

Incidence, Duration, and Determinants of Cervical Human Papillomavirus Infection in a Cohort of Colombian Women with Normal Cytological Results

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Data on the incidence and determinants of human papillomavirus (HPV) infection in women >30 years old are scarce. To address this, a cohort of 1610 women—15–85 years old, HPV negative, and with normal cytological results at baseline—was monitored every 6 months for an average of 4.1 years. Information on risk factors and cervical samples for cytological testing and detection and typing of HPV DNA were obtained at each visit. The incidence of high-risk types was higher than that of low-risk types (5.0 vs. 2.0 cases/100 woman-years). The age-specific incidence curve for high-risk types was bimodal, whereas the incidence of low-risk types gradually decreased with age. Infections with high-risk types lasted longer than infections with low-risk types (14.8 vs. 11.1 months). In this cohort of cytologically normal women, the incidence of cervical HPV infection was high, and the epidemiological profile of high-risk HPV types was different from that of low-risk types.

Certain types of genital human papillomavirus (HPV) are the etiological agents of cervical cancer [1] and its precursor lesions and of genital warts [2]. HPV infections are transmitted by sexual contact, are highly prevalent, and are mostly transient [3]. Studies in the United States and Europe have reported incidence rates and risk factors for HPV infection among young women, but there is a

paucity of data for women >30 years old, who are particularly affected by HPV in Latin America [4].

Two patterns of the age-specific prevalence curve of the presence of HPV DNA have been reported. In some populations, a peak in women <20 years old has been observed, with a sharp decline thereafter resulting in very low levels at older ages [3, 5–8]. In other populations, especially those of countries with populations at high risk for cervical cancer [9–11], a bimodal distribution has been observed, with a first peak at age <20 years and a second peak around age 45–50 years [9–11]. However, the shape of the age-specific incidence curve of HPV DNA is unknown, and very limited information exists on the incidence and determinants of HPV infection in middle-aged women.

It has also been suggested that high-risk HPV types have a longer duration than low-risk HPV types [4]; however, this observation has not been confirmed in other studies [12, 13]. Here, we present data on the incidence, duration, and determinants of HPV infection in a large cohort of Colombian women, 15–85 years

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Table 1. Incidence rates of human papillomavirus (HPV) infection, by oncogenic type, in Bogotá, Colombia.

HPV type	No. of subjects with incident infections	No. of woman-years	Incidence rate × 100 (Poisson exact 95% CI)
All HPV	316	5107.4	6.2 (5.5–6.9)
All HR	269	5405.3	5.0 (4.4–5.6)
16	64	6339.7	1.0 (0.8–1.3)
18	43	6524.1	0.7 (0.5–0.9)
31	44	6535.2	0.7 (0.5–0.9)
33	24	6568.7	0.4 (0.2–0.5)
45	34	6548.2	0.5 (0.4–0.7)
52	30	6615.5	0.5 (0.3–0.6)
56	35	6605.5	0.5 (0.3–0.7)
58	45	6495.4	0.7 (0.5–0.9)
Other	86	6416.6	1.3 (1.1–1.7)
All LR	124	6135.5	2.0 (1.7–2.4)
6	12	6891.9	0.2 (0.1–0.3)
11	10	6664.8	0.2 (0.1–0.3)
42	26	6588.6	0.4 (0.3–0.6)
43	19	6664.3	0.3 (0.2–0.4)
70	15	6641.2	0.2 (0.1–0.4)
Other	65	6396.6	1.0 (0.8–1.3)

NOTE. CI, confidence interval; LR, low risk; HR, high risk.

old, who were monitored at regularly scheduled visits for an average of 4.1 years.

SUBJECTS, MATERIALS, AND METHODS

Study population and data collection. Between November 1993 and November 1995, a total of 2200 sexually active women, 13–85 years old, who presented to cervical cancer screening centers and family-planning clinics in low socioeconomic settings in Bogotá, Colombia, were invited to participate in a prospective study of cervical HPV infection. Eligible women were those residing in Bogotá, without a history of preneoplastic or neoplastic lesions of the cervix or of conization or hysterectomy, who were willing to participate and who signed an informed consent form. At study entry, participants responded to a questionnaire on the risk factors for cervical cancer and underwent a pelvic examination for the collection of cervical cells for cytological testing and detection of HPV DNA. Follow-up visits were scheduled every 6 months—here, we present data for the first 6 visits, up to December 1999, for almost 80% of them and up to 2001 for the remaining 20%. At each follow-up visit, a questionnaire on lifestyle and sexual behavior was administered, a pelvic examination was performed, and cervical specimens were collected for cytological testing and HPV DNA detection. Cervical scrapes were collected by use of Ayre spatulas and endocervical brushes. The cells were eluted in PBS plus 0.05% thimerosal. Smears were classified by use of the Bethesda classification. Only samples from women with normal cytological results were included in the analysis. Women with atypical squamous cells of

undetermined significance and with atypical cells were excluded. All study participants signed an informed consent form, in compliance with the clinical research guidelines of the authors' institutions.

Of the 2200 women who were invited to participate in the cohort study, 53 (2.4%) refused participation, 8 (0.4%) were considered to be ineligible (because of mental illness, hysterectomy, or a history of cervical cancer), 29 (1.3%) did not provide adequate specimens for HPV detection, 94 (4.3%) had inadequate HPV results because of poor DNA quality (i.e., failure to amplify the β -globin gene and a negative HPV result), 147 (6.7%) had abnormal cytological results at baseline, and 12 (0.5%) were <15 years old or attended only 1 visit and were excluded, leaving 1857 women. At baseline, 247 women tested positive for HPV, leaving 1610 women who constituted the population of the study.

