Research Article

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Incidence, Duration, and Reappearance of Type-Specific Cervical Human Papillomavirus Infections in Young Women

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Abstract

Background: We describe the incidence and duration of cervical human papillomavirus (HPV) infection episodes along with the risk of infection reappearance following a period of nondetection.

Methods: Women (1,788) ages 16 to 23 years underwent cytologic testing and PCR-based testing of cervical swab samples for HPV DNA (HPV-16/18/31/33/35/45/52/58/59) at ~6-month intervals for up to 4 years in the context of a phase 3 clinical trial (placebo arm). HPV type–specific incidence rates were estimated per 100 person-years. Duration of type-specific cervical infection episodes and risk of reappearance following a period of nondetection were estimated using Kaplan-Meier methods.

Results: HPV-16 exhibited the highest (5.9), and HPV-35 and HPV-33 exhibited the lowest (1.0) incidence rates per 100 person-years. Mean cervical infection durations ranged from 13 months for HPV-59 to 20 months for HPV-16 and 58 (with ongoing infections censored at the time of treatment, if done). The risk of cervical infection reappearance within ~3 years following a period of nondetection ranged from 0% to 16% across HPV types, with a mean of 8%. Limited evidence was found for a role of false-positive HPV tests, missed infections that were above the threshold for detection, or new acquisition of infection in accounting for patterns of infection reappearance.

Conclusions: Incidence of high-risk cervical infection was observed to vary considerably more across HPV types than infection duration. A nontrivial proportion of women exhibited infection reappearance following a period of nondetection, with a potential explanation for many such events observed within this analysis being a return to detectable levels of a previously acquired infection.

Impact: The risk of HPV infection reappearance following a period of nondetection has not been previously reported for individual HPV types, and this study finds that a nontrivial proportion of infected women exhibit reappearances. Future studies could ascertain subject-level factors that potentially modify the risk of infection reappearance. *Cancer Epidemiol Biomarkers Prev; 19(6); 1585–94. ©2010 AACR.*

Introduction

Human papillomavirus (HPV) infections can cause cervical, anal, penile, vaginal, vulvar, and head and neck cancers, anogenital warts, and recurrent respiratory papillomatosis (1, 2). HPV types 16 and 18 are often the most commonly observed types among these cancers, with types 6 and 11 accounting for the vast majority of anogenital warts and recurrent respiratory papillomatosis (3). However, other HPV types also contribute to disease, particularly cancers of cervix (4-6). There have been studies to suggest that prophylactic HPV vaccines confer a degree of cross-protection against some of these nonvaccine types (7-9), and in the future, coverage of vaccines may be broadened to incorporate additional HPV types (10). Thus, an understanding of their natural histories will be important for forthcoming policy evaluations.

We have previously described the incidence and duration of cervical HPV-6, 11, 16, and 18 infections based on data from the placebo arm of a phase II HPV-16 vaccine

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trial (11). In the present analysis, we examine these parameters for a broader array of HPV types, using data from the placebo arm of a phase III HPV-6/11/16/18 vaccine trial (9). Several previous studies have estimated the median duration of non–HPV-6/11/16/18 types (12-16), but only two analyses have described mean durations (14, 15), a potentially useful parameter for infectious disease modeling.

Epidemiologic models of HPV infection and disease typically separate time spent with HPV infection in the absence of detectable cervical intraepithelial neoplasia (CIN) from time with CIN (17). A unique feature of the present analysis is the incorporation of this perspective within the analytic methods. We also examine the frequency of reappearance of type-specific cervical HPV infection following a period of nondetection and explore the potential roles of four specific events in contributing to infection reappearance: (*a*) false-positive HPV test results, (*b*) missed infections that were above the threshold for detection, (*c*) new acquisition of HPV infection, and (*d*) a return to detectable levels of a previously acquired HPV infection. The frequency of infection reappearance following a period of nondetection has not been previously described for specific HPV types.

Materials and Methods

Study participants and procedures

The current evaluation focuses on women enrolled in the placebo arm of a multinational, randomized, double-blind clinical trial of an HPV-6/11/16/18 vaccine (Gardasil/Silgard, Merck and Co., Inc.). The study population of Merck Protocol V501-012 and trial design have been described elsewhere (9, 18). Briefly, the placebo arm population consisted of 1,788 women who on their first day of the trial were 16 to 23 years of age and did not report a history of clinical HPV disease (e.g., CIN or genital warts) or more than four lifetime sex partners. The study was conducted in conformance with applicable federal and local requirements regarding ethical committee review and protection of human subjects participating in biomedical research.

Women underwent type-specific endo/ectocervical and labial/vulvar/perineal/perianal (LVPP) swab HPV PCR testing for "high-risk" HPV-16/18/31/33/35/45/52/58/59 infections at day 1, month 3, month 7, and at ~6-month intervals thereafter through 48 months as described (18-20). Briefly, swabs were prepared for PCR using a QIAamp DNA Blood kit (QIAGEN, Inc.). DNA was analyzed by qualitative PCR using type-specific and gene-specific primers. β-Globin PCR assay was done to verify that purified samples contained a sufficient quality and quantity of DNA for PCR amplification. All PCR assays were done at Merck Research Laboratories.

