duration or HIV-1 PVL or CD4+. High risk**	CD4 <200 vs. ≥500 cells/mm^3	aPR=2.40 (95%CI:1.70-5.90), adjusted for age, type of community, LTSP, marital status
Reddy, 2014	All women coming to clinic for routine ART or pre-ART care were given the opportunity to participate in the study. Large number on ART (82%) limits power to test association between ART and HR-HPV. **Medium risk**	PCR based (MY09/MY11). **Low risk**	Age, nadir CD4_, time since HIV diagnosis. **Low risk**	-	-
De Vuyst, 2012	WLHIV attending Coptic Hope Centre for Infectious Diseases for HIV related conditions invited to participate in a study comparing cervical cancer screening methods. **Low risk**	PCR based (GP5+/6+). **Low risk**	Age, CD4 count and ART duration. **Low risk**	Long duration ART (≥2yrs) vs. ART-naive	aPR=0.77 (95%CI:0.61-0.96), adjusted for age
				CD4<250 vs. ≥500 cells/µl among ART-naive	aPR=1.51 (95%CI:1.02-2.25), adjusted for age
Jaquet, 2012	Women with no cervical neoplastic lesion (determined by visual inspection), consecutively enrolled, attending HIV clinic and recruited through cervical cancer screening programme in Abidjan. **Low risk**	Linear Array. **Low risk**	Age, marital status, age at first sex, CD4+. **Low risk**	CD4<200 vs. ≥500 cells/µl	aOR=2.8 (95%CI: 1.1-8.1), adjusted for age, marital status, age at first sex
Veldhuijzen, 2011	'High-risk' women testing HIV positive as part of a HIV prevalence survey in Kigali, Rwanda were invited to participate in a survey for HPV prevalence. Women recruited via community meetings in three districts, study conducted in an international non-governmental organisation. Low risk.	Linear Array**. Low risk**	Unadjusted analysis undertaken by authors. **High risk**	-	-
Asia					
Menezes, 2015	Consecutive women receiving care at Y.R. Gaitonde Center for AIDS Education and Research in Chennai, India. Low sample number (n=50). **Medium risk**	PCR based (TS-E7-MPG). **Low risk**	Unadjusted analysis due to small numbers. **High risk**	-	-

First author, year	Participant selection	HR-HPV test method	Adjustment for confounding	Other significant published findings related to HIV	
				Comparison groups	Effect estimate, adjustment
Zhang, 2014	Recruitment to HIV clinic based on linkages with VCT centers and referrals of known HIV infected women from provincial and prefecture level CDC affiliated ART clinics. Representative of HIV-positive women seeking cervical cancer screening services. **Low risk**	HC2; has good sensitivity for clinically relevant HR-HPV. **Low risk**	Adjusted for age, CD4+ and ART duration. **Low risk**	-	-
Mane, 2012	Women attending outpatient gynaecology clinic in tertiary care hospital in Pune, India, recruited consecutively. **Low risk**	PCR based (Linear Array). **Low risk**	age, marital status, education, family income, parity, age 1st sex, LTSP, past STI, smoking, CD4; Other typical confounding factors such as smoking, parity were not associated with HPV infection; **Low risk.**	-	-
Aggarwal, 2012	HIV-positive women randomly enrolled from ARV clinic. **Low risk**	Hybribio GenoArray. Low prevalence of HR-HPV suggests poor sensitivity of test. **Unclear risk**	Unadjusted analysis undertaken by authors. **High risk**	-	-
Latin America					
Rocha-Brischiliari, 2014	Women aged 18-66 yrs attending Specialized Assistance Service for STD/AIDS of Maringa city/Southern Brazil from April to Oct 2011. **Low risk.**	PCR based (MY09/MY11/HypCH4V). **Low risk**	Unadjusted analysis undertaken by authors. **High risk**	-	-
Dames, 2014	HIV sero-positive women ≥18yrs consecutively enrolled from Infectious Disease Clinic at the Princess Margaret Hospital in New Providence, Nassau, Bahamas Feb-Sep 2008. Clinic caters for all Bahaman islands, see 1500 HIV-positive each year but at time of this study, there was no cervical cancer screening progamme (may explain high disease). **Low risk**	PCR based (HC2+Linear Array). **Low risk**	Unadjusted analysis undertaken by authors. **High risk**	CD4 ≤200 cells/µl vs. >200 cells/µl	aOR=7.27 (95%CI:1.41-37.53), adjusted for age, duration on ART, HIV PVL and cytological abnormality
Grinsztejn, 2009	Prospective open cohort (IPEC-Fiocruz) established in 1996, women followed up in a clinical research hospital in Rio de Janeiro. **Low risk**	Not PCR based(HC2) but HC2 has good sensitivity for HR-HPV. **Low risk**	Age, marital status, drug use, age at first sex, LTSP, history of HPV, condom use, nadir CD4+. **Only Prevalence Ratio available. HIgh risk**	Nadir CD4 <100 vs. ≥250 cells/ µl	aPR=1.56 (95%CI: 1.18-2.06), adjusted for age, marital status, drug use, age at first sex, LTSP, history of HPV, condom use
Europe/North America					
Konopnicki, 2013	Women consecutively enrolled from cervical cancer screening program at AIDS reference centre in Saint-Pierre University Hospital from 2002 to 2010. Those who developed lesions during study period were censored. **84% of women were of SSA origin. Low risk**	Not PCR based (HC2) but HC2 has good sensitivity for HR-HPV. **Low risk**	Age, current and nadir CD4+ count, CDC stage, duration of HIV follow-up, ART duration, HIV-1 viral suppression. **Low risk**	Long duration ART (≥2yrs) vs. short duration (<2yrs) or ART-naive	aPR=0.60 (95%CI:0.37-0.99), adjusted for age, current and nadir CD4+, CDC stage during HIV infection, ART status, HIV-1 viral suppression
				HIV VL suppression ≥2yrs vs. <2yrs	aPR=0.28 (95%CI:0.17-0.49), , adjusted for age, current and nadir CD4+, CDC stage during HIV infection, ART status, ART duration

First author, year	Participant selection	HR-HPV test method	Adjustment for confounding	Other significant published findings related to HIV	
				Comparison groups	Effect estimate, adjustment
				Nadir CD4 <500 vs. ≥500 cells/ µl	aPR=3.31 (95%CI:1.51-7.24), , adjusted for age, current CD4+, CDC stage during HIV infection, ART status, ART duration, HIV-1 viral suppression
				Per 100 CD4 cells/µl increase	aRR=0.89 (95%CI:0.85-0.93), , adjusted for age, current and nadir CD4+, CDC stage during HIV infection, ART status, ART duration, HIV-1 viral suppression
Blitz, 2013	HIV-positive women recruited from community-based or tertiary care centres. 71% of women attended >1 follow-up visit. **Medium risk -significant LTFU**	PCR based (MY09/MY11/HNB01 & PGMY). **Low risk**	Unadjusted analysis undertaken by authors. **High risk**	-	-
Minkoff, 2010	Prospective follow-up of ART initiators in the Women's Interagency HIV Study (WIHS). **Selection bias of ART initiators.**	PCR based (MY09/MY11/HMB01). **Low risk**	Treatment of CIN, CD4 pre- and post-HAART. Women acted as their own comparator group. Minimises bias due to fact that women starting ART are sicker than those who do not yet need ART. **Low risk.**	-	-
Fife, 2009	Subjects enrolled when they were about to begin ART; either in controlled clinical trial or by perscription. 18% LTFU at 6 months and 36% loss to follow-up at 24 months. Hispanic subjects more likely to have HPV data at all time points. A higher rate of HR-HPV DNA detection at baseline associated (p=0.089) with with missing HPV data for at least one visit. **Selection bias of ART initiators. High risk**	PCR based (Roche). **Low risk**	Age, sexual activity at baseline and current, LSIL+ at baseline, CD4 count, HIV-1 PVL. **Prevalence reported after compared to before - participants did not act as their own comparator group. High risk**	-	-

NR=not reported

Appendix 3. Quality assessment of studies reporting the effect of ART on high-grade cervical lesion prevalence, and other significant findings reported

First author, year	Participant selection	Adjustment for confounding	Endpoint determination & Biopsy decision	Other significant findings related to HIV	
				Comparison groups	Effect estimate, adjustment factors
Africa					
Kelly, 2016	WLHIV attending HIV outpatient and treatment centres in Ouagadougou and Johannesburg invited to participate in a study comparing cervical cancer screening methods. **Low risk**	In BF: age, BV, cervical ectopy, CD4+ and ART duration. In SA: age at first pregnancy, injectable contraception, LTSP, CD4+ and ART duration. **Low risk**	All participants were referred for colposcopy performed by trained colposcopists. Systematic 4-quadrant cervical biopsy, including directed biopsy of any suspicious lesions, was performed for participants who had abnormalities detected by cytology, VIA/VILI or colposcopy, or who were HR-HPV DNA positive (Digene HC-II). **Low risk**	Short duration ART (≤2yrs) vs. Long duration ART (≥2yrs)	SA: aPR=1.99 (95%CI:1.12-3.54), adjusted for CD4+
				ART-naive vs. Long duration ART (≥2yrs)	SA: aPR=1.87 (95%CI:1.11-3.17), adjusted for CD4+
Memiah, 2015	Consecutive enrolment of HIV positive women attending ART treatment clinic in Kiambu district, Kenya. Study site is a faith based hospital offering care and treatment to ~4000 HIV infected persons. **Medium risk**	Unadjusted analysis undertaken by authors. **High risk**	Women positive on VILI were indicated for biopsy. No details on histology readings or any QA involved. **High risk.**	-	-
Huchko, 2014	Screening offered to women enrolled in care in two HIV clinics (Family AIDS Care and Education Services) in Kisumu, Kenya, no prior screening programmes were in place. **Low risk.**	age and site. No adjustment for ART duration, HIV-1 PVL or CD4+. **High risk.**	Screening in 3 clinics; 814 (25%) women were referred for colposcopy if: abnormal VIA (main clinic) ; abnormal VIA and VILI (staellite clinic 1);and cytology result of ≥ASCUS+ (satellite clinic 2) . Biopsies were taken when colposcopy abnormal or unsatisfactory (544 women (16.8% of all enrolled)). Histology results available for 15% of all enrolled women (n=488). **High risk - only 15% have histology result; 25% with colposcopy, remainder have combined VI/ cytology endpoint (possible underreporting of cervical disease)**	CD4 . ≥500 vs. <200 among ART-naive	aOR=0.42 (95%CI:0.22-0.80), adjusted for hormonal contraception
				Nadir CD4<500 vs. <200 cells/µl	aOR=0.61 (95%CI:0.38-0.97), adjusted for months in HIV care and hormonal contraception
				Months on HAART	aOR=0.98 (95%CI: 0.95-1.01), adjusted for current CD4+, hormonal contraception
De Vuyst, 2012	HIV positive women attending Coptic Hope Centre for Infectious Diseases for HIV related conditions invited to participate in a study comparing cervical cancer screening methods. **Low risk**	Age, CD4+ and ART duration. **Low risk**	All women biopsied (from most abnormal area of cervix, or if no lesion was visualised at 12 o clock). Cytology slides and biopsies read by single pathologist at Aga Khan University of Nairobi. No information on QA. **All with histology result. Low risk**	CD4<250 vs. ≥500 among ART-naive	aPR=4.23 (95%CI:1.27-14.0), adjusted for age
				Long duration ART (≥2yrs) vs. ART-naive	aPR=0.88 (95%CI:0.57-1.35), adjusted for age
Mabeya, 2012	Women recruited from waiting rooms of HIV clinics at Moi University school of Medicine in Eldoret, Kenya. **Low risk**	Unadjusted analysis undertaken by authors. **High risk**	All participants underwent VIA and cytology pap smear done by nurse. A single punch biopsy taken from visible abnormal lesion with aid of colposcope; if no visible lesion, a single punch biopsy taken from eith 6 or 12 oclock. Blinding screening and reading. 10% of pap smears and biopsies read by an external pathologist. **All with histology result. Low risk**	-	-

