HPV Types in Cervical Precancer by HIV Status and Birth Region: A Population-Based Register Study

Christina Carlander[1,2,3], Camilla Lagheden[4], Carina Eklund[4], Sara Nordqvist Kleppe[4], Mensur Dzabic[4], Philippe Wagner[2], Aylin Yilmaz[5], Kristina Elfgren[6], Anders Sönnerborg[1], Pär Sparén[3], and Joakim Dillner[4]

ABSTRACT

Background: Data are lacking regarding which human papillomavirus (HPV) types cause high-grade cervical neoplasia (CIN2+) in people with HIV in Europe. We assessed which HPV types are associated with CIN2+ in women living in Sweden by HIV status.

Methods: The Swedish National HIV Registry, the Swedish Population Registry, and the Swedish National Cervical Screening Registry were linked. CIN2+ tissue blocks of 130 women living with HIV (WLWH) and 234 HIV-negative women, matched for country of birth (1:2), were retrieved from bio-banks and HPV genotyped. Adjusted ORs (adjOR), stratified by country of birth, were calculated using conditional logistic regression. Matching was broken for cross-group comparisons.

Results: WLWH with CIN2 were less likely to have HPV16 [14% vs. 40%; adjOR 0.1; 95% confidence interval (CI), 0.04–0.56] than HIV-negative women, but among women with CIN3, there was no difference in HPV16 prevalence by HIV status (adjOR 0.9; 95% CI, 0.51–1.70). WLWH were six times more likely to have HPV35 in CIN3 than HIV-negative women (adjOR 6.2; 95% CI, 1.3–30.4). WLWH from sub-Saharan Africa (SSA) had less 9-valent vaccine types, compared with both HIV-negative women born in Sweden (adjOR 0.1; 95% CI, 0.02–0.44) and WLWH born in Sweden (adjOR 0.1; 95% CI, 0.01–0.73), mostly because of decreased HPV16 and increased HPV35.

Conclusions: WLWH from SSA were less likely to be covered by the 9-valent vaccine, mostly due to less HPV16 and more HPV35.

Impact: This could have implications for HPV vaccines, currently not including HPV35, and for HPV-screening algorithms in women with origin from SSA.

Introduction

Women living with HIV (WLWH) have a higher prevalence and cumulative incidence of human papillomavirus (HPV), and are more likely to have persistent high-risk HPV (HR HPV) cervical infections than HIV-negative women (1–3). WLWH also have an increased risk of developing high-grade cervical intraepithelial lesions and invasive cervical cancer (CIN2+; refs. 4–7).

HR HPV types differ greatly in their carcinogenicity, and in their geographical distribution, and it has been suggested that migrant women carry the HPV types present in their countries of origin (8–10). Meanwhile, HIV seems to affect some HR HPV types more than others (9). HPV16 is best at evading a competent immune system, and with increasing immunosuppression the proportion of non-HPV16 types increases (11). Migrant women, particularly from sub-Saharan Africa (SSA), constitute the largest proportion of WLWH in most HIV cohorts in the European Union/European Economic Area and large proportions of Canadian cohorts, but there is a lack of data of which HPV types causes CIN2+ in this population (9, 12, 13).

We conducted a population-based study with the aim of assessing which HR HPV types are associated with CIN2+ in the Swedish HIV cohort, a cohort that is dominated by migrants from SSA, compared with HIV-negative women from the same country of origin. This could have implications for HPV-screening algorithms and for management strategies after HPV vaccination in this population.

Materials and Methods

Study design, participants, and data sources

The personal identification number, assigned to all individuals in Sweden at birth or upon immigration, was used to link the Swedish National HIV Registry (InfCareHIV) and the Swedish Population Registry (SPR) with the Swedish National Cervical Screening Registry (NKCx; ref. 14). The study population has been described previously (15).

All WLWH, born between 1942 and 1989, living in the counties of Stockholm and Gothenburg sometime between 1983 and 2014 were identified from InfCareHIV (n = 1,926; **Fig. 1**). This registry includes > 99% of Swedish residents diagnosed with HIV (16). Patients are consecutively enrolled to the registry at time of HIV diagnosis and demographic, therapeutic, and laboratory data are registered at least every 6 months. We extracted data regarding antiretroviral therapy (ART), HIV-RNA, $CD4^+$ T-cell counts, date of HIV diagnosis, mode of HIV transmission, and country of birth.

All HIV-negative women, born between 1942 and 1989, living in the counties of Stockholm and Gothenburg, sometime between 1983 and 2014, were identified from the SPR (n = 1,189,835; **Fig. 1**) and data regarding date of birth and country of birth were extracted. This registry contains all individuals residing in Sweden on a permanent basis (17).