Cervical samples were also collected for liquid-based cytology (ThinPrep, Cytyc) testing at day 1, month 7, and at ~6-month intervals thereafter. Subjects were referred for colposcopy, and biopsy when indicated, if the cytology result showed (*a*) atypical squamous cells of undetermined significance along with a positive reflex Hybrid Capture II test (Digene) for high- and/or low-risk HPV types from residual ThinPrep material or (*b*) low-grade squamous intraepithelial lesion or worse cytology. All women with normal cytology results up to the last scheduled trial visit, who had an abnormal Pap test at that visit, were referred for colposcopy. Cervical tissue specimens were processed, and adjacent histologic sections were first read for clinical management by pathologists at a central laboratory (Diagnostic Cytology Laboratories) and then read for end point determination by a panel of up to four pathologists who were blinded to central laboratory and clinical diagnoses, treatment group, and HPV status. Histologic specimens were typed by PCR for HPV-6/11/16/18/31/33/35/39/45/51/52/56/58/59 as described (20, 21).

Measures

Incidence

Incident cervical HPV-16/18/31/33/35/45/52/58/59 infections were identified from cervical swab or tissue specimens among women negative for the relevant HPV type on their first two study cervical swabs and any cervical biopsy specimens obtained on or before the second swab. Only women meeting these criteria and with at least one subsequent cervical swab specimen available were eligible to contribute person-time to each type-specific analysis. Consistent with others' and our earlier work (11, 22, 23), we assumed cervical HPV infections occurred at the midpoint between the initial positive test date and the previous negative test (Fig. 1). Time elapsed following the point of incident infection was not included in estimates of person-time at risk. We focused our analysis specifically on the cervical HPV infections because the incidence, duration, and reappearance of HPV infections can be site specific (24-26), with cervical HPV infections potentially leading to cervical precancers and cancers.

Duration

The monthly duration of each incident cervical HPV infection was examined from two analytic perspectives, which we have previously termed as "health state" (infection alone) and "natural history" (infection and disease) perspectives (Fig. 1; ref. 11). Because it could not be definitively ascertained whether women may have previously been infected and become negative for the same HPV type before the start of the study, and incident infections observed could reappear following a period of nondetection, we refer to these as incident infection "episodes" when discussing duration. Under the *health state perspective*, incident HPV infection episode duration was measured until either the detection of a CIN1-3 lesion for which the cervical tissue specimen tested PCR positive for the relevant HPV type, or negative cervical swab HPV test results for that type (Fig. 1). This health state perspective is consistent with how HPV infection is often