First author, year	Participant selection	Adjustment for confounding	Endpoint determination & Biopsy decision	Other significant findings related to HIV	
				Comparison groups	Effect estimate, adjustment factors
Ezechi, 2014	Participants recruited at cervical cancer screening clinic, NIMR, Lagos and 10 communities of Lagos and Ogun States of Nigeria during community outreach programmes. Women who presented for cervical cancer screening at the NIMR clinic and during community outreach programmes were screened for eligibility for recruitment. **Low risk**	Unadjusted analysis undertaken by authors. **High risk.** Original authors report the OR of HSIL+ vs Normal, and excludes those with ASCUS/LSIL from the analysis. Reported unadjusted analysis includes all women. **High risk (unadjusted)**	Cytology only. Interpretation of pap at Anapath laboratory using Bethesda system. Second reading by senior pathologist of all abnormal cases and 15% normal cases (n=358 [31%] in total). In event of discrepency (5% -all ASCUS), slides sent to a second senior pathologist for independent review and final diagnosis attributed to this final review. Of 17 ASCUS cases, 1 was upgraded and 5 were downgraded. **Low risk.**	-	-
Firnhaber, 2010	Cross-sectional cohort recruitment from adult HIV outpatient clinic in teaching hospital affiliated with University of Witwatersand. **Low risk**	Unadjusted OR undertaken by authors. **High risk**	Cytology only, read and analysed according to Bethesda system. 10% of cytology sldes sent to University of North Carolina for blinded double reading on two occasions; high rate of concordance observed (81-85%). Of the 182 cases graded as HSIL+, 83 had pathology results; most HSIL cases were histologically confirmed as CIN2 (30%) ot CIN3 (47%); 23% were classified as CIN1. **Low risk.**	CD4 <200 vs. ≥500	aPR=2.4 (95%CI:1.4-4.2), adjusted for age and ART
Mogtomo, 2009	Participants recruited in a day care centre at Bonassama hospital in Douala for HIV therapy. **Low risk**	Unadjusted analysis undertaken by authors. **High risk**	Cytology only, read by single pathologist. Unclear whether QA was used. **High risk.**	-	-
Asia					
Feng, 2012	Recruitment through outreach to Women's and Children's Hospital of Luxi County in Mangshi, Dehong Perfecture by hospital personnel familiar to the local HIV-infected population. Unpublished data. **High risk**.	Age, CD4+ and ART duration. **Low risk.**	Colposcopy performed on all. Biopsy performed on consenting participants with clinical evidence of cervical abnormalities (does not report N who had histology). Final diagnosis based on histology where biopsy was taken, and on colposcopic diagnosis where biopsy was not indicated or not taken. CIN2+ was 8.4% while HSIL+ was 1.1%. **High risk**	-	-
Sahasrabuddhe, 2010	Study participation offered to consecutive HIV-infected women in a public-sector ART centre in hospital premises. Participants also recruited through outreach efforts. No participant had previously been screened or treated for cervical abnormality. **Low risk**	Age, education, income, age at first sex, lifetime sex partners, parity, current CD4+, WHO stage of HIV disease, HR-HPV. No adjustment for ART duration or HIV-1 PVL. **High risk.**	Colposcopy performed on all. Biopsy (cervical punch, ECC or LEEP) performed on consenting participants with clinical evidence of cervical abnormalities (24.1%). Final diagnosis based on histology where biopsy was taken, and on colposcopic diagnosis where biopsy was not indicated or not taken. Colposcopy results served as final diagnosis for 81.2% (246/303) participants. Final histopathology results were available for 18.8% (57/303). QA of colposcopic images by a senior experienced gynaecologist. **High risk - only 19% have histology result**	-	-

First author, year	Participant selection	Adjustment for confounding	Endpoint determination & Biopsy decision	Other significant findings related to HIV	
				Comparison groups	Effect estimate, adjustment factors
Latin America					
De Andrade, 2011	Prospective open cohort at Evandro Chagas Clinical Research Institute, Oswaldo Cruz Foundation. **Low risk**	Unadjusted (reported by original authors). **High risk.**	Directed biopsies performed when minor colposcopic changes were observed but it was not possible to exclude CIN2+ and the pap test showed LSIL, ASC or normal. No information on how many had histology endpoint but authors report that all CIN2+/ICC were histopathologically reported. **High risk**	Nadir CD4<350 vs. ≥350 cells/µl	aPR=6.03 (95%CI:1.50-24.3), adjusted for Age, smoking, VIN and/or VAIN
Europe					
Patrellli, 2013	HIV-infected women admitted to the Department of Obstetrics and Gynaecology of the University of Parma, referred from Infectious Disease Clinic for early detection of HPV-related disease. No details on recruitment procedures or selection. **Medium risk**		Cytology by pap smear and colposcopy. Those with abnormal pap and colposcopy underwent targeted biopsy (48% of all women). All colposcopy performed by same examiner. Cytology and histology examined by gynaecological pathologists. No information on how many, or whether there was independent reading or QC. **Medium risk.** Authors mention bias could be attributed to period effect of cytology reporting over a 17 year period (frequency and reliability changed in subsequent years) and introduction of ASCUS class could have reduced number of high grade cases reported.	-	-

Appendix 4. Quality assessment of studies reporting the effect of ART on high-grade cervical lesion incidence, progression and regression and other significant findings reported

First author, year	Participant selection & ART status	Statistical methods used	Adjustment for confounding	Endpoint assessment	Other significant findings related to HIV	
					(Outcome) Comparison groups	Effect estimate, adjustment
Africa (in alphabetical order)						
Adler, 2012	Operational cohort of treatment-naive HIV infected women set up to transition patients onto ARV, receiving a package of care between 2003-2010. **Selection bias of ART initiators**	For incidence: Survival analysis using generalised estimating equation, accounts for changes over time. **Low risk** For progression/regression: marginal models which assumes no pattern in the correlation of observations within an individual; i.e. assess differences between individuals (a comparison of women on ART vs. women not on ART). The survival analysis was restricted to the women not on HAART at baseline who had a normal baseline cervical smear result (n=767) and assessed the risk of progression to any abnormal result. Women started on HAART during the study period were included in the survival analysis after they had been receiving treatment for 180 days.	BMI, sex history, STI symptom, time since enrolment, current CD4+ count, time-varying CD4+ and ART. **Low risk**.	Cytology on all women analysed at NHLS which is accredited by South African National Accreditation System. All smears verified by second reader. **Low risk.**	(Incidence): Current CD4 ≤200 vs. >500	aHR=1.73 (95%CI:1.15-2.61), adjusted for BMI, LTSP, STI, smoking, ART
					(Progression): Current CD4 <200 vs. >500	aOR=2.50 (95%CI:1.67-3.73, adjusted for BMI, LTSP, STI, smoking, ART
Kelly, 2016	WLHIV attending HIV outpatient and treatment centres in Ouagadougou and Johannesburg invited to participate in a study comparing cervical cancer screening methods. **Low risk**	Logistic regression. Not included in the meta-analysis.	Baseline CD4+ and lifetime number of sex partners. **Medium risk**	All participants were referred for colposcopy performed by trained colposcopists. Systematic 4-quadrant cervical biopsy, including directed biopsy of any suspicious lesions, was performed for participants who had abnormalities detected by cytology, VIA/VILI or colposcopy, or who were HR-HPV DNA positive (Digene HC2). All histological slides from women with a local diagnosis of CIN2+ and approximately 10% of slides from women with ≤CIN1 histological findings were reviewed by the HARP Endpoint Committee of five pathologists, for consensus classification. **Low risk**		-

First author, year	Participant selection & ART status	Statistical methods used	Adjustment for confounding	Endpoint assessment	Other significant findings related to HIV	
					(Outcome) Comparison groups	Effect estimate, adjustment
Firnhaber, 2012	Observational longitudinal study included women 18-65yrs recruited from adult HIV outpatient clinic affiliated with teaching hospital in Johannesburg. **Low risk.**	For both incidence and progression: Poisson regression reporting Incidence Rate Ratio. Intent-to-treat analysis, so no adjustment for change in ART exposure after baseline were considered -**Low risk**	Age, CD4, age 1st sex, LTSP, history of STI, hormonal contraception, condom at last sex, employment, current smoking, snuff, education. No adjustment for change in ART exposure after baseline. **Medium risk**	Cytology on all; 10% of cytology sldes sent to University of North Carolina for blinded double reading on two occasions; high rate of concordance observed (81-85%). Of the 182 cases graded as HSIL+, 83 had pathology results; most HSIL cases were histologically confirmed as CIN2 (30%) or CIN3 (47%); 23% were classified as CIN1. **Low risk.**	n/a	n/a
Omar, 2011	Operational cohort of treatment naive HIV infected women set up to transition patients onto ARV between 2003-2010. **Although guidelines for annual screening have been established, implementation has been poor - authors do not think over-diagnosis bias exists.**	Cox proportional hazards model. **Low risk**	Age, baseline CD4, baseline smear result, smoking, baseline weight, time-varying ART. **Low risk**	Cytology on all; performed at National Health Laboratory Service - accredited by South Africa National Accreditation System. Women referred to colposcopy if cytology abnormal. Smear readers not blinded to previous smears which could have resulted in spuriously higher rates of premalignant lesions than if readers were blinded. Each smear was verified by second reader and all previous smears with higher/lower grade diagnosis to the current were then re-reviewed by a senior technologist. For women diagnosed with ASCUS (n=16), their subsequent smear was used instead of their baseline smear in longitudinal analysis of progression and regression. ASCUS diagnosed at endline were excluded.Medium risk of overdiagnosis.	(Progression): Baseline CD4 <200 vs. >500	aHR=1.96 (95%CI: 1.33-2.88), adjusted for age, baseline smear result, smoking history and time-varying ART
Zeier, 2012	Retrospective cohort analysis of records from Colposcopy Clinic and Infectious Disease Clinic in Tygerberg Hospital, Cape Town, information collected from 2004-2009. Authors identified 1960 cases that had LSIL at first abnormal smear, of these 1720 had follow-up data available; only women with FU visit > 6mths after first LSIL detected were included, but they were included if they experienced progression event <6mths. **Low risk**	Multivariable Cox proportional hazards regresion model. **Low risk**	Age, CD4+ count at first LSIL, virological failure (2 consecutive HIV VL measurements of >1000 copies/ml at least 4 weeks apart) and excision treatment. ART considered as started before LSIL development (so no requirement for adjustment for duration). **Low risk.**	Cytology on all; quality of cytology assessed by determining the percentage of smears with endocervical cells present. Cytology result read by pathologist and checked by a second pathologist. **Low risk.**	n/a	n/a

