Original research article

INTERNATIONAL JOURNAL OF STD & AIDS

International Journal of STD & AIDS
2022, Vol. 33(9) 847–855

DOI: 10.1177/0956462422109686
journals.sagepub.com/home/std
SAGE

Vaginal microbiota associated with oncogenic HPV in a cohort of HPV-vaccinated women living with HIV

Elisabeth McClymont[1,2], Arianne Y Albert[3], Christine Wang[4], Scott J Dos Santos[5], François Coutlée[6], Marette Lee[1], Sharon Walmsley[7,8], Nancy Lipsky[3], Mona Loutfy[9], Sylvie Trottier[10], Fiona Smaill[11], Marina B Klein[12], Mark H Yudin[9,13], Marianne Harris[4,14], Wendy Wobeser[15], Janet E Hill[5], Deborah M Money[1,3] and the CTN 236 HPV in HIV Study Team

Abstract

Background: Women living with HIV (WLWH) experience higher rates of human papillomavirus (HPV) infection and cervical cancer than women without HIV. Changes in the vaginal microbiome have been implicated in HPV-related disease processes such as persistence of high-risk HPV infection but this has not been well defined in a population living with HIV.
Methods: Four hundred and 20 girls and WLWH, age ≥9, across 14 clinical sites in Canada were enrolled to receive three doses of quadrivalent HPV vaccine for assessment of vaccine immunogenicity. Blood, cervical cytology, and cervico-vaginal swabs were collected. Cervico-vaginal samples were tested for HPV DNA and underwent microbiota sequencing.
Results: Principal component analysis (PCA) and hierarchical clustering generated community state types (CSTs). Relationships between taxa and CSTs with HPV infection were examined using mixed-effects logistic regressions, Poisson regressions, or generalized linear mixed-effects models, as appropriate. Three hundred and fifty-six cervico-vaginal microbiota samples from 172 women were sequenced. Human papillomavirus DNA was detected in 211 (59%) samples; 110 (31%) contained oncogenic HPV. Sixty-five samples (18%) were taken concurrently with incident oncogenic HPV infection and 56 (16%) were collected from women with concurrent persistent oncogenic HPV infection.
Conclusions: No significant associations between taxa, CST, or microbial diversity and HPV-related outcomes were found. However, we observed weak associations between a dysbiotic microbiome and specific species, including *Gardnerella*, *Porphyromonas*, and *Prevotella* species, with incident HPV infection.

[1] Department of Obstetrics and Gynecology, University of British Columbia, Vancouver, BC, Canada
[2] Canadian HIV Trials Network, Vancouver, BC, Canada
[3] Women's Health Research Institute, Vancouver, BC, Canada
[4] Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada
[5] Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, SK, Canada
[6] Département de Microbiologie Médicale et Infectiologie, l'Université de Montréal, Montréal, QC, Canada
[7] Toronto General Hospital Research Institute, University of Toronto, University Health Network, Toronto, ON, Canada
[8] Dalla Lana School of Public Health, University of Toronto, Toronto, ON, Canada
[9] Women's College Research Institute, University of Toronto, Toronto, ON, Canada
[10] Infectious Diseases Research Centre, Université Laval, Québec City, QC, Canada
[11] Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada
[12] McGill University Health Centre, Montreal, QC, Canada
[13] Department of Obstetrics and Gynecology, University of Toronto, St. Michael's Hospital, Toronto, ON, Canada
[14] British Columbia Centre for Excellence in HIV/AIDS, Vancouver, BC, Canada
[15] Departments of Public Health and Molecular & Biomedical Sciences, Queen's University, Kingston, ON, Canada

Corresponding author:
Deborah M Money, Department of Obstetrics and Gynecology, University of British Columbia, C425-4500 Oak Street, Vancouver, BC V6H 3N1, Canada.
Email: deborah.money@ubc.ca

Keywords
Human papillomavirus, HIV, vaginal microbiome, cervical cancer, women

Date received: 4 September 2021; accepted: 30 May 2022

Background

There are higher rates of both incident and persistent human papillomavirus (HPV) infections in women living with HIV (WLWH) compared to women without HIV.[1–3] Women living with HIV also thereby experience increased rates of cervical intraepithelial neoplasia (CIN), faster progression of CIN, and increased rates of cervical cancer.[4–6] Changes in the vaginal microbiome have been associated with infections and disease states such as HPV, HIV, bacterial vaginosis, and cervical cancer.[7–12] The healthy vaginal microbiome is typically dominated by a *Lactobacillus species*, most commonly *L. crispatus or L. gasseri,* which creates an acidic environment and keeps the growth of other bacterial species at bay.[10,11] Increased diversity and the dominance of anaerobic bacteria including *Gardnerella, Prevotella,* and *Atopoium*[13] increase the vaginal pH due to the loss of the *Lactobacillus* predominance. The lactic acid produced by lactobacilli has a role in inhibition of pro-inflammatory cytokines, increased degradation of pathogens through autophagy,[14] and inactivation of pathogens such as HIV.[15,16] Thus, the higher pH seen in some vaginal microbiomes creates a permissible environment for deleterious processes such as increased survival of cell-associated HIV in leukocytes and increased inflammatory cytokines which can disrupt the vaginal epithelium.[17]

The role of the vaginal microbiome with respect to HPV infection in women with and without HIV is not well understood. Studies in women without HIV have shown increased biological diversity[18] and a greater proportion of community state type (CST) III (*L. iners* dominated – considered an intermediary state type) and CST IV-B (low *Lactobacillus*)[18,19] microbiota contributing to HPV infection.[20,21] In fact, the clearance of high-risk HPV (HR-HPV) in individuals without HIV has been associated with a specific increase in *L. crispatus,*[22] a decrease in dysbiosis, and a decrease in inflammatory cytokines, compared to those with persistent HR-HPV.[23] However, the literature is conflicting in that a lack of association between HPV persistence and cervical microbiota has also been reported.[24]

