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Variant-Specific Persistence of Infections with Human Papillomavirus Types 31, 33, 45, 56 and 58 and Risk of Cervical Intraepithelial Neoplasia

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Abstract

In our previous study of the etiologic role of oncogenic human papillomavirus (HPV) types other than HPV16 and 18, we observed a significantly higher risk of cervical intraepithelial neoplasia grades 2-3 (CIN2/3) associated with certain lineages of HPV types 31/33/45/56/58 (called high-risk (HR) variants) compared with non-HR variants. This study was to examine whether these intra-type variants differ in persistence of the infection and persistence-associated risk of CIN2/3. Study subjects were women who had any of HPV types 31/33/45/56/58 newly detected during a 2-year follow-up with 6-month intervals. For each type, the first positive sample was used for variant characterization. The association of reverting-to-negativity with group of the variants and CIN2/3 with length of positivity was assessed using discrete Cox regression and logistic regression, respectively. Of the 598 newly detected, type-specific HPV infections, 312 became undetectable during follow-up. Infections with HR, compared with non-HR, variants were marginally more likely to become negative (adjusted hazard ratio = 1.3; 95% confidence interval (CI), 0.9-1.8). The adjusted odds ratio associating with the development of CIN2/3 was 3.0 (95% CI, 1.2-7.4) for persistent infections with HR variants for 6 months and 10.0 (95% CI, 3.8-38.0) for persistent infections with HR variants for 12-18 months as compared with the first positive detection of HR variants. Among women with non-HR variants, there were no appreciable differences in risk of

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CIN2/3 by length of positivity. Findings suggest that the lineage-associated risk of CIN2/3 was not mediated through a prolonged persistent infection, but oncogenic heterogeneity of the variants.

Keywords

Human Papillomavirus; Variants; Persistence; Cervical Intraepithelial Neoplasia

Introduction

To date, over 170 human papillomavirus (HPV) types have been fully characterized ¹ with 12 epidemiologically defined as oncogenic types ². For a given type of HPV, viral isolates are further subdivided into finer phylogenetic categories called variants ³, lineages or sublineages ⁴. Despite a high degree of DNA sequence homology between HPV variants, some differ in their group phenotypic traits. We previously analyzed intratypic diversity of oncogenic types other than HPV16 and HPV18, showing that risks of cervical precancer, defined clinically as cervical intraepithelial neoplasia grades 2-3 (CIN2/3) differed significantly by variant lineages of five types (i.e., HPV31, HPV33, HPV45, HPV56, and HPV58) ⁵. We have postulated that genetic changes accumulated in the long-term viral evolution may have altered certain biologic properties of the virus which are responsible for the observed risk difference.

Results from studies of the natural history of HPV infection indicate that transiently detectable infections are common ⁶⁻⁸; a persistent infection with an oncogenic isolate is a presumed prerequisite for the development of cervical precancer and cancer ⁹⁻²⁰. Given the etiologic importance of HPV persistence, a question raised is whether our previously observed, lineage-associated risk of CIN2/3 could be in part attributable to differences in persistence of the infection. Data on intratypic variations of the likelihood of persistent infections with non-HPV16/18 oncogenic types are rare ²¹⁻²⁵.

Previous studies have also shown that the association between risk of cervical neoplasia and persistence of the infection differs significantly by types of HPV, with HPV16 persistence conferring the highest risk ^{9, 10}. Those with non-oncogenic types tend to regress or remain unchanged during follow-up ¹¹. It remains largely undetermined whether for a given type of oncogenic HPVs, risks of CIN2/3 associated with length of positive duration differ by group of the variants.

To address these questions of interest, we extended our previous observation of risk of CIN2/3 by lineages of non-HPV16/18 oncogenic types to examine variant-specific persistence of newly detected infections as well its relation to risk of CIN2/3.

Materials and Methods

Subjects and study design

Study subjects were women enrolled in the Atypical Squamous Cells of Undetermined Significance (ASC-US) and Low-Grade Squamous Intraepithelial Lesion (LSIL) Triage Study (ALTS), a randomized clinical trial designed to compare different strategies for the

management of women with a screening diagnosis of ASC-US or LSIL. ALTS participants were followed every 6 months for 2 years for detection of HPV infection and cervical lesions. A detailed description of the ALTS design and study population is available elsewhere²⁶. The ALTS protocol was approved by the institutional review board at National Cancer Institute and each of the four clinical sites involved in the trial.

