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Modeling the Time Dependence of the Association between Human Papillomavirus Infection and Cervical Cancer Precursor Lesions

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The authors studied the time-dependent association between human papillomavirus (HPV) infection and squamous intraepithelial lesions (SIL) among women enrolled in a cohort study in Brazil (1993–2002), using repeated Papanicolaou cytologic examination and HPV testing by polymerase chain reaction. Through simulation with conceivable alternative cohort designs, they investigated different regression modeling approaches using time-varying covariates, time-varying hazard ratio functions, and repeated events to assess the effect of delay in lesion detection. Associations between HPV and early SIL were of high magnitude. The age-adjusted hazard ratios for the association between HPV at enrollment and low-grade SIL decreased gradually with time until 72 months for both oncogenic types of HPV (hazard ratio = 3.96, 95% confidence interval (CI): 2.5, 6.4) and nononcogenic types (hazard ratio = 2.37, 95% CI: 1.3, 4.3). The hazard ratio for incident high-grade SIL remained constant, ranging from 7.15 (95% CI: 2.0, 25.1) at 12 months to 6.26 (95% CI: 2.7, 14.5) at 72 months for oncogenic types of HPV. With oncogenic HPV as the time-dependent predictor variable, the hazard ratios for incident SIL and high-grade SIL events were 14.2 (95% CI: 8.7, 23.1) and 32.7 (95% CI: 8.4, 127.3), respectively. Investigators may underestimate the prognostic value of HPV detection using designs that rely on HPV ascertainment at a single time point. The waning in hazard ratios should be considered in the implementation of HPV testing-based screening programs.

cervix neoplasms; longitudinal studies; papillomavirus, human; precancerous conditions; statistics; survival analysis

Abbreviations: CI, confidence interval; HPV, human papillomavirus; SIL, squamous intraepithelial lesion.

The association between human papillomavirus (HPV) DNA and cervical cancer has been well documented worldwide (1). In the natural history of the disease, preinvasive lesions of cervical neoplasia can be transient and can reoccur over time. Women may develop low- and high-grade squamous intraepithelial lesions (SILs); low-grade SIL may progress to high-grade SIL or may regress back to a normal state (2). HPV infection, particularly infection with the 13 or more types known to be oncogenic, is the main etiologic agent in the initiation and maintenance of this process, and it may ultimately progress to cancer (1). The strength of the observed association with HPV can be influenced by methodological factors, such as the measures of cumulative exposure that are used in the study design and analysis (3, 4).

To date, cohort studies of HPV and SIL have differed in the frequency with which subjects are reevaluated over time for exposure and outcome status (5–13), as well as in the amount of time elapsed between these two measurements (14–17). While all of these studies have supported a causal relation between HPV and the incidence of SIL, there is a

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lack of consensus concerning the magnitude of the effect and the possible waning of the risk association over time. Furthermore, studies that rely on single measures of HPV infection may be more susceptible to misclassification of viral exposure, given the transient nature of many HPV infections (7).

Longitudinal studies with repeated measures present a unique challenge for the statistical analysis of observational data and the investigation of disease associations because of the inherent correlation between measures (18, 19). Newer statistical approaches have been developed for the analysis of such data that contrast with the simplistic approach, which involves collapsing the information on repeated events into one or two summary measures (20). The latter approach does not make use of all available data collected at repeated intervals for each individual, and it ignores the time dependence of the epidemiologic association between events.

In the study described here, we analyzed data from an epidemiologic investigation involving repeated measurements of HPV infection status and cervical lesions over time among women in a maternal and child health program catering to low-income families in São Paulo, Brazil (the Ludwig-McGill Cohort Study). Using algorithms that simulated different study approaches possible in our cohort investigation, we analyzed the longitudinal relation between HPV and cervical neoplasia to examine the strength of this association as a function of time between exposure assessment and outcome events.

MATERIALS AND METHODS

Subject recruitment

A detailed description of the design and methods of the Ludwig-McGill Cohort Study has been published previously (21, 22). In brief, from November 1993 to March 1997, we selected a systematic sample of female outpatients from the family medicine, gynecology, and family planning clinics at the Vila Nova Cachoeirinha Municipal Hospital in São Paulo. Eligibility criteria included: 1) being aged 18–60 years; 2) being a permanent resident of the city of São Paulo; 3) not currently being pregnant and having no intention of becoming pregnant during the next 12 months; 4) having an intact uterus and no current referral for hysterectomy; 5) not having used vaginal medication in the previous 2 days; and 6) not having been treated for cervical disease in the previous 6 months. In addition to these criteria, women were considered ineligible if they were not interested in complying with all scheduled return visits, at least for the subsequent 2 years.