First author, year	Participant selection & ART status	Statistical methods used	Adjustment for confounding	Endpoint assessment	Other significant findings related to HIV	
					(Outcome) Comparison groups	Effect estimate, adjustment
Latin America						
Kreitchmann, 2013	Enrolled all WLHIV referred for gynaecological exam at reference center for STI/AIDS in Porto Algere, Brazil. Low risk	Multivariable Cox proportional hazards regresion model. **Low risk**	Age, CD4+ count, log viral load, years at school. **Medium risk.**	Cytology on all; all pap smears evaluated in one reference laboratory and interpreted by a certified cytopathologist. Approx. 10% of negative pap smears, chosen at random, and all positive smears for any cytologic abnormality were independenttly interpreted by another cytopathologist. Low risk	(Incidence): Current CD4 ≤200 vs. >500	aHR=3.0 (95%CI:1.2-7.2), adjusted for Age, ART, viral load, race, education
					(Incidence): Log viral load (copies/ml)	aHR=1.4 (1.0-1.9), adjusted for Age, ART, CD4+, race, education
EuropeNorth America (in alphabetical order)						
Blitz, 2013	Women recruited from 28 community based or tertiary care centres across Canada; 58% Canadian, 31% from HIV-endemic country, from 1993-2002. There was 29% loss to follow-up after baseline visit. **Medium risk of selection bias. 19% were on HAART at start of study; 64% by end of study -includes initiators**	Multistate time-homogenous Markov models used to model bidirectional transitions through different disease states by individuals over time; assumes that transition from one state to another is constant over timeand the probabilty of of transition depends on time between observations, rather than the overall time of observation. **Low risk**	Unadjusted -univariate results presented only. **High risk**.	Cytology on all. Women with abnormal findings referred for colposcopy and biopsy and treatment as necessary. Only cytology results used as endpoint. **Medium risk of reporting bias. Sensitivity analysis which groups ASCUS with normal found similar results.**	n/a	n/a
Clifford, 2016	Swiss HIV cohort study (SHCS): nationwide prospective cohort enrolling PLHIV ≥16yrs. Case control study including cases of CIN2/3 and ICC record in the SHCS and matched to ≤CIN1 controls using incidence density sampling, matched on age, location, HIV transmission category and year of enrolment. **Low risk**	Logistic regression. Not included in meta-analysis	Nadir CD4+. **Low risk**	NR	(Incidence): per 100 cell/µl decrease in nadir CD4+	OR=1.15, 95%CI: 1.08-1.22
					(Incidence): per 100 cell/µl decrease in current CD4+ (at time of CIN2/3 diagnosis)	OR=1.10, 95%CI: 1.04-1.16
Del Mistro, 2004	All HIV infected women attending the Infectious Disease Units of Vicenza and Padova City Hospitals were offered gynaecologic consultation from 1994-2002. HIV infection acquired by injection drug use or heterosexual contact (in near equal proportions). Mortality during FU was 9.5%, cause of death linked to HIV infection, except for 2 which were related by hysterectomy complictaions and breast cancer. **Unclear Risk?**	Logistic regression was used to estimate odds of lesion regression. **High risk- does not take into account changes over time.**	Unadjusted analysis undertaken by authors. High risk.	Cytology on all; colposcopy driven biopsy for high grade SILs. Unclear if quality checks used for cytology reading. **Unclear risk.**	n/a	n/a

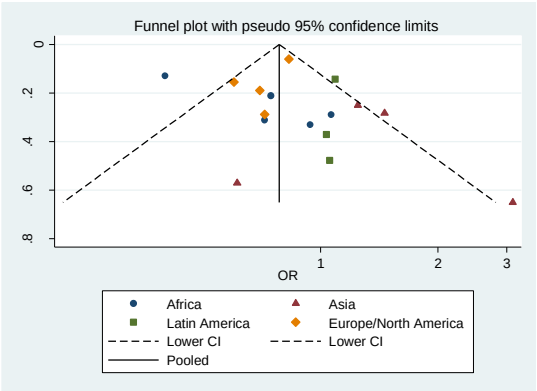
First author, year	Participant selection & ART status	Statistical methods used	Adjustment for confounding	Endpoint assessment	Other significant findings related to HIV	
					(Outcome) Comparison groups	Effect estimate, adjustment
Ellerbrock, 2000	Prospective cohort study of women enrolled in the New York Cervical Disease Study, recruited from HIV clinics between 1991-1996. Women recruited from HIV clinics directly by health care provider, without regard of risk factors for cervical disease or clinical HIV status. Enrolled women likely to report history of prostitution (24%) and intravenous drug use (44%). Study conducted prior to introduction of protease inhibitors. **High risk – possible bias given the population not generalizable and period effect (early ARV use).**	Cox proportional hazard model. Assumption of proportionality was tested and met in multivariate analysis. **Low risk.**	Age, smoking, transient and persistent HPV, CD4 at enrolment. **ART was included as a time-dependent variable. Low risk**	Cytology on all, confirmed by histology (428 biopsies read). **Low risk of misclassification.**	n/a	n/a
Heard, 2002	Prospective study among HIV positive women attending outpatient HIV clinics in Paris initiated in 1993, during period 1993-1999. **Period effect - unclear risk.**	Multivariate analysis of lesion regression and risj factors performed using Cox's proportional hazard model. **Low risk**	HAART was considered as time-dependent variable using intention-to-continue treatment approach. Women starting HAART during follow-up were classified as off-HAART until time of first prescription and on-HAART thereafter. Other adjustments for CD4+ count at detection, calender priod of CIN detection (1997-1999 vs. 1996-1996), lesion grade. **Low risk**	Cytology on all, standardised colposcopic exam with biopsy if necessary. Smears and biopsies read by same pathologist. CIN defined as high grade on basis of histologically confirmed high grade CIN or low grade CIN associated with smear showing HGSIL, similar for low grade. Normal smear and colposcopy, minor colposcopic changes associated with normal smear were considered as no evidence fo CIN. Biopsies taken for 57% of all participants. R**eader bias?**	n/a	n/a
Heard, 2006	Prospective study enrolling women attending gynecology outpatient clinic of the HIV dept of Hopital Europeen Georges Pompidou and Hopital Cohin, Paris between 1993-2005 . Women with SIL diagnosed before or at time of enrolment were excluded, including those with abnormal colposcopic finding at enrolment. **Low risk**	Multiple Cox regression model accounting for duration of follow-up. Relative risk reported. **Low risk**	Age, ethnicity, smoking, LTSP, contraception, condom use, CD4+ count, inclusion period. Hormonal contraception, condom use, CD4+ and HAART as time-dependent variables. **Low risk**	Cytology on all; patients with major colposcopic abnormalities or high grade SIL underwent a biopsy, unless they refused or were not compliant for follow-up. All smears and biopsies read by the same patholgist. **Possible reader bias?**	n/a	n/a
Kim, 2013	20year Retrospective study among HIV infected women cared for at Strong Memorial Hospital AIDS Centre with ≥2 pap smears between 1991-2011; 800-1061 individuals with HIV were followed of which 30% were women. Mean nadir CD4=206	Cox proportional hazards modelallowing for the possibility of a patient having multiple events. Sandwich estimator used to adjust for the correlation. **Low risk**	Time-dependent covariates assessed at visit prior to event or censoring:CD4+ count, duration of HIV infection, menopausal status, drug use, smoking . **Low risk**	Cytology on all (Bethesda 1988 and 2001). Women with abnormal pap tests were referred to gynaecology clinic for colposcopy and further management. Unclear whether only cytology results used. **Medium risk.**	(Progression): Per 100 CD4 cells/mm^3 increase	aHR=0.91 (95%CI: 0.86-0.96), adjusted for ART, Duration of HIV infection, menopausal status, drug use, smoking

First author, year	Participant selection & ART status	Statistical methods used	Adjustment for confounding	Endpoint assessment	Other significant findings related to HIV	
					(Outcome) Comparison groups	Effect estimate, adjustment
Lehtovirta, 2006	Retrospective study of HIV positive women attending Dept of Obstetrics and Gynaecology in the University of Helsinki from 1989 to 2003, including all WLHA followed up with >1 visit; 65% of Finnish, 21% of African origin, origin. **Low risk.**	Cox proportional hazard analysis used for calculating Relative Risk. **Unclear risk.**	Age. No adjustment for ART duration during follow-up. **High risk.**	Cytology on all; in event of LSIL+, patients had colposcopy and biopsy	n/a	n/a
Lillo, 2001	HIV positive women recruited from a patient care program that included gynecologic monitoring from 1995-1997	Odds Ratios estimated using logistic regression. **High risk- does not take into account changes over time.**	CD4+ count, HIV RNA and gynecologic treatment. **No adjustment for duration on ART- High risk**	Cytology on all; slides with abnormal smears were regularly seen by 2 cytopathologists and 2 physicians. 53 women (33%) had colposcopy driven biopsy at baseline: 68% had LSIL and 13% had HSIL. **Low risk or reporting bias.**	n/a	n/a
Minkoff, 2001	HIV positive women enrolled in six clinical consortia: Bronx, Brooklyn, Chicago, Los Aneles, San Francisco, Washington between 1994-1995	Odds Ratios estimated using logistic regression. As each participant could contribute >1 pair of smears, inferences were based on robust statistical methods that adjust for correlation inherent in such repeated measures. **High risk**- does not take into account changes over time.	CD4+ count and pap smear status at baseline. No adjustment for ART duration. **High risk**	Cytology (Bethesda 1994). All smears read by two cytotechnologists who were blinded to participants HIV status.All abnormal smears and 10% of all negative smears were confirmed by cytopathologist. **Low risk**	n/a	n/a
Minkoff, 2010	Prospective follow-up of ART initiators in the Women's Interagency HIV Study (WIHS) from 1994-2002; ≥2 semiannual visits during the 2.5 years just prior to HAART initiation and ≥2 semiannual visits during the 2.5 years immediately following HAART initiation. **Selection bias of ART initiators.**	Within-woman analysis using random effects model -controlled for the fact that the data involved repeat observations of the same women over time and each woman acted as their own comparison group (after compared to before ART initiation). **Low risk**	Adjusted for time-dependent variable, CD4+ count pre- and post-HAART. **Low risk**	Cytology on all. Oncogenic SIL (SIL that was HR-HPV DNA positive) was used as endpoint. HPV DNA testing was performed using cervicovaginal lavage sample tested on PCR. There is a possibility that some SIL lesions could have been missed (incorrectly classified as HR-HPV negative). **However, when analysis was repeated with any SIL, results were similar. Low risk.**	(Regression): Adherence vs. non-adherence	aHR=3.75 (95%CI: 1.43-9.88), adjusted for treatment of CIN using a time-dependent variable, and CD4+ count at the start of each period
Paramsothy, 2009	HIV Epidemiology Research Study (HERS): a prospective cohort study of women with HIV without AIDS–defining conditions were selected between 1996-2000; study sites in Bronx, Providence and Detroit and Baltimore. **Low risk**	Cox proportional hazards model. Women never on HAART had time calculated from first until their last study visit. Women on HAART; first study visit when they report HAART use until their last study visit. Women on HAART were placed in the model for the time before they started HAART as pre-HAART; measured from first study visit until visit before HAART use (pre-HAART combined with never HAART as comparison group). **Low risk**	CD4+ count, HPV DNA status, baseline pap test result. Intent-to-treat analysis: once a woman reported HAART use, she was considered to be on HAART through her last visit; effect of discontinuation, changes in regimen or adherence were not evaluated. Adjusted for time-varying CD4+. **Medium risk**	Cytology on all (Bethesda 1988). Women with abnormal pap in Bronx and Providence were referred for colposcopy. In Detroit and Baltimore, all women received colposcopy. No information about quality measures for reading. **Unclear risk.**	n/a	n/a

