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Characterizing the Temporal Dynamics of Human Papillomavirus DNA Detectability Using Short-Interval Sampling

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Abstract

Background—Variable detection of human papillomavirus (HPV) DNA can result in misclassification of infection status, but the extent of misclassification has not been quantitatively evaluated.

Methods—In 2005–2007, 33 women aged 22–53 self-collected vaginal swabs twice per week for 16 consecutive weeks. Each of the 955 swabs collected was tested for 37 HPV types/subtypes. Assuming that a woman's underlying infection status did not change over the short study period, biases in prevalence estimates obtained from single versus multiple swabs were calculated. Using event history analysis methods, time to recurrent gain and loss of at least one HPV type was determined, separately. Baseline any- and high risk-HPV prevalence was 60.6% and 24.2%, respectively. Cumulative any- and high risk-HPV prevalence over the 16-week period was 84.8% and 60.6%, separately.

Results—Overall, there were 319 events of detection and 313 events of loss of detection. Median times to a recurrent detection and loss of detection was 11 and 7 days, respectively. Neither vaginal sex nor condom use during follow-up was associated with recurrent viral detection or loss of detection. Assuming the cumulative 16-week prevalence reflects the true prevalence of infection, the baseline any-HPV prevalence under-estimated infection status by 24.2%, with a bootstrapped mean of 20.2% (95% confidence interval [CI]: 8.9%, 29.6%).

Conclusions—These findings suggest that a substantial proportion of HPV-infected women are misclassified as being un-infected when using a single-time DNA measurement.

Impact—Short-term variation in detectable HPV DNA needs to be considered while interpreting the natural history of infections using single samples collected at long intervals.

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Keywords

Epidemiology; Human papillomavirus; Interval sampling; Misclassification bias; Prevalence

INTRODUCTION

Outcome assessment in HPV natural history studies relies on viral DNA detection from cervical samples collected at fixed intervals, usually every 4–6 months. Several recent studies using highly sensitive PCR-based tests to measure HPV DNA have reported that recurrent detection of an HPV type after a period of non-detection is common, ranging from around 8% to 19.4% (1–3). Weaver, et al, reported that samples testing HPV16 negative in between runs of HPV16 DNA positivity by standard consensus primer PCR (linear array, LA) were found to be HPV16 positive when more sensitive type-specific amplification assays were used, suggesting that recurrent detection is more likely to represent viral load fluctuations of a persistent infection or periods of latency and reactivation, rather than re-infection of a previously cleared viral type (4).

Because HPV incidence rates are based on person-time at risk for new infection, accurate estimates require valid assessment of baseline infection status at enrollment into a natural history study. Because HPV natural history studies suffer from left truncation bias (unobserved HPV DNA status prior to study entry) and interval sampling (unobserved HPV DNA status between study visits or sample collections), it is likely that a substantial fraction of newly detected HPV in observational studies is misclassified as an initial infection, rather than recurrent detection of a persistent infection (5).

In this study, we sought to characterize the temporal variability of detectable HPV DNA based on frequent self-sampling by women over a 16-week period. Under an assumption that fluctuation in DNA detection was largely a result of variable detection in women with low viral load (4), we estimated biases between prevalence estimates based on a single versus multiple samples using the 16-week cumulative prevalence as the ‘true’ prevalence. Under an alternative assumption that the loss and gain of DNA detection reflected acquisition, clearance, and reinfection or deposition of viral DNA from a male partner, we investigated whether recurrent detection or its subsequent loss of detection was associated with sexual activity at follow-up. In the absence of molecular markers to assess the validity of each hypothesis tested, these analyses were intended to evaluate the alternative assumptions for the best fit to the observed data.

