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

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Title of the Article: **HPV infection in HIV infected women in Belgium: Implications for prophylaxis vaccines within this sub population**

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HPV infection in HIV infected women in Belgium: Implications for prophylaxis vaccines within this sub population

Whilst high risk (HR) 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 (LR) 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 of pHR/HR HPV genotypes.

Methods

This cross-sectional analysis reports the findings of a 593 HIV-infected women, who underwent a cytological examination and HPV genotyping. A logistic regression model was fitted to adjust for age and co-infection of pHR/ HR HPV genotypes.

Results:

In the 143 women with abnormal cytology, a multiple pHR/HR HPV genotype prevalence of 64.1% (95%CI: 44.6%-57.6%) was observed. A combined prevalence of HPV16 and 18 of 29.6% (95%CI: 22.2%-37.8%) was exhibited. HPV 6 and 66 were found in two cases of LSIL as stand-alone genotypes and HPV 53 in a HSIL case. Pairing involving HPV 31 with HPV 16 and 58 were found in HSIL cases.

Significant associations were observed between abnormal cytology, multiple HPV, HPV 39 and 53 (aOR; 2.02; p=0.01; 95%CI 1.2-3.5; aOR: 3.8; p=0.01 95%CI OR1.4-10.7 and aOR: 0.5; p=0.03; 95%CI: 0.2-0.9 respectively).

Conclusion:

Co-infection with pHR/HR HPV genotypes, HPV 39 and 53 were significantly associated with abnormal cytology. Research into the imputed role of HPV 31 in pairings, LR and pHR HPV genotypes in HIV-infected women is warranted.

Introduction:

Human Papilloma Viruses (HPV) are double-stranded DNA viruses, considered to be the primary etiological agents in cervical intraepithelial neoplasias and cancers.¹ High-risk (HR) genotypes are associated with cervical cancer,² and include HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68.^{3 4}

According to the recent review of the International Agency for Research on Cancer (IARC 2009), which reassessed carcinogenicity of biological agents, HPV types 26, 53, 67, 70, 73 and 82 are now classified as possible carcinogenic. However, there is only insufficient evidence of their involvement in cervical cancer. This also includes HPV 66, which although is now being reconsidered as being possibly carcinogenic, in most commercial screening tests is still considered to be “high-risk”.⁵ LR HPV genotypes, which are considered benign include, 6, 11, 42, 43, and 44.⁶

Distinct precancerous stages or pre-invasive precursor lesions to invasive squamous carcinoma of the cervix, called cervical intraepithelial neoplasia (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).⁷ Moderate and severe dysplasia has 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 2 genotypes are associated with approximately two thirds of all invasive cervical carcinomas.⁸ A recent meta-analysis found HPV 16 and HPV 18 in 57% and 16% of cervical carcinomas, respectively.⁹ After HPV16/18, data confirm HPV31/33/35/45/52/58 as

the most frequently detected genotypes in Invasive Cervical Cancer (ICC) worldwide.^{10 11} The bivalent HPV vaccines against high risk 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 co-infection and therefore the development of CIN and genital cancer,^{12 13} as well as the persistence and recurrence of pre-invasive cervical lesions, CIN 2 or CIN 3.¹⁴ HIV also significantly increases the prevalence and the persistence of HR HPV infection due to the similarity of transmission, which in turn results more often in cervical high-grade squamous intraepithelial lesions (HSIL) and cervical cancer.¹⁵

In HIV positive populations, HPV16 has shown to be frequent, but not as predominant as seen in most HIV negative populations.^{16 17} Although the bivalent/quadrivalent vaccines, including HPV 6 and 11 constitute an important breakthrough in cervical cancer control for managing a successful vaccination program 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.¹⁸ Also, epidemiological knowledge of potential high risk (pHR) HPV types is highly limited, mainly because commercial molecular assays focus on HR HPV genotypes only. There is a scarcity of data on these genotypes in HIV positive women with abnormal cytology, albeit it is likely that they may have an enhanced role in cervical dysplasia development in HIV positives patients.^{19 20}

Apart from a higher prevalence and broader range of HR HPV, HIV immunosuppression has been linked to multiple HPV infection.^{21 22} 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.^{23 24 25 26} A recent Brazilian study reported that only co-infections with HR genotypes were a predictor of abnormal cytology.²⁷

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 through synergistic mechanisms and second, that pairing of HPV 31 with alpha 9 phylogenetically-related HPV types is the most prevalent. The objectives of this analysis are threefold, 1) to assess genotype-specific distribution of pHR/HR HPV in HIV-infected women in Belgium, which has an adult prevalence of 0.3%,²⁸ as well as the pairing prevalence of certain pHR/HR HPV genotypes found in women with abnormal cytology 2) to investigate risk factors associated with abnormal cytology (ASC-U or higher), 3) discuss bottlenecks in assessing the potential efficacy of the current bivalent/quadrivalent HPV prophylactic vaccines in this study population.

