Predictors of Cervical Coinfection with Multiple Human Papillomavirus Types[1]

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Abstract

Women infected with multiple human papillomavirus (HPV) types seem to be at higher risk of cervical intraepithelial neoplasia, although there is controversy about whether coinfections are associated with lower or higher grades of dysplasia. There is no established risk factor profile for infection with multiple HPV types. We analyzed data from a prospective cohort of 2075 Brazilian women to identify determinants of HPV coinfection. Cervical specimens were collected for cytology and HPV DNA detection. Data on baseline and time-dependent putative risk factors were obtained by interview. Baseline predictors of HPV coinfection included younger age, greater number of recent sexual partners, a history of condyloma but not of other sexually transmitted diseases, and younger age at first sexual intercourse. In repeated measures analyses, there was a weak positive association between the number of sexual partners in the time interval between two study visits and the risk of coinfection. Our results suggest that the risk factor profile for HPV coinfection among HPV-infected women shares several similarities with risk factors for any HPV infection.

Introduction

HPV[3] is the central etiological agent of cervical cancer (1, 2). Research on factors associated with HPV infection first focused on infection with any HPV type (3–7). More recent studies suggested that risk factor profiles may differ according to the oncogenic potential of HPV (8–13).

Coinfection with multiple HPV types has been observed more frequently among younger women (14, 15) and among those with cytological abnormalities (15–18). HPV coinfections also occur more commonly among women with an impaired immune response (19). In some studies, infections with multiple HPV types have been associated with a higher risk of cervical intraepithelial neoplasia (16, 20, 21). In other studies, no increased risk of cervical intraepithelial neoplasia or cervical cancer was reported among women with multiple infections compared with women with single HPV infections (17, 22).

With the recent promising results from preliminary trials of HPV vaccine efficacy (23, 24), it becomes imperative to obtain a solid knowledge base of epidemiological determinants of infections with multiple HPV types. This will help in the interpretation of the results of large scale trials of monovalent and polyvalent vaccines.

We analyzed data from a cohort study of HPV infection and precursors of cervical cancer to identify determinants of coinfection with multiple HPV types compared with infection with single HPV types. More specifically, we have assessed risk of coinfection in association with baseline and updated values of putative risk factors, using two approaches for defining HPV coinfection.

Materials and Methods

Study Population and Design. The subjects included in this study are enrolled in the Ludwig-McGill Cohort, an ongoing longitudinal investigation of HPV infection and precursor lesions of cervical cancer. The design and methods were described previously (25). Briefly, women attending a maternal and child health program in São Paulo, Brazil, were eligible if they: (*a*) were aged between 18 and 60 years; (*b*) were permanent residents of São Paulo (city); (*c*) were not currently pregnant nor planning to become pregnant for a year; (*d*) had an intact uterus and no current referral for hysterectomy; (*e*) reported no use of vaginal medication in the previous 2 days; and (*f*) reported no treatment for cervical disease in the previous 6 months. The study began in 1993 and recruitment ended in 1997. Women returned every 4 months in the first year of follow-up and every 6 months thereafter. HPV typing and Pap cytology were performed on cervical samples collected at every visit. A baseline interviewer-administered questionnaire elicited information on sociodemographic characteristics, sexual behavior, reproductive and contraceptive history, and some lifestyle habits (*e.g.*, smoking, diet). Interviews at return visits were used to gather information on sexual behavior, contraceptive use, and smoking since the last visit. In the present analysis, we used a subset of 2075 subjects from the Ludwig-McGill Cohort Study (82% of the original 2528 women enrolled) for

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[3] The abbreviations used are: HPV, human papillomavirus; CI, confidence interval; GEE, generalized estimating equations; OR, odds ratio; STD, sexually transmitted disease.

whom we had HPV test results for one or more visits (maximum: six visits).

We used two definitions of HPV coinfection status: cumulative and concurrent. First, for the cumulative HPV status, multiple infection was defined as the detection of more than one HPV type over the entire follow-up, whereas single HPV infection was defined as having only one type detected. For example, a subject infected by HPV-16 at one study visit and HPV-18 at another visit was considered to have a cumulative coinfection. The control group for each set of analyses was comprised of subjects who had not developed the outcome of interest (infection with any HPV type, infection with multiple HPV types) but who had attended at least four visits (residual noncases). We excluded women who did not develop the outcome of interest and had fewer than four visits because attending fewer visits decreased the opportunity that coinfection could be detected, possibly resulting in misclassification. Cases (*i.e.,* women with any HPV detected for the analysis of any HPV infection *versus* HPV-negative, women with multiple HPV types detected for the contrast between multiple *versus* single infection) were included regardless of the number of visits because their status was already determined. The analyses on associations between baseline variables and cumulative HPV status included: (*a*) 1867 subjects for the models contrasting those with any HPV type with HPV-negative; (*b*) 718 subjects when studying multiple infections as compared with single infections; (*c*) 1853 subjects when comparing positivity for any oncogenic HPV with HPV-negative; and (*d*) 443 subjects when comparing those with multiple oncogenic HPV types with those with single oncogenic HPVs.

In the second approach, HPV coinfection was defined as concurrent detection of more than one HPV type at any visit. Subjects who had one HPV type were classified as having a single-type infection, even if the type changed from one visit to another. All visits from the 2075 subjects with HPV test results ($n = 9425$ visits) were included in a longitudinal analysis that accounted for repeated measures on a single subject.