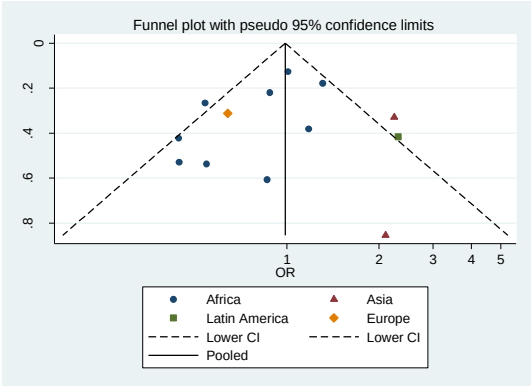
First author, year	Participant selection & ART status	Statistical methods used	Adjustment for confounding	Endpoint assessment	Other significant findings related to HIV	
					(Outcome) Comparison groups	Effect estimate, adjustment
Sirera, 2008	Retrospective cohort study including all patients in the database of the HIV Clinical Unit of the Germans Trias i Pujol University Hospital between 1997-2006, including only women with CD4+ >350 cells/mm3 at baseline. **High selection bias.**	Multivariate proportional hazard regression (Cox regression) using log-rank test. **Low risk**	CD4+ count. Not adjusted fir time-varying ART. Medium risk	Cytology on all. Most samples were checked by two cytopathologists. When a patient was diagnosed with LSIL+, a colposcopy and biopsy were proposed to verify cytology result. **Low risk.**	n/a	n/a
Soncini, 2007	Screening data from pap smears and colposcopic exams were collected from HIV positive women at Colposcopy and Cervical Pathology Service at Parma University Hospital between 1993-2003 within a year of being admitted to a screening and early-diagnosis program for cervical cancer. Only patients without prior diagnosis of CIN at first visit were enrolled. 81% of Italian orgin, 14% of African origin. **Low risk**	Cox regression model and log-rank test. **Low risk**	HAART was considered as time-dependent variable adjusted for CD4+ at first visit. **Low risk**	Cytology and colposcopy (93% of visits had combined cytology and colposcopy, remainder were colposcopy only). If pap and/or colposcopy reveal abormality, targeted biopsy performed. Cytology and histology reviewed by gynecologic pathologists at the hospital. Colposcopy by two expert colposcopists, according to IFCPC classification. **Low risk.**	(Incidence): Low vs. high CD4 at enrolment (unclear reporting)	aHR=2.38 (95%CI:1.44-3.96), adjusted for time-dependent ART
Schuman, 2003	HERS study: women 16-55yrs eligible if they reported injection use or high risk sex and no history of AIDS defining illness between 1993-1995. **Selection bias for a hIgh risk population.**	Discrete time survival analysis with complementary log-log model link allowing a Relative Risk interpretation of the coefficients. Repeated measures multivariate logistic regression models used for progression/regression, accounting for within-subject correlation. **Low risk**	Cofactors permitted to have different values at each visit. Adjusted for time (visit), study site, age, race, education. Not possible to model ART duration ART; reported ART at each visit was considered; would not have taken into account new, intermittent or prolonged duration on ART. **High risk**	Cytology (Bethesda 1988) on all. A senior cytopathologist read all tests originally classified as abnormal and 10% of the normal. **Low risk.**	(Incidence): CD4 <200 vs >500 at baseline	aRR=2.13 (95%CI: 1.27-3.64), adjusted for time (visit), site and age
					(Progression): CD4 <200 vs >500 at baseline	aRR=1.76 (95%CI: 1.09-2.84), adjusted for time (visit), site and age, race and education
					(Regression): HIV-1 viral load (status at previous visit)	aRR=0.78 (95%CI: 0.65-0.94), adjusted for time (visit), site and age, race and education

Appendix 5. Funnel plots of publication bias among studies evaluating the association of ART with HR-HPV and cervical lesions

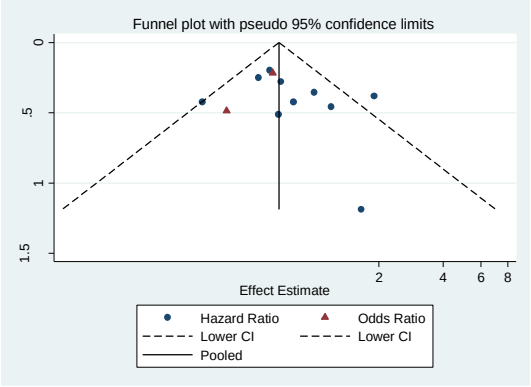
A. HR-HPV Prevalence (OR=Odds Ratio)



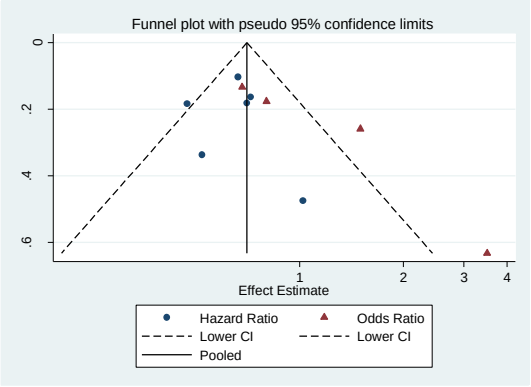
B. High-grade lesion prevalence (OR=Odds Ratio)



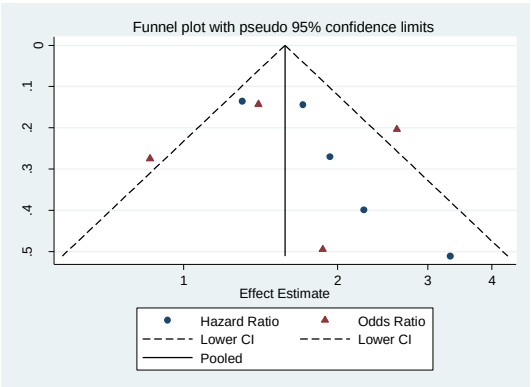
C. Cervical lesion incidence



D. Cervical lesion progression



E. Cervical lesion regression



Appendix 6.Description of included studies for systematic review of DNA methylation and CIN2+ (by gene marker with most recent first)

Author	Methylation marker	Country	Study design	Population	Sample size	Median age	Sample	HPV DNA	Methylation
CADM1/MAL/MIR									
Kim, 2016	CADM1, MAL, PAX1, ADCYAP1	South Korea-Seoul	Case-control	Samples collected at Cheil General Hospital & Women's Healthcare Centre ;**HPV positive** with concordant biopsy and pap result; biopsy-matched LBP sample and not population based screening	170	NR	Cervical scraping/Liquid based cytology sample	Seeplex HPV4 ACE/GG HPV genotyping Chip Kit	PSQ
VanBaars, 2016	CADM1, MAL	Spain-Barcelona	Case-control	Women aged ≥17y referred for colposcopy to Hospital Clinic, Barcelona because of abnormal cervical cytology. Random selection of 65 women with varying grades of CIN; **irrespective of HPV positivity**	60	33 [range:19-92]	Cervical scraping/Liquid based cytology sample	SPF10-PCR-DEIA-LIPA	qMSP
DeVuyst, 2015	CADM1, MAL, MIR	Kenya-Nairobi	Cohort	HIV positive +**HR-HPV positive** recruited as part of a cervical screening study at Coptic Hope Center for Infectious Diseases, Nairobi, Kenya	248	37 [33-42]	Cytobrush	PCR	qMSP
Verhoef, 2015	CADM1/MAL	The Netherlands	RCT	**HPV positive** women from Dutch screening programme (PROHTECT3), cytology triage group, self-samples [non-responder population]. Exclusion of previous hysterectomy, or CIN2+ or abnormal cytology in previous 2 yrs. All women from this group with HPV+ result and valid sample [28 CIN2; 56 CIN3; 6 ICC]	364	42 [38-48]	Cervical scrapes	Diassay EIA HPV	qMSP
DeStrooper, 2014	CADM1/MAL	The Netherlands	Cohort	**HR-HPV+ Women** participating in population-based screening (similar to POBASCAM trial)	234	34-40	Cervical scrapes	PCR	qMSP
DeStrooper, 2014	CADM1/MAL/MIR124-2	The Netherlands	Case-control	Women participating in population based screening or attending gynaecological outpatient clinic [79 ICC, 16 CIN3, 32 CIN2; 120 <CIN2] ; **irrespective of HPV positivity**	247	37-48	Cervical scrapes		qMSP
Hesselink, 2014	CADM1, MAL, MIR	The Netherlands	Cohort	**HR-HPV+** Non-attending women from Dutch screening programme (PROHTECT1) using self-samples [20 CIN2; 74 CIN3+, includes 4 SCC and 1 ADC]	355	36-41	Cervical scrapes	PCR	qMSP
Hesselink, 2011	CADM1, MAL	The Netherlands	Case-control (Training+ validation set)	Cervical scraping of 300 of total 1102 **HR-HPV+** women participating in intervention arm of the population-based RCT POBASCAM; 51 women selected with CIN3 detected within first screening + 224 women without evidence of CIN2 lesions after 2 screening rounds	275		Cervical scrapes	PCR	qMSP
Overmeer, 2011	CADM1, MAL	The Netherlands	Case-control	Random selection of 40 cervical scrapings with **HR-HPV+** normal cytology with no evidence of CIN disease + 30 scrapings with moderate dyskaryosis or worse of HR-HPV+ women with CIN3+ obtained from population-based cervical screening trial POBASCAM	70	32-34	Cervical scrapes	PCR	qMSP
MAL/MIR									
Verhoef, 2014	MAL/MIR	The Netherlands	Prospective cohort	**HPV positive** women from Dutch screening programme (PROHTECT3) returned self-samples and with study endpoint	1019	43 [33-63]	Cervical scrapes-self sample	PCR	qMSP
FAM194A									
DeStrooper, 2014	FAM194A	The Netherlands	Case-control	Validation set of samples comprised **hrHPV-positive** cervical scrapes of 52 women with CIN2+; including 33 CIN3+, 19 CIN2, and 166 women with CIN1.	52	NR	Cervical scrapes	PCR	qMSP

