Journal of Infectious Diseases Advance Access published April 26, 2013

Evaluation of HIV and HAART on the natural history of HPV infection and cervical cytopathology in HIV-positive and high risk HIV-negative women

Sandra Blitz[1], Joanna Baxter[2,3], Janet Raboud[1,4], Sharon Walmsley[1,5,a], Anita Rachlis[5,6,a], Fiona Smaill[7,a], Alex Ferenczy[8,9], François Coutlée[10,11,a], Catherine Hankins[12,13,14,a], Deborah Money[2,3,a], for the Canadian Women's HIV Study Group[a]

[1]Toronto General Research Institute, University Health Network, Toronto, Ontario, Canada M5G 2C4

[2]Department of Obstetrics and Gynecology, University of British Columbia, Vancouver, British Columbia, Canada V6Z 2K5

[3]Women's Health Research Institute, BC Women's Hospital, Vancouver, British Columbia, Canada V6H 3N1

[4]Dalla Lana School of Public Health, University of Toronto, Toronto, Ontario, Canada M5T 3M7

[5]Department of Medicine, University of Toronto, Toronto, Ontario, Canada M5G 2C4

[6]Sunnybrook Research Institute, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada M4M 3M5

[7]Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5

[8]Departments of Pathology and Obstetrics and Gynecology, McGill University, Montreal, Quebec, Canada H3A 2T5

[9]Jewish General Hospital, Montreal, Quebec, Canada, H3T 1E2

[10]Département de Microbiologie et Infectiologie, l'Université de Montréal, Montréal, Québec, Canada H3C 3J7

[11]Centre de recherche du CHUM, Centre Hospitalier de l'Université de Montréal, Montréal, Québec, Canada H2L 4M1

[12]Department of Global Health, Academic Medical Centre, University of Amsterdam and Amsterdam Institute for Global Health and Development, 1100 DE Amsterdam, The Netherlands

[13]Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, United Kingdom WC1E 7HT

[14]Département de Médecine Sociale et Préventive, Université de Montréal, Montréal, Québec, Canada H2V 4P3

Corresponding Author: Deborah Money, MD, Rm B327B, 4500 Oak Street, Vancouver, BC, V6H 3N1, PH: 604-875-3459, Fax: 604-875-3954, Email: dmoney@cw.bc.ca

[a]Members of the Canadian Women's HIV Study Group are listed in the Footnotes section.

ABSTRACT

Background: The Canadian Women's HIV Study (CWHS) enrolled HIV-positive and high-risk HIV-negative women in a longitudinal cohort. This analysis considered the effects of HIV and HAART on HPV persistence and cervical squamous intraepithelial lesion (SIL).

Methods: Longitudinal cytopathology and HPV-DNA results were analyzed using multi-state models. States of cervical SIL were defined as absent, present and treatment; HPV states as negative or positive. Demographic variables and markers of sexual activity were considered predictors. Results were calculated based on transition probabilities and reported as hazard ratios.

Results: The CWHS followed 750 HIV-positive and 323 HIV-negative women between 1993-2002. 467 and 456 women were included in the longitudinal cervical cytopathology and HPV-DNA analyses, respectively. HIV-positive women had increased prevalence (46.6% vs. 28.7% p<.0001), increased acquisition (HR=2.3, p=0.03), and decreased clearance (HR=0.4, p<0.001) of oncogenic-HPV compared to HIV-negative women. Oncogenic HPV infection predicted progression of cervical dysplasia from normal to abnormal SIL (HR: 2.8, p=0.002). Among HIV-positive participants, HAART increased the likelihood of regression (present to absent) of cervical SIL (HR: 3.3, p=0.02) and increased the clearance of oncogenic HPV types other than HPV16 or HPV18 (HR=2.2, p=0.01).

Conclusion: This analysis demonstrated beneficial effects of HAART on cervical SIL in HIV-positive women.

INTRODUCTION

It is now well established that high risk human papillomaviruses (HR-HPV) are the causative agents of invasive cervical cancer (ICC).[1-3] Twelve HR-HPV types are identified and 14 additional types considered probable carcinogens.[4-6] In North America, HPV16 and HPV18 are responsible for 55.2% and 21.3% of cervical cancers, respectively, and together account for about 70% of cervical cancers worldwide.[4-7]

The disease burden of HPV is further confounded by co-infection with HIV, particularly in resource-constrained settings. All HPV-related urogenital malignancies (including ICC, vulvar cancer and anal cancer) are more prevalent in populations with high HIV prevalence.[8, 9] Although studies have shown that HIV-infected women are at high risk of HPV infection, increased HPV prevalence alone does not account for the rate of cervical disease observed.[9-12] It is hypothesized that HIV infection and the consequent immunodeficiency negatively affects HPV clearance time [10, 13] and prolonged persistence accounts for the development of cervical high grade SIL (HSIL) and the increased risk of ICC seen in this population.[1, 9, 14-16]

Type-specific distributions of HPV in HIV-positive women differ from HIV-negative women in that the former display a broader range of genotypes that are more likely to include HR-types, [1, 17, 18] and be multiple-type infections. [19] New evidence also suggests there is less association of HPV16 with cervical SIL/ICC in HIV-positive women than the general population.[19-21] It is speculated that the natural history and prolonged persistence of HPV16 and HPV18 could behave independently of HIV-serostatus [22] and CD4 count [23], whereas other HR-HPV types (e.g. 51, 52, 58), multiple types, and co-infection with low-risk types may be more prominently featured in ICC in HIV-positive women.[19, 20]