MATERIALS AND METHODS

Study population and design

We utilized archived vaginal swabs from a prior study of vaginal douching cessation. The study design and characteristics of the study population were previously described elsewhere (6). In brief, the 16-week study began with a 4-week observational period (phase I) followed by a 12-week douching cessation where women were asked to withhold their douching practice (phase II). Forty-seven eligible women were enrolled between December 2005 and March 2007. Ethical approval for the primary study was obtained from the Institutional Review Boards (IRB) of the Johns Hopkins University School of Medicine. There was no evidence suggesting any effect of douching on HPV DNA detectability (7), and we did not observe any difference in HPV detection between the two study phases within the same subjects. Therefore, the current analysis included women who completed both phases I and II of the original study.

Data collection

After consenting, women were interviewed at baseline and at the end of phase II to collect demographic information such as age, race, highest education levels and reproductive health history including oral contraceptive use, sexual partnerships and behaviors. Women were also asked to keep daily diaries on menstrual bleeding and sexual activity during the 16 week follow-up. In both phases, participants self-collected mid-vaginal samples twice per week (every 3–4 days). Instructions were provided at baseline on how to perform the self-sampling and to transport the swabs back to the study laboratory. DNA was extracted from the vaginal swab as previously described (8), and genotyped using the Roche HPV LA kit according to the manufacturer's instructions. The detection limit of LA is 10–100 viral copies for each of the genotypes tested. Each PCR test included 81 study specimens, 4 characterized positive controls (25 and 100 copies of HPV 16 and 18 plasmid DNA in a background of roughly 30,000 human cellular DNA equivalents); the LA kit extraction controls: HPV-DNA negative (containing 1.0×10^6 K562 cells/mL), high HPV-DNA positive (1.25×10^5 SiHa cells and 1.0×10^6 K562 cells/mL) and low HPV-DNA positive controls (5.0×10^3 SiHa cells and 1.0×10^6 K562 cells/mL); 6 negative (no DNA) controls (9). A PCR batch was considered valid if all positive controls were positive for human β -globin and the intended PCR genotype (and no others) and the negative controls were completely negative. Any deviation would result in an invalid run and the batch would be repeated. All runs included in the current study were valid.

Statistical Analysis

Comparisons of demographic and behavioral characteristics of the study population were achieved by Wilcoxon rank sum test for continuous variables and by Chi-squared test or Fisher's exact test for categorical variables. HPV DNA positivity at the woman-level (for any type) and at the genotype-level (for a specific type) was evaluated at each pre-specified sampling time (point prevalence) and over the study period (cumulative prevalence), respectively. Numbers of detected viral types at each sampling time were summarized graphically for each individual over time (10). In addition, type-specific temporal variability in viral detection was visualized using history transition matrices (11).

Assuming that the cumulative prevalence reflected the underlying infection status, absolute bias was calculated by taking the difference between the baseline and the cumulative prevalence, whereas relative bias was determined by the ratio of baseline to cumulative prevalence minus 100%. The baseline result was excluded from the cumulative prevalence while performing the exact McNemar's test to examine whether point and cumulative prevalence estimates were statistically different from each other. Applying the bootstrapping technique, the uncertainty surrounding, as well as the representativeness of, the baseline estimate was further quantified by randomly assigning a single time point during the 16-week period as the baseline and then obtaining the corresponding bias estimates. This procedure was repeated 400 times to determine the distribution of bias estimates for prevalence of any type and of high-risk (HR) types. In addition, 100,000 bootstraps were performed to determine the minimal number of consecutive swabs needed from the same woman in order to reduce the median absolute bias to less than 10%.

Recognizing the left-truncation nature of prevalent cases, the current analysis excluded prevalent HPV DNA detected at baseline. Women may have experienced one or multiple episodes of gain or loss of HPV DNA detection before they were recruited and, therefore, the first incident detection (or loss of detection) under observation may simply be one of the many recurrent events. As such, we included the first incidence along with other recurring events of detection (loss of detection) in the following survival analysis. For detection events, the time origin began at the mid-interval of a positive type-specific test and a

