American Journal of Epidemiology
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Vol. 158, No. 5
Printed in U.S.A.
DOI: 10.1093/aje/kwg171

Determinants of Clearance of Human Papillomavirus Infections in Colombian Women with Normal Cytology: A Population-based, 5-Year Follow-up Study

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Received for publication November 22, 2002; accepted for publication February 26, 2003.

Little is known about the factors that influence clearance of human papillomavirus (HPV), the primary cause of cervical carcinoma. A total of 227 women cytologically normal and HPV positive at baseline were identified from a population-based cohort of 1,995 Bogota, Colombia, women aged 13–85 years followed between 1993 and 2000 (mean follow-up, 5.3 years). HPV DNA detection and viral load determination were based on a GP5+/GP6+ polymerase chain reaction enzyme immunoassay. Rate ratio estimates for HPV clearance were calculated by using methods for interval-censored survival time data. Analyses were based on 316 type-specific HPV infections. HPV 16 had a significantly lower clearance rate than infections with low-risk types (rate ratio (RR) = 0.47, 95% confidence interval (CI): 0.32, 0.72), HPV types related to HPV 16 (types 31, 33, 35, 52, 58) had intermediate clearance rates (RR = 0.62, 95% CI: 0.47, 0.94), and other high-risk types did not show evidence of slower clearance compared with low-risk types. Infections with single and multiple HPV types had similar clearance rates. There was no evidence of a dose-response relation between clearance and viral load. Observed was slower clearance in parous women (RR = 0.64, 95% CI: 0.47, 0.89) and faster clearance in ever users of oral contraceptives (RR = 1.38, 95% CI: 1.07, 1.77).

cervix neoplasms; cohort studies; cytology; oral contraceptives; papillomavirus

Abbreviations: CIN III, cervical intraepithelial neoplasia, grade III; EIA, enzyme immunoassay; HPV, human papillomavirus; PCR, polymerase chain reaction.

Epidemiologic and molecular studies have shown a causal relation between human papillomavirus (HPV) infection (principally high-risk types) and cervical cancer (1, 2). However, HPV is also one of the most common sexually transmitted agents and has been detected in the cervical epithelia of 10–40 percent of women who have no cytologic abnormalities (3, 4). The prevalence of HPV is age dependent, with a peak in young women after they start sexual activity (3, 5–7).

HPV infection is mostly a transient phenomenon resulting in no cervical lesions or leading to low-grade lesions that often regress spontaneously (8, 9). HPV presence is thus a necessary, but not sufficient cause of cervical neoplasia development (1). Persistence of an HPV infection appears to be a prerequisite for the development of cervical intraepithelial neoplasia, grade III (CIN III), and cervical cancer (10–13). Viral, host, and environmental factors may influence the course of HPV infection (2, 9, 14).

In 1993, the National Cancer Institute, Colombia, in collaboration with the International Agency for Research on Cancer, started a population-based cohort study on the natural history of HPV infections and cervical neoplasia in a group of low-income women from Bogota, Colombia. The country has one of the highest incidences of cervical cancer in the world (age-standardized rate, 34.4/100,000) (15), and the present study cohort showed a high prevalence of HPV at enrollment (15 percent among cytologically normal women, 26 percent among women aged <20 years) (3).

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We present here results from a 5-year follow-up analysis of HPV-positive women who had normal cervical cytology at baseline. Analysis focuses on the possible role of HPV types, viral load, and various women's characteristics on the clearance rate of HPV infection.