Author	Methylation marker	Country	Study design	Population	Sample size	Median age	Sample	HPV DNA	Methylation
EPB41L3									
Lorincz, 2016	EPB41L3, HPV16-L1	UK-London	Case-control	Samples randomly selected from UK screening group (selection based on HR-HPV positivity, cytology results and CIN status)	341	NR	Exfoliated cervical specimen	Aptima HPV positive +Abbott RT for genotyping	PSQ
Louvanto, 2015	EPB41L3, LMX1, HPV16	Canada-Montreal	Case-control	Women with abnormal pap referred for colposcopy and matched with women presenting for annual pap matched by age and site; **irrespective of HPV positivity**	244	NR	Exfoliated cervical specimen	Linear Array	PSQ
Vasiljevic, 2014	EPB41L3, EDNRB, LMX1, DPYS, MAL, CADM1	UK-London	Populaton-based screening study	P1: **HR-HPV positive** women, Colposcopy referral sample, referred due to abnormal smear (Predictors)	572	mean	Exfoliated cervical specimen	Linear Array + BD HPV test	PSQ
Boers, 2014	EPB41L3, JAM3, TERT, C13ORF18	The Netherlands	Case-control	**HR-HPV positive** women from Dutch screening programme (PROHTECT3b), self-sample. Sample selected based on false positive/false negative cytology vs. histology	128	NR	Physician collected cytobrush	Diassay EIA HPV	qMSP
Eijinsk, 2012	EPB41L3, JAM3, TERT, C13ORF18	The Netherlands-Groningen	Case-control	Patients referred to colposcopy with abnormal smear **irrespective of HR-HPV**	143	20-85	Cervical scraping	PCR	qMSP
PAX1/SOX									
Xu, 2015	PAX1	China-Shanghai	Case-control	Women referred for colposcopy or known ICC at the central Hospital of Minhang District. Healthy women were included as healthy controls with pap test done at same time	121	range of mean:36-44	Cervical scrape	PCR	PSQ
Kan, 2014	PAX1	Taiwan-Taipei	Case-control	443 women were recruited from the Yuan's General Hospital	96	NR	Cervical scrape		
Lai, 2014	PAX1, SOX1	Taiwan	Case-control	Women aged ≥20y attending 11 medical centres in Taiwan, referred for low and high grade lesions identified by cytology and underwent biopsy; controls recruited from healthy women who underwent routine pap screening; final diagnosis made by histopathology for cases and cytology for controls	346	Controls=46yrs	Cervical scrape	HC2	qMSP
Wang , 2014	PAX1	China-Shan dong	Case-control	Women participating in screening programme at Weifang city people's hospital and with an ASC-H diagnosis	130	mean age 46 years (range 25-68)	Cervical scrape		MS-HRM
Lin, 2011	PAX1	Taiwan	ASCUS triage sample set	Women with ASCUS nested in multicentre study attending 11 medical centres in Taiwan,	220	NR	Cervical scrape	HC2	MSP
Huang, 2010	PAX1	Taiwan	ASC-H triage	Data extracted from an earlier meta-analysis [535]	73				
Lai, 2010	PAX1, SOX1, LMX1	Taiwan	Case control	Women referred for low and high grade lesions identified by cytology and underwent biopsy; controls recruited from healthy women who underwent routine pap screening; final diagnosis made by histopathology for cases an cytology for controls	185	range of means by CIN: 40-54 yrs.	Cervical scrape	NR	qMSP
HPV16									
Bryant, 2015	HPV16-L1/L2/E2	Uk-Cardiff	Case-control	Women aged 20-22 attending first call for cervical screening as part of a cervical screening programme; this study included all women who **screened positive for HPV16** and were referred for colposcopy (n=267), a	200	mean-21yrs	LBC	PCR	PSQ

Author	Methylation marker	Country	Study design	Population	Sample size	Median age	Sample	HPV DNA	Methylation
				further 22 who tested positive for HPV16 and with cytology normal					
Mirabello, 2015	HPV16-L1	USA-California	Case-control	Women aged 30 yrs. and older screening routinely using HC2 and cytology at Kaiser Permanente California; women aged <30 yrs. with ASCUS are triaged using HC2. HPV persistence and progression cohort is repository of residual cervical specimens; cases=women **with HPV16 at enrolment** with CIN2/3 at enrolment; controls- HPV16 positive at enrolment with <CIN2 and <LSIL during FU	99	Cases: 33 [range:22-68]; controls=34 [range:21-64]	Exfoliated cervical specimen in Specimen transport medium (STM)	HC2 + PCR	PSQ+NGS
Qiu, 2015	HPV16-L1	China-Zhengzhou	Convenience sample	Patients undergoing routine LBC test at Third Affiliated Hospital of Zhengzhou University; women with abnormal cytology referred for cervical biopsy; **HPV16**+	114	37 [range: 25-74]	Cervical scrape	PCR	qPCR/MS-HRM (me-sensitive high-resolution melting
Simanaviciene, 2015	HPV16-L1, LCR	Lithuania-Vilnius	Case-control (unclear from the methods)	Samples from a prior study estimating prevalence of HR-HPV among Lithuanian women with cervical pathology. **HPV16**+	157	NR	Cervical swab	PCR	Bisulfite sequencing
Brandsma, 2014	HPV16-L1/L2/E2	Senegal-Dakar/USA-New Haven	Case-control (unclear from methods)	Two populations enrolled-21 women from routine ccx screening program in New Haven+12 women in Dakar never before screened attending community health clinic for reasons unrelated to ccx. **All HPV+**	33	African women=44 yrs (range:27-60); Usa-34 yrs (range: 23-65)	Cytology samples	PCR	Bisulfite sequencing
Brentnall, 2014	HPV16, 18, 31	UK-London	Population-based Screening sample	Colposcopy referral sample, referred due to abnormal smear; **HPV16**+	1493	NR	Exfoliated cervical specimen	Linear Array+ BD HPV test	PSQ
Bryant, 2014	HPV16-L1/L2/E2	UK-South Wales	Population-based screened sample	**HPV16**+ samples from population based screening	37	26-31	LBC	PCR	
Lorincz, 2013	HPV16-L1/L2/E6/URR	UK-Wales	Prospective	**HPV16+** Samples from clinical trial among women with newly diagnosed LG (borderline changes or mild dysplasia) cytological abnormalities; all women invited for colposcopy at 6 months with biopsy	73	NR	Exfoliated cervical specimen	PCR	PSQ
Mirabello, 2013	HPV16	Costa-Rica-Guanacaste	Case-control	Samples from population based cohort of 10,049 individuals recruited for screening and followed up as part of natural history study of HPV among women >18 yrs. Women referred to colposcopy if cytology abnormal. This study includes **HPV16+ women**; 100 with clearance in >2 yrs., 38 with persistence w/o CIN2+; 67 with HR-HPV persistence and CIN2+	273	NR	Cervical swab	PCR	PSQ
Xi, 2011	HPV16-LCR	USA	Case-control	Women (18-50 yrs.) attending Planned Parenthood clinics in Western Washington for routine Papanicolaou screening who **were HPV16 positive** at the screening and/or subsequent colposcopy visit	211	NR	Cervical swab	PCR	SequencerTM
Hublarova, 2009	HPV16-E6 Promoter	NK	Unclear (abstract only retrieved)	Abstract only retrieved; cervical smears from asymptomatic women with no cytological and colposcopic abnormalities, 20.4% were **HPV-16-positive**.	103	NR	NR	PCR	specific restriction endonuclease McrBC

Appendix 7.Performance measures of *CADM1* methylation assays for the detection of CIN2/3 and CIN3+ compared to ≤CIN1 in 7 studies

Author	Country	Sample size	Case definition	N Cases	Sensitivity	Specificity	PPV	NPV	AUC
CIN2/3									
Kim, 2016 [329]	South Korea-Seoul	170	CIN2/3	48	12.5%	95.9%	66.7%	62.8%	
VanBaars, 2016 [330]	Spain-Barcelona	60	CIN2/3	25	20.0%	94.3%	71.4%	62.3%	
DeVuyst, 2015 [17]	Kenya-Nairobi	248	CIN2/3	93	64.0%	70.0%	56.1%	76.4%	0.740
CIN3									
VanBaars, 2016 [330]	Spain-Barcelona	60	CIN3	12	33.3%	94.3%	66.7%	80.5%	
DeVuyst, 2015 [17]	Kenya-Nairobi	248	CIN3	43	75.0%	70.0%	41.0%	91.0%	0.800
Overmeer, 2011[4] [333]	The Netherlands	70	CIN3	30	86.7%	92.5%	89.7%	90.2%	
CIN3+									
Hesselink, 2014[2] [331]	The Netherlands	355	CIN3+	74	47.0%	70.0%	29.2%	83.4%	0.637
Hesselink, 2011[3] [332]	The Netherlands	275	CIN3+	38	79.0%	70.0%	36.0%	94.0%	0.682

PPV=Positive Predictive Value; NPV=Negative Predictive Value; AUC=Area under the operating receiving characteristic (not reported by all studies);[2]includes 4 squamous cell carcinoma and 1 Adenocarcinoma; [3]includes 3 squamous cell carcinoma and 1 Adenocarcinoma in situ; [4] includes 2 cancer cases

Appendix 8.Performance measures of *MAL* methylation assays for the detection of CIN2/3 and CIN3+ compared to ≤CIN1 in 7 studies

Author	Country	Sample size	Case definition	N cases	Sensitivity	Specificity	PPV	NPV	AUC
CIN2/3									
Kim, 2016 [329]	South Korea-Seoul	170	CIN2/3	48	2.1%	91.9%	14.3%	59.1%	
VanBaars, 2016 [330]	Spain-Barcelona	60	CIN2/3	25	32.0%	77.1%	50.0%	61.4%	
DeVuyst, 2015 [17]	Kenya-Nairobi	248	CIN2/3	93	64.0%	70.0%	56.1%	76.4%	0.72
Vasiljevic, 2014 [328]	UK-London	572	CIN2/3	208	90.0%	12.0%	36.9%	67.7%	0.62
CIN3									
VanBaars, 2016 [330]	Spain-Barcelona	60	CIN3	12	41.7%	77.1%	38.5%	79.4%	
DeVuyst, 2015 [17]	Kenya-Nairobi	248	CIN3	43	70.0%	70.0%	39.3%	89.4%	0.77
Overmeer, 2011[4] [333]	The Netherlands	70	CIN3	30	83.3%	87.5%	83.3%	87.5%	
CIN3+									
Hesselink, 2014[2] [331]	The Netherlands	355	CIN3+	74	67.0%	70.0%	37.0%	89.0%	0.77
Hesselink, 2011[3] [332]	The Netherlands	275	CIN3+	38	60.0%	70.0%	29.9%	89.1%	0.57

PPV=Positive Predictive Value; NPV=Negative Predictive Value; AUC=Area under the operating receiving characteristic curve (not reported by all studies); [2]includes 4 squamous cell carcinoma and 1 Adenocarcinoma; [3]includes 3 squamous cell carcinoma and 1 Adenocarcinoma in situ; [4] includes 2 cancer cases

Appendix 9.Performance measures of combination methylation panel assays of *CADM1*, *MAL* and *MIR* for detection of CIN2/3 and CIN3+ compared to ≤CIN1 in 7 studies

Author	Country	Sample size	Case definition	N cases	Sensitivity	Specificity	PPV	NPV	AUC*
CIN2/3									
CADM1/MAL									
VanBaars, 2016 [330]	Spain-Barcelona	60	CIN2/3	25	36.0%	71.4%	47.4%	61.0%	
Verhoef, 2015 [351]	The Netherlands	358	CIN2/3	84	57.1%	72.3%	38.7%	91.2%	
DeStrooper, 2014 [352][2]	The Netherlands	234	CIN2+	58	62.1%	78.4%	48.6%	86.3%	
Hesselink, 2011 [332][3]	The Netherlands	236	CIN2+	58	62.1%	78.1%	48.0%	86.3%	
MAL/MIR									
Verhoef, 2014 [353]	The Netherlands	1019	CIN2/3	212	30.7%	83.0%	32.5%	81.8%	
Hesselink, 2014 [4]	The Netherlands	355	CIN2+	94	59.6%	70.0%	41.7%	82.8%	
CADM1/MAL/MIR									
DeVuyst, 2015 [17]	Kenya-Nairobi	248	CIN2/3	93	65.0%	80.0%	66.0%		0.80
DeStrooper, 2014 [354]	The Netherlands	247	CIN2/3	48	47.9%	80.8%	50.0%	79.5%	
CIN3+									
CADM1/MAL									
VanBaars, 2016 [330]	Spain-Barcelona	60	CIN3	12	50.0%	71.4%	37.5%	80.6%	
Verhoef, 2015 [351]	The Netherlands	330	CIN3	56	66.1%	72.3%	32.7%	79.5%	
Overmeer, 2011 [333]	The Netherlands	70	CIN3	30	90.0%	87.5%	84.4%	92.1%	
DeStrooper, 2014 [352][2]	The Netherlands	234	CIN3+	38	68.4%	75.5%	35.1%	92.5%	
Hesselink, 2011 [332][3]	The Netherlands	236	CIN3+	38	68.4%	75.3%	34.7%	92.5%	
MAL/MIR									
Verhoef, 2014 [353]	The Netherlands	1019	CIN3	134	41.0%	83.0%	28.9%	89.3%	
Hesselink, 2014	The Netherlands	355	CIN3+	74	64.9%	70.0%	36.3%	88.3%	
CADM1/MAL/MIR									
DeVuyst, 2015 [17]	Kenya-Nairobi	248	CIN3	43	77.0%	80.0%	52.0%		0.85
DeStrooper, 2014 [354]	The Netherlands	247	CIN3	16	68.8%	80.8%	32.4%	95.9%	
DeStrooper, 2014 [354][5]	The Netherlands	247	CIN3+	95	94.7%	77.0%	72.0%	95.9%	

