IDENTIFYING RISK FACTORS FOR ANAL HUMAN PAPILLOMAVIRUS TYPE 16 INFECTION AND ASSESSING THE ACCEPTABILITY OF SCREENING FOR ANAL CANCER AND ITS PRECURSORS IN WOMEN LIVING WITH HIV

Elaina Kaufman

Department of Family Medicine, McGill University, Montreal

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Supervised by: Dr. Alexandra de Pokomandy

Co-supervised by: Dr. Tyler Williamson

To my mother, Barbara

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PREFACE & CONTRIBUTION OF CO-AUTHORS

This research is presented in the form of a manuscript-based thesis. It includes extended versions of two articles, to be submitted for publication. The thesis was prepared according to the McGill University requirements for preparation of a manuscript-based thesis, which are provided in Appendix C. Several individuals have contributed directly to the development of this thesis and the manuscripts.

Elaina Kaufman BA&Sc., as the MSc. Candidate, was responsible for conducting the literature searches and writing the literature review; conceiving the topics and objectives for the manuscripts; planning, conducting, and presenting the statistical analyses; interpreting the findings; presenting the findings at scientific conferences, and writing the manuscripts. From November 2013 to March 2014, Elaina also assisted the EVVA study coordinator with chart reviews, entry of questionnaire and chart review information into the database, and recruitment tasks such as preparation of medical and interview supplies for study visits.

Dr. Alexandra de Pokomandy MDCM MSc., the Principal Investigator of the EVVA Study and MSc. supervisor of Elaina Kaufman, was responsible for the overall supervision and management of the EVVA study. She supervised all work carried out by Elaina Kaufman towards the completion of this thesis, and provided essential feedback on all aspects of the manuscripts including planning, writing, data analysis, and interpretation of results.

Christina de Castro BSc., Coordinator of the EVVA study, conducted on-site coordination and recruitment, including all administrative tasks, questionnaire administration, communication

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Dr. Tyler Williamson PhD, Co-supervisor of Elaina Kaufman and Assistant Professor in the Department of Community Health Sciences at the University of Calgary's Cumming School of Medicine, provided guidance and review of the statistical analyses as well as review of the manuscripts.

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Dr. Francois Coutlée MD, an infectious disease specialist and microbiologist with expertise in HPV laboratory techniques and high-resolution anoscopy (HRA), was responsible for the sample handling, HPV testing, and performance of HRA.

GLOSSARY OF ABBREVIATIONS

AIDS: Acquired immunodeficiency syndrome

AIN: Anal intraepithelial neoplasia

ANCHOR: The ANCHOR Study, "Anal Cancer/HSIL Outcomes Research"

ART: Antiretroviral therapy

AZT: Azidothymidine, or zidovudine (ZDV)

CIHR: Canadian Institutes of Health Research

DARE: Digital anorectal examination

EVVA: The EVVA Study, "Evaluation of HIV, HPV, and AIN in Women"

HIV: Human immunodeficiency virus

HPV: Human papillomavirus

HRA: High-resolution anoscopy

HSIL(s): High-grade squamous intraepithelial lesion(s)

IRC: Infra-red coagulation

LOPAC: The LOPAC Trial, "Laser Ablation vs. Observation to Prevent Anal Cancer"

LSIL(s): Low-grade squamous intraepithelial lesion(s)

MSM: Men who have sex with men

PLHIV: People living with HIV

RCT: Randomized controlled trial

SIL(s): Squamous intraepithelial lesion(s)

LAST: The LAST Project, "Lower Anogenital Squamous Terminology Standardization"

WHO: World Health Organization

WIHS: Women's Interagency Cohort Study

WLHIV: Women living with HIV

ABSTRACT

Background

Anal infection with oncogenic human papillomavirus (HPV) can lead to anal cancer. HPV type 16 is both highly oncogenic and highly prevalent. Given the high rate of anal cancer in women living with HIV (WLHIV), routine screening is being considered. Decisions regarding screening and prevention require knowledge of the risk factors at each step in the development of anal cancer, and confirmation of the acceptability of screening tests.

Objectives

The first manuscript aims to elucidate the risk factors for prevalent anal HPV 16 infection in WLHIV. The second manuscript aims to assess the acceptability of 3 anal cancer screening procedures (anal swabs for cytology or HPV testing, high-resolution anoscopy with biopsies, and digital anorectal examination) in WLHIV.

Methods

This manuscript-based thesis is based in the EVVA study ("Evaluation of HIV, HPV, and Anal Intraepithelial Neoplasia in women"), an epidemiological cohort study in 151 WLHIV. Risk factors for anal HPV 16 were identified using logistic regression. Acceptability questionnaires were analyzed using descriptive and inferential statistics.

Results

In age-adjusted multivariable analyses, factors associated with prevalent anal HPV 16 infection were past anogenital herpes (Odds Ratio[OR]=6.6; 95% Confidence Interval [CI]: 1.8-23.9; p=0.004), nadir CD4 count ≤200 cells/μL (OR=5.9; 95% CI: 1.6-21.2; p=0.007), current

smoking (OR=5.1; 95% CI: 1.4-18.9; p=0.014), and concurrent cervical HPV 16 infection (OR=27.7; 95% CI: 2.3-326.7; p=0.008). Effect modification was observed between past anogenital herpes and lifetime number of anal sex partners (OR for interaction = 0.045; 95% CI: 0.003-0.773 p=0.003). All 3 screening procedures were considered necessary and very acceptable by the majority of women. Screening intervals of 2 years for digital anorectal examinations and anal swabs, and 5 years for high-resolution anoscopy, were considered acceptable by over 90% of women. Pain accounted for most low acceptability responses.

Conclusions

Our results can help identify women at greatest risk of anal HPV 16 infection. These women are more likely to benefit from screening, through either anal HPV testing or cytology. If screening programs were to be considered, currently available anal cancer screening procedures would be acceptable for most WLHIV, particularly with improvements to pain management.

RESUME

Contexte

Une infection anale par un virus du papillome humain (VPH) de type oncogène peut mener au cancer anal. Le VPH 16 est hautement oncogène et très prévalent. Compte tenu du taux élevé de cancer anal chez les femmes vivant avec le VIH (FVVIH), le dépistage systématique est envisagé. Les décisions concernant le dépistage et la prévention exigent la connaissance des facteurs de risque pour chaque étape du développement du cancer anal et la confirmation de l'acceptabilité des procédures de dépistage.

Objectifs

Le premier manuscrit vise à identifier les facteurs de risque pour l'infection prévalente anale du VPH 16 chez les FVVIH. Le deuxième manuscrit vise à évaluer l'acceptabilité de trois procédures de dépistage (écouvillon anal pour la cytologie ou les tests VPH, anuscopie sous haute resolution avec biopsies, et toucher ano-rectal) chez les FVVIH.

Méthodes

Ce mémoire est basée sur l'étude EVVA («Évaluation du VIH, VPH, et néoplasie intraépithéliale anale chez les femmes»), une étude de cohorte chez 151 FVVIH. Les facteurs de risque pour le VPH 16 anal ont été identifiés à partir d'analyses par régression logistique. Les questionnaires menant sur l'acceptabilité ont été analysés avec des méthodes descriptives et inférentielles.

Résultats

Dans le modèle final multivarié ajusté pour l'âge, les variables significativement associés à l'infection anale au VPH 16 étaient un antécédent d'herpès anogénital (Odds Ratio (OR)=6.6;

Intervalle de confiance [IC] 95%: 1.8-23.9; p=0.004), un nadir de CD4 ≤200 cellules/μL (OR=5.9; IC 95%: 1.6-21.2; p=0.007), le tabagisme actuel (OR=5.1; IC 95%: 1.4-18.9; p=0.014), et une infection concurrente par le VPH 16 au col utérin (OR=27.7; IC 95%: 2.3-326.7; p=0.008). Les antécédants d'herpes anogénital modifiaent l'effet d'avoir eu plus qu'un partenaire de relations sexuelles anales à vie (OR pour l'intéraction = 0.045; 95% CI: 0.003-0.773 p=0.003). Selon la majorité des femmes, les trois procédures sont nécessaires et très acceptables. Des dépistages au 2 ans pour les écouvillons anaux et touchers ano-rectaux, et aux 5 ans pour l'anuscopie sous haute résolution, étaient acceptables selon plus de 90 % des femmes. La majorité des réponses indiquant une faible acceptabilité étaient dues à la douleur.

Conclusions

Nos résultats peuvent aider à identifier les femmes à plus haut risque d'infection anal au VPH 16. Ces femmes pourraient avoir les plus fortes chances de bénéficier d'un dépistage, soit par test VPH anal ou par cytologie. Si les programmes de dépistages auraient à être considérés, les procédures de dépistage du cancer anal actuellement disponibles seraient acceptables pour la plupart des FFVIH, surtout avec des améliorations au niveau de la gestion de la douleur.

1. INTRODUCTION

1.1 BACKGROUND

Human immunodeficiency virus (HIV) in Women

Of the estimated 36.9 million people living with HIV (PLHIV) worldwide[1], over 71,000 live in Canada [2]. Today, on a global scale, equal numbers of men and women are living with the virus, and, in Canada, one in four PLHIV is female. A growing 26% of new HIV infections in Canada are in women, and well over half of these occur in Aboriginal women, incarcerated women, and women from countries where HIV is endemic[3].

Success of Antiretroviral Therapy (ART)

In the early years of the HIV epidemic (1980 to 1995), a positive HIV diagnosis implied that a person was unlikely to survive more than a few years. In contrast, HIV is now considered a manageable, long-term chronic illness. This shift is attributable to the development of successful Antiretroviral Therapy (ART), which consists of a combination of three antiretroviral drugs (tritherapy) that hinder various stages of the virus' replication cycle[4]. HIV compromises the health of affected individuals by infecting and destroying CD4 cells ("helper" T lymphocytes)[5], which are essential to immune system function. In the absence of treatment, the CD4 count (number of CD4 cells per microliter [μL] of blood) decreases to such an extent that the immune system becomes unable to defend the individual against potentially life-threatening opportunistic infections[6]. Successful treatment with ART decreases the amount of HIV in the body (viral load, measured in number of HIV RNA copies per mL of blood) so that the CD4 cells are replenished and effective immune functioning is restored[6]. Use of combination ART to suppress viral load in PLHIV became widespread after its superiority to single-drug therapy (monotherapy with AZT) was emphasized at the 11th International AIDS Conference in 1996 in

Vancouver[7]. In the following years, there were stark and swift improvements in life expectancy and quality of life for PLHIV[8, 9].

PLHIV experience comorbidities despite use of ART

Increased longevity since the development of ART is primarily attributable to a marked reduction in the incidence of AIDS-defining illnesses, including opportunistic infections and AIDS-related cancers (Kaposi's sarcoma, non-Hodgkin's lymphoma, and invasive cervical cancer) [10, 11]. These illnesses generally occur when CD4 counts fall below 200 cells/μL. Yet, despite success of ART to restore the immune system in PLHIV, the incidence of certain non-AIDS defining cancers, including anal cancer, Hodgkin lymphoma, and liver cancer, has risen[11]. In the case of anal cancer, the 5-year cumulative incidence in one cohort study of nearly 500,000 PLHIV in the United States rose across the three successive calendar periods of 1980-1989 to 1990-1995 to 1996-2006, from 0.02% to 0.07% to 0.09%[11].

Anal cancer survival and treatment

The most common type of anal cancer occurs in the squamous cells of the anus, the terminal portion of the large intestine[12]. According to the Canadian Cancer Society, the 5-year survival rate for anal cancer is 62%[13]. This survival rate is similar to that of colorectal cancer (64%)[14], but well below that of cervical cancer (74%)[15] and breast cancer(88%)[16]. The standard course of treatment for anal cancer depends on the size and location of the tumour and whether or not the cancer has metastasized[17]. Minor surgery involving local excision, which leaves the anal sphincter intact in most cases, is considered the optimal approach for treating small (<2 cm diameter and <3 mm thick) and minimally invasive anal cancers [17]. The treatment of choice for anal cancers between 2 and 5 cm in diameter is combined chemoradiation

[18], which also preserves the anal sphincter. Abdominoperineal resection, a more extensive surgery involving removal of the anus, rectum, and a portion of the colon, is used for recurrent or persistent anal cancers[18]. In a multi-cohort study of 235 patients in Denmark, 26% (95% CI, 21%-32%) of anal cancer patients had a tumour-related colostomy by 5 years post-diagnosis[19].

Some groups face a high risk of anal cancer

Although anal cancer is a relatively rare disease in the general population[13], its incidence is disproportionately high in certain groups, including men who have sex with men (MSM)[20], solid organ transplant recipients[21], and PLHIV of any gender[22, 23]. The incidence of anal cancer among PLHIV had been rising steadily since 1992[24, 25], until it began to show signs of leveling off as of 2003 in the United States[26] and 2006 in the Netherlands[26, 27]. This purported stabilization may or may not be a lasting trend, as one cohort of 86,620 PLHIV in the United States showed a steady increase in cumulative incidence of anal cancer by age 75 of 6% per year for the period of 1996 to 2009[28]. Although it is possible that anal cancer incidence in high-income countries will decline as initiation of treatment with ART is now recommended at an earlier stage of infection [29], PLHIV currently face an approximately 30-fold increased risk of anal cancer compared to the general population[21]. The increased anal cancer incidence since the advent of ART can be attributed to the fact that increased longevity on ART has given rise to an aging population of PLHIV who, due to their age, face a higher risk of most cancers compared to their younger counterparts[8, 28].

Anal cancer in WLHIV

In the general population, the risk of anal cancer in women is approximately twice as high as the risk in men[13]. In contrast, in PLHIV, anal cancer affects more men than women due to the

staggeringly high rate of anal cancer among MSM who are living with HIV [30-32]. While the overall incidence of anal cancer in men living with HIV compared to the general population is 32 times greater, the incidence of anal cancer in MSM living with HIV is estimated to be 78 times greater than in the general population[20]. Because of the extremely high incidence in this group, most anal cancer research to date has focused on these men. Yet, the incidence is also disproportionately high among WLHIV: 24 times greater than the general population[33]. Although some research findings regarding anal cancer in HIV-positive MSM may be generalizable to people of all genders, findings in men and women may differ due to dissimilarities in anatomy, social context, lifestyle, and sexual practices. Due to the high rate of anal cancer in WLHIV and the paucity of research investigating anal cancer and related conditions in this population, there is a pronounced need for research in this field.

Anal cancer screening

There is strong evidence that early detection and treatment of anal cancer reduces mortality. According to US National Cancer Institute data, the 5-year survival rate for anal tumours of less than 2 cm in diameter at diagnosis is 80%, compared to only 45-65% for larger tumours[34]. Yet, the benefit of screening for potentially precancerous lesions is less clear. Biological parallels[i] between cervical and anal cancer suggest that treating potentially precancerous anal lesions can decrease the incidence of anal cancer. Proponents of anal cancer screening highlight these parallels and the dramatic decrease in cervical cancer incidence after the implementation of screening and treatment for potentially precancerous cervical lesions in the 1960s.

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[i] Details of the biological parallels between cervical and anal cancer are provided on page 32.

[ii] Details of the prevalence and oncogenic potential of HPV-16 are provided on pages 27-28.

[iii] A summary of current anal cancer screening recommendations is provided on pages 36-39.

Although some regional and organizational screening guidelines exist[35-38], there are no national-level guidelines for anal cancer screening. Current screening guidelines, such as those proffered by the New York State Department of Health[35], European AIDS Clinical Society[36], German AIDS Society[37], and Italian society of colo-rectal surgery[38] draw on expert opinion as the highest available form of evidence. Despite a lack of perfect consensus among screening guidelines, trends are apparent: The New York[35], European[36], and German-Austrian[37] guidelines uniformly recommend anal cancer screening approaches involving initial screening with anal cytology or digital anorectal examination (DARE) followed by referral to high-resolution anoscopy (HRA) for patients considered at high risk of anal cancer. Similarly, the Italian society of colo-rectal surgery recommends cytology and HRA as anal cancer screening in PLHIV[38]. Guidelines vary slightly, however, in their stance concerning which specific groups and subgroups require screening and which patients should be considered at highest risk. For example, the European AIDS Clinical Society guidelines recommend anal cytological screening for the subset of PLHIV who practise anal sex, while the New York State Department of Health, German AIDS Society, and Italian society of colo-rectal surgery all recommend some form of anal screening (DARE or cytology) in *all* adults living with HIV and explicitly include women with cervical HSIL among those who should be referred for HRA.

Many experts believe there is already sufficient evidence to support screening for anal cancer and treatment of potentially precancerous anal lesions in HIV-positive MSM[39] and other high-risk groups; however, controversy persists due to the absence of evidence from randomized controlled trials (RCTs) confirming that treating these lesions does, indeed, prevent anal cancer. Despite the ethical concerns associated with having a non-intervention group for a condition that many experts believe to be treatable[40], two trials were recently launched with the aim of

resolving this controversy: The "ANCHOR" study [41], in the United States, and the LOPAC trial in the United Kingdom[42].

Proof of effectiveness and efficacy are not the only requirements for implementation of a new screening program. According to the World Health Organization (WHO), a new screening program must meet 10 requirements[43]: 1) The condition sought should be an important health problem; 2) There should be an accepted treatment for patients with recognized disease; 3) Facilities for diagnosis and treatment should be available; 4) There should be a recognizable latent or early symptomatic stage; 5) There should be a suitable test or examination; 6) The test should be acceptable to the population; 7) The natural history of the condition, including development from latent to declared disease, should be adequately understood; 8) There should be an agreed policy on whom to treat as patients; 9) The cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole; 10) Case-finding should be a continuing process and not a "once and for all" project.

This thesis project aims to contribute knowledge to the domains of several of the WHO requirements: "1) The condition sought should be an *important health problem*"; "5) There should be a *suitable test* or examination"; "6) The *test should be acceptable* to the population"; "7) [Adequate understanding of] the *natural history* of the condition" (risk factors for prevalent anal HPV 16, in this case). Specifically, this knowledge will pertain to WLHIV, a population that is not well understood in the context of anal cancer despite their high risk for the disease.

Since the initial development of the WHO's 10 requirements for screening programs in 1968[43], these guidelines have been revisited[44] and new paradigms for the evaluation of proposed screening programs have been presented. In 2011, Harris et al. proposed systematically reviewing the evidence that is indicative of benefits or harms of the proposed screening, and comparing the two collections of evidence in order to determine their relative magnitude[45]. If this newer strategy were to be employed instead of or in addition to the WHO checklist in decision-making processes regarding anal cancer screening, the aforementioned knowledge domains to be addressed in this thesis would nevertheless contribute to our understanding of the magnitude of potential benefits and harms of anal cancer screening. Although we expect the LOPAC trial to provide answers regarding the benefit of screening for potentially precancerous anal lesions in HIV-positive MSM, there will be a delay of five to eight years before follow-up results from the ANCHOR study, which includes WLHIV, become available[46].

Anal cancer and human papillomavirus (HPV)

Sexually transmitted types of HPV will infect three out of every four Canadians over their lifetime[47]. While some types of HPV have little or no effect on humans, others can lead to disease. Like cervical cancer, the vast majority of cases of anal cancer have been associated with persistent infection with oncogenic ("high-risk") types of HPV[48, 49], which can cause potentially precancerous cell changes that progress in some cases and become invasive cancer[50]. Anal HPV infection may arise from sexual activity involving anal penetration, but it may also occur due to incidental contact during vaginal or non-penetrative sexual activity[51], as well as via autoinoculation from the hands, genitals or, possibly, urine[52].

1.2. RATIONALE AND OBJECTIVES

Risk factors for anal HPV in WLHIV

Understanding the natural history and risk factors at play in each step of the causal pathway towards anal cancer will help identify which patients may benefit most from screening. This knowledge may also help determine the most beneficial frequency of screening, in order to optimize health outcomes while minimizing discomfort and inconvenience to the patient as well as clinical resource intensiveness. The risk factors for anal HPV infection are an essential element of this complex puzzle. HPV 16 is associated with over 75% of anal cancers[53]. It is also considered the most prevalent and most oncogenic HPV type[ii]. For these reasons, it is necessary to identify the risk factors for anal HPV 16 in WLHIV.

Although a substantial body of research has examined risk factors for anal HPV, precancerous lesions, and anal cancer in HIV-positive MSM, knowledge of these risk factors in WLHIV is sparse. The high prevalence of anal HPV in MSM, especially those who are HIV-positive, has been attributed in part to anal sexual activity. In comparison, far fewer WLHIV regularly practise anal sex and their most important risk factors may, therefore, be different. Risk factors for anal HPV in WLHIV may also differ from those affecting HIV-negative women in Canada, due to potential differences in immunity, behaviour, and past or present barriers to health care.

Acceptability of anal cancer screening in WLHIV

Although some research has investigated anal cancer screening acceptability in MSM, little is known about anal cancer screening acceptability in women. Results from research on anal cancer screening in MSM cannot necessarily be extrapolated to WLHIV, because of divergences in sexual practices, life experiences, and social context between these two groups. The lower

[ii] Details of the prevalence and oncogenic potential of HPV-16 are provided on pages 27-28.

frequency with which anal intercourse is practised among WLHIV compared to among MSM may prompt corresponding differences in the level of comfort, both physically and psychologically, with screening procedures of the anal region. Furthermore, although MSM and WLHIV are both groups that experience a disproportionately high level of marginalization, the issues they face are not identical; WLHIV experience socioeconomic challenges and violence differently from MSM. Predictors of acceptability and unacceptability may also differ between these groups. Knowledge of these predictors is needed to assist physicians in the identification of patients who are more likely to be reticent to screening or potentially at risk of psychological harms from screening procedures, and to ensure that screening tools and procedures are acceptable to as many patients as possible.

Despite some dissent, most anal cancer and HIV experts support some form of screening for anal cancer and its potential precursors in high-risk patients. The manuscript addressing screening acceptability within this thesis is not a definitive call for screening for anal cancer or potentially precancerous anal lesions in all WLHIV or in the general population. Rather, examining acceptability of screening *now* serves as a preparatory measure to pinpoint potential physical or psychological harms of screening, confirm screening acceptability among candidate populations, and identify barriers to acceptability in the event that screening should become more widely recommended in the future[iii]. It can also provide physicians who do choose to screen WLHIV for anal cancer at the present time[iv] with information about the acceptability of the procedures[v], including areas of concern. Furthermore, some anal cancer screening procedures are less

[iii] A summary of current anal cancer screening recommendations is provided on pages 36-39.

