

# Modifiable Risk Factors Associated with Clearance of Type-Specific Cervical Human Papillomavirus Infections in a Cohort of University Students

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## Abstract

**Background:** Previous findings regarding risk factors for human papillomavirus (HPV) persistence, other than viral determinants, identified from prospective cohort studies have been inconsistent in part because study designs have differed with respect to differing HPV detection methods and varying lengths of follow-up time. Therefore, the objectives of this study were to continue the search for epidemiologic risk factors of persistent cervical HPV infections and determine what behaviors differed between those women with transient HPV infections and those women who cannot clear their type-specific HPV infections. **Methods:** Female university students ( $n = 621$ ) in Montreal were followed for 24 months at 6-month intervals. At each visit, a cervical cell specimen was collected. HPV DNA was detected using the MY09/MY11 PCR protocol and 27 HPV genotypes were identified by the line blot assay

(Roche Molecular Systems, Inc., Alameda, CA). Proportional hazards regression was used to estimate the crude and adjusted hazard ratios of clearing a type-specific high-risk ( $n = 222$ ) or low-risk ( $n = 105$ ) HPV infection over time according to specific baseline and time-dependent covariates.

**Results:** Daily consumption of vegetables seemed to increase the rate of HPV clearance independent of type. The use of tampons was associated with a reduced rate of high-risk HPV clearance, whereas regular condom use was associated with an increased rate of low-risk HPV clearance only.

**Conclusion:** Some proactive measures can be taken to increase the rate of HPV clearance, and there may be some differences between the sets of predictors of low-risk and high-risk HPV clearance. (Cancer Epidemiol Biomarkers Prev 2005;14(5):1149–56)

## Introduction

The identification of certain sexually acquired human papillomavirus (HPV) infections as the necessary etiologic agent for cervical cancer (1, 2) has helped to explain many of the classic risk factors originally identified for cervical cancer, such as age at first intercourse and number of lifetime partners (3). However, given that the evidence from longitudinal cohort studies show that most HPV infections are transient and only women who harbor a persistent HPV infection are likely to develop a cervical squamous intraepithelial lesion (SIL; refs. 4–6), other factors are likely to influence the natural history of HPV infections (7).

Viral determinants, including HPV type and viral load, have been identified as markers of HPV persistence or cervical neoplasia (7–9). Nonetheless, environmental risk factors, some of which are modifiable, are also thought to play a role in cervical carcinogenesis (10). Recent epidemiologic studies have shown that the cofactors most consistently identified in the etiology of cervical neoplasia, after adjusting for the presence of HPV, include high parity and long-term oral contraceptive use (11–14), smoking (15–17), and a coinfection with another sexually transmitted disease (18, 19).

However, it is not clear at which biological stage in HPV carcinogenesis these cofactors are most influential, and results from prospective cohort studies that have attempted to evaluate the effect of hormone contraceptives, diet, alcohol, and tobacco consumption on HPV persistence have been inconsistent (8, 20–22). Furthermore, the definition of HPV persistence has varied significantly between studies, and study designs have differed with respect to differing HPV detection methods and varying lengths of follow-up time (5, 8, 20–23). Consequently, comparison of results in the literature can be challenging.

The objectives of this study were to continue the search for epidemiologic determinants of persistent cervical HPV infections in a cohort of young women attending university in Montreal, Canada. This article presents the results from the analysis of determinants of clearance of type-specific high oncogenic risk and low oncogenic risk HPV infections.

## Materials and Methods

**Subjects.** Female students attending either the McGill University or the Concordia University Health Clinic were invited to participate if they intended to remain in Montreal, Canada for the next 2 years and had not required treatment for cervical disease in the last 12 months. Recruitment was initiated in November 1996 and accrual was completed in December 1998.

**Procedures.** All eligible women were asked to return to the clinic every 6 months over a period of 2 years for a total of five visits. At each visit, a questionnaire was completed and

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endocervical and ectocervical cells from the uterine cervix were collected with two accelon cervical biosamplers (Meds-cand, Inc., Hollywood, FL). A Papanicolaou smear was prepared with the first sampler and the remaining cells along with cervical cells collected with a second sampler were used for HPV DNA testing. At enrollment, information from a detailed, self-administered questionnaire was obtained on potential risk factors, such as sociodemographic status, race, diet, smoking history, sexual behavior, reproductive history, contraceptive and medical history, and personal hygiene. An abridged (follow-up) questionnaire designed to measure changes in recent sexual practices and other lifestyle factors was completed at each subsequent visit.