All WLWH with a diagnosis of cervical intraepithelial neoplasia grade 2, grade 3, adenocarcinoma *in situ*, or invasive cervical cancer

[1]Unit of Infectious Diseases, Department of Medicine, Huddinge, Karolinska Institutet, Stockholm, Sweden. [2]Centre for Clinical Research Västmanland, Västmanland County Hospital, Uppsala University, Uppsala, Sweden. [3]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden. [4]Division of Clinical Pathology, Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden. [5]Department of Infectious Diseases, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden. [6]CLINTEC, Department of Obstetrics and Gynaecology, Karolinska University Hospital, Huddinge, Stockholm, Sweden.

Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

Corresponding Author: Christina Carlander, Karolinska Institute, Stockholm 72461, Sweden. Phone: 467-0883-0761; E-mail: christina.carlander@ki.se

Cancer Epidemiol Biomarkers Prev 2020;29:2662–8

doi: 10.1158/1055-9965.EPI-20-0969

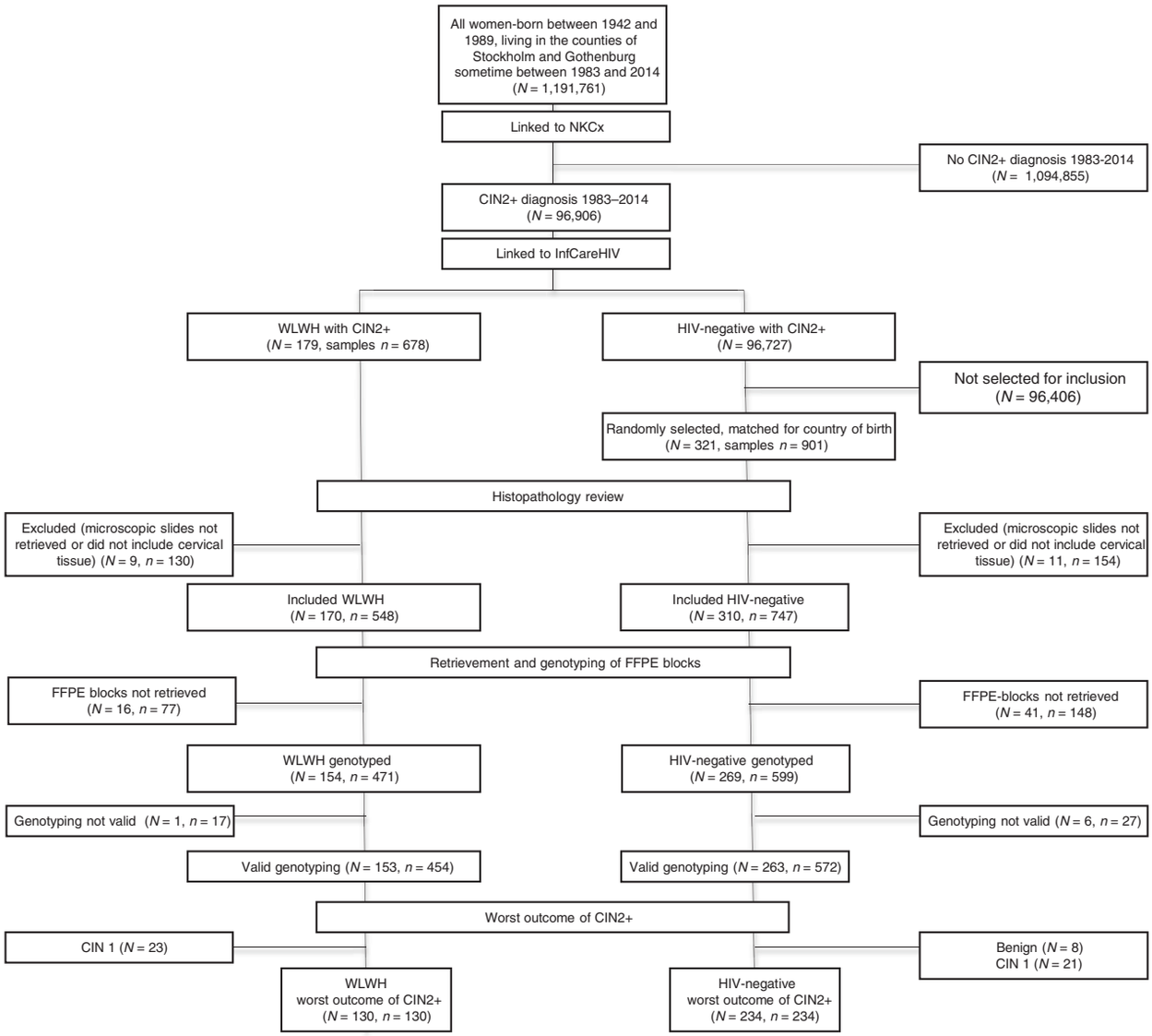

Figure 1.

