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Progression of HPV infection to detectable cervical lesions or clearance in adult women: Analysis of the control arm of the VIVIANE study

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Abbreviations: 6MPI: 6-month persistent infection; 12MPI: 12-month persistent infection; CI: confidence interval; CIN: cervical intraepithelial neoplasia; CIN1+: cervical intraepithelial neoplasia grade 1 or greater; CIN2+: cervical intraepithelial neoplasia grade 2 or greater; CIN3+: cervical intraepithelial neoplasia grade 3 or greater; FUTURE: *Females United* To Unilaterally Reduce Endo/Ectocervical Disease; HPV: human papillomavirus; HPVI: HPV infection of any duration; HR: hazard ratio; PATRICIA: PApilloma TRIal against Cancer In young Adults; PCR: polymerase chain reaction; TVC: total vaccinated cohort; VIVIANE: Human PapillomaVIrus Vaccine Immunogenicity ANd Efficacy

Additional Supporting Information may be found in the online version of this article.

Conflict of interest: LB, DR, FS, BG are employed by the GSK group of companies. MCB is a consultant outsourced from 4Clinics to the GSK group of companies. GD was employed by the GSK group of companies at the time of the study and has several relevant patents and received GSK shares. GD is currently a full time employee of Takeda Pharmaceuticals, Deerfield, Illinois and receives salary and stock shares. DR, FS, LB and BG own shares and stock options in the GSK group of companies. AC received grant funding for clinical trials, served on the speakers' bureau and advisory boards for the GSK group of companies and Merck. SMG has received advisory board fees and grants support from CSL and the GSK group of companies, and lectures fees from Merck, the GSK group of companies and Sanofi Pasteur. In addition, she also received funding through her institution to conduct HPV vaccines studies for MSD and the GSK group of companies. She is a member of the Merck Global Advisory Board as well as the Merck Scientific Advisory Committee for HPV. ELP received fees to conduct HPV vaccines studies from the GSK group of companies and Merck. MJL received research funds from the GSK group of companies and chairs an adjudication committee for the GSK group of companies. SMN has received research grants from the GSK group of companies, Pfizer and Sanofi Pasteur. RDRR received honoraria as investigator and travel support for attendance at investigator meetings from the GSK group of companies. BR received research grants, travel support and speaker honoraria from the GSK group of companies. RS received funds through her institution from the GSK group of companies to cover expenses involved in the collection of data for this study. The GSK group of companies provided funds to reimburse expenses incurred with travel to conference to present data from this study and other studies, and paid honoraria to her institution for work conducted in the context of Advisory Board and educational meetings. CMW received funding through her institution, the University of New Mexico (UNM), to conduct HPV vaccine trials or travel reimbursements for publication activities from the GSK group of companies and Merck and Co., Inc. and UNM also received equipment and reagents to perform HPV genotyping assays from Roche Molecular Systems. XC received research funding through his institution (ICO) from Merck & Co, SPMSD, the GSK group of companies and Genticel. He also received honoraria for conferences from Vianex and SPMSD. GM, as investigator at a study clinical site, received fees from the GSK group of companies through her institution to do the study protocol. She also received funding from Merck Sharp & Dohme to participate as principal investigator in efficacy trials. She received travel support to attend scientific meetings, honoraria for speaking engagements and participation in advisory board meetings and consulting fees from the GSK group of companies and Merck Sharp & Dohme. VP, VR, IG, CV, DM, DMH and DPDS declare that they have no conflicts of interest.

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The control arm of the phase III VIVIANE (Human PapillomaVIrus: Vaccine Immunogenicity ANd Efficacy; NCT00294047) study in women >25 years was studied to assess risk of progression from cervical HPV infection to detectable cervical intraepithelial neoplasia (CIN). The risk of detecting CIN associated with the same HPV type as the reference infection was analysed using Kaplan–Meier and multivariable Cox models. Infections were categorised depending upon persistence as 6-month persistent infection (6MPI) or infection of any duration. The 4-year interim analysis included 2,838 women, of whom 1,073 (37.8%) experienced 2,615 infections of any duration and 708 (24.9%) experienced 1,130 6MPIs. Infection with oncogenic HPV types significantly increased the risk of detecting CIN grade 2 or greater (CIN2+) versus non-oncogenic types. For 6MPI, the highest risk was associated with HPV-33 (hazard ratio [HR]: 31.9 [8.3–122.2, $p < 0.0001$]). The next highest risk was with HPV-16 (21.1 [6.3–70.0], $p < 0.0001$). Similar findings were seen for infections of any duration. Significant risk was also observed for HPV-18, HPV-31, and HPV-45. Concomitant HPV infection or CIN grade 1 or greater associated with a different oncogenic HPV type increased risk. Most women (79.3%) with an HPV infection at baseline cleared detectable infections of any duration, and 69.9% cleared a 6MPI. The risk of progression of HPV infection to CIN2+ in women >25 years in this study was similar to that in women 15–25 years in PATRICIA.

What's new?

Which HPV infections lead to cancer in women over 25 years? Most cervical cancer follows persistent oncogenic HPV infection, but most HPV infections clear naturally. Thus, to best predict patient outcomes, it's imperative to understand how HPV infections progress to CINs. This study confirmed that in women over 25 years, persistent infection with HPV-33 or HPV-16 meant the greatest chance of developing a CIN—the same as was found in women 15–25 years, in an earlier analysis.

