Incidence and Duration of Cervical Human Papillomavirus 6, 11, 16, and 18 Infections in Young Women: An Evaluation from Multiple Analytic Perspectives

Ralph P. Insinga,[1] Erik J. Dasbach,[1] Elamin H. Elbasha,[1] Kai-Li Liaw,[2] and Eliav Barr[3]

Departments of [1]Health Economic Statistics, [2]Epidemiology, and [3]Clinical Research-Vaccines and Biologics, Merck Research Laboratories, North Wales, Pennsylvania

Abstract

Objective: **To estimate the incidence and duration of cervical human papillomavirus (HPV)-6, HPV-11, HPV-16, and HPV-18 infections in a population of young American women.**
Methods: **The study population consisted of U.S. women who at baseline were 16 to 23 years of age, reported zero to five lifetime sexual partners, never having been pregnant, and never having had a prior abnormal Papanicolaou test and were enrolled in the placebo arm of a randomized multicenter clinical trial of a HPV-16 L1 virus-like particle vaccine. Women underwent type-specific endocervical/ectocervical swab HPV DNA testing at ~6-month intervals for up to 48 months of follow-up. To contribute person-time in the analyses of type-specific HPV incidence, a woman must have had at least three satisfactory swab specimens available and been negative for the relevant HPV type (HPV-6, HPV-11, HPV-16, or HPV-18) on her first two trial swabs. The duration of incident HPV infections was estimated using Kaplan-Meier survival analysis methods.**
Results: **Person-years of exposure ranged by type-specific analysis from 2,645 to 3,188, with an incidence rate per 100 person-years of 3.6 for HPV-6, 0.4 for HPV-11, 5.4 for HPV-16, and 2.1 for HPV-18. With censoring at the time of treatment for cervical intraepithelial neoplasia, where done, the mean duration of incident infections was 9.3, 8.4, 18.2, and 16.4 months, respectively, for HPV-6 (n = 103), HPV-11 (n = 13), HPV-16 (n = 142), and HPV-18 (n = 62). When the duration of HPV infections was truncated at the time of cervical intraepithelial neoplasia detection (any grade), where applicable, mean duration figures were 8.4, 8.1, 14.0, and 15.1 months for HPV-6, HPV-11, HPV-16, and HPV-18 infections, respectively.**
Conclusions: **Previous studies of the mean duration of cervical HPV infection have been based on prevalent infections and/or featured relatively short duration of follow-up. This study tested women for HPV infection over a period of up to 48 months and observed a mean duration of incident HPV-16/HPV-18 infections approximately twice that of HPV-6/HPV-11.** (Cancer Epidemiol Biomarkers Prev 2007;16(4):709–15)

Introduction

Human papillomavirus (HPV) infection is the most commonly occurring sexually transmitted infection in the United States (1). HPV infections can lead to cancers and precancers of the cervix (2), anus, penis, vagina and vulva (3, 4), anogenital warts (5, 6), and recurrent respiratory papillomatosis (7, 8), with growing evidence for a role in the pathogenesis of cancers of the head and neck (9). A diagnosis of HPV can lead to distress (10), shame, and anger and negatively affect sexual activity and enjoyment (11).

A vaccine targeting HPV-6, HPV-11, HPV-16, and HPV-18 (12) has recently been approved by the U.S. Food and Drug Administration, with a second vaccine targeting HPV-16 and HPV-18 also in development (13). HPV-16 and HPV-18 have been observed in ~70% of U.S. cervical cancer cases (14), with HPV-6 and HPV-11 detected in >90% of anogenital warts (5, 6) and in 10% of low-grade cervical intraepithelial neoplasia (CIN; refs. 15-18). Data on the natural history of HPV infection are critical for policy evaluations of these and other emerging technologies (19-21).

The present study describes the type-specific incidence, mean and median duration, and clearance rates of cervical HPV-6, HPV-11, HPV-16, and HPV-18 infections among U.S. young women enrolled in the placebo arm of a 4-year randomized double-blind clinical trial of a HPV-16 vaccine (22, 23). Several previous studies have examined the duration or clearance over time of cervical HPV infections from other perspectives, with analysis of prevalent rather than incident HPV infections (or mixture of prevalent and incident infections; refs. 24-28), aggregation of data across multiple HPV types (29-31), and/or type-specific data for HPV-16 only (32). Some analyses have featured follow-up of <2 years, which has generally been adequate for assessing clearance and mean duration of prevalent HPV infections (24, 27, 31), but resulted in a greater degree of right censoring and potential underestimation in studies of incident infections (33). Two prior studies with >2 years of postinfection follow-up have estimated the median duration of incident type-specific HPV infections; however, mean infection duration was not described (34, 35). Data on the type-specific incidence of HPV infection in the United States are sparse, with data from the two prior studies conducted among women recruited in Arizona and Washington state yielding somewhat disparate results (24, 36). Additional data on the incidence and mean duration of type-specific HPV infections from various perspectives can be of value to clinicians and patients as well as those conducting policy evaluations.

Materials and Methods

Study Participants and Procedures. Data were analyzed from women enrolled in the placebo arm of a randomized double-blind clinical trial of a HPV-16 L1 virus-like particle vaccine (Merck Research Laboratories, West Point, PA). The study population and trial design have been described in detail elsewhere (22, 23, 37). Briefly, the trial population consisted of

Received 10/5/06; revised 2/2/07; accepted 2/7/07.

Grant support: Merck Research Laboratories.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Ralph P. Insinga, Merck & Co., Inc., UG1C-60, P. O. Box 1000, North Wales, PA 19454-1099. Phone: 267-305-7992; Fax: 267-305-6455. E-mail: ralph_insinga@merck.com

doi:10.1158/1055-9965.EPI-06-0846

2,391 U.S. women who at enrollment were 16 to 23 years of age, nonpregnant, without a prior abnormal Papanicolaou test, and with no more than five lifetime male sexual partners. Virgins were enrolled if they were seeking contraception. Women in the placebo arm received i.m. injections of 225 μg of aluminum adjuvant in a total carrier volume of 0.5 mL visually indistinguishable from vaccine.