The effect of immune suppression on HPV infection is unclear. In some studies, low CD4+ counts and increased HIV viral loads have been independently associated with the incidence and delayed clearance of HPV infections, as well as with the risk of progression of cervical SIL [11, 12, 24-26]. Since highly active anti-retroviral therapy (HAART) is known to improve CD4+ counts and decrease HIV viral loads, it was expected that HAART would improve outcomes of HPV infection. Patel demonstrated no significant change in the overall incidence of cervical cancer and an increase in anal cancer among HIV-positive individuals between 1992 and 2003, and reported that antiretroviral therapy (ARV) significantly decreased the risk of cervical cancer but not anal cancer.[27] Conversely, earlier epidemiological evidence has failed to show a decrease in the incidence of ICC since the introduction of HAART in 1996.[28, 29] This may be explained by the increased longevity of antiretroviral treated HIV-positive patients allowing time for increased exposure to oncogenic mutations and for the progression of HPV-related disease[9] or to increased awareness and surveillance in this population.

The Canadian Women's HIV Study (CWHS) was a prospective, multi-centered cohort study of HIV-positive and high risk HIV-negative women conducted between 1993 and 2002.[12] The period of data collection covered the beginning of the HAART era, enabling a unique opportunity to provide evidence-based data of the impact of HAART on the natural progression of HPV in HIV-positive women. The objective of this analysis was to determine the effects of HIV status, HAART use, CD4 cell recovery and other factors on the acquisition/clearance of HPV and the progression/regression of SIL by cytology.

MATERIALS AND METHODS

Participants: HIV-positive and high risk HIV-negative women aged 15-44 were followed in the CWHS. In brief, this multi-centered prospective cohort study collected data from 1993 to 2002 at 28 institutions across Canada. Ethics approval was obtained from each participating institution. The cohort has been previously described in more detail. [12]

Recruitment: HIV-positive participants were recruited from community-based or tertiary care centers while high risk HIV-negative women were recruited from sexually transmitted infection (STI) clinics or birth control centers. HIV-negative women were required to have >3 lifetime sexual partners. Questionnaires, administered by study nurses at enrollment and semi-annually, collected data on demographics, gynecologic and reproductive history (including sexual history). For HIV-positive participants additional questions addressed symptomatology, AIDS related illnesses, and HIV and non-HIV drug treatments. Routine clinical HIV blood work, including CD4 counts and HIV viral load tests, were recorded from local sources when available.

All participants were sampled semi-annually with conventional Pap cytology and vaginal HPV testing. Cytology smears were reviewed at a central reference laboratory and classified according to the 1991 Bethesda classification system.[30] As per local standardized guidelines, women with abnormal cytology were referred for colposcopy and biopsy and treatment as appropriate.

HPV sampling was conducted by use of vaginal tampons and/or cervicovaginal lavages (CVL) and processed as previously described.[31] Sample lysates were tested for HPV detection with MY09/MY11/HNB01 primers and radioactive probes for 14 types

including 12 oncogenic types (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58) from 1994 to 2001, and with the PGMY-line blot assay (Roche Molecular Systems, Alameda, California, US) for 27 types including 12 oncogenic types (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59) from 2001 to 2003.[12, 31, 32] We reported previously a concordance of 99.8% between these assays of genotyping results.[32]

Statistical Analysis: Patient characteristics were summarized by HIV status using medians with interquartile ranges for continuous variables, and frequencies with percentages for categorical variables. Continuous variables were compared between groups with the Wilcoxon test and categorical variables were compared between groups with the chi square test or Fisher's exact test, as appropriate. We report unadjusted p-values for the pairwise comparisons for the prevalence of each HPV type.

Multi-state time-homogenous Markov models were used to analyze the longitudinal cytology and HPV results. Generally, these models can be used to model bi-directional transitions through different disease stages by individuals over time.[33, 34] The two basic assumptions for time-homogenous multi-state models are the transition rate from one state to another is constant over time, and the probability of a transition between two states depends on the time between observations, not the specific time of the observation. We applied this modeling strategy to the progression and regression of cervical SIL and the acquisition and clearance of HPV types over follow-up. Covariate effects are reported as hazard ratios calculated from the estimated transition intensities under a time-homogenous assumption.[35] Although planned, multivariate modeling was not carried out due to the low number of observed transitions.

Cervical Squamous Intraepithelial Lesion (SIL)

For the progression and regression of SIL we considered a 3-state Markov model. Cytopathology results from each visit were categorized as Absent (A) and Present (P). The Present category included atypical squamous cells of undetermined significance (ASC-US), LSIL and HSIL. Low rates observed in each category precluded modeling a less heterogeneous grouping. Treatment (T) for SIL and invasive cancer (IC) was a unique absorbing state. All subsequent cytopathology results for women following treatment for SIL were censored at the date of treatment initiation. Cytopathology results were also censored after any non-SIL-related ablative or excisional cervical procedures (e.g. hysterectomy for other diagnosis). Participants were included if they had at least 2 Pap test results or 1 Pap test result followed by SIL treatment. Since the actual cervical disease status of women with an ASC-US result is unknown, sensitivity analyses were conducted with ASC-US included in the Absent results, ie. ASC-US was grouped with normal results, restricting Present results as LSIL, HSIL and IC.

Hazard ratios were estimated for transitions from absent-to-present states, present-to-absent states, and from present states to treatment. The observed absent-to-treatment transitions were assumed to have progressed through unobserved absent-to-present and present-to-treatment transitions. That is, the probability of a transition from a normal state to treatment was *a priori* set to 0.

