

Persistent Human Papillomavirus Infection as a Predictor of Cervical Intraepithelial Neoplasia

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HUMAN PAPILLOMAVIRUS (HPV) infection is believed to be the central cause of cervical cancer, although most of the epidemiological evidence has come from retrospective, case-control studies,¹ which do not provide information on the dynamics of cumulative or persistent exposure to cervical HPV infection. The social and economic implications of cervical cancer for public health programs worldwide and the recent interest in the development of HPV vaccines have compelled the initiation of prospective, long-term multidisciplinary studies of the natural history of cervical cancer to investigate the role of HPV infection in the development of preinvasive cervical lesions. To date, several studies have reported results on the prospective relationship between HPV infection and the incidence of squamous intraepithe-

Context Human papillomavirus (HPV) infection is believed to be the central cause of cervical cancer, although most of the epidemiological evidence has come from retrospective, case-control studies, which do not provide information on the dynamics of cumulative or persistent exposure to HPV infection.

Objective To assess the risks of cervical neoplasia related to prior persistent HPV infections.

Design and Setting Longitudinal study of the natural history of HPV infection and cervical neoplasia in women residing in the city of São Paulo, Brazil, which was conducted between November 1993 and March 1997 and involved repeated measurements of HPV and lesions with follow-up until June 2000.

Participants A total of 1611 women with no cytological lesions at enrollment and HPV test results available from the first 2 visits.

Main Outcome Measure Cervical specimens taken for Papanicolaou cytology and HPV testing every 4 months in the first year and twice yearly thereafter. Incident cervical cancer precursor lesions ascertained by expert review of all cytology smears.

Results The incidence rate of squamous intraepithelial lesions (SILs) was 0.73 per 1000 women-months (95% confidence interval [CI], 0.5-0.9) among women free of HPV at the 2 initial visits and 8.68 (95% CI, 2.3-15.1) among women with HPV type 16 or 18 infections persisting over both visits. Relative to those negative for HPV oncogenic types at both initial visits, the relative risk (RR) of incident SIL was 10.19 (95% CI, 5.9-17.6) for persistent infections with any known oncogenic HPV types. The equivalent RR of incident high-grade SIL was 11.67 (95% CI, 4.1-33.3). The RRs of lesions were considerably higher for persistent infections with HPV type 16 or 18.

Conclusion A strong relationship exists between persistent HPV infections and SIL incidence, particularly for HPV types 16 and 18.

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lial lesions (SILs)²⁻⁹; however, only 2 of these studies have reported on HPV persistence and subsequent SIL incidence over an extended period of time.^{8,9}

In 1993, we began a longitudinal investigation (the Ludwig-McGill Cohort Study) of the natural history of HPV infection and cervical neoplasia in the city of São Paulo, Brazil, a high-

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risk area for cervical cancer. Women enrolled in this study were followed up over a period of several years at scheduled return visits during which they were screened concurrently for cervical lesions and HPV infection. This allowed us to assess the risks of SIL related to prior cumulative and persistent HPV positivity. We were also able to focus on persistence of HPV types 16 and 18, which have been linked with increased incidence of high-grade lesions^{2,5,7,9} and higher probability of lesion persistence.¹⁰

METHODS

Subject Recruitment and Follow-up

Since 1993, we have carried out a cohort study involving repeated measurements on women attending a comprehensive maternal and child health program catering to low-income families living in neighborhoods located in the northern sector of the city of São Paulo, Brazil (population, 12 million). Using rosters of outpatients in the family medicine, gynecology, and family planning clinics at the Vila Nova Cachoeirinha municipal hospital, 2 nurses specifically trained for the study selected a systematic sample of 4990 women to be approached for interview. Of these, 3589 initially met the eligibility criteria, were given a detailed overview of the study, and were invited to participate. Between November 1993 and March 1997, a total of 2528 women were enrolled into the study, representing a response rate of 70.4%. Another 52 women who did not fit the eligibility criteria were excluded after enrollment. Subjects entered the study only after giving signed informed consent. The study protocol was approved by institutional ethical and research review boards of the participating institutions in Canada and Brazil.

Women were eligible to participate if they (1) were between 18 and 60 years of age; (2) were permanent residents of São Paulo (city); (3) were not currently pregnant and had no intention of becoming pregnant during the next

12 months; (4) had an intact uterus and no current referral for hysterectomy; (5) reported no use of vaginal medication in the previous 2 days; and (6) had not had treatment for cervical disease by electrocoagulation, cryotherapy, or conization in the previous 6 months. In addition to these criteria, women were considered ineligible if they were not interested in complying with all scheduled returns, at least for the subsequent 2 years.