Women underwent type-specific endocervical/ectocervical swab HPV PCR testing for HPV-6, HPV-11, HPV-16, and HPV-18 at ~6-month intervals through 48 months of follow-up. At these visits, cervical samples were also collected for thin-layer (ThinPrep, Cytyc) Papanicolaou testing.

An algorithm was used to guide further evaluation for cytologic findings. A single Papanicolaou test result of high-grade squamous intraepithelial lesion, or repeated Papanicolaou tests showing low-grade squamous intraepithelial lesions or atypical squamous cells of undetermined significance, prompted colposcopy. Investigators were allowed to manage single atypical squamous cells of undetermined significance and low-grade squamous intraepithelial lesion results based on local standards of care, with some women referred for colposcopy. All women attending the month 48 trial visit were referred for colposcopy, with biopsy done if a CIN lesion was suspected. If more than one biopsy was obtained during colposcopy, then each tissue sample was processed separately. Cervical biopsy specimens were processed and assigned histologic diagnoses for purposes of medical management by central laboratory pathologists and were typed by PCR for HPV-6, HPV-11, HPV-16, and HPV-18 (37). Women diagnosed with CIN grades 2 to 3 or adenocarcinoma *in situ* underwent loop electrosurgical excision procedure. Women with repeated biopsy diagnoses of CIN 1 were referred for therapy at the discretion of the physician.

HPV Testing Methods. The HPV testing methods used have been described in detail elsewhere (22, 23). Briefly, cervical swabs were prepared for PCR using a QIAamp DNA Blood kit (Qiagen, Inc., Valencia, CA). DNA was analyzed by qualitative PCR using HPV-6, HPV-11, HPV-16, and HPV-18 type-specific and gene-specific primers based on the *L1, E6*, and *E7* genes for these types (37). β-Globin PCR assay was done to verify that purified samples contained a sufficient quality and quantity of DNA for PCR amplification. PCR products were dot blotted, hybridized to the corresponding ^{32}P-labeled β-globin or HPV-6/HPV-11/HPV-16/HPV-18 gene-specific oligonucleotide, and visualized by autoradiography. Appropriate negative and positive controls were run with each assay, and any specimen testing positive for at least two of the three genes was considered positive. Specimens testing positive for only one gene were considered positive if, on retesting, they were positive for two or three genes or the same single gene. Laboratory validation studies rigorously evaluated assay sensitivity against known copy number type- and gene-specific plasmids. The assay was shown to have a >95% probability of detecting at least 13 copies per sample, with 95% upper confidence bounds for sample false negativity and false positivity of 0.7% and 0.8%, respectively. All PCR assays were done at Merck Research Laboratories.

Measures. The type-specific incidence of HPV-6, HPV-11, HPV-16, and HPV-18 infections per 100 person-years was estimated. To contribute person-time to the analyses, a woman was required to have had at least three satisfactory cervical swab specimens available and to have been negative for the relevant HPV type (HPV-6, HPV-11, HPV-16, or HPV-18) on her first two trial swabs and any cervical biopsy specimens obtained on or before the date of her second swab. The selection of eligible enrollees for analyses of the incidence of each HPV type is illustrated in Fig. 1.

For the purposes of this analysis, incident HPV-6, HPV-11, HPV-16, and HPV-18 infections were defined by a positive test for the relevant HPV type on at least one cervical swab or biopsy specimen. Because HPV testing occurred at discrete intervals (typically every 6 months), it is likely that a positive HPV test result observed on a particular date was preceded by an unobserved period of HPV positivity of indeterminate length. For the calculation of person-time at risk, it was therefore assumed that the HPV infection occurred at the midpoint between the initial positive test date and the previous negative test. Time elapsed following the point of incident HPV infection was not included in estimates of person-time at risk. For individuals testing negative for a specific HPV type throughout the trial, person-time was estimated through the time point of the last HPV test, rather than the trial conclusion date, as this was the last opportunity to observe a HPV infection.

The monthly duration of each incident HPV infection was examined from three perspectives (Fig. 2). First, the time elapsed from incident infection until the clearance of a given HPV type, as measured by negative cervical swab specimen(s), was estimated. This ''clinical practice'' perspective describes most completely the course of each infection under the clinical management practices within the trial, without statistical adjustment for the treatment of disease. These data can provide insight into the actual duration of HPV infections in a screened population. Second, the time elapsed from the incident infection, until either the detection of a CIN grade 1 to 3 lesion testing positive for the relevant HPV type or infection

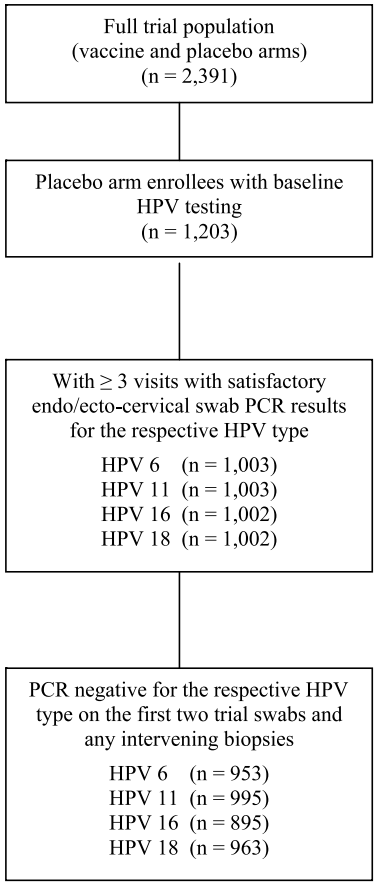

Figure 1. Sample selection criteria for analyses of HPV-6, HPV-11, HPV-16, and HPV-18 incidence. The final samples eligible for each type-specific analysis are represented within the *bottom box*, labeled by HPV type, reflecting placebo arm women negative on PCR testing for the relevant HPV type on the first two trial endocervical/ectocervical swabs and any intervening cervical biopsies.

