CERVICAL PRECANCEROUS LESIONS IN HIV-POSITIVE WOMEN IN CAMEROON:

PREVALENCE, PREDICTORS AND POTENTIAL IMPACT OF SCREENING

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ABSTRACT

JULIUS ATASHILI: Cervical precancerous lesions in HIV-positive women in Cameroon: prevalence, predictors and potential impact of screening

(Under the direction of William C. Miller, MD, PhD, MPH)

Cervical cancer is the most common cancer in women in low-income countries. Although cervical cancer incidence and mortality is higher in HIV-positive women, resource limitations restrict the implementation of systematic screening programs in these women. The purpose of this dissertation was to explore the potential for targeted screening by assessing the prevalence and clinical predictors of cervical squamous intra-epithelial lesions (SIL) in HIV-positive women in Cameroon. Furthermore, we sought to explore the potential impact of antiretroviral therapy and screening on mortality from cervical cancer.

We initially conducted a cross-sectional study of HIV-positive women in Cameroon. A total of 282 women, aged 19 to 68 years with a median CD4 cell count of 179 cells/microliter, were enrolled. SIL were detected in 43.5% of the 276 women with satisfactory samples: including high-grade SIL (HSIL) in 3.3%. None of the clinical factors assessed significantly predicted the presence of any lesion. Among patients with CD4 counts less than 200 cells/microliter, the prevalence of SIL was higher in patients aged 26-59 years compared to younger women, while there was essentially no difference amongst women with CD4 counts greater than 200 cells/microliter.

Using a Markov state-transition model of a cohort of HIV-positive women in Cameroon, we examined the potential impact of scenarios including: no HAART and no screening (NHNS); HAART and no screening (HNS); and HAART and screening once at

age 35 (HS35). Compared to NHNS, lifetime cumulative cervical cancer mortality doubled with HNS. It will require 202 women being screened at age 35 to prevent one cervical cancer death amongst women on HAART.

The high prevalence of SIL in women initiating antiretroviral therapy in Cameroon underscores the need for screening in this population. With neither age nor any other clinical factor being a good predictor of SIL, alternative affordable screening options need to be explored. Furthermore, the long-term evolution of SIL needs to be assessed in prospective studies of these women. Screening has the potential of reducing cervical cancer mortality in HIV-positive women in Africa. The cost of achieving such an effect needs to be assessed.

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AIDS	Acquired Immune Deficiency Syndrome
ANOVA	Analysis of variance
aOR	Adjusted odds ratio
ASC-H	ASCUS, cannot exclude a high grade lesion
ASCUS	Atypical Squamous Cells of Unknown Significance
ATC	AIDS treatment center
AUC	Area under the curve
CD	Cluster designate
CDC	Center for Disease Control and prevention
CE	Cost-effective
CEA	Cost-effectiveness analysis
CI	Confidence Interval
CIN	Cervical Intraepithelial Neoplasia
CSCCD	Center for the Study and Control of Communicable Diseases
DAG	Directed acyclic graph
DNA	Deoxyribonucleic acid
GDP	Gross Domestic Product
HAART	Highly Active Antiretroviral Therapy
HC	Hybrid Capture
HIV	Human Immunodeficiency Virus
HNS	HAART and No screen
HPV	Human Papillomavirus
HR	Hazard ratio
HR-HPV	High-risk Human Papilloma Virus

HS35	HAART and Screen at age 35
HSHI	HAART and Screen at HAART initiation
HSIL	High-grade Squamous Intraepithelial Lesion
IARC	International Agency for Research on Cancer
ICC	Invasive Cervical Cancer
ICER	Incremental cost-effectiveness ratio
LBC	Liquid Based Cytology
LEEP	Loop electrosurgical excision procedure
LOWESS	Locally-weighted Sums of Squares methods
LRT	Likelihood ratio test
LSIL	Low-grade Squamous Intraepithelial Lesion
MLE	Maximum Likelihood Estimation
NA	Not applicable
NHNS	No HAART and No Screen
NPV	Negative predictive value
OR	Odds ratio
PCR	Polymerase Chain Reaction
PD	Prevalence difference
PMTCT	Prevention of Mother-to-Child Transmission of HIV
PPV	Positive predictive value
QALY	Quality-adjusted life years
RTI	Reproductive Tract Infection
SCC	Squamous Cell Carcinoma
SCJ	Squamocolumnar Junction
SIL	Squamous intraepithelial Lesions

STI	Sexually Transmitted Infection
UK	United Kingdom
UNAIDS	Joint United Nations Program on HIV/AIDS
US	United States
USD	United States Dollars
VIA	Visual Inspection with Acetic acid
VIAM	Magnified visual inspection with acetic acid
VILI	Visual Inspection with Lugol’s Iodine
WHO	World Health Organization

CHAPTER ONE: INTRODUCTION, SPECIFIC AIMS AND HYPOTHESES

1.1 Introduction

Cervical cancer is the most common cancer in women in low-income countries [WHO, 2006] and the second most common cancer in women worldwide [Stewart and Kleihues, 2003]. Compared to immuno-competent women, HIV-positive women have a higher prevalence, incidence and progression rate of precancerous cervical lesions [Palefsky, 2006]. By the end of 2007, women accounted for 50% of the estimated 33 million people living with HIV worldwide, and close to 59% of the 22 million in sub-Saharan Africa [UNAIDS, 2008]. With the recent increase in access to antiretroviral therapy these women are expected to live longer thus potentially allowing sufficient time for cervical cancer to develop. In addition to longer life expectancy, antiretroviral therapy is associated with a reduction of competing causes of death, such as Kaposi sarcoma and tuberculosis, thus potentially increasing the proportion of morbidity and mortality attributable to cervical cancer [Franceschi and Jaffe, 2007]. Enhancing early detection and treatment of precancerous lesions, through screening could reduce the burden of cervical cancer in these HIV-positive women [Goldie et al, 2005; Franceschi and Jaffe, 2007].

Despite the relatively high association of HIV with precancerous and cancerous cervical lesions, unlike other opportunistic affections, the current management of women initiating

HAART in most developing countries (including Cameroon) does not include a systematic screen for cervical cancer or precancerous lesions. We hypothesize that cervical cancer goes undiagnosed and that early diagnosis of precancerous lesions by a screen at HAART-initiation could be cost-effective in reducing overall morbidity and mortality rates in these HIV-positive women. Targeted screening could potentially increase the cost-effectiveness of screening in these resource-limited settings. However, for targeted screening to be effective the factors associated with a higher prevalence and severity of lesions need to be identified.

Age has been a common consideration in the targeted screening for precancerous lesions in the general population. In effect, current guidelines for screening the general population of women in the United States (US) suggest screening commence no later than at age 21 years, reducing the frequency of screening at age 30 and stopping screening at age 65 (or 70 in some guidelines) [USPSTF, 2008; ACOG 2003, Saslow et al, 2002]. World Health Organization (WHO) guidelines aimed primarily at resource-limited settings are less stringent, recommending screening begin at age 30, need not be annual and need not be done over the age of 65 [WHO, 2006]. These age considerations may not necessarily be ideal for HIV-positive women in whom higher human papilloma virus (HPV) prevalence, higher HPV persistence, and a faster progression of lesions could mean an earlier occurrence and or a longer persistence of precancerous lesions. The optimal age for screening in HIV positive individuals could differ substantially from those in the general population and needs to be better described.

This dissertation sought to provide data aimed at improving the detection and management of cervical cancer in HIV-positive women. In Cameroon, and other resource-limited settings, the period of HAART initiation may be a critical time during which HIV-positive women could be screened as these women already undergo a series of clinical and laboratory assessment required for HAART. Age may also be an important consideration to further target screening.

To assess the need and potential effect of a screening program in a resource-limited setting like Cameroon, this dissertation had the following specific aims:

1.2 Specific Aim 1

To determine the prevalence, severity and predictors of cervical squamous intraepithelial lesions in HIV-positive women initiating antiretroviral therapy in Cameroon.

Rationale: Targeted screening may be a potential cost-effective alternative for the screening of HIV-positive women in resource-limited settings. Determining clinical characteristics that predict cervical lesions will help identify sub-populations which could be targeted for screening and thus potentially increase the ratio of the number of cases detected per screening test. We will develop a predictive model for prevalent cervical epithelial lesions in women initiating HAART in Cameroon and assess the use of risk-scores to guide screening.

Hypothesis: We hypothesize that cervical precancerous lesions are prevalent in women initiating HAART in Cameroon and that readily available socio-demographic and clinical characteristics can be used to predict the presence of lesions. We further hypothesize that these characteristics could be used to develop a score that could in turn be used to reliably predict which women need to be screen.

1.3 Specific Aim 2

To describe the age trends in the prevalence of cervical squamous intraepithelial lesions in HIV-positive women on antiretroviral therapy in Cameroon.

Rationale: The optimal ages to begin and discontinue cervical cancer screening in HIV positive women are unknown. By assessing the age-specific prevalence of lesions we would estimate the minimum age at which lesions occur, the age with maximum occurrence and the latest age at which lesions occur. This information on age-specific prevalence could be

useful in the development of age-targeted screening guidelines in HIV positive women in Cameroon.

Hypothesis: We hypothesize that the prevalence of lesions is dependent on age and that age alone could be used as a criterion for targeted screening.

1.4 Specific Aim 3

To quantify the potential effect of antiretroviral therapy and screening, on mortality from cervical cancer in HIV-positive women in Cameroon

Rationale: The long term effect of cervical cancer screening in HIV-positive women in Cameroon is unknown. Mortality from cervical cancer and mortality from HIV can be competing risks in the evolution of each other disease. The increased survival that is expected to result from the increased access to HAART in Cameroon may also be accompanied by an increase in the incidence and mortality due to cervical cancer. By assessing the potential impact of modifiable factors such as antiretroviral therapy and screening, we would quantify the potential gains that could be expected from these interventions and help policymakers in the allocation of limited resources.

Hypothesis: We hypothesize that the cumulative mortality due to cervical cancer would increase with antiretroviral therapy while screening would substantially reduce this mortality.

1.5 Overview

To achieve these aims we conducted two distinct studies: a cross-sectional descriptive study of women in HIV clinics in Cameroon who were interviewed on demographic and clinical characteristics and then screened for cervical precancerous lesions; and a computer-simulated analysis of the potential outcomes in a group of HIV-positive women in Cameroon.

CHAPTER TWO: BACKGROUND AND SIGNIFICANCE

In this section we present the burden of cervical cancer, its pathogenesis and risk factors, and the role of screening in reducing cervical cancer mortality in the general population. We then summarize the literature on the peculiarities of cervical cancer in HIV-infected women, the occurrence of precancerous and cancerous lesions by age and end by reviewing published studies of the prevalence of precancerous lesions in HIV in developing countries, and studies of the impact of age on cervical cancer.

2.1 Cervical cancer

2.1.1 Classification and epidemiology

The term 'cervical cancer' is generally used in reference to squamous cell carcinoma of the uterine cervix although other histological forms are plausible. The cervix is covered by two types of epithelia: a squamous cell epithelium usually limited to the ectocervix and a columnar (glandular) epithelium usually limited to endocervix. The pathogenesis of squamous cell carcinoma (SCC) involves the exposure and subsequent squamous metaplasia of the squamocolumnar ('transformation') zone, the intersection of the ecto- and endocervix. Other histological forms of cervical neoplasia include adenocarcinomas and other non-squamous, non-glandular epithelial tumors such as

adenosquamous carcinoma and small cell carcinoma amongst others [Silverberg and Loffe 2003]. SCC is however by far the most common form of cancer accounting for 80% of primary cervical cancers [Waggoner, 2003]. Any unspecified use of 'cancer of the cervix' in this document will be a reference to SCC**.**

Cervical cancer is the second cause of cancer in all-age women worldwide and the second cause of cancer death in sub-Saharan Africa. It is estimated that 500,000 new cases are diagnosed yearly with 85% of these in the developing world [Waggoner, 2003; Ferlay et al, 2004]. Cervical cancer is also estimated to cause over 270,000 deaths annually with the majority of deaths occurring in developing countries. Worldwide these represent incidence and mortality rates of 16.0 and 8.9 per 100,000 respectively. The incidence and mortality rates in Africa (19 and 14.8 per 100,000 respectively) [WHO/ICO 2007] are higher than those observed in South-East Asia (15.9 and 8.4 per 100,000 respectively) and substantially higher than those observed in North America (9.0 and 3.6 per 100,000 respectively) [Ferlay et al, 2004]. These rank cervical cancer the first cause of cancer incidence and mortality in women of all ages in Africa.

In Cameroon, cervical cancer is estimated to be the first cause of cancer and cancer mortality in women of all-ages (and the third cause of cancer mortality in women aged 15-44 years, after breast cancer and Kaposi sarcoma) [WHO/ICO, 2007]. The incidence and mortality rates of 22.6 and 18.2 per 100,000 women in Cameroon more than double the rates observed in the US (9.0 and 3.2 per 100,000 women respectively) where cervical cancer is the 13th most frequent cancer.

2.1.2 Pathogenesis

Cervical cancer pathogenesis is characterized by the role of infection with oncogenic HPV and the progression over a long time of precancerous lesions prior to the development of invasive cancer per se.

HPV is increasingly recognized as a necessary, albeit not a sufficient, cause of cervical cancer [Castellsague, 2008]. The etiologic role of HPV was first suggested when case-control studies worldwide, detected HPV DNA in 90-100% of adequately collected and preserved cervical tissue from cases as opposed to 5-20% of cervical samples in controls [Bosch et al, 2002]. These findings were confirmed in prospective studies in which women with HPV-16 had higher incidence of advanced cervical intraepithelial neoplasia (CIN III) [Schiffman and Castle, 2003; Munoz et al, 2006].

HPV are DNA viruses with more than 100 types identified based on the DNA diversity. Anogenital tissues are infected by close to 40 HPV types. Based on epidemiologic data 13 types (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) have been established to have high oncogenic risk [Munoz et al, 2003; Munoz et al, 2006]; six other types (types 26, 53, 66, 68, 73, 82) are considered by some to probably have a high risk[Munoz et al, 2006]. Other types are generally considered to be of low oncogenic risk.

HPV-16 and 18 are estimated to account for 70% of cervical cancers. There is however a variability in the frequency and geographic distribution of the different oncogenic types. While HPV 16, 31,51 and 53 were the most common types in low-grade lesions worldwide, HPV-16 appears more frequent in Europe (2-fold compared to Africa) while HPV-18 appears more frequent in North America than in Europe and South/Central America [Clifford et al, 2005]. Furthermore, a recent meta-analysis reports HPV 16/18 in 74-77% of high-grade squamous intra-epithelial/invasive cancer cases in North America, Europe and Australia versus a prevalence of 65-70% in Africa, Asia and South/Central America[Smith et al, 2007].

The HPV virion has a circular genome enclosed in a capsid shell made of two proteins: a major capside protein L1 and a minor protein L2 [Steben and Duarte-Franco, 2007]. The L refers to ‘Late’ proteins, a reference to when these proteins are synthesized in

the virus' life cycle. The viral genome also codes for six early proteins (E1, E2, E4, E5, E6 and E7) that interact with host cell genomes and are involved in viral transcription, replication and assembly[Munoz et al, 2006]. HPV infects squamous epithelial cells, particularly in differentiating cells such as those of the skin or the squamocolumnar junction. The complex interaction between viral (particularly E6/E7) proteins and host proteins may result in changes the regulation of host cell differentiation and neoplasia.

HPV infection is not considered sufficient for the development of cervical cancer as despite its high prevalence very few infections lead to cancer. **The development of precancerous and cancerous lesions is a function of persistent infection**. In multiple studies the relative risk of high grade lesions in women with persistent HPV infection compared to HPV negative women ranged from 1.3 (95%CI: 1.1, 1.5) to 813 (95%CI: 168.2, 3229.2) [Koshiol et al, 2008]. Persistence appears to be multi-factorial depending on viral factors (type, viral load), exogenous factors and host factors.

HPV-persistence may lead to precancerous lesions. Multiple systems have been proposed to classify these precancerous lesions. The most frequently used Bethesda 2001 system classifies lesions based on cervical cytology as unsatisfactory, negative (normal), atypical squamous cells of uncertain significance (ASCUS), atypical squamous cells (ASC-H), low-grade squamous intraepithelial lesions (LSIL), high-grade SIL (HSIL), or invasive cervical cancer[Solomon et al, 2002]. The correspondence between this system and other classification systems is shown in Table 2.1 below.

Table 2.1: Terminologies/classification of cervical precancerous/cancerous lesions [source: WHO, 2006]

Cytological classification (screening)		**Histological classification (diagnosis)**	
Pap	**Bethesda System**	**CIN**	**WHO**
Class I	Normal	Normal	Normal
Class II	ASC-US ASC-H	Atypia	Atypia
Class III	LSIL	CIN 1 including flat condyloma	Koilocytosis
Class III	HSIL	CIN2	Moderate dysplasia
Class III	HSIL	CIN3	Severe dysplasia
Class IV	HSIL	CIN3	Carcinoma in situ
Class V	Invasive carcinoma	Invasive carcinoma	Invasive carcinoma

2.1.3 Risk factors for cervical cancer

Other factors, in addition to persistent infection with oncogenic HPV, are associated with the development of cervical cancer. With varying magnitudes of association these include factors such as the lifetime number of sex partners (more than four), early onset of sexual activity (<16 years), high parity, history of genital warts, Chlamydia, or HSV infection, immunosuppression, smoking and exposure to environmental tobacco, oral contraceptive use (>5 years) [Waggoner, 2003; WHO, 2006].

These factors may influence the risk of cervical lesions indirectly with HPV infection as an intermediate. Factors such as early onset of sexual activity, lifetime number of partners, parity, history of STIs and oral contraceptive use may simply be indicative of a higher exposure to HPV. On the other hand factors such as immunosuppresion and oral contraceptive use may be indicative of a lower ability to clear HPV infections.

Other factors may have a more direct effect on the cell differentiation and maturation. Tobacco-specific carcinogens found in the epithelium of smokers can bind and damage host DNA and produce neoplastic transformations. In pooled analysis by the International Agency for Research on Cancer (IARC) cancer was twice as likely in women who ever smoked compared to those who did not [Bosch and Sanjose, 2007].

Epidemiological evidence for the association between **oral contraceptive use** and cervical cancer is not consistent. In IARC's analysis, though ever use of oral contraceptives was associated with an increased risk of cervical cancer OR=1.47 (95%CI: 1.02, 2.12), the use of oral contraceptives for less than 5 years was not associated with cervical cancer (OR=0.77, 95%CI: 0.46, 1.29). The risk however increased with increased use of oral contraceptives: OR=2.72 (95%CI: 1.36, 5.46) for 5-9 years of use, and OR= 4.48(95%CI: 2.24, 9.36) for 10+ years of use [Bosch and Sanjose, 2007]. Furthermore, the risk also seems to return to normal after 5-10 years of cessation of contraceptives. Multiple mechanisms have been postulated for the potential role of oral contraceptives in the onset of cancer. High hormonal levels may accelerate the progression from premalignant lesions to malignant cervical lesions by promoting integration of HPV DNA into the host genome, with deregulation of E6 and E7 expression [Castellsague and Munoz, JNCI 2003]. Estradiol may also stimulate the transcription of HPV16 E6 and E7 proteins.

Closely related to oral contraceptive use is the effect of **parity** which also involves higher levels of circulating estrogens. In IARC's studies HPV positive women with 7+ full term pregnancies were four times as likely to have cervical cancer as HPV positive nulliparous women and two times as likely to have cancer as women with 1-2 full term pregnancies [Munoz et al, Lancet 2002]. Parity may increase cancer risk by maintaining the squamocolumnar junction in the ectocervix and thus increasing exposure to HPV. The hormonal changes associated with pregnancy are associated with immunosuppression and this could possible reduce the ability to clear HPV infection.

The independent effect of **co-infections with other reproductive tract infections (RTIs)** is rather difficult to assess because of their colinearity with HPV infection and other RTIs. These RTIs may have an independent effect arising from the observation that, among HPV positive women, even non-specific inflammatory changes are associated with a modest increase in the risk of precancerous cervical lesions [Bosch and Sanjose, 2007]. Large IARC multicenter studies found that women with cancer were twice as likely to have antibodies to *Chlamydia trachomatis* as those with no cancer (OR=2.1, 95%CI: 1.1, 4.0) [Smith et al, JID 2002]. Similarly women with cancer were also twice as likely to have antibodies to HSV-2 (OR=2.2, 95%CI: 1.4, 3.4) [Smith et al, JNCI 2002].

Co-infection with HIV is also a risk factor; see below for a more detailed discussion of the role of HIV.

2.1.4 Tests for cervical cancer and role for cytology screening

Multiple methods can be employed for the diagnosis of cervical cancer and precancerous lesions. **Screening tests include tests aimed at identifying HPV DNA (PCR, DNA Hybridization), to those aimed at identifying precancerous/cancerous lesions: cytology (conventional or adapted such as liquid based), Visual methods (Visual Inspection with Acetic acid (VIA), Visual Inspection with Lugol's Iodine (VILI), magnified visual inspection with acetic acid (VIAM)**.

Conventional cytology ("Pap smear") has been the most used historically and involves collecting cervical samples, making a slide, staining and microscopy. Trained personnel are needed both for accurate sample collection, and cytology. There is also a need for infrastructure for sample collection and laboratory analysis. In review studies, the sensitivity and specificity of conventional cytology for the detection of CIN2-3 ranged from 47-62% and 60-95% respectively [Sankaranarayanan et al, 2005]. This accuracy was maintained in developing countries. **The low sensitivity of cytology leaves a potential for**

high false negative-rates. However this is compensated for by using repeated cytology so that cases missed in previous tests can be detected in subsequent tests. This low sensitivity (and the recognition of the etiologic role that HPV plays) has been the driving force behind the continuous search for alternative testing methods. Nevertheless conventional cytology remains the only method that has been proven to be effective in reducing mortality in developed countries with high quality screening with high coverage and reliable follow-up of women [Sankaranarayanan et al, 2005].

There is no epidemiologic evidence that conventional cytology is effective in reducing cancer morbidity or mortality in developing countries. Major barriers include the absence of trained personnel, lack of infrastructure and the need for regular follow-up, thus encouraging the search for alternative testing methods.

Liquid-based cytology is one of the methods that have been proposed to improve the sensitivity of conventional cytology at the level of sample collection storage. It involves using a liquid conservation medium to preserve the cells collected and allowing for a more accurate microscopy. Though sensitivity is increased, it is more expensive and requires more infrastructure/instruments to prepare smears, limiting its utility in resource-limited settings.

HPV testing may involve polymerase chain (PCR) amplification of HPV DNA and detection or hybridization methods through a second generation Hybrid Capture II (HCII) assay that includes RNA probes for high-risk HPV types. Overall the accuracy of these DNA detection methods depends on the primers and probes used. In a review of studies in developed countries, the sensitivity and specificity of HCII in detecting CIN 2-3 ranged respectively from 66-100% and 61-96% [Franco, JNCI 2003]. The sensitivity in developing countries has been lower ranging from 50-80% [Sankaranarayanan et al, 2005]. HPV testing has been proposed to be used as screening test on its own (so-called primary screening) or in conjunction with cytology (“triage”).

Visual screening tests, VIA, VILI, VIAM involve visualizing the cervix and using chemicals to differentiate neoplastic areas from normal areas. They can be combined with immediate care of lesions either by cauterization of excision. They thus have the advantage of offering the ability to do 'one-visit' screen and care. However they need trained personnel and can be very subjective. VIA is the most frequently used and its sensitivity and specificity range from 67-79% and 49-86% respectively, which is generally midway between cytology and HPV testing [Sankaranarayanan et al, 2005].

Innovative methods being developed for potential use in cancer detection include methods to automate conventional analysis, using markers to enhance cytology and or assays that detect markers specific to cancer pathogenesis. These markers include those that indicate cell aneuploidy, loss of heterozygosity, telomerase activity or DNA methylation [Nijhuis et al, 2006].

2.1.5 Management.

The primary prevention of cervical cancer lesions rests in the prevention of infection with HPV. This involves general methods to prevent STIs (reducing exposure by late onset of sexual activity, few partners, safer-sex practices) and a more specific HPV-targeted intervention: vaccination. Two HPV vaccines have been developed one targeting HPV 16 and 18, the other targeting HPV 6,11, 16 and 18. By preventing infection with HPV 16 and 18 both are expected to prevent the 70% of cancers that are attributed to these two types. However the existence of other high-risk HPV types and the fact that not all women would have been vaccinated prior to the onset of sexual activity justifies the continuous need for screening programs in addition to vaccinations programs.

Though vaccines have been shown to be efficacious in reducing the frequency of HPV infection, HPV persistence, low-grade and high-grade lesions in previously uninfected

women aged 15-25 years [Rambout et al, 2007] the extent of long-term prevention offered by these vaccines and the best administration regimen remain to be ascertained.

Secondary prevention is aimed at the early detection of precancerous lesions and their treatment. Various guidelines exist depending on the target population and the issuing agency. Screening involves the various tests discussed in section 2.1.4 in different combinations of onset of screening, frequency of screening and care provided with screening. Guidelines for cervical screening in the US are presented in Table 2.2 below.

WHO guidelines for screening targeted mainly for developing countries recommend at least one screen in the 4^{th} and 5^{th} decade or a screen every 3 years where possible [WHO, 2006]. Conventional cytology is considered the standard method, though this could be replaced with visual methods if possible.

Screening would result in the detection of precancerous lesions which need to be treated. Treatment modalities include outpatient treatments such as cryotherapy, loop electrosurgical excision procedure (LEEP), and cold knife conization.

Tertiary prevention involves the reduction of cancer mortality: treatment options are varied and include surgery (hysterectomy), radiology and/or chemotherapy.

Table 2.2: Summary of cervical cancer screening guidelines in the US

[Source CDC, From http://www.cdc.gov/std/hpv/ScreeningTables.pdf]

	American Cancer Society	**US Preventive Services Task Forces**	**American College of Obstetricians and Gynecologists**
	ACS, Nov 2002	**USPSTF, Jan 2003**	**ACOG, Aug 2003**
When to start	Approximately 3 years after onset of vaginal intercourse, but no later than age 21	Within 3 years of onset of sexual activity or age 21, whichever comes first	Approximately 3 years after onset of vaginal intercourse, but no later than age 21
Intervals			
Conventional Pap test	Annually; every 2-3 years for women aged 30+ with 3 negative cytology tests*	At least every 3 years	Annually; every 2-3 years for women aged 30+ with 3 negative cytology tests*
If liquid-based cytology	Every 2 years; every 2-3 years for women aged 30+ with 3 negative cytology tests*	Insufficient evidence	Annually; every 2-3 years for women aged 30+ with 3 negative cytology tests*
If HPV testing used	Every 3 years if HPV negative, cytology negative	Insufficient evidence	Every 3 years if HPV negative, cytology negative
When to stop	Women 70+ years with 3+ recent, consecutive negative tests and no abnormal tests in prior 10 years*	Women >65 years with negative tests and not otherwise at high risk for cervical cancer	Inconclusive evidence to establish upper age limit
Post-total hysterectomy	Discontinue if for benign reasons and no prior history of high-grade CIN*	Discontinue if for benign reasons	Discontinue if for benign reasons and no prior history of high-grade CIN*

*some exceptions apply (for example women who are immunocompromised, have a history of prenatal exposure to DES etc).

2.2 Cervical cancer and precancerous lesions in HIV

An estimated 33 million people were living with HIV by the end of 2007, about half of whom are women and about two-thirds of whom live in sub-Saharan Africa [UNAIDS, 2008]. The proportion of women with HIV is much higher in developing countries (where the heterosexual route is predominant) compared to most developed countries (with

a substantial role for homosexual and intravenous routes). In Cameroon, which has an estimated HIV prevalence of 5.5%, women constitute up to 300,000 of the 490,000 people 15+ years living with HIV. In the US, with a prevalence of 0.6%, only 230,000 of the 1,100,000 people 15+ living with HIV are women.

The early recognition of the frequency of cervical cancer in patients with advanced HIV disease led to its definition as an AIDS-defining condition. It is now recognized that HIV-positive women have: a higher prevalence of HPV and that the risk of infection increases with the extent of immunosuppression; a higher prevalence of persistent infection and infection with multiple HR-HPV types; a greater risk of precancerous lesions; a higher risk of developing cancer, with diagnosis up to 10 years earlier than in the general population and a faster progression to advanced disease with poor prognosis [WHO, 2006].

HIV positive women have a higher prevalence and incidence of cervical HPV infection [Palefsky, 2006]. A recent review of over 30 studies showed that the ratio of HPV prevalence in HIV positive to negative women ranged from 1 to 9.3, with most estimates being between 1 and 3.6 [De Vuyst et al, 2008]. In an analysis of baseline data from the HIV Epidemiology Research Study (HERS) cohort in the US, 64% of 851 HIV positive women had HPV infection compared to 28% of 434 HIV negative women [Cu-Uvin 1999]. In a longitudinal study of 284 women in the US (186 of whom were HIV positive) with semi-annual HPV testing, HPV positivity among HIV- women and HIV+ women with CD4+ > or =200 and <200 cells/uL was 47.5%, 78.7%, and 92.9% respectively [Ahdieh et al, 2000]. While about half of these infections may be attributed to the similar sexual transmission routes, the other half occurred in women who did not report any recent sexual exposure yet had incident HPV-infection, suggesting that there may be a reactivation of previously controlled HPV-infection in immunosuppressed HIV positive women [Strickler et al, 2005].

HIV positive women are less likely to clear HPV infection, ie have a higher HPV persistence. Compared with HIV negative women, the relative incidence of HPV clearance

was 0.29 and 0.10 among HIV+ women with CD4+ > or =200 and <200 cells/uL [Ahdieh et al, 2000]. In another study of 220 HIV positive and 231 HIV negative women in New York, HPV was persistent in 24% of HIV positives versus only 4% of HIV negatives [Sun et al, 1997].

HIV positive women are more likely to have infection with high-risk HPV. HIV-positive women were 1.8, 2.1, and 2.7 times more likely to have high-, intermediate-, and low-risk HPV infections, respectively, compared with HIV-negative women [Ahdieh et al, 2001]. In a recent meta-analysis of 3230 women with no cytological abnormalities any HPV prevalence was 36.3%, multiple HPV type prevalence was 11.9% while the most common HPV types were HPV-16, 58, 18, 52, 31 and 33 (respective prevalence of 4.5, 3.6, 3.1, 2.8 2.0 and 2.0%) [Clifford et al, 2006]. Curiously HIV positive women with HSIL were less likely to have HPV-16 compared to other women in the general population with HSIL (OR=0.6; 95%CI: 0.4, 0.7). Also in studies from Africa and North America, HPV type 58 was more prevalent than type 18. Furthermore HIV positive women with HSIL had a higher prevalence of low risk –HPV types 11, 53, and 61 suggesting that these supposedly **low-risk types may have a potential to cause neoplastic changes in immunodepressed women**. **It is also plausible that lower but undetectable levels of high risk types could be leading to HSIL.**

Both low grade and high grade precancerous lesions are more prevalent in HIV positive women. In a recent review of published studies the prevalence of LSIL in HIV positive women ranged from 9.7% to 19.0% and LSIL was 1.6 to 8.8 times as prevalent in HIV positive women as in HIV negative women [De Vuyst et al, 2008]. HSIL was also more prevalent in HIV positive women who had prevalence rates ranging from 2.3% to 17.6%, representing an occurrence 1.9 to 11.7 times as prevalent in HIV positive women as in HIV negative women. The highest prevalence of lesions was reported in Zambia recently: 76%

of HIV positive women had lesions with LSIL in 23%, HSIL in 32% and lesion suspicious of ICC in 20% of 150 HIV positive women [Parham et al, 2006].

HIV positive women have a higher incidence of precancerous lesions. Three cohort studies conducted in the US reported four-fold incidence of SIL in HIV positive women compared to HIV negative women [Six et al, 1998; Massad et al, 2001; Schuman et al, 2003]. In another study conducted in Senegal 71/627 (11%) of women developed HSIL after a median 2.2 years of follow-up[Hawes et al, 2006]. HIV-2 positive appeared less likely to develop HSIL compared to HIV-1 infected women (HR=0.3, 95%CI: 0.1, 0.9). HIV+ women with each of CD4 counts <200 high HIV viral load appeared more likely to develop HSIL (HR for CD4<200 versus >200=5.5, 95%CI: 2.0, 15.2; HR for each log increase in viral load=1.4, 95%CI: 1.1, 1.7). These factors were however not significantly associated with incident HSIL in multivariate adjustments.