Exposures and outcomes of interest. We evaluated the association of the following factors with the acquisition of a new HPV infection: age, number of sex partners, age at first sexual intercourse, contraceptive use, pregnancy, parity, and smoking. All of these exposures were considered to be time-variant in the analysis, except for age at first sexual intercourse.

The analysis included the incidence and duration of HPV infections. For the analysis of incidence, women found to be HPV negative and to have normal cytological results at baseline were monitored to detect the occurrence of new infections. Subsequently, the subcohort of women who developed incident infections was monitored to determine the duration of infection. The type-specific incidence and duration of infection were

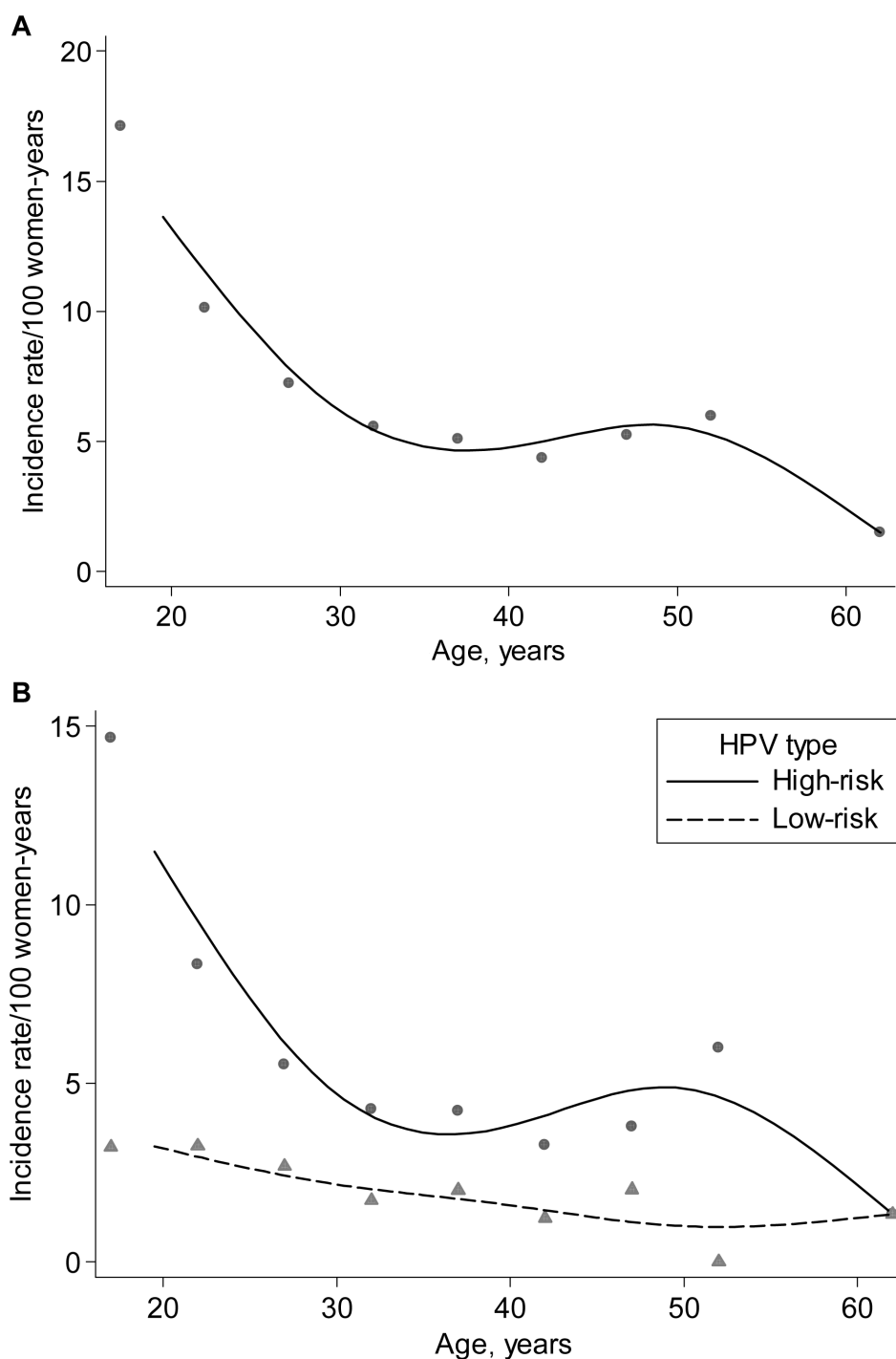


Figure 1. Incidence rate of human papillomavirus (HPV) infection, by age, among cytologically normal women in Bogotá, Colombia, 1993–2001. *A*, All HPV types; *B*, by oncogenic type of virus.

analyzed on the basis of the epidemiological categorization proposed by Muñoz et al. [1] for high- and low-risk HPV types.

HPV detection. Testing for HPV was conducted by use of a standard GP5⁺/GP6⁺ polymerase chain reaction (PCR) EIA, as described elsewhere [9]. Briefly, HPV samples that tested positive by GP5⁺/GP6⁺ PCR were subjected to EIA-HPV group-

specific analysis by use of a mixture of probes for high- and low-risk HPV types [14]. The high-risk HPV probe mixture consisted of oligoprobes for HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68; the low-risk HPV probe mixture consisted of oligoprobes for HPV types 6, 11, 26, 34, 40, 42, 43, 44, 53, 54, 55, 57, 61, 70, 71 (CP8061), 72, 73, 81 (CP8304), 82

Table 2. Type-specific cumulative risk of human papillomavirus (HPV) infection, by age.