An ALTS participant was eligible for the present study if she had HPV31, HPV33, HPV45, HPV56, and/or HPV58 newly detected in her cervical swab sample by polymerase chain reaction (PCR)-based reverse line blot assay²⁷ during follow-up but not at enrollment. For each type of HPV infection, the first positive sample was chosen for variant testing. Overall, 888 type-specific HPV infections were identified; 241 of them excluded due to lack of samples for variant testing or failure to PCR-generate DNA fragments for sequencing. To minimize a bias possibly introduced by uncertainty on the time of infection onset or reverting-to-negativity, we additionally excluded 39 infections that had 2 consecutive missing visits prior to the first positive detection and 10 infections that had 2 consecutive missing visits prior to the negative reversion or redetection. This left 598 newly detected type-specific HPV infections (among 537 women) in analysis, including 158 with HPV31, 75 with HPV33, 121 with HPV45, 127 with HPV56, and 117 with HPV58.

Data on HPV typing results, cytologic and histologic diagnoses of cervical lesion, and characteristics of study subjects were obtained from the ALTS database. The protocol for the present study was approved by the institutional review board at the University of Washington.

Testing for and classification of HPV variants

A detailed protocol for characterization and classification of HPV variants was reported previously⁵. Briefly, sequence variations of the 3' part of the long control region and the entire E6 and E7 region were determined by PCR-based direct DNA sequencing. The sequencing reaction was run from both directions using a BigDyeTM Sequencing kit (Applied Biosystems, Foster City, CA). A viral isolate was defined as a distinct variant if, as compared with the prototype and other isolates detected in the study, there was one or more nucleotide alterations in the region analyzed. These variants were phylogenetically classified and named as lineages or sublineages according to the alphanumeric nomenclature²⁸. As reported previously⁵, infections with variant lineages of HPV31 A or B, HPV33 A1, HPV45 A2, A3, or B2, HPV56 A1 or B and HPV58 A1 or A3 conferred a 2-10 fold higher risk of CIN2/3 than those with other lineages of the corresponding types; thus, these lineages were epidemiologically classified as HR variants. The remaining lineages of these five HPV types were categorized as non-HR variants. Because this study was designed to address whether these intra-type variants differ in persistence of the infection and persistence-associated risk of CIN2/3, two groups of the variants defined previously were used for present analyses.

Statistical analyses

Statistical analyses were performed at the infection level. A woman would be counted multiple times if she was positive for 2 types of interest. In ALTS, HPV status was assessed

only at visits that were scheduled every 6 months. Thus, the precise time of the onset and clearance of the infection was unknown. For analysis of time to loss of the infection, we assumed that the newly detected infection occurred at one third of the time interval prior to the first type-specific positive visit; the event of reverting-to-negativity occurred at one third of the time interval subsequent to the last positive visit. Results remained similar when the event was assumed to occur at the mid-point between the visits. For simplicity, these results were not presented. Women who completed the follow-up without clearing their type-specific infection or were lost to follow-up were censored at their last available positive visit. The median times to loss of the infection were estimated using Kaplan-Meier analyses. A log-rank test was used to assess the significance of differences in the likelihood of becoming negative between infections with HR and non-HR variants.

The events of loss of the infection were not ascertained in continuous time; rather, most were detected around the scheduled visit time. Therefore, a discrete Cox regression model²⁹ was used to estimate hazard ratios and their 95% confidence intervals (CIs) for the association of reverting-to-negativity with group of the variants. Factors potentially associated with loss of the infection, such as age, race/ethnicity, sexual behavior, smoking status, use of hormonal contraceptives, treatment for CIN2/3, and coinfection with other oncogenic types, were initially examined by univariate analyses. Those with $p < 0.2$ in univariate analyses were entered into multivariate models as covariates. We used a backward stepwise regression to construct the final model, with $p < 0.20$ as the criterion for entering and removing variables. Covariates included in the final model were age (18-24 or 25), post treatment for CIN2/3 (yes or no) and a time-dependent variable of coinfection with other oncogenic types (yes or no for all oncogenic HPVs except for the target type). For analysis of five HPV types together, the hazard ratio was additionally adjusted for type of HPV; robust variance estimates were used to account for correlation within subjects.