[iv] Some physicians have begun screening for anal cancer and HSIL in high-risk groups. See details page 34.

[v] Screening procedures are described on pages 35-36 and 86-87.

controversial than others. Compared to HRA and anal cytology, DARE has been more widely recommended. DARE has been proposed as an effective and minimally invasive method for identifying early or small invasive anal cancer[35, 54, 55], even in the general population[56]. As such, there is a need to confirm the acceptability of DARE in WLHIV regardless of the degree of consensus regarding screening for precancerous anal lesions.

This article-based thesis is based on data from the EVVA study ("Evaluation of HIV, HPV, and AIN in women"), an ongoing CIHR-funded prospective cohort study of 151 WLHIV in Montreal, Canada. EVVA study participants attend biannual visits involving questionnaires, chart review, and screening for HPV, anal SILs, and anal cancer, for two years. Given the paucity of published sources addressing risk factors for anal HPV in WLHIV and acceptability of anal cancer screening in this population, I use EVVA study data to examine and address the following two research objectives via two manuscripts:

1) Identify risk factors for prevalent anal HPV 16 infection among WLHIV participating in the EVVA study.

2) Assess the acceptability of anal cancer screening procedures among WLHIV participating in the EVVA study and explore factors that may predict low acceptability in some women.

1.3. TERMINOLOGY OF ANAL HPV-RELATED LESIONS

Two main terminologies have been used to describe lower anogenital tract lesions associated with HPV. One, developed by physicians specialized in dermatology and dermatopathology, has been used to describe precancerous cutaneous lesions. The other, developed mainly by pathologists and gynecologists, for mucosal lesions[57, 58]. Cutaneous lesions have been called squamous intraepithelial lesions (SILs) and mucosal lesions have been called intraepithelial neoplasia (IN) 1, 2, and 3, in order of increasing severity. These terminologies are now considered comparable on a histological level[58]. Semantic differences may lead to clinical miscommunications or contribute to uncertainty regarding patient care decisions.

The "LAST" Project[vi] was conceived by the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology to standardize the terminology[57]. A resulting two-tiered system is now used to describe precancerous HPV-related anogenital lesions, with LSIL referring to Low Grade Squamous Intraepithelial lesions (previously called AIN-1), which will usually resolve themselves spontaneously, and HSIL referring to High Grade Squamous Intraepithelial Lesions (previously called AIN-2 or AIN-3), which are at risk of progressing to invasive cancer[57]. This system permits the use of previous terminology for mucosal lesions as qualifiers to complement the two-tiered terminology. In addition, a category of superficially invasive squamous cell carcinoma has been defined to designate minimally invasive carcinomas that might respond to simple local excision, a significantly more conservative treatment than that typically used for invasive anal cancers [57]. In this thesis, the terms LSIL and HSIL are used to describe low- and high-grade HPV-related anal lesions previously. The -IN terminology is used when citing literature in which it has been employed.

[vi] The "LAST" Project: Lower Anogenital Squamous Terminology Standardization Project

CHAPTER 2. LITERATURE REVIEW

2.1 NARRATIVE REVIEW: ANAL CANCER AND ANAL HPV IN WLHIV

2.1.1. THE LINK BETWEEN HPV AND CANCER

HPV-associated cancers

Globally, an estimated 5.2% of cancers were associated with HPV in the year 2002[59]. Infection with high-risk HPV plays a causal role in 90-100% of all cervical and anal cancers[60, 61], as well as 50-70% of vulvar and vaginal cancers[62, 63], 60% of penile cancers, and 70% of oropharyngeal cancers[63]. Sexually transmitted HPV types are classified as "low risk" and "high risk"; types that cause benign warts (condylomata) are considered "low risk", whereas oncogenic types are considered "high risk". This distinction has become nuanced due to the recent discovery that condylomata are occasionally linked to oncogenic HPV types and associated with HSILs[64] and that, conversely, it is possible for HSILs to contain only low-risk types of HPV[64]. Nonetheless, high-risk HPV types are typically defined as those that can cause potentially precancerous squamous cell changes, known as squamous intraepithelial lesions (SILs, encompassing the aforementioned LSILs and HSILs), which may progress to invasive cancer.

The two most common high-risk HPV types, HPV 16 and 18, are associated with approximately 90% of anal cancers[65, 66] and 70% of cervical cancers. The cancer risk associated with HPV-16 is ten times higher than that of other high-risk HPV types[67]. A 2009 meta-analysis by de Vuyst et al. found that 84.3% of cases of anal cancer were associated with HPV. HPV type 16 was associated with 73.4% of cases, while types 18, 31, 33, and 45 were associated with 12. 4% of cases[66]. Two years later, a multi-site study in France reported an even higher prevalence of HPV in anal cancer: 91% of histological specimens were found to contain one or more high risk

HPV types, with HPV 16 present in over 75% of cases[53]. Only 3.3% of squamous cell carcinomas of the anus were found to be HPV-negative in this study. Furthermore, it is possible that some tumours that appear to be HPV-negative are, in fact, initiated by an HPV infection that has subsequently been cleared or masked in the cells[68].

In addition to its major role in causing anal and cervical cancers, HPV type 16 is associated with more than 50% of all oropharyngeal cancers[69], as well as a significant proportion of penile, vaginal and vulvar cancers[65]. Two "low-risk" types, HPV 6 and 11, account for 90% of all cases of anogenital warts[70]; these are costly to treat[71, 72] and can negatively affect psychological well-being[73]. HPV 6 and 11 can also cause recurrent respiratory papillomatosis, a rare medical condition that requires many surgeries to remove wart-like tumours from the air passages of the nose and mouth[74].

Mechanism of HPV oncogenesis

HPV types that infect the mucosa of the oral cavity, oropharynx, anus, and genitals belong to the alpha genus of the virus[75]. These mucosal HPV types are those that are generally considered sexually transmitted; they comprise approximately 40 of the more than 170 types of HPV that have been identified [76, 77]. Of these 40 mucosal HPV types, 14 or more are considered oncogenic and a further 13 are possibly oncogenic or have an undetermined oncogenic potential[78].

Alpha papillomaviruses infect basal epithelial stem cells and interfere with the normal functioning of numerous proteins in the host cell[79]. Notably, the DNA of alpha papillomaviruses contains the viral proteins E6 and E7, which bind to and inactivate pRB and

p53, two tumor suppressor proteins that are essential for control of the cell cycle[79, 80]. The interaction of E7 with pRB and its related proteins produces an effect on E2F transcription factors that instigates DNA replication, prematurely launching the S-phase of the cell cycle[79, 80]. In tandem with this action, the E6 protein inactivates p53, the host cell protein that would initiate apoptosis to counteract this malfunctioning of pRB if the cell were fully functional[81].

In malignant HPV-related tumours, high-risk HPV DNA is usually integrated into the host genome[67]. HPV DNA is circular, but it linearizes during integration[82]. This integration facilitates continued action of E6 and E7, which contribute to instability of the host cell chromosomes and copy-number abnormalities[82]. As the action of E6 and E7 persists, uncontrolled cell growth and an increasingly large proportion of cells with chromosomal instability and copy-number abnormalities are observed[80, 82]. Under these conditions, in combination with other influences and in the absence of interference due to immune system activity or medical intervention, invasive cancer may develop[80, 82, 83]. In contrast, in benign HPV-associated lesions such as warts and LSILs, which are usually associated with low-risk HPV types, DNA integration into the genome of infected host cells is far less common[67]. Integration of viral DNA into the host cell genome is considered to be a key aspect of HPV-induced carcinogenesis[67].

2.1.2. ACTIVITY OF HPV IN PLHIV

In PLHIV, the risk of anogenital and oral HPV as well as HPV-related HSIL and cancers is higher than in the general population[82]. PLHIV with lower CD4 counts have been demonstrated to experience a higher prevalence of HPV and incidence of HSIL, but a link between low CD4 count and progression to invasive cancer is not apparent[82]. This discrepancy

in the role of CD4 count at different stages of the causal pathway suggests that the high rate of HPV-related cancers in PLHIV may be due to the effect of immunosuppression on initial HPV infection, persistence of infection, and progression to HSIL, but that immunosuppression may play a less direct role in the progression from HSIL to cancer[82, 84]. The viral proteins tat and gp120, which are secreted by immune cells infected with HIV, may facilitate initial mucosal HPV infection by disrupting tight junctions between epithelial cells[85]. The tat protein may also favour development of HSIL, which, once it has developed, may be more likely to persist and progress to invasive cancer in PLHIV due to inhibitory effects of HIV on HPV-specific immune responses responsible for regression of HPV-associated lesions in healthy individuals[82, 84].

2.1.3. NATURAL HISTORY OF ANAL CANCER

Early evidence of causality

As early as the 1960s, physicians observed a correlation between anal cancer and lower genital tract cancers in women, raising the possibility that this correlation was due to exposure of these contiguous anatomical surfaces to a shared carcinogen[86]. In 1979, Cooper et al published the first article[87] suggesting a link between sexually transmissible carcinogens and anal cancer, although another research team had previously documented a case of squamous cell carcinoma of the anus in a group of MSM[88]. Soon, additional publications discussed the possible link between anal cancer and receptive anal intercourse or sexually transmitted infection, including mention of a possible association with human papillomavirus[89, 90]. In 1987, Daling et al. published results indicating an association between genital condylomata and anal cancer, as well as a strong association between receptive anal intercourse and anal cancer, which appeared to be strong in men and weak in women[91]. Based on these associations, Daling et al. posited that human papillomavirus infection was a cause of anal cancer[91]. The same year, Palmer et al.

published preliminary results from a study that demonstrated the high prevalence of HPV type 16 DNA in squamous cell carcinomas of the anus compared to control tissues. Using hybridization with DNA fragments separated by electrophoresis, Palmer et al. found HPV 16 in 6 out of 10 squamous cell carcinomas of the anus and no HPV 16 in the tissues taken from the 10 control patients[92].

Role of HPV in the development of anal cancer

Oncogenic HPV infection of the anus is the most significant risk factor for anal cancer[46]. Anal SILs develop as a result of persistent HPV infection and progress through multiple stages, from LSILs to HSILs. HSILs have long been considered the immediate precursors to invasive anal cancer, although it was not until recently that evidence was published confirming that individual HSILs can progress to invasive cancer if left untreated[50]. It must be noted that these lesions do not always progress and might, potentially, spontaneously regress to a lower grade in some cases[93]. Although there is a lack of prospective data regarding progression of HSIL, a recent meta-analysis estimated the rate of progression of anal HSIL in MSM to be well below the rate of progression of cervical pre-cancerous lesions[94]. A recent longitudinal analysis of PLHIV who had been diagnosed with anal HSIL revealed a five-year cumulative incidence of invasive anal cancer of 1.65%, with an upper 95% confidence limit of 4.5%, after adjusting for use of ART, HIV viral load, smoking status, and infrared photocoagulation (IRC) ablation therapy[95].

2.1.4. COMPARING CERVICAL AND ANAL CANCER

Parallels between cervical cancer and anal cancer

There are numerous biological parallels between cervical and anal cancer. These cancers develop in mucosa that are alike in structure and arise from a shared embryological origin, the cloacogenic membrane[86]. Like cervical cancer, for which HPV infection is considered a necessary condition, the vast majority of cases of anal cancer are associated with high-risk types of HPV[53]. A parallel can further be noted in how these two cancers develop; both arise when these high-risk HPV types persist and cause potentially precancerous cell changes in the transformation zone of the anal or endocervical canal[96]. This transition or "transformation" zone is an area of cell metaplasia where squamous epithelium meets columnar epithelium[12, 96]. Potentially precancerous cervical and anal lesions are analogous to one another in that each arise in this zone in the cervix or anus, progress through similar stages, and can eventually progress to invasive cancer[96].

Differences between the cervix and the anus

Despite the many parallels between cervical and anal cancer, there are microanatomical differences between the cervix and the anus. Recent research by Yang et al has identified two important differences. One difference is that the transformation zone of the anal canal consists of up to nine layers of columnar to cuboidal cells at the surface whereas the equivalent part of the cervix is comprised of a single layer of undifferentiated embryonic cells[97]. The other difference is that the cells of the anal transformation zone appear to form metaplastic squamous epithelium, in contrast to the squamocolumnar junction in the transformation zone of the cervix[97]. This research indicates that anal transformation zone cells are similar to vaginal and

vulvar epithelium. This would explain why the anus is less susceptible to HPV-induced cancers than the cervix in immunocompetent individuals[97].

2.1.5. ANAL CANCER SCREENING

The anal cancer screening debate

As discussed above, the five-year survival rate for people diagnosed with anal cancer is higher when the disease is diagnosed at an early stage[34]. In addition, there is mounting evidence that diagnosis and treatment of anal HSIL may further reduce mortality by reducing the incidence of progression to invasive anal cancer. When understood as a body of complementary studies in dialogue with one another, available literature regarding progression of HSIL lesions shows a greater persistence of HSIL and higher probability of progression to invasive cancer among individuals whose HSIL has not been treated compared to those who have received treatment[98-101], even when treatment must be repeated due to HSIL recurrence[98]. Results from a retrospective cohort study reported by Goldstone et al. show a cure rate of 82% for HSIL in HIV-positive MSM and 90% in HIV-negative MSM using infrared coagulation, although some participants required up to four treatments due to HSIL recurrence[102]. After only one ablation, the cure rate for an individual lesion in this study was 67%. Similarly, most other studies have reported success rates of around 70% for treatment of individual HSIL lesions[103]. In light of the recent evidence confirming that individual HSIL lesions can progress to invasive anal cancer if left untreated[50], the high cure rate for HSIL offers potential support to the hypothesis that treating HSIL will reduce the incidence of anal cancer.

The many parallels between cervical and anal cancer etiology and progression also provide support for the view that treatment of HSIL may produce clinical benefits. The widespread

implementation in the 1960s of cytological screening programs for cervical cancer, with referral to colposcopy to treat potentially precancerous cervical lesions, reduced cervical cancer rates by 80%[104]; some experts hold the opinion that routine screening for and treatment of HSIL will have a similar effect on rates of anal cancer in high-risk populations[105]. Some clinicians who are highly specialized in HIV and/or anal health have already decided to conduct such screening and treatment in their clinical practice and plan to continue doing so until research determines that this approach is not beneficial[103]. They believe that there is sufficient evidence to begin treating HSIL[39], and they emphasize the importance of only conducting screening for HSIL in locations in which there is access to physicians and tools needed for follow-up of patients with HSIL[103]. According to a Markov model developed by Goldie et al., anal cytological screening in MSM would be cost effective[106].

Nevertheless, it is possible that early treatment of precancerous anal lesions does not prevent anal cancer. Given this, there are clinicians and researchers who do not consider screening and treatment of HSIL to be justifiable without further research. They argue that differences between cervical and anal cancer are too great to transfer screening strategies from the former to the latter outside of a research setting[94] without stronger evidence that treatment of HSIL reduces the incidence of anal cancer and that the benefits of screening for precancerous anal lesions outweigh the harms. Proponents of this view remind us that most studies in this area have been retrospective in nature[107], and cite a need for more evidence regarding the natural history of anal SILs, target populations and best practices for screening, potential harms of screening and treatment, and response to treatment[107]. In 2012, Wentzensen published a comment arguing that it is necessary to characterize, at the molecular level, HSILs most likely to progress to cancer; this would render it possible to establish a "good surrogate endpoint" identifying lesions

most at risk of progression to cancer, before moving forward with trials investigating screening and treatment options[108]. Similarly, health insurance companies remain skeptical: they will not provide coverage for routine anal cancer screening and treatment of HSIL until the benefit of this strategy has been proven[41]. It is due to this controversy and uncertainty that two multi-site RCTs, the ANCHOR study led by Dr. Joel Palefsky, and the LOPAC trial led by Dr. Mayura Nathan, were recently launched to compare progression of HSIL to invasive anal cancer among patients undergoing HSIL treatment compared to active monitoring[41, 42].

Anal cancer screening procedures[vii]

Given the gradual progression from HPV infection to potentially precancerous cell changes and, finally, to invasive anal cancer, anal cancer screening can target various stages of the causal pathway. Screening may involve the use of anal swabs to test for anal HPV or to detect anal SILs via anal cytology. A single method is used for anal cytology (anal Pap smear), and anal HPV testing: for each procedure, a moistened Dacron swab is inserted and rotated in the anus to collect squamous epithelial cells from the anal walls. This method and the analysis of these anal samples are analogous to those used for cervical HPV testing and Pap smears, which involve the insertion of a speculum into the vagina in order to collect cells from the cervix using a swab. An important difference between the two clinical procedures is that anal swabs do not require the use of a speculum, due to the high accessibility of the anus compared to the cervix. HRA is another potential screening tool; histological examination of the biopsies taken through HRA is considered the "gold standard" for anal HSIL diagnosis[96]. In HRA, the anal canal is visualized via a hollow tube called an anoscope and biopsies are taken for subsequent histological analysis. The use of HRA for detection of anal cancer and anal SILs is analogous to the use of colposcopy

[vii] Screening procedures are described in greater detail on pages 86-87.

for cervical cancer and its precursors[96]. Finally, the least resource-intensive screening procedure for anal cancer, which can only be used to detect cancer that is already invasive and palpable, is DARE. In DARE, the perianal region is examined visually and the anal canal is palpated with a gloved finger to detect lumps that may be indicative of anal cancer.

Biomarkers for anal cancer

Due to the limited sensitivity and specificity of cytology and HRA for detecting anal HSIL, some research has examined the role of biomarkers[109]. These could be useful for distinguishing between anal SILs and benign conditions when results from cytology or HRA are ambiguous[110]. Various biomarkers have been considered. Biomarker techniques which appear to have high sensitivity and specificity include Ki-67 and ProEx C immunostaining for LSIL and condyloma, as well as E6/E7mRNA detection and p16 immunostaining for HSIL[110]. Because diagnosis of anal SILs is subjective, interrater agreement may be improved when a portion of the diagnostic information is drawn from more objective biomarkers[111].

Current screening guidelines and recommendations

Various strategies have been proposed for anal cancer screening. Routine anal HPV testing is not considered useful or practical as a screening strategy for anal HSIL and anal cancer in PLHIV, due to the high prevalence of anal HPV infection in this population[112, 113]. Screening with HRA, without pre-screening using other methods, appears to be the most cost-effective approach, due to the large proportion of false positive results when anal cytology is used[114] as well as the fact that abnormal cytological results of any grade may be found to represent HSIL when examined histologically[115, 116]. According to Dr. Joel Palefsky, President of the International Papillomavirus Society as well as founder and former President of the International

Anal Neoplasia Society, this approach is theoretically desirable but is not currently feasible due to the scarcity of physicians trained in HRA techniques[117].

For this reason, some experts have recommended screening high-risk individuals routinely with DARE and anal pap smears, and referring patients with abnormal cytology or other risk factors (condylomata, cancers, or SILs,) for HRA[39, 54, 117]. This approach is comparable to that which is now widely used for cervical cancer screening and prevention. It has been adopted by the New York State Department of Health; their clinical guidelines for HIV care recommend screening all HIV-positive adults with DARE, and screening with anal cytology in MSM, patients with past or present anogenital condylomata, and women with a history of abnormal cervical or vulvar histology[35]. These guidelines also recommend referral to HRA for any woman with cervical HSIL and any patient with other abnormal anal findings[35]. Similarly, despite stating that the evidence of benefit is not known, the European AIDS Clinical Society Guidelines recommend considering anal HPV testing and cytology in all PLHIV who practise anal sex, followed by referral for HRA when cytological findings are abnormal[36], and the Italian society of colo-rectal surgery recommends screening with anal cytology and HRA in all adults living with HIV, people who practise anal sex, MSM, and women with a history of cervical HSIL [38]. Regarding screening for anal HSIL in WLHIV, anal cytology may be the preferred screening option due to the ease with which it can be performed[118].

Some clinicians do not have access to the necessary resources for anal cytological testing and HRA. In these contexts, DARE has been recommended for all individuals at high risk for anal cancer, including PLHIV[54]. DARE is inexpensive, requiring no resources besides a latex glove, and is easily learned by physicians. Early detection, though not prevention, of anal cancer

could be achieved through performance of DARE every 3 to 4 months in patients who are most at risk within the population of PLHIV[55]. It is noteworthy that the Canadian Cancer Society suggests that DARE may be performed as part of routine physical examination in adults in the general population, although not specifically for the purpose of detecting anal cancer[56]. In 2014, Ong et al. published a systematic review of regional and national guidelines for DARE in HIV-positive MSM; they examined English-language HIV prevention and management guidelines and found only two that recommended DARE. For both of these recommendations, expert opinion was the highest level of supporting evidence.

At present, there are no national or international screening guidelines addressing screening and treatment of anal cancer or potentially precancerous anal lesions, although some regional guidelines have been implemented and some organizations have published recommendations. Guidelines differ regarding screening schedules and treatment methods, as well as which groups and sub-groups need anal cancer screening most. A 2015 systematic review of guidelines for management of high-grade anal intraepithelial neoplasia (AIN), the HSIL equivalent, found only three guidelines regarding screening for AIN; two of these were from European organizations and one was from the American Society of Colon and Rectal Surgeons[119]. The reviewers noted a lack of consensus between these three sets of guidelines with regards to treatment and surveillance strategies[119].

Guidelines have also evolved to include a broader subsection of the population. For example, the 2012 European AIDS Clinical Society Guidelines only recommended screening for anal cancer in HIV-positive MSM[120], while the 2014 guidelines from the same organization state that anal HPV testing and cytology should be considered in all PLHIV who practise anal sex[121]. Most

recently, the German AIDS Society and working groups in Germany and Austria published an English language version of their collaboratively developed German-Austrian guidelines[37]. These guidelines recommend annual genital and anal physical inspection and palpation, as well as anal cytology in all PLHIV and additional screening with HRA (or regular anoscopy if HRA is unavailable) in patients who are considered at particularly high risk for anal cancer. The identification of subgroups of women most at risk of anal cancer may be facilitated by information about the risk factors for high-risk anal HPV infection in women.