Flowchart of the study population. CIN2+, cervical intraepithelial neoplasia grade 2, grade 3, adenocarcinoma *in situ*, and invasive cervical cancer; FFPE, formalin-fixed paraffin embedded; InfCare HIV, Swedish National HIV Registry; *N*, number of individuals; *n*, number of cervical samples; NKCx, Swedish National Cervical Screening Registry; WLWH, women living HIV.

(CIN2+) were identified from the NKCx ($n = 179$; **Fig. 1**). This registry includes all cervical cytology and histopathology results in Sweden since 1993, irrespective of where screening or colposcopy has taken place. For each included WLWH, two HIV-negative women diagnosed with CIN2+, living in the same counties sometime between 1983 and 2014, were randomly selected and matched for country of birth ($n = 321$; **Fig. 1**). For some WLWH, only one or no HIV-negative women from the same country of birth could be identified. These women were then matched to HIV-negative women from a neighboring country (Supplementary Table S1). During the study period, all women living in Sweden were invited to cervical cancer screening every 3 (ages 23–50) to 5 years (ages 51–60), according to national guidelines. WLWH were recommended annual screening. Colposcopy was used when indicated.

Final outcome was defined as type-specific HPV, detected in first occurrence of most severe CIN2+ of included women.

Women were classified into six regions of birth: Sweden, Western Europe except Sweden, Eastern Europe and Central Asia, Sub-Saharan Africa, Asia and Pacific, or Latin America and Caribbean (Supplementary Table S1).

HPV genotyping

Formalin-fixed paraffin-embedded (FFPE) blocks and corresponding diagnostic slides from cervical biopsies/conizations are stored at local pathology laboratories (bio-banks). Diagnostic slides of included women were retrieved and reviewed by an experienced pathologist. Confirmed cases of CIN2+ were included in the final study population (**Fig. 1**).

All cases were sectioned according to a contamination-proof procedure, as described previously (18). The first and last sections for each case were stained with hematoxylin and eosin for later rereview if needed. In between each case-block, a blank-block was sectioned as contamination control. All sections, blank-blocks and case-blocks, were extracted with a xylene-free method and HPV genotyped using PCR with modified general primers–PCR (primer targeting L1) and hybridization with type-specific probes in Luminex, as described previously (18–20). Forty-two beads, encompassing 37 different HPV types, three HPV variants, and two "universal" HPV probes, were included in the Luminex. If the case was HPV-negative, extracted material from both the blank-block and case-block was diluted 1/10 and retested. Blank-blocks and the corresponding case-blocks were treated in exactly the same way during the whole process. All samples were analyzed for β-globin by quantitative real-time PCR to confirm sample adequacy. The blank-block had to be negative for both HPV and β-globin and the case-block positive for β-globin. Cases that were β-globin negative were classified as inadequate samples and were not analyzed. Cases with a contaminated blank-block were not analyzed.

Cases that were HPV-negative in genotyping were analyzed for the E7/E6 regions of the two common oncogenic HPV types, HPV16 (primer targeting E7) and HPV18 (primer targeting E6) using real-time PCR with 1 μL DNA used in both assays with a total volume of 25 μL (21).

Ethical approval

The Regional Ethics Committee in Stockholm, Sweden granted ethical approval for this study and declared that written informed consent from patients was not needed for this national population-based register study (212/70-31/1, 2012/1776-32, 2013/2032-32, 2015/1970-1970-32, 2016/1618-32). This study was conducted in accordance with ethical guidelines in the Declaration of Helsinki and was approved by an institutional review board.

Statistical analysis

Conditional logistic regression, stratified by country of birth, was used to compare differences in type-specific HPV, detected in first occurrence of most severe CIN2+, for each category defined by HIV status and grade of cervical lesion. ORs of any multiple HPV, single/multiple HPV16, single/multiple HPV35 were calculated with 95% confidence intervals (CI). All models were adjusted (adjOR) for age (continuous, at time of first occurrence of most severe CIN2+ diagnosis).