The causal relationship between persistent HPV infection and cervical cancer is well established.[25–28] The prevalence of HPV is doubled in WLWH compared to women without HIV at approximately 50%, while the rate of persistent HPV is 3–6 fold higher among WLWH at approximately 20–24%.[29–31] This highlights the importance of understanding factors contributing to HPV persistence in WLWH. Alterations in the vaginal microbiome in the setting of both prevalent HPV infection and CIN include a decreased abundance of *L. crispatus* and a predominance of species such as *L. iners, Atopobium vaginae, Gardnerella vaginalis,* and *Mycoplasma.*[19,21,32,33] However, the contribution of the cervico-vaginal microbiome to the incidence and persistence of HPV infection is still unclear, particularly in the presence of HIV co-infection. The objective of this analysis was to assess the relationship between the vaginal microbiota and HPV-related outcomes, including incidence and persistence, in WLWH. We hypothesized that WLWH with oncogenic HPV infection would be more likely to have non-*Lactobacillus* dominated microbiota than WLWH without oncogenic HPV infection.

Methods

As part of an HPV vaccine study, 420 girls and WLWH aged nine and over from 14 clinical sites across Canada were enrolled to receive three doses of quadrivalent HPV vaccine at months 0, 2, and 6. Study visits took place at months −3, 0, 2, 6, 12, 18, 24, and annually thereafter, until a maximum of 8 years of follow up. Blood samples for serology and cervical cytology samples (ThinPrep liquid based cytology) were collected throughout the study with HPV DNA testing on samples collected at months −3, 0, 6, 12, 18, 24, and annually thereafter. An aliquot of the cytology samples in PreservCyt underwent HPV DNA testing. Extracted DNA was tested with the Linear array (Roche Diagnostic, Laval, Qc, Canada) for the detection of 36 genotypes of HPV and for β-globin to determine the adequacy of the sample. Weak positive HPV controls were included in each amplification run. Samples negative for HPV DNA and β-globin were considered inadequate. The HPV types detected have previously been published.[34,35] Cervico-vaginal swab samples were collected by physicians during genital examination, prior to pap collection, at up to three visits between years three and eight of the study. Physicians rolled sterile flocked swabs against the lateral vaginal wall three times.

Total genomic DNA was purified from cervico-vaginal swabs using the MagMAX Total Nucleic Acid Isolation Kit (Applied Biosystems, Life Technologies, Burlington, ON, Canada). Extraction negative controls including only kit reagents were included to monitor for contaminants. *cpn*60 barcode PCR and sequencing library preparation was performed as described in detail elsewhere.[36] No template

controls were included with each batch of PCR reactions. Indexed amplicon libraries from samples and all negative controls were pooled and sequenced on the MiSeq platform (500 cycles, with 400 cycles for read 1; only read 1 used in downstream analysis).

Amplification primer sequences were removed using CUTADAPT. Quality trimming was then performed using TRIMMOMATIC with a quality cut-off of 30 and minimum length of 150. Quality trimmed reads were loaded into QIIME2 for sequence variant calling and read frequency calculation with DADA2[37] and a truncation length of 150.[38] Variant sequences were aligned with the cpnDB_nr reference database (version 20190305, downloaded from www.cpndb.ca) using WATERED-BLAST for taxonomic identification.[39] In instances where sequence variants had the same best database reference, they were grouped together into nearest neighbour 'species' by summing their total read counts within samples. The nearest neighbour taxonomic labels were used in this analysis.

We used compositional data analysis methods including centre log-ratio transformation of data in ALDEx2,[40,41] then visualised communities with principal component analysis (PCA) and hierarchical clustering to generate the CSTs. Human papillomavirus variables we investigated included any HPV positivity, incident oncogenic HPV infection (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, and 82; defined as detection of a new HPV genotype not detected in the prior visit), persistent oncogenic HPV infection (defined as the same HPV genotype detected in ≥2 consecutive samples taken ≥6 months apart), clearance of any oncogenic HPV (defined as presence of a type in one visit followed by absence at the subsequent visit), HIV viral load (suppressed at <50 copies/mL vs. not suppressed), and the total number of oncogenic HPV types. The relationship between taxa abundance or CSTs and incident or persistent oncogenic HPV infection and HIV viral load was investigated with mixed-effects logistic regressions. The relationship between taxon relative abundance or CSTs with number of oncogenic HPV types or HPV positivity was examined using Poisson regressions. Generalized linear mixed-effects models were used to determine if there was any relationship between microbiota diversity and any of the HPV-associated outcome variables listed above.

Results

The demographics of the study population eligible for this sub-analysis are shown in Table 1. The median age at study baseline was 37.8 years (interquartile range [IQR]: 31.9–44.3, range = 13.6–58.6), and most women received all three doses of quadrivalent HPV vaccine (94.2%). The median baseline $CD4^+$ T-cell count was 489 cells/mm^3 (IQR: 370–675), and a modest majority had an HIV viral load under 50 copies/mL (64.5%). The baseline antiretroviral therapy was variable, including integrase inhibitor-based regimens in 39%, protease inhibitor (PI)-based regimens in 34.9%, and non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimens in 24.4% of study participants.