A persistent oncogenic human papillomavirus (HPV) infection is a prerequisite for development of most cervical intraepithelial neoplasia (CIN) and cervical cancer.[1,2] Together, HPV types HPV-16, HPV-18, HPV-45, HPV-31, and HPV-33 account for ~85% of invasive cervical cancer worldwide.[3] Several determinants have been found to promote progression of oncogenic HPV infection to a CIN, including tobacco exposure, higher number of sexual partners, contraceptive use and previous pregnancy,[4–6] as well as individual immune responses and infection with other sexually transmitted pathogens such as *Chlamydia trachomatis* and herpes simplex virus.[7–9]

Although new HPV infections are most common in young sexually active women, women aged over 25 years remain at risk of HPV infection.[10–13] Type-specific HPV infections can be redetected after a period of negativity, reflecting either persistent infection that has temporarily fallen below detectable HPV DNA levels, redetection of a potential latent infection or acquisition of a new infection. A true incident infection is more likely in the setting of new sexual partners.[14,15]

Most HPV infections clear naturally. However, the natural history of clearance of a cervical HPV infection or its progression to a CIN needs to be better understood, both in young women and those aged over 25 years in order to predict likely outcomes. The control arm of prophylactic HPV vaccine trials systematically collected data on HPV types, histological lesions and potential modifiers of disease progression, and are therefore useful vehicles for such analyses. We have previously presented analyses of the natural history of HPV infection in the **PA**pilloma **TRI**al against **C**ancer In young **A**dults (PATRICIA) in women aged 15–25 years.[16–18] The present study describes the natural history of HPV infection, including persistence, clearance and progression to CIN in the Human Papilloma**VI**rus: Vaccine Immunogenicity **AN**d **E**fficacy (VIVIANE) study, a phase III trial of the HPV-16/18 AS04-adjuvanted vaccine (*Cervarix*™, GSK) in women aged over 25 years.

Material and Methods

This analysis was based on data collected during a 4-year follow-up period in the placebo arm of the ongoing VIVIANE trial (NCT00294047). The first participant was enrolled in February 2011. Data from the trial remain blinded. The objectives of the analysis were to investigate the risk of progression from detection of an HPV infection to detection of a CIN lesion associated with the same HPV type, or natural clearance of infection (*i.e.*, not detectable), and to identify modifiers of these relationships.

Study participants and procedures

The trial methodology has been previously reported.[19] Briefly, we enrolled healthy women aged over 25 years from Asia Pacific, Europe, North America and Latin America, which included a subset of up to 15% of women with a history of HPV-associated infection/disease (defined as two or more abnormal smears in sequence; abnormal colposcopy; or biopsy/treatment of the cervix). We performed HPV DNA typing every 6 months and cytological examination (Bethesda system) every 12 months using liquid-based cervical cytology samples. Women were referred for colposcopy if they had a single abnormal cytology finding of atypical squamous cell of undetermined significance, low grade squamous intraepithelial lesion associated with an oncogenic HPV type, atypical squamous cells—cannot exclude high-grade squamous intraepithelial lesion, atypical glandular cell, and high grade intraepithelial lesion or worse. Histological classification was performed on any biopsies taken. We used a broad spectrum polymerase chain reaction (PCR) SPF_{10}-DEIA/LiPA25 (version 1) assay to test for HPV DNA from 14 HPV oncogenic types,[20] and tested oncogenic HPV-positive samples by multiplex type-specific PCR and reverse hybridisation assay to detect HPV types HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, HPV-45, HPV-52, HPV-58, and HPV-59. Women completed a similar questionnaire as previously described, asking about sexual behaviour and lifestyle factors known to influence acquisition of HPV infection.[21]

Written informed consent was obtained from all participants, and the protocol and other materials were approved by independent ethics committees or institutional review boards.

Endpoint definitions and statistical analysis

A similar analysis has been previously reported using data from the PATRICIA trial in women aged 15–25 years, the methodology of which has been reported in detail.[18]

Definitions related to HPV infections

HPV infections were classified as a transient infection (HPV DNA detected at any single point, followed by a negative sample for the same HPV type at the next evaluation, including infections detected at baseline only), 6-month persistent infection (6MPI) (same HPV type detected at two consecutive evaluations over at least a 6-month period), 12-month persistent infection (12MPI) (same HPV type detected at two consecutive evaluations over at least a 12-month period), less than 6MPI (two consecutive positive samples $\leq$150 days apart), and infection detected only at the last visit of the study. The time to clearance was defined as the time between the date of the first sample positive for type-specific HPV DNA and the date of the first subsequent sample negative for the type-specific HPV DNA. However, at least two type-specific negative samples taken at two consecutive intervals of $\sim$6 months following a positive sample were required to confirm clearance. Although we recognise that apparent clearance could in reality be an inability to detect the infection, we use the term clearance for simplicity. Histologically confirmed lesions were categorised as CIN grade 1 or greater (CIN1+), CIN grade 2 or greater (CIN2+), and CIN grade 3 or greater (CIN3+). CIN1+ included CIN1, CIN2, CIN3 and adenocarcinoma *in situ* identified by standard methods. If more than one HPV type was found in the lesion, causality was attributed based on detection of the same HPV type in preceding samples, as previously described.[22] If more than one HPV type was found in preceding samples, each infection was treated as a separate observation.