Subjects gave signed informed consent. The study protocol was approved by the institutional ethical and research review boards of the participating institutions in Canada and Brazil. All participants were seen every 4 months during the first year (at 0, 4, 8, and 12 months) and twice yearly thereafter. Cervical specimens were taken for Papanicolaou cytologic examination and HPV testing at every visit. The study nurses also performed a detailed interview to collect information on sociodemographic factors, reproductive health, sexual activity, smoking, and diet at enrollment and on selected risk factors at subsequent return visits.

Cervical cell specimens

An Accelon biosampler (Medscand, Inc., Hollywood, Florida) was used to collect ecto- and endocervical samples at each of the visits. After the cells had been smeared onto a glass slide and fixed for cytologic examination, the sampler containing the residual exfoliated cells was immersed in a tube containing Tris-ethylenediaminetetraacetic acid buffer, pH 7.4. Cytopathology reports were based on the 1992 Bethesda system for cytologic diagnoses (23). Readers were blinded to previous cytologic outcomes and to HPV results for the same women.

Detection of HPV DNA

Cervical specimens were tested for the presence of HPV DNA by means of the standardized MY09/11 polymerase chain reaction protocol (24, 25). Typing of the amplified products was performed through hybridization with individual oligonucleotide probes specific for 27 genital types of HPV (25). Amplified products that hybridized with the generic probe but with none of the type-specific probes were tested further by restriction fragment length polymorphism analysis (26) to increase the number of identifiable HPV types. 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, we also included in the assays primers to amplify the β-globin gene (24). HPV types were separated into two groups by presumed level of oncogenicity. Oncogenic types included HPVs 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68; nononcogenic types included HPVs 6/11, 26, 32, 34, 40, 42, 44, 53–55, 57, 62, 64, 66, 67, 69–73, and 81–84, CP6108, and unknown types. HPV status at each visit was categorized hierarchically as follows: 1) no HPV DNA detected; 2) only nononcogenic types of HPV detected; and 3) any oncogenic type of HPV detected. HPV assays were carried out on coded specimens with no identification linking specimens from the same woman. Appropriate precautions were taken to reduce the possibility of specimen contamination.

Statistical analyses

For the results reported here, follow-up continued until March 2002, the development of neoplasia requiring treatment, death, or loss to follow-up, whichever occurred first. Time-to-event was measured from the date of enrollment to the date of first occurrence of a lesion (as defined below) or the date of the last recorded return visit for censored subjects.

To assess the time dependence of the association between HPV and lesion outcomes, we evaluated different analysis approaches based on distinct designs that were conceivable as part of the repeated-measurement layout of the Ludwig-McGill cohort (figure 1). We first assessed the association between HPV infection at enrollment and the occurrence of