HIV-positive women may have a higher progression and a lower regression of precancerous lesions. Few studies have assessed the long-term evolution of lesions in HIV positive women. In the US, Six et al [1998] and Massad et al [2001] both reported faster progression from LSIL to HSIL in HIV-positive women. In the former study, 38.1% of LSIL had progressed to HSIL over a year in HIV positive women while none had progressed in HIV negative women. All the progression occurred in women with CD4 counts less than 500/uL. In the latter study, the 6-months progression was 13.6 in HIV-positive versus 6.8% in HIV negative women. The regression rate was also slower in HIV positive women (43% versus 66%). These studies were however not sufficiently powered to assess the role of other factors such as CD4 count, HIV viral load on evolution.

The prevalence and incidence of invasive cervical cancer appear higher in HIV positive women. Though cervical cancer was the most frequent cancer observed in early HIV positive women, early comparative studies did not find an increased incidence/prevalence of invasive cancer in HIV positive women compared to the general

population. A few cohort studies such as the Women Interagency HIV study (WIHS) did not find a difference in cervical cancer incidence by HIV status. Initial analysis by IARC in 1996 did not find any relationship either [De Vuyst, 2008]. However the rarity of cervical cancer suggests the best evidence of association could only be gleaned from case-control studies or much larger population based cohort studies. The WIHS observed no case of cancer in HIV negatives and only one case of confirmed cervical cancer in HIV positives, thus having a very low power to detect any difference [Massad et al, 2004]. Furthermore, high HIV-associated mortality prior to HAART may have prevented the development and subsequent detection of cervical cancer in HIV positive women in cancer registries. In a larger population based study, the Sentinel Hospital Surveillance System for HIV infection, the prevalence of cancer was slightly higher in HIV positive women (10.4 versus 6.2 cases per 1000 women) [Chin et al, 1998]. A case-control study in South Africa found a relative risk for cervical cancer of 1.6 (95%CI 1.1, 2.3) [Sitas et al, 2000], while another study in Kenya found that among women aged 35 and less women with cervical cancer had a higher HIV prevalence (35% versus 17% in women without cancer) [Gichangi, 2002]. Multiple other studies have shown an increased risk of cervical cancer in HIV positive women [De Vuyst, 2008].

While HIV related immunosuppression could account for most of the aforementioned characteristics of cervical lesions in HIV, other mechanisms, such as HIV-HPV interactions, have been postulated to play a role. Multiple studies reported a higher prevalence of any HPV, HR-HPV types, persistent HPV, SIL and CD4 count less than 200/uL (or 500/ul in some studies) [De Vuyst et al, 2008]. In some studies, high HIV viral load was independently associated with increased incidence and progression of SIL [Massad et al, 2001] suggesting that there may be direct HIV-HPV viral interactions. The HIV tat gene may increase the expression of HPV E1 and L1 viral genes as well as HPV-16

E7 transcription. Furthermore, it is postulated that the inflammatory responses associated with HIV may interfere with the effectiveness of the anti-HPV immune response.

The effect of antiretroviral therapy on HPV and HPV-associated disease is not clearly understood. Highly active anti-retroviral therapy (HAART) does not appear to reduce HPV prevalence or incidence. In a French cohort the prevalence of HPV remained at 81% 5 months after HAART initiation [Heard et al, 2001]. In some studies, highly active anti-retroviral therapy (HAART) has been shown to reduce the progression to high-grade lesions and increase the regression of lesions to normal [Heard et al, 2004], however this has not been consistent. The effect of HAART appears to be best with higher CD4 levels [Palefsky, 2003].

2.3 Need to screen for cervical cancer in HIV in resource poor settings including Cameroon

Cervical cancer could potentially be a major cause of mortality in HIV-positive women if their life expectancy is increased with the use of antiretroviral treatment and they are never screened. In developed countries effective screening and early treatment of precancerous lesions have been key in preventing cervical cancer [Franceschi and Jaffe, 2007]. In the US 81% of women on antiretrovirals successfully receive annual Pap smears [Stein et al, 2001] and up to 94% of cervical cancers are detected at an early stage of carcinoma-in-situ [Frisch et al, 2000]. Furthermore the long interval between precancerous lesions and cancers allows for time to screen for cancer. In Italy 50% of HIV positive women diagnosed with cervical cancer in 1996-2004 had had their HIV test results at least 10 years prior to cancer diagnosis [Franceschi et al, 2006], sufficient time for the women to have been screened and received treatment for precancerous lesions.

Access to antiretrovirals has dramatically increased in developing countries. While the increased survival that is expected to accompany this increased access with an expected reduction of the burden certain opportunistic affections such as Kaposi sarcoma,

the effect on cervical is expected to be moderate at best, as HAART has a very limited effect, if any, on HPV persistence and the progression of lesions [Heard, 2004]. Even while on antiretrovirals women would still need to be screened or receive other preventive care for cervical cancer.

One of the reasons why women in resource-limited areas are not regularly screened is the limited access to health providers or services. Presumably this could be overcome in women on antiretrovirals who because of the need for follow-up of their HIV disease have a greater contact with health services. This regular contact could be taken advantage of to propose regular screening services. Once the women can be seen regularly in hospitals the next potential barrier would be the cost of screening and the chance that most tests would be negative. Screening could be made more cost-effective by targeting. Screening could be targeted based on clinical demographic and or behavioral characteristics. HPV DNA tests could also be used for screening, however the accuracy of HPV DNA testing in HIV positive women is still unclear and it is plausible that HPV types not included in the current tests and which are not considered high risk in the general population could well turn out to be oncogenic in HIV-positive women. Furthermore the current costs of HPV DNA testing remains a barrier for its widespread use in resource-limited settings. Other screening methods such as VIA and VILI also have limited assessment in HIV positive women.

Recently developed HPV vaccines could potentially be useful, however so far their efficacy has only been demonstrated in immunocompetent women. Screening with cervical cytology thus offers better prospects for effectiveness in reducing cervical morbidity and mortality in HIV positive women. Knowing the risk factors for cervical lesions could help in reducing the cost of screening resource-limited settings.

2.4 Age and cervical cancer

The occurrence of cervical cancer and precancerous appears to be age dependent and may reflect the interaction of multiple physiological and pathological factors.

Physiological changes in the size and anatomical position of the cervical squamocolumnar junction (SCJ) may play a role in the age at which lesions occur. Prior to puberty the SCJ is located at the external cervical os, at the intersection between the endocervix (covered by a single-layered and thus relatively fragile columnar epithelium) and the ectocervix (covered by a stratified squamous epithelium). At puberty, under the influence of higher estrogen levels, the uterus and cervix grow in size, a growth that results in the original SCJ being pushed externally such that a substantial proportion of columnar epithelium is exposed to the vaginal atmosphere and its consequent acidity. Under the influence of this acidity and increased estrogen levels, this area of columnar epithelium begins undergoing a process called squamous metaplasia (which is considered normal) with a gradual change of the columnar epithelium into a squamous epithelium. This occurs until women are in their thirties and both the original and a new SCJ become visible on colposcopy. The area between the new and the original SCJ is known as the transformation zone and is the site where most precancerous lesions are detected. As women age into menopause the influence of estrogen decreases, with a resultant decrease in the size of the cervix that leads to the transformation zone retracting to the endocervical canal. In the postmenopausal women both the new SCJ and the transformation zone have completely retracted into the cervical canal and only the original SCJ is visible on colposcopy [WHO, 2006].

It can be inferred from these physiological changes that squamous lesions would be very rare in prepubescent girls as there is no transformation zone. Lesions occur after

puberty and incidence could increase until reaching a peak in the premenopausal ages and then decrease after menopause with a reduction in the amount of transformation zone exposed to the ectocervix. Although the retraction of the transformation zone in the canal after menopause may imply a reduction in the occurrence of new lesions, it may also imply that, particularly in settings with little or no systematic screening, lesions that occurred prior to menopause may be retracted into the endocervical canal rendering detection by cytology smears more difficult and thus resulting in the detection of cancers at a relatively advanced stage in older women. It may also be inferred that, in countries with repeated regular screening, in the absence of any lesions in the early post-menopausal period, it will be unlikely to detect new lesions at older ages (as the transformation zone is less exposed).

The age at which lesions occur may also be related to the age-specific incidence, prevalence and persistence of HPV infection. Potentially reflecting higher exposure to HPV and increased susceptibility to HPV-infection, multiple studies have shown that the incidence of HPV peaks amongst adolescents and women aged less than 25 years then decreased in women in their third to fifth decades (35 to 55 years) only to have a second peak after age 55 [Baseman and Koutsky, 2005; Herrero et al, 2000]. The latter increase is thought to be reflective of the lesser ability to clear HPV infections, a reactivation of latent HPV infection or a cohort effect at older ages. A recent review of age-specific HPV prevalence, confirmed these trends to be consistent across geographical regions, with peak ages varying to reflect sexual activity at different ages [Smith et al, 2008].

The age-specific occurrence of cervical lesions appears to reflect that of HPV infection albeit with a delay. Numerous studies report peak prevalence of lesions in women under 40[Chang et al, 1991; Sadeghi et al, 1989; Gupta et al, 2008]. The median or mean age of occurrence however increases with severity of lesion [Chung et al, 1982; Gupta et al, 2008]. There is no evidence that the progression or regression rates of lesions are a function of age: Winn and Jones [2005], Giannopoulos et al [2005] and Wright et al.

[2005] found no age differences in regression or progression. However in one study of HSIL cases in India, lesions in older women (as well as women with high parity) were more likely to progress than those in younger women [Misra et al, 2006].

Unlike HPV-infection and precancerous lesions, the incidence of and the mortality due to cervical cancer increases with age as shown in figure 2.1 below. While the increase is gradual in countries with a well established screening system, it is very marked in less developed countries peaking after the fourth decade of life.

The relationship between age and the occurrence of precancerous or cancerous lesions led to the consideration of age as targeting factor in screening the general population of women. As described in Table 2.2 all the major screening guidelines consider the age at which to begin screening, the age at which to modify the interval between screening tests and the age to discontinue screening. Such is not the case for guidelines in HIV positive women at least not explicitly. Differences in the pathogenesis of cervical SIL and age specific mortality may imply different age considerations in HIV positive women. The age of occurrence of lesions in HIV-positive women, as well as the progression of lesions by age and the cost-effectiveness of age-targeted screening needs to be better described.

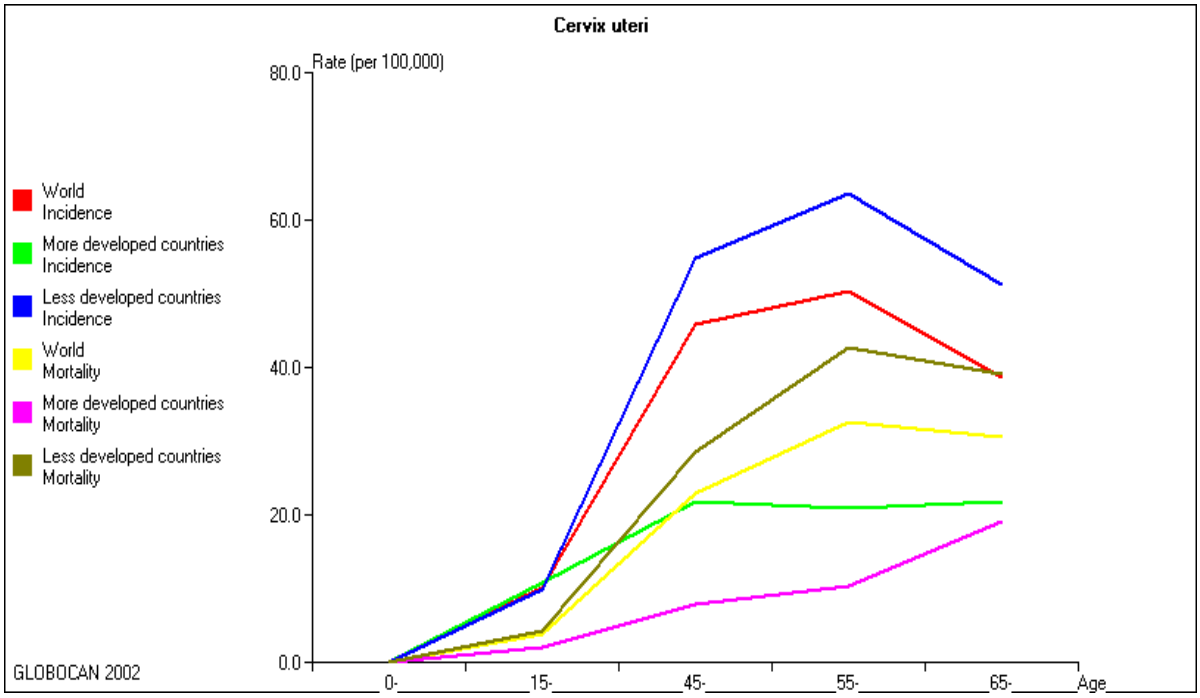


Figure 2.1: Age-specific incidence and mortality of cervical cancer (Source: Globocan 2002, [Ferlay et al, 2004])

2.5 Review of previous studies

2.5.1 Studies of the prevalence and predictors of cervical lesions in HIV-positive women developing countries

Though numerous studies have documented the epidemiology of cervical squamous intraepithelial lesions in HIV positive women in developed countries few do so for developing countries. Table 2.3 summarizes the main studies conducted in the latter. The prevalence of SIL was quite varied ranging from 1.2% [Mbakop et al, 1996] to 76% [Parham et al, 2006]. Because most studies were conducted to establish the association between HIV infection and the presence of SIL, very few assessed the risk factors specific to HIV positive women. Amongst these, immunosuppression (low CD4 count), high HIV viral load and or infection with high-risk HPV types tended to be associated with the presence of SIL. Only one prospective study in sub-Saharan Africa was identified in the literature and this study indicated a SIL incidence rate of 11% over a mean of 2.2 years of follow-up [Hawes et al, 2006].

Table 2.3: Summary epidemiology of HPV, cervical precancerous and cancerous lesions in HIV positive women in developing countries

Author(s), year, study population, site, subject	Aim and methods	Results
SUB-SAHARAN AFRICA		
Adam et al, 2008. South Africa. Predictors and progression of lesions	To assess predictors of lesions following LEEP in patients with HSIL or worse. Follow-up Pap smears were conducted in 575 of these patients.	The median time between LEEP and follow-up Pap was 122 days. 49% of patients still had abnormal lesions: 6.3 ASCUS, 47.6% LSIL, 1.8% ASC-H, 0.4% AGUS, 43.2% HSIL, 0.7 ICC. The odds of persistence in HIV positive women was 8 fold that in women self reporting as HIV-negative. Predictors of persistence in HIV positive women included the low CD4 count and the presence of disease at excision margins.
Mbu et al, 2008. Cameroon. Prevalence of lesions	Prevalence of lesions in 198 HIV positive pregnant women.	HIV positive women were more likely to have LSIL (18.2% vs 4.4%) and HSIL (12.1% vs 1.5%).
Yamada et al, 2008. Kenya. Prevalence of HPV and lesions	HPV prevalence and cervical lesions studied in 488 women visiting a clinic in Kenya. HPV diagnosed by PCR.	32% (155/488) of women were HIV positive, 23% of whom were on antiretrovirals. Cervical HPV was more prevalent in HIV positives (49% vs 17%). LSIL and HSIL were also more frequent in HIV positive: 21% vs 6.9% and 5.8% vs 0.6% respectively. Infection with HR-HPV and low CD4 counts were risk factors for cervical lesions
Ng'andwe et al 2007. Zambia. Prevalence of HPV	Cross-sectional study to describe HPV prevalence, genotype and risk factors in a cohort of patients in Lusaka, Zambia. Cross sectional study. HPV diagnosed by PCR	Overall HPV 16 and 18 each had a prevalence of 21.6% HIV positive patients were two times as likely to have HR-HPV as HIV-negatives. Meanwhile the prevalence of LR-HPV was similar in HIV positive and negatives.
Sahasrabuddhe et al, 2007. Zambia Prevalence of HPV and lesions	In a cross-sectional study 145 HIV positive women were screened using LBC and HPV genotyped.	HR-HPV were more frequent in women with CD4<200 (OR: 4.9, 95%CI: 1.4, 16.7) and in women with HSIL or squamous cell carcinoma (OR: 8.0, 95%CI: 1.7, 37.4). In women with HSIL or squamous cell carcinoma HPV HR type proportion was as follows: HPV-52=37.2%, 58=24.1%, 53=20.7%, 16=17.2%, 18=13.1%. High prevalence of non-16/18 HR types suggest probable inadequacy of HPV 16/18 vaccines.
Gaym et al, 2007. South Africa. Prevalence of lesions.	Cross-sectional study to assess the association between HIV and cervical dysplasia and a cross sectional study was	24.5% of patients were HIV positive. The overall prevalence of ASCUS=6.4%, LSIL=9.2%, HSIL=1.3%. Among the 114 HIV positive patients the prevalence of

	conducted in 466 women at a primary health care clinic in KwaZulu-Natal.	ASCUS=10.5%, LSIL=21.0%, HSIL=4.4%.
Parham et al, 2006. Zambia. Prevalence of lesions.	Cross-sectional study to evaluate the prevalence and predictors of SIL in 150 HIV-infected women in Zambia (age 23-49). Screening was done using LBC and HPV typing by Roche linear array PCR	CD4 ranged from 7 – 942 (median 165)/uL. 76% had SIL: of which LSIL=23.3%, HSIL=32.6% and 20% had lesions suspicious of SCC. 85.3% had HR-HPV. Overall very high prevalence of SIL and HR-HPV. The highest in any study population. The HR-HPV type independently predicted the presence of HSIL/SCC (adjusted OR: 12.4, 95%CI: 2.62, 58.1).
Didelot-Rousseae et al, 2006. Burkina Faso. Prevaence of HPV and lesions	Cross-sectional study of HPV and cervical SIL in 379 high-risk women.	HIV-1 seroprevalence=36.0%. Overall HPV prevalence=66.1% of 360 validly tested. HPV prevalence was higher in HIV (87% vs 54%; PR=1.61, 95%CI: 1.4, 1.8). Similarly HR-HPV types and multiple HPV infections were more frequent in HIV positive women. Prevalence of SIL in 126 HIV+ women= 48.4%; LSIL=38%, HSIL= 10%
Hawes et al, 2006. Senegal. Incidence of lesions	627 women were assessed for the persistence of HPV and incidence of HSIL over a mean follow-up time of 2.2 years.	71/627 (11%) of women developed HSIL. HIV-2 positive appeared less likely to develop HSIL compared to HIV-1 infected (HR=0.3, 95%CI: 0.1, 0.9). HIV+ women with each of CD4 counts <200 high HIV viral load appeared more likely to develop HSIL (HR for CD4<200 vs>200=5.5, 95%CI: 2.0, 15.2; HR for each log increase in viral load=1.4, 95%CI: 1.1, 1.7). These factors were however not significantly associated with incident HSIL in multivariate adjustments.
Moodley and Garib, 2004. South Africa. Prevalence of lesions	To determine the prevalence of SIL in 160 HPV positive women in South Africa.	HIV prevalence=41.9%; SIL prevalence=36.9%. Biopsy confirmation of SIL in HIV positive=49.3% vs 28% in HIV negative. But no difference by grade. Authors suggest similar management of HPV positive women whether they are HIV-positive or not.
Hawes et al, 2003. Senegal. Prevalence of HPV and lesions	Cross-sectional study of prevalence and predictors of HSIL/ICC in 4114 outpatient women in Senegal.	HIV prevalence =10.5%, 433 women. HIV-1 only=8.1%, HIV-2=1.7%, HIV1 and 2= 0.7%. Prevalence of HR-HPV in HIV-1 only (N=330), -2only (N=68), -1 and -2 (N=28) was respectively 53%, 41%, and 60%. In all 3 groups about 1/3rd did not have HPV.

		Prevalence of LSIL or worse was 17.2%, 19.5% and 34.5% respectively (vs 4% in HIV negative). Prevalence of HSIL or worse was 4.5%, 10.5% and 13.8% respectively (vs 1.4% in HIV negative). The association between HIV and HSIL/ICC was restricted only to women with HR-HPV infection. Among HIV positive women factors associated with HSIL/ICC and HR-HPV included high HIV viral load and low CD4 count
Chirenje et al, 2002. Zimbabwe. Prevalence of lesions	Cross-sectional study in 554 women in Harare.	HIV prevalence = 36.8%. Prevalence of lesions in HIV+ = 25.6% (vs 6.7% in HIV negative): with ASCUS=12.6%, LSIL=9.7%, HSIL=3.4%.
Mayaud et al, 2001. Tanzania. Prevalence of lesions	To determine the prevalence and interrelation of HPV genotypes, SIL and other reproductive tract infections in 660 urban ANC attendees in Mwanza.	HIV prevalence was 15%. HPV prevalence was 34%. 86% of HPV-typable samples had HR-HPV. No association between HIV and HR-HPV (OR=1.02, 95%CI: 0.6, 1.6). Higher prevalence of HPV in HIV women at older age. Prevalence of SIL overall was 7% with HSIL in 3%. Prevalence of SIL in HIV positive= 9/90 = 10%.
Kapiga et al, 1999. Tanzania Prevalence of lesions	To determine the prevalence and risk factors for SIL in 691 HIV positive women in Dar es Salam (1996-1997).	Prevalence of SIL = 2.9% (20/686). LSIL=1.6%, HSIL=1.3%. Presence of SIL was associated with CD4<200 (adjusted OR=6.15, 95%CI: 1.19, 41.37) and a decrease in mid-upper arm circumference (adjusted OR=0.32, 95%CI: 0.10, 0.93 per 5cm increase in circumference). The number of lifetime sexual partners and parity were marginally and non-significantly associated with SIL.
Womack et al, 2000. Zimbabwe Prevalence of HPV and lesions	To determine the utility of the HCII test in primary screening of 466 women at risk of HIV in Zimbabwe	HIV positive proportion=53.5% Prevalence of HPV in HIV = 64.3% (vs 27.6% in HIV negative). Prevalence of HSIL in HIV= 17.3% (vs 5.9%) Strong association between HSIL and HPV in both HIV-negative and positive. Sensitivity and specificity of HCII for detecting HSIL in HIV = 90.7% and 41.3% (vs 61.5% and 74.5%). Utility of HCII test depends on prevalence and availability of resources.
Leroy et al, 1999.	To assess the prevalence of SIL and their	Prevalence of SIL in HIV =24.3% (vs 6.5%)

Rwanda Prevalence of lesions	association with HIV in pregnant women in Kigali. (1992-1993). 103 were HIV positive while 107 were HIV negative.	Women with SIL tended to have a lower CD4 count.
Temmerman et al, 1999. Kenya Prevalence of HPV and lesions	To identify risk factors for HPV and SIL and determine the role of HIV in 513 family planning clinic attendees in Nairobi.	An analysis stratified by HIV-1 showed a stronger association between HPV and HSIL in HIV-1 negative women (OR: 17.0, 95%CI: 6.4, 46.3) than in HIV-1 positive women (OR:4.5, 95%CI: 0.8, 27.4).
La Ruche et al, 1998. Cote d'Ivoire Prevalence of lesions	To assess the prevalence and factors associated with SIL and ICC in 2170 women in Abidjan.	HIV prevalence= 21.7%. In HIV-1 positive women, the prevalence of LSIL increased slightly with the clinical stage of HIV-related disease, from 14.4% in Stage I to 19.8% in Stage III, without reaching statistical significance (very few women (5) were in stage IV). HSIL prevalence increased from 10.3 to 13.8%. Prevalence of LSIL increased markedly and significantly with a decrease in CD4 cell count, from 14.1% in women with CD4 count >500/mL to 41.9% in women with CD4 count<200/mL. The prevalence of HSIL also increased with a decrease in CD4 count.
Langley et al, 1996. Senegal. Prevalence of HPV and Lesions	To determine the effect of HIV 1 and 2 on the prevalence of HPV and SIL a cross sectional study was conducted in 759 female commercial sex workers in Dakar	68 women had HIV-1 only, 58 HIV-2 only, 14 HIV- and -2. Among with HIV, women with HPV had lower CD4/CD8 ratio.
Motti et al, 1996. Malawi Prevalence of HPV and lesions.	To assess cervical abnormalities, HPV and HIV infection in women in Malawi.	Of 132 HIV positive women SIL prevalence=15%. 60% of HIV women with CD4<300 had HPV DNA.
Mbakop et al, 1996. Cameroon. Prevalence of lesions	To present the cytological aspects of smears in HIV positive versus HIV negative women in Cameroon.	Only 1 of 65 (1.5% HIV positive women had SIL and it was LSIL. Unexpectedly lower than 4% of 50 HIV negative who had SIL. But note small sample sizes.
OTHER DEVELOPING COUNTRIES		
Mangclaviraj et al, 2008. Thailand. Predictors of lesions	Cross sectional study of 385 HIV positive women in Bangkok. Pap smears were done in these women.	Prevalence of LSIL=11.2%. HSIL=4.7% and invasive cell carcinoma=0.5%. Only nadir CD4 count and income were significantly associated with the presence of cervical anomalies (women with nadir CD4<200 and income <$125 each

		had twice the odds of having anomalies)
Gheit et al, 2006. Brazil. HPV type	A comparison of HPV-16 variants in 19 HIV positive women to 22 HIV negative women.	Non-European variants of HPV-16 were approximately 3 times as frequent in HIV positive women (36.8% vs 13.6%) as in HIV negative women. Association of European variants with HSIL in HIV negatives but no association of non-European variants with HSIL in both populations. Very small numbers though.
Chalermchockcharoenkit et al, 2006. Thailand. Prevalence lesions	To assess the prevalence of lesions iat post-partum visit of 636 women in a PMTCT program n Siriraj(1996-2004).	Prevalence of SIL=13.3%, 90% of these were LSIL. Women with CD4<200 had a higher prevalence of lesions (21.2% vs 12.2%).
Sirivongrangson et al, 2007. Thailand. Prevalent lesions and prevalent HPV	Cross-sectional study (2003-2004) with Pap smear and HC2 testing for HR-HPV in 210 HIV-infected women	Prevalence of HR-HPV=38.6%. Prevalence of abnormal cytology=20.4% Estimated prevalence of cervical cancer was 1.9% (4 of 210) however this was based only on 23 women having a pathology result.
Levi et al, 2004. Brazil. Prevalence of HPV	To determine the prevalence of HPV genotypes in cervical samples from 255 HIV infected women and compare to a control of 36 HIV negative women.	Prevalence of abnormal lesions in HIV+=18% HPV-DNA prevalence = 87% Multiple HPV types in 45%. All HIV negative had HPV but only 3/36 had multiple types. Thus an increased rate of multiple HPV infection in HIV. But no association between the presence of multiple HPV and SIL. The authors however do not distinguish between multiple infection with HR-HPV and multiple infection with any HPV.
Goncalves et al, 2003. Brazil. Predictors of lesions	Cross-sectional study of 141 HIV-positive women. Outcome was anogenital lesions (not just cervical lesions)	Not clear how many had abnormal lesions. However biopsy conducted in 35, 10 had low grade lesions while 8 had high grade lesions. Both undetermined HPV and HR-HPV type were associated with the presence of lesions
Volkow et al, 2001. Mexico. Prevalence of HPV	To determine the prevalence of HPV and SIL in 85 HIV positive in Mexico.	Prevalence of HPV 69%, of HR-HPV = 33%. Prevalence of LSIL=17.8%, HSIL=8.2% No association with CD4 counts and antiretroviral therapy.
Goncalves et al, 1999. Brazil. Prevalence of HPV	To characterize HPV prevalence and types in 141 HIV positive women	Prevalence of HPV=80.8% Prevalence of multiple (2+) HPV types = 45%. Prevalence of HPV 16, 18 = 30.5%; HPV 61, 53 = 24.4%, unidentified types = 18.7%.

2.5.2 Studies of the relationship between age and cervical lesions

While the age of onset and occurrence of precancerous and cancerous lesions in HIV women is not well described, the literature is crammed with studies exploring these characteristics in the general population. These studies are summarized in Table 2.4. As soon as the 1980's, studies were published documenting the prevalence of lesions in young women. Subsequent studies showed that the peak of occurrence of precancerous lesions was in the third to fourth decade while malignant lesions tended to occur later in the fourth or fifth decade. Lesions also appeared to be less frequent in women far past menopause. The implementation of successful frequent screening resulted in a decrease of the mean ages at which patients have dysplasias, though this may simply reflect detection bias. Most studies also noted similar progression rates irrespective of age. More recent studies have confirmed the higher prevalence of HPV in younger sexually active women.

Table 2.4: Summary of studies of that addressed the impact of age on the prevalence of lesions, the progression of lesions, the prevalence of HPV or diagnostic work-up.

