

Cervical Coinfection with Human Papillomavirus (HPV) Types as a Predictor of Acquisition and Persistence of HPV Infection

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Interest in coinfection with multiple types of human papillomavirus (HPV) has increased in response to the possibility of vaccination and the discovery that the host immune response appears to be mainly type specific. This study attempts to document the occurrence of coinfection with multiple HPV types and to determine whether these coinfections predicted acquisition or persistence of other HPV types in a prospective cohort of women in Brazil. Multiple HPV types were detected at the same visit in one-fifth of all women who tested positive for HPV at any time. Acquisition of an HPV infection was more likely among women with any HPV type detected on study entry. Persistence of HPV infection, the true precursor of cervical abnormalities, was independent of coinfection with other HPV types. Given the increasing prominence of HPV vaccination as a potential preventive approach, it is imperative that additional insights on cross-type protection be obtained from longer-term longitudinal investigations.

Human papillomaviruses (HPVs) are generally recognized to be the central causative agent of cervical cancer [1]. HPV DNA has been detected in virtually all invasive cervical tumors, with types 16, 18, 31, 33, and 45 identified in >80% of the HPV-harboring specimens [2, 3]. However, HPV is also the most common sexually transmitted virus and is often present in the cervical epithelia of women who have no cytologic abnormalities. Screening has substantially reduced the incidence of and mortality due to cervical cancer, but organized screening programs are particularly difficult to implement in nonindustrialized countries, where the majority of invasive cancers are diagnosed [4]. Vaccines to prevent infections in young men and women would have the potential to further reduce the burden of cervical cancer. Several clinical trials aimed at determining the safety and immunogenicity of prophylactic HPV vaccines are under way or have been reported [5, 6]. Humoral immunity

against HPV infection is primarily type specific [7–10], although some evidence of cross-protection has been found, depending on the antigens used for immunization [11]. Potential vaccines will, therefore, either target HPV-16, which is detected in 50% of cervical tumors, or multiple HPV types, such as HPV-16, HPV-18, HPV-31, and HPV-45.

To date, little attention has been given to coinfection with >1 HPV type and to the effect that infection with some HPV types may have on acquisition or loss of other types. Recently, interest in coinfections has been heightened because of the imminent availability of vaccination against HPV infection. The introduction of vaccination into a population may modify an established equilibrium in the distribution of HPV types [12]. In addition to immunologic factors involved in the interplay between the types, competition or synergy could exist among HPV types infecting the same epithelium. A conceivable outcome of successful HPV vaccination is that the decreased prevalence and incidence of the HPV types targeted by vaccines would influence the distribution of infection with other HPVs. Two studies have addressed the dynamics of acquisition and loss of HPV infections in the presence of other HPV types [12, 13]. In a study on concurrent and sequential acquisition of multiple HPV types in a population of young, female, American university students, Thomas et al. [13] concluded that coinfection with multiple HPV types occurred more often than would be expected to result from chance alone, but they did not identify types that were more likely to be detected together. Liaw et al. [12] observed women who tested positive or negative for HPV-16 at the enrollment visit, to document subsequent acquisition, clearance, and persistence of other HPV types. Presence of HPV-16 in the enrollment specimen was associated with an increased risk of acquisition

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of other types, but no effect on clearance or persistence was found.

We analyzed data from our ongoing cohort study of HPV infection in Brazil to assess the phenomenon of coinfection among women who are slightly older than those included in the 2 studies just cited [12, 13]. In addition, we obtained information from 4 consecutive visits for most of the subjects, which allowed us to look at both acquisition and loss over a relatively long follow-up period with frequent HPV DNA detection. Our objectives were to document the occurrence of cervical coinfection with multiple HPV types; to estimate the risk of subsequent infection, judging by baseline HPV status (status at enrollment); and to study the loss of individual infections associated with the presence of coinfection with other HPV types.

Subjects, Materials, and Methods

Study population. Our subjects are a subset of women enrolled in the Ludwig-McGill Cohort Study, a longitudinal investigation of the natural history of HPV infection and precursor lesions of cervical cancer, with repeated assessments of viral and cytologic end points and of lifestyle, nutritional, and behavioral risk factors. A detailed description of the study design and methods has been published elsewhere [14]. In brief, women attending a comprehensive maternal and child health program (Maternidade Escola Vila Nova Cachoeirinha) for low-income families in São Paulo, Brazil, were eligible to participate if they met the following criteria: (1) age 18–60 years, (2) permanent residence in São Paulo City, (3) no current pregnancy and no plans to become pregnant for 1 year, (4) intact uterus and no current referral for hysterectomy, (5) no reported use of vaginal medication in the previous 2 days, and (6) no reported treatment for cervical disease in the previous 6 months. In addition, women had to indicate that they would comply with scheduled visits for at least 2 years after entering the study.

Recruitment of the women enrolled in this study occurred between November 1993 and March 1997. Follow-up consisted of 1 visit every 4 months for the first year and 2 visits per year for the next 4 years, for a total follow-up period of 5 years. At each visit, subjects answered an interviewer-administered questionnaire designed to collect information on sociodemographic, lifestyle, sexual, reproductive, and contraceptive factors. Cervical specimens were also collected at each visit for Papanicolaou cytologic examination and HPV testing.