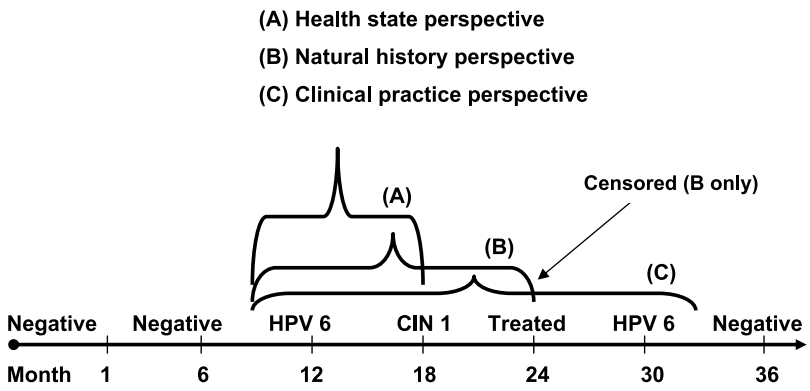

Figure 2. Health state, clinical practice, and natural history perspectives for analyzing duration of type-specific HPV infections. This example illustrates how the duration of a hypothetical HPV-6 infection would be categorized based on the health state, clinical practice, and natural history analytic perspectives. From all three perspectives, infection duration begins at the midpoint between the prior negative and the first positive HPV test result; in this example, at month 9. For the health state perspective (**A**), HPV infection duration continues until either clearance or detection of CIN; in this case, until CIN 1 is detected at month 18. For the natural history perspective (**B**), infection duration either ends with clearance or is censored at the time of disease treatment; in this case, with censoring at treatment in month 24. For the clinical practice perspective (**C**), the duration of infection ends at the midpoint between the last positive and the first negative HPV test irrespective of treatment; in this case, at month 33.

clearance, was examined. This ''health state'' perspective is consistent with how the duration of HPV infection is often conceptualized in cost-effectiveness models and other policy evaluations of technologies to prevent and diagnose HPV disease (19, 20), where women may transition from a health state characterized by infection in the absence of clinically detectable CIN to clinically detectable CIN grades 1, 2, and 3. Third, the time elapsed from incident infection until infection clearance was analyzed, with censoring of infection duration at the time of treatment, if done. Given that treatment may eliminate infection (38), by censoring on-going infections at the time of therapy, this ''natural history'' perspective can yield estimates more consistent with the duration of infection in the absence of treatment. This perspective can be useful for understanding the complete course of HPV infections as would be observed among unscreened populations.

Women were required to have two consecutive negative endocervical/ectocervical swabs for a given HPV type before the start of an incident infection. For consistency, if two consecutive negative swabs for that type were subsequently observed, the infection was assumed to have cleared.

Several women were found to have a positive HPV swab sample(s) for a given type followed by only a single negative swab, as of their last available sample. Among women with incident HPV-6 infections, 19 (18.4%) fell into this category compared with 2 (15.4%) with HPV-11, 16 (11.3%) with HPV-16, and 9 (14.5%) with HPV-18. Because these women lacked two consecutive negative swabs, some previous analyses have evaluated them as censored at the date of the negative swab (28). However, this approach would be expected to considerably overestimate the actual duration of infection for these women because, in actuality, many would likely have cleared their infection around the time of their last positive swab and differ with respect to their subsequent course from women who were otherwise censored with a positive swab at their last trial visit. For instance, among 63 women with an incident HPV-6 infection followed by a negative swab sample, and at least one additional swab in the trial, only 2 (3.2%) had a nonnegative HPV-6 result on the second swab or a concurrent biopsy specimen. Similarly, for incident HPV-11 infections, the proportion was 0/6 (0.0%), for HPV-16 it was 8/76 (10.5%), and for HPV-18 it was 3/31 (9.7%). This would suggest that nearly all of the women with only a single negative HPV test at the conclusion of their trial follow-up would have previously cleared their HPV infections, and we therefore evaluated these women as having cleared their infections following their final positive test. Women observed to have had positive swab or biopsy specimens followed by a single negative swab followed by a swab or biopsy specimen positive for the same HPV type were analyzed as having persistent infections. Women with a positive test on the date of their final trial swab sample were evaluated as censored.

Consistent with previous studies (25, 32), in the primary analysis, it was assumed that women acquired type-specific HPV infections at the midpoint in time between their most recent prior negative HPV test and their initial positive test and cleared infections at the midpoint in time between their final positive and their first subsequent negative swab tests. For women with only a single positive HPV test, this yielded a length of infection of $\sim$6 months. In sensitivity analyses, we explored the effect on results of selecting lower and upper bounds reflecting 50% longer and 50% shorter intervals for the estimated time from first positive HPV test back to incident infection and from last positive HPV test to infection clearance (e.g., among women with only a single positive HPV test, the lower and upper bounds would simulate approximately 3- and 9-month infections, respectively).

Statistical Analysis. HPV type-specific incidence rates per 100 person-years were estimated along with 95% confidence intervals (95% CI). The mean and median duration of HPV infections and the proportion of women clearing infections at 12, 24, and 36 months of follow-up were estimated using Kaplan-Meier methods (39). SEs for the Kaplan-Meier survivorship function $S(t)$ are typically estimated using Greenwood's formula:

$$\mathrm{SE}\{\hat{S}(t)\} \approx [\hat{S}(t)]\left\{\sum_{j=1}^{k}\frac{d_j}{n_j(n_j-d_j)}\right\}^{\frac{1}{2}},$$

where n_j is the number surviving to the start of interval j and d_j is the number of deaths during interval j (40). However, when the survivorship function nears 0, SEs calculated using Greenwood's formula will lead to confidence intervals that overlap zero. Therefore, in these instances, confidence intervals in the present analysis were estimated using a log(-log) transformation of the survivorship function as described by Collett (41):

Table 1. Incidence of HPV-6, HPV-11, HPV-16, and HPV-18 infections

	HPV-6 (n = 953)	HPV-11 (n = 995)	HPV-16 (n = 895)	HPV-18 (n = 963)
Mean person-years at risk	3.0	3.2	3.0	3.1
Cases/total person-years at risk	103/2,889	13/3,188	142/2,645	62/3,007
Incidence per 100 person-years (95% CI)	3.6 (2.9-4.3)	0.4 (0.2-0.6)	5.4 (4.5-6.3)	2.1 (1.5-2.6)

$$\mathrm{SE}[\log\{-\log \hat{S}(t)\}] = \left\{ \frac{1}{[\log \hat{S}(t)]^2} \sum_{j=1}^{k} \frac{d_j}{n_j(n_j - d_j)} \right\}^{1/2}$$

These SEs were then back transformed to obtain confidence intervals using the formula: $(t)^{\exp[\pm z_{\alpha/2}\mathrm{SElog}[-\log(t)]]}$, where $z_{\alpha/2}$ is the upper $\alpha/2$ point of the standard normal distribution.