Acquisition and Clearance of HPV

A 2-state Markov model was used to assess acquisition and clearance probabilities of HPV over time. We considered 3 outcomes: (1) presence of 1 or more oncogenic HPV types; (2) presence of HPV16; (3) presence of at least 1 oncogenic HPV other than 16 or 18. Participants were included if they had at least 2 HPV genotype

results prior to any cervical procedures including those for SIL. Only results from the cervicalvaginal lavage samples were used and observations after any cervical procedures were excluded.

Covariates

Variables considered for association with multi-state transitions were: HIV status; age; ethnicity (white versus non-white); number of lifetime sexual partners (<5 versus > 5); and, for HIV-positive women, CD4+ count and antiretroviral (ARV) treatment (HAART: yes/no). Current ARV therapy was recorded at every visit, and HAART was defined according to the contemporary definition of 2 nucleoside reverse transcriptase inhibitors (NRTI) and at least one of the following: a protease inhibitor (PI), a non-nucleoside reverse transcriptase inhibitor (NNRTI), or an additional NRTI. HAART was set to no when a participant was not on any ARV or a pre-HAART era ARV regimen.

Statistical summaries were conducted using SAS Statistical Software, version 9.3 by SAS Institute Inc, Cary, NC. Multi-state models were fit using the *msm* package for R (http://www.r-project.org).

RESULTS

Study Population

In total, 1073 women were enrolled in the CWHS between 1993 and 2002. Approximately 70% of the cohort was HIV-positive. The HIV-positive and HIV-negative populations differed on many characteristics (Table 1). HIV-negative women were younger, more likely to be white, less likely to be an immigrant from an HIV-endemic country, more likely to have a smoking history and were more likely to have ever used illicit drugs. HIV-positive women had fewer lifetime sexual partners and were more

likely to have one or more oncogenic HPV types detected in the first available vaginal sample.

Among HIV-positive women, the median age at HIV diagnosis was 30 years (IQR: 25-35) and median duration of HIV infection was 2 years (IQR: 1-5 years) at the time of enrolment. Median CD4 at enrollment was 336 cells/mm^3 (IQR: 180-515). Of the 54% on ARV (n=408), 38% were on NRTI-monotherapy, 26% were on NRTI-dual therapy and 35% were on HAART. By the end of the study 64% of all HIV-positive participants had documented HAART use.

In this cohort, 307 (29%) did not return after enrolment (Supplemental Figure 1). Only 456/870 women with at least one cervical Pap result had sufficient data for inclusion in the multi-state model for progression/regression of SIL. There were no clinically important differences in those excluded due to insufficient data. Only 467/930 women with at least one HPV result had the >2 results to permit inclusion in the multi-state model for acquisition/clearance of HPV. Although no difference in the number of oncogenic HPV types identified at their first visit, participants with insufficient follow-up for inclusion in the HPV transition models had worse baseline cervical cytology results and were more likely to have previous abnormal cytology results.

Demographic differences between the HIV-positive and HIV-negative groups noted for the entire sample were similar to those in the data subsets in the cervical SIL and HPV transition models.

Cervical SIL

Four hundred fifty-six participants (130 HIV-negative/326 HIV-positive) were included in the longitudinal analysis. Median (range) follow-up was 24 (5-109) months for HIV-positive women compared to 14 (5-51) months for HIV-negative (p<0.001).

During ollow-up 23 (5%) women had treatment for SIL, 86 (19%) had progression of SIL (ie. higher grades of SIL observed in follow-up), 299 (66%) had stable SIL results and 48 (10.5%) had regression of SIL (ie. lower grades of SIL were observed in follow-up) (Table 2).

Multi-state model

The 456 participants contributed 1572 PAP results (or 1116 transitions; shown in Table 3) to the multi-state model. The median (interquartile range; IQR) time between cervical exams was 243 (195, 391) days; about half of the women (52%) only had 2 PAP results, 19% had 3 results, 10% had 4 results, and the remaining 18% had between 5 and 15 results.

In univariate multi-state models, cytological progression (absent to present transitions) was more likely among women with > 5 lifetime sexual partners (HR=1.70, p=0.04), coincident HPV16 infection (HR=2.50, p=0.04), or any other coincident HR-HPV other than 16/18 (HR=2.76, p<0.01). Cervical SIL regression (present to absent transitions) was more likely among women with > 5 lifetime sexual partners (HR=2.07, p<0.01). Transitions to the treatment state were more likely among those with HPV16 present (HR=3.21, p<0.01). HIV-positive women on HAART were found to have a higher likelihood of cervical lesion regression compared to HIV-positive women with similar characteristics but not on HAART (HR=3.32, p=0.02). (Table 4)

The sensitivity analyses that grouped ASC-US within normal cytology yielded similar results (data not shown).

HPV

Each of the 11 oncogenic HPV types tested was more prevalent among HIV-positive than HIV-negative women and statistically significant differences were detected in 8 types (HPV-31, 33, 35, 39, 45, 52, 56 and 58) (Figure 1). Although HPV16 was the most prevalent, found in 10.8% of samples, there was no difference in HPV16 prevalence between the HIV-positive and HIV-negative women. Among HIV- positive women the prevalence of oncogenic HPV was 39% among those on HAART at enrollment compared to 48% among those not on ARVs or on a pre-HAART regimen (p=0.09).