Similar to the above analyses, we compared the proportion of HR HPV types covered by 2-valent/4-valent (HPV 16 and/or 18), and 9-valent HPV (16/18/31/33/45/52/58) vaccines. In this calculation, we included multiple infections with both vaccine genotypes and other HR genotypes, that is, at least one vaccine genotype.

In a sensitivity analysis, using ordinary logistic regression, we broke matching to perform cross-group comparisons, categorizing women with CIN3 by HIV status and birth region. In a second sensitivity analysis, we included only women born in SSA.

For WLWH, we assessed the effect of HIV-specific covariates [undetectable HIV-RNA (HIV-RNA < 50 copies/mL or < 400 copies/mL before year 2004) at time of CIN2+, $CD4^+$ T-cell count at time of CIN2+, nadir $CD4^+$ cell count) associated with any multiple HPV, single/multiple HPV16, single/multiple HPV35, and genotypes covered by HPV vaccines, using ordinary logistic regression, we broke matching to perform cross-group comparisons, presented as adjOR or *P* for trend (P_{trend}). Calculations were done using STATA 13 software.

Results

Of 500 included women (179 WLWH and 321 HIV-negative), registered with CIN2+ in NKCx, valid HPV genotyping of the most severe histologically confirmed CIN2+ lesion was obtained for 364 women (130 WLWH and 234 HIV-negative; **Fig. 1**; **Table 1**). Diagnostic microscopic slides from 18 women (9 WLWH and 11 HIV-negative) could not be retrieved from the bio-bank or samples retrieved were not confirmed CIN2+ and these women were excluded. FFPE blocks from 57 women (16 WLWH and 41 HIV-negative) with eligible samples were never received from the bio-banks. In total, 1,070 blocks from 154 WLWH and 269 HIV-negative were sectioned and HPV

Table 1. Characteristics of participants with histology-confirmed CIN2+ and valid HPV genotyping performed.

	WLWH *N* (%)	HIV-negative *N* (%)
All, *n*	130	234
HPV status		
HPV positive	125 (96)	214 (91)
Lesion grade		
CIN2	47 (36)	66 (28)
CIN3	79 (61)	154 (66)
AIS	—	4 (2)
Squamous cell carcinoma	4 (3)	8 (3)
Adenocarcinoma	—	2 (1)
Year of CIN2+ diagnosis		
1983–1995	21 (16)	24 (10)
1996–2005	49 (38)	78 (33)
2006–2015	60 (46)	132 (56)
Age, years, mean (SD)	36 (8)	35 (8)
Birth region		
Sweden	41 (31)	82 (35)
Sub-Saharan Africa	60 (46)	83 (35)
Asia and Pacific	19 (15)	48 (20)
Other[a]	10 (8)	21 (9)
Time since HIV diagnosis		
Years, median (IQR)	4.4 (1.6–9.3)	—
Level of immunosuppression		
Nadir CD4, cells per μL, median (IQR)[b]	140 (50–240)	—
CD4, cells per μL, median (IQR)	332 (185–497)	—
HIV-RNA level		
Undetectable[c]	71 (55)	—
Detectable	59 (45)	—
Year of HIV diagnosis		
1984–1993	46 (35)	—
1994–2003	49 (38)	—
2004–2013	35 (27)	—
Mode of HIV transmission		
Heterosexual	107 (82)	—
Intravenous drug use	20 (15)	—
Other[d]	3 (2)	—

Note: Measurements closest to date of first occurrence of most severe CIN2+, unless otherwise stated. Data are number *N* (%) or median (IQR), unless otherwise indicated. Percentages do not always add up to 100 due to rounding. Abbreviations: AIS, adenocarcinoma *in situ*; CIN, cervical intraepithelial neoplasia; CIN2+, CIN2, CIN3, AIS, and ICC; WLWH, women living with human immunodeficiency virus.

[a]Western Europe except Sweden, Eastern Europe and Central Asia, Latin America and Caribbean.

[b]Lowest CD4 count measured since the diagnosis of HIV.

[c]HIV-RNA <50 copies/mL (<500 copies/mL before 2004).

[d]Defined as blood products, homosexual/bisexual and unknown.

Table 2. Prevalence of single and multiple HPV infection and potential HPV vaccine coverage categorized by HIV status and grade of lesion.

