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A Randomized Controlled Trial for Treatment of High-Grade Cervical Lesions
Among HIV-Infected Women in Kenya

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Abstract

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HIV and cervical cancer are co-epidemics that disproportionately impact women living in low resource settings. Women infected with HIV have consistently been reported to have higher burden of human papillomavirus (HPV) infection, persistent HPV infection, and cervical pre-cancerous lesions than HIV-uninfected women. These lesions, if left untreated, can progress to cervical cancer. For women with high grade cervical pre-cancer, current World Health Organization (WHO) guidelines recommend treatment with either cryotherapy or loop electrosurgical excision procedure (LEEP). A key challenge is the specificity of these guidelines to address the complex needs of women infected with human immunodeficiency virus (HIV). Published evidence suggests that cervical pre-cancer has a different disease course among HIV-infected women than women in the general population.

Previous studies have shown that treatment with cryotherapy or LEEP in HIV-infected women may stimulate cervical shedding of HIV and subsequently increase the risk of HIV sexual transmission from treated women to HIV-uninfected partners. Cryotherapy and LEEP have both been associated with cervical shedding of HIV, but they have never been directly compared to each other and few studies have rigorously studied treatment outcomes among HIV-infected women. Our findings support the current guidelines that HIV-infected women should either avoid sexual intercourse for one month or practice risk reduction strategies including condom use to minimize risk of HIV-infection.

High grade cervical pre-cancer can be difficult to treat and recurs in approximately 5-14% of women after treatment and among HIV-infected women, recurrence has been reported as high as 23%. There have been conflicting results and conclusions from observational studies and randomized trials that assessed effectiveness of cryotherapy or LEEP. The consistency and quality of evidence varies because the data are pooled from observational studies that define different end points, post-treatment follow-up times are too short to accurately measure the impact of treatment on recurrence, or the sample sizes have not been large enough to precisely measure how recurrence is modified HIV-cofactors. We addressed these limitations by using a robust study design, gold-standard outcome definition, and a long duration of follow-up. We found that, cryotherapy was associated with significantly higher risk of recurrent pre-cancerous cervical disease, suggesting that LEEP should be adopted as the standard of care for HIV-infected women.

This research has contributed to an improved understanding of how treatment for high grade cervical lesions in HIV-infected women affects short-term risk of HIV transmission and the long-term risk of recurrence of cervical disease. For secondary prevention of cervical cancer to be programmatically effective, HIV-status must be addressed by treatment guidelines for high grade cervical pre-cancer.

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Chapter 1: Introduction

Chapter 1: Introduction

There is growing consensus that cervical cancer elimination is achievable, but there remain significant knowledge gaps in secondary prevention of cervical cancer [1]. Annually, more than half a million women will develop cervical cancer and almost half of these women will die from the disease [2]. The majority of these women live in resource constrained settings, particularly in sub-Saharan Africa (SSA) where the burden of cervical cancer has been intensified by the contribution of HIV. Women infected with HIV experience a higher prevalence of human papillomavirus (HPV) infection, which often goes undetected, and develops into cervical pre-cancer [3–5]. These lesions, if left untreated, can ultimately progress to cervical cancer. Yet, cervical cancer is a preventable disease. Early diagnosis of cervical cancer precursor lesions, treatment, and continued clinical follow-up is associated with reduction in incidence of cervical cancer [6].

In settings with high uptake of cervical cancer screening and appropriate laboratory infrastructure to support secondary prevention programs, the incidence of cervical cancer is similar among HIV-infected and uninfected women [7]. In low resource settings, screening and treatment programs have been a challenge to implement and sustain. Underscoring this challenge, the incidence of cervical cancer is projected to increase over the next 20 years due in part to increases in population size, burden of HIV, and lack of HPV vaccination programs [2]. Improved access to HIV care and antiretroviral therapy (ART) has extended the lives of HIV-infected women and appropriate treatment strategies need to be defined for this vulnerable population so that they receive appropriate treatment.

Treatment algorithms for cervical pre-cancer vary by region and resource level [8]. A notable solution to expand secondary prevention services is a screen-and-treat approach using visual inspection with acetic acid (VIA) or high-risk HPV DNA screening, coupled with treatment of

precancerous lesions. Common treatments for cervical pre-cancer include cryotherapy and loop electrosurgical excision procedure (LEEP). Both procedures have similar treatment successes in HIV-uninfected women. Cryotherapy is an ablative treatment option that is often used in screen and treat programs because it is inexpensive and can be performed in primary care centers. LEEP is an excisional procedure, which necessitates both expensive equipment and trained providers, typically in a tertiary care setting [9].

There are several important considerations required for policy makers and program managers to adopt either cryotherapy or LEEP as standard of care for HIV-infected women. For women with high grade cervical pre-cancer, current World Health Organization (WHO) guidelines recommend treatment with either cryotherapy or LEEP [10]. One challenge is the specificity of these guidelines to address the complex needs of women infected with human immunodeficiency virus (HIV). Specifically, more data are needed to comprehensively understand the risk of HIV sexual transmission following treatment for cervical pre-cancer. Further additional data are necessary to guide decisions on the optimal treatment procedure to minimize the risk of post-treatment disease recurrence for HIV-infected women. This dissertation addresses several of the key scientific questions to inform evidence-based guidelines for programmatic delivery of secondary cervical cancer prevention. Here we provide an overview of each research question.

Chapter 2. Increased cervical HIV shedding after LEEP

An important research question to address is whether treatment of cervical pre-cancer in HIV-infected women has the potential for increased infectivity. This is especially concerning in countries such as Kenya, where HIV-prevention strategies are paramount as healthcare resources remain limited and the majority of HIV-infected individuals are partnered in sero-

discordant relationships [11]. Understanding factors impacting HIV-transmission risk is essential to developing and implementing effective prevention strategies.

Treatment of cervical pre-cancer can cause inflammation or ulceration of the cervix [12,13]. In HIV-infected women, these interventions may stimulate cervical shedding of HIV and subsequently increase the risk of HIV sexual transmission from treated women to HIV-uninfected partners [14]. While the risk of sexual HIV transmission from women on ART is low, cervical HIV levels can differ from plasma HIV levels [15–20]. Both cryotherapy and LEEP have been associated with cervical shedding of HIV but these studies have been limited in size and did not address potential for effect modification by HIV co-factors including ART [21,22]. In Chapter 2, we nested a sub-study in a randomized controlled trial to compare the levels of cervical shedding at one, two, and three weeks following treatment with cryotherapy or cryotherapy. Our results provide evidence that there is a higher risk of cervical HIV shedding in three weeks after treatment for cervical pre-cancer associated with LEEP. Further ART may modulate changes in HIV shedding and the risk of sexual transmission of HIV is low for women who used ART prior to cervical treatment.

Chapter 3. Cryotherapy associated with high rate of recurrence of cervical pre-cancer in HIV-infected women

In resource-poor settings where cervical cancer screening may only take place once or twice in a woman's lifetime, ensuring treatment is effective must be prioritized. Decision-makers need information on the optimal treatment procedures to maximize the life-saving benefit of cervical pre-cancer treatment for HIV-infected women.

After treatment for high grade cervical pre-cancer the risk of post-treatment disease remains higher than that of women who screened negative for cervical cancer. Post-treatment detection

of disease occurs in approximately 5% of women [23–29]. For HIV-infected women, recurrence of disease was reported as high as 27% in a recent clinical trial comparing cryotherapy and LEEP [30]. Though HIV-infected women experience more frequent disease recurrence, the WHO guideline states the data is inadequate to make specific treatment recommendations for HIV-infected women. Rates of recurrence among HIV-infected women estimates vary considerably according to the extent of pre-treatment disease, length of follow-up time, and by immune status [31]. In Chapter 3, we addressed these limitations by using a robust study design, gold-standard outcome definition, and a long duration of follow-up. Our results conclusively establish that cryotherapy is associated with significantly higher risk of recurrent pre-cancerous cervical disease suggesting that LEEP should be adopted as the standard of care for HIV-infected women.

Summary

To advance the scientific understanding of how HIV-infection modifies the impact of treatment for cervical pre-cancer, the current dissertation focuses on the effect of cervical treatment with cryotherapy or LEEP. Specifically, the scientific research presented in this dissertation 1) quantifies the risk of HIV shedding in the three weeks following cervical treatment, and 2) evaluates the rate of cervical disease recurrence over two years of follow-up. This study has practical implications for the management of HIV-infected women with high grade cervical lesions. In the past two decades, there have been widespread efforts to expand the delivery of secondary prevention services using single-visit, screen-and-treat approaches to manage cervical pre-cancer. While there has been a considerable body of evidence to support screen-and-treat approaches, the public health recommendations were not based on rigorous evidence of treatment impact and efficacy among HIV-infected women. The study was designed to address the research gaps on how choice of treatment procedure affects the risk of HIV-transmission and post-treatment disease recurrence among HIV-infected women. Collectively,

our results highlight the individual and public health risks associated with treatment for cervical pre-cancer and the need to modify treatment guidelines to account for HIV-status.

Chapter 2: Increased Cervical HIV Shedding After LEEP

Increased Cervical HIV RNA Shedding Among HIV-Infected Women Randomized to Loop Electrosurgical Excision Procedure (LEEP) compared to Cryotherapy for Cervical Intraepithelial Neoplasia 2/3

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ABSTRACT

Background. Treatment of human immunodeficiency virus (HIV)–infected women to prevent cervical cancer may stimulate HIV RNA cervical shedding and risk HIV transmission.

Methods. From 2011-2014, 400 HIV-infected women diagnosed with cervical intraepithelial neoplasia 2/3 in Kenya were randomized to loop electrosurgical excision procedure (LEEP) or cryotherapy. Cervical samples were collected at baseline and 3 weekly intervals. Samples were tested for HIV RNA using the Gen-Probe Aptima HIV assay with a minimum detection level of 60 copies/swab and analyzed using generalized estimating equations.

Results. Women who received LEEP had significantly higher cervical HIV RNA levels than those who received cryotherapy at weeks 2 (adjusted incident rate ratio [aIRR], 1.07; $P = .038$) and 3 (aIRR, 1.08; $P = .046$). Within LEEP, significantly higher cervical shedding was found at weeks 2 (2.03 log₁₀ copies/swab; $P < .001$) and 3 (2.04 log₁₀ copies/swab; $P < .001$) compared to baseline (1.80 log₁₀ copies/swab). Cervical HIV RNA was significantly higher following LEEP for up to 3 weeks among women on antiretroviral treatment (ART) (0.18 log₁₀ copies/swab increase; $P = .003$) and in ART-naive women (1.13 log₁₀ copies/swab increase; $P < .001$) compared to baseline. Within cryotherapy, cervical shedding increased in ART-naive women (0.72 log₁₀ copies/swab increase; $P = 0.004$) but did not increase in women on ART.

Conclusions. Women randomized to LEEP had a larger increase in post-procedural cervical HIV shedding than cryotherapy. Benefits of cervical cancer prevention outweigh the risk of HIV sexual transmission; our findings underscore the importance of risk-reduction counseling.

INTRODUCTION

Women infected with HIV experience a higher burden of cervical cancer than HIV-uninfected women [32,33]. Cervical cancer prevention depends on early diagnosis, treatment, and continued clinical follow-up. Globally, standard treatment modalities for cervical intraepithelial neoplasia (CIN) include loop electrosurgical excision procedure (LEEP) and cryotherapy. LEEP is the standard of care for treatment of CIN in the United States and Europe [34,35], and cryotherapy is commonly used in resource-limited settings using a single-visit “see-and-treat” approach [36–41].

Treatment of CIN can cause inflammation or ulceration of the cervix [12,13]. Increased risk of hemorrhage associated with LEEP and watery discharge with cryotherapy have been reported [25]. In HIV-infected women, these interventions may stimulate cervical shedding of HIV and subsequently increase the risk of HIV sexual transmission from treated women to HIV-uninfected partners [14]. While the risk of sexual HIV transmission from women on antiretroviral therapy (ART) is low, cervical HIV levels can differ from plasma HIV levels [15–20]. LEEP and cryotherapy have both been associated with cervical shedding of HIV [12,21,22,42], but they have never been directly compared to each other and few studies have rigorously examined cervical shedding after these procedures in association with ART use, plasma viral load, and immunodeficiency [43].

We conducted a randomized controlled trial (RCT) comparing recurrence of cervical disease among women with high-grade pre-cancerous cervical lesions randomized to LEEP or cryotherapy (NCT01298596) [44]. Within the parent trial, we nested a sub-study to compare the cervical HIV RNA levels at 1, 2, and 3 weeks following LEEP or cryotherapy, and hypothesized that LEEP would be more likely to increase cervical HIV RNA levels compared to cryotherapy.

METHODS

Study Population

This study was nested within a RCT to compare recurrence of cervical lesions between LEEP and cryotherapy over two-year follow-up. The RCT was performed at the Coptic Hope Center for Infectious Diseases, an urban HIV clinic in Nairobi, Kenya that offers free, comprehensive HIV care and treatment with support from the President's Emergency Plan for AIDS Relief (PEPFAR) [45]. Eligible HIV-infected women enrolled at the Coptic Hope Center who agreed to participate in the study were consented and screened for cervical cancer using Papanicolaou (Pap) smear and colposcopy-directed biopsy. Women were eligible for the study if they were HIV-infected, at least 18 years of age, had initiated sexual activity, were not pregnant, and had an intact cervix without history of hysterectomy. Women were excluded if they had a history of treatment for cervical lesions, or if they had a lesion that was larger than 75% of the cervix surface or extended more than 2 mm into the endocervical canal. Women with a high grade squamous intraepithelial lesion or squamous cell carcinoma by Pap smear and histologically confirmed CIN2/3 disease were randomized.

Randomization and Follow-up

Randomization was prepared using a computer-generated sequence in permuted blocks of 10, and assignment was in a 1:1 allocation to receive LEEP or cryotherapy. Study investigators and participants were not blinded to the interventions. During the baseline visit, women completed a standardized questionnaire detailing medical, sexual, and reproductive histories. ART use and duration were abstracted from the patient health record at the Coptic Hope Center.

Prior to treatment, endocervical swabs for HIV RNA were obtained by placing a sterile Dacron swab (Puritan, Guilford, Maine, USA) on the outer part of the endocervix and rotating 360 degrees in the cervical os. One swab was kept dry and another swab was expressed into a

cryotube containing 1 mL of freezing media (70% RPMI, 20% fetal bovine serum, and 10% dimethyl sulfoxide) (Sigma Life Science, St. Louis, Missouri, USA). A blood sample was also collected for CD4 lymphocyte counts and plasma HIV RNA.