Results

Among all 1,203 women enrolled in the placebo arm of the trial who underwent HPV testing at baseline, the prevalence of individual HPV types on endocervical/ectocervical swab PCR testing was as follows: HPV-6, 3.2%; HPV-11, 0.5%; HPV-16, 6.8%; and HPV-18, 2.6%. The combined baseline prevalence of HPV-6, HPV-11, HPV-16, or HPV-18 infections was 12.1%.

Baseline characteristics were generally similar across samples eligible for each type-specific analysis of HPV incidence, with sample sizes ranging from 895 to 995 women. The size of each sample was directly correlated with the baseline prevalence of the relevant HPV type. Approximately 41% of eligible women were between the ages of 16 and 19 years and the remainder between the ages of 20 and 23 years. By self-reported race, 74% were White, 16% were Black or Hispanic, and 10% were of other racial designation. Nearly 15% reported a history of pregnancy and 24% as being current smokers. Five percent reported being virgins, with 24%, 38%, and 33% reporting one, two to three, and four to five lifetime sexual partners, respectively.

Table 1 reports incidence rates of HPV-6, HPV-11, HPV-16, and HPV-18 infections, per 100 person-years, among eligible women. Excluding the time covering the first two trial cervical swabs, women had an average of 3.0 to 3.2 person-years at risk for each HPV type. Of the four types examined, HPV-16 infection was the most commonly occurring (5.4 per 100 person-years) followed by HPV-6 infection (3.6 per 100 person-years). Women with incident HPV-6 (n = 103), HPV-11 (n = 13), HPV-16 (n = 142), and HPV-18 (n = 62) infections contributed a total of 675 unique sampling dates with a positive HPV test for the relevant HPV type. Of the 320 incident infections analyzed, 266 had at least one subsequent cervical swab result, with 59% of the latter group having multiple HPV tests positive for a given type versus 41% for a single positive test.

The duration and clearance of HPV infections, inclusive of the detection of CIN and treatment, are reported in Table 2. HPV-16 infection was observed to have the longest duration (17.1 months), with the duration of HPV-6 and HPV-11 infections roughly one half that of HPV-16 and HPV-18. Adjusting the time point for the incidence and clearance of HPV infections from the midpoint to one fourth and three fourths of the interval between discordant tests resulted in lower and upper bound estimates for the duration of infection of approximately ±3 months the mean duration estimated in the primary analyses. The mean duration of infection exceeded the median by 25% to 37% depending on HPV type. Whereas an estimated 70% of women were cleared of their incident HPV-6/HPV-11 infections within 12 months, only 40% of women cleared their HPV-16/HPV-18 infections over this period. By 36 months, nearly all women had cleared their incident HPV-6/HPV-11 infections, with 7.5% still infected with HPV-16 or HPV-18.

Given that a small proportion of infections were estimated to persist beyond the conclusion of the observed follow-up (3.8% of all infections), the mean duration of infection is underestimated for all types, except HPV-11, where ascertainment was complete. However, even for HPV-18, which exhibited the highest degree of persistence (9.0%) of the four types at 36 months, a sensitivity analysis suggests that the degree of underestimation may well be minimal. In the data, 62.5% of HPV18 infections persisting beyond 12 months cleared between months 12 and 24 and 60% of infections persisting beyond 24 months cleared between months 24 and 36. Assuming a similar 60% annual clearance rate for the 9.0% of infections persisting beyond 36 months, the estimated mean duration of HPV-18 infections (16.6 months) would increase by ~1 month if the full duration of infections persisting beyond 36 months is to be added. For HPV-6 and HPV-16 infections, the increment would be considerably smaller.

Table 3 displays the duration and persistence of HPV infections up to the time of detection of CIN grades 1 to 3 or clearance. The following numbers of women with incident HPV infections were observed to develop CIN positive for the same

Table 2. The monthly duration and clearance of HPV-6, HPV-11, HPV-16, and HPV-18 infections inclusive of the detection of CIN and treatment

	Mean duration,* mo (95% CI)	Lower bound† mean uration,* mo (95% CI)	Upper bound† mean duration,* mo (95% CI)	Median duration, mo (95% CI)	Proportion cleared at 12 mo (95% CI)	Proportion cleared at 24 mo (95% CI)	Proportion cleared at 36 mo (95% CI)
HPV-6 (n = 103)	9.2 (8.3-10.1)	6.2 (5.3-7.1)	12.1 (11.2-13.0)	6.7 (6.2-9.1)	68.2% (58.2-78.1)	98.3% (98.0-98.5)	98.3% (98.0-98.5)
HPV-11 (n = 13)	8.1 (5.5-10.7)	4.7 (2.5-6.9)	11.4 (8.2-14.6)	6.5 (5.7-11.9)	87.5% (73.8-94.2)	100.0% (—)	100.0% (—)
HPV-16 (n = 142)	17.1 (15.0-19.2)	14.3 (12.2-16.4)	20.0 (17.8-22.1)	13.2 (12.0-17.6)	41.3% (32.2-50.5)	72.3% (63.0-82.9)	93.1% (90.8-94.9)
HPV-18 (n = 62)	16.6 (13.4-19.7)	13.7 (10.4-17.0)	19.2 (16.2-22.2)	13.2 (11.7-17.7)	40.0% (26.3-54.6)	77.5% (64.4-90.5)	91.0% (82.4-94.7)

*Because the individual observation with the longest follow-up time was censored for the analyses of HPV-6, HPV-16, and HPV-18 infections, the mean duration of infection is underestimated.