Analyses were restricted to cervico-vaginal microbiota samples that had at least 1000 quality-filtered sequence reads, of which there were a total of 356 samples from 172 women (28% women had one sample sequenced, 37% had two samples, and 35% had three samples). Samples were taken between 3 and 8 years post-HPV vaccination (8% at 3 years, 12% at 4 years, 17% at 5 years, 28% at 6 years, 21% at 7 years, and 14% at 8 years). The presence of HPV DNA was detected in 211 samples (59% of samples) from 122 women (Table 2). One hundred and 10 samples (31%) from 73 women had detectable oncogenic HPV. Incident oncogenic HPV infection was found in 65 samples (18%) from 59 women, persistent oncogenic HPV infection was found in 56 samples (16%) from 32 women, while

Table 1. Participant characteristics at baseline (n = 172).

Characteristic	n (%) or median (IQR)
Age at vaccination, years	37.8 (31.9–44.3)
Age at vaccination, categorical	
14–19	6 (3.5%)
20–24	10 (5.8%)
25–29	17 (9.9%)
30–34	28 (16.3%)
35–39	37 (21.5%)
40–44	33 (19.2%)
45+	40 (23.3%)
Ethnicity	
African/Black/Caribbean	66 (38%)
White	63 (37%)
Indigenous	30 (17%)
Other	12 (7%)
Number of HPV vaccine doses	
1	4 (2.3%)
2	5 (2.9%)
3	162 (94.2%)
Missing	1 (0.6%)
Baseline HIV viral load	
>50 copies/mL	55 (32.0%)
<50 copies/mL	111 (64.5%)
Missing	13 (7.6%)
Baseline antiretroviral therapy	
Integrase inhibitor-based	67 (39.0%)
PI Based-based	60 (34.9%)
NNRTI based-based	42 (24.4%)
NRTI only	1 (0.6%)
None	1 (0.6%)
Missing	1 (0.6%)
Baseline CD4 count, cells/mm^3	489 (370–675)
CD4 nadir, cells/mm^3	230 (120–360)

clearance of any oncogenic HPV was found in 24 samples (6.7%) from 24 women.

Principal component analysis resulted in three principal component axes that explained 42% of the variance in taxon relative abundance (Figure 1; PC1 = 26%, PC2 = 9%, PC3 = 7%). Higher scores on PCA axis 1 (PC1) indicated greater relative abundance of *Clostridiales* sp., *Megasphaera* genomosp type 1, *Prevotella timonensis, Prevotella buccalis, Porphyromonas uenonis, Prevotella amnii,* and *Dialister pneumosintes*. Lower scores on PC1 indicated a greater relative abundance of *Lactobacillus crispatus, L. iners,* and *L. jensenii*. Higher scores on PCA axis 2 (PC2) indicated a greater relative abundance of *L. crispatus,* while lower scores indicated a greater relative abundance of *L. iners* and *Gardnerella vaginalis*.

Table 2. HPV results for samples sequenced.

HPV result	*n* (%)
Incident oncogenic HPV	65 (18)
Persistent oncogenic HPV	56 (16)
Any oncogenic HPV	110 (31)
Any HPV	211 (59)

Hierarchical clustering analysis on the Euclidean distances in relative abundance had relatively good support for six clusters. Both silhouette width and Pearson-Gamma indicated that six clusters had the most support (Figures 1 and 2). The six clusters are as follows: (i) CST IVA with a mixture of profiles with diverse dominant bacterial types, but very little *Lactobacillus* or *Megasphaera,* (ii) CST IVC has communities with high relative abundance of *Gardnerella vaginalis,* and *G. swidsinskii*, (iii) CST IVD.1 contains communities with high relative abundance of *Megasphaera, Clostridiales* sp*., Prevotella* spp*., Dialister pneumosintes* and *Porphyromonas* uenonis, (iv) CST IVD.2 contains very little *Megasphaera*, with appreciable abundance of *Clostridiales* sp*., Prevotella* spp*.,* and *Porphyromonas uenonis*, (v) CST III/V with high relative abundance of *L. iners,* and/or *L. jensenii*, (vi) CST I with communities dominated mainly by *L. crispatus*.

A number of weak and non-significant associations to specific taxa were found. Incident oncogenic HPV infection was non-significantly increased with greater relative abundance of *Gardnerella swidsinskii* (OR = 1.10, 95%CI = 0.98–1.22, p = .08) and decreased with greater relative abundance of *L. crispatus* (OR = 0.91, 95%CI = 0.84–1.01, p = .09). Greater total number of oncogenic HPV types were associated with greater relative abundance of *Porphyromonas uenonis* (p = .09) and *Prevotella timonesis* (p = .02). No taxa were significantly associated with HPV persistence or clearance.