2.1.6. HPV VACCINATION

Amidst attempts to identify and define preferred screening tools and schedules, HPV vaccination has emerged as a potentially effective anal cancer prevention strategy. In 2006, a quadrivalent vaccine marketed under the brand name "Gardasil", consisting of three doses and covering the four most common types of HPV (types 6, 11, 16, and 18), was approved for use in Canada in females between 9 and 26 years of age[122]. In 2010, this vaccine was authorized for use in males of the same age group[122]. A bivalent vaccine, "Cervarix", covering the two most common oncogenic HPV types, was also approved in Canada after having already been approved in nearly 100 other countries[122].

School vaccination programs initially included only girls[122], but some have since begun to include boys as well. For example, HPV vaccination of boys has been implemented nationally in Australia[123]. Vaccination of school-aged males has also been implemented regionally in several Canadian provinces[124], including, Quebec. The province of Quebec will now offer free HPV vaccination to boys in grade 4 (as of September 2016) and to all self-identified MSM under the age of 26 (as of January 2016)[125].

In 2015, a nonavalent HPV vaccine ("Gardasil 9"), covering five less common high-risk types in addition to the two high-risk and two low-risk types covered by the quadrivalent vaccine, was also approved for use in Canada[126]. Calculations based on the distribution of HPV types in six multi-centre retrospective studies in France determined that, assuming high coverage of teenage girls, nonavalent HPV vaccination could prevent 90% of invasive cervical cancers, CIN2/3 (equivalent to cervical HSIL), genital warts, and anal cancers in France[127]. Indeed, although initially touted as a vaccine against the virus that causes cervical cancer and genital warts, the potential impact of HPV vaccination on non-cervical cancers, including anal cancer, is immense[65].

Studies to date have also shown HPV vaccination to be safe and cost-effective. An overview of 15 studies spanning the nine years since the quadrivalent vaccine was licensed found no evidence of increased incidence of serious adverse events such as stroke and autoimmune disease in vaccinated individuals[128]. Regarding Gardasil 9, Health Canada recently announced that it considers the benefits to outweigh the risks of vaccination in girls and women ages 9 to 45 and boys and men ages 9 to 26[129]. Although the HPV vaccines are expensive compared to most other vaccines[130], public health strategies involving widespread quadrivalent HPV vaccination, including programs that incorporate vaccination of boys and MSM in addition to girls, have been found to produce a direct benefit that outweighs the associated costs[131-133].

Positive outcomes of HPV vaccination are already becoming apparent and the potential large-scale public health benefit is promising[134-136]. As HPV vaccination coverage becomes more widespread in the population and adolescents who are currently being vaccinated begin to reach

adulthood, screening strategies are likely to adapt to the needs of a changing population whose HPV prevalence is lower than that of previous generations. As HPV-vaccinated populations reach maturity in the next 25 to 30 years, this new era of screening for HPV-related cancers will begin[137]. In populations with widespread vaccine coverage and, consequently, lower HPV prevalence, it may be practical to conduct initial pre-testing for HPV in order to identify individuals requiring cancer screening[137]. In this scenario, individuals who are HPV-negative might not require further screening whereas individuals who have high-risk HPV infection might be referred for screening or other monitoring.

There remains considerable disparity between populations with regards to uptake of HPV vaccination, and some groups at highest risk for anogenital cancers may be the least likely to be vaccinated against HPV[138]. In light of this disparity, it is reasonable to conclude that high-risk HPV infection will not be eradicated in the foreseeable future. Thus, the importance of investigating the acceptability of anal cancer screening remains high despite the potentially monumental impact of HPV vaccination on future anal cancer incidence. Similarly, it remains necessary to investigate the risk factors for anal HPV in order to ensure that individuals most at risk receive vaccination and/or appropriate screening.

2.2. COMPREHENSIVE LITERATURE REVIEWS

I conducted two comprehensive literature reviews to assess the scientific literature associated with the topics of my two manuscripts. Condensed versions of these literature reviews are presented in the two manuscripts, while more detailed literature reviews are presented here.

2.2.1. LITERATURE REVIEW FOR MANUSCRIPT 1: RISK FACTORS FOR ANAL HPV IN WLHIV

I conducted a literature search in Pubmed on November 28, 2015, to assess the state of knowledge of risk factors for prevalent anal HPV infection in WLHIV. The search strategy was constructed to locate articles addressing anal HPV infection *or* precancerous anal conditions in WLHIV, because articles about anal SILs were needed to understand the context of my topic. Also, articles about anal SILs often discussed one or more aspects of anal HPV infection, including prevalence and/or predictors, and I did not want to overlook articles that were highly relevant to my search. The search strategy is presented in Appendix A.

This search strategy located 405 original references. Of these, 274 had been published in the last 10 years. I did not exclude older articles, but placed the greatest emphasis on these 274 newer articles. I exported all references to Endnote X7. I read all article titles, beginning with the most recent, and flagged those that were relevant to prevalence of anal HPV or anal SILs in women, or risk factors for anal HPV in men or women. I included only published scientific journal articles that were written in, or translated into, English. I considered the most relevant titles those that mentioned risk factors or predictors for anal HPV or anal SILs. I also examined articles pertaining to anal HPV prevalence because many of these also discussed risk factors. I did not include articles focusing on anogenital condylomata, unless these also mentioned risk factors for anal HPV. I also employed a "snowballing"[139] technique, whereby I consulted relevant

publications that were cited by articles in the original list of results. Recent reviews by Moscicki et al.[140] and Stier et al.[141] were particularly helpful in this respect. In total, I found six articles that directly addressed my query regarding risk factors for anal HPV in WLHIV.

Prevalence of anal HPV in WLHIV

Compared to HIV-negative women, it is estimated that WLHIV face a 1.8 fold greater risk of anal HPV infection[142]. The high prevalence of anal high-risk HPV infection in WLHIV is also well documented, with cross-sectional studies reporting a prevalence between 16%[143] and 85%[144] in WLHIV compared to between 4%[145] and 17%[146] in HIV-negative women. It may be due to improved HPV detection methods, including the power of newer PCR methodology to detect a larger number of HPV types, that some recent studies show a higher prevalence of HPV than older studies [144]. The significantly higher HPV prevalence in WLHIV compared to HIV-negative women is not unique to adults; it has also been demonstrated in adolescent girls. Baseline results from one cohort study showed that anal HPV was twice as prevalent in girls ages 13-18 living with HIV than it was in their HIV-negative peers[147].

With occasional exceptions, such as some studies restricted to HIV-negative women with lower genital tract HPV lesions or abnormal cervical cytology[148, 149], research studies have tended to report a higher prevalence of anal HPV than cervical HPV among WLHIV as well as among HIV-negative women. As early as 1994, research teams posited that anal HPV infection and associated lesions were at least as common as their cervical equivalents in WLHIV[150]. In 2001, Palefsky et al. reported results from 200 women participating in the Women's Interagency HIV Study (WIHS). In this study, the prevalence of anal HPV and cervical HPV in WLHIV were 79% and 53%, and the corresponding prevalence of anal and cervical HPV in "high-risk" HIV-

negative women were 43% and 24%[142]. Subsequent studies that recruited women from within the WIHS, which is considered representative of the population of WLHIV in the United States, produced similar results[146, 151]. A prospective cohort study of HIV-negative women in Hawaii also found that anal HPV infection was slightly more common than cervical HPV infection[152].

High-risk HPV types also appear to be more common anally than cervically in WLHIV; one recent American study reported prevalences of high-risk anal and cervical HPV of 85% and 75%, respectively, in WLHIV[144]. However, this pattern may not be as consistent in HIV-negative women; Goodman et al. reported that high-risk HPV was more common in the cervix than in the anus of the 431 HIV-negative women in their cohort[152]. According to Hessol et al., discrepancies in HPV prevalence between the anus and the cervix indicate either that anal HPV infection is, indeed, more common than cervical infection, or that anal HPV is easier to detect due to higher levels of HPV in the anus than the cervix[146].

Prevalence of anal SILs in WLHIV

Research findings vary regarding the prevalence of abnormal anal cytology in WLHIV; however, studies in WLHIV have commonly reported a higher prevalence of abnormal anal cytology than studies in HIV-negative women[145, 146, 153, 154]. Efforts to estimate the prevalence of anal SILs in WLHIV are hindered by the fact that HRA has not been performed systematically in all participants. More often, anal cytology has been performed as a stand-alone procedure[155] or HRA has been done only in participants with abnormal anal cytology results[146, 153, 156-158].

An audit of paired cytology and histopathology outcomes published by Williams et al. in 2010 reported that anal cytology had a 96% sensitivity for detecting anal cellular abnormalities when compared with histopathological results[159]. Yet, most other studies have contradicted this finding. As Dietrich et al. remark, "anal cytology as a solitary screening tool for anal cancer fails to detect anal dysplasia in a considerable number of patients" [160]. In Dietrich et al's cohort of 123 PLHIV (88.6% men), histological results indicated that four (23.5%) of 17 participants with normal anal cytology had AIN 1 (equivalent to anal LSIL) and as many as five (29.4%) had AIN 2 or 3 (equivalent to anal HSIL). In a prospective study of anal SILs by Palefsky et al., the sensitivity of anal cytology for detecting biopsy-proven anal SILs at a single visit was 69% in HIV positive and 47% in HIV-negative MSM[161]. The majority of published studies of anal cytology in WLHIV to date[153, 156, 158, 162-164] have reported a total prevalence of abnormal anal cytology between 10.5% and 42%; however, given the apparently imperfect sensitivity of anal cytology, limiting HRA to participants with abnormal anal cytology produces estimates that are likely to be considerably lower than the true prevalence of SILs.

Risk factors for anal HPV in WLHIV

Few studies investigating risk factors for anal HPV infection have included WLHIV. Of those articles which have included other demographics (i.e. Heterosexual men and/or MSM) in addition to WLHIV, two have included fewer than 50 WLHIV and published only analyses pertaining to the overall sample[165, 166]. Others did not specify the HIV status of participating women[148] or assessed risk factors for abnormal anal cytology but HPV[167]. Considerable diversity of findings is present among the six remaining studies assessing risk factors for anal HPV in WLHIV. In the following subsections, these findings are presented and compared with risk factors for anal HPV previously identified in MSM and HIV-negative women.

Age

Data regarding a potential association between age and prevalent anal HPV in WLHIV are meager. To my knowledge, the only study examining this association in WLHIV was the WIHS-based cohort by Palefsky et al. mentioned above. In this study, younger age (<36 years) was identified as a statistically significant risk factor for anal HPV infection[142]. In variance with this finding, the prevalence of anal HPV infection in HIV-negative MSM does not appear to be dependent on age[168].

Race/ethnicity

While Moscicki et al's cohort study of adolescent WLHIV showed no association between race and anal HPV infection[147], in Palefsky et al.'s cohort of WLHIV from the WIHS study an association was found between being non-Hispanic white and detection of HPV DNA in anal specimens[142]. African American women in their study had a significantly lower risk of prevalent anal HPV infection compared to white women. In contrast, a 2015 systematic review of racial disparities in HPV-related diseases in MSM found that the prevalence of "group 2" high-risk anal HPV types (probable or possible carcinogens) was 1.3 times lower among white MSM than among black MSM[169].

Cigarette smoking

Findings are divided regarding the possible association of cigarette smoking with anal HPV infection in WLHIV. Palefsky et al. and Moscicki et al. both found no association[142, 147]. However, Kojic et al. found smoking to be associated both with having any prevalent anal HPV infection and with prevalent high-risk anal HPV infection. Furthermore, given that they did not

an observe an association between smoking and cervical HPV infection, it does not appear likely that this association was due to confounding by cervical HPV infection[144]. An association between smoking and anal HPV has also been observed in MSM. In a German cohort study by Wieland et al., the baseline prevalence of high-risk HPV DNA was significantly higher in smokers than in non-smokers[170].

Legal and illegal drugs

Although it is possible that prescription medication may influence HPV infection or clearance in WLHIV, thereby influencing the prevalence of HPV, there are minimal data regarding this question. Palefsky et al. found a significant association between anal HPV and ever having used the antiretroviral medication zidovudine, but no association with lifetime or current use of numerous other medications[142]. They also found no association with injection drug use or other use of illegal drugs, including marijuana and cocaine.

Number of vaginal or anal sex partners

In this literature search, no studies were found that commented on the association between number of vaginal or anal sex partners and anal HPV infection in WLHIV. However, both Castro et al. and Schlecht et al. found that both number of vaginal sex partners and number of anal sex partners were associated with anal HPV infection in HIV-negative women[171, 172].

Age at first intercourse

Moscicki et al. found no association of anal HPV prevalence with the age of initiation of vaginal or anal sexual activity in adolescent WLHIV. In young HIV-negative women, Schlecht et al. found an association between younger age at first intercourse and anal HPV infection[172].

Anal sex

Given the observed relationship between vaginal sex and cervical HPV infection in a large number of studies, it has often been presumed that a significant association is present between anal sex and anal HPV infection. This presumption is not entirely unfounded and explains, at least in part, the high rates of anal HPV infection in MSM. Anal sex has been identified as a risk factor for anal HPV among both HIV-positive and HIV-negative MSM[173]. However, anal sexual activity is by no means a necessary condition for anal HPV infection. Piketty et al. published results from a cross sectional study of 50 HIV-positive heterosexual male injection drug users and 67 HIV-positive MSM indicating a high prevalence of anal HPV in men living with HIV, including those who do not engage in receptive anal sex[174].

In addition to not being a necessary factor for anal HPV infection, a history of anal sex is not sufficient to predict the presence of anal HPV infection. In one small study in which only 4 women reported having had receptive anal sex in the previous year, all 4 of these women (2 of whom were WLHIV), had anal HPV infection. However, this was not a statistically meaningful association. Subsequently, much larger studies found no significant association between history of receptive anal intercourse and anal HPV infection in WLHIV[142, 144, 147, 162].

In contrast to these findings, anal sex has been identified as a risk factor for anal HPV infection in HIV-negative women in some studies. Notably, Castro et al. found that women who reported having a history of anal intercourse had nearly twice the prevalence of anal HPV infection (43.4% vs. 28.4%; $p < 0.001$), and anal infection with multiple HPV types (21.2% vs. 11.2%; $p < 0.001$) as women who did not report having ever had anal intercourse[171]. In an article reporting results from the same Hawaiian cohort study by Goodman et al. mentioned above, anal

intercourse was only associated with anal HPV in women who did not have cervical HPV[175]. In this same cohort, anal sex was also associated with an increased risk of incident anal HPV during follow-up, although it was not associated with an increased risk of acquisition of high-risk anal HPV[152].

CD4 count

A link between low CD4 count and higher prevalence of anogenital HPV infection, as well as between HPV infection and presence of HIV antibodies, was identified in MSM as early as the 1980s[176]. Recent studies in HIV-positive MSM have also identified low CD4 count as a predictor of anal oncogenic HPV infection[177, 178]. With some exceptions [150, 179], this inverse relationship has been observed in WLHIV. Palefsky et al observed an association with anal HPV for CD4 counts below 200 cells/μL [142] and Moscicki et al observed significant associations in a cohort study of 183 adolescent girls living with HIV for CD4 counts less than 200 cells/μL versus above 500 cells/μL, as well as CD4 counts in the range of 200-499 cells/μL versus above 500 cells/μL [147]. Despite the apparent significance of CD4 count, a relationship between anal HPV prevalence and high HIV viral load was not observed by Palefsky et al.[142].

Anogenital health

Palefsky et al. reported no association of anal HPV infection with history of sexually transmitted infections (including genital herpes) and, likewise, no association with history of anal health problems, such as hemorrhoids and fissures, in WLHIV[142]. In contrast, Schlecht et al. found that chlamydia was associated with anal HPV infection in cross-sectional analyses based on their cohort of 645 young HIV-negative women[172], and Castro et al. found an association between anal fissures and anal HPV infection among 2017 sexually active HIV-negative young women in

Costa Rica participating in an HPV vaccination trial[171]. An association between previous chlamydia infection and current anal oncogenic HPV infection has also been observed in HIV-positive MSM[178].

Genital/cervical HPV and genital condylomata

An association between cervical HPV infection and anal HPV infection has been observed in several studies. Palefsky et al. found that prevalent cervical HPV infection was associated with prevalent anal HPV infection in both univariable and multivariable analyses[142]. An association between cervical and anal *high-risk* HPV infection has likewise been observed in WLHIV[157]. Cervical HPV infection has also been shown to be associated with anal HPV infection in HIV-negative women[171, 175].

It is likely that associations between cervical and anal HPV infection can be explained by the fact that similar modes of acquisition, notably sexual contact, can lead to simultaneous HPV acquisition at different anatomical sites. In addition, anal HPV in WLHIV may be acquired from cervical or vaginal fluids via autoinoculation. There is already research suggesting that autoinoculation between anatomical sites occurs in HIV-negative women. As Goodman et al. explain regarding consecutive cervical and anal HPV infections, high concordance of HPV genotypes in the cervix and anus suggests that these areas "may serve as reservoirs for HPV infection at the other anatomical site"[180]. Goodman et al. also found that infection with multiple types of HPV in the cervix further increased the risk of anal HPV infection[181].

The potential for simultaneous acquisition at more than one anatomical site, and for autoinoculation, is also reflected in the finding that genital condylomata are a significant

predictor of anal HPV infection. A multivariable logistic regression model reported by Moscicki et al. indicated that prevalent genital warts were significantly associated with anal HPV infection in a cohort of 183 adolescent girls. This same analysis also indicated that anal warts were associated with anal HPV infection, although this finding is expected given knowledge that anal HPV infection is a causal factor in development of anal warts[147]. Genital warts have also been associated with anal HPV infection in young HIV-negative women[172].

2.2.2. LITERATURE REVIEW FOR MANUSCRIPT 2: ACCEPTABILITY OF SCREENING FOR ANAL CANCER IN MEN AND WOMEN

I conducted a comprehensive narrative literature search in Embase Current (via the Ovid platform) and Pubmed on December 20, 2015 to assess the state of knowledge of the acceptability of screening for anal HPV, HSIL/AIN, and anal cancer in WLHIV. The search strategy was developed with the aim of including articles about acceptability of anal cancer screening in men or women, because I did not expect a large number of sources discussing only women. After duplicate references were removed, 141 original results remained. Of these, 9 were relevant to anal cancer screening acceptability in HIV-positive or HIV-negative women or men as defined at the top of page 53 of this thesis. The two search strategies used to investigate this topic, one in Embase Current (via Ovid) and one in Pubmed, are presented in Appendix A.

Understanding the extent to which medical procedures are acceptable to patients, as well as the factors affecting this acceptability, is widely recognized as important for ensuring optimal patient care. Knowledge of the acceptability of screening tools can inform efforts to optimize patients' experiences of screening and mitigate potential harms. Acceptability has been investigated for many screening and testing services, including testing for sexually transmitted infections such as syphilis[182] and cervical HPV[183], and screening for diseases such as colorectal cancer[184].

Some studies have addressed the question of acceptance, in terms of the proportion of individuals who agree to be screened with a given procedure[185, 186], at times using the term acceptability to refer to this concept[187-189]. This concept relates to uptake of screening, willingness to be screened, or openness to the idea of being screened, all of which are distinct from the concept of acceptability as it is defined for the purposes of this literature review. Here,

acceptability is defined as the extent to which women who have already experienced the screening procedures say they would describe the procedure as "acceptable" as part of routine care for WLHIV at various proposed frequencies. Anal cancer screening is understood as encompassing screening for anal HPV, anal SILs, and invasive anal cancer.

Acceptability of Anal Cancer Screening

Like cervical cancer screening, anal cancer screening poses unique challenges because it involves the complexities of both cancer screening and sexually transmitted infection screening. Yet, because anal cancer screening is a relatively new area of research, there is a paucity of literature on its acceptability. The limited research that has been conducted on this topic has centered on men.

Overall, studies of anal cancer screening acceptability in men (primarily HIV-positive MSM) have reported a high level of acceptability for anal pap smears, DARE (performed by self or physician), and HRA with or without biopsy[190-195]. However, this literature search found scant sources addressing anal cancer screening acceptability in women, and some researchers reported significant difficulty recruiting WLHIV. In one striking example, 164 WLHIV were screened for participation in an HRA screening study and only two consented to participate[196]. Only one article was found that reported results pertaining to acceptability among women of screening procedures performed by physicians. Of the 28 women included in this study, only 4 were WLHIV[197]. A cross-sectional study by Ortiz et al. reported a high level of acceptability of anal HPV self-sampling in a group of 100 women; however, all participating women were HIV-negative[198]. This research team also highlighted an important difference between screening acceptability in men and women: The level of acceptability of clinician-collected

sampling in women was higher than that of self-collected sampling, in contrast to studies in MSM in which participants preferred self-sampling to clinician-collected samples[199, 200].

The level of acceptability of anal cancer screening in WLHIV may be affected by the fact that a high proportion of women in this population are survivors of sexual assault or face emotional and mental health problems[201, 202]. In a report from a survey conducted by Battaglia et al., the barriers to anal Pap testing among WLHIV were fear of pain or sexual assault flashbacks[203] and lack of social support[157]. Women with higher levels of past trauma were more likely to believe that sexual assault flashbacks could occur as a result of anal Pap testing[203]. In addition, fear and symptoms of depression have been cited by WLHIV as barriers to accessing cervical cancer screening[204]. Rosa Cunha et al. note that these barriers may also affect the extent to which WLHIV attend anoscopy appointments[193].

Supplementary Search: Psychosocial Consequences of Anal and Cervical Cancer Screening

Even when screening procedures are considered acceptable to patients, there is a potential for adverse psychosocial consequences to arise from screening. Moreover, potential psychosocial consequences of screening may be compounded by socioeconomic and cultural factors[205]. Psychosocial risks are under study within screening programs for numerous diseases, including breast cancer, colorectal cancer, and cervical cancer [206-209]. Given the many similarities between cervical and anal cancer, and the fact that these similarities have served as a motivating factor for anal cancer screening initiatives and research, research investigating psychosocial effects of cervical cancer screening may shed light on potential psychosocial consequences of anal cancer screening. For this reason, I conducted a supplementary search for articles discussing psychosocial consequences of either anal or cervical cancer screening.

With this supplementary search, I did not find any articles that directly addressed psychosocial effects of anal cancer screening in women. However, a 2012 literature review by Landstra et al. examined 7 articles about the psychosocial consequences of anal cancer screening in MSM [210]. While screening did not appear to pose a major threat to overall mental health, it caused an increase in "cancer-specific worry", especially in participants who were younger, were already somewhat distressed at baseline, or had concerning test results [210]. In February 2013, Landstra published a doctoral thesis on the psychosocial effects of anal cancer screening in MSM. Landstra's research found no evidence that screening for anal cancer in HIV-positive MSM had an effect on general anxiety, depression, or quality of life[211].