† The lower bound for mean duration was estimated assuming that HPV infections began one fourth of the way between the initial positive test and the last prior negative cervical swab and ended one fourth of the way between the last positive test and the first subsequent negative cervical swab. For the upper bound, the cut point was changed from one fourth to three fourths, with the midpoint used for the primary analyses.

Table 3. The monthly duration and persistence of HPV-6, HPV-11, HPV-16, and HPV-18 infections up to the time of diagnosis of CIN or clearance

	Mean duration,* mo (95% CI)	Lower bound † mean duration,* mo (95% CI)	Upper bound † mean duration,* mo (95% CI)	Median duration, mo (95% CI)	Proportion persistent at 12 mo (95% CI)	Proportion persistent at 24 mo (95% CI)	Proportion persistent at 36 mo (95% CI)
HPV-6 (n = 103)	8.4 (7.5-9.3)	5.8 (5.0-6.7)	11.0 (10.0-11.9)	6.2 (6.1-7.1)	26.0% (16.8-35.3)	0.0% (—)	0.0% (—)
HPV-11 (n = 13)	8.1 (5.5-10.7)	4.7 (2.5-6.9)	11.4 (8.2-14.6)	6.5 (5.7-11.9)	12.5% (5.7-26.2)	0.0% (—)	0.0% (—)
HPV-16 (n = 142)	13.9 (12.1-15.7)	11.9 (10.1-13.7)	16.1 (14.2-18.0)	11.7 (9.8-14.3)	47.2% (38.0-56.4)	15.8% (7.9-23.7)	0.0% (—)
HPV-18 (n = 62)	14.9 (11.9-18.0)	12.4 (9.3-15.5)	17.4 (14.4-20.5)	12.4 (10.7-16.3)	52.9% (39.3-66.5)	16.3% (4.9-27.8)	8.2% (4.9-13.6)

*Because the individual observation with the longest follow-up time was censored for the analysis of HPV-18 infections, the mean duration of infection is underestimated.

† The lower bound for mean duration was estimated assuming that HPV infections began one fourth of the way between the initial positive test and the last prior negative cervical swab and ended one fourth of the way between the last positive test and the first subsequent negative cervical swab. For the upper bound, the cut point was changed from one fourth to three fourths, with the midpoint used for the primary analyses.

HPV type during the course of follow-up: HPV-6, n = 15; HPV-11, n = 0; HPV-16, n = 37; and HPV-18, n = 9. The mean durations of infections excluding persistence following the detection of CIN are generally shorter than estimates from Table 2, which include infection progression to CIN. For instance, for HPV-16, the duration was roughly 20% shorter (13.9 versus 17.1 months). Virtually all women with incident HPV infections were observed to either develop CIN or clear the infection within 36 months (within 24 months for HPV-6/HPV-11).

Although women routinely underwent colposcopy at their month 48 trial visits, CIN diagnoses observed at earlier trial time points were based solely on biopsies done as follow-up for an abnormal Papanicolaou smear. Consequently, among women diagnosed with CIN, it is likely that the actual period from incident infection to initial CIN development is shorter than that based on the actual date of detection. Although it is not possible to determine the exact time of development of clinically detectable CIN, a sensitivity analysis was conducted in which the time from incident HPV infection to CIN development was reduced by half for women with HPV-16, representing the infection group with the largest proportion of women developing CIN. This resulted in a minor decrease in the estimated mean duration of HPV-16 infection across all infected women (12.5 versus 13.9 months).

Few women who were HPV-6, HPV-11, HPV-16, or HPV-18 positive at the time of the month 48 trial visit, and underwent routine colposcopy at month 48, had clinically detectable CIN. For instance, among women with HPV-16 infection on endocervical/ectocervical swab (n = 45), four (8.9%) were found to have CIN on colposcopically directed biopsy. Similarly, among women with HPV-6 (n = 16), HPV-11 (n = 5), and HPV-18 (n = 21) infections at month 48, two (10.0%), zero (0.0%), and three (14.3%), respectively, were found to harbor CIN.

An additional sensitivity analysis was conducted to examine the effect of not distinguishing between HPV infection and CIN 1 and truncating infection duration only with the detection of CIN 2 or CIN 3. Treated CIN 1 lesions (n = 4) were censored at the time of treatment. Mean (95% CI) infection durations by type were 9.1 (8.2-10.0) months for HPV-6, 8.1 (5.5-10.7) months for HPV-11, 16.1 months (14.1-18.1) for HPV-16, and 15.8 months (12.7-18.9) for HPV-18. The mean duration of HPV infections where CIN 1 was diagnosed (16.9 months) was longer than for HPV infections without histologic diagnosis of CIN (12.9 months; P = 0.0008).

Table 4 reports the duration and clearance of HPV infections, with censoring of infections at the time of treatment, where done. During the period of their on-going infections, 2 women with HPV-6 underwent treatment for a CIN lesion compared with 1 with HPV-11, 14 with HPV-16, and 3 with HPV-18 infections. The mean duration of HPV infection from this perspective was 1% to 6% longer, depending on the HPV type, than when infection duration was not censored at the time of treatment (Table 2).

Among the 17 women with HPV-16/HPV-18 infections undergoing treatment, 4 (23.5%) failed to clear their respective HPV infections by the time of their next cervical swab sample, collected an average of 3.3 months following the date of treatment. All of the women with persistent infection after therapy had a pretherapy cervical biopsy specimen testing positive for HPV-16 or HPV-18 infection, an average of 1.2 months before undergoing treatment.