Multi-state model

467 participants (134 HIV-/333 HIV +) with 1531 HPV genotype results (or 1064 transitions) were included in the model, as shown in Table 5. In the multi-state models for acquisition (from none to detection of one or more oncogenic HPV types) and clearance (from one or more oncogenic HPV types to none) (Table 6), acquisition was more likely among HIV-positive women (HR=2.28, p=0.03) and less likely as age increased (HR=0.70 per 10 years, p=0.03). Clearance was less likely among HIV-positive women (HR=0.41, p<0.001). When considered separately, the likelihood of HPV16 acquisition decreased with age (HR=0.47, p<0.001) but was higher among white women (HR=2.31, p=0.04) and HIV-positive women with CD4 cell counts <200 cells/mm^3 (HR=4.82, p<0.01). Clearance of HR-HPV types other than 16/18 was less likely among HIV-positive women (HR=0.32, p<0.001) but more likely among HIV-positive women on HAART (HR=2.20, p=0.01).The low prevalence and frequency of HPV18 transitions precluded model-fitting for this outcome.

DISCUSSION

This study conducted over a period of transition into HAART, shows that in a context of adequate access to cervical screening and treatment, HAART is associated with a greater rate of regression of cervical SIL as determined by cytology. Similar cohort studies have reported trends toward improved outcomes for cervical disease with HAART, although many failed to reach statistical significance.[36, 37]

Despite the causal link between HPV and cervical disease, improvement in HPV clearance in the presence of HAART have not been widely observed.[36] Paramsothy demonstrated HAART increased the likelihood of clearing HPV among women with LSIL at study entry.[37] Although a non-statistically significant trend towards improved HPV clearance was noted for women with normal or ASC-US cytology at baseline, Fife demonstrated a small decline in the proportion of subjects in whom at least 1 HPV type was detected from 66% at baseline to 49% 96 weeks post-HAART initiation, and a significant decrease in HR-HPV types from 62% to 39%.[38]

Overall, our study revealed higher acquisition and reduced clearance of oncogenic HR-HPV among HIV-positive compared to HIV-negative women. The effect estimates were similar in the subset of non-HR-HPV 16/18 types and for the acquisition of HPV16, but not the clearance of HPV16 alone. One strength of our study is that the current ARV regimen was recorded at each visit, and we demonstrated that HAART improved clearance of non-HR-HPV 16/18 types. The effect size was smaller and non-significant when all HR-HPV types were grouped, possibly because the interaction between HAART and HPV16 clearance is different than other HR-HPV types. This hypothesis is supported by data published by Strickler, who reported that HPV16 prevalence was more

weakly associated with immune status (as measured by CD4 cell counts) than other HPV types. [23]

Our decision to censor all HPV results after any cervical procedure resulted in the exclusion of women who had worse cervical cytology prior to and at the beginning of follow-up (data not shown). However, sensitivity analyses which did not censor this data, gave similar effect estimates for HIV status and HAART.

The validity of our models is strengthened by the fact that the estimates indicated that progression of cervical disease (absent to present transitions) increased in the presence of HR-HPV. The lack of a relationship between HPV18 and progression may be due to the low prevalence of this HPV type in our cohort. HPV16 was significantly associated with present-to-treatment transitions. Physicians were not aware of the real time type-specific HPV results during the study, so treatment decisions were made independently of knowing that this HR-HPV type was present.

Our findings also demonstrated the probabilities of a progression from normal to abnormal cytology result and of regression from abnormal to normal cytology increased with the number of sexual partners. This effect was no longer significant in the sensitivity analysis where ASCUS results were grouped in the normal cytology category suggesting the relationship between the number of sexual partners and cervical cytology transitions was largely driven by changes between normal and ASCUS.

There are limitations to this analysis. In this cohort, only 71% of participants attended >1 follow-up visits and among those, many had insufficient HPV or cytology samples to be included in the longitudinal analysis. The median follow-up for HIV-positive participants was approximately 10 months longer than HIV-negative participants. This differential follow-up means that we cannot ascertain if a) HIV status

would become a significant predictor of any transitions in the cytopathology multi-state models or b) the significance of HIV status in the HPV models would be reduced if the HIV-negative women had been followed for the same amount of time.

Further, only cervical cytology results were available and we lack the gold standard histological diagnoses. Clinical decisions regarding the timing of treatment for cervical disease may have been different for HIV-positive and HIV-negative women. This may have resulted in a biased estimate for the abnormal cytology-to-treatment transition for this covariate in the multi-state model, however the estimate in our model does not reflect such a bias.

Finally, as HIV viral load monitoring was introduced after our cohort study began, many early observations did not have these data available, precluding an assessment of viral load suppression as a predictor of cytological transition.. We used multi-state models because we believe they are reflective of the patterns observed for individual participants where cervical cytology may show worsening and improvement of SIL and the acquisition and clearance of HPV over follow up. However, these models also have limitations. The power of their analysis depends not only on the number of observations, but specifically on the number of transitions between states. Only 15-20% of the observations available for analysis were between state transitions. This precluded fitting multi-variable models and may explain why a relationship between HAART and HPV16 clearance or acquisition was not observed. Although other authors have noted that the time homogeneity assumption for multi-state models was not valid for HPV, this was deemed adequate when assessed for our sample.[39, 40] The grouped HPV outcomes (any HR-HPV and any not HPV16/HPV18 HR-HPV type) were operationalized as “any positive” versus “all negative”, which does not take any unique

HPV type acquisition and/or clearance into account. While this may have underestimated the total number of transitions among all HPV types, we proceeded with this definition in order to classify visits where one HPV type was acquired and a different one was cleared. Hense our outcome reflects the presence or absence of any HPV type at each visit. Although *a priori* we planned to analyze HPV18 transitions, the low prevalence of this genotype precluded fitting a multi-state model to this outcome.