Exposures and determinants

The main determinants considered were HPV type (for all endpoints) and duration of detected HPV infection (clearance only). Other covariates were the cumulative tobacco exposure measured as number of pack-years (one pack-year was equivalent to 365 packs of 20 cigarettes) and as smoking history at baseline (yes or no), age at onset of the HPV infection, age at first sexual intercourse, marital/partner status, education, number of lifetime sexual partners, number of sexual partners during the 12-month period prior to the reference HPV infection, use of hormones for contraception or other indication, surgical sterilisation, use of an intrauterine device, previous pregnancy, menopausal status and history of *Chlamydia trachomatis* during the past 12 months.

In addition, we examined the potential effect of previous cervical HPV infection, cervical HPV co-infection, previous CIN1+ associated with an HPV type different to the reference infection (*i.e.*, CIN1+ preceding the onset of the reference infection), concomitant CIN1+ associated with an HPV type different to the reference infection (*i.e.*, CIN1+ following the onset of the reference infection and preceding its end) and history of HPV infection/disease or a non-intact cervix (history of cauterisation or surgical treatment involving damage to the transformation zone of the cervix). Cervical HPV co-infections and concomitant CIN1+ associated with an HPV type different to the reference HPV infection were included in the models as time-varying covariates.

Statistical analysis

The analysis was performed in the total vaccinated cohort (TVC), excluding women with high grade cytology or missing cytology data at baseline. As the trial is ongoing, some data remain blinded and are therefore not presented.

The Kaplan–Meier method and univariate and multivariable Cox proportional-hazards models were used.[23,24] The statistical unit was the infection, and variance estimates adjusted for the correlation within subjects were obtained using the robust estimation method.[23,24] Hazard ratios (HR) with 95% confidence intervals (CI) were calculated. All data were censored at the last recorded visit, occurrence of an endpoint event, or at 48 months, whichever occurred first. Covariates with a p value <0.2 in the univariate model were included in the multivariable model, with the exception of region which was always included. Infections or lesions with a missing covariate value were excluded from the multivariable analysis. For lesions in which multiple HPV types were detected, each HPV type was considered as a different observation. This was also the case for the analysis of clearance.

All analyses were performed using SAS version 9.2. The analysis was performed by an external statistician to maintain the study blind.

Results

The analysis population included 2,838 women with no high grade or missing cytology data at baseline (Fig. 1a). Women who acquired an HPV infection were generally younger, had first sexual intercourse at a younger age, had more sexual partners, were more likely to smoke, were more likely to have a history of *Chlamydia trachomatis* infection, and were less likely to have been pregnant compared with women who did not acquire an infection (Supporting Information Table 1). Median follow-up in the study was 47.9 months.

A total of 1,073 (37.8%) women experienced 2,615 HPV infections of any duration before the last study visit; 708 (24.9%) women experienced 1,130 6MPIs and 465 (16.4%) women experienced 611 12MPIs (Fig. 1a). At baseline, 507 (17.9%) women had a prevalent HPV infection; of these, 319 (11.2%) women were subsequently identified as having a 6MPI and 214 (7.5%) as having a 12MPI (Fig. 1b). During follow-up, 888 (31.3%) women experienced an HPV infection, including 528 (18.6%) with a subsequently identified 6MPI and 311 (11.0%) with a subsequently identified 12MPI (Fig. 1c).

Risk of detecting a CIN lesion associated with a 6MPI or 12MPI

Among 708 women with 6MPI, 90 (12.7%), 49 (6.9%) and 18 (2.5%) women, respectively, had a CIN1+, CIN2+ or CIN3+ lesion associated with the same HPV type within 48 months (Fig. 1a). More CIN lesions detected following a 6MPI arose from infections first detected at baseline than from infections first detected during follow-up. Of the 319 women with a 6MPI first detected at baseline, 49 (15.3%) had CIN1+ detected, 32 (10.0%) CIN2+, and 14 (4.4%) CIN3+ (Fig. 1b). Of the 528 women in whom 6MPI was first detected during follow-up, 48 (9.1%) had CIN1+ detected, 22 (4.2%) CIN2+ and 6 (1.1%) CIN3+ (Fig. 1c).

A similar pattern was seen for the 465 women with 12MPIs, with CIN1+, CIN2+, or CIN3+ lesions associated with the same HPV type as the reference 12MPI detected in 71 (15.3%), 43 (9.2%) and 18 (3.9%) women, respectively (Fig. 1a). Again, more lesions were detected following infections first detected at baseline. Of the 214 women with a 12MPI first detected at baseline, 40 (18.7%) had CIN1+ detected, 28 (13.1%) CIN2+ and 14 (6.5%) CIN3+ (Fig. 1b). Of the 311 women in whom a 12MPI was first detected during follow-up, 34 (10.9%) had CIN1+ detected, 18 (5.8%) CIN2+ and 5 (1.6%) CIN3+ (Fig. 1c).