The analyses described here were carried out on data from a subset of the Ludwig-McGill Cohort Study, namely, the first 1860 women who had completed up to 4 visits and whose specimens were adequate for the testing of HPV DNA (i.e., were β -globin positive). On average, 2.4% of the specimens tested per visit were β -globin negative. For analysis of data on acquisition of HPV, women were included if they had HPV testing results for the baseline visit and at least 1 consecutive return visit ($n = 1690$). For analysis of data on loss of any HPV infection, women were included if they had positive results of testing for HPV DNA in at least 1 visit, followed by an informative (available) result of HPV testing in at least 1 visit ($n = 426$). For analysis of data on loss of type-specific HPV, only

women who harbored that type during follow-up and who attended at least 1 follow-up visit were considered.

Detection of HPV DNA. Ectocervical and endocervical cells were collected with an Accelon biosampler (Medscand) and immersed in Tris-EDTA buffer (pH 7.4). The DNA from these samples was purified by spin column chromatography and amplified with the MY09/11 polymerase chain reaction (PCR) protocol, which targets a conserved 450-bp region of the L1 gene [15, 16]. Hybridization of the amplified products with generic and type-specific oligonucleotide probes and restriction fragment–length polymorphism analyses were used to identify >40 different types of genital HPV [17]. Amplified products that hybridized only with the generic probe were considered to be positive for unknown types. Quality of the DNA specimens was verified by amplification of a 268-bp region of the human β -globin gene [15]. All specimens were tested blindly. Standard precautions were taken to prevent specimen contamination.

HPV types were analyzed either individually or in groups based on oncogenic potential. We classified HPV-16 and HPV-18 together (HPV-16/18), because they are the types most commonly found in cervical tumors [2] and are frequently targeted in vaccine studies. Other oncogenic HPV types were those most commonly found in cervical tumors, after HPV-16 and HPV-18 (types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) [2]. All other individual HPV types were classified as nononcogenic (types 6, 11, 26, 32, 34, 40, 42, 44, 53–55, 57, 61, 62, 64, 66, 67, 69, 70–73, 81–84, CP6108, and IS39).

Statistical analyses. We produced simple frequency distributions to describe the patterns of HPV positivity among women who had complete follow-up and HPV results for the first 4 visits in the study (typically 0, 4, 8, and 12 months). The prevalence of coinfection at each visit was calculated by dividing the number of specimens in which multiple HPV types were identified by the number of informative specimens for that visit. We used the Kaplan-Meier technique to plot the cumulative probability of acquiring an infection (of any type, nononcogenic type, or oncogenic type) against the length of follow-up. We carried out Cox proportional hazards regression analyses to estimate the hazard ratios (HRs) and 95% confidence intervals (CIs) of acquisition of HPV infection, according to baseline HPV status (HPV negative, nononcogenic type, oncogenic type other than HPV-16/18, HPV-16/18), and according to HPV status at the first 2 visits. Time zero was visit 1 for the former and visit 2 for the latter sets of analyses. Empirical confounding variables were included in the adjusted Cox models of HPV acquisition. Empirical confounding variables were defined as those variables that resulted in a $\geq 10\%$ change in the HR for at least 1 of the levels of the main exposure variable (baseline HPV status) [18]. Sociodemographic characteristics, markers of sexual behavior, and reproductive, contraceptive, and lifestyle factors were evaluated as potential confounding variables. We also used Cox regression to estimate the likelihood that an index infection would be cleared, according to the presence or absence of coinfections by other HPV types, with time zero set at the visit at which the index infection was first detected.

Table 1. Frequency distribution of patterns of overall human papillomavirus (HPV) infection and infection with HPV-16 or HPV-18 (HPV-16/18), among all women tested for HPV, in the first 4 visits in the Ludwig-McGill Cohort Study.

Results of testing for HPV					
Visit 1 (study entry)	Visit 2 (4 months)	Visit 3 (8 months)	Visit 4 (12 months)	Overall HPV infection	HPV-16/18
—	—	—	—	958 (68.9)	1308 (94.1)
+	—	—	—	62 (4.5)	16 (1.2)
—	+	—	—	39 (2.8)	8 (0.6)
—	—	+	—	41 (2.9)	6 (0.4)
—	—	—	+	50 (3.6)	18 (1.3)
+	+	—	—	31 (2.2)	6 (0.4)
—	+	+	—	11 (0.8)	0
—	—	+	+	26 (1.9)	4 (0.3)
+	—	+	—	8 (0.6)	1 (0.1)
—	+	—	+	13 (0.9)	0
+	—	—	+	5 (0.4)	0
+	+	+	—	32 (2.3)	6 (0.4)
—	+	+	+	29 (2.1)	7 (0.5)
+	+	—	+	14 (1.0)	0
+	—	+	+	12 (0.9)	0
+	+	+	+	59 (4.2)	10 (0.7)

NOTE. Data are no. (%) of subjects. Total no. of subjects was 1860; for 470 of those, data were incomplete (subjects had <4 informative results; either they were yet to be tested or ≥1 specimen yielded negative results on testing for β-globin). The no. of subjects with complete data (1390) was used to calculate percentages.