This study provides additional evidence that HAART has a beneficial impact on the outcome of cervical SIL. This analysis further supports the growing evidence of differential prevalence of HR-HPV types in HIV-positive women compared to HIV-negative women. Our results demonstrate the favorable impact of HAART on non-16/18 HR-HPV but not on HPV16, underscoring the importance of primary prevention of HPV16 but also the potential impact of including women in this high risk population into currently available vaccination programs using genotype-specific vaccines containing HPV16 and HPV18 virus-like particles. In light of the recognized need to increase accessibility to HPV vaccines in countries with poor access to cervical cancer screening and treatment, it is critical to understand the potential effect that all types of HR-HPV have in HIV-positive women, particularly HPV16 and HPV18. Further research is required to better clarify HPV genotype-specific characteristics in women with HIV infection in order to inform effective strategies to prevent cervical cancer in this population.

Table 1: Baseline demographic and clinical characteristics of the CWHS cohort

Variables	HIV-Positive n=750	HIV-Negative n=323	p-value
Age	33 (28-38)	26 (22-33)	<.0001
Race/Ethnicity			
White	430 (57.4%)	279 (86.7%)	<.0001
Black	242 (32.3%)	25 (7.7%)	
Aboriginal	46 (6.1%)	0 (0.0%)	
Other	32 (4.3%)	19 (5.9%)	
Married	314 (42.0%)	105 (32.4%)	<.01
Total Years of Education	12 (10-14)	15 (12-17)	<.0001
Birth Country			<.0001
Canada	435(58.0%)	274(84.8%)	
Endemic Country	233(31.1%)	15(4.6%)	
Non-Endemic Country	82(10.9%)	34(10.5%)	
Risk Factors for HIV			
Heterosexual Contact	629 (83.9%)	308 (95.4%)	<.0001
Injection Drug Use	109 (14.5%)	3 (0.9%)	<.0001
Blood Product	168 (22.4%)	75 (23.1%)	0.77
Sex with other women	7 (0.9%)	23 (7.1%)	<.0001
Unknown	35 (4.7%)	9 (2.8%)	0.15
Ever smoked	432 (57.6%)	217 (67.2%)	<.01
Drink alcohol in the last 6 months	404 (53.9%)	280 (86.7%)	<.0001
Ever used illicit drugs	356 (47.5%)	209 (64.7%)	<.0001

Variables	HIV-Positive n=750	HIV-Negative n=323	p-value
Used illicit drugs in the last 6 months	187 (24.9%)	87 (26.9%)	0.49
14 or older at first menses	253 (34.9%)	85 (26.5%)	0.01
Age at first sexual relationship	17 (15-19)	17 (16-18)	0.37
Ever pregnant	636 (84.8%)	164 (50.8%)	<.0001
# of lifetime sexual partners	5 (3-12)	7 (5-14)	<.0001
Condom Use			<.0001
Always in the past 6 months	187 (24.9%)	244 (75.5%)	
Inconsistently in past 6 months	268 (35.7%)	53 (16.4%)	
Not currently sexually active	264 (35.2%)	25 (7.7%)	
Number of Oncogenic HPV types in first sample			<.0001
0	338 (53.4%)	211 (71.3%)	
1	171 (27.0%)	60 (20.3%)	
2	71 (11.2%)	18 (6.1%)	
3 or more	53 (8.4%)	7 (2.4)	
Any abnormal cytology results prior to study entry	259 (34.5%)	138 (42.7%)	0.01
Cytology Results on first sample			
Normal	419 (73.5%)	230 (76.7%)	0.05
AS-CUS	53 (9.3%)	38 (12.7%)	
LSIL	78 (13.7%)	25 (8.3%)	
HSIL	20 (3.5%)	7 (2.3%)	

Table 2: Frequencies of worst cytology result or treatment for SIL during study follow-up by cytology result at first visit

First Cytology Result	Worst Cytology Result during Study Follow-up						
	Normal	ASC-US	LSIL	HSIL	IC	Treatment for SIL	Total
Normal	264 (76.7%)	34 (9.9%)	36 (10.5%)	3 (0.9%)	1 (0.3%)	6 (1.7%)	344
ASC-US	24 (46.2%)	13 (25.0%)	10 (19.2%)	1 (1.9%)	0	4 (7.7%)	52
LSIL	15 (29.4%)	6 (11.8%)	19 (37.3%)	1 (2.0%)	0	10 (19.6%)	51
HSIL	1 (11.1%)	0	2 (22.2%)	3 (33.3%)	0	3 (33.3%)	9

Table 3: Number of Transitions between Cytopathologic States by HIV Status

HIV-Positive		PAP Result at Next Visit (i+1)					
			SIL Present				
		Normal	AS-CUS	LSIL	HSIL	IC	Any Treatment
PAP Result at Visit (i)	Normal	685	41	39	2	1	7
	ASC-US	42	12	11	1	0	7
	LSIL	36	10	23	2	0	9
	HSIL	3	0	4	6	0	4
	IC	0	0	0	0	0	1
HIV-Negative		PAP Result at Next Visit (i+1)					
			SIL Present				
		Normal	ASC-US	LSIL	HSIL	IC	Any Treatment
PAP Result at Visit (i)	Normal	120	9	4	0	0	2
	ASC-US	14	5	1	0	0	1
	LSIL	7	1	3	0	0	3
	HSIL	0	0	0	0	0	0
	IC	0	0	0	0	0	0

