Title:

Rates of new human papillomavirus detection and loss of detection in middle-aged women by recent and past sexual behavior

Running title:

HPV detection in middle-aged women

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Word count

Manuscript: 3447

Abstract: 191

Tables and Figures: 3 Tables and 3 Figures

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Footnote Page

1. Conflicts of interest statement:

   Potential conflicts of interest: AH has received speaker's fee from Astra Zeneca, Denmark, outside of the submitted work. PP, AFR, NC, AB, RV, MIS, AOY, PG: no conflicts of interest.
2. Funding: This work was supported by the National Institutes of Health R01CA123467.

   Anne Hammer was funded by a grant from the Danish Cancer Society.
3. Not applicable
4. Requests for reprints:

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Abstract

Background: Understanding the source of newly detected human papillomavirus (HPV) in middle-aged women is important to inform preventive strategies, such as screening and HPV vaccination.

Methods: We conducted a prospective cohort study in Baltimore, Maryland. Women aged 35-60 underwent HPV testing and completed health and sexual behavior questionnaires every 6-months over a 2-year period. New detection/loss of detection rates were calculated and adjusted hazard ratios (aHR) were used to identify risk factors for new detection.

Results: 731 women and 104 high-risk (HR) HPV-positive women were included in the new and loss of detection analyses, respectively. The rate of new HR HPV detection was 5.0/1000 woman-months. Reporting a new sex partner was associated with higher detection rates (aHR 8.1; 95% CI 3.5, 18.6), but accounted only for 19.4% of all new detections. Among monogamous and sexually abstinent

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women, new detection was higher in women reporting ≥5 lifetime sexual partners compared to women reporting <5 (aHR 2.2; 95% CI 1.2, 4.2).

Conclusion: While women remain at risk of HPV acquisition from new sex partners as they age, our results suggest that most new detections in mid-adult women reflect recurrence of previously acquired HPV.

Keywords:

Human papillomavirus, cervical neoplasia, epidemiology, sexual behavior, cervical cancer screening

Introduction

Human papillomavirus (HPV) is the most common sexually transmitted virus [1]. The prevalence of HPV peaks shortly after sexual debut, and the risk of having a new HPV infection detected increases with increasing number of sexual partners [2, 3]. Most HPV infections become undetectable within 1-2 years, a phenomenon typically defined as viral clearance [4]; however, recent studies have suggested that the virus may not be completely eradicated but rather may enter a latent state in the basal cell layer of the cervical epithelium [5, 6], with subsequent loss of immune control possibly resulting in re-detection of the virus [7, 8]. Thus, new HPV detection may represent a mixture of new acquisition and redetection of previously acquired infections, particularly in middle-aged women many years past sexual debut [9, 10]. Understanding the source of new HPV detection may be useful for clinical counseling of adult women participating in HPV-based screening and when evaluating the potential benefits of HPV vaccination, which is now approved for use up to age 45 years [11]. Further, HPV

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natural history models used to evaluate the cost-effectiveness of alternative vaccination and screening strategies have been shown to be sensitive to assumptions about redetection vs. new acquisition [12].

We previously reported the rates of new HPV detection in an interim analysis of a large prospective cohort of well-screened, mid-adult women in Baltimore, MD, USA (13). In this previous analysis, relative risk of new detection by current and past sexual behavior was estimated using an infection-level analysis, which allows all women with HPV to be at-risk for new HPV genotypes. In the current analysis, we report (a) the risk of new high-risk (HR) HPV detection using complete follow-up time, from a clinical screening perspective, where women move from "HR-HPV negative" to "HR-HPV positive" and (b) a new loss-of-detection analysis to provide a more complete understanding of the women-level transitions in HPV detectability by current and past sexual behavior.