The analyses with cumulative HPV status and with repeated measures were then redone considering only 13 oncogenic HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68; Ref. 26). Women were defined as having no infection if none of these HPV types was detected, a single-type infection if only one, and a multiple infection if two or more oncogenic types were detected. This approach was used to examine whether risk factors identified for coinfections with all HPV types were also predictive of coinfections with the most important types for cervical cancer etiology.

HPV DNA Detection. Ectocervical and endocervical cells were collected with an Accelon biosampler (Medscand, Inc.) and immersed in Tris-EDTA buffer at pH 7.4. The DNA was purified by spin column chromatography and amplified by PCR using MY09/11 primers (27, 28). Hybridization of the amplified products with oligonucleotide probes and RFLP analyses were used to identify >40 different genital HPV types (29). Amplified products that hybridized only with a generic probe and did not have a discernible pattern in RFLP analysis were considered positive for unknown types. The quality of the DNA specimens was verified by the amplification of a 268-bp region of the human *β-globin* gene (27). Specimens were tested blindly, and standard precautions were taken to prevent contamination.

Statistical Analysis. Unconditional multiple logistic regression was used to estimate the adjusted ORs, with 95% CIs, for potential risk factors assessed at baseline and any HPV infection (as compared with HPV-negative) among all women. We then repeated the analysis using only data from HPV-positive women to assess baseline predictors of cumulative multiple HPV infection contrasted with single HPV infection. Similarly, separate models were estimated for any HPV infection (compared with HPV-negative) and multiple infection (compared with single infection), restricting the detection to oncogenic HPV types. Variables with univariate Ps < 0.25 were considered as candidates for inclusion in multivariable models (30). Selection of independent variables into the final model was based on the likelihood ratio test and a backward elimination strategy using a P of 0.15. Multivariable models are presented for four outcomes: any HPV infection and multiple infection considering all HPV types; any HPV infection and multiple infection with detection limited to oncogenic HPV types. To investigate if the associations with prevalent infections at baseline were the same as associations related to prediction of incident infections, we carried out an analysis stratified according to whether the HPV infection and coinfection were prevalent at baseline or incident.

The GEEs (31) extension of logistic regression was used to estimate the ORs and 95% CI for the associations of baseline and time-dependent variables with HPV infection status at consecutive visits. In these longitudinal analyses, the binary variable indicating the subject's HPV status at each consecutive visit provided repeated measures of the outcome. The GEE procedure with a first-order autoregressive working correlation matrix was used to adjust the SEs for the correlation between multiple observations on the same subject, assuming that observations from more distant visits were less correlated. Baseline variables identified as predictive of any HPV infection and coinfection, based on cumulative HPV status (previous analyses), were used as a starting point in the longitudinal models. Baseline variables that did not show statistically significant associations with HPV status in the GEE models were then excluded (education, number of sexual partners ≥5 years before baseline). In addition, two baseline variables (smoking and sexual partners in the past year) were excluded because they were inherently correlated with the corresponding time-dependent variables (smoking and number of sexual partners since last visit). For an outcome measured at visit *t*, the time-dependent variables referred to exposure in the interval between visit *t-1* and visit *t*. These variables included number of sexual partners, number of new sexual partners, condom use, oral contraceptive use, and smoking.

In the subset of 1867 subjects used in the analysis of cumulative HPV status, the following proportions of subjects were missing values for: education (0.1%); history of STDs (0.3%); sexual partners >5 years before baseline (0.2%); sexual partners 2–5 years before baseline (0.8%); sexual partners in past year (0.8%); and number of pregnancies (0.5%). Individuals missing data on number of sexual partners and number of pregnancies were assigned the median value for the other individuals, whereas the mode was used for education and history of STDs. For time-dependent variables, the last available previous value was carried forward when an answer was missing.

Results

A total of 43,431 woman-months of follow-up was accumulated for the subset of 2075 subjects included in this study, with a mean follow-up of 20.9 months (4.5 visits) and a median of 24 months (5 visits)/woman.

Cumulative HPV Status. In the subset of 1867 subjects, 760 (41%) harbored an HPV infection. When contrasting multiple *versus* single infection, single HPV types were observed in 438 (24%) subjects, and multiple HPV infections were identified in 280 (15%). For this analysis, 42 (2%) subjects with single infections had completed fewer than four visits and were excluded from the control group. When considering only oncogenic HPV types, 14 (0.7%) subjects who harbored only non-oncogenic HPV types and had fewer than four visits were excluded. Among the remaining 1853 subjects, 481 (26%) had an oncogenic HPV infection. For the comparison between subjects with multiple and single oncogenic HPV infections, 331 (18%) had a single HPV infection, whereas 112 (6%) had a multiple infection. We additionally excluded 38 (2%) subjects from the control group because they had a single oncogenic HPV infection but fewer than four visits completed.