Results

Of the 1865 women who had HPV test results from multiple visits, 5 women were excluded, 2 because they did not provide questionnaire information and 3 because their specimens were invalid (either the specimen was β-globin negative or it had not yet been tested). The participants' mean age was 33.1 years (median, 33.0; SD, 8.8). One-fifth of the women were <25 years old (n = 360), 39% were 25–34 years old (n = 726), 30% were 35–44 years old (n = 560), and 11.5% were 45–60 years old (n = 214). Among the 1860 women who had multiple valid results of testing for HPV in the first year of follow-up, information from 4 consecutive visits was available from 1390 women (results shown in table 1). HPV testing was negative at all 4 visits in 958 women (68.9%) and was positive at only 1 visit in 192 women (13.8%), at 2 visits in 94 women (6.8%), at 3 visits in 87 women (6.3%), and at all 4 visits in 59 women (4.2%). Simi-

larly, 82 women (5.9%) tested positive for HPV-16 or HPV-18 in at least 1 visit during follow-up, and 48 (3.5%) of those women harbored HPV at only 1 visit, whereas the other 34 women (2.4%) tested positive for HPV-16 or HPV-18 in >1 visit.

Among all women who tested positive for HPV at any point (n = 536) during the first 4 visits, 102 (19%) harbored an HPV coinfection with multiple HPV types at least once during follow-up. When each visit was considered individually, the prevalence of HPV coinfection was 2.0%–2.8%, representing 13.3%–17.5% of all HPV infections at that visit (table 2). The maximum number of HPV types detected at 1 visit was 4, but >75% of the coinfections involved only 2 HPV types. When data were stratified by age (≤25 vs. >25 years), we observed a higher point prevalence of HPV infection and a higher prevalence of coinfection in younger women. Among younger women (n = 415), the mean point prevalence of HPV infection (visits 1–4) was 20.6%. On average, 20% of these infections were

Table 2. Prevalence of coinfection by multiple human papillomavirus (HPV) types and number of types detected, by visit.

Visit	No. of specimens tested	No. (%) of subjects with HPV infection	No. (%) of subjects with coinfection			
			Total	2 Types	3 Types	4 Types
1 (Study entry)	1789	286 (16.0)	50 (2.8)	41 (2.3)	7 (0.4)	2 (0.1)
2 (4 months)	1715	283 (16.5)	42 (2.4)	36 (2.1)	5 (0.3)	1 (0.06)
3 (8 months)	1574	240 (15.2)	35 (2.2)	26 (1.7)	8 (0.5)	1 (0.06)
4 (12 months)	1528	226 (15.0)	31 (2.0)	24 (1.6)	7 (0.5)	0

NOTE. Differences between the sum of percentages of subjects with coinfection and the total percentage are due to rounding error.

coinfections (27%, 16%, 19%, and 20% for visits 1, 2, 3, and 4, respectively). Among women >25 years old ($n = 1445$), the mean point prevalence of HPV infection was 14.1%. The proportion of coinfections was a mean of 13% of all HPV infections in this group (13%, 14%, 13%, and 11% for visits 1, 2, 3, and 4, respectively), somewhat lower than the mean proportion among women ≤ 25 years old. The period prevalence (detection of coinfection at least once during the 4 visits) was 24.4% among women ≤ 25 years old and 16.8% among women >25 years old ($P = .052$).

Figure 1 shows the cumulative probabilities of acquisition of HPV infections (any type, oncogenic type, nononcogenic type) according to baseline HPV status. Women free of HPV infection at entry had a lower probability of acquiring an infection (any type, oncogenic type, nononcogenic type) during follow-up, compared with women who were infected at enrollment. No striking differences were seen in the probability of acquisition of any HPV type and of acquisition of nononcogenic types among women who harbored HPV-16 or HPV-18, other oncogenic types, or nononcogenic types at baseline until 12 months of follow-up (figure 1A and 1B). Women who harbored HPV at baseline, regardless of type, were more likely to acquire an infection with any different type and with a (new) nononcogenic type during follow-up than were women who were HPV negative at baseline. Among women who were infected with HPV-16 or HPV-18 at baseline, the greater probability of detection of infection with a different type after the first 12 months of follow-up was based on small numbers. Women with HPV-16 or HPV-18 at baseline had a greater cumulative probability of acquiring an oncogenic HPV infection during the first 12 months of follow-up than those who were infected with other types at entry (figure 1C), but the cumulative probabilities tended to converge with extended follow-up.

Table 3 summarizes the results obtained from Cox models that compared time to acquisition of HPV by baseline HPV status. Even after adjustment for empirical confounding variables, the hazards of acquisition of any HPV type, nononcogenic types, and oncogenic types were higher among women who were infected with HPV at baseline, regardless of the type or oncogenic potential of the index infection, compared with women who were HPV negative at the baseline visit. Women harboring HPV-16 or HPV-18 infections at baseline were at the greatest risk of acquiring infections with any type, nononcogenic types, and oncogenic types other than HPV-16/18, although the point estimates did not differ substantially from those for women in whom other HPV types were detected at baseline. No increased risk of acquisition of HPV-16 or HPV-18 was observed among women who were infected with HPV at baseline. Adjusted HRs were consistent with a decreased risk of acquiring HPV-16 or HPV-18 among women with HPV infections at baseline, compared with the risk for women who were initially HPV negative, although this finding was not statistically significant and was based on few events.