	CIN2			CIN3		
	WLWH *N* (%)	HIV-negative *N* (%)	adjOR[a]	WLWH *N* (%)	HIV-negative *N* (%)	adjOR[a]
Any positive HPV	44	60	—	77	141	—
Single and multiple HPV						
Any multiple HPV	17 (38.6)	13 (21.7)	3.17 (1.19–8.45)	23 (29.9)	28 (19.9)	1.48 (0.73–2.98)
Single or multiple 16 (any HPV)	6 (13.6)	24 (40.0)	**0.14** (0.04–0.56)	39 (50.6)	75 (53.2)	0.93 (0.51–1.70)
Single or multiple 18 (any non-16)	0 (0)	3 (5.0)	NA	3 (2.13)	3 (3.9)	2.96 (0.42–20.78)
Single or multiple 35 (any non-16/18)	8 (18.2)	3 (5.0)	**9.71** (1.06–88.52)	10 (13.0)	4 (2.8)	**6.25** (1.28–30.43)
Potential HPV vaccine coverage						
At least one of 16/18	6 (13.6)	27 (45.0)	**0.13** (0.03–0.51)	42 (54.6)	78 (55.3)	1.05 (0.57–1.93)
At least one of 16/18/31/33/45/52/58	27 (61.4)	45 (75.0)	**0.53** (0.20–1.39)	64 (83.1)	133 (94.3)	**0.33** (0.11–1.00)

Note: Numbers are *N* (% of any positive HPV). Percentages do not always add up to a hundred due to rounding. Significant results in bold.
Abbreviations: NA, not applicable; WLWH, women living with HIV.
[a]OR (HIV-infected vs. HIV-negative women) calculated using conditional logistic regression analysis, stratified by country of birth and adjusted for age.

genotyped. Seven women (one WLWH and six HIV-negative) were excluded because of nonvalid genotyping (β-globin negative or contaminated blank block) and 52 women were excluded because the histopathologic rereview found only lesions less than CIN2 (one CIN1 among WLWH and 21 CIN1 and eight benign among HIV-negative).

Mean age at time of CIN2+ diagnosis in women with valid HPV genotyping was 36 years in WLWH and 35 years in HIV-negative (**Table 1**). Most WLWH had acquired HIV heterosexually (82%), a majority were migrants from SSA (46%), and had at one point been highly immunosuppressed [median nadir CD4 140 cells (interquartile range 50–240)]. About half of WLWH had undetectable HIV-RNA at time of CIN2+; a majority of WLWH was diagnosed with CIN2+ before ART was recommended to everyone at time of HIV diagnosis (**Table 1**; Supplementary Table S2).

WLWH with CIN2 were less likely to have single HPV16 (9% vs. 30%, $P = 0.011$; Supplementary Table S3) and single/multiple HPV16 (14% vs. 40%; adjOR 0.14; 95% CI, 0.04–0.56; **Table 2**) than HIV-negative. In women with CIN3, no significant difference was seen in single HPV16 (38% vs. 42%; $P = 0.837$; Supplementary Table S3) or single/multiple HPV16 (51% vs. 53%; adjOR 0.93; 95% CI, 0.51–1.70; **Table 2**) by HIV status.

When women with CIN3 were categorized by birth region in cross-region analysis, there was no difference in the prevalence of single/multiple HPV16 infections in Swedish women by HIV status (**Table 3**). All women born in SSA (regardless of their HIV status) were less likely to have single/multiple HPV16 infections than HIV-negative born in Sweden (**Table 3**). This difference in single/multiple HPV16 infections in women from SSA compared with Swedish women remained when restricting the analysis to WLWH, adjusting for CD4 count and age (WLWH born in SSA, adjOR 0.18; 95% CI, 0.05–0.65, reference WLWH born in Sweden), and similar results were seen when adjusting for CD4 nadir (adjOR 0.17; 95% CI, 0.05–0.62, reference WLWH born in Sweden).

The prevalence of HPV18 was low irrespective of HIV status and grade of lesion (Supplementary Table S3). There was no significant difference in the prevalence of HPV 45 by HIV status.

Table 3. Prevalence of single and multiple HPV infection in CIN3 by HIV status and birth region.