With respect to cervical cancer screening, multiple studies have investigated the psychological impact using scales and psychometric testing [212-217]. Such studies tended to indicate a lack of knowledge about the purpose and nature of the test, and greater negative psychosocial outcomes in the women with the greatest lack of knowledge[215]. Moreover, for certain participants, even supplementary information might not lessen anxiety; one study found that some participants adhered to certain erroneous beliefs even after receiving accurate information [218]. This raises the question of whether changes in the mode of delivery of information might affect whether and how it is understood and absorbed by participants. Indeed, in-depth qualitative interviews conducted by Waller et al. in 2005 suggested that the way in which information was presented to patients was paramount in preventing psychosocial harms related to screening [219].

One study found no negative consequences of cervical cancer screening on quality of life when results of screening were normal, despite a barrage of unpleasant effects experienced by the

participants. However, this study may have missed important nuance and detail related to the experiences of women, as it employed scoring measures that were designed to pinpoint only serious psychological difficulties [220]. All other studies reviewed indicated some adverse psychosocial consequences to screening for cervical HPV infection, cervical precancer, and cervical cancer in women [216, 221-224].

Although screening programs for cervical cancer have been highly effective, there have been numerous adjustments along the way. These adjustments have included integrating greater consideration for the psychosocial aspects of patients' lives, and integrating patient perspectives into guidelines for the provision of health care[225]. There is no doubt that the development of anal cancer screening strategies will be an iterative process as well, requiring attention to patients' perfectives for guidance and understanding.

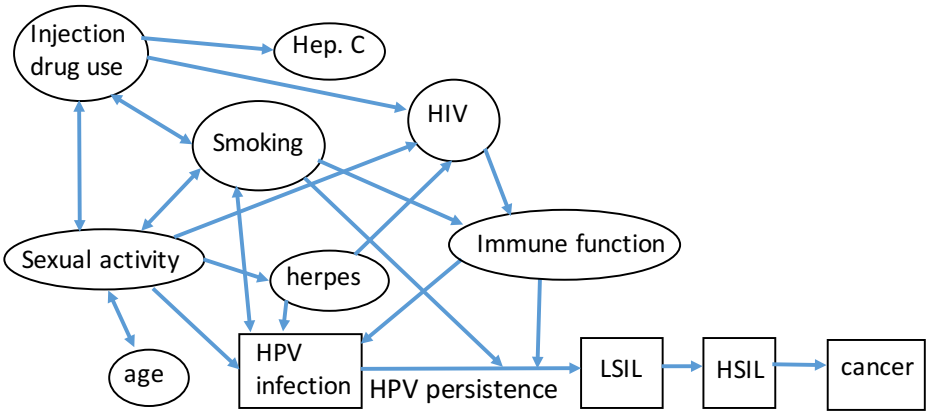
CHAPTER 3: MANUSCRIPT 1

3.1. PREFACE TO THE FIRST MANUSCRIPT

The impetus behind the first manuscript (to be submitted for publication in 2016) was the lack of published data regarding risk factors for anal HPV infection in WLHIV. Anal HPV 16 infection is the most important cause of anal cancer in WLHIV, and its risk factors are poorly understood. Knowledge of risk factors is needed to inform anal cancer screening and prevention strategies and determine which WLHIV are most likely to acquire anal HPV 16. These women are an important target group for HPV vaccination and are likely to be among those considered top priority if anal cancer screening becomes standard of care in WLHIV.

I considered potential causal and correlational relationships, confounders, and effect modifiers in detail (Schema 1, below), and subsequently tested these using logistic regression. I sought to identify risk factors at the first step of the causal pathway depicted below, when HPV infects healthy anal squamous epithelial cells and persists at a detectable level. As shown, associations of lifestyle factors, such as injection drug use, may be due to confounding by more biologically plausible causes, such as immune function and sexual activity.

Schema 1. Causal diagram of potential factors involved in anal cancer [viii]



[viii] Unidirectional arrows: expected causation; bidirectional arrows: expected association

3.2. LIST OF CO-AUTHORS

Identifying risk factors for prevalent anal human papillomavirus type 16 infection in women living with HIV

E. Kaufman[1], C. de Castro[2], T. Williamson[3], M. Munoz[1,2,4], B. Lessard[1,2,5], M.-H. Mayrand[6], L. Charest[4], M. Auger[7], V. Marcus[7], A. Burchell[8], M. Klein[2], F. Coutlée[2,9], A. de Pokomandy[1,2]

1. McGill University, Faculty of Medicine, Family Medicine Department, Montreal, Canada
2. McGill University Health Centre (MUHC), Chronic Viral Illness Service, Montreal, Canada
3. University of Calgary, Department of Community Health Sciences, Calgary, Canada
4. Clinique Médicale l'Actuel, Montreal, Canada
5. Clinique Médicale du Quartier Latin, Montreal, Canada
6. Centre Hospitalier de l'Université de Montréal (CHUM), Department of Obstetrics & Gynaecology, Montreal, Canada
7. McGill University Health Centre (MUHC), Pathology Department, Montreal, Canada
8. Ontario HIV Treatment Network, Toronto, Canada
9. Centre Hospitalier de l'Université de Montréal (CHUM), Department of Microbiology & Infectious Diseases, Montreal, Canada

3.3. ABSTRACT

Background

Women living with HIV (WLHIV) face a high risk of anal cancer. Identifying risk factors for anal oncogenic HPV infection, the most significant risk factor for anal cancer, is essential for prevention and screening strategies. The oncogenic potential of HPV 16 is an order of magnitude greater than other oncogenic HPV types.

Methods

To identify risk factors for prevalent anal HPV 16 infection in WLHIV, baseline data from a cohort of 151 WLHIV (the EVVA study) were analyzed using univariable and multivariable logistic regression. HPV testing samples were collected using cervical and anal swabs. Demographic and clinical data were collected via questionnaires and chart reviews.

Results

Among 150 women with adequate HPV test results, HPV 16 DNA was detected anally in 23 (15.3%; 95%CI:10.4-22.1) and cervically in 5 (3.3%; 95%CI:1.4-7.8). In crude analyses, significant predictors of anal HPV 16 included current smoking (OR=4.2; 95%CI:1.6-11.1; p=0.004), ever smoking (OR=3.4; 95%CI:1.4-8.5; p=0.008), lifetime number of vaginal sex partners >10 (vs. ≤10) (OR=4.1; 95%CI:1.6-11.0; p=0.005) or anal sex partners >1 (vs. 0 or 1) (OR=5.1; 95%CI:1.7-15.3; p=0.004), past anogenital herpes (OR=3.9; 95%CI:1.6-10.0; p=0.004), current CD4 ≤ 200 cells/μL (6.6; 95%CI:1.7-25.1; p=0.006), nadir CD4 ≤ 200 cells/μL (5.2; 95%CI:1.8-14.9; p=0.002), and prevalent cervical HPV 16 (26.5; 95%CI:2.8-250.1; p=0.004). In the final multivariable model, current smoking (OR=5.1; 95%CI:1.4-18.9; p=0.014), nadir CD4 count ≤ 200 cells/μL (OR=5.9; 95%CI:1.6-21.2; p=0.007), and prevalent

cervical HPV 16 (OR=27.7; 95%CI:2.3-326.7; p=0.008) were significant, and effect modification was observed between total number of anal sex partners and past anogenital herpes (OR for interaction=0.045; 95%CI:0.003-0.773; p=0.033). In women with no history of anogenital herpes, having >1 anal sex partners over lifetime was significantly associated with prevalent anal HPV 16 infection (OR=10.1; 95%CI:1.9-53.3; p=0.006).

Conclusions

These findings add important knowledge of the risk factors for anal HPV 16 to the scant literature regarding anal HPV infection in WLHIV. Knowledge of risk factors can help identify women at greatest risk of anal HPV 16 infection. These women are most likely to benefit from screening, through either anal HPV testing or cytology.

3.4. MANUSCRIPT 1: IDENTIFYING RISK FACTORS FOR PREVALENT ANAL HUMAN PAPILLOMAVIRUS TYPE 16 INFECTION IN WOMEN LIVING WTIH HIV

Among women living with HIV (WLHIV), the incidence of anal cancer is 24 times greater than in the general population[33]. The incidence of anal cancer in HIV positive men who have sex with men (MSM) is even higher: 78-fold greater than in the general population[20]. Most anal cancer research to date has focused on HIV-positive MSM due to the extremely high incidence in this group. Yet, there is also a need for research investigating anal cancer and related conditions in WLHIV.

Like cervical cancer, anal cancer occurs when infections with high-risk types of human papillomavirus (HPV) persist to cause squamous intraepithelial lesions (SILs). Akin to cervical high-grade SILs (HSILs), the precursors to invasive cervical cancer, individual anal HSILs can progress and become malignant[50]. Although they do not always progress[95], and may spontaneously regress in some cases[93], anal HSILs are considered the immediate precursors to invasive squamous cell carcinoma of the anus. Oncogenic ("high-risk") human papillomavirus (HPV) infection of the anus is the most significant risk factor for anal cancer[46].

Compared to other high-risk HPV types, the cancer risk associated with HPV type 16 is an order of magnitude higher[67]. Based on prevalence data from over 30,000 invasive cervical cancers, Li et al. estimated that the worldwide prevalence of HPV-16 in cervical cancer is 57%. They estimated that the prevalence in cervical cancers of the second most common type, HPV 18, was only 16%[226]. HPV-16 is also associated with more than 50% of all oropharyngeal cancers[69], as well as a significant proportion of penile, vaginal and vulvar cancers[65]. This high level of

oncogenicity is even more notable with respect to invasive anal cancer, with HPV-16 associated with approximately 75% of cases[53, 66].

Understanding risk factors, from initial anal HPV infection to invasive anal cancer, is essential to the development of prevention and screening strategies. Risk factors for HPV may differ between men and women living with HIV in Canada, due to sociocultural and anatomical differences. Knowledge of risk factors in WLHIV for anal HPV, including HPV 16, is scant.

Cigarette smoking in one study was associated with prevalent anal HPV infection, including with high-risk types, in WLHIV[144], but two other studies found no association[142, 147]. Low CD4 count and younger age have been identified as risk factors for anal HPV in WLHIV[142, 147]. Although an association between anal sex and anal HPV infection has been observed in MSM[173], as well as in HIV-negative women[171], multiple studies in WLHIV have identified no such association[142, 144, 147, 162]. Associations have been observed between younger age at first vaginal intercourse[172], as well as number of vaginal or anal sex partners[171, 172] and prevalent anal HPV infection in young HIV-negative women, but not in WLHIV[147].

To increase our understanding of the risk factors for prevalent anal HPV 16 infection, we analyzed baseline data from a cohort of WLHIV.

Methods

Study design and population

The EVVA study ("Evaluation of HIV, HPV, and AIN in Women") was an epidemiological prospective observational cohort study, which followed 151 WLHIV attending study visits every

6 months for 2 years. The overall aim of the EVVA study was to investigate anal cancer screening and the natural history of both anal HPV infection and anal SILs in WLHIV. The aim of the analyses conducted for this manuscript was to identify risk factors for prevalent anal HPV 16 infection among WLHIV who participated in the EVVA cohort study. The findings presented here are based exclusively on analyses of baseline EVVA study results. Participants were recruited during routine HIV care at four HIV clinics in Montreal, Canada. Participants were women age 18 or older with confirmed positive serology for HIV, who had a cervix, were not pregnant at recruitment, and had never been diagnosed with anal cancer. To be eligible, participants were required to possess sufficient knowledge of English or French to provide informed consent and understand the questionnaire. All study materials were available to participants in both French and English, and assistance was available for participants for whom literacy or language level posed a barrier to understanding the study materials. All participants gave informed written consent. Ethics approval was obtained from the McGill University Health Centre Research Ethics Board/Institutional Review Board.

HPV testing,

HPV testing was performed by a registered nurse. Cervical cell samples were collected by inserting and rotating a cytobrush in the cervical os through a vaginal speculum. For anal cell samples, a saline-moistened Dacron swab was inserted 3-5 cm into the anal canal and rotated upon removal to collect epithelial cells.

Analysis of cervical and anal samples for HPV testing

The cervical and anal epithelial cell samples from cervical and anal swabs were agitated in 1.5 mL of PreservCyt (Hologic), a methanol-based transport medium used to preserve the integrity

of the cells, and stored at 4°C. At the time of analysis, cell suspensions were centrifuged at 13,000 *xg* for 15 minutes at 22 °C. Pellets obtained were later re-suspended in 300 μL of 22 mml/L Tris buffer at pH=8.3 and DNA was purified using a Master Pure Kit (Epicentre)[227]. Extracted DNA was subsequently tested using primers PC04 and GH20, which target a fragment of β-globin. Samples determined to be β-globin-positive were then amplified with the L1 consensus HPV PGMY09/PGMY11 primer set and screened with a generic probe mix with a sensitivity of 99.3% (95% CI 97.7-99.8) and specificity of 64.2 (95% CI 59.3-68.8) [228] to minimize the probability of overlooking samples positive for HPV. To confirm positivity and determine the HPV genotype(s) present in each sample, specimens positive for HPV DNA according to the generic probe mix were subsequently tested with the reverse Linear Array detection system. Given the known cross-reactivity of HPV 52 with HPV 33, 35, and 58, samples positive for HPV 52 on Linear Array were re-tested with a validated PCR assay for HPV type 52[229].

Questionnaires and chart reviews

Data regarding putative risk factors for anal HPV-16 infection were collected from participant questionnaires and chart review forms. Questionnaires were administered in clinic by the study coordinator, or self-administered if preferred, and included questions about sexual practices, medical history pertaining to HIV, hepatitis, HPV-related disease, HPV vaccination, anal health, sexually transmitted infections, injection drug use, cigarette smoking, and socio-demographic characteristics. Chart review forms were completed based on electronic and paper patient charts by the study coordinator or research assistant and examined clinical factors including history of sexually transmitted infections, HPV vaccination, nadir CD4 count, current CD4 count, and current HIV viral load. Some questions, such as those regarding sexually transmitted infections,

were addressed in both questionnaires and chart review forms. Combined formats of these variables, defined as "yes" if either source of information was affirmative, were used in the analyses.

Statistical Analysis

Descriptive and inferential statistics were used to describe demographic and clinical characteristics. Some clinical markers, including CD4 count and HIV viral load, displayed nonlinear relationships with anal HPV 16 infection and were, therefore, dichotomized or categorized according to clinically relevant cutoffs. Age was analyzed as a continuous linear variable, as there was no evidence of nonlinearity of the effect of this predictor. Univariable (binary) logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for putative predictors of the primary outcome, prevalent anal HPV 16 infection. The analyses were restricted to HPV 16 due to the higher prevalence and oncogenic potential of this HPV type compared to other types. For perfect predictors, chi-squared p-values were used to assess the difference between groups. Putative risk factors that were statistically significant in univariable analyses and clinically plausible were included in the preliminary multivariable logistic regression model. Variables that were considered a result of HPV (i.e. Condylomata or SILs), or unlikely to be a direct cause of initial HPV infection or persistence (i.e. Active hepatitis C infection), were excluded from multivariable analyses. Potential correlations were assessed using the Spearman correlation coefficient (Spearman's ρ) and corresponding p values. A final parsimonious multivariable model was elicited via manual selection. A backward selection approach was used to minimize the potential for omission of important predictors, given the paucity of knowledge regarding risk factors for anal HPV 16 infection in WLHIV and the understanding that relationships between predictors and outcomes may be complex. Variables

identified as significant in the final model were examined for evidence of effect modification. A statistical cutoff of $p<0.05$ at the univariable analysis stage was used as a criterion for inclusion of predictors in the preliminary multivariable model. This relatively conservative criterion was adopted due to the high number of statistically significant predictors that, while correlated with the outcome as possible markers for other factors, were not considered clinically or biologically plausible as causes of anal HPV 16 infection.

Missing values for questionnaire variables were assumed to be missing not at random, due to the sensitive nature of many topics. Some missing values may have depended upon unmeasured variables. This mechanism of missingness could not be modelled. Chart review variables such as active hepatitis C infection were more likely to be missing completely at random and therefore theoretically replaceable through multiple imputation or mean substitution; however, a slight loss of power was not a concern for the univariable analyses and no values were missing for chart review variables in multivariable analyses. Listwise deletion and complete case analysis were therefore conducted. All statistical tests were two-sided and considered significant at $p<0.05$. Analyses were performed using the statistical analysis software Stata/IC 14.1 for Mac (64-bit Intel).

Results

Baseline characteristics of the 151 WLHIV participating in EVVA are presented in Table 1. Of 151 participants, the analysis included only the 150 women from whom adequate anal HPV testing samples had been obtained. The mean age was 45.2 years. HPV 16 DNA was detected anally in 23 (15.3%; 95%CI:10.4-22.1) and cervically in 5 (3.3%; 95%CI:1.4-7.8) women. The vast majority (96%) stated a sexual preference for men. Approximately one quarter (23%) were

born in Canada, 44% in an African country, and 28% in the Caribbean. Only 2 women (1%) born in Canada self-identified as Aboriginal. Most women (78%) had an undetectable HIV viral load (≤39 copies/mL).

Univariable logistic regression

Findings from univariable logistic regression analyses are presented in Table 2. Having had a total number of vaginal sex partners in one's lifetime >10 (vs. ≤10) was associated with a four times greater odds of prevalent anal HPV 16 (OR=4.1; 95% CI 1.6-11.0; p=0.005). A history of anal sex (ever) was not associated with the odds of prevalent anal HPV 16 infection (OR=1.2; 95% CI: 0.5-3.3; p=0.657). Among the 40 women who reported a history of anal sex, the number of lifetime anal sex partners ranged from 1 to 20. None of the 23 women with only 1 lifetime anal sex partner had prevalent anal HPV 16 (0%; 95% CI: 0-15%), compared to 7 of the 17 women who had 2 or more anal sex partners (41%; 95% CI: 18-67%) (p value for difference = 0.001). Condom use during anal sex was not significantly protective against anal HPV 16.

The crude effect of past anogenital herpes infection was a nearly 4-fold increase in the odds of anal HPV 16 infection (OR=3.9; 95% CI: 1.6-10.0; p=0.004). Active hepatitis C infection was identified as another significant risk factor (OR=12.4; 95% CI: 4.0-38.2; p<0.001). Concurrent prevalent cervical HPV 16 was associated with a 26.5-fold increase in the odds of anal HPV 16 (95% CI: 2.8-250.1; p=0.004).

Baseline (current) CD4 count and nadir CD4 count both showed significant crude associations with anal HPV 16, particularly when dichotomized as CD4 count ≤200 cells/μL (OR=6.6; 95%

CI: 1.7-25.1; p=0.006) and nadir CD4 count ≤200 cells/μL (OR=5.2; 95% CI: 1.8-14.9; p=0.002).

Additional variables not found to have significant crude associations with anal HPV 16 infection in univariable analyses (p>0.05) included: age at baseline, marital status, monogamy, age at first anal sex, condom use during last anal sex, number of days since last anal sex, number of days since last vaginal sex, lifetime frequency of vaginal sex, frequency of vaginal sex in last 6 months, HIV viral load, and history of: gonorrhea, chlamydia, yeast infections, hemorrhoids, anal bleeding, or anal discharge (data not shown)

Multivariable logistic regression

Several variables that were initially significant in crude analyses were not significant after adjustment for lifetime number of vaginal sex partners (>10 vs. ≤10). These included being born in Canada, age at first vaginal sex (<17 vs. ≥ 17 years), lifetime frequency of anal sex, exchanging sex for goods or money, history of sexual abuse involving penile-anal or penile-vaginal penetration, ever smoking, and the total lifetime number of pack-years smoked (defined as the number of packs of cigarettes smoked per day multiplied by the number of years the participant smoked). Ever having used injection drugs was not significant (OR=1.7; 95% CI: 0.3-9.7; p=0.555) after accounting for sex-related variables. In contrast, the effect of past anogenital herpes infection and concurrent cervical HPV 16 infection remained significant after adjustment for lifetime number of vaginal sex partners. The effect of active hepatitis C infection also remained significant after accounting for numerous potential confounders, including intravenous drug use and number of sexual partners.

In the final age-adjusted multivariable model, current smoking (OR=5.1; 95% CI: 1.4-18.9; p=0.014, nadir CD4 count ≤ 200 cells/μL (OR=5.9; 95% CI: 1.6-21.2; p=0.007), and prevalent cervical HPV 16 infection (OR=27.7; 95% CI: 2.3-326.7; p=0.008) were significantly associated with the odds of prevalent anal HPV 16 infection and a statistically significant effect modification was observed between past anogenital herpes and total number of anal sex partners >1 (OR for interaction = 0.045; 95% CI: 0.003-0.773 p=0.003). In women without a history of anogenital herpes, lifetime number of anal sex partners >1 was associated with a 10 fold greater odds of anal HPV 16 (OR=10.1 95% CI: 1.9-53.5; p=0.006). By symmetry, past anogenital herpes was significantly associated with anal HPV 16 in women who did not have multiple past anal sex partners (OR=6.6; 95% CI: 1.8-23.9; p=0.004). Among women for whom one of these two factors was present, the added effect of the other factor was not statistically significant. The Hosmer-Lemeshow goodness of fit test did not provide significant evidence of poor fit for the final model (Hosmer-Lemeshow chi-square statistic=4.80; p=0.78).

Discussion/Conclusion

These analyses based on the EVVA cohort contribute to the scant literature on risk factors for anal HPV infection in WLHIV. To our knowledge, this is the first study to report on risk factors specific to anal HPV 16 in this population, although others have reported on anal infection with high-risk HPV.

The crude effect observed among women born in Canada compared to women born in Africa and the Caribbean parallels previous findings of reduced risk in African American women compared with white women[142]; loss of effect upon adjustment indicates confounding by number of vaginal sex partners. Unlike some reports of risk factors for anal HPV [142] and cervical HPV in

larger cohorts of WLHIV in the United States and Brazil[230], we did not observe a significant effect of age.

The significance of current smoking in the multivariable model adds to ongoing discussions of the potential link between smoking and anal HPV, for which some studies in WLHIV and MSM have found no evidence [142, 147, 178] and one study in WLHIV has observed an association[144].