The cervix was colposcopically visualized and Lugol's solution was applied to outline the lesion and transformation zone. Prior to LEEP, the cervix was injected with 3-5 mL of anesthetic and Lugol's solution. A high frequency electrosurgical generator, Finesse II (Utah Medical Products Inc., Midvale, Utah, USA) was inserted at a depth of 4-5 mm and drawn laterally across the cervix, and an additional pass with the loop was made only for large lesions to ensure complete excision. Cryotherapy was performed by the study doctor using nitrous oxide with a 3-minute freeze, 5-minute thaw, and 3-minute freeze method. The duration of treatment allowed for an ice crystal formation that extended 4-5 mm beyond the tip of the cryotherapy to ensure tissue destruction of up to 5 mm in depth. Appropriate 25 mm or 19 mm probes with either a flat surface or a shallow nipple were utilized according to the size and location of the lesion. Hemostasis was achieved following LEEP with electrocoagulation or application of Monsel's paste, or both; following cryotherapy, Monsel's paste was applied if bleeding was observed. Women were advised to abstain from sexual activity for four weeks following treatment. All clinical procedures were performed by the same study doctor.

Women were scheduled for follow-up in the study clinic at 7, 14, and 21 days after treatment. At each time point, cervical and plasma samples were collected, a physical examination was performed, and a standardized questionnaire was administered on acceptability of treatment and sexual activity.

Laboratory Testing

Cervical and plasma samples were stored at -80°C and shipped to the Fred Hutchinson Cancer Research Center in Seattle, Washington, on dry ice. HIV-1 RNA was quantified in cervical and plasma specimens using Gen-Probe APTIMA HIV assay (Hologic Gen-Probe Inc., San Diego, California, USA). Specimens with less than 300 μl of cervical fluid were not sufficient for testing. The lower limit of HIV RNA detection was 60 copies/swab for cervical and 60 copies/ml for plasma samples. Samples measured below the lower limit of detection were assigned the median value, 30 ($1.48 \log_{10}$) copies, between zero and the lower limit of detection. CD4 lymphocyte counts were determined using flow cytometry (Becton Dickinson Biosciences, San Jose, California, USA).

Statistical Analysis

The targeted sample size of the parent RCT (400 women; 200 per arm) was designed to detect a 10% difference in recurrence of CIN between treatment arms. This sample size was sufficient to detect a $0.25 \log_{10}$ difference in cervical HIV RNA levels with greater than 90% power. Cervical HIV shedding was compared between arms using intent-to-treat analysis.

Statistical analyses were performed using Stata version 14.1 (Stata Corp., College Station, Texas, USA). Generalized estimating equations (GEE) with robust standard errors were used to model the mean change in cervical HIV RNA levels over time. We assumed an exchangeable correlation structure to account for intra-subject correlation and used a log link to interpret relative change and an identity link for absolute change in risk.

Cervical HIV RNA was modeled as a log-transformed, continuous outcome. Weekly follow-up study visits were categorical variables defined as any study visit ± 3 days of 7, 14, or 21-day time points post-treatment. The multivariate model included baseline cervical HIV RNA, an

interaction term for treatment and time, detectable plasma HIV RNA, and dichotomous ART (on/off). We conducted sub-analyses to evaluate change in cervical HIV RNA levels by treatment arm. Each treatment arm was also stratified by women on or off ART because it was expected HIV RNA levels would vary by ART exposure.

The ethical review committees of the University of Washington (USA) and Kenyatta National Hospital (Kenya) approved the protocol.

RESULTS

Baseline Characteristics

Demographic and behavioral characteristics were balanced between the treatment arms (Table 1). Fewer women treated with cryotherapy had baseline CIN 3, 54.5% (n=109) compared to 64.0% (n=128) among women treated with LEEP. Baseline median CD4 count was 380 [interquartile range (IQR) 215-525] cells per mm³ in the overall cohort and did not differ significantly between the arms. Seventeen women (8.5%) in LEEP and 28 (14%) in cryotherapy were off ART at the time of intervention. Most women (79.8%) reported exposure to ART for longer than 6 months. At baseline, plasma viral load was similar between LEEP and cryotherapy (mean log₁₀ HIV RNA, 2.17 vs. 2.33; *P*=0.224) and cervical shedding was significantly lower in LEEP (mean log₁₀ HIV RNA, 1.80 vs. 2.02 log₁₀ copies; *P*=0.009).

Cervical HIV RNA following LEEP vs. Cryotherapy

Among 400 women randomized to LEEP or cryotherapy in the parent RCT, two women (1.0%) in the LEEP and three (1.5%) in the cryotherapy arm had inadequate specimens at baseline. Of 600 possible scheduled follow-up visits in the LEEP arm, 18 (3.0%) visits were missed, and 51 (8.5%) were outside the ±3-day window, and 9 specimens were inadequate (1.5%). In the cryotherapy arm, 17 (2.8%) visits were missed, 51 (8.5%) visits were outside the ±3-day

window, and 9 (1.5%) had inadequate specimens of <300 µl of cervical fluid (Figure 1). Fewer women in the LEEP arm engaged in sexual intercourse in the three weeks following cervical treatment, 33 (16.6%) compared to 42 (21.1%) in cryotherapy.

There was no difference in mean cervical HIV RNA levels between arms in the first (Incident Rate Ratio [IRR], 0.95; 95% Confidence Interval [CI], 0.87-1.04; $P=0.297$), second (IRR 0.98, 95% CI, 0.88-1.09; $P=0.727$), and third weeks (IRR 0.98; 95% CI: 0.87-1.10; $P=0.701$) after cervical treatment in the unadjusted analysis (Figure 2).

Adjusting for baseline cervical HIV RNA, ART exposure, and plasma viral load, the mean rate of change in cervical shedding from baseline was similar between treatments at week 1 (aIRR 1.04; 95% CI: 0.98-1.10; $P=0.203$). However, the risk of HIV cervical shedding in the LEEP arm was significantly increased compared to the cryotherapy arm at week 2 (aIRR 1.07; 95% CI: 1.00-1.15; $P=0.038$) and remained significant at week 3 (aIRR 1.08; 95% CI: 1.00-1.16; $P=0.046$). The magnitude of difference in mean cervical HIV RNA levels between the treatment arms in the adjusted analysis was consistently higher in the LEEP arm and ranged between 0.03-0.12 \log_{10} copies during follow up.

Cervical HIV Shedding Within LEEP Arm

Women treated with LEEP showed a significant increase in mean cervical HIV RNA between week 1 and baseline from 1.80 to 1.92 \log_{10} copies ($P=0.009$). Cervical HIV RNA levels further increased at week 2 to 2.03 \log_{10} copies ($P<0.001$) and week 3 to 2.04 \log_{10} copies ($P<0.001$). LEEP's effect on cervical HIV RNA levels remained statistically significant after adjusting for plasma HIV RNA levels in multivariate analysis. Among the subset of women on ART who were treated with LEEP, the mean cervical viral load increased from baseline at weeks 2 and 3 [week

2 mean HIV RNA increased to 1.88 log₁₀ copies ($P=0.002$) and at week 3, 1.93 log₁₀ copies ($P=0.002$]. Among women off ART, cervical HIV RNA levels increased from a baseline of 2.29 log₁₀ copies to 3.02 log₁₀ copies ($P<0.001$) at week 1, 3.56 log₁₀ copies ($P<0.001$) at week 2, and 3.36 log₁₀ copies ($P<0.001$) at week 3 (Table 2).

Cervical HIV Shedding Within Cryotherapy Arm

Mean cervical HIV RNA levels for women treated with cryotherapy were 2.02 log₁₀ copies at baseline and did not significantly increase at week 1 (1.96 log₁₀ copies; $P=0.566$), week 2 (2.04 log₁₀ copies; $P=0.150$), or week 3 (2.12 log₁₀ copies; $P=0.122$) (Table 3). The lack of association persisted in analyses adjusting for plasma viral load. Among the subset of women on ART randomized to cryotherapy, cervical HIV RNA levels did not increase at weeks 1 (1.82 log₁₀ copies; $P=0.627$), 2 (1.86 log₁₀ copies; $P=0.877$) 2, or 3 (1.86 log₁₀ copies; $P=0.831$) above baseline. Among women off ART, mean cervical HIV RNA was unchanged at week 1 (2.83 log₁₀ copies; $P=0.812$) following cryotherapy. However, at weeks 2 and 3, mean HIV RNA levels significantly increased to 3.31 log₁₀ copies ($P=0.011$) and 3.80 log₁₀ copies ($P=0.002$), respectively.

DISCUSSION

In this study examining the impact of LEEP and cryotherapy on cervical HIV shedding, women treated with LEEP had significantly higher mean cervical HIV RNA levels compared to cryotherapy for three weeks following treatment. Women treated with LEEP had higher cervical HIV RNA levels throughout the follow-up period compared to baseline, regardless of ART status or plasma viral load. In contrast, cervical HIV RNA levels did not increase after treatment with cryotherapy or among women on ART; only women off ART had higher HIV RNA levels during follow-up.

The results of our study are consistent with a recent study demonstrating LEEP treatment resulted in a significant, short-term increase in HIV shedding at two weeks following treatment which returned to baseline by four weeks [22]. Immune activation in the genital tract recruits CD4+ T-cells to the area of tissue damage, increasing HIV target cells [46]. Pro-inflammatory cytokines predict HIV shedding [18,47], and higher levels of cervical HIV RNA and pro-inflammatory cytokines have been measured following CIN treatment [42]. As an excisional procedure, LEEP removes both the lesion and healthy cervical tissue around the lesion [22]. The subsequent ulceration resulting from LEEP treatment covers a greater surface area and may thereby promote a greater inflammatory response than cryotherapy [12]. Depending on the size of the loop electrode used, tissue should be excised to a depth of 6-7 mm, but it is not uncommon for the depth of tissue removal to exceed 10 mm [35,48]. By removing cells of both the ecto- and endocervix, LEEP may contribute to HIV-shedding from the upper genital tract in addition to the lower tract [49]. In contrast, cryotherapy causes necrosis and damage to ectocervical cells by freezing tissue up to 5-7 mm deep [50]. Typical CIN2/3 lesions are less than 5 mm in depth from the surface [51]. Compared to LEEP, the inflammation following cryotherapy treatment may be more localized and therefore cause less HIV shedding.

The transmission risk of female-to-male HIV transmission has been shown to be 1.56-fold higher for every 1- \log_{10} increase in cervical HIV RNA after adjusting for plasma HIV RNA [14]. In our study, the magnitude of change in cervical HIV RNA from baseline was less than 0.20 \log_{10} among women on ART in both LEEP and cryotherapy cohorts. However, localized inflammation is significantly associated with increased cervical HIV shedding [52], and women on ART with undetectable plasma HIV RNA have still been found to shed HIV from the cervix [53,54]. Women not on ART experienced a mean change of greater than 1- \log change following LEEP and 0.68 \log_{10} following cryotherapy. ART appears to modulate changes in cervical HIV levels, and thus the risk of sexual HIV transmission may not pose a substantial risk for women on ART,

but there may be increased infectivity among women who are not receiving ART at the time of cervical treatment particularly among those receiving LEEP.

Our study has several limitations. First, we reported an imbalance between arms in cervical HIV RNA at baseline, and we addressed this by adjusting for baseline cervical viral load. Second, the study was limited by the number of women not taking ART which decreased the precision of the study to evaluate the effect of treatment and the role of ART. While this data is informative for clinical decision-making, further research on the role of ART in mitigating post-treatment cervical HIV shedding is warranted to more effectively assess the risk of HIV-transmission. We only measured cervical HIV shedding for three weeks after treatment and did not measure when cervical viral load returned to baseline. However, prior studies have shown that cervical HIV RNA levels return to baseline after four weeks [21,22], suggesting that uncomplicated treatment results only in short-term changes to the cervix that were likely captured within the course of the study.

As access to ART continues to expand globally and HIV-infected women are living healthier and more productive lives, screening and treatment for cervical cancer may be a critical component in further extending their quality of life. The results of our study support the current guidelines that HIV-infected women should avoid sexual intercourse for one month to allow the treatment wound to heal or to practice risk reduction strategies to minimize risk of HIV-transmission. Our data indicate that there is an increase in cervical HIV shedding which differs by treatment procedure and ART status and HIV-infected women should be counseled on the risk of HIV sexual transmission. Our results suggest that cryotherapy used in resource-limited settings likely does not contribute to HIV transmission during the immediate post-procedure period and is optimally performed when women are on ART to minimize cervical shedding. LEEP treatment

may contribute to a time-limited increase in HIV infectivity among women not on ART and these changes likely occur within the one month interval following treatment.

Table 1. Baseline Demographic, Behavioral, and Clinical Characteristics by Treatment Arm

Characteristic	Cryotherapy		LEEP	
	n	Median (IQR) ^a or %	n	Median (IQR) ^a or %
Age (years)	200	38.2 (32.0-42.5)	200	37.4 (31.7-44.9)
Education (years)	200	11.0 (8.0-12.0)	200	11.0 (8.0-12.0)
Marital Status	200		200	
Single	58	29.0	52	26.0
Committed Relationship	79	39.5	84	42.0
Divorced/Separated/Widowed	63	31.5	64	32.0
Salaried Employment	53	26.5	63	31.5
Monthly Income (USD)	186		193	
<\$50	44	23.7	48	24.9
\$50-\$150	65	35.0	69	35.8
>\$150	77	41.4	76	39.4
Lifetime Sexual Partners	197		198	
1-2	88	44.6	67	33.8
≥3	93	47.2	113	57.0
Any Hormonal Contraception	36	18.1	32	16.0
Menses in Last 7 days	39	20.1	33	16.8
CIN 3	109	54.5	128	64.0
CD4 (cells/mm ³)	200	371 (213-532)	199	385 (221-522)
<250	59	29.5	59	29.7
250-499	83	41.5	84	42.2
≥500	58	29.0	56	28.1
No ART Exposure	28	14.0	17	8.5
ART Exposure <30 days	3	1.5	6	3.0
ART Exposure 30 days – 6 months	12	6.0	15	7.5
ART Exposure >6 months	157	78.5	162	81.0
Median ART Exposure (months) ^b	172	29.4 (14.1-60.2)	183	34.0 (12.4-74.3)
Plasma HIV RNA ≥1.48 log ₁₀ copies/ml ^c	72	37.0	67	34.7
Cervical HIV RNA ≥1.48 log ₁₀ copies/swab ^c	61	31.0	42	21.2

Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; IQR, interquartile range; RNA, ribonucleic acid; USD, dollars (United States).

^aInterquartile range.

^bAmong those on ART prior to treatment.

^cMinimum limit of detection of HIV RNA was 1.48 log₁₀ copies per ml for plasma and 1.48 log₁₀ copies per swab for cervical swabs.