All participants were seen every 4 months in the first year (0, 4, 8, and 12 months) and twice yearly thereafter. Delays in returning for a given appointment were allowed, with information and specimens collected during any postdue visits being assigned to the delayed follow-up return, which precluded the occurrence of missing interval visits. Cervical specimens were taken for Papanicolaou cytology and HPV testing at every visit. An in-person interview was also performed at enrollment to collect information on risk factors for HPV infection and cervical neoplasia, including sociodemographics, reproductive health, sexual practices, smoking, and diet. For the analyses reported here, follow-up continued until June 2000, the development of SIL, death, or loss to follow-up, whichever occurred first. A detailed description of the design and methods of the study has been published.¹¹

Cervical Cell Specimens

A cervical Papanicolaou smear was performed using an Accelon biosampler (Medscand, Inc, Hollywood, Fla) to collect a standardized sample of ectocervical and endocervical cells. After the smear was prepared on a glass slide and fixed in 95% ethanol, the sampler containing the exfoliated cells was immersed in a tube containing Tris-EDTA buffer (pH 7.4) and agitated to release the cells. Samples were then sent to the laboratory at the Ludwig Institute for Cancer Research (São Paulo) for storage and HPV testing. The cervical smear slides were read locally and then shipped to Montreal, where they

were coded and read specifically for the study by an expert cytopathologist (A. F.) who was unaware of any other results from the subjects. Cytopathology reports were based on the Bethesda system for cytological diagnoses.¹² For the purpose of this analysis, the following categories were used: within normal limits or benign cellular changes (normal); atypical squamous cells of undetermined significance (ASCUS) or atypical glandular cells of undetermined significance (AGUS); low-grade SIL (LSIL); and high-grade SIL (HSIL); or cancer. All detected events of HSIL were referred for colposcopic follow-up and biopsy if required according to National Institutes of Health approved guidelines.

HPV DNA Detection

DNA was extracted from all cervical specimens using digestion with 100 µg/mL of proteinase K for 3 hours at 55°C, and the DNA samples were then purified by spin column chromatography. Cervical specimens were tested for the presence of HPV DNA by a previously described polymerase chain reaction (PCR) protocol amplifying a highly conserved 450-base pair (bp) segment in the L1 viral gene (flanked by primers MY09/11).^{13,14} Typing of the amplified products was performed by hybridization with individual oligonucleotide probes specific for 27 HPV genital types.¹⁴ The PCR amplification products that hybridized with the generic probe but with none of the type-specific probes were tested further by restriction fragment length polymorphism analysis of the L1 fragment¹⁵ to distinguish among unknown HPVs. To verify the specificity of the hybridizations, we included more than 30 type-specific positive controls in all membranes. To check the integrity of the host DNA material extracted from the specimens, assays also included an additional set of primers (GH20 and PC04) to amplify a 268-bp region of the β-globin gene.¹³ All HPV assays were done blindly on coded specimens with no identification linking specimens from the same woman.

Table 1. Distribution of Selected Characteristics Among 1789 Subjects With Human Papillomavirus (HPV) Test Results at Enrollment*

Demographic Variable	Subjects, No. (%)
Age, y	
18-24	343 (19.2)
25-34	700 (39.1)
35-44	538 (30.1)
≥45	208 (11.6)
Ethnicity	
White	1158 (64.8)
Nonwhite	630 (35.2)
Education	
Less than elementary	412 (23.1)
Elementary	1046 (58.5)
High school	280 (15.7)
College/university	49 (2.7)
Marital status	
Single	167 (9.3)
Married or living together	1468 (82.1)
Divorced or widowed	154 (8.6)
Smoking	
Never	884 (49.4)
Current	601 (33.6)
Former	304 (17.0)
Age at first intercourse, y	
≤15	486 (27.2)
16-17	456 (25.5)
18-19	371 (20.7)
≥20	476 (26.6)
Lifetime No. of sexual partners	
0-1	799 (44.7)
2-3	624 (34.9)
≥4	365 (20.4)
Duration of oral contraceptive use, y	
Never	281 (15.7)
≤5	980 (54.8)
>5	528 (29.5)
No. of pregnancies	
0-1	275 (15.5)
2-3	769 (43.3)
4-6	546 (30.8)
≥7	184 (10.4)
Menopausal status	
Premenopausal or perimenopausal	1724 (96.4)
Postmenopausal	65 (3.6)
Condom use	
Never	716 (40.0)
Rarely	629 (35.2)
Frequently or always	444 (24.8)
Douching	
Never	1191 (66.7)
Occasionally	413 (23.1)
Frequently	182 (10.2)
History of sexually transmitted diseases	
Not reported	1372 (77.0)
HPV-associated	87 (4.9)
Other diseases	322 (18.1)

*Subjects with missing information excluded.

Statistical Analysis

Lesion incidence rates were calculated over the accrued women-months of follow-up according to the HPV infection status determined at the enrollment and first follow-up visits combined to ascertain initial persistence of HPV infec-

tion. Human papillomavirus types were grouped by oncogenic potential: non-oncogenic HPV types included types 6/11, 26, 32, 34, 40, 42, 44, 53, 54, 55, 57, 62, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, 83, 84, CP6108, IS39, and other unknown types; oncogenic HPV types included 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68, based on an expanded classification of Bauer et al.¹⁶ Stratification by HPV oncogenicity was hierarchical and based on mutually exclusive categories. We also treated HPV types 16 and 18 separately in some analyses. Following a conservative approach to exposure classification, subjects with invalid HPV test results at any of the visits were excluded from the analyses.