Method

To examine the epidemiology of type-specific HPV infections, we performed a cross-sectional analysis of all 592 HIV-infected women, who underwent HPV genotype testing 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.²⁹

The only inclusion criterion was presence of HIV infection and 18 years of age or above; history of hysterectomy was the sole exclusion criterion. Other variables included age and coinfections of pHR/HR HPV genotypes. Data were entered in 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 level training, supervised by a pathologist. An external cytopathologist provided quality control. The Bethesda Reporting System was used for cytological classification.³⁰

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 executed as described by Depuydt et al (2006) Micalessi IM et al (2012) in an accredited laboratory (ISO certification: ISO15189).^{31 32} 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 (100µg/ml) for 3 hours 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, USA) probes for HR HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66 and 68 IARC (2006). Low risk (LR) HPV types 6, 11 and 67 were also detected. HPV DNA was tested according to de Meijers' et al (2009) guidelines for HPV DNA test requirements.³³

.Ethical consideration

Overall ethical approval is granted for using leftover anonymized material.

Statistical analysis

Following data checking and cleaning, analysis was undertaken using STATA version 12. Corporation, College Station, TX, 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 based upon normal distributions were calculated.

To examine patterns of clustering of high-risk HPV types, the prevalence pHR/HR HPV type in 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/HR HPV genotypes. The prevalence of pairings detected in women with HSIL was also calculated.

Women over 30 years have a higher risk of abnormal cytology, hence, age was dichotomized into two categories, ≥ 30 years and < 30 years. The number of pHR/HR HPV co-infections was also dichotomized as a categorical variable with 1 and ≥ 2 genotypes. Based on women over 30 years of age being more at risk for abnormal cytology, we tested this variable as a potential effect modifier

The variables were explored by means of 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 co-infection with pHR/HR genotypes and each pHR/HR HPV genotype separately on abnormal cytology. A multivariable logistic regression analysis was performed to simultaneously control for potential confounders, including age and the presence of co-infections and assess other variables as possible independent factors.

In building the regression models, a variable was kept in the model if appeared to be an independent risk factor or a confounder. A multivariable logistic regression analysis was performed to assess the association between pHR/HR HPV genotypes and abnormal cytology, ASC-U 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 a LRT. Statistical significance was considered at $p \leq 0.05$.

Results:

Of the 593 HIV infected women, 450 had normal cytology (78.0%; 95%CI: 71.0-78.2%) and 143 (24.1%; 95%CI: 21%-28%) had abnormal cytology, or $\geq$ ASC-U. No missing data were observed.

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	

Table (1) reports the prevalence of cervical abnormalities observed in the sample (N=592)

The median age was 30 years (IQR: 26-38 years). 318 women (53.6%) were aged less than 30 years and 275 women (46.4%) were aged 30 or more.

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 which 20 (3.4%; 95%CI: 2.1-5.2) were LR HPV, 64 (10.8%; 95%CI: 8.4-13.6%) pHR; and 138 (23.3%, 95%CI: 19.9-26.9%) HR HPV and 117 (51.1%, 95%CI: 44.6%-57.6%) had multiple pHR/HR HPV genotypes.

Normal and Abnormal cytology and each HPV genotypes.

Out of the 143 women with abnormal cytology, we observed a multiple pHR/HR HPV genotype prevalence of 64.1% (n=xx). In 14 (9.8%) women with abnormal cytology, no HR HPV was detected. The combined prevalence of HPV16 and HPV18 was 29.6% (95%CI: 22.2- 37.8 %) and a pHR prevalence of 27.5 % (95%CI: 11.7-24.9%). Table 2, 3, and 4 report the prevalence of each HPV genotype in HIV infected women in normal cytology, in abnormal cytology and according to the cytological status, respectively.