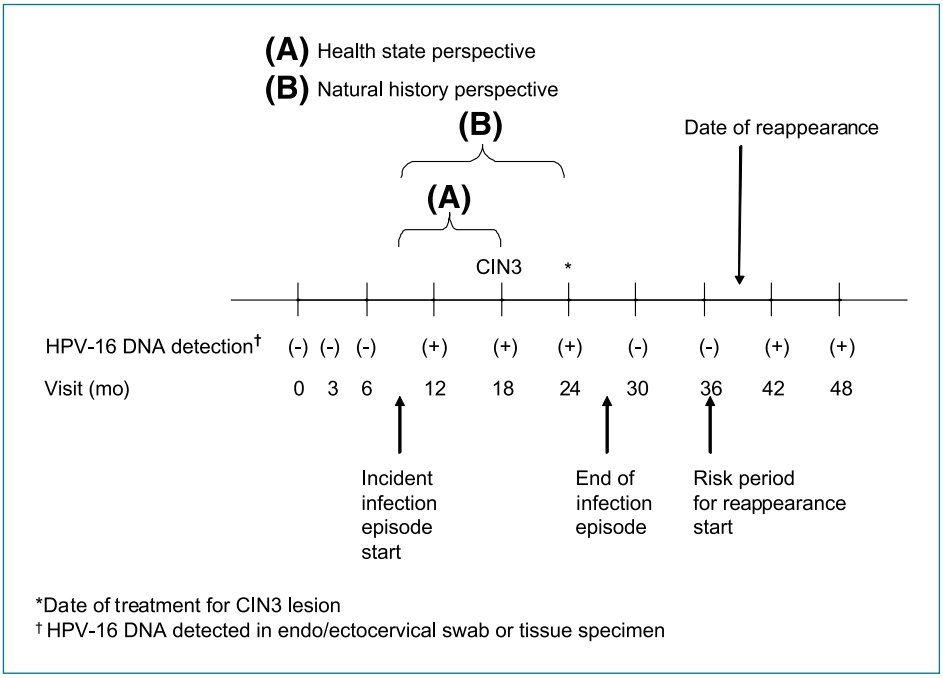

Figure 1. Incidence, duration, and reappearance of HPV infection. This example illustrates how the duration of a hypothetical HPV-16 infection would be categorized based on the health state and natural history analytic perspectives. For both perspectives, infection duration begins at the midpoint between the prior negative and the first HPV-positive HPV-16 test result; in this example, it is at month 9. For the health state perspective (A), HPV infection duration continues until either a period of no HPV detection or until the development of CIN; in this case, it is until the development of CIN3 at month 18. For the natural history perspective (B), infection duration either ends with a period of no HPV DNA detection or is censored at the time of disease treatment; in this case, it is with censoring at treatment at month 24. For the analysis of infection reappearance, the end of the initial infection episode occurs at month 27, representing the midpoint in time between the last positive test at month 24 and the first negative swab at month 30. The risk period for assessing reappearance begins following the date of the second negative swab at month 36. In this instance, this risk period ends on the estimated date of reappearance (month 39), estimated as the midpoint in time between the last negative swab and first subsequent sample testing positive for HPV 16.

conceptualized in policy evaluations of interventions to prevent and diagnose HPV disease (17, 27), in which women may transition from a health state characterized by infection in the absence of clinically detectable CIN to development of a clinically detectable CIN lesion.

Following an incident infection, if two consecutive negative swabs for that type were subsequently observed, the infection was assumed to have gone below detectable levels at the midpoint in time between the final positive test and the first negative swab result (Fig. 1). As previously reviewed in greater conceptual detail (11), for women with a single negative swab at the last visit on record (following an incident infection episode), it was assumed that the infection had gone below detectable levels at the midpoint in time between the final positive test and the negative swab. Positive swab or biopsy specimens, followed by a single negative swab, followed by a positive swab or biopsy specimen (occurring among 4.1% of incident infections), were analyzed as persistent infections. Ongoing infections characterized by a positive test on the date of the final trial swab were evaluated as censored. Analytic methods were similar for the *natural history perspective*, with the exception that ongoing infections were censored at the time of treatment, if done, rather than truncated on the date of a CIN diagnosis positive for the relevant HPV type (Fig. 1). Given that treatment of a lesion may eliminate infection, by censoring duration at the time of therapy, this perspective can yield estimates of infection episode duration more consistent with its complete natural course.

Reappearance

Analyses of the risk of type-specific infection reappearance following a period of nondetection included all women (regardless of baseline HPV status and regardless of treatment status) with endo/ectocervical swab or tissue specimen(s) testing PCR positive for a given HPV type who then had at least two consecutive negative swab tests (Fig. 1). Person-time was defined to begin after the date of the second negative swab as, by definition, women were not at risk for infection reappearance before this time point. For women with only negative PCR test results for a given HPV type on all subsequent endo/ectocervical swab and tissue specimens, person-time was defined to end on the date of the last swab. For women with a subsequent swab or tissue specimen testing positive for a given HPV type, the date of infection reappearance was defined as the midpoint in time between the positive test and the previous negative swab, with person-time truncated on this interpolated date. Four potential hypotheses about the reasons for infection

reappearance were considered as follows: (*a*) false-positive HPV test results, (*b*) false-negative HPV test results, (*c*) new acquisition of HPV infection, and (*d*) return of previously acquired HPV infection to detectable levels.

False-positive results

The potential for false-positive HPV test results to contribute to the observed infection patterns was first examined by descriptively assessing the point prevalence estimates for individual HPV types and comparing them to the degree of multiplicity of HPV-positive results during the initial and reappearing HPV infections. A low point prevalence for a given type, combined with a moderate to large number of consecutive HPV-positive results during both the initial and reappearing infections, would suggest a relatively low likelihood of the observed patterns being primarily due to false-positive test results. Next, type-specific test results were examined for LVPP swabs collected at the same visits at which positive cervical swab results were observed within the initial and reappearing infections. A consistent pattern of discordance in type-specific swab results collected at the two sites could suggest the potential presence of false-positive cervical swab results. Finally, to be scored positive for HPV, a sample must have tested positive on at least two of three genes (*L1*, *E6*, or *E7*) or on one gene, followed by a positive result for the same gene on a retest of the same sample. We investigated whether single-gene positive samples were more likely to be seen within single-time HPV-positive results in either the initial or reappearing infection than within infection runs characterized by multiple positive tests.

Missed infections above the threshold for detection

We also explored whether cervical HPV DNA may have been present at a detectable level but was not detected within a given sample (e.g., due to incomplete swabbing of the cervix). The potential contribution of this type of false-negative HPV test result was explored by examining intervening negative swab specimens observed between the conclusion of the initial HPV infection and its reappearance, and evaluating type-specific test results for LVPP swabs collected at the same visits. Analogous to assessments of potentially false-positive results, a high degree of type-specific discordance in swab results collected at the two sites could suggest the potential presence of this type of false-negative swab result.