**HPV DNA Detection.** HPV DNA testing by a PCR in this study has been described previously elsewhere (24). DNA (5  $\mu$ L) purified with the use of QIAamp columns (Qiagen, Inc., Valencia, CA) was first amplified for  $\beta$ -globin DNA with PC04 and GH20 primers to show the absence of inhibitors and the integrity of processed DNA (25). Specimens that were  $\beta$ -globin positive were further tested with the L1 consensus HPV primers MY09/MY11 and HMB01 with Amplitag Gold (TaqGold, Perkin-Elmer-Cetus, Norwalk, CT) and with the line blot assay (Roche Molecular Systems, Inc., Alameda, CA) for the detection of 27 genital HPV genotypes (26, 27). HPV types were analyzed individually or in groups according to a classification based on their oncogenic potential. High-risk HPV types included those genotypes that are most frequently found in cervical tumors: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 (1). All other individual types that were identified with the line blot assay were classified as low-risk HPV types: 6, 11, 26, 40, 42, 53, 54, 55, 57, 66, 73, 82, 83, and 84.

**Statistical Methods.** Stratified Cox proportional hazards regression (28) was used to estimate the adjusted relative risk of clearance of prevalent and incident high-risk or low-risk HPV infections over time according to specific baseline characteristics and behaviors. Separate analyses were carried out for clearance of type-specific high-risk and low-risk HPV infections. Variables with repeated measures were represented by their most recent value using time-dependent covariates (28).

The proportional hazards assumption was tested based on a flexible generalization of the Cox proportional hazards model (29). The flexible model allows the hazard ratio (HR) for selected exposures to change over time according to an arbitrary function, the shape of which is estimated from the data using a quadratic regression spline with 5 *df*. The 4-*df* likelihood ratio test, comparing the fit of the conventional 1-*df* proportional hazards model and the flexible 5-*df* regression spline model, was used to verify the null hypothesis that the HR between those exposed and those not exposed did not change with increasing time since start of infection. All hypotheses were tested at the 0.05 significance level.

Time to an event was defined as the time between the first HPV-positive visit (detected at enrollment or at any follow-up visit) and the first visit when a subject was no longer positive for her longest enduring, type-specific, high-risk or low-risk HPV infection. Subjects with both high-risk and low-risk HPV types at the index visit were assigned to either the high-risk or the low-risk group based on the type with the longest duration. If there was a tie, the index visit was assigned to the high-risk group to make the two groups mutually exclusive. In case of a tie within the high-risk or low-risk group, all longest persisting infections had to have cleared to be assigned "clearance" status. Those women who completed the study without clearing their infection or were lost to follow-up were censored at their last available visit. Similarly, follow-up time was censored at the date of any biopsy for a low-grade SIL ( $n = 4$ ) and/or treatment for a high-grade SIL ( $n = 4$ ).

We wanted to investigate the role of smoking, alcohol consumption, oral contraceptive, and condom use and certain

aspects of diet and personal hygiene habits in clearance of HPV infection. These and other variables with at least one category, with  $P < 0.20$ , after controlling for age were included in later multivariable models so that their independent effects could be evaluated. All analyses were done with SAS version 8.0.

## Results

There were 621 women enrolled in the study that completed a questionnaire and consented to HPV DNA testing. The analysis was based on data collected from 2,650 completed visits (mean of 4.3 visits/subject) of which 2,570 (97.6%) of the cervical specimens were suitable for HPV DNA testing. Women returned every 6 months on average (65% returning between 5 and 8 months; range, 2-29 months).

A total of 327 (52.7%) women had a HPV infection detected at one or more visits and 124 (38%) of those women had at least one visit with a coinfection with high-risk and low-risk HPV types. There were 259 women with a high-risk HPV infection and of those, 37 (14%) had a longer persisting low-risk HPV infection and were excluded from the high-risk group, so that 222 (86%) of the women with a high-risk HPV infection were included in the analysis for clearance of high-risk HPV infections. Of those, 91 (48%) women eventually cleared their infection, whereas 131 remained positive at their last visit or were lost to follow-up and thus were censored. There were 215 women with a low-risk HPV infection, and of those, 110 (51%) had a longer persisting high-risk HPV infection and were thus excluded from the low-risk group, so that only 105 (49%) of the women with a low-risk type infection were included in the analysis for clearance of low-risk HPV infections. Of those, 59 (56%) women cleared their infection, whereas 46 women were censored.