Discussion

This study has reported the type-specific incidence and duration of cervical HPV-6, HPV-11, HPV-16, and HPV-18 infections among young women. Unique features of the analysis have included the examination of the duration and clearance of HPV infection over time from multiple perspectives (clinical

Table 4. The monthly duration and clearance of HPV-6, HPV-11, HPV-16, and HPV-18 infections with censoring at the time of treatment

	Mean* duration, mo (95% CI)	Lower bound † mean duration,* mo (95% CI)	Upper bound † mean duration,* mo (95% CI)	Median duration, mo (95% CI)	Proportion cleared at 12 mo (95% CI)	Proportion cleared at 24 mo (95% CI)	Proportion cleared at 36 mo (95% CI)
HPV-6 (n = 103)	9.3 (8.4-10.2)	6.3 (5.4-7.3)	12.2 (11.2-13.1)	6.8 (6.2-10.8)	66.5% (56.3-76.7)	98.2% (97.9-98.5)	98.2% (97.9-98.5)
HPV-11 (n = 13)	8.4 (5.4-11.4)	5.6 (3.3-7.8)	11.7 (8.1-15.3)	6.5 (5.7-11.9)	85.4% (66.0-94.2)	100.0% (—)	100.0% (—)
HPV-16 (n = 142)	18.2 (15.9-20.6)	15.1 (13.0-17.3)	21.3 (18.8-23.8)	15.6 (12.1-18.2)	40.1% (30.8-49.4)	69.0% (58.9-79.2)	85.3% (75.0-91.5)
HPV-18 (n = 62)	16.4 (13.1-19.6)	13.6 (10.2-17.0)	19.1 (16.0-22.3)	12.8 (11.7-17.5)	41.5% (27.4-55.7)	77.7% (64.0-91.4)	91.1% (84.6-94.9)

*Because the individual observation with the longest follow-up time was censored for the analyses of HPV-6, HPV-16, and HPV-18 infections, the mean duration of infection is underestimated.

† The lower bound for mean duration was estimated assuming that HPV infections began one fourth of the way between the initial positive test and the last prior negative cervical swab and ended one fourth of the way between the last positive test and the first subsequent negative cervical swab. For the upper bound, the cut point was changed from one fourth to three fourths, with the midpoint used for the primary analyses.

practice, health state, and natural history) and longer follow-up than prior studies estimating the mean duration of incident HPV infection (27, 33), allowing for more complete ascertainment. Excluding studies in recognized high-risk (e.g., women infected with HIV) or specialized (e.g., women first initiating sexual activity) populations, relatively few prior analyses have described the type-specific incidence of HPV infection in the U.S. population. In a smaller study, with ~200 total person-years at risk, Giuliano et al. (24) reported incidence rates (converted to per 100 person-years) for HPV-6 (1.0), HPV-11 (1.4), HPV-16 (7.1), and HPV-18 (1.0) among women ages 18 to 35 years attending a Planned Parenthood clinic for routine gynecologic care in southern Arizona. A second study, among female students ages 18 to 20 years attending the University of Washington (n = 553), with >800 person-years at risk, reported incidence rates (converted to per 100 person-years) for HPV-6 (3.9), HPV-11 (0.5), HPV-16 (5.5), and HPV-18 (2.1) virtually identical to those estimated in the present study (36). One additional U.S. study has reported the cumulative incidence of type-specific HPV infections over time (34).

With women enrolled from 16 academic centers from across the United States, the study population in the present analysis is more geographically diverse than that of prior analyses of HPV incidence. However, similar to earlier studies, it does not reflect a nationally representative sample of the population. The 2002 National Survey of Family Growth reported the following distribution of lifetime male sexual partners among U.S. females ages 15 to 24 years: 0 (23.4%), 1 to 2 (36.9%), 3 to 6 (25.7%), and 7+ (14.1%; ref. 42). For comparison, across all females ages 16 to 23 years enrolled in the placebo arm of the present study, the distribution of lifetime sexual partners was as follows: 0 (4.5%), 1 to 2 (43.2%), 3 to 5 (52.3%), and 6+ (0.0%). Thus, the present study includes fewer women with the lowest and highest numbers of lifetime sexual partners compared with the general U.S. population. Nonetheless, the baseline prevalence of HPV-6, HPV-11, HPV-16, and HPV-18 infections was observed to be relatively similar to that obtained from a nationally representative sample of 18- to 25-year-old U.S. women (43).

Although populations may differ across studies, the high incidence of HPV infection among young women is a common theme with, for instance, a cumulative 2-year incidence of HPV due to any type among university aged women of 39% to 43% and for HPV-16 of 7% to 10% reported in prior analyses (34, 36). U.S. data comparing the type-specific incidence and duration of HPV infection among women at different ages are generally unavailable and constitute an area for future research.

Several studies have reported the type-specific median duration of incident HPV infections from a perspective most similar to the natural history perspective in Table 4. Using cervicovaginal lavage specimens and a 6-month testing interval, Ho et al. (34) estimated median durations of incident HPV-6 (6 months) and HPV-18 (12 months) infections that were similar to those reported in this study, with a comparatively shorter duration for HPV-16 (11 months). In contrast, based on testing of cervical smear samples taken at 6-month intervals, Woodman et al. (35) reported a relatively longer median duration of incident HPV-6/HPV-11 (9.4 months) infections and a relatively shorter duration for HPV-16 (10.3 months) and HPV-18 infections (7.8 months). Results obtained by Richardson et al. (33) and Xi et al. (32) were generally more consistent with the present analysis. The former study collected samples using an Accelon cervical biosampler with a 6-month testing interval, whereas the latter used cervical and vulvovaginal swabs and a 4-month testing interval. Subjects in each of the preceding studies were U.S. female university students, with the exception of the analysis by Woodman et al., which was conducted among women ages 15 to 19 years attending a center for sexual and reproductive health in the United Kingdom.

The mean durations of incident type-specific HPV infections have not generally been reported in prior analyses, with the lone exception being abbreviated estimates from a study with <2 years of follow-up after infection (33). In the natural history perspective of the present study, a substantial proportion of HPV-16 and HPV-18 infections were observed to persist beyond 2 years, with more than half of these estimated to resolve between years 2 and 3. A consistent finding across all analyses was that the mean duration of incident HPV infection exceeded the median duration, with the differential varying from 15% to 40% depending on the HPV type and perspective adopted.

