Human papilloma virus infection in HIV-infected women in Belgium: implications for prophylactic vaccines within this subpopulation

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Although high-risk (HR) human papilloma virus (HPV) infection is the primary causative factor for cervical squamous intraepithelial lesions and invasive cervical cancer, the epidemiology of potentially HR (pHR) and low-risk HPV still remains to be elucidated in HIV-infected women. In addition, the synergistic potential of the multiplicity of HPV infections harboured renders it difficult to model the impact of vaccines. This cross-sectional analysis of HIV-infected women explores the epidemiology of abnormal cytology, thereby profiling and pairing pHR/HR HPV genotypes. This cross-sectional analysis reports the findings of 593 HIV-infected women, who underwent a cytological examination and HPV genotyping. A logistic regression model was fitted to adjust for age and coinfection with pHR/HR HPV genotypes. In the 143 women with abnormal cytology, a multiple pHR/HR HPV genotype prevalence of 64.1% [95% confidence interval (CI): 44.6–57.6%] was observed. A combined prevalence of HPV 16 and HPV 18 of 29.6% (95% CI: 22.2–37.8%) was found. HPV 6 and HPV 66 were found in two cases of low-grade squamous intraepithelial lesions as stand-alone genotypes and HPV 53 in a high-grade squamous intraepithelial lesion case. Pairing involving HPV 31 with HPV 16 and HPV 58 was found in high-grade squamous intraepithelial lesion cases. Significant associations were observed between abnormal cytology, multiple HPV, HPV 39 and HPV 53 [adjusted odds ratio (aOR): 2.02; $P = 0.01$; 95% CI: 1.2–3.5; aOR: 3.8; $P = 0.01$; 95% CI: 1.4–10.7; and aOR: 0.5; $P = 0.03$; 95% CI: 0.2–0.9, respectively]. Coinfection with pHR/HR HPV genotypes HPV 39 and 53 was significantly associated with abnormal cytology. Research into the imputed role of HPV 31 in pairings, low-risk and pHR HPV genotypes in HIV-infected women is warranted. ***European Journal of Cancer Prevention*** **27:46–53**

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Introduction

Human papilloma viruses (HPV) are double-stranded DNA viruses, considered to be the primary aetiological agents in cervical intraepithelial neoplasias (CIN) and cancers (Ng'andwe *et al.*, 2007). High-risk (HR) genotypes are associated with cervical cancer (Muñoz *et al.*, 2003) and include HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 (Muñoz *et al.*, 2003; Cogliano *et al.*, 2005).

According to the review of the International Agency for Research on Cancer (IARC, 2007), which reassessed carcinogenicity of biological agents, HPV types 26, 53, 67, 70, 73 and 82 are now classified as possibly carcinogenic. However, there is insufficient evidence of their involvement in cervical cancer. This also includes HPV 66, which, although now being reconsidered as being possibly carcinogenic, in most commercial screening tests is still considered to be 'HR' (Bouvard *et al.*, 2009). Low-risk (LR) HPV genotypes, which are considered benign, include 6, 11, 42, 43 and 44 (Burd, 2003).

Distinct precancerous stages or preinvasive precursor lesions to invasive squamous carcinoma of the cervix, called CIN, or dysplasia, can be distinguished. CIN can be histologically graded into mild dysplasia (CIN 1), moderate dysplasia (CIN 2) and severe dysplasia to carcinoma in-situ (CIN 3) (National Cancer Institute at the National Institutes of Health, 2015). Moderate and severe dysplasia have a higher rate of progression to cervical cancer.

It is well recognized that among the 14 (HR)-HPV genotypes, HPV 16 and HPV 18 confer the greatest risk for CIN 2 or more advanced stages; in fact, these two genotypes are associated with approximately two-thirds of all invasive cervical carcinomas (Bosch and de Sanjose, 2003). A recent meta-analysis found HPV 16 and HPV 18

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in 57 and 16% of cervical carcinomas, respectively (Li *et al.*, 2011). After HPV 16/18, data confirm HPV 31/33/35/45/52/58 as the most frequently detected genotypes in invasive cervical cancer (ICC) worldwide (Li *et al.*, 2011; Rahman *et al.*, 2011). The bivalent HPV vaccines against HR genotypes 16 and 18 are designed to prevent about 70% of cervical cancers.