The distribution of risk factors (Table 1) varied significantly between the cohort members from the two participating university clinics. Participants from university clinic 2 had a significantly higher frequency of persistent high-risk or low-risk HPV infections, when persistence was defined as three or more consecutive visits with the same high-risk type or low-risk type. The proportional hazards assumption, according to which the HR is constant over time, was confirmed for all variables of interest (data not shown), except the two university clinics. Thus, given the heterogeneity of the two populations and the evidence that the effect of clinic on clearance of HPV was not constant over time, stratified, time-dependent proportional hazards Cox models were used throughout, with a "no-interaction assumption," to adjust for clinic (30).

There was no significant difference between those women lost to follow-up and among those women who completed follow-up concerning their distribution of risk factors and HPV status at baseline (data not shown). Furthermore, there was no consistent relationship between age and HPV clearance rates (Table 2). Despite this, all models were adjusted for age because it was considered *a priori* as a confounder of most associations we were interested in exploring. Table 2 presents the age-adjusted HRs for high-risk or low-risk HPV clearance according to putative risk factors. The top part of Table 2 shows estimates for baseline variables identified as time-independent variables, whereas the bottom part of Table 2 shows estimates for variables with repeated measures identified as time-dependent variables. Measures of tobacco, alcohol, condom, and oral contraceptive use along with remaining variables, with  $P$ s  $< 0.20$ , in the age-adjusted models (Table 2) were assessed in a single multivariable model (Table 2). Cumulative lifetime exposure to tobacco, expressed as "pack-years," and duration of oral contraceptive use in the participant's lifetime were the dimensions of tobacco and oral contraceptive use retained in the final multivariable models (Table 2).

**Table 1. Distribution of baseline characteristics between participants from the two participating university health clinics**

Baseline characteristics	Total cohort (n = 621), n (%)	University clinic 1 (n = 421), %	University clinic 2 (n = 200), %
Age (y)			
17-20	187 (30.1)	41.3	6.5
21-23	228 (36.7)	34.4	41.5
24-26	102 (16.4)	11.2	27.5
27+	104 (16.7)	13.1	24.5
		$P_{\chi^2} < 0.001$	
Race			
White (Canadian/European descent)	513 (82.6)	82.6	82.5
Black (African/Caribbean descent)	62 (10.0)	11.9	6.0
Hispanic (South American descent)	29 (4.7)	3.6	7.0
Asian (South and East Asian descent)	17 (2.7)	1.9	4.5
		$P_{\chi^2} = 0.009$	
Age at first sex encounter (y)			
19+	174 (28.0)	29.2	25.5
16-18	314 (50.6)	52.5	46.5
<16	133 (21.4)	18.3	28.0
		$P_{\chi^2} = 0.025$	
No. lifetime sex partners			
1	147 (23.7)	27.6	15.5
2-4	196 (31.6)	34.2	26.0
4-9	161 (25.9)	25.7	26.5
10+	117 (18.8)	12.6	32.0
		$P_{\chi^2} < 0.001$	
Pack-years smoked			
Nonsmoker	378 (60.9)	67.7	46.5
<1	101 (16.3)	16.6	15.5
1-2	58 (9.3)	7.1	14.0
>2	84 (13.5)	8.6	24.0
		$P_{\chi^2} < 0.001$	
Alcohol consumption (drinks/wk)			
0	226 (36.4)	39.2	30.5
1-3	179 (28.8)	29.2	28.0
>3	216 (34.8)	31.6	41.5
		$P_{\chi^2} = 0.035$	
Duration of oral contraceptive use (y)			
0	145 (23.3)	25.7	18.5
<1	121 (19.5)	21.6	15.0
1-5	252 (40.6)	40.1	41.5
>5	103 (16.6)	12.6	25.0
		$P_{\chi^2} < 0.001$	
Condom use			
Never	54 (8.7)	8.1	10.0
Sometimes	208 (33.5)	30.9	39.0
Always	359 (57.8)	61.0	51.0
		$P_{\chi^2} = 0.061$	
History of <i>Chlamydia</i>			
Never	588 (94.8)	97.1	90.0
Ever	32 (5.2)	2.9	10.0
		$P_{\chi^2} < 0.001$	
History of warts			
Never	537 (86.5)	89.8	79.5
Ever	84 (13.5)	10.2	20.5
		$P_{\chi^2} < 0.001$	
Cumulative HPV status			
HPV negative (at all visits)	293 (47.3)	53.4	34.5
HPV positive (at 1+ visits)	32 (5.2)	46.6	65.5
		$P_{\chi^2} < 0.001$	
Persistent high-risk HPV			
Negative or transient	548 (88.2)	90.5	83.5
≥3 consecutive visits high-risk HPV positive	73 (11.8)	9.5	16.5
		$P_{\chi^2} = 0.011$	
Persistent low-risk HPV			
Negative or transient	583 (93.9)	95.5	90.5
≥3 consecutive visits low-risk HPV positive	38 (6.1)	4.5	9.5
		$P_{\chi^2} = 0.015$	
Cumulative SIL status			
Normal cytology (at all visits)	575 (92.6)	91.9	94.0
SIL (at ≥1 visit)	46 (7.4)	8.1	6.0
		$P_{\chi^2} < 0.001$	