previous negative result; the event time was similarly calculated as the mid-interval of the positive visit and a subsequent visit with a negative test. Likewise, for recurrent events of loss of detection of a specific type, women began to enter the risk set at the mid-interval of a positive test and the following negative test. While recurrent events of detection and loss of detection for all 37 types within the same individual were observed over the study period, event history analysis methods were employed for these repeated events (12). Survival functions were estimated by the generalized product limit estimator developed by Peña et al. to account for correlation among multiple events of the same genotype over time as well as a potential non-independence among different genotypes within the same woman (13, 14). Stratum-specific survival functions were also determined by stratifying on potential risk factors. Woman-level risk factors included older age (>40 years), number of lifetime sex partners and self-reported casual sex history at baseline. Self-reported sexual activity at follow-up, such as vaginal intercourse and condom use, were summarized as time-invariant covariates at the end of the study. All descriptive analyses were performed using Stata for Windows version 11.2 and the “survrec” package (version 1.2–2) of the R statistical program, version 3.0.0 at a two-sided significance level of 0.05 unless otherwise specified (15, 16).

RESULTS

Over the study period, 28 out of 33 women completing both phases I and II of the original study were positive for at least one type of HPV DNA (Table 1). On average, each of these 28 women contributed a median number of 30 mid-vaginal specimens (inter-quartile range [IQR]: 29–31, total: 955), recorded 4 menstrual cycles (median: 4, IQR: 4–5) and kept 113 daily diary records (median; range: 112–115). There was little evidence of risk of new exposures during the 16-week period for these 33 women. Most women reported being in a monogamous relationship. The only exception was three women (9%) reporting ever having casual partners in the prior 12 months at baseline, and one (3%) reported a new partnership at follow-up (Table 1).

Variable detectability and associated biases

The temporal dynamics of detectable viral DNA is shown in Figure 1 (also see online Supplementary Figures 1 & 2). Overall, a median number of four unique HPV genotypes (IQR: 3–7, range: 1–13) were detected per ever-positive woman (Figure 1). By comparison, the median number of HPV types detected on one randomly-collected swab was two (IQR: 3–4). Within the same subject, an ever-positive genotype was only detectable in 49.3% (95%CI: 23.7%, 90.3%) of all swabs that were positive for at least one HPV type, suggesting that single-time swab results have a low-to-intermediate reliability for type-specific HPV DNA detection.

Table 2 shows comparisons between point and cumulative prevalence of detectable viral DNA. At baseline, 20 women had at least one genotype of HPV DNA detected in the first available swab, corresponding to a point prevalence of 60.6%. The 16-week cumulative prevalence was higher at 84.8%. Assuming that cumulative prevalence represented the ‘true’ underlying infection status, the absolute bias comparing point versus cumulative prevalence was –24.2% and the relative bias was –28.6% (Table 2). Similarly, the prevalence of HR-types was under-estimated by 36.4% (absolute) and 60.0% (relative), respectively. Results of pair-wise exact McNemar’s tests were all statistically significant, indicating that the point prevalence estimate was substantially different from the corresponding cumulative prevalence. Bootstrapped results provided a wide range of possible biases when the ‘baseline’ sample used for point prevalence estimation was randomly sampled over the 16-week period. In general, point prevalence under-estimated cumulative prevalence by 20–36% in absolute values and by 24–55% in relative terms (Table 2). To reduce the median

absolute bias in point prevalence of any-type HPV to less than –10%, four or more consecutive swabs would be needed given the cumulative prevalence in the current study and the sampling interval employed (Table 2). Similar results to reduce biases when requiring non-consecutive sampling are provided online (see Supplementary Table 1).

To assess the robustness of our results, we excluded genotypes that appeared only once (“sporadic detections”) for each woman over the study period and repeated analyses for the bias quantification. Among 136 ever-positive person-types, 29% were positive on single occasion (n=40) whereas 96 person-types (71%) were detected at least twice (see Supplementary Table 2). After excluding these “sporadic” detections, the cumulative prevalence of any type HPV or of HR-types remained the same. The only difference observed was a lower cumulative prevalence of women with multiple-type HPV infection than previously estimated (Table 2), but this difference was not statistically significant (63.6% versus 66.7%; p: 1.000 for exact McNemar’s test). As a result, empirical or bootstrapped estimates for misclassification bias were overlapping with those based on all ever-positive results (data not shown).