		CIN3					
		HIV-negative born in Sweden (reference)	HIV-negative born in SSA	HIV-negative born in other[a]	WLWH born in Sweden	WLWH born in SSA	WLWH born in other[a]
Any positive HPV	*N*	43	51	47	23	34	20
Any multiple HPV	*N* (%)	10 (23%)	7 (14%)	11 (23%)	3 (13%)	14 (41%)	6 (30%)
	adjOR[b]	1.0	0.62 (0.21–1.85)	1.16 (0.43–3.16)	0.58 (0.14–2.43)	**2.87** (1.02–8.08)	1.66 (0.49–5.59)
Single or multiple 16	*N* (%)	26 (60%)	21 (41%)	28 (59%)	16 (69%)	12 (35%)	11 (55%)
	adjOR[b]	1.0	**0.42** (0.18–0.99)	0.91 (0.39–2.14)	1.39 (0.46–4.13)	**0.32** (0.12–0.85)	0.75 (0.25–2.21)
Single or multiple 35 (any non-16/18)	*N* (%)	0 (0%)	3 (6%)	1 (2%)	1 (4%)	7 (21%)	2 (10%)
	adjOR[b]	NA	NA	NA	NA	NA	NA
At least one of 16/18	*N* (%)	26 (60%)	23 (45%)	29 (62%)	16 (70%)	13 (38%)	13 (65%)
	adjOR[b]	1.0	0.52 (0.22–1.21)	1.03 (0.44–2.43)	1.45 (0.49–4.31)	**0.39** (0.15–1.00)	1.18 (0.39–3.60)
At least one of 16/18/31/33/45/52/58	*N* (%)	41 (95%)	46 (90%)	46 (98%)	22 (96%)	23 (53%)	19 (95%)
	adjOR[b]	1.0	0.39 (0.07–2.18)	1.99 (1.17–23.24)	0.93 (0.78–11.08)	**0.08** (0.02–0.44)	0.82 (0.07–9.76)

Note: Numbers are *N* (% of any positive HPV). Significant results in bold.
Abbreviations: SSA, sub-Saharan Africa; WLWH, women living with HIV.
[a]Western Europe except Sweden, Eastern Europe and Central Asia, Asia and Pacific, Latin America and Caribbean.
[b]OR, adjusted for age, calculated using ordinary logistic regression (matching was broken to perform cross-group comparisons).

WLWH with CIN3 were six times more likely to have single/multiple HPV 35 infection than HIV-negative (adjOR 6.2; 95% CI, 1.28–30.43). Similar results were seen for CIN2 (**Table 2**). Almost all cases of HPV35 were found in women born in SSA (70% of WLWH, 75% of HIV-negative) but while single/multiple HPV35 represented 6% of CIN3 in HIV-negative born in SSA they represented 21% in WLWH born in SSA (**Table 3**). We were not able to stratify by birth region in cross-region analysis due to the low number of HPV35 in women born in Sweden. When restricting the analysis to only women born in SSA, differences by HIV status remained (adjOR 4.2; 95% CI, 1.00–17.72).

The likelihood of having any multiple HPV infection was increased in WLWH compared with HIV-negative in CIN2 (39% vs. 22%; adjOR 3.2; 95% CI, 1.19–8.45) but not in CIN3 (30% vs. 20%; adjOR 1.5; 95% CI, 0.73–2.98; **Table 2**). In cross-region analysis, it was only WLWH born in SSA that had significant increase of multiple HPV infections (adjOR 2.9; 95% CI, 1.02–8.08), reference HIV-negative born in Sweden; **Table 3**). When restricting the analysis to WLWH, adjusting for CD4 count and age, results by birth region remained similar but not statistically significant (WLWH born in SSA adjOR 3.0; 95% CI, 0.69–12.87, reference WLWH born in Sweden).

WLWH with CIN3 were less likely to have genotypes covered by the 9-valent vaccine (83% vs. 94%; adjOR 0.33; 95% CI, 0.11–1.00), compared with HIV-negative (**Table 2**).

When performing cross-region analysis, WLWH with CIN3 born in SSA were the only women with reduced prevalence of 9-valent types compared with HIV-negative born in Sweden (**Table 3**). When restricting the analysis to women born in SSA, WLWH remained less likely to have 9-valent types compared with HIV-negative (adjOR 0.23; 95% CI, 0.07–0.74, reference HIV-negative born in SSA). When restricting the analysis to WLWH, women from SSA remained less likely to have 9-valent types compared with WLWH born in Sweden, adjusting for CD4 count and age (WLWH born in SSA adjOR 0.08; 95% CI, 0.01–0.73). Similar result was reached adjusting for CD4 nadir (adjOR 0.06; 95% CI, 0.01–0.68).

Neither the level of CD4 cell count (at time of CIN2+/nadir CD4 cell count) nor the level of HIV-RNA was significantly associated with the detection of any multiple HPV, single or multiple HPV16, single or multiple HPV 35, HPV 16/18 or of having genotypes covered by the 9-valent vaccine, (Supplementary Table S4).