Table 2. Change in Mean Cervical HIV RNA [\log_{10} copies/swab] Among Women Treated with LEEP

Time	Mean (copies/swab)	Absolute mean change from baseline (95% CI) ^c	Unadjusted IRR ^d (95% CI)	<i>P</i>	Adjusted IRR ^d (95% CI)	<i>P</i>
Overall^a (n=200)						
Baseline	1.80	-	-		-	-
Week 1	1.92	0.10 (0.02-0.18)	1.06 (1.01-1.10)	0.009	1.06 (1.01-1.11)	0.010
Week 2	2.03	0.25 (0.14-0.37)	1.14 (1.08-1.21)	<0.001	1.17 (1.10-1.24)	<0.001
Week 3	2.04	0.26 (0.14-0.38)	1.14 (1.08-1.21)	<0.001	1.17 (1.10-1.25)	<0.001
On ART^b (n=183)						
Baseline	1.76	-	-		-	-
Week 1	1.81	0.05 (-0.02-0.12)	1.02 (0.98-1.06)	0.281	1.03 (0.98-1.07)	0.220
Week 2	1.88	0.16 (0.07-0.26)	1.09 (1.03-1.15)	0.002	1.11 (1.05-1.18)	<0.001
Week 3	1.93	0.18 (0.08-0.29)	1.10 (1.04-1.17)	0.002	1.13 (1.06-1.21)	<0.001
Off ART^b (n=17)						
Baseline	2.29	-	-		-	-
Week 1	3.02	0.74 (0.27-1.20)	1.32 (1.16-1.52)	0.000	1.35 (1.13-1.61)	0.001
Week 2	3.56	1.22 (0.70-1.74)	1.51 (1.27-1.80)	<0.001	1.57 (1.32-1.87)	<0.001
Week 3	3.36	1.08 (0.54-1.62)	1.49 (1.26-1.77)	<0.001	1.51 (1.26-1.80)	<0.001

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; IRR, incident rate ratio; LEEP, loop electrosurgical excision procedure.

^aAdjusted for antiretroviral treatment (ART) and detectable plasma HIV RNA.

^bAdjusted for detectable plasma HIV RNA.

^cAbsolute change and 95% confidence intervals (CI) of cervical \log_{10} copies/swab HIV RNA in comparison to baseline.

^dIncident rate ratio (IRR) and 95% confidence interval (CI) in comparison to baseline.

Table 3. Change in Mean Cervical HIV RNA [\log_{10} copies/swab] Among Women Treated with Cryotherapy

Time	Mean \log_{10} copies	Absolute mean change from baseline (95% CI) ^c	Unadjusted IRR ^d (95% CI)	<i>P</i>	Adjusted IRR ^d (95% CI)	<i>P</i>
Overall^a (n=200)						
Baseline	2.02	-	-		-	-
Week 1	1.96	-0.02 (-0.11-0.06)	0.99 (0.95-1.03)	0.566	0.98 (0.94-1.03)	0.390
Week 2	2.04	0.07 (-0.02-0.16)	1.04 (0.99-1.09)	0.150	1.04 (0.99-1.10)	0.102
Week 3	2.12	0.05 (-0.05-0.14)	1.04 (0.99-1.10)	0.122	1.05 (0.99-1.11)	0.104
On ART^b (n=172)						
Baseline	1.88	-	-		-	-
Week 1	1.82	-0.02 (-0.11-0.06)	0.99 (0.95-1.03)	0.627	0.98 (0.93-1.03)	0.386
Week 2	1.86	0.03 (-0.06-0.12)	1.00 (0.96-1.05)	0.877	1.01 (0.96-1.07)	0.658
Week 3	1.86	-0.01 (-0.11-0.08)	0.99 (0.94-1.05)	0.831	1.00 (0.94-1.05)	0.899
Off ART^b (n=28)						
Baseline	2.88	-	-		-	-
Week 1	2.83	-0.04 (-0.33-0.26)	0.99 (0.89-1.09)	0.812	0.99 (0.88-1.11)	0.864
Week 2	3.31	0.45 (0.03-0.86)	1.18 (1.04-1.35)	0.011	1.17 (1.02-1.34)	0.024
Week 3	3.80	0.68 (0.18-1.17)	1.25 (1.08-1.44)	0.002	1.25 (1.07-1.45)	0.004

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; IRR, incident rate ratio.

^aAdjusted for antiretroviral treatment (ART) and detectable plasma HIV RNA.

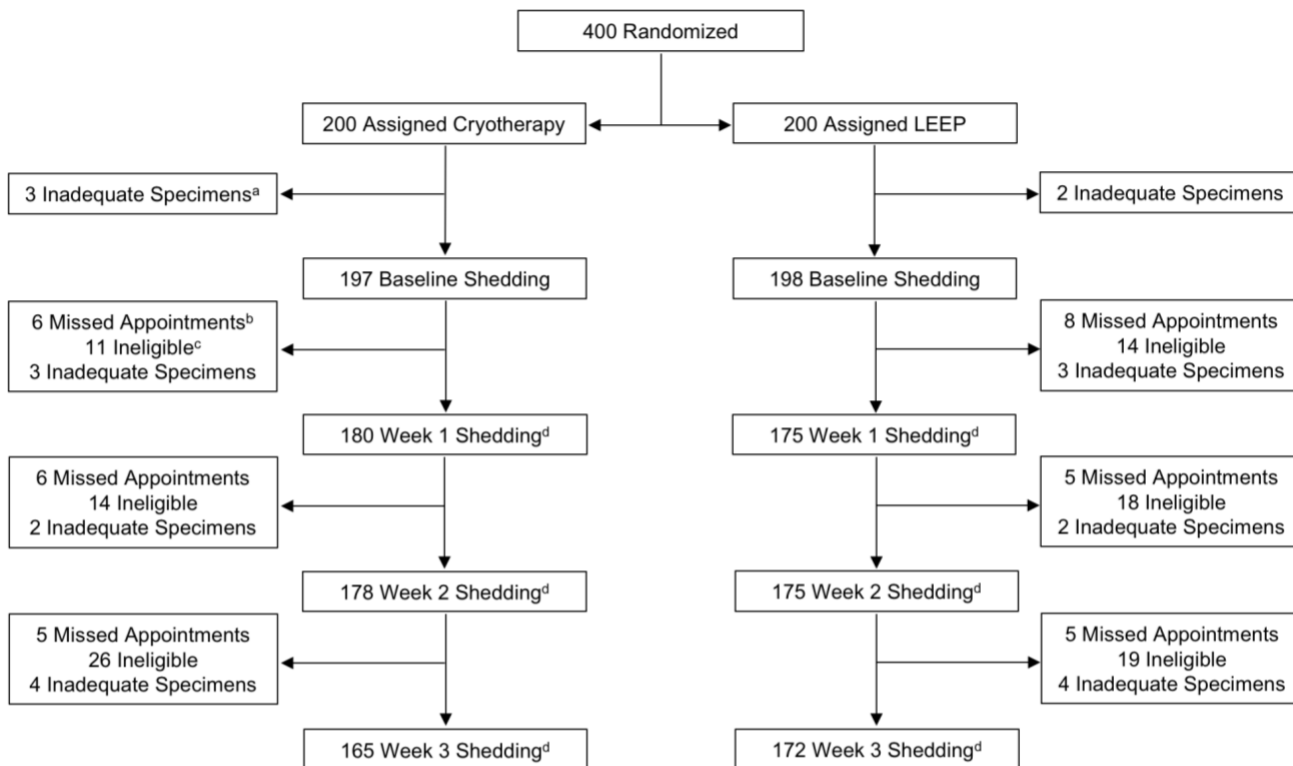
^bAdjusted for detectable plasma HIV RNA.

^cAbsolute change and 95% confidence intervals (CI) of cervical \log_{10} copies/swab HIV RNA in comparison to baseline.

^dIncident rate ratio (IRR) and 95% confidence interval (CI) of cervical \log_{10} copies/swab HIV RNA in comparison to baseline.

FIGURES

Figure 1. Participant Flow of Randomization and Follow-up in the Trial.



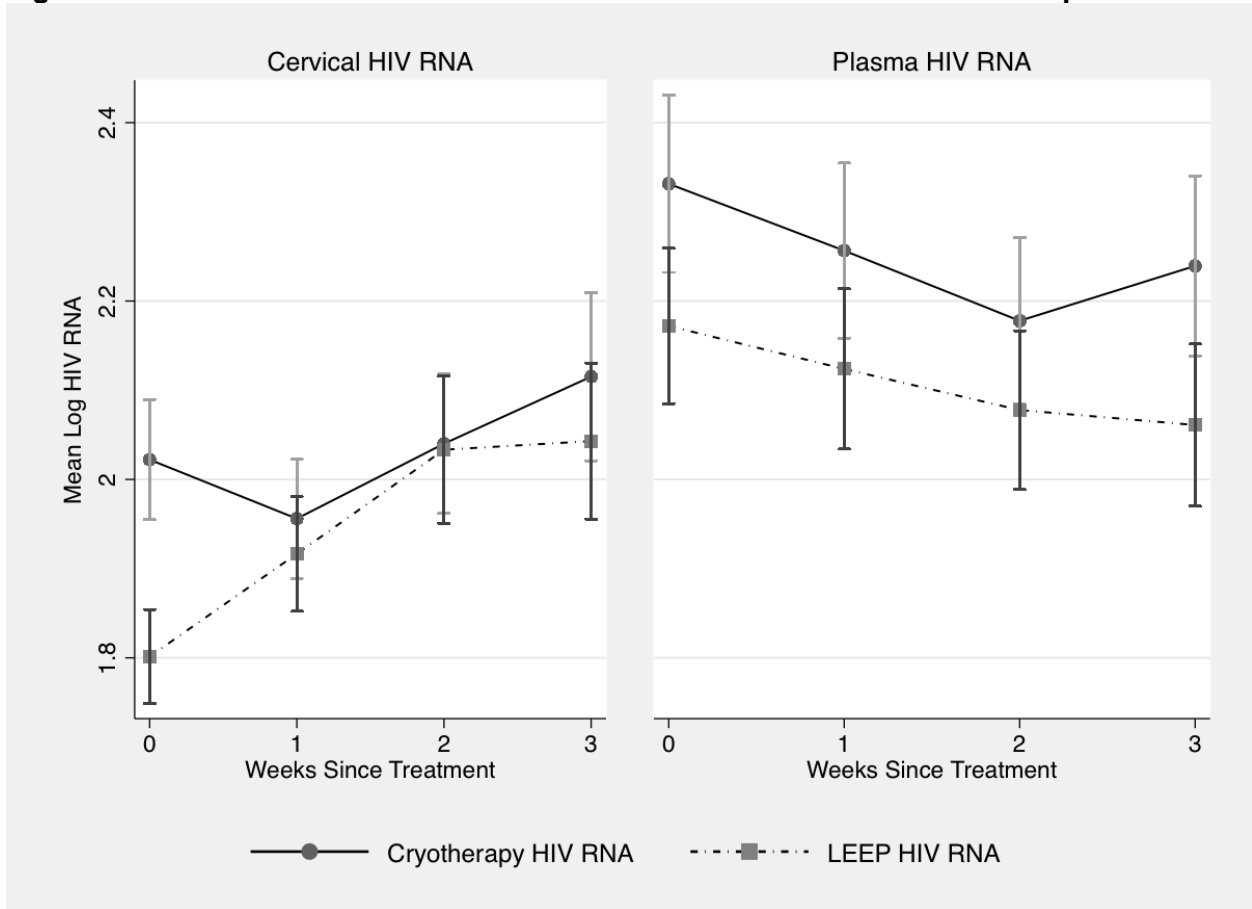
^aThe cervical swab volume collected was less than 300 μ l.

^bThe study participant did not attend a follow-up study visit at weeks 1, week 2, or week 3.

^cFollow-up study visits did not occur within ± 3 days of 7, 14, or 21 days post-treatment.

^dThe expected number of women at weeks 1, 2, and 3 was 200 for each arm.

Figure 2. Mean Cervical and Plasma HIV RNA Over Three Weeks of Follow-up.



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DISCLAIMER

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the funding agencies.

CONFLICT OF INTEREST

All authors: No reported conflicts.

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Chapter 3: Cryotherapy Associated with High Rate of Recurrence of Cervical Pre-Cancer

Effectiveness of Cryotherapy versus Loop Electrosurgical Excision Procedure Among HIV-Infected Women: A Randomized Controlled Trial

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ABSTRACT

Background

WHO guidelines recommend cryotherapy or loop electrosurgical excisional procedure (LEEP) for histologically confirmed cervical intraepithelial neoplasia 2/3 (CIN2+) regardless of HIV infection. Cryotherapy is more feasible in resource-limited settings but may be less effective for HIV-infected women.

Methods

We conducted a randomized trial among HIV-infected women to compare cryotherapy to LEEP and evaluated risk of recurrence of HSIL+ and CIN2+ over 2-year follow-up (NCT01298596). From June 2011 to July 2014, HIV-infected women enrolled at the Coptic Hope Center for Infectious Diseases in Nairobi, Kenya underwent cervical screening with Papanicolaou smear and confirmatory biopsy. Four hundred women with CIN2+ were randomized 1:1 to receive cryotherapy or LEEP, and were followed every 6 months with a Pap smear and confirmatory biopsy for 2 years. Recurrence was defined as high grade squamous intraepithelial lesions (HSIL) or greater on cytology and CIN 2+ on histology, and outcomes were compared between arms using Chi-square tests and Cox proportional hazards regression.

Findings

Among women randomized to cryotherapy, 60 (30%) women had recurrent CIN2+ compared to 37 (19%) recurrences in the LEEP arm. The cumulative incidence of CIN2+ recurrence was 19.2 (95% CI 14.9-24.7) per 100 person-years after cryotherapy and 11.2 (95% CI 8.1-15.5) per 100 woman-years after LEEP ($p=0.005$). At 24 months, CIN2+ was significantly higher in the cryotherapy arm compared to LEEP (hazard ratio [HR] 1.69; 95% CI 1.12-2.55; $p=0.012$) and after adjusting for ART, CD4 and baseline CIN (HR 1.76, 95% CI 1.17-2.64; $p=0.007$). Women on ART for less than 2 years (HR 2.18; 95% CI 1.14-4.19; $p=0.019$), with low CD4 (<250

cells/mm³) (HR 2.58; 95% CI 1.12-5.94; p=0.026), or detectable plasma viral load (≥60 copies/ml) (HR 2.44; 95% CI 1.28-4.66; p=0.007) had significantly higher risk of CIN2+ recurrence following cryotherapy.

Interpretation

Among HIV-infected women, cryotherapy was associated with higher risk of recurrent pre-cancerous cervical disease than LEEP. In settings with HIV and cervical cancer, LEEP should be the standard of care for CIN treatment.

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INTRODUCTION

Over half a million women develop cervical cancer each year and 85% of cases are estimated to occur in low resource settings [2]. Disparities between high and low resource settings include the availability of effective screening and treatment programs, prevalence of high-risk human papillomavirus (HPV) types, and burden of HIV, which contributes to higher incidence of disease, later presentation, and increased mortality [32,55]. Due to underlying immunosuppression, HIV-infected women have a higher burden of cervical intraepithelial neoplasia (CIN) that is more likely to persist and progress to cervical cancer than women without HIV [4,5,23,56].

Current World Health Organization guidelines for the treatment of CIN grade 2/3 recommend cryotherapy or loop electrosurgical excision procedure (LEEP) for women regardless of HIV status [10]. Cryotherapy is an ablative treatment option that is low cost, can be performed in primary care centers, and has been shown to be efficacious among HIV uninfected women [36,57]. Many low-resource settings use cryotherapy in a single-visit, screen-and-treat approach which reduces both the number of clinic visits and risk of loss to follow-up [36,38,58]. LEEP is an excisional procedure that is the first choice of treatment in high resource settings regardless of HIV status because it provides tissue for histopathology [8]. The technology is less common in low resource settings because LEEP requires expensive equipment and trained providers.