MATERIALS AND METHODS

Patients and study design

Between November 1993 and November 1995, the National Cancer Institute, Colombia, conducted a population census in four health districts of Bogota (3). Two thousand women aged 18–85 years were randomly identified and were invited to participate in the cohort study. Additionally, to increase information on sexually active adolescents, 200 sexually active women aged 13–17 years who self-referred to an adolescent clinic for contraceptive counseling were also invited to participate. At recruitment, the women answered a structured questionnaire on sociodemographic characteristics, sexual behavior, reproductive history, smoking, and dietary habits. After interview, all women were asked to undergo a gynecologic examination, to provide a cervical scrape (for cytologic evaluation and HPV DNA testing), and to give 10 ml of blood. Of 2,200 women invited to join the study, 53 refused to participate, eight were considered ineligible (on account of mental illness, hysterectomy, or history of cervical cancer), 29 did not provide cell specimens for HPV detection, and 14 did not fill in the epidemiologic questionnaire. In addition, the HPV test was inadequate in 101 women because of poor DNA quality (i.e., failure to amplify the β-globin gene).

A total of 1,995 (91 percent) women who had a valid questionnaire, a valid HPV test, and adequate cervical cytology were thus included in the cohort study (3). Informed consent was obtained from all study participants. The ethical committees at the National Cancer Institute, Colombia, and the International Agency for Research on Cancer approved the study protocol and the manner in which informed consent was obtained from subjects.

Follow-up consisted of a visit every 6–9 months. At each visit, a short follow-up questionnaire was administered, and a cervical scrape was obtained for cytologic evaluation and HPV testing. Follow-up ended in December 2000 or at the diagnosis of CIN III, whichever occurred first. Women who had a CIN III diagnosis underwent confirmatory biopsies and treatment. Women's HPV status was not known during the follow-up and did not influence clinical management.

The analysis described here was carried out on a subset of the study cohort. We selected 330 women who were positive for one or more HPV types at baseline. We then restricted the analysis to women whose cytology was normal (i.e., we excluded three women with inadequate cytology and 51 women with abnormal cytology; the majority (39/51) evidenced low-grade lesions). Finally, we excluded 49 women who did not have at least one follow-up visit, thus yielding a total of 227 women. Since each woman could have one or more infections with different types of HPV, we based the analyses on type-specific HPV infections rather than on individual women. Clearance of a given HPV type was defined as disappearance of the HPV type detected at enrollment. At any visit, if the HPV test was inadequate, clearance status was considered unknown and follow-up was continued.

HPV detection by polymerase chain reaction

Testing for HPV was conducted by using a standard GP5+/GP6+ polymerase chain reaction (PCR) enzyme immunoassay (EIA) (3). Briefly, HPV-positive samples were subjected to EIA-HPV group-specific analysis by using cocktail probes for high-risk and low-risk HPVs (16). The high-risk HPV cocktail probe consisted of oligoprobes for HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68; the low-risk HPV probe consisted of oligoprobes for HPV 6, 11, 26, 34, 40, 42, 43, 44, 53, 54, 55, 57, 61, 70, 71 (CP8061), 72, 73, 81 (CP8304), 82 (MM4), 83 (MM7), 84 (MM8), IS39, and CP6108. Additionally, HPV positivity was assessed by Southern blot hybridization of GP5+/GP6+ PCR products with the general probe of specific DNA fragments from cloned DNA of HPV 6, 11, 16, 18, 31, and 33 (17). Samples positive by Southern blot analyses and negative by high-risk/low-risk EIA were considered HPV X or undetermined type and were classified as low risk. The low-risk cocktail probe contained some HPV types—namely, 26, 34, 53, 73, and IS39—of unknown oncogenic potential. These types were classified as "high-risk" for analysis purposes.

During follow-up, a new GP5+/GP6+ PCR reverse line blot analysis was developed and was used to type the same 37 different HPV types detected by EIA. The findings from the GP5+/GP6+ PCR reverse line blot analysis were compared with those from the PCR-EIA assay and were found to agree in 96 percent of cases (kappa = 0.77) (18).

Viral load analysis

PCR-EIA can be used as a semiquantitative method to assess the relative amount of HPV DNA in cervical scrapes because of the linear relation between the amount of DNA and the optical density in the range of $10–10^6$ genome equivalents. A semiquantitative, noncompetitive GP5+/GP6+ PCR-EIA was carried out according to the method described by Jacobs et al. (19). Viral load of the samples was analyzed in two ways: 1) by using an a priori classification of low (optical density, <0.5), medium (optical density, ≥0.5–<1.5), and high (optical density, ≥1.5); and 2) by classifying the optical density into quintile groups to obtain a clearer picture of the dose-response relation.