Ethnicity was associated with clearance of high-risk HPV but not low-risk HPV infections (Table 2). However, although Black, Hispanic, and Asian women seemed to clear their high-risk HPV infections more rapidly than White women, only

Black women were significantly more likely to clear their high-risk HPV infection compared with White women [HR, 2.86; 95% confidence interval (95% CI), 1.18-6.95]. Surprisingly, increased alcohol consumption was associated with a 2-fold

**Table 2. Determinants of clearance of type-specific high-risk and low-risk HPV infections: age-adjusted and final multivariable model**

Time-independent variables	High oncogenic risk ( <i>n</i> = 222)		Low oncogenic risk ( <i>n</i> = 105)	
	Age adjusted, HR (95% CI)	Multivariable model, HR (95% CI)	Age adjusted, HR (95% CI)	Multivariable model, HR (95% CI)
Age (y)				
17-20	Reference	Reference	Reference	Reference
21-23	1.1 (0.7-1.7)	1.3 (0.7-2.5)	0.9 (0.5-1.7)	1.4 (0.5-3.9)
24-26	0.6 (0.3-1.1)	0.7 (0.3-1.6)	0.8 (0.4-1.6)	0.9 (0.3-2.8)
27+	0.8 (0.4-1.7)	0.7 (0.3-1.9)	0.9 (0.4-1.8)	1.3 (0.4-4.6)
Age at first sex encounter (y)				
19+	Reference	Not included in final model*	Reference	Not included in final model*
16-18	0.8 (0.5-1.3)		1.2 (0.6-2.1)	
<16	0.8 (0.5-1.5)		0.9 (0.4-1.9)	
Ethnicity				
White (Canadian/European descent)	Reference <sup>†</sup>	Reference	Reference	Not included in final model*
Black (African/Caribbean descent)	1.8 (0.8-3.7)	3.5 (1.2-9.4)	0.7 (0.2-2.2)	
Hispanic (South American descent)	1.9 (0.6-6.0)	3.7 (0.6-22.3)	3.0 (0.4-22.3)	
Asian (South and East Asian descent)	1.2 (0.5-6.0)	1.5 (0.6-4.0)	1.0 (0.3-2.7)	
Sanitary products				
Tampons	Reference <sup>†</sup>	Reference	Reference	Not included in final model*
Sanitary napkins	3.3 (1.7-10.0)	5.0 (2.5-10.0)	1.0 (0.5-2.0)	
Sanitary napkins and tampons	2.0 (1.1-3.3)	2.5 (1.4-5.0)	1.3 (0.7-2.5)	
Vegetable consumption (daily servings)				
<1	Reference <sup>†</sup>	Reference	Reference <sup>†</sup>	Reference
1+	1.7 (1.1-3.3)	2.5 (1.4-5.0)	1.6 (0.9-2.5)	2.0 (0.9-5.0)
Duration of oral contraceptive use (y)				
0	Reference <sup>†</sup>	Reference	Reference	Reference
<1	1.4 (0.8-2.5)	1.7 (0.8-3.5)	0.7 (0.2-2.0)	1.2 (0.3-4.2)
1-5	0.8 (0.5-1.4)	1.2 (0.6-2.4)	1.0 (0.5-2.0)	1.9 (0.7-5.5)
>5	0.8 (0.4-1.7)	1.0 (0.4-2.5)	1.4 (0.6-3.1)	2.3 (0.6-9.2)
Smoking status				
Never	Reference	Not included in final model <sup>§</sup>	Reference	Not included in final model*
Former	1.0 (0.5-2.0)		0.7 (0.3-1.8)	
Current	0.7 (0.5-1.2)		1.1 (0.6-2.1)	
No. cigarettes daily				
Nonsmoker	Reference <sup>†</sup>	Not included in final model <sup>§</sup>	Reference	Not included in final model <sup>§</sup>
<1	0.4 (0.2-1.0)		1.2 (0.6-2.6)	
1-5	0.9 (0.5-1.8)		1.1 (0.5-2.5)	
>5	0.8 (0.4-1.5)		1.4 (0.6-2.9)	
Pack-years smoked				
Nonsmoker	Reference <sup>†</sup>	Reference	Reference	Reference
<1	0.8 (0.5-1.5)	0.7 (0.4-1.4)	1.3 (0.7-2.5)	0.8 (0.3-1.8)
1-2	0.6 (0.3-1.2)	0.5 (0.2-1.1)	0.5 (0.2-1.9)	0.3 (0.1-1.1)
>2	0.7 (0.4-1.4)	0.6 (0.3-1.3)	1.1 (0.5-2.2)	0.7 (0.3-1.9)
Alcohol consumption (drinks/wk)				
0	Reference	Reference	Reference	Reference
1-3	1.1 (0.6-1.9)	2.1 (1.0-4.5)	1.4 (0.6-3.2)	1.1 (0.4-2.7)
>3	1.0 (0.5-1.8)	2.0 (1.0-4.9)	1.9 (0.9-4.3)	1.9 (0.7-5.3)
Oral contraceptive use				
Never	Reference	Not included in final model <sup>§</sup>	Reference	Not included in final model <sup>§</sup>
Sometimes	0.8 (0.3-2.2)		0.0 (0.0-)	
Always	0.8 (0.3-1.3)		1.1 (0.6-1.9)	
Condom use				
Never	Reference	Reference	Reference <sup>†</sup>	Reference
Sometimes	0.8 (0.5-1.5)	0.8 (0.4-1.5)	1.2 (0.5-2.5)	2.3 (0.9-5.8)
Always	1.1 (0.6-1.7)	1.0 (0.6-1.7)	2.3 (1.1-4.7)	4.2 (1.7-10.3)
History of <i>Chlamydia</i>				
No	Reference	Not included in final model*	Not possible to calculate	Not possible to calculate
Yes	1.4 (0.3-5.9)			
Wash after sex				
Never/rarely	Reference	Not included in final model*	Reference	Not included in final model*