Author(s), year, study population, site, subject	Aim and methods	Results
PREVALENCE OF LESIONS		
Gupta et al, 2008. India. Prevalence of lesions	To identify target age groups were screening efforts could be concentrated a retrospective analysis of hospital-based cytology screening data from 29 475 women in India (2001-2004) was conducted.	The prevalence of abnormal lesions was 5.6%. the highest incidence of SIL was observed in the age group 30-39, while that of malignancies was in the age group >60. the mean ages for LSIL, HSIL and cancer were 34.7, 37.7 and 51.8 respectively. Screening in both the fourth and fifth decades of life could detect 2/3rd of SIL.
Tanaka et al, 2001. Japan. Prevalence of lesions.	To assess the prevalence of premalignant or malignant lesions in patients HPV 16 positive. 207 women referred for colposcopy during a 20-month period between 10/1994 – 05/1996.	Amongst HPV 16 positive women, premalignant or malignant cervical diseases were approximately 8 times as frequent in women 44 years or younger (n=111) as in women 45 year or more. HPV testing thus recommended in younger women.
Sujathan et al, 1995. India. Prevalence of lesions	Assess the prevalence of cervical cytology abnormalities and risk factors at early stages of a screening program in South India.	Only low-grade lesions were found in women aged less than 40 years.
Vishnevskii et al, 1994. Russia. Prevalence of lesions	Prevalence of HPV associated lesions	Females of reproductive age revealed a significantly higher frequency of HPV-associated dysplasia and preinvasive carcinoma (4.2%) than in pre- and postmenopausal women (2.8%).
Carson and DeMay, 1993. USA. Prevalence of lesions	To describe the age distribution of 1947 cases with dysplasia or carcinoma at a pathology unit in the US.	Only the age of women with carcinoma was normally distributed. The ages of women with dysplasia are not distributed normally, but are asymmetrically skewed to younger women; authors suggest that the mode better describes the central tendency for ages of women with dysplasia.
Das et al, 1992. India. Prevalence of lesions	To assess the efficacy of hospital based cytology in six hospitals in India. From 1976 to 1986 117471 women were screened with cytology	Dysplasia was present in 1.6% of patients while 0.2% had malignant lesions (confirmed by histology in 90.1% of these). The median age of detection of mild/moderate, severe dysplasia, carcinoma in situ and invasive cancers was 34, 37.9, 38.6 and 47.8 years respectively. Estimated 15 years between onset of precursor lesions

		and occurrence of invasive disease.
Chang, 1991. New Zealand. Prevalence of lesions.	Analysis of histology samples from 1371 women on colposcopy in New Zealand. 1982-1988	Women <29 years accounted for 58.3% of CIN3 lesions.
Mitchell and Medley, 1990. Australia Prevalence of lesions	Assessed the trends in prevalence of neoplasia un a Victorian Cytology service between 1970 and 1988.	The age group having the highest prevalence of “definite” CIN decreased from 40-49 in the years 1970-1973 to 25-29 in the years 1982-1988.
Kaminski et al, 1989. USA. Prevalence of lesions	To evaluate the influence of age on colposcopy following the detection of squamous dysplasia on cytology in 1074 women	Abnormal biopsy findings were detected in 19.6% of 787 women aged <41 had versus only 6.3% of 287 women aged >40
The New Zealand Contraception and Health Study group, 1989. Prevalence of lesions	Study of cytology smears from 9430 women seen between 1980 and 1986 in New Zealand	4.3% of women had abnormal lesions. The prevalence of dysplasia decreased slightly with increasing age (over ages 20-39), but the prevalence of carcinoma in situ/invasive cancer increased from 0.18% at ages 20-24 to 0.74% at ages 35-39.
Sadeghi et al, 1989. USA. Prevalence of lesions.	Study of prevalence and risk factors for cervical neoplasia in a population of 1,672,847 women screened over a 3-year period in the US.	Mild-to-moderate dysplasias were most frequent between ages 25-29, while severe dysplasia and carcinoma in situ were most frequent between ages 35 to 39.
Wheat et al, 1988. USA. Prevalence of lesions	To assess the need to screen women aged 65+. Pap smear results of women in two San Francisco hospitals were reviewed.	Only 5 of 140 (3.5%) women aged 65+ had class II atypia. None had dysplasia or carcinoma in situ. Thus suggesting screening in this age group may not be indispensable particularly in women who have had multiple previous screens.
Benmoura et al, 1986. France. Prevalence of lesions	To describe the epidemiology of cervical lesions in a sample of 870 adolescents (<21 years) screened by cytology.	11% had mild or moderate dysplasias. With such a high prevalence the authors recommend early screening.
Chung et al, 1982. USA. Prevalence of lesions	To describe the prevalence and incidence rates of cervical dysplasia and invasive carcinoma in 58,053 patients in Maryland Medical Center.	The mean age for mild to moderate dysplasia was 25.7 years, for moderate to severe dysplasia, 29.29 years and for Carcinoma in situ, 33.25 years. Lesions were present at young age.
Macgregor and Teper, 1978. USA. Prevalence of lesions	9000 women aged <=20 years in 1967-1976 period.	1.6% (145) of women had abnormal smears. On follow-up for up-to 10 years, 50% of abnormalities regressed to normal while 19/145 had smears suggestive of malignancy. No carcinoma-in –situ or invasive carcinoma

		was found in women <21.
PROGRESSION OF LESIONS		
Misra et al, 2006. India. Progression	To assess factors associated with the progression of lesions	571 HSIL cases out of 33,658 smears over 35 years. High age and parity each played significant role in progression of SIL
Winn and Jones 2005. UK. Regression	To identify the proportion of 1484 women (between 1996 -1998) with first abnormal smear who returned to normal in later cytology. And also to see if this was influenced by age or HPV status.	50.9% of women returned to normal without colposcopy. This was not influenced by age (<36 vs >35) or HPV status.
Giannopoulos et al, 2005 UK. Progression	To estimate the incidence of CINII and III in 510 women with mildy dyskaryotic smears and verify if this was a function of age.	Overall incidence of CINII and CINIII in women with mild dyskaryosis was 28.7% and was similar in all 3 age groups (<20, 20-25 and >25 years). Age would thus not be a suggested criterion for targeting referral. Also 5-yearly screen may result in young women carrying CIN for 5 years before they are first screened.
Wright et al, 2005. USA. Regression, Progression and Persistence	Adolescents (<=18 years) seen between 1997-2003 with a diagnosis of LSIL or HSIL.	477 LSIL and 55 HSIL cases had follow-up results. At follow-up of girls with LSIL 47% were normal, 47% had ASCUS/LSIL/CINI while 18% had HSIL/CINII/III. After 36 months 62% had regressed while 21.8% progressed. At follow-up of girls with HSIL, 21.8% were negative, 27% had ASCUS/LSIL/CINI while 50.9% had HSIL/CINII/III. After 36 months 31% had progressed to CINIII. Progression rates in adolescents similar to that in adults.
Knudsen et al, 2003. Denmark. Progression	To assess compliance to screening, progression, treatment and follow-up after treatment in a 993 women in a hospital database, between 1990-1991.	Age was not significantly associated with progression of lesions.
Konno et al, 1998. Japan. Progression	To assess the significance of HPV infection, grade of CIN and age on CIN progression. Study of follow-up data from 194 patients.	Age did not impact progression rate (while HPV status and CIN grade were independent predictors of progression).
HPV PREVALENCE		
Gonzalez-Bosquet et al 2006. Spain. HPV prevalence	To assess the prevalence of HPV in 215 women with abnormal cytology results.	Women aged ≤ 35 years had a higher HPV prevalence (85.6%) compared to women over 35 years (54%)
De Villiers et al, 1992. Germany.	To determine the prevalence of HPV 6, 11, 16, and 18 in a population of 11,667 women	8.8% of women with normal cytology were positive for HPV. HPV prevalence was much lower in women aged

HPV prevalence.	without abnormalities attending 3 clinics in Germany.	>=55 (98/3062 = 3.2%) than in women <55 (852/7716 = 11%).
Tideman et al, 2003. Australia. HPV prevalence	Cross-sectional study to assess risk factors and prevalence of HPV and Pap smear abnormalities in 288 commercial sex workers and 266 controls	HPV prevalence of 31.6% in CSWs and 24.4% in controls. Age less than 36 was associated with higher HPV prevalence.
Hankins et al, 1999. Canada. HPV prevalence in HIV	To assess risk factors for HPV infection in a group of 375 HIV positive women.	HPV was prevalent in 67.2% of women; intermediate or HR-HPV in 49.1%. HPV infection was associated with CD4 count less than 200/uL, non-white race, inconsistent condom use in the 6 months preceding study and lower age (<30 years), with women aged 30-39 years having an adjusted OR 0.51 [95% Cl 0.30-0.87] and women aged 40 years or older having an adjusted OR 0.52 [95% Cl 0.26-1.01] compared to women aged <30 years.
Gjooen et al, 1996. Norway. HPV prevalence	To assess HPV prevalence in 231 women with no lesions ad 103 women with histologically confirmed CINII-III.	In both groups HPV prevalence was higher in patients aged less than 30 years.
DIAGNOSIS		
Crowther et al 2008. Ireland. Colposcopy	To assess the ratio of HSIL to LSIL by age strata. To determine if colposcopy recommendations following the detection of cytology could be a function of age.	34,180 Pap smears conducted between 07/2004 and 06/2005, 2326 had abnormalities, 67% low grade and 33% high grade. The ratio of low grade to high grade remained 2:1 across age strata suggesting that age targeted colposcopy may not be useful.
Cibas et al, 2005. USA Cytology	To assess the role of 'PM' cells ie cells with band nuclear enlargement, smooth nuclear membranes and fine chromatin (common in perimenopausal women) in the interpretation of cytology as ASCUS. Cytology results from 100 women aged 40-55 with ASCUS as result were re-assessed	15% of 'ASCUS' actually had PM cells and should have been classified as negative. PM cells were identified as a significant cause of ASCUS overdiagnosis in women 40 to 55 years old.
Melnikow et al, 1997. USA. Cytology	To estimate age-specific positive predictive values and likelihood ratios for the diagnosis of High grade lesions using Pap smears.	Women under age 25 were less likely to have high-grade biopsies (positive predictive value, 7.3%; likelihood ratio 0.7). Repeat Pap smears for ASCUS and LSIL showing only HPV in women under age 30 would have reduced the immediate colposcopy rate by 60% and delayed diagnosis of high-grade lesions by 23%. Age could be factor in determining extent of follow-up care.

2.5.3 Cost effectiveness studies: in HIV positive women, targeting age, and in developing versus developed countries.

Practical and ethical difficulties limit the use of randomized studies to identify optimal screening strategies. In addition to the very long observation period that would be needed to implement a clinical trial with cancer mortality as outcome, a multitude of other parameters (such as age of onset, type of screening test used, screening frequency, age of screening discontinuation) would need to be randomized for such an endeavor. Cost-effectiveness analysis (CEA) and other simulation methods thus have a prominent role in directing cancer prevention policy. CEA have been used with varying purposes over the past decade, reflecting the changes in tests available and then the availability of HPV vaccines. Initial CEA focused on the impact of improving the sensitivity of conventional Pap smears with methods such as repeat testing or liquid based cytology. Later CEA assessed the impact of HPV DNA testing either as a primary test, alone or in conjunction with conventional cytology. Most recent CEA have sought to assess the need for screening in populations exposed to HPV vaccinations.

A summary review of each of the published CEA is shown in Table 2.5 (below). The exact costs and cost-savings were quite varied reflecting the diversity in the target study populations (HIV positive, populations in less-developed countries and populations in developed countries), and the strategies assessed. We summarize the major trends that could be noted below.

Very few CEA focused on screening in HIV-positive women [Goldie et al 1999; and Goldie et al 2001]. In comparing six strategies for screening in HIV-positive women in the US (no screening, annual Pap smears, annual Pap smears after two negative 6-monthly smears, semi-annual Pap smears, annual colposcopy and semi-annual colposcopy), Goldie et al[1999] recommended annual smears after two negative semi-annual screens as the most cost-effective strategy. This strategy (compared to annual screening) cost $14,800 per

QALY saved and is the currently recommended strategy for screening in HIV positive women. A later analysis to assess the impact of adding HPV-testing in HIV positive women in the US showed that compared to no screening, a targeted screen (with cytology every 6 months in high-risk HPV positive women and annual cytology in HR-HPV-negative women) cost between $10,000-14,000 per QALY gained [Goldie et al, 2001]. We could not find any CEA of screening in HIV positive women in less-developed settings, care in these women being extrapolated from studies in developed countries.

Age has been a frequent consideration of CEA (albeit in HIV negative populations). In developed countries with existing effective screening programs, screening women as from age 21-25[Goldie et al, Vaccine 2006b] followed with frequent screening was found CE as well as was reducing the screen frequency to 2-yearly or 3-yearly once the age of 30 was reached. Screening women aged 65 or more who had consistently had previous negative screens was not CE while screening those who had not previously had screen was CE. In less developed settings one-time lifetime screening targeting women aged 35 or more, or two- or three-times in the lifetime screening of women between 30-50 years with 5-yearly screening tended to be CE.

Other trends in CEA of screening in developed settings include that: CE reduces as screening becomes more frequent than every 2-3 years; increasing the sensitivity of pap smear/screening tests would be CE if screening interval was increased to 3-4 years; strategies that use HPV testing and reduce screening frequency can be CE [Goldie et al 2006]. Adding HPV vaccination to current screening procedures was still CE [Kulasingam et al, 2007; Szucs et al, 2008], however the screening frequency may need to be reduced [Myers et al, 2008].

In less developed settings CE was influenced by the choice of test: HPV testing (effective), visual screening methods (inexpensive) or cytology (sensitive); and the need to have fewer visits that offer screen and treat. Increasing sensitivity could be more CE when

there was infrequent testing, while specificity only had an impact when there was frequent screening [Goldie et al 2006]. The combination of vaccination and screening three-times in the lifetime appeared CE [Diaz et al, 2008].

Table 2.5: Summary of cost-effectiveness studies of the cervical cancer screening.

Author(s), year, study population, site	Aim and methods	Results
HIV POSITIVE		
Goldie et al, 2001 HIV positive women, USA.	To assess the cost effectiveness of adding high-risk HPV-testing in screening for cervical cancer in HIV positive women. Used a Markov model to assess two strategies: 1)targeted screening in which HPV testing is added to the two initial cytology results to determine subsequent follow-up. 2)Universal screening involving no HPV testing.	Compared to no screening a targeted screen (with cytology every 6 months in high-risk HPV positive women and annual cytology in HR-HPV-negative women) cost between $10,000-14,000 per QALY gained. Annual cytology regardless of HPV results was 15% less effective than targeted screening. A screening strategy based on initial HPV results to determine whether subsequent cytology will be annual or every 6 months appeared to be cost-effective.
Goldie et al, 1999. HIV positive women. USA	To assess the cost-effectiveness of various screening strategies, used a Markov model to simulate a clinical practice in the US. Assessed six strategies: no screening, annual Pap smears, annual Pap smears after two negative 6-monthly smears, semi-annual Pap smears, annual colposcopy and semi-annual colposcopy	Compared to no screen, annual Pap smear gained 2.1 months in QALY's, at a of cost $12,800 per QALY saved. Annual Pap smears after 2 negative semi-annual smears cost 14,800 per QALY saved (with an average gain of 0.04 QALY) compared to annual screen. Semi-annual smears cost $ 27,600 per QALY saved (with an average gain of 0.17 QALYs) compared to annual screening after 2 negative semi-annual screen. Colposcopy-based strategies were much more costly. This study recommended annual smears after two negative semi-annual smears as the most cost effective and as having a cost similar to other clinical preventive interventions such as mammogram etc.
HIV NEGATIVE		
Diaz et al, 2008 General population, India	To assess the potential impact of HPV-16/18 vaccination and cervical screening in India (where 25% of worldwide cases of cervical cancer occur). Strategies assessed: vaccination of girls before age 12, screening of women aged >30, combined vaccination and screening. Screening strategies were differed by test (cytology, visual inspection, HPV DNA testing), number of clinic	With a 70% coverage vaccine alone resulted in a 44% (range 28-57%) reduction in lifetime risk of cancer while screening by conventional cytology 3x per lifetime resulted in 21-33% reduction in lifetime risk; a combination of vaccination plus 3-visit cytology resulted in 56% reduction and a combination of vaccination plus 2-visit HPV DNA testing resulted in 63% reduction in risk. A combination of vaccination (cost per vaccinated girl of $10) and screening 3x in the lifetime was considered

	visits (1, 2 or 3), frequency (1 x , 2 x , 3 x per lifetime), and age range (35-45).	cost-effective as costing less than the countries per capita GDP.
Szucs et al, 2008 General population Switzerland	To assess the cost-effectiveness of adding a quadrivalent HPV vaccine to the cervical screening program in Switzerland. Used a Markov model of a simulated cohort of 41,200 girls aged 11 years for their lifetime. Compared conventional cytology only and HPV vaccination plus conventional cytology.	Assuming an 80% coverage rate and lifetime protection from vaccine, adding vaccine to screening could prevent 62% of cervical cancer and related deaths. The incremental cost-effectiveness ratio was CHF 26,005 per QALY gained. The ICER was sensitive to the need for boosters and discount rates. Authors conclude that adding quadrivalent HPV is likely to be cost-effectiveness in Switzerland.
Kulasingam et al, 2007 General population Australia	To assess the cost-effectiveness of adding a HPV vaccine to the Australian National Cervical Cancer Screening Program. A Markov model of a cohort of girls. Strategies compared: vaccination at age 12, assuming 80% coverage and lifetime efficacy in conjunction with screening versus screening only	Compared to screening only, vaccination (with cost per dose of $115) plus screening had an ICER of $18 735 per QALY. Accounting for herd immunity this ICER reduced to $13 316 per QALY. Vaccinating both boys and girls resulted in an ICER of $33 644. A vaccination with catch-up dose for 14-year-olds had an ICER of $16 727 per QALY. A vaccination with catch-up dose for 26-year-olds had an ICER of $34 536 per QALY. Adding vaccination to current screening was thus cost-effective compared to screening only.
Kulasingam et al, 2006 Women with prior normal tests, USA	To assess the cost-effectiveness of screening women with 3 or more prior normal test compared to screening those with no prior tests. Cost-effectiveness model and data from the CDC National Breast and Cervical Cancer Early Detection Program.	As the number of prior normal tests increased, the cost per life year saved increased substantially. For example for women aged 30-44 years; with no prior test the ICER for screening annually and triennially were respectively $331,837 and $20,533 per life year saved (compared to no screening). Meanwhile in same aged women with 3 prior normal tests the costs for annual and triennial screening were respectively $709,067 and $60,028 per life year saved.
Goldie et al, 2005 India, Kenya, South Africa, Peru, Thailand.	To assess the cost-effectiveness of screening strategies in developing countries (in which conventional cytology is judged to be impractical). Strategies assessed included single-visit, two-visit and three-visit strategies.	VIA or HPV DNA testing in one or two-visits are cost-effective alternatives to conventional cytology with three-visits in resource-limited settings. One-time screening of women at age 35, with one-visit or two-visit VIA or HPV testing reduced lifetime risk by 25 to 36% and cost <$500 per year of life saved. Two lifetime

		screens at age 35 and 40 years, further reduced lifetime risk by 40% and was very cost-effective.
Kim et al, 2005 UK, Netherlands, France, Italy	To assess cost-effectiveness of adding HPV DNA testing in countries with established cytology –based screening programs. Strategies assessed 1. Each countries ongoing strategy versus 2. HPV as triage for equivocal cytology results in a lifetime cytology program; 3. Cytology until age 30, then HPV combined with cytology in women aged >30years.	Both strategies with HPV testing as triage or in combination with cytology were more effective than ongoing strategies with respective ICER of $13,000 and $9800-75,900 (depending n screening interval) per year of life saved.
Sherlaw-Johnson and Philips, 2004. UK.	Assess liquid-based cytology (LBC) versus HPV testing (either as triage for borderline cases or as a primary test) in the UK cervical cancer screening program.	Unless the marginal cost of LBC is higher than that of conventional cytology, then LBC is as cost-effective as conventional cytology. Five-yearly combined LBC and HPV testing has similar cost-effectiveness to 3-yearly pap smear or HPV testing alone. Combined testing and HPV testing alone reduced false positive cases.
Goldie et al, 2004 USA	To assess the CE of HPV testing in combination with cytology in women aged 30 years or m ore. Markov model simulating a cohort of US women. Strategies compared: no screening, screening at different frequencies with conventional cytology, LBC with HPV for triage of equivocal results and HPV testing plus cytology in women after age 30.	For women aged >30 years, 2 or 3-yearly screening with a combination of cytology and HPV testing (either primary or as triage) is more CE than annual conventional cytology. With ICER of $95,300 and $228700 per year of life gained, all age 3-yearly LBC and 3-yearly HPV testing plus cytology in women aged >30 years had equal or greater benefits when compared to annual conventional cytology. Annual cytology plus HPV testing was not very beneficial with a ICER of $2,000,000 per year of life gained.
Mandelblatt et al, 2002 Thailand	To assess the costs and benefits of different screening strategies in less-developed countries. A population based model assessment of 7 strategies in Thailand: 1) 1-visit VIA; 2) 2-visit VIA; 3) HPV DNA testing; 4) Pap smear screening; 5) Pap smear screening with HPV DNA testing, followed by evaluation of women with an abnormal result from either test; 6) VIA followed by HPV DNA testing, with women who are found to have VIA-detected lesions that are not appropriate for immediate	Costs ranged from $121 to $6720 per life year saved. 5-yearly 'screen and treat' VIA in women aged 35-55 was the most CE strategy with a cost of $517 per life year saved. HPV testing had similar CE if the HPV test cost $5 and if 90% of patients returned for follow-up. Cytology would result in similar results if the sensitivity was >80% and 90% of women underwent follow-up. A combination of 5-yearly HPV and Pap in women aged 20-70 years could reduce mortality by 90% at a cost of $1683 per life year saved; while VIA could reduce mortality by 83% at a cost of $524 per life year saved.

	treatment being referred for appropriate care (work-up of lesion), regardless of HPV test result; and 7) no screening (but treatment of symptomatic disease).	
Maxwell et al, 2002. Military beneficiaries in the US.	To assess the costs and CE of new screening methods for cervical cancer in the military. Used a Markow model on a simulated cohort of 100000 military beneficiaries aged 18-85. Strategies compared include: conventional cytology, LBC, LBC plus HPV triage with 1-, 2-, and 3-year screening intervals.	Cancer incidence and mortality reduced with increased sensitivity of screening method. Both LBC and LBC plus HPV triage are CE (with <$50,000 per life year saved) when conducted 3-yearly but not CE when conducted more frequently than 3-yearly. A more sensitive test performed less frequently may be more CE than annual conventional cytology.
Kim et al, 2002 US	To determine the most CE management strategy for women with ASCUS in the US. Strategies assessed: 1. immediate colposcopy; HPV triage (1-visit or 2-visit) with colposcopy if HR-HPV detected; repeat cytology involving follow-up cytology at 6 and 12 months and then colposcopy if repeat abnormal result detected; reclassifying ASCUS as normal and ignore.	Reclassifying ASCUS as normal was least costly, reducing the lifetime cancer incidence by 75% when conventional cytology is used. HPV testing and immediate colposcopy each respectively reduced cancer incidence by 86% and 87%. Biennial (versus triennial) LBC with reflex HPV testing costs $174200 per years of life saved. Similarly triennial (vs 5-yearly) LBC cost $59600 per YLS and was more CE compared to biennial conventional cytology with repeat cytology or immediate colposcopy. Reflex HPV is more CE than other management strategies.
Mandelblatt et al, 2002 USA	To compare the CE of HPV testing, Pap smears and their combination. 18 strategies assessed distinguished by 3 possible tests (Pap plus HPV testing, Pap testing alone, and HPV testing alone); 2 possible frequencies (every 2 or every 3 years); all beginning at age 20 years but distinguished by the age at discontinuation (65 years, 75 years, or at death).	Compared to biennial Pap smears alone, biennial HPV testing plus Pap smears (both until death) had an ICER of $76183 per QALY. Stopping HPV and Pap testing at age 75 and 65 years respectively captured 97.8% and 86.6% of the benefits of lifetime screening. HPV testing alone was equally as effective as Pap testing alone, (irrespective of age and frequency) but was more costly. HPV testing would be more CE than Pap testing if it cost $5 or less per HPV test. Costs can be reduced by using HPV plus Pap test biennially (compared with Pap smear alone); Applying age limits maintain benefits while reducing costs.
Van den Akker-van Marle et al, 2002	To compare the CE of various cancer screening strategies in high-income countries.	15 efficient screening policies (with no alternative less costly policy) were identified. For these policies the

High-income countries.	Used a microsimulation screening analysis to model approximately 500 potential strategies that differed with respect to the recommended number of screenings, screening intervals, and targeted age ranges.	screening age ranged from 40-52 to 20-80 years, and the screening interval decreased from 12 to 1.5 years. The average ICER ranged from $6700 to $23900 per life year gained. CE could generally be improved by reducing the frequency of screening or starting screening at a later age.
Philips and Whynes, 2001. UK	To assess the CE of early withdrawal from screening.	Median cost savings were always less than £1000 per life-year lost. The estimates were sensitive to the age at which cancer occurred and the rate of cancer progression. The authors did not think the cost-savings were worth the life lost with early withdrawal.
Goldie et al, 2001 South African women	To assess the CE of several screening strategies a hypothetical cohort of previously unscreened 30-year –old South African women. Strategies assessed include; direct visualization of cervix (DVI), cytology and HPV testing. These strategies also differed by the number of visits, screening frequency and response to a positive test result.	Amongst one time screening strategies, HPV testing with treatment of screen-positive women at a second visit was the most CE, reducing cancer incidence by 27% and costing $39 per life-year saved; DVI with immediate treatment of positive cases reduced cancer incidence by 26% and cost less than cytology. Cytology with treatment of positives at a second visit was least effective reducing cancer incidence by 19% only at a cost of $81 per life-year saved. In general HPV testing was more effective but more costly than DVI, and also more effective and less costly than cytology.
Montz et al, 2001.	To assess the impact of increasing Pap smear sensitivity and compliance using a Markov model of a cohort of women followed from age 20 to 80 years. Pap smear was compared to LBC using 3 different compliance rates. Analyses were conducted for all women then limited to each of white and black women	Increasing compliance from the Healthy People 2000 rates (5% never compliant, 85% fully compliant, and 10% partially compliant) to the Healthy People 2010 target compliance rates (3% never compliant, 90% fully compliant, and 7% partially compliant) resulted in cancer incidence reductions of 23%, 21.7% and 17% in all women, Whites and Blacks respectively. Using LBC instead of conventional cytology resulted in reductions in cancer incidence of 32-33%. The ICER for LBC (compared to conventional cytology) in black women was $10,335 while it was $17,967 per life-year save in white women.
Suba et al 2001; Vietnam	To assess the CE of cytology screening in developing country, Vietnam	A total annual cost of $148,400 was needed to establish a nationally cytology screening program with 5-yearly testing. A 70% program coverage resulted in a reduction

		of cancer incidence from 26 to 14.8 per 100,000 inhabitants, costing $725 per life year saved. Pap smear cytology in developing countries could be cost-effective and relatively inexpensive, despite perceptions of it being otherwise.
Myers et al, 2000	To assess the potential effects of sensitivity, specificity and screening frequency on the cost-effectiveness. The baseline sensitivity and specificity of Pap smear was estimated to be 51% and 97% respectively.	Increasing sensitivity, while holding specificity constant increased life expectancy and cost. Decreasing specificity increased cost. Costs were further increased with higher screening frequencies. Most caused were attributed to the diagnosis and care of low-grade lesions. Authors recommend tests that can detect lesions highly predictive of invasive cancer.
Sherlaw-Johnson and Gallivan, 2000. Eastern Europe.	To compare HPV testing to conventional Pap smears in Eastern Europe. Various strategies varying test used, age of onset, frequency and coverage were assessed. However costs were based on estimates from the UK.	Cost reduced with increased coverage. This is partly because cost of increasing coverage was assumed to be negligible compared to the cost of tests and care. The ICER increased as screening frequency decreased from 10-yearly to 5 yearly and as HPV testing was used in lieu of Pap smears.
Hutchinson et al, 2000 USA	To assess the impact of test sensitivity on the cost-effectiveness of cancer screening. Used a model of women who were screened between ages 20 to 65 years. New technologies assessed included the ThinPrep Pap Test which addresses problems in specimen preparation by providing a standardized fluid for collection and automates the process of transferring the specimen to a microscope slide; the AutoPap Primary Screening System that automatically screens the slides before human screening and selects the 75% most likely to contain abnormalities for subsequent screening by a cytotechnologist. The AutoPap QC 300 rescreening device which rescreens all slides found negative by the human screeners and flags those most likely to contain abnormalities for review by a cytotechnologist.	Increasing test sensitivity by 50% had the potential of reducing cancer incidence by 45-60% depending on the screening frequency. With this increased sensitivity, the cost effectiveness ratio was below $50,000 when the screening interval was 2 years or more. Increasing sensitivity may be realistic and cost-effective in screening for cervical cancer.

Brown and Garber, 1999.	To estimate the cost-effectiveness of 3 enhancements (with improved sensitivity) to the conventional Pap smear test (ThinPrep, AutoPap, and Papnet). Modeled a cohort of US women from age 20 to age 65.	All 3 technologies increased both cost and life expectancy. In general Autopap was the most CE, costing $7777 with quadrennial screening and $166000 with annual screening. CE increased with increased sensitivity of new method, increased disease prevalence and lower frequency of screening.
Sherlaw-Johnson et al 1997. Resource-limited settings	To evaluate the CE of screening programs in resource-scarce settings. HPV testing compared to cytology as one-time lifetime screen with varied coverage.	One-time screening of women age 30-59 reduced cancer incidence by 30%. Inconclusive/speculative on the CE of HPV testing; would be a function of the prevalence of HPV infection, and the cost of the HPV test.
Gustafsson and Adami, 1992	To investigate how a screening program interacts with the natural history of cervical neoplasia.	Effect of screening is sensitive to age at first screen and age at last screen (though to a lesser extent).
Mandelblatt and Fahs, 1988. Low-income elderly women. US	To assess the CE of cervical screening in infrequently seen elderly women in an urban municipal clinic.	11 of 816 women had an abnormal Pap smear. This resulted in savings of $5907 and 3.7 years of life per 100 pap smears. Further including medical cost per year of life extended, the program cost $2874 per year of life saved. The program appeared cost-effective (with benefits of screening offsetting the cost).
REVIEWS		
Stanley, 2008	Review study to compare HPV vaccination to screening	HPV vaccination considered most effective in developing countries with unavailable or ineffective screening programs. In countries with well developed screening programs these screening programs would still be needed since only two of 15 oncogenic types are included in vaccines and for at least 3 decades unvaccinated women will still need to be screened. A combination of vaccination and screening is thus recommended in such settings
Myers et al 2008,	Review on the role of screening in the era of HPV vaccination.	Screening will still be needed to further reduce cancer incidence. However screening guidelines may need to be modified and cost-effectiveness of different screening scenario may depend on the age and effectiveness of vaccination.
Bosch et al, 2008	Review of the role of screening versus vaccination in the prevention of cervical cancer. HPV and cervical cancer: screening or	Both but not a straightforward answer. Screening will be needed because of the 25-30% of cancers due to types other than HPV 16 and 18.

	vaccination?	Argues that vaccination and screening will be needed in scenarios in which an effective screening program already exists. There might be a role for vaccination only (if affordable) in developing settings were screening is ineffective and poorly implemented. Otherwise in these settings other efficient screening strategies such as use of low-cost HPV tests, and 'screen and treat' approaches will be needed. Finally, a potential polyvalent vaccine that could prevent 90% HR-HPV could be the answer for both settings.
Schiffman 2007,	Review	HPV 16 and 18 vaccines could prevent 70% of cancers. Universal vaccination of adolescents may affect cytology and HPV testing. HPV infections are common particularly at a young age. 90% of infections are cleared within 2 years Infection with oncogenic HPV is more predictive of cancer than are grade1/2 precancerous lesions. Controlling for HR-HPV infection the risk of CIN3 is equal for ASCUS and LSIL. Vaccination would reduce the positive predictive values of cytology and HPV tests.
Goldie, 2006	Review considering screening versus vaccination strategies to cervical cancer control as well as the methodologic issues involved in decision analyses.	Cervical cancer is still a substantial cause of cancer and death in developing countries. Conventional cytology remains difficult to implement successfully in developing countries. Alternative non-cytologic approaches for HPV detection, visualization of lesions and vaccination are needed.
Wright et al, 2006	A summary of a workshop to review the role of HPV vaccines and screening in the prevention of cervical cancer and how HPV vaccines and new diagnostic methods can be integrated to provide maximum benefit to women.	Next step issues age of vaccination, vaccinating boys, introduction in developing countries
Holmes et al 2005 Review	Review of modeling studies of the cost-effectiveness of HPV screening for cervical cancer. Most models assumed screening starts at 18 or 20 years; HPV prevalence of 10% in those aged 18 years to 20% in those aged 20-25	Cost per QALY varied in the range $ 12400-16600. The highest cost was observed with a strategy that involved annual screening with liquid cytology and HPV testing. Cost of triennial HPV testing is less than that of biennial pap smear cytology. Screening using HPV testing in women after age 30 is

	years, then drops after age 30. Costs for diagnosis and treatment vary considerably.	recommended.
Fahs et al, 1996.	Review of cost-effectiveness studies.	CE programs have the following characteristics: Centrally organized, having guidelines allowing for spontaneous discretionary screening, begin at age 25 to 35 and end at age 65 to 70 years, older women need to get 3 negative screens before discontinuation, screening interval between 3 and 5 years, better to screen more women less frequently than screen fewer women more frequently; screening previously unscreened women very cost-effective.

CHAPTER THREE: RESEARCH DESIGN AND METHODS

3.1 Methods to determine the prevalence, severity and predictors of cervical squamous intraepithelial lesions in HIV-positive women on antiretroviral therapy in Cameroon.

3.1.a Study design and population

Study design: Descriptive cross-sectional study.

Study setting: Participants were enrolled in HIV care clinics in Cameroon. These clinics were chosen such that on average 20 women on HAART will be recruited per week and achieve the required sample size in a reasonably short time. Cytological slide analysis, cervical swab storage and data entry were conducted in the Center for the Study and Control of Communicable Disease (CSCCD) of the Faculty of Medicine and Biomedical Sciences, Yaoundé, Cameroon.

Study population: Women initiating HAART in Cameroon.

Eligibility criteria: Women aged 18 years or more, initiating HAART and consenting to study procedures.

Sample size considerations: Our primary aim was to determine the prevalence of cervical epithelial lesions in women initiating HAART. Valid cytology results were obtained for 276 women. Figure 3.1 shows the precision (measured as the width of the 95% confidence

interval) of the prevalence estimates that would be obtained with a sample size of 276 women. This was estimated using the formula

$d = 4p(1-p)Z_{\alpha}{}^{2}/n$. Where d= precision (width of 95%CI), p=prevalence estimate and n=sample size.

For example with 276 women we will be able to detect a prevalence of 10% with a 95%CI width of 0.5% and a prevalence of 50% with a 95%CI width of 1.4%. Overall, irrespective of the prevalence obtained in our sample the width of the 95%CI around the prevalence estimate will be less than 1.5%.