The associations between baseline variables and cumulative HPV status are presented in Table 1. The following baseline variables were associated with higher risk of any HPV infection: younger age; smoking; history of condyloma; younger age at first sexual intercourse; and a higher number of sexual partners. Among HPV-positive women, younger age, history of condyloma, and higher number of sexual partners in the recent past were statistically significant risk factors, whereas current smoking and younger age at first intercourse were marginally significant risk factors for multiple infection, relative to single HPV infection. Associations with younger age, history of condyloma, and higher number of sexual partners in the recent past were also observed when HPV detection was limited to oncogenic types (rightmost columns in Table 1). However, women with a college or university degree were at higher risk of multiple infection with oncogenic HPV types as compared with single HPV infection. Although statistically significant, this finding was based on small frequencies, as indicated by the wide confidence interval.

Table 2 shows the results of multivariable analyses for all variables retained in the four final regression models. Accordingly, all ORs in Table 2 are adjusted for all other variables in the table. Younger age, a history of condyloma, a younger age at first sexual intercourse, and a higher number of sexual partners increased the risk of both infection with any HPV type and multiple HPV infection. In contrast, smoking and number of sexual partners >5 years before baseline were not associated with the risk of multiple HPV infection. Associations with the same variables were observed when detection was limited to oncogenic HPV types, although the CIs were wider because of the smaller sample size especially in the analyses of multiple *versus* single infection. However, women who attended college or university had a statistically significant higher risk of multiple infection with oncogenic HPVs, even after adjusting for several potential confounders.

A higher number of sexual partners was associated with an increase in risk of any HPV infection and of multiple HPV infection, but the predictive ability of more recent data were stronger than for values in the distant past (>5 years before baseline). This relationship with number of recent sexual partners was observed for all four outcomes considered, although not statistically significant for multiple *versus* single oncogenic HPV types. The number of sexual partners >5 years before baseline was only marginally associated with risk of any HPV infection and not associated with HPV coinfection.

Repeated Measures Analysis of Concurrent HPV Status. The 2075 subjects generated 9425 visits with available HPV status. Of these, 760 (36.6%) had HPV detected at least once at a total of 1521 follow-up visits (median: two visits/woman) where HPV DNA was present. An HPV coinfection was detected among 158 (7.6%) women for a total of 239 visits with concurrent coinfections (median: one visit/woman).

Table 3 presents the results of multivariable repeated measures analyses of the associations between baseline as well as time-dependent risk factors and the risk of either any HPV infection or concurrent HPV coinfection. These analyses attempted to explain the presence of the infection at a given visit not only by baseline risk factors but also updated information on number of sexual partners, smoking status, and condom use in the interval since the last visit. Number of new sexual partners and oral contraceptive use were not associated with any of the outcomes and are thus not included in the models. The associations observed for baseline variables such as age, age at first sexual intercourse, and number of sexual partners were similar to the associations in models based on cumulative HPV status. In contrast, the effect of history of condyloma was much weaker than in the models using cumulative HPV status. Reporting one or more sexual partner since last visit was not associated with risk of HPV infection but marginally increased the risk of concurrent HPV coinfection as compared with women who reported no sexual partners. Women who used condoms since their last study visit had a higher risk of HPV infection with all HPV types and oncogenic HPVs. Those who smoked since their last study visit were significantly more likely to have any HPV infections detected. However, condom use and smoking did not predict concurrent coinfections among HPV-positive women.

Changes Over Time in the Predictive Ability of Baseline Variables. We investigated if the associations of baseline variables with prevalent infections were the same as those with incident infections. The predictive ability of two baseline variables, history of STDs, and number of sexual partners in the last 5 years, varied based on whether the HPV infections were prevalent or incident. Single and multiple HPV infections based on cumulative HPV status were divided into prevalent (detected at baseline visit) and incident. Fig. 1 shows the ORs and 95% CIs for the associations for history of STDs with HPV infection (Fig. 1*A*) and HPV coinfection (Fig. 1*B*) adjusted for age and number of sexual partners in the past 5 years. A history of condyloma was clearly associated with an increased risk of prevalent HPV infection but was not a predictor of incident HPV infections (Fig. 1*A*). In contrast, among HPV-positive women, history of condyloma was associated with both prevalent and incident HPV coinfection, although these associations were of borderline statistical significance, with wide CIs (Fig. 1*B*).

Similarly, we also investigated whether the impact of the number of sexual partners in the 5 years before study entry on the risks of HPV infection/coinfection was different for prevalent and incident infections. Fig. 2 shows the ORs and 95% CIs for number of sexual partners in the 5 years before study entry with HPV infection (Fig. 1*A*) and HPV coinfection (Fig. 1*B*), adjusted for age and history of STDs. Both the associations for one sexual partner and for two or more sexual partners compared with no partner were slightly stronger for HPV infections prevalent at baseline than for incident HPV infections, but there was substantial overlap of CIs (Fig. 2*A*). Finally, in the analyses restricted to HPV-positive women, the impact of one or more sex partners on increasing the risk of multiple, rather than single infection, was very similar for prevalent and incident infections (Fig. 2*B*).