Age group	HPV type																	
	HR									LR								
	16	18	31	33	45	52	56	58	Other HR	Any HR	6	11	42	43	70	Other LR	Any LR	Any HPV
15–19 years, cumulative risk at																		
1 year	3.7	1.3	3.0	1.2	2.5	1.8	1.2	3.0	4.3	17.4	1.2	0.6	1.2	0.0	1.9	1.2	2.6	17.2
3 years	7.2	1.3	5.8	1.9	4.6	5.3	4.1	3.8	7.1	32.0	1.8	0.6	1.2	1.4	1.9	2.8	6.5	35.7
5 years	9.8	2.2	7.6	4.2	5.4	6.3	5.2	6.3	13.1	38.0	2.9	0.6	2.0	3.6	1.9	5.0	13.3	42.5
20–24 years, cumulative risk at																		
1 year	2.3	2.2	1.1	0.0	0.5	0.0	0.0	1.1	2.2	9.5	0.6	0.5	0.0	0.0	0.0	2.2	3.4	11.3
3 years	2.9	4.5	3.3	0.6	1.7	1.7	0.6	4.0	4.6	21.0	1.1	1.7	0.6	1.1	0.6	3.9	9.5	24.1
5 years	6.5	6.3	6.8	3.3	3.2	3.1	3.3	4.7	10.0	34.4	1.8	2.4	0.6	2.7	2.2	5.9	14.5	36.9
25–29 years, cumulative risk at																		
1 year	2.0	1.3	0.7	0.7	1.0	0.3	0.0	0.0	2.4	6.9	0.0	0.0	1.0	0.7	0.0	2.0	3.7	9.5
3 years	3.5	2.8	1.7	1.0	1.3	0.7	1.1	0.8	5.9	15.5	0.0	0.0	2.8	1.0	0.3	2.7	7.0	20.1
5 years	5.2	3.9	4.0	1.0	2.1	0.7	2.1	1.3	9.8	22.3	0.0	0.7	2.8	1.7	1.3	5.4	10.7	30.0
30–44 years, cumulative risk at																		
1 year	1.3	0.9	0.4	0.2	0.5	0.6	0.2	0.6	0.6	4.1	0.1	0.3	0.3	0.1	0.6	0.9	1.8	5.4
3 years	2.1	1.5	1.3	1.2	1.2	1.0	1.2	2.2	2.8	10.8	0.5	0.4	1.2	0.4	0.6	3.4	5.6	14.2
5 years	4.2	2.0	2.2	1.4	2.2	1.2	2.3	3.4	5.2	18.2	0.5	0.4	2.3	1.0	0.6	4.7	8.5	21.9
≥45 years, cumulative risk at																		
1 year	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.7	0.7	1.4
3 years	1.4	2.2	2.0	0.7	0.0	0.0	2.8	1.3	0.0	2.3	0.0	0.0	0.0	0.0	0.7	1.3	1.4	8.1
5 years	3.2	3.4	2.0	0.7	0.0	2.0	7.9	1.3	0.0	2.7	0.0	0.0	0.0	0.0	2.5	1.3	3.3	12.4
All ages, cumulative risk at																		
1 year	1.6	1.1	0.7	0.4	0.7	0.6	0.3	0.9	1.4	6.1	0.2	0.2	0.4	0.2	0.5	1.2	2.3	7.5
3 years	2.9	2.1	2.1	1.1	1.5	1.3	1.5	2.2	3.7	14.4	0.6	0.4	1.3	0.7	0.7	3.1	6.0	17.7
5 years	5.2	3.1	3.7	1.9	2.4	1.9	3.0	3.2	7.2	22.2	0.8	0.7	1.9	1.6	1.2	4.8	9.7	26.3

NOTE. LR, low risk; HR, high risk.

(MM4 and IS39 subtypes), 83 (MM7), 84 (MM8), and CP6108. The low-risk probe mixtures contained HPV types 73 and 82, which have been classified as high-risk HPV types epidemiologically, and some HPV types (26, 34, and 53) that have been classified as probably high-risk HPV types [1]. These types were classified as high risk for the purposes of the present analysis.

Additionally, HPV positivity was assessed by Southern-blot hybridization of GP5⁺/GP6⁺ PCR products, under low stringent conditions, with the general probe of specific DNA fragments from cloned DNA of HPV types 6, 11, 16, 18, 31, and 33 [15]. Samples that tested positive by Southern blot analysis and negative by high-risk/low-risk EIA were considered to be of undetermined type and were classified as low risk.

During follow-up, a new GP5⁺/GP6⁺ PCR reverse line-blot (PCR-RLB) analysis was developed and was used to type the same 37 different HPV types detected by PCR-EIA. Specimens collected during the first 4 visits were typed with PCR-EIA, and those from visits 5 and 6 were typed with PCR-RLB. Agreement between the PCR-RLB analysis and the PCR-EIA result was found in 96% of cases [16].

Statistical analysis. Person-time methods were used to calculate the HPV incidence density. When interval-censored principles were used, a new infection was assumed to occur at the

midpoint between the last negative and the first positive test result. Women continued to make follow-up visits until they developed a new infection or until the last visit, if they had consistently negative test results. Incidence rates were estimated for high- and low-risk HPV types. The age-specific incidence of high- and low-risk HPV infections was calculated as the number of new cases of infection per woman-years observed at 5-year intervals (grouped by ages 15–19 to 50–54 and ≥55 years). We estimated exact confidence intervals on the basis of the number of HPV infections, modeled as a Poisson variable.