Statistical methods

The time of clearance of an HPV infection was modeled by using methods for interval-censored survival time data. Doing so is necessary since clearance times are never observed precisely but are known only to have occurred between two visits. The survival function, which describes the probability that an HPV infection has cleared as a function of time, was estimated by using the nonparametric maximum likelihood estimator (20, 21), which is the natural

generalization of the Kaplan-Meier estimator to interval-censored data. Rate ratio estimates were derived by applying Cox regression to imputed clearance times, as described by Pan (22). The analyses were implemented in R language (23) by using the survival package. Custom software was written to calculate the survival function and carry out the multiple imputation.

The following factors potentially associated with HPV clearance were considered: HPV type in which a classification (2) based on phylogenetic groups was used (low-risk types, HPV 16, types linked to HPV 16 (31, 33, 35, 52, 58), HPV 18 and types linked to HPV 18 (18, 39, 45, 59, 68), other high-risk types), multiplicity of infection, viral load (in quintile groups), age (<18, 18–24, 25–34, ≥35 years or older), educational level (none or primary, secondary, higher), number of regular sexual partners (1, ≥2), parity (0, 1–2, ≥3 children), use of oral contraceptives (ever, never), intrauterine device use (ever, never), and smoking (ever, never). For each factor, the value at baseline was used. The basic unit of analysis when modeling clearance times was an HPV infection. To account for possible correlations in the clearance of times of multiple infections in the same woman, a cluster term was included in the model, and standard errors were calculated that are robust to correlations within clusters (24).

HPV infection may clear as a consequence of treatment of cervical lesions. Follow-up time was therefore censored at the date of any biopsy or treatment and, in any case, when a cytologic diagnosis of CIN III or worse was observed. This choice led to the censoring of five women. Three were infected with HPV 16 only; two developed CIN III (at months 17 and 54 after enrollment), and one developed invasive cervical carcinoma (at month 58). The other two women had single infections with HPV 52 and HPV 58 and developed one CIN III at month 5 and one CIN III at month 10 after enrollment, respectively.

RESULTS

Characteristics of the cohort

This paper presents data on 227 HPV-positive, cytologically normal women for whom a total of 1,373 cervical specimens were tested at enrollment and during follow-up visits. The median duration of follow-up for this group of women was 5.3 years, the median time interval between visits was 7 months, and the median number of visits was six. Seventy-seven percent of the women had at least four visits.

Table 1 shows the characteristics of the study population subdivided into three age groups. Median age of the participants was 29 years. Twenty-eight percent of the women reported a primary education only, 76 percent had one lifelong sexual partner, and 20 percent had not given birth to any children. Ever use of oral contraceptives was reported by 54 percent and ever use of an intrauterine device by 57 percent of the women. Twenty-nine percent of the women reported ever smoking.

In total, 316 infections were detected in the 227 studied women at the start of the study. HPV 16 was the most frequently detected HPV type (16 percent of all infections) (table 2). HPV types phylogenetically linked to HPV 16 accounted for 23 percent of infections, those linked to HPV 18 for 17 percent, and other high-risk HPV types for 15 percent. After HPV 16, HPV 58 (8 percent), HPV 18 (5 percent), and HPV 45 (5 percent) were the most common high-risk types. Low-risk types represented 30 percent of all infections. Among the low-risk HPV types, the most frequently detected were HPV 42 (5 percent) followed by HPV 81 (CP8304) (4 percent). Compared with women aged 35 years or older, women younger than age 25 years tended to have proportionally fewer HPV 16 infections but more infections with other high-risk HPV types, whereas low-risk HPV types were distributed approximately equally across the three age groups considered. Fifty-one percent of the HPV infections detected were single infections, whereas 20 percent of infections were present along with two or more other HPV types. Multiple infections were more common in women younger than age 25 years versus age 25 years or older (table 2). High viral load was found in 42 percent of HPV infections and tended to decrease slightly with women's age (table 2).