Prior studies of the course of incident type-specific HPV infections (32-35) have not examined infection duration from a health state perspective. This has represented a gap in the literature, as data from this perspective are particularly needed for cost-effectiveness models and other policy evaluations of technologies to prevent and diagnose HPV disease, which typically consider HPV infection without progression to clinically detectable CIN as a separate health state from that of clinically detectable CIN grades 1, 2, and 3 due to HPV (19, 20). In this study, the mean duration of HPV-16 infection was observed to be >30% higher when evaluated from the natural history perspective than when infection alone was evaluated as a separate health state from CIN due to HPV-16. Differences in duration were of a lesser magnitude for other HPV types evaluated. As some natural history models used in policy analyses have modeled persistent HPV infection in the absence of CIN as a separate health state from transient infection (44), it is also interesting to note that persistent HPV infections without detected CIN were generally not found to exist beyond 18 months for HPV-6 and HPV-11 infections or beyond 36 months for HPV-16 and HPV-18 infections.

Our study has several potential limitations. First, similar to prior studies of HPV infection duration, it is possible that a proportion of infections of shortest duration would be missed by the analysis testing interval, which would tend toward overestimating infection duration (34). A trade-off may exist, however, in that increasing numbers of false-positive test results in studies with relatively more frequent testing would tend toward underestimation of mean duration.

Second, consistent with previous natural history studies of the duration of incident type-specific HPV infections (32, 33, 35), women with high-grade or otherwise persistent CIN were referred for treatment. Although these women were evaluated as censored at the time of treatment in the natural history analysis, it is possible that the subsequent course of their HPV infections differed from those of women with HPV infections of similar duration not undergoing treatment. Informative censoring of this type could potentially lead to an underestimate or overestimate of infection duration in the natural history analysis of this and other prior studies.

Third, because of the additional censoring at the time of treatment, the natural history analysis estimate of mean infection duration for HPV-16 (14.7% persisting at the conclusion of follow-up) is likely to be underestimated by at least a few months. The degree of censoring is likely to be much less of a concern for the other analyses as described in Results.

Fourth, estimates from the clinical practice perspective are reflective of infection duration under the screening and follow-up practices occurring in the trial. Results may vary somewhat in other settings to the extent that women are managed more or less aggressively. However, with most infections not resulting in clinically treated CIN, the degree of variation may be relatively modest, as the mean infection duration estimated from the clinical practice perspective only differed by 1% to 6%, depending on the HPV type, from that in which no treatment was assumed.

Finally, some prior analyses of HPV natural history have censored women before the point of CIN treatment, such as at the time of initial colposcopy/cervical biopsy (32, 33). We

instead elected to censor women at the time of treatment in the natural history analyses, as a randomized clinical trial of women undergoing no biopsy, central biopsy, and peripheral biopsy at baseline found no difference across groups in change in lesion size at 6-week follow-up, with colposcopic and histologic confirmation of CIN grade 1 to 3 persistence (45). This allowed for more complete ascertainment of infection natural history, particularly in instances where treatment was not subsequently done.

This analysis has estimated the type-specific incidence and duration of cervical HPV-6, HPV-11, HPV-16, and HPV-18 infections. The development of type-specific HPV vaccines and HPV DNA tests has amplified the need for descriptive data on HPV infection for policymakers, physicians, and patients. Through assessment of the duration of HPV infection from clinical practice, health state, and natural history perspectives, the results of this analysis can be useful for a variety of purposes.

Acknowledgments

We thank Christine K. Gause, Ph.D. (Merck Research Laboratories) for statistical consultation in the preparation of this manuscript.