In the multivariable analysis of 6MPI, infection with an oncogenic HPV type was significantly associated with a higher risk of detecting a lesion (Table 1). The highest risk was observed with HPV-33, with an HR (versus a non-oncogenic HPV type) of 39.5 (95% CI: 11.7–132.9, $p < 0.0001$) for CIN1+ and 31.9 (8.3–122.2, $p < 0.0001$) for CIN2+. It was followed by HPV-16 (HR 17.9 [6.2–51.7] for CIN1+ and 21.1 [6.3–70.0] for CIN2+, $p < 0.0001$) (Table 1). Infection with HPV-18, HPV-31 and HPV-45 also significantly increased the risk versus non-oncogenic types of detecting CIN1+ or CIN2+ (Table 1). There was a trend for an association between the risk of detecting CIN1+ and co-infection with an oncogenic HPV type different to the reference infection or presence of a concomitant CIN1+ lesion associated with an HPV type different to the reference infection (HR: 1.5 [1.0–2.4], $p = 0.067$ and HR: 2.2 [0.9–5.6], $p = 0.102$, respectively) (Table 1). Both factors were significantly associated with the risk of CIN2+ (HR: 2.2 [1.2–4.1], $p = 0.013$ and 2.9 [1.2–6.8], $p = 0.014$, respectively) (Table 1). The analysis did not show an effect of previous cervical HPV infections or previous precancerous lesions.

Several other determinants influenced the risk of detecting lesions associated with the same HPV type as the reference 6MPI in the multivariable analysis (Supporting Information Tables 2 and 3). Peri- or post-menopausal status was associated with a lower risk of detecting a CIN1+ (HR: 0.1 [95%

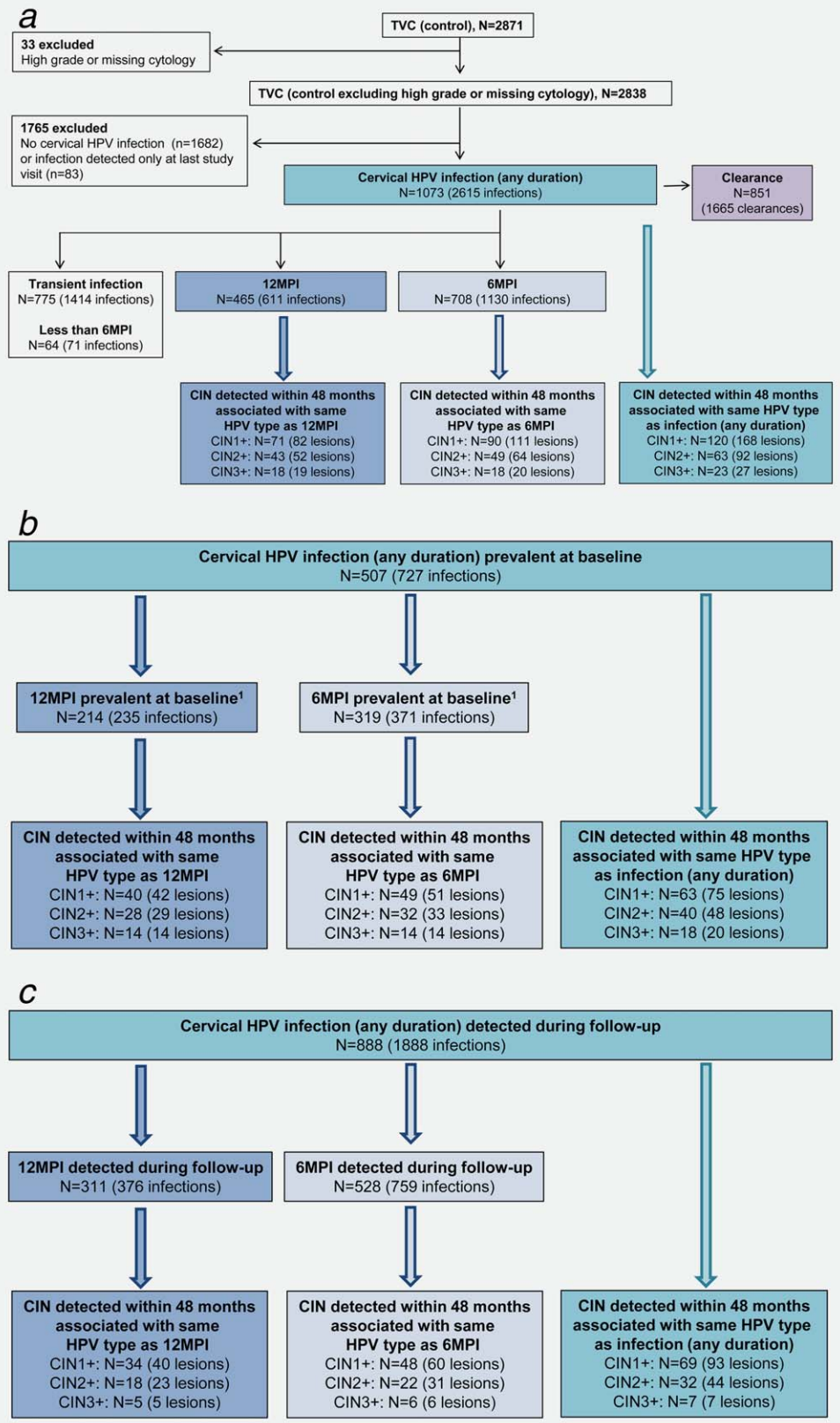

Figure 1. Study flow chart: detection of HPV infections and CIN. A. Throughout study. B. Prevalent infection at baseline. C. Infection first detected during follow-up. [1]Infection detected at baseline and subsequently identified as being a 6MPI or 12MPI. 12MPI: 12-month persistent infection; 6MPI: 6-month persistent infection; CIN: cervical intraepithelial neoplasia; HPV: human papillomavirus; TVC: total vaccinated cohort.