The cumulative risk of acquiring a new HPV infection was estimated by use of the Kaplan-Meier method, under the assumption that infections occurred at the midpoint between the last negative and the first positive test result. Time to infection was measured from the date of study entry until the date of a new infection, with censoring at the visit with the last negative result. We estimated risk by specific HPV types and age groups at 1, 3, and 5 years of follow-up. To describe the tendency of the hazard of HPV infection over time, the hazard rate (HR) was modeled by parametric methods by use of Weibull regression, and differences in the HRs were tested between age groups.

To determine the risk factors for incident infections, incorporating the time-varying nature of the factors of interest, we

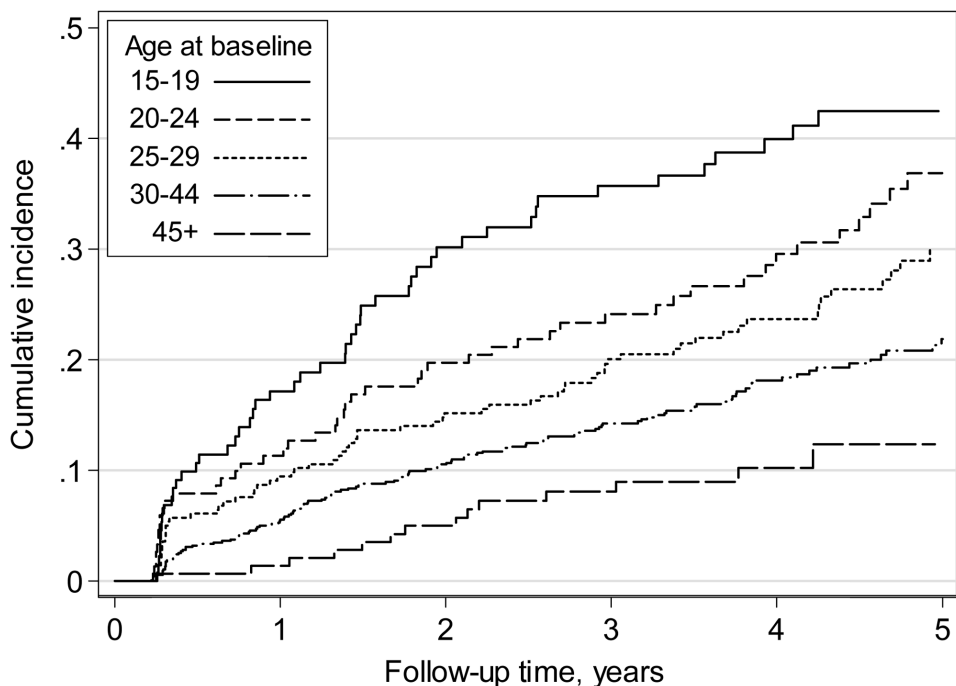


Figure 2. Cumulative risk of any human papillomavirus infection, by age

used pairs of visits as outcome units for the data analysis. Specifically, we grouped consecutive HPV results from each individual into pairs of HPV results, defined by the index test result and a follow-up test result. This analytical approach al-

lowed us to highlight the dynamic nature of HPV status and provided the basis to determine which exposures were associated with the occurrence of new infections. Among the pairs of tests whose index results were negative for HPV, the outcome

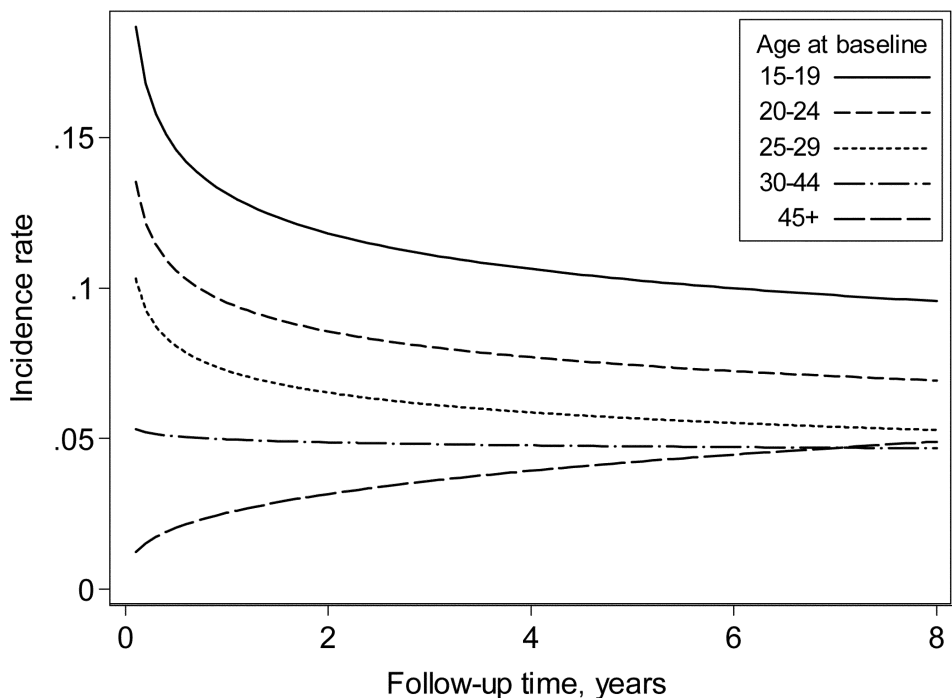


Figure 3. Incidence rate (per 100 woman-years) of human papillomavirus infection, by age at baseline

Table 3. Duration of incident human papillomavirus (HPV) infection, in months.