Association of sexual behavior and time to recurrent detection and loss of detection

During follow-up, 319 type-specific viral detections were recorded among 27 women (768 person-swabs, Table 3). One woman was persistently positive for the same type over the entire study period. Overall, each woman experienced a median number of 10 incident events (IQR: 5–14, Table 3). The estimated median time to a new detection was 11 days (95%CI: 10, 11). Women with 5 or more lifetime sex partners experienced a 3-day shorter interval between any two events than those without (11 vs. 14 days), suggesting that more recurrent events (median: 10 vs. 9 events) were marginally associated with a higher cumulative sexual exposure. Similarly, women reporting casual sex partners in the previous 12 months were more likely to have frequent incident detections than those without (median time to incident detection: 7 vs. 11 days). Older age (>40 years) did not seem to affect women’s risk of HPV DNA detection (Table 3).

Meanwhile, 313 losses of type-specific detection were documented among these 27 women; the median number of loss-of-detection events was 10 (IQR: 5–14, Table 4). The median time to a recurrent loss of detection was 7 days and varied little across strata of selected woman-level covariates, except for history of casual sex reported at baseline. Women with a prior exposure to casual sex tended to have a marginally longer detectable duration for HPV DNA than those without (median [IQR]: 7 [4,7] vs. 10 [7,14] days, Table 4). For recurrent detection and loss of detection, we did not find significant associations between these recurring events and any sexual intercourse recorded during the sampling interval.

DISCUSSION

We analyzed 955 vaginal swabs from 33 predominately monogamous women over a 16-week period and revealed substantial variation in detectable HPV DNA over time. Consistent with the growing body of literature, we found that point prevalence estimates generally underestimate cumulative prevalence by at least 20% (17–22). Under an assumption that the variability reflects gain and loss of prevalent DNA detection among infected individuals, we estimated that at least four consecutive swabs per woman would be required to minimize the (absolute) median bias of ‘true’ infection status to < 10%. However, even if the assumption of variable detection in truly infected individuals is valid, we recognize that collection and testing of multiple samples during each sampling interval is not feasible in large natural history studies. This analysis was therefore conducted to quantify possible misclassification in infection status to aid in interpretation of long-interval sampled data, and not to suggest new sampling algorithms.

An alternative explanation for the variability in repeated HPV testing results is that these variations represent new acquisitions, from either transient depositions of viral material from a recent sex act (23), or re-infections of the same genotype (5, 24). In this study, most women were in a monogamous relationship during the study period and thus had a relatively low risk for new infections. While frequent re-infection and then clearance from the single male partner could explain the observed variability, we found that the temporal changes were no less frequent among abstinent women than those who were sexually active throughout the follow-up period. Yet, like many others, we were unable to make a clear distinction between persistent infections of low viral load fluctuating around the assay detection limit and re-infections of the same type. Even if women were conferred non-ignorable risks of re-infection by their partners' behavior (about which we had no information), women would also have to be susceptible to the same type that they were infected with as recently as less than 2 weeks ago. However, according to animal models, it generally requires 2–3 weeks for HPV to establish a successful life cycle in tropic tissue cells (25). Because of our uncertainty about the normal duration of natural immunity following a transient infection, we cannot definitively rule out the possibility of repeated acquisition and clearance events with frequent exposure to an infected male partner. Nevertheless, the short interval between two new detections found in the current study (1–6 weeks, type-dependent) reduces the probability of re-infection as the only probable cause of the variable HPV detection.

As a result, we considered low-level persistent infections or intermittent reactivation from viral latency as an equally probable cause of the observed variability in HPV detection. This explanation would be consistent with our observation that women who remained abstinent over the study period and were thus not at risk for new acquisition or re-infection had similarly high variations in viral detection (and non-detection). We have also recently found that, using 6-month interval sampling, risk of new HPV detection in peri-menopausal women was similar in sexually abstinent and monogamous women, who contributed 85% of all new HPV detection (26). In that study, the HPV population attributable risk (PAR) due to a higher number of lifetime sex partners (PAR=72%) was higher than that associated with recent new sex partners (PAR=13–27%), consistent with variable detection of low-level persistent infection or reactivation from latency (26). These observations are further supported by prior work showing that detecting a previously-found, type-specific HPV DNA following a period of non-detection is often a result of re-detection of the same HPV variant rather than re-infection of the same genotype even among sexually active women (1, 4, 27).