We did a similar analysis, focusing on type-specific acquisition of HPV and restricted to types with a cumulative incidence of $\geq 1\%$ in the first year of follow-up, specifically types 16 (2.4%), 31 (1.1%), 51 (1.5%), 52 (1.1%), 53 (2.3%), 58 (1.2%), and 84 (1.0%). Women who had HPV infections at baseline were just as likely as HPV-negative women to acquire HPV-16 during follow-up. This lack of association was observed only with acquisition of HPV-16. The risk of type-specific acquisition

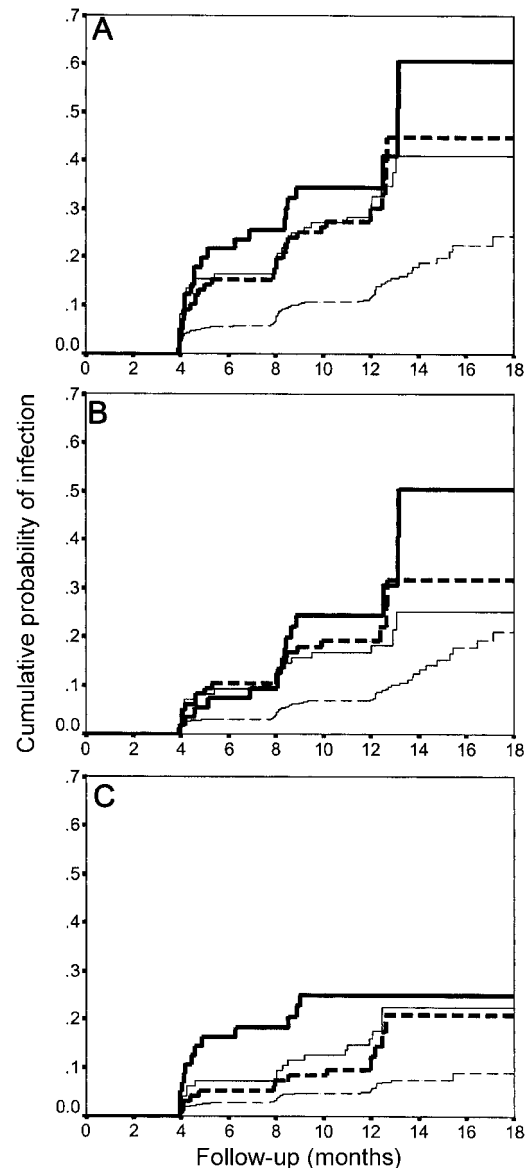


Figure 1. Cumulative probability of acquisition of an infection with (A) any HPV type, (B) nononcogenic HPV types, and (C) oncogenic HPV types, by HPV status at enrollment. Thick solid lines, HPV-16 or HPV-18 detected at enrollment; thick dashed lines, oncogenic HPV types other than HPV-16 or HPV-18 detected at enrollment; thin solid lines, nononcogenic HPV types detected at enrollment; thin dashed lines, no HPV detected at enrollment.

Table 3. Hazard ratios (HRs) and 95% confidence intervals (CIs) for acquisition of human papillomavirus (HPV) infection, by HPV status at enrollment.

HPV status at enrollment	No. of subjects with incident infection	Woman-months	Crude HR (95% CI)	Adjusted HR (95% CI) ^a
Acquisition of any HPV type ^b				
Negative	188	16,326	Reference	Reference
Nononcogenic	33	1080	2.6 (1.8–3.8)	2.1 (1.5–3.1)
Oncogenic, except HPV-16/18	33	1066	2.7 (1.9–3.9)	2.2 (1.5–3.2)
HPV-16/18	21	562	3.4 (2.2–5.3)	2.6 (1.7–4.2)
Acquisition of nononcogenic HPV types ^c				
Negative	121	16,768	Reference	Reference
Nononcogenic	19	1153	2.2 (1.3–3.5)	1.9 (1.1–3.1)
Oncogenic, except HPV-16/18	22	1109	2.8 (1.8–4.5)	2.3 (1.4–3.7)
HPV-16/18	15	609	3.5 (2.1–6.0)	3.0 (1.7–5.1)
Acquisition of oncogenic HPV types, except HPV-16/18 ^d				
Negative	87	16,793	Reference	Reference
Nononcogenic	19	1203	3.0 (1.8–5.0)	2.1 (1.2–3.5)
Oncogenic, except HPV-16/18	14	1181	2.3 (1.3–4.0)	1.4 (0.8–2.6)
HPV-16/18	13	593	4.5 (2.5–8.0)	3.7 (2.0–6.7)
Acquisition of HPV-16/18 ^e				
Negative	43	17,118	Reference	Reference
Nononcogenic	3	1289	0.8 (0.2–2.6)	0.6 (0.2–2.1)
Oncogenic, except HPV-16/18	3	1209	1.0 (0.3–3.3)	0.8 (0.2–2.5)
Acquisition of HPV-16 ^e				
Negative	35	17,126	Reference	Reference
Nononcogenic	3	1289	1.0 (0.3–3.2)	0.9 (0.3–2.9)
Oncogenic, except HPV-16/18	3	1209	1.3 (0.4–4.1)	1.0 (0.3–3.3)
HPV-16/18	0	126	—	—
Acquisition of HPV-31 ^f				
Negative	15	17,238	Reference	Reference
Nononcogenic	2	1281	1.8 (0.4–7.8)	1.1 (0.2–5.2)
Oncogenic, except HPV-16/18	0	1051	—	—
HPV-16/18	4	636	6.8 (2.3–20.7)	8.1 (2.5–26.5)
Acquisition of HPV-51 ^g				
Negative	15	17,208	Reference	Reference
Nononcogenic	6	1260	5.5 (2.1–14.2)	3.4 (1.2–9.3)
Oncogenic, except HPV-16/18	6	991	6.9 (2.7–17.8)	3.5 (1.2–10.0)
HPV-16/18	0	631	—	—