Acquisition of new infections

To investigate whether reappearing infections might have been due to new acquisition of the same HPV type, first, sexual activity data were examined for women with infection reappearance. Self-reported data on the number of new sex partners (male or female) since the previous routinely scheduled trial visit before the date of detected reappearance among these women was examined. In most instances (88% of all reappearing infections), this look-back period (~6 mo) corresponded to the time from the visit concurrent with the date of infection reappearance, to the scheduled visit immediately prior, when the woman had last tested negative for the relevant HPV type on a swab sample. However, in cases in which sexual activity data from a concurrent routinely scheduled visit was not available, the look-back period for sexual activity corresponded to the time elapsed between routinely scheduled visits straddling the date of infection reappearance. A sensitivity analysis was done, extending the look-back period by one additional visit (~12-mo window). These visits were examined to provide information on sexual activity before the date of infection reappearance. If infection reappearances were primarily due to newly acquired infections of the same HPV type, one would expect to observe new sex partners during the months before infection reappearance for a substantive proportion of women (28, 29).

Next, the association between having had a new sex partner since the last scheduled visit and infection reappearance was examined in multivariate regression analyses. Regression analyses were restricted to women whose HPV infections had gone below detectable levels, as evidenced by at least two consecutive negative swabs, with the risk period for reappearance starting from the date of the second negative swab. Control variables included HPV type, age, region, lifetime number of sex partners (at start of risk period), tobacco use since prior scheduled visit (never used tobacco products, ex-user, or current user), contraceptive use since prior scheduled visit (barrier method, chemical method, both barrier and chemical method, or neither barrier nor chemical method), treatment for CIN (on or following the date of detection of the initial HPV infection, and before any reappearance of infection), and sexual activity since the prior scheduled visit (no sex partner, same sex partner as in a prior period, or new sex partner).

Swabs were not routinely tested for HPV-6 or 11 throughout the trial, and the risk of reappearance of these types could not be evaluated therein. However, routine swab testing for these types was conducted in an earlier Merck trial of an HPV-16 vaccine as previously described, from which infection incidence and duration, but not reappearance, was reported (11). Placebo arm data (n = 1,203) from that trial were therefore assessed for examining the risk of HPV-6/11 infection reappearance. These data were excluded from analyses examining potential reasons for infection reappearance, however, due to incomplete availability of LVPP swab results and female sexual partnership information from this earlier trial.

Statistical analysis

All analyses was conducted using SAS version 9.1. HPV type–specific incidence rates per 100 person-years were estimated, along with Poisson-based 95% confidence intervals (95% CI). Poisson regression was used to test for differences in incidence rates between HPV types, accounting for within-subject correlations. Mean

and median durations of HPV infection episodes, and the risk of HPV infection reappearance over time were estimated using Kaplan-Meier methods (30). Potential differences in the mean duration of HPV infection across types were analyzed using an accelerated failure time regression model (PROC LIFEREG), specifying a generalized γ distribution (as was observed to exhibit the best model fit; ref. 31). Comparisons in the risk of infection reappearance between various time intervals, and for incident versus prevalent infections, were estimated using a Cox regression model (32) controlling for HPV type, with indicator variables for time period and incident status, respectively. The robust sandwich estimator of Lin and Wei (33) was used to account for within-subject correlation. A Cox regression model was also used for multivariate analyses of the risk of infection reappearance associated with having had a new sex partner. Tobacco use, contraceptive method, CIN treatment, and sexual activity were included as time-dependent covariates, whereas all other independent variables corresponded to fixed covariates.

Results

Baseline

Among 1,788 women in the placebo arm of the trial who underwent endo/ectocervical swab HPV PCR testing at day 1, the prevalence of individual HPV types was as follows: HPV-16 (6.6%), HPV-31 (3.8%), HPV-52 (3.4%), HPV-59 (3.3%), HPV-58 (2.8%), HPV-18 (2.6%), HPV-45 (2.0%), HPV-33 (1.0%), and HPV-35 (0.9%). There were 1,694 women potentially eligible for one or more analyses of type-specific HPV incidence who had at least three trial visits with satisfactory endo/ectocervical swab PCR test results for a given HPV type, with the exception of for HPV-58 and 59 (n = 1,693). Baseline characteristics for these women are presented in Table 1.