Material and methods

We conducted a prospective cohort study in Baltimore, Maryland from March 2008 to March 2011. Women were enrolled in the HPV in Perimenopause (HIP) Study if they were aged 35-60 years, had an intact cervix, and provided informed consent. Women were excluded if they were pregnant, had plans to become pregnant within the next two years, had a history of organ transplantation, or were HIV seropositive. Women were followed every six months for two years. At baseline and every six-month visit, a trained study physician or registered nurse collected an exfoliated cell sample from the cervix using the Digene HPV cervical brush (Digene, United States). Information on sociodemographic

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characteristics, reproductive health, and sexual history was collected using questionnaires at baseline (administered via telephone) and at each follow-up visit (administered face-to-face or by telephone).

All study protocols were approved by the Johns Hopkins Bloomberg School of Public Health Institutional Review Board. To conduct this analysis, additional approval was obtained from the University of Pittsburgh Institutional Review Board.

Exfoliated cervical cell samples were HPV genotyped using the Roche HPV Linear Array PCR-based assay (Roche Diagnostics), as described elsewhere [10]. The Roche HPV Linear Array detects 37 distinct HPV types, including all HR HPV types. For the current analysis, any HPV type refers to any of the following 37 HPV types: 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73 (MM9), 81, 82 (MM4), 83 (MM7), 84 (MM8), IS39, and CP6108; any HR HPV type refers to any of the following 13 HR HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68; any nonavalent HPV type refers to any of the following 2 low risk HPV types or 7 HR HPV types: 6, 11, 16, 18, 31, 33, 45, 52, 58.

At enrollment, serum samples were collected to determine serostatus for 8 HPV types (6, 11, 16, 18, 31, 33, 45, and 52) using a virus-like-particle (VLP) based enzyme-linked immunosorbent assay as described previously [13, 14]. Of women enrolled, a total of 719 (75.6%) had serum samples available, 447 (62.2%) of which had at least one HPV type detected. As illustrated in Figure 1, the number of HPV types detected by serology was positively correlated to the number of self-reported lifetime sexual

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partners, suggesting that the lifetime number of sexual partners can be used as surrogate measure of cumulative exposure to HPV. Based on these findings, we stratified previous exposure to HPV in two risk categories: 1-4 lifetime sexual partners vs. ≥5 lifetime sexual partners.

Statistical analysis

For the current analysis, women were included if they had completed the baseline questionnaire, had valid HPV DNA results at baseline, and at least one follow-up visit. In the analysis of new detections, women were considered at risk for a maximum of 37 HPV types. A woman would not be considered at risk for detection of any new HPV, any new HR HPV, or any new nonavalent HPV if she tested positive at baseline for an HPV type in a given group. Rates of new detection in a given group (i.e., any HPV, any HR HPV, and any nonavalent HPV) were calculated by dividing the number of women with ≥1 new HPV detections by person-time at risk. Women contributed time at risk starting at baseline (if negative for the relavent HPV types in the group analysis) and ending at the date of the first HPV detection in a given group or at the last study visit if they remained HPV negative. Loss of HPV detection was defined as two-consecutive type-specific negative results or if HPV was undetectable at the last study visit. Rates of loss of detection were calculated by dividing the number of women with loss of detection by person-time at risk (HR HPV only). Women contributed time at risk starting on the date of HPV detection (new or prevalent) and ending at the date of loss of detection or at the last study visit. Infections detected at the last study visit did not contribute person-time to the loss of detection

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analysis. If an HPV result was missing between two non-missing results, the prior non-missing HPV DNA result was carried forward. Results were similar when using carry-backward imputation. Cumulative
probability of new HPV detection or loss of detection was estimated using the Kaplan Meier method.

We calculated the relative risk of new HPV detection according to self-reported current sexual activity as outlined in Figure 2; (A) no sexual activity in the previous 6 months, (B) sexual activity with same partner as previous time period, and (C) sexual activity with a new partner in past 6 months. For group A, we assumed that 100% of new HPV detections occurred as a result of recurrent detection of a previously acquired infection (i.e., reactivation, autoinoculation, or increase in viral load to detectable limits). For group B, the source of new detection was considered uncertain because acquisition could have occurred through the unmeasured sexual behavior of the male partner, while new detection in Group C was conservatively assumed to occur as a result of acquisition from the new partner.