To determine the relationship between risk of CIN2/3 and persistent infection, the time of the first HPV-positive test was rescaled as month 0, the next visit as month 6, and the following visit as month 12. In ALTS, cervical cytology and histology were initially diagnosed by the clinical center pathologists and then reviewed by a panel of expert pathologists. The clinical endpoint of this study was the first episode of CIN2/3 histologically confirmed by the panel of expert pathologists. CIN2/3 diagnosed at the colposcopy visit immediately following the first HPV-positive detection was considered to be related to a short-term positivity. Women without CIN2/3 at the first positive visit who continued to be positive at the next visit were at risk for CIN2/3 that was related to 2 consecutive positive visits (a surrogate of 6-month persistent infection), and so on for those with 3 or 4 consecutive type-specific positive visits. Polytomous logistic regression³⁰ (with CIN2 and CIN3 as separate outcomes) or unconditional logistic regression (with CIN2/3 as outcome) with generalized estimating equations³¹ was used to estimate odds ratios (ORs) and their 95% CIs for the association of CIN2/3 with length of type-specific positivity. Considering the small number of CIN2/3 cases, the 95% CIs were computed using a bootstrap method with 1,000 repetitions. If one or more parameters could not be estimated in 10% bootstrap replicates, the 95% CIs were estimated by jackknife logistic regression clustering on the individual woman³². The ORs were adjusted for age, type of HPV, and coinfection with other oncogenic types.

A robust *t*-test was used to compare mean lengths of follow-up following the first positive detection between women with HR and non-HR variants. We used a robust chi-square test to compare proportions of completing the scheduled exit visit and proportions of coinfection with HPV16/18 or HR variants of other types between women with HR and non-HR variants. All statistical analyses were performed using STATA statistical software version 11 (StataCorp, College Station, TX) at the 5% two-sided significance level.

Results

Of the 598 type-specific HPV infections newly detected during a 2-year follow-up, 319 (53.3%) were positive for HR variants. Table 1 shows distribution of the lineage-specific infection by group of the HR and non-HR variants for each type of HPVs. The mean length ($\pm$ SD) of follow-up since the first positive detection was 12.1 ($\pm$ 7.4) and 12.0 ($\pm$ 7.0) months for infections with HR and non-HR variants, respectively ($P = 0.89$). There was no appreciable difference in proportions of completing the scheduled exit visit at month 24 between those with HR and non-HR variants (88.1% versus 88.5%, $P = 0.86$).

During follow-up, 312 type-specific HPV infections became undetectable. Table 2 shows the median time to loss of the infection for individual types. With all five types taken into account, the median time to reverting-to-negativity was 8.0 (95% CI, 5.6-10.5) months for infections with HR variants and 10.0 (95% CI, 8.9-11.1) months for infections with non-HR variants (Figure 1, log rank test, $P = 0.06$). After adjusting for age, treatment for CIN2/3, and coinfection with other oncogenic types, the hazard ratio for the association of reversion to negativity with HPV31 HR, compared with non-HR, variants was 1.9 (95% CI, 1.0-3.9). The differences in the likelihood of negative reversion by variants of other four HPV types were not statistically significant (Table 3). Overall, infections with HR, compared with non-HR, variants tended to be more likely to resolve although the difference was not statistically significant (adjusted hazard ratio = 1.3; 95% CI, 0.9-1.8).

Sixty-four women had a diagnosis of CIN2/3 prior to loss of the infection. The estimates did not change substantially by either censoring cases at the time of CIN2/3 diagnosis or treating them as being positive through the last available visit (data not shown). Restricting the analysis to women without a diagnosis of CIN2/3 yielded an adjusted hazard ratio of 1.5 (95% CI, 1.0-2.1) for the association of reversion to negativity with HR, compared with non-HR, variants. An intervening negativity between positive visits was detected in 9 (2.8%) of 319 infections with HR variants and 12 (4.3%) of 279 infections with non-HR variants ($p = 0.33$). Arbitrarily treating these negative tests as being positive did not appreciably alter the results (data not shown).