References

1. Cates W, Jr. Estimates of the incidence and prevalence of sexually transmitted diseases in the United States. American Social Health Association Panel. Sex Transm Dis 1999;26:S2–7.
2. Walboomers JM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999;189:12–9.
3. Carter JJ, Madeleine MM, Shera K, et al. Human papillomavirus 16 and 18 L1 serology compared across anogenital cancer sites. Cancer Res 2001;61:1934–40.
4. Schiffman M, Kjaer SK. Chapter 2: Natural history of anogenital human papillomavirus infection and neoplasia. J Natl Cancer Inst Monogr 2003;31:14–9.
5. Brown DR, Schroeder JM, Bryan JT, Stoler MH, Fife KH. Detection of multiple human papillomavirus types in condylomata acuminata lesions from otherwise healthy and immunosuppressed patients. J Clin Microbiol 1999;37:3316–22.
6. Greer CE, Wheeler CM, Ladner MB, et al. Human papillomavirus (HPV) type distribution and serological response to HPV type 6 virus-like particles in patients with genital warts. J Clin Microbiol 1995;33:2058–63.
7. Pou AM, Rimell FL, Jordan JA, et al. Adult respiratory papillomatosis: human papillomavirus type and viral coinfections as predictors of prognosis. Ann Otol Rhinol Laryngol 1995;104:758–62.
8. Rimell FL, Shoemaker DL, Pou AM, et al. Pediatric respiratory papillomatosis: prognostic role of viral typing and cofactors. Laryngoscope 1997;107:915–8.
9. Herrero R. Chapter 7: Human papillomavirus and cancer of the upper aerodigestive tract. J Natl Cancer Inst Monogr 2003;31:47–51.
10. Maissi E, Marteau TM, Hankins M, et al. Psychological impact of human papillomavirus testing in women with borderline or mildly dyskaryotic cervical smear test results: cross sectional questionnaire study. BMJ 2004;328:1293.
11. Clarke P, Ebel C, Catotti DN, Stewart S. The psychosocial impact of human papillomavirus infection: implications for health care providers. Int J STD AIDS 1996;7:197–200.
12. Villa LL, Costa RL, Petta CA, et al. Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial. Lancet Oncol 2005;6:271–8.
13. Harper DM, Franco EL, Wheeler C, et al. Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. Lancet 2004;364:1757–65.
14. Clifford GM, Smith JS, Plummer M, Munoz N, Franceschi S. Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. Br J Cancer 2003;88:63–73.
15. Aoyama C, Peters J, Senadheera S, Liu P, Shimada H. Uterine cervical dysplasia and cancer: identification of c-myc status by quantitative polymerase chain reaction. Diagn Mol Pathol 1998;7:324–30.
16. Evans MF, Mount SL, Beatty BG, Cooper K. Biotinyl-tyramide-based *in situ* hybridization signal patterns distinguish human papillomavirus type and grade of cervical intraepithelial neoplasia. Mod Pathol 2002;15:1339–47.
17. Isacson C, Kessis TD, Hedrick L, Cho KR. Both cell proliferation and apoptosis increase with lesion grade in cervical neoplasia but do not correlate with human papillomavirus type. Cancer Res 1996;56:669–74.
18. Quade BJ, Park JJ, Crum CP, Sun D, Dutta A. *In vivo* cyclin E expression as a marker for early cervical neoplasia. Mod Pathol 1998;11:1238–46.
19. Dasbach EJ, Elbasha EH, Insinga RP. Mathematical models for predicting the epidemiologic and economic impact of vaccination against human papillomavirus infection and disease. Epidemiol Rev 2006;28:88–100.
20. Holmes J, Hemmett L, Garfield S. The cost-effectiveness of human papillomavirus screening for cervical cancer. A review of recent modelling studies. Eur J Health Econ 2005;6:30–7.
21. McCrory DC, Myers ER, Nanda K, et al. Evaluation of cervical cytology. Evidence report/technology assessment no. 5. AHCPR publication no. 99-E010. Rockville (MD): Agency for Healthcare Policy and Research; 1999.
22. Koutsky LA, Ault KA, Wheeler CM, et al. A controlled trial of a human papillomavirus type 16 vaccine. N Engl J Med 2002;347:1645–51.
23. Mao C, Koutsky LA, Ault KA, et al. Efficacy of human papillomavirus-16 vaccine to prevent cervical intraepithelial neoplasia: a randomized controlled trial. Obstet Gynecol 2006;107:18–27.
24. Giuliano AR, Harris R, Sedjo RL, et al. Incidence, prevalence, and clearance of type-specific human papillomavirus infections: The Young Women's Health Study. J Infect Dis 2002;186:462–9.
25. Hildesheim A, Schiffman MH, Gravitt PE, et al. Persistence of type-specific human papillomavirus infection among cytologically normal women. J Infect Dis 1994;169:235–40.
26. Molano M, Van den BA, Plummer M, et al. Determinants of clearance of human papillomavirus infections in Colombian women with normal cytology: a population-based, 5-year follow-up study. Am J Epidemiol 2003;158:486–94.
27. Franco EL, Villa LL, Sobrinho JP, et al. Epidemiology of acquisition and clearance of cervical human papillomavirus infection in women from a high-risk area for cervical cancer. J Infect Dis 1999;180:1415–23.
28. Koshiol JE, Schroeder JC, Jamieson DJ, et al. Time to clearance of human papillomavirus infection by type and human immunodeficiency virus serostatus. Int J Cancer 2006;119:1623–9.
29. Brown DR, Shew ML, Qadadri B, et al. A longitudinal study of genital human papillomavirus infection in a cohort of closely followed adolescent women. J Infect Dis 2005;191:182–92.
30. Syrjanen S, Shabalova I, Petrovichev N, et al. Age-specific incidence and clearance of high-risk human papillomavirus infections in women in the former Soviet Union. Int J STD AIDS 2005;16:217–23.
31. Kotloff KL, Wasserman SS, Russ K, et al. Detection of genital human papillomavirus and associated cytological abnormalities among college women. Sex Transm Dis 1998;25:243–50.
32. Xi LF, Carter JJ, Galloway DA, et al. Acquisition and natural history of human papillomavirus type 16 variant infection among a cohort of female university students. Cancer Epidemiol Biomarkers Prev 2002;11:343–51.
33. Richardson H, Kelsall G, Tellier P, et al. The natural history of type-specific human papillomavirus infections in female university students. Cancer Epidemiol Biomarkers Prev 2003;12:485–90.
34. Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. N Engl J Med 1998;338:423–8.
35. Woodman CB, Collins S, Winter H, et al. Natural history of cervical human papillomavirus infection in young women: a longitudinal cohort study. Lancet 2001;357:1831–6.
36. Winer RL, Lee SK, Hughes JP, et al. Genital human papillomavirus infection: incidence and risk factors in a cohort of female university students. Am J Epidemiol 2003;157:218–26.
37. Brown DR, Fife KH, Wheeler CM, et al. Early assessment of the efficacy of a human papillomavirus type 16 L1 virus-like particle vaccine. Vaccine 2004;22:2936–42.
38. Cruickshank ME, Sharp L, Chambers G, Smart L, Murray G. Persistent infection with human papillomavirus following the successful treatment of high grade cervical intraepithelial neoplasia. BJOG 2002;109:579–81.
39. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958;53:457–81.
40. Greenwood M. The errors of sampling of the survivorship tables. Reports on public health and statistical subjects. Report no. 33, Appendix 1. London: HMSO; 1926.
41. Collett D. Modelling survival data in medical research. Boca Raton (FL): Chapman & Hall/CRC; 1994.
42. Mosher WD, Chandra A, Jones J. Sexual behavior and selected health measures: men and women 15-44 years of age, United States, 2002. Adv Data 2005;362:1–55.
43. Manhart LE, Holmes KK, Koutsky LA, et al. Human papillomavirus infection among sexually active young women in the United States: implications for developing a vaccination strategy. Sex Transm Dis 2006;33:502–8.
44. Goldie SJ, Kohli M, Grima D, et al. Projected clinical benefits and cost-effectiveness of a human papillomavirus 16/18 vaccine. J Natl Cancer Inst 2004;96:604–15.
45. Chenoy R, Billingham L, Irani S, et al. The effect of directed biopsy on the atypical cervical transformation zone: assessed by digital imaging colposcopy. Br J Obstet Gynaecol 1996;103:457–62.

Incidence and Duration of Cervical Human Papillomavirus 6, 11, 16, and 18 Infections in Young Women: An Evaluation from Multiple Analytic Perspectives

Ralph P. Insinga, Erik J. Dasbach, Elamin H. Elbasha, et al.

Cancer Epidemiol Biomarkers Prev 2007;16:709-715.