Several studies have shown a strong and consistent association between HIV and HPV coinfection and therefore the development of CIN and genital cancer (Hawes *et al.*, 2003; De Sanjose *et al.*, 2007) as well as the persistence and recurrence of preinvasive cervical lesions, CIN 2 or CIN 3 (Russomano *et al.*, 2013). HIV also significantly increases the prevalence and the persistence of HR HPV infection because of the similarity of transmission, which in turn results more often in cervical high-grade squamous intraepithelial lesions (HSIL) and cervical cancer (Ahdieh *et al.*, 2001).

In HIV-positive populations, HPV 16 has been shown to be frequent, but not as predominant as observed in most HIV-negative populations (Ahdieh *et al.*, 2001; Didelot-Rousseau *et al.*, 2006). Although the bivalent/quadrivalent vaccines, including HPV 6 and HPV 11, constitute an important breakthrough in cervical cancer control for managing a successful vaccination programme in HIV-negative women, epidemiological data available suggest that a much wider variety of HPV types may be involved in the pathogenesis of cervical neoplasia (Merck and Co. Inc. Annual report, 2012). Also, epidemiological knowledge of potential HR (pHR) HPV types is greatly limited, mainly because commercial molecular assays focus only on HR HPV genotypes. There is a scarcity of data on these genotypes in HIV-positive women with abnormal cytology, albeit it is likely that they may play an enhanced role in cervical dysplasia development in HIV-positive patients (Schopp *et al.*, 2010; Barcellos *et al.*, 2011).

Apart from a higher prevalence and broader range of HR HPV, HIV immunosuppression has been linked to multiple HPV infections (Levi *et al.*, 2002; Moscicki *et al.*, 2004). Concomitant infection with multiple HPV genotypes has been found to be attributable to the inability to clear HPV infections as well as to the reactivation of latent HPV infections, both occurring as a result of immune suppression (Palefsky *et al.*, 1999; Levi *et al.*, 2002; Strickler *et al.*, 2003; Strickler *et al.*, 2005). A recent Brazilian study reported that only coinfections with HR genotypes were a predictor of abnormal cytology (Rocha-Brischiliari *et al.*, 2014).

This analysis of HIV-positive women with abnormal cytology purports to test our two a priori hypotheses, first that single pHR/HR HPV genotypes in HIV-infected women are not independent predictors of abnormal cytology, but rather involve synergistic mechanisms, and second, that pairing of HPV 31 with phylogenetically related HPV types is the most prevalent. The objectives of this analysis are three-fold: (a) to assess genotype-specific distribution of pHR/HR HPV in HIV-infected women in Belgium, which has an adult prevalence of 0.3% (ECDC, 2007), as well as the pairing prevalence of certain pHR/HR HPV genotypes found in women with abnormal cytology, (b) to investigate the risk factors associated with abnormal cytology [atypical cells of undetermined significance (ASC-US) or higher] and (c) discuss bottlenecks in assessing the potential efficacy of the current bivalent/quadrivalent HPV prophylactic vaccines in this study population.

Methods

To examine the epidemiology of type-specific HPV infections, we carried out a cross-sectional analysis of all 593 HIV-infected women who underwent HPV genotype testing at the AML laboratory in Antwerpen, Belgium. This cross-sectional study based on record reviews adhered to the methodological guidelines recommended in the STROBE document on observational studies (von Elm *et al.*, 2007).

The only inclusion criteria were the presence of HIV infection and age 18 years or older; a history of hysterectomy was the only exclusion criterion. Other variables included age and coinfections with pHR/HR HPV genotypes. Data were entered into the lab 400 system (CEGCGK).