CIN2/3 was histologically confirmed in 26 (4.3%) of 598 women at the first positive visit (rescaled as month 0). A woman was counted more than once if she was positive for 2 types of interest. Among those without a diagnosis of CIN2/3 at previous visits, CIN2/3 was confirmed in 19 (8.5%) of 224 women at month 6, 16 (18.0%) of 89 women at month 12 and 3 of (11.1%) of 27 women at month 18 who continued to be positive at these visits. After adjusting for age, type of HPV, and coinfection with other oncogenic types, the OR associating with CIN2/3 was 3.0 (95% CI, 1.2-7.4) for persistent infections with HR variants

for 6 months and 10.0 (95% CI, 3.8-38.0) for persistent infections with HR variants for 12 or 18 months as compared with the first positive detection of HR variants. Among women with non-HR variants, however, there were no appreciable differences in risk of CIN2/3 by length of the positive duration (Table 4). When the analysis was restricted to visits without coinfection by HPV16 and/or HPV18, the OR_{adjusted} for the association of CIN2/3 with the HR variants persisting for 6 months relative to the first positive detection was 4.3 (95% CI, 1.7-10.9); it was 0.7 (95% CI, 0.1-3.7) for the association of CIN2/3 with the non-HR variants persisting for 6 months relative to the first positive detection.

Coinfection with other oncogenic types at the time of CIN2/3 diagnosis was common, detected in 19 (86.4%) of 22 cases with non-HR variants and 34 (81.0%) of 42 cases with HR variants. CIN2/3 cases positive for non-HR variants were marginally more likely to have a coinfection with HPV16/18 or HR variants of other types detected at the time of CIN2/3 diagnosis than were those with HR variants, although the difference was not statistically significant (16/22 = 72.7% versus 21/42 = 50.0%, $P = 0.08$). Among women without a concurrent diagnosis of CIN2/3, proportions of coinfection with HPV16/18 or HR of other types at the first positive visit were comparable between those with HR and non-HR variants (85/304 = 28.0% versus 82/268 = 30.6%, $P = 0.49$).

Discussion

The present study is an extension of our previous observation that risks of CIN2/3 differed significantly by variant lineages of HPV types 31/33/45/56/58⁵. In this study of women with these HPV types newly detected during a 2-year follow-up, we found that infections with HR, compared with non-HR, variants tended to be marginally more likely to become negative. Adjustment for factors that correlated to the likelihood of loss of the infection did not appreciably alter the estimates. Our results could not be explained by an ascertainment bias because testing for variant was performed without knowledge of epidemiologic information. Also, differential loss to follow-up was not an issue as the mean length of follow-up since the first positive detection was comparable between women with HR and non-HR variants, as was the proportion of completing the exit visit at month 24. Notably, by restricting the analysis to women with newly detected infections, a bias possibly introduced by uncertainty of the time of infection onset was minimized.

One issue complicating our results is the treatment for CIN2/3. As reported previously³³, an HPV infection may resolve as a consequence of the therapeutic procedure. In ALTS, virtually all CIN2/3 cases were treated with a loop electrosurgical excision procedure. This procedure might differentially influence estimates of the likelihood of persistence because women with HR, compared with non-HR, variants were more likely to have a diagnosis of CIN2/3 leading to subsequent treatment and often to HPV negativity. While this interruption of natural history was unavoidable, we performed various sensitivity tests by classifying CIN2/3 cases as either censored at the time of diagnosis or positive through the last available visit or restricting the analysis to women without a diagnosis of CIN2/3. All analyses yielded comparable results, suggesting that our findings were not materially distorted by treatments.

Few prior studies examined the variant-specific persistence of non-HPV16/18 oncogenic types²¹⁻²⁵. It was reported that the presence of a deletion of 78 base pairs in the long control region of HPV33 genome²³ or the LCR-MTL-21 variant of HPV56²⁵ was more frequently detected in women with a persistent infection than in those with a transient infection. A case-control study nested in the population-based Guanacaste Cohort reported detection of lineages of various HPV types in the α genus among women with a persistent infection or a diagnosis of CIN2 or CIN3, as compared to controls²¹. Because of differences in variants compared across studies, we were unable to directly compare our results with those by others for all types with the exception of HPV31 seen in the Guanacaste Cohort²¹; in that study, HPV31 lineage A and B (defined as HR variants in this study) relative to C (non-HR variants) were less likely to be detected in women with a persistent infection. This result is similar to what we observed, although the difference in that study was not statistically significant.