PPV=Positive Predictive Value; NPV=Negative Predictive Value; AUC=Area under the operating receiving characteristic curve (not reported by all studies); [2]includes 3 squamous cell carcinoma (SCC) and 1 Adenocarcinoma *in situ* (AdCaIS); [3]includes 3 SCC and 1 AdCaIS; [4])[4]includes 4 squamous cell carcinoma and 1 Adenocarcinoma; [5] includes 62 SCC, 12 AdCa, 2 Adenosquamous carcinoma and 3 undifferentiated carcinoma; *few studies provided AUC estimates

Appendix 10.Performance measures of *EPB41L3* methylation for the detection of CIN2/3 and CIN3+ compared to ≤CIN1 in 5 studies

Author	Country	Sample size	Case definition	N cases	Sensitivity	Specificity	PPV	NPV	AUC
CIN2/3									
Louvanto, 2015[2] [334]	Canada-Montreal	210	CIN2/3	94	60.0%	70.0%	76.7%	51.5%	0.67
Boers, 2014[3] [254]	The Netherlands	119	CIN2/3	40	60.0%	57.0%	41.4%	73.8%	
Vasiljevic, 2014[2] [328]	UK-London	884	CIN2/3	323	61.0%	70.0%	55.6%	74.5%	
Eijinsk, 2012 [335]	The Netherlands-Groningen	200	CIN2/3	66	50.0%	88.1%	67.3%	78.1%	
CIN2+									
Lorincz, 2016 [256][5]	UK-London	341	CIN2+	39	74.0%	65.0%	21.5%	95.1%	0.73
Louvanto, 2015[2,4] [334]	Canada-Montreal	210	CIN2+	153	78.4%	35.1%	76.4%	37.7%	0.73
Eijinsk, 2012 [335][6]	The Netherlands-Groningen	200	CIN2+		65.0%	79.0%			
CIN3+									
Boers, 2014[3] [254]	The Netherlands	104	CIN3	25	84.0%	57.0%	38.2%	91.8%	0.88
Eijinsk, 2012 [335]	The Netherlands-Groningen	171	CIN3	37	67.6%	88.1%	61.0%	90.8%	
Eijinsk, 2012 [335][6]	The Netherlands-Groningen	171	CIN3+		82.0%	73.0%			
Lorincz, 2016 [256][5]	UK-London	341	CIN3+	19	84.0%	63.0%	11.9%	98.5%	0.80

PPV=Positive Predictive Value; NPV=Negative Predictive Value; AUC=Area under the operating receiving characteristic curve (not reported by all studies); [2]Read from ROC curve for 70% specificity; [3]Invasive cancer cases removed from analysis; [4]includes 31 SCC and 28 ADC; [5]in combination with HPV16, 18, 31 and 33; 6 in combination with TERT (telomerase reverse transcriptase), JAM3 (junctional adhesion molecule 3) and C13ORF18 (Chromosome 13 Open Reading Frame 18)

Appendix 11.Performance measures of *PAX1* for the detection of CIN2/3 and CIN3+ in 8 studies

Author	Country	Sample size	Case definition	Reference	N cases	Sensitivity	Specificity	PPV	NPV
CIN2/3									
Kim, 2016 [329]	South Korea-Seoul	170	CIN2/3	≤CIN1	48	14.6%	97.3%	77.8%	63.7%
Xu, 2015 [336]	China-Shanghai	121	CIN2/3	≤CIN1	34	44.1%	95.0%	83.3%	75.0%
Wang, 2014 [337][3]	China-Shan dong	130	CIN2/3	≤CIN1	29	79.3%	94.9%	82.1%	85.5%
Lin, 2011 [338]	Taiwan	220	CIN2/3	≤CIN1	31	22.6%	90.4%	29.2%	87.0%
CIN2+									
Lai, 2014 [339][1]	Taiwan	346	CIN2+	≤CIN1	62	37.1%	89.8%	46.9%	85.4%
Kan, 2014 [340][2]	Taiwan-Taipei	96	CIN2+	≤CIN1	43	74.4%	88.7%	84.2%	81.0%
Huang, 2010 [341][4]	Taiwan	73	CIN2+	≤CIN1	39	56.4%	100.0%	100.0%	62.2%
Lai, 2010 [342][5]	Taiwan	185	CIN2+	≤CIN1	90	77.8%	100.0%	100.0%	72.2%
CIN3									
Wang, 2014 [337][3]	China-Shan dong	130	CIN3	≤CIN1	12	91.7%	94.9%	68.8%	98.9%
Lin, 2011 [338]	Taiwan	220	CIN3	≤CIN1	14	35.7%	90.4%	22.7%	93.1%
CIN3+									
Kan, 2014 [340][2]	Taiwan-Taipei	89	CIN3+	≤CIN1	36	86.1%	88.7%	83.8%	90.4%
Lin, 2011 [338]	Taiwan	220	CIN3+	≤CIN1	25	52.0%	90.4%	43.3%	93.1%
Huang, 2010 [341][4]	Taiwan	73	CIN3+	≤CIN1	37	54.1%	99.9%	97.6%	41.4%
Lai, 2010 [342][5]	Taiwan	185	CIN3+	≤CIN1	73	94.5%	99.9%	99.3%	92.9%
Lai, 2014 [339][1]	Taiwan	346	CIN3+	≤CIN1	62	74.2%	89.8%	63.9%	89.8%

PPV=Positive Predictive Value; NPV=Negative Predictive Value; AUC=Area under the operating receiving characteristic curve was not reported in any study; [1] Lai 2014 includes unknown carcinoma in situ (CIS); [2] Kan includes 4 Adenocarcinoma (AC)/squamous cell carcinoma (SCC); [3] Wang includes 2 AC/SCC; [4] Huang includes 14 AC/SCC; [5] Lai 2010 includes 27 AC/SCC;

Appendix 12.Performance measures of *SOX1* for the detection of CIN2/3 and CIN3+ in 2 studies

Author	Country	Sample size	Case definition	Reference	N cases	Sensitivity	Specificity	PPV	NPV
CIN2+									
Lai, 2014[339][1]	Taiwan	346	CIN2+	≤CIN1	62	43.5%	79.9%	34.6%	85.3%
CIN3+									
Lai, 2014 [339][1]	Taiwan	346	CIN3+	≤CIN1	62	80.6%	79.9%	49.5%	90.2%
Lai, 2010 [342][2]	Taiwan	185	CIN3+	≤CIN1	73	87.7%	81.6%	77.2%	90.3%

PPV=Positive Predictive Value; NPV=Negative Predictive Value; AUC=Area under the operating receiving characteristic curve was not reported by any study; [1] Lai 2014 includes unknown carcinoma in situ (CIS); [2]Lai 2010 includes 27 AC/SCC;

Appendix 13.Summary of studies reporting performance estimates of methylation of HPV16 L1, L2, E2 and LCR regions for detection of CIN2/3, CIN2+ and CIN3+ relative to ≤CIN1, in 10 studies

Author	HPV	Country	Sample size	Case definition	N cases	Sensitivity	Specificity	PPV	NPV	AUC
L1/L2 regions										
CIN2/3										
Louvanto, 2015 [334][1]	HPV16-L1	Canada-Montreal	244	CIN2/3	94	62.5%	70.0%	77.5%	53.1%	
Mirabello, 2015 [343]	HPV16-L1	USA-California	99	CIN2/3	59	92.5%	73.1%	83.5%	86.9%	
Simanaviciene, 2015 [344]	HPV16-L1	Lithuania-Vilnius	157	CIN2/3	87	20.7%	97.4%	94.7%	35.5%	
Brentnall, 2014 [345]	HPV16 -L1/L2	UK-London	556	CIN2/3	323	90.0%	38.0%	66.8%	73.3%	
Brandsma, 2014 [346]	HPV16-L1/L2/E2	Senegal-Dakar/USA-New Haven	33	CIN2/3	12	75.0%	75.0%	69.2%	80.0%	
Lorincz, 2013 [347]	HPV16-L1/L2	UK-Wales	73	CIN2/3	25	92.0%	40.0%	44.4%	90.6%	
Lorincz, 2013 [347]	HPV16-L1/L2/E6/URR	UK-Wales	73	CIN2/3	25	92.0%	40.0%	44.4%	90.6%	
CIN2+										
Lorincz, 2016 [256]	HPV16-L1	UK-London	99	CIN2+	39					0.69
Mirabello, 2013 [348]	HPV16-L1	Costa-Rica-Guanacaste	93	CIN2+	57	91.1%	60.2%	78.4%	81.0%	0.82
Qiu, 2015 [349]	HPV16-L1	China-Zhengzhou	114	CIN2+	72	91.7%	59.5%	79.5%	80.7%	0.978
Lorincz, 2016 [256]	HPV16-L2	UK-London	99	CIN2+	39					0.69
Bryant, 2015 [350]	HPV16-L1/L2	UK-Cardiff	200	CIN2+	145	61.9%	66.0%	82.8%	39.7%	
Bryant, 2015 [350]	HPV16-L1/L2/E2	UK-Cardiff	200	CIN2+	145	60.3%	61.7%	80.6%	37.1%	
CIN3/CIN3+										
Lorincz, 2016 [256]	HPV16-L1	UK-London	99	CIN3+	19					0.72
Lorincz, 2016 [256]	HPV16-L2	UK-London	99	CIN3+	19					0.73
Bryant, 2015 [350]	HPV16-L1/L2	UK-Cardiff	200	CIN3+	145	60.5%	70.3%	84.3%	40.3%	
Bryant, 2015 [350]	HPV16-L1/L2/E2	UK-Cardiff	200	CIN3+	145	60.5%	65.9%	82.4%	38.8%	
Brandsma, 2014 [346]	HPV16-L1/L2/E2	Senegal-Dakar/USA-New Haven	33	CIN3	5	80.0%	75.0%	50.0%	80.0%	

PPV=Positive Predictive Value; NPV=Negative Predictive Value; AUC=Area under the operating receiving characteristic curve (not reported by all studies) [1] read from ROC curve

Appendix 14. Table of denominators for all analyses in Chapters 6, 7, 8, 9 and 10