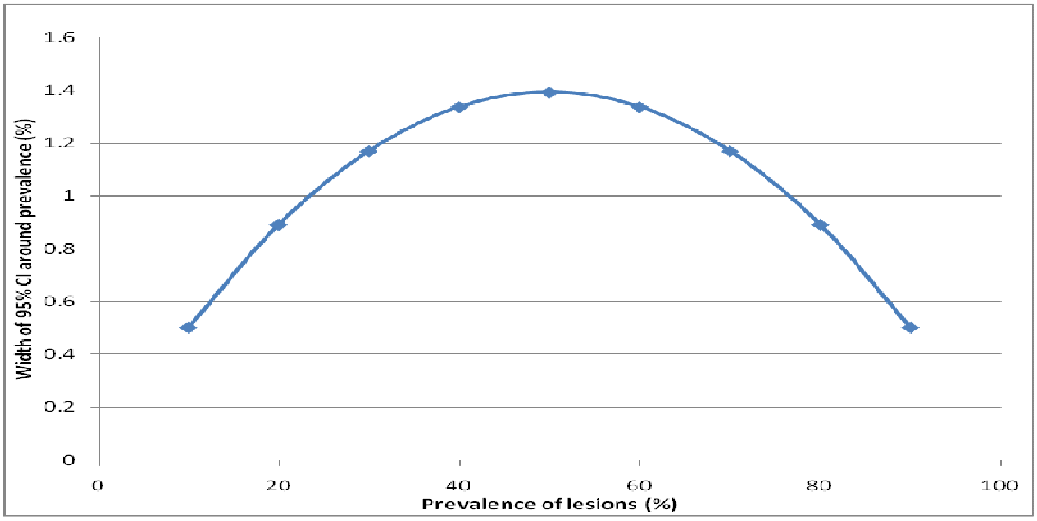


Figure 3.1: Precision of prevalence estimates based on a sample size of 276 women

3.1.b Study procedures

After obtaining administrative and IRB approval we trained three interviewers (with a previous medical training) to ensure that study procedures and cervical sampling in particular are being implemented correctly. This was necessary for an optimal quality of cervical smears.

Participant recruitment: At each site clinicians, after providing usual care referred the patients to our study interviewers who then explained the study to each eligible patient.

Interview: After obtaining written informed consent, the interviewers questioned and collected data on demographic and clinical characteristics with the aid of a standardized questionnaire (see appendix).

Sample collection: The interviewers collected cervical smear samples using conventional methods. For each participant, two slides were labeled with a unique identification number. Cervical smear samples were collected by placing the sharp tip of a spatula in the endocervical canal while the blunt tip of the spatula was on the squamocolumnar junction. The spatula was then rotated for 360 degrees and the sample collected immediately smeared on pre-labeled slides. The slides were immediately fixed using a 95% ethanol solution. The slides were stored at room temperatures while the swabs in STM media were stored in refrigerators (at 2-8 degrees Celsius) for the duration of patient enrollment (1 month). These samples (swab and slides) were sent for analysis and storage in the laboratory of the CSCCD which has experience in the staining and reading of cervical smears.

Laboratory analysis: Cervical samples will be stained using the conventional Papanicolau method. Briefly, this method involves staining the nuclei with hematoxylin and then using Orange G and Eosin and Light Green SF yellowish as counterstains. The stained slides were observed under the microscope (at 400X) and then scored according to the Bethesda 2001 system, as unsatisfactory, negative, atypical squamous cells of uncertain significance, low-grade squamous intraepithelial lesions (LSIL), high-grade SIL (HSIL), or invasive cervical cancer[Solomon et al, 2002].

Cytology results were returned to the clinicians who transmitted them to participants. Patients with LSIL or less were advised to repeat screen within a year. Participants with HSIL or more severe precancerous lesions are being invited back to the clinic for specialized care of the lesions involving a colposcopy with biopsy and cryotherapy/LEEP.

Participants with cancer in situ or invasive cancer were referred to gynecologists for specialized care.

3.1.c Data analysis

Data collected were entered into MS Access interface on Epi-info. Statistical analysis were conducted using SAS version 9.2 (SAS institute inc, Cary NC) and STATA version 10 (STATA corps, Texas USA).

Outcomes: Two outcomes were considered for this analysis: 1) Prevalent cervical lesions (defined as the proportion of women who had *any* cervical epithelial lesions); 2) Prevalent severe cervical lesions (defined as the proportion of women who had ASC-H/HSIL or worse cervical lesions, requiring coloscopy and biopsy).

We assessed the univariate distribution of both predictors and outcomes. From this analysis we discerned the prevalence and severity of precancerous lesions in this population.

Predictors: marital status, parity, number of lifetime sex partners, age, history of hormonal contraception, smoking history, CD4 count, AIDS clinical stage. These predictors were chosen based on the ease with which they can be elicited and recorded in a clinical setting and previous literature describing their association with the presence of cervical lesions or a plausible etiological role. The maximum number of predictors that could be included in a multivariate logistic regression was determined using the formula $3*N_1*N_0/10(N_1+N_0)$ where N_1 is the number of participants with the outcome being analyzed and N_0 is the number of participants without the outcome [Harrell, 2001].

We described the univariate distribution of predictors. To determine predictors of lesions we conducted bivariable and multivariable (adjusted) analyses of the association of each predictor with each outcome. For bivariable analysis, proportions were compared using chi-square statistics while continuous variables were compared using t-test (when

comparing the means of two-groups) or ANOVA test (when comparing the means of more than two groups). Prevalence odds ratios and their 95% confidence intervals, comparing the odds of each outcome between predictor groups were also estimated.

Assessment of linearity: The linearity of the odds of the outcomes for each continuous variable (such as age and CD4 count) were assessed prior to their being included in regression models that assume the linearity of the outcome. The assessment was done graphically and statistically. In graphical analysis we plotted the log of the odds of cervical lesions (on the y-axis) by the continuous variable (on the x-axis). The plots were smoothed using Lowess estimation methods that are locally weighted moving averages of the odds within small categories of continuous variables. The statistical significance of improving the model fit by including non-linear (quadratic terms) for the continuous variable was assessed. A model with the continuous variable and a non-linear (quadratic) term and one with only the continuous variable were compared using a likelihood ratio test. When there was evidence against linearity, continuous variables were coded as categorical variables using clinically meaningful cut-offs such as 200 cells/uL for CD4 counts or based on the lowess trend estimates (example 26-59 versus other women for age).

Predictive models: All models were unconditional logistic regression models. For each outcome we created an initial (full) model that included all the predictors described above. A parsimonious subset of clinically or statistically significant variables were then determined based on a backward elimination strategy in which we removed one variable at a time from the initial model, based on the largest p-value [Harell, 2001]. The predictive value of each variable was assessed by a likelihood ratio test (LRT) comparing two successive models with or without the variable being assessed, and a comparison of the area under the curve (AUC) of the ROC plot (a plot of the sensitivity, on the y-axis, by 1-specificity, on the x-axis, at potential cut-off point) of two successive models with or without the variable. Variables were dropped from the model if the LRT of their removal had a p-value >0.2 and the AUC

changed by <10%. A final reduced model was thus obtained retaining only predictors that when dropped resulted in a LRT <0.2 or a 10% changed in the AUC.

For each outcome, the models developed were based on the full sample. However the internal validity of the model performance was ascertained by repeating the analysis within data subsets corresponding to each study site.

Development of risk scores: For each outcome we attempted to develop two sets of risk scores (one each from the full model and the reduced model). The numeric score assigned to each predictor was based on the model slope coefficients. To allow for a simple and feasible application in clinical settings, each predictor score was obtained by multiplying the model slope coefficients by a constant (for example 10 or 100) and then rounding to the nearest integer. The aggregate risk score was based on the sum total of each predictor score. The overall accuracy of the score was assessed using the area under an ROC curve (ie a plot of the sensitivity, on the y-axis, by 1-specificity, on the x-axis, at potential cut-off point). The best cut-off values for each risk score model was assessed by calculating the number of false positives and false negatives that would result from using each cut-off point.

Sensitivity analyses: We conducted sensitivity analyses of the error in estimating prevalence based on the sensitivity and specificity of conventional cytology.

We used the following formula:

Observed prevalence = (true positive rate + false positive rate)

If P' designates the observed prevalence and P designates the "true", unobserved prevalence then

$$P' = sens * P + (1 - spec) * (1 - P)$$
$$P' = sens * P + 1 - P - spec + spec * P$$
$$P' = P(sens + spec - 1) + 1 - spec$$
and
$$P = \frac{P' + spec - 1}{sens + spec - 1}$$

The above formula was applied on a spreadsheet for different combinations of sensitivity (range 0.5 -1) and specificity (0.7-1).

Limitations and strengths: Though we sampled in three different clinics, the sample may not be representative of all HIV women in Cameroon as the clinics were conveniently selected and not randomly chosen. There may be measurement errors for the outcomes as the limited Pap smear has a low sensitivity and we did not perform confirmatory histology on the lesions detected. While the sample size was adequate for estimating prevalence, only a limited number of covariates could be considered as potential predictors. The study was however strengthened by the fact that sample size was adequate for prevalence estimation, multiple sites were used to reflect some of the diversity of women in Cameroon, interviewers were trained to insure adequate sample collection, the primary data collection insured that questions pertaining to this study were directly ascertained, two slides were collected per patient to increase the sensitivity of the pap smear which were read by a trained cytologist. The statistical analysis using only clinically feasible predictors would allow for results that could be easily implemented by clinicians.

3.2 Methods to describe the age trends in the prevalence of cervical squamous intraepithelial lesions in HIV-positive women on antiretroviral therapy in Cameroon.

This objective focused on modeling the prevalence of cervical lesions by age and determining the optimal model relating age to prevalent cervical epithelial lesions. We also attempted to estimate the minimum and maximum age at which cervical lesions are present as well as the age (or age group) with the maximum prevalence of lesions. We refer to these three as critical ages.

3.2.a Study design and population

These were identical to those for aim1 and described in section 3.1.a above.

3.2.b Study procedures

These were identical to those for aim1 and described in section 3.1.b above.

3.2.c Data analysis

Data collected were entered into MS Access interface on Epi-info. Statistical analysis were conducted using SAS version 9.2 (SAS institute inc, Cary NC) and STATA version 10 (STATA corps, Texas USA).

Outcomes: 1) Prevalent cervical lesions (defined as the proportion of women who had *any* cervical epithelial lesions); 2) Prevalent severe cervical lesions (defined as the proportion of women who had *ASC-H/HSIL*, requiring coloscopy and biopsy).

Predictors: Age. Age was analyzed in following forms:

- age as a binary variable (<30 versus ≥30, based on current recommendations for cervical cancer screening),
- age as a continuous linear variable (assuming that the prevalence of cervical lesions increase linearly and monotonically with age),
- age as a continuous quadratic variable (assuming that the prevalence of cervical lesions has a quadratic shape ie initially increasing with age, reaches a maximum and then decreases).
- Age coded as (<26, 26-59, 60+) based on the lowess plot
- Age coded as binary (26-59 versus <26 and 60+) as age <25 and 60+ had similar characteristics and we had few women aged 60+.

Potential modifiers of the prevalence ratio: marital status, parity, number of lifetime sex partners, history of hormonal contraception, smoking, CD4 count, and AIDS clinical stage.

Potential confounders: None. As shown in the Directed Acyclic Graph (DAG) below, in the absence of any covariate that affects age, there is no unblocked backdoor path between age and prevalent cervical lesions, thus no confounding of the effect of age.

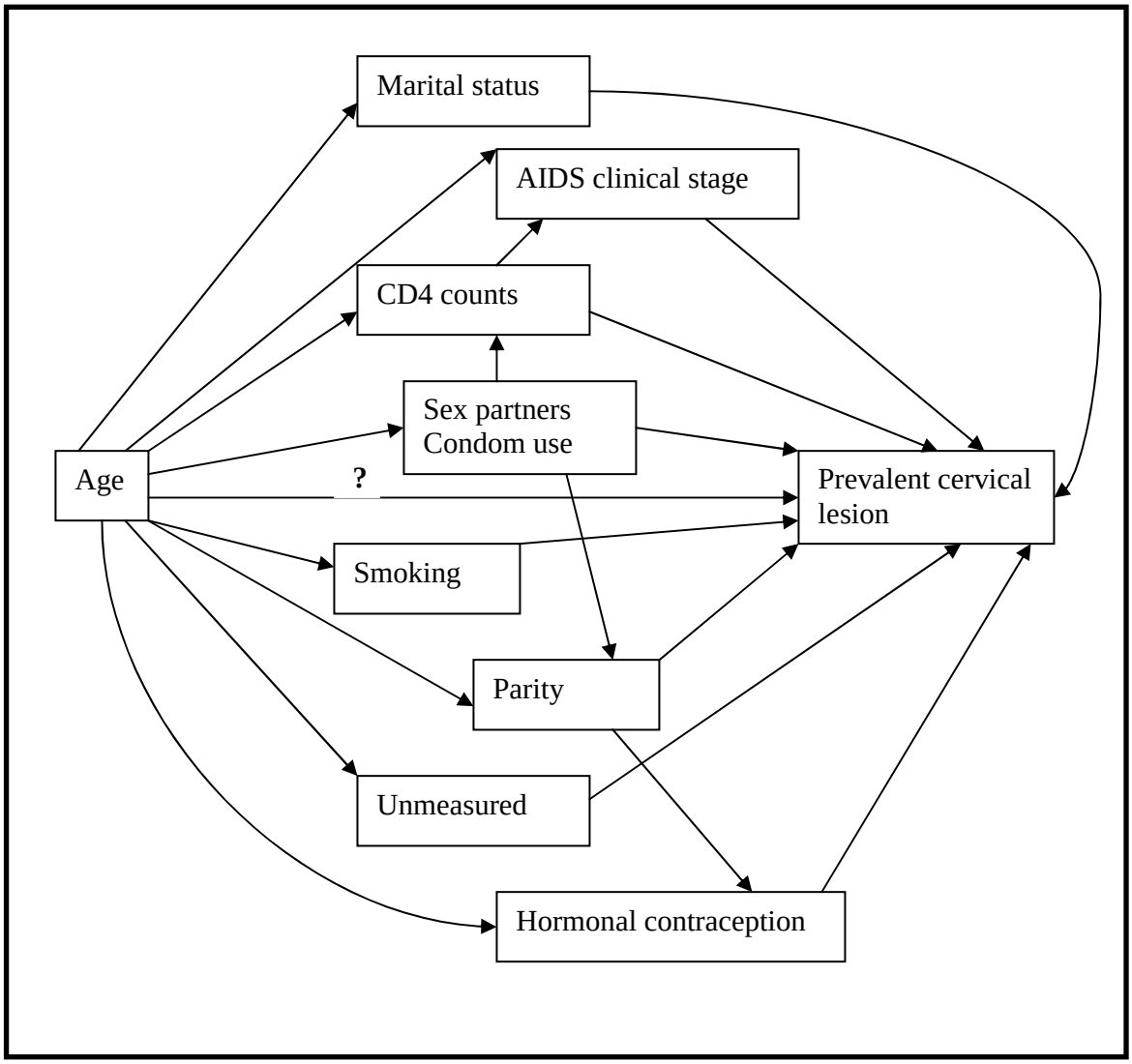


Figure 3.2: Directed acyclic graph (DAG) of the relationship between age, prevalent cervical lesions and other covariates.

Bivariable model estimation

We initially assessed the univariate distribution of predictors, outcomes and covariates.

In bivariable analysis, we used a linear risk binomial model (ie a form of generalized linear model that assumes a binomial distribution for the outcome, and an identity link between predictors and outcome) to model the prevalence of cervical lesions by age. Using the 3 types representations of the age variable, these models can be summarized mathematically viz.

A binary age model: $Pr(Y) = \alpha_1 + \beta_1 \text{ age30}$ ……Equation 3.2.1

A linear age model: $Pr(Y) = \alpha_2 + \beta_2 \text{ agec}$ ……Equation 3.2.2

A quadratic age model: $Pr(Y) = \alpha_3 + \beta_3 \text{ agec} + \beta_4 \text{ agec}^2$ …… Equation 3.2.3

Where

Y is a variable indicating prevalent cervical lesion (Yes/No)

age30 is a variable that takes a value of 1 when age ≥30 and a value of 0 when age<30

agec is a variable with participant's age centralized (ie age – mean age)

α_1 is the prevalence of cervical lesions in participants aged <30

β_1 is the prevalence difference (PD) comparing the prevalence of cervical lesions in participants aged≥30 to those aged <30.

α_2 is the prevalence of cervical lesions in participants with mean age

β_2 is the increase in prevalence of cervical lesions for every one year increase in age (ie the annual change in prevalence)

α_3 is the prevalence of cervical lesions in participants with mean age

β_3 and β_4 do not have any meaningful epidemiologic interpretation.

All α and β parameters for each model were estimated using maximum likelihood estimation (MLE). The relative fit of these models to our data (and thus the best of the models) were assessed using Likelihood ratio tests comparing pairs of nested models and Akaike information criteria (AIC) for non-nested models.

Estimation of critical ages

The minimum and maximum ages at which cervical lesions are present as well as the age with the maximum prevalence of lesions were estimated primarily from the quadratic age model. The minimum/maximum age at which cervical lesions are present could also be estimated from the linear age model while the binary age models is not suitable for estimating any of these parameters.

To estimate minimum and maximum ages at which lesions are prevalent using the quadratic model, we equated the right hand side of Equation 3 to 0 (after substituting the parameters obtained by MLE) and then solved for agec.

We thus obtained the quadratic equation:

$\alpha_3 + \beta_3\ agec + \beta_4\ agec^2 = 0$ …… Equation 3.2.4

Just as the solution of a general quadratic equation $ax^2 + bx + c=0$ is given by $x = \left(-b \pm \sqrt{b^2 - 4ac}\right) \Big/ 2a$, the solution to Equation 3.2.4 will be given by

$$agec = \left(-\beta_3 \pm \sqrt{\beta_3{}^2 - 4\beta_4\alpha_3}\right) \Big/ 2\beta_4$$

The age of maximum prevalence will be the age at which the first differential of Equation 3.2.4, with respect to agec, takes a value of 0 ie when $\beta_3 + 2^* \beta_4\ agec = 0$.

The centralized age of maximum prevalence will thus be solved as $agec = -\beta_3/(2^* \beta_4)$.

The minimum/maximum age at which lesions are prevalent can also be estimated using the linear age model. After substituting the parameters obtained by MLE, we equated the right hand side of equation 3.2.2 to 0 and then solved for agec.

We thus obtained the equation:

$\alpha_2 + \beta_2$ agec =0 …… Equation 3.2.5

The solution was given by agec= - α_2 / β_2.

The 95% confidence intervals for each of these estimates of the minimum and maximum ages at which cervical lesions are present as well as the age with the maximum prevalence of lesions were obtained using post-model estimation commands in Stata.

Multivariable analysis

In multivariate analysis, modifiers of the prevalence difference were assessed by comparing each of the models in equations 1- 3 with similar models that include product interaction terms between the age variable(s) and the potential modifier. All potential modifiers were made binary (and coded 0/1) to allow for an understandable analysis. As an example, for a potential modifier, Z, the models in equations 3.2.1- 3.2.3 will become:

A binary age model: Pr (Y) = $\alpha_1 + \beta_1$ age30 + γ_1 Z + $\delta_1$age*Z ……Equation 3.2.6

A linear age model: Pr (Y) = $\alpha_2 + \beta_2$ agec + γ_2Z + $\delta_2$age*Z …… Equation 3.2.7

A quadratic age model: Pr (Y) = $\alpha_3 + \beta_3$ agec + $\beta_4$agec^2 + γ_3Z + $\delta_4$age*Z + δ_5 Z*age^2 ……Equation 3.2.8

Where

α_1 is the prevalence of cervical lesions in participants aged <30 in whom Z is coded 0.

β_1 is the prevalence difference comparing the prevalence of cervical lesions in participants aged≥30 to those aged <30, when Z is coded as 0.

γ_1 is the increase in the prevalence of cervical lesions for a unit increase in the coding of Z, when age takes its mean value

δ_1 is the difference in the prevalence difference comparing the prevalence of cervical lesions in participants aged≥30 to those aged <30, when Z is coded 1, to the same prevalence difference when Z is coded 0.

α_2 is the prevalence of cervical lesions in participants with mean age and Z coded 0.

β_2 is the increase in the prevalence of cervical lesions for every one year increase in age (or the annual change in prevalence), when Z is coded as 0.

γ_2 is the increase in the prevalence of cervical lesions for a unit increase in the coding of Z, when age takes its mean value

δ_2 is the difference between the annual change in prevalence when Z is coded 1, and the annual change in prevalence when Z is coded 0.

α_3 is the prevalence of cervical lesions in participants with mean age and Z coded 0

γ_3 is the increase in prevalence of cervical lesions for a unit increase in the coding of Z, when age takes its mean value

β_3 , β_4 , δ_4 , and δ_5 do not have direct epidemiologic interpretations (because of the quadratic terms involved).

Modification of the prevalence difference was assessed statistically by a likelihood ratio test comparing a model with the age variable, the potential modifier and a product interaction term to a similar nested model without the product interaction term. A p-value of <0.1 was considered statistically significant and evidence of heterogeneous prevalence

differences. The detection of any significant prevalence difference modifier led to a repeat of the bivariable analysis but this time stratified by categories of the modifier.

Sensitivity analysis: We conducted sensitivity analysis of non-differential misclassification of prevalent lesions in estimating the prevalence difference between age-categories.

Assumptions

- P_i indicates true unobserved prevalence in 'exposure' group i.
- P_i' denotes the observed prevalence in 'exposure' group i.
- Non-differential misclassification implies sensitivity and specificity are equal within 'exposure' groups, ie $sens_i$ = sens and $spec_i$ = spec, where sens and spec represent the sensitivity and specificity of detecting the outcome in each 'exposure' group.

Based on formula above

$$P_i = \frac{P_i' + spec_i - 1}{sens_i + spec_i - 1}$$

And the prevalence difference:

$$P_1 - P_0 = \frac{P_1' + spec_1 - 1}{sens_1 + spec_1 - 1} - \frac{P_0' + spec_0 - 1}{sens_0 + spec_0 - 1}$$

Because of the non-differential nature of misclassification the above formula simplifies to:

$$P_1 - P_0 = \frac{P_1' + spec - 1 - P_0' - spec + 1}{sens + spec - 1}$$

$$P_1 - P_0 = \frac{P_1' - P_0'}{sens + spec - 1}$$

$$PD = \frac{PD'}{sens + spec - 1}$$

Limitations and strengths: Though we sampled in three different clinics, the sample may not be representative of all HIV women in Cameroon as the clinics were conveniently selected

and not randomly chosen. Furthermore, because of the cross-sectional design of the study, the age-specific prevalence described here reflects the age of detection of lesions and not necessarily the age of incidence or the age-specific prevalence in the population. Age differences in access to clinics may result in artificially increased prevalence in older women who are more likely to be in the health care system. The latter detection bias is however expected to be minimal in a study population of HIV positive women in whom access to care is largely driven by worsening HIV disease rather an age. There may also be measurement errors for the outcomes as the limited Pap smear has a low sensitivity and we did not perform confirmatory histology on the lesions detected. Because all models are inherently smoothed mathematical summaries of real life data, the mathematical estimates of the critical ages will simply be approximate estimates.

The study was however strengthened by the fact that multiple sites were used to reflect some of the diversity of women in Cameroon, interviewers were trained to insure adequate sample collection, the primary data collection insured that questions pertaining to this study were directly ascertained, two slides were collected per patient to increase the sensitivity of the pap smear which were read by a trained cytologist. The statistical analysis using models that permit flexible non-linear shapes of age-trends allowed for the detection of a more accurate description of the trends and permitted us to propose a better method for analyzing age in subsequent models of the age at which lesions are detected in HIV positive women.

3.3 Methods to quantify the potential effect of antiretroviral therapy and screening, on mortality from cervical cancer in HIV-positive women in Cameroon

3.3.a Study design and population

Study design: A mathematical simulation.

Study setting: We simulated a clinical practice in Cameroon.

Study population: HIV-infected women in Cameroon.

Model type: We used a state-transition Markov model. This type of simulation model allows analysts to model transitions of a cohort of patients among a number of health states over a long period of time subdivided into a series of short intervals [Nainmark et al, 1997]. We developed a computer model of cervical neoplasia in HIV similar to that previously developed, validated and published by Goldie et al [1999]. The form of the model is shown below.

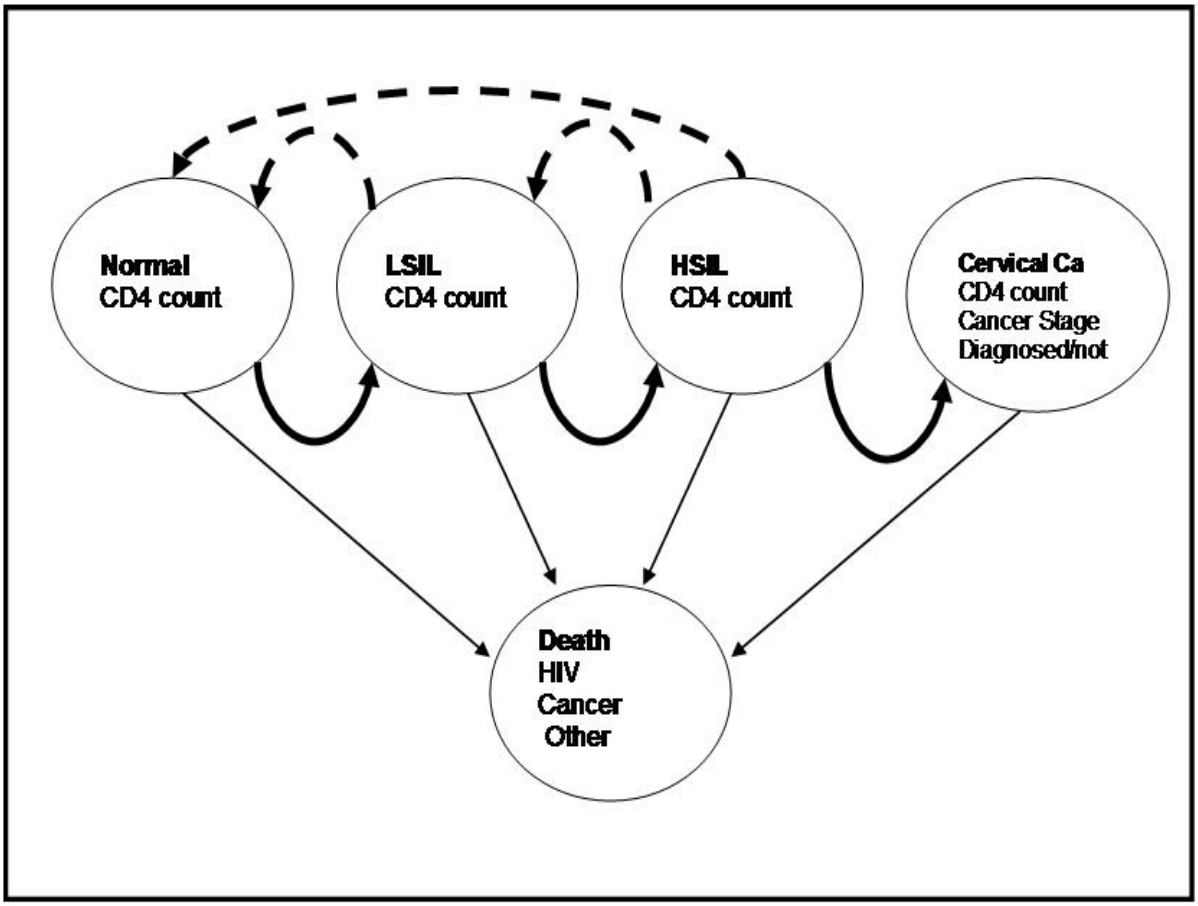


Figure 3.3: Summary of states in the Markov model (Adapted from an original depiction by Goldie et al, [1999])

The model summarizes the progression of cervical neoplasia in HIV in five states: Normal with no lesion, LSIL, HSIL, cervical cancer and death. Each of the four (non-death) states is stratified by CD4 cell count. The cancer stage is further stratified by stage of cervical cancer and whether this has been diagnosed (and thus being treated) or not. During their lifetime women's disease state can progress from normal to LSIL to HSIL to cervical cancer. Women in the HSIL and LSIL states can regress to lower states. Death can occur to women in any of the four states and can result from cervical cancer, HIV related-causes or other causes of death.

The plausible transitions between states are depicted in Table 3.1 below.

Assumptions: As with every simulation assumptions were made in order to have a model that is analytically manageable and easier to summarize. We adopted the following assumptions included in Goldie et al's model: 1)The natural history of cervical cancer involves progress from normal to LSIL to HSIL to local cancer to regional/distant cancer to death from cancer, without skipping. 2) The regression of neoplasia can only be from HSIL to normal or LSIL, of from LSIL to normal. A patient cannot regress from cancer. Cancer stages also cannot regress. 3) HIV diseases progression is only from CD4 500+ to 200-500 to <200. This is a historic parameter, indicating the advance in HIV disease. Once a patient has CD4 <200 she will always be classified in the CD4 <200 category, even if her actual CD4 count improved with treatment. In other words worsening HIV-disease cannot regress. The latter assumption is consistent with data that show that improvements in antiretroviral therapy do not appear to reduce the progression of precancerous lesions even amongst women with improved CD4 counts.

In addition to these assumptions, unlike in Goldie et al's [1999] original paper, we assumed that the progression/regression rate of cervical precancerous lesions is not dependent on a previous history of precancerous lesions. This is because even though Goldie et al originally assumed a higher transition probability from a precancerous lesion to cancer in those with a

previous history of treated lesions Goldie and Kuntz [2003] later showed this could be erroneous: the source of the error being the fact that the parameters used in the model are from heterogeneous populations (that include patients with and without a previous history of lesions).

3.3.b Study procedures

In the absence of prospective data on HIV-progression, cervical precancerous disease progression and regression for Cameroonian women, we developed a Markov chain model using parameters published in Goldie et al's validated model for cervical cancer in HIV. We recognize that the parameters were primarily based on US cohorts at the initiation of HAART. However the model was calibrated to reflect the shape of age-specific cancer incidence and mortality in the Cameroon, as estimated by the WHO.

3.3.c Data analysis

The model was constructed and analyzed using the Healthcare module of Treeage Pro 2008, a software meant primarily for decision analysis including those based on Markov models.

Model parameters: Parameters used in the model were abstracted from the published literature and reflected data for Cameroon as much as possible. The values used in the baseline model are shown in Table 3 2. The baseline model was designed to simulate the progression over time of a cohort of HIV-positive women aged 25 with CD4 count >500, 30% of whom had precancerous lesions (one-third of which were high grade lesions). All cause age-specific mortality rates were estimated based on abridged life tables for women in Cameroon [WHO, 2008]. HIV mortality rates were estimated based on WHO data [UNAIDS, 2007]. The proportion of HIV-mortality that occurs in each CD4 category was estimated based on data by Goldie et al [1999]. Cervical cancer mortality rates were also abstracted from WHO estimates of annual cervical cancer incidence and deaths in Cameroon [Ferlay et al., 2004]. Age-specific mortality rates from other causes were

estimated by adjusting (using another Markov analysis) all-cause age-specific mortality rates to deduct mortality from cervical cancer and mortality from HIV.

In the absence of published data on long-term progression or regression rates of precancerous lesions in HIV positive women in Cameroon, we used published estimates from women in the pre-HAART era from Goldie et al [1999].

Scenarios and outcomes assessed: We assessed the projected lifetime cumulative mortality due to cervical cancer in four plausible scenarios of HIV and cervical cancer care in Cameroon: no HAART and no screening (NHNS), HAART when indicated and no screening (HNS), HAART when indicated and screening once at age 35 (HS35), and HAART when indicated and screening on HAART initiation (HSHI).

