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Prevalence of human papillomavirus in women with invasive cervical carcinoma by HIV status in Kenya and South Africa

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Data on the prevalence of human papillomavirus (HPV) types in cervical carcinoma in women with HIV are scarce but are essential to elucidate the influence of immunity on the carcinogenicity of different HPV types, and the potential impact of prophylactic HPV vaccines in populations with high HIV prevalence. We conducted a multicentre case–case study in Kenya and South Africa. During 2007–2009, frozen tissue biopsies from women with cervical carcinoma were tested for HPV DNA using GP5+/6+-PCR assay. One hundred and six HIV-positive (mean age 40.8 years) and 129 HIV-negative women (mean age 45.7) with squamous cell carcinoma were included. Among HIV-positive women, the mean CD4 count was 334 cells/μL and 48.1% were on combined antiretroviral therapy. HIV-positive women had many more multiple HPV infections (21.6% of HPV-positive carcinomas) compared with HIV-negative women (3.3%) ($p < 0.001$) and the proportion of multiple infections was inversely related to CD4 level. An excess of HPV18 of borderline statistical significance was found in HIV-positive compared with HIV-negative cases (Prevalence ratio (PR) = 1.9, 95% confidence interval (CI): 1.0–3.7, adjusted for study centre, age and multiplicity of infection). HPV16 and/or 18 prevalence combined, however, was similar in HIV-positive (66.7%) and HIV-negative cases (69.1%) (PR = 1.0, 95% CI: 0.9–1.2). No significant difference was found for other HPV types. Our data suggest that current prophylactic HPV vaccines against HPV16 and 18 may prevent similar proportions of cervical SCC in HIV-positive as in HIV-negative women provided that vaccine-related protection is sustained after HIV infection.

Infection with high-risk (HR) human papillomavirus (HPV) is a necessary cause for invasive cervical carcinoma. World-wide HPV16 and 18 are found in 57% and 16% of cervical carcinomas, respectively, according to the findings of a recent meta-analysis[1] and are targeted by the current prophylactic HPV vaccines for cervical carcinoma prevention. HIV-positive women are at increased risk for HPV infection and progression to cervical intraepithelial neoplasia grade 3 (CIN3).[2] Linkage studies between HIV/AIDS and cancer registries have shown a 2- to 22-fold increased invasive cervical carcinoma incidence in HIV-positive women compared with the general female population from the same area, depending upon the life expectancy of HIV-positive women and the coverage and quality of cervical cancer screening in different countries.[3,4]

HPV16 infection and HPV16-associated precancerous lesions were reported to be less dependent on a woman's immune status compared with other HR HPV types.[5] Indeed, a meta-analysis of HPV prevalence in HIV-positive women worldwide showed a relative underrepresentation of HPV16 and overrepresentation of the other HR HPV types in HIV-positive compared with HIV-negative women with or without cervical abnormalities.[6] The lower prevalence of HPV16 in CIN2/3 (the endpoint lesions used in the evaluation of HPV vaccine efficacy) raised the fear that vaccination may prevent a smaller proportion of invasive cervical carcinomas in HIV-positive than HIV-negative women.

Information on HPV prevalence in HIV-positive women with invasive cervical carcinoma is scarce.[7] The aim of this

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study was, therefore, to further elucidate whether any difference exists in HPV type distribution between HIV-positive and HIV-negative women with invasive cervical carcinoma in two sub-Saharan African countries (Kenya and South Africa) where the HIV epidemic has been especially severe for several decades.[8] The study was approved by ethical review committees from the International Agency for Research on Cancer (IARC) and locally from both study sites in Kenya and South Africa.