Age group	HPV type, median (95% CI) [no.]				
	16	18	31	33	45
<30 years	10.2 (6.9–18.8) [22]	12.8 (7.8–20.3) [11]	16.5 (7.5–23.2) [17]	10.0 (6.2–13.6) [7]	14.2 (7.3–19.8) [14]
≥30 years	14.8 (7.9–27.6) [25]	10.5 (7.9–16.6) [23]	14.2 (7.5–22.0) [18]	16.2 (5.9–23.3) [10]	10.4 (7.3–23.1) [14]
Total	13.7 (8.4–18.8) [47]	11.9 (9.1–16.6) [34]	16.5 (11.2–20.5) [35]	13.4 (7.0–17.0) [17]	12.2 (7.7–19.3) [28]

NOTE. CI, confidence interval; LR, low risk; HR, high risk.

of interest was the possible positivity of the testing done at the subsequent visit (i.e., a new infection), and the aim of the analysis was to identify the variables that differentiate samples showing new infections from those that remained negative. The type-specific incidence was evaluated with the same possible outcome for specific HPV types, irrespective of concomitant infection with another HPV type. When subjects missed study visits, the HPV status reported at the preceding visit was carried forward, up to a maximum of 3 years, after which they did not contribute person-years to the analysis of incidence. We used this criterion to include in the analysis ~90% of the incident infections with follow-up windows of >1 year; however, 85% of the incident cases had windows of <1.5 years' duration. We used logistic regression for the analysis of pairs of samples, and, because each woman could contribute >1 pair of HPV tests, the units of analysis (i.e., pairs) do not conform to the assumption of statistical independence. To include the statistical dependence of pairs of samples contributed by each woman, inferences were based on robust statistical methods that adjust for the correlation inherent in such repeated measures [17]. We estimated the duration of new infections using survival analysis methods and the assumption that infections cleared at the midpoint between the last consecutive positive result and the first subsequent negative test.

RESULTS

A total of 1610 women, 15–85 years old, with normal cytological results and a negative HPV test at study entry, were included in the analysis. At enrollment and during follow-up, 9207 cervical specimens were tested for HPV DNA at visits scheduled to occur every 6 months. The median duration of follow-up was 4.1 years (interquartile range [IQR], 3.2–5.0 years), and the median interval between visits was 7 months (IQR, 6.0–12.0 months). More than 65% of the women had at least 4 visits (IQR, 2.0–5.0 visits).

The baseline characteristics of this subcohort of 1610 women were very similar to those of the entire cohort, as described elsewhere [9]. At baseline, the median age was 32.3 years (IQR, 26.5–39.2 years); all subjects were sexually active, and 20% reported having had >1 regular sex partner; the median number

of history of full-term pregnancies was 2 (IQR, 1–3 pregnancies); 47.5% had ever used oral contraceptives at some time before baseline; 18.6% were current smokers, and 11.2% were former smokers.

Of the 1610 women at risk, 316 presented incident infections with ≥1 HPV types during follow-up. Table 1 shows that the overall incidence rate of infection for any HPV was 6.2 cases/100 woman-years, the incidence rate for high-risk HPV types was 5.0 cases/100 woman-years, and the incidence rate for low-risk HPV types was 2.0 cases/100 woman-years, with the incidence of high-risk HPV types being significantly ($P < .001$) higher than the incidence of low-risk HPV types. Among the 405 women who were infected with high-risk HPV types (table 1), the highest incidence rates were for HPV types 16, 58, 31, and 18, representing 15.8%, 11.2%, 10.9%, and 10.6% of all infections, respectively. Among the 147 low-risk incident infections (table 1), the most frequent HPV types were 42 (17.7%) and 43 (12.9%).

To describe the incidence of HPV infection according to age and to determine whether there was a steady trend or a bimodal pattern, figure 1A shows the incidence of HPV in 5-year intervals from ages 15–19 to 50–54 and >55 years. The incidence of HPV infection steeply decreased with age, from ~17 cases/100 woman-years (17%) in women 15–19 years old to ~10%, 7%, 5%, and 1.5% in women 20–24, 25–29, 30–54, and >55 years old, respectively. A secondary minor increase in incidence was seen around age 50 years, before the subsequent decline in HPV infection after age 55 years.

Figure 1B shows that the incidence rates of infection with high-risk HPV types reproduce the bimodal trend described for all HPV. In contrast, infection with low-risk HPV types, with comparatively lower levels of incidence, gradually decline, from 4% in younger women to ~1% in women >50 years old.

The age-specific cumulative risk of HPV infection for the most common HPV types is summarized in table 2 and figure 2. For any HPV, the highest 5-year cumulative risk (42.5%) was observed in the 15–19-year age group; incidence thereafter decreased monotonically with age to reach its lowest levels (12.4%) in women >45 years old. A similar pattern was observed for HPV type 16, for HPV types phylogenetically related

HPV type, median (95% CI) [no.]				
52	56	58	All HR	All LR
9.7 (6.3–11.3) [11]	10.3 (8.1–12.3) [10]	17.0 (6.1–34.3) [11]	14.2 (10.2–17.2) [81]	8.7 (7.3–16.2) [33]
8.9 (6.6–43.0) [10]	16.2 (7.5–29.3) [20]	11.2 (7.8–28.6) [20]	16.2 (13.0–17.7) [130]	12.0 (8.9–21.4) [60]
9.7 (6.9–14.6) [21]	14.6 (9.0–18.1) [30]	14.8 (8.4–23.0) [31]	14.8 (13.1–17.0) [211]	11.1 (8.2–16.5) [93]

to HPV type 16 (31, 33, 52, and 58), and for HPV type 45 (which is phylogenetically related to type 18) but not for HPV types 18, 56, or low-risk types (table 2).