We analyzed the risk of postenrollment occurrence of SIL as an incident finding in relation to HPV infection status at enrollment and during the first 2 visits. The longitudinal relative risks (RRs) and respective 95% confidence intervals (CIs) of incident cervical lesions over time were modeled by Cox proportional hazards regression and graphically represented by Kaplan-Meier curves depicting the actuarially estimated cumulative incidence over the entire follow-up period from 4 months to 6 years postenrollment. Time to event was measured from enrollment to the first instance of a lesion event or to the last recorded return visit date for censored subjects. We investigated the potential effect of confounding by marital status, education, smoking history, feminine hygiene practices, parity, oral contraceptive use, condom use, menopausal status, age at first intercourse, number of sexual partners, history of sexually transmitted diseases, and occurrence of other genital infections that could influence the development of SIL using a change in point estimate cutoff of 10%. We adjusted a priori for age using 4 groups contrasted as dummy variables (18-24, 25-34, 35-44, and 45-60 years of age) and for ethnicity (white and nonwhite); no other variables were identified as confounders and so were not included. Incidence of SIL was estimated both by lesion severity (first confirmed SIL event of any

grade and first occurrence of HSIL) and by persistence of SIL for 2 or more consecutive visits allowing for at most 1 negative intermediate visit. All incident cases of SIL were compared with subjects who had no detected lesions or ASCUS during the entire period of follow-up. Prevalent cases of lesions detected at enrollment were excluded from all longitudinal analyses.

Extending the period of observation of HPV status to 3 visits, we also assessed the effect of loss of a persistent HPV infection. Subjects with persistent HPV positivity over 3 visits were compared with subjects persistent for the same HPV types at the first 2 visits only. Adjusted odds ratios (ORs) of incident SIL events occurring within 2 years of assessment of HPV status at the third visit were evaluated by unconditional logistic regression using subjects with no infections at all 3 visits as the reference group.

We performed tests of trend by including categorized risk factors as ordinal variables in the multivariate models. Relative risks of incident SIL events by HPV infection status during the first 2 visits were compared by age group, stratifying for subjects 30 years of age and younger vs older than 30 years at enrollment. Interaction between age and HPV was assessed by comparing multivariate models assuming independence of effects to the same models further incorporating a cross-product term for interaction using log likelihood ratio tests based on the χ^2 distribution with *df* equal to the number of parameters of interest. All statistical analyses were performed using the statistical program SPSS, version 10.0 (SPSS, Chicago, Ill).

RESULTS

Subject Characteristics

At the time of analysis, valid HPV typing results (excluding β -globin-negative samples) were available for the enrollment and up to 3 follow-up samples of 1862 women. Among the 1791 women with typing information at enrollment, 286 (16.0%) were found to be positive for at least 1 HPV type.

TABLE 1 shows the descriptive statistics on the primary factors that could influence the relationship between HPV persistence and cervical lesion incidence for the 1789 women with HPV test results and a complete questionnaire at enrollment. All women in the study (except 1) had initiated sexual activity by the time of their first follow-up visit interview and therefore had potentially been exposed to HPV through sexual transmission. The majority of women had only 1 to 2 partners in their lifetime. The mean age at enrollment was 33.1 years (median age, 33 years). The actuarial proportions of women who have been compliant with all scheduled follow-up visits were 89% at 12 months, 84% at 24 months, 79% at 36 months, 74% at 48 months, and 69% at 60 months.

Forty-one women were found to have cervical lesions at enrollment, and smears from 4 women were deemed inconclusive or were lost. Among women free of lesions at enrollment, the mean age at diagnosis of a first SIL was 32.7 years (SD, 8.9), whereas the mean age at first diagnosis for HSIL was 32.1 years (SD, 9.3).

Incidence Rates of SIL Events

TABLE 2 shows the frequencies and incidence rates of SIL by HPV infection status during the first 2 visits (including enrollment visit) for 1611 women with valid HPV test results at both initial vis-

its and no cytologically detected lesions at enrollment. Subjects with invalid HPV test results at either of the 2 visits were excluded from the analyses. Rates of any-grade SIL were high among women testing positive for oncogenic HPV types at enrollment and higher for those who tested positive for either HPV types 16 or 18 (data not shown). These rates further increased when infections persisted to the second visit for the same HPV types. For the most part, the patterns were similar for HSIL and persistent SIL. No HSIL or persistent SIL cases were observed for women testing positive only once for HPV type 16 or 18.

Rates of any-grade SIL were highest in women between 18 and 24 years of age (2.44 per 1000 women-months; 95% CI, 1.7-3.2) and lowest for women 35 to 44 years of age (1.01 per 1000 women-months; 95% CI, 0.6-1.4), then increasing marginally to 1.08 per 1000 women-months (95% CI, 0.4-1.7) in women 45 to 60 years of age. The downward trend in incidence rates of persistent SIL continued to the oldest age group, decreasing gradually from 0.58 per 1000 women-months (95% CI, 0.2-0.9) for 18- to 24-year-olds to 0.19 per 1000 women-months (95% CI, 0.1-0.5) for women 45 to 60 years old (*P* value for trend=.04).