Sample size calculation

We included all 593 available samples.

Biologic specimens

In 2014, cervical samples were collected using a cervix brush (Cervex-Brush; Rovers, Oss, the Netherlands) and cervical cytology was assessed with conventional Papanicolaou (Pap) smears. Slides were read by a cytologist with master's level training, supervised by a pathologist. An external cytopathologist provided quality control. The Bethesda Reporting System was used for cytological classification (Davey, 2003).

The cervix brush tips were preserved in a liquid-based cytology collection medium (SurePath; Tripath Imaging Inc., Burlington, North Carolina, USA) and stored at 4°C until further processing.

HPV DNA extraction, detection and typing

HPV testing was performed as described by Depuydt *et al.* (2006) and Micalessi *et al.* (2012) in an accredited laboratory (ISO certification: ISO15189). Briefly, HPV DNA was extracted from exfoliated cervical cells using the standard proteinase K-based digestion protocol following the manufacturer's instructions. Cells were incubated with proteinase K solution (100 μg/ml) for 3 h at 55°C. DNA was then further purified by spin column chromatography. HPV types were determined using a

series of real-time PCR reactions with specific primers and TaqMan (Invitrogen, La Jolla, California, USA) probes for HR HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66 and 68 (IARC, 2007). LR HPV types 6, 11 and 67 were also detected. HPV DNA was tested according to the guidelines for HPV DNA test requirements (Meijer *et al.*, 2009).

Ethical consideration

Overall ethical approval was granted for using leftover anonymized material.

Statistical analysis

Following data checking and cleaning, an analysis was carried out using STATA (version 12; Stata Corporation, College Station, Texas, USA).

We first described the distribution of low, pHR/HR HPV types observed among women with both normal cytology and abnormal cytology, for which the overall prevalence and 95% confidence intervals on the basis of normal distributions were calculated.

To examine patterns of clustering of HR HPV types, the prevalence of the pHR/HR HPV type in the presence of another pHR/HR HPV by abnormal cytology was calculated, which was defined as the proportion of women with abnormal cytology who were positive for the pHR/HPV genotypes. The prevalence of pairings detected in women with HSIL was also calculated.

Women older than 30 years of age have a higher risk of abnormal cytology; hence, age was dichotomized into two categories: at least 30 years and younger than 30 years. The number of pHR/HR HPV coinfections was also dichotomized as a categorical variable with 1 and at least 2 genotypes. Considering women older than 30 years of age as being at a higher risk for abnormal cytology, we tested this variable as a potential effect modifier.

The variables were explored by tabulation and cross-tabulation. The χ^2-test was used to assess whether there was an association between CIN 2+ and various risk factors. For the univariate analysis, a logistic regression was fitted to measure the strength of the association between coinfection with pHR/HR genotypes and each pHR/HR HPV genotype separately on abnormal cytology. A multivariable logistic regression analysis was carried out to simultaneously control for potential confounders, including age and the presence of coinfections, and assess other variables as possible independent factors.

In constructing the regression models, a variable was retained in the model if it appeared to be an independent risk factor or a confounder. A multivariable logistic regression analysis was carried out to assess the association between pHR/HR HPV genotypes and abnormal cytology, ASC-US or higher, the main outcome of interest and to simultaneously control for potential confounders. The likelihood ratio test (LRT) was used to measure the association of each variable with the outcome.

To assess for a potential interaction effect due to age, logistic regression models were fitted with and without the interaction term; significance for interaction was then checked through an LRT. Statistical significance was considered at P of at least 0.05.

Results

Of the 593 HIV-infected women, 450 had normal cytology [78.0%; 95% confidence interval (CI): 71.0–78.2%] and 143 (24.1%; 95% CI: 21–28%) had abnormal cytology, or less than or equal to ASC-US. No missing data were observed (Table 1).

The median age of the women was 30 years (interquartile range: 26–38 years). A total of 318 women (53.6%) were younger than 30 years of age and 275 women (46.4%) were 30 years of age or older.