Modeling the cumulative probability of HPV infection shown in figure 2 with a Weibull distribution provided the means to describe the HRs depicted in figure 3. The HRs show that, in women <30 years old, the force of infection with any HPV type is higher during the first year of follow-up and thereafter gradually decreases. In women 30–44 years old, the HR of HPV infection tended to be constant during follow-up, whereas, in women >45 years old, the HR was lower during the first year and progressively increased thereafter. Using Weibull regression, we found that the decreasing shape of the HR in women <30 years old ($\sigma = 1.18$) was not significantly different ($P = .222$) from the constant HR of women 30–44 years old ($\sigma = 1.03$) but was statistically different ($P = .037$) from the increasing trend observed in women >45 years old ($\sigma = 0.76$).

Table 3 shows that the median duration of a new HPV infection was longer for high-risk HPV types (14.8 months) than for low-risk HPV types (11.1 months). The longest duration (>13 months) was observed for HPV types 31, 58, 56, 16, and 33, whereas, for the low-risk HPV types and HPV type 52, the duration of infection was shortest. For HPV types 16, 33, and 56, the median duration of infection was longer for women >30 years old, whereas, for HPV types 45 and 58, the median duration was longer in women <30 years old (table 3).

To identify the determinants of incident HPV infection, we used logistic regression for the analysis of pairs, as described in Subjects, Materials, and Methods. The age-adjusted and multivariate odds ratios of incident infection are summarized in table 4. An increased risk of incident infection with high-risk HPV types was associated with younger age, pregnancy during follow-up, and new sex partners during follow-up but not with the number of partners at study entry. A long-term relationship with the first sex partner was associated with a reduced risk of incident infection with high-risk HPV types. Recent pregnancy was positively associated with infection with low-risk HPV types, especially for women 20–29 years old who had stable relationships (>5 years with the same partner) and no new sex partner during follow-up (data not shown). A reduced risk of

infection with low-risk HPV types was observed among women who had no new sex partners during follow-up, those who ever used injectable contraceptives, and parous women.

Given the bimodal mixture of the incidence curve shown in figure 1 and because pregnancy was shown to be relevant only in women <40 years old, we stratified the multivariate analysis by those below and above age 40 years (table 5). The most striking finding was that an increase in the number of partners during follow-up had a stronger effect in women >40 years old (i.e., for high-risk HPV types, the relative odds increased from 2.4 among those <40 years old to 7.6 among those ≥ 40 years old). The stratification by age also suggested that the observed association with pregnancy at a previous follow-up visit mainly occurred in women <40 years old.

DISCUSSION

The results of our prospective study describe the natural history of HPV infection in a cohort of women from Bogotá, Colombia, of a broad age range and from a population at high risk for cervical cancer. Except for a Brazilian study that recruited a population very similar to ours [4], all cohort studies to date have, to our knowledge, only included young women from low-risk countries [12, 18].

Our cohort included women 15–85 years old who were monitored, on average, for >4.1 years. We were able to estimate the age-specific incidence rates of the various HPV types. As far as we know, this is the first time that the age-specific incidence curves of cervical HPV infection has been provided. The shape of this curve is remarkably similar to the curve of prevalent infections reported previously in the same population [9]. For high-risk HPV types, the highest level was clearly seen in women <20 years old, followed by a sharp decrease to the lowest levels in women 35–39 years old, after which the curve tended to increase again in postmenopausal women. In contrast, for low-risk HPV types, the incidence was slightly higher in younger women and decreased progressively with age. This difference in the age-specific incidence curve of high- and low-risk HPV types is intriguing and could suggest differences in the immune response to these types.

Table 4. Risk factors associated with incident human papillomavirus (HPV) infection.