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**Table 2. Determinants of clearance of type-specific high-risk and low-risk HPV infections: age-adjusted and final multivariable model (Cont'd)**

Time-independent variables	High oncogenic risk (n = 222)		Low oncogenic risk (n = 105)	
	Age adjusted, HR (95% CI)	Multivariable model, HR (95% CI)	Age adjusted, HR (95% CI)	Multivariable model, HR (95% CI)
Sometimes	1.1 (0.7-1.8)		0.8 (0.5-1.4)	
Always	0.8 (0.4-1.6)		0.7 (0.3-1.9)	
Vaginal irritation				
Never	Reference <sup>†</sup>	Reference	Reference	Not included in final model*
1-2 since last visit	1.5 (0.9-2.4)	1.6 (0.9-2.8)	1.1 (0.6-2.0)	
>2 since last visit	1.6 (0.9-3.1)	1.9 (0.9-3.9)	1.1 (0.4-2.6)	

NOTE: All variables were adjusted for each other in the final model.

\*These variables were not included in the final model for clearance of high-risk or low-risk infections because of a clear lack of association in earlier models.

<sup>†</sup>P < 0.20.

<sup>‡</sup>P < 0.05.

<sup>§</sup>These variables were not included in the final model for clearance because other indices of the same exposure were selected instead.

increased rate of clearance for both high-risk and low-risk HPV infections when adjusted for other predictors of clearance, including tobacco use. However, women who smoked the equivalent of one or two packs of cigarettes per day (e.g., 20 cigarettes per pack) for at least 1 year were less likely to clear their high-risk HPV (HR, 0.5; 95% CI, 0.2-1.1) or low-risk HPV (HR, 0.3; 95% CI, 0.1-1.1) infections compared with women who never smoked. Similarly, compared with women who did not eat vegetables daily, women who had one or more daily servings of vegetables (carrots, broccoli, cabbage, salad, or green beans) cleared their high-risk HPV (HR, 2.5; 95% CI, 1.4-5.0) or low-risk HPV (HR, 2.0; 95% CI, 0.9-5.0) infections more rapidly (Table 2).