There were no cases of adenocarcinoma *in situ* in WLWH and four cases in HIV-negative (one with multiple infection, HPV16, 45, and 66; two with HPV18; and one HPV-negative). There were 4 cases of invasive cervical cancer (ICC) in WLWH of which all were squamous cell carcinoma (2 HPV16, 1 HPV45, and 1 HPV68) and 10 cases in HIV-negative; 8 cases of squamous cell carcinoma (7 HPV16 and 1 HPV18), and 2 cases of adenocarcinoma (1 HPV16 and 1 HPV18; Supplementary Table S5).

Discussion

In this population-based study on HPV types in cervical biopsies of CIN2+, we found the prevalence of HPV16 to be decreased in WLWH diagnosed with CIN2 compared with HIV-negative women. However, in women with CIN3, we found similar prevalence of HPV16, irrespective of HIV status, when controlled for country of birth. WLWH born in SSA were less likely covered by the 9-valent vaccine compared with women with HIV born in Sweden, mostly because of decreased prevalence of HPV16 and increased prevalence of HPV35.

Unlike most previous studies on CIN3 performed in the United States or in Europe, but similar to recent studies based in SSA, we did not find a significant difference in the prevalence of HPV16 by HIV status in CIN3 (22–25). Earlier studies on HPV in WLWH with CIN3 are almost all based on PCR from cervical lavage specimens, which may explain why we found a higher proportion of HPV16 in our biopsy-based study (9). When performing cross-region analysis, all women born in SSA had lower prevalence of HPV16 in CIN3, irrespective of HIV status, compared with women born in Sweden. This is consistent with results from earlier meta-analyses that have found women from SSA to have the lowest proportion globally of HPV16 in all grades of cervical lesions, regardless of HIV status (8, 9, 26, 27). Meanwhile, U.S. studies have found lower HPV16 prevalence in CIN3+ in African American women compared with Caucasian women, regardless of HIV status and independent of immune status (28, 29).

The second most common single infection in WLWH was HPV35 and HIV status was associated with HPV35 in adjusted models. Most of single/multiple HPV35 were detected in women born in SSA, which agrees with earlier studies that have shown an increase of HPV 35 in all grades of cervical lesions in women born in SSA compared with other regions of the world in both HIV-negative women and WLWH (8, 9, 30). A recent U.S. study (HIV status not stated) found African American women to have more HPV35 and more HPV35-associated precancers (30). In addition, a recent study based in SSA found persistent HPV35 (and 56) to be the nonvaccine types most commonly associated with incident CIN2+ in WLWH (31). However, given that HPV35 only caused 7% of 770 cases of ICC in a meta-analysis based on WLWH diagnosed with ICC in SSA, it is likely that some of CIN3 caused by HPV35 in our study would not proceed to ICC, even when untreated (27). Nonetheless, HPV35 was the fifth most common cause of ICC in WLWH in the previously mentioned meta-analysis based in SSA. Adding HPV35 to the HPV vaccine may thus improve vaccine effectiveness in WLWH with SSA origin/ancestry.

We found a lower than expected prevalence of HPV18 in CIN3 in all women, but, in particular, in WLWH who have been previously found to have a higher proportion of HPV18 compared with HIV-negative women (9). The detection of HPV45 was also lower than expected, given that women born in SSA, who have earlier been found to have more HPV45, dominated our study population (24, 27). One reason for lower than expected HPV18/45 may be the few cases of ICC included in this study as it has been suggested that the carcinogenicity of HPV 18/45 is underestimated in studies based on CIN2/CIN3, rather than ICC, due to the fact that the increase of HPV 18/45 is mainly seen in the interim between the development of CIN3 to ICC (8).

We did not find as high rates of multiple HPV infection in WLWH with CIN3 as have been seen in earlier studies (32–34). One explanation may be that our study was based on biopsies rather than cell samples, and testing for HPV from biopsies is known to reduce the prevalence of multiple infections (8, 9, 27, 35). The clinical importance of multiple infections is unclear as prospective evidence on this issue is limited (36). A U.S. study with long-term follow-up did not find multiple HPV infection associated with an increase of CIN3, adenocarcinoma *in situ* or ICC (34). In another study, including only HIV-negative women, coinfection with both low- and high-risk HPV was even associated with a reduced risk of future invasive disease and slower progression to ICC (37).

It has been suggested that the increased prevalence of non-HPV16 (i.e., less carcinogenic HR HPVs) detected in WLWH diagnosed with CIN2/CIN3 may partly explain why, although cervical cancer is increased in WLWH, the absolute risk is not as great as for other virally associated AIDS-defining cancers such as Kaposi sarcoma or

non-Hodgkin lymphoma (22, 38, 39). In Sweden, HPV testing includes all HR HPVs but triaging for HPV16/18 is often recommended, especially in low resource settings (40, 41). The question remains whether to include HR HPV other than HPV16/18 in HPV-based cervical cancer screening in WLWH with the risk of reducing the positive predictive value of this test.