Of the single infections, 52 (32 percent) were low-risk types, 73 (45 percent) were high-risk types other than HPV 16, and 37 (23 percent) were HPV 16. Of the multiple infections, 14 (9 percent) were HPV 16, 97 (63 percent) were infections with high-risk HPV types other than HPV 16, and 43 (28 percent) were infections with low-risk HPV types. Some correlation was found between the presence of high-risk types and viral load. High viral load was detected in 55 percent of HPV 16 infections, 46 percent of infections with high-risk HPV types other than HPV 16, and 27 percent of low-risk HPV infections (data not shown).

HPV-DNA clearance

Figure 1 shows the proportion of persistent HPV infection as a function of time, overall and according to HPV phylogenetic groups. The clearance rate was not constant, but it was highest in the first 6 months of follow-up. Globally, 23 percent of HPV infections were still present at 1 year and 7 percent at 5 years. Clearance rates were lower for HPV 16 than for low-risk HPV types.

Table 3 shows age-adjusted and multivariate rate ratios of HPV clearance by several viral and host characteristics. Regarding viral characteristics, noticeable differences were found in clearance rates between different HPV types but not with multiplicity of infection or viral load. Both HPV 16 and its related types (31, 33, 35, 52, 58) showed reduced clearance rates compared with low-risk types. Conversely, there was no evidence of reduced clearance for HPV 18-linked types. The difference in clearance rate between HPV 16- and HPV 18-linked types was statistically significant ($p = 0.001$). In terms of host characteristics, reduced clearance was observed among parous women and increased clearance among ever users of oral contraceptives. Weak trends with education and age were evident, but they were not significant (table 3).

HPV type, oral contraceptive use, and parity were then combined in a multivariate model. Age and multiplicity of infection were also included as potential confounders. The

TABLE 1. Distribution of various characteristics* of 227 HPV†-positive, cytologically normal women, by age group, Bogota, Colombia, 1993–2000

	Age (years)						Total	
	≤24		25–34		≥35			
	No.	%	No.	%	No.	%	No.	%
Education								
Primary or less	9	10.8	25	26.6	30	60.0	64	28.2
Secondary, incomplete	46	55.4	38	40.4	10	20.0	94	41.4
Secondary or more	28	33.7	31	33.0	10	20.0	69	30.4
Age at first intercourse (years)								
≤15	35	42.2	14	14.9	7	14.0	56	24.7
16–18	36	43.4	32	34.0	20	40.0	88	38.8
≥19	12	14.5	48	51.1	23	46.0	83	36.6
No. of regular sexual partners								
1	63	81.8	62	68.1	40	81.6	165	76.0
≥2	14	18.2	29	31.9	9	18.4	52	24.0
Parity								
0	33	39.8	11	11.7	2	4.0	46	20.3
1–2	44	53.0	54	57.5	13	26.0	111	48.9
≥3	6	7.2	29	30.9	35	70.0	70	30.8
Oral contraceptive use								
Never	49	59.0	36	38.3	19	38.8	104	46.0
Ever	34	41.0	58	61.7	30	61.2	122	54.0
Intrauterine device use								
Never	49	59.0	34	36.2	15	30.0	98	43.2
Ever	34	41.0	60	63.8	35	70.0	129	56.8
Smoking								
Never	70	84.3	60	63.8	32	64.0	162	71.4
Ever	13	15.7	34	36.2	18	36.0	65	28.6

* Some percentages do not total 100 because of rounding.
† HPV, human papillomavirus.

multivariate model confirmed the independent effects of HPV type, oral contraceptive use, and parity.

DISCUSSION

To the best of our knowledge, our study of the natural history of HPV infection includes the longest follow-up reported so far. It also shows that clearance of HPV infection occurs chiefly in the 2 years after HPV is first detected, but rarely afterwards.