HPV genotype	Frequency (N=450)	%	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%	

Table (2) reports the frequency and prevalence of each HPV genotypes in women with **normal** cytology

HPV genotype	Frequency (N=143)	%	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-.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

Table (3) reports the frequency and prevalence of each HPV genotype in women with **abnormal** cytology

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%)

Table (4) reports the frequency and prevalence of each HPV genotype in HIV infected women according to the cytological status

186

187 *LR HPV in abnormal cytology:*

188 HPV 6 was found in two cases of LSIL as a stand-alone HPV genotype

189 *Potential HR HPV in abnormal cytology*

190 HPV 53 was found as a stand-alone pHR HPV genotype in a HSIL case and HPV 66 as a stand-alone
191 genotype in 2 LSIL cases.

192 *Pairings pHR/HR genotypes in abnormal cytology (table 5)*

193 The most frequently observed pairings were HPV 31 and HPV 66, $n = 20$ occurrences (14%) of the 143
194 women with abnormal cytology , HPV 39 and HPV 52, $n = 12$ occurrences (8.4%); HPV 31 and 52,
195 HPV 39 and 66, and HPV 51 and 66, each pair with $n=11$ occurrences (7.7% of the total). Less
196 frequent pairs but observed in a high number of HSIL were, HPV 16 and 31 of which 5 of 6 cases were
197 found in HSIL cases, (41.6%); HPV 31 and 58 of which 3 of 8 cases were found in HSIL cases (25%).

Most prevalent pairings in abnormal cytology	Occurrences (n)	% 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%

198

199 *Table (5) most prevalent pairing occurrences in women with abnormal cytology and HSIL*

200 *Risk factors for abnormal cytology:*

201 Women under 30 years of age have a crude OR=2.3 (95%CI: 1.9-4.3; p<0.0001) of having abnormal
 202 cytology compared to older women. A crude statistically significant OR was found for multiple HPV and
 203 abnormal cytology compared to single HPV genotype infection (OR=2.2; 95%CI: 1.2-3.7; p=0.005);
 204 when adjusted for age, this association slightly decreased but remained significant (OR=2.02; 95%CI:
 205 1.2-3.5; p=0.01).

206 Whilst all pHR/HR HPV had a significant association with abnormal cytology when adjusted for age,
 207 when this association was adjusted for co-infection with pHR/HR HPV co-infections, the associations
 208 became statistically insignificant, except for HPV 39 and HPV 53.

209

	Age-adjusted OR for abnormal cytology (95%CI)	P-value§	OR for abnormal cytology adjusted for age and pHR/HR coinfections (95%CI)	P-value§
Age 30 and above	2.3 (95%CI: 1.9-4.3)*	<0.0001		
pHR/HR Co-infections 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

210 *Crude OR

211 § P-value from LRT

Table 3 Age-Adjusted association between specific pHR/HR HPV genotypes and abnormal cytology; Age and pHR/HR co-infection adjusted OR between various HR HPV genotypes and abnormal cytology. OR from logistic regression;

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 have a high prevalence of potential high risk and HR HPV genotypes, 39.5%, which is higher than the 33.2% prevalence found in a recent multicenter study performed in Catalonia. Of this HIV-HPV co-infected population, 51.1 % of HIV-1–infected women had multiple infections, consistent with results of several studies illustrating that HIV-1–infected women not only have a greater prevalence of HR-HPV infection but multiple coinfections.^{34 35}

In our study group, HIV positive women with both normal and 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 in Europe.^{36 37} In our study, a significant association between < 30 years and abnormal cytology was observed (OR 2.3; p<0.0001; 95%CI: 1.9-4.3) revealing that in HIV-infected women < 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 and older.^{38 39}

Moreover, in contrast, to the 0.5% prevalence of HSIL in the general population,⁴⁰ in our study, 2% of all HIV infected women have HSIL.

The three most common pHR/ HR HPV in abnormal cytology was HPV 31, HPV 66, and HPV 16, found in abnormal cytology. Albeit 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.⁴¹

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 co-infected women in a HIV clinic in Mombasa, Kenya, Menon et al (in press 2016), which may be due to the greater level of immunosuppression of our study population in Mombasa. Although a marginal association was revealed between HPV 53 and abnormal cytology when it was adjusted for age, when it was adjusted for HR co-infection, a protective marginal OR was yielded. However, as found in our Kenyan study population of co-infected 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 disagreement with Maranga et al's (2012) observations that pHR HPV was only detected in low-grade lesions.⁴² Also our findings are incongruent with a recent Brazilian study on HIV infected women, which found only one woman with HPV 53 infection without co-infection by a high-risk type was found to have ASC-US on cytology. Nor is our finding of the LR HPV 6 as a stand-alone genotype in cases of LSIL congruent with available literature.