The total lifetime number of vaginal sex partners and age at first vaginal sex were not significant in adjusted analyses, as previously observed in adolescent WLHIV[147] and in contrast to the findings of some studies in HIV negative-women, in which total numbers of vaginal and anal sex partners were significant[171, 172]. Like other studies in WLHIV[142, 144, 147, 162], history of anal sex (ever vs. never) was not associated with an increased odds of anal HPV 16 in our dataset. This contrasts with some studies in MSM [173] and HIV-negative women[171]. It was not possible to examine the effect of the frequency of recent anal sex (past 6 months), as only 1 woman with anal HPV 16 reported any recent anal sex.

In contrast to previous publications [142], we observed an effect of anogenital herpes in both crude and adjusted analyses. Given the small sample size of our study, it is unlikely that our finding of an effect for herpes is due to type 1 error; rather, this difference between study findings may be due to variations in herpes prevalence and other clinical and demographic characteristics between study populations. Herpes may increase the odds of anal HPV 16 infection by causing mucosal ulcers and providing a potential entry point for HPV, increasing the odds of both direct anal HPV infection and autoinoculation from other anogenital sites. Herpes

may also be more prevalent in women with higher levels of past exposure to anogenital HPV, and could hence be considered a proxy variable for past exposure to sexually transmitted skin infections. The statistical effect modification between multiple lifetime anal sex partners and herpes suggests that the effect of multiple lifetime anal sex partners on the risk of anal HPV-16 becomes less meaningful for women with a history of herpes because herpes drives most of the effect. If the association of herpes with anal HPV is due to an indirect link, whereby herpes increases the risk of cervical and other anogenital HPV which is then autoinoculated to the anus, then our finding that herpes modifies the effect of having >1 anal sex partners parallels a Hawaiian cohort study in HIV-negative women, in which anal sex was only associated with anal HPV infection in women who did not have cervical HPV[175].

The strong effect of cervical HPV 16 infection is expected given the same mode of acquisition, proximity of the two anatomical sites, and potential for autoinoculation. The consistently higher prevalence of HPV in the anus than in the cervix across studies in women may be due to increased clearance at the cervix or a greater propensity for anal HPV to be detected due to the greater area swabbed during anal sampling.

The absence of crude associations for marital status, monogamy, history of gonorrhoea, chlamydia, yeast infections, hemorrhoids, or anal bleeding is similar to the findings from Palefsky et al.[142]. The anal HPV 16 risk associated with sex work, abuse, and hepatitis C infection in crude analyses is likely to be due to confounding by unmeasured differences in socioeconomic status, lifestyle or sexual practices.

PLHIV with lower CD4 counts, including young WLHIV, have been shown to have a higher prevalence of anogenital HPV infection[82, 142, 147]. Our results confirm this crude association for anal HPV 16 in WLHIV ages 19-67, but raise the possibility of confounding by nadir CD4 count. The identification of nadir CD4 count as a potential risk factor for anal HPV 16 is important, given the well-documented effect of nadir CD4 count on long-term health in PLHIV. An association between nadir CD4 count (<100 cells/μL) and cervical HPV infection has previously been identified in WLHIV[230].

This study has certain limitations. Some important risk factor effects might not have reached statistical significance due to type 2 error, and bias may have been introduced into the sample due to differential participation. For example, the low prevalence of certain variables (such as intravenous drug use) in our sample may have been due to reticence to participate among women who have experienced marginalization, and, due to the low prevalence of these variables, it was difficult to assess their predictive value. It is also possible that the prevalence of HPV in our sample was slightly amplified, particularly if women with histories of HPV-related issues were more inclined to participate. However, this potential source of bias may have been balanced by the low number of participants who use intravenous drugs or who have certain other characteristics that were found to be strongly (though not necessarily causally) associated with anal HPV 16 infection. The EVVA cohort study was designed with awareness of the need to mitigate such bias caused by differential participation; the biannual visit schedule was intended to be suitable for participants' busy schedules, and compensation was provided for their time and participation expenses. However, this compensation was modest, barely covering the cost of parking or childcare if needed. An additional limitation concerns the cross-sectional nature of the analyses; observed associations may not be causal, although some variables identified are

biologically plausible risk factors, and causality cannot be inferred directly from these correlational associations. Although our sample is representative of the population of WLHIV in Québec, the generalizability of our findings to other populations may be limited, especially due to the low number of Aboriginal women and women who have injected drugs.

In summary, our cross-sectional analyses confirm the high prevalence of anal HPV 16 in WLHIV and identify several clinically plausible risk factors including current smoking, nadir CD4 count ≤200, and concurrent cervical HPV 16 infection. Our analyses also found that history of anogenital herpes modified the effect of having more than 1 anal sex partner on prevalent anal HPV 16 infection. Given the scarcity of studies addressing risk factors for prevalent anal HPV infection in WLHIV and, to our knowledge, the absence of literature specific to anal HPV 16 in this population, our findings are of great importance to anal cancer prevention efforts. Knowledge of risk factors for prevalent anal HPV 16 infection can help identify women at greatest risk of infection. These women are most likely to benefit from screening, through either anal HPV testing or cytology. Research would benefit from investigation of the risk factors for other high-risk HPV types, especially types that are highly prevalent among WLHIV.

As the effects of HPV vaccination intensify in the coming years, it will be necessary to evaluate the evolving risk factors for prevalent and incident HPV infections. Women immigrating to Canada from countries without HPV vaccination programs may continue to face the same risk factors for HPV as in previous decades. However, the risk profile for anal HPV 16, and for all HPV types at all body sites, may change for women born in Canada and other countries with HPV vaccination coverage. The prevalence of HPV types included in the vaccines is also expected to decrease among these women. This change will most likely be accompanied by an

increased focus on monitoring risk factors for high-risk HPV types not included in current vaccines, and a need to compare risk factors for vaccine and non-vaccine HPV types.

3.5. TABLES AND FIGURES

Table 1. Baseline characteristics of 150 women living with HIV, by anal HPV 16 status

Participant characteristic	**N (%) (Total n=151)**	
	Anal HPV 16+ (n=23)	**Anal HPV 16- (n=127*)**
Age group (Years)		
19-39	6 (26.1)	33 (26.0)
40-49	10 (43.5)	55 (43.3)
50-69	7 (30.4)	39 (30.7)
Place of birth		
Canada	11 (47.8)	24 (18.9)
Africa	8 (34.8)	58 (45.7)
Caribbean	3 (13.0)	40 (31.5)
Other	1 (4.4)	5 (3.9)
Education completed		
Less than high school	3 (13.0)	24 (18.9)
High school	16 (69.6)	54 (42.5)
College, or university	4 (17.4)	49 (38.6)
Smoking		
Pack-years, past smokers (mean, range)	31.4 (2-50)	9.8 (0.1-37.5)
Pack-years, current smokers (mean, range)	30.2 (1.2-105)	18.2 (2-65)
Never	10 (43.5)	92 (72.4)
Current	9 (39.1)	17 (13.4)
Past	4 (17.4)	18 (14.2)
Years since quit smoking (mean, range)	5.5 (0.5-16)	12.1 (1-31)
Intravenous drug use (Ever)	8 (34.8)	7 (5.5) **(n=126)**
Sexual activity		
>10 lifetime vaginal sex partners	9 (39.1)	17 (13.4) **(n=126)**
Age at first vaginal intercourse ≥17 years	10 (43.5)	91 (71.7) **(n=125)**
Ever had anal sex	7 (30.4)	33 (26.0)
>1 lifetime anal sex partners (vs. 0 or 1)	7 (30.4)	10 (7.9)
Average frequency (times per year) of anal sex during sexually active life, for women who have had anal sex (mean, range)	31.9 (0.5-104)	8.2 (0.5-52)
Condom use at last anal sex	1 (4.4) **(n=6)**	17 (13.4) **(n=33)**
Past sexual abuse/assault (Yes, any)	15 (65.2)	53 (41.7) **(n=123)**
Past vaginal sexual abuse (vs. no sexual abuse)	13 (56.5) **(n=21)**	38 (29.9) **(n=108)**
Past anal sexual abuse (vs. no sexual abuse)	3 (13.0) **(n=11)**	4 (3.2) **(n=74)**
Gonorrhoea ever	3 (13.0)	9 (7.1)
Chlamydia ever	5 (21.7)	11 (8.7)
Anogenital herpes ever	11 (47.8)	24 (18.9)
Active hepatitis C infection	10 (43.5)	7 (5.5) **(n=120)**

CD4 cell count ≤200 (cells/μL of blood)	5 (21.7)	5 (3.9) **(n=124)**
Nadir CD4 cell count ≤200 (cells/μL of blood)	18 (78.3)	52 (40.9)
HIV viral load (RNA copies/mL of plasma)		
Undetectable (<40)	15 (65.2)	101 (79.5) **(n=125)**
Prevalent Cervical HPV		
Any (besides type 16)	19 (82.6)	56 (44.1)
High-risk (besides type 16)	17 (73.9)	36 (28.4)
Type 16	4 (17.4)	1 (0.8)
Prevalent Anal HPV		
Any (besides type 16)	22 (95.7)	90 (70.9)
High-risk (besides type 16)	19 (82.6)	59 (46.5)

Note. Pack-years, the number of packs of cigarettes smoked per day multiplied by the number of years the participant has smoked in her lifetime; HPV, human papillomavirus; High-risk HPV, types for which there is limited or sufficient (but not merely analogous) evidence of a link with cervical cancer (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 26, 53, 66, 67, 70, 73, 82)[231].

*Unless otherwise specified.

Table 2. Factors associated* with prevalent anal HPV 16 infection (univariable analyses)

	Odds Ratio	95% CI	p
Demographic characteristics			
Age	1.01	0.96-1.05	0.775
Born in Canada	3.93	1.55-9.98	0.004*
Tobacco use			
Current smoking (vs. past or never)	4.16	1.56-11.09	0.004*
Ever smoking (vs. never)	3.42	1.37-8.50	0.008*
Years since quit smoking	0.89	0.74-1.06	0.197
Lifetime pack-years (past smokers)	1.10	1.01-1.20	0.033*
Lifetime pack-years (current smokers)	1.02	0.98-1.06	0.274
Injection drug use			
Ever injecting drugs (vs. never)	9.07	2.88-28.57	0.000*
Sexual activity			
>10 lifetime vaginal sex partners	4.12	1.55-10.99	0.005*
Age at first vaginal intercourse (<17 vs. ≥17 years)	3.48	1.40-8.68	0.007*
Ever had anal sex	1.25	0.47-3.30	0.657
>1 lifetime anal sex partners (vs. 0 or 1)	5.12	1.71-15.35	0.004*
Avg. frequency of anal sex during sexually active life (times per year, not incl. 0)	1.04	1.00-1.07	0.037*
Condom use at last anal sex	0.19	0.02-1.79	0.146
Past sexual abuse/assault			
Past vaginal sexual abuse/assault (vs. no sexual abuse/assault)	2.99	1.14-7.86	0.026*
Past anal sexual abuse (vs. no sexual abuse/assault)	6.56	1.24-34.72	0.027*
Comorbidities			
Gonorrhoea ever	1.97	0.49-7.90	0.340
Chlamydia ever	2.93	0.91-9.42	0.071
Anogenital herpes ever	3.93	1.55-9.98	0.004*
Active hepatitis C infection	12.42	4.04-38.19	0.000*
Markers of HIV and immune function			
HIV viral load >39 copies/mL (vs. ≤39)	2.24	0.85-5.90	0.101
Nadir CD4 count ≤200 cells/μL (vs. >200)	5.19	1.81-14.87	0.002*
CD4 count ≤200 cells/μL (vs. >200)	6.61	1.74-25.12	0.006*
HPV 16 infection at other anatomical sites			
Cervical HPV 16	26.5	2.81-250.11	0.004*

Note. HPV, human papillomavirus

Note. Pack-years, the number of packs of cigarettes smoked per day multiplied by the number of years the participant has smoked.

* Denotes statistical significance at $p<0.05$

Table 3. Putative risk factors for anal HPV 16 identified with multivariable logistic regression

Independent variables	Final multivariable model		
	OR	95%CI	p
Age at baseline in years	1.0	0.9-1.0	0.696
Current smoking (vs. past or never)	5.1	1.4-18.9	0.014
Nadir CD4 count ≤200 cells/μL (vs. >200 cells/μL)	5.9	1.6-21.2	0.007
Past anogenital herpes among women with 1 or 0 past anal sex partners	6.6	1.8-23.9	0.004
>1 anal sex partners ever (vs. 0 or 1) among women with no past anogenital herpes	10.1	1.9-53.3	0.006
Strength of interaction between anogenital herpes and >1 anal sex partners	0.045	<0.01-0.77	0.033
Prevalent cervical HPV 16	27.7	2.3-326.7	0.008

Note. HPV, human papillomavirus; CI, confidence interval; OR, odds ratio

Note. The dependent variable, anal HPV 16 infection, was coded as 0="No HPV type 16 DNA detected in anal canal" and 1="HPV type 16 DNA detected in anal canal"

CHAPTER 4: MANUSCRIPT 2

4.1. PREFACE TO THE SECOND MANUSCRIPT

This manuscript describes the acceptability, pain, worry, and wish for screening associated with three anal cancer screening procedures based on responses to an acceptability questionnaire administered to 98 of the 151 WLHIV who experienced the procedures through the EVVA study. After the remaining 53 participants have completed the acceptability questionnaire, the manuscript will be updated and submitted for publication.

The knowledge of risk factors for anal HPV 16 presented in the first manuscript is an important component of the overall understanding of risk factors and natural history that would be needed for the development of anal cancer screening programs. Yet, even if ongoing RCTs provide conclusive evidence that treatment of HSIL prevents anal cancer, it will be impossible to implement screening for HSIL and anal cancer without understanding screening acceptability in candidate populations. Given the possibility that routine screening for anal cancer may be recommended for WLHIV within the next 5-8 years, knowledge of the acceptability of these screening tools must be available to physicians and policy makers to ensure that misconceptions about acceptability do not serve as unwarranted barriers or facilitators to screening. As some physicians have already begun screening WLHIV for anal cancer in select clinical and research settings, knowledge of screening acceptability will also serve to identify potential predictors of unacceptability to support their efforts to optimize patients' experiences of care and mitigate potential harms of anal cancer screening.

4.2. LIST OF CO-AUTHORS

Assessing the acceptability of three anal cancer screening tests in women living with the HIV

E. Kaufman[1], C. de Castro[2], M. Munoz[1,2,3], B. Lessard[1,2,4], M.-H. Mayrand[5], L. Charest[3], T. Williamson[6], M. Auger[7], V. Marcus[7], A. Burchell[8], M. Klein[2], F. Coutlée[2,9,] A. de Pokomandy[1,2]

1. McGill University, Faculty of Medicine, Family Medicine Department, Montreal, Canada
2. McGill University Health Centre (MUHC), Chronic Viral Illness Service, Montreal, Canada
3. Clinique Médicale l'Actuel, Montreal, Canada
4. Clinique Médicale du Quartier Latin, Montreal, Canada
5. Centre Hospitalier de l'Université de Montréal (CHUM), Department of Obstetrics & Gynaecology, Montreal, Canada
6. University of Calgary, Department of Community Health Sciences, Calgary, Canada
7. McGill University Health Centre (MUHC), Pathology Department, Montreal, Canada
8. Ontario HIV Treatment Network, Toronto, Canada
9. Centre Hospitalier de l'Université de Montréal (CHUM), Department of Microbiology & Infectious Diseases, Montreal, Canada

4.3. ABSTRACT

Background

Given the high rate of anal cancer among people living with HIV, routine anal cancer screening is being considered. Verification of the acceptability of procedures is a requirement for any screening program. A comprehensive literature review identified no published articles investigating the acceptability of anal cancer screening procedures according to WLHIV who had experienced them.

Methods

The EVVA study ("Evaluation of Human Immunodeficiency Virus, Human papillomavirus, and Anal Intraepithelial Neoplasia in Women") was a prospective cohort study of 151 WLHIV (≥18 years old) in Montreal. Participants were screened with cervical/anal human papillomavirus (HPV) testing and cytology (Pap tests) biannually for 2 years. A systematic high-resolution anoscopy (HRA) and digital anorectal examination (DARE) were performed at baseline and 2 years. Using descriptive and inferential statistics, we analyzed data from the "acceptability questionnaire", which was administered at 2 years or study withdrawal.

Results

In total, 98 acceptability questionnaires were complete by December 2015. At proposed yearly screening intervals, Pap tests were judged very acceptable by 85% of women (83/98; 95% CI: 76-91), anal swabs by 78% (76/98; 95% CI: 68-85) and DARE by 83% (81/98; 95% CI: 74-89). HRA every 2 years (the most frequent proposed interval) was considered very acceptable by 83% (80/98; 95% CI: 73-88). Screening intervals of 2-years for DARE and anal swabs and 5-years for HRA were acceptable to over 90% of women. Compared to women who found one or

more procedures not acceptable, women who found all procedures acceptable considered the screening more necessary and less painful. Routine screening was considered an "absolute necessity" by 73% of women (55/75; 95% CI: 63-84). Pain of anal swabs and DARE was similar to cervical swabs (median: 1/10), but pain was greater for HRA (median: 5/10). Low acceptability was usually due to pain.

Conclusion: The vast majority of participating WLHIV consider anal cancer screening necessary and very acceptable. Procedures are considered more acceptable when they are performed less often and viewed as necessary. Pain is a potential barrier to acceptability, particularly for HRA. Pain management can be improved and potential adverse psychological or physical effects of screening should be explored.

4.4. MANUSCRIPT 2: ASSESSING THE ACCEPTABILITY OF THREE ANAL CANCER SCREENING TESTS IN WOMEN LIVING WITH HIV

The incidence of anal cancer in women living with HIV (WLHIV) is approximately 24-fold greater than in the general population[33]. Yet, due to the even higher rate of anal cancer among HIV-positive men who have sex with men (MSM), an estimated 78 times greater than the general population[20], most anal cancer research to date has focused on men living with HIV. Although anal cancer screening acceptability has been verified in men, it has not been confirmed in WLHIV.

Like cervical cancers, anal cancers are associated with persistent infection with oncogenic types of human papillomavirus (HPV)[48, 49]. These infections can cause cell changes, called squamous intraepithelial lesions (SILs; equivalent to anal intraepithelial neoplasia or AIN), which are classified as "low-grade" (LSIL) or "high-grade" (HSIL)[57]. Cervical HSIL, the precursor to cervical cancer, is considered analogous to anal HSIL, the immediate precursor to invasive anal cancer[50], although progression of HSIL is never guaranteed and spontaneous regression to LSIL may occasionally occur[93]. The many biological parallels between cervical and anal cancer suggest that treating anal HSIL could reduce the incidence of anal cancer, akin to the remarkable reduction in cervical cancer incidence after the implementation of cervical screening programs in the 1960s[104]. There is mounting clinical evidence that treatment of anal HSIL does, indeed, reduce the incidence of progression to cancer[98-101]. In addition, we know that detection and treatment of invasive anal cancer at earlier stages reduces mortality[34].

There are no national screening guidelines for anal HSIL and anal cancer, although regional and

organizational guidelines exist and have been published by various entities including the American Society of Colon and Rectal Surgeons, European AIDS Clinical Society, New York State Department of Health, and German AIDS Society[35, 37, 119, 121]. Some specialists consider current evidence to be adequate and have already begun treating HSIL[39, 103]. They emphasize the necessity of restricting HSIL screening to settings with the appropriate tools and access to trained anoscopists[103]. However, screening and treatment of HSIL are controversial due to an absence of evidence from randomized controlled trials (RCTs) demonstrating that treating HSIL prevents anal cancer. Despite ethical concerns associated with having a non-intervention group for a condition which many experts believe to be treatable[40], two multi-site RCTs (the "ANCHOR" and "LOPAC" trials) were recently launched to resolve this controversy and assess the safety and effectiveness of treatments for anal HSIL[41, 42].

According to the World Health Organization, a screening test (procedure) must be suitable and acceptable to the population[232]. Yet, the acceptability of anal cancer screening procedures in WLHIV has not been confirmed. Some studies have assessed screening *acceptance*, understood as *willingness* to be screened[185, 186], at times using the term *acceptability* to refer to this concept[187-189]. Participants in such studies did not experience the procedures first-hand. In this manuscript, acceptability is defined as the extent to which individuals who have experienced screening procedures rate the procedures as "acceptable" as part of routine care at various proposed frequencies and would be willing to be screened again with those specific procedures.

Minimal research has addressed anal cancer screening acceptability in women. A literature search in Pubmed on December 20, 2016, for the acceptability or tolerance of screening for anal HPV, HSIL (and related terms), or anal cancer, found no published full-length articles pertaining

to acceptability in WLHIV. While studies of anal cancer screening in HIV-positive MSM have found high acceptability[190-195], acceptability in WLHIV and MSM living with HIV may differ due to dissimilarities in sexual practices and life experiences between these populations. Ortiz et al.[198] have already highlighted one difference between anal HPV testing acceptability in men and women; in their cross-sectional study of 100 HIV-negative women, clinician-collected HPV testing was more acceptable than self-collected sampling, whereas MSM in another study preferred self-sampling[199, 200].

The aim of these analyses was to assess the acceptability of three anal cancer screening procedures in a cohort of WLHIV who experienced the procedures through the EVVA study in Montreal, Canada. A secondary aim is to examine whether the women who found the procedures not acceptable differed with respect to any key characteristics from those who found all procedures "acceptable". This research may help optimize screening for WLHIV who are presently being screened for anal cancer and anal HSIL in some research and clinical settings. It is also an essential preparatory measure to confirm acceptability, pinpoint potential harms of screening, confirm that at-risk populations are amenable to long-term screening on a larger scale, and identify barriers to acceptability, in the event that routine HSIL screening should become more widely recommended in the future.