Several limitations are noted while interpreting our findings. First, HPV prevalence estimates were based on mid-vaginal swabs using sampling devices and DNA extraction methods not typically used in HPV natural studies, which may raise concerns regarding the representativeness of the more clinically relevant cervical infections and probability of sampling errors. However, a systematic review published in 2005 has concluded the agreement of HPV prevalence as determined by self-collected vaginal specimens with that by physician-collected cervical samples (28). This statement was further supported by two more recent reviews (29, 30). Second, we have ignored the potential phase dependence while estimating bias distributions (31, 32), and our bootstrapping approach may have introduced additional sampling errors while performing re-sampling. Third, lack of statistical power because of the highly correlated nature of multiple events per subject was another concern in interpreting stratified results of the current analysis. For the same reason, we were unable to assess other potential time-dependent factors that might influence HPV detection, such as the composition of the vaginal bacterial communities (33). Lastly, our duration estimates should be interpreted as duration of incident detection events, and not as overall duration of HPV infection as Weaver et al. have shown that estimated durations of

low-level persistent infections using standard assays are likely to be severely underestimated (4).

In summary, we found that HPV DNA detection was extremely variable over very short intervals. We quantified biases in point estimates as compared to cumulative prevalence when assuming the variability reflected changes in detectability rather than in infection status. We reasoned that, in addition to new acquisitions or re-infection of the same type via sexual exposures, re-detection of low-copy persistent infections or reactivated latent infections seemed equally plausible in explaining the dynamics of variable HPV DNA detection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Detectable HPV DNA for Each Woman Over Time

Detectability of HPV DNA in each individual (divided by a Divider above and below) over the 16-week follow-up period is visualized as a transition history matrix, which comprises of sampling times in the X-axis and participants' detectability profiles for ever-positive genotypes along the Y-axis. Women are sorted by the total number of unique types ever detected (Y-axis). For each individual, each row indicates positive and negative testing results for the same type over time, using black and gray colors, respectively. Blanks represent missing swabs for HPV DNA tests. Within the same subject, genotypes are ordered by the overall number of positive testing results. "a" indicating women who self-reported abstinence throughout the 16-week study period (N=5); "n" indicating woman who self-reported ever having had a new sex partner during the follow-up period (N=1); "c" indicating women who self-reported ever having had casual sex partners in the prior 12 months at baseline (N=3).

Characteristics at Baseline and Over 16 Weeks among 28 HPV-positive Women in the Baltimore-Washington Metropolitan Area, Maryland, 2005–2007

Table 1

	All (N=33)		Ever positive (N=28)		Never positive (N=5)	
	n	(%)	n	(%)	n	(%)
Baseline						
Age (years), Median* (IQR)	38	(31–42)	39	(23–42.5)	31	(31–35)
Age > 40 years	11	(33)	11	(39)	0	(0)
Years with current partner ^a , median (IQR)	7	(2.5–14)	7	(2–13)	10	(7–18)
Race: White versus non-White	14	(42)	11	(39)	3	(60)
College or higher versus high school or less	14	(42)	10	(36)	4	(80)
Ever smoked	16	(48)	15	(54)	1	(20)
Frequency of drinking alcohol, last 12 months						
<1 versus ≥1/week	7	(21)	6	(21)	1	(20)
Num lifetime partners ^b , median (IQR)	6	(4–10)	6	(4–10)	3	(2–7)
5+ lifetime partners	22	(67)	20	(71)	2	(40)
Ever had casual partner(s) ^b	3	(9)	3	(11)	0	(0)
Current user of condom	11	(33)	9	(32)	2	(40)
Use of oral contraceptive or equivalent	7	(21)	6	(21)	1	(20)
Had tubal ligation	15	(45)	13	(46)	2	(40)
Monogamous with current partner	28	(85)	23	(82)	5	(100)
Sexually active with a male partner	30	(91)	25	(89)	5	(100)
History of Herpes simplex virus-related ulcer ^b	2	(6)	1	(4)	1	(20)
History of other STD ^d , last 12 months ^b	3	(9)	3	(11)	0	(0)
Follow-up over a 16-week period						
Ever use tampons+/-pads ^b	25	(78)	22	(81)	3	(60)
Ever had vaginal intercourse ^b	27	(84)	22	(81)	5	(100)
No vaginal sex	5	(16)	5	(19)	0	(0)
1–4 times/16weeks	8	(25)	7	(26)	1	(20)
5–8 times/16weeks	9	(28)	6	(22)	3	(60)