High grade CIN can be difficult to treat and recurs in approximately 5-14% of women after treatment and among HIV-infected women, recurrence has been reported as high as 23% [23–25,28,59]. Though HIV-infected women experience more frequent disease recurrence, the WHO guideline notes that data are inadequate to make specific treatment recommendations for HIV-infected women [10]. Recurrence among HIV-infected women estimates vary considerably according to the extent of pre-treatment disease, length of follow-up time, and by immune status

[31]. Few longitudinal studies have sufficient follow-up time necessary to measure long-term recurrence risk or assess how recurrence is modified by CD4 count and antiretroviral therapy (ART). Improved understanding of the interactions between CIN, HIV, and treatment is needed to guide secondary prevention programs for HIV-infected women.

The aim of this randomized controlled trial was to assess the incidence of disease recurrence among HIV-infected women treated with either cryotherapy or LEEP over two years of follow-up

METHODS

Study design and participants

The Cervical Treatment Study was an unmasked randomized controlled trial of cryotherapy versus LEEP treatment among HIV-infected women with biopsy confirmed CIN grade 2/3 in Nairobi, Kenya. The protocol is available online. We recruited women receiving HIV care and treatment at the Coptic Hope Center for Infectious Diseases, a President's Emergency Plan for AIDS Relief (PEPFAR) supported urban HIV care clinic [45]. Eligible women were ≥ 18 years of age, HIV-infected, had initiated sexual activity, were not pregnant, and had an intact cervix without history of hysterectomy. Exclusion criteria included a history of treatment for cervical lesions, or if the woman had a lesion that was not eligible for cryotherapy including a lesion that was larger than 75% of the cervix surface, extended more than 2 mm into the endocervical canal, or could not be fully visualized on colposcopy.

We obtained approval to do the study from the Kenyatta National Hospital and University of Nairobi Ethics Research Committee, the Centers for Disease Control and Prevention, and the University of Washington Institutional Review Board. All participants provided written informed consent to participate in the study.

Randomization and masking

A permuted, block-randomization scheme with block sizes of 10 was used to randomly assign women in a 1:1 allocation to receive either cryotherapy or LEEP treatment. All study investigators were masked to block number, block size, and sequence in the block. The allocation sequence was concealed in sequentially numbered opaque sealed envelopes. Study investigators and participants were not blinded to the interventions.

Procedures

Women were consented and screened for cervical cancer using Papanicolaou (Pap) smear. Cervical smears were collected using a cervical brush that was inserted into the ectocervix and rotated anticlockwise 5 times to collect cells from the endo- and ectocervix and then inserted in Thin-Prep solution (Hologic, Inc; Marlborough, Massachusetts, USA). Women with squamous cell carcinoma (SCC), high-grade squamous intraepithelial lesion (HSIL), or atypical squamous cells which cannot exclude high-grade squamous intraepithelial lesion (ASC-H) underwent colposcopy-directed biopsy for histology. Punch biopsies were collected using biopsy forceps at the site exhibiting the most severe changes. An endocervical curettage was performed on women with cytology results that indicated atypical glandular epithelium abnormalities.

Cervical samples for Pap testing and biopsies were prepared and processed by the laboratory technologist. Cellular changes were classified according to the Bethesda System [60]. Biopsy specimens were fixed in 10% formalin and embedded in paraffin. Slides were stained with hematoxylin and eosin. All specimens were reviewed by a certified pathologist who had no knowledge of the women's treatment arm or research data and reported based on three-tiered CIN classification (CIN 1, 2, and 3). A quality assurance protocol was implemented where a separate pathologist employed by the study reviewed a random sample of 10% of abnormal and

3% of normal Pap smears as well as all abnormal cervical biopsies. If there were discrepant results, then a third pathologist reviewed for consensus. All reported data are consensus results; some women were randomized and received treatment based on initial results read by the first pathologist.

Women with histologically confirmed CIN 2/3 lesions that were eligible for cryotherapy returned to the clinic to undergo treatment. The same medical doctor performed colposcopic examinations, collected biopsies, and performed treatment procedures. Lugol's solution was applied to the cervix to outline the lesion and transformation zone. Prior to LEEP, the cervix was injected with 3-5 mls of anesthetic. A high frequency electrosurgical generator, Finesse II (Utah Medical Products Inc., Midvale, Utah, USA) was inserted at a depth of 4-5 mm and drawn laterally across the cervix, and an additional pass with the loop was made only for large lesions to ensure complete excision. Hemostasis was achieved following LEEP with electrocoagulation or application of Monsel's paste, or both. The LEEP tissue specimen was fixed in 10% buffered formalin solution and specimens were evaluated for morphological changes by the pathologist.

Cryotherapy was performed using nitrous oxide and a cryosurgical unit (LL100; Wallach Surgical Devices, Inc, Orange, Connecticut, USA) with a 3-minute freeze, 5-minute thaw, and 3-minute freeze method which was necessary to observe the ice ball form 5 mm beyond the edge of the cryoprobe. Flat probes or probes with a shallow nipple measuring either 19 mm or 25 mm were selected to cover the entire lesion. Following cryotherapy, Monsel's paste was applied if bleeding was observed.

Oral norfloxacin, metronidazole, and/or doxycycline were prescribed to protect against infection after cervical treatment or as treatment for sexually transmitted infection detected on pelvic exam. Possible complications were described and women were encouraged to return to the

clinic immediately if they experienced post-operative symptoms. Women were counseled to abstain from sexual intercourse for at least four weeks after treatment and were provided condoms to reduce risk of infection or HIV-transmission.

Follow-up visits occurred at 6, 12, 18, and 24 months after cervical treatment. At each visit, Pap smears and plasma samples were collected. If cytology results were HSIL+, then on a subsequent visit, women underwent a cervical biopsy and LEEP treatment. Women were defined as lost to follow-up if they missed a bi-annual Pap smear visit and did not return for additional follow-up visits. Women who met the criteria for disease recurrence and had received two prior LEEP treatments or had histological evidence of invasive disease were referred to the national referral hospital for further management. Women received a physical examination and completed a standardized questionnaire on both acceptability and complications of treatment on

Women were scheduled for follow-up in the study clinic at 7, 14, and 21 days after baseline treatment for a nested sub-study on HIV shedding after cervical treatment [CITE CID article]. During these visits, women had a physical examination and completed a standardized questionnaire on both acceptability and complications of treatment. All reported adverse events were graded according to the Division of AIDS Table for Grading the Severity of Adult Adverse Events version 1.0 [61].

Outcomes

The primary outcomes were cumulative HSIL+ and CIN2+. We defined HSIL+ as ASC-H, HSIL, or SCC on consensus cytology interpretation at any of the 6-month intervals during the 2-year follow-up. We defined CIN2+ as consensus interpretation of CIN 2, CIN 3, or invasive cervical cancer (ICC) on either cervical biopsy or endocervical curettage. Secondary outcome measures included acceptability of treatment and the number of adverse events.

Statistical analysis

In the study design, we assumed a difference in cervical pre-cancer recurrence of at least 10% based on subgroup analysis of an earlier study which reported CIN 2/3 disease recurrence among HIV-infected women treated with cryotherapy was 14.3% compared to 4.0% after LEEP [26]. We estimated that randomization of 400 HIV-infected women, 200 in each arm, accounting for 20% attrition over 24-month follow-up, would provide a power of more than 80% to detect a 10% difference in disease recurrence between LEEP and cryotherapy at a two-sided alpha level of 0.05.

The primary analysis was an intention-to-treat analysis of the participants' randomization assignment to assess the primary end points. Baseline characteristics were compared using medians, interquartile ranges (IQR), and proportions. We compared proportions with chi-square tests and Fisher's exact tests, as appropriate. Kaplan-Meier method was used to provide estimates of the cumulative incidence of disease recurrence at 24 months and to compute the number needed to treat [62]. Women contributed at-risk time from date of cervical treatment to either detection of disease recurrence or date of final Pap smear, whichever occurred first. Women were censored at the date of treatment or last completed Pap smear, if they were lost to follow-up, withdrew from the study, died, or if they had a total abdominal hysterectomy during follow-up. To assess the risk of recurrence between the intervention arms, we performed log-rank tests and Cox proportional hazards regression analysis. The model was adjusted for potential confounders of antecedent histology of CIN 3, antiretroviral therapy (ART) exposure, and CD4 cell count was included as a time-dependent covariate. Analyses were conducted using STATA software, version 14.2 (College Station, TX: StataCorp LP).

An independent Data Safety and Monitoring Board (DSMB) reviewed the study results after the first 100 women received treatment and then every 6 months until the completion of the study.

Five pre-specified interim analyses were conducted to assess safety and evaluate the primary endpoint. The O'Brien-Fleming approach was used to define stopping boundaries of statistical significance. The study is registered with clinicaltrials.gov, number NCT01298596.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author (SAG) had access to all the data in the study, and the senior author (MHC) had final responsibility for the decision to submit for publication.

RESULTS

Enrollment began on June 6, 2011 and the last follow-up visit occurred on August 8, 2016. We assessed 5,330 HIV-infected women for cervical cancer and study eligibility. Most women (82.2%) had no evidence of cervical pre-cancer and 4.1% had low grade results on cytology. For 738 (13.8%) women with either high grade cervical lesions on cytology or who received a cervical biopsy, the main exclusion criteria were either a diagnostic colposcopy biopsy of less than CIN 2 (30.7%) or a CIN2+ lesion that was not amenable to cryotherapy (14.6%). Among women with CIN2+, 400 were randomly assigned to a treatment intervention.

Socioeconomic characteristics were balanced between the trial arms. Women had a median age was 38 years (interquartile range [IQR]: 32-44) and almost all women had one or more children. The median age at sexual debut was 18 years (IQR: 16-20) and more than half of women reported 3 or more lifetime sexual partners. Half of the women did not complete secondary school, and more than half of women lived in households earning less than \$150 USD per month. Most women had controlled HIV disease. Almost 90% of women were treated

with ART and more than 50% were prolonged ART users (≥ 2 years). Two thirds of women had CD4 counts above 250 cells/mm³ and had undetectable plasma viral load at baseline (Table 1).

One hundred and ninety-five women in both arms had at least one follow-up Pap smear. The rate of retention in the study, defined as completion of either end-of-study visit procedures at 24 months or when disease recurrence was detected during follow-up, was 91% and did not differ significantly between the treatment groups (Figure 1). There were 296 person-years at risk of recurrence in the cryotherapy cohort and a mean follow-up time of 1.48 years (Standard deviation [SD]: 0.68) per person. In the LEEP arm, there were 311 person-years at risk and a mean follow-up time of 1.56 years (SD: 0.68). During follow-up, cervical biopsies were collected on 195 of 254 (76.8%) women with either HSIL+ or had atypical glandular cells on cytology, 21 women were referred for further management outside the study, 19 received biopsy and LEEP treatment based on an earlier cytology result, and 19 women were pregnant, declined, or did not return to the study clinic until the subsequent biannual study visit.

Cumulative incidence of HSIL+ and CIN 2+ endpoints are summarized in table 2. Over 24 months of follow-up, 74 (37%) women had recurrent HSIL+ disease in the cryotherapy arm compared to 52 (26%) recurrences in the LEEP arm ($p=0.013$). At 6 months, HSIL+ recurrence was greater in cryotherapy (21%) than LEEP (13%) ($p=0.024$) and at 12 months (30%) in cryotherapy and (21%) in LEEP ($p=0.030$). Median time to HSIL+ recurrence was 196 days in cryotherapy arm and 215 in the LEEP arm (log rank $p=0.033$). Incidence of CIN2+ over 24 months was also significantly higher in the cryotherapy ($n=60$) arm compared to LEEP ($n=37$) over two years, 30% vs 19% ($p=0.005$). At 6 months, CIN2+ recurrence was greater in cryotherapy (15%) than LEEP (8%) ($p=0.0123$) and at 12 months (22%) in cryotherapy and (12%) in LEEP ($p=0.006$).

Women treated with cryotherapy had a 47% increased risk of HSIL+ recurrence (95% CI 1.03-2.09; $p=0.034$) and a 69% increased risk of CIN2+ recurrence (95% CI 1.12-2.55; $p=0.012$) over two years (figure 2). This is equivalent to an absolute difference in CIN2+ event rates of 21.3% and corresponds to an average of 6.5 (95% CI: 4.1-27.0) women with CIN 2+ need to be treated to observe one CIN2+ recurrence free woman more in the LEEP arm than in the cryotherapy arm over two years of follow-up. Adjusting for ART, CD4, and histology of treated lesion further strengthened this association, women treated with cryotherapy 53% more likely to experience HSIL+ recurrence ($p=0.019$) and 76% more likely to experience CIN2+ recurrence ($p=0.007$).

We assessed recurrence of high-grade disease according to HIV cofactors. Figure 3 shows treatment effects stratified by HIV and clinical characteristics over 24 months. The treatment benefit with LEEP was observed among women who had not achieved HIV suppression. In particular, women either not on ART or on ART for less than 2 years, those with low CD4 cells (<250 cells/mm³), or detectable viral load (≥ 60 copies/ml) at baseline had significantly higher CIN2+ recurrence when treated with cryotherapy. Women on ART for less than 2 years were twice as likely to experience CIN2+ recurrence following cryotherapy ($p=0.019$); among prolonged ART users, there was no treatment difference ($p=0.261$). For women with low CD4 counts at the time of treatment, cryotherapy resulted in a 2.5-fold increased risk of CIN2+ recurrence compared to women treated with LEEP ($p=0.026$). In contrast, the difference in CIN2+ recurrence was not statistically different between cryotherapy and LEEP for women with CD4 greater than 250 cells/mm³ ($p=0.152$). Similarly, women with detectable plasma viral load had more than a 2-fold increase in risk of CIN2+ recurrence when treated with cryotherapy ($p=0.007$) while there were no treatment differences for women with undetectable viral load at baseline ($p=0.249$).

Treatment acceptability was assessed at 7, 14, and 21 days after the initial treatment procedure. Over 95% of women in both treatment groups reported that they either strongly agreed or agreed that the procedure was an acceptable method to treat cervical lesions. Among women treated with cryotherapy, all women reported a having a positive reaction to the procedure, and all but one woman would recommend the procedure to a friend or family member. Among women treated with LEEP, 198 (99%) reported having a positive reaction to the procedure and would be willing to recommend the procedure.

Complications following treatment were mild and did not require medical intervention. Following baseline procedure, 185 (93%) women treated with cryotherapy and 176 (88%) women treated with LEEP reported experiencing any symptom after the procedure. A higher proportion of women treated with cryotherapy reported a heavy discharge volume which required a sanitary pad, 81.3% at 7 days and 92.9% at 14 days compared to 56.3% and 50.9% among women treated with LEEP ($P < 0.001$). The number of women reporting mild vaginal bleeding was similar between cryotherapy and LEEP, 17.1% vs 23.6% ($p = 0.106$).

There were no withdrawals from participation as a result of study procedures or adverse events. More than half (59.0%) of 83 reported adverse events were grade 1 or 2 in severity, 45 (54%) in cryotherapy and 38 (46%) in LEEP. Two serious adverse events related to the study procedures were reported during the study. The first was an intraoperative complication during a LEEP procedure for CIN 3 recurrence and the lateral vaginal wall and anterior bladder were perforated. The study participant was hospitalized, and the vaginal lining was repaired. The second complication occurred following a LEEP procedure for CIN 3 recurrence. The study participant reported heavy post-operative bleeding. A repeat application of topical Monsel's paste controlled the cervical bleeding.