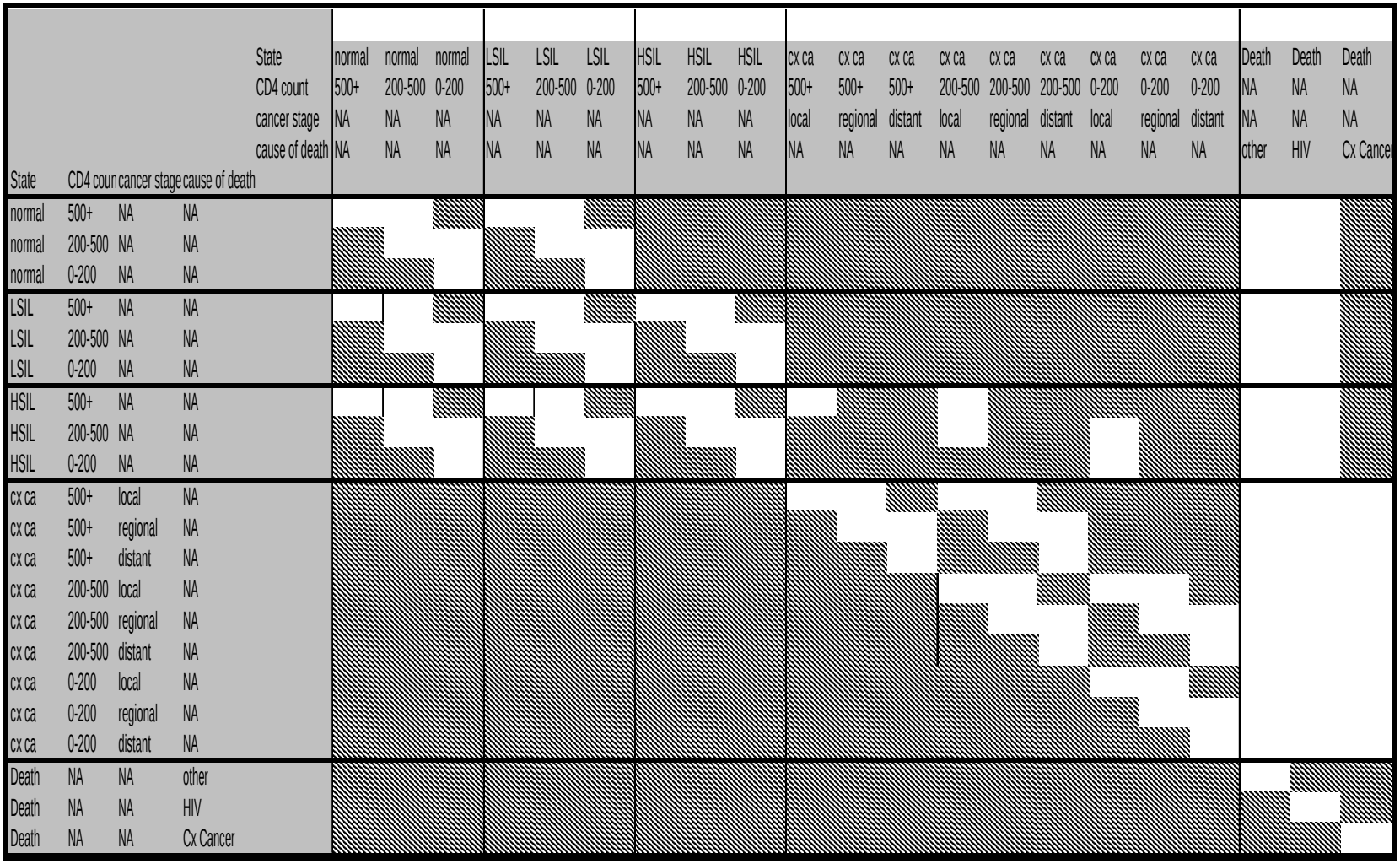
Sensitivity analyses: The sensitivity of cumulative cervical cancer mortality to parameter estimates was analyzed in one-way sensitivity analyses. We were particularly interested in the sensitivity of HAART effectiveness in lowering HIV-mortality and SIL progression rates since the baseline values used were all external to the study and these values are likely to vary substantially depending on the study population.

Limitations and strengths: As with every model, this model will be limited by the veracity of the model assumptions and parameters.

The analysis is strengthened by the use of a previously validated model of cervical cancer screening in HIV positive women. The use of a Markov model allows for time changes such as the progression and or regression of lesions in individuals in the cohort.

Table 3.1: Plausible transitions (blank cells) between stages in the Markov model.

State	CD4 coun	cancer stage	cause of death
normal	500+	NA	NA
normal	200-500	NA	NA
normal	0-200	NA	NA
LSIL	500+	NA	NA
LSIL	200-500	NA	NA
LSIL	0-200	NA	NA
HSIL	500+	NA	NA
HSIL	200-500	NA	NA
HSIL	0-200	NA	NA
cx ca	500+	local	NA
cx ca	500+	regional	NA
cx ca	500+	distant	NA
cx ca	200-500	local	NA
cx ca	200-500	regional	NA
cx ca	200-500	distant	NA
cx ca	0-200	local	NA
cx ca	0-200	regional	NA
cx ca	0-200	distant	NA
Death	NA	NA	other
Death	NA	NA	HIV
Death	NA	NA	Cx Cancer



Rows and columns respectively represent initial and final stages at each transition time. (cx ca: Cervical Cancer, NA: Not applicable).

Table 3.2 Baseline values

Variable	CD4 >500	CD4 200-500	CD4 <200	Source
Initial prevalence of lesions, %	30	NA	NA	Mbu et al, 2008
Initial proportion of lesions that were HSIL (%)	33	NA	NA	Mbu et al, 2008
HIV infection				
HIV mortality rate (per 1000 per year)*	0.05	6.06	48.6	UNAIDS, 2007
Effect of HAART in reducing HIV mortality	NA	NA	4-fold	Murphy et al, 2001; Mermin et al, 2008
HIV progression rate (per 100 per year)**	18.1	27.5	NA	Goldie et al, 1999
Cervical Cancer				
Cancer mortality rate (per 1000 per year)*				[Ferlay et al., 2004
Local invasive cancer	41.1	41.1	41.1	
Regional invasive cancer	222.1	222.1	222.1	
Distant invasive cancer	543.5	543.5	543.5	
Progression rate (per 100 per year)				Goldie et al, 1999
Normal to LSIL	0.016	0.67	0.67	
LSIL to HSIL	0.73	2.93	2.93	
HSIL to local invasive cancer	2.0	2.42	2.42	
Local to regional invasive cancer	4.03	4.03	4.03	
Regional to distant invasive cancer	4.03	4.03	4.03	
Regression rate (per 100 per year)				Goldie et al, 1999
LSIL to normal	2.99	2.99	2.99	
HSIL to normal	0.30	0.30	0.30	
Screening test				Goldie et al, 1999
Sensitivity, %	70	70	70	
Specificity, %	90	90	90	

* Mortality in each CD4 category or cervical cancer stage were determined by weighting crude estimates by the proportions due to each category or stage from Goldie et al[1999]. ** estimated from mean duration at each stage

CHAPTER FOUR: CERVICAL SQUAMOUS INTRAEPITHELIAL LESIONS IN WOMEN INITIATING ANTIRETROVIRAL THERAPY IN CAMEROON: PREVALENCE AND PREDICTORS

4.1 ABSTRACT

Background: Cervical cancer is the most common cancer in women in low-income countries. Although cervical cancer incidence and mortality is higher in HIV-positive women, resource limitations restrict the implementation of systematic screening programs in these women.

Objectives: We explored the potential for targeted screening by assessing the prevalence, severity and predictors of cervical squamous intra-epithelial lesions (SIL) in HIV-positive women in Cameroon.

Methods: We conducted a cross-sectional study of women initiating antiretroviral therapy between August and September of 2008 in three clinics in Cameroon. Socio-demographic, behavioral, and clinical information was obtained from eligible women by trained interviewers. Cervical exfoliated cells were then collected, a conventional cytology performed and epithelial lesions classified according to the Bethesda 2001 system.

Results: A total of 282 women, aged 19 to 68 years, were enrolled in this study. The median CD4 count was 179 cells/microliter (interquartile range: 100 to 271). SIL were detected in 43.5% of the 276 women with satisfactory samples: including atypical squamous

cells of unknown significance (ASCUS) 0.7%, low-grade SIL (LSIL) 25.0%, atypical squamous cells, cannot exclude high grade lesions (ASC-H) 14.5%, and high-grade SIL (HSIL) 3.3%. None of the demographic or clinical characteristics considered significantly predicted the presence of any SIL or the presence of severe lesions requiring colposcopy. However, compared to women from urban areas, SIL were more frequent in women from rural areas (adjusted OR: 1.68; 95%CI: 0.88, 3.18). SIL were also slightly more frequent in women aged 26-59 (aOR: 1.57; 0.65, 3.81).

Conclusion: The prevalence of SIL in women initiating antiretroviral therapy in Cameroon was high underscoring the need for screening in this population. In the absence of any accurate demographic or clinical predictor of SIL, alternative affordable screening options need to be explored.

4.2 INTRODUCTION

Cervical cancer is the most common cancer in women in low-income countries [WHO, 2006]. Compared to immuno-competent women, HIV-positive women have a higher prevalence, incidence and progression rate of precancerous cervical lesions [Palefsky, 2006; De Vuyst et al, 2008]. By the end of 2007, women accounted for 50% of the estimated 33 million people living with HIV worldwide, and close to 59% of the 22 million in sub-Saharan Africa [UNAIDS, 2008]. With the recent increase in access to highly-active antiretroviral therapy (HAART), these women are expected to live longer thus potentially allowing sufficient time for cervical cancer to develop and progress to be a clinical burden. In addition to a longer life expectancy, HAART is associated with a reduction of competing causes of death, such as Kaposi sarcoma and tuberculosis, while appearing to have little or no impact on the prevalence and progression of cervical precancerous lesions [Franceschi and Jaffe, 2007]. The proportion of morbidity and mortality attributable to cervical cancer in women on HAART is thus expected to increase. Enhancing early detection and treatment of

precancerous lesions through screening could reduce the burden of cervical cancer in these HIV-positive women [Goldie et al, 2005; Franceschi and Jaffe, 2007].

Despite the relatively high risk of precancerous and cancerous cervical lesions in HIV-positive women, unlike many other opportunistic infections, the current management of women initiating HAART in most low-income countries does not include a systematic screen for cervical cancer or precancerous lesions. We hypothesize that cervical cancer goes undiagnosed and that early diagnosis of precancerous lesions by a screen at HAART-initiation could be cost-effective in reducing overall morbidity and mortality rates in these HIV-positive women. Targeted screening among HIV-positive women could potentially increase the cost-effectiveness of screening in these resource-limited settings by increasing the ratio of the number of cases detected per screening test. However, for targeted screening to be effective socio-demographic and clinical factors associated with a higher prevalence and severity of lesions need to be identified.

In this paper, we describe the prevalence and severity of cervical epithelial lesions in women initiating HAART in Cameroon and assess the clinical predictors of lesions in these women. Clinical risk scores are also developed based on the aforementioned predictors and their potential performance assessed.

4.3 METHODS

Study design and study population

We conducted a cross-sectional study of HIV-positive women recruited from three HIV-care clinics in Cameroon: the Bamenda Provincial Hospital AIDS Treatment Center (ATC), the Limbe Provincial Hospital ATC and the Nylon District Hospital ATC in Douala. The clinics are all located in urban areas in Cameroon but provide regular care to patients from surrounding urban and peripheral rural areas. Consecutive HIV-positive women receiving care in these clinics, between August and September 2008, were invited to

participate in the study. Women aged 18 years or more, who initiated HAART within a year of study enrollment and consented to study procedures, were eligible. Women who were either pregnant, having menses or had a previous total hysterectomy were excluded. Study procedures were approved by the relevant ethical review boards in Cameroon and the University of North Carolina, USA.

Study procedures

Three research assistants with previous medical training were trained on study procedures and cervical sampling to optimize the quality of cervical smears. After providing usual care clinicians at each site referred the patients to our research assistants who then explained the study to each eligible patient. After obtaining written informed consent, data on demographic and clinical characteristics was collected with the aid of a standardized questionnaire. The assistants then collected cervical smear samples using conventional methods. Two slides were made for each participant. The slides were immediately fixed using a 95% ethanol solution and stored at room temperature for the duration of patient enrollment. These slides were sent for analysis and storage in the laboratory of the Center for the Study and Control of Communicable Disease (CSCCD) of the Faculty of Medicine and Biomedical Sciences, Yaoundé, Cameroon.

Slides were stained by the Papanicolau's method and examined under the microscope by a trained cytologist. Each slide was scored according to the Bethesda 2001 system as unsatisfactory, negative, atypical squamous cells of uncertain significance (ASCUS), low-grade squamous intraepithelial lesions (LSIL), atypical squamous cells, cannot exclude high grade lesions (ASC-H), high-grade SIL (HSIL), or invasive cervical cancer[Solomon et al, 2002]. Cytology readings were conducted blinded of clinical characteristics. For quality control purposes, both research assistants and cytologists received specific training related to the study, two slides were made and analyzed for each patient (the most severe result was considered the final result, in case of differences

between both slides), and slides with lesions were double-checked by a cytologist external to the study (differences were resolved by consensus). Furthermore, a subset of 25 slides were reviewed by an experienced cytologist at the University of North Carolina at Chapel Hill – the percentage agreement on the presence of lesions was 76%, (kappa=0.49) while the percentage agreement on lesions being ASC-H/HSIL was 60%(kappa=0.26). The potential impact of these limitations with conventional cytology was assessed in sensitivity (bias) analyses [Rothman et al, 2008].

Statistical analysis

Data collected were entered into MS Access interface on Epi-info 2000. Statistical analyses were conducted using SAS version 9.2 (SAS institute inc, Cary NC) and STATA version 10 (STATA corps, Texas USA). Two outcomes, based on cervical cytology, were considered: 1) Prevalent cervical lesions (defined as the presence of *any* cervical epithelial lesions); 2) Prevalent ASC-H/HSIL (lesions requiring colposcopy). Participant's age, marital status, parity, number of lifetime sex partners, age at first sexual intercourse, history of hormonal contraception, history of exposure to cigarette smoke, CD4 count, and AIDS clinical stage were considered as potential clinical predictors of lesions. These characteristics were chosen based on the ease with which they can be elicited and recorded in a clinical setting and previous literature describing their association with the presence of cervical lesions or a plausible etiological role.

We assessed the univariate distribution of predictors and outcomes. We conducted bivariable and multivariable (adjusted) analyses to assess the association of each predictor with the outcomes. Prevalence odds ratios (OR) and their 95% confidence intervals(CI), comparing the odds of each outcome between predictor levels were estimated using unconditional logistic regression models. Continuous variables, such as age and CD4 count, were assessed graphically and statistically for the linearity of the logit. Graphically, we plotted the log-odds of cervical lesions by the continuous variable. The plots were smoothed

using locally weighted estimation methods with a smoothing parameter of 0.5[Rothman et al, 2008]. We also used a likelihood ratio test of the improved model fit with non-linear (quadratic terms) for the continuous variable. In the presence of evidence against linearity, continuous variables were coded as categorical variables using clinically meaningful cut-offs (for example 200 cells/uL for CD4 counts) or as a function of the shape of the graph of the log-odds of the outcome (for example 25-60 years versus others for age).

All predictors considered were included in multivariable analyses. For each outcome, an initial (full) model that includes all the predictors was created. We then attempted to determine a reduced model based on a parsimonious subset of clinically and statistically significant predictors identified by a stepwise backward elimination strategy [Harell, 2001]. A final reduced model was thus obtained retaining only predictors that when dropped resulted in a likelihood ratio test p-value<0.2 or a more than 10% change in the area under the curve of the ROC plot (c-statistic). A reduced model was possible only for the prediction of prevalent SIL (and not for the prediction of ASC-H/HSIL).

The models developed were based on the full sample. However the internal validity of the models' performance was ascertained by implementing the models to three subsets of the sample corresponding to each study site. The c-statistics for the models were all within 20% of the model implemented on the full sample.

One objective of this analysis was to identify if any clinical predictor(s) could be used in a resource-limited settings to develop a targeted screening approach. We thus developed and assessed potential risk scores for targeting screening only to patients more likely to have lesions. Risk scores were developed from each of the three final models: the two models for predicting the presence of any lesion (the full model and the reduced model) and the full model for predicting the presence of ASC-H/HSIL. The numeric score assigned to each predictor was based on the model slope coefficients. To allow for a simple and feasible application in clinical settings, each predictor score was obtained by multiplying the model

slope coefficients by 10 and then rounding to the lower integer. The aggregate risk score was based on the sum total of each predictor score. We assessed the performance (sensitivity, specificity, positive and negative predictive values) of each risk score for targeting 25%, 50% and 75% of women. We also evaluated the total errors that would result from implementing either targeted screening based on the risk scores versus universal or no screening. Total unweighted errors were estimated as the sum of 'false negative' and 'false positive' errors, respectively defined as the number of patients with no lesion being screened and the number of patients with lesions not being screened. Total weighted errors were also estimated taking into account the relative cost (both monetary and non-monetary) associated with having a 'false negative' versus a 'false positive' error.

4.4 RESULTS

Study population

Altogether 282 women were enrolled in this study. Participants' age ranged from 19 to 68 years, with a mean of 36 years (Table 4.1). Most participants (73.4%) were from urban areas. As many as 26.9% were widowed, while 21.3% had never been married. The median parity was 2 (range 0-11). Active tobacco exposure (2.5%) and oral contraceptive pill usage (23.8%) was relatively infrequent. The number of lifetime partners exceeded 5 in 25.2% of participants. A history of genital warts was reported by 7.1% of participants while 43.3% could not say if they previously had genital warts or not.

HIV diagnosis had preceded study enrolment by 18.5 months on average (range 0-136 months). The median CD4 count was 179 cells/microliter (interquartile range: 100 to 271). The vast majority of patients (80.9%) had advanced HIV disease (WHO HIV clinical stages III/IV). Only 2.1% were certain they had previously had a Pap smear.

Prevalence and severity of lesions

The prevalence of SIL was 43.5% (95%CI: 37.5, 49.6%) in the 276 women with satisfactory samples. The prevalence of specific abnormalities was ASCUS 0.7% (95%CI:

0.09, 2.3%), LSIL 25.0% (95%CI: 20.0, 30.5%), ASC-H 14.5% (95%CI: 10.6, 19.2%), and HSIL 3.3% (95%CI: 1.5, 6.1%). The overall prevalence of ASC-H/HSIL was 17.8% (95% CI: 13.4, 22.8%).

Association of clinical predictors and prevalent SIL

Most of the clinical factors assessed were either weakly associated with prevalent SIL or had a poor precision (Table 4.2). Compared to women from urban areas, SIL were more frequent in women from rural areas (adjusted OR: 1.68; 95%CI: 0.88, 3.18). SIL were also slightly more frequent in women aged 26-59 (aOR: 1.57; 0.65, 3.81). Although estimates were relatively imprecise, the odds of SIL were lower in women with HIV stage III/IV compared to women in stage I/II (aOR: 0.63; 95%CI: 032, 1.23). The odds of SIL did not differ substantially by marital status, educational status, previous exposure to tobacco smoke, previous pill usage, parity, age at first sex, time since HIV diagnosis or CD4 count (all aORs were more than 0.8 and less than 1.25).

The overall predictive value of the model with all these variables was relatively low with a c-statistic of 0.60. A reduced model included only two predictors: participants' residence (rural vs. urban aOR 1.58 (95% CI: 0.87, 2.87) and HIV clinical stage (HIV stage III/IV vs. I/II aOR 0.61 (95% CI: 0.31, 1.19)), with a c-statistic of 0.58.

Association of clinical predictors and lesions requiring colposcopy/biopsy

Bivariable and multivariable associations with ASC-H/HSIL were relatively weak (Table 4.2). However, ASC-H/HSIL were somewhat more frequent in women who reported age at first sexual intercourse less than 16 years (aOR: 1.27; 95%CI: 0.64, 2.53) as well as in patients with CD4 counts below 200 (aOR: 1.49; 95%CI: 0.72, 3.07). ASC-H/HSIL were less frequent in women self-reporting exposure to tobacco smoke (aOR: 0.66, 95%CI: 0.33, 1.34). The odds of ASC-H/HSIL did not differ substantially by age, marital status, education, residence, age, previous pill usage, parity, HIV clinical stage, or time since HIV diagnosis (all

aORs were more than 0.8 and less than 1.25). With a c-statistic of 0.59, the overall predictive value of the model with all these variables was also low.

Assessment of clinical risk scores for targeted screening

The clinical risk scores developed are defined and assessed in Tables 4.3(A and B). Overall the classification accuracy based on these scores were all less than 70%. If the risk score based on the full model for any SIL was used to target the screening of 25% of women, then only 35.7% of women with lesions would have been screened while 23% of women with no lesions would be screened as well. If the target was to screen 25% of women with ASC-H/HSIL, then using the score based on the full model predicting ASC-H/HSIL would result in 38.1% of women with ASC-H/HSIL lesions being screened while 26.7% of women with no ASC-H/HSIL would also be screened.

It is worth noting that despite the relatively poor performance of these scores, their value compared to universal screening or no screening was a function of the relative weight given to 'false negative' errors compared to 'false positive' errors. In screening for any lesion, when 'false negative' errors were considered equal to 'false positive' errors, then the total error rates associated with targeting 50% (406 errors per 1000 women) or 25% (410 errors per 1000 women) of women was lower than each of universal screening (565 errors per 1000 women) or no screening (435 errors per 1000 women). However, as the relative cost of 'false negative' errors (compared to 'false positive' errors) increased, universal screening tended towards having the least total errors while the total errors associated with screening fewer proportions of patients increased (Figure 4.1). Similar trends were observed in assessing potential screening for ASC-H/HSIL only (Figure 4.2). While no screening was associated with the least errors when 'false negative' errors were considered equal to 'false positive' errors, increasing the proportion of women screened was a preferable option when the former errors were at least five times the latter.

Bias analyses

We conducted bias analyses assessing what the true population prevalence of lesions could be considering the inaccuracies in conventional cytology. Our analysis showed that a lower sensitivity of cytology would mean that the study tended to underestimate the true prevalence while a lower specificity would have resulted in the study overestimating the true prevalence. Our data were compatible with a true population prevalence of lesions ranging from 19.3% (when the sensitivity was 100% and the specificity 70%) to 87.0% (when the sensitivity was 50% and specificity 100%). Meanwhile the prevalence of ASC-H/HSIL could be as low as 0% (if the specificity was 80% or less) and as high as 35.6% (when sensitivity was 50% and specificity 100%).

4.5 DISCUSSION

To appraise the need and potential for targeted screening for cervical cancer in HIV-positive women in resource-limited settings, we assessed the prevalence, severity and predictors of SIL in women initiating antiretroviral therapy in Cameroon. We document that the prevalence of precancerous lesions is high in these women. Approximately twenty percent of these women have lesions severe enough to warrant colposcopy. In this study population, readily available demographic and clinical factors, both individually and as a group, did not accurately distinguish women with lesions from those without.

While the overall prevalence of any cervical lesion in this population appears high (43%), it is within the range described in women in similar conditions. Among HIV-positive pregnant women in Yaounde, Cameroon, lesions were detected in 40%, including 12% with high grade lesions [Mbu et al, 2008]. The prevalence of SIL in HIV-seropositive women living in sub-Saharan Africa has ranged from 15 to 48% in Burkina Faso[Didelot-Rousseau et al, 2006], Kenya [Yamada et al, 2008], South Africa[Moodley and Garib, 2004; Gaym et al, 2007], Zimbabwe [Chirenje et al, 2002], Rwanda [Leroy et al, 1999], Malawi [Motti et al 1996] and Cote d'Ivoire [LaRuche et al]. The highest prevalence of SIL (76%) so far was

detected in a study of 150 HIV-positive women in Zambia who had a median CD4 count of 165/uL. Although an unusually low prevalence of 7% was reported in a study of 691 women in Tanzania, the vast majority (86%) of these women were in WHO clinical stage I and only 12% had CD4 count less than 200[Kapiga et al, 1999].

The prevalence in our study could be influenced by study population characteristics or the accuracy of cytology results. Though we sampled in three different clinics, the sample may not have been exactly representative of all HIV-positive women in Cameroon as the clinics were conveniently selected and not randomly chosen. We do not however expect the difference to be substantial as the clinics sampled offer care to a very high number of patients in regions with the highest HIV-prevalences and our participant characteristics were similar to those reported nationwide [Mosoko et al, 2009]. Potential errors due to the Pap smear's low sensitivity could actually mean that our prevalence is an underestimate. Nevertheless, the sensitivity of cytology in this study was assured by research assistants being trained to insure adequate sample collection, collecting two slides per patient to reduce sampling error and having the interpretations validated by an experienced cytologist. The latter review suggested a tendency of the initial cytologist to overclassify participants as having lesions. Our bias analyses however shows that the lowest true population prevalence of lesions compatible with our data was in the order of 19% which is still relatively high.

The high prevalence of cervical lesions in this population reiterates the current need to offer screening and care for cervical precancerous lesions in HIV-positive women. Despite being at increased risk for cervical cancer, less than 5% of participants in our study had previously been screened. Access to antiretrovirals has dramatically increased in developing countries. While the increased survival that is expected to accompany this increased access the effect on cervical precancerous lesions is expected to be moderate at best, as HAART has a very limited effect, if any, on HPV persistence and the progression of lesions [Heard,

2004]. Even while on antiretrovirals women will still need to be screened or receive other preventive care for cervical cancer.

We are not aware of any other study that assessed the potential for clinical risk scores for targeted cervical cancer screening in HIV-positive women. Because most studies were conducted to establish the association between HIV infection and the presence of SIL, very few assessed the risk factors specific to HIV-positive women. Amongst the few that did, immunosuppression (low CD4 count), high HIV viral load and or infection with high-risk HPV types tended to be associated with the presence of SIL[Kapiga et al, 1999; Yamada et al, 2008; LaRuche et al, 1998; Leroy et al, 1999]. While our sample size was adequate for estimating prevalence, only a limited number of covariates could be considered as potential predictors. Predictors were thus judiciously chosen taking into account the established literature and the ease of clinical assessment. Women with lower CD4 counts and younger age at first sex thus appeared more likely to have severe lesions. We did not consider HIV viral load as resource limitations render it difficult to ascertain in most patients, making it of little clinical use in this setting. Unexpectedly, women who self-reported exposure to cigarette smoke or a history of taking contraceptive pills tended to be less likely to have lesions. We attribute these apparent lower odds to these factors being indicators of higher socio-economic status in these settings. It is not clear why women with more advanced clinical HIV disease appeared less likely to have lesions. Nevertheless this association was relatively imprecise.

Considered as a group, the clinical predictors only slightly performed better than chance in differentiating women who had lesions (or women with severe lesions) from those without. For comparison the commonly used Framingham cardiovascular risk score had a c-statistic in the order of 0.65 -0.70 in a population of diabetics in the UK [Guzder et al, 2005]. Nonetheless, the choice between universal screening, targeted screening or no screening would depend on how much policy makers value the cost of ‘false negative’ errors relative to

the cost of ‘false positive’ errors. We expect that most would attribute a higher long-term cost to ‘false negative’ errors than to ‘false positive’ errors as cancer can develop in the former, while the latter would only result in an unnecessary pap smear. With this being the case, strategies advocating for more screening (thus with higher sensitivity and lower ‘false negatives’) will tend to be better options. Formal cost-effectiveness analyses may aid in further clarifying which option is best in each setting.

In addition to prevalence, the need to screen would also depend on the progression of lesions towards self-resolution or cancer. It was not possible to assess the progression or regression of lesions, given the cross-sectional nature of our study. Further studies would need to assess the long-term progression of lesions in HIV-positive women in this setting.

In conclusion, the prevalence of SIL in women initiating antiretroviral therapy in Cameroon was high. This high prevalence, in light of the potential to treat precancerous lesions when detected early and the limited role of HAART on the progression of lesions, underscores the need for screening in this population. In the absence of any accurate demographic or clinical predictor of SIL, alternative affordable screening options need to be explored. A prospective study of the long-term evolution of these lesions and their determinants is needed to further guide policy decisions.

Table 4.1: Socio-demographic and clinical characteristics in 282 women initiating HAART in Cameroon

Characteristic	Level	N	% or Mean (SD)*	Median*	Range *
Marital status	Never married	60	21.3		
	Married monogamous	56	19.9		
	Married polygamous	13	4.6		
	Living with a partner	30	10.6		
	Separated	31	11.0		
	Divorce	16	5.7		
	Widow	76	26.9		
Education	None	14	5.0		
	Primary	135	47.9		
	Secondary	121	42.9		
	Tertiary	12	4.3		
Residence	Urban	207	73.4		
	Rural	75	26.6		
Previous use of hormonal pills	Yes	67	23.8		
	No	215	76.2		
Exposure to tobacco smoke	No	141	50.0		
	Active	7	2.5		
	Passive	133	47.2		
	Missing	1	0.3		
WHO HIV clinical stage	I	8	2.8		
	II	46	16.3		
	III	168	59.6		
	IV	60	21.3		
Previous Pap smear	No	269	95.4		
	Yes	6	2.1		
	Don't know/missing	7	2.5		
Lifetime sex partners	1	9	3.2		
	2	29	10.3		
	3	56	19.9		
	4	34	12.1		
	5	35	12.4		
	6+	71	25.2		
	Missing	48	17.0		
Sex partners since HIV diagnosis	0	127	45.0		
	1	136	48.2		
	2+	19	6.8		

Characteristic	Level	N	% or Mean (SD)*	Median*	Range *
Lifetime condom use	Never	102	36.2		
	Less than 50% of the time	138	48.9		
	More than 50% of the time	39	13.8		
	Always	3	1.1		
Condom use since HIV diagnosis	Never	29	10.3		
	Less than 50% of the time	37	13.1		
	More than 50% of the time	19	6.7		
	Always	69	24.5		
	No new partner/missing	128	45.4		
History of genital warts	No	140	49.6		
	Yes	20	7.1		
	Don't Know/missing	122	43.3		
Age (years)		281	36.3 (9.6)	34	19 - 68
Age at first sex (years)		272	16.9 (2.4)	17	12 – 27
Parity		282	3.1 (2.6)	2	0 - 11
Time since HIV diagnosis (months)		282	18.5 (19.0)	12	0 – 136
CD4 count (per uL)		267	206 (170)	179	1 - 1759

* Mean (standard deviation), median and range for continuous characteristics.

Table 4.2: Association of clinical predictors with prevalent cervical precancerous lesions in 282 women initiating HAART in Cameroon

Characteristic	N	ANY CERVICAL PRECANCEROUS LESION					ASC-H/HSIL				
		Prev (%)	OR*	95% CI	aOR**	95% CI	Prev (%)	OR*	95% CI	aOR**	95% CI
Marital status											
Single, never married	59	42.4	1.	-	1.	-	18.6	1.	-	1.	-
Married/in partnership	98	43.9	1.06	0.55, 2.04	0.99	0.47, 2.11	17.4	0.92	0.40, 2.12	0.95	0.37, 2.49
Separated/divorced/widowed	119	43.7	1.05	0.56, 1.98	0.95	0.45, 2.01	17.7	0.94	0.42, 2.10	0.89	0.34, 2.34
Education											
None/primary	144	43.1	1.	-	1.	-	17.4	1.	-	1.	-
Secondary/tertiary	132	43.9	1.04	0.64, 1.67	1.24	0.71, 2.16	18.2	1.06	0.57, 1.96	1.05	0.51, 2.18
Residence											
Urban	204	39.7	1.	-	1.	-	16.7	1.	-	1.	-
Rural	72	54.2	1.79	1.04, 3.09	1.68	0.88, 3.18	20.8	1.32	0.67, 2.59	1.20	0.52, 2.74
Age (years)											
25 and less or 60+	32	37.5	1.	-	1.	-	21.9	1.	-	1.	-
26-59	243	44.0	1.31	0.61, 2.80	1.57	0.65, 3.81	16.9	0.72	0.29, 1.79	1.07	0.35, 3.24
Previous use of pills											
No	210	43.8	1.	-	1.	-	18.6	1.	-	1.	-
Yes	66	42.4	0.95	0.54, 1.65	0.95	0.50, 1.83	15.2	0.78	0.37, 1.67	0.83	0.34, 2.01
Exposure to tobacco smoke											
No	137	46.0	1.	-	1.	-	19.7	1.	-	1.	-
Yes	139	41.0	0.82	0.51,	0.86	0.51,	15.8	0.77	0.41,	0.66	0.33,

				1.32		1.46			1.42		1.34
Age at first sex (years)											
16 or less	126	45.2	1.18	0.71, 1.98	1.17	0.69, 2.01	19.1	1.27	0.64, 2.53	1.31	0.65, 2.63
More than 16	141	41.1	1.	-	1.	-	15.6	1.	-	1.	-
Parity											
2 or less	152	42.1	1.	-	1.	-	17.8	1.	-	1.	-
More than 2	124	45.2	1.13	0.70, 1.83	1.04	0.59, 1.83	17.7	1.00	0.54, 1.86	0.95	0.45, 2.00
Time since HIV diagnosis											
1 year or less	165	44.2	1.	-	1.	-	18.2	1.	-	1.	-
More than 1 year	111	42.3	0.93	0.57, 1.50	0.83	0.49, 1.42	17.1	0.93	0.49, 1.75	0.97	0.48, 1.97
WHO HIV stage											
I/II	51	54.9	1.	-	1.	-	19.6	1.	-	1.	-
III/IV	225	40.9	0.57	0.31, 1.05	0.63	0.32, 1.23	17.3	0.86	0.40, 1.86	0.95	0.39, 2.27
CD4 count (/µL)											
Less than 200	155	42.6	1.04	0.61, 1.78	1.10	0.64, 1.88	19.4	1.46	0.71, 3.09	1.49	0.72, 3.07
200 or more	106	41.5	1.	-	1.	-	14.2	1.	-	1.	-

*OR: unadjusted odds ratio; **aOR: adjusted for all other covariates in table; OR: Prev: Prevalence; Odds ratio; CI: confidence interval.