Methods

Study design and population

We assessed acceptability within the EVVA study ("Evaluation of HIV, HPV, and AIN[ix] in

[ix] "SIL" terminology (HSIL, LSIL) is now used for both cytologically and histologically identified lesions and has replaced the "AIN" terminology previously used for histologically identified anal HPV-related lesions.

women"), an ongoing prospective observational cohort study investigating anal cancer screening in 151 WLHIV in Montreal, Canada. The study involves the collection of 5 biannual repeated measurements over two years, via questionnaires, chart reviews, cervical cytology and HPV testing, and anal cytology and HPV testing. All participants also undergo high-resolution anoscopy (HRA) with targeted biopsies at baseline and 2 years (or more often if clinically indicated). Participants are women 18 years and older, with confirmed positive serology for HIV and sufficient knowledge of English or French to provide informed consent and understand the questionnaire. All study materials are offered to participants in French and English, and assistance is available if participants experience difficulty with literacy or language level. Exclusion criteria are defined as pregnancy at recruitment, invasive anal cancer diagnosis previously or at recruitment, or not having a cervix (as data collection included testing of the cervical area). Women were recruited during routine HIV care between February 2012 and July 2015 at four HIV clinics in Montreal, and provided informed and voluntary written consent at the time of recruitment. Ethics approval was obtained from the McGill University Health Centre Research Ethics Board/Institutional Review Board.

Screening procedures

At each visit, cervical and anal samples were collected for HPV testing and cytology. To obtain cervical samples for cytology, a cytobrush and wooden cervical spatula were sequentially inserted through a vaginal speculum and rotated in the cervix. For cervical HPV testing, a second cytobrush was used to collect cells in the same manner. To obtain anal samples, two consecutive Dacron swabs moistened with saline were inserted 3-5 cm into the anal canal and removed with a twirling motion to gently remove epithelial cells from the canal walls. One was used for cytology and the other for HPV testing. HRA was performed systematically in all participants at the

baseline and final (24-month) study visits. If HSIL was revealed at any point, HRA was repeated 6 months later. HRA involved examining the anal canal through a clear plastic anoscope with a magnifying lens (a colposcope) after application of 5% acetic acid and lugol's iodine. Two or more targeted biopsies were taken using disposable bronchoscopy forceps or reusable baby tischler forceps, and a 2% xylocaine gel was applied. In the absence of visual abnormalities, biopsies were targeted to areas of greatest concern. HRA visits included a digital anorectal examination (DARE), consisting of visual observation of the perianal region and insertion of a gloved finger into the anal canal to check for palpable invasive cancer. Participants were given Colace (docusate sodium) and 5% xylocaine ointment for as-needed home use after the procedures. HRA, biopsies, and DARE were performed by a trained anoscopist and swabs were performed by a registered nurse.

Questionnaires and chart review

Data on sociodemographic, lifestyle, and medical variables was drawn from chart reviews and detailed questionnaires administered at baseline and follow-up visits. A questionnaire addressing screening acceptability and related concepts was integrated into the study in May 2013 as a pilot study of acceptability. This "acceptability questionnaire" was administered after the final visit or, when possible, in the case of study withdrawal. Using semantic differential (binary) 0-to-10 scales, the questionnaire assessed the level of pain felt during procedures, degree of worry about anal cancer, degree to which participants consider anal cancer screening in WLHIV a necessity, and level of acceptability of screening procedures at various hypothetical screening frequencies proposed for the screening procedures. The questionnaire also assessed the pain and acceptability of cervical Pap testing as a benchmark for comparison with anal screening procedures, because cervical Pap tests are considered highly acceptable and are currently recommended as routine

screening every 1-3 years for most women. When a participant rated a procedure's acceptability as low (<5/10), she was asked to provide a reason. Participants were also invited to provide comments at the end of the questionnaire. The study coordinator administered most questionnaires in person and recorded comments in writing. Some participants chose to complete the questionnaire privately. The EVVA study co-investigators and collaborators confirmed the face validity of the questionnaire when designing this pilot study of acceptability.

Statistical Analysis

Standard descriptive statistical methods were used to describe demographic and clinical characteristics as well as acceptability-related variables. The distribution of responses for acceptability was non-normal, showing nearly complete dichotomization of responses to the extremes. Consequently, this variable was dichotomized as a new binary variable such that acceptability ≥5/10 was considered "acceptable" and acceptability <5/10 was considered "not acceptable". Frequencies and proportions were used to compare participants who considered all anal cancer screening procedures acceptable at all frequencies with participants who considered any one or more procedure(s) not acceptable at any one or more proposed frequencies. Potential correlations were assessed using Spearman's ρ for comparisons involving one or more categorical variables and Pearson's *r* for continuous variables. Pearson's chi-square test and Student's t-tests were used to assess the significance of the differences in characteristics between the two groups; for these tests, p values <0.05 were considered significant. Missing values were few in number and are portrayed in the tables. It is possible that some missing values, such as those for abuse, depended on unobserved data. As this mechanism of missingness could not be modelled, complete case analysis was performed. All analyses were conducted using the statistical analysis software Stata/IC 14.1 for Mac (64-bit Intel).

Results

Participant characteristics and response rate

Table 1 presents the baseline characteristics of women in the EVVA study compared with the subsample of women who had completed the acceptability questionnaire by December 2015. Of note, our sample includes few women who have used intravenous drugs and only two Aboriginal women, one of whom had completed the acceptability questionnaire by this date. We observed no major differences in characteristics between the study sample of 151 WLHIV and the 98 women who had completed the acceptability questionnaire by December 2015.

The progress of participants in our rolling cohort through their follow-up visits as of December 2015 is depicted in Figure 1. Of 98 completed acceptability questionnaires, 85 (87%) were completed after the final study visit, while 13 (13%) were completed at study withdrawal (5 after the baseline visit, 1 after 6 months, 2 after 12 months and 5 after 18 months). There were no statistically significant differences between acceptability questionnaire respondents and participants who withdrew without completing the acceptability questionnaire, although the proportion of participants with >1 anal sex partners was higher among participants who completed the acceptability questionnaire (12/98, 12.2% vs. 0/24, 0%; p for difference = 0.071).

Anal cancer worry and wish for routine screening

Participants' perceptions of the need for routine anal cancer screening in WLHIV were assessed via questions addressing worry about anal cancer as well as the wish for routine screening. Regarding worry, 26% (95% confidence interval [CI]: 15-35) described their worry as 0/10 ("not worried at all") while 43% (95% CI: 31-54) described their worry as 10/10 ("extremely

worried"). When asked to what extent they want anal cancer screening to become part of routine care for all WLHIV, only 1% (95% CI: 0-4) responded that they were "against" routine screening, rating their wish for screening as 0/10, while screening was regarded as "an absolute necessity" (10/10) by 73% (95% CI: 63-84). Although these two variables were correlated, with Pearson's r=0.26 (p=0.009), worry was not a necessary condition for considering screening "an absolute necessity".

Pain

Pain was rated on a scale from 0 ("No pain at all") to 10 ("Worst pain ever felt"). Pain felt during anal swabs, DARE and HRA was compared to pain of cervical swabs. Anal swabs were considered less painful than cervical swabs by 25.5% of participants (95% CI: 17.7-35.2), equally painful by 51.0% (95% CI: 41.0-60.9), and more painful by 23.5% (95% CI: 16.0-33.0). DARE was considered less painful than cervical swabs by 28.6% (95% CI: 20.4-38.5), equally painful by 33.7% (95% CI: 24.9-43.7), and more painful by 37.8% (95% CI: 28.6-47.9). HRA was considered less painful than cervical swabs by 5.1% (95% CI:2.1-11.8), equally painful by 12.2% (95% CI: 7.0-20.5), and more painful by 82.7% (95% CI:73.7-89.0). The median pain for cervical/anal swabs and DARE was low (1/10), while the median pain for HRA was higher (5/10). Pain felt during HRA varied widely. Figure 2 shows the distribution of pain responses.

Acceptability of procedures

Acceptability was judged on a scale from 0 ("Not acceptable; don't want to do it ever again") to 10 ("Very acceptable; so easy I could do it even more often"). The acceptability of anal swabs, DARE, and HRA at various proposed frequencies was compared with that of yearly cervical Pap tests (swabs), which 80% of participants (95% CI: 71-89) described as very acceptable. Sections

a, b, and c of Figure 3 each depict the acceptability of an anal screening procedure at three proposed frequencies, compared to yearly cervical Pap tests.

Most women considered anal swabs and DARE similar in acceptability to yearly cervical pap tests, a routine and accepted screening test in WLHIV. Overall, acceptability increased as the proposed frequency of screening decreased, although this trend was statistically insignificant due to overlapping confidence intervals. At the less frequent proposed screening intervals of 2 and 5 years, the acceptability of anal swabs and DARE was considerably higher than that of yearly cervical Pap tests. Despite higher pain scores for HRA compared to other procedures, the acceptability of HRA was also high; at a proposed screening interval of 2 years, its acceptability was only 3 percentage points lower than that of yearly cervical Pap tests. Like anal swabs and DARE, HRA was more acceptable than yearly cervical Pap tests when the proposed screening interval was less frequent (every 5 or 10 years). For 3 women, reduced frequency of screening did not render HRA acceptable; 1 woman considered HRA not acceptable every 5 years, and 2 considered it not acceptable every 10 years. All 3 of these women cited pain as the reason.

Participant characteristics suggestive of low acceptability

In Table 5, women who considered all screening procedures and proposed frequencies acceptable are compared with women who considered one or more procedures or frequencies not acceptable (acceptability <5/10). Most p values for differences between the two groups were insignificant. Given the small sample size, this may be due to type II (β) error.

As expected, the difference in acceptability between groups was significant (p=0.028) for pain; women who considered pain >5/10 for any procedure, including cervical Pap tests, more often

found one or more anal cancer screening procedures not acceptable. Women who considered all procedures acceptable at all frequencies also tended to consider anal cancer screening necessary in WLHIV (p=0.004). The predictive value of past sexual abuse/assault was also significant (p=0.045); screening procedures were slightly more acceptable to women with histories of sexual abuse. This finding, and others, may change with completion of the 53 remaining acceptability questionnaires. Even where p values are insignificant, the proportions suggest some clinically plausible trends, such as a greater probability of having completed post-secondary education, and of having 1 or fewer lifetime anal sex partners, in the group of women who viewed one or more anal screening procedures as not acceptable.

Reasons for low acceptability

When participants did not consider a given anal cancer screening procedure acceptable at a proposed frequency (i.e. Acceptability <5/10), they were encouraged to provide a reason. This question was open-ended. The reasons stated for low acceptability are presented here, with the number of participants who stated each reason shown in parentheses. Some women provided more than 1 reason.

Regarding yearly anal swabs, the reasons given for low acceptability were related to discomfort or pain (4), opinion that it is unnecessary to screen so often or in the absence of sexual activity (4), or embarrassment (1). Two women found the swab too rigid and suggested using a softer or more flexible swab. For yearly DARE, reasons were related to discomfort/pain (3), duration of the procedure ("too long") (1), or opinion that it is unnecessary to screen so often or in absence of sexual activity (1). For HRA every 2 years, reasons given were discomfort/pain during and/or after the screening (7), travel distance and parking (1), and concern about the biopsies causing

new lesions (1). One woman, who did not report a history of sexual abuse or anal sex, also felt an intense and painful "pulsating sensation" for 1 day after the biopsies and said she felt like she "had been raped". All 3 women who found HRA not acceptable every 5 or 10 years cited pain as a reason. One also stated that it took "too long".

Discussion

The EVVA cohort of 151 WLHIV has provided a unique opportunity to inquire about screening acceptability in WLHIV; to our knowledge, EVVA is the only substantially sized cohort of WLHIV, worldwide, that has performed HRA twice, systematically, in all participants, and the only published study examining the acceptability of anal cancer screening procedures in WLHIV. We found that most participants conceived of anal cancer screening in WLHIV as an "absolute necessity" and found anal swabs, DARE, and HRA very acceptable. For all anal screening procedures, as well as for cervical Pap tests, acceptability increased as the proposed frequency of screening decreased. Spaces been confidence intervals within this trend may become apparent after the 53 remaining acceptability questionnaires have been completed. As expected, Pain during procedures was significantly associated in stratified analyses with finding one or more of the proposed screening procedures or frequencies not acceptable. Pain was also the main reason given for low acceptability in open-ended comments.

The finding that HRA was more painful (median 5/10) than DARE and swabs (median 1/10) was expected, because HRA involves biopsies. In a small subset of our cohort, HRA was considered not acceptable even if performed every 10 years; the characteristics and views of this subset are likely to be very informative for identifying and addressing possible challenges to acceptability in this population. With regards to DARE and anal swabs, there were also multiple participants

who stated that pain was the reason they considered the procedure not acceptable. Regardless of the precise nature of future anal cancer screening recommendations in WLHIV, strategies to improve pain management will be essential to increasing the acceptability of anal cancer screening tests such that they may be performed when indicated.

Contrary to what might be expected, histories of sexual assault/abuse were *less* common in the low acceptability group. Nonetheless, the high prevalence of emotional and mental health problems and histories of sexual assault among WLHIV[201, 202] must be accorded careful attention when considering screening, particularly anogenital screening, in this population. A survey by Battaglia et al. identified fear of sexual assault flashbacks and fear of pain as barriers to anal Pap testing in WLHIV[203]. Women with high levels of past trauma were more likely to believe that sexual assault flashbacks could arise from anal Pap testing[203]. In addition, fear and symptoms of depression have been cited by WLHIV as barriers to accessing cervical cancer screening[204]; Rosa Cunha et al. note that these barriers may also affect the extent to which WLHIV attend anoscopy appointments[193].

In light of the knowledge that treating anal cancer early decreases mortality, recent evidence that HSIL lesions can, indeed, progress to invasive cancer if left untreated, and pending confirmation that treating HSIL prevents invasive anal cancer, it is valuable to begin addressing potential barriers to screening now. Acceptability of screening is one of the many factors that must be understood for successful screening program implementation. As such, this research will help prevent unnecessary delays in the implementation of routine anal cancer screening programs in WLHIV in the event that ongoing research soon finds such programs to be warranted.

Confirming acceptability of screening also has the potential to curb unnecessary barriers to screening that may be introduced by well-meaning physicians who wish to protect their patients. Results from a 2013 questionnaire administered to physicians by Ong et al. showed that approximately one third of physicians who were not performing DRE in MSM cited concern about patient acceptability as a reason behind their reluctance to screen[233], and semi-structured qualitative interviews by this same team in 2015 revealed similar concerns. Yet, studies soliciting the views of men and women in high-risk groups have found that the majority of patients are open to anal cancer screening[189, 234-236]. The present results confirm that anal cancer screening procedures are acceptable to most WLHIV who have experienced the procedures through the EVVA study.

Compared to one survey in 404 HIV-negative women[188], in which only 28% were "very interested" in undergoing screening with HPV testing and cytology, our results indicate much higher acceptability (willingness to undergo *further* screening) in WLHIV who have already experienced repeat screening with anal swabs, DARE, and HRA. These results are consistent with Battaglia et al.'s finding that prior experience with HRA increases willingness to participate in screening[237] and imply that, for many women, anal cancer screening may be less uncomfortable and embarrassing than they expect.

Women's oral and written comments were also valuable. Women suggested potential strategies to improve acceptability (such as providing cleaning supplies), spoke frankly about their experiences being screened, and provided insight and detail that would otherwise have been overlooked. These varied and thoughtful comments suggest that a qualitative study of women's

experiences with anal cancer screening might add depth to our understanding of anal cancer screening acceptability.

This study has certain limitations. Although we recorded reasons for loss to follow-up when possible, we did not record reasons for refusal to participate. As such, we cannot pinpoint differential participation that may have introduced bias. In addition, the level of worry and wish for routine screening measured in EVVA study participants are most likely higher than in WLHIV outside the study, because information about anal cancer is included in the informed consent form. This increased worry and wish for screening could, in turn, affect acceptability. Thus, while possibly not representative of the opinion of *all* WLHIV regardless of prior knowledge, the results of this study are representative of WLHIV who have received the accurate information about anal cancer screening provided in the informed consent form. It must also be noted that the study procedures changed in May 2013. After this date, xylocaine gel replaced xylocaine injections as the anesthetic for HRA. The acceptability questionnaire was also introduced at this time. In conversations with the study coordinator, participants expressed a strong preference for the gel over the injections, but we did not collect quantitative data regarding acceptability of HRA using xylocaine injections prior to the change in procedures. Although the tone and content of women's comments suggests that they expressed themselves frankly, it is possible that some participants were reluctant to criticize the procedures. To reduce the likelihood of social desirability bias, questionnaires were administered in the absence of the anoscopist and nurse and participants were given the option of filling out the questionnaire alone. The study coordinator emphasized our firm adherence to confidentiality and the importance of honest answers in this research in order to prevent harms and improve health care for WLHIV.

In contrast with many populations of WLHIV, our study population was largely comprised of women born in Africa and the Caribbean, with few participants identifying as Aboriginal or reporting a history of injecting drugs. As place of birth and intravenous drug use were not found to be significant predictors of pain and acceptability in our cohort, the generalizability of our findings to other WLHIV is expected to be strong. Nonetheless, given possible psychosocial effects of screening, caution should be heightened when applying these findings to any population with notably different sexual experiences, life histories, or demographic characteristics. As well, there is no gold standard for measuring screening acceptability and there was no existing validated questionnaire of acceptability of anal cancer screening in WLHIV prior to this study. Thus, to create our pilot questionnaire, we drew upon other validated questionnaires to create a measurement tool possessing the greatest possible validity. Finally, some factors potentially affecting acceptability, such as anal cancer knowledge and discussions with peers, were not measured in this study and may warrant future investigation using quantitative or qualitative methods.

In summary, the majority of the WLHIV participating in the EVVA study who had filled out the acceptability questionnaire by December 2015 considered anal cancer screening necessary and very acceptable. According to these women, HRA was more painful than DRE and anal swabs. The median level of pain they felt during DARE and anal swabs was no greater than that which they felt during cervical Pap tests. Pain was the primary reason for low acceptability of anal swabs, DARE, or HRA. This strongly suggests that improved pain management strategies will further increase acceptability. Despite pain, most participating WLHIV found HRA acceptable. The least frequent screening intervals were the most acceptable. In order to prevent potential

harms and optimize screening experiences for WLHIV, further exploration of the reasons that accounted for low acceptability in some participants is warranted. It is also necessary to investigate potential adverse effects of both anal cancer screening procedures and screening results in WLHIV.

4.5. TABLES AND FIGURES

Table 1. Baseline demographic and clinical characteristics of EVVA cohort compared with baseline characteristics of acceptability questionnaire (AQ) respondents as of December 31, 2015

Participant Characteristic	No. (%) of cohort (n=151)	No. (%) of completed AQ (n=98)
Age at baseline in years		
19-39	40(26.5)	24(24.5)
40-49	65(43.0)	44(44.9)
50-69	46(30.5)	30(30.6)
Place of birth		
Canada	35(23.2)	23(23.5)
Africa	66(43.7)	44(44.9)
Caribbean	43(28.5)	29(29.6)
Central America or elsewhere	7(3.6)	2(2.0)
Education completed		
High school or less	97(64.2)	64(65.3)
CEGEP, college, or university	54(35.8)	34(34.7)
Currently smoke cigarettes	26(17.2)	15(15.3)
Ever injected drugs	15(90.0)(n=150)	11(11.3)(n=97)
CD4 cell count ≤200 cells/μL	10 (6.8) (n=148)	6(6.2) (n=97)
HIV VL Undetectable (<40 RNA copies/mL)	117(78.5) (n=149)	73(75.3.0)(n=97)
Prevalent anal high-risk HPV	90(60.0)	62(63.1)
Lifetime # of anal sex partners (range: 0-20)		
None	110(72.8)	70(71.4)
1	23(15.2)	16(16.3)
≥2	18(11.9)	12(12.2)
Number of vaginal sex partners during 6 months pre-baseline		
0	70(46.0)	48(49.0)
1	77(51.0)	46(46.9)
>1	4(3.0)	4(4.1)
Recent anal sex partners (Last 6 months)		
0	145(96.0)	94(95.9)
1	6(4.0)	4(4.1)
Sexual preference		
Men	136(90.1)	91(92.9)
Women	2(1.3)	2(2.0)
Both men and women	4(2.7)	1(1.0)
Missing/Uncertain/Declined to answer	9(6.0)	4(4.1)

Note. AQ, acceptability questionnaire; CEGEP, pre-university college in Quebec, Canada; VL, viral load; HPV, human papillomavirus; High-risk HPV, types for which there is limited or sufficient (but not merely analogous) evidence of a link with cervical cancer (Types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 26, 53, 66, 67, 70, 73, 82)[231]; VL, viral load

Table 2. Characteristics of women living with HIV who found screening with anal swabs, DARE, and HRA *acceptable (acceptability ≥ 5/10)* compared with women who considered one or more procedures or proposed screening intervals *not acceptable (acceptability <5/10)*.

	n=79 Acceptability ≥ 5	**n=19 Acceptability <5**	**P value for difference (t-test or chi-square)**
	n (%)	**n (%)**	
Born in Canada	17(21.5)	6(31.6)	0.35
Age			
Median	46	45	0.92
36 and older	64(81.0)	17(89.5)	0.38
Pain >5/10 for any anal screening procedure *or* cervical Pap test	36(45.6)	14(73.7)	0.03
Worry about anal cancer >5/10	49(62.0)	8(42.1)	0.11
Wish for routine screening >5/10	75(94.9)	14(73.7)	0.004
Past anal sex (ever)	22(27.8)	6(31.6)	0.75
>1 lifetime anal sex partners (vs. 0 or 1)	11(13.9)	1(5.3)	0.30
Any past sexual abuse or assault	39(49.4) (n=75)	5(26.3)	0.045
Completed college, CEGEP*, or university	25(31.6)	9(47.4)	0.20
Ever injected drugs	8(10.1)(n=78)	3(15.8)	0.50

Note. DARE, digital anorectal examination; HRA, high-resolution anoscopy; CEGEP, pre-university college in Quebec

Note. Acceptability was judged on a scale of 0 (“Not acceptable; don’t want to do it ever again”) to 10 (“Very acceptable; so easy I could do it even more often”).