Table 4: Univariate Hazard Ratios (95% Confidence Intervals) for cervical cytopathology multi-state models

	SIL Absent -> SIL Present		SIL Present -> SIL Absent		SIL Present-> Treatment	
	Hazard Ratio	95% Confidence Limit	Hazard Ratio	95% Confidence Limit	Hazard Ratio	95% Confidence Limit
HIV-positive	0.93	(0.43-2.03)	0.60	(0.30-1.19)	0.77	(0.31-1.91)
Age (per 10 years)	0.80	(0.58-1.09)	0.83	(0.56-1.23)	0.61	(0.35-1.04)
More than 5 sexual partners	**1.70**	**(1.03-2.80)**	**2.07**	**(1.25-3.44)**	0.61	(0.29-1.27)
White Ethnicity (vs all others)	0.90	(0.55-1.46)	1.48	(0.85-2.56)	0.61	(0.3-1.24)
Presence of any oncogenic HPV	**2.79**	**(1.46-5.34)**	0.54	(0.28-1.03)	1.61	(0.63-4.08)
Presence of HPV16	**2.50**	**(1.07-5.86)**	1.57	(0.66-3.75)	**3.21**	**(1.37-7.53)**
Presence of HPV18	3.16	(0.57-17.41)	1.72	(0.34-8.81)	1.88	(0.53-6.70)
Presence of any other oncogenic HPV (not 16/18)	**2.76**	**(1.42-5.36)**	0.52	(0.26-1.01)	1.7	(0.71-4.12)

	SIL Absent -> SIL Present		SIL Present -> SIL Absent		SIL Present-> Treatment	
	Hazard Ratio	95% Confidence Limit	Hazard Ratio	95% Confidence Limit	Hazard Ratio	95% Confidence Limit
Among HIV-positive Women						
HAART	1.02	(0.4-2.59)	**3.32**	**(1.22-9.04)**	1.15	(0.38-3.48)
CD4 Cell count						
>500 cells/mm^3(Reference)	1.00		1.00		1.00	
200-500 cells/mm^3	1.07	(0.58-1.98)	1.47	(0.73-2.93)	0.50	(0.19-1.36)
<200 cells/mm^3	1.38	(0.66-2.90)	0.89	(0.39-2.04)	0.49	(0.16-1.55)

Table 5: Number of Transitions between HPV States by for HIV Status

HIV-Positive Women				
0=Negative 1=Positive	No HPV: 0 at visit(i) -> 0 visit(i+1)	Acquisition: 0 at visit(i) -> 1 at visit(i+1)	Clearance: 1 at visit(i) -> 0 at visit(i+1)	Persistence: 1 at visit(i) -> 1 at visit(i+1)
Any oncogenic HPV type	419	75	79	295
HPV16	754	34	35	45
HPV18	826	13	10	19
Any other oncogenic HPV (not 16/18)	485	56	65	262
HIV-Negative Women				
Any oncogenic HPV type	130	9	28	29
HPV16	178	5	5	8
HPV18	189	0	6	1
Any other oncogenic HPV (not 16/18)	142	9	23	22

Table 6: Univariate Hazard Ratios (95% Confidence Intervals) for Acquisition and Clearance Transitions for HPV

	Any Oncogenic HPV		HPV16		Other Oncogenic HPV (Not 16/18)	
	Acquisition Hazard Ratio (95% CI)	Clearance Hazard Ratio (95% CI)	Acquisition Hazard Ratio (95% CI)	Clearance Hazard Ratio (95% CI)	Acquisition Hazard Ratio (95% CI)	Clearance Hazard Ratio (95% CI)
HIV-positive	**2.28 (1.09-4.77)**	**0.41 (0.25-0.65)**	2.03 (0.75-5.52)	1.36 (0.52-3.62)	1.56 (0.73-3.32)	**0.32 (0.19-0.55)**
Age (per 10 years)	**0.70 (0.52-0.96)**	0.88 (0.67-1.15)	**0.47 (0.28-0.79)**	0.78 (0.55-1.11)	0.74 (0.53-1.02)	0.77 (0.56-1.07)
More than 5 sexual partners	0.73 (0.46-1.16)	1.14 (0.76-1.72)	1.38 (0.69-2.76)	0.99 (0.50-1.95)	0.90 (0.54-1.51)	1.28 (0.82-2.00)
White Ethnicity (vs all others)	0.84 (0.52-1.35)	1.53 (0.99-2.37)	**2.31 (1.04-5.13)**	1.90 (0.83-4.38)	0.70 (0.41-1.18)	1.32 (0.84-2.07)

	Any Oncogenic HPV		HPV16		Other Oncogenic HPV (Not 16/18)	
	Acquisition Hazard Ratio (95% CI)	Clearance Hazard Ratio (95% CI)	Acquisition Hazard Ratio (95% CI)	Clearance Hazard Ratio (95% CI)	Acquisition Hazard Ratio (95% CI)	Clearance Hazard Ratio (95% CI)
Among HIV-positive Women						
HAART	0.56 (0.25-1.27)	1.50 (0.84-2.68)	0.52 (0.15-1.81)	0.69 (0.28-1.70)	0.94 (0.41-2.16)	**2.20 (1.22-3.98)**
CD4 cell count						
>500 cells/mm^3(Reference)	1.00		1.00		1.00	
200-500 cells/mm^3	0.98 (0.52-1.85)	1.35 (0.76-2.41)	1.20 (0.41-3.56)	**2.80 (1.06-7.36)**	0.84 (0.42-1.71)	1.09 (0.59-2.02)
<200 cells/mm^3	1.78 (0.85-3.74)	1.13 (0.53-2.42)	**4.82 (1.51-15.41)**	2.97 (0.78-11.28)	1.01 (0.39-2.60)	0.92 (0.42-2.03)

Figure Legend

Figure 1: Prevalence of HPV types at the first study visit

REFERENCES

1. Ellerbrock TV, Chiasson MA, Bush TJ, et al. Incidence of cervical squamous intraepithelial lesions in HIV-infected women. JAMA **2000**; 283:1031-7.