Risk factor	High risk		Low risk		Any HPV	
	Univariate	Multivariate	Univariate	Multivariate	Univariate	Multivariate
Age						
15–19 years	1	1	1	...	1	1
20–29 years	0.4 (0.3–0.6)	0.6 (0.4–0.9)	0.9 (0.4–1.8)	...	0.5 (0.3–0.8)	0.8 (0.5–1.3)
30–54 years	0.2 (0.1–0.3)	0.4 (0.2–0.6)	0.5 (0.2–0.9)	...	0.3 (0.2–0.4)	0.5 (0.3–0.8)
≥55 years	0.1 (0.0–0.2)	0.2 (0.1–0.4)	0.4 (0.1–1.4)	...	0.2 (0.1–0.3)	0.2 (0.1–0.5)
Sexual behavior						
Age at first sexual intercourse, by 2-year increase	1.0 (0.9–1.0)	...	1.1 (1.0–1.3)	...	1.0 (0.9–1.1)	...
1 partner at entry/0 new during follow-up	1	1	1	1	1	1
≥2 partners at entry/0 new during follow-up	1.2 (0.9–1.8)	1.0 (0.7–1.5)	1.1 (0.6–1.8)	1.0 (0.6–1.7)	1.2 (0.9–1.7)	1.0 (0.7–1.5)
1 partner at entry / ≥1 new during follow-up	2.9 (1.8–4.6)	2.5 (1.5–4.2)	2.3 (1.2–4.7)	2.7 (1.3–5.5)	2.6 (1.7–4.1)	2.3 (1.4–3.8)
≥2 partners at entry/≥1 new during follow-up	3.3 (1.6–6.8)	2.4 (1.1–5.4)	2.3 (0.8–7.3)	2.3 (0.8–7.0)	3.1 (1.6–6.0)	2.2 (1.1–4.5)
Duration of relationship with first partner						
<2 years	1	1	1	...	1	1
2–5 years	0.8 (0.5–1.1)	0.9 (0.6–1.3)	0.7 (0.4–1.3)	...	0.8 (0.6–1.1)	1.0 (0.7–1.4)
>5 years	0.5 (0.4–0.7)	0.6 (0.4–0.9)	0.6 (0.4–1.0)	...	0.5 (0.4–0.7)	0.7 (0.5–1.0)
Contraceptive use						
Age at start, by 10-year increase	1.0 (0.7–1.4)	...	1.5 (0.9–2.5)	...	1.2 (0.9–1.6)	...
Ever used oral contraceptives	1.0 (0.8–1.3)	...	1.0 (0.7–1.4)	...	1.0 (0.8–1.3)	...
Ever used intrauterine device	0.8 (0.7–1.1)	...	0.6 (0.4–0.9)	...	0.8 (0.6–1.0)	...
Ever used injections	1.2 (0.8–1.6)	...	0.4 (0.2–0.8)	0.5 (0.3–0.9)	1.0 (0.8–1.4)	...
Ever used Norplant	0.7 (0.3–1.3)	...	0.5 (0.2–1.7)	...	0.5 (0.3–1.0)	...
Ever used condoms	1.0 (0.8–1.6)	...	1.1 (0.8–1.6)	...	1.0 (0.8–1.3)	...
Duration of exposure to oral contraceptives						
None	1	...	1	...	1	...
≤2 years	1.1 (0.8–1.4)	...	1.2 (0.8–1.8)	...	1.1 (0.8–1.4)	...
>2 years	0.9 (0.6–1.2)	...	0.6 (0.4–1.1)	...	0.9 (0.6–1.2)	...
Current use of oral contraceptives						
No	1	...	1	...	1	...
Yes	1.0 (0.6–1.8)	...	1.0 (0.5–2.2)	...	1.1 (0.7–1.7)	...
Years since started, by 5-year increase	1.0 (0.9–1.2)	...	0.9 (0.7–1.1)	...	0.9 (0.8–1.1)	...
Years since last use, by 5-year increase	1.2 (1.0–1.5)	...	1.2 (0.9–1.5)	...	1.1 (0.9–1.3)	...
Parity						
None	1	...	1	1	1	1
≥1	0.5 (0.4–0.7)	...	0.4 (0.3–0.8)	0.4 (0.2–0.7)	0.5 (0.4–0.7)	0.6 (0.4–0.9)
Pregnancy during follow-up						
None	1	1	1	...	1	1
Current	1.5 (1.0–2.4)	1.6 (1.0–2.5)	1.9 (1.0–3.5)	...	1.7 (1.1–2.6)	1.7 (1.1–2.6)
Smoking						
Smoking at enrollment						
Never	1	...	1	...	1	...
Former	0.7 (0.4–1.2)	...	1.1 (0.6–2.1)	...	0.8 (0.5–1.3)	...
Current	1.2 (0.8–1.7)	...	1.1 (0.6–1.8)	...	1.1 (0.8–1.5)	...
Smoking during follow-up						
Never	1	...	1	...	1	...
Former	1.3 (0.9–1.7)	...	1.3 (0.8–2.0)	...	1.2 (0.9–1.6)	...
Current	0.7 (0.5–1.1)	...	1.2 (0.7–2.1)	...	0.8 (0.6–1.2)	...

NOTE. Bold type, $P < .05$; regular type, $.05 < P < .20$.

Our estimates of the cumulative risk for young women after 1, 3, and 5 years of follow-up were similar to those reported by other investigators [12, 13]. The 5-year cumulative risk of acquiring any HPV infection declined monotonically from 43% in women 15–19 years old to 12% in women >45 years old. A

remarkable difference by age was observed in the shape of the HR curve of HPV infection with time. In women <30 years old, the incidence tended to decrease with time, whereas, in women >45 years old, the incidence tended to increase with time, albeit at a significantly lower rate of occurrence.

Table 5. Risk factors associated with human papillomavirus (HPV) infection, according to age strata.

Risk factor	Age stratum, years					
	High risk		Low risk		Any HPV	
	<40	≥40	<40	≥40	<40	≥40
Age, by 10-year increase	0.5 (0.4–0.7)	0.8 (0.5–1.1)	...	0.6 (0.3–1.1)	0.5 (0.4–0.7)	0.6 (0.4–0.9)
Sexual behavior						
1 partner at entry/0 new during follow-up	1	1	1	...	1	1
≥2 partners at entry/0 new during follow-up	1.4 (0.9–2.0)	1.0 (0.4–2.1)	1.0 (0.5–1.7)	...	1.3 (0.9–1.9)	1.1 (0.6–2.1)
1 partner at entry / ≥1 new during follow-up	2.4 (1.4–4.0)	7.6 (1.6–36.2)	2.4 (1.1–4.9)	...	2.2 (1.4–3.6)	6.4 (1.5–27.1)
≥2 partners at entry/≥1 new during follow-up	3.5 (1.6–7.8)	...	2.3 (0.8–6.9)	...	3.4 (0.6–6.0)	...
Parity						
None	1	...	1	1	1	1
≥1	0.7 (0.4–0.9)	...	0.4 (0.3–0.7)	0.2 (0.1–0.3)	0.6 (0.4–0.9)	0.3 (0.1–1.1)
Pregnancy during follow-up						
None	1	...	1	...	1	...
Current	1.6 (1.0–2.5)	...	1.8 (1.0–3.5)	...	1.7 (1.1–2.6)	...

NOTE. Bold type, $P < .05$; regular type, $.05 < P < .20$.