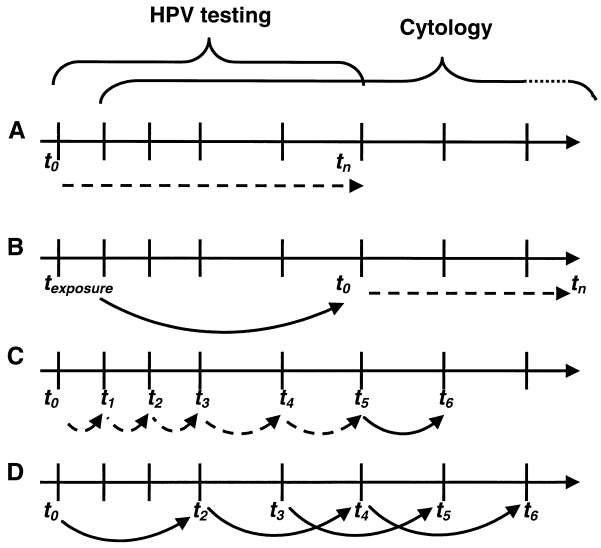

FIGURE 1. Graphic representation of different underlying models for data analysis that are conceivable in a cohort study with staggered and repeated assessments of human papillomavirus (HPV) infection (the exposure) and squamous intraepithelial lesion (SIL) events determined by cytologic examination (the outcome), such as the Ludwig-McGill Cohort Study. Model A illustrates a traditional cohort study layout with HPV assessed at enrollment and SIL documented exclusively within a specified period of time, with further follow-up ignored after that date. Model B illustrates the same kind of cohort study where ascertainment of SIL is delayed until after a prespecified period elapses. Model C illustrates a time-dependent cohort analysis approach for repeated HPV data and first occurrence of SIL. Model D illustrates cohort analyses with multiple observations per person, assuming fixed follow-up intervals and repeated SIL events. Solid lines indicate the widths of predefined fixed intervals; dashed lines indicate periods of relevant follow-up; t_0 represents the beginning of follow-up for outcome events; t_1–t_6 represent repeated scheduled visits where HPV status (exposure) and cytologic results (outcome) are assessed over the course of follow-up; t_n represents the end of the outcome follow-up period; and $t_{exposure}$ represents the time at which HPV infection status is determined before follow-up for outcome events is initiated in model B (i.e., the enrollment visit). See text for details.

cervical lesions that could be documented exclusively within a specified period of time, with follow-up ending (i.e., ignored) after that date (figure 1, model A). A second approach involved initiating follow-up for outcome events later in the study, after a period of delay, and associating HPV infection status at enrollment with cervical lesions detected after a specified period of time (i.e., outcomes occurring before that time were ignored) (figure 1, model B). Models A and B assumed traditional cohort analyses (i.e., Cox regression) of single-point assessment of exposure at enrollment and a first documented instance of outcome based on the respective layout restrictions.

To produce a cumulative estimate of association over time while representing the transient nature of HPV infection, we extended the Cox model to incorporate a time-varying measure of changing HPV status at each visit (figure 1, model C). The constraint of model C is that instances of SIL after the first event are not considered. Therefore, we used a fourth approach (figure 1, model D) that involved correlating HPV infection status and lesion incidence at different, fixed follow-up returns. This was performed using a moving time window defined by t_i, corresponding to exposure time, and t_{i+n}, at which time outcome is assessed a specified number of months later. We conducted separate analyses with increasing time intervals (n) between exposure and outcome assessment. In this layout, subjects could contribute multiple time windows in each series of analysis (e.g., enrollment to 12 months, 12–24 months, 18–30 months, etc., as shown in figure 1, model D). Two approaches, generalized estimating equations (27) and marginal hazards regression (19), were used to estimate relative risks of SIL while taking into account the clustering within each individual implied in model D. In the generalized estimating equations approach, correlations between outcome events are treated as nuisance parameters, thereby allowing for inference based on the coefficients for the covariates in the model that can be either time-dependent or time-independent—in this case, HPV infection status at each visit and age at enrollment, respectively. All models incorporated an exchangeable or equal correlation pattern for the repeated events. We adapted the marginal hazards model approach described by Therneau and Grambsch (19) to the analysis of pairwise associations (model D). Stratification in this model was performed to allow the baseline hazard to vary with each period of observation (time window) defined by the index visit for each period in which HPV status was tested. The hazard function $h[t, X(t)]$ at time t for an individual with the vector of explanatory variables $X(t)$ is characterized by the following formula:

$$h[t, X(t)] = h_{0j}(t) \times e^{\sum_{i=1}^{p} \beta_i X_i(t)},$$

where = $h_{0j}(t)$ is an arbitrary and unspecified baseline hazard function for each period of observation (j), β_i is the regression parameter associated with the ith explanatory variable, and $X_i(t)$ is the ith explanatory variable, $i = 1, \ldots, p$ (i.e., HPV status at time t).

For all models (except generalized estimating equations), we estimated the relative risk of lesion occurrence given HPV infection status by computing hazard ratios and 95 percent confidence intervals. A robust variance was also computed in all models, using a sandwich estimate obtained by incorporating the clustering within each individual. We examined proportionality of the hazards for the traditional Cox models using Schoenfeld residuals and fit models for nonproportional hazards using the extended Cox model for time-varying covariates (19). Analyses were performed with the following software: SPSS, version 11.0 (SPSS, Inc., Chicago, Illinois); Stata, versions 6 and 7 (Stata Corporation, College Station, Texas); S-Plus for Windows (Insightful Corporation, Seattle, Washington); and R, version 1.4.1 (R Collaborative Group; available at www.http://www.r-project.org).