Given the previously observed, 2-10 fold higher risk of CIN2/3 associated with HR, compared to non-HR, variants, it would be expected that infections with HR variants might be more likely to persist than those with non-HR variants. Data from this study indicate that this is not the case. Although the underlying mechanisms for a marginally more frequent resolution of infections with HR, compared to non-HR, variants remains unknown, it is clear that the previously observed, higher risk of CIN2/3 associated with the HR variants was not mediated through a prolonged persistence of the infection. Thus, a more plausible explanation is that HR and non-HR variants may differ in their oncogenic potential. This interpretation is in part supported by our observation of risk of CIN2/3 by length of type-specific positive duration between women with HR and non-HR variants.

The present study revealed that the persistence-associated risk of CIN2/3 was evident for infections with HR but not non-HR variants. The association seen among women with HR variants is in line with previous reports regarding the etiologic role of persistent infections with oncogenic types⁹⁻²⁰. The reason for lack of the association among those with non-HR variants is not clear, but a possibility of the reduced oncogenicity of the non-HR variants deserves consideration. This is similar to a scenario of oncogenic versus non-oncogenic genotypes: whereas the prolonged persistence of infections with oncogenic types pose a woman at higher risk of cervical lesions, it may not be true for those with non-oncogenic types. If some non-HR variants possess a reduced oncogenicity, they may tend to exist more as bystanders in the lesion development, thereby attenuating the association between risk of CIN2/3 and a persistent infection with non-HR variants towards null.

Our results further support this possibility with the observation that most CIN2/3 cases were positive for multiple oncogenic types at the time of diagnosis. Particularly, those with non-HR, compared with HR, variants tended to be marginally more likely to have a coinfection with HPV16/18 (types with the highest oncogenicity) or HR variants of other types. Previous studies of HPVs in microdissected tissue samples reported that most cervical lesions contained only one type of HPV, suggesting a possibility of one virus-to-one lesion^{34, 35}. In ALTS, HPV testing was performed on cervical swab samples but not on tissue samples. Thus, an HPV type was considered to be CIN2/3-related in the present study, if it was detected in the cervical swab sample at the screening visit immediately preceding

the colposcopic visit yielding the histologic diagnosis. It is possible that some types found in cervical swabs may not exist in the actual CIN2/3 lesional tissue³⁶; thus, not all infections defined as CIN2/3-related were actually responsible for the lesion development. Consequently, the lack of the association between risk of CIN2/3 and a persistent infection with non-HR variants might be in part explained by an overestimation of CIN2/3-related infections that were detected in swab samples. In this study, the number of CIN2/3 events among women positive for a single type of HPV was too small to be allowed for a valid assessment of risk association. More studies are warranted to examine the consistency of observations in different settings using different methods, ideally in a large-scale longitudinal setting of women with only a single type of HPV detected during a study period.

Several limitations of the study should be addressed. We are aware that ALTS participants had a cytologic diagnosis of ASC-US or LSIL within 6 months prior to enrollment. Thus, a newly detected infection could be from new acquisition, reactivation of a pre-existing latent infection, or intermittent persistent detection. It is currently unknown whether these three kinds of new infections differ by variants, persistence, and/or progression. Second, because of a scheme of follow-up every 6 months, we were unable to count infections that occurred and disappeared within the interval, thereby leading to overestimation of the length of persistence. On the other hand, as in all studies of the newly detected infection, the average length of persistence might be underestimated due to exclusion of infections initially detected at enrollment that may represent part of those with a prolonged persistence. Presently, however, no evidence suggests that this would have occurred differentially by variants. Third, the limited number of follow-up visits in ALTS does not allow us to define a newly detection infection and negative reversion using a more stringent criterion of 2 consecutive negative visits preceding the first and following the last positive visit, respectively. It is possible that a single negative test could result from sampling issue or viral load fluctuation (rather than a true negativity). If this had occurred differentially between infections with HR and non-HR variants, results of the variant-specific persistent infection might have been distorted. Arguing against this is the observation of a small number of an intervening negativity between positive visits and the fact that proportions of an intervening negativity did not differ substantially between infections with HR and non-HR variants. Lastly, we defined variant lineages by Sanger sequencing of PCR products of a variable fraction of the HPV genome. In fact, we are now learning through application of next-generation sequencing methods that this approach mainly detects predominant variants of the infection. How the ongoing application of next-generation sequencing methods to studies of HPV genomics, coexistence of multiple variants, and associations with natural history and carcinogenesis remains to be seen.