Cumulative Risks of SIL Events

We evaluated the cumulative incidence of cytologically detected SIL over

time in women free of lesions at enrollment. The FIGURE illustrates the cumulative risk of any-grade SIL as a function of HPV infection status at enrollment alone (Figure 1A) or during the first 2 visits including the enrollment visit (Figure 1B). Subjects with oncogenic HPV infections at enrollment were more likely to develop SIL compared with those with nononcogenic infections or those who were HPV negative (Figure 1A). The cumulative risk was somewhat more pronounced for women with HPV types 16 and 18 at entry compared with those who had other oncogenic types, but there was substantial overlap between the 2 curves. Persistent detection of HPV types 16 or 18 at the enrollment and first follow-up visits was associated with a greater cumulative incidence of SIL compared with persistent infections with other types or transient infections. The cumulative detection of SILs among women with both initial visits positive for HPV types 16 or 18 approached 40% after 4 years (Figure 1B).

Relative Risks of First Instance of SIL Events

Using Cox regression we estimated age- and ethnicity-adjusted RRs of a first instance of any SIL, HSIL, or persistent SIL over the entire period of follow-up among the 1611 women free of lesions at enrollment (TABLE 3). The highest RRs for any SIL and HSIL were ob-

Table 2. Frequencies of New Cervical Lesions After Extended Follow-up of 1611 Women According to Human Papillomavirus (HPV) Infection Assessed at the First 2 Visits*

Definition of Persistent HPV Infection Based on First 2 Visits	No. of Subjects	Women-Months†	Any SIL		High-Grade SIL		Persistent SIL‡	
			No. (%) of Events	Rate/1000 Women-Months (95% CI)	No. (%) of Events	Rate/1000 Women-Months (95% CI)	No. (%) of Events	Rate/1000 Women-Months (95% CI)
Negative	1268	61 948.7	44 (3.5)	0.73 (0.5-0.9)	9 (0.7)	0.15 (0.1-0.2)	12 (0.9)	0.19 (0.1-0.3)
Positive either visit for any type§	198	9638.2	29 (14.6)	3.40 (2.2-4.6)	6 (3.0)	0.64 (0.1-1.1)	6 (3.0)	0.64 (0.1-1.2)
HPV types 16 or 18 once	36	1434.4	3 (8.3)	2.25 (0.0-4.8)	0 (0)	0.0	0 (0)	0.0
Positive on 2 visits for same nononcogenic types	47	2212.5	7 (14.9)	3.52 (0.9-6.1)	0 (0)	0.0	3 (6.4)	1.39 (0.0-3.0)
Positive on 2 visits for same oncogenic types except 16 or 18	38	1867.6	12 (31.6)	8.27 (3.6-13.0)	2 (5.3)	1.08 (0.0-2.6)	6 (15.8)	3.51 (0.7-6.3)
HPV types 16 or 18 (same) both visits	24	1037.8	7 (29.2)	8.68 (2.3-15.1)	2 (8.3)	2.06 (0.0-4.9)	1 (4.2)	1.00 (0.0-3.0)