Table 1. Baseline characteristics of women potentially eligible for one or more analyses of HPV type–specific incidence (with satisfactory swab results at three or more trial visits)

Variable	*n* = 1,694 *n* (%)
Age group, y	
16-19	594 (35.1)
20-23	1,100 (64.9)
Region	
Asia	154 (9.1)
Australia/New Zealand	139 (8.2)
Europe	315 (18.6)
South/Central America	403 (23.8)
North America	683 (40.3)
Tobacco use	
Current user	411 (24.3)
Ex-user	195 (11.5)
No reported use	1,088 (64.2)
Contraceptive use	
Barrier method (no chemical method)	490 (28.9)
Chemical method (no barrier method)	728 (43.0)
Barrier and chemical methods	158 (9.3)
Other/no method	318 (18.8)
Lifetime number of sex partners	
0	107 (6.3)
1-2	949 (56.0)
3-4	638 (37.7)
Past pregnancy	
Yes	311 (18.4)
No	1,383 (81.6)

Incidence

HPV-16 was the most commonly occurring HPV type (5.9 per 100 person-years), with an incidence rate of >70% higher (P = 0.006) than the high-risk HPV type with the next highest incidence, HPV-52 (Table 2). HPV-35 and HPV-33 were the least commonly occurring HPV types with incidence rates near 1 per 100 person-years that were observed to be below (P = 0.05 and 0.07, respectively) the incidence rate for the next least common type (1.5 per 100 person-years for HPV-45).

Duration

Available follow-up, after incident HPV infection, varied by woman and HPV type (range in mean follow-up times across HPV types was 19.0-22.8 mo). Mean and median durations for incident HPV infection episodes from the health state and natural history perspectives are described in Table 3. Although a >5-fold difference was previously seen among incidence rates for the various HPV types, the variation across types in mean duration of detectable infection from either perspective was not nearly so great (<1.6:1). For instance, the longest mean durations from the natural history perspective were observed for HPV-16 and 58 (~20 mo); however, these were proximate to, and did not differ statistically from, those for HPV-31 or 33 (P > 0.05 for all comparisons). The shortest mean duration from the natural history perspective was observed for HPV-59 (13.0 mo), which was statistically similar (P = 0.31) to that for HPV-35 (13.8 mo). As would be expected, mean durations estimated from the natural history perspective were uniformly longer than those from the health state perspective, in which duration was truncated with the diagnosis of a CIN lesion testing positive for the relevant HPV type (by 7-30% across HPV types). Mean infection episode duration is slightly underestimated for certain HPV types due to infections of longest duration persisting at the conclusion of follow-up as noted in Table 3. In the health state perspective analysis, 3.7% of all infections persisted beyond the longest follow-up time (range by type, 0.0-10.9%) compared with 9.0% for the natural history perspective (range by type, 0.0-19.0%).

Table 2. Incidence rates by HPV type

HPV type	*n*	Mean exposure time, y	Cases/person-years	Incidence per 100 person-years	(95% CI)
HPV-16	1,558	3.0	273/4,606	5.9	5.2-6.6
HPV-18	1,642	3.1	113/5,162	2.2	1.8-2.6
HPV-31	1,603	3.1	157/4,978	3.2	2.7-3.6
HPV-33	1,677	3.2	57/5,386	1.1	0.8-1.3
HPV-35	1,671	3.2	52/5,392	1.0	0.7-1.2
HPV-45	1,649	3.2	77/5,267	1.5	1.1-1.8
HPV-52	1,614	3.1	173/4,987	3.5	3.0-4.0
HPV-58	1,641	3.2	109/5,215	2.1	1.7-2.5
HPV-59	1,617	3.1	172/5,006	3.4	2.9-3.9

Reappearance

The frequency of HPV infection reappearance following a period of nondetection is reported in Table 4. It should be noted that person-time at risk (overall denominator = 827) was defined to begin after the date of the second consecutive negative swab result for a given HPV type following nondetection (mean interval of 8.0 mo for reappearing infections). Following this wash out period, a cumulative total of 4.9%, 5.9%, and 8.1% of type-specific infections reappeared within 12 (n = 38), 24 (n = 43), and 36 (n = 48) months, respectively, after an additional 13.3 months on average. The risk of infection reappearance was higher in the first 12-month period than during either of the subsequent two 12-month periods ($P < 0.01$). Within 36 months following the date of the second negative swab following nondetection, type-specific infection reappearance rates exceeded 10% for both HPV-6 and 16 and were <5% for HPV-35, 45, and 58. However, there was considerable overlap in CIs across individual types. When evaluating only incident HPV infection (episodes overall denominator = 294), overall cumulative risks of infection reappearance were 4.3% and 4.9%, respectively, within 12 months (n = 12) and 24 months (n = 13; data were insufficient for evaluating 36 mo), with risk not differing from that observed for baseline prevalent infections (P = 0.58).