(continued)

of HPV-31, HPV-52, HPV-58, or HPV-84, but not of HPV-51 or HPV-53, was generally highest among women who harbored HPV-16 or HPV-18 at baseline. The risk of type-specific acquisition of HPV among women who were infected with nononcogenic types at baseline, compared to that for HPV-negative women, was elevated for acquisition of some types (HPV-51 and HPV-52), decreased for the acquisition of others (HPV-53 and HPV-58), and similar to that of the remaining groups at baseline for HPV-31 and HPV-84. Presence of oncogenic HPV types other than HPV-16/18 at baseline was associated with a greater risk of acquiring only HPV-51 and HPV-53, compared with the risk for women who were HPV negative at baseline. Adjustment for confounding variables generally moved the estimates closer to the null value, but substantial associations were seen, even after extensive control for empirical confounding variables. Because of the small numbers of type-specific incident infections included

in the analyses, CIs were generally wide, and few HRs reached statistical significance.

Because most HPV infections are transient [19], the detection of an HPV type at baseline may have limited value as a predictor for acquisition of a subsequent infection. We therefore assessed HPV status at the first 2 visits as a predictor of acquisition of HPV infection at subsequent visits (table 4). In general, women in whom HPV was detected at either of the first 2 visits were more likely to acquire other HPV infections during follow-up than were women who were HPV negative at both visits. Adjusted HRs indicated a similarly increased risk of acquisition of any HPV type and of nononcogenic types among women with transient infection, persistent infection with nononcogenic types, and persistent infection with oncogenic types other than HPV-16/18. Among women who harbored persistent infections with either HPV-16 or HPV-18, the adjusted HRs for acquiring any HPV type and for acquiring nononcogenic types were even

Table 3. (Continued.)

HPV status at enrollment	No. of subjects with incident infection	Woman-months	Crude HR (95% CI)	Adjusted HR (95% CI) ^a
Acquisition of HPV-52 ^h				
Negative	13	17,238	Reference	Reference
Nononcogenic	4	1271	4.4 (1.4–13.4)	2.3 (0.7–8.2)
Oncogenic, except HPV-16/18	1	1052	1.3 (0.2–9.7)	0.8 (0.1–7.5)
HPV-16/18	2	644	4.6 (1.0–20.4)	2.8 (0.6–13.7)
Acquisition of HPV-53 ⁱ				
Negative	28	17,198	Reference	Reference
Nononcogenic	1	1062	0.6 (0.1–4.2)	0.5 (0.1–3.6)
Oncogenic, except HPV-16/18	10	1136	5.7 (2.8–11.7)	4.8 (2.3–10.2)
HPV-16/18	2	592	2.1 (0.5–8.8)	1.9 (0.4–8.0)
Acquisition of HPV-58 ^j				
Negative	13	17,235	Reference	Reference
Nononcogenic	1	1289	1.0 (0.1–8.0)	0.7 (0.1–5.7)
Oncogenic, except HPV-16/18	1	987	1.4 (0.2–10.5)	1.0 (0.1–8.1)
HPV-16/18	3	656	6.2 (1.8–21.7)	4.9 (1.3–18.2)
Acquisition of HPV-84 ^k				
Negative	11	17,253	Reference	Reference
Nononcogenic	2	1154	2.1 (0.4–10.3)	0.9 (0.1–7.3)
Oncogenic, except HPV-16/18	1	1225	1.2 (0.1–9.1)	0.7 (0.1–5.9)
HPV-16/18	3	657	7.4 (2.1–26.7)	5.4 (1.3–23.0)

NOTE. HPV-16/18, HPV-16 or HPV-18; STDs, sexually transmitted diseases.

^a HRs and 95% CIs were adjusted for empirical confounding variables.

^b Adjusted for age and no. of sex partners (lifetime, past 5 years).

^c Adjusted for age and no. of sex partners (lifetime, past 5 years, past year).

^d Adjusted for age, history of STDs, no. of sex partners (lifetime, past 5 years, past year), and no. of years since first sexual intercourse.

^e Adjusted for age and no. of sex partners (past 5 years, past year, in interval between first and second visit).

^f Adjusted for age, education, smoking status, use of tampons, history of STDs, no. of sex partners (past 5 years, in interval between first and second visit), and no. of years since first sexual intercourse.

^g Adjusted for age, use of noncommercial absorbents during menstruation, history of STDs, no. of sex partners (past 5 years, past year, in interval between first and second visit), and no. of years since first sexual intercourse.