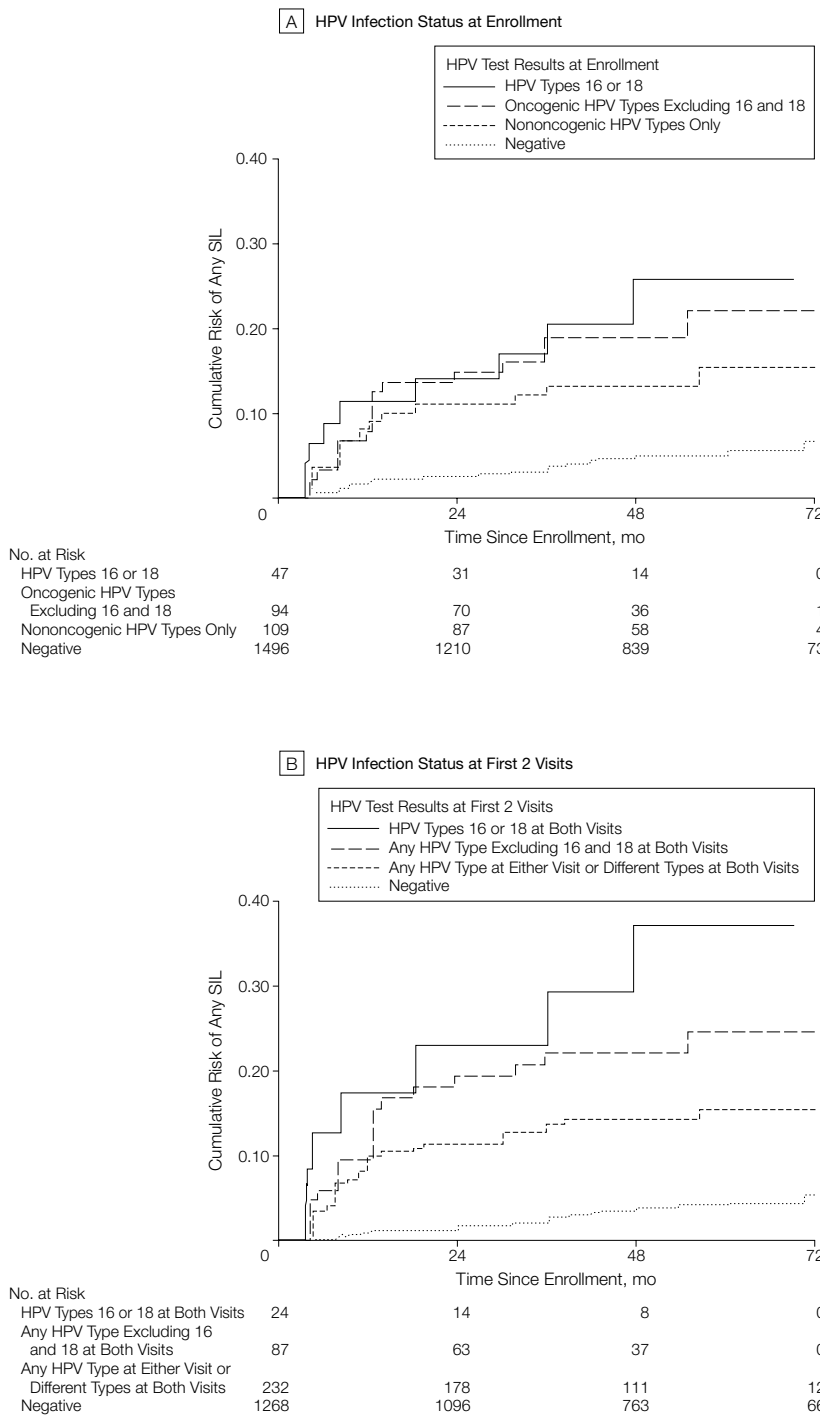
*Excluding subjects with squamous intraepithelial lesions (SILs) detected at enrollment or missing HPV test results. CI indicates confidence interval.

†Total study follow-up time for all enrolled women.

‡Two or more visits with SIL during follow-up allowing for 1 negative interval visit.

§Includes women with 2 positive visits but different HPV types in each, except types 16 or 18.

Figure. Kaplan-Meier Estimates of Cumulative Incidence of Any Grade of Squamous Intraepithelial Lesions (SILs)



Mutually exclusive categories for human papillomavirus (HPV) infection among women free of cervical lesions at enrollment are displayed. A, HPV positivity at enrollment by oncogenicity among 1746 women, and B, HPV persistence for the first 2 visits by oncogenicity among 1611 women. Women with lesions detected at enrollment or missing HPV test results are excluded from the analyses.

served for women testing positive for oncogenic HPV types at enrollment and first follow-up visit compared with women testing negative for any HPV type at both visits. These RRs increased slightly when restricted to women with persistent infection with HPV types 16 or 18. Relative risks tended to be higher for persistent SIL compared with any SIL events, when considering persistence for oncogenic types except 16 or 18. Subjects with missing HPV test results at either visit, grouped as a separate category, showed slightly elevated RRs compared with the referent group only for any SIL (data not shown).

Human papillomavirus persistence was also defined using a less stringent method that grouped types by level of oncogenicity rather than by taxonomic classification. We distinguished subjects positive for oncogenic types at both enrollment and first follow-up visit from those displaying persistent infections with the same type at both visits. Although the RR of SIL was high for women with 2 positive visits with different oncogenic types, no cases of HSIL or persistent SIL were observed. Comparatively, elevated RRs for any SIL were also observed for women with transient infections involving an oncogenic HPV type at one of the visits and a nononcogenic one at the other. Most subjects (64/68) with oncogenic types detected at both visits, however, consisted of persistent infections with the same HPV types. We were also able to distinguish subjects with transient infections but positive at both visits for different HPV types. Twenty-five subjects were classified as having repeatedly positive visits, with one of them revealing an oncogenic type.

We also investigated whether RRs differed for younger and older women by stratifying on age at enrollment. Separate analyses were performed for women 30 years of age and younger and older than 30 years. Higher RRs of HSIL were observed for older women with persistent oncogenic infections at the first 2 visits (RR, 29.35; 95% CI, 7.3-118.0) compared with younger women (RR, 5.26; 95% CI, 1.0-27.1), but this dif-

ference was not significant. Women 30 years old and younger who harbored persistent infections for oncogenic HPV types during the first 2 visits were more likely, albeit nonsignificantly, to develop persistent SILs (RR, 19.87; 95% CI, 5.0-79.5) than older women (RR, 13.85; 95% CI, 3.7-52.4). There was no interaction between age and HPV infection, regardless of outcome definition. Using a different cut point (25 years) for defining the 2 age strata yielded similar conclusions (data not shown).