False-positive results

Regarding the potential contribution of false-positive HPV test results to patterns of infection reappearance, the baseline point prevalence for the 11 HPV types included in Table 4 ranged from 0.5% to 6.6% (11), effectively ruling out a false-positive detection rate much in excess of a few percent. Data were reviewed from all 43 instances in which HPV-16/18/31/33/35/45/52/58/59 infections reappeared following a period of nondetection. There were 12 instances for which positive HPV test results were observed on multiple dates within both the initial infection as well as the reappearing infection, and 2 instances for which three separate runs of positive

Table 3. Duration of incident cervical HPV-16, 18, 31, 33, 35, 45, 52, 58, and 59 infection episodes

	Health state perspective		Natural history perspective	
	Mean* duration, mo (95% CI)	Median duration, mo (95% CI)	Mean† duration, mo (95% CI)	Median duration, mo (95% CI)
HPV-16 (n = 273)	15.5 (14.2-16.8)	12.8 (11.9-15.1)	20.2 (18.4-22.0)	17.1 (15.1-20.2)
HPV-18 (n = 113)	12.5 (11.0-14.0)	9.6 (8.9-12.1)	16.1 (13.9-18.2)	12.4 (11.0-17.7)
HPV-31 (n = 157)	14.2 (12.6-15.7)	11.2 (10.3-12.6)	18.2 (16.1-20.2)	16.8 (14.1-20.3)
HPV-33 (n = 57)	15.0 (12.5-17.5)	13.8 (11.0-16.3)	17.6 (14.4-20.7)	14.9 (11.2-18.1)
HPV-35 (n = 52)	12.6 (10.2-15.0)	9.6 (7.1-14.5)	13.8 (11.6-16.0)	12.8 (8.4-20.2)
HPV-45 (n = 77)	13.3 (11.1-15.5)	10.7 (9.8-13.7)	15.4 (13.0-17.8)	12.2 (11.1-17.2)
HPV-52 (n = 173)	14.8 (13.3-16.3)	12.6 (10.9-16.2)	17.2 (15.7-18.7)	17.7 (14.9-21.5)
HPV-58 (n = 109)	16.3 (13.6-19.0)	12.6 (11.4-16.9)	20.0 (16.7-22.5)	15.9 (13.5-22.2)
HPV-59 (n = 172)	12.1 (10.9-13.3)	10.6 (8.9-11.9)	13.0 (11.8-14.3)	11.7 (10.0-12.5)

*Because individual observations with the longest follow-up times were censored in analyses for HPV-16, 18, 31, 35, and 52, mean durations of infections are slightly underestimated.

†Because individual observations with the longest follow-up times were censored in analyses of HPV-16, 18, 35, 52, and 59, mean durations of infections are slightly underestimated.

Table 4. Reappearance of cervical HPV-6,11, 16, 18, 31, 33, 35, 45, 52, 58, and 59 infections following a period nondetection

HPV type (n = 827)	Proportion of infections reappearing by 12 mo* (95% CI)	Proportion of infections reappearing by 24 mo* (95% CI)	Proportion of infections reappearing by 36 mo* (95% CI)
HPV-6 (n = 89)	0.0 (–)	1.7 (0.2-11.2)	16.1 (5.6-41.1)
HPV-11 (n = 11)	9.1 (1.3-49.2)	9.1 (1.3-49.2)	9.1 (1.3-49.2)
HPV-16 (n = 162)	6.6 (3.6-11.9)	7.5 (5.2-13.3)	11.0 (6.1-19.4)
HPV-18 (n = 70)	5.8 (2.2-14.8)	8.8 (3.5-20.8)	8.8 (3.5-20.8)
HPV-31 (n = 112)	6.8 (3.3-13.7)	6.8 (3.3-13.7)	6.8 (3.3-13.7)
HPV-33 (n = 33)	3.0 (0.4-19.6)	7.9 (1.9-29.0)	7.9 (1.9-29.0)
HPV-35 (n = 26)	0.0 (–)	0.0 (–)	0.0 (–)
HPV-45 (n = 53)	3.8 (1.0-14.3)	3.8 (1.0-14.3)	3.8 (1.0-14.3)
HPV-52 (n = 100)	6.4 (2.9-13.6)	6.4 (2.9-13.6)	6.4 (2.9-13.6)
HPV-58 (n = 55)	2.3 (0.3-15.4)	2.3 (0.3-15.4)	2.3 (0.3-15.4)
HPV-59 (n = 116)	5.4 (2.5-11.7)	7.5 (3.4-16.0)	7.5 (3.4-16.0)

*These monthly intervals refer to time from the date of the second negative cervical swab following the initial infection. The actual time from infection nondetection corresponding to these data is therefore ~8 mo longer (is 9-21, 21-33, and 33-45 mo following nondetection).

tests were observed (that is, positive test results after two separate periods of nondetection). These 14 instances would seem least likely to be the product of false-positive results, and a comparison with LVPP swab results for the same HPV types at the same visits with positive cervical swabs revealed at least some concordance in HPV positivity within both the initial and reappearing infections in 10 cases (71.4%), completely negative LVPP swabs in 3 cases (21.4%), and positive (initial)/negative (reappearing) results in 1 case (7.1%). Overall concordance in positivity between cervical and LVPP swabs in this subgroup was 56%. From all cervical samples scored as HPV positive for these cases (n = 79), 98.7% tested positive for two or more genes.