Table 4.3A: Definition of clinical risk scores developed and assessed for predicting the prevalence of cervical precancerous lesions in women initiating HAART in Cameroon

Characteristic	Score
Full model for predicting prevalent SIL	
HIV diagnosed within 1 year	2
Age at first sex less than or equal 16 years	2
Not exposed to tobacco	2
Secondary/tertiary education	2
Rural residence	5
WHO HIV Stage I/II	5
Age 26-59 years	5
TOTAL	**23**
Full model for predicting prevalent ASC-H/HSIL	
Neither separated/widowed/divorced	1
Aged 26-59	1
Rural residence	2
No previous use of pills	2
Age at first sex less than or equal 16 years	3
Not-exposed to tobacco	4
CD4 count less than 200	4
TOTAL	**17**

Table 4.3B: Performance of potential clinical risk scores for targeting cervical screening in 282 women initiating HAART in Cameroon

Targeted proportion to screen	Cut-off predictive probability	Cut-off score	SENS (%)	SPEC (%)	PPV (%)	NPV (%)	Total unweighted** errors (per 1000 women)
Detection of any SIL							
Risk score for any SIL							
Screen 25%	> 0.500	> 14	35.7	77.0	54.0	61.3	410
Screen 50%	> 0.434	> 11	45.2	70.4	53.6	62.9	406
Screen 75%	> 0.381	> 8	81.7	30.9	47.2	69.1	470
Universal screening (100%)*	None	None	100.0	0.0	43.5	NA	565
No screening (0%)*	None	None	0.0	100.0	NA	56.5	435
Detection of ASC-H/HSIL							
Risk score for ASC-H/HSIL							
Screen 25%	> 0.201	> 12	38.1	73.3	22.2	85.6	330
Screen 50%	> 0.171	> 10	61.9	51.4	20.3	87.1	467
Screen 75%	> 0.134	> 7	76.2	32.9	18.5	87.3	594
Universal screening (100%)*	None	None	100.0	0.0	17.8	NA	822
No screening (0%)*	None	None	0.0	100.0	NA	82.2	178

SENS: Sensitivity; SPEC: Specificity; PPV: Positive predictive value; NPV: Negative predictive value; SIL: Squamous intra-epithelial lesion; NA: Not applicable because using cut-off resulted in every woman being targeted for screening.
*included for comparative purposes; ** total unweighted errors = sum of 'false positives' and 'false negatives'

Figures

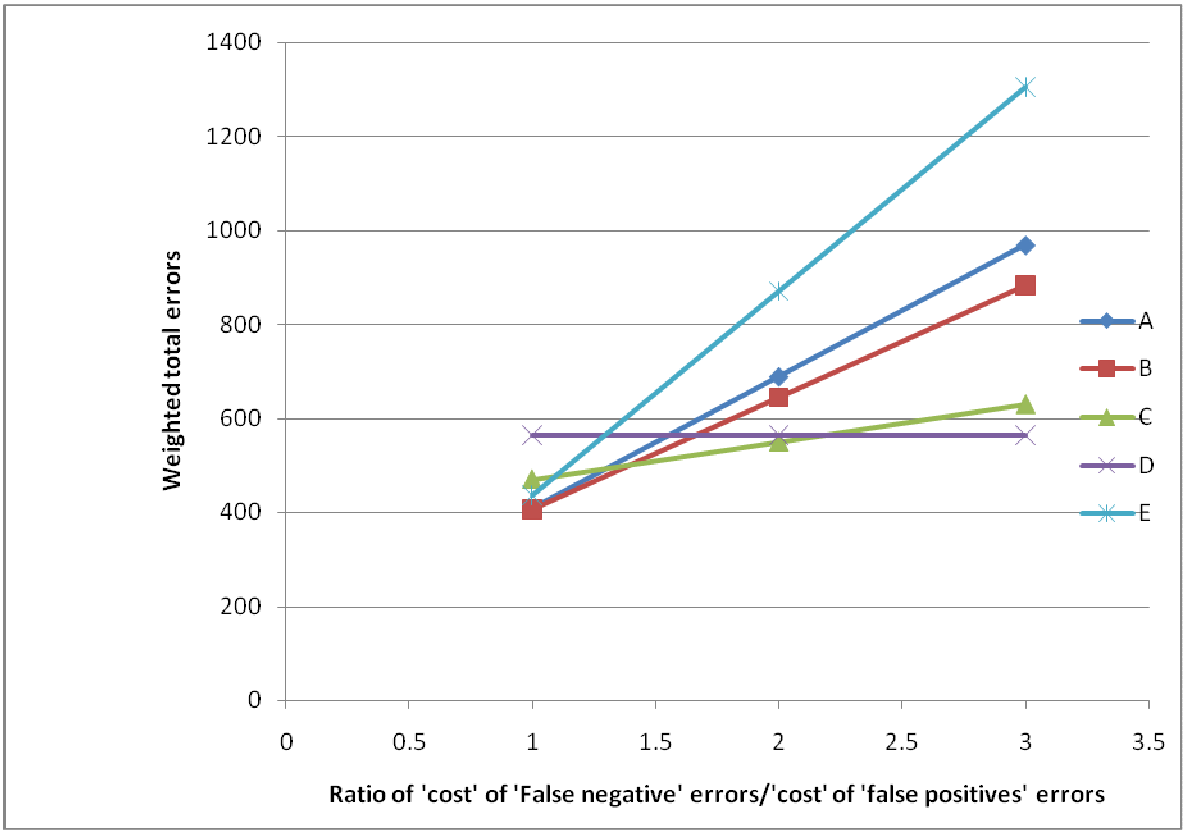


Figure 4.1: Total weighted errors associated with screening for any precancerous lesion by the relative 'cost' of 'false negative' errors compared to 'false positive' errors. (A-C: Screening based on risk score with a cut-off targeting 25%, 50% and 75% of women for A,B and C respectively; D: Universal screening; E: No screening). Note that 'cost' is used as a generic term, not just limited to monetary value, while 'false positive' and 'false negative' respectively refer to screening a patient with no lesion and not screening a patient with lesions.

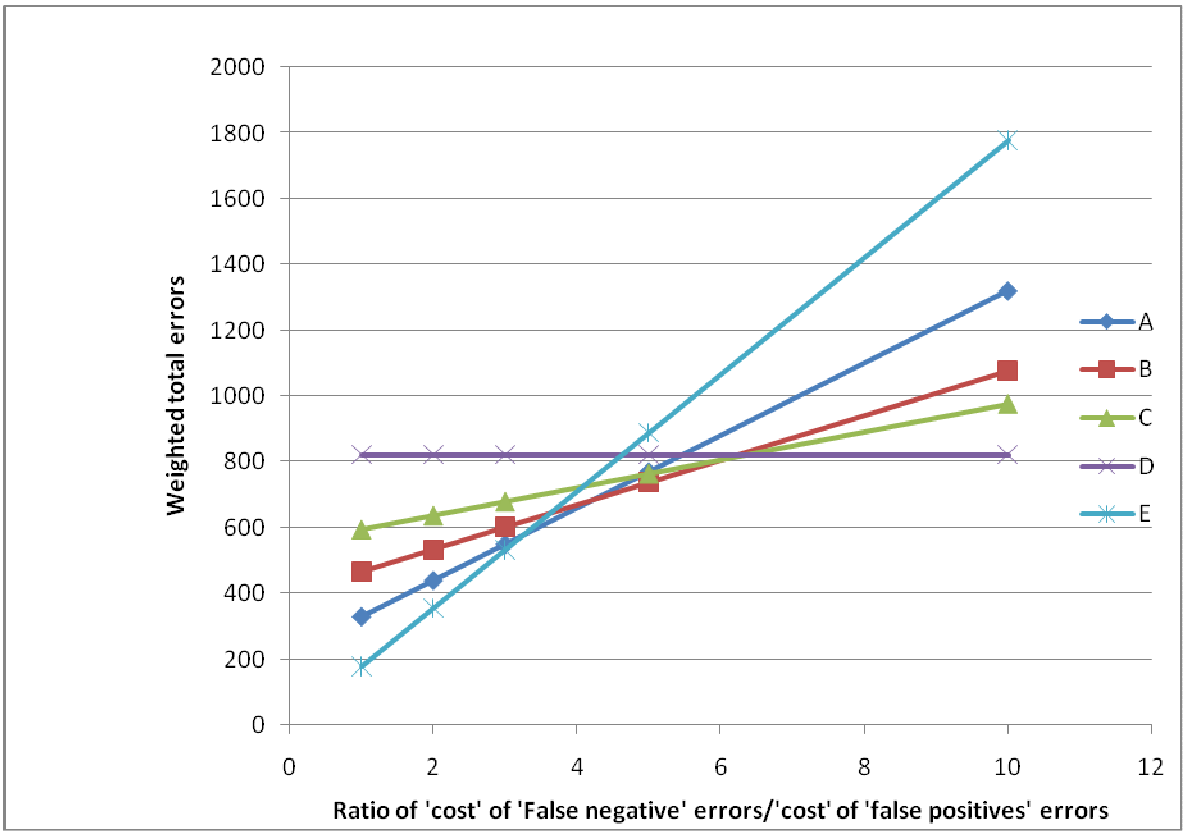


Figure 4.2: Total weighted errors associated with screening for ASC-H/HSIL by the relative 'cost' of 'false negative' errors compared to 'false positive' errors. (A-C: Screening based on risk score with a cut-off targeting 25%, 50% and 75% of women for A,B and C respectively; D: Universal screening; E: No screening). Note that 'cost' is used as a generic term, not just limited to monetary value, while 'false positive' and 'false negative' respectively refer to screening a patient with no severe lesion and not screening a patient with severe lesions.

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CHAPTER FIVE: AGE AND THE PREVALENCE OF CERVICAL SQUAMOUS INTRAEPITHELIAL LESIONS AMONG HIV-POSITIVE WOMEN IN CAMEROON

5.1 ABSTRACT

Background: Cervical squamous intra-epithelial lesions (SIL) are more frequent in HIV-positive women overall. However the appropriate age at which to begin and end cervical cancer screening for early detection of lesions in HIV-positive women is not clear. We assessed the age-specific prevalence of any SIL and SIL requiring colposcopy in HIV-positive women in Cameroon.

Methods: We enrolled, interviewed and conducted conventional cervical cytology in 282 women, aged 19-68 years, initiating antiretroviral therapy in three clinics in Cameroon.

Results: SIL were detected in 43.5% of the 276 women with satisfactory samples, 17.8% of whom had ASC-H/HSIL. On average, women aged 26 to 59 tended to have a slightly higher prevalence of any SIL than other women (Prevalence difference PD: 6.5%; 95%CI: -11.4, 24.4%). This PD was a function of CD4 count (heterogeneity test p-value =0.09): amongst patients with CD4 counts less than 200cells/uL, the prevalence was higher in patients aged 26-59, while there was essentially no difference amongst women with CD4 counts greater than 200 cells/uL. ASC-H/HSIL were present in women as young as 19 and as old as 62.

Overall the prevalence of ASC-H/HSIL increased by 0.7% (95%CI: -3.8%, 5.1%) per decade increase in age.

Conclusion: Both severe and less severe lesions were prevalent at all ages suggesting little utility of age-targeted screening among HIV-positive women. Nevertheless, the long-term evolution of these lesions needs to be assessed in prospective studies.

5.2 INTRODUCTION

Cervical cancer is the second most common cancer in women worldwide [Stewart and Kleihues, 2003]. Although cervical cancer incidence and mortality is higher in HIV-positive women, resource limitations restrict the implementation of systematic screening programs in these women in developing countries. With the recent increase in access to antiretroviral therapy HIV-positive women are expected to live longer, potentially allowing sufficient time for cervical cancer to develop. Targeted screening could potentially alleviate the strain on resources needed to screen these women.

Age has been a common consideration in the targeted screening for precancerous lesions in the general population. Current guidelines for screening the general population of women in the United States (US) suggest screening commence no later than age 21 years, reducing the frequency of screening at age 30 among women with previously negative cytology results and stopping screening at age 65 (or 70 in some guidelines) [Saslow et al, 2002; ACOG 2003; USPSTF, 2008]. World Health Organization (WHO) guidelines aimed primarily at resource-limited settings are less stringent, recommending screening begin at age 30, need not be annual and need not be done over the age of 65 [WHO, 2006]. These age considerations may not necessarily be ideal for HIV-positive women among whom higher human papilloma virus (HPV) prevalence, higher HPV persistence, and a faster

progression of lesions [Sun et al, 1997; Six et al, 1998; Cu-Uvin et al, 1999; Ahdieh et al, 2000; Massad et al, 2001; Palefsky, 2006; De Vuyst et al, 2008;] could mean an earlier occurrence and or a longer persistence of precancerous lesions. The optimal age for screening in HIV-positive individuals could thus be younger than for women in the general population.

We describe here the age-specific prevalence of lesions in HIV-positive women initiating antiretroviral therapy in Cameroon, with the aim of estimating the minimum age at which lesions occur, the age with maximum occurrence and the latest age at which lesions occur.

5.3 METHODS

Study design and population

In this cross-sectional study, HIV-positive women were recruited from three HIV-care clinics in Cameroon: the Bamenda Provincial Hospital AIDS Treatment Center (ATC), the Limbe Provincial Hospital ATC and the Nylon District Hospital ATC in Douala. These are all located in urban areas in Cameroon and provide regular care to patients from surrounding urban areas and peripheral rural areas. Consecutive HIV-positive women receiving care in these clinics, between August and September 2008, were invited to participate in the study. Women aged 18 years or more, who initiated HAART within a year of study enrollment and consenting to study procedures were eligible. Women who were either pregnant, bleeding due to menses or had a previous total hysterectomy were excluded. After obtaining written consent from each participant, socio-demographic and clinical data were collected using a structured interview, a clinical examination and a review of medical records. Cervical cell samples were then collected using Ayre's spatula, and smeared into two pre-labeled slides.

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Conventional cytology slides collected were transported to the laboratory of the Center for the Study and Control of Communicable Diseases (CSCCD) in Yaounde,

Cameroon where they were stained by the Papanicolau's method and examined under the microscope by a trained cytologist. The stained slides were observed under the microscope (at 400X) and then scored according to the Bethesda 2001 system, as unsatisfactory; negative; atypical squamous cells of uncertain significance (ASCUS); low-grade squamous intraepithelial lesions (LSIL); atypical squamous cells, cannot exclude high grade lesions (ASC-H); high-grade SIL (HSIL); or invasive cervical cancer [Solomon et al, 2002]. For quality control purposes, both research assistants and cytologist received specific training related to the study, two slides were made and analyzed for each patient (the most severe result was considered the final result, in case of differences between both slides), and slides with lesions were double-checked by a cytologist external to the study (differences were resolved by consensus). Furthermore, a subset of 10% slides were reviewed by an experienced cytologist at the University of North Carolina at Chapel Hill – the percentage agreement was on the presence of lesions was 76%, (kappa=0.49) while the percentage agreement on lesions being ASC-H/HSIL was 60%(kappa=0.26). The potential impact of these limitations with conventional cytology were assessed in sensitivity (bias) analyses [Rothman et al, 2008]. The study was approved by relevant ethical committees in Cameroon and the University of North Carolina, Chapel Hill (USA).

Data analysis

Data collected were entered into MS Access interface on Epi-info 2000. Statistical analysis were conducted using SAS version 9.2 (SAS institute inc, Cary NC) and Stata version 10 (Stata corps, Texas USA). Two outcomes were considered for this analysis: 1) Prevalent cervical lesions (defined as the presence of *any* cervical epithelial lesions); 2) Prevalent ASC-H/HSIL. Age (in years) was the independent predictor considered. Other covariates considered in this analysis included marital status, education level, parity, history of hormonal contraception, smoking history, CD4 count, and AIDS clinical stage. Univariable

distributions of these characteristics were determined by computing means, median and ranges (for continuous variables) and proportions at different levels (for categorical variables).

In bivariable analyses, the crude relationship between each outcome and age was assessed using locally weighted regression (LOWESS) methods with a smoothing parameter of 0.5 [Rothman et al, 2008]. In subsequent analyses, we used generalized linear models with prevalence as the outcome, an identity link and a binomial distribution, as we sought to estimate prevalence differences [Rothman et al, 2008]. We explored coding age as a continuous variable (linear or quadratic) or coding age as a categorical variable with cut-offs based on the LOWESS-smoothed curve. For each outcome, the coding of age that resulted in the best model fit (or least deviance), as assessed by a likelihood ratio test (for nested models) or the Akaike's Information criterion (for non-nested models) was selected. Age coded as a binary variable (age 26-59 or not) had the best fit in modeling the association of age and any lesion, while age coded as a continuous linear variable had the best fit in modeling the association of age and ASC-H/HSIL.

In multivariable analysis all covariates other than age and the outcomes were assessed as potential modifiers of the prevalence difference. Each covariate was coded as a binary variable and a product interaction term created between age and each covariate. Covariates were considered modifiers if a likelihood ratio test of the product interaction term had a p-value less than 0.1, (a higher cut-off point set *a priori* to account for the low power associated with tests of homogeneity) or if the stratum-specific prevalence differences varied by 20% or more.

Although all covariates were also considered as potential confounders, a Directed Acyclic Graph (DAG) analysis revealed that none of the variables should be considered a confounder [Rothman et al, 2008]. We attempted to mathematically estimate the minimum and maximum ages at which cervical lesions are present as well as the age with the

maximum prevalence of lesions assuming a quadratic relationship between age and prevalent lesions. Only the age of maximum prevalence could however be estimated as all other models resulted in extrapolations beyond biologically plausible ages. Age was centered in these models to allow for a meaningful interpretation of all model parameters [Rothman et al, 2008].

5.4 RESULTS

Altogether 282 women were enrolled in this study. Participants' age ranged from 19 to 68 years (with a mean of 36 years). The median CD4 count was 179 cells/microliter (interquartile range: 100 to 271). SIL were detected in 43.5% of the 276 women with satisfactory samples: 0.7% as ASCUS, 25.0% as LSIL, 14.5% as ASC-H, and 3.3% as HSIL.

Prevalence of lesions by age

The age of participants with no lesions ranged from 19 to 68 (with a mean of 36.3) years while that of participants with any lesion ranged from 19 to 62 (with a mean of 35.7) years. The prevalence of any lesion tended to increased from age 19 to a peak at about 25 years (Table 5.1), from which it stabilized between 40% and 50% until the age of 60, after which it reduced among the small number of women surveyed (Figure 5.1).

On average women aged 26 to 59 had a slightly higher prevalence than relatively younger or older women (Prevalence difference PD: 6.5%; 95%CI: -11.4, 24.4%). However this PD was a function of CD4 count (heterogeneity test p-value =0.09). Amongst patients with CD4 counts less than 200cells/uL, women aged 25-59 had a substantially higher prevalence (PD= 21.0%; 95% CI: -0.8%, 42.8%). In contrast, there was only a little difference in prevalence by age among women with CD4 counts greater than 200 cells/uL (PD= -9.8%; 95%CI: -37.8%, 18.3%).

We conducted bias analyses assessing what the true population prevalence difference of lesions could be considering the inaccuracies in conventional cytology. The misclassification of the outcome resulting from these inaccuracies was assumed to be non-differential as the cytologist was masked from participants' ages. Our analysis showed that a lower cytology sensitivity or specificity would mean that the study tended to underestimate the magnitude of the prevalence difference between age groups (Figure 5.2). For example with a sensitivity of 70% and a specificity of 90% among women with CD4 counts less than 200, the prevalence of lesions in women aged 25-59 could be 35% higher than in younger or older women. A similar sensitivity and specificity among women with CD4 counts more than 200, could correspond to a 16.3% lower prevalence of lesions in women aged 25-59 compared to younger or older women.

Assuming a quadratic relationship between age and the prevalence of SIL, the age with maximum prevalence was estimated to be 34.9 (95% CI: 11.6, 58.1) years.

Prevalence of ASC-H/HSIL by age

The age of participants with ASC-H/HSIL ranged from 19 to 62 (with a mean of 36.5) years. In contrast to any lesion, the age-specific prevalence of ASC_H/HSIL increased slowly but more or less monotonically with age (Figure 5.1). On average, the prevalence of ASC-H/HSIL increased by 0.7% (95%CI: -3.8%, 5.1%) per decade increase in age. The age-specific prevalence of ASC-H/HSIL did not appear to differ by CD4 count.

5.5 DISCUSSION

Data on age-specific prevalence of SIL are needed if age-targeted screening is to be considered in HIV-positive women. In this paper, we show that while the prevalence of SIL appeared highest in the third and fourth decades of life, and the prevalence of ASC-H/HSIL gradually increased with age, the prevalence of lesions did not appear to be age-limited.

The epidemiology of SIL in the general population of (mainly HIV-negative) women has been at the origin of age-targeted screening in these women. Studies conducted in the 1980s, documented the prevalence of lesions in young women [Macgregor and Teper, 1978; Chung et al, 1982; Benmoura et al, 1986]. Subsequent studies showed that the peak of occurrence of precancerous lesions was in the third to fourth decade while malignant lesions tended to occur later in the fourth or fifth decade[Sadeghi et al, 1989; The New Zealand Contraception and Health Study group, 1989; Das et al, 1992; Gupta et al, 2008]. Lesions also appeared to be less frequent in women far past menopause [Wheat et al, 1988].

In this study limited to HIV-positive women, the prevalence of lesions was only slightly higher in all women aged 25-59 compared to other women. This suggests that, unlike in HIV-negative women, age only may not be a good criterion for targeted screening. Age differences in the prevalence of lesions, however, appeared to depend on CD4 counts. Amongst women with low CD4 counts, middle-aged women had a higher prevalence than younger or older women, suggesting that screening efforts are particularly needed in these women. To the best of our knowledge few studies have discussed the age-specific prevalence of lesions and severe lesions in HIV-positive women. Unlike our study in which the prevalence of ASC-H/HSIL lesions increased with age, Parham et al [2006] described an inverse-U trend among 691 HIV-positive women aged 23-49 years in Zambia, with a peak prevalence of HSIL/invasive cancer between age 35 and 40 years. It is not clear why these findings differ but the variations in study population age, the relatively low CD4 counts (median of 165) and slightly different outcomes may have contributed to this difference.

While we document prevalent ASC-H/HSIL at all ages in HIV-positive women, it is not clear what the long-term outcome of these lesions would be and this may depend on age. It is conceivable that if lesions were less likely to progress in younger versus older women then targeting older women would be justified or vice versa. Prospective studies in

HIV-negative women have had inconsistent results: while lesions were more likely to progress in older women in some studies[Misra et al, 2006] the majority of studies noted similar progression rates irrespective of age [Konno et al, 1998; Knudsen et al, 2003; Wright et al, 2005; Giannopoulos et al, 2005; Winn and Jones, 2005]. Similar studies need to be conducted in HIV-positive women with limited access to systematic screening.

Our findings are susceptible to bias from misclassification of outcomes as conventional cytology typically has a low sensitivity [Sankaranarayanan et al, 2005]. Nonetheless, because the cytologists were masked from participants' age information, these errors are expected to be independent of age (non-differential) potentially biasing our effect estimates towards the null (resulting in an underestimate of the difference in prevalence by age groups).

Secondly, because of the cross-sectional design of the study, the age-specific prevalence described here reflects the age of lesion detection and not necessarily the age of incidence or the age-specific prevalence in the population. Age differences in access to clinics may result in artificially increased prevalence in older women who are more likely to be in the health care system. The latter detection bias is however expected to be minimal in a study population of HIV-positive women in whom access to care is largely driven by worsening HIV disease rather than age. Four in five women in this study had advanced HIV diseases (WHO stage III or IV) and the small number of women aged 50 or more than limited the influence of these women on study estimates.

In conclusion, cervical precancerous lesions were prevalent at all ages in this population of HIV-positive women, suggesting little utility of age-targeted screening. A better understanding of the value of age-targeted screening would require an assessment of the age-specific long-term evolution of untreated non-severe lesions and treated severe lesions using prospective studies. The potential costs and benefits associated with age-targeted screening will also need to be evaluated in formal cost-effectiveness analyses.

Table 5.1: Age-specific prevalence of cervical precancerous epithelial lesion in 276 women initiating HAART in Cameroon

Age (Years)	N	Prevalence of any lesion		Prevalence of ASC-H/HSIL	
		%	95% CI	%	95% CI
18-24	16	31.3	11.0, 58.7	6.3	01.6, 30.2
25-34	124	45.2	36.2, 54.3	18.5	12.1, 26.5
35-44	80	43.8	32.7, 55.3	15.0	08.0, 24.7
45-54	42	40.5	25.6, 56.7	16.7	07.0, 31.4
55-59	7	57.1	18.4, 90.1	42.9	09.9, 81.6
60+	6	33.3	04.3, 77.7	33.3	04.3, 77.7

FIGURES

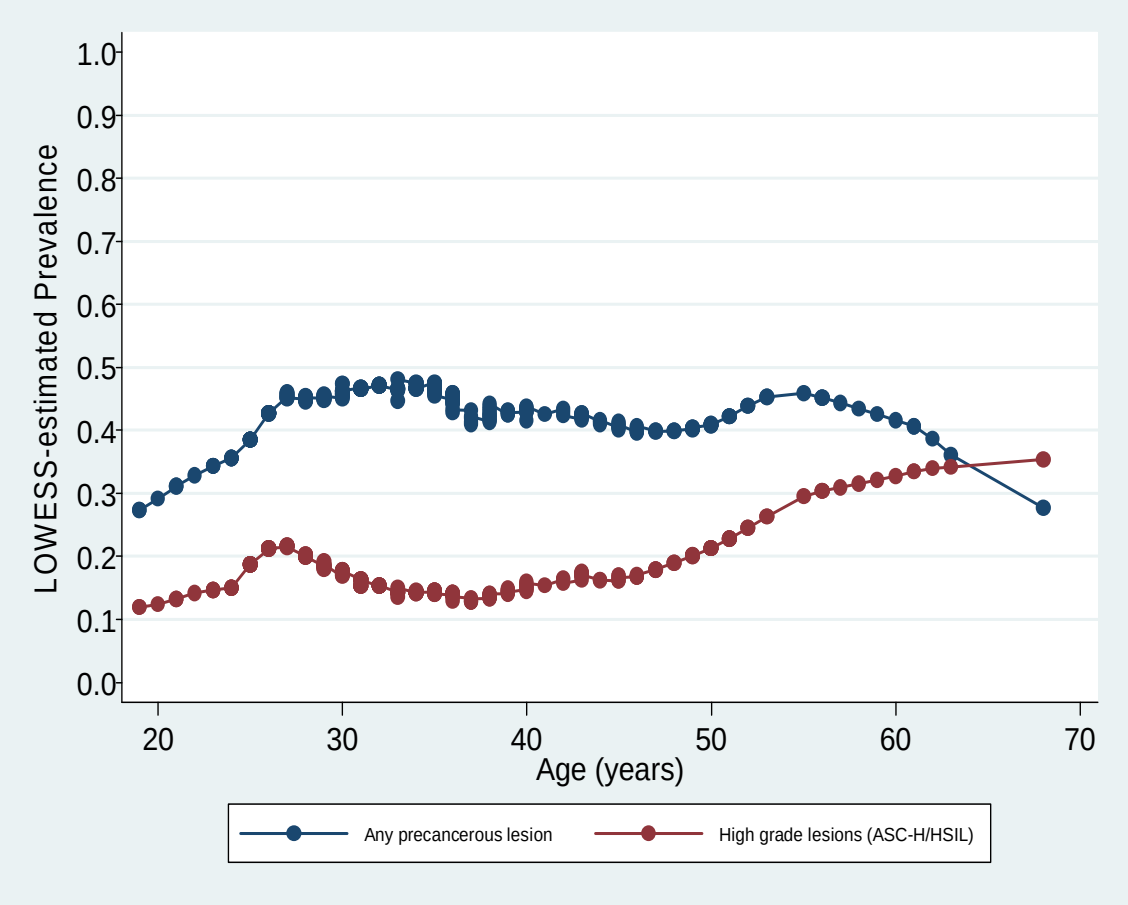

Figure 5.1: Trends in age-specific prevalence of precancerous lesions and ASC_H/HSIL in 276 women initiating HAART in Cameroon (estimates based on locally weighted regression models).

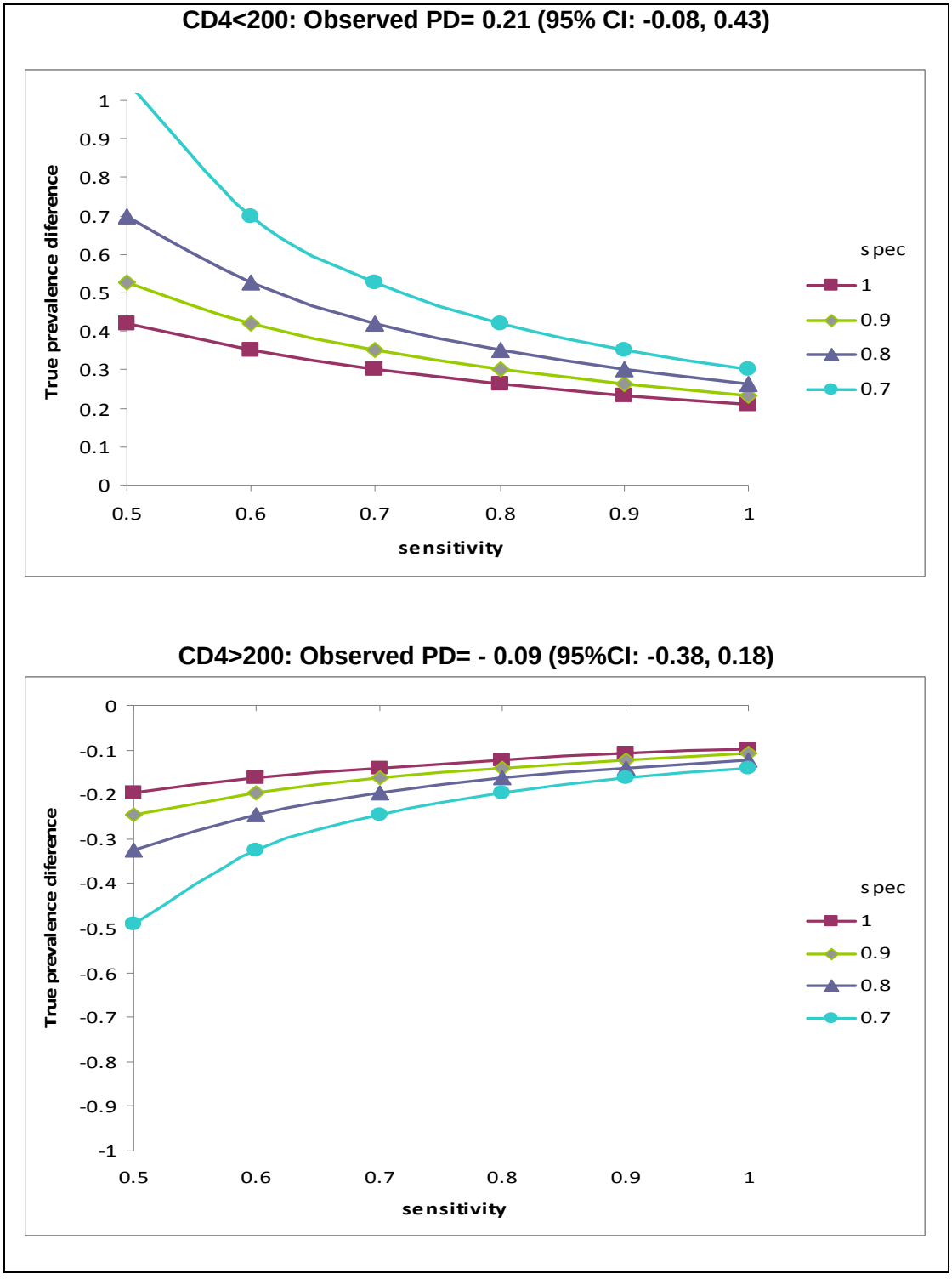

Figure 5.2: Sensitivity analysis of outcome misclassification on the observed prevalence difference between age groups (26-59 versus 18-25 and 60+ years) by CD4 count

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CHAPTER SIX: POTENTIAL IMPACT OF ANTIRETROVIRAL THERAPY AND SCREENING ON CERVICAL CANCER MORTALITY IN HIV-POSITIVE WOMEN IN SUB-SAHARAN AFRICA

6.1 ABSTRACT

Background: Despite having high cervical cancer incidence and mortality rates, screening for cervical precancerous lesions remains infrequent in sub-Saharan Africa. The need to screen HIV-positive women because of the higher prevalence and faster progression of cervical precancerous lesions may be heightened by the increased access to highly-active antiretroviral therapy (HAART). Policymakers need quantitative data on the effect of HAART and screening to better allocate limited resources. Our aim was to quantify the potential effect of these interventions on cervical cancer mortality.