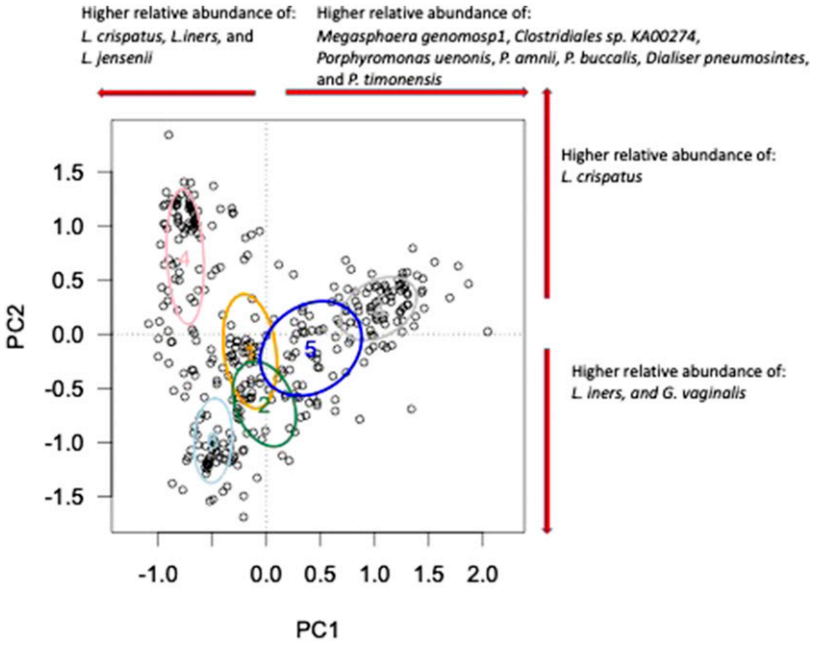

Figure 1. Hierarchical clustering results with PCA axes 1 and 2. The ellipses indicate the clusters and they extend to 1SD in both directions. Grey = CST IVD.1, orange = CST IVA, green = CST IVC, blue = CST IVD.2, light blue = CST III + V (mixed lactobacilli), and pink = CST I.

Investigations at the level of CST suggested that individuals with incident oncogenic HPV infection, a higher number of oncogenic HPV types, and a higher number of total HPV types were more likely to be classified into CST IVD.2 (*Porphyromonas, Clostridiales,* and *Prevotella*), but this did not remain significant after controlling for repeated measures. There were no significant relationships between any of the HPV variables and diversity either as Shannon's diversity index (H) (Figure 3), or the number of detected species.

Discussion

In our study of HPV-vaccinated WLWH, we detected incident oncogenic HPV DNA in 31% of cervico-vaginal swabs and persistent oncogenic HPV DNA in 16% of cervico-vaginal swabs. We did not find any strongly significant associations between taxa, CST, or microbial diversity and HPV variables including incident or persistent oncogenic HPV infection, especially given the large number of tests conducted. However, the trends we found were consistent with expectations based on the current understanding of the roles of the specific taxa investigated and were consistent with the associations found in some other studies. For example, we found a non-significant association of decreased incident oncogenic HPV infection with greater relative abundance of *L. crispatus*. Prior evidence has shown that D-lactate produced by *L. crispatus* increases the viscosity of the cervico-vaginal mucus, thereby enhancing its viral particle trapping potential; such enhancement could represent a mechanism behind the reduction in incident oncogenic HPV infection we observed with increasing relative abundance of these bacteria.[42] Additionally, the associations between oncogenic HPV infection and greater relative abundance of *Gardnerella* and *Prevotella* were not unexpected, as it is known that some of