DISCUSSION

In this randomized control trial of 400 HIV-infected women, treatment with cryotherapy was associated with significantly higher risk of recurrent pre-cancerous cervical disease among HIV-infected women compared to LEEP over two years of follow-up. The data support LEEP treatment in particular for women on ART for less than 2 years, with low CD4, or detectable plasma viral load. Cumulative CIN 2+ recurrence was high in both treatment arms, demonstrating that HIV-infected women treated for high-grade CIN experience elevated rates of recurrence and warrant close follow-up after treatment.

After 12 months, we found CIN2+ recurrence was 20% among women treated with cryotherapy and 11% among those treated with LEEP; after two years of follow-up, recurrent CIN2+ lesions were detected among 30% of women treated with cryotherapy and 19% among women treated with LEEP. Lower treatment effectiveness of cryotherapy compared to LEEP has been demonstrated in two other trials. Our data demonstrate higher rates of recurrence than a study was conducted among a largely HIV-uninfected cohort in Zimbabwe which reported that recurrence of a composite outcome, HSIL+ or CIN2+, after cryotherapy and LEEP treatment of 8% and 2.4%, respectively [25]. More recently, a small trial of HIV-infected women in South Africa reported recurrent CIN2+ at 12 months to be 27% among women treated with cryotherapy and 19% among women treated with LEEP [30]. While the rate of recurrence at 12 months in this study was similar to what we report at 24 months, the difference in recurrence rate between cryotherapy and LEEP was not significant. There are several differences between our study and the two prior studies. First, the duration of follow-up the previous studies was only 12 months and this time window may not be sufficient to fully detect disease recurrence because subsequent diagnosis of cervical disease is highest in the first two years following treatment and remains elevated for at least ten years [63,64]. Second, the trial in South Africa was designed to detect differences between study arms for a more common outcome, CIN1+

and the smaller samples sizes reduced the likelihood of detecting true differences in a CIN2+ outcome. Finally, our study was able to analyze how recurrence of CIN2+ was modified by HIV cofactors. The results of the current study reflect an enhanced precision to make subgroup comparisons given the larger sample size and longer duration of follow-up.

In this study, many women were on ART for longer than 6 months and had controlled HIV. Despite virological suppression at the time of cervical treatment, recurrence was high. The observed rate of CIN2+ recurrence for HIV-infected women at 12 months was 19.9% (95% CI 15.7 to 25.3%), exceeding the CIN 2-3 recurrence rate of 5.3% in a pooled analysis in the general population by almost 4-fold [57]. The reasons for the difference in recurrence rates are poorly understood but varies not only by grade of treated CIN, treatment modality, and length of follow-up but is also impacted by interactions between HIV and HPV [65]. ART initiation in our study population followed older guidelines and women likely started ART at lower CD4 thresholds. Women with prolonged ART use or higher CD4 counts had similar treatment outcomes between cryotherapy and LEEP and overall had lower rates of recurrence than women with less ART exposure or lower CD4 counts. The interaction between immune reconstitution, ART, and CD4 on CIN recurrence and progression is complex and may be important indicators to guide treatment recommendations.

Treatment success with either ablation or excision procedures depends on both the effective removal of the lesion and an acquired HPV type-specific immune response to protect against recurrent infection [66]. Prior research has shown that incomplete excision or inadequate freezing of the abnormal tissue, lesion size, and multifocal lesions located outside the transformation zone have been associated as independent risk factors for disease recurrence [67,68]. Surgical treatment with LEEP removes both the lesion and healthy cervical tissue around the lesion. Typically, the excised tissue measures at least 6-7 mm and the depth of

tissue removal may exceed 10 mm [35,48]. Cryotherapy causes necrosis to a depth up to 5-7 mm but may not always achieve therapeutic depth, notably if lesions are located on the posterior lip of the cervix [50,68]. The mean depth of CIN2-3 lesions in screened populations is less than 5 mm from the surface the cervical epithelium and have a linear extent of less than 8 mm [51]. However, in under-screened populations, the maximal depth of CIN 3 has been found to be deeper which may contribute to the high recurrence rate observed in this study [69]. Compared to cryotherapy, the higher effectiveness of LEEP may reflect a more complete removal of the abnormal tissue, particularly if the lesion is large or multifocal.

HIV-infection results in a progressive loss of CD4 T-cells from cervical mucosal barriers which alters the immune response to HPV-induced CIN [66,70]. In HIV-infected women, the cell-mediated immune response to HPV may be suppressed following cervical treatment due in part to the lack of memory T-cells that recognize HPV viral antigens and respond to viral replication [71]. The high rates of disease recurrence reported in this study and previously reported among HIV-infected women may thus be an indication that immune reconstitution following ART may not have occurred early enough after HPV infection to activate a sufficient immune response to clear CIN following surgical treatment [65]. In our study, women on shorter duration of ART, with low CD4, or detectable HIV viral load, had higher risk of recurrence following cryotherapy. The impact of cryotherapy may be to decrease but not eliminate HPV viral burden and a functional immune environment is required to eliminate recurrent CIN [72]. In contrast, there were no treatment differences among women on ART for longer than two years and high nadir CD4 count. Prolonged ART duration and HIV viral suppression may restore functional cervical mucosal immunity to respond to HPV-induced CIN [65]. This observation also aligns with recent epidemiological research suggesting that early ART initiation, high ART adherence, and sustained viral suppression reduces risk of CIN2+ [73–75].

There are several limitations in our study. First, we do not have HPV-type specific results at baseline or during follow-up. Given the randomization, high risk HPV subtypes would likely have been balanced between the treatment arms, but this data would help to better characterize the risk of recurrent CIN and to distinguish between recurrent and incident infection. The length of follow-up time in this study was two years which was adequate to accrue a substantial amount of CIN recurrence in this population, however, we are not able to describe the long-term risk of recurrent CIN and cervical cancer. Lastly, we censored women from follow-up at the time of HSIL recurrence and the majority of these women underwent repeat treatment so we cannot determine whether some women who recurred with histology of CIN 1 would have later progressed to high-grade disease. Nevertheless, even among women with CIN2+ biopsy results, LEEP was a significantly more effective procedure.

This study has practical implications for the management of HIV-infected women with high grade cervical lesions. In the past two decades, there have been widespread efforts to expand the delivery of secondary prevention services using VIA for screening and cryotherapy for managing of cervical pre-cancer. While there has been a considerable body of evidence to support this screen-and-treat approach, the public health recommendations were not based on rigorous evidence of treatment efficacy among HIV-infected women. The current randomized trial was designed specifically to address the research gap on how treatment modality modifies post-treatment disease recurrence among HIV-infected women. As countries update comprehensive national cervical cancer strategies, evidence-based management algorithms for HIV-infected women must be developed to improve the impact of secondary prevention programs.

For HIV-infected women who screen positive for cervical pre-cancer, the results from this randomized trial provides compelling evidence for WHO and ministries of health to promote of

LEEP as a standard of care for all HIV-infected women. Combined with frequent post-procedure screening, cryotherapy can be an important management tool where resources are limited or for prolonged ART users with high CD4 and low HIV viral loads. LEEP treatment resulted in fewer recurrences and these benefits should outweigh the additional resources required for management with LEEP. In high HIV-burden settings, a screen-and-treat approach coupled with HIV testing and referral for LEEP may be an important programmatic approach to ensure HIV-infected women receive more effective treatment.

There is need to evaluate the frequency and intensity of post-treatment screening to define the optimal screening algorithm to detect recurrence early before cervical cancer progression. Current WHO guidelines recommend post-treatment follow-up one year after treatment for CIN2-3 for both HIV-infected and uninfected women [10]. Given the majority of disease recurrence in this study was detected within six months of treatment emphasizes the need for more frequent intervals for repeat screening as a necessary component of cervical cancer prevention. Future research directions should focus on more effective triage methods for HIV-infected women including the use of point-of-care HPV detection or other biomarkers to differentiate between lesions that are likely progress to cervical cancer. This may help predict which lesions have a higher risk of recurring and require more frequent repeat screenings.

The combined effect of cervical cancer and HIV is complex and highlights the urgent need to develop innovative and sustainable solutions to address secondary prevention of cervical cancer. High HIV burden settings are often the same settings with limited resources for routine screening and treatment. One feasible strategy to ensure HIV-infected women gain access to routine cervical cancer screening and treatment is through service integration. HIV-infected women are engaged in routine care and this offers a promising opportunity to integrate appropriate cervical cancer prevention, screening, and treatment strategies [76–78]. Modifying

cervical cancer prevention guidelines to account for HIV-status has the potential to mobilize a more coordinated response to cervical cancer prevention and promote the implementation of evidence-based approaches into routine service delivery.

Table 4. Baseline Demographic, Behavioral, and Clinical Characteristics by Treatment Arm

Characteristic	Cryotherapy		LEEP	
	n	Median (IQR) ^a or %	n	Median (IQR) ^a or %
Age (years)	200	38.2 (32.0-42.5)	200	37.4 (31.7-44.9)
Education	200		200	
None	23	11.5	12	6.0
Primary	70	35.0	79	39.5
Secondary	58	29.0	64	32.0
University	49	24.5	45	22.5
Monthly Household Income <\$150 USD	109	54.5	117	58.5
Relationship Status	200		200	
No current partner	120	60.0	112	56.0
At least one current partner	80	40.0	88	44.0
Lifetime Sexual Partners	200		200	
1-2	97	48.5	72	36.0
≥3	103	51.5	128	64.0
Any Hormonal Contraception	36	18.1	32	16.0
CD4 (cells/mm ³) ^b	200	371 (211-532)	199	385 (221-522)
<250	59	29.5	59	29.7
250-499	83	41.5	85	42.7
≥500	58	29.0	55	27.6
ART-naïve	28	14.0	17	8.5
Median Duration on ART (months) ^c	172	29.4 (14.1-60.2)	183	34.0 (12.4-74.3)
Undetectable HIV-1 Plasma Viral Load ^d	123	63.1	126	65.3

^a Interquartile range

^b One woman in the LEEP arm had a missing CD4 test

^c Among those on ART prior to treatment.

^d Minimum detection level <60 copies/ml

Table 5. Incidence of HSIL+ and CIN2+ Disease Recurrence after Two-year Follow-up by Baseline HIV Factors

	Cryotherapy			LEEP			P-value
	Number of recurrences	Person years of follow-up	Rate per 100 person-years (95% CI)	Number of recurrences	Person years of follow-up	Rate per 100 person-years (95% CI)	
HSIL							
Overall	74	296.2	25.0 (19.9-31.4)	52	311.2	16.7 (12.7-21.9)	0.013
ART ≥2 years	35	144.9	24.2 (17.3-33.6)	33	163.5	20.2 (14.4-28.4)	0.231
ART <2 years	28	108.1	25.9 (17.9-37.5)	15	117.9	12.7 (7.7-21.1)	0.012
No ART	11	43.2	25.4 (14.1-45.9)	4	29.9	13.4 (5.0-35.7)	0.139
Plasma VL <60 copies ml ¹	42	187.3	22.4 (16.6-30.3)	29	197.4	14.7 (10.2-21.1)	0.040
Plasma VL ≥60 copies ml ¹	31	100.3	30.9 (21.7-43.9)	19	104.9	18.1 (11.6-28.4)	0.033
CD4 <250	22	54.5	40.3 (26.6-61.3)	13	48.9	26.6 (15.4-45.8)	0.118
CD4 250-499	29	121.9	23.8 (16.5-34.2)	19	126.6	15.0 (9.6-23.5)	0.060
CD4 ≥500	23	118.7	19.4 (12.9-29.2)	20	135.2	14.8 (9.5-22.9)	0.190
CIN 2+							
Overall	60	313.2	19.2 (14.9-24.7)	37	329.9	11.2 (8.1-15.5)	0.005
ART ≥2 years	30	154.6	19.4 (13.6-27.8)	24	178.6	13.4 (9.0-20.1)	0.091
ART <2 years	21	113.2	18.5 (12.1-28.4)	11	121.9	9.0 (5.0-16.3)	0.025
No ART	9	45.3	19.8 (10.3-38.1)	2	29.3	6.8 (1.7-27.3)	0.081
Plasma VL <60 copies ml ¹	31	203.7	15.2 (10.7-21.6)	22	206.7	10.6 (7.0-16.2)	0.101
Plasma VL ≥60 copies ml ¹	28	101.0	27.7 (19.1-40.2)	13	111.7	11.6 (6.8-20.0)	0.004
CD4 <250	20	56.5	35.4 (22.8-54.9)	7	52.1	13.4 (6.4-28.2)	0.011
CD4 250-499	21	132.2	15.9 (10.4-24.4)	15	135.1	11.1 (6.7-18.4)	0.146
CD4 ≥500	19	123.4	15.4 (9.8-24.1)	15	142.1	10.6 (6.4-17.5)	0.139

¹Twelve women had missing plasma viral load at baseline.

ART=antiretroviral therapy. VL=viral load.