In 13 instances, only a single positive HPV test was observed in isolation [that is, preceded and followed by negative test(s) for that type] for either the initial infection, the reappearing infection, or both infections. These 13 instances would seem most likely to reflect false-positive results; however, a comparison with LVPP swab results for the "one-time positive" cervical swab results within 11 evaluable cases revealed concordance in HPV positivity in 7 cases (63.6%), which is similar to what was observed for reappearing infections with multiple positive samples. Two cases were nonevaluable due to one-time positive cervical infections being detected in a tissue specimen, without a corresponding LVPP swab sample. All single-time HPV-positive results within the initial and reappearing HPV infections for these cases (n = 15) tested positive for two or more genes. Finally, the remaining 16 instances reflected cases in which observation either preceding or following a single positive test was censored, preventing the assessment of whether one or both infection runs reflected single or multiple positive tests. It would therefore seem unlikely that many of the observed patterns of infection reappearances are the product of false-positive test results as, among the 27 instances for which multiplicity of positive test results could be evaluated, 14 (52%) reflected either multiple positive tests within two separate infection runs or three separate infection runs. Furthermore, the degree of correspondence with LVPP swabs did not differ between one-time positive and "multi-time positive" cervical swab runs, and there was no evidence of an excessive rate of single-gene positive test results. Considering all trial cervical swabs testing positive for one of the nine HPV types, in which an evaluable LVPP swab on the same date was available (n = 4,197), in 85.2% of instances, the LVPP swab result was also positive.

Missed infections above the threshold for detection

Between the last positive test within an initial HPV infection and the first positive test of a reappearance (minimum two consecutive negative tests), there were 124 cervical swab samples testing negative for the relevant HPV type. Analysis of the type-specific concordance between the 124 intervening negative cervical swab results and LVPP swabs collected at the same visit revealed a modest degree of discordance. There were 5 LVPP swabs for which test results were unavailable, with 21 of the remaining 119 (17.7%) testing positive for the relevant HPV type, originating from 14 of the 43 (32.6%) reappearing infections. A detailed review of LVPP swabs from these 14 cases revealed 5 (11.6%) instances in which all or most intervening swabs tested positive, 7 (16.3%) instances in which half (1 of 2) tested positive, and 2 (4.7%) instances in which a minority tested positive. Thus, false-negative specimens, in which infection was present at detectable levels, but failed to be detected, could potentially account for up to roughly 25% of infection reappearances; however, there may be

alternate interpretations for these findings as articulated in the Discussion.

Acquisition of new infections

To assist in assessing whether a newly acquired HPV infection of the same type may have contributed to infection reappearances, sexual activity data before infection reappearance were examined. Most women reported no new sex partners within the ~6-month periods (88.4% with no new sex partners) and ~12-month periods (83.7% with no new sex partners) before the date of detection of reappearance of their HPV infections. In a multivariate regression analysis including both instances in which infections were (n = 43) and were not (n = 651) detected as reappearing following a period of nondetection, neither the reporting of a new sex partner since the previous scheduled visit or the same sex partner as in a prior period (versus no sex partner) was significantly associated with the risk of infection reappearance.

Discussion

This analysis has described rates and durations of incident cervical HPV infections along with risks of infection reappearance following a period of nondetection. Consistent with several large studies conducted in other geographic settings (13, 15, 16), there was a multifold difference in incidence rates across high-risk HPV types, with HPV-16 observed to have the highest incidence among these. From the natural history perspective, mean infection episode durations for most HPV types were longer than previously estimated for populations in Montreal, Canada (14) and São Paulo, Brazil (15). Differences with respect to the former study might be due to a longer follow-up period in the current analysis. The latter study evaluated single-time negative HPV test results interspersed among positive ones as clearance of infection, whereas the present study considered these infections as persisting, which may help explain the longer durations reported here. In general, mean durations of infection episodes whether evaluated from the health state or natural history perspectives exhibited far less variation across types than that seen in incidence rates. The precise reason(s) for the large variation in incidence rates are poorly understood.

Data on the risk of HPV infection reappearance following a period of nondetection have not been previously reported for individual HPV types. The risk of reappearance within ~3 years following a period of nondetection ranged from 0% to 16% across HPV types, with a mean of 8%. Either modest or no evidence was found to support false-positive HPV test results, missed infections that were above the threshold for detection, or newly acquired HPV infections as contributors to the reappearance of HPV infections. This therefore leaves open the possibility that a return to detectable levels, among HPV infections that have fallen below the threshold for detection, is a contributor to infection reappearance, although we were not able to conduct a formal investigation of this hypothesis. A previous study also concluded that reappearing infections reflect a single persistent infection with wavering positivity, rather than acquisition of a new infection, on the basis of the same HPV type variant being detected over time (34). In that analysis, one or more negative swab results were evaluated as evidence of intercurrent negativity, whereas the present study required at least two consecutive negative swab results between positive tests. Reasons for the risk of infection reappearance in the present study being somewhat higher in the near term than in subsequent years are not known. However, it is possible that an important fraction of women whose infections go below detectable levels who are destined to have a reappearance will tend to do so in the near term if a return of the infection to detectable levels is triggered by a commonly occurring immunologic event (e.g., fluctuations in immunocompetence levels based on common life-style factors). Longer term follow-up studies would be needed to investigate this, including potentially an analysis of HPV variants (e.g., to investigate the presence of a common variant over time) and ascertainment of additional subject-level factors that could modify the risk of infection reappearance.