Risk of SIL Events With Long-term HPV Infection

To measure the effects of longer-term persistence and of loss of the initial 2-visit persistence of HPV infection, we distinguished the 1611 women with persistent infections for the first 2 scheduled visits according to their HPV status at the third visit if available (TABLE 4). Odds ratios estimated by logistic regression for women with persistent infections and testing positive at the third visit were compared with those derived for women with the same persistent infections but showing no HPV DNA at the third (referent, no HPV at visits 1-3). Only events occurring within 48 months following the ascertainment of long-term HPV persistence (visits 1-3) were included. The OR of incident SIL of any grades for women remaining HPV positive following a persistent infection for oncogenic types was 6.69 times (22.02/3.29) that for those eliminating their infections at the third visit. This effect was also observed among women with infections for HPV types 16 and 18 at both initial visits, though to a lesser degree, for whom the OR was 1.15 times (12.27/10.71) that of nonpersistors. Conversely, there was no incremental risk associated with continued positivity after a persistent non-oncogenic infection (3.25/3.55). Corresponding ORs of developing a persistent lesion for women maintaining HPV infections after being initially persistent were also high. No HSIL or persistent lesion events were observed for women eliminating their infections by the third visit regardless of HPV classification.

Table 3. Relative Risks (RRs) of Cervical Lesions Among 1611 Women Over 5 Years of Follow-up According to Human Papillomavirus (HPV) Positivity During the First 2 Visits*

Definition of HPV Infection Status Based on First 2 Visits	No. of Subjects	RR (95% CI)		
		Any SIL	High-Grade SIL	Persistent SIL†
Emphasis on same type persistence				
Negative	1268	1.0 (Referent)	1.0 (Referent)	1.0 (Referent)
Positive either visit for any type‡	198	4.23 (2.6-6.8)	4.08 (1.4-11.7)	3.53 (1.3-9.5)
HPV types 16 or 18 once	36	2.69 (0.8-8.7)	3.85 (0.5-31.1)	0.00
Positive on 2 visits for same nononcogenic types	47	4.49 (2.0-10.0)	0.00	7.93 (2.2-28.3)
Positive on 2 visits for same oncogenic types except 16 or 18	38	9.92 (5.2-18.9)	9.68 (2.6-36.2)	18.89 (7.0-51.1)
HPV types 16 or 18 (same) both visits	24	11.15 (5.0-24.9)	12.27 (2.6-57.5)	7.40 (1.0-57.3)
Persistence based on oncogenic types				
Negative	1268	1.0 (Referent)	1.0 (Referent)	1.0 (Referent)
Positive at only 1 visit	197	2.92 (1.7-5.0)	2.64 (0.8-8.7)	2.78 (1.0-8.0)
Positive both visits for any type	53	4.89 (2.3-10.4)	2.48 (0.3-19.7)	7.26 (2.0-25.9)
Positive both visits with an oncogenic type in 1 visit	25	10.57 (5.0-22.5)	5.24 (0.7-41.8)	5.47 (0.7-42.4)
Positive both visits with different oncogenic types	4	24.55 (5.8-103.5)	0.0	0.0
Positive both visits for the same oncogenic types	64	10.19 (5.9-17.6)	11.67 (4.1-33.3)	14.92 (5.8-38.3)

*Excluding subjects with squamous intraepithelial lesions (SILs) detected at enrollment. RR estimates and 95% confidence intervals (CIs) by Cox proportional hazards regression adjusting for age and ethnicity. Missing categories not shown.

†Two or more visits with SIL during follow-up allowing for 1 negative interval visit.

‡Includes women with 2 positive visits but different HPV types in each, except types 16 or 18.

COMMENT

The traditional epidemiological study designs of single-opportunity assessment of exposure and outcome do not allow questions of viral persistence or regression of cervical lesions to be addressed.¹⁷ To understand the role of HPV and the pattern of the dynamic changes in the natural history of cervical dysplasia, studies that collect data repeatedly on risk factors (HPV) and screen for cervical lesions on multiple occasions during follow-up must be conducted. In our study, we have provided evidence that persistent HPV infection, particularly with oncogenic types, is associated with a much greater risk of incident cervical cancer precursor lesions than when HPV positivity is defined on the basis of a single-assessment measurement at enrollment.

Our study has a number of strengths and weaknesses. Among the former we include more elaborate algorithms to define type-specific viral persistence

(and loss thereof) on the basis of multiple initial visits in the cohort and the assessment of long-term incidence of initial and recurrent SIL as a function of HPV persistence. However, the definition of a persistent infection was based on detecting viral DNA of the same taxonomic type using a consensus PCR protocol. Fluctuations in viral load below the detection threshold of PCR may have caused some cases of persistent infection to be misclassified as transient due to false-negative test results. In addition, it is impossible to ascertain via HPV DNA detection alone if test positivity is equated with true (active, albeit latent) viral infection. On the other hand, it is reassuring to note that PCR typing alone may be sufficient for defining persistent infections. Longitudinal testing for molecular variants of HPVs 16 and 18, while providing an enhanced level of taxonomic detail for ascertaining true persistence,¹⁸ indicated that persistently detected HPVs 16 or 18 were of the same molecular

variant in each case.¹⁹ This observation suggests that persistent detection of the same viral type may truly represent a persistent infection.