After adjusting for age and co-infection with potential HR/HR HPV genotypes, no significant association was observed between any single potential HR/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 significantly increased risk of CIN 2+ when compared with those with single

infections, and those of a recent Brazilian study, in which only co-infections with HR genotypes were a predictor of abnormal cytology.

When adjusting for both age and co-infection 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.⁴³ In our study HPV 31 and HPV 58, like, HPV 16 and HPV 31, observed in a high number of HSIL cases suggest 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 cohort of HIV-infected women in Botswana and Zambia,^{44 45 46} and has been found to be among the eight most common HPV types in HIV infected women below age 30 in Rumania.⁴⁷

Strengths and limitations:

Our major strengths were the large sample size to explore risk factors for abnormal cytology and the sensitive screening HPV diagnostics employed. However, due to our lack of clinico-epidemiological data, including CD4 count and HAART, we have not been able to adjust for these potential confounders. Although our sample was large, it was not large enough to explore co-infection 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 concerning age of acquisition of HIV infection since it is possible this may have occurred too late in life for 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 met. This may have an impact as immunologic responses may differ following concurrent

acquisition of multiple HPV from when infections are acquired sequentially. These limitations may result in a sub-optimal internal validity of our study.

Implications for vaccination programmes

Our combined HPV 16 and 18 prevalence (29.6%) in women with abnormal cytology suggests that the 70% expected reduction of ICC through HPV 16 and 18 protection conferred by the quadrivalent/bivalent HPV vaccine may not be met 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%) suggest that a 70% HPV vaccine protection rate may not be attained lest there is an effective cross protection to HPV 31. In light of a high prevalence of multiple HR HPV infections, especially with HPV 31, which was often detected in presence of other pHR/HR genotypes, an effective cross protection may be hampered by the additional burden of competing with other synergistic relationships among HR HPV genotypes present. In addition, it may have to face emerging synergistic interactions induced by non-vaccine genotypes that previously remained clinically elusive possibly due to HPV 16/ 18 related lesions. A recent systematic review and meta-analysis⁴⁸ found that bivalent Cervarix© vaccine from GlaxoSmithKline had better cross protection against HPV 31 in persistent infection, but that efficacy against persistent infections with types 31 appeared to decrease with longer follow-up, suggesting a waning of cross-protection. It still remains to be determined whether a cross protection can be extrapolated to HIV-infected women and in 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 stand-alone genotypes in two LSIL cases suggests that a quadrivalent vaccine may confer more adequate protection for a 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 to cervical carcinoma be established. This is pivotal for estimating how much cervical disease will be prevented by current

vaccination programs and to determine which genotypes should be targeted by the next generation of vaccines. Our results can be generalized to other women receiving HIV care management.

Conclusion:

Co-infection with potential HR HPV genotypes was more strongly associated with abnormal cytology after adjusting for age, than any single high-risk 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 in order for the vaccine efficacy to be assessed.

Our combined HPV 16 and 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 meet its objective 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 genesis.

The magnitude of pHR HPV infections in HIV infected women in presence of HR HPV genotypes should prompt 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 genotypes 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 vaccinations. The current recommendation for a screening algorithm for high at risk women in Belgium to screen every year should be considered good clinical practice.

327 **Abbreviations:**

328 HIV= Human immunodeficiency virus

329 HPV= Human Papilloma virus

330 LR HPV= low risk HPV

331 pHR HPV= potential high risk

332 ASC-U= Atypical cells of undetermined significance

333 LSIL= Low grade squamous intraepithelial lesion.

334 ASC-H= Typical squamous cells-cannot exclude high-grade squamous intraepithelial lesion HSIL= High
335 grade squamous intraepithelial lesion.