The association between sexually transmitted diseases and HPV persistence could not be well evaluated because there were too few women with a HPV infection and a past or recent sexually transmitted disease, such as *Chlamydia trachomatis* or a herpes simplex virus-2 infection (Table 2). Barrier methods of contraception were associated with faster clearance of low-risk HPV infections (HR, 4.2; 95% CI, 1.7-10.3), whereas increased duration of oral contraceptive use was also associated with a faster rate of low-risk HPV clearance but was not statistically significant.

Most interestingly, the use of tampons was a strong predictor of high-risk HPV persistence, and women who used sanitary napkins, exclusively, were five times (HR, 5.0; 95% CI, 2.5-10.0) more likely to clear their high-risk HPV infection compared with women who used tampons, exclusively.

## Discussion

The viral-host interaction is believed to play a crucial role in HPV persistence. Furthermore, functional differences between oncoproteins of viral HPV subgroups are believed to explain the ability of high-risk HPV types to cause cellular transformation, proliferation, and lesion progression (31-33). For example, estradiol has been shown to stimulate transcription of E6 and E7 gene products of HPV 16 (34). However, it remains to be shown that hormonal stimuli can also influence transcriptional activity of other HPV types, particularly low-risk types. If there are biological distinctions between the molecular mechanisms of low-risk HPV and high-risk HPV-induced infections, there may also be distinct sets of risk factors for persistence associated with these HPV subgroups. When we did an analysis without grouping type-specific infections into high-risk and low-risk groups, many of the associations that we observed in the stratified (on oncogenic group) analyses disappeared or were diluted (data not shown).

Thus, certain effects may be masked if all type-specific infections are grouped together (35).

The rationale for studying mutually exclusive groups by dividing women with coinfections into the HPV-risk group according to her most persistent type infection was to avoid mixing a preexisting infection and a newly acquired (different type) infection in the definition of persistence (or clearance; ref. 24). We also wanted to study determinants of a woman's most persistent HPV infection, because the evidence suggests that it is the most persistent type-specific infections that best predict progression to high-grade SIL (6) and cervical cancer.

Results from our study suggest that regular condom use is significantly protective against low-risk HPV persistence. Conflicting reports from cross-sectional studies demonstrating protective effects of condom use against low-risk HPV (36) or high-risk HPV infection only (37, 38) suggest that condoms may confer varying levels of protection against different HPV types. However, whether this means that genital HPV types have altered biological tropisms and can be found, differentially, on specific anatomic genital sites that are not all protected by a condom still needs to be clarified (37-39).

Although condom use and other barrier contraceptives have not been shown to be associated with HPV persistence in previous studies (20, 23), regular use of condoms has been associated with a reduction in risk for high-grade SILs among HPV-positive women in two separate cohorts (8, 12). These results are fairly unexpected, because it has generally been thought that if condoms do confer protection it would be further upstream, by preventing transmission of HPV, not lesion development. However, it is possible that, if condoms do protect against acquisition of a new HPV infection, then extended persistent HPV episodes (reinfection with the same type) may be prevented. Another possibility is that, although condoms may not entirely protect against transmission, they may help reduce the viral load of HPV or protect against other sexually transmitted diseases that may interact with HPV and worsen the natural course of infection (12). This hypothesis is further supported with results from a recent clinical trial in the Netherlands that showed condom use, for at least 3 months, helped to promote regression of cervical intraepithelial neoplasia and clearance of HPV among women with cervical intraepithelial neoplasia (40).

The carcinogenic effects of tobacco exposure on the development of cervical disease have been studied extensively in both epidemiologic and experimental studies (41). It has been shown that tobacco exposure affects the host immunosurveillance system against viral infections as shown by reduced markers of immune function *in vitro* (42). In addition, tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone has been measured in cervical mucosa

(43), and there is also preliminary laboratory evidence to suggest that certain HPV-infected cells may be more susceptible to DNA damage from specific tobacco carcinogens (44).