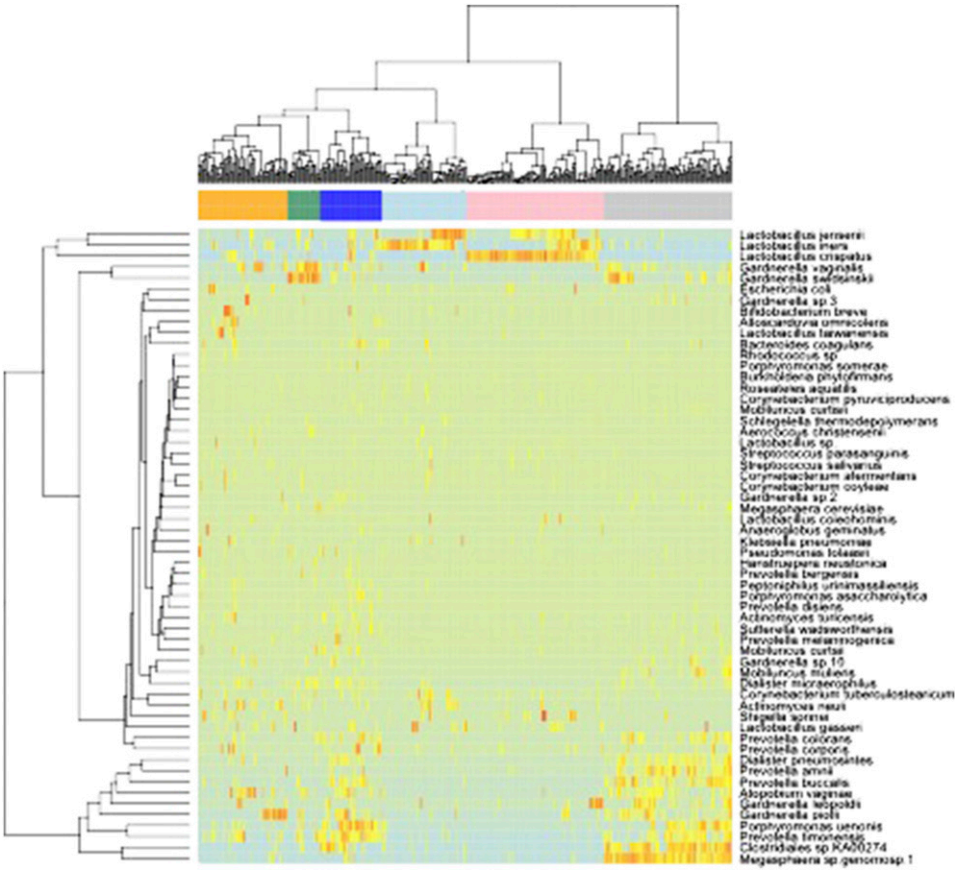

Figure 2. Heatmap of centred log-ratio-transformed relative abundance showing six clusters. The orange cluster corresponds to IVA with a mixture of profiles with diverse dominant bacterial types, but very little *Lactobacillus* or *Megasphaera*. The green cluster has communities with high relative abundance of *Gardnerella vaginalis,* and *G. swidsinskii* which is similar to CST IVC. The dark blue cluster and the grey cluster appear to be what was collectively IVD previously. The grey cluster, IVD.1, contains communities with high relative abundance of *Megasphaera, Clostridiales* sp., *Prevotella* spp., *Dialister pneumosintes* and *Porphyromonas uenonis*. The dark blue cluster, IVD.2, contains very little *Megasphaera*, with appreciable abundance of *Clostridiales sp., Prevotella* spp., and *Porphyromonas uenonis*. Finally, the light blue cluster is a mix of CST III and V with high relative abundance of *L. iners,* and/or *L. jensenii*, while the pink cluster corresponds to CST I with communities dominated mainly by *L. crispatus*.

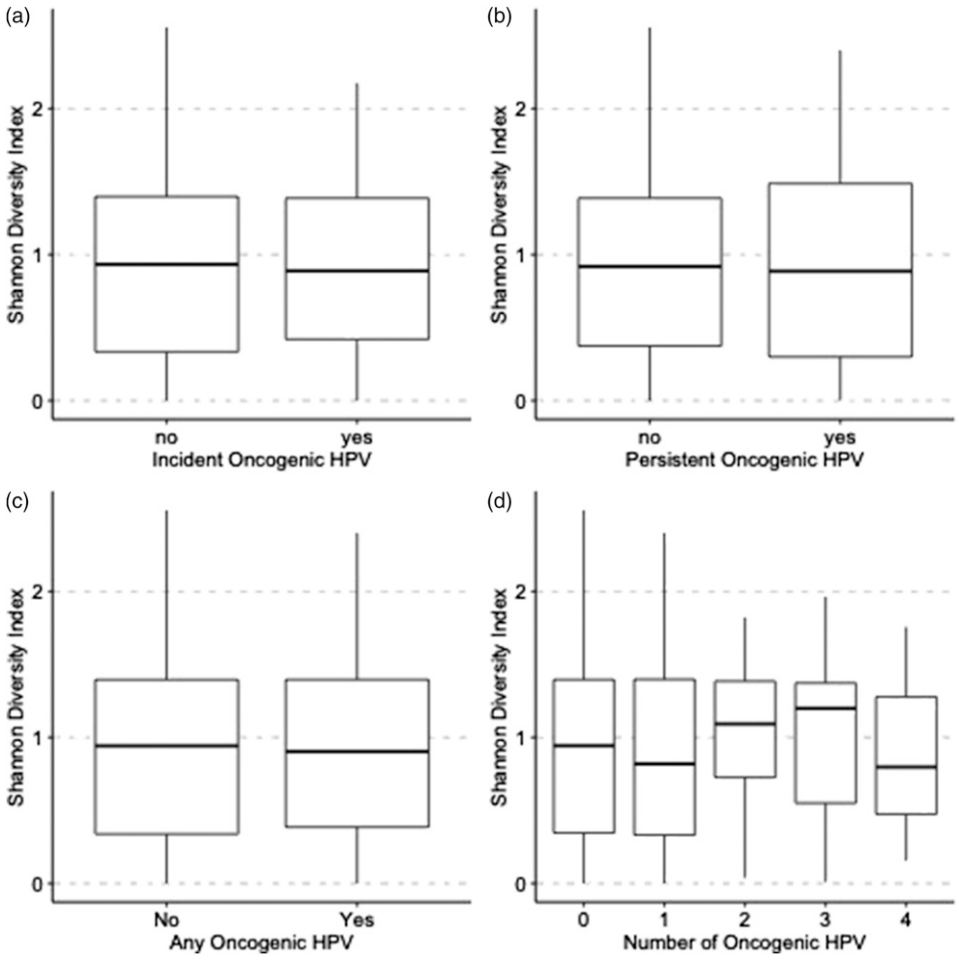

Figure 3. Shannon Diversity Index (H) by oncogenic HPV. (A) Incident oncogenic HPV, (B) Persistent oncogenic HPV, (C) Any oncogenic HPV, (D) Number of oncogenic HPV types. Dark lines indicate the medians, boxes indicate the interquartile ranges, and whiskers extend to 1.5 times the interquartile range.

these species exhibit sialidase activity,[43] which can negatively impact the cervical mucosa and reduce viral trapping via mucin degradation, potentially facilitating HPV infection. *Gardnerella* species have further been implicated in disrupting vaginal epithelial cytoskeleton proteins, causing damage and desquamation which may facilitate HPV entry into its target basal epithelial cells.[44] The lack of associations between bacterial taxa and HPV persistence is consistent with recent findings from a Norwegian study.[24]

This cohort included some WLWH who did not have a suppressed HIV viral load at baseline (32%). However, this rate of HIV viral load suppression is consistent with other studies among WLWH in Canada and therefore appears to be representative of the Canadian context.[45] A small proportion of participants included in this analysis acquired HIV through perinatal infection (n = 10, 5.8%), and therefore may have differing risks for HPV acquisition. The lack of strong associations between vaginal microbiota and HPV infection outcomes in this analysis may have been due to the small overall number of HPV infection outcomes, particularly given that this cohort of WLWH had been previously vaccinated against HPV. Future studies with larger sample sizes are needed to fully elucidate the role of the vaginal microbiome in HPV infection and disease within WLWH. Such improvements in understanding are critically important, as they could lead to interventions to reduce the high burden of HPV among WLWH, and ultimately contribute to the global elimination of cervical cancer.