Outcome	Analysis	Obj.	Chap.	Table/fig	Denominator (N women)			Reason for exclusions
					Total	BF	SA	
STUDY 1: Epidemiology of HR-HPV and cervical lesions in WLHIV in BF and SA								
Population characteristics	Description of study population at baseline	n/a	6	Table 6.2 Fig. 6.1	1,238	615	623	No exclusions
HR-HPV prevalence	HR-HPV prevalence description	2.1	6	Table 6.2	1215	594	621	All women with genotyping data at baseline
	Risk factor analysis for HR-HPV prevalence	2.3	6	Table 6.5	1183	570	613	All women with genotyping data available; 32 women who were ART-naive and with undetectable HIV-1 PVL were excluded from analysis
	HIV-related factors associated with HR-HPV prevalence	2.4	7	Table 7.2	1183	570	613	As above
CIN2+ prevalence	CIN2+ prevalence description	2.2	6	Table 6.2	1128	554	574	All women with histology data at baseline
	Risk factor analysis for CIN2+ prevalence	2.3	6	Table 6.9	1096	530	566	All women with histology data available; 32 women who were ART-naive and with undetectable HIV-1 PVL were excluded from analysis
	HIV-related factors associated with CIN2+ prevalence	2.4	7	Table 7.6	1096	530	566	As above
Population characteristics	Description of study population at endline	2.1	6	Table 6.3 Fig 6.1	963	512	451	Women with ≤CIN1 at baseline who returned for endline visit
HR-HPV incidence	HR-HPV incidence description	2.1	6	Table 6.3 Fig 6.1	922	476	446	Women with ≤CIN1 at baseline with genotyping at baseline and endline at risk for acquiring any HR-HPV (no woman was positive for ALL HR-HPV types)
	Risk factor analysis for HR-HPV incidence	2.3	6	Table 6.6	900	460	440	As above, but excluding 22 women who were ART-naive and with undetectable HIV-1 PVL at baseline
	HIV-related factors associated with HR-HPV incidence	2.3	7	Table 7.3	900	460	440	As above
HR-HPV persistence	HR-HPV type-specific persistence description	2.1	6	Table 6.3 Fig 6.1	610	270	340	Women with ≤CIN1 and HR-HPV positive at baseline
	Risk factor analysis for HR-HPV persistence	2.3	6	Table 6.7	597	263	334	As above, but excluding 13 women who were ART-naive and with undetectable HIV-1 PVL at baseline
	HIV-related factors associated with HR-HPV persistence	2.4	7	Table 7.4	1002	404	598	Same number of women as above, but total number of infections was used as denominator

Outcome	Analysis	Obj.	Chap.	Table/fig	Denominator (N women)			Reason for exclusions
					Total	BF	SA	
HR-HPV complete clearance	HR-HPV complete clearance description	2.1	6	Table 6.3 Fig 6.1	610	270	340	As above
	Risk factor analysis for HR-HPV complete clearance	2.3	6	Table 6.8	597	263	334	As above, but excluding 13 women who were ART-naive and with undetectable HIV-1 PVL at baseline
	HIV-related factors associated with HR-HPV complete clearance	2.4	7	Table 7.5	597	263	334	As above
CN2+ Incidence	Incidence of CIN2+ description	2.2	6	Table 6.3 Fig 6.1	809	430	379	Women with ≤CIN1 at baseline who returned for endline visit with histology data at both baseline and endline
	Risk factor analysis for CIN2+ incidence	2.3	6	Table 6.10	785	412	373	As above but excluding 24 women who were ART-naive and with undetectable HIV-1 PVL at baseline
	HIV-related factors associated with CIN2+ incidence	2.4	7	Table 7.7	785	412	373	As above
STUDY 2: HPV type-specific infection and serodynamics among WLHIV in Burkina Faso and South Africa								
HPV-type specific infection	HPV genotype prevalence	3.1	8	Table 8.1	1215	594	621	All women with genotyping data at baseline
	The association of prevalent HPV genotypes with prevalent CIN2+	3.2	8	Figure 8.1 Figure 8.2 Figure 8.3 Figure 8.4	1119	546	573	All women with genotyping and histology data at baseline
	HPV type incidence	3.1	8	Table 8.1	922	476	446	Women with ≤CIN1 at baseline with genotyping at baseline and endline at risk for acquiring any HR-HPV
	HPV type persistence	3.1	8	Table 8.1	610	270	340	Women with ≤CIN1 and HR-HPV positive at baseline
	HR-HPV type persistence associated with incidence CIN2/3	3.2	8	Table 8.3	780	405	375	Women with ≤CIN1 at baseline who returned for endline visit with histology and genotyping data at both baseline and endline
	Association of HIV-related factors with HR-HPV type prevalence	3.3	8	Table 8.4 Table 8.5	1183	570	613	All women with genotyping data available; 32 women who were ART-naive and with undetectable HIV-1 PVL were excluded from analysis

Outcome	Analysis	Obj.	Chap.	Table/fig	Denominator (N women)			Reason for exclusions
					Total	BF	SA	
STUDY 2: HPV type-specific infection and serodynamics among WLHIV in Burkina Faso and South Africa								
HPV serology	HPV seroprevalence description	3.4	9	Fig 9.2/9.3	604	-	604	All women enrolled in SA with matched genotyping and serology data at baseline
	Correlation of HPV DNA and serology at baseline	3.4	9	Table 9.1	604	-	604	As above
	Risk factors associated with HPV seroprevalence	3.4	9	Appendix (App.) 13	600	-	600	As above, but excluding 4 women who were ART-naive and with undetectable HIV-1 PVL at baseline
	HPV seroincidence description	3.5	9	Narrative	433	-	433	Women with ≤CIN1 at baseline who returned for endline visit and with genotyping and serology data at both baseline and endline
	Risk factors associated with HPV seroincidence, including HIV-related	3.5	9	Not shown	425	-	425	As above, excluding 4 women who had seropositivity for all 15 types at baseline, and 4 women who were ART-naive and with undetectable HIV-1 PVL at baseline
	HPV seroconversion description	3.5	9	Table 9.2	219	-	219	Women with ≤CIN1 at baseline who returned for endline visit ; who were HV DNA positive and same type seronegative at baseline
	Risk related factors associated with HPV seroconversion	3.5	9	App.14	214	-	214	As above, excluding 4 women who were ART-naive and with undetectable HIV-1 PVL at baseline
	HIV related factors associated with HPV seroconversion	3.5	9	Table 9.3	319	-	319	As above but using total baseline infections as denominators (319 baseline infections among 214 women)
	Risk of type-specific re-infection following same-type seropositivity at baseline	3.6	9	Table 9.4	433	-	433	Women with ≤CIN1 at baseline with genotyping and serology at baseline and endline at risk for acquiring any HR-HPV (no woman was positive for ALL HR-HPV types)
	Risk of type-specific DNA persistence following same-type seropositivity at baseline	3.6	9	App.15	148	-	148	Women with ≤CIN1 at baseline with genotyping and serology at baseline and endline at risk and positive for any HPV at baseline – includes a total of 577 infections
	Association of baseline seropositivity and seropositivity at endline with CIN2/3 incidence	-	9	App.16	365		365	Women with ≤CIN1 at baseline with histology and serology at baseline and endline

Outcome	Analysis	Obj.	Chap.	Table/fig	Denominator (N women)			Reason for exclusions
					Total	BF	SA	
STUDY 3: Association of DNA methylation with prevalent and incident CIN2+ among WLHIV in Burkina Faso and South Africa								
DNA methylation	Determining the association of DNA methylation of a human gene *EPB41L3* with CIN2+ prevalence	4.1	11	Fig 10.4, Table 10.4 Table 10.5 App. 17 App. 18	362	94	268	All CIN2+ cases at baseline matched 1:1 with ≤CIN1 in each country for *EPB41L3* DNA methylation
	Evaluating the role of socio-demographic, behavioral and HIV-related factors on the *EPB41L3* DNA methylation at enrolment	4.2	11	Table 10.8 App. 19 App. 20	360	94	266	As above excluding 2 ART-naïve women with undetectable HIV-1 PVL
	Determining the association of baseline and endline *EPB41L3* DNA methylation with CIN2+ incidence	4.1	11	Table 10.5 Table 10.6	185	57	128	All ≤CIN1 controls at baseline with histology and *EPB41L3* DNA methylation at endline
	Monitoring the change in *EPB41L3* DNA methylation levels over time among CIN2+ that progress vs. regress	4.3	11	Table 10.7	36	-	36	All prevalent CIN2+ in SA that did not receive treatment by endline visit
	Determining the association of DNA methylation of a HPV16 L1 with CIN2+ prevalence	4.1	11	App. 21	81	23	58	All HPV16-positive CIN2+ cases at baseline matched 1:1 with HPV16-positive ≤CIN1 in each country

Appendix 15. Multivariate analysis of HPV seroprevalence among 600 WLHIV[1] in South Africa: associations with baseline sociodemographic factors, behavioural factors, HIV-related factors, clinical symptoms/signs and STIs

			Univariate analysis		Multivariate analysis	
	N	n (%)	PR (95%CI)	p-value	aPR (95%CI)*	p-value
Ever used injectable contraceptives						
Never	165	146 (88.5)	1		1	
Past	322	305 (94.7)	1.07 (1.01-1.14)	0.03	**1.07 (1.01-1.13)**	**0.03**
Current	113	109 (94.5)	1.09 (1.02-1.16)	0.01	**1.09 (1.03-1.16)**	**0.01**
ART status						
ART >2 years	224	216 (94.6)	1		1	
ART ≤2 years	174	164 (94.3)	0.98 (0.93-1.02)	0.32	0.98 (0.92-1.04)	0.412
ART-naïve	202	180 (89.1)	0.92 (0.88-0.98)	0.004	0.98 (0.92-1.03)	0.412
HIV-1 viral suppression						
<1000 copies/ml	342	331 (96.8)	1		1	
≥1000 copies/ml	258	229 (88.8)	0.92 (0.87-0.96)	<0.001	**0.93 (0.87-0.99)**	**0.02**
CD4+ count (cells/mm^3)						
>500	220	206 (93.6)	1		1	
351-500	181	174 (96.1)	1.03 (0.98-1.07)	0.25	1.03 (0.99-1.08)	0.17
200-350	143	127 (88.8)	0.95 (0.89-1.01)	0.13	0.96 (0.90-1.03)	0.27
<200	56	53 (94.6)	1.01 (0.94-1.09)	0.77	1.02 (0.95-1.10)	0.58

Adjusted Prevalence Ratio (aPR); *All factors in table adjusted for each other; [1]4 women excluded from analysis as they were ART-naïve but had detectable HIV-1 PVL

Appendix 16. HPV DNA persistence among 148 WLHIV, measured over 16 months follow-up, stratified by same type seropositivity at baseline