In some previous studies (25), two or more consecutive HPV-negative tests were required before HPV infection was considered cleared. We considered an infection cleared when it was absent during a single visit; in our study, the time interval between visits was relatively long (6–9 months) and thus probably sufficient to clear the infection. Indeed, only five of 223 HPV infections that had cleared and could be reevaluated during a subsequent visit were positive again for infection with the same HPV type. Defining HPV clearance as two consecutive HPV-negative tests, rather than one only, therefore would not have materially altered our results.

In our analysis, the clearance rate of HPV 16 was lower than that of low-risk HPV types. High-risk HPV types phylogenetically linked with HPV 16 showed clearance rates intermediate between those of HPV 16 and low-risk types, whereas HPV types linked with HPV 18 and other high-risk types had clearance rates similar to those of low-risk types. Franco et al. (9), in a follow-up study from Brazil (Ludwig-McGill cohort), showed that 12-month clearance was higher for low-risk HPV types (12.2 percent, 95 percent confidence interval: 9.6, 15.4) than for high-risk HPV types (9.5 percent, 95 percent confidence interval: 7.5, 11.9), but no clear difference was found between infections with HPV 16 and those with high-risk types other than HPV 16 (8.9 percent, 95 percent confidence interval: 5.8, 13.1) (9). A tendency for HPV 16, but not HPV 18, to persist longer than other HPV types has been noted in previous follow-up studies (7, 8, 26, 27), but the reasons for this phenomenon remain unclear. HPV 16 is by far the predominant type in invasive cervical cancer (28), although HPV 18 is suspected to induce a more rapid transition to malignancy than HPV 16 does (29).

TABLE 2. Distribution of 316 infections in study participants,* by viral characteristics and age group, Bogota, Colombia, 1993–2000

	Age (years)						Total	
	≤24		25–34		≥35			
	No.	%	No.	%	No.	%	No.	%
HPV† types								
HPV 16	13	10.1	21	17.4	17	25.8	51	16.1
HPV 31, 33, 35, 52, 58	30	23.3	32	26.5	10	15.2	72	22.8
HPV 18, 39, 45, 59, 68	24	18.6	19	15.7	9	13.6	52	16.5
Other high-risk	22	17.1	16	13.2	8	12.1	46	14.6
Low-risk	40	31.0	33	27.3	22	33.3	95	30.1
Multiplicity of infection								
Single	52	40.3	72	59.5	38	57.6	162	51.3
Multiple								
2 types	38	29.5	36	29.8	16	24.2	90	28.5
≥3 types	39	30.2	13	10.7	12	18.2	64	20.2
Viral load (optical density)								
Low (<0.5)	36	28.1	41	35.0	28	43.1	105	33.9
Medium (≥0.5–<1.5)	37	28.9	23	19.7	15	23.1	75	24.2
High (≥1.5)	55	43.0	53	45.3	22	33.9	130	41.9

* Some percentages do not total 100 because of rounding.
† HPV, human papillomavirus.

Studies using DNA sequencing have shown a considerable intratypic diversity for HPV 16 compared with other high-risk types, which may be critical to understanding the greater ability of HPV 16 to escape immunologic surveillance (30). Several studies have suggested that non-European variants of HPV 16 could be associated with longer persistence than European variants (31, 32) and more frequent progression into clinically relevant cervical lesions (33–35). Unfortunately, we did not have information on HPV 16 variants.

Numerous authors have suggested an association between viral load and persistence of HPV infection (11, 36–38). We used a semiquantitative PCR-EIA method to distinguish between high- and low-viral-load infections but found no evidence of a trend in persistence or a threshold effect. Other studies showed associations between high viral load and persistent cytologic abnormalities (39, 40). van Duin et al. (38) observed that high-viral-load infections in women with normal cytology conferred an increased risk of developing a CIN, most notably a high-grade CIN. Conversely, Lorincz et al. (41) reported that high viral load for 13 high-risk types of HPV, evaluated by using the Hybrid Capture 2 test, did not predict risk of CIN III.