Table 1 ORs and 95% CIs for the associations between baseline variables and cumulative HPV status, for all HPV types and for detection restricted to oncogenic HPVs

Variable	All HPV types[a]						Oncogenic HPV types[a]					
	No. HPV−	No. HPV+	Any infection *versus* negative OR[b] (95% CI)	No. single HPV	No. multiple HPV	Multiple *versus* single infection OR[b] (95% CI)	No. HPV−	No. HPV+	Any infection *versus* negative OR[b] (95% CI)	No. single HPV	No. multiple HPV	Multiple *versus* single infection OR[b] (95% CI)
Age, years												
18–24	167	208	1.00	95	105	1.00	230	140	1.00	83	50	1.00
25–34	436	301	0.55 (0.43–0.71)	171	107	0.57 (0.39–0.82)	526	206	0.64 (0.49–0.84)	141	42	0.49 (0.30–0.81)
35–44	365	184	0.40 (0.31–0.53)	123	53	0.39 (0.25–0.60)	447	98	0.36 (0.27–0.49)	78	15	0.32 (0.17–0.61)
45–60	139	67	0.39 (0.27–0.55)	49	15	0.28 (0.15–0.53)	169	37	0.36 (0.24–0.54)	29	5	0.29 (0.10–0.79)
Education												
<Elementary	255	151	1.00	89	52	1.00	308	95	1.00	65	18	1.00
Elementary	651	457	1.06 (0.84–1.35)	264	167	0.90 (0.60–1.35)	807	293	1.02 (0.78–1.34)	209	63	0.92 (0.50–1.70)
High school	171	134	1.10 (0.80–1.50)	76	53	0.95 (0.57–1.58)	219	83	0.96 (0.67–1.37)	54	25	1.29 (0.62–2.67)
College/university	30	18	0.95 (0.51–1.78)	9	8	1.49 (0.52–4.25)	38	10	0.78 (0.37–1.64)	3	6	6.46 (1.42–29.35)
Smoking												
Never	573	342	1.00	203	120	1.00	695	215	1.00	145	52	1.00
Current	349	286	1.47 (1.19–1.82)	156	110	1.28 (0.91–1.81)	441	186	1.47 (1.16–1.86)	126	44	1.08 (0.67–1.76)
Former	185	132	1.28 (0.98–1.66)	79	50	1.17 (0.76–1.80)	236	80	1.17 (0.87–1.58)	60	16	0.83 (0.44–1.60)
Vaginal douching												
Never/occasional	988	690	1.00	396	256	1.00	1226	440	1.00	299	106	1.00
Frequent	119	70	0.91 (0.66–1.24)	42	24	0.97 (0.56–1.66)	146	41	0.86 (0.60–1.25)	32	6	0.60 (0.24–1.49)
History of STDs												
None	854	566	1.00	337	200	1.00	1059	356	1.00	244	82	1.00
Condyloma	32	51	2.24 (1.41–3.56)	20	27	2.09 (1.13–3.88)	47	31	1.83 (1.13–2.95)	19	10	1.45 (0.63–3.30)
Other STDs[c]	221	143	1.07 (0.84–1.36)	81	53	1.29 (0.86–1.92)	266	94	1.16 (0.89–1.52)	68	20	1.10 (0.62–1.96)
Age at first intercourse (yrs)												
≥20	345	153	1.00	110	37	1.00	416	80	1.00	63	11	1.00
18–19	235	155	1.30 (0.98–1.73)	84	64	1.74 (1.04–2.93)	292	97	1.47 (1.05–2.07)	64	27	1.65 (0.71–3.84)
16–17	250	227	1.71 (1.30–2.25)	124	87	1.54 (0.94–2.52)	316	156	2.10 (1.52–2.89)	105	39	1.44 (0.64–3.21)
≤15	277	225	1.46 (1.11–1.92)	120	92	1.55 (0.94–2.58)	348	148	1.73 (1.25–2.40)	99	35	1.28 (0.56–2.93)
Sexual partners >5 years before baseline												
0	623	383	1.00	216	147	1.00	759	242	1.00	158	66	1.00
1	222	163	1.34 (1.05–1.71)	91	60	1.15 (0.77–1.72)	267	115	1.52 (1.16–1.99)	78	23	0.84 (0.47–1.48)
≥2	262	214	1.63 (1.29–2.06)	131	73	1.02 (0.70–1.49)	346	124	1.38 (1.06–1.80)	95	23	0.74 (0.42–1.32)
Sexual partners 2–5 years before baseline												
0	934	514	1.00	336	155	1.00	1125	317	1.00	232	64	1.00
1	119	141	1.98 (1.52–2.61)	62	67	2.13 (1.42–3.18)	166	89	1.76 (1.31–2.35)	54	25	1.49 (0.85–2.61)
≥2	54	105	3.07 (2.16–4.36)	40	58	2.77 (1.76–4.38)	81	75	2.80 (1.98–3.96)	45	23	1.50 (0.83–2.72)
Sexual partners in past year												
0–1	1087	695	1.00	418	240	1.00	1341	430	1.00	303	92	1.00
≥2	20	65	4.70 (2.80–7.87)	20	40	3.17 (1.79–5.61)	31	51	4.72 (2.95–7.53)	28	20	2.18 (1.15–4.14)
Anal intercourse												
Never	683	472	1.00	275	174	1.00	855	293	1.00	205	65	1.00
Ever	424	288	0.97 (0.80–1.18)	163	106	1.03 (0.75–1.41)	517	188	1.04 (0.84–1.29)	126	47	1.22 (0.78–1.92)
Oral sex												
Never	526	326	1.00	191	112	1.00	642	205	1.00	134	48	1.00
Ever	581	434	1.12 (0.93–1.36)	247	168	1.05 (0.77–1.44)	730	276	1.08 (0.87–1.34)	197	64	0.80 (0.51–1.25)
Oral contraceptives												
Never	160	128	1.00	70	51	1.00	198	89	1.00	53	26	1.00
<6 years	597	433	0.92 (0.70–1.20)	247	164	0.99 (0.64–1.51)	757	263	0.76 (0.56–1.02)	183	63	0.77 (0.44–1.36)
≥6 years	350	199	0.91 (0.67–1.24)	121	65	1.08 (0.65–1.81)	417	129	0.91 (0.65–1.28)	95	23	0.76 (0.37–1.56)
Condom use												
Never/occasional	1071	726	1.00	421	267	1.00	1326	459	1.00	316	107	1.00
Always	36	34	1.21 (0.74–1.97)	17	13	0.98 (0.46–2.11)	46	22	1.22 (0.72–2.08)	15	5	0.71 (0.25–2.08)
Pregnancies												
0–1	164	143	1.00	72	66	1.00	202	103	1.00	68	29	1.00
2–3	495	321	0.96 (0.72–1.27)	183	120	0.89 (0.58–1.37)	613	196	0.79 (0.58–1.06)	137	44	0.97 (0.54–1.74)
4–6	333	223	1.17 (0.86–1.60)	135	75	0.91 (0.55–1.49)	413	139	0.99 (0.70–1.39)	96	32	1.45 (0.72–2.90)
≥7	115	73	1.25 (0.84–1.88)	48	19	0.78 (0.39–1.54)	144	43	1.04 (0.66–1.65)	30	7	1.19 (0.43–3.34)