Material and Methods
Participants and study procedures
Between August 2007 and June 2009, a case–case study of invasive cervical carcinoma in HIV-positive and HIV-negative women was conducted at two teaching hospitals that concentrate a large proportion of local invasive cervical carcinoma cases: the Kenyatta National Hospital (KNH), Nairobi, Kenya and the Inkosi Albert Luthuli Central Hospital (IALCH), Durban, South Africa. Women who presented at the gynecology departments of those hospitals with a suspected or confirmed diagnosis of invasive cervical carcinoma received information about the study and were invited to participate ($n = 274$). We initially restricted our present study to women aged 50 years or younger, but we eventually also included some older women. Two hundred fifty-one women agreed to participate.

After an informed consent had been signed, a clinical report form was filled out, including clinical and demographical information. A venous blood sample was taken by a trained nurse to allow HIV testing and CD4 count. Information on HIV viral load was not available. Appropriate procedures for HIV testing including pre- and post-test counselling were followed. At the Durban site, HIV testing was not repeated if the medical records clearly indicated that a woman had previously been tested HIV-positive. Similarly, a CD4 count was not always repeated if a recent CD4 result (within 6 months of the study visit) was available. At a subsequent visit, a trained gynecologist obtained a biopsy from the suspected cervical lesion. The tissue sample was placed dry in a cryotube, immediately immersed in liquid nitrogen and then stored at $-80°C$ until transportation. A second biopsy was obtained for local histological examination. A frozen sample was not obtained from five participants from Nairobi due to temporary unavailability of liquid nitrogen, and HPV testing was performed instead on a buffered formaldehyde-fixated and paraffin-embedded biopsy.

HPV DNA testing
HPV DNA testing on frozen and paraffin-embedded biopsies was performed at the Department of Pathology of the Vrije University Medical Center (VUMC), Amsterdam, according to a protocol similar to that used in previous IARC HPV Prevalence Surveys.[9] One or more 5 μM sections representing ~ 1 cm^2 of tissue were predigested with Proteinase K, after

which DNA was extracted using magnetic beads (Macherey-Nagel, Düren, Germany). Beta-globin PCR analysis was performed in order to assess the quality of the DNA to be submitted to HPV PCR. HPV DNA was first determined using general primer GP5+/6+-mediated PCR.[10] PCR products were hybridized using an enzyme immunoassay (EIA) that included two oligoprobes: one for HR HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 and another for low-risk (LR) HPV types 6, 11, 26, 30, 32, 34, 40, 42, 43, 44, 53, 54, 55, 57, 61, 64, 67, 69, 70, 71, 72, 73, 81, 82/mm^4, 82/is39, 83, 84, 85, 86, cp6108 and jc9710. Subsequent HPV typing was performed by reverse-line blot hybridization of PCR products, as described previously.[11] HPV types of IARC classification group 1 "carcinogenic to humans" (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59) and group 2A "probably carcinogenic to humans" (HPV68) were considered as HR types for this analysis.[12,13] All other HPV types were considered LR types. Samples that were positive at HR EIA, but did not reveal positivity in the typing assay were considered to be uncharacterized high-risk types (HRx). Two women who were negative for beta-globin and HPV DNA were excluded.

Histological diagnosis
Histological examinations were performed at the Pathology Departments of the KNH and IALCH. An additional histological examination was done on the frozen samples at the VUMC for those with indeterminate histological results (30 from Nairobi and 24 from Durban).

Ultimately, valid histological diagnosis and HPV findings were available for 129 squamous cell carcinoma (SCC) cases and six adeno/adenosquamous cell carcinoma (ADC) cases from Kenya, and 106 SCC cases and 12 ADC cases from South Africa. Since HPV type distribution in ADC is different compared with SCC cases, and only few ADC were found, we focused this report on the 235 women with SCC and briefly described the 18 ADC cases separately.