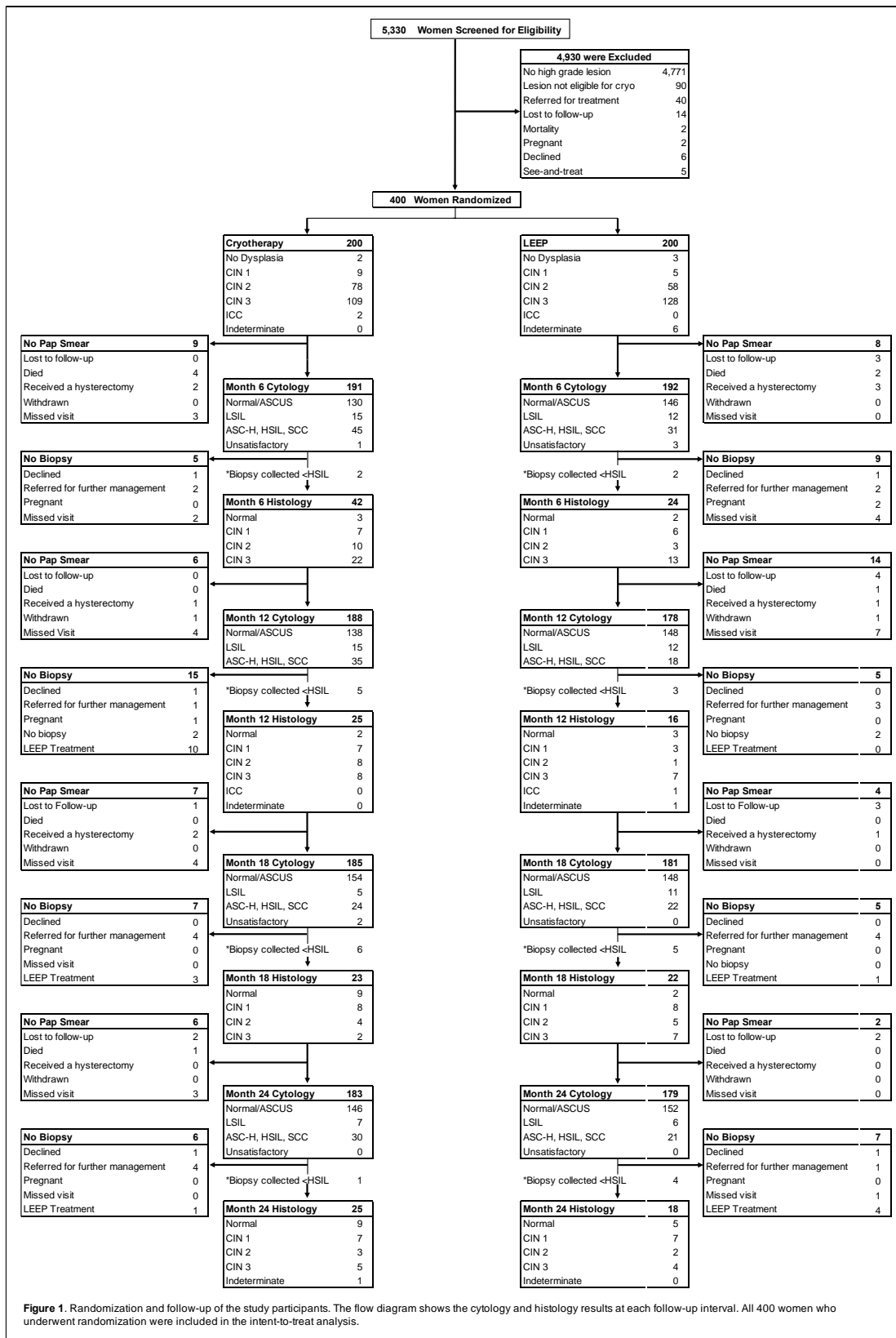
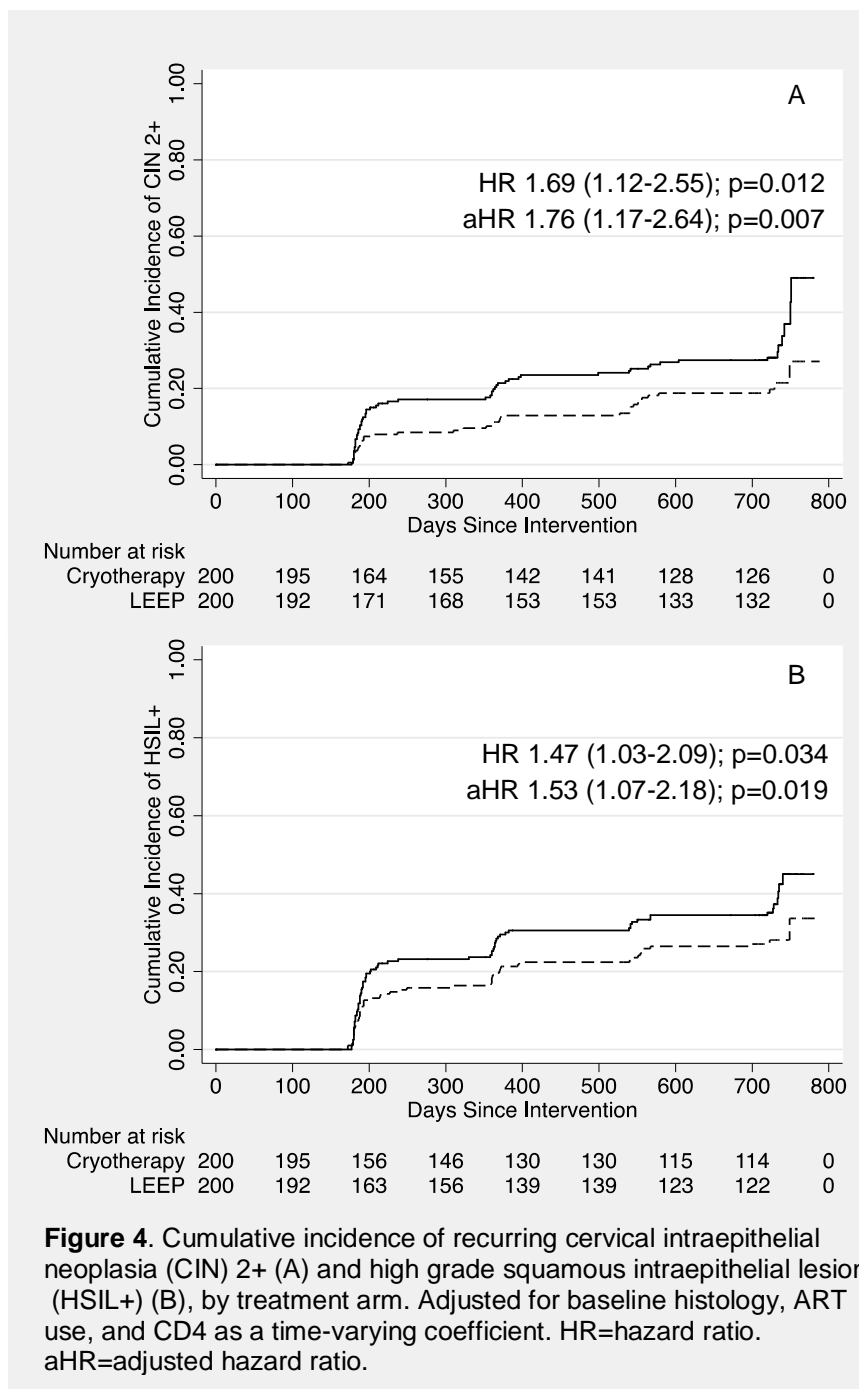
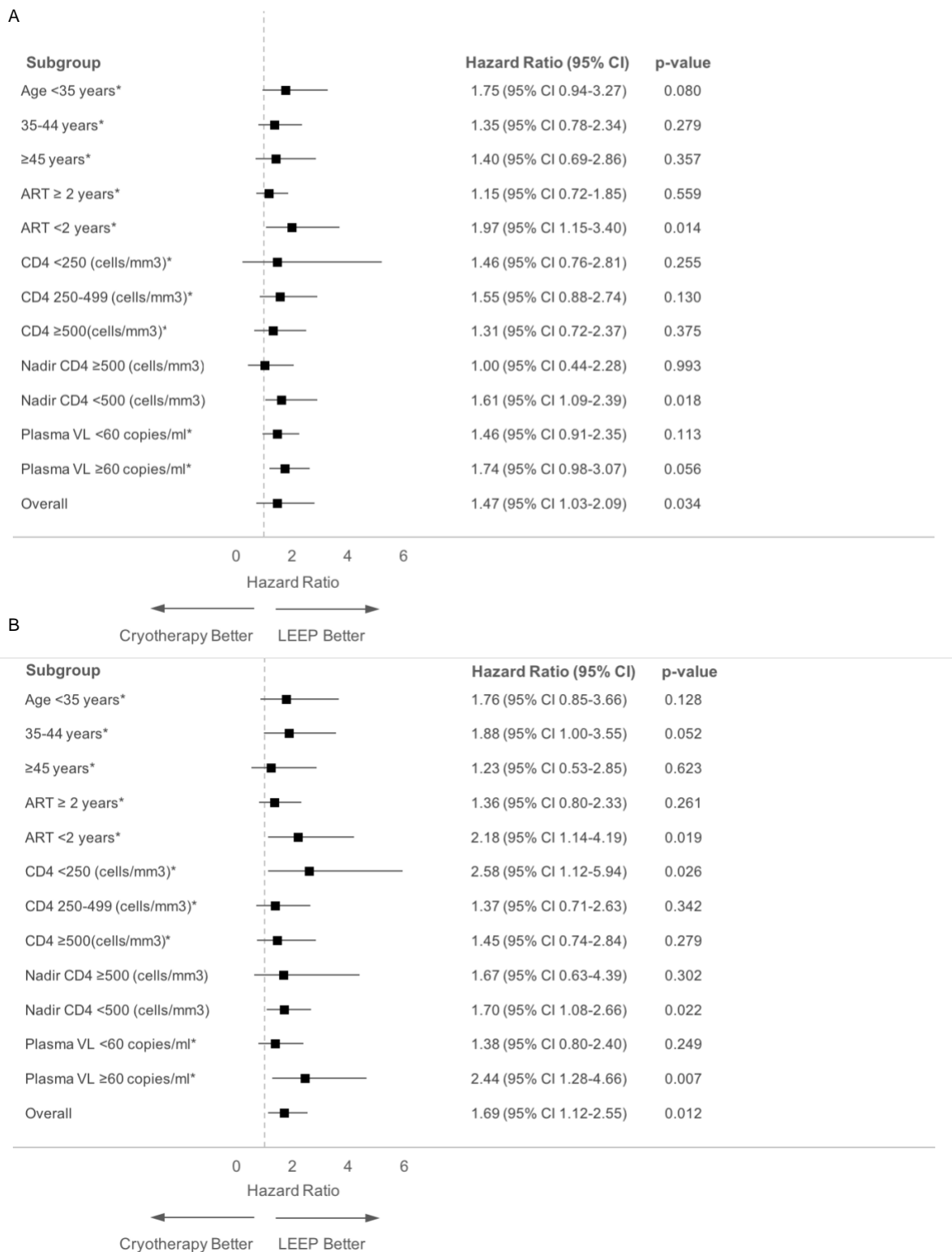


Figure 3: Randomization and Follow-up of Study Participants

Figure 4. Cumulative Incidence of Recurring Cervical Intraepithelial Neoplasia (CIN) 2+





ART=antiretroviral therapy. VL=viral load. *Categorization based on baseline values.

Figure 5. Unadjusted Hazard Ratios for Recurrence of HSIL+ (A) or CIN2+ (B) Stratified by Key Subgroups

Chapter 4: Conclusion

Conclusion

The two studies presented in this dissertation contribute to the understanding of the risks and benefits of cervical pre-cancer treatment with cryotherapy and LEEP. At present, there is no clear consensus as to how best manage cervical pre-cancer among HIV-infected women. This dissertation analyzed the data of the primary aims from a randomized controlled trial of 400 HIV-infected women with biopsy confirmed, high-grade cervical pre-cancer. Combined, these two aims generated much needed causal evidence for decision-makers to make informed assessments on the impact and efficacy of cryotherapy and LEEP for HIV-infected women.

This research is motivated by several converging areas of clinical and implementation science. First, epidemiology has established that HIV adversely impacts the natural history of HPV infection, a necessary cause of cervical cancer [79]. We know that HIV-infected women are more likely to be infected with HPV and HIV is associated with not only a higher occurrence of cervical pre-cancer, persistence, and accelerated progression of lesions to invasive cervical cancer, but also HIV-infected women have significantly lower survival from invasive cancer [3–5,80]. For the past three decades, research has consistently shown the linkages between HIV and HPV, and cervical cancer is recognized as an AIDS-defining condition [81]. Despite this research, effective treatment procedures for HIV-infected women have not been identified.

Second, as countries like Kenya continue to scale ART through test and start guidelines, HIV-infected women are living healthier, more productive lives [82]. Despite immune reconstitution associated with ART initiation, there has not been a corresponding decline in the incidence of cervical cancer [83]. Moreover, as ART extends life expectancy, HIV-infected women will have a longer time horizon for either incident or reactivation of an existing HPV infection to progress toward cervical cancer. Until primary prevention through HPV vaccination is routinely

administered before sexual debut, cervical cancer prevention will heavily depend on screening and treatment programs.

Third, the interactions between HIV-cofactors and HPV infection are not well understood. There is a high degree of heterogeneity among observational studies with respect to study design, HPV subtype, timing of ART initiation in relation to HIV and HPV acquisition, and time-varying effects of CD4 and HIV viral load. More recently, research suggests that HIV suppression measured by high CD4 and low viral loads is associated with reduced HPV persistence and that ART may confer limited benefit among women who have been taking ART for longer than two years [74,75,84,85]. A clear understanding of the role of immune dysfunction on both the post-treatment cervical HIV shedding and recurrence of cervical disease will help to further stratify cervical cancer risk and result in more effective guidelines for HIV-infected women.

Finally, secondary cervical prevention programs rely in large part on reliable health infrastructure for sustainability and to achieve appropriate coverage. In Kenya, for example, screening with VIA and treating with either cryotherapy or LEEP has not been widely adopted because while all district level hospitals and some health centers have cervical cancer screening with VIA available, only 10% of the 300 screening sites have outpatient treatment. Sites that do offer treatment are faced with shortages of compressed gas for cryotherapy and trained providers to administer the treatment [86]. Alongside the lack of supply, the need for cervical cancer screening and treatment will continue to rise as the prevalence of HIV-infected women increases. Women of reproductive age are the fastest growing group testing positive for HIV and this concurrently puts further strain on health systems to address both epidemics for the next 10-20 years [87]. In Kenya, it is estimated that 80-90% of women have never had a pelvic exam or screening for cervical cancer [88]. As access to cervical cancer screening increases, the incidence high grade cervical cancer among HIV-infected women will also initially

increase but with effective screening programs, will be expected to decrease over time [7].

Higher initial detection rates are expected because women have either delayed or do not have access to routine screening services.

The combined effect of HIV and cervical cancer on quality of life, morbidity, and mortality highlight the urgent need to develop innovative and sustainable solutions to address secondary prevention of cervical cancer in low resource settings where there are not routine screening and treatment programs. The results of this clinical trial can help provide comprehensive data for programs looking to integrate HIV treatment and care with screening and treatment for cervical cancer.

HIV-Infected Women Should Receive Risk Reduction Counseling Following Cervical Treatment

The work presented in Chapter 2 addresses whether cryotherapy or LEEP interventions may stimulate cervical shedding of HIV and subsequently increase the risk of HIV sexual transmission from treated women to HIV-uninfected partners. Current clinical guidelines recommend that HIV-infected women treated for cervical pre-cancer abstain from sexual activity for up to one month to allow for the wound to heal and reduce risk of HIV shedding. The recommendation is based on a study conducted in the pre-HAART era that quantified HIV shedding before and after treatment with either cryotherapy, LEEP, or cold-knife cone biopsies for cervical pre-cancer among 14 women. There has not been a comprehensive evaluation of appropriateness of this guideline or a direct comparison of HIV shedding between cryotherapy and LEEP. The impact of treatment is critical in settings like Kenya where HIV-infected individuals are often partnered in sero-discordant relationships, access to pre-exposure HIV prophylaxis is uncommon, and women may not be empowered to negotiate the timing of sexual intercourse or condom use.

Our results are the most robust estimates of risk available to date on HIV shedding following WHO recommended treatments of cryotherapy and LEEP. We reported that women treated with LEEP had higher post-treatment levels of HIV shedding, particularly among women not taking ART. Importantly, the impact of LEEP was reduced among women taking ART at the time of treatment. For ART users, cervical treatment with LEEP did not pose a substantial increase in HIV shedding. In contrast, women treated with cryotherapy had similar levels of HIV shedding before and after treatment regardless of ART exposure.