Although just over 25% of reappearing infections exhibited a 50% or greater discordance in intervening cervical and LVPP swab results between initial and reappearing cervical infections, it is difficult to definitively regard these instances as the product of false-negative cervical swabs. HPV infection detection in the vulva/vagina has been shown to precede that in the cervix in some women (26), and concordance in HPV positivity between the cervix and vulva seems to be substantially poorer than between the cervix and vagina (24). Thus, it is plausible that at least some of these discordant instances may reflect truly different natural courses of HPV infections at cervical and external genital sites rather than false-negative cervical swabs.

Our analysis has several limitations. As in virtually all HPV natural history studies, women with confirmed CIN lesions could undergo treatment, and their infection durations were censored at the time of therapy. As alluded to previously, if the subsequent course of their HPV infections differed from those for women with infections of similar duration who were not treated, then infection durations as reported from the natural history perspective would differ from those expected in a screening naïve population. Although a variety of methods were used to examine the potential plausibility of different hypotheses (that is, false-positive tests, false-negative tests, and new acquisition of infection) with regard to HPV infection reappearances, it was not possible to definitively assign a given explanation to a particular infection. It was also not possible to definitively ascertain whether a woman was previously infected with the same HPV type before the start of the study. It is therefore possible that at least a portion of "incident" or "initial" infections in the present study could have been preceded by HPV infections of the same type before the start of the trial. If this were the case, our estimates of the true incidence of HPV

infection would be overestimated and of the risk of infection reappearance would be underestimated. Because swab samples were typically collected every 6 months, infections of shorter duration may have been missed. In addition, we evaluated infection reappearances occurring after at least two consecutive negative cervical swab results (typically >12 mo) following an initially observed HPV infection episode. The relative importance of potential contributors to infection reappearance could differ if using a different time frame for defining reappearance. We did not have information on the sexual behavior of women's sex partners within the trial, and reporting of sexual behavior may have been imperfect. Finally, the proportion of all incident infections found to have just a single negative swab at the conclusion of follow-up (20.5%) was moderately higher than that reported in our previous analyses (14.4%; ref. 11), but this did not appreciably affect estimates of mean duration (data not shown).

In summary, the incidence of cervical infection was observed to vary considerably more across high-risk HPV types than duration of infection episodes. A nontrivial proportion of women exhibits type-specific infection reappearance following a period of nondetection. A potential explanation for many such events is deemed to be the return to detectable levels of previously acquired infection, which has decreased below the threshold for detection for a period of time.

Disclosure of Potential Conflicts of Interest

G. Perez has received lecture fees and consultancy fees from Merck and Co., Inc. and Sanofi Pasteur MSD, and is now an employee of the Sponsor, Merck and Co., Inc. C.M. Wheeler has received funding through her institution for reagents and equipment for Roche Molecular Systems in support of HPV genotyping studies and to conduct HPV vaccine studies for GlaxoSmithKline. D.G. Ferris has received consultancy, lecture, and advisory board fees from Merck and Co., Inc., and has received funding through his institution to conduct HPV studies for GlaxoSmithKline. S.M. Garland has received advisory board fees and grant support from Commonwealth Serum Laboratories and GlaxoSmithKline, lecture fees from Merck and Co., Inc., and research funds through her institution to conduct HPV vaccine studies for GlaxoSmithKline. S.M. Garland is a member of the Merck Global Advisory Board as well as the Merck Scientific Advisory Committee for HPV. S. Leodolter has received lecture fees from Merck and Sanofi Pasteur MSD. E.A. Joura has received advisory board fees from Merck and Sanofi Pasteur MSD; has received funding through his institution to conduct epidemiologic HPV studies for GlaxoSmithKline; and has received lecture fees from Merck and Co., Inc., Sanofi Pasteur MSD, and GlaxoSmithKline. D.R. Brown has received lecture fees, advisory board fees, and intellectual property fees from Merck and Co., Inc. M. Steben has received lecture fees and grant support from Merck and Co., Inc. J. Paavonen has received advisory board fees, and lecture fees from Merck and Co., Inc. In addition, G. Perez, C.M. Wheeler, D.G. Ferris, L.A. Koutsky, S.M.Garland, S.Leodolter, E.A. Joura, M. Steben, D.R. Brown, and J. Paavonen have received funding through their institutions to conduct HPV vaccine studies for Merck. R.P. Insinga, E.H. Elbasha, and R.M. Haupt are employees of Merck and potentially own stock and/or stock options in the company.

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