[a] Sample sizes are 1867 for all HPV types and 1853 for oncogenic HPV types. Fourteen subjects who only harbored nononcogenic HPVs and had completed fewer than four visits were excluded from the analyzes of oncogenic HPV types (not eligible as controls).
[b] Adjusted for age where appropriate.
[c] Gonorrhea, chancre, syphilis, herpes, trichomoniasis, or candidiasis.

Table 2 Multivariate ORs[a] and 95% CIs of baseline variables for HPV infection among all women and for coinfections with multiple types among HPV-positive women

Variable	All HPV types		Oncogenic HPV types	
	Any infection *versus* negative (n = 1867)	Multiple *versus* single infection (n = 718)	Any infection *versus* negative (n = 1853)	Multiple *versus* single infection (n = 443)
Age (yrs)				
18–24	1.00	1.00	1.00	1.00
25–34	0.61 (0.46–0.80)	0.69 (0.45–1.05)	0.74 (0.55–1.00)	0.65 (0.37–1.15)
35–44	0.46 (0.33–0.63)	0.49 (0.30–0.82)	0.44 (0.31–0.63)	0.45 (0.21–0.95)
45–60	0.53 (0.35–0.80)	0.41 (0.19–0.87)	0.54 (0.34–0.87)	0.52 (0.16–1.68)
	$P_{trend} < 0.001$	$P_{trend} = 0.003$	$P_{trend} < 0.001$	$P_{trend} = 0.046$
Education				
<Elementary	1.00	1.00	1.00	1.00
Elementary	1.05 (0.82–1.35)	0.84 (0.55–1.29)	1.02 (0.77–1.36)	0.92 (0.49–1.72)
High school	1.11 (0.80–1.54)	0.90 (0.52–1.55)	1.01 (0.69–1.46)	1.34 (0.63–2.86)
College/university	1.09 (0.57–2.09)	1.70 (0.57–5.10)	0.89 (0.41–1.94)	7.41 (1.55–35.55)
Smoking				
Never	1.00	1.00	1.00	1.00
Current	1.30 (1.04–1.63)	1.15 (0.80–1.66)	1.29 (1.00–1.65)	1.11 (0.66–1.87)
Former	1.19 (0.90–1.57)	1.17 (0.74–1.85)	1.09 (0.80–1.50)	1.04 (0.52–2.08)
History of STDs				
None	1.00	1.00	1.00	1.00
Condyloma	1.78 (1.10–2.89)	1.80 (0.95–3.44)	1.54 (0.93–2.55)	1.48 (0.63–3.45)
Other STDs[b]	0.93 (0.72–1.19)	1.14 (0.75–1.74)	1.04 (0.78–1.38)	0.97 (0.53–1.80)
Age at first intercourse (yrs)				
≥20	1.00	1.00	1.00	1.00
18–19	1.16 (0.87–1.57)	1.59 (0.92–2.74)	1.32 (0.93–1.87)	1.77 (0.72–4.34)
16–17	1.46 (1.09–1.95)	1.42 (0.84–2.40)	1.82 (1.30–2.54)	1.59 (0.68–3.74)
≤15	1.11 (0.82–1.52)	1.37 (0.78–2.41)	1.34 (0.96–1.97)	1.64 (0.66–4.04)
Sexual partners >5 years before baseline				
0	1.00	1.00	1.00	1.00
1	1.21 (0.93–1.57)	1.06 (0.69–1.64)	1.35 (1.02–1.80)	0.79 (0.43–1.44)
≥2	1.31 (1.01–1.70)	0.86 (0.57–1.32)	1.04 (0.77–1.40)	0.67 (0.36–1.26)
Sexual partners 2–5 years before baseline				
0	1.00	1.00	1.00	1.00
1	1.78 (1.35–2.36)	1.99 (1.31–3.01)	1.57 (1.16–2.12)	1.41 (0.79–2.54)
≥2	2.52 (1.75–3.63)	2.46 (1.53–3.95)	2.29 (1.60–3.30)	1.43 (0.77–2.66)
Sexual partners in past year				
0–1	1.00	1.00	1.00	1.00
≥2	3.56 (2.10–6.05)	2.45 (1.35–4.43)	3.71 (2.29–6.02)	1.90 (0.97–3.71)

[a] Mutually adjusted for all variables in a column.
[b] Gonorrhea, chancre, syphilis, herpes, trichomoniasis, or candidiasis.