Figure 1. Completed visits, Acceptability Questionnaires (AQ), and withdrawals, Dec. 2015

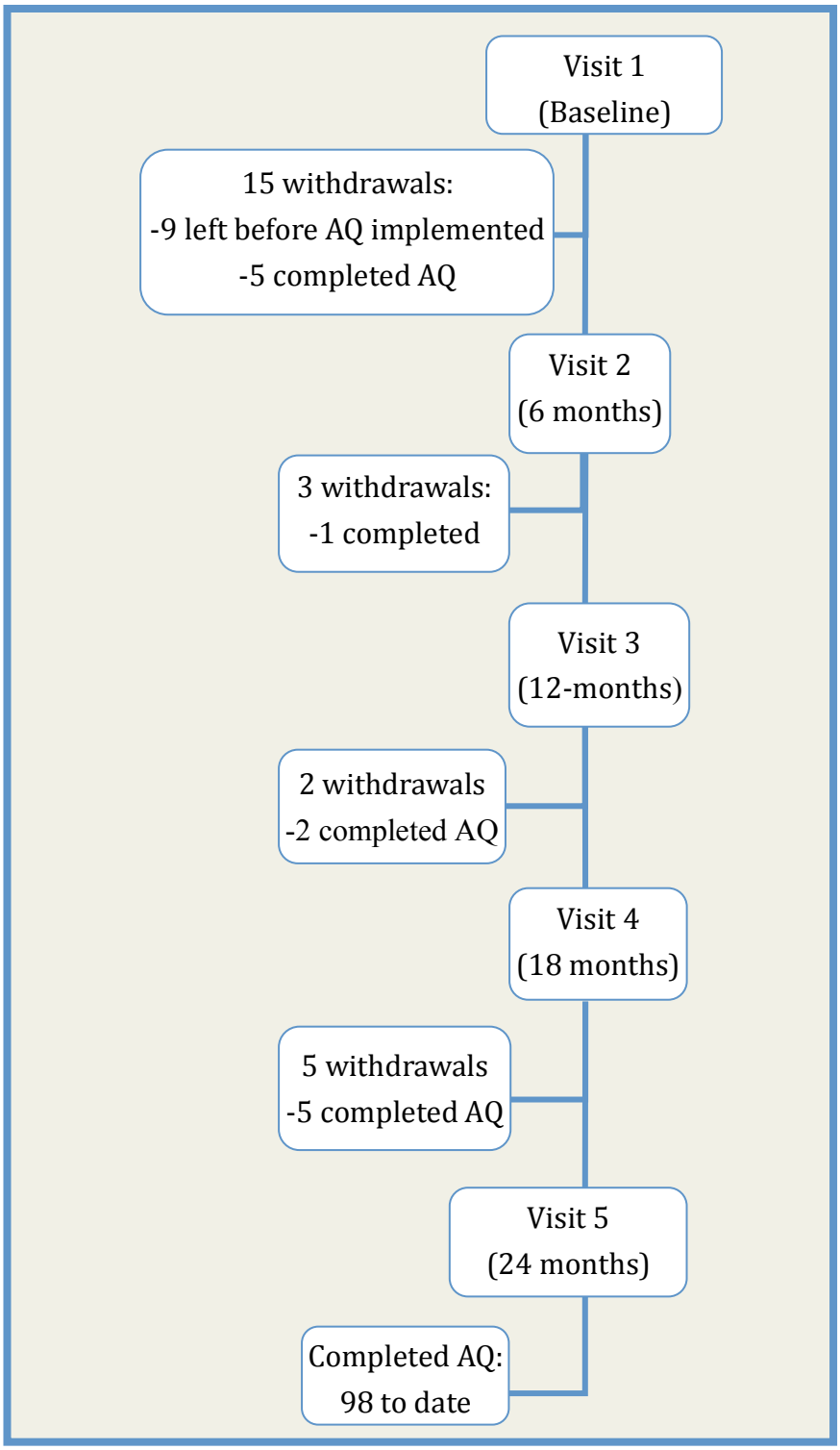
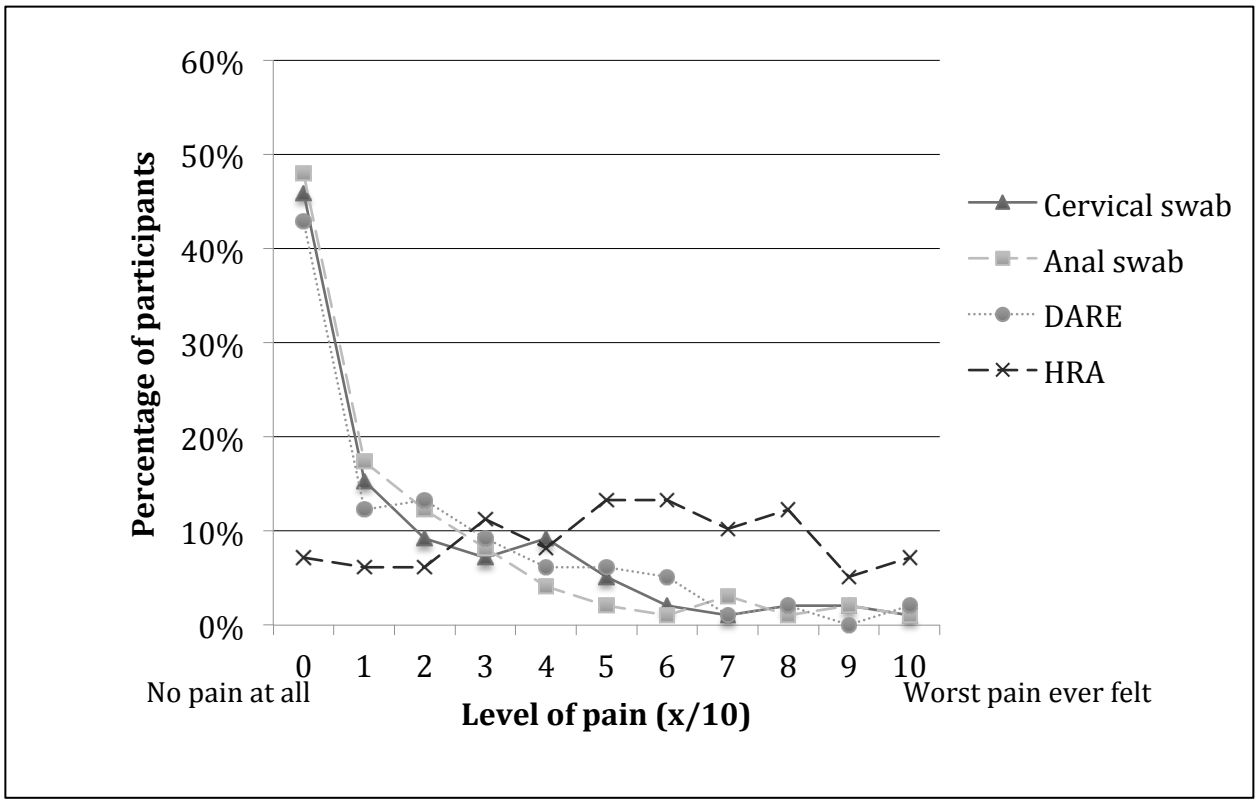


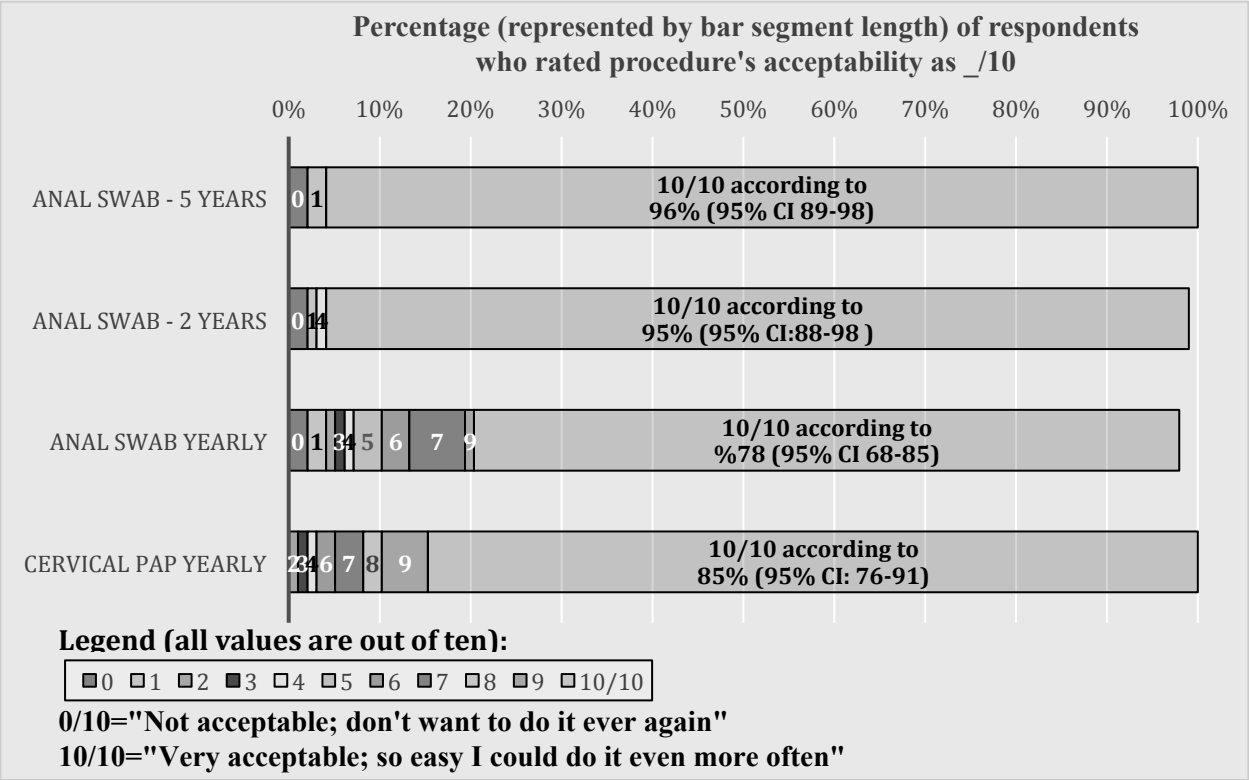
Figure 2. Percentage of EVVA study participants who felt a level of pain of x/10 during screening procedures, where 0= “No pain at all” and 10= “Worst pain ever felt”



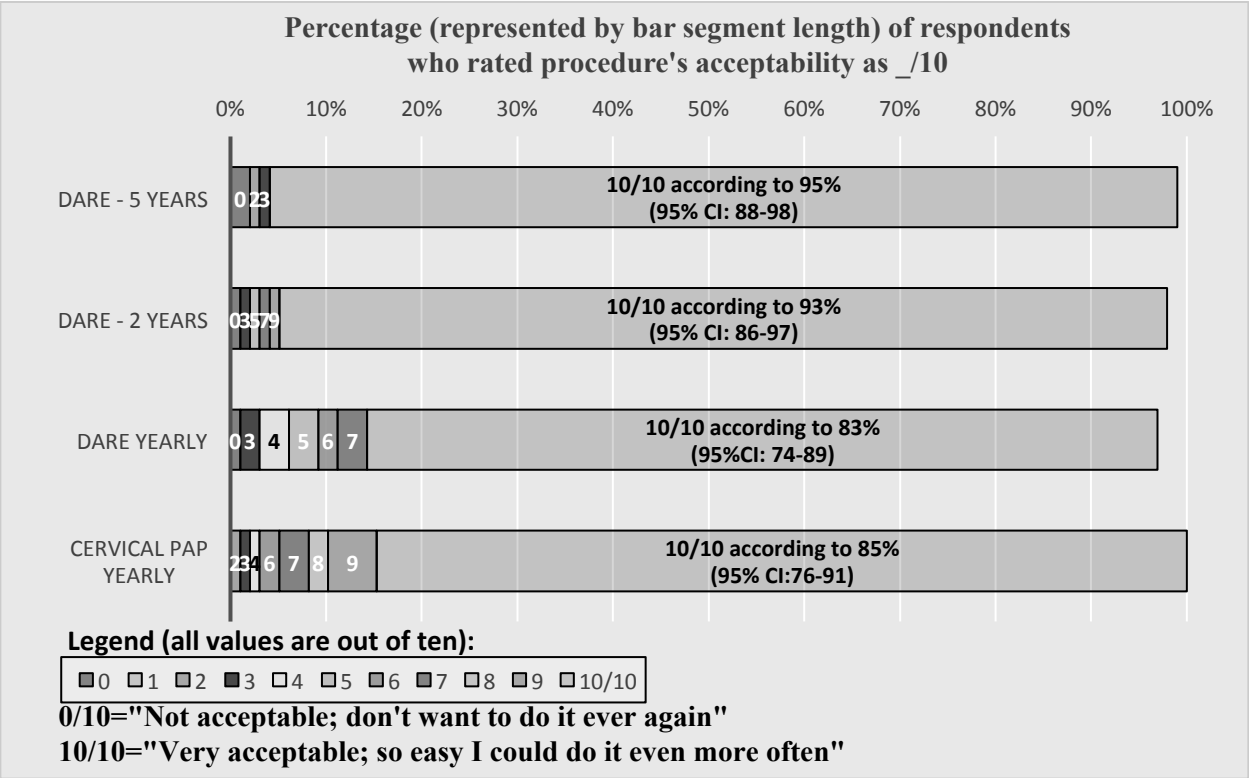
Note. DARE, digital anorectal examination; HRA, high-resolution anoscopy

Figure 3. Acceptability of anal swab (a), digital anorectal examination (b), and high-resolution anoscopy (c) at various proposed screening frequencies compared to yearly cervical Pap tests

a) Acceptability of **anal swab** every 1, 2, or 5 years compared to yearly cervical Pap tests

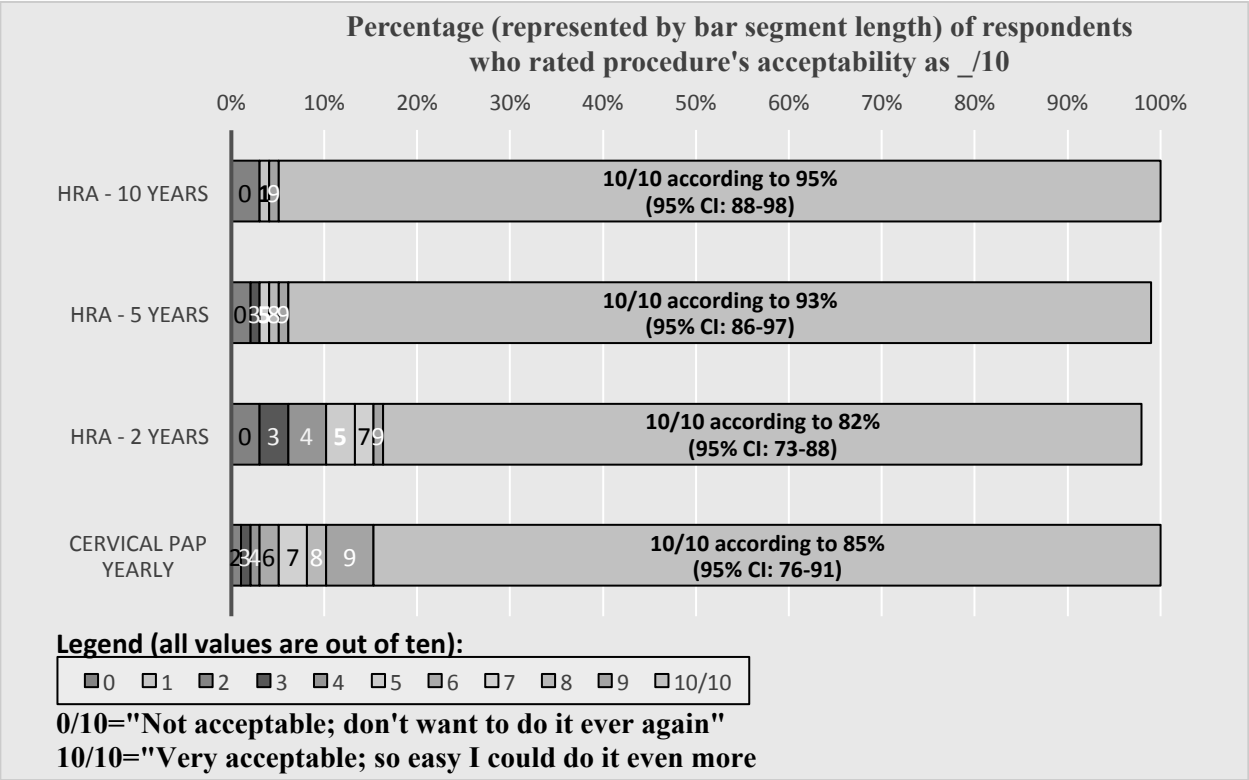


b) Acceptability of **digital anorectal examination (DARE)** every 1, 2, and 5 years compared to yearly cervical pap



Note. DARE; digital anorectal examination

c) Acceptability of **high-resolution anoscopy (HRA) with biopsies,** every 1, 2, and 5 years compared to yearly cervical pap



Note. HRA; high-resolution anoscopy

CHAPTER 5: CONCLUSION AND SUMMARY

Summary

For this thesis project, I analyzed baseline and acceptability questionnaire data from the EVVA study of WLHIV. To my knowledge, EVVA is the only study in WLHIV to perform HRA twice, systematically, in all participants, as well as the only study to assess the acceptability of anal cytology/HPV testing, DARE, and HRA in a substantially-sized cohort of WLHIV who have experienced these procedures first-hand. The main sections of my thesis were a literature review and two manuscripts, the first presenting analyses of risk factors for prevalent anal HPV 16 and the second presenting the findings of the acceptability questionnaire. These manuscripts will be submitted for publication in 2016.

The literature review was conducted in three parts. The first was in-depth narrative review and provided extensive background knowledge of the biology and epidemiology of anal cancer and HPV in WLHIV and other populations. Next, two comprehensive literature searches were performed. The first comprehensive search sought to identify documented risk factors for anal HPV in WLHIV. Age, CD4 count, and smoking were identified in the literature as risk factors for anal HPV in WLHIV. The literature in men and HIV-negative women also suggested an association with anal and vaginal sexual activity although evidence of this was not found in WLHIV. The second, comprehensive search was intended to assess the state of knowledge regarding the acceptability of anal cancer screening tests (procedures) in WLHIV. Given that knowledge of anal cancer screening acceptability in WLHIV is extremely scant, this literature search quickly exhausted the few publications in this area. A supplementary search for studies investigating psychosocial consequences of anal cancer screening was therefore performed. Both comprehensive searches, as well as the supplementary search, revealed a paucity of sources

investigating the risk factors for anal HPV in WLHIV and the acceptability of anal cancer screening tests in this population.

The analyses for the first article were conducted with the goal of identifying risk factors for anal HPV 16 in WLHIV. Numerous clinical and demographic variables were analyzed via univariable and adjusted logistic regression analyses. Descriptive analyses confirmed, as is expected in women, a higher prevalence of anal HPV compared to cervical HPV. Like other studies, we found that history of any anal sex ever was not a significant risk factor for anal HPV 16 infection in WLHIV. However, in our data, history of anogenital herpes modified the effect of having multiple (compared to 1 or 0) lifetime anal sex partners; having multiple lifetime anal sex partners was associated with an increased odds of anal HPV 16 among women who did not have a history of anogenital herpes, but not among women who did have a history of anogenital herpes. This effect modification may be due to an increased susceptibility of the anogenital area to HPV infection where the skin is thin or broken because of a herpes outbreak; the implication is that having had multiple anal sex partners may not be a statistically significant risk factor for anal HPV 16 in women with a history of anogenital herpes outbreaks because herpes drives most of the effect. Smoking, nadir CD4 count, and concurrent prevalent cervical HPV 16 infection were also associated with increased odds of anal HPV 16 in adjusted analyses.

The second article, assessing the acceptability of anal cancer screening in WLHIV, confirmed that most WLHIV in the EVVA study considered anal cancer screening necessary and very acceptable. It also found that pain is presently the main barrier to acceptability in this population, that HRA is usually considered acceptable despite higher pain than DARE and anal swabs, and that participating WLHIV considered proposals for future screening increasingly acceptable with

decreasing frequency of the proposed screening. Women's comments about the screening procedures provided richness and depth of understanding, and reflected the importance of attention to their diverse and complex issues and busy lives when considering routine screening. They included poignant suggestions about simple changes that could be made to the screening procedures in order to increase comfort and overall acceptability.

Clinical implications

The clinical implications of the findings of both manuscripts are substantial. Understanding the risk factors for anal HPV, including the particularly prevalent and oncogenic type 16, is paramount to developing prevention and screening strategies for anal cancer. The oncogenic potential of HPV 16 is considered an order of magnitude higher than other high-risk HPV types[67]. With respect to anal cancer, HPV 16 is a cause of approximately 75% of cases[53, 66]. Given the disproportionately high rate of anal cancer in WLHIV, contributions to screening and prevention efforts are of great importance. Knowledge of the risk factors for anal HPV 16 in this population will contribute to efforts to identify which WLHIV are likely to be a top priority for HPV vaccination and anal cancer screening.

As we await results from ongoing RCTs about the potential benefit of treating HSIL, the immediate precursor to anal cancer, developing our understanding of screening acceptability will ensure that screening efforts are neither hindered nor unduly facilitated should HSIL screening become standard of care. Furthermore, if screening in the EVVA study had been found to be not acceptable to WLHIV, the clinical relevance of investigating the effectiveness of screening for anal cancer in this population would have been greatly diminished. Even if screening for anal HSIL and anal HPV is not implemented, understanding the acceptability of screening with

DARE is of great importance as DARE has already been recommended as a means of detecting early palpable invasive anal cancer in WLHIV and the general population. Although generalizability of findings should never be assumed, these findings of high acceptability in WLHIV participating in the EVVA study may shed light on anal cancer screening acceptability in other populations.