Methods: We constructed a Markov state-transition model of a cohort of HIV-positive women in Cameroon. Published data on the prevalence, progression and regression of lesions as well as mortality rates from HIV, cervical cancer and other causes were incorporated into the model. We examined the potential impact of four possible scenarios: no HAART and no screening (NHNS), HAART and no screening (HNS), HAART and screening once on HAART initiation (HSHI), and HAART and screening once at age 35 (HS35).

Findings: Our model projected that, compared to NHNS, lifetime cumulative cervical cancer mortality approximately doubled with HNS. It will require 262 women being screened on HAART initiation to prevent one cervical cancer death amongst women on HAART. The magnitudes of these effects were most sensitive to the rate of progression of precancerous lesions.

Interpretation: Screening has the potential of reducing cervical cancer mortality among HIV-positive women in Africa. The most feasible and cost-effective screening strategy needs to be determined in each setting.

6.2 INTRODUCTION

Cervical cancer is one of the leading causes of cancer death among women in sub-Saharan Africa [Ferlay et al., 2004]. More than 10 million HIV-infected women also live in this region [UNAIDS, 2008]. Compared to HIV-negative women, HIV-positive women have a higher prevalence of cervical precancerous lesions as well as a faster progression of these lesions to invasive cancer[Six et al, 1998; Massad et al, 2001; Schuman et al, 2003; Hawes et al, 2006; De Vuyst et al, 2008].

Mortality from cervical cancer and mortality from other HIV-associated diseases can be competing risks in the evolution of each other disease. Death from cervical cancer would prevent further progression of HIV disease and, prior to the advent of highly active antiretroviral therapy (HAART), the progression of cervical precancerous lesions to cancer was largely averted by early death from AIDS and other opportunistic infections. We hypothesize that the increased survival that is expected to result from increased access to HAART may be large enough to allow for lesions to progress to invasive cervical cancer and thus is likely to be followed by an increase in cervical cancer incidence and mortality.

In developed countries, the potential increase in cervical cancer mortality in HIV-positive women is curtailed by systematic and frequent screening in these women [Franceschi and Jaffe, 2007]. Despite having a higher HIV prevalence and cervical cancer incidence, screening remains very infrequent in developing countries presumably because of resource-limitations. Policymakers need data on the effect of screening on cervical cancer mortality to better allocate limited resources.

The long term effect of cervical cancer screening in HIV-positive women in sub-Saharan Africa, particularly in the era of HAART remains unknown. Although HAART is expected to increase cervical cancer mortality while screening is expected to reduce this mortality, the magnitude of these effects need to be estimated to better guide policy. In this paper, we estimate the size the potential effect of HAART therapy with or without screening on the mortality due to cervical cancer in Cameroon.

6.3 METHODS

Model structure

We developed a state-transition Markov model, using TreeAge Pro™ 2008 Healthcare Module (TreeAge Software Inc., Williamstown, MA, USA). This type of model allows analysts to model transitions of a cohort of patients among a number of health states over a long period of time subdivided into a series of short intervals [Nainmark et al, 1997]. Our model was designed to simulate the evolution over time of HIV infection and cervical precancerous and cancerous lesions in a cohort of HIV-positive women in Cameroon. The primary structure of the Markov model was based on a previous description of a model implemented by Goldie et al [1999] in HIV-positive women in the US (Figure 6.1). In brief, the model summarizes the progression of cervical neoplasia in HIV in five states: normal with no lesion, low-grade squamous intra-epithelial lesions (LSIL), high-grade squamous

intra-epithelial lesions (HSIL), invasive cervical cancer and death. Each of the four (non-death) states is stratified by CD4 cell count. The cancer stage is further stratified by stage of cervical cancer and whether cancer has been diagnosed (thus being treated) or not. During their lifetime, women's disease state can progress from normal to LSIL to HSIL to cervical cancer. Women in the HSIL and LSIL states can regress to lower states. Death can occur to women in any of the four states and can result from cervical cancer, HIV related-causes or other causes of death.

Model parameters

Parameters used in the model were abstracted from the published literature and reflected data for Cameroon as much as possible. The values used in the baseline model are shown in Table 6.1. The baseline model was designed to simulate the progression over time of a cohort of HIV-positive women aged 25 with CD4 count >500, 30% of whom had precancerous lesions (one-third of which were high grade lesions). All cause age-specific mortality rates were estimated based on abridged life tables for women in Cameroon [WHO, 2008]. HIV mortality rates were estimated based on WHO data [UNAIDS, 2007].The proportion of HIV-mortality that occurs in each CD4 category was estimated based on data by Goldie et al [1999]. Cervical cancer mortality rates were also abstracted from WHO estimates of annual cervical cancer incidence and deaths in Cameroon [Ferlay et al., 2004]. Age-specific mortality rates from other causes were estimated by adjusting (using another Markov analysis) all-cause age-specific mortality rates to deduct mortality from cervical cancer and mortality from HIV.

In the absence of published data on long-term progression or regression rates of precancerous lesions in HIV positive women in Cameroon, we used published estimates from women in the pre-HAART era from Goldie et al [1999]. The effect of HAART on

HIV/AIDS-related mortality was also estimated based on the published literature [Murphy et al, 2001; Mermin et al, 2008]

Model assumptions

Key simplifying assumptions of the model include: 1)The natural history of cervical cancer involves progress from normal to LSIL to HSIL to local cancer to regional/distant cancer to death from cancer, without skipping. 2) The regression of neoplasia can only be from HSIL to normal or LSIL, of from LSIL to normal. A patient cannot regress from cancer. Cancer stages also cannot regress. 3) HIV diseases progression is only from CD4 500+ to 200-500 to <200. This is a historic parameter, indicating the advance in HIV disease. Once a patient has CD4 <200 she will always be classified in the CD4 <200 category, even if her actual CD4 count improved with treatment. In other words worsening HIV-disease cannot regress. This assumption is consistent with data that show that improvements in antiretroviral therapy do not appear to reduce the progression of precancerous lesions even amongst women with improved CD4 counts [Palefsky, 2003]. 4) The progression/regression rate of cervical precancerous lesions is not dependent on a previous history of precancerous lesions as the parameters used in the model are from heterogeneous populations (that include patients with and without a previous history of lesions) [Goldie and Kuntz, 2003].

Scenarios and outcomes assessed

We assessed the projected lifetime cumulative mortality due to cervical cancer in four plausible scenarios of HIV and cervical cancer care in Cameroon: no HAART and no screening (NHNS), HAART when indicated and no screening (HNS), HAART when indicated and screening on HAART initiation (HSHI), and HAART when indicated and a single screen at age 35. The age 35 was selected based on WHO recommendations for screening in resource-limited settings [WHO, 2006].

Sensitivity analyses

The sensitivity of cumulative cervical cancer mortality to parameter estimates was analyzed in one-way sensitivity analyses. We were particularly interested in the sensitivity of HAART effectiveness in lowering HIV-mortality and SIL progression rates since the baseline values used were all external to the study and these values are likely to vary substantially depending on the study population.

6.4 RESULTS

Baseline model

A substantial proportion of women were expected to die from cervical cancer. The baseline model projected a lifetime cumulative cervical cancer mortality of 25.4 per 1000 HIV-positive women who were infected at age 25 and neither received HAART nor were screened for cervical cancer (Table 6.2). Cumulative cervical cancer mortality doubled to 46.6 per 1000 HIV-positive women who were infected at age 25, were placed on HAART when their CD4 went below 200cells/mm3 and had no screening for cervical cancer. If the latter women were screened either once at HAART initiation or once when they were aged 35, then mortality could reduce to 42.8 and 41.7 per 1000 women respectively. Interestingly, cervical cancer mortality following HAART and screening once at HAART initiation was still higher than cervical cancer mortality associated with no HAART and no screening.

In absolute measures, these mortality projections meant that compared to no HAART and no screening, an additional cervical cancer death would occur for every 47 women put on HAART when indicated, but not screened. Conversely, once women were put on HAART as indicated, then screening once at HAART initiation would prevent one case of cancer for

every 262 women screened. Screening once at age 35 was projected to prevent one cancer death per 202 women screened.

The timing of cervical cancer deaths was also influenced by the type of intervention received (Figure 6.2A). With NHNS the majority of cervical cancer deaths occurred within the second and third decade after diagnosis. In patients on HAART the occurrence of deaths was further delayed beyond the third decade after infection.

Only HAART had a substantial impact on the overall survival from all causes of death after infection (Figure 6.2B and Table 6.3). Compared to no HAART and no screening, all three scenarios with HAART (HAART with no screening, HAART with one screen at HAART initiation and HAART with one screen at age 35) resulted in gains in life-expectancy. However, there was only minimal difference in survival from all causes under the three scenarios with HAART, with one time screening only slightly increasing survival (Table 6.3).

Sensitivity analysis

The projected cervical cancer mortality estimates were robust to the magnitude of the effect of HAART in reducing HIV-related mortality (Figure 6.3A). All four mortality estimates were within 10% of their baseline value unless the effect of HAART was below a two-fold decrease in HIV mortality. Further increasing the effect of HAART only slightly increased cumulative cervical cancer mortality.

Mortality estimates were most sensitive to the rate of progression of precancerous lesions to more severe lesions or invasive cancer (Figure 6.3B). In all four scenarios cumulative mortality increased with faster progression rates. Doubling the progression rate resulted in a near doubling of cervical cancer mortality. Nevertheless, the relative mortality between each of the scenarios remained constant across progression rates.

Compared to the sensitivity to the progression rate of lesions, the model mortality estimates were rather robust to other parameters such as cancer mortality rates and other

baseline parameters. The cumulative cervical cancer mortality only slightly increased with increases in cervical cancer mortality rates (Figure 6.3B). Cumulative cervical cancer mortality was also robust to the baseline prevalence of lesions (Figure 6.4A) and the proportion of lesions that were high grade lesions (Figure 6.4B). As expected, mortality from cervical cancer decreased as the age of HIV infection (the initial age) of the cohort increased (Figure 6.4C). Screening test sensitivity had very little impact on cervical cancer mortality with mortality only slightly decreasing as sensitivity increased. Life expectancy also increased only slightly with improved sensitivity - a gain of 0.06 years as sensitivity increased from 50% to 100%. Meanwhile, screening test specificity had no impact on cervical cancer mortality or life expectancy.

6.5 DISCUSSION

The magnitude and the impact of HAART treatment and screening, on cervical cancer mortality in HIV-positive women in sub-Saharan Africa remain unknown. The ethical and practical complexities of potentially denying care to patients and following women for their lifetime respectively make it unfeasible to conduct a study to quantify this impact. Computer-based simulation models, however, provide an alternative means of quantifying the potential impact of these interventions. These models are of even greater importance in sub-Saharan Africa, where real-life estimation is hampered by limitations in the long-term follow-up of patients, in cancer diagnosis and in the determination of cause of death.

In this paper, we used a mathematical model to project that mortality due to cervical cancer in HIV positive women in Africa is potentially very high. While this mortality can be worsened by providing HAART without screening, screening can be associated with non-neglibible reductions in mortality from cervical cancer. These data confirm and quantify the potential gains of cervical cancer screening in HIV-positive women in regions with high

cancer incidence and mortality such as Cameroon. With an estimated 200,000 HIV-infected women in Cameroon [UNAIDS, 2007] our projections imply that screening these women once at HAART initiation would prevent close to 763 deaths due to cervical cancer, while screening these women once at age 35 could prevent approximately 990 deaths in these women.

WHO guidelines for screening in resource-limited settings include at least a one-time screening in the third or fourth decade of life [WHO, 2006]. Our projected potential gains with one-time screening in HIV-positive women are much smaller than those in other settings where screening is systematic and more frequent. For example, screening in the UK was estimated to prevent one death for every 65 women screened systematically [Peto et al, 2004]. The reductions in cervical cancer mortality associated with a single screen in HIV-positive women were also less compared to the estimated 25-36% reductions in lifetime risk reported in models of HIV-negative women in five other developing countries [Goldie et al, 2005]. These differences could be due to a higher incidence and faster progression of precancerous lesions in HIV-positive women compared to HIV-negative women [De Vuyst et al, 2008].

Although we show potential benefits of screening, our analysis did not take the cost of screening into account. A formal assessment of the cost-effectiveness of screening in this and other settings will provide additional information regarding resource needs and potential effects for cervical cancer screening. Our data show that even one time screening at HAART initiation or at age 35 would potentially be beneficial. According to guidelines from the Commission on Macroeconomics and Health, a policy is generally considered cost-effective if the incremental cost per life saved is less than the country's GDP per capita [WHO, 2001]. Our analyses estimate that one-time screen policies would improve life expectancy by approximately 0.10 years. With Cameroon's GDP per capita being estimated at USD 1019[UNSD, 2009], we further estimated that a one-time screening strategy will need to

have a cost of 101 USD per screen at most for it to be considered cost-effective. More formal cost-effectiveness analyses including appropriate weighting for disability and discounting will be needed to confirm this.

In the absence of prospective data on cervical cancer progression and regression rates for Cameroonian women, we used published data from the pre-HAART era in the US and assessed the impact of this choice in sensitivity analyses. A faster progression rate in developing countries could translate to even higher mortality due to cervical cancer. Nevertheless, the relative effects of HAART and screening were projected to remain similar, while the number of deaths averted with screening would increase as progression rates increase. On the other hand, if HAART was shown to have an effect on the progression of lesions, a finding that has been inconsistent [Palefsky, 2003], then the mortality due to cancer as well as the gains associated with screening would be reduced.

While cervical cancer screening could substantially reduce mortality due to cervical cancer, it was projected to have very little effect on all cause mortality. We did not assess the quality of life gains, but these data indicate that even with screening further gains in overall life expectancy will depend on the extent of prevention and care for other causes of mortality including opportunistic infections.

In conclusion, cervical cancer could account for a high proportion of deaths among HIV- positive women in Africa once they have access to HAART. These deaths could be reduced with screening, even when done just once. Antiretroviral treatment scale-up activities need to be followed by strategies to systematically increase access to screening services as well as treatment for cervical precancerous and cancerous lesions as needed. While the feasibility and cost-effectiveness of more frequent screening is still to be assessed, women need to be provided the opportunity to get screened at least once on initiating HAART or at age 35.

TABLES

Table 6.1: Baseline parameters used in modeling cervical cancer mortality in HIV positive women

Variable	CD4 >500	CD4 200-500	CD4 <200	Source
Initial prevalence of lesions, %	30	NA	NA	Mbu et al, 2008
Initial proportion of lesions that were HSIL (%)	33	NA	NA	Mbu et al, 2008
HIV infection				
HIV mortality rate (per 1000 per year)*	0.05	6.06	48.6	UNAIDS, 2007
Effect of HAART in reducing HIV mortality	NA	NA	4-fold	Murphy et al, 2001; Mermin et al, 2008
HIV progression rate (per 100 per year)**	18.1	27.5	NA	Goldie et al, 1999
Cervical Cancer				
Cancer mortality rate (per 1000 per year)*				[Ferlay et al., 2004
Local invasive cancer	41.1	41.1	41.1	
Regional invasive cancer	222.1	222.1	222.1	
Distant invasive cancer	543.5	543.5	543.5	
Progression rate (per 100 per year)				Goldie et al, 1999
Normal to LSIL	0.016	0.67	0.67	
LSIL to HSIL	0.73	2.93	2.93	
HSIL to local invasive cancer	2.0	2.42	2.42	
Local to regional invasive cancer	4.03	4.03	4.03	
Regional to distant invasive cancer	4.03	4.03	4.03	
Regression rate (per 100 per year)				Goldie et al, 1999
LSIL to normal	2.99	2.99	2.99	
HSIL to normal	0.30	0.30	0.30	
Screening test				Goldie et al, 1999
Sensitivity, %	70	70	70	
Specificity, %	90	90	90	

* Mortality in each CD4 category or cervical cancer stage were determined by weighting crude estimates by the proportions due to each category or stage from Goldie et al[1999]. ** estimated from mean duration at each stage

Table 6.2: Projected cumulative (lifetime) cervical cancer mortality in HIV-positive women in Cameroon on HAART and or screened for cervical cancer

Intervention	Mortality per 1000	NNT	NNS
No HAART, No Screen	25.4	Ref.	-
HAART, No Screen	46.6	47	Ref
HAART+ Screen once at HAART initiation	42.8	-	262
HAART+ Screen once at age 35	41.7	-	202

NNT - Number of women who need to receive HAART for each additional cancer death.
NNS - Number of women who need to be screened for each cancer death prevented.

Table 6.3: Projected cumulative cause of mortality and gains in life expectancy in HIV-positive women in Cameroon on HAART and or screened for cervical cancer.

Intervention	Cause of mortality (proportion)			Gains in life expectancy (years)	
	HIV/ AIDS	Cervical cancer	Other cause	Due to HAART	Due to Screening
No HAART, No Screen	63.3%	2.5%	34.1%	Ref.	-
HAART, No Screen	28.3%	4.7%	66.8%	10.6	Ref.
HAART+ Screen once at HAART initiation	28.4%	4.3%	67.1%	-	0.09
HAART+ Screen at age 35	28.4%	4.2%	67.2%	-	0.11

Ref: Referent

FIGURE LEGENDS

Figure 6.1: Summary of states in the Markov model (Adapted from an original depiction by Goldie et al, 1999)

Figure 6.2: Cumulative mortality from cervical cancer (A) or from all causes of death (B) by intervention in a cohort of HIV positive women getting infected at age 25. (NHNS: No HAART No Screening; HNS: HAART but No Screening; HSHI: HAART and one screen at HAART initiation; HS35: HAART and one screen at age 35).

Figure 6.3: Sensitivity of cumulative cervical cancer mortality to the relative effect of HAART in reducing HIV-mortality (A); the progression rate of precancerous lesions (B) and cervical cancer mortality rate(C). (NHNS: No HAART No Screening; HNS: HAART but No Screening; HSHI: HAART and one screen at HAART initiation; HS35: HAART and one screen at age 35).

Figure 6.4: Sensitivity of the cumulative cervical cancer mortality to baseline parameters: the prevalence of squamous intraepithelial lesions at beginning of cohort (A); the proportion of lesions that are high grade squamous intraepithelial lesions at the beginning of cohort (B); and the initial age of cohort (C). (NHNS: No HAART No Screening; HNS: HAART but No Screening; HSHI: HAART and one screen at HAART initiation; HS35: HAART and one screen at age 35).

FIGURES

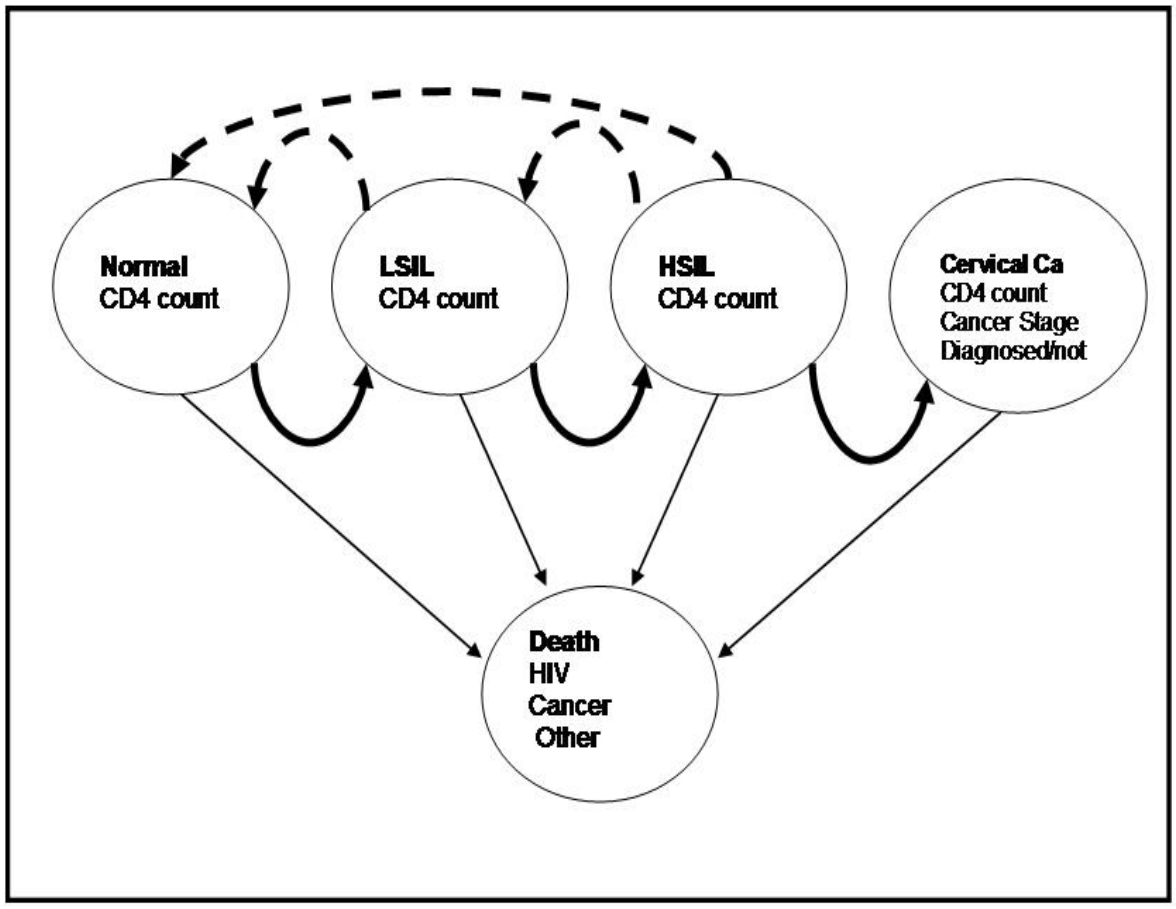
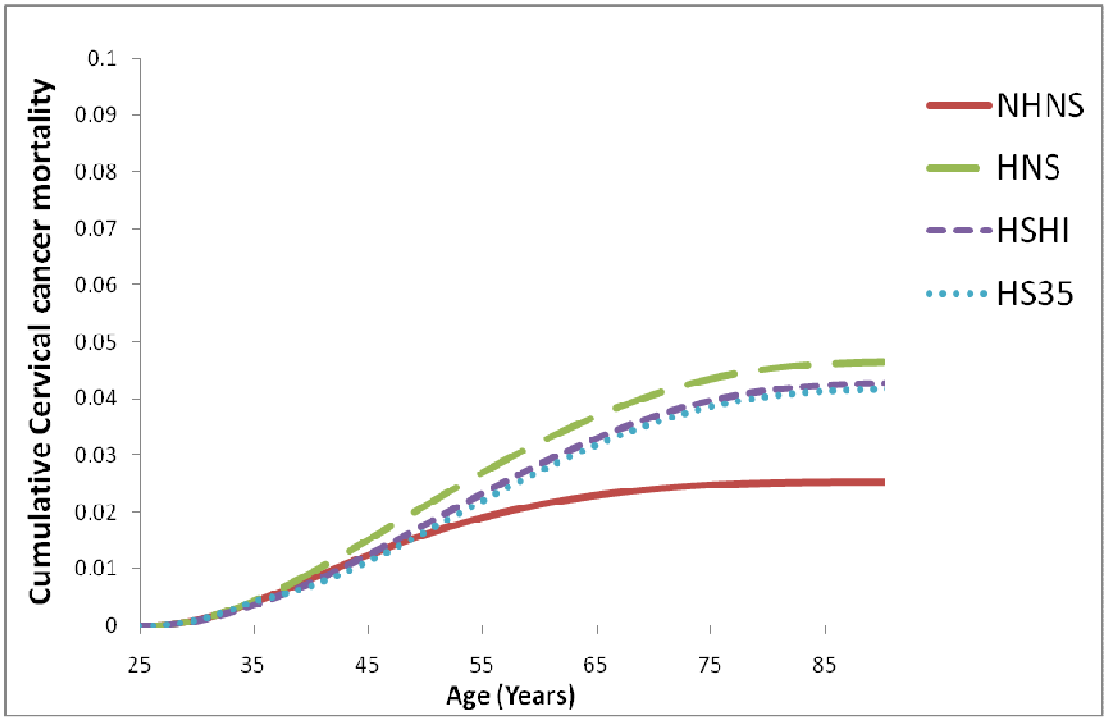


Figure 6.1

A


Cumulative Cervical cancer mortality
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
25
35
45
55
65
75
85
Age (Years)
NHNS
HNS
HSHI
HS35

B

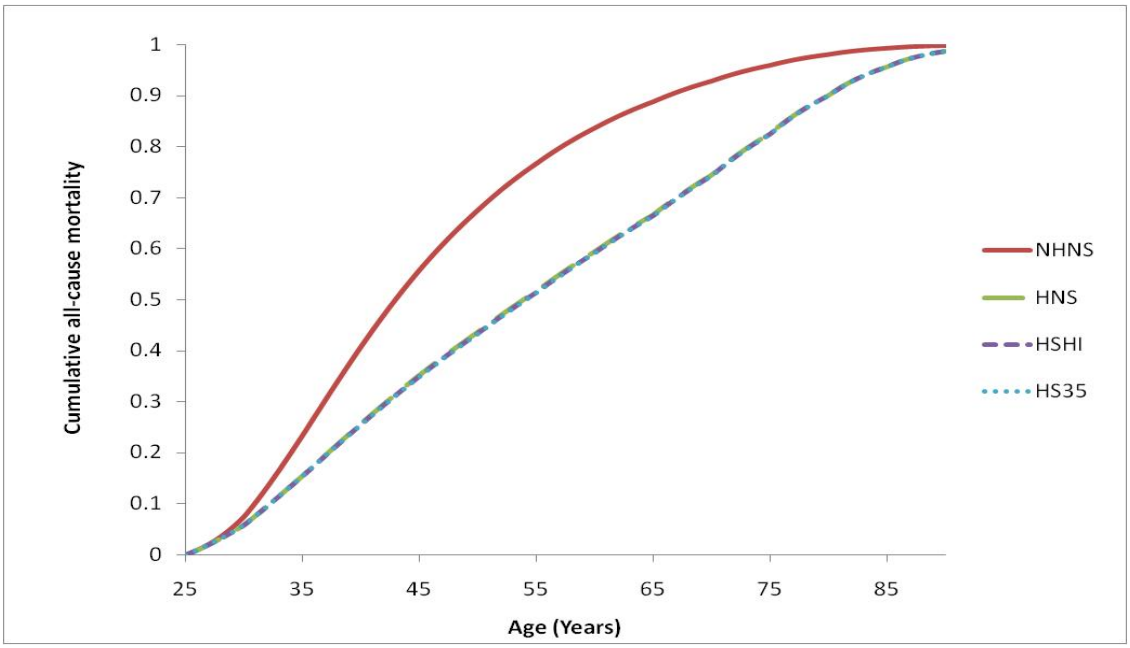
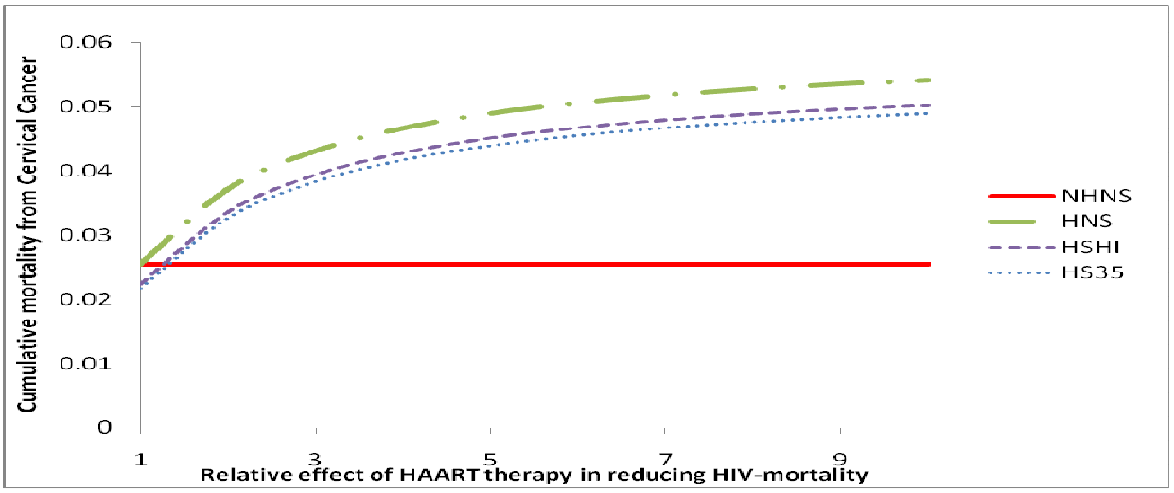

Cumulative all-cause mortality
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
25
35
45
55
65
75
85
Age (Years)
NHNS
HNS
HSHI
HS35

Figure 6.2

A



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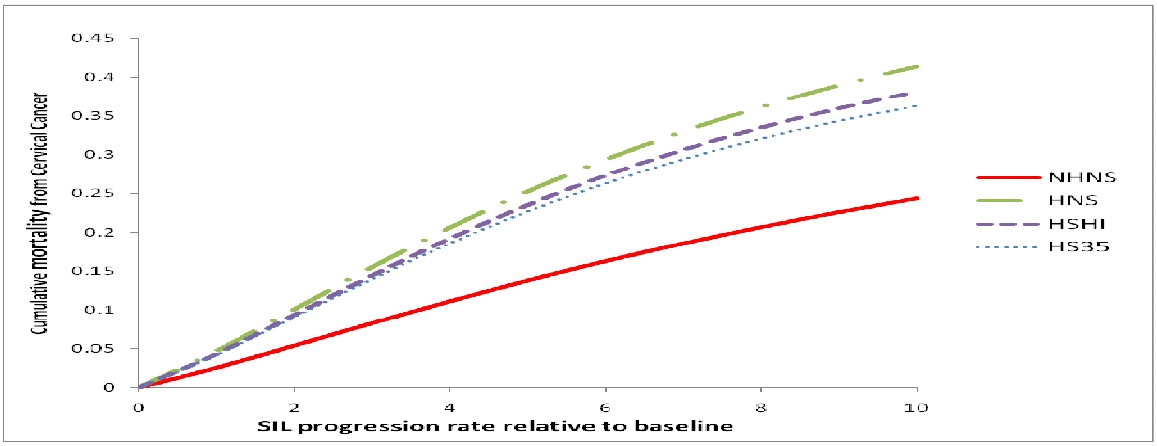

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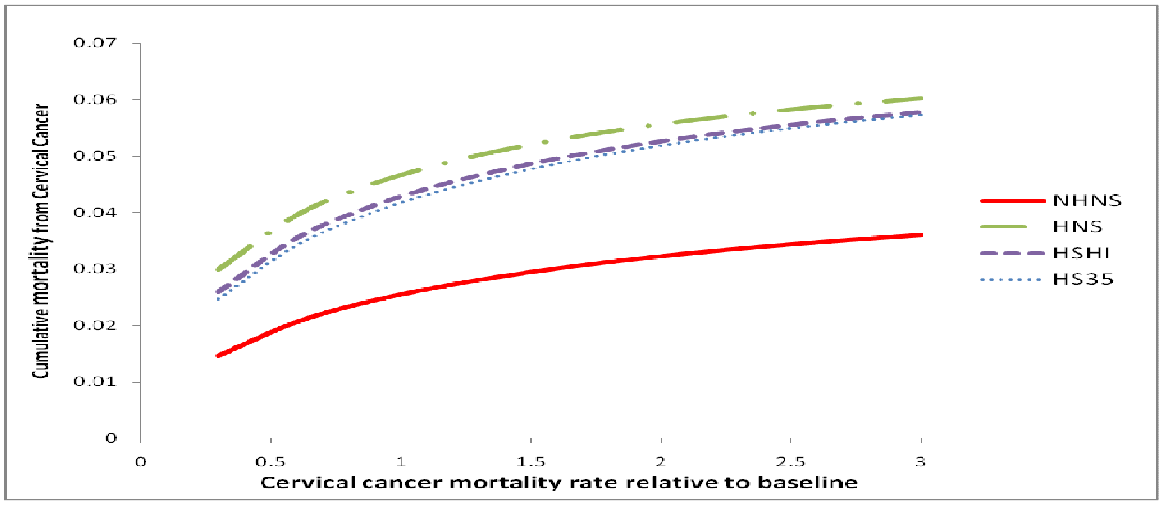
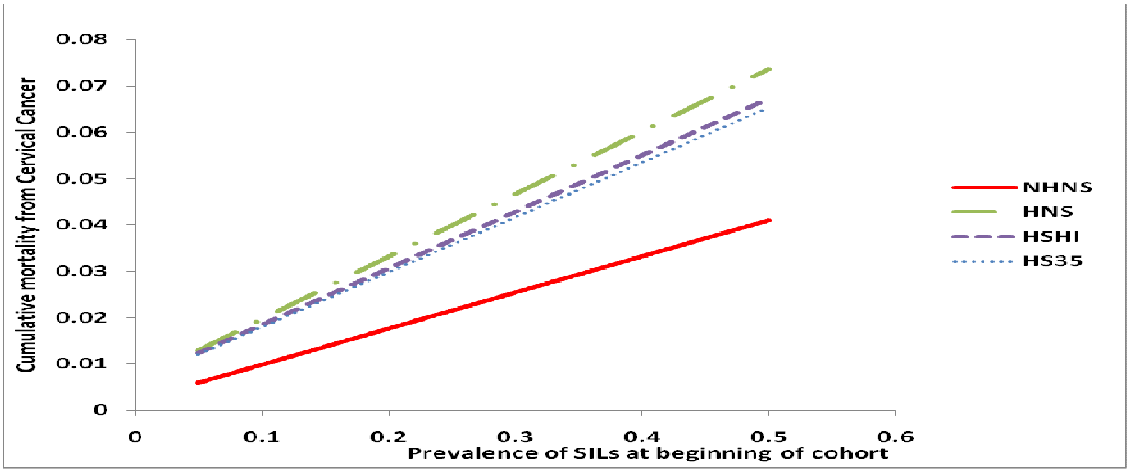