336 **Conflict of interest:** The authors declare that they have no competing financial, political, personal,
337 religious, ideological, academic, intellectual, commercial or any other interests

338 **Authors' contribution**

339 SM lead author conceived and drafted the manuscript, performed the statistical analysis, and interpreted
340 the findings

341 RR participated in the data analysis, interpretation of findings, and in the revision of the article

342 IB participated in the revision and validation of the article

343 JP participated in the revision and validation of the article

344 DB participated in the conception of the paper, revision and validation of the article

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References:

¹ Ng'andwe C, Lowe JJ, Richards PJ, Hause L, Wood C, Angeletti PC. The distribution of sexually-transmitted Human Papillomaviruses in HIV positive and negative patients in Zambia, Africa. *BMC Infectious Diseases*. 2007;7:77. doi:10.1186/1471-2334-7-77.

² Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV, et al. International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med*. 2003;348(6):518–27.

³ Muñoz N, Bosch FX, de Sanjosé S, et al. International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med*. 2003;348:518–27

⁴ Coglianò V, Baan R, Straif K, et al. WHO International Agency for Research on Cancer. Carcinogenicity of human papillomaviruses. *Lancet Oncol*. 2005;6:204.

⁵ Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, Benbrahim-Tallaa L, Guha N, Freeman, Galichet L, Coglianò V; WHO International Agency for Research on Cancer Monograph Working Group(2009) A review of human carcinogens—Part B: biological agents. *Lancet Oncol* 10:321-2

⁶ Burd EM (2003) Human papillomavirus and cervical cancer. *Clinical Microbiology Reviews* 16: 1–17.

⁷ National Cancer Institute at the National Institutes of Health

⁸ Bosch FX, de Sanjose S. Chapter 1: Human papillomavirus and cervical cancer: burden and assessment of causality. *J Natl Cancer Inst Monogr*. 2003:3-13

⁹ Li N, Franceschi S, Howell-Jones R, Snijders PJ, Clifford GM: Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: Variation by geographical region, histological type and year of publication. *Int J Cancer* 2011, **128**(4):927-935

-
- ¹⁰ Li N, Franceschi S, Howell-Jones R, Snijders PJ, Clifford GM: Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: Variation by geographical region, histological type and year of publication. *Int J Cancer* 2011, **128**(4):927-935
- ¹¹ Rahman M, Sasagawa T, Yamada R, Kingoro A, Ichimura H, Makinoda S: High prevalence of intermediate-risk human papillomavirus infection in uterine cervixes of Kenyan women infected with human immunodeficiency virus. *J Med Virol* 2011, **83**:1988 – 1996
- ¹² Hawes SE. *et al*: Increased risk of high-grade cervical squamous intraepithelial lesions and invasive cervical cancer among African women with human immunodeficiency virus type 1 and 2 infections. *J Infect Dis* 2003, **188**(4):555-63
- ¹³ De Sanjose S, Diaz M, Castellsague X *et al*. Worldwide prevalence and genotype distribution of cervical human papilloma DNA in women with normal cytology : a meta-analysis. *Lancet Infect Dis* 2007, **7**:453-459
- ¹⁴ Russomano F, Paz BR *et al* Recurrence of cervical intraepithelial neoplasia in human immunodeficiency virus-infected women treated by means of electrosurgical excision of the transformation zone (LLETZ) in Rio de Janeiro, Brazil.
- ¹⁵ Ahdieh L, Klein R, Burk R, Cu-Uvin S, Schuman P, Duerr A, *et al*. Prevalence, incidence, and type-specific persistence of human papillomavirus in human immunodeficiency virus (HIV)-positive and HIV-negative women. *J Infect Dis*. 2001;**184**:682–90
- ¹⁶ Didelot-Rousseau MN, Nagot N, Costes-Martineau V, Valles X, Ouedraogo A, Konate I, Weiss HA, Van de Perre P, Mayaud P, Segondy M, Yerelon Study Group Human papillomavirus genotype distribution and cervical squamous intraepithelial lesions among high-risk women with and without HIV-1 infection in Burkina Faso. *Br J Cancer*. 2006;**95**:355–362.
- ¹⁷ Ahdieh, L., Klein, R. S., Burk, R. & 7 other authors . Prevalence, incidence, and type-specific persistence of human papillomavirus in Human Immunodeficiency Virus (HIV)-positive and HIV-negative women. *J Infect Dis* 2001 **184** , 682–690
- ¹⁸ Merck&Co.,Inc: *Annual report*. 2012 accessed online on July 23th, 2015
- ¹⁹ Schopp B, Holz B, Zago M, Stubenrauch F, Petry KU, Kjaer SK, *et al*.: Evaluation of the performance of the novel PapilloCheck HPV genotyping test by comparison with two other genotyping systems and the HC2 test. *J Med Virol* 2010, **82**:605-615
- ²⁰ Barcellos RB, Almeida SE, Sperhake RD, Verza M, Rosso F, Medeiros RM, *et al*.: Evaluation of a novel microplate colorimetric hybridization genotyping assay for human papillomavirus. *J Virol Methods* 2011, **77**:38-43.
- ²¹ Levi JE, Kleter B, Quint WG, Fink MC, Canto CL, Matsubara R, *et al* . (2002). High prevalence of human papillomavirus (HPV) infections and high frequency of multiple HPV genotypes in human immunodeficiency virus-infected women in Brazil. *J Clin Microbiol* **40** :3341–3345.
- ²² Moscicki AB, Ellenberg JH, Farhat S, Xu J. Persistence of human papillomavirus infection in HIV-infected and -uninfected adolescent girls: risk factors and differences, by phylogenetic type. *J Infect Dis*. 2004;**190**:37–45.
- ²³ Strickler HD, Burk RD, Fazzari M, Anastos K, Minkoff H, Massad LS, Hall C, Bacon M, Levine AM, Watts DH, Silverberg MJ, Xue X, Schlecht NF, Melnick S, Palefsky JM. Natural history and possible reactivation of human papillomavirus in human immunodeficiency virus-positive women. *J Natl Cancer Inst*. 2005;**97**:577–586