Conclusions

This analysis supports previous associations of dysbiotic microbiota and specific bacterial taxa, including *Gardnerella*, *Porphyromonas*, and *Prevotella* species, with incident HPV infection. The lack of association between dysbiosis and HPV persistence may be related to low numbers of events in this cohort of HPV-vaccinated WLWH.

Acknowledgements

We would like to thank the participants without whom this research would not be possible. The authors would also like to acknowledge the CTN 236 HPV in HIV Study Team, in alphabetical order: Ariane Alimenti, MD (University of British Columbia), Arezou Azampanah, MSc (Women's Health Research Institute), Ari Bitnun, MD (University of Toronto), Sandra Blitz, MSc (University Health Network), Jason Brophy, MD (University of Ottawa), Jan Christilaw, MD (University of British Columbia), Jeffrey Cohen, MD (Windsor Regional Hospital HIV Care Program), Andrew Coldman, PhD (British Columbia Cancer Agency), Simon Dobson, MD (Vaccine Evaluation Centre), Laurie Edmiston (Canadian AIDS Treatment Information Exchange), Catherine Hankins, MD, PhD (Amsterdam Institute for Global Health and Development), Christos Karatzios, MD (McGill University Health Centre), Mel Krajden, MD (British Columbia Centre for Disease Control), Normand Lapointe, MD (CHU Sainte Justine), Jessica McAlpine, MD (University of British Columbia), Dianne Miller, MD (University of British Columbia), Dirk van Niekerk, MD (British Columbia Cancer Agency), Gina Ogilvie, MD, DrPH (University of British Columbia), Neora Pick, MD (University of British Columbia), Lindy Samson, MD (University of Ottawa), Julie van Schalkwyk, MD (University of British Columbia), David Scheifele, MD (Vaccine Evaluation Centre), Joel Singer, PhD (CIHR Clinical Trials Network), Sarah Stone, MD (British Columbia Centre for Excellence in HIV/AIDS), Gavin Stuart, MD (University of British Columbia), Marcie Summers (Positive Women's Network), Laura Vicol, MN, NP (University of British Columbia), and Melissa Watt (Women's Health Research Institute). The authors wish to thank all of the additional clinicians and research staff for their important contributions to participant enrollment and study visits, as well as Julie Guenoun and Émilie Comète for sample processing and HPV testing.

Declaration of conflicting interests

The author(s) declared have the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: EM, AA, CW, SD, MY, NL, and JH has no conflicts to declare. FC has received grants for research projects given to his institution from Roche Diagnostics, Becton Dickenson, and Merck, Sharp, and Dome, honoraria for presentations from Merck, Sharp, and Dome and Roche Diagnostics, and has participated in an expert group by Merck, Sharp, and Dome, outside the submitted work. MLee has received honoraria from Merck Canada Inc. SW has received grants, personal fees and non-financial support from Merck Canada Inc., ViiV Healthcare, Gilead, AbbVie, Janssen and Bristol Meyers Squibb for participation on advisory boards, presentations, meetings, studies, workshops and symposia for each, outside the submitted work. MLoutfy has received grants, personal fees and non-financial support from Merck Canada Inc., ViiV Healthcare, Gilead Sciences, and Janssen for studies, meetings, and presentations, outside the submitted work. ST has received grants from ViiV Healthcare, Gilead, GlaxoSmithKline, and Merck, outside the submitted work. FS received grant and honoraria funding from Merck Canada Inc., ViiV Healthcare, and Gilead, unrelated to the submitted work. MK has received funding for investigator-initiated research from ViiV and Merck, unrelated to this work, and honoraria for participation in advisory boards from Merck, ViiV, and BMS. MH has received consulting fees and honoraria from Gilead Sciences Canada Inc., Merck Canada Inc., and ViiV Healthcare for participation in advisory boards, outside the submitted work. WW has received grants, paid to the institution, from CIHR and Merck Canada Inc., as well as honoraria for consultancy and/or speaking engagements from ViiV Healthcare, Gilead, AbbVie, and Janssen outside of the submitted work. DM has received grants from GSK and Merck Canada Inc. for conducting sponsored vaccine trials. She also reports grants from Novartis and Sanofi for conducting sponsored vaccine trials in an unrelated area. She has received personal fees for symposium participation from Merck Canada Inc., outside the submitted work.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Canadian Institutes for Health Research (CIHR) [funding reference number: MOP 136784]; CIHR Canadian HIV Trials Network (CTN 236); Chair in Clinical Management and Aging from the Ontario HIV Treatment Network to SW; CANFAR/CTN Postdoctoral Fellowship and MSFHR Trainee Award to EM; and in-kind contribution from Merck Canada Inc. The opinions expressed in this paper are those of the authors and do not necessarily represent those of Merck Canada Inc.

Ethics approval

Ethical approval for central study coordination was obtained from the University of British Columbia Clinical Research Ethics Board (approval H08-00997) and all recruiting clinical sites received research ethics approval locally.