Misclassification of lesion outcome history is a noteworthy weakness since our results were based on cytological ascertainment, however carefully conducted in a reference laboratory following a strict quality control protocol. We opted for an intensive, expert cytological follow-up every 6 months of all SILs found in the study to avoid having to perform unnecessary biopsies, which would have interfered with the natural history of early lesions. It is conceivable, however, that the magnitude of the associations would have been much greater if we had used histological ascertainment of all lesions detected in the study, an observation that we will make at a later phase of the investigation after we are able to define HPV persistence using algorithms that encompass at least 2 or more years' worth of follow-up visits with complete HPV testing and after more lesions are documented during long-term follow-up. Differential misclassification is unlikely because all HPV and Papanicolaou tests were performed blindly with respect to each other and by different laboratories. Therefore, being nondifferential, it is unlikely that the putative lesion misclassification bias would have elevated the observed associations.¹⁷

The HPV measurements in our study were collected at several return visits over

a period of 1 year, allowing us to be more stringent with our definition of exposure to a persistent infection. Previous studies have relied on only 2 points of measurement, sometimes over a period of several years until a diagnosis of SIL.^{5,6} The restriction to prevalence measures in case-control studies produces similarly elevated risk associations for concurrent HPV infection and lesion development.¹ When we emulated this restricted approach by ascertaining persistence using HPV test results taken at enrollment and at the time of diagnosis for an incident SIL during the first year of follow-up, our ORs increased to 94.9 (95% CI, 27.6-325.7) for "persistent" oncogenic infections and to 56.3 (95% CI, 16.5-192.6) for "persistence" of non-oncogenic types. We even observed an increase in OR for transient infections to 24.1 (95% CI, 10.1-57.7) when relying on such an algorithm for HPV exposure.

There are some underlying differences between our study and previous cohort studies with respect to defining HPV persistence. The first approach is that described above where HPV status was evaluated at 2 points in time, the first at enrollment and the second being a prevalence measure collected at the same moment as the outcome is diagnosed.^{5,6} A second approach is that HPV was measured at repeated intervals before the onset of disease,^{2,4,7-9,20} although Ho et al³ used a time-dependent algorithm for exposure assessment similar to that of the

above studies. A variation on this latter method is to take into account the transient nature of precursor lesions in the cause of cervical neoplasia allowing for a woman to contribute more than once to the analysis.¹⁰ Potentially more efficient when multiple events occur, this approach must take into account the sequential interdependencies between repeated events within subjects as they are followed up over time. However, a repeated analysis approach does not lend itself to an evaluation of severe outcomes such as HSIL because of the need for intervention.

Among those studies that evaluated oncogenic HPV infection status through consecutive scheduled visits,^{2,4,7-9} few investigated the RR association for incidence of cervical neoplasia following a repeated HPV infection.^{2,4,8,9} Using a nonamplified hybridization method for typing HPVs, Koutsky et al² observed an RR of 26 (95% CI, 6.5-112) for incident HSIL among women with multiple positive visits for HPV. Relative risk associations for single-point infections with oncogenic HPV types 16 or 18 were lower (RR, 11; 95% CI, 4.6-26). We observed a similar dose-response relationship in ORs with level of oncogenicity for persistent infections, although our RR associations, extended over a longer period of time, were lower. This observation has been part of a trend in decreasing RRs with increasing interval period between HPV exposure and SIL incidence in our study (data not shown). In 2 studies on populations of adoles-

Table 4. Odds Ratios (ORs) of Incident Cervical Lesions Among 1162 Women Within 48 Months Following a Persistent Infection for Human Papillomavirus (HPV) According to Infection Status at the Third Visit*

Assessment of HPV Infection		Any SIL			Persistent SIL†		
Status at Visits 1 and 2	Status at Visit 3	Lesion	No Lesion	OR (95% CI)	Lesion	No Lesion	OR (95% CI)
Negative all 3 visits		25	1046	1.0 (Referent)	8	1008	1.0 (Referent)
Positive for 2 visits with same nononcogenic types	Negative	1	12	3.55 (0.4-28.5)	0	10	
	Positive	2	25	3.25 (0.7-14.5)	2	21	12.03 (2.4-60.8)
Positive for 2 visits with same oncogenic types‡	Negative	1	12	3.29 (0.4-26.5)	0	12	
	Positive	7	13	22.02 (8.1-60.2)	4	12	41.16 (10.7-158.3)
Positive for 2 visits for HPV type 16 or 18	Negative	1	4	10.71 (1.1-101.2)	0	4	
	Positive	3	10	12.27 (3.2-47.6)	1	8	17.21 (1.9-158.4)

*Excluding subjects with squamous intraepithelial lesions (SILs) detected at enrollment or first follow-up visit. Odds ratios and 95% confidence intervals (CIs) by logistic regression with analyses restricted to events occurring within 48 months of assessment of HPV persistence adjusting for age and ethnicity.