Statistical analysis
Mean ages were compared using Student's two-group mean-comparison test. Odds ratios (ORs) and corresponding 95% confidence intervals (CI) for multiple *versus* single HPV infection were estimated using logistic regression. The potential risk factors for multiple infections investigated were HIV status and, among HIV-positive women, CD4 cell count (<200; 200–349; ≥350 cells/μL) and combined antiretroviral therapy (cART) use. All unconditional regression models included age (<40; 40–49; ≥50 years) and study centre as cofactors. Among HIV-positive women, the prevalence of the most common HPV types (HPV16, 18, 35, 45) were compared between HIV-positive and HIV-negative women. Prevalence ratios (PRs) adjusted for study centre, age and multiplicity of infection, and corresponding 95% CIs were estimated using a binomial regression model with a log link instead of the logistic link used for estimating odds ratios.[14]

Table 1. Characteristics of 235 women with squamous cell carcinoma of the cervix by study centre and HIV status

	Kenya		South Africa		Overall	
	HIV-pos (N = 46) n (%)	HIV-neg (N = 80) n (%)	HIV-pos (N = 60) n (%)	HIV-neg (N = 49) n (%)	HIV-pos (N = 106) n (%)	HIV-neg (N = 129) n (%)
Age (years)						
<40	22 (47.8)	24 (30.0)	29 (48.3)	6 (12.2)	51 (48.1)	30 (23.3)
40–49	20 (43.5)	37 (46.3)	22 (36.7)	29 (59.2)	42 (39.6)	66 (51.2)
≥50	4 (8.7)	19 (23.8)	9 (15.0)	14 (28.6)	13 (12.3)	33 (25.6)
HPV infection						
Single	30 (65.2)	73 (91.3)	50 (83.3)	46 (93.9)	80 (75.5)	119 (92.3)
Multiple	13 (28.3)	3 (3.8)	9 (15.0)	1 (2.0)	22 (20.8)	4 (3.1)
HPV-negative	3 (6.5)	4 (5.0)	1 (1.7)	2 (4.1)	4 (3.8)	6 (4.7)
CD4 (cells/μL)						
≥500	4 (9.1)		10 (22.7)		14 (15.9)	
350–499	7 (15.9)		8 (18.2)		15 (17.1)	
200–349	19 (43.2)		15 (34.1)		34 (38.6)	
<200	14 (31.8)		11 (25.0)		25 (28.4)	
Missing	*2*		*16*		*18*	
Women on cART						
No	21 (46.7)		33 (55.9)		54 (51.9)	
Yes	24 (53.3)		26 (44.1)		50 (48.1)	
Missing	*1*		*1*		*2*	

The italic values indicate the missing CD4 and cART values.
Abbreviations: cART: combined antiretroviral therapy; HPV: human papillomavirus.

Results

Table 1 shows the distribution of selected characteristics of 235 SCC cases by HIV status and study centre, and overall. The mean age was 40.8 for HIV-positive and 45.7 years for HIV-negative women ($p < 0.001$). HIV-positive women were younger than HIV-negative women, both in Kenya (p-value for mean age = 0.01), and South Africa (p-value for mean age < 0.001). Almost all samples were HPV-positive: 96.2% among HIV-positive and 95.3% among HIV-negative women. Multiple HPV infections were present in 20.8% of HIV-positive women and 3.1% of HIV-negative women ($p < 0.001$). The proportion of multiple HPV infections among HIV-positive women was higher in Kenya compared with South Africa but the difference was not statistically significant after adjustment for CD4 level. Overall, 15.9% of HIV-positive women had CD4 ≥500, 17.1% between 350 and 499, 38.6% between 200 and 349 and 28.4% <200 cells/μL. cART use was reported by 48.1% of HIV-positive women. CD4 level was lower and cART use less frequently reported in Kenya than in South Africa, but the differences were not statistically significant.

Among HPV-positive SCC cases, HIV-positive cases had a much higher risk of harbouring multiple HPV infections (21.6%) than HIV-negative cases (3.3%) (OR = 9.7, 95% CI: 3.1–30.6) (Table 2). The OR for multiple infections increased significantly with the decrease in CD4 level ($p_{\text{trend}} < 0.001$).