In summary, our data indicated that the previously observed, lineage-associated risk of CIN2/3 was not mediated through a prolonged persistence of the infection. The finding of the variant-dependent, persistence-associated risk of CIN2/3 further suggests intratypic oncogenic heterogeneity of the virus.

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Glossary

HPV	human papillomavirus
CIN	cervical intraepithelial neoplasia
ASC-US	atypical squamous cells of undetermined significance
LSIL	low-grade squamous intraepithelial lesion
HSIL	high-grade squamous intraepithelial lesion

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Novelty and Impact Statements

The report is one of the first, if not the first study, to examine the impact of persistent infections with oncogenic HPV types 31, 33, 45, 56 and 58 on risk of CIN2/3, stratified by group of the variants. Clarification of the variant-dependent, persistence-associated risk of CIN2/3 is important as a proportion of infection with non-HR variants among women positive for these oncogenic HPV types is not trivial.

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Figure 1.

Figure 1 Kaplan-Meier estimates of proportion of women remaining positive for type-specific human papillomavirus infection from the time of the first positive detection among those with high-risk (HR, dashed line) and non-HR (solid line) variants Log rank test: $P = 0.06$ +. Censor indicator.

Table 1

Lineage distribution of newly detected type-specific human papillomavirus (HPV) infections by group of the variants *

Type	Group of the variants	Lineage or sublineage	No. (%) of women [#]
HPV31	Non-HR	C	158
		A	64 (40.5)
	HR	B	67 (42.4)
			27 (17.1)
HPV33	Non-HR	A2	75
		B	3 (4.0)
	HR	A1	17 (22.7)
			55 (73.3)
HPV45	Non-HR	A1	121
		B1	45 (37.2)
		C	19 (15.7)
		A2	2 (1.7)
	HR	A3	17 (14.0)
		B2	10 (8.3)
			28 (23.1)
HPV56	Non-HR	A2	127
		A1	33 (26.0)
	HR	B	10 (7.9)
			84 (66.1)
HPV58	Non-HR	A2	117
		B1	68 (58.1)
		B2	1 (0.9)
		C	7 (6.0)
	HR	D2	17 (14.5)
		A1	3 (2.6)
		A3	18 (15.4)
			3 (2.6)

* Information on sequence variation of individual lineages and classification of the variants was reported previously (5).

[#] A woman was counted more than once if she was positive for 2 types of interest. HR = high-risk variants, non-HR = non-high-risk variants.

Table 2

Median time to reverting-to-negativity from the onset of type-specific human papillomavirus (HPV) infection with high-risk (HR) and non-HR variants

<u>Months to reverting-to-negativity from the onset of infection with</u>					
Type	Non-HR variants		HR variants		<i>P</i> -value [*]
	No.	Median (95% CI)	No.	Median (95% CI)	
HPV31	64	11.0 (9.9-12.1)	94	9.9 (8.7-11.1)	0.05
HPV33	20	12.8 (3.9-21.7)	55	6.5 (1.8-11.2)	0.33
HPV45	66	11.2 (4.5-17.9)	55	9.8 (3.5-16.1)	0.57
HPV56	33	4.8 (3.4-6.2)	94	4.7 (3.9-5.5)	0.83
HPV58	96	9.4 (6.7-12.2)	21	9.3 (3.8-14.8)	0.99

* Log-rank test for a null hypothesis that there is no difference between infections with HR and non-HR variants in the probability of becoming negativity at any time point.