RESULTS

The study enrolled 2,528 women, corresponding to a 70 percent response rate. Women subsequently found ineligible (n = 66) were excluded. The remaining 2,462 women partic-

TABLE 1. Hazard ratios for the association between nononcogenic and oncogenic human papillomavirus infection at study enrollment and low- or high-grade squamous intraepithelial lesions detected within specified periods of follow-up, Ludwig-McGill Cohort Study, São Paulo, Brazil, 1993–2002*

Period of follow-up (months) *within which* SIL† was detected	No. of events		Nononcogenic types of HPV†				No. of events		Oncogenic types of HPV			
			Low-grade SIL		High-grade SIL				Low-grade SIL		High-grade SIL	
	Low-grade SIL	High-grade SIL	HR†	95% CI†	HR	95% CI	Low-grade SIL	High-grade SIL	HR	95% CI	HR	95% CI
Enrollment‡	3	0	9.33	2.0, 42.5			19	16	44.34	14.8, 133.1	49.5	16.1, 152.2
8	8	1	4.29	1.9, 9.6	10.65	0.7, 172.8	12	3	4.60	2.3, 9.3	23.53	2.3, 238.5
12	10	1	4.11	2.0, 8.5	1.92	0.2, 16.5	16	5	5.51	3.0, 10.2	7.15	2.0, 25.1
24	11	2	4.03	2.0, 8.0	3.00	0.6, 14.5	18	7	5.49	3.1, 9.8	7.83	2.7, 22.8
36	11	3	2.63	1.4, 5.1	3.78	1.0, 14.4	22	8	4.52	2.7, 7.5	7.57	2.8, 20.5
48	11	3	2.33	1.2, 4.5	2.85	0.8, 10.3	22	10	4.04	2.5, 6.6	7.15	3.0, 17.1
60	13	3	2.42	1.3, 4.4	2.67	0.7, 9.5	23	10	3.82	2.4, 6.2	6.69	2.9, 15.7
72	13	3	2.37	1.3, 4.3	2.50	0.7, 8.8	24	10	3.96	2.5, 6.4	6.26	2.7, 14.5

* Results from a traditional Cox proportional hazards model with robust variance for events occurring by the scheduled return visit. Data were adjusted for age. Prevalent cases at enrollment were excluded. Events occurring during the period up to and including the indicated scheduled visit were considered, according to model A (see text for details).

† SIL, squamous intraepithelial lesion; HPV, human papillomavirus; HR, hazard ratio; CI, confidence interval.

‡ Cross-sectional relation between HPV status and cytologic findings determined at enrollment (adjusted for age). The resulting model reduces to traditional logistic regression.

ipating in the study were followed at repeated scheduled return visits for a period of up to 8 years. Fifty-one women (2.1 percent) with a prevalent lesion at enrollment and six women with inconclusive cytologic results were excluded from the analysis. The total follow-up time for subjects with no lesions at enrollment was 128,129 woman-months. The total time-to-event used in the regression models was 122,299 woman-months. With respect to HPV status at enrollment, follow-up times were 99,725 and 15,886 woman-months for HPV-negative and HPV-positive subjects, respectively (80 and 47 subjects, respectively, were censored at the time of their first SIL).

A decrease in the effect of HPV over time was evident when we examined the association between HPV infection at enrollment and the occurrence of low-grade SIL (table 1). This approach is analogous to performing different prospective cohort studies with varying follow-up times from 4 months to 72 months in which multiple cervical samplings were made within the assumed study duration (figure 1, model A). When we consider only incident low-grade SIL events that occur over time until the indicated time has elapsed, we see a decreasing trend in the hazard ratio with time, from 4.29 for nononcogenic HPV types after an interval of 8 months to 2.37 after 72 months. A similar trend is observed for oncogenic HPV types, with hazard ratios decreasing from 4.60 to 3.96 over the same time intervals. We fitted a time-varying covariate Cox model using follow-up data for the entire period of study follow-up. The p values for interaction terms between HPV and time (centered on the median value) and Schoenfeld residuals were less than 0.01 and less than 0.08, respectively, suggesting nonproportionality in hazards. Point estimates for different periods of follow-up were higher for the association with incident high-grade SIL over time. Although the initial association for oncogenic HPVs was very high over the first 8 months of follow-up, the hazard ratio for the follow-up periods from 12 months to 72 months remained between 6.26 and 7.83. While hazard ratios for nononcogenic HPVs were lower, a similar pattern of hazard ratios was observed with increasing follow-up time.