2. Walboomers JM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol **1999**; 189:12-9.

3. Bosch FX, Manos MM, Munoz N, et al. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. J Natl Cancer Inst **1995**; 87:796-802.

4. Munoz N, Bosch FX, de Sanjose S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med **2003**; 348:518-27.

5. Clifford GM, Gallus S, Herrero R, et al. Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. Lancet **2005**; 366:991-8.

6. Smith JS, Lindsay L, Hoots B, et al. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: A meta-analysis update. International Journal of Cancer **2007**; 121:621-32.

7. Coutlee F, Ratnam S, Ramanakumar AV, et al. Distribution of human papillomavirus genotypes in cervical intraepithelial neoplasia and invasive cervical cancer in Canada. J Med Virol **2011**; 83:1034-41.

8. Ferenczy A, Coutlee F, Franco E, Hankins C. Human papillomavirus and HIV coinfection and the risk of neoplasias of the lower genital tract: a review of recent developments. Canadian Medical Association Journal **2003**; 169:431-4.

9. Adler DH. The Impact of HAART on HPV-related Cervical Disease. Curr HIV Res **2010**; 8:493-7.

10. Minkoff H, Feldman J, DeHovitz J, Landesman S, Burk R. A longitudinal study of human papillomavirus carriage in human immunodeficiency virus-infected and human immunodeficiency virus-uninfected women. Am J Obstet Gynecol **1998**; 178:982-6.

11. Ahdieh-Grant L, Li R, Levine AM, et al. Highly active antiretroviral therapy and cervical squamous intraepithelial lesions in human immunodeficiency virus-positive women. J Natl Cancer Inst **2004**; 96:1070-6.

12. Hankins C, Coutlee F, Lapointe N, et al. Prevalence of risk factors associated with human papillomavirus infection in women living with HIV. Canadian Women's HIV Study Group. CMAJ **1999**; 160:185-91.

13. Hankins C CF, Girard M, Pourreaux K, Lapointe N and the Canadian Women's HIV Study Group. Immunosuppression and sontraceptive practices associated with persistence of human papillomavirus in HIV-positive and HIV-negative women. XIIIth International AIDS Conference. Durban, South Africa, **2000**.

14. Hawes SE, Critchlow CW, Faye Niang MA, et al. Increased risk of high-grade cervical squamous intraepithelial lesions and invasive cervical cancer among African women with human immunodeficiency virus type 1 and 2 infections. J Infect Dis **2003**; 188:555-63.

15. Conti M, Agarossi A, Parazzini F, et al. HPV, HIV-infection, and risk of cervical intraepithelial neoplasia in former intravenous drug-abusers. Gynecol Oncol **1993**; 49:344-8.

16. Branca M, Costa S, Mariani L, et al. Assessment of risk factors and human papillomavirus (HPV) related pathogenetic mechanisms of CIN in HIV-positive and HIV-negative women. Study design and baseline data of the HPV-PathogenISS study. Eur J Gynaecol Oncol **2004**; 25:689-98.

17. Marais DJ, Passmore JAS, Denny L, Sampson C, Allan BR, Williamson AL. Cervical and oral human papillomavirus types in HIV-1 positive and negative women with cervical disease in south Africa. J Med Virol **2008**; 80:953-9.

18. Palefsky JM. Anal squamous intraepithelial lesions: relation to HIV and human papillomavirus infection. J Acquir Immune Defic Syndr **1999**; 21 Suppl 1:S42-8.

19. Luque AE, Jabeen M, Messing S, et al. Prevalence of human papillomavirus genotypes and related abnormalities of cervical cytological results among HIV-1-infected women in Rochester, New York. J Infect Dis **2006**; 194:428-34.

20. Clifford GM, Goncalves MAG, Franceschi S. Human papillomavirus types among women infected with HIV: a meta-analysis. Aids **2006**; 20:2337-44.

21. McKenzie ND, Kobetz EN, Hnatyszyn J, Twiggs LB, Lucci JA. Women with HIV are more commonly infected with non-16 and-18 high-risk HPV types. Gynecol Oncol **2010**; 116:572-7.

22. Koshiol JE, Schroeder JC, Jamieson DJ, et al. Time to clearance of human papillomavirus infection by type and human immunodeficiency virus serostatus. Int J Cancer **2006**; 119:1623-9.

23. Strickler HD, Palefsky JM, Shah KV, et al. Human papillomavirus type 16 and immune status in human immunodeficiency virus-seropositive women. Journal of the National Cancer Institute **2003**; 95:1062-71.

24. Firnhaber C, Van Le H, Pettifor A, et al. Association between cervical dysplasia and human papillomavirus in HIV seropositive women from Johannesburg South Africa. Cancer Causes & Control **2010**; 21:433-43.

25. Heard I, Tassie JM, Schmitz V, Mandelbrot L, Kazatchkine MD, Orth G. Increased risk of cervical disease among human immunodeficiency virus-infected women with severe immunosuppression and high human papillomavirus load. Obstetrics and Gynecology **2000**; 96:403-9.