	All participants		Seronegative at baseline		Seropositive at baseline		aOR (95%CI)[2]	Seropersistence at endline		Seroreversion at endline	
	N[1]	n (%)	N[1]	n (%)	N[1]	n (%)		N[1]	n (%)	N[1]	n (%)
Any Alpha-9 HR-HPV types											
HPV16	67	22 (32.8)	41	11 (26.8)	26	11 (42.3)	2.35 (0.74-7.75)	23	8 (34.8)	3	3 (100.0)
HPV31	39	5 (12.8)	13	2 (15.4)	26	3 (11.5)	0.76 (0.10-5.76)	24	3 (12.5)	2	0 (0.0)
HPV33	25	4 (16.0)	15	1 (6.7)	10	3 (30.0)	4.50 (0.25-80.57)	9	3 (33.3)	1	0 (0.0)
HPV35	59	24 (40.7)	26	11 (42.3)	33	13 (39.4)	1.00 (0.34-2.95)	30	12 (40.0)	3	1 (33.3)
HPV52	106	38 (35.9)	63	21 (33.3)	43	17 (39.5)	1.49 (0.64-3.50)	39	16 (41.0)	4	1 (25.0)
HPV58	29	11 (37.9)	12	4 (33.3)	17	7 (41.2)	1.35 (0.29-6.40)	15	7 (46.7)	2	0 (0.0)
Any Alpha-7 HR-HPV types											
HPV18	66	22 (33.3)	41	14 (34.2)	25	8 (32.0)	0.94 (0.31-2.86)	18	6 (33.3)	7	2 (28.6)
HPV39	36	8 (22.2)	19	2 (10.5)	17	6 (35.3)	4.75 (0.70-32.19)	10	3 (30.0)	7	3 (42.9)
HPV45	30	11 (36.7)	25	8 (32.0)	5	3 (60.0)	2.00 (0.20-20.08)	1	0 (0.0)	4	3 (75.0)
HPV59	9	2 (22.2)	4	1 (25.0)	5	1 (20.0)	0.77 (0.03-18.30)	3	0 (0.0)	2	1 (50.0)
HPV68	21	4 (19.1)	13	3 (23.1)	8	1 (12.5)	0.16 (0.01-2.83)	8	1 (12.5)	0	0 (0.0)
Other HPV types											
HPV56	37	9 (24.3)	26	4 (15.4)	11	5 (45.6)	**12.79 (1.14-143.23)**	8	5 (62.5)	3	0 (0.0)
LR-HPV											
HPV6	23	2 (8.7)	13	0 (0.0)	10	2 (20.0)	-	10	2 (20.0)	0	0 (0.0)
HPV11	25	4 (16.0)	13	3 (23.1)	12	1 (8.3)	0.16 (0.09-2.91)	11	1 (9.1)	1	0 (0.0)
HPV73	5	0 (0.0)	2	0 (0.0)	3	0 (0.0)	-	3	0 (0.0)	-	-
Any HPV type	**577**	**166 (28.8)**	**326**	**85 (26.1)**	**251**	**81 (32.3)**		**212**	**67 (31.6)**	**39**	**14 (35.9)**

[1]Denominator is total number of infections detected at baseline; [2]adjusted Odds Ratio (OR) for DNA persistence among same-type seropositive vs. seronegative at baseline, adjusted for ART status at baseline

Appendix 17. Risk of incident CIN2+ according to HPV seropersistence over 16 months among 365 WLHIV in South Africa

	Seronegative at baseline		Seropositive at baseline		aOR (95%CI)[1]	Seroreversion or seronegative at baseline		Seropersistence		aOR (95%CI)[2]
	N	n (%)	N	n (%)		N	n (%)	N	n(%)	
Any Alpha-9 HR-HPV types										
HPV16	207	10 (4.8)	158	12 (7.6)	2.20 (0.88-5.49)	**234**	13 (5.6)	131	9 (6.9)	1.76 (0.69-4.48)
HPV31	152	5 (3.3)	213	17 (8.0)	**3.45 (1.19-10.07)**	**171**	6 (3.5)	194	16 (8.3)	**3.32 (1.21-9.11)**
HPV33	223	11 (4.9)	142	11 (7.8)	1.93 (0.79-4.73)	**238**	14 (5.9)	127	8 (6.3)	1.23 (0.49-3.08)
HPV35	212	13 (6.1)	153	9 (5.9)	1.07 (0.43-2.67)	**245**	15 (6.1)	120	7 (5.8)	1.03 (0.39-2.71)
HPV52	247	15 (6.1)	118	7 (5.9)	1.09 (0.42-2.85)	**261**	16 (6.1)	104	6 (5.8)	1.04 (0.38-2.85)
HPV58	158	3 (1.9)	207	19 (9.2)	**6.60 (1.85-23.51)**	**183**	3 (3.3)	182	16 (8.8)	**3.53 (1.30-9.58)**
Any Alpha-7 HR-HPV types										
HPV18	231	15 (6.5)	134	7 (5.2)	0.94 (0.36-2.47)	**279**	18 (6.5)	86	4 (4.7)	0.79 (0.25-2.49)
HPV39	229	14 (6.1)	136	8 (5.9)	1.14 (0.45-2.85)	**288**	17 (5.9)	77	5 (6.5)	1.33 (0.46-3.88)
HPV45	297	17 (5.7)	68	5 (7.4)	1.47 (0.50-4.27)	**331**	19 (5.7)	34	3 (8.8)	1.81 (0.49-6.74)
HPV59	238	10 (4.2)	127	12 (9.5)	**2.81 (1.14-6.91)**	**270**	14 (5.2)	95	8 (8.4)	1.94 (0.77-4.91)
HPV68	262	12 (4.6)	103	10 (9.7)	**2.71 (1.09-6.77)**	**284**	16 (5.6)	81	6 (7.4)	1.61 (0.58-4.50)
Other HR-HPV types										
HPV56	247	9 (3.6)	118	13 (11.0)	**3.79 (1.53-9.39)**	**274**	10 (3.7)	91	12 (13.2)	**4.58 (1.85-11.37)**
LR-HPV										
HPV6	213	15 (7.0)	152	7 (4.6)	0.75 (0.29-1.94)	**238**	18 (7.6)	127	4 (3.2)	0.49 (0.16-1.52)
HPV11	236	14 (5.9)	129	8 (6.2)	1.31 (0.51-3.37)	**255**	14 (5.5)	110	8 (7.3)	1.81 (0.69-4.75)
HPV73	277	17 (6.1)	88	5 (5.7)	1.17 (0.40-3.45)	**301**	20 (6.6)	64	2 (3.1)	0.55 (0.12-2.52)
Any HPV	28	0 (0.0)	337	22 (6.5)		45	0 (0.0)	320	22 (6.9)	-

[1]adjusted Odds Ratio (OR) for CIN2/3 incidence among seropositive vs. seronegative at baseline; [2]adjusted OR for CIN2/3 incidence among seropersistent vs. seroreverted or seronegative at baseline, adjusted for injectable contraception, cervicitis, ART status (associated with incident CIN2/3, Chapter 7) and same-type DNA detection at baseline

Appendix 18. Multivariate risk factor analysis for 'high' methylation among 94 WLHIV in Burkina Faso

	N	Low methylation n (%)	High methylation n (%)	aOR (95%CI)
Education				
Primary or less	60	44 (73.3)	16 (26.7)	1
More than primary	33	11 (33.3)	22 (66.7)	**3.89 (1.20-12.62)**
Partner status				
Never married	11	3 (27.3)	8 (72.7)	1
Divorced/separated/widowed	46	32 (69.6)	14 (30.4)	0.38 (0.04-3.34)
Married/cohabiting	36	20 (55.6)	16 (44.4)	0.75 (0.09-6.06)
CD4+ count (cells/mm^3)				
350+	63	41 (65.1)	22 (34.9)	1
201-349	16	9 (56.3)	7 (43.8)	1.84 (0.45-7.48)
≤200	15	5 (33.3)	10 (66.7)	**8.29 (1.30-52.79)**
Candida albicans				
Negative	73	42 (57.5)	31 (42.5)	1
Positive	13	11 (84.6)	2 (15.4)	**0.08 (0.01-0.99)**
HR-HPV (by INNO-LiPA)				
Negative	16	12 (75.0)	4 (25.0)	1
Positive	77	43 (55.8)	34 (44.2)	2.18 (0.39-12.11)
CIN status				
<CIN1	34	24 (70.6)	10 (29.4)	1
CIN1	32	21 (65.6)	11 (34.4)	0.75 (0.19-2.98)
CIN2	17	7 (41.2)	10 (58.8)	1.14 (0.225.94)
CIN3	11	3 (27.3)	8 (72.7)	2.94 (0.34-25.48)

[1]adjusted Odds Ratio (aOR); adjusted for all covariates in table

Appendix 19. Multivariate risk factor analysis for 'high' methylation among 266 WLHIV* in South Africa

	N	Low methylation n(%)	High methylation n(%)	aOR (95%CI)
Education				
Less than complete secondary	145	70 (48.3)	75 (51.7)	1.00
Minimum secondary	112	69 (61.6)	43 (38.4)	0.48 (0.21-1.10)
Age at 1st pregnancy				
<20 years	135	67 (49.6)	68 (50.4)	1.00
≥20 years	117	69 (59.0)	48 (41.0)	1.07 (0.46-2.51)
ART status				
>2 years	79	36 (45.6)	43 (54.4)	1.00
≤2 years	83	40 (48.2)	43 (51.8)	**0.26 (0.10-0.67)**
ART-naïve	104	67 (64.4)	37 (35.6)	-
ART adherence				
60-90%	138	69 (50.0)	69 (50.0)	1.00
<60%	22	7 (31.8)	15 (68.2)	1.68 (0.48-5.89)
HIV-1 Viral suppression				
≥1000 copies/ml	127	75 (59.1)	52 (40.9)	1.00
<1000 copies	139	68 (48.9)	71 (51.1)	1.02 (0.33-3.14)
CD4+ count (cells/mm^3)				
>350	160	97 (60.6)	63 (39.4)	1.00
201-349	73	35 (48.0)	38 (52.1)	1.66 (0.58-4.75)
≤200	32	10 (31.3)	22 (68.8)	3.18 (0.95-10.69)
Chlamydia trachomatis				
Negative	253	132 (52.2)	121 (47.8)	1.00
Positive	13	11 (84.6)	2 (15.4)	-
Anogenital warts				
No	243	135 (55.6)	108 (44.4)	1.00
Yes	23	8 (34.8)	15 (65.2)	1.55 (0.37-6.40)
CIN status				
<CIN1	84	62 (73.8)	22 (26.2)	1.00
CIN1	59	36 (61.0)	23 (39.0)	2.68 (0.94-7.64)
CIN2	72	28 (38.9)	44 (61.1)	**19.28 (5.47-68.03)**
CIN3	51	17 (33.3)	34 (66.7)	**9.31 (2.77-31.29)**

*2 WLHIV who were ART-naïve and with undetectable HIV-1 PVL were excluded from the analysis; adjusted Prevalence Ratio (aPR); adjusted for all covariates in table

Appendix 20. Median methylation levels (%) for HPV16 L1 among 23 HPV16 positive WLHIV in BF and 58 in SA, by CIN grade.

	Burkina Faso			South Africa			Sites combined		
	N	**Median % (IQR)**	***p*[1]**	**N**	**Median % (IQR)**	***p*[1]**	**N**	**Median % (IQR)**	***p*[1]**
<CIN1	6	6.88 (2.65-22.5)	Ref	14	9.95 (0.0-19.15)	Ref	20	8.68 (0.78-20.83)	Ref
CIN1	9	7.20 (4.90-14.60)	0.77	14	9.28 (3.3-33.40)	0.50	23	8.40 (3.30-20.75)	0.59
CIN2	3	3.75 (4.45-4.60)	0.30	14	5.28 (2.60-8.00)	0.64	17	4.95 (2.60-7.85)	0.44
CIN3	5	39.90 (14.70-46.85)	0.20	16	7.83 (3.28-14.40)	0.97	21	8.90 (4.80-26.55)	0.44
p-trend[2]			0.24			0.79			0.72

[1]Mann-Whitney U test, p-value for difference in median values of CIN2/3 relative to <CIN1;
[2]Cuzick test for trend; p-value for trend in methylation levels by CIN grade