A second cohort analysis approach involves initiating follow-up of subjects later in the study, after a delay (table 2). This approach simulates a study design with delayed initiation of follow-up, whereby occurrences of low-grade or high-grade SIL are associated with the result of an HPV test taken several months or years earlier (model B). The magnitude of the association between HPV infection at enrollment and low-grade SIL is lower than that seen in equivalent intervals in table 1, and it decreases as the interval between exposure and first assessment of outcome is lengthened. For a persistent oncogenic infection, defined as positivity for the same oncogenic types at both enrollment and the first follow-up visit, the hazard ratio for any SIL decreased from 8.35 (95 percent confidence interval (CI): 5.0, 13.9) after 4 months to 5.60 (95 percent CI: 1.6, 19.8) after 48 months (data not shown). The effect was weaker for persistent nononcogenic infections (hazard ratios were 3.14 (95 percent CI: 1.6, 3.9) and 1.87 (95 percent CI: 0.2, 14.3) for 4- and 48-month delays, respectively). For the occurrence of high-grade SIL, no decreasing trend in the hazard ratio was demonstrated with increasing delay interval. While the stability of the point estimates decreased with increasing interval period, the hazard ratio for oncogenic HPV remained high: 5.4 (95 percent CI: 2.3, 12.6) after a delay of only 4 months and 5.6 (95 percent CI: 0.5, 59.4) after 60 months.

TABLE 2. Hazard ratios for the association between nononcogenic and oncogenic human papillomavirus infection at enrollment and low- and high-grade squamous intraepithelial lesions detected after specified periods of postponement during follow-up, Ludwig-McGill Cohort Study, São Paulo, Brazil, 1993–2002*

Period of follow-up (months) *after which* SIL† was detected	No. of events		Nononcogenic types of HPV†				No. of events		Oncogenic types of HPV			
			Low-grade SIL		High-grade SIL				Low-grade SIL		High-grade SIL	
	Low-grade SIL	High-grade SIL	HR†	95% CI†	HR	95% CI	Low-grade SIL	High-grade SIL	HR	95% CI	HR	95% CI
4	11	3	2.00	1.1, 3.8	2.42	0.7, 8.6	26	9	4.20	2.7, 6.6	5.35	2.3, 12.6
8	9	3	1.97	1.0, 4.0	2.64	0.7, 9.4	19	8	3.82	2.3, 6.5	5.23	2.1, 12.9
12	5	2	1.29	0.5, 3.3	1.95	0.4, 8.8	16	8	3.90	2.2, 6.9	5.81	2.3, 14.6
24	2	2	0.65	0.2, 2.7	2.78	0.6, 13.2	8	4	2.54	1.2, 5.5	4.30	1.3, 14.5
36	1	1	0.40	0.1, 3.0	2.20	0.3, 19.0	6	4	2.41	1.0, 5.9	6.96	1.8, 26.4
48	1	0	0.91	0.1, 7.0			2	3	1.97	0.4, 8.9	14.89	2.4, 91.8
60	0	0					0	2			5.61	0.5, 59.4

* Results from a traditional Cox proportional hazards model with robust variance for events occurring only after the indicated amount of follow-up time. Data were adjusted for age. Prevalent cases at enrollment were excluded. Interval SIL events occurring after enrollment but before the indicated period of follow-up were not considered, according to model B (see text for details).

† SIL, squamous intraepithelial lesion; HPV, human papillomavirus; HR, hazard ratio; CI, confidence interval.

Cox models with time-dependent covariates incorporate the transient nature of HPV infection by updating the results to reflect the latest infection status observed at the previous visit (model C). The maximum duration of follow-up was 3 years, because HPV testing results were available only for the first 2 years of follow-up. Compared with the previous analyses using a single assessment of HPV status, table 3 shows substantially higher hazard ratios for SIL events, with hazard ratios of 5.7 for nononcogenic types and 14.2 for oncogenic types. The equivalent associations with high-grade SIL were of much greater magnitude.

Model D analyses considered multiple measurements of HPV infection and lesions within individuals. Among persons with no indication of a prevalent lesion by cytologic examination at enrollment, 38 had two or more SIL events during the follow-up period. The number of subjects contributing multiple high-grade SIL events was lower (eight people), and events were concentrated in a few persons (of the eight subjects, six had two events, one had four, and one had seven). Although correlations between multiple events are unspecified, we allowed for a varying baseline hazard for each observation period, depending on the follow-up visit being considered. We observed associations for the occurrence of lesions 4 or 6 months after a positive HPV result (table 4) of a magnitude similar to those observed in the traditional analyses for first occurrence of SIL. Results obtained by the two regression models (generalized estimating equations and marginal hazards) were generally comparable, although they differed in definition—that is, marginal odds ratios for generalized estimating equations and hazard ratios for Cox regression for marginal data. Odds ratios begin somewhat higher for oncogenic HPV types than for nononcogenic types after an average period of 4 months, followed by a drop in relative risk at 8 months. A similar