The prevalence of pHR/HR HPV infection in the 593 HIV-positive women was 39.9% ($n=222$; 95% CI: 33.5–41.5%), of whom 20 (3.4%; 95% CI: 2.1–5.2) had LR HPV and 64 had pHR (10.8%; 95% CI: 8.4–13.6%) and 138 (23.3%; 95% CI: 19.9–26.9%) had HR HPV and 117 had multiple pHR/HR HPV genotypes (51.1%; 95% CI: 44.6–57.6%).

Normal and abnormal cytology and each HPV genotype

Of the 143 women with abnormal cytology, we observed a multiple pHR/HR HPV genotype prevalence of 64.1% ($n=117$). In 14 (9.8%) women with abnormal cytology, no HR HPV was detected. The combined prevalence of HPV 16 and HPV 18 was 29.6% (95% CI: 22.2–37.8%) and the prevalence of pHR was 27.5% (95% CI: 11.7–24.9%). Tables 2–4 report the prevalence of each HPV genotype in HIV-infected women with normal cytology, with abnormal cytology and according to the cytological status, respectively.

Table 1 Prevalence of cervical abnormalities observed in the sample ($N=593$)

Cytological status	*n*	% Of the total sample (95% CI)
Normal cytology	450	75.8 (71.0–78.2)
ASC-US	41	6.9 (5.0–9.3)
LSIL	90	15.2 (12.4–18.4)
ASC-H	1	0.2 (0.0–0.9)
HSIL	11	1.9 (0.9–3.3)
Total	593	

ASC-H, typical squamous cells – cannot exclude high-grade squamous intraepithelial lesion; ASC-US, atypical cells of undetermined significance; CI, confidence interval; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.

Table 2 Frequency and prevalence of each HPV genotype in women with normal cytology

HPV genotype	Frequency ($N=450$) [n (%)]	95% CI (%)
HPV 6	6 (1.3)	0.5–2.9
HPV 11	0 (0.0)	
HPV 16	16 (3.6)	2.0–5.7
HPV 18	6 (1.3)	4.9–2.9
HPV 31	28 (6.2)	4.2–8.9
HPV 33	3 (0.7)	1.4–1.9
HPV 35	3 (0.7)	1.4–1.9
HPV 39	7 (1.6)	0.6–3.2
HPV 45	0 (0.0)	
HPV 51	5 (1.1)	0.4–2.6
HPV 52	21 (4.7)	2.9–7.0
HPV 53	17 (3.8)	2.2–6.0
HPV 56	4 (0.9)	0.2–2.3
HPV 58	9 (2.00)	0.9–3.8
HPV 59	9 (2.00)	0.9–3.8
HPV 66	12 (2.70)	1.4–4.6
HPV 67	15 (3.30)	1.9–5.4
HPV 68	0 (0.00)	

CI, confidence interval; HPV, human papilloma virus.

Table 3 Frequency and prevalence of each HPV genotype in women with abnormal cytology

HPV genotype	Frequency ($N=143$) [n (%)]	95% CI (%)
HPV 6	18 (12.6)	7.6–19.2
HPV 11	1 (0.2)	0.005–1.1
HPV 16	21 (14.8)	9.4–21.7
HPV 18	21 (14.8)	9.4–21.7
HPV 31	51 (35.9)	26.9–42.2
HPV 33	3 (2.1)	0.4–6.0
HPV 35	5 (3.5)	1.2–8.0
HPV 39	26 (18.2)	12.3–25.7
HPV 45	2 (1.4)	0.2–5.0
HPV 51	18 (12.6)	7.7–19.3
HPV 52	47 (32.9)	25.2–41.2
HPV 53	11 (7.7)	3.9–13.4
HPV 56	5 (3.5)	1.2–8.0
HPV 58	19 (13.9)	8.3–0.20.1
HPV 59	15 (10.5)	6.0–16.8
HPV 66	28 (19.6)	39.4–56.4
HPV 67	14 (9.8)	5.5–16.0
HPV 68	8 (5.6)	2.5–10.8

CI, confidence interval; HPV, human papilloma virus.