Multiple HPV infections were also more frequent among cART users than among nonusers (OR = 6.7, 95% CI: 2.0–22.0, adjusted for study centre and age) but were not influenced by age among HIV-positive women ($p_{\text{trend}} = 0.8$, adjusted for study centre) (data not shown). Of note, cART users had on average a lower CD4 count (mean 298 cells/μL) than nonusers (mean 371 cells/μL).

Figure 1 shows a comparison of the prevalence of individual HR HPV types and the combination of LR HPV types by HIV status. The most frequently detected types in HIV-positive and HIV-negative women were similar: HPV16 (46.1% and 57.7%, respectively), HPV18 (22.6% and 11.4%), HPV45 (16.7% and 13.0%) and HPV35 (9.8% and 5.7%). On account of the higher frequency of multiple infections, all other HR and LR HPV types were detected more often in HIV-positive than HIV-negative women. The prevalence of single infections with LR types was, however, low and similar in HIV-positive (3.9%, including HPV42, 43, 66 and 73) and HIV-negative women (2.4%, including HPV26, 67 and 69).

Table 3 shows the distribution of HR HPV types by HIV status and multiplicity of infection. An excess of borderline statistical significance was found for HPV18 (PR = 1.9, 95% CI: 1.0–3.7) among HIV-positive women compared with HIV-negative women. The distribution of HPV16 and other HR HPV types did not differ by HIV status. When HPV16 and/or 18 were considered their combined prevalence was

similar in HIV-positive (66.7%) and HIV-negative women (69.1%) (PR = 1.0, 95% CI: 0.9–1.2). The PR for HPV16 and/or 18 among HIV-positive compared with HIV-negative women was similar in Kenya and South Africa (PR = 1.0, 95% CI: 0.8–1.4 and PR = 1.1, 95% CI: 0.8–1.4, respectively, adjusted for age and multiplicity) (data not shown).

In respect to the 18 ADC found in this study, HPV16 (n = 2), 18 (4), and 45 (4) infections were detected in 10 HIV-

Table 2. Odds ratios (ORs) for multiple human papillomavirus infection in HIV-positive *versus* HIV-negative women with cervical squamous cell carcinoma overall, and by CD4 level and combined antiretroviral therapy (cART) use[1]

	Single n (%)	Multiple n (%)	OR (95% CI)[2]
HIV-Negative	119 (96.8)	4 (3.3)	1
HIV-Positive	80 (78.4)	22 (21.6)	9.7 (3.1–30.6)
CD4 (cells/μL)[3]			
≥350	24 (88.9)	3 (11.1)	3.8 (0.8–18.8)
200–349	23 (69.7)	10 (30.3)	14.7 (4.0–53.8)
<200	16 (66.7)	8 (33.3)	18.0 (4.5–72.0)
X^2(trend)			*p < 0.001*
cART use[3]			
No	48 (92.3)	4 (7.7)	2.9 (0.7–12.3)
Yes	31 (64.6)	17 (35.4)	20.4 (6.0–69.3)

The italic values indicate X^2 for trend in OR for multiple human papillomavirus infection in HIV-positive versus HIV-negative women by CD4 level.
[1]10 HPV-negative women were excluded. [2]Adjusted for study centre and age. [3]Among HIV-positive women only. Abbreviations: CI: confidence interval.

positive women and HPV16 (1), HPV18 (2), HPV45 (4), and one mixed infection with HPV16 and 45 in eight HIV-negative women (data not shown).