†Two or more visits with SIL during follow-up allowing for 1 negative interval visit.

‡Excluding HPV type 16 or 18.

cent women, Moscicki et al⁸ and Woodman et al⁹ also observed a decreasing cumulative risk with time since first exposure to HPV. It has been suggested that latent SIL events occurring several years following an HPV infection found at baseline may correlate better with more recent infections yet to be detected.^{6,7} This remains to be confirmed by continued follow-up and HPV typing at repeated visits.

Persistence of HPV infection was monitored by detection of individual HPV types by PCR, which provides a much finer level of detail than that afforded by a commercially available HPV testing method such as the Hybrid Capture assay (Digene Corporation, Gaithersburg, Md), the only HPV assay approved by the US Food and Drug Administration. Studies that rely on the Hybrid Capture test are limited to testing for multiple oncogenic HPVs collectively without distinguishing among types. Among those with repeated positive test results, we found that persistent HPV infections for both nononcogenic and oncogenic types were associated with substantially elevated RRs for SIL incidence and persistence. Such risk associations would have been missed if only the 13 oncogenic types had been tested for, in combined form. Furthermore, considering subjects with nononcogenic infections as negative would have diminished the strength of the associations between HPV infections and lesions. Interestingly, for women with repeated positivity for oncogenic types (one of the highest risk categories in our study), almost all harbored persistent infections with the same types. No instances of HSIL were observed among women with nonpersistent (implying different type) yet with repeatedly positive HPV test results.

Some studies have demonstrated elevated RRs associated with HPV types 16 and 18 detected 2 years^{2,21} or more^{7,9} prior to the development of a high-grade lesion. In this study we were able to give particular attention to persistence of HPV types 16 and 18 and its relationship to incidence of HSIL. We found that the RR associations inten-

sified with repeated detection of HPV 16 and 18 both in Cox regression analyses and by actuarial analysis for all SIL events. Ellerbrock et al²⁰ observed similar increases in RR for persistent HPV type 16 or 18 infections (RR, 11.6; 95% CI, 2.7-50.7) after adjustment for HIV seropositivity.

We also looked at the risk of developing a persistent lesion after an HPV infection. We noted particularly high RRs for persistent infections by oncogenic types. Of the 29 women with persistent lesions, 13 (44.8%) involved a diagnosis of HSIL. Due to the small number of subjects with persistent lesions, we could not speculate on the relationship with HPV type 16 or 18 infections. Other studies have attempted to look for predictors of lesion persistence or regression^{10,22} basing their comparisons on a control group of women with previously detected lesions. Ho et al¹⁰ observed ORs above 1 for lesion persistence among women who were positive for HPV at 2 prior consecutive visits, although they were not able to differentiate between oncogenic and nononcogenic HPV types.

The increase in ORs for incidence of SIL in women harboring long-term oncogenic HPV infections adds to the evidence for HPV persistence as a key determinant of lesion development. Although few SIL events were available for analysis after restriction for HPV positivity at the first 3 scheduled visits, proportionally fewer lesions were found in women who eventually eliminated their infection within 8 months. No persistent SIL cases or incident HSIL events were detected among those who cleared their infection at the third visit in the study. In a study of adolescent women with HPV infection at enrollment, Moscicki et al⁴ observed an OR of 14.1 (95% CI, 2.3-84.5) for the incidence of HSIL in women positive for oncogenic HPV at 3 of 4 preceding visits compared with those who lost their infection after enrollment into the study.

In our study, we do not know the proportion of HPV-positive women identified at enrollment who were already harboring persistent infections before en-

tering the investigation. Loss of persistence at the third visit could merely indicate that the 2 previous positive visits were of a transient nature, whereas those with the initial 3 visits being positive for oncogenic HPVs might have represented true long-term persistence that began before they entered the study. As we extend typing to subsequent follow-up visits in the study, we will be able to more accurately evaluate the effects of long-term persistence and of loss of positivity to HPV on the subsequent development of SIL.

In conclusion, our study adds to the body of evidence strongly implicating persistent HPV infections, particularly with oncogenic types and more prominently with HPV types 16 and 18, in the cause of SIL. Using a longitudinal, repeated measurement cohort investigation we were able to assess more refined algorithms of cumulative HPV exposure with respect to their prognostic value in determining lesion outcome history. However, further analyses for repeated measures remain to be done to evaluate the transient nature of the disease and to investigate the long-term natural history of HPV infection and cervical intraepithelial neoplasia in our cohort. Our results, however, would support the proposal for the application of repeated type-specific HPV DNA testing in screening and for the potential use of vaccines for HPV types 16 and 18 to prevent the development of clinically relevant cervical lesions.

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Descartes said: "I think, therefore I am." Helvetius wants to say: "I feel, therefore I want to feel pleasantly." I prefer Hobbes who claims that in order to draw a conclusion which takes us somewhere, we must say, "I feel, I think, I judge; therefore, a part of organized matter like me is capable of feeling, thinking, and judging.

—Denis Diderot (1713-1784)