LR HPV in abnormal cytology

HPV 6 was found in two cases of low-grade squamous intraepithelial lesion (LSIL) as a stand-alone HPV genotype.

Potential HR HPV in abnormal cytology

HPV 53 was found as a stand-alone pHR HPV genotype in an HSIL case and HPV 66 as a stand-alone genotype in two LSIL cases.

Pairings of pHR/HR genotypes in abnormal cytology

The most frequently observed pairings were HPV 31 and HPV 66, $n=20$ occurrences (14%) of the 143 women with abnormal cytology; HPV 39 and HPV 52, $n=12$ occurrences (8.4%); HPV 31 and HPV 52, HPV 39 and HPV 66, and HPV 51 and HPV 66, each pair with $n=11$ occurrences (7.7% of the total) (Table 5). Less frequent pairs, but observed in a high number of HSIL, were HPV 16 and HPV 31, of which five of six cases were found in HSIL cases (41.6%), and HPV 31 and HPV 58, of which three of eight cases were found in HSIL cases (25%).

Table 4 Frequency and prevalence of each HPV genotype in HIV-infected women according to the cytological status

	n (%)			
HPV genotype	ASC-US ($N=41$)	LSIL ($N=90$)	ASC-H ($N=1$)	HSIL ($N=11$)
HPV 6	3 (7.3)	15 (16.7)	0	0
HPV 16	5 (12.2)	12 (13.3)	0	4 (36.4)
HPV 18	1 (2.4)	17 (18.9)	0	3 (27.3)
HPV 31	12 (29.3)	29 (32.2)	1 (100.0)	9 (81.8)
HPV 33	1 (2.4)	2 (2.2)	0	0
HPV 39	3 (7.3)	22 (24.4)	0	1 (9.1)
HPV 45	0	2 (2.2)	0	0
HPV 51	2 (4.9)	15 (16.7)	0	1 (9.1)
HPV 52	11 (26.8)	32 (35.6)	0	4 (36.4)
HPV 53	3 (7.3)	6 (6.7)	1 (100)	1 (9.1)
HPV 56	1 (2.4)	4 (4.4)	0	0
HPV 58	3 (7.3)	12 (13.3)	0	4 (36.4)
HPV 66	3 (7.3)	22 (24.4)	0	3 (27.3)
HPV 67	3 (7.3)	9 (10)	1 (100)	1 (9.1)
HPV 68	0	7 (7.8)	0	1 (9.1)

ASC-H, typical squamous cells – cannot exclude high-grade squamous intraepithelial lesion; ASC-US, atypical cells of undetermined significance; HPV, human papilloma virus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.

Table 5 Most prevalent pairing occurrences in women with abnormal cytology and HSIL

	Occurrences (n) (% in abnormal cytology)
Most prevalent pairings in abnormal cytology	
HPV 31 and 66	20 (14)
HPV 39 and 52	12 (8.4)
HPV 31 and 52	11 (7.7)
HPV 39 and 66	11 (7.7)
HPV 51 and 66	11 (7.7)
Most prevalent pairings specifically in HSIL (% in HSIL)	
HPV 16 and 31	5 (41.7)
HPV 31 and 58	3 (25)

HPV, human papilloma virus; HSIL, high-grade squamous intraepithelial lesion.

Risk factors for abnormal cytology

Women younger than 30 years of age have a crude OR = 2.3 (95% CI: 1.9–4.3; $P<0.0001$) of having abnormal cytology compared with older women. A crude statistically significant OR was found for multiple HPV and abnormal cytology compared with a single HPV genotype infection (OR = 2.2; 95% CI: 1.2–3.7; $P=0.005$); when adjusted for age, this association decreased slightly, but remained significant (OR = 2.02; 95% CI: 1.2–3.5; $P=0.01$).