Discussion

Our present study is the largest comparison of HPV type distribution in invasive cervical carcinomas in HIV-positive women with HIV-negative women to date. We found no difference in the proportion of cervical SCC associated with HPV16 and/or 18 by HIV status, which is reassuring for the potential impact of HPV16/18 vaccines on HIV-positive women. We confirmed that HIV-positive SCC cases have a 10-fold risk of multiple infections compared with HIV-negative cases.[7] As suggested before,[15] the proportion of multiple HPV infections was inversely correlated to CD4 level. Women who were using cART were also at increased risk of harbouring multiple HPV types, compared with women who were not using cART. It is plausible to assume that cART in Africa is a marker of longer duration of HIV infection, and hence more protracted immune impairment, notwithstanding a partial immune recovery due to the treatment. The frequency of multiple infections was not related to age in HIV-positive women.

We found no difference in HPV16 prevalence and an excess of HPV18 of borderline statistical significance among HIV-positive women. The presence of HPV16 was not significantly associated with CD4 count or cART status but these findings were based on small numbers. Likewise, the comparison of the prevalence of HR HPV types other than HPV16 and 18 was hampered by broad confidence intervals and the influence of the relatively large proportion of multiple infections among HIV-positive women. The prevalence of HPV45
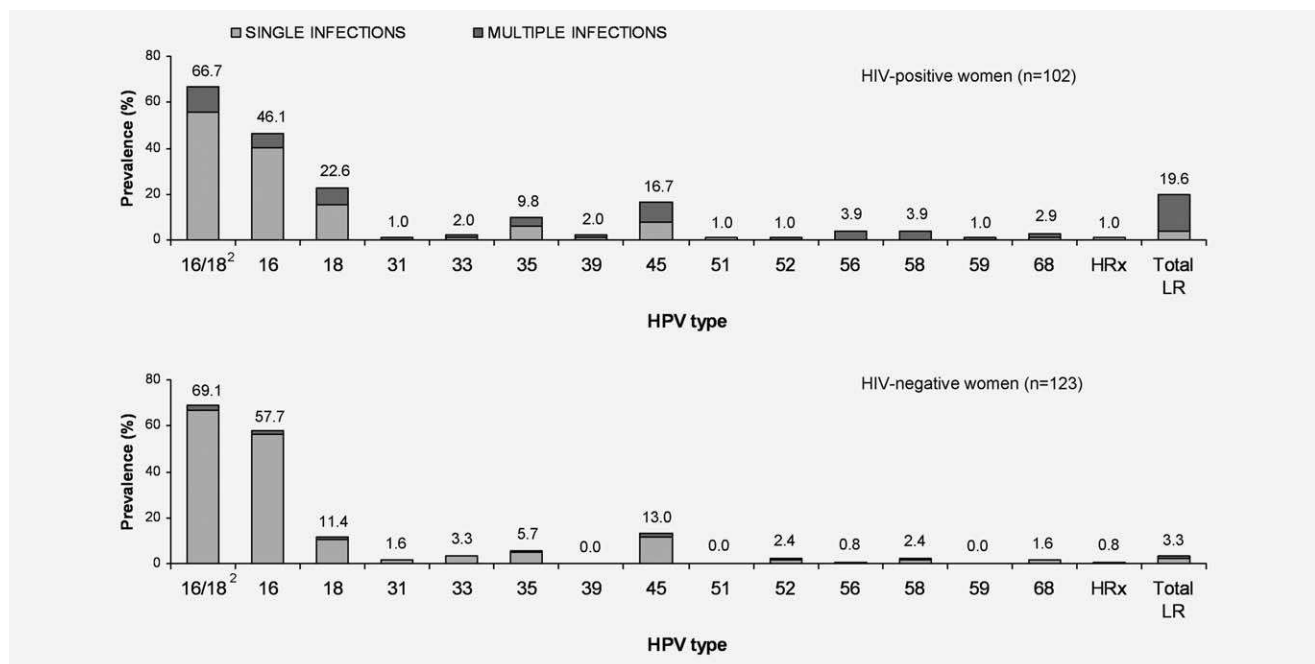

Figure 1. Prevalence of human papillomavirus (HPV) in 225 women with cervical squamous cell carcinoma by HIV status and multiplicity of HPV infection[1]. [1]10 HPV-negative women were excluded. [2]Either 16 or 18 as single infection or in combination with any type as multiple infection. HRx: uncharacterized high-risk type; LR: low-risk.