^h Adjusted for age, education, history of STDs, no. of sex partners (lifetime, in interval between first and second visit), no. of pregnancies, and no. of years since first sexual intercourse.

ⁱ Adjusted for age and no. of sex partners (past 5 years).

^j Adjusted for age, smoking status, history of STDs, no. of sex partners (past 5 years, past year), and no. of years since first sexual intercourse.

^k Adjusted for age, education, age at menarche, history of STDs, no. of sex partners (lifetime, past 5 years, past year), oral contraceptive use, no. of pregnancies, and history of anal sex.

higher. The risk of acquisition of oncogenic types other than HPV-16/18 was similarly elevated among women who had transient infections at the first 2 visits or who had persistent infection with nononcogenic types. HRs were slightly higher among women who harbored persistent infection with an oncogenic HPV. Our extensive adjustment for confounding variables usually brought the HR estimates closer to unity, but we still observed statistically significant associations in most categories.

Finally, we investigated whether the time to clearance of an infection was associated with the presence of coinfection with other HPV types at the index visit (table 5). The index visit was defined as the first visit at which an HPV infection with an identifiable type was detected. The presence or absence of coinfection with other HPV types at the index visit was assessed. Time to loss of the index infection was compared among women with and without coinfection with other HPV types at the index visit, and the analysis was adjusted for age and sexual activity (lifetime number of sex partners). None of the HRs for this analysis

were statistically significantly different from unity. The point estimates for clearance of HPV-6/11 and HPV-59 when other types were present at the index visit suggested that women infected with these types were less likely to clear the infection. Conversely, women infected with HPV-84 and another HPV type seemed to clear HPV-84 more rapidly than women who harbored HPV-84 alone.

Discussion

In this study, we investigated the occurrence of coinfection with multiple HPV types within a large longitudinal study of HPV and cervical cancer. To our knowledge, this analysis used the largest sample size to date to examine the association between an index HPV infection and its effect on acquisition and persistence of other types. Almost 1400 women in our study population had 4 consecutive visits at 4-month intervals at which valid information on HPV status and typing was obtained;

Table 4. Hazard ratios (HRs) and 95% confidence intervals (CIs) for acquisition of human papillomavirus (HPV) infection, by HPV status at first 2 study visits.

HPV status at first 2 visits	No. of subjects with incident infection	Woman-months	Crude HR (95% CI)	Adjusted HR (95% CI) ^a
Acquisition of any HPV type ^b				
Negative both visits	93	9553	Reference	Reference
Transient, not HPV-16/18	47	1584	2.9 (2.0–4.1)	2.1 (1.4–3.0)
Transient, HPV-16/18	10	255	3.8 (2.0–7.3)	2.7 (1.4–5.4)
Persistent nononcogenic	9	372	2.3 (1.1–4.6)	2.1 (1.0–4.3)
Persistent oncogenic, not HPV-16/18	9	326	3.0 (1.5–5.9)	2.2 (1.1–4.4)
Persistent, HPV-16/18	8	192	4.6 (2.3–9.6)	3.8 (1.8–8.0)
Acquisition of nononcogenic HPV types ^c				
Negative both visits	62	9634	Reference	Reference
Transient, not HPV-16/18	27	1633	2.4 (1.5–3.7)	1.9 (1.2–3.0)
Transient, HPV-16/18	7	260	3.9 (1.8–8.5)	2.8 (1.3–6.3)
Persistent nononcogenic	7	376	2.8 (1.3–6.1)	2.3 (1.0–5.1)
Persistent oncogenic, not HPV-16/18	3	335	1.4 (0.5–4.6)	1.2 (0.4–3.7)
Persistent, HPV-16/18	6	197	5.0 (2.2–11.6)	4.3 (1.8–10.1)
Acquisition of oncogenic HPV types, except HPV-16/18 ^d				
Negative both visits	40	9686	Reference	Reference
Transient, not HPV-16/18	22	1653	3.0 (1.8–5.1)	2.0 (1.2–3.6)
Transient, HPV-16/18	3	268	2.5 (0.8–8.2)	1.8 (0.5–6.2)
Persistent nononcogenic	3	391	1.6 (0.5–5.4)	1.6 (0.5–5.3)
Persistent oncogenic, not HPV-16/18	6	330	4.6 (1.9–10.7)	3.2 (1.3–8.0)
Persistent, HPV-16/18	3	208	3.8 (1.2–12.2)	3.0 (0.9–9.7)
Acquisition of HPV-16/18 ^e				
Negative both visits	22	9755	Reference	Reference
Transient, not HPV-16/18	5	1682	1.1 (0.4–2.9)	0.8 (0.3–2.4)
Transient, HPV-16/18	0	273	—	—
Persistent nononcogenic	3	395	2.6 (0.7–9.2)	2.7 (0.8–9.6)
Persistent oncogenic, not HPV-16/18	0	339	—	—
Persistent, HPV-16/18	0	215	—	—

NOTE. HPV-16/18, HPV-16 or HPV-18.

^a HRs and 95% CIs were adjusted for empirical confounding variables.

^b Adjusted for age and no. of sex partners (lifetime, past 5 years, past year, in interval between first and second visit).

^c Adjusted for no. of sex partners (lifetime, past 5 years, past year, in interval between first and second visit).

^d Adjusted for age and no. of sex partners (lifetime, past year, in interval between first and second visit).