Discussion

We used a large longitudinal cohort followed for a median of 24 months to assess the role of several putative risk factors for HPV infection. Specifically, we investigated whether risk factors for HPV coinfection were the same as for any HPV infection, using two definitions of coinfection, and assessed baseline and time-varying putative risk factors.

We first defined the HPV status with a cumulative approach, using all visits available (4–6) to classify subjects as having either no HPV infection, an infection with a single HPV, or accumulating multiple HPV types during their available follow-up. For variables collected at baseline, our results were generally consistent with literature on predictors of any HPV infection (3–7), as well as of oncogenic HPV types (8, 10, 11), in showing associations with age, number of sexual partners, and age at first sexual intercourse. However, in contrast to some previous studies, we have identified recent sexual partners as a stronger predictor of oncogenic HPV infection than lifetime partners (9, 12, 13). When the analyses were restricted to women who had tested positive for HPV at least once during follow-up, the variables associated with multiple HPV types relative to single type were similar to the classical predictors of any HPV infection. The strongest baseline predictors of HPV coinfections were younger age and the number of sexual partners 2–5 years before baseline, as well as in the past year. The use of three variables describing the number of sexual partners allowed us to assess which time periods were relevant for HPV infections and for coinfections. Number of sexual partners ≥5 years before the baseline did not have much effect on the risk of HPV infections and coinfections. In contrast, sexual activity in periods closer to the beginning of the study were relevant for infection and coinfection risk, as much in the 2–5 years before baseline as during the last year before study entry.

Results obtained from the repeated measures analyses of HPV status at subsequent visits were generally consistent with those using the cumulative HPV status. However, the added benefit of the repeated measures analyses was that they allowed us to assess factors that could vary over time. We observed an interesting pattern with number of sexual partners since the last visit: having one or more partners did not increase the risk of any HPV infection but showed a tendency toward increased risk of coinfection among HPV-positive women. The reasons for this pattern (no increase in risk of HPV infection, tendency toward increased risk of coinfection) observed both with all HPV types and with oncogenic types are not clear. Condom use was associated with an increased risk of HPV infection with all

Table 3 Baseline and time-dependent predictors of HPV infection among all women and of coinfections with multiple types among HPV-positive women

Variable	All HPV types, OR (95% CI)[a]		Oncogenic HPV, OR (95% CI)[a]	
	Any infection *versus* negative (n = 9425)	Multiple *versus* single infection (n = 1521)	Any infection *versus* negative (n = 9425)	Multiple *versus* single infection (n = 859)
Baseline				
Age (yrs)				
18–24	1.00	1.00	1.00	1.00
25–34	0.73 (0.59–0.90)	0.67 (0.45–1.01)	0.74 (0.57–0.95)	0.60 (0.33–1.07)
35–44	0.56 (0.43–0.72)	0.60 (0.34–1.04)	0.51 (0.37–0.71)	0.45 (0.16–1.31)
45–60	0.62 (0.43–0.90)	0.35 (0.14–0.83)	0.53 (0.34–0.83)	0.17 (0.02–1.19)
	$P_{trend} < 0.001$	$P_{trend} = 0.011$	$P_{trend} < 0.001$	$P_{trend} = 0.035$
History of STDs				
None	1.00	1.00	1.00	1.00
Condyloma	1.69 (1.19–2.40)	0.97 (0.51–1.87)	1.22 (0.77–1.93)	1.22 (0.45–3.30)
Other STDs[b]	0.97 (0.78–1.21)	1.00 (0.62–1.60)	0.96 (0.73–1.26)	1.05 (0.51–2.18)
Age at first intercourse (yrs)				
≥20	1.00	1.00	1.00	1.00
18–19	1.31 (1.00–1.73)	1.36 (0.72–2.60)	1.45 (1.03–2.04)	1.85 (0.62–5.50)
16–17	1.47 (1.14–1.90)	1.71 (0.94–3.12)	1.80 (1.31–2.47)	2.19 (0.81–5.94)
≤15	1.17 (0.90–1.53)	1.29 (0.68–2.48)	1.42 (1.02–1.98)	1.41 (0.44–4.47)
Sexual partners >5 years before baseline				
0	1.00	1.00	1.00	1.00
1	1.90 (1.50–2.40)	1.04 (0.66–1.64)	1.61 (1.22–2.12)	0.63 (0.30–1.32)
≥2	2.66 (2.04–3.47)	2.06 (1.28–3.30)	2.56 (1.87–3.51)	1.46 (0.74–2.88)
Time dependent[c]				
Sexual partners since last visit				
0	1.00	1.00	1.00	1.00
≥1	0.82 (0.63–1.06)	1.58 (0.86–2.91)	0.92 (0.67–1.26)	1.98 (0.77–5.13)
Condom use since last visit				
No	1.00	1.00	1.00	1.00
Yes	1.19 (1.01–1.40)	1.01 (0.69–1.47)	1.24 (1.01–1.52)	1.06 (0.54–2.08)
Smoking since last visit				
No	1.00	1.00	1.00	1.00
Yes	1.27 (1.09–1.49)	0.76 (0.53–1.08)	1.33 (1.08–1.62)	0.80 (0.48–1.35)