This study has some limitations. Although all pathology bio-banks were (in principle) open access not all cervical blocks of included women were sent. This had different reasons such as lack of tissue left in the block, the bio-bank not willing to provide the material requested or diagnostic slides needed for review having been discarded (19). We did not adjust for smoking, as this information was not available. It is unlikely though that confounding by smoking entirely explains our results.

To the best of our knowledge, we present the largest population-based study, outside SSA, on HPV types in cervical biopsies from WLWH with CIN2+ (9). We have had access to high quality data from a nationwide register of cervical histopathology and a virtually complete national HIV register. A unique factor is that we have had access to registry data regarding country of birth of both WLWH and HIV-negative women, which enabled us to match on this variable thus reducing the impact of confounding factors associated with origin of birth. An experienced pathologist reviewed all slides to ensure that only confirmed CIN2+ diagnoses were included and contamination-proof standardized and quality-assured sectioning of FFPE blocks was performed followed by quality-controlled genotyping, altogether adding to the high validity of this study.

In conclusion, this study adds to the body of knowledge regarding HPV type-specific prevalence, by HIV status and country of birth, in women with cervical precancer. We found no difference in the prevalence of HPV16 in CIN3, when country of birth was controlled for, which is consistent with recent studies establishing HPV16 as the priority target for the prevention of cervical cancer irrespective of HIV status (25, 27). In particular, this study adds to the growing literature that HPV35 make up a greater proportion of CIN3+ among women from SSA than women from European origins. This could have implications for HPV vaccine, which currently does not include HPV 35, and for HPV-screening algorithms in women of SSA origin/ancestry.

Disclosure of Potential Conflicts of Interest

C. Carlander reports grants from Västmanland County Council during the conduct of the study, as well as personal fees from Gilead Sciences (lectures), AbbVie (lectures), and GlaxoSmithKline/ViiV (advisory board) outside the submitted work. M. Dzabic reports personal fees from MSD outside the submitted work. J. Dillner reports grants from Merck (to institute; not related to the present work), Roche (to hospital; not related to the present work), and Genomica (to hospital; not related to the present work) during the conduct of the study. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

C. Carlander: Conceptualization, resources, data curation, formal analysis, supervision, funding acquisition, validation, investigation, visualization, methodology, writing–original draft, project administration, writing–review and editing. **C. Lagheden:** Data curation, formal analysis, validation, investigation, methodology, writing–original draft, performed laboratory work (HPV genotyping). **C. Eklund:** Data curation, formal analysis, validation, investigation, methodology, writing–original draft, performed laboratory work (HPV genotyping). **S. Nordqvist Kleppe:** Data curation, software, validation, methodology, writing–original draft, project administration. **M. Dzabic:** Validation, methodology, writing–original draft. **P. Wagner:** Conceptualization, formal analysis, validation, methodology, writing–original draft, performed statistical analyses with Christina Carlander. **A. Yilmaz:** Conceptualization, supervision, visualization, writing–original draft, critically revised original draft from the point of HIV specialist. **K. Elfgren:** Supervision, visualization, writing–original draft, critically revised original draft from the point of gynecologist specializing in HPV. **A. Sönnerborg:** Conceptualization, supervision, visualization, writing–original draft. **P. Sparén:** Conceptualization, data curation, software, formal analysis, supervision, validation, investigation, visualization, methodology, writing–original draft. **J. Dillner:** Conceptualization, resources, supervision, funding acquisition, validation, investigation, visualization, methodology, writing–original draft, writing–review and editing.

Acknowledgments

C. Carlander received funding from the Västmanland County Council and J. Dillner from the Swedish Cancer Society. The funders had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

C. Carlander, P. Wagner, A. Sönnerborg, P. Sparén, and J. Dillner contributed to the design. S. Nordqvist Kleppe collected slides and blocks, and performed data management. M. Dzabic performed pathological review. C. Lagheden and C. Eklund performed laboratory work. C. Carlander and P. Wagner performed statistical analysis. C. Carlander drafted the manuscript. All authors contributed to the interpretation of data, critically reviewed the article, and approved the final version of the report.

The authors thank Pouran Almstedt and Emmi Andersson for help with extracting data from NKCx and InfCareHIV, respectively.

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Received June 24, 2020; revised September 1, 2020; accepted September 18, 2020; published first September 23, 2020.

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