	All (N=33)		Ever positive (N=28)		Never positive (N=5)	
	n	(%)	n	(%)	n	(%)
9+ times/16weeks	10	(31)	9	(33)	1	(20)
Ever had anal sex ^b	8	(25)	7	(26)	1	(20)
Ever used condom ^b	13	(41)	11	(41)	2	(40)
No	20	(63)	17	(63)	3	(60)
1-4 times/16weeks	9	(28)	8	(30)	1	(20)
5+ times/16weeks	3	(9)	2	(7)	1	(20)
Ever had casual sex ^b	3	(9)	3	(11)	0	(0)
Ever had new sex partners ^c	1	(3)	1	(4)	0	(0)

* P<0.05

^a 5 women who were ever positive did not answer the question

^b Self-reported, 1 woman who was ever positive did not answer the question

^c Self-reported, 3 woman who was ever positive did not answer the question

^d Self-reported, other STDs included gonorrhea, chlamydia, syphilis and other reportable STDs

Table 2

Comparing Prevalence Estimates Between Baseline and All Swab Samples Among 33 Women in the Baltimore-Washington Metropolitan Area, Maryland, 2005–2007

HPV Prevalence	Baseline Swab ^d		All Swabs		Empirical Bias	Bootstrapped Bias ^b			Minimum No Swabs Needed ^c
	n/N	%	n/N	%		Mean	95%CI	Median (IQR)	
Absolute bias ^d									
Any type	20/33	60.6	28/33	84.8	-24.2%*	-20.2%	-29.6%, -8.9%	-20.5% (-24.1%, -16.9%)	4
High-risk types	8/33	24.2	20/33	60.6	-36.4%**	-32.9%	-42.7%, -20.6%	-33.9% (-36.5%, -30.6%)	12
Multiple types	9/33	27.3	22/33	66.7	-39.4%**	-35.6%	-48.0%, -21.9%	-35.7% (-40.9%, -32.2%)	8
Relative bias ^d									
Any type					-28.6%	-23.8%	-34.9%, -10.5%	-24.2% (-28.4%, -20.0%)	6
High-risk types					-60.0%	-54.3%	-70.5%, -34.0%	-56.0% (-60.2%, -48.8%)	16
Multiple types					-59.1%	-53.4%	-71.9%, -32.8%	-53.5% (-61.3%, -48.3%)	11

Abbreviations: CI, confidence interval; HPV, human papillomavirus; IQR, inter-quartile range; No., number

* $P < 0.01$ for exact McNemar test comparing baseline and period (excluding baseline) prevalence in 33 women

** $P < 0.001$ for exact McNemar test comparing baseline and period (excluding baseline) prevalence in 33 women

^a First available swab

^b Bootstrapping results showed a wide range of possible under-estimation when one random sample was treated as if it were obtained at the baseline.