Nonetheless, based on the current literature, it is still difficult to conclude at which stage in the natural history of HPV cervical carcinogenesis tobacco exposure would exert most influence. Although a consistent relationship between tobacco use and *in situ* or invasive cervical carcinoma has been observed in case-control and cohort studies as reviewed in ref. 41, the association between smoking tobacco and HPV persistence has not been consistently shown in the literature (5, 8, 21). Nonsmokers were surprisingly more likely to have a persistent HPV infection than current smokers among a cohort of university students in New York followed every 6 months for >2 years (8) and among a cohort of middle-aged women in Oregon who returned for one follow-up visit (5). Conversely, Giuliano et al. (21) observed that women who smoked were less likely to clear a HPV infection than nonsmokers among a cohort of women who returned for two follow-up visits over an average of 10 months. Others have not observed any association between tobacco use and HPV persistence (23) or clearance (20, 22). However, only one of these studies (21) evaluated the cumulative intensity of tobacco exposure (pack-years).

Cumulative intensity of exposure to tobacco metabolites (often measured in "pack-years") may be a better predictor of genomic damage in squamous epithelial cells and risk of subsequent development of cervical disease (17). However, if exposure to tobacco is also associated with HPV clearance (a rather rapidly evolving event), suppression of the immune response is more likely to be the mechanistic explanation rather than DNA damage (42), and presumably recent and cumulative tobacco exposure would have similar influences on cell-mediated immunity against viral infections. Nonetheless, we observed that women who smoked one or two packs of cigarettes per day for at least 1 year were nearly half as likely to clear their HPV infections than nonsmokers, although the association was marginally nonsignificant, and there was not a clear dose response. However, a dose response with tobacco use may have been hard to observe in this study because of the relatively low levels of tobacco exposure in this young cohort. When we evaluated the effect of recent number of cigarettes smoked or smoking status (never, former, or current) in the final multivariable model, neither variable was associated with HPV clearance (data not shown).

Other factors that may reduce immune function or affect the health of the cervical squamous epithelial environment could include oral contraceptive use (22, 45), excessive alcohol consumption (46, 47), and dietary patterns deficient in essential micronutrients (48). Although oral contraceptives seem to be a cofactor in cervical carcinogenesis (41), the majority of cohort studies have not observed an association with duration of oral contraceptive use and HPV persistence (5, 8, 20). In our study, however, we observed that women who used oral contraceptives regularly for at least 1 year cleared their low-risk HPV infections more rapidly than women who did not use oral contraceptives. These findings partially support results from a recent population-based cohort study conducted in Bogota, Colombia (22). Women were followed for at least 5 years and the authors observed that women who had ever used oral contraceptives experienced faster clearance of any type-specific HPV infection compared with women who were nonusers of oral contraceptives (22). However, because oral contraceptive use may be a proxy for monogamous sexual relationships, more epidemiologic and laboratory studies are required to clarify whether oral contraceptives exert a real protective biological effect within the cervical microenvironment or whether our results are confounded by "safe partners."

A limited food frequency questionnaire was completed by our cohort members at enrollment only, and the main goal was

to characterize general eating patterns with respect to dairy products and fruits and vegetables that were high in vitamins C and E, carotenoids, and folate. Two different mechanistic pathways have been proposed to support the protective effects of a healthy diet against cervical carcinogenesis. Antioxidant nutrients, such as vitamins C and E, lycopene, and carotenoids, may act early in cervical carcinogenesis by helping to prevent cellular and DNA damage from reactive oxygen species caused from exposure to tobacco or cervical inflammation (48). Folate as well as vitamins B<sub>12</sub> and B<sub>6</sub> and methionine may also play a role in decreasing viral proliferation through their role in DNA methylation (49).

Despite our crude measurement of dietary patterns, we observed that women who consumed one or more servings of vegetables per day cleared their HPV infections significantly more quickly than women who did not consume vegetables daily. Our results add to the mounting evidence that fresh vegetables may play a protective role against persistent HPV infections. A recent study that measured food intake with a food frequency questionnaire and also measured levels of specific micronutrients in blood samples observed that a higher level of vegetable consumption was associated with a 54% decrease risk of HPV persistence in a cohort of young women from Arizona (49). Furthermore, lower levels of important micronutrients found in vegetables, such as  $\beta$ -cryptoxanthin and lutein/zeaxanthin, were associated with increased HPV persistence in the Arizona cohort (50) and similarly among a cohort of women in Brazil (51).

Whereas fruits and vegetables may protect against HPV persistence, excessive alcohol use may increase the risk of HPV persistence by interfering with folate absorption and metabolism (52). Paradoxically, in our study, alcohol use seemed to be associated with a more rapid rate of HPV clearance. These results are not in agreement with other cohort studies among young women who showed that increased alcohol consumption was either associated with HPV persistence (8) or not associated with HPV clearance at all (53). However, ~90% of the cohort members in our study drank less than one drink per day. Therefore, the effect of heavy drinking on HPV clearance could not be adequately assessed.