The lower risk observed in women 30–40 years old might be the result of lower exposure to HPV because of more stable sexual behavior or of acquired immunity to HPV from past exposure. The subsequent slight increase in incidence observed in women around age 50 years might be explained by women having new high-risk sex partners, as is suggested by the increase in ratios shown in table 5. Other alternative mechanisms include a promiscuous sex partner or a decreased age-related immune response caused by hormonal changes related to menopause that result in the reactivation of latent HPV infections. We do not have information on the behavior of sex partners and HPV variants that would help us to discriminate between these possible explanations. However, we found that the odds ratio of incident high-risk infection increased by >7-fold among women >40 years old who had a new sex partner during follow-up, which suggests that new partners of women >40 years old may carry a higher risk of HPV infection. This is in agreement with the findings of a population-based HPV prevalence survey in Thailand, in which having a husband with extramarital sex partners was one of the main determinants of cervical HPV infection in their wives [6].

With regard to the type-specific cumulative risk of infection (table 2), it is of interest to note that the incidence of HPV type 18 was lower in women <20 years old than in older women. This suggests that the glandular epithelium in young women may have fewer receptors for HPV type 18.

Consistent with the results of previous studies [6, 12, 18–20], we found that the main determinants of incident HPV infection were age and number of sex partners. In addition, we observed that recent pregnancy was positively associated with incident infection, whereas parity was a protective factor, especially for low-risk HPV types. This paradoxical finding may be explained by the transient nature of HPV infection or, in

part, by these variables being markers of sexual behavior. Some reports have shown a higher prevalence and persistence of high-risk HPV types during pregnancy, compared with those of the postpartum period, and other researchers have found reduced HPV clearance during the first trimester of pregnancy and faster HPV clearance during the postpartum period [21–24]. Our finding of an increased risk of incident infection with recent pregnancy might indicate that the eversion or ectropion of the transformation zone that occurs during pregnancy may facilitate the acquisition of HPV infection, mainly by low-risk HPV types, during sexual encounters. This could also be the result of an increased viral load of latent HPV infections caused by hormonal changes during pregnancy. We also cannot exclude the possibility that some of these associations may be due to chance or that they are spurious and do not reflect biological events.

Specific differences were observed in the risk-factor profile for high- and low-risk HPV types. Age was inversely associated with risk for both high- and low-risk HPV types, but, given the described gradual decline in incidence with age among low-risk infections, the association remained statistically significant only for high-risk HPV types in the multivariate model. Having new sex partners during follow-up was a consistent predictor of both high- and low-risk HPV types, even after adjustment. The protective effect of a long-term relationship with the first sex partner and the observed association with pregnancy during follow-up remained statistically significant in the multivariate model only for high-risk HPV types. In contrast, a protective effect of the use of injectable contraceptives and parity were observed in the multivariate model only for low-risk HPV types.

Differences were also observed when the women were stratified in age groups of <40 and ≥40 years: For high-risk HPV types, age, new sex partners, parity, and recent pregnancy were associated with infection among younger women, whereas, among

women >40 years old, having new sex partners appeared to be the main determinant of infection. For low-risk HPV types, the associations with number of sex partners and recent pregnancy were observed only in women <40 years old. The associations that we observed for age, number of sex partners, and stability of relationships with infection with high-risk HPV types are consistent with the findings of most previous studies [4, 25, 26].

With regard to low-risk HPV types, our results are partially in agreement with those of previous reports. The number of sex partners was associated with risk, as was also reported by Rousseau et al. and Krüger-Kjær et al. [19, 25]. Although an inverse association with age was apparent in our univariate analysis, it was not statistically significant in the multivariate model, which is again in agreement with other findings [19, 25] and might be explained by the gradual decline in incidence with age. We detected novel inverse associations with the use of injectable contraceptives and with parity. These 2 associations may be surrogate markers for more stable sexual behavior of the women or their partners.

Like the majority of previous studies, we did not find an association between smoking and HPV infection. However, most of the studies that have explored this association have been cross-sectional studies of prevalent HPV infections [6, 27–30]. Among the 4 cohort studies that have assessed the role of smoking on incident HPV infections, one reported an association with ever smoking and infection with low-risk HPV types [19] and another with current smoking [20]. These inconsistent associations with smoking may represent residual confounding by unmeasured sexual behaviors.

With regard to the duration of infection, we found that infections with high-risk HPV types tend to last longer than infections with low-risk HPV types, which is in agreement with the results of the Brazilian cohort study [4] but not with those of 2 cohort studies of young women from the United States [12] and the United Kingdom [13]. The longer duration of infection with high-risk HPV types could be the result of a lack of or milder immune response to these types or of higher intratypic diversity that allow these types to more easily escape immunological surveillance. In fact, it has been suggested that HPV type 16 is less immunogenic than other HPV types [31, 32] and that it has higher intratypic diversity than do other HPV types [33].

The main limitations of our study are the lack of information on sexual behavior of the sex partners of the women included in our cohort and the lack of information on host susceptibility. In particular, information on biologically relevant groups of HLA alleles could have been important to assess how much genetic susceptibility influences or modulates the acquisition of HPV types.

Our cohort study had several strengths, including the relatively large number and broad age range of women enrolled,

the very low proportion of those refusing to participate, the long follow-up period, the comprehensive information available on risk factors, and the use of sensitive and well-validated PCR-based assays for the detection of HPV DNA in a central laboratory. In conclusion, we have shown that, in this cohort of Colombian women with normal cytological results and of a broad age range, the incidence of cervical HPV infection is high and the epidemiological profile of high-risk HPV types is different from that of low-risk types.

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