^c Minimal numbers of consecutive swab samples needed in order to reduce median absolute or relative bias to $< -10\%$

^d Absolute bias=baseline or bootstrapped point prevalence- period prevalence; relative bias=(baseline or bootstrapped point prevalence/period prevalence) - 100%

Time to Recurrent Detection of HPV DNA (All Types) by Selected Baseline and Follow-up Covariates in 27 Women^a (319 Events) Over 16 Weeks in the Baltimore- Washington Metropolitan Area, Maryland, 2005–2007

Table 3

	No. subjects	No. events	No. recurrences		Time to a recurrent detection (days) ^b	
			Median	Min.-Max.	Median	95%CI ^c
Overall	27	319	10	2–43	11	(10,11)
Baseline						
Age						
40	17	173	9	2–24	11	(7,14)
> 40	10	146	12.5	3–43	11	(10,12.6)
No. lifetime partners ^d						
< 5	7	62	9	2–13	14	(11,21)
5	19	233	10	3–43	11	(7,11)
Had casual sex partners, last 12 months						
No	24	265	9	2–43	11	(11,14)
Yes	3	54	14	12–28	7	(7,11)
Follow-up						
No. types detected						
<4	16	107	6	2–14	11	(10,18)
5	11	212	15	10–43	11	(7,11)
Ever had vaginal intercourse during follow-up ^d						
No vaginal sex	5	54	11	6–14	11	(7,14)
1–4 times/16weeks	7	72	6	3–28	11	(11,17)
5–8 times/16weeks	5	43	9	6–10	7	(4,17)
9+ times/16weeks	9	146	12	2–43	11	(7,14)
Ever used condom ^d						
No	17	220	11	4–43	11	(10,14)
1–4 times/16weeks	7	83	9	3–28	11	(7,15.6)
5+ times/16weeks	2	12	6	2–10	11	(7,94)

Abbreviations: CI, confidence interval; HPV, human papillomavirus; No., number

^aOne woman who was persistently positive for a single type was not included in the analysis

^bAssuming inter-occurrence intervals were iid following an unknown continuous distribution F , which was estimated by estimation-maximization (EM) algorithm (see reference 13)

^cEstimated from 500 bootstrapped samples using nonparametric procedure (see reference 14)

^dSelf-reported, 1 woman did not answer the question

Time to Recurrent Loss of Detection of HPV DNA (All Types) by Selected Baseline and Follow-up Covariates in 27 Women^a (313 Events) Over 16 Weeks in the Baltimore- Washington Metropolitan Area, Maryland, 2005–2007

Table 4

	No. subjects	No. events	No. recurrences		Time to a recurrent loss of detection (days) ^b	
			Median	Min.-Max.	Median	95%CI ^c
Overall	27	313	10	1–39	7	(7,7)
Baseline						
Age						
40	17	173	9	1–23	7	(4,7)
> 40	10	140	12	3–39	7	(4,7)
No. lifetime partners ^d						
< 5	7	60	9	1–14	7	(4,7)
5	19	230	11	2–39	7	(4,7)
Had casual sex partners, last 12 months						
No	24	258	9	1–39	7	(4,7)
Yes	3	3	14	14–27	10	(7,14)
Follow-up						
No. types detected						
<4	16	104	6	1–14	7	(4,7)
5	11	209	15	11–39	7	(7,7)
Ever had vaginal intercourse ^d						
No vaginal sex	5	56	11	6–14	7	(7,11)
1–4 times/16weeks	7	70	6	2–27	7	(4,10)
5–8 times/16weeks	5	43	9	6–11	7	(7,11)
9+ times/16weeks	9	140	14	1–39	7	(4,7)
Ever used condom ^d						
No	17	216	11	4–39	7	(7,7)
1–4 times/16weeks	7	81	8	2–27	7	(4,11)
5+ times/16weeks	2	12	6	1–11	7	(4,17)

Abbreviations: CI, confidence interval; HPV, human papillomavirus; No., number

^aOne woman who was persistently positive for a single type was not included in the analysis

^bAssuming inter-occurrence intervals were iid following an unknown continuous distribution F , which was estimated by estimation-maximization (EM) algorithm (see reference 13)

^cEstimated from 500 bootstrapped samples using nonparametric procedure (see reference 14)

^dSelf-reported, 1 woman did not answer the question