^e Adjusted for age, education, and no. of sex partners (past 5 years, past year).

another group of >400 women had sufficient visits to contribute to the analyses of acquisition of HPV.

Before discussing specific results, we must address the limitations of our study. First, acquisition of infection was defined as the time of first detection of a specific HPV type that differed from the type(s) detected at baseline. We defined persistence of infection as the sustained detection of HPV DNA of a specific type in ≥ 2 consecutive visits. Because on average there is an interval of 4 months between each visit and because most women reported having sexual intercourse between visits, we cannot confirm that the sustained detection of a specific type in 2 consecutive visits was not, in fact, a reacquisition of the same HPV type that occurred in the interval between 2 visits. Notably, data on molecular variants of HPV-16 and HPV-18 in this study suggest that the same molecular variants are present in the vast majority of cases in which these 2 types are persistently detected [20]. Second, despite its recognized high molecular sensitivity, the PCR protocol used in this study may have missed cases in

which the virus load was lower than the threshold of detection (possibly misclassifying some persistent cases as transient). Third, we defined the time to an event (acquisition or loss of an HPV type) as the time from the baseline visit to the first subsequent visit at which the type was (acquisition) or was not (loss) detected. This could result in an overestimation of the time to acquisition or loss. However, we are not making inferences about or estimating the time to an event but, rather, are comparing groups of women based on their baseline HPV status. By treating every subject in the same manner, no bias in estimation of HRs should be introduced by this procedure. Moreover, because the interval between visits is relatively short (approximately 4 months), any overestimation is likely relatively small.

In a sizable proportion (19%) of all women who tested positive for HPV at any time, coinfection with other types was detected at the same visit. On average, 82% of all coinfections consisted of only 2 HPV types that were detected concurrently. Most women participating in our study were not in the age group at the highest

Table 5. Hazard ratios (HRs) and 95% confidence intervals (CIs) for clearance of human papillomavirus (HPV) infection, by presence or absence of coinfection with other HPV types at the index visit.

HPV type cleared, presence of coinfection ^a	No. of subjects with clearance of the index HPV infection	Woman-months	Crude HR (95% CI)	Adjusted HR (95% CI) ^b
Clearance of HPV-6 or HPV-11				
No	20	103	Reference	Reference
Yes	6	65	0.6 (0.2–1.5)	0.4 (0.1–1.2)
Clearance of HPV-16				
No	35	389	Reference	Reference
Yes	14	159	1.0 (0.5–1.9)	1.0 (0.5–1.8)
Clearance of HPV-18				
No	9	103	Reference	Reference
Yes	2	93	0.6 (0.1–2.9)	0.8 (0.1–4.4)
Clearance of HPV-31				
No	14	162	Reference	Reference
Yes	8	195	0.7 (0.3–1.6)	0.6 (0.3–1.6)
Clearance of HPV-33				
No	9	116	Reference	Reference
Yes	4	46	0.9 (0.3–2.9)	0.9 (0.3–3.4)
Clearance of HPV-45				
No	6	34	Reference	Reference
Yes	7	49	0.6 (0.2–1.9)	0.6 (0.2–2.1)
Clearance of HPV-51				
No	21	199	Reference	Reference
Yes	7	46	1.3 (0.6–3.2)	1.3 (0.5–3.2)
Clearance of HPV-52				
No	16	109	Reference	Reference
Yes	8	60	0.8 (0.3–1.9)	0.9 (0.3–2.5)
Clearance of HPV-53				
No	27	242	Reference	Reference
Yes	19	135	1.2 (0.7–2.2)	1.2 (0.6–2.2)
Clearance of HPV-56				
No	7	66	Reference	Reference
Yes	4	23	4.1 (1.0–17.2)	1.2 (0.1–18.7)
Clearance of HPV-58				
No	14	155	Reference	Reference
Yes	5	155	0.5 (0.2–1.5)	0.6 (0.2–1.8)
Clearance of HPV-59				
No	10	57	Reference	Reference
Yes	4	34	0.6 (0.2–2.3)	0.4 (0.1–1.6)
Clearance of HPV-68				
No	8	42	Reference	Reference
Yes	5	48	0.5 (0.2–1.8)	0.9 (0.2–3.7)
Clearance of HPV-84				
No	12	108	Reference	Reference
Yes	2	23	1.7 (0.4–8.3)	1.7 (0.3–9.3)

^a Presence or absence of coinfection with other HPV types at time of detection of index HPV infection.

^b HRs and 95% CIs were adjusted for age and lifetime no. of sex partners.

risk for HPV infections (early 20s). Therefore, our estimates of the proportion of coinfections and the number of HPV types detected at the same visit are lower than would be expected in a younger study population. Our results suggest that, even among women who are considered to be at a lower risk of HPV infection (because they presumably have been exposed before and may have developed immunity), a substantial proportion of coinfections occur. The proportion of detected coinfections with multiple HPV types in a specific setting likely varies according to many factors, such

as age, sexual behavior, HPV detection method, and variables affecting immune response (e.g., immunosuppressive conditions and HLA genotypes) [21, 22].