ORCID iDs

Elisabeth McClymont https://orcid.org/0000-0002-6869-2870
Scott J Dos Santos https://orcid.org/0000-0001-7793-3501
Mona Loutfy https://orcid.org/0000-0001-7887-8997
Fiona Smaill https://orcid.org/0000-0002-1305-6260

References

1. Adler D, Wallace M, Bennie T, et al. High risk human papillomavirus persistence among HIV-infected young women in South Africa. *Int J Infect Dis* 2015; 33: 219–221. DOI: 10.1016/j.ijid.2015.02.009
2. Liu G, Sharma M, Tan N, et al. HIV-positive women have higher risk of human papilloma virus infection, precancerous lesions, and cervical cancer. *AIDS* 2018; 32: 795–808. DOI: 10.1097/QAD.0000000000001765
3. Sohn AH, Kerr SJ, Hansudewechakul R, et al. Risk factors for human papillomavirus infection and abnormal cervical cytology among perinatally human immunodeficiency virus-infected and uninfected Asian youth. *Clin Infect Dis* 2018; 67: 606–613. DOI: 10.1093/cid/ciy144
4. Nappi L, Carriero C, Bettocchi S, et al. Cervical squamous intraepithelial lesions of low-grade in HIV-infected women: recurrence, persistence, and progression, in treated and untreated women. *Eur J Obstet Gynecol Reprod Biol* 2005; 121: 226–232. DOI: 10.1016/j.ejogrb.2004.12.003
5. Wright TC, Ellerbrock TV, Chiasson MA, et al. Cervical intraepithelial neoplasia in women infected with human

immunodeficiency virus prevalence, risk factors and validity of papanicolaou smears. *Obstet Gynecol* 1994; 84: 591–597.
6. Hleyhel M, Belot A, Bouvier AM, et al. Risk of AIDS-defining cancers among HIV-1-infected patients in France between 1992 and 2009: results from the FHDH-ANRS CO4 cohort. *Clin Infect Dis* 2013; 57: 1638–1647. DOI: 10.1093/cid/cit497
7. Ma B, Forney LJ and Ravel J. Vaginal microbiome: rethinking health and disease. *Annu Rev Microbiol* 2012; 66: 371–389. DOI: 10.1146/annurev-micro-092611-150157
8. Fredricks DN, Fiedler TL and Marrazzo JM. Molecular identification of bacteria associated with bacterial vaginosis. *New Engl J Med* 2005; 353: 1899–1911. DOI: 10.1056/NEJMoa043802
9. Anahtar Melis N, Byrne Elizabeth H, Doherty Kathleen E, et al. Cervicovaginal bacteria are a major modulator of host inflammatory responses in the female genital tract. *Immunity* 2015; 42: 965–976. DOI: 10.1016/j.immuni.2015.04.019
10. Huang B, Fettweis JM, Brooks JP, et al. The changing landscape of the vaginal microbiome. *Clin Lab Med* 2014; 34: 747–761. DOI: 10.1016/j.cll.2014.08.006
11. Champer M, Wong AM, Champer J, et al. The role of the vaginal microbiome in gynaecological cancer. *BJOG* 2018; 125: 309–315. DOI: 10.1111/1471-0528.14631
12. Houlihan CF, Larke NL, Watson-Jones D, et al. Human papillomavirus infection and increased risk of HIV acquisition. A systematic review and meta-analysis. *AIDS* 2012; 26: 2211–2222. DOI: 10.1097/QAD.0b013e328358d908
13. Cone RA. Vaginal microbiota and sexually transmitted infections that may influence transmission of cell-associated HIV. *J Infect Dis* 2014; 210(Suppl 3): S616–S621. DOI: 10.1093/infdis/jiu459
14. Witkin SS and Linhares IM. Why do lactobacilli dominate the human vaginal microbiota? *BJOG* 2017; 124: 606–611. DOI: 10.1111/1471-0528.14390
15. Aldunate M, Tyssen D, Johnson A, et al. Vaginal concentrations of lactic acid potently inactivate HIV. *J Antimicrob Chemother* 2013; 68: 2015–2025. DOI: 10.1093/jac/dkt156
16. Mirmonsef P and Spear GT. The barrier to HIV transmission provided by genital tract Lactobacillus colonization. *Am J Reprod Immunol* 2014; 71: 531–536. DOI: 10.1111/aji.12232
17. Olmsted SS, Khanna KV, Ng EM, et al. Low pH immobilizes and kills human leukocytes and prevents transmission of cell-associated HIV in a mouse model. *BMC Infect Dis* 2005; 5: 79. DOI: 10.1186/1471-2334-5-79
18. Gao W, Weng J., Gao Y., et al. Comparison of the vaginal microbiota diversity of women with and without human papillomavirus infection: a cross-sectional study. *BMC Infect Dis* 2013; 13: 271.
19. Mitra A, MacIntyre DA, Lee YS, et al. Cervical intraepithelial neoplasia disease progression is associated with increased vaginal microbiome diversity. *Sci Rep* 2015; 5: 16865. DOI: 10.1038/srep16865.
20. Wei Z-T, Chen H-L, Wang C-F, et al. Depiction of vaginal microbiota in women with high-risk human papillomavirus infection. *Front Public Health* 2020; 8: 587298. DOI: 10.3389/fpubh.2020.587298
21. Cheng L, Norenhag J, Hu YOO, et al. Vaginal microbiota and human papillomavirus infection among young Swedish women. *NPJ Biofilms Microbiomes* 2020; 6: 39. DOI: 10.1038/s41522-020-00146-8
22. Carter KA, Srinivasan S, Fiedler TL, et al. Vaginal bacteria and risk of incident and persistent infection with high risk subtypes of human papillomavirus: a cohort study among Kenyan women. *Sex Transm Dis* 2020. Publish Ahead of Print. DOI: 10.1097/OLQ.0000000000001343
23. Caselli E, D'Accolti M, Santi E, et al. Vaginal microbiota and cytokine microenvironment in HPV clearance/persistence in women surgically treated for cervical intraepithelial neoplasia: an observational prospective study. *Front Cel Infect Microbiol* 2020; 10: 540900. DOI: 10.3389/fcimb.2020.540900
24. Wiik J, Sengpiel V, Kyrgiou M, et al. Cervical microbiota in women with cervical intra-epithelial neoplasia, prior to and after local excisional treatment, a Norwegian cohort study. *BMC Womens Health* 2019; 19: 30. DOI: 10.1186/s12905-019-0727-0
25. Bosch FX, Lorincz A, Munoz N, et al. The causal relation between human papillomavirus and cervical cancer. *J Clin Pathol* 2002; 55: 244–265.
26. Schiffman M, Castle PE, Jeronimo J, et al. Human papillomavirus and cervical cancer. *Lancet* 2007; 370: 890–907. DOI: 10.1016/s0140-6736(07)61416-0
27. de Sanjosé S, Brotons M and Pavón MA. The natural history of human papillomavirus infection. *Best Pract Res Clin Obstet Gynaecol* 2018; 47: 2–13. DOI: 10.1016/j.bpobgyn.2017.08.015
28. Mpunga T, Chantal Umulisa M, Tenet V, et al. Human papillomavirus genotypes in cervical and other HPV-related anogenital cancer in Rwanda, according to HIV status. *Int J Cancer* 2020; 146: 1514–1522. DOI: 10.1002/ijc.32491
29. Sun X-W, Kuhn L, Ellerbrock TV, et al. Human papillomavirus infection in women infected with the human immunodeficiency virus. *New Engl J Med* 1997; 337: 1343–1349. DOI: 10.1056/NEJM199711063371903
30. Blitz S, Baxter J, Raboud J, et al. Evaluation of HIV and highly active antiretroviral therapy on the natural history of human papillomavirus infection and cervical cytopathologic findings in HIV-positive and high-risk HIV-negative women. *J Infect Dis* 2013; 208: 454–462.
31. Kriek J-M, Jaumdally SZ, Masson L, et al. Female genital tract inflammation, HIV co-infection and persistent mucosal human papillomavirus (HPV) infections. *Virology* 2016; 493: 247–254. DOI: 10.1016/j.virol.2016.03.022
32. Oh HY, Kim BS, Seo SS, et al. The association of uterine cervical microbiota with an increased risk for cervical intraepithelial neoplasia in Korea. *Clin Microbiol Infect* 2015; 21: 674.e1. DOI: 10.1016/j.cmi.2015.02.026
33. Klein C, Gonzalez D, Samwel K, et al. Relationship between the cervical microbiome, HIV status, and precancerous lesions. *mBio* 2019; 10: e02785. DOI: 10.1128/mBio.02785-18
34. McClymont E, Lee M, Raboud J, et al. The efficacy of the quadrivalent human papillomavirus vaccine in girls and women living with human immunodeficiency virus. *Clin Infect Dis* 2019; 68: 788–794. DOI: 10.1093/cid/ciy575
35. McClymont E, Coutlée F, Lee M, et al. Brief report: persistence of non-vaccine oncogenic HPV genotypes in quadrivalent HPV-vaccinated women living with HIV. *J Acquir Immune Defic Syndr* 2020; 83: 230–234. DOI: 10.1097/QAI.0000000000002258