It is worth noting that most previous studies (39) focused on HPV 16 only or measured overall viral load, whereas we had information on HPV type-specific viral load. Unfortunately, it is as yet unclear whether high viral load is the result of a few cells with a large number of virions or a large number of cells with a few virions.

Another interesting finding of our study was the similar clearance rate of single and multiple HPV infections. Some studies have shown an association between persistent HPV infection and the presence of multiple types (8). These studies have been interpreted as if women who have multiple types (e.g., women infected with human immunodeficiency virus) (42) had certain characteristics, such as a deficient immune response to HPV, that could predispose to persistent infection. Other studies (27), like ours, have shown that clearance of a type-specific HPV infection seems to be independent of the presence of a coinfection with other types, at least in immunocompetent women.

Several cross-sectional studies have shown that a woman's age is the strongest determinant of HPV prevalence, with a peak in HPV infections generally observed in women younger than age 25 years (3–5, 43). Some studies have shown that HPV infections in older women may be more persistent than those in younger women (8, 44). Possible explanations for this difference are selection of persistent infections, a decrease in the immune response (14), hormonal changes, and specific lifestyle characteristics of older women (or of their sexual partners). However, we did not confirm any unfavorable effect of age on clearance, at least among women with a normal cytologic smear. We did find a steady increase in the proportion of HPV 16 infections, but not of high-risk types other than HPV 16, across the three age groups considered.

Women's characteristics associated with persistence and/or clearance of HPV infections are not totally consistent across studies (8, 9, 11, 44) and do not always coincide with characteristics found to increase cervical cancer risk. Use of oral contraceptives, for instance, is a risk factor for CIN III and cervical cancer (45). In our study, however, HPV infections were less persistent in women who had ever used oral contraceptives than in never users. A consistent association is lacking between oral contraceptive use and the prevalence