Hygienic practices, such as douching and use of tampons during menstruation, have frequently been proposed as explanatory variables for observed differences in HPV prevalence, but associations have been inconsistent (8, 35, 38). Our data showed that women who used tampons instead of sanitary napkins were significantly less likely to clear their high-risk HPV infection. One other cohort study observed a similar elevated risk for cumulative high-risk HPV among women who used tampons instead of sanitary napkins during menstruation (35). Although the mechanism is currently unknown, it is possible that tampons will serve to spread the viral infection to different locations in the cervix, thereby increasing the area and possibly the severity of the HPV infection. Cervical inflammation has also recently been proposed as a relevant cofactor in HPV carcinogenesis (48) and perhaps the premature removal of tampons may induce some form of epithelial irritation that may facilitate persistence of a high-risk HPV infection. Nonetheless, regardless of the mechanism, if future studies among populations with equally high exposure to tampons reveal similar measures of effect, then discouraging the use of tampons during the course of a HPV infection may prove to be one of the more easily modifiable determinants of rapid HPV clearance.

The observation that Black women from African or Caribbean descent cleared their high-risk HPV infections more rapidly than White women (from European descent) was unexpected and could not be explained by the student's perceived level of their family's financial situation ("difficult," "moderate," or "very comfortable"; data not shown).

However, White subjects were also more likely to have engaged in sexual activities at an earlier age (<16 years) compared with other women ( $P < 0.001$ ). Age at first intercourse may be associated with latent infections because it could be a proxy for time since first possible exposure to HPV. One recent cohort study observed a slower clearance rate (relative risk, 0.74; 95% CI, 0.52-1.06) for women who engaged in sexual relationships at age  $\leq 15$  years (22). However, we did not observe a clear relationship with age at first intercourse and HPV clearance in our study, and it did not alter the estimates of effect for ethnicity substantially when added to a regression model that only included ethnicity.

There are some limitations in our study that should be addressed. Both prevalent and incident infections were included in our analysis of clearance. One concern with combining prevalent and incident HPV data is that the time at which a woman is first HPV positive cannot be easily approximated for prevalent infections, even if the time of clearance is well approximated, and leads to a form of left censoring. Furthermore, including prevalent cases may introduce a type of survival bias because a greater proportion of the prevalent cases may represent persistent infections compared with the incident cases. However, when we evaluated the association between selected risk factors and clearance rates of incident HPV infections, the HRs did not differ materially from the point estimates presented in Table 2, although the 95% CIs were wider (data not shown). Therefore, the potential length bias introduced by including prevalent cases in the analysis appeared minimal and it was decided to combine both incident and prevalent infections in the analysis to improve the power of the analyses.

Etiologic studies on HPV persistence have varied widely with respect to length of time between return visits. This difference in time can affect the results as women with a longer time interval between two visits (>12 months) are significantly less likely to have a persistent HPV infection (12). Extreme variation in length of time between visits could also have biased our estimated time to HPV clearance. Although the majority of women in our cohort returned within 6 months of each visit, there was a small proportion of women whose time interval between visits was >1 year. If this subgroup of women had differentially cleared their infection at a much earlier date before their return visit date, the average time to clearance could be inflated. However, the association between putative risk factors and the clearance rate of HPV would only be distorted if certain exposure-outcome combinations were associated differentially with time between visits. Given that the participants were unaware of their HPV status until the end of the study, this is an unlikely scenario.

In conclusion, viral characteristics and the host environment (i.e., genetic susceptibility, and immunosuppression) are currently the most important determinants of HPV persistence (7). Therefore, until a vaccine or antiviral medication is available to treat HPV infections, there seems to be little that a physician can do for a HPV-positive patient other than see her regularly for cervical cytology screening. However, our results suggest that there may be some proactive measures that a woman can take to modify her risk for HPV persistence. The cessation of tobacco use, increased vegetable consumption, and use of sanitary napkins instead of tampons during the course of a HPV infection are actions that may potentially lead to a faster rate of HPV clearance.

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# BLOOD CANCER DISCOVERY

## Modifiable Risk Factors Associated with Clearance of Type-Specific Cervical Human Papillomavirus Infections in a Cohort of University Students

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