Although all pHR/HR HPV showed a significant association with abnormal cytology when adjusted for age, when this association was adjusted for coinfection with pHR/HR HPV, the associations became statistically insignificant, except for HPV 39 and HPV 53 (Table 6).

Table 6 Age-adjusted association between specific pHR/HR HPV genotypes and abnormal cytology; age and pHR/HR coinfection-adjusted OR between various HR HPV genotypes and abnormal cytology

	Age-adjusted OR for abnormal cytology (95% CI)	*P*-value[b]	OR for abnormal cytology adjusted for age and pHR/HR coinfections (95% CI)	*P*-value[b]
Age 30 years and older	2.3 (1.9–4.3)[a]	< 0.0001		
pHR/HR Coinfections prevalence	2.0 (1.2–3.5)	0.01		
HPV 16	5.3 (2.6–10.7)	< 0.001	1.3 (0.6–2.7)	0.6
HPV 18	10.1 (4.0–26.1)	< 0.001	2.3 (0.9–6.2)	0.09
HPV 31	6.9 *P* (4.1–11.7)	< 0.001	1.4 (0.8–2.6)	0.3
HPV 33	2.1 (0.4–10.5)	0.4	0.3 (0.0–2.7)	0.3
HPV 35	5.4 (1.2–23.8)	0.03	1.3 (0.3–5.9)	0.7
HPV 39	11.0 (4.6–26.2)	< 0.001	3.8 (1.4–10.7)	0.01
HPV 51	12.2 (4.4–34.5)	< 0.001	2.6 (0.9–7.7)	0.08
HPV 52	8.0 (4.5–14.2)	< 0.001	1.8 (0.9–3.4)	0.07
HPV 53	2.2 (1.0–4.9)	0.06	0.5 (0.2–0.9)	0.03
HPV 56	5.0 (1.2–19.2)	0.03	1.1 (0.3–4.2)	0.9
HPV 58	7.4 (3.2–17.3)	< 0.001	1.6 (0.7–3.9)	0.3
HPV 66	8.5 (4.1–17.6)	< 0.001	1.6 (0.8–3.6)	0.2
HPV 67	2.2 (1.0–4.8)	0.05	0.4 (0.2–1.02)	0.06

OR from logistic regression.
CI, confidence interval; HPV, human papilloma virus; HR, high-risk; LRT, likelihood ratio test; OR, odds ratio; pHR, potential high-risk.
[a]Crude OR.
[b]*P*-value from LRT references

As no interaction terms were significant, no result reflecting the differential impact for that particular outcome was presented.

Discussion

Summary of results and comparison with other studies

In the present study, we found a high prevalence of pHR and HR HPV genotypes, 39.5%, which is higher than the 33.2% prevalence found in a recent multicentre study carried out in Catalonia. Of this HIV–HPV-coinfected population, 51.1% of HIV-1 infected women had multiple infections, consistent with the results of several studies reporting that HIV-1 infected women not only have a higher prevalence of HR-HPV infection, but multiple coinfections (Temmerman *et al.*, 1999; Grinsztejn *et al.*, 2008).

In our study group, in HIV-positive women with either normal or abnormal cytology, the overall prevalence of HPV was 39.9%, of which 34.6% had pHR/HR genotypes. This is more than three times the prevalence of 12% found in the general population in Belgium and elsewhere in Europe (Arbyn *et al.*, 2009; De Vuyst *et al.*, 2009). In our study, a significant association between age younger than 30 years and abnormal cytology was observed (OR 2.3; $P < 0.0001$; 95% CI: 1.9–4.3), indicating that HIV-infected women younger than 30 years are at risk for abnormal cytology. These results contrast with the current consensus guidelines from the American Society for Colposcopy and Cervical Pathology and the American College of Obstetricians and Gynecologists, which recommend that HR-HPV testing can be performed in addition to routine cytology screening in women 30 years of age and older (Wright *et al.*, 2007; Clark *et al.*, 2009).