-
- ²⁴ Palefsky JM, Minkoff H, Kalish LA, Levine A, Sacks HS, Garcia P, Young M, Melnick S, Miotti P, Burk R. Cervicovaginal human papillomavirus infection in human immunodeficiency virus-1 (HIV)-positive and high-risk HIV-negative women. *J Natl Cancer Inst.* 1999;91:226–236.
- ²⁵ Strickler HD, Palefsky JM, Shah KV, Anastos K, Klein RS, Minkoff H, Duerr A, Massad LS, Celentano DD, Hall C, Fazzari M, Cu-Uvin S, Bacon M, Schuman P, Levine AM, Durante AJ, Gange S, Melnick S, Burk RD: Human papillomavirus type 16 and immune status in human immunodeficiency virus-seropositive women. *J Natl Cancer Inst* 2003, **95**(14):1062-71
- ²⁶ Levi JE, Kleter B, Quint WG, Fink MC, Canto CL, Matsubara R, et al . (2002). High prevalence of human papillomavirus (HPV) infections and high frequency of multiple HPV genotypes in human immunodeficiency virus-infected women in Brazil. *J Clin Microbiol* 40 :3341–3345.
- ²⁷ Rocha-Brischiliari SC, Gimenès F, de Abreu AL, Irie MM, Souza RP, Santana RG, et al. Risk factors for cervical HPV infection and genotypes distribution in HIV-infected South Brazilian women. *Infect Agents Cancer.* 2014;9(1):6.
- ²⁸ ECDC (2007) Technical Report HIV infection in Europe: 25 years into the pandemic, Background paper prepared for the conference “Responsibility and Partnership: Together Against HIV/AIDS” Bremen, 12–13 March 2007 accessed online on October 29th, 2015
- ²⁹ von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, the STROBE initiative: The Strengthening of Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet* 2007, **370**:1453-1457
- ³⁰ Davey DD: Cervical cytology classification and the Bethesda System. *Cancer J* 2003, **9**(5):327-334.
- ³¹ Depuydt CE, Benoy IH, Bailleul EJ, Vandepitte J, Vereecken AJ, Bogers JJ: Improved endocervical sampling and HPV viral load detection by Cervex-Brush Combi. *Cytopathology* 2006, **17**(6):374-381
- ³² Micalessi IM, Boulet GA, Bogers JJ, Benoy IH, Depuydt CE. 2012. High-throughput detection, genotyping and quantification of the human papillomavirus using real-time PCR. *Clin. Chem. Lab. Med.* **50**:655–661
- ³³ Meijer CJLM, Berkhof J, Castle PE, et al. Guidelines for human papillomavirus DNA test requirements for primary cervical cancer screening in women of 30 years and older. *International journal of cancer Journal international du cancer.* 2009;124(3):516-520. doi:10.1002/ijc.24010.
- ³⁴ Grinsztejn B, Veloso VG, Levi JE, Velasque L, Luz PM, et al. (2008) Factors associated with increased prevalence of human papillomavirus infection in a cohort of HIV-1-infected Brazilian women. *Int J Infect Dis* 13(1)72–80
- ³⁵ Temmerman M, Tyndall MW, Kidula N, Claeys P, Muchiri L, et al. (1999) Risk factors for human papillomavirus and cervical precancerous lesions, and the role of concurrent HIV-1 infection. *Int J Gynaecol Obstet* 65(2)171–81
- ³⁶ Arbyn M, Benoy I, Simoons-Schot J, Bogers J, Beutels P, Depuydt C. Prevalence distribution of human papillomavirus types in women attending at cervical cancer screening in Belgium. *Cancer Epidemiol Markers Prev.* 2009;18(1):321–30
- ³⁷ De Vuyst H, Clifford G, Li N, Franceschi S. HPV infection in Europe. *Eur J Cancer.* 2009;45(15):2632–9