Table 1. Multivariable analysis of the risk of detecting a CIN lesion associated with the same HPV type for 6-month persistent HPV infections

	CIN1+			CIN2+		
	1126 infections in 704 women 111 lesions[2]			1128 infections in 706 women 64 lesions[2]		
	No. CIN1+	Hazard ratio[1] (95% CI)	*p* values	No. CIN2+	Hazard ratio[1] (95% CI)	*p* values
HPV type						
Non-oncogenic type	4	1	–	3	1	–
HPV-16	19	17.9 (6.2–51.7)	<0.0001	17	21.1 (6.3–70.0)	<0.0001
HPV-18	6	12.8 (4.0–41.2)	<0.0001	3	8.3 (1.6–43.3)	0.012
HPV-31	11	13.8 (4.3–44.2)	<0.0001	7	13.6 (3.7–49.6)	<0.0001
HPV-33	11	39.5 (11.7–132.9)	<0.0001	8	31.9 (8.3–122.2)	<0.0001
HPV-45	5	8.8 (2.5–31.8)	0.001	3	7.1 (1.5–33.5)	0.013
Other oncogenic type	55	10.6 (4.0–28.3)	<0.0001	23	5.3 (1.7–16.2)	0.004
		<0.0001			*<0.0001*	
Previous cervical HPV infection						
No	75	1	–	44	Not included	
Yes (at least 1 oncogenic HPV type)	NA[3]	1.2 (0.7–1.9)	0.556	NA[3]		
Yes (only non-oncogenic HPV types)	NA[3]	0.4 (0.1–1.9)	0.263	NA[3]		
		0.400				
Cervical HPV co-infection						
No	49	1	-	26	1	-
Yes (at least 1 oncogenic HPV type)	55	1.5 (1.0–2.4)	0.067	36	2.2 (1.2–4.1)	0.013
Yes (only non-oncogenic HPV types)	7	0.9 (0.4–1.9)	0.704	2	0.4 (0.1–1.9)	0.260
		0.120			*0.004*	
Previous CIN1+[4]						
No	NA[3]	Not included		NA[3]	Not included	
Yes (any oncogenic or non-oncogenic HPV type)	NA[3]			NA[3]		
Concomitant CIN1+[5]						
No	105	1	–	57	1	–
Yes (any oncogenic or non-oncogenic HPV type)	6	2.2 (0.9–5.6)	0.102	7	2.9 (1.2–6.8)	0.014
		0.102			*0.014*	

[1]Covariates were included in the multivariable analysis if they had a global *p* value <0.2 in the univariate analysis (except region which was always included); covariates were: region, tobacco exposure measured as number of pack-years, age at onset of the 6MPI, age at first sexual intercourse, marital/partner status, education, number of lifetime sexual partners, number of sexual partners during the past 12 months, use of hormones for contraception or other indication, surgical sterilisation, use of an intrauterine device, previous pregnancy, menopausal status, and history of *Chlamydia trachomatis* during the past 12 months. Full analysis is shown in Supporting Information Tables 1 and 2.
[2]Infections or lesions with a missing value for a covariate included in the analysis were excluded from the multivariable analysis.
[3]Time-varying covariate.
[4]CIN1+ associated with an HPV type different to the reference infection, preceding the onset of the 6MPI.
[5]CIN1+ associated with an HPV type different to the reference infection, concomitant to the 6MPI (following its onset and preceding its end).
Values in italics show the global p-value.
6MPI: 6-month persistent infection; CIN: cervical intraepithelial neoplasia; HPV: human papillomavirus; CI: confidence interval.

CI: 0.0–0.9], $p = 0.037$), whilst previous pregnancy was associated with a higher risk of CIN2+ (HR 2.0 [1.1–3.7], $p = 0.023$). There was some indication that women aged ≥36 years at onset of the 6MPI had a lower risk of lesion detection compared with women aged 26–35 years, with HRs of 0.7 (0.4–1.0), $p = 0.060$ for CIN1+ and 0.6 (0.3–1.1), $p = 0.090$ for CIN2+. Smoking at baseline was associated nonsignficantly with an increased risk: HR: 1.3 (0.7–2.3), $p = 0.369$ for CIN1+ and 1.5 (1.0–3.3), $p = 0.304$ for CIN2+.

Risk of detecting a CIN lesion associated with an HPV infection of any duration

Among the 1,073 women with an HPV infection of any duration, 120 (11.2%), 63 (5.9%), and 23 (2.1%), women, respectively, developed a CIN1+, CIN2+, or CIN3+ lesion

associated with the same HPV type within 48 months (Fig. 1a). Of 507 women with an HPV infection at baseline, CIN1+, CIN2+, or CIN3+ associated with the same HPV type were detected in 63 (12.4%), 40 (7.9%), and 18 (3.6%), respectively (Fig. 1b). Of 888 women with an HPV infection detected during follow-up, CIN1+, CIN2+, or CIN3+ associated with the same HPV type were detected in 69 (7.8%), 32 (3.6%), and 7 (0.8%), respectively (Fig. 1c).