36. Champika F and Janet EH. cpn60 metagenomic amplicon library preparation for the illumina miseq platform. *Protoc Exchange* 2021. DOI: 10.21203/rs.3.pex-1438/v1
37. Callahan BJ, McMurdie PJ, Rosen MJ, et al. DADA2: high-resolution sample inference from Illumina amplicon data. *Nat Methods* 2016; 13: 581–583. DOI: 10.1038/nmeth.3869
38. Vancuren SJ, Dos Santos SJ, Hill JE, et al. Evaluation of variant calling for cpn60 barcode sequence-based microbiome profiling. *PloS One* 2020; 15: e0235682. DOI: 10.1371/journal.pone.0235682
39. Schellenberg J, Links MG, Hill JE, et al. Pyrosequencing of the chaperonin-60 universal target as a tool for determining microbial community composition. *Appl Environ Microbiol* 2009; 75: 2889–2898. DOI: 10.1128/AEM.01640-08
40. Gloor GB, Macklaim JM, Pawlowsky-Glahn V, et al. Microbiome datasets are compositional: and this is not optional. *Front Microbiol* 2017; 8: 2224. DOI: 10.3389/fmicb.2017.02224
41. Mandal S, Van Treuren W, White RA, et al. Analysis of composition of microbiomes: a novel method for studying microbial composition. *Microb Ecol Health Dis* 2015; 26: 27663–27667. DOI: 10.3402/mehd.v26.27663
42. Nunn KL, Wang Y-Y, Harit D, et al. Enhanced trapping of HIV-1 by human cervicovaginal mucus is associated with lactobacillus crispatus-dominant microbiota. *mBio* 2015; 6: e01084. DOI: 10.1128/mBio.01084-15
43. Schellenberg JJ, Paramel Jayaprakash T, Withana Gamage N, et al. Gardnerella vaginalis subgroups defined by cpn60 sequencing and sialidase activity in isolates from Canada, Belgium and Kenya. *PloS One* 2016; 11: e0146510. DOI: 10.1371/journal.pone.0146510
44. Borgdorff H, Gautam R, Armstrong SD, et al. Cervicovaginal microbiome dysbiosis is associated with proteome changes related to alterations of the cervicovaginal mucosal barrier. *Mucosal Immunol* 2016; 9: 621–633. DOI: 10.1038/mi.2015.86
45. Kerkerian G, Kestler M, Carter A, et al. Attrition across the HIV cascade of care among a diverse cohort of women living with HIV in Canada. *J Acquir Immune Defic Syndr* 2018; 79: 226–236. DOI: 10.1097/QAI.0000000000001775