Figure 6.3

A



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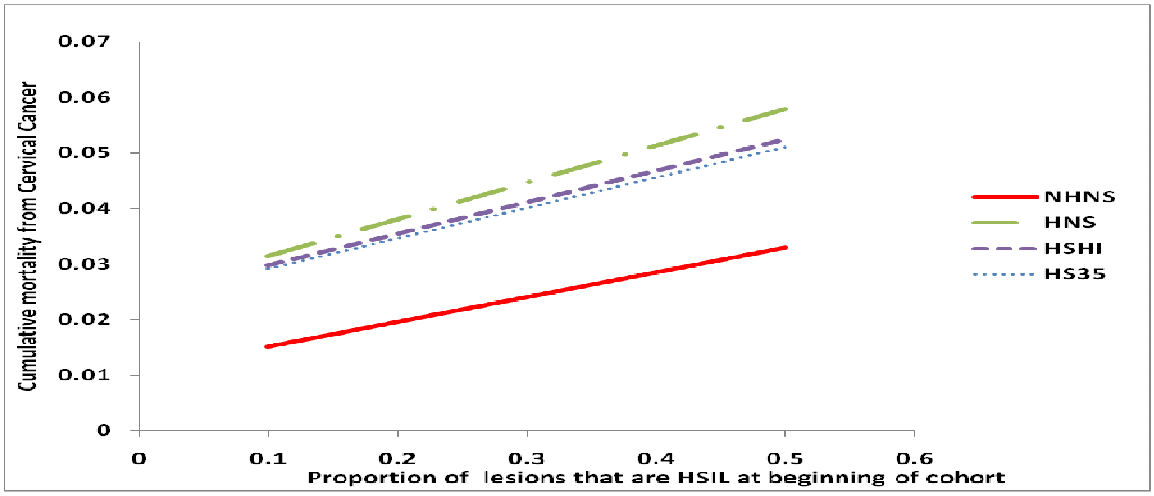

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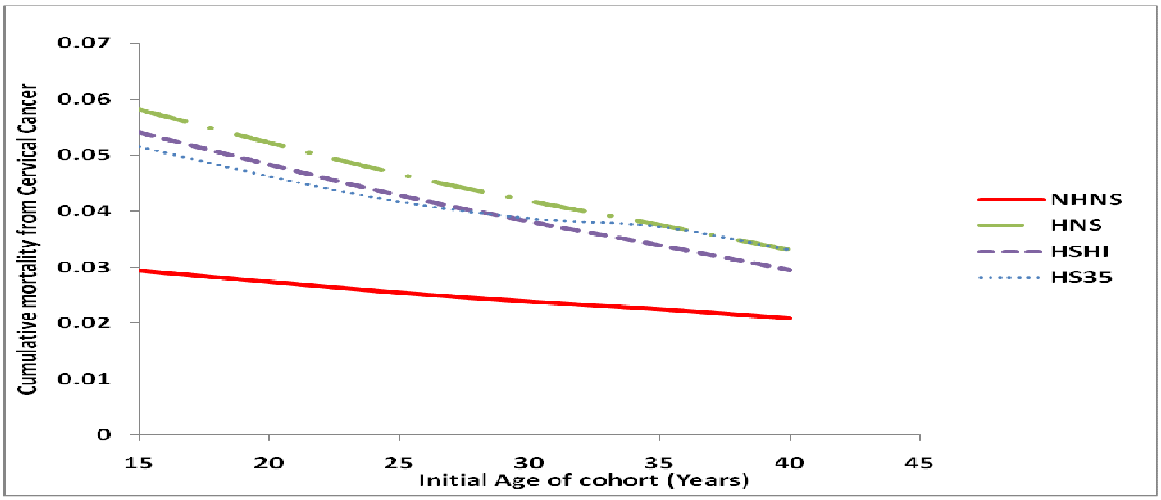

Figure 6.4

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CHAPTER SEVEN: CONCLUSION

This dissertation was premised on the hypotheses that cervical precancerous lesions are prevalent in HIV-positive women in Cameroon and that these were not being adequately managed as there was no systematic screening and thus no care. We sought to provide data that could assist clinicians and policy makers with better options for caring for precancerous lesions particularly in an era of increased access to antiretroviral therapy.

We conducted a cross-sectional study in which we collected primary data from Cameroon and screened women for precancerous lesions using conventional cytology methods. While we successfully assured the adequacy of the vast majority of samples collected, the accurate reading of cytology smears turned out to be a challenge with little agreement between the first cytologist and a more experienced cytologist. The resultant potential for misclassification bias was however explored in bias analyses of prevalence of SIL.

We confirmed our initial hypothesis of a high prevalence of precancerous lesions. The prevalence in our study was similar to that in women in similar contexts. Unfortunately, the predictive value of the clinical characteristics considered either individually or as a group, was poor. Women's age, a characteristic that has been used to somewhat target women in the general population, was not a good predictor either.

Interestingly, despite the relatively poor diagnostic performance of the risk scores developed to predict the presence of lesions or the presence of lesions requiring follow-up

care (ASC-H/HSIL), these scores resulted in fewer numbers of diagnostic errors (assuming false positives and false negatives all had the same cost) when compared to universal screening or no screening. However, the balance was tilted towards favoring universal screening as the relative cost of a false negative decision to not screen increased (a scenario which is probably more realistic).

Overall, we could not identify any single or group of readily available clinical factor(s) on which to target the decision to screen or not.

We then proceeded to quantify the potential impact on cervical cancer mortality of HAART in women who are not being screened and then assess the potential impact of screening in women who are put on HAART when it is indicated. We projected that the cumulative mortality due to cervical cancer could double once HAART was made available to women who previously were not screened and do not have access to HAART. In the population we simulated this could translate into one additional cancer death for every 47 women who are placed on HAART. This mortality could be reduced by screening. A single screen at age 35 would prevent one cervical cancer death per 202 women screened. The number of cervical cancer deaths that could be prevented would increase with even more frequent screening, but frequent screening is probably not affordable in settings such as Cameroon.

The public health implications of these findings are important. First, cervical cancer prevention (and eventual care) needs to be part of any comprehensive program seeking to increase access to antiretrovirals, as cervical cancer may be a long-term unintended secondary effect of antiretroviral therapy. Patients may need to be monitored for precancerous lesions in a similar way to which they are monitored for drug side-effects and the development of drug resistances. The challenge to policy-makers would be how to effectively implement such monitoring in a cost-effective manner. Potential strategies to reduce screening costs include targeted screening, the use of cheaper and accessible

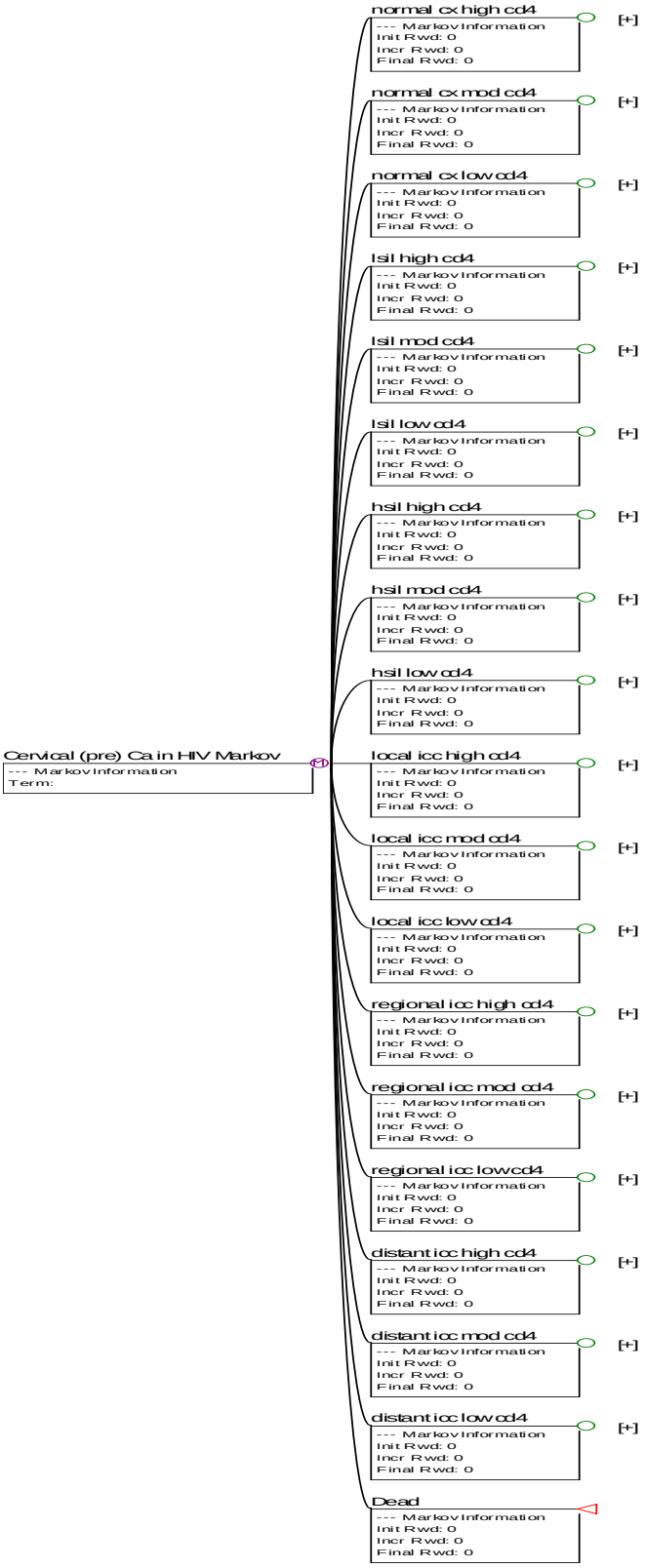
screening tests with high sensitivity and specificity, or less frequent screening. Our data suggest a limited utility of targeted screening. The alternatives to cervical cytology being considered including visual inspection techniques and or targeted HPV-DNA testing could be of use but these remain expensive and inaccessible. The development of a more accurate and cheaper screening test, that could preferably be used at the point-of-care could go a long way to improve the early detection (and subsequent treatment) of lesions that are likely progress to lesions.

Secondly, there needs to be an overhaul of resources (including human resources) dedicated to cancer prevention and care in these settings. Diagnosed precancerous and cancerous lesions will need to be treated for screening to have any impact on cervical cancer mortality.

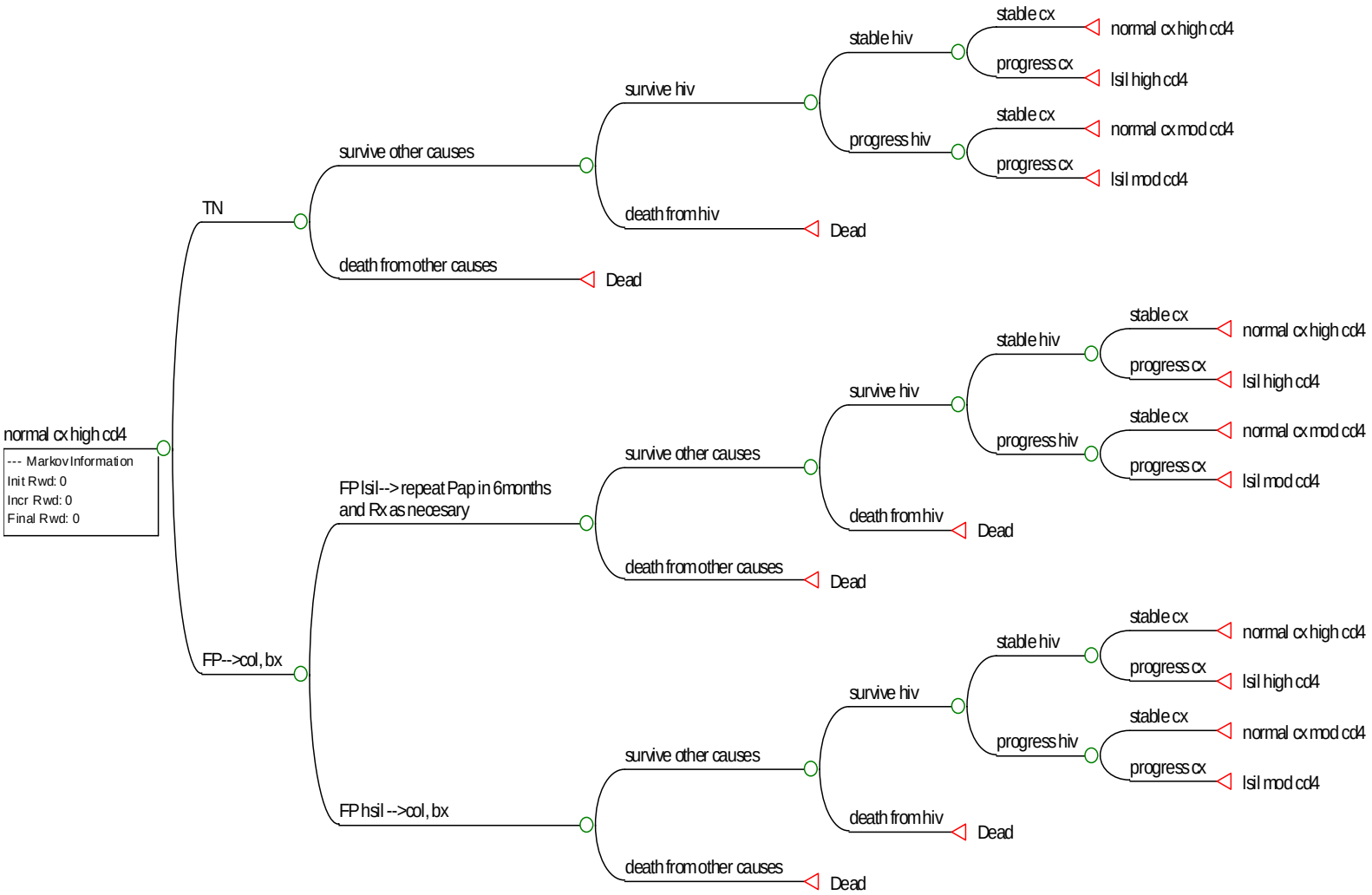
Future studies will need to assess the long-term evolution of precancerous lesions in HIV-positive women in Africa, including in women who are screened and treated. Because cost remains a barrier and a key determinant, formal analyses of the cost-effectiveness of various screening strategies will be imperative to better guide policy.

APPENDICES

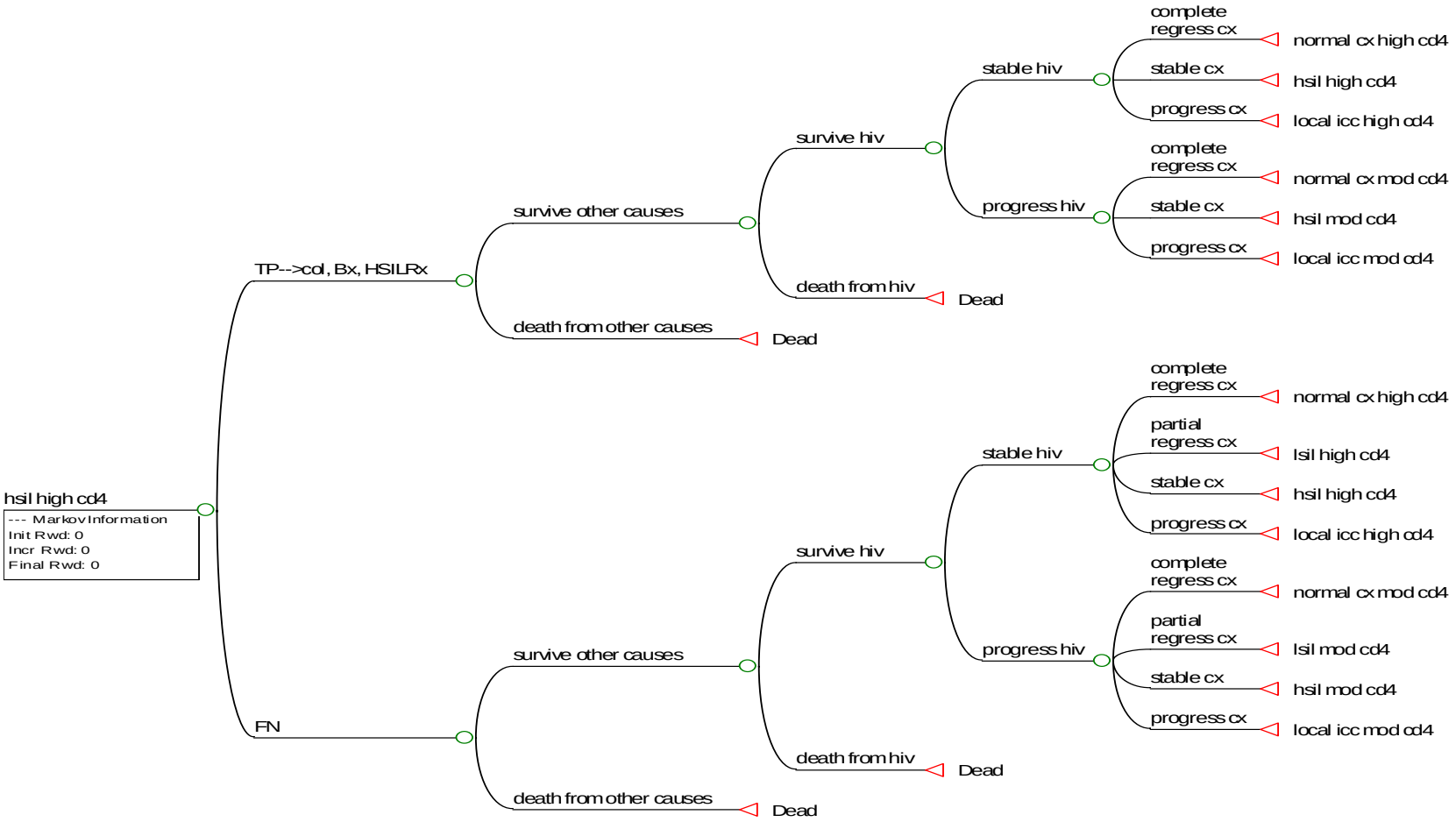
Appendix 1: Sample Markov node from TreeAge pro 2008



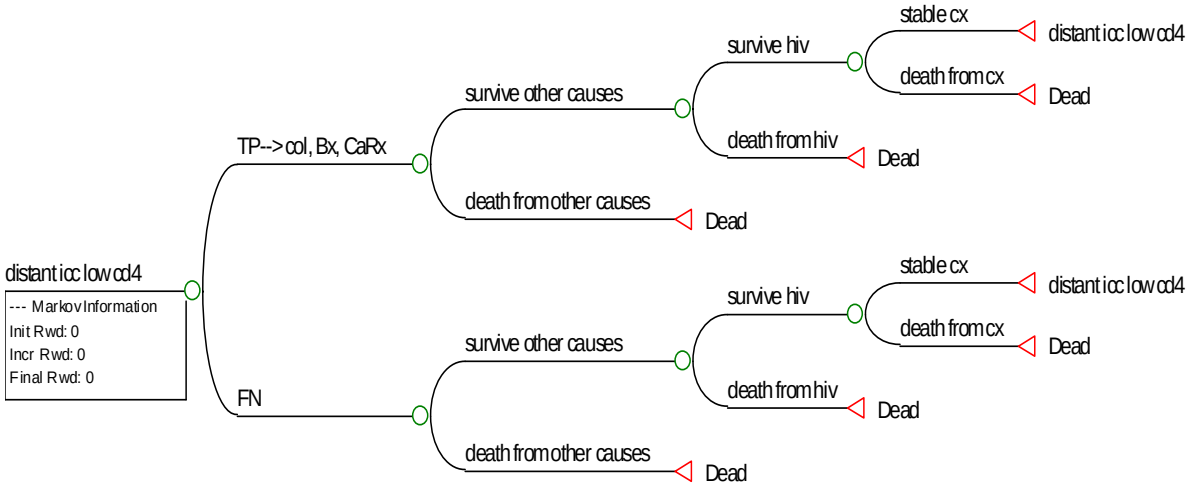
Appendix 2: Sample normal cervix high CD4 (>500/uL) subtree



Appendix 3: Sample HSIL moderate CD4 (200-500/uL) subtree



Appendix 4: Sample distant ICC low CD4 (<200/uL) subtree



Appendix 5: Study questionnaire

Cervical Cytology at HAART Initiation in Cameroon (CCHIC) Study

(Prevalence, severity and predictors of cervical epithelial lesions among women initiating HAART in Cameroon)

Questionnaire

Date: _ _/_ _/_ _ (dd/mm/yy)

Part I: Identification
File Number _ _ _ _ _ _, Study IDNUM __ _ _

Part II: Medical History

HIV History

1. How long ago were you first diagnosed as HIV+ ? _ _ years, _ _ months
   (If does not know, please ask date diagnosed and estimate duration)

2. Have you taken any other medication aimed at your HIV infection? ***(select one)***
   0 = Pills/tablets 1 = Herbs 2 = Injections 3 = other ……

Gyneco-Obstetrical History

3. Have you ever been screened for cervical cancer (also called a "pap smear")? ***(select one)***
   0= Never 1 = Yes, No lesion detected 2 = Yes, lesion detected 9 = Don't know

4. How manytimes have you been pregnant? _ _

5. How many pregnancies that lasted **more than 6 months** have you had? _ _

6. Have you ever ***had an abortion***? ***(select one)***
   0 = No 1 = Yes, 9= Don't know

7. Have you ever used Oral contraceptive pills? ***(select one)***
   0 = Never 1=Yes, Only prior to HIV diagnosis 2 = Yes, only after HIV diagnosis
   4 = Yes, both prior to and after HIV diagnosis 9 = Don't know

8. Have you ever used Injectable Hormones (e.g., Depo-provera or Norplant)? ***(select one)***
   0 = Never 1=Yes, Only prior to HIV diagnosis 2 = Yes, only after HIV diagnosis
   4 = Yes, both prior to and after HIV diagnosis 9 = Don't know

9. Have you ever used an intrauterine device? ***(select one)***
   0 = Never 1=Yes, Only prior to HIV diagnosis 2 = Yes, only after HIV diagnosis
   4 = Yes, both prior to and after HIV diagnosis 9 = Don't know

10. Have you ever used a diaphragm/cervical cap? ***(select one)***
    0 = Never 1=Yes, Only prior to HIV diagnosis 2 = Yes, only after HIV diagnosis
    4 = Yes, both prior to and after HIV diagnosis 9 = Don't know

11. Have you ever had Surgery for contraception (Tubal Ligation/Hysterectomy)? ***(select one)***
    0 = Never 1=Yes, prior to HIV diagnosis 2 = Yes, after HIV diagnosis 9 = Don't know

Other medical History

12. Have you been frequently exposed to cigarette smoke? ***(select one)***
    0 = No
    1= Yes, I have smoked for at least a year (estimate of packet years of smoking _ _ packets/day for _ _ years)
    2 = Yes, exposed for at least a year to someone else in household/jobsite who smoked
    3 = Yes, other_____

Sex History

13. How old were you when you first had sex? _ _ years

(Please select one in each and all of the following 14 cells)

	a. Lifetime	**b. One year prior to HIV diagnosis**	**c. Since HIV diagnosis**	**d. In the past 6 months with HIV**
14. How many sex partners did you have in the following periods	0 1 2 3 4 5 6 = >5 9 = Cannot estimate	0 1 2 3 4 5 6 = >5 9 = Cannot estimate	0 1 2 3 4 5 6 = >5 9 = Cannot estimate	0 1 2 3 4 5 6 = >5 8 =HIV diagnosis < 6 months 9=Cannot estimate
15. How often did you use a condom with these partners	0 = Never 1 = <50 %time 2 = ≥50 % time 3 = Always 8 = No partner 9 = Cannot estimate	0 = Never 1 = <50 %time 2 = ≥50 % time 3 = Always 8 = No partner 9 = Cannot estimate	0 = Never 1 = <50 %time 2 = ≥50 % time 3 = Always 8 = No partner 9 = Cannot estimate	0 = Never 1 = <50 %time 2 = ≥50 % time 3 = Always 8 = No partner or HIV diagnosis < 6 mnths 9 = Cannot estimate
16. How many NEW sex partners did you have in the following periods	NA	0 1 2 3 4 5 6 = >5 9 = Cannot estimate	0 1 2 3 4 5 6 = >5 9 = Cannot estimate	0 1 2 3 4 5 6 = >5 8 =HIV diagnosis < 6 months 9=Cannot estimate
17. How often did you use a condom with these *new* partners	NA	0 = Never 1 = <50 %time 2 = ≥50 % time 3 = Always 8 = No partner 9 = Cannot estimate	0 = Never 1 = <50 %time 2 = ≥50 % time 3 = Always 8 = No partner 9 = Cannot estimate	0 = Never 1 = <50 %time 2 = ≥50 % time 3 = Always 8 = No partner or HIV diagnosis < 6 mnths 9 = Cannot estimate

18. On average, how often did you have sex in the 6 months preceding your HIV diagnosis:
    _ _ per month (or _ _ per week)

19. On average, how often did you have sex in the past 6 months (OR since you were diagnosed of HIV if this was less than 6 months ago):
    _ _ per month (or _ _ per week)

20. Have you ever been diagnosed with the following sexually transmitted (venereal) diseases?
    a. Chlamydia: 0 = No 1=Yes 9 = Don't Know
    b. Gonorrhoea: 0 = No 1=Yes 9 = Don't Know
    c. Herpes (genital): 0 = No 1=Yes 9 = Don't Know
    d. Warts (genital): 0 = No 1=Yes 9 = Don't Know
    e. Other ……

Part III: Demographics

21. What was your age at your last birthday? _ _ years

22. What is your current relationship status? ***(select one)***
    0 = Never married and not living with a partner 1 = married monogamous 2 = married polygamous
    3 = Living with a partner 4 = Separated 5 = Divorced 6 = Widow

23. What is the highest level of education you have ever attended? ***(select one)***
    0 = None 1 = Primary 2 = Secondary 3 = University

24. What province do you **currently** live in? ***(select one)***
0 = Adamawa 1 = Center 2 = East 3 = Extreme-North 4 = Littoral
5 = North 6 = North-West 7 = South 8 = South-West 9 = West

25. Do you **currently** live in an urban (town) or rural (village) area? ***(select one)***
0 = Urban 1 = Rural

26. What province have you lived in the ***longest***? ***(select one)***
0 = Adamawa 1 = Center 2 = East 3 = Extreme-North 4 = Littoral
5 = North 6 = North-West 7 = South 8 = South-West 9 = West

27. Have you lived **longer** in an urban (town) or a rural (village) area? ***(select one)***
0 = Urban 1 = Rural

28. What is your province of **origin**? ***(select one)***
0 = Adamawa 1 = Center 2 = East 3 = Extreme-North 4 = Littoral
5 = North 6 = North-West 7 = South 8 = South-West 9 = West

Part IV: Symptoms

29. Have you ever had **vaginal discharge** in the past 6 months? ***(select one)***
0 = Never 1=don't have it now, but had it before 2 = Yes, have it now 9 = Don't know

30. Have you ever had **vaginal odor or smell** in the past 6 months? ***(select one)***
0 = Never 1=don't have it now, but had it before 2 = Yes, have it now 9 = Don't know

31. Have you ever had **itching in or around the vagina** in the past 6 months? ***(select one)***
0 = Never 1=don't have it now, but had it before 2 = Yes, have it now 9 = Don't know

32. Have you ever had **ulcer or sore in genital area** in the past 6 months? ***(select one)***
0 = Never 1=don't have it now, but had it before 2 = Yes, have it now 9 = Don't know

33. Have you ever had **pain or burning during urination** in the past 6 months? ***(select one)***
0 = Never 1=don't have it now, but had it before 2 = Yes, have it now 9 = Don't know

34. Have you ever had **more frequent urination** in the past 6 months? ***(select one)***
0 = Never 1=don't have it now, but had it before 2 = Yes, have it now 9 = Don't know

35. Have you ever had **pain in lower abdomen** in the past 6 months? ***(select one)***
0 = Never 1=don't have it now, but had it before 2 = Yes, have it now 9 = Don't know

36. Have you ever had **vaginal bleeding between periods** in the past 6 months? ***(select one)***
0 = Never 1=don't have it now, but had it before 2 = Yes, have it now 9 = Don't know

37. Have you **missed any periods** in the past 6 months? ***(select one)***
0 = Never 1=don't have it now, but had it before 2 = Yes, missed my last period
8 = did not expect periods (pregnant, menopause…) 9 = Don't know

38. Have you ever had **pain during vaginal sex** in the past 6 months? ***(select one)***
0 = Never 1=don't have it now, but had it before 2 = Yes, have it now
8 = Have not had sex 9 = Don't know

Part V: Signs

39. What is present on inguinal exam?
a. Is it Normal? 0= No 1 = Yes
b. Enlarged lymphnodes? 0= No 1 = Yes
c. Other …….

40. What is present on vulvo/vaginal exam?
a. Is it Normal? 0= No 1 = Yes
b. Warts? 0= No 1 = Yes
c. Ulcers? 0= No 1 = Yes
d. papules/vesicules? 0= No 1 = Yes

e. Abnormal vaginal erythema? 0= No 1 = Yes
f. Abnormal vaginal discharge? 0= No 1 = Yes
g. Other ……

41. What is present on cervical exam?
a. Is it Normal? 0= No 1 = Yes
b. Abnormal cervical erythema? 0= No 1 = Yes
c. Abnormal cervical discharge? 0= No 1 = Yes
d. Cervical friability? 0= No 1 = Yes
e. Other ……

42. What is present on bimanual pelvic exam?
a. Is it Normal? 0= No 1 = Yes
b. Cervical motion tenderness? 0= No 1 = Yes
c. Adnexal tenderness? 0= No 1 = Yes
d. Other ……

43. What is present on abdominal exam?
a. Is it Normal? 0= No 1 = Yes
b. Tenderness to palpation? 0= No 1 = Yes
c. Other ……

Part VI: Overall assessment of HIV disease

44. What is the patients Clinical HIV Stage (WHO Classification)? ***(select one)***
1 = Stage I 2 = Stage II 3 = Stage III 4 = Stage IV

45. What is the patients CD4 level count? _ _ _ _cells/mm3

46. What is the patients HIV viral load? _ _ _ _logs/ml

Part VII: Screening results

47. Cervical smear cytology findings ***(select one)***
0 = Normal
1 = ASC-US (Atypical squamous cell of undetermined significance)
2 = LSIL (low-grade squamous intraepithelial lesion)
3 = HSIL (high-grade squamous intraepithelial lesion)
4 = Adenocarcinoma in situ
5 = Other ……..
9 = unsatisfactory

48. Biopsy findings ***(select one)***
0 = Normal 1 = CIN 1 2 = CIN 2 3 = CIN 3
4 = Adenocarcinoma in situ 5 = Invasive cancer 6 = Other ……..

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