[a] Adjusted for all variables, SE adjusted for the correlation introduced by use of multiple measurements/subject by generalized estimating equations with an autoregressive (type 1) correlation matrix.
[b] Gonorrhea, chancre, syphilis, herpes, trichomoniasis, or candidiasis.
[c] Outcome measured at visit *t*, time-dependent variables refer to exposure in the interval between visit *t-1* and visit *t*.

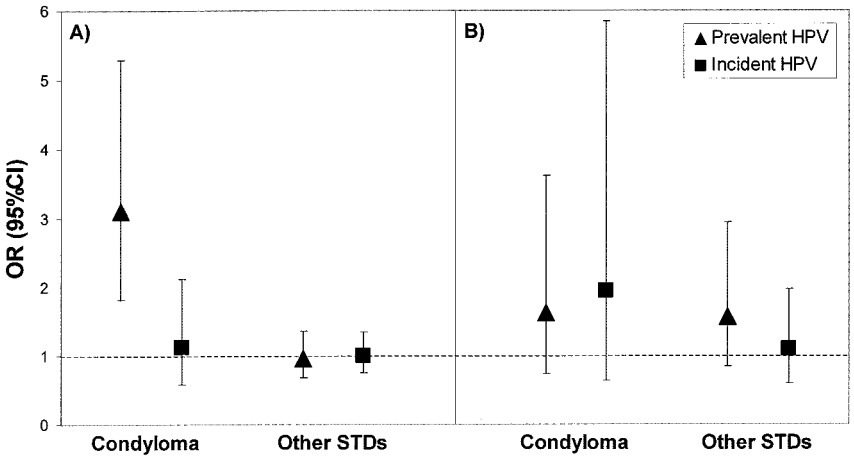

Fig. 1. ORs and 95% CIs for the associations between history of STDs and HPV infection (*A*) and HPV coinfection (*B*), adjusted for age and number of sexual partners in past 5 years. HPV infection and coinfection are classified as either prevalent (denoted by ▲) or incident (denoted by ■).

types and oncogenic types but had no association with multiple HPV infections. Some published studies also have produced similar, apparently counterintuitive results, suggesting that condom use was associated with increased likelihood of infection among users (9, 11, 32, 33). Some have raised the difficulty in adequately measuring some potentially relevant aspects of condom use (9, 11). Misclassification related to a late application of the condom during intercourse could dilute the protective effect of condom use. The possible association of condom use with different sexual behaviors not captured by other variables might result in confounding if condom use served as a marker for such behaviors. Smoking was associated with an increased risk of HPV infection but not coinfection. If it is presumed that the mode of action would be through a decreased immune

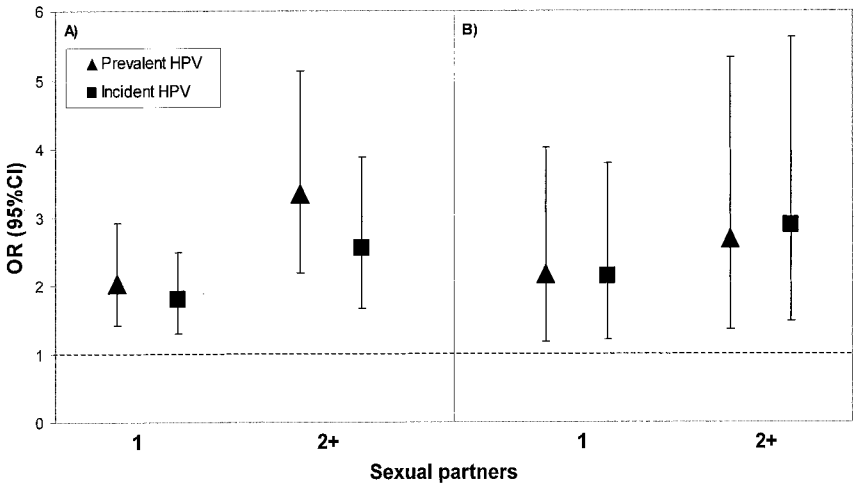

Fig. 2. ORs and 95% CIs for the associations between number of sexual partners in the past 5 years and HPV infection (*A*) and HPV coinfection (*B*), adjusted for age and history of STDs. HPV infection and coinfection are classified as either prevalent (denoted by ▲) or incident (denoted by ■).

response, it is possible that smoking would increase the likelihood of HPV infection. When restricting the analysis to women who were HPV-positive, it is possible that we restricted attention to women whose immune response to HPV was at least partially inadequate and, in whom, therefore, smoking status would not have had an additional effect.

We observed that the association between history of condyloma and risk of HPV infection decreased with increasing time since baseline. This suggests that the HPV infection detected at baseline could have been a direct result of the reported condyloma. Because the temporal sequence between history of condyloma and prevalent HPV infection is not clearly defined, reverse causality cannot be excluded, *i.e.*, the reported condyloma could be a result of a persistent HPV infection detected at baseline. However, this interaction between time since baseline and history of condyloma was not observed when the outcome was multiple HPV infection among HPV-positive women. This underscores the importance of questioning cross-sectional relationships obtained between putative risk factors and prevalent HPV infection.