Table 3. Distribution of selected human papillomavirus (HPV) types in single and multiple infections, and prevalence ratios (PRs) in 102 HIV-positive compared with 123 HIV-negative women with squamous cell carcinoma (SCC) of the cervix[1]

| | Number of SCC infected | | |
HPV types	HIV-pos s/m (%)[2]	HIV-neg s/m (%)[2]	PR (95% CI)[3]
16	41/6 (46.1)	69/2 (57.7)	0.9 (0.7–1.1)
18	16/7 (22.6)	13/1 (11.4)	1.9 (1.0–3.7)
35	6/4 (9.8)	6/1 (5.7)	1.4 (0.5–4.1)
45	8/9 (16.7)	14/2 (13.0)	0.9 (0.5–1.9)
16 and/or 18	57/11 (66.7)	82/3 (69.1)	1.0 (0.9–1.2)
Other HR than 16/18	18/10 (27.5)	33/1 (27.6)	0.9 (0.6–1.5)

[1]10 HPV-negative women were excluded. [2]Overall prevalence of HPV types. [3]Adjusted for study centre, age and multiplicity of infection. Abbreviations: CI, confidence interval; s, single infection; m, multiple infection.

in SCC was similar to that of HPV18 in both HIV-positive and HIV-negative women. In HIV-positive women, however, HPV45 was found more frequently in multiple infections than single infections. LR types were found many-fold more often in HIV-positive than HIV-negative women but in both groups they were rarely detected in the absence of a HR HPV type. Interestingly, among the seven LR types found in single infections, five were classified in group 2B (i.e., limited evidence of carcinogenesis in humans for cervical carcinoma) by an international panel of experts gathered at IARC in 2009.[12,13,16] Two of these types (HPV66 and 73) were found in HIV-positive cases, and three (HPV26, 67 and 69) in HIV-negative cases.

To date, only a few small-sized studies have reported HPV type distribution in invasive cervical carcinomas in HIV-positive women (Table 4). Comparisons of HPV type distribution in invasive cervical carcinomas in HIV-positive women to invasive cervical carcinomas in HIV-negative women have been carried out by us previously in an independent series of cases from one of the study centres included in the present report (Kenya)[7] and in a recent report from Uganda by Odida et al. (2011).[17] HPV type prevalence in invasive cervical carcinomas among HIV-positive women only was also reported by Sahasrabuddhe et al. (2007)[18] (n = 28) and in a few smaller studies[5,19–23] that are summarized in Table 4. The proportion of HPV16 and/or 18 in previous studies ranged between 53.6%[18] to 86.0%[17] and is, therefore, compatible with our current finding of 66.7% and not incompatible with the 71% prevalence of HPV16 and/or 18 seen in invasive cervical carcinomas in the general female population in sub-Saharan Africa.[1,24] These proportions are, however, lower than the prevalence range found in developed countries (Europe and North America combined: 73.2%[24]—75.7%)[1].

Chance variations in small-sized studies and important differences in diagnostic standards, sampling methods and HPV testing, however, hamper comparison of the findings of different studies. SPF10, for instance, is known to be more sensitive than the GP5+/6+ test we used in our present

Table 4. Published and present study findings on the prevalence of human papillomavirus (HPV) types and multiple infections in HIV-positive women with cervical carcinoma among HPV-positive samples