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APPENDIX A: SEARCH STRATEGIES

Search 1: Anal HPV or AIN/anal HSIL in WLHIV

Database: Pubmed

Latest search date: November 28, 2015

Number of original results: 405; with 274 in last 10 years

(((("HIV Infections"[mesh]) OR ("hiv"[tw])) AND (("Women"[mesh:noexp]) OR ("Female"[mesh]) OR (women[tiab] OR woman[tiab] OR females[tiab] OR female[tiab]))) AND ((((("Papillomavirus Infections"[mesh:noexp] OR "Papillomaviridae"[mesh:noexp] OR "human papillomavirus"[tw] OR "Papilloma"[mesh:noexp]) OR ("HPV"[tw])) OR ("anal intraepithelial lesions"[tw] OR "Precancerous Conditions"[mesh:noexp] OR "anal intraepithelial neoplasia"[tw]) OR ("anal dysplasia"[tw]) OR ("HSIL"[tw]) OR ("squamous intraepithelial lesions"[tw])) AND (("anus"[tw] OR "Anal Canal"[mesh:noexp]) OR ("anal"[tw]))))

Search 2(a): Acceptability of screening for anal HPV, or anal cancer in WLHIV

Database: Pubmed

Latest search date: December 20, 2015

Number of original results: 89

("anus neoplasms"[MeSH Terms] OR ("anus"[All Fields] AND "neoplasms"[All Fields]) OR "anus neoplasms"[All Fields] OR ("anal"[All Fields] AND "cancer"[All Fields]) OR "anal cancer"[All Fields]) AND ("screening"[All Fields] OR "mass screening"[MeSH Terms] OR ("mass"[All Fields] AND "screening"[All Fields]) OR "mass screening"[All Fields] OR "screening"[All Fields] OR "early detection of cancer"[MeSH Terms] OR ("early"[All Fields] AND "detection"[All Fields] AND "cancer"[All Fields]) OR "early detection of cancer"[All Fields]) AND (acceptability[All Fields] OR acceptance[all fields] OR tolerability[all fields] OR tolerance[all fields] OR "Patient acceptance of Health Care"[MeSH Terms])

Search 2(b): Acceptability of screening for anal HPV or AIN/HSIL or anal cancer in WLHIV

Database: Embase using the Ovid platform

Latest search date: December 20, 2015

Number of original results: 52

1. anal cancer.mp. or Anus Neoplasms/
2. mass screening/
3. screen*.mp.
4. 2 or 3
5. acceptability.mp. or "Patient Acceptance of Health Care"/
6. accept*.mp.
7. toler*.mp.
8. 5 or 6 or 7
9. squamous cell carcinoma of the anus.mp.
10. anal carcinoma.mp.
11. Papillomavirus Infections/ or Papillomaviridae/ or human papillomavirus.mp. or Papilloma/
12. HPV.mp.
13. 11 or 12
14. anal intraepithelial lesions.mp. or Precancerous Conditions/
15. anal dysplasia.mp.
16. HSIL.mp.
17. high-grade squamous intraepithelial lesions.mp.
18. 13 or 14 or 15 or 16 or 17
19. anus.mp. or Anal Canal/
20. anal.mp.
21. 19 or 20
22. 1 or 9 or 10 or 18
23. 21 and 22
24. 4 and 8 and 23

APPENDIX B:

PREPARATION OF MANUSCRIPT-BASED (ARTICLE-BASED) THESES

The following is a direct reproduction of the McGill University guidelines for the preparation of manuscript-based (article based) theses (Copyright © 2016 McGill University). Sections of text that have been abridged or removed are indicated by "[…]".

Manuscript-Based (Article-Based) Theses

As an alternative to the traditional thesis format, the thesis research may be presented as a collection of scholarly papers of which the student is the author or co-author; that is, it can include the text of one or more manuscripts, submitted or to be submitted for publication, and/or published articles reformatted according to thesis requirements as described below. […]

The thesis must contain additional text that will connect them, producing a cohesive, unitary focus, and documenting a single program of research. A Manuscript- (or Article-) based thesis will be judged by the examiners as a unified, logically coherent document in the same way a traditional thesis is judged.

The structure for the manuscript-based thesis must conform to the following:

- Just as in the traditional format, the thesis must be presented as a unified whole with respect to font size, line spacing and margin sizes (see thesis format).
- The thesis must conform to all other requirements listed under thesis components […].
- The thesis must be more than a collection of manuscripts. All components must be integrated into a cohesive unit with a logical progression from one chapter to the next, providing a cohesive, unitary focus, documenting a single program of research. Connecting text must be provided so that the completed thesis functions as an integrated whole.

- There is no specified number of manuscripts or articles required for a Master's or a Doctoral thesis, nor is prior publication or acceptance for publication of the manuscripts a requirement. Publication or acceptance for publication of research results before presentation of the thesis in no way supersedes the University's evaluation and judgment of the work during the thesis examination process (i.e., it does not guarantee that the thesis will be found acceptable for the degree).
- In the case of multiple-authored articles, the student must be the primary author. Multiple-authored articles cannot be used in more than one thesis. In the case of students who have worked collaboratively on projects, it may be preferable for both students to write a standard format thesis, identifying individual contributions.

APPENDIX C: ACCEPTABILITY QUESTIONNAIRE

EVVA STUDY: ACCEPTABILITY QUESTIONNAIRE

(To complete at the end of the last visit or when a participant choses to exit the study.)

Date: _____(YYYY/MM/DD)

If anal cancer screening becomes part of routine care for women living with HIV, we need to know what could be an acceptable procedure at an acceptable frequency.
Since you experienced these procedures through your participation in this study, your opinion is very important.
All questions use a scale from 0 to 10. Please indicate an X in the box below the number that best represents your personal opinion.

1. How worried are you about anal cancer (if no screening program is available)?

0	1	2	3	4	5	6	7	8	9	10
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Not worried at all — Extremely worried

2. To what extent would you want anal cancer screening to become part of routine care for all women living with HIV?

0	1	2	3	4	5	6	7	8	9	10
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

I'm against it — It's an absolute necessity

Assessment of pain felt during procedures

3. How would you describe the pain felt during the CERVICAL procedures performed by the NURSE (insertion of speculum in vagina and cervical cell sampling for PAP and HPV tests)? Base your answer on the most recent visit.

0	1	2	3	4	5	6	7	8	9	10
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

No pain at all — Worst pain ever felt

4. How would you describe the pain felt during the ANAL procedures done by the NURSE (cotton-tip swabs in the anal canal)? Base your answer on the most recent visit.

0	1	2	3	4	5	6	7	8	9	10
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

No pain at all — Worst pain ever felt

5. How would you describe the pain felt during the DIGITAL RECTAL EXAM done by the ANOSCOPIST (when Dr Coutlée or Dr Muñoz inserted a finger in the anal canal to check for masses or palpable abnormalities, before the High Resolution Anoscopy)? Base your answer on the most recent visit.

0	1	2	3	4	5	6	7	8	9	10
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

No pain at all — Worst pain ever felt

6. How would you describe the pain felt during the HIGH RESOLUTION ANOSCOPY procedure done by the ANOSCOPIST (examination of the anal canal through a plastic tube and biopsies done by Dr Coutlée or Dr Muñoz)? Base your answer on the most recent visit.

0	1	2	3	4	5	6	7	8	9	10
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

No pain at all — Worst pain ever felt

Acceptability of procedures as screening tools

7. Cervical PAP tests are currently recommended every year in women living with HIV to screen for cervical cancer. How acceptable do you consider YEARLY CERVICAL PAP TESTS as a screening tool for cervical cancer?

0	1	2	3	4	5	6	7	8	9	10
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Not acceptable (Don't want to do it ever again) — Very acceptable (So easy I could even do it more often)

8. How acceptable would you consider the ANAL PAP TEST and/or HPV TEST as screening tools at the following frequencies? This implies 1 or 2 cotton-tip swabs in the anus. A High Resolution Anoscopy would only be done only if the results were abnormal.

	0	1	2	3	4	5	6	7	8	9	10
Every year:	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Every 2 years:	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Every 5 years:	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Not acceptable (Don't want to do it ever again) — Very acceptable (So easy I could even do it more often)

We know that PAP tests and HPV tests are not perfect tests and they sometimes don't detect all precancerous lesions. Therefore, some experts suggest that it would be safer to use High Resolution Anoscopy directly as a screening tool.

9. How acceptable would you consider HIGH RESOLUTION ANOSCOPY as a screening tool (i.e. to be done as prevention, when no symptoms are felt) at the following frequencies? This is the procedure that was performed through the clear plastic tube by Dr Coutlée or Dr Muñoz.

	0	1	2	3	4	5	6	7	8	9	10
Every 2 years:	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Every 5 years:	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Every 10 years:	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Not acceptable — Very acceptable

(Don't want to do it ever again) (So easy I could even do it more often)

Finally, even if screening for precancerous lesions does not become part of routine care, some experts suggest that screening for established anal cancer should be part of routine care through a yearly digital rectal exam in people living with HIV.

10. How acceptable would you consider DIGITAL RECTAL EXAM as a screening test for anal cancer at the following frequencies?

	0	1	2	3	4	5	6	7	8	9	10
Every year:	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Every 2 years:	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Every 5 years:	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Not acceptable Very acceptable
(Don't want to do it ever again) (So easy I could even do it more often)

If you scored any item below 5 in questions 7-10, please specify why you don't think it's an acceptable procedure at that frequency. Here are some examples of answers: too painful, too embarrassing, too time consuming, not convinced of the utility, etc…

Q7, every year :_____

Q8, every year: _____
Q8, every 2 years: _____
Q8, every 5 years: _____

Q9, every 2 years: _____
Q9, every 5 years: _____
Q9, every 10 years: _____

Q10, every year: _____
Q10, every 2 years: _____
Q10, every 5 years: _____

Other comments :

_____

_____

_____

Thank you for your participation in the EVVA study.
We really appreciate your help.

APPENDIX D: EVVA STUDY INFORMED CONSENT FORM

INFORMED CONSENT DOCUMENT

Study Title: Evaluation of Human Immunodeficiency Virus, Human Papilloma Virus, and Anal Intraepithelial Neoplasia in Women: EVVA study

Principal Investigator: Dr Alexandra de Pokomandy (MUHC)

Sponsor: Canadian Institute of Health Research (CIHR)
Grant title: Is human papillomavirus causing an epidemic of anal dysplasia in HIV-positive women?

Study site: Montreal Chest Institute
3650 St-Urbain Street, 8th Floor
Montreal (Quebec) H2X 2P4

INTRODUCTION

You are being invited to take part in this research study because you are a woman infected with HIV (human immunodeficiency virus). This is a study which will evaluate the evolution of human papilloma virus (HPV) infections in a population of HIV-infected women.

Before you decide to take part in this study, you should read the following document. Please take your time to make your decision about taking part. You may discuss your decision with your friends and family. You can also discuss it with your health care team. If you have any questions, you can ask the members of the study staff to answer your questions and explain any word or information that you do not understand.

BACKGROUND

Human papillomaviruses (HPV) are responsible for oral, vulvar, vaginal, cervical and anal cancers. HIV-positive men and women are more at risk of anal cancer than HIV-negative populations. However, most of the infections caused by HPV are short-lived. We would like to study the evolution of anal infection and anal lesions caused by HPV taking into account some factors such as: your HIV viral load (the number of HIV copies in your blood), your CD4 cell count (the number of immune cells), the fact that you smoke cigarettes or not. Some sexual behaviours are also taken into account, such as anal penetration, the number of sexual partners and condom use.

PURPOSE OF THE STUDY:
The purpose of this study is to evaluate the presence and evolution of HPV anal infections and anal precancer over 2 years.

STUDY DESCRIPTION
This study will include 180 HIV-positive women in Montreal. Participants will be followed at 6 months intervals for 2 years, for a total of 5 study visits.

In order to be eligible for this study, you must be an HIV-positive woman, aged more than 18 years old, not currently pregnant and have never received the diagnosis of anal cancer in the past or at the first study visit.

STUDY PROCEDURES

Screening visit- (Week -8 to -1)
Eight weeks prior to being enrolled into the study, you will be assessed to determine if your health status meets the eligibility criteria to take part in this study. If you do not meet all the eligible criteria, you will be excluded from the study.

If you are eligible and agree to take part in this study, you will be asked to return to the clinic to meet with one of our research staff at the start of the study (Baseline -Week 0) and subsequently at every 6 months during 2 years. You will be advised to abstain from anal douching, vaginal sex and anal sex in the 48 hours prior to any study visit.

Baseline- Week 0
This study visit will last approximately 2 hours and you will undergo the following procedures:

- You will be asked to complete a questionnaire about factors that could increase your risk of HPV infection (general characteristics, smoking, drug use, medical history and sexual practices). If you do not feel comfortable completing any of the questions, please speak with the research staff and she/he will assist you.
- You will undergo a regular cervical PAP test and HPV test. You will undergo an anal PAP test and HPV test. You will undergo an anus examination through a high resolution anoscopy for biopsy samples collection. A review of your medical chart (at your usual HIV clinic) will be done to collect data about your medical history.

Follow-up visits- (Month 6, 8 and 12)
These study visits will last approximately 1.5 hours and you will undergo the following procedures:

- All tests and procedures performed at the Baseline visit, excluding the high resolution anoscopy.

Final study visit- (Month 24)
This study visit will last approximately 2 hours and you will undergo the following procedures:

- All tests and procedures performed at the Baseline visit.

PAP tests and HPV tests

These procedures will be conducted at each study visit.
You will be asked to get undress and lie on the examining table. The nurse will insert a speculum in your vagina to do a regular PAP test. She will collect cells by scraping a wooden spatula and a very small brush on your cervix. She will also collect cells from your cervix with a swab to test for the presence of HPV. The speculum will be removed and she will insert two consecutive cotton-tipped swabs in your anus to collect cells from the anal canal. This sample will be used to evaluate the presence of precancerous cells (anal PAP test) and the presence of HPV, and it does not cause any pain.

At the first visit of the study and at the last visit of the study, you will meet a trained physician who will examine your anus through a high resolution anoscopy (HRA). This procedure is the equivalent to the colposcopy for the cervix if you ever had one. The trained physician needs to do this procedure to verify for the presence of anal precancer lesions. He will first insert a swab filled with vinegar in your anus for approximately 3 minutes. This allows the lesions caused by HPV to become white and be more easily identified by the physician. He will then introduce a short and clear plastic tube in your anus (called an anoscope), and will look inside your anus through a magnifying lens equipped with a light. He will then perform an anal biopsy. A biopsy is the collection of a small sample of the anal mucosa. Biopsies will be used to detect the presence of precancerous cells.

- If an invasive cancer is found, you will rapidly be referred to a specialist and your participation in the study will end.
- If we find precancer on the biopsy, you will be offered additional HRA during the study according to your personal results. We will offer you treatment if the lesion persists.
- If an anal PAP test (analysis of your anal cells) shows a high suspicion of precancer, you will be offered an additional HRA at the next visit.
- If your cervical PAP test is abnormal, you will be referred to a gynaecologist for investigation.

POTENTIAL RISKS AND/OR DISCOMFORTS

High Resolution Anoscopy
The HRA may cause some discomfort when the anoscope is introduced into your anus. During the exam, you may feel pressure in the rectum similar to the sensation you feel with the urge to have a bowel movement. You may also feel a small amount of cramping in your abdomen as well. The biopsy is an invasive procedure. The most serious risk of the procedure is poking a hole in the lining of the intestine. However, it is extremely uncommon, occurring once out of every 10,000 procedures. Some bleeding will likely occur when the biopsies are done. If you should experience severe discomfort during the biopsy, the procedure will be stopped immediately; the physician and the research nurse will monitor you until you are ready to go home. You may feel a slight discomfort 24 hours after the biopsy and you may have some light bleeding for 24 hours. Overall, other than temporary discomfort, the proposed procedures present no risk for your health.

Pregnancy
Women who are already pregnant are not allowed to participate in this study. However, women planning pregnancy may participate; if you become pregnant during the study, you should inform your treating physician (Gynecologist/Obstetrician) about your participation. You may continue your participation in the study, but no High Resolution Anoscopy will be performed during your pregnancy.

POTENTIAL BENEFITS
You should not expect any direct benefit from taking part in this study, besides being screened for anal cancer. However the information gained from this study will help add medical knowledge about anal HPV infection and anal precancer in HIV-positive women. It will allow us to decide if we should recommend routine anal cancer screening or not as part of women-specific HIV health care, and will help to identify which women are at higher risk of developing precancerous anal lesions due to HPV.

SIGNIFICANTS FINDINGS
You will be told by the study doctor or his/her staff of any significant new findings that develop during the course of this study that may affect your willingness to continue participating in this study. You may then use this information to make a decision about remaining in the study. Also, at the end of the study, your physician will inform you of the research findings.

ALTERNATIVE TO STUDY RESEARCH
You do not have to join this study to receive treatment for your condition. In addition, there may be other research studies looking at other ways of managing this stage of HIV infection. Your doctor will discuss any of these options with you.

STORAGE AND USE OF SPECIMENS
At each study visit, collected cervical and anal specimens will be processed and stored at Dr. Coutlée's laboratory (Centre Hospitalier de l'Université de Montréal (CHUM)). Participants may decide at any point not to have their specimens stored. In this case, the principal investigator will destroy all known remaining specimens and report what was done to both the participant and to the Ethics Committee of the Institution. The samples will not be made available to any commercial enterprise.

We will protect the confidentiality of your samples. Any personal identification will be coded, upon the assignment of a unique identifier. Scientists working on the sample will only be able to identify a sample by its assigned number but will not know who you are. This unique identifier will be used to store your sample and any corresponding data until the final study report has been written. No human genetic testing will be performed on your sample. At the end of this study, your sample will be kept in storage by the investigator for a period up to 15 years.

COST AND COMPENSATION
You will receive a $40 compensation for each of the study visits with a high resolution anoscopy and 20$ for each of the other visits to reimburse your parking or taxi fees and child care. You will not be charged for any research procedure.

INDEMNIFICATION / COMPENSATION IN CASE OF INJURY
In the event you suffer injury as a direct result of participating in this study, necessary medical treatment not covered by provincial health insurance will be made available at no additional cost to you. No other form of compensation will be awarded for injuries or complications related to this research. However, by signing this consent form, you are not waiving any of your legal rights nor are you freeing the investigators, sponsors, or the health establishment where the study takes place from their legal and professional responsibilities.

VOLUNTARY PARTICIPATION AND STUDY WITHDRAWAL
Your participation in this study is entirely voluntary. If you decide not to take part or to withdraw from the study at any time, you will not be penalized nor lose any benefits to which you are otherwise entitled and your medical care and treatment will in no way be affected. If you choose to participate, you may change your mind and withdraw at any time and may refuse to answer any question you do not want to answer.

CONFIDENTIALITY
If you decide to participate in this study, the study doctor and research team will have access to your medical file to collect information that identifies you. This may include your name, address, phone number, health plan number, date of birth, medical history, and medical-related information, which shall be collected from your treating physician (or other health care workers). All personal information obtained during this study will be kept confidential.

All information shared in the questionnaires and HPV tests will remain confidential. They will not be included in your medical record. The results of the biopsies and the cytologies will however be included in your medical record. Should any information be of importance for your clinical care, the pertinent results will be provided to your HIV physician.

You will have a separate study file that will be kept in a locked cabinet under strict supervision. This file will only be consulted by the research team. Your name will be coded and the code list will be kept in a locked filing cabinet at the study site with limited access. This code will be the only identifying information on all your forms. Finally, when all the research data is entered on computer for final analysis, your code number will be the only identifying information used.

The Principal Investigator will use the study data for research purposes to support the scientific objectives of the study described in the consent document, to better understand the disease(s) included in the study, or to improve the design of future studies.

Your study data might be provided to the following groups, if required:

- Representatives of the Sponsor
- Government regulatory agencies, including Health Canada, the United States FDA and similar government agencies from other countries;
- MUHC-MGH Research Ethics Board (people who review the research study to protect your rights);
- Any other entity as required by law.

To verify the research data, other physicians participating in this research study at this institution and monitors from the following organizations may review your original medical chart (contains information that can directly identify you) for quality assurance and data analysis:

- The Sponsor or its representatives (e.g., clinical monitors and auditors) may inspect research and medical records in the presence of the investigator or study staff, however, they will not be able to record any information and no such records will be allowed to leave the investigator's office;
- The Quality Assurance Officer from the MUHC-MGH Research Ethics Board;
- Government regulatory agencies, including Health Canada, the United States FDA, or their authorized representatives. Information may be provided to these agencies in a way that maintains your privacy according to United States and Canadian regulations.
- Any other entity as required by law.

By signing this consent form, you give us permission to release and disclose your personal health information as described above, and to inform your treating physician of your study participation. The results from this study may be published, however your identity will not be revealed in the combined results. Your confidentiality will be protected to the extent permitted by applicable laws and regulations.

CONTROL OF ETHICAL ASPECTS OF THE RESEARCH PROJECT

The Research Ethics Board of the MUHC approved this research project and ensures the follow-up. In addition, it will first approve any review and amendment made to the information/consent form and to the study protocol.

QUALITY ASSURANCE PROGRAM

The MUHC implemented a Quality Assurance Program that includes active continuing review of projects (on site visits) conducted within our establishment. Therefore, it must be noted that all human subject research conducted at the MUHC or elsewhere by its staff, is subject to MUHC Routine and Directed Quality Improvement Visits.

FUNDING OF THE RESEARCH PROJECT

This research study is being funded by the Canadian Institute of Health Research (CIHR) and will be run by Dr. Alexandra de Pokomandy. The study doctor named above will be looking after you during your participation in this study.

INFORMATION AND CONTACTS

- If you have questions about this clinical research study, you may contact, **Dr. Alexandra de Pokomandy** during working hours at **(514) 843-2090 or the study coordinator, Christina de Castro** at **514-934-1934 extension 32225**.

- In case of emergency during clinic hours (8:00-16:00), contact **Dr. Alexandra de Pokomandy at 514 843-2090**. After working hours, call **514 934-1934, ext. 33333** and ask for the physician-on-call for the immunodeficiency service.

- If you have questions concerning your rights as a Research Participant and wish to discuss them with someone not connected to the clinical research study, please contact the **Ombudsman of the McGill University Health Centre (514) 934-1934 extension 35655.**

- If you believe you have been injured as a result of participating in this study, you may contact the **Director of Professional Services at (514) 934-1934, ext. 34329**

Study Title: Evaluation of Human Immunodeficiency Virus, Human PapillomaVirus, and Anal Intraepithelial Neoplasia in Women: EVVA study

DECLARATION OF CONSENT

I have read this 8-page informed consent form and I voluntarily agree to participate in this research study, understanding that I may withdraw my participation at any time. I have had the opportunity to ask questions and all of my questions have been answered to my satisfaction. I have been given sufficient time to consider the above information and to seek advice.

I grant direct access to my study and medical records. I will be given a copy of this signed and dated Informed Consent Form.

By signing this consent form, I am not waiving any of my legal rights nor am I freeing the investigators, sponsors, or the health establishment where the study takes place from their legal and professional responsibilities.

_____	_____	_____
Participant's signature	Name (in block letters)	Date

I confirm having met with the participant at the time of enrolment to answer questions about this study

_____	_____	_____
Signature of Person Administering Informed Consent	Name (in block letters)	Date

_____	_____	_____
Signature of Investigator or Co-Investigator	Name (in block letters)	Date