TABLE 3. Hazard ratios for the association between current nononcogenic and oncogenic human papillomavirus infection at a given study visit and first instance of squamous intraepithelial lesions (any grade and high-grade) during the first 3 years of follow-up, Ludwig-McGill Cohort Study, São Paulo, Brazil, 1993–2002*

HPV† status *at a given visit*	First SIL† event		First high-grade SIL event	
	HR†	95% CI†	HR	95% CI
Negative	1.0‡		1.0‡	
Only nononcogenic types of HPV detected	5.68	3.0, 10.7	11.33	2.2, 57.3
Any oncogenic type of HPV detected	14.20	8.7, 23.1	32.69	8.4, 127.3

* Results from a traditional time-dependent Cox proportional hazards model for the first lesion detected at any scheduled return visit, with time-dependent variables for HPV status, according to model C (see text for details). Data were adjusted for age. Prevalent cases at enrollment were excluded.

† HPV, human papillomavirus; SIL, squamous intraepithelial lesion; HR, hazard ratio; CI, confidence interval.

‡ Referent.

TABLE 4. Relative risk estimates for the associations between nononcogenic and oncogenic human papillomavirus infection at a given study visit and detection of a squamous intraepithelial lesion of any grade at a scheduled follow-up return visit, Ludwig-McGill Cohort Study, São Paulo, Brazil, 1993–2002

Point during follow-up (months) *at which* SIL* was detected	Marginal hazards model†				Generalized estimating equations regression model‡			
	Nononcogenic types of HPV*		Oncogenic types of HPV		Nononcogenic types of HPV		Oncogenic types of HPV	
	HR*	95% CI*	HR	95% CI	OR*	95% CI	OR	95% CI
0 (same visit)	NA*		NA		31.09	14.2, 67.8	48.57	23.7, 99.5
4	6.14	3.1, 12.1	13.03	7.6, 22.5	6.60	3.2, 13.5	15.76	9.0, 27.5
6	4.39	0.7, 25.8	14.32	4.6, 44.9	1.92	0.1, 42.1	10.60	3.2, 35.5
8	3.08	1.3, 7.4	5.33	2.8, 10.2	3.13	1.2, 8.0	5.74	2.7, 12.1
12	5.56	2.5, 12.2	11.81	6.4, 21.9	4.10	1.8, 9.3	10.60	5.6, 20.1
18	6.97	2.8, 17.2	8.95	4.0, 20.1	6.78	2.7, 16.9	9.94	4.5, 22.2
24	3.85	1.6, 9.5	7.34	3.6, 15.1	3.19	1.3, 7.7	5.58	2.5, 12.4
30	1.90	0.4, 8.9	7.78	3.8, 16.0	1.46	0.2, 8.7	8.29	4.0, 17.3
36	2.62	1.0, 6.7	3.35	1.6, 7.0	2.24	0.8, 6.1	2.57	1.1, 6.1
42	2.47	0.8, 7.4	5.69	2.9, 11.3	2.48	0.8, 7.7	5.78	2.8, 11.9
48	2.63	0.5, 13.0	4.40	1.6, 11.8	2.32	0.5, 10.6	4.47	1.7, 11.9
54	4.23	1.3, 13.6	1.98	0.5, 7.4	5.07	1.8, 14.2	1.82	0.5, 6.7
60	0.73	0.1, 6.3	3.37	1.0, 11.1	1.13	0.2, 7.2	2.30	0.5, 10.8

* SIL, squamous intraepithelial lesion; HPV, human papillomavirus; HR, hazard ratio; CI, confidence interval; OR, odds ratio; NA, not applicable.

† Results from a marginal hazards Cox regression analysis for repeated outcomes over unequal intervals of time with robust variance. Data were adjusted for age. Prevalent cases of SIL at enrollment were excluded. Interval SIL events occurring before or after the indicated return visit were not considered, according to model D (see text for details).

‡ Results from a generalized estimating equations regression analysis with a logit link for binary outcomes (exchangeable correlation between repeated events). Data were adjusted for age. Prevalent cases of SIL at enrollment were excluded.

drop is seen at an average follow-up time of 6 months after testing for nononcogenic HPV types. More importantly, table 4 also shows a trend of decreasing relative risks for both nononcogenic and oncogenic HPV types as cohort interval time increases.