Study	Location	Samples	PCR primers	HPV-pos (n)	HPV16 (n)	HPV18 (n)	HPV16 and/or 18 (%)	HPV31 (n)	HPV35 (n)	HPV45 (n)	HPV52 (n)	HPV58 (n)	Multiple types (%)
Odida et al., 2011[17]	Africa	Exfol. cells	SPF10	43	25	12	86.0[1]	2	0	5	2	0	16.3
De Vuyst et al., 2008[7]	Africa	Exfol. cells	SPF10	51	21	14	64.7	3	4	4	10	0	37.3
Sahasrabuddhe et al., 2007[18]	Africa	Exfol. cells	PGMY-LB	28	10	5	53.6[1]	6	8	5	13	10	78.6
Other studies combined[2]	Africa and US	Mix	Mix	17	9	1	58.8[1]	0	1	3	1	0	–
Total (n (%))				139 (100)	65 (46.8)	32 (23.0)	(68.3)	11 (7.9)	13 (9.4)	17 (12.2)	26 (18.7)	10 (7.2)	(39.4)
Our current study[3]	Africa	Frozen biopsies	GP5+/6+	102	47	23	66.7	1	10	17	1	4	21.6

[1]We report the sum of individual prevalence of HPV16 and 18, as details on combination of HPV16 and 18 were not provided. [2]Includes studies with fewer than six ICC cases each: Strickler et al., 2003[5]; La Ruche et al., 1998[19]; Hawes et al., 2003[20]; Jamieson et al., 2002[21]; De Vuyst et al., 2003[22]; Keita et al., 2009[23]. [3]Among squamous cell carcinoma.

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study.[25,26] This was also evident from the higher proportion of multiple HPV infections (37.3%) found by one SPF10-based study[7] than in our present study in invasive cervical carcinoma, a disease which is understood to arise from a single HPV-infected cell. An additional explanation for the lower proportion of multiple infections in our present study compared with most other studies can be the use of carcinoma tissue biopsies instead of exfoliated cells. Biopsies may be better for the detection of HPV types present in cancer cells, compared with exfoliated cervical cells,[5,18–22] which can lead to the detection of noncancer-associated HPV infections in the cervix and lower genital tract.

The prevalence of HPV52 (1.0%) in our present study was substantially lower than in the combination of previous studies in HIV-positive women (18.7%) and in a meta-analysis of HPV prevalence in CIN2/3 that showed an excess of HPV52 in HIV-positive compared with HIV-negative women.[6] This difference is likely to be attributable to a relatively low sensitivity of the GP5+/6+-based assay to detect HPV52, especially in the presence of multiple HPV infection.[26,27]

Strengths of our present study include the use of frozen tumor biopsies that avoid DNA degradation, and the accurate histological confirmation, including the distinction of SCC from ADC. An additional strength was the use of a well-validated HPV test that allows comparisons with many previous studies.[1] The lower sensitivity of the GP5+/6+-based assay compared with other PCR assays may have actually been an asset of this study as it allowed to reduce the concurrent detection of noncancer-associated HPV infections in studies.

Unfortunately, also in our present study, the number of SCC in HIV-positive women was too low to draw conclusions on the prevalence of less frequent HPV types.

The most important weakness of our study as well as of all other smaller reports on HPV types in HIV-positive women with ICC is, however, lack of information on the time of acquisition of HIV infection. It is, therefore, always possible that any difference in the relative importance of different HR HPV types in HIV-positive and HIV-negative women has been attenuated by later acquisition of HIV infection compared with HPV infection. In that case, HIV-associated immune impairment would not have had the possibility to influence the HPV type that caused invasive cervical carcinoma (a process that typically takes decades).[28]

In conclusion, our present data suggest that the current prophylactic HPV vaccines against HPV16 and 18 may prevent a similar proportion of SCC, regardless of HIV status, provided that vaccine-related protection is sustained after HIV infection. The evolution of the HIV epidemic (*i.e.*, the increase in the proportion of women who will have acquired HIV infection early in life but survived long time because of cART) will provide the ultimate confirmation of the similarity in the importance of different HPV types in the causation of invasive cervical carcinoma in HIV-positive and HIV-negative women.

Acknowledgements

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