The results from our study indicate that clinical providers should be aware that there is a temporary increase in HIV viral load after treatment for high grade cervical pre-cancer and the magnitude differs by treatment and ART status. Current guidelines to counsel women on the risk of HIV transmission appear to be appropriate as LEEP treatment may contribute to a time limited increase in HIV shedding. We reported in Chapter 2 that for women taking ART, the absolute change in HIV shedding did not increase in a clinically meaningful way. For this group of women, the risk of HIV transmission is low, but there is still risk that HIV transmission can occur. In contrast, for women not taking ART, the risk of HIV transmission was nearly twice as high in the three-week window following LEEP. This demonstrates a considerable range of risk and while we used ART use as a way to stratify and communicate differences among women, cervical levels of HIV may vary due to factors that were not measured including sexually transmitted infections, menstrual cycle, oral contraceptive use, or diet or there may be additional factors that we were not able to analyze including the difference in shedding by women recently initiated to ART compared to prolonged ART users [19,89–93]. Therefore, secondary cervical cancer prevention programs should provide standardized counseling for all HIV-infected women on the potential short-term risk of HIV transmission following treatment for cervical pre-cancer. When planning for safe and effective cervical cancer prevention strategies, clinicians and

program managers must be aware of the potential impact cervical treatment on HIV shedding. The choice of treatment procedure should not be dependent on the risk of HIV shedding, particularly among women on ART because the benefits of cervical pre-cancer treatment outweigh the potential risk of HIV sexual transmission.

Cervical Pre-Treatment Guidelines Should Account for HIV Status

Cervical cancer treatment guidelines for HIV-infected women have been limited because there is very little existing primary data on treatment interventions specifically designed for this population. Instead, the risks and benefits of treatment among HIV-infected have largely been estimated based on extrapolations of data from the HIV-uninfected population or from single-arm, observational studies. While this extrapolation may generally be true for some diseases, the relationship between HIV, HPV, and cervical cancer is complex. HIV-infected women are more likely to experience recurrence of cervical disease, making post-treatment follow-up an essential component of prevention programs.

Epidemiologic studies have consistently shown that treatment for cervical pre-cancer is less effective among HIV-infected women [26,31]. In Chapter 3, we report causal evidence that cryotherapy resulted in significantly higher risk in post-treatment disease recurrence. The validation of a specific treatment for high grade cervical pre-cancer is important because decision-makers lacked rigorous evidence of treatment efficacy among HIV-infected women. For HIV-infected women who screen positive for cervical pre-cancer, the results from this randomized trial provides compelling evidence for WHO and ministries of health to promote of LEEP as a standard of care for all HIV-infected women.

Further, we found that the difference between cryotherapy and LEEP was attenuated for prolonged ART users or women with high CD4 counts. Treatment success with either

cryotherapy or LEEP depends on both the effective removal of the pre-cancerous lesion and an acquired HPV type-specific immune response to protect against recurrent infection [66]. Long-term, effective ART use contributes to high CD4 and low HIV replication, which represent functional immune restoration. For women with controlled HIV infection, screen and treat with cryotherapy may be an effective alternative in settings where LEEP is not available or requires a referral to receive care. Cervical cancer treatment guidelines may assess additional risk stratification by nadir CD4 cell counts or ART duration.

Successful cervical cancer prevention programs require the effective treatment of cervical pre-cancer because the management of invasive cervical cancer is a major challenge in resource constrained settings. Currently, LEEP is more expensive to perform than cryotherapy because equipment must be imported and there is a shortage of trained providers [94]. One strategy to contain the cost is through service integration with either HIV care clinics or routine maternal health services [77,78,95]. The results reported in Chapter 3 support the adoption of LEEP in HIV care clinics implementing cervical cancer screening programs. Providing high impact care in a routine manner will generate more public awareness, catalyze advocacy, increase the pool of trained clinicians, and help establish the infrastructure necessary for quality control measures. Further including cervical cancer in these larger health service structures will place a higher priority on cervical cancer prevention. Building this type of service delivery system will also facilitate the implementation of newer screening and treatment technologies as they become validated. The dual burden of HIV and cervical cancer underscores that the health needs of HIV-infected women must be prioritized and demand innovative solutions.

Key Gaps in Knowledge for Secondary Cervical Cancer Prevention for HIV-infected Women

Ongoing clinical research and program implementation will provide data on cervical cancer screening, diagnosis, and treatment effectiveness for HIV-infected women. There remain several outstanding challenges for secondary prevention of cervical cancer. One challenge is the optimal screening intervals and frequency for HIV-infected women. There has been a considerable amount of research into the optimal screening frequency for HIV-uninfected women [34,96,97]. Moreover, current decision models do not simulate the interactions between HIV and cervical cancer as an altered natural history despite the strong epidemiological link due limitations in the data [10,98]. In resource-limited settings, decision-makers need further epidemiological data to model the optimal screening strategies for HIV-infected women to maximize the health benefits and resource allocation.

WHO guidelines recommend follow-up within three years following a negative screening test in populations with a high burden of HIV instead of three to five years for HIV-uninfected women [99]. More recently, a model simulated that among HIV-uninfected women, screening three times in a lifetime at adequate intervals, 5- and 10-year intervals, and at critical ages, between ages 30 and 45 years, was optimal [98]. Among HIV-infected women, cervical pre-cancer is detected at younger ages and this population experiences an accelerated progression toward cervical cancer. Further, our results indicate that the development of pre-cancerous lesions may be different among women who are long-term ART users or have sustained viral suppression and that this population may require less frequent screening than HIV-infected women with less functional immune recovery. Therefore, it will be important for mathematical simulations to model the natural history for cervical cancer progression in HIV-infected women to determine the when and how often to screen this population and further primary data will be necessary to inform these estimates and future screening guidelines.

For women who are treated for cervical pre-cancer, WHO guidelines recommend post-treatment follow-up one year after treatment [99]. Results presented in Chapter 3 demonstrate that disease recurrence was as high as 27% among women treated with cryotherapy at 12 months and the majority of these events occurred within 6 months of treatment. The length of follow-up time in our study was two years which was adequate to accrue a substantial number of recurrence events among HIV-infected women, however, there is no data about the long-term risk of recurrent cervical pre-cancer and cancer. In order to inform screening frequency for women treated for cervical cancer it will be necessary to understand when an HIV-infected women's post-treatment risk of cervical pre-cancer returns to baseline and can return to routine screening frequency.

Finally, for women with advanced cervical pre-cancer who require treatment, the use of molecular biomarkers may predict which lesions may be at higher risk of disease recurrence and therefore warrant closer post-treatment surveillance. Persistence of high risk HPV is a significant prognostic factor associated with recurrence cervical pre-cancer [100]. Further, risk-stratification using HPV DNA testing has been proposed as a test of cure after treatment for high grade cervical pre-cancer because HPV DNA is a highly sensitive test. The main advantage is that a negative HPV DNA test would identify women who have a low risk of disease recurrence. This approach has been shown to be successful in HIV-uninfected women but has not been tested as a strategy for HIV-infected women. A validation study will be critical because the HPV specificity has been shown to be lower in HIV-infected women, particularly for younger women or women with little or no exposure to ART [58]. If the clinical performance test is validated, HPV testing may have an important role in not only reducing the cost and anxiety associated with intensive follow-up but also triaging women at risk for recurrence for closer clinical management.

Conclusion

This dissertation has contributed to our understanding of the short and long-term risks associated with treatment for cervical pre-cancer. These results also contribute a more nuanced understanding of the effect of HIV-cofactors including ART, CD4 cell count, and viral load have on post-treatment changes in viral shedding and disease recurrence. Modifying cervical cancer prevention guidelines to account for HIV-status has the potential to mobilize a more coordinated response to cervical cancer prevention and promote the implementation of evidence-based approaches into routine service delivery.

References

1. United Nations Programme on HIV/AIDS. HPV, HIV and Cervical Cancer. **2016**; :1–8.
2. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* **2014**; 136:E359–E386.
3. Jamieson DJ, Duerr A, Burk R, et al. Characterization of genital human papillomavirus infection in women who have or who are at risk of having HIV infection. *Am J Obs Gynecol* **2002**; 186:21–7.
4. Ellerbrock T V, Chiasson MA, Bush TJ, et al. Incidence of cervical squamous intraepithelial lesions in HIV-infected women. *JAMA* **2000**; 283:1031–1037 7p.
5. Denny LA, Franceschi S, de Sanjosé S, Heard I, Moscicki AB, Palefsky J. Human papillomavirus, human immunodeficiency virus and immunosuppression. *Vaccine* **2012**; 30:F168–F174.
6. Denny L, de Sanjose S, Mutebi M, et al. Interventions to close the divide for women with breast and cervical cancer between low-income and middle-income countries and high-income countries. *Lancet* **2016**; 6736:21–30.
7. Massad SL, Xie X, D’Souza G, et al. Incidence of cervical precancers among HIV-seropositive women. *Am J Obstet Gynecol* **2015**; 212:606.e1-606.e8.
8. Jeronimo J, Castle PE, Temin S, et al. Secondary Prevention of Cervical Cancer: ASCO Resource-Stratified Clinical Practice Guideline. *J Glob Oncol* **2016**;
9. Ministry of Public Health and Sanitation, Ministry of Medical Services. National cervical cancer prevention program strategic plan 2012-2015. 2012: 38. Available at: <https://www.k4health.org/toolkits/kenya-health/national-cervical-cancer-prevention-program-strategic-plan-2012-2015>. Accessed 15 July 2016.
10. World Health Organization. WHO guidelines for treatment of cervical intraepithelial neoplasia 2–3 and adenocarcinoma in situ. *WHO Guidel* **2014**; :52 p.
11. Kaiser R, Bunnell R, Hightower A, et al. Factors associated with HIV infection in married or cohabitating couples in Kenya: Results from a nationally representative study. *PLoS One* **2011**; 6.
12. Wright TC, Subbarao S, Ellerbrock T V, et al. Human immunodeficiency virus 1 expression in the female genital tract in association with cervical inflammation and ulceration. *Am J Obstet Gynecol* **2001**; 184:279–285.
13. Wang CC, McClelland RS, Reilly M, et al. The effect of treatment of vaginal infections on shedding of human immunodeficiency virus type 1. *J Infect Dis* **2001**; 183:1017–1022.
14. Baeten JM, Kahle E, Lingappa JR, et al. Genital HIV-1 RNA quantity predicts risk of heterosexual HIV-1 transmission. *Sci Transl Med* **2011**; 3:1–15.

15. Launay O, Tod M, Tschope I, et al. Residual HIV-1 RNA and HIV-1 DNA production in the genital tract reservoir of women treated with HAART: the prospective ANRS EP24 GYNODYN study. *Antivir Ther* **2011**; 16:843–852.
16. Mhatre M, McAndrew T, Carpenter C, Burk RD, Einstein MH, Herold BC. Cervical intraepithelial neoplasia is associated with genital tract mucosal inflammation. *Sex Transm Dis* **2012**; 39:591–597.
17. Johnson LF, Lewis DA. The effect of genital tract infections on HIV-1 shedding in the genital tract: a systematic review and meta-analysis. *Sex Transm Dis* **2008**; 35:946–959.
18. Mitchell C, Hitti J, Paul K, et al. Cervicovaginal shedding of HIV type 1 is related to genital tract inflammation independent of changes in vaginal microbiota. *AIDS Res Hum Retroviruses* **2011**; 27:35–39.
19. Gitau RW, Graham SM, Masese LN, et al. Effect of acquisition and treatment of cervical infections on HIV-1 shedding in women on antiretroviral therapy. *AIDS* **2010**; 24:2733–7.
20. Fiore JR, Suligoj B, Saracino A, et al. Correlates of HIV-1 shedding in cervicovaginal secretions and effects of antiretroviral therapies. *AIDS* **2003**; 17:2169–76.
21. Chung MH, McKenzie KP, Richardson BA, et al. Cervical HIV-1 RNA shedding after cryotherapy among HIV-positive women with cervical intraepithelial neoplasia stage 2 or 3. *AIDS* **2011**; 25:1915–9.
22. Huchko MJ, Woo VG, Liegler T, et al. Impact of loop electrosurgical excision procedure for cervical intraepithelial neoplasia on HIV-1 genital shedding: a prospective cohort study. *BJOG* **2013**; 120:1233–1239.
23. De Vuyst H, Mugo NR, Franceschi S, et al. Residual Disease and HPV Persistence after Cryotherapy for Cervical Intraepithelial Neoplasia Grade 2/3 in HIV-Positive Women in Kenya. *PLoS One* **2014**; 9:e1111037.
24. Huchko MJ, Leslie H, Maloba M, et al. Outcomes up to 12 months after treatment with loop electrosurgical excision procedure for cervical intraepithelial neoplasia among HIV-infected women. *JAIDS, J Acquir Immune Defic Syndr*. 2015; 69:200–205.
25. Chirenje ZM, Rusakaniko S, Akino V, Mlingo M. A randomised clinical trial of loop electrosurgical excision procedure (LEEP) versus cryotherapy in the treatment of cervical intraepithelial neoplasia. *J Obstet Gynaecol* **2001**; 21:617–621.
26. Chirenje ZM, Rusakaniko S, Akino V, Munjoma M, Mlingo M. Effect of HIV Disease in Treatment Outcome of Cervical Squamous Intraepithelial Lesions Among Zimbabwean Women. *J Low Genit Tract Dis* **2003**; 7:16–21.
27. Santesso N, Mustafa RA, Wiercioch W, et al. International Journal of Gynecology and Obstetrics Systematic reviews and meta-analyses of benefits and harms of cryotherapy, LEEP, and cold knife conization to treat cervical intraepithelial neoplasia. *Int J Gynecol Obstet* **2015**; 132:4–9.
28. Martin-Hirsch-Pierre PL, Paraskevaidis E, Bryant A, Dickinson HO. Surgery for cervical

- intraepithelial neoplasia. *Cochrane Database Syst Rev* **2013**;
29. Sauvaget C, Muwonge R, Sankaranarayanan R. Meta-Analysis of the effectiveness of cryotherapy in the treatment of cervical intraepithelial neoplasia. *Int J Gynecol Obstet* **2013**; 120:218–223.
 30. Smith JS, Sanusi B, Swarts A, et al. A randomized clinical trial comparing cervical dysplasia treatment with cryotherapy vs loop electrosurgical excision procedure in HIV-seropositive women from Johannesburg, South Africa. *Am J Obstet Gynecol* **2017**; 217:183.e1-183.e11.
 31. Massad LS, Fazzari MJ, Anastos K, et al. Outcomes after treatment of cervical intraepithelial neoplasia among women with HIV. *J Low Genit Tract Dis* **2007**; 11:90–7.
 32. De Vuyst H, Alemany L, Lacey C, et al. The burden of human papillomavirus infections and related diseases in sub-saharan Africa. *Vaccine* **2013**; 31:F32–F46.
 33. Harris TG, Burk RD, Palefsky JM, et al. Incidence of cervical squamous intraepithelial lesions associated with HIV serostatus, CD4 cell counts, and human papillomavirus test results. *JAMA* **2005**; 293:1471–1476.
 34. Massad LS, Einstein MH, Huh WK, et al. 2012 Updated consensus guidelines for the management of cervical cancer screening test and cancer precursors. *Am Soc Colposc Cerv Pathol* **2013**; 17:S1–S27.
 35. Jordan J, Martin-Hirsch P, Arbyn M, et al. European guidelines for clinical management of abnormal cervical cytology, Part 2. *Cytopathology* **2009**; 20:5–16.
 36. Denny LE, Kuhn L, Souza M De, et al. Screen-and-treat approaches for cervical cancer prevention in low-resource settings: A randomized controlled trial. *JAMA* **2005**; 294:2173–2181.
 37. Goldie SJ, Gaffikin L, Goldhaber-Fiebert JD, et al. Cost-effectiveness of cervical-cancer screening in five developing countries. *N Engl J Med* **2005**; 353:2158–2168.
 38. Sankaranarayanan R, Anorlu R, Sangwa-Lugoma G, Denny L. Infrastructure requirements for human papillomavirus vaccination and cervical cancer screening in Sub-Saharan Africa. *Vaccine* **2013**; 31:F47–F52.
 39. Sankaranarayanan R, Nene BM, Dinshaw KA, et al. A cluster randomized controlled trial of visual, cytology and human papillomavirus screening for cancer of the cervix in rural India. *Int J Cancer* **2005**; 116:617–623.
 40. Lince-Deroche N, Phiri J, Michelow P, Smith JS, Firnhaber C. Costs and cost effectiveness of three approaches for cervical cancer screening among HIV-positive women in Johannesburg, South Africa. *PLoS One* **2015**; 10:e0141969.
 41. Chamot E, Kristensen S, Stringer JS, Mwanahamuntu MH. Are treatments for cervical precancerous lesions in less-developed countries safe enough to promote scaling-up of cervical screening programs? A systematic review. *BMC Womens Health* **2010**; 10:11.