DISCUSSION

Using the repeated-measurements design of our study, we were able to analyze the dynamic status of cervical lesion outcomes with respect to similar changes in status over time for HPV testing results. Previous studies of the natural history of HPV and cervical cancer have also used statistical methods for longitudinal data, to a limited extent, to analyze either HPV infections (28) or precursor lesions (8, 9, 29) as outcomes. Although they are potentially more powerful, such statistical approaches have some additional conditions and assumptions that are not required in traditional cohort study designs (30).

The objective of this study was to evaluate the time dependence of the association between HPV infection and risk of incident SIL by decomposing our cohort data to simulate different cohort study designs. First, we replicated traditional cohort designs of baseline assessment of exposure and delayed outcome incidence with increasing durations of follow-up that either assumed a termination date (outcomes after that date being ignored, i.e., model A) or imposed a specified period of time during which no outcome ascertainment was made (outcomes before this period of time being ignored, i.e., model B). These two approaches are based on a single time assessment of HPV exposure (at enrollment) and first incident lesion (at a given interval restriction) and thus ignore most of the exposure and lesion information collected during the study. We then resorted to models that supplemented enrollment HPV status with postenrollment HPV testing data and correlated the resulting combined information with risk of incident lesions (i.e., model C). Finally, we used longitudinal designs with repeated assessments of HPV and SIL over time and varying time intervals between exposure and outcome (model D).

The hallmark of our findings is the decline in the magnitude of the association between baseline HPV status and risk of subsequent low-grade SIL with increasing duration of follow-up. Relative risks might be attenuated by the occurrence of remote SIL events (i.e., events later in the study) as a result of new (as-yet-undetected) HPV infections occurring after enrollment. Thus, traditional cohort analyses that rely on single measures of exposure may underestimate the relative risk, because of misclassification that arises when we try to associate prevalent HPV infections with SIL events occur-

ring several years later (model A). The hazard associated with initial HPV status is high early on but declines with time, suggesting that HPV status at baseline is closely associated with early events but is almost unassociated with later events. The slight association with exclusively distant events (model B) could be ascribed to the close connection between current HPV and new infections after the primary visit. However, we cannot exclude the possibility that the point estimates may be influenced by false-negative HPV tests, since HPV infections are mostly transient.

When we evaluate the pairwise associations between HPV and SIL at different points in time following an HPV measurement (model D), we see the magnitude of the association drop at 6 and 8 months after HPV assessment for nononcogenic HPV types and at 8 months after HPV assessment for oncogenic types. These fluctuations in relative risk coincide with our previous estimations of retention time for oncogenic and nononcogenic types of HPV (31). A return to initial levels of association does not occur until after 12 months for either group of HPV types. Although we used a standard polymerase chain reaction protocol, small technical variants have been introduced over the long time span (nearly 10 years) involving laboratory work in this project. Enrollment HPV results were generated 1–4 years before HPV tests done on later follow-up specimens. The fact that model D results largely replicated the findings from models A and B suggests that the quality of our viral testing did not drift over time.

Traditional cohort analyses that rely on a single measure of HPV exposure obtained at enrollment present a design situation analogous to that of nested case-control studies carried out on registries of screening data with exposure assessment made at the beginning of assembly of the parent cohort (model A). Two such studies by Wallin et al. (10) and Liaw et al. (11) observed odds ratios of 15.0 (95 percent CI: 0.8, 1,541) for incident cervical cancer an average of 6 years after an HPV-positive normal Papanicolaou smear and 12.7 (95 percent CI: 6.2, 25.9) for incident high-grade SIL within 5 years of follow-up after an HPV-positive normal Papanicolaou smear, respectively. Taking an alternative follow-up approach, we witness a decline in hazard ratio associations as the time interval between positive HPV results and cervical lesion incidence becomes longer. That is, the likelihood of a new SIL event or a repeat low-grade SIL event decreases as the opportunity for detecting such events by cytologic examination is delayed. This method reproduces the situation encountered in studies with fixed (model D) (16, 32, 33) or delayed (model B) (34) follow-up of subjects. We observed decreasing hazard ratios associated with SIL occurring later in follow-up for both oncogenic and nononcogenic HPV infections at enrollment. Considering persistent type-specific oncogenic HPV infections for the first 4 months of follow-up, the association with incident SIL events or repeat SIL events continues for a longer period of time, with appreciably higher hazard ratios being observed for incident events 48 months later. This is not seen for nononcogenic persistent infections (data not shown).