Moreover, in contrast to the 0.5% prevalence of HSIL in the general population (Massad *et al.*, 2009), in our study 2% of all HIV-infected women had HSIL.

The three most common pHR/HR HPV in abnormal cytology were HPV 31, HPV 66 and HPV. Despite different endpoints, our findings contrast with those from a meta-analysis, whose pooled estimates showed a dominance of HPV 16 (32%), HPV 31 (22%) and HPV 39/52 (11%) in women with HSIL (Arbyn *et al.*, 2009).

Our pHR prevalence in HIV-positive women with abnormal cytology of 24.1% is lower than the 39.2% observed in a baseline study of 74 coinfected women in an HIV clinic in Mombasa, Kenya, (Menon *et al.*, 2016), which may be because of the greater level of immunosuppression of our study population in Mombasa. Although a marginal association was found between HPV 53 and abnormal cytology when it was adjusted for age, when it was adjusted for HR coinfection a protective marginal OR was yielded. However, as found in our Kenyan study of a population of coinfected women, in which HPV 53 was found as a stand-alone genotype in ICC, HPV 53 in this study population in Belgium was observed as a stand-alone genotype in a HSIL case. This finding is in not in agreement with the observations of Maranga *et al.* (2013) that pHR HPV was only detected in low-grade lesions. Also, our findings are incongruent with a recent Brazilian study on HIV-infected women, which found that only one woman with HPV 53 infection without coinfection by a HR type was found to have ASC-US on cytology. Our finding of the LR HPV 6 as a stand-alone genotype in cases of LSIL is also not congruent with the available literature.

After adjusting for age and coinfection with pHR/HR HPV genotypes, no significant association was observed between any single pHR/HR HPV and abnormal

cytology, except for HPV 39 and HPV 53, for which a protective effect was found. Our observations are in agreement with those of a recent large study on multiple HPV infections in Costa Rica in which young healthy women with multiple infections were at a significantly increased risk of CIN 2+ compared with those with single infections, and those of a recent Brazilian study in which only coinfections with HR genotypes were a predictor of abnormal cytology (Castilho *et al.*, 2015).

When adjusting for both age and coinfection with pHR/HR genotypes, the crude association between HPV 66 and abnormal cytology became statistically insignificant (OR: 1.7; $P=0.2$; 95% CI: 0.8–3.7). HPV 66 was mostly found in conjunction with HPV 31 and HPV 39. The results from a recent study in Brazil also observed frequent pairing between HPV 31 and HPV 58 in women with abnormal cytology (Castilho *et al.*, 2015). In our study, HPV 31 and HPV 58, like HPV 16 and HPV 31, were observed in a high number of HSIL cases, suggesting that synergistic effects may be at play between HPV 31 and HPV genotypes related to the alpha family. HPV 58 has also been observed at high rates in multiple studies of cervical infection in women in Brazil and in a cohort of HIV-infected women in Botswana and Zambia (Sahasrabuddhe *et al.*, 2007; Macleod *et al.*, 2011; Rocha-Brischiliari *et al.*, 2014), and has been found to be among the eight most common HPV types in HIV-infected women younger than 30 years old in Romania (Ursu *et al.*, 2015).

Strengths and limitations

Our major strengths were the large sample size available to explore risk factors for abnormal cytology and the sensitive screening HPV diagnostics used. However, because of the lack of clinicoepidemiological data, including CD4 count and highly active antiretroviral therapy, we could not adjust for these potential confounders. Although our sample was large, it was not large enough to explore coinfection patterns as a risk factor, resulting in some associations appearing as not significant even if they may be biologically plausible.

A limitation related to the cross-sectional design may be the lack of data on the age of acquisition of HIV infection as it is possible that this may have occurred too late in life, in some of the women in our study, to influence abnormal cytology. Similarly, an analysis of a cross-sectional study for exploring associations between multiple HPV and abnormal cytology may not be adequate to assess whether infections were acquired concurrently or sequentially; therefore, the criterion of temporality for causation is not fulfilled. This may have an impact as immunological responses may differ following concurrent acquisition of multiple HPV from when infections are acquired sequentially. These limitations may result in a suboptimal internal validity of our study.