42. Lawn SD, Subbarao S, Wright TC, et al. Correlation between human immunodeficiency virus type 1 RNA levels in the female genital tract and immune activation associated with ulceration of the cervix. *J Infect Dis* **2000**; 181:1950–1956.
43. Forhan SE, Godfrey CC, Watts DH, Langley CL. A systematic review of the effects of visual inspection with acetic acid, cryotherapy, and loop electrosurgical excision procedures for cervical dysplasia in HIV-infected women in low- and middle-income countries. *J Acquir Immune Defic Syndr* **2015**; 68 Suppl 3:S350–S356.
44. Greene SA, Nyongesa-Malava E, Richardson BA, et al. Randomized Trial of LEEP vs Cryotherapy to Treat CIN 2/3 in HIV-Infected Women. In: Conference on Retroviruses and Opportunistic Infections. Seattle, Washington: 2017.
45. Chung MH, Drake AL, Richardson BA, et al. Impact of prior HAART use on clinical outcomes in a large Kenyan HIV treatment program. *Curr HIV Res* **2009**; 7:441–6.
46. Jaspan HB, Liebenberg L, Hanekom W, et al. Immune activation in the female genital tract during HIV infection predicts mucosal CD4 depletion and HIV shedding. *J Infect Dis* **2011**; 204:1550–1556.
47. Nkwanyana NN, Gumbi PP, Roberts L, et al. Impact of human immunodeficiency virus 1 infection and inflammation on the composition and yield of cervical mononuclear cells in the female genital tract. *Immunology* **2009**; 128.
48. Noehr B, Jensen A, Frederiksen K, Tabor A, Kjaer SK. Depth of cervical cone removed by loop electrosurgical excision procedure and subsequent risk of spontaneous preterm delivery. *Obstet Gynecol* **2009**; 114:1232–8.
49. Coleman JS, Hitti J, Bukusi EA, et al. Infectious correlates of HIV-1 shedding in the female upper and lower genital tracts. *AIDS* **2007**; 21:755–9.
50. Khan MJ, Smith-McCune KK. Treatment of cervical precancers: back to basics. *Obstet Gynecol* **2014**; 123:1339–43.
51. Abdul-Karim FW, Fu YS, Reagan JW, Wentz WB. Morphometric study of intraepithelial neoplasia of the uterine cervix. *Obstet Gynecol*. 1982; 60:210–4.
52. Blish CA, McClelland RS, Richardson B a, et al. Genital inflammation predicts HIV-1 shedding independent of plasma viral load and systemic inflammation. *J Acquir Immune Defic Syndr* **2012**; 61:436–40.
53. Neely MN, Benning L, Xu J, et al. Cervical shedding of HIV-1 RNA among women with low levels of viremia while receiving highly active antiretroviral therapy. *J Acquir Immune Defic Syndr* **2007**; 44:38–42.
54. Cu-Uvin S, DeLong AK, Venkatesh KK, et al. Genital tract HIV-1 RNA shedding among women with below detectable plasma viral load. *AIDS* **2010**; 24:2489–2497.
55. Awolude OA, Morhason-Bello IO, Denny LA, Adewole IF. Human papillomavirus infection and related cancers in sub-saharan Africa: Burden and tools for prevention. *Vaccine* **2013**; 31:7–10.

56. Reimers LL, Sotardi S, Daniel D, et al. Outcomes after an excisional procedure for cervical intraepithelial neoplasia in HIV-infected women. *Gynecol Oncol* **2010**; 119:92–97.
57. Santesso N, Mustafa RA, Wiercioch W, et al. Systematic reviews and meta-analyses of benefits and harms of cryotherapy, LEEP, and cold knife conization to treat cervical intraepithelial neoplasia. *Int J Gynaecol Obstet* **2015**; 132:4–9.
58. Chung MH, McKenzie KP, De Vuyst H, et al. Comparing Papanicolaou smear, visual inspection with acetic acid and human papillomavirus cervical cancer screening methods among HIV-positive women by immune status and antiretroviral therapy. *AIDS* **2013**; 27:2909–19.
59. Wesley RS, Muwonge R, Sauvaget C, Thara S, Sankaranarayanan R. Effectiveness of cryotherapy for histologically confirmed cervical intraepithelial neoplasia grades 1 and 2 in an Indian setting. *Int J Gynecol Obstet* **2013**; 123:16–20.
60. Solomon D, Davey D, Kurman R, et al. The 2001 Bethesda System: terminology for reporting results of cervical cytology. *Jama* **2002**; 287:2114–9.
61. U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS. Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1.0. [Updated August 2009]. 2009. Available at: <https://rsc.tech-res.com/clinical-research-sites/safety-reporting/daids-grading-tables>. Accessed 26 January 2018.
62. Altman DG, Andersen PK. Calculating the number needed to treat for trials where the outcome is time to an event. *Bmj* **1999**; 319:1492–1495.
63. Melnikow J, McGahan C, Sawaya GF, Ehlen T, Coldman A. Cervical intraepithelial neoplasia outcomes after treatment: Long-term follow-up from the british columbia cohort study. *J Natl Cancer Inst* **2009**; 101:721–728.
64. Katki HA, Schiffman M, Castle PE, et al. Five-year risk of recurrence following treatment of CIN2, CIN3, or AIS: Performance of HPV and Pap cotesting in post-treatment management. *J Low Genit Tract Dis* **2013**; 17:S78–S84.
65. Kelly H, Weiss HA, Benavente Y, et al. Association of antiretroviral therapy with high-risk human papillomavirus, cervical intraepithelial neoplasia, and invasive cervical cancer in women living with HIV: a systematic review and meta-analysis. *Lancet HIV* **2017**; 3018:5–7.
66. De Vos Van Steenwijk PJ, Piersma SJ, Welters MJP, et al. Surgery followed by persistence of high-grade squamous intraepithelial lesions is associated with the induction of a dysfunctional HPV16-specific T-Cell response. *Clin Cancer Res* **2008**; 14:7188–7195.
67. Paraskevaidis E, Lolis ED, Koliopoulos G, Alamanos Y, Fotiou S, Kitchener HC. Cervical intraepithelial neoplasia outcomes after large loop excision with clear margins. *Obstet Gynecol* **2000**; 95:828–31.
68. Mariategui J, Santos C, Taxa L, Jeronimo J, Castle PE. Comparison of depth of necrosis

- achieved by CO₂- and N₂O-cryotherapy. *Int J Gynecol Obstet* **2008**; 100:24–26.
69. Taxa L, Jeronimo J, Alonzo TA, et al. Depth of Cervical Intraepithelial Neoplasia Grade 3 in Peruvian Women. *J Low Genit Tract Dis* **2018**; 22:27–30.
 70. van der Burg SH, Palefsky JM. Human immunodeficiency virus and human papilloma virus - why HPV-induced lesions do not spontaneously resolve and why therapeutic vaccination can be successful. *J Transl Med* **2009**; 7:108.
 71. Brickman C, Palefsky JM. Human Papillomavirus in the HIV-Infected Host: Epidemiology and Pathogenesis in the Antiretroviral Era. *Curr HIV/AIDS Rep* **2015**; 12:6–15.
 72. Kobayashi A, Greenblatt RM, Anastos K, et al. Functional Attributes of Mucosal Immunity in Cervical Intraepithelial Neoplasia and Effects of HIV Infection Functional Attributes of Mucosal Immunity in Cervical Intraepithelial Neoplasia and Effects of HIV Infection. **2004**; :6766–6774.
 73. Clifford GM, Franceschi S, Keiser O, et al. Immunodeficiency and the risk of cervical intraepithelial neoplasia 2/3 and cervical cancer: A nested case-control study in the Swiss HIV cohort study. *Int J Cancer* **2016**; 138:1732–1740.
 74. Konopnicki D, Manigart Y, Gilles C, et al. Sustained viral suppression and higher CD4+ T-cell count reduces the risk of persistent cervical high-risk human papillomavirus infection in HIV-positive women. *J Infect Dis* **2013**; 207:1723–1729.
 75. Minkoff H, Zhong Y, Burk RD, et al. Influence of adherent and effective antiretroviral therapy use on human papillomavirus infection and squamous intraepithelial lesions in human immunodeficiency virus-positive women. *J Infect Dis* **2010**; 201:681–90.
 76. Parham GP, Mwanahamuntu MH, Kapambwe S, et al. Population-Level Scale-Up of Cervical Cancer Prevention Services in a Low-Resource Setting: Development, Implementation, and Evaluation of the Cervical Cancer Prevention Program in Zambia. *PLoS One* **2015**; 10:e0122169.
 77. Huchko MJ, Bukusi EA, Cohen CR. Building capacity for cervical cancer screening in outpatient HIV clinics in the Nyanza province of western Kenya. *Int J Gynecol Obstet* **2011**; 114:106–110.
 78. Sigfrid L, Murphy G, Haldane V, et al. Integrating cervical cancer with HIV healthcare services: A systematic review. *PLoS One* **2017**; 12:e0181156.
 79. Bosch FX, Lorincz A, Muñoz N, Meijer CJLM, Shah K V. The causal relation between human papillomavirus and cervical cancer. *J Clin Pathol* 2002 Apr; 55(4) 244–265 **2002**; :244–265.
 80. Dryden-Peterson S, Bvochora-Nsingo M, Suneja G, et al. HIV Infection and Survival Among Women With Cervical Cancer. *J Clin Oncol* **2016**; 34:JCO679613.
 81. UNAIDS. Global Aids Monitoring 2017: Indicators for monitoring the 2016 United Nations Political Declaration on HIV and AIDS. 2017.

82. World Health Organisation. Guidelines Guideline on When To Start Antiretroviral Therapy and on Pre-Exposure Prophylaxis for Hiv. World Heal Organ **2015**; :78.
83. Chaturvedi AK, Madeleine MM, Biggar RJ, Engels EA. Risk of human papillomavirus-associated cancers among persons with AIDS. *J Natl Cancer Inst* **2009**; 101:1120–30.
84. Clifford GM, de Vuyst H, Tenet V, Plummer M, Tully S, Franceschi S. Effect of HIV infection on human papillomavirus types causing invasive cervical cancer in Africa. *J Acquir Immune Defic Syndr* **2016**; 73:332–339.
85. Heard I, Potard V, Foulot H, Chapron C, Costagliola D, Kazatchkine MD. High rate of recurrence of cervical intraepithelial neoplasia after surgery in HIV-positive women. *J Acquir Immune Defic Syndr* **2005**; 39:412–8.
86. Topazian H. Cervical Cancer Prevention in Kenya. A Qualitative Assessment of the National Cervical Cancer Prevention Program. 2016.
87. National AIDS Control Council, Kenya Ministry of Health. Kenya AIDS Strategic Framework 2014/2015-2018/2019. 2014.
88. Gakidou E, Nordhagen S, Obermeyer Z. Coverage of cervical cancer screening in 57 countries: Low average levels and large inequalities. *PLoS Med* **2008**; 5:0863–0868.
89. Larke NL, Weiss H a., Mayaud P, et al. Design of epidemiological studies measuring genital and plasma HIV-1 outcomes: Lessons from a randomised controlled trial. *Trop Med Int Heal* **2009**; 14:267–275.
90. Mcclelland RS, Wang CC, Overbaugh J, et al. Association between cervical shedding of herpes simplex virus and HIV-1. *AIDS* **2002**; 16:2425–2430.
91. Mcclelland RS, Wang CC, Mandaliya K, et al. Treatment of cervicitis is associated with decreased cervical shedding of HIV-1. *AIDS* **2001**; 15:105–10.
92. Mcclelland RS, Baeten JM, Overbaugh J, et al. Micronutrient supplementation increases genital tract shedding of HIV-1 in women: results of a randomized trial. *J Acquir Immune Defic Syndr* **2004**; 37:1657–63.
93. Curlin ME, Leelawiwat W, Dunne EF, et al. Cyclic changes in HIV shedding from the female genital tract during the menstrual cycle. *J Infect Dis* **2013**; 207:1616–20.
94. Holme F, Kapambwe S, Nessa A, Basu P, Murillo R, Jeronimo J. Scaling up proven innovative cervical cancer screening strategies: Challenges and opportunities in implementation at the population level in low- and lower-middle-income countries. *Int J Gynecol Obstet* **2017**; 138:63–68.
95. Coleman JS, Cespedes MS, Cu-Uvin S, et al. An Insight Into Cervical Cancer Screening and Treatment Capacity in Sub Saharan Africa. *J Low Genit Tract Dis* **2015**; 20:1.
96. Saslow D, Solomon D, Lawson HW, et al. American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer. *CA Cancer*

- J Clin **2012**; 62:147–172.
97. Jeronimo J, Bansil P, Lim J, et al. A multicountry evaluation of careHPV testing, visual inspection with acetic acid, and papanicolaou testing for the detection of cervical cancer. *Int J Gynecol Cancer* **2014**; 24:576–585.
 98. Campos NG, Tsu V, Jeronimo J, Mvundura M, Lee K, Kim JJ. When and how often to screen for cervical cancer in three low- and middle-income countries : A cost-effectiveness analysis. *Papillomavirus Res* **2015**; 1:38–58.
 99. World Health Organization. Guidelines for screening and treatment of precancerous lesions for cervical cancer prevention. 2013.
 100. Kreimer AR, Schiffman M, Herrero R, et al. Long-term risk of recurrent cervical HPV infection and precancer and cancer following excisional treatment. *Int J Cancer Int J Cancer Jul* **2012**; 1:211–218.

Vita

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