Acquisition of an HPV infection was more likely among women in whom any type of HPV was detected at the baseline visit (figure 1). It is highly plausible that acquisition of HPV types during the study period would not be independent of prior HPV status, due to the sharing of risk factors and mode of transmission. We further adjusted this analysis for the main determi-

nants of HPV infection, in an attempt to isolate the predictive effect of an HPV infection at baseline on acquisition of other types. Our results are in concordance with those already published. Acquisition of other types has been reported as being more likely among women who previously had harbored HPV-16 [12]. A generally higher risk of sequential acquisition of HPV was also observed with most pairwise combinations of types 6, 16, 18, 31, and 45, although the associations were not statistically significant [13].

Our results expand on other findings by suggesting that infection at baseline, not only with the HPV types already cited but with any HPV type, is associated with an increased likelihood of acquisition of other HPV types at a later time. Previous HPV infection (HPV at baseline) may be a marker of higher-risk sexual behavior, but such an association should disappear once the analysis is adjusted for sexual activity variables. Sexual behavior is multidimensional and difficult to describe, both quantitatively and qualitatively. Residual confounding may remain, despite our best efforts to eliminate it by adjusting not only for a priori confounding variables but also for empirical confounding variables. However, host factors that have not been taken into account may make some women more susceptible to infection by HPV and might explain the associations that we observed [12, 13]. An inadequate immune response to HPV secondary to immunosuppressive conditions may be among these host factors [23].

Acquisition of individual HPV type was somewhat more likely in women infected with HPV-16 or HPV-18 at baseline than in women infected with nononcogenic or oncogenic types other than HPV-16 or HPV-18, even after the analysis was controlled for confounding variables. However, this observation must be interpreted cautiously, because the CIs for the HR estimates largely overlap across HPV status categories.

Defining the baseline HPV status on the first 2 visits added little information to that obtained from baseline HPV status only. In general, all of the estimates seemed to indicate a greater likelihood of acquisition when women had previously harbored an infection, regardless of the type or oncogenic potential of the infection. A transient initial HPV infection and a persistent initial infection with a nononcogenic type were similarly predictive of later HPV acquisition. The fact that either a persistent infection with HPV-16 or HPV-18 or a persistent type-specific infection with any oncogenic HPV predicted a slightly higher likelihood of acquisition of HPV may suggest that some women have a higher susceptibility, immunologic or otherwise, to acquisition and persistence of HPV infections. The fact that no reduction in risk was observed suggests that there is no competition between established (persistent) and new infections.

Our analyses of clearance of HPV infection did not suggest that some types were more or less likely to be cleared if other types were detected at the same time as the index infection. In this sense, whereas acquisition of HPV seems to occur in a non-independent manner, clearance or persistence of a type-specific

infection seems to be independent of other infections. However, if certain specific combinations of types are more likely to colonize the cervical epithelium together (synergism), then our analysis would result in misclassification of the exposure variable, because it does not identify the type detected in combination with the index infection. Despite this caveat, our results seem to point in the same direction as those of Liaw et al. [12], who noted that the presence or absence of HPV-16 did not seem to influence the persistence of type-specific infections.

Few researchers have addressed the natural history of coinfection with multiple types of HPV, although some studies have considered them to be a risk factor for HPV persistence and for preinvasive and invasive cervical lesions. Schiffman et al. [24] reported that women infected with multiple HPV types were more likely than women infected with single types to have cervical intraepithelial neoplasia of grade 1 or worse. Conversely, no difference in the proportion of coinfections with multiple HPV types was observed among the different cytologic diagnoses in a large population-based study in Costa Rica [25]. In a study of female university students in New Jersey, detection of multiple infections at a prior visit was a more important predictor of HPV persistence at ≥ 6 months into the study (odds ratio [OR], 4.1; 95% CI, 2.7–6.3) than detection of an oncogenic HPV at a prior visit (OR, 1.5; 95% CI, 1.1–2.2) [26].

Multiple infections could have a different meaning, depending on whether they are detected in younger or in older women. Younger women, especially adolescents and those in their early adult years, are in the process of building immunity against HPV, whereas older women may already have established an immune response against at least some HPV types. No obvious effect modification was observed between initial HPV status and age (≤ 25 vs. > 25 years) in the analysis of acquisition of any HPV, nononcogenic or oncogenic type (data not shown). However, this analysis was based on small frequencies. The exact meaning and implications of multiple infections among women of different ages still need to be investigated, as does the role of such infections as predictors of HPV persistence and preinvasive lesion development.

If competition exists among some HPV types for colonization of the cervical epithelium, and if a specific type (e.g., HPV-16) is targeted by vaccination, prevalence of the competing types could increase. Reassuringly, our results do not suggest that this is the case. A lower risk of acquisition of HPV associated with a given index infection at baseline would have suggested competition between HPV types. In contrast, our results suggest that prior infection with HPV increases the likelihood of acquiring another infection. Whether the observed associations are based on shared risk factors, differential type-specific host susceptibility, or synergism is not yet clear. A likely hypothesis is that increased risk arises as a result of shared risk factors, although we adjusted extensively for confounding variables in the analysis. Our results suggest that persistence of HPV infection, the true precursor of cervical abnormalities, is independent

of the presence of coinfection with multiple types. However, because HPV vaccination is gaining increasing attention as a potential preventive approach, it is imperative that we obtain additional insight from longer-term longitudinal investigations.

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