26. Levi JE, Fernandes S, Tateno AF, et al. Presence of multiple human papillomavirus types in cervical samples from HIV-infected women. Gynecol Oncol **2004**; 92:225-31.

27. Patel P, Hanson DL, Sullivan PS, et al. Incidence of types of cancer among HIV-infected persons compared with the general population in the United States, 1992-2003. Annals of internal medicine **2008**; 148:728-36.

28. Chaturvedi AK, Madeleine MM, Biggar RJ, Engels EA. Risk of Human Papillomavirus-Associated Cancers Among Persons With AIDS. Journal of the National Cancer Institute **2009**; 101:1120-30.

29. Shrestha S, Bachmann LH, Wilson CM, Kempf MC. Pre- and post-HAART prevalence, incidence, and type-specific HPV persistence among HIV-positive adolescents. Pharmacoepidemiology and Drug Safety **2008**; 17.

30. Broder S. THE BETHESDA SYSTEM FOR REPORTING CERVICAL VAGINAL CYTOLOGIC DIAGNOSES - REPORT OF THE 1991 BETHESDA WORKSHOP. Jama-Journal of the American Medical Association **1992**; 267:1892-.

31. Coutlee F, Hankins C, Lapointe N. Comparison between vaginal tampon and cervicovaginal lavage specimen collection for detection of human papillomavirus DNA

by the polymerase chain reaction. The Canadian Women's HIV Study Group. J Med Virol **1997**; 51:42-7.

32. Coutlee F, Gravitt P, Richardson H, et al. Nonisotopic detection and typing of human papillomavirus DNA in genital samples by the line blot assay. The Canadian Women's HIV study group. J Clin Microbiol **1999**; 37:1852-7.

33. Cox D, Miller H. The theory of stochastic processes. London: Chapman and Hall, **1965**.

34. Kalbfleisch JD, Lawless JF. The analysis of panel data under a Markov assumption. Journal of the American Statistical Association **1985**; 80:863-71.

35. Jackson CH, Sharples LD, Thompson SG, Duffy SW, Couto E. Multistate Markov models for disease progression with classification error. Journal of the Royal Statistical Society Series D-the Statistician **2003**; 52:193-209.

36. Heard I, Palefsky JM, Kazatchkine MD. The impact of HIV antiviral therapy on human papillomavirus (HPV) infections and HPV-related diseases. Antivir Ther **2004**; 9:13-22.

37. Paramsothy P, Jamieson DJ, Heilig CM, et al. The effect of highly active antiretroviral therapy on human papillomavirus clearance and cervical cytology. Obstetrics and Gynecology **2009**; 113:26-31.

38. Fife KH, Wu JW, Squires KE, Watts DH, Andersen JW, Brown DR. Prevalence and persistence of cervical human papillomavirus infection in HIV-positive women initiating highly active antiretroviral therapy. J Acquir Immune Defic Syndr **2009**; 51:274-82.

39. Saint-Pierre P, Combescure C, Daures JP, Godard P. The analysis of asthma control under a Markov assumption with use of covariates. Stat Med **2003**; 22:3755-70.

40. Kang M, Lagakos SW. Statistical methods for panel data from a semi-Markov process, with application to HPV. Biostatistics **2007**; 8:252-64.

NOTES

Disclosure: There are no conflicts of interest related to this paper and project.

Funding: The Canadian Women's HIV Study was funded by National Health Research and Development Program Grant 6605-4164-AIDS. SW and JR have career support through the Ontario HIV Treatment Network. This project was also supported by the CIHR-Canadian HIV Trials Network.

Meetings: These analyses were presented in part at the Canadian Association of HIV Research Meeting, April 14-17, 2011, Toronto, Canada and the Infectious Diseases Society for Obstetrics and Gynecology Meeting, August 11-13, 2011, Chicago, USA.

Canadian Women's HIV Study Group

Principal investigators: Catherine Hankins and Normand Lapointe. Montreal: François Beaudoin, Marc Boucher, Ngoc Bui, Alena Capek, Michel Châteauvert, Manon Côté, François Coutlée, Douglas Dalton, Gretty Deutsch, Julian Falutz, Diane Francoeur, Lisa Hallman, Eleanor Hew, Lina Karayan, Louise Labrecque, Richard Lalonde, Christianne Lavoie, Catherine Lounsbury, John MacLeod, Nicole Marceau, Gail Myhr, Grégoire Noël, Robert Piché, Manisha Raut, Chantal Rondeau, Jean-Pierre Routy, Karoon Samikian, Pierre Simard, Christina Smeja, Graham Smith, Paul-Pierre Tellier and Émil Toma. Toronto: Donna Keystone, K. Joan Murphy, Anne Phillips, Marion Powell, Anita Rachlis, Pat Rockman, Irving Salit, Cheryl Wagner and Sharon Walmsley. Vancouver: Penny Ballem, David Burdge, Marianne Harris, Deborah Money, Julio Montaner and Janice Veenhuizen. Calgary: John Gill. Edmonton: Barbara Romanowski and Stephen

Shafran. Québec: Louise Côté, Édith Guilbert, Michel Morissette, Hélène Senay and Sylvie Trottier. Ottawa: Gary Victor. Halifax: Rob Grimshaw, David Haase and Wally Schlech. Sudbury: Roger Sandre. Hamilton: Stephan Landis, John Sellors and Fiona Smaill. Saskatoon: Kurt Williams.