-
- ³⁸ Wright TC, Jr, Massad LS, Dunton CJ, Spitzer M, Wilkinson EJ, Solomon D. 2006 consensus guidelines for the management of women with abnormal cervical cancer screening tests. *Am J Obstet Gynecol*. 2007;197(4):346–355
- ³⁹ . Clark JL, Lescano AG, Konda KA, Leon SR, Jones FR, Klausner JD, et al. Syndromic management and STI control in urban Peru. *PLoS One*. 2009;4(9):e7201.
- ⁴⁰ Massad LS, Einstein M, Myers E, Wheeler CM, Wentzensen N, Solomon D. The impact of human papillomavirus vaccination on cervical cancer prevention efforts. *Gynecologic oncology*. 2009;114(2):360-364. doi:10.1016/j.ygyno.2009.04.005.
- ⁴¹ Arbyn M, Benoy I, Simoens C, Bogers J, Beutels P, Depuydt C. Prevaccination distribution of human papillomavirus types in women attending at cervical cancer screening in Belgium. *Cancer Epidemiol Markers Prev*. 2009;18(1):321–30
- ⁴² Maranga IO, Hampson L, Oliver AW, He X, Gichangi P, Rana F, et al. HIV Infection Alters the Spectrum of HPV Subtypes Found in Cervical Smears and Carcinomas from Kenyan Women. *Open Virol J*. 2013;7:19–27
- ⁴³ Castilho JL, Levi JE, Luz PM, et al. A cross-sectional study of high-risk human papillomavirus clustering and cervical outcomes in HIV-infected women in Rio de Janeiro, Brazil. *BMC Cancer*. 2015;15:478. doi:10.1186/s12885-015-1486-4.
- ⁴⁴ Rocha-Brischiliari SC, Gimenes F, de Abreu AL, Irie MM, Souza RP, Santana RG, et al. Risk factors for cervical HPV infection and genotypes distribution in HIV-infected South Brazilian women. *Infect Agents Cancer*. 2014;9(1):6.
- ⁴⁵ Macleod IJ, O'Donnell B, Moyo S, Lockman S, Shapiro RL, Kayembe M, et al. Prevalence of human papillomavirus genotypes and associated cervical squamous intraepithelial lesions in HIV-infected women in Botswana. *J Med Virol*. 2011;83(10):1689–95.
- ⁴⁶ Sahasrabuddhe VV, Mwanahamuntu MH, Vermund SH, Huh WK, Lyon MD, Stringer JS, et al. Prevalence and distribution of HPV genotypes among HIV-infected women in Zambia. *Br J Cancer*. 2007;96(9):1480–3.
- ⁴⁷ Ursu RG, Onofriescu M, Luca A, Prisecariu LJ, Sălceanu SO, Nemescu D, et al. (2015) The Need for Cervical Cancer Control in HIV-Positive and HIV-Negative Women from Romania by Primary Prevention and by Early Detection Using Clinically Validated HPV/DNA Tests. *PLoS ONE* 10(7): e0132271. doi:10.1371/journal.pone.0132271
- ⁴⁸ Malagón T, Drolet M, Boily MC, et al. Cross-protective efficacy of two human papillomavirus vaccines: a systematic review and meta-analysis. *Lancet Infect Dis* 2012