Again, infection with an oncogenic HPV type was the strongest predictor of lesion detection. HPV-33 was associated with the highest risk for CIN1+ and CIN2+, followed by HPV-18, HPV-16, HPV-31, and HPV-45 for CIN1+, and by HPV-16, HPV-18, HPV-31, and HPV-45 for CIN2+ (Table 2). Infections of at least 6 months' duration were associated with a higher risk than infections of shorter duration for both CIN1+ and CIN2+ (Table 2). Co-infection with an oncogenic HPV type or a concomitant CIN1+ was significantly associated with an increased risk of detecting CIN1+ and CIN2+ (Table 2). The analysis did not show an effect of previous HPV infection or previous CIN1+.

Other determinants also influenced risk in the multivariable analysis. Previous pregnancy was associated with a higher risk of detecting CIN1+ (HR: 1.9 [1.3–2.9], $p = 0.003$) and CIN2+ (HR: 2.9 [1.6–5.5], $p = 0.001$), whilst peri- or post-menopausal status was associated with a lower risk (HR: 0.2 [0.1–0.7], $p = 0.014$ for CIN1+ and HR: 0.2 [0.0–1.3], $p = 0.093$ for CIN2+). Smoking at baseline was also associated with an increased, but nonsignificant risk of detecting CIN1+ (HR: 1.3 [0.8–2.0], $p = 0.278$) and CIN2+ (HR: 1.5 [0.8–2.9], $p = 0.260$). Women aged $\geq$36 years at onset of the HPV infection had a lower but nonsignificant risk compared with women aged 26–35 years of detection of CIN1+ (HR: 0.7 [0.5–1.1], $p = 0.112$) and CIN2+ (HR: 0.7 [0.4–1.2], $p = 0.185$).

Apparent clearance of HPV infection

A total of 851 women cleared 1,665 infections (Fig. 1a). Out of 507 women with an HPV infection at baseline and follow-up, 402 (79.3%) cleared the infection. Of 319 women with a 6MPI at baseline, 223 (69.9%) cleared the infection. Overall, there was a 77% (95% CI: 75–79) chance of clearing an HPV infection at 24 months and 89% (87–91) at 48 months.

The median duration of all HPV infections (present at baseline or detected during study follow-up) was 11.5 months. Median duration of infection was 17.4 months for HPV-31, 12.5 months for HPV-16, 12.0 months for HPV-45, 11.8 months for HPV-18, 11.7 months for HPV-33, 11.3 months for other oncogenic HPV types and 11.2 months for non-oncogenic HPV types (log rank test $p = 0.006$) (Fig. 2). However, the difference between HPV types was no longer significant after adjustment for other covariates.

Women who smoked at baseline were significantly less likely to clear an infection than nonsmokers (HR: 0.8 [0.7–0.9], $p = 0.004$). The effect of age at onset of the HPV infection was not significant in the univariate analysis and was not included in the multivariable analysis.

Discussion

The analysis confirmed that persistent infection with an oncogenic HPV type was the main risk factor for detecting a CIN lesion in our study population. HPV-33 and HPV-16 were associated with the highest risk, followed by HPV-18, HPV-31 and HPV-45. Compared with a 6MPI with a non-oncogenic HPV type, the risk of lesion detection was 30–40 times higher for HPV-33 and approximately 20 times higher for HPV-16. Clearance rates were high, and overall, only one tenth of HPV infections failed to clear by 4 years. The median duration of all HPV infections was ~1 year. HPV-31 had the longest duration of detectable infection, followed by HPV-16, HPV-45, and HPV-18.

These findings are consistent with other studies in a younger age group. In parallel with the present analysis, we conducted a post-hoc analysis of the cumulative incidence of lesions in women aged over 25 years in VIVIANE compared with women aged 15–25 years in the previous PATRICIA study. Overall, the risk of detecting a CIN1+ or CIN2+ following an HPV infection was similar in VIVIANE and PATRICIA (Supporting Information Fig. 1). Analyses of the PATRICIA study and the FUTURE (Females United To Unilaterally Reduce Endo/Ectocervical Disease) study of the HPV-6/11/16/18 vaccine in young women also showed that HPV-33 and HPV-16 have the strongest association with lesion detection, including CIN3+ in PATRICIA.[18,25] In PATRICIA, the risk of detecting CIN1+ was approximately 4-fold higher for HPV-16 and HPV-33 versus nononcogenic HPV types, and approximately 10-fold higher for CIN2+ after 4 years.[18]

A higher risk of progression associated with HPV-16 and HPV-33 has also been shown in population-based studies. In a cross-sectional study in the Netherlands of women (30–60 years of age) participating in a cervical cancer screening programme who were infected with an oncogenic HPV type, women with CIN2+ and CIN3+ were significantly more likely to be positive for HPV-16 and HPV-33 than women with normal cytology.[26] A case-control study in New Mexico, US showed that women of all ages positive for HPV-16 and HPV-33 had an equal risk of developing carcinoma *in situ* or adenocarcinoma *in situ*.[27] Also in New Mexico, a surveillance programme of women of any age attending for cervical screening showed that HPV-16 and HPV-33 were the types most often detected in high-grade cytological abnormalities.[28,29] In the United Kingdom, a study of women with abnormal cytology referred for colposcopy found that HPV-33 had a very high positive predictive value for CIN2+ and suggested that women with HPV-33 infections should be managed similarly to women with HPV-16 infections.[30] A prospective, population-based study of 10,000 adult women ($\geq$18 years) in Guanacaste, Costa Rica concluded that HPV-16 remains the most carcinogenic HPV type overall.[31]