Table 3

Hazard ratios for the association between loss of newly detected type-specific infections and variants of human papillomavirus (HPV) types

Type	Group of the variants	Person-visits at risk [*]	Loss of the infection No. (per 100 person-visits)	Hazard ratio _{crude} (95% CI)	Hazard ratio _{adjusted} (95% CI) [#]
All	Non-HR	321	138 (43.0)	1.0	1.0
	HR	342	174 (50.9)	1.4 (1.0-1.9)	1.3 (0.9-1.8)
HPV31	Non-HR	79	27 (34.2)	1.0	1.0
	HR	101	48 (47.5)	1.9 (1.0-3.5)	1.9 (1.0-3.9)
HPV33	Non-HR	25	10 (40.0)	1.0	1.0
	HR	62	33 (53.2)	1.6 (0.6-4.1)	1.8 (0.6-4.9)
HPV45	Non-HR	65	28 (43.1)	1.0	1.0
	HR	55	25 (45.5)	1.1 (0.5-2.4)	1.3 (0.6-2.9)
HPV56	Non-HR	34	22 (64.7)	1.0	1.0
	HR	96	54 (56.3)	0.7 (0.3-1.7)	0.7 (0.3-1.6)
HPV58	Non-HR	118	51 (43.2)	1.0	1.0
	HR	28	14 (50.0)	1.3 (0.5-2.9)	1.2 (0.5-2.8)

^{*} indicating numbers of visits following the first positive detection at which women became negative or were at risk for loss of the infection. A woman was counted more than once if she was positive for 2 types of interest.

[#] Adjusted for age, treatment for CIN2/3 and coinfection with other oncogenic types. For analysis of all types, the hazard ratio was additionally adjusted for type of HPV. CI = confidence interval, HR = high-risk variants, non-HR = non-high-risk variants.

Table 4

Risk of cervical intraepithelial neoplasia grades 2 or 3 (CIN2/3) associated with lengths of positivity among women with newly detected type-specific human papillomavirus (HPV) infection

Variants	Remaining HPV-positive at month [*]	No. of Women at risk [#]	CIN2		CIN3		CIN2/3	
			No. (%)	OR _{crude} (95% CI)	No. (%)	OR _{crude} (95% CI)	OR _{crude} (95% CI)	OR _{adjusted} (95% CI) [†]
Non-HR	0	279	4 (1.4)	1.0	7 (2.5)	1.0	1.0	1.0
	6	115	4 (3.5)	2.5 (0.5-12.4)	2 (1.7)	0.7 (0.1-5.7)	1.3 (0.4-3.6)	1.4 (0.5-4.1)
	12-18	64	3 (4.7)	3.4 (0.6-20.4)	2 (3.1)	1.3 (0.2-10.8)	2.1 (0.5-6.8)	2.1 (0.7-6.9)
HR	0	319	7 (2.2)	1.0	8 (2.5)	1.0	1.0	1.0
	6	109	4 (3.7)	1.8 (0.4-7.4)	9 (8.3)	3.6 (1.2-11.0)	2.7 (1.2-6.1)	3.0 (1.2-7.4)
	12-18	52	3 (5.8)	3.4 (0.7-17.8)	11 (21.2)	11.0 (3.4-35.3)	7.5 (2.9-19.4)	10.0 (3.8-38.0)

* Rescaling the first positive visit as month 0, the next visit as month 6, the visit after the next as month 12, and the exit visit as month 18. Because of a small sample size, data on the last two visits were combined together.

A woman was counted more than once if she was positive for 2 types of interest. Of those without CIN2/3 at the first positive visit, 101 exited the cohort, 193 tested HPV-negative, 54 did not return and 224 continued to be positive at month 6. Of those without CIN2/3 but HPV-positive or non-return at month 6, 65 exited the cohort, 80 tested HPV-negative, 25 did not return and 89 continued to be positive at month 12. Of those without CIN2/3 but HPV-positive or non-return at month 12, 50 exited the cohort, 21 tested HPV-negative and 27 continued to be positive at month 18.

† Adjusted for age, type of HPV, and coinfection with other oncogenic types. OR = odds ratio, CI = confidence interval, HR = high-risk variants, non-HR = non-high-risk variants.