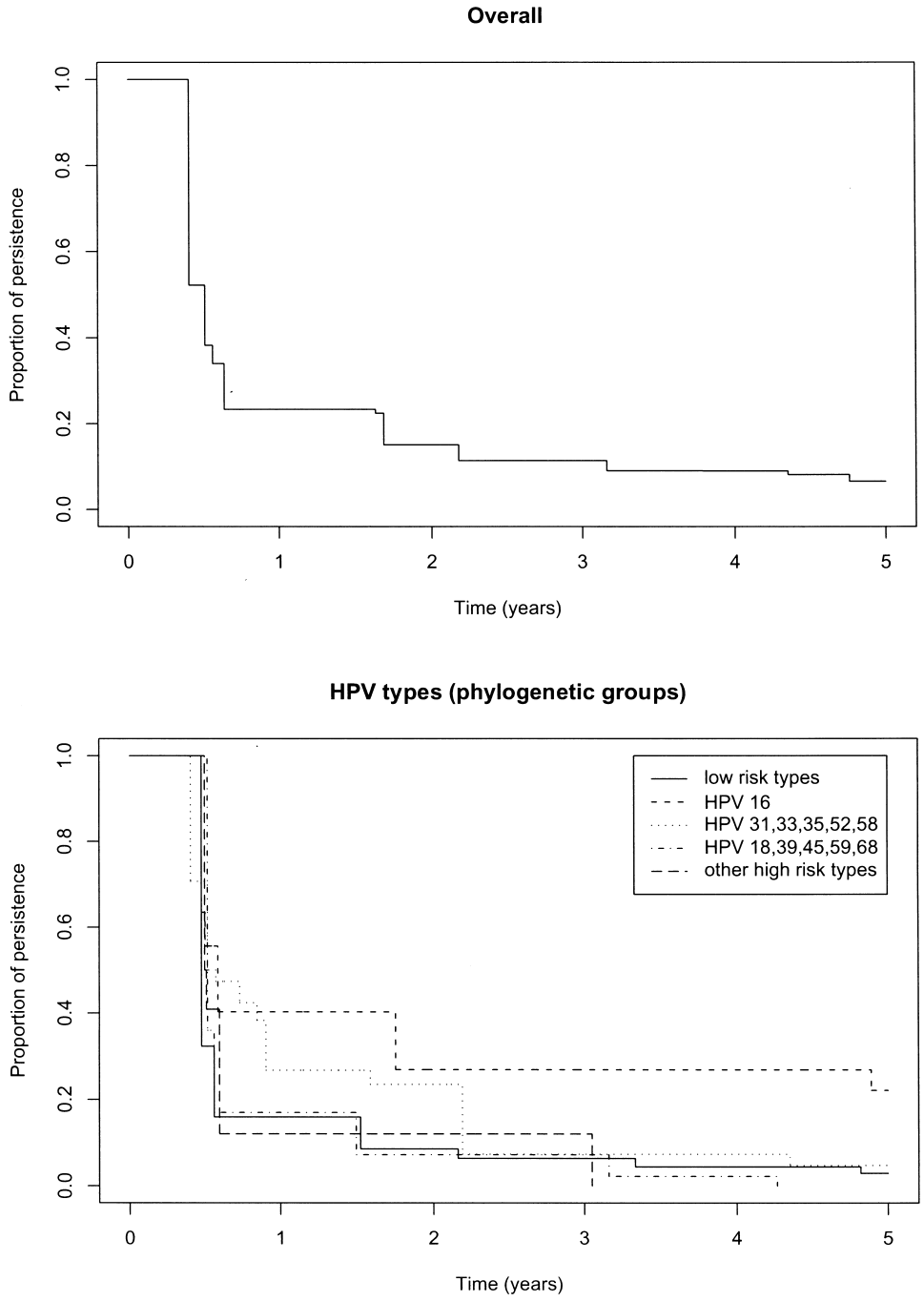

FIGURE 1. Persistence of human papillomavirus (HPV) infection, overall and by viral characteristics, Bogota, Colombia, 1993–2000.

of HPV infection in cross-sectional studies (6, 46) and among controls in case-control studies (45). In prospective studies, Moscicki et al. (25) found no association between oral contraceptive use and clearance of HPV infection, and Castle et al. (47) found no association between oral contraceptive use by HPV-positive women and subsequent incidence of CIN III or cervical cancer. High parity is associated with an increased risk of CIN III and cervical cancer (48). Our findings suggest that HPV infection in parous women may persist longer than among nulliparae.

Our follow-up study has several strengths, including the large number of women involved, the low proportion of those refusing to participate, and the presence of information on a broad range of viral and lifestyle factors. HPV testing and typing were conducted in a central laboratory by means of well-validated, sensitive methods. Some misclassification

TABLE 3. Rate ratios and 95% confidence intervals for clearance of 316 HPV* infections in study participants, Bogota, Colombia, 1993–2000

	No. of subjects	No. of infections	Age-adjusted model		Multivariate model†	
			RR*	95% CI*	RR	95% CI
HPV types						
Low-risk‡		95	1.00		1.00	
HPV 16		51	0.47	0.31, 0.69	0.48	0.32, 0.72
HPV 31, 33, 35, 52, 58		72	0.67	0.47, 0.94	0.66	0.47, 0.94
HPV 18, 39, 45, 59, 68		52	0.98	0.70, 1.39	1.09	0.75, 1.55
Other high-risk		46	0.87	0.56, 1.35	0.89	0.55, 1.36
Multiplicity of infection						
Single‡	162	162	1.00		1.00	
Multiple	65	154	1.05	0.81, 1.36	0.97	0.74, 1.28
Viral load (quintile)						
I (lowest)‡		67	1.00			
II		55	1.24	0.87, 1.76		
III		61	0.89	0.56, 1.20		
IV		63	0.60	0.42, 0.85		
V (highest)		69	1.11	0.79, 1.57		
			p for trend = 0.11			
Age (years)						
13–17‡	32	57	1.00		1.00	
18–24	51	72	1.02	0.70, 1.48	1.06	0.72, 1.56
25–34	94	121	0.74	0.52, 1.05	0.90	0.62, 1.30
≥35	50	66	0.79	0.52, 1.22	0.92	0.59, 1.43
			p for trend = 0.10		*p* for trend = 0.53	
Education						
Higher‡	69	104	1.00			
Secondary	94	127	0.81	0.59, 1.10		
None or primary	64	85	0.77	0.54, 1.11		
			p for trend = 0.11			
Age at first intercourse (years)						
≤15‡	56	86	0.74	0.52, 1.06		
>15	171	230	1.00			
No. of regular sexual partners						
1‡	165	223	1.00			
≥2	52	73	1.14	0.86, 1.51		
Parity						
Nulliparous‡	46	79	1.00		1.00	
Parous	181	237	0.64	0.48, 0.88	0.64	0.47, 0.89
Oral contraceptive use						
Never‡	104	150	1.00		1.0	
Ever	122	164	1.30	1.00, 1.67	1.38	1.07, 1.77
Intrauterine device use						
Never‡	98	147	1.00			
Ever	129	169	0.87	0.66, 1.11		
Smoking						
Never‡	162	227	1.00			
Ever	65	89	0.98	0.74, 1.30		