Table 2. Multivariable analysis of the risk of detecting a CIN lesion associated with the same HPV type for HPV infections of any duration

	CIN1+			CIN2+		
	2,601 infections in 1,068 women 168 lesions[2]			2,601 infections in 1,068 women 92 lesions[2]		
	No. CIN1+	Hazard ratio[1] (95% CI)	*p* values	No. CIN2+	Hazard ratio[1] (95% CI)	*p* values
HPV type						
Non-oncogenic type	10	1	–	4	1	–
HPV-16	26	11.1 (5.1–24.3)	<0.0001	23	23.0 (8.6–62.0)	<0.0001
HPV-18	13	11.6 (5.0-27.0)	<0.0001	8	16.7 (5.4–51.5)	<0.0001
HPV-31	15	10.3 (4.5–23.9)	<0.0001	10	16.4 (5.1–52.9)	<0.0001
HPV-33	17	21.8 (9.3–51.0)	<0.0001	12	31.2 (10.2–95.3)	<0.0001
HPV-45	7	6.4 (2.2–18.4)	0.001	4	9.1 (2.2–37.5)	0.002
Other oncogenic type	80	6.6 (3.2–13.5)	<0.0001	31	5.6 (2.1–15.0)	0.001
		<0.0001			*<0.0001*	
Duration of infection						
Transient and less than 6MPI	57	1	–	28	1	–
6MPI	111	2.2 (1.6–3.1)	<0.0001	64	2.3 (1.4–3.8)	0.001
		<0.0001			*0.001*	
Previous cervical HPV infection						
No	106	1	–	61	1	
Yes (at least 1 oncogenic HPV type)	56	1.2 (0.8–1.9)	0.343	NA[3]	1.5 (0.8–2.7)	0.227
Yes (only non-oncogenic HPV types)	6	0.9 (0.4–2.1)	0.808	NA[3]	0.4 (0.1–3.4)	0.428
		0.576			*0.294*	
Cervical HPV co-infection						
No	69	1	–	37	1	–
Yes (at least 1 oncogenic HPV type)	89	1.8 (1.2–2.6)	0.003	53	2.1 (1.3–3.5)	0.005
Yes (only non-oncogenic HPV types)	10	0.8 (0.4–1.5)	0.473	2	0.3 (0.1–1.4)	0.125
		0.003			*0.001*	
Previous CIN1+[4]						
No	160	Not included		NA[3]	Not included	
Yes (any oncogenic or non-oncogenic HPV type)	8			NA[3]		
Concomitant CIN1+[5]						
No	155	1	–	80	1	–
Yes (any oncogenic or non-oncogenic HPV type)	13	2.8 (1.4–5.6)	0.005	12	3.4 (1.7–6.8)	0.001
		0.005			*0.001*	

[1]Covariates were included in the multivariable analysis if they had a global *p* value <0.2 in the univariate analysis (except region which was always included); covariates were: region, tobacco exposure measured as number of pack-years, age at onset of the HPV infection, age at first sexual intercourse, marital/partner status, education, number of lifetime sexual partners, number of sexual partners during the past 12 months, use of hormones for contraception or other indication, surgical sterilisation, use of an intrauterine device, previous pregnancy, menopausal status, and history of *Chlamydia trachomatis* during the past 12 months.
[2]Infections or lesions with a missing value for a covariate included in the analysis were excluded from the multivariable analysis.
[3]Time-varying covariate.
[4]CIN1+ associated with an HPV type different to the reference infection, preceding the onset of the 6MPI.
[5]CIN1+ associated with an HPV type different to the reference infection, concomitant to the 6MPI (following its onset and preceding its end)Values in italics show the global *p* value.
6MPI: 6-month persistent infection; CIN: cervical intraepithelial neoplasia; CI: confidence interval.

Although HPV-18 has a high prevalence in invasive cervical cancer,[3] women in VIVIANE infected with HPV-18 had a lower risk of developing CIN2+ than women infected with HPV-16 or HPV-33, and a similar risk as women infected with HPV-31 and HPV-45. These findings are consistent with PATRICIA and other studies.[18,26,27] In women of all

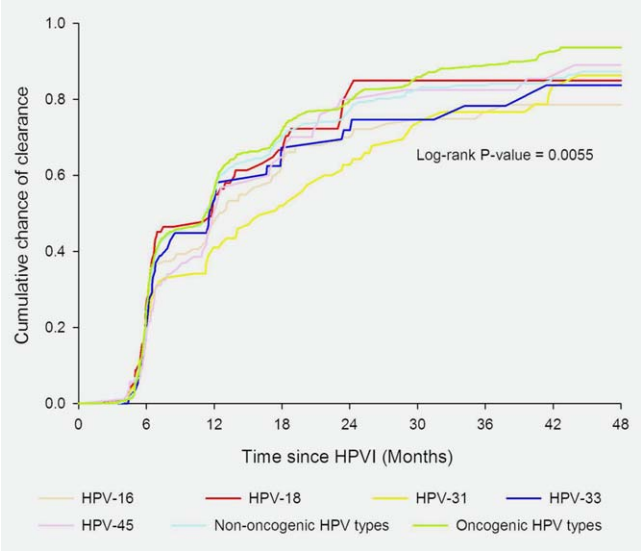

Figure 2. Chance of clearance of an HPV infection of any duration according to HPV type. HPV: human papillomavirus; HPVI: HPV infection of any duration.

ages, HPV-18 infection is reported to have a relatively low risk of detection in CIN, but a higher risk of subsequent progression to invasive cervical cancer, especially to adenocarcinoma.[32,33] This may be potentially attributable to a number of factors including the anatomic distribution of HPV-18-related cancers, which may be more difficult to sample if located higher in the cervical canal.