The predictors of multiple HPV infections were substantially the same whether HPV detection was performed for all HPV types or limited to oncogenic HPVs, although estimates were less precise in the latter analyses because of the smaller sample size. However, one striking difference was observed. Women who attended college or university were at substantially higher risk of coinfections than women who had not completed elementary school. Although these estimates were adjusted for age and number of sexual partners, it is possible that this reflects some unmeasured aspect of sexual behavior such as contacts with higher risk partners from a different social milieu. Additional studies should investigate the replicability of this intriguing finding in different populations.

Our approach in identifying predictors of coinfections with multiple HPV types was different from that of previous studies (14, 34). Instead of comparing predictors of single infections to those of multiple infections, we directly contrasted multiple to single-type infections by restricting the analysis to HPV-positive women. We observed that the strongest predictors of infection with multiple HPV types, relative to single infections, were younger age and a larger number of sexual partners in the 5 years before study entry. Young age was a stronger risk factor for multiple than single infection in a study conducted in Colombia (14). Younger age could be a proxy for a less efficient immune response to HPV because type-specific antibodies will be generated as new HPV types are encountered through sexual transmission. Individuals closer in time to onset of sexual activity would presumably have encountered less HPV types and would only be protected against those with which they have already been infected. The development of an immune response has often been suggested as the explanation for the decreasing prevalence of HPV infection with increasing age (35) and could also be related to infection with multiple HPV types. It is conceivable that qualitative differences between sexual partners of younger women as compared with partners of older women may also explain part of the association with age. Number of sexual partners was not associated with multiple HPV infections in previous studies. However, these studies have measured either the regular (14) or lifetime (34) number of sexual partners, which may not be the most relevant measures of sexual activity for multiple HPV infections. We observed that HPV coinfections were associated with number of sexual partners in the past 5 years but not with lifetime partners. Having numerous sexual partners increases an individual's likelihood of HPV exposure and thus HPV infection (3, 36). Our results suggest that by increasing the number of different HPV types to which a woman may be exposed, having numerous sexual partners could also lead to a greater risk of infection with multiple HPV types.

In other studies that assessed risk factors of HPV coinfections, these infections were defined as concurrent, *i.e.*, detected in the same specimen (14, 34). The longitudinal design of our study allowed us to define the HPV status not only in a concurrent fashion with the added benefit of using multiple measurements/subject but also as a cumulative measure over a median follow-up of 2 years. We had hypothesized that the cumulative HPV status may be less prone to misclassification resulting from limitations in sensitivity of cervical cell sampling and HPV tests. For example, a coinfection missed at one visit because only one HPV type had been detected may be captured at the next visit. Comparisons of analyses with a single outcome representing cumulative infection, with repeated measures analyses of concurrent coinfection showed, on average, stronger associations with cumulative HPV status (data not shown). This pattern of results suggests that our misclassification hypothesis may have been correct. On the other hand, repeated measures analyses allowed us to study variables whose

values changed over time, an issue not addressed in previous studies (14, 34).

We carried out some sensitivity analyses to assess the potential impact of some of our methodological decisions. Specifically, our analyses of cumulative HPV status were limited to a subset of women with either the outcome of interest (any HPV infection, HPV coinfection) or at least four completed visits to account for the fact that more frequent visits would increase the opportunity of infection or coinfection being detected. When the analyses were redone with all subjects while adjusting for the number of visits, results were very similar to those limited to a subset of women (data not shown). Also, we did not exclude subjects from these analyses based on cytological abnormalities. Cervical cytological abnormalities result from progression of persistent rather than transient HPV infections (37–39). Therefore, excluding women with abnormal Pap smears could have resulted in a study subset where transient infections would have been overrepresented and persistent ones underrepresented, which could have biased the estimates of association. Given the current thinking that HPV infection occurs in a pathway that can eventually lead to cervical dysplasia (22), it is pertinent to include these highest risk subjects in a study focusing on predictors of HPV coinfection. Nonetheless, all models were rerun excluding women with any level of cytological abnormalities and similar conclusions were obtained.

We acknowledge the limitations of our study. It is possible that some HPV types were preferentially amplified and detected by the laboratory protocol that we used and that some types were underdetected. The impact would have been an underascertainment of multiple infections. There is no reason to suspect that this did not occur randomly according to levels of putative risk factor variables, therefore resulting in unbiased relative risk estimates. Misclassification of risk factor information may result in biased estimates of association. However, a recent study found acceptable reproducibility for information on sexual behavior, although the follow-up period was relatively short (40).

In conclusion, we have observed that predictors of HPV coinfection include younger age, greater number of sexual partners (and particularly recent rather than lifetime sexual partners), a history of condyloma but not of other STDs, and a younger age at first sexual intercourse. These results may help in the interpretation and planning of the upcoming large scale trials of mono- and polyvalent vaccines by providing a better characterization of subjects susceptible to multiple HPV infections. Our results suggest that the risk factor profile for HPV coinfection among HPV-infected women shares several similarities with risk factors for any HPV infection.

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