* HPV, human papillomavirus; RR, rate ratio; CI, confidence interval.
† Controlling for all other variables in the model.
‡ Reference category.

in our semiquantitative evaluation of viral load is possible, which would attenuate any underlying dose-response relation between viral load and HPV clearance. In the present study, follow-up visits were less frequent than those in some other investigations. Although this difference may have hampered to some extent our ability to capture rapid variations in HPV status, it enabled us to reduce the number of unnecessary treatments resulting from the cytologic manifestations of transient HPV infections. Finally, the information on HPV status became available only at the end of the present follow-up, ruling out any influence of this knowledge on the clinical management of study participants.

The decision to analyze prevalent rather than incident HPV infections has both advantages and disadvantages. An advantage is that we were able to include infections that had persisted for many years prior to the start of the study, especially in older women. A disadvantage is that we did not have the complete natural history of the infections studied. For this reason, the clearance curve in figure 1 should not be interpreted as the clearance of incident infections. Note that the clearance rate was high in the first 6 months of follow-up and diminished thereafter. This finding may be taken as evidence of some heterogeneity in the potential for persistence among prevalent infections. Our estimate of the median clearance time (6 months) is lower than in previously reported studies. This estimate correctly accounts for the uncertainty in the clearance time, which is not known exactly, but is known to only have occurred between two visits. An analysis that equates the clearance date with the visit at which the subject was HPV negative is incorrect and leads to an overestimation of persistence. In our study, such an analysis yields an (incorrect) estimate of median clearance time of 19.5 months.

In conclusion, we found that less than half of prevalent HPV infections persist after 6 months and only 7 percent after 5 years. The strongest risk factor for persistence of infection was the presence of HPV 16.

ACKNOWLEDGMENTS

Financial support was received from the Department of Pathology, Vrije Universiteit Medical Centre, the Netherlands; the Department of Epidemiology, Instituto Nacional de Cancerologia, Colombia; the International Agency for Research on Cancer, France; and the United Nations Development Programme/United Nations Population Fund/World Health Organization/World Bank Special Program of Research, Development and Research Training in Human Reproduction, Department of Reproductive Health and Research, World Health Organization, Switzerland (grant 94053A).

Dr. Molano obtained a fellowship from Colciencias of the Colombian government.

The authors are indebted to all of the gynecologists, nurses, and social workers who collaborated on the fieldwork. They also thank Dr. L. Rozendaal for comments and Drs. R. Pol, N. Fransen-Daalmeijer, R. Van Andel, and H. Schrijnemakers for technical support.

HPV Study Group: Drs. Mauricio Gonzalez, Joaquin Luna, Gilberto Martinez, Edmundo Mora, Gonzalo Perez, Jose Maria Fuentes, Constanza Gomez, Eva Klaus, Constanza Camargo, Cecilia Tobon, Teodolinda Palacio, Carolina Suarez, and Claudia Molina.

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