We used number of lifetime sexual partners as a surrogate measure of cumulative exposure to HPV infection and risk of harboring a latent infection (Figure 2). We calculated unadjusted and adjusted hazard ratios of new HPV detection using group A (no sexual activity in the previous 6 months) as the reference group. To focus on the most parsimonious risk estimation for the primary exposures of sexual behavior, we only adjusted for variables that changed the point estimate by 10% (i.e. marital status). Results were reported overall and stratified by lifetime number of sex partners (i.e., <5 or 5+ LTSP), to

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determine whether risk of new HPV detection differed by cumulative HPV exposure. For simplicity, only results for HR HPV are reported in the text, unless otherwise stated. Statistical analyses were conducting using Stata 15 (StatCorp, College Station, Texas).

Results

A total of 951 women were enrolled in the study, 731 of which were included in the new detection analysis (Supplementary Figure 1). The loss of detection analysis was restricted to 104 women who had at least one HR HPV type detected at baseline or during follow-up, excluding women who had HR HPV detected at the last study visit (n= 26).). Over half of the women completed all study visits, 175 (23.9%) had at least one missing in-between visit, and 165 (22.6%) completed visits until loss to follow-up. Of these, 88 (12.0%) had only one follow-up visit. The median follow-up time was 24.5 months (interquartile range (IQ): 19.0 – 25.9 months. Frequency distributions of the cohort demographics, sexual, and reproductive characteristics overall and by age category are reported in Table 1.

At baseline, the overall prevalence of any HPV type was 18.6%, while the prevalence of any HR HPV type and any nonavalent HPV type was 8.5% and 5.8%, respectively (Table 2). HPV 16 was the most prevalent HPV type. Crude prevalence rates were slightly higher in women aged 35-49 compared to women aged 50-60.

Detection rates of new HPV were 9.6 (95% CI 8.0 – 11.6) per 1000 woman-months (any HPV type),

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5.0 (95% CI 3.9 – 6.3) per 1000 woman-months (any HR HPV type), and 3.8 (95% CI 2.9 – 4.9) per 1000 woman-months (any nonavalent HPV type). HPV16 was the most common newly detected HPV type, with a new detection rate of 1.0 (95% CI 0.6 – 1.6) per 1000 woman-months (Table 2).

Among new HR HPV positive women, 13 (19.4%) of new detections occurred among women who reported having had a new sexual partner, 46 (68.7%) among monogamous women, and 8 (11.9%) among sexually abstinent women. Recent sexual activity with a new partner was associated with the highest rate of new HR HPV detection, regardless of past sexual behavior and age (Table 3, Supplementary Table 1). There was no significant difference in new HR HPV detection rates in women who reported no recent sex and women who reported having sex with the same partner (Figure 3). When exploring the impact of past sexual behavior on risk of new HR HPV detection among women who reported having no new sex partner, women with ≥5 LTSP were 2.2-fold more likely to have HPV detected compared to women with <5 LTSP ($p<0.001$) (Table 3 and Figure 3).

With respect to the most commonly detected genotype, HPV 16, rates of new detection were highest in women reporting a new sex partner (3.1 per 1000 woman-months, 95% CI 0.8 – 12.4), followed by women reporting having sex with the same partner (1.0 per 1000 woman-months, 95% CI 0.6 – 1.8), and sexually abstinent women (0.6 per 1000 woman-months, 95% CI 0.2 – 2.4).