Implications for vaccination programmes

Our combined HPV 16 and HPV 18 prevalence (29.6%) in women with abnormal cytology suggests that the 70% expected reduction of ICC through HPV 16 and HPV 18 protection conferred by the quadrivalent/bivalent HPV vaccine may not be fulfilled in HIV-positive girls in Belgium, unless these genotypes are more prevalent in HIV-positive women with ICC. Our high prevalence of HPV 31 in HIV women with abnormal cytology (35.9%) makes it uncertain whether the available bivalent prophylactic vaccine will be able to meet its objective of reducing cervical cancer incidence by 70%; this may depend on the efficacy of cross protection against HPV 31. In light of a high prevalence of multiple HR HPV infections, especially with HPV 31, which was often detected in the presence of other pHR/HR genotypes, an effective cross protection may be hampered by the additional burden of competing with other synergistic relationships among the HR HPV genotypes present. In addition, it may have to face emerging synergistic interactions induced by nonvaccine genotypes that previously remained clinically elusive possibly because of HPV 16/18 related lesions. A recent systematic review and meta-analysis (Malagón *et al.*, 2012) found that the bivalent Cervarix vaccine from GlaxoSmithKline (London, UK) had better cross protection against HPV 31 in persistent infection, but that efficacy against persistent infections with type 31 appeared to decrease with longer follow-up, suggesting a waning of cross protection. It still remains to be determined whether cross protection can be extrapolated to HIV-infected women and in the presence of multiple HR HPV genotypes, especially HPV 58, which, together with HPV 31, has been found in a relatively high number of HSIL cases. The presence of HPV 6 as a stand-alone genotype in two LSIL cases suggests that a quadrivalent vaccine may confer more adequate protection for an HIV-infected population.

In light of the higher prevalence of multiple HR HPV genotypes in HIV-infected women, an effective strategy may require that the contribution of individual HPV genotypes towards cervical carcinoma be established. This is pivotal for estimating how much cervical disease will be prevented by current vaccination programmes and to determine which genotypes should be targeted by the next generation of vaccines. Our results can be generalized to other women receiving HIV management.

Conclusion

Coinfection with pHR HPV genotypes was more strongly associated with abnormal cytology, after adjusting for age, than any single HR HPV except for HPV 39 in this HIV-infected population. Therefore, although elucidation of the epidemiology of specific HPV types in this population is pivotal for estimating the efficacy of the bivalent/quadrivalent vaccine, in light of the high prevalence of multiple HR HPV genotypes harboured by HIV-positive

women, its micro epidemiology in cervical carcinoma in HIV-positive women needs to be explored for the vaccine efficacy to be assessed.

Our combined HPV 16 and HPV 18 prevalence in women with abnormal cytology in this population suggests that the 70% expected HPV vaccine protection rate may not be met in this population. Whether the available bivalent prophylactic vaccine will be able to achieve its aim of reducing cervical cancer incidence by 70% may depend on the efficacy of cross protection against HPV 31 in HIV-infected women and the synergies with other HR HPV genotypes in inducing cervical cancer.

The magnitude of pHR HPV infections in HIV-infected women in the presence of HR HPV genotypes should lead to the investigation of its epidemiology. In particular, the cervical carcinoma genesis potential of HPV 53 as a stand-alone genotype should be explored, notwithstanding the significant protective effect detected for abnormal cytology. In addition, the presence of HPV 6 as a stand-alone genotype in women with LSIL gives rise to the notion that the clinico-epidemiology of LR HPV genotypes in HIV-infected women needs to be elucidated.

These current gaps in epidemiology underscore the need for an intense screening algorithm, despite HPV vaccination. The current recommendation for high-risk women in Belgium to be screened every year should be considered good clinical practice.

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Conflicts of interest

There are no conflicts of interest.

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