A coinfection with an oncogenic HPV type different to the referent infection and a concomitant CIN1+ lesion increased the risk of detecting CIN1+ and CIN2+. However, the analysis did not show an effect of previous infections or previous lesions on risk of lesion detection. In PATRICIA, risk of detection was increased by concomitant infection, but not by previous infection, and was also increased by both concomitant and previous CIN1+.[18] The role of multiple oncogenic infections in the natural history of HPV infection is controversial, with some studies showing a higher risk of acquiring a new HPV type if already infected,[34,35] and others showing that infections with different HPV types occur independently of one another.[36,37] Two recent studies have shown no evidence of a synergistic association of infection with multiple HPV genotypes and risk of high-grade squamous intraepithelial lesions or CIN2+.[28,38] In addition, laser microdissection of CIN lesions has shown that each component of the lesion is associated with a single HPV type *i.e.*, one virus causing one lesion.[39]

Behavioural risk factors for lesion detection in an older age group may differ compared with those in a sample of younger women, as the prevalence of these behaviours changes over the lifespan. In VIVIANE, previous pregnancy increased the risk of lesion detection and there was some evidence of increased risk with increasing number of sexual partners over the past 12 months. Smoking was associated with a small but nonsignificant increase in risk. Several studies have demonstrated an association between smoking and HPV infection, cervical abnormalities or cervical cancer,[40–43] whilst other studies have shown a relationship between smoking reduction and cervical lesion size or changes in cervical immune cell counts.[44,45] Peri- or postmenopausal status was associated with lower risk compared with premenopausal status. In PATRICIA, previous pregnancy was also associated with increased risk of incident lesion detection; other determinants associated with increased risk in PATRICIA included tobacco exposure, use of hormones for contraception or other purposes and younger age at first sexual intercourse.[18]

Despite the success of the Pap test in reducing cervical cancer rates in countries where screening is effectively implemented, cervical cancer prevention in the future may shift towards use of the more sensitive HPV-based screening. Various methods are now available for primary screening for cervical cancer and precancer, triage of women with equivocal or low-grade cytologic abnormalities and prediction of treatment outcomes.[46–48] Identification of HPV types with a higher risk of progression to cervical lesions may help to improve the specificity of HPV testing in cervical cancer screening, leading to follow-up algorithms and time intervals tailored to the particular HPV type, and subsequent improved efficiency and cost-effectiveness.[49] Our findings help to provide a better understanding of the natural history of HPV infection to inform these developments.

The analysis had some strengths and limitations. Only women with a confirmed HPV infection were included, so the analysis evaluated only factors potentially affecting the risk of lesion detection following infection, and was not confounded by factors affecting the risk of HPV acquisition. Another strength was the high follow-up rate and collection of well-characterised virological and histological samples which is not often feasible in an observational epidemiological study. The study recruited a broad population of women, with no restriction on the number of lifetime sexual partners and including ~15% of women with previous HPV infection/disease. The population of VIVIANE had a higher exposure at baseline to HPV-16/18 than would be expected in the general population of a similar age and as seen in a previous clinical trial of the HPV-6/11/16/18 vaccine in this age group.[11,19,50]

A limitation of the analysis is that CIN1 reflects a state of infection rather than a stage in disease development. Detection of CIN1+ following HPV infection does not therefore automatically represent disease progression. Nevertheless, the CIN1+ endpoint provides valuable information on the natural history of HPV infection. In addition, apparent clearance may relate to an inability to detect the infection; clearance rates should therefore be interpreted with caution. A further limitation is that misclassification of HPV infection below the threshold for detection (a false-negative result) might have underestimated persistent infection rates. However, a very sensitive HPV PCR algorithm was used. CIN detection rates may have been underestimated because the 4-year follow-up period was not long enough to detect all lesions, especially

those associated with an HPV type with a slower rate of progression from infection to lesion. More frequent follow-up would have allowed earlier detection of events, enabling more accurate estimates of the time between detection of infection and detection of a lesion or clearance. Lastly, most lesions were detected from what could be considered prevalent infections when persistent infection was first seen at baseline. For these cases, age when the infection first occurred could not be accurately determined and indeed many may have been present from adolescence and young adulthood. This limitation may have contributed to the lack of statistically significant association of lesion detection by age group and when comparing rates of lesion detection with those in PATRICIA.

In conclusion, persistent infection with an oncogenic HPV type was the main risk factor for CIN1+ and CIN2+ detection in women aged over 25 years, with HPV-33 and HPV-16 being associated with the highest risk. Concomitant HPV infection or CIN1+ due to an HPV type different to the reference infection also increased the risk of lesion development. Compared with women aged 15–25 years in PATRICIA, the risk of CIN detection following a 6MPI or HPVI was similar in women aged >25 years. Overall, clearance rates were high. These findings may contribute towards a better understanding of the natural history of HPV infections and CIN lesions at different ages.

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