A second important observation in this study is the divergent pattern of risk association observed for high-grade SIL as compared with low-grade SIL. While point estimates for high-grade SIL were less stable because of the small number of events, they were indicative of a constant risk relation over time for both HPV infection variables. The majority of incident high-grade SIL events (18/27) were preceded by a low-grade SIL (14/27) or an "ASCUS" (atypical squamous cells of undetermined significance) smear (9/27). The first instance of detection of any lesion was used in the analyses for any SIL (models C and D), and therefore the observed declines in hazard ratios are dominated by the effect of incident low-grade SILs. While the attenuation of the association with low-grade SIL may be due to nondifferential misclassification, the magnitude and persistence of the hazard ratio for high-grade SIL over time is consistent with a causal hypothesis. To our knowledge, this is the first study showing this distinction. Woodman et al. (9) observed a decline in risk of high-grade SIL by cytologic examination in relation to time since first detection of HPV from less than 6 months (relative risk = 25.33, 95 percent CI: 8.81, 72.83) to more than 12 months (relative risk = 6.42, 95 percent CI: 2.10, 19.65). Ylitalo et al. (16) showed decreasing odds ratios across levels of HPV 16 viral load measured 1–9 years before diagnosis of carcinoma in situ from histologic samples.

Investigators in several studies have tried to address this attenuation in effect with time by incorporating time-varying measures of HPV status into their statistical models of cervical neoplasia (model C) (7, 13, 29). Use of statistical methods that allow time-dependent covariates (e.g., generalized estimating equations) in these studies demonstrates a clinical utility of HPV testing in predicting the incidence of a new or recurrent cervical lesion following a recent HPV infection. In similar cohort studies by Ho et al. (7) and Moscicki et al. (12), the authors observed elevated odds ratios for SIL following a positive infection for oncogenic HPV types detected 4–6 months earlier.

Although outcome assessment in our study was carefully conducted in a reference laboratory following strict quality control procedures and without knowledge of exposure status, cytologic misclassification may have resulted in attenuation of the relative risk estimates. We opted for an intensive, expert cytologic follow-up of all Papanicolaou smears collected in the study every 4–6 months to avoid having to perform unnecessary biopsies, which may interfere with the early natural history of lesions. The inclusion of intensive cytologic testing over time also permitted us to evaluate repeated occurrences of lesion events. However, we cannot be sure that a repeat detection of an event represented recurrence or persistence, even if no lesion was detected at interval assessments by cytologic examination.

In cohort studies of SIL involving postcoital observations of subjects, we rarely know the disease history of women following a first exposure to HPV. As a result, we and other investigators often exclude prevalent cases detected at enrollment from the analysis (6, 7, 11, 12, 22, 35, 36) and assume that women with negative cytologic lesions at enrollment are at risk of lesion incidence. The majority of the SIL events in our study began as low-grade SIL. While the progression of low-grade SIL to high-grade SIL to cancer may depend on other factors in conjunction with HPV, the detection of low-grade SIL could simply fluctuate with HPV

status. The short-term association observed between HPV and low-grade SIL may result from the relatively easy recognition by cytologic examination of cytopathic effects induced by HPV (37–39). However, a relation between HPV and incidence of SIL over an extended period of time has also been observed (13), suggesting that a temporal risk effect exists. When estimating the association between HPV and SIL events, we can gain precision by taking into account the transient nature of the disease. The incorporation of repeat SIL events in our analyses, achievable because of the length of follow-up in this study, marginally improved model power (as indicated by the narrowing of the confidence intervals) without changing the estimation of effects (data not shown).

In conclusion, we observed a decrease in the magnitude of the association between baseline HPV detection and low-grade SIL over time. However, allowing for the dynamic nature of both exposure and outcome yielded larger relative risk estimates for corresponding time points. Thus, use of traditional cohort design and analytical techniques relying on ascertainment of exposure at a single point in time may result in underestimation of the effects of HPV, even when testing only for high-risk, oncogenic viral types. Future assessments of the empirical effects of HPV infection in long-term follow-up studies may require more complex algorithms to quantify past exposure to the virus (e.g., type-specific information, long-term persistence, and inclusion of viral load). In addition, in future implementation of screening programs based on HPV testing, researchers will have to consider the transient nature of HPV infections over time and the clinical significance of detected lesions (i.e., high-grade lesions vs. repeat low-grade lesions).

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