Among 104 women with a baseline or new HR HPV infection, 58 loss of detection events were observed (44.1 per 1000 women-months (95% CI 34.1, 57.1)) (Table 4). Loss of detection rate was

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33.3 per 1000 women-months (95% CI 23.6, 47.1) for women with prevalent infections at baseline compared to 73.4 per 1000 women-months (95% CI 50.0, 107.8) for women negative for HR HPV at baseline with new HR HPV detected during follow-up. Differences in loss of detection were observed across genotypes, with HPV16 and HPV45 having the lowest loss of detection rates while HPV33 and HPV58 had the highest. The loss of detection rate was slightly lower in women aged 50-60 compared to women aged 35-49. We found no difference in loss of detection rates by past and recent sexual behavior (Supplementary Table 2).

Discussion

Mid-adult women with a recent new sexual partner have higher rates of new HPV detection, supporting the premise that individuals remain at risk for HPV acquisition throughout the lifespan. Given the high clearance rates observed from newly detected HPV, rapid control to undetectable levels appears to be the usual response. However, only 15% of all newly detected HPV infections occurred in women reporting a new sexual partner, while 23% occurred in sexually inactive women and 62% in women having sex with the same partner. Because the rates of new HPV detection in women reporting no sexual activity were similar to rates in women reporting sex with the same partner, new exposure from the unreported sexual behavior of the male partner appears to be minimal in this study population. Rates of new HPV detection in women without a new sexual partner were over 2-fold higher among those reporting five or more lifetime sexual partners who are at higher risk of harboring a non-productive or latent HPV. Taken together, these data may suggest that a substantial proportion of new HPV detection in mid-adult women reflects recurrent detectability of a previously acquired infection.

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These results confirm data from an interim analysis of the HIP cohort [15], a higher-risk cohort of older women in Seattle, USA [9], and data from a large cohort of unvaccinated men [16]. Recurrent detection may reflect reactivation of infection from a latent state, autoinoculation from another epithelial site (e.g., vulvovaginal or anal infection), or false negative/false positive test results. The differential risk in women with a higher number of LTSP suggests that reactivation and autoinoculation are more likely explanations than misclassification of test results, which would be expected to be non-differential by cumulative exposure risk. Data reporting high anal HPV prevalence and widespread infection in the vulvovaginal epithelium and vice versa [17] would support the possibility of autoinoculation, while recent reports by us [5] and others [18], as well as elegant animal papillomavirus models of latency [6, 8] support the possibility of focal latent infection of the cervix. High density sampling of anal, vulvovaginal, and cervical HPV with daily sexual behavior data will be needed to differentiate these two mechanisms of recurrent detectability.

Our findings have several important clinical implications. First, as HPV testing becomes a routine part of early detection and treatment programs, women will be accumulating their own individual HPV natural history profiles. Our data may help with counseling sexually abstinent women and women in monogamous relationships who may be concerned about the source of new HPV infection. These data also reinforce the need for continued routine screening in sexually inactive or sexually monogamous women, even if their last screening test was HPV negative. Second, HPV vaccines are now approved by the US Food and Drug Association for use in individuals up to age 45 years, though most

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professional organizations including the Advisory Committee on Immunization Practices and the American College of Obstetricians and Gynecologists have not made specific recommendations for vaccination from 27-45 years; instead they recommend shared decision-making between patient and provider to weigh risks and benefits on a case-by-case basis. While there is no definitive study to show whether prophylactic HPV vaccination may prevent recurrent detection, the significantly reduced population-effectiveness in women receiving the vaccine in early adulthood, presumably after sexual debut [20, 21], suggests that the vaccine may have minimal benefit in reducing reactivation risk. On the other hand, some [22, 23] but not all [24, 25] studies have shown that vaccination of individuals after conization reduced risk of disease recurrence, suggesting some immunologic boosting or possible prevention of lateral spread of infection. Randomized controlled trials will be needed to confirm the benefit of reducing recurrent disease in treated individuals and evaluate whether this can be extended to increased control of latent infection in asymptomatic women.

The clinically important question is whether there is a differential risk of cervical precancer and cancer (CIN3+) in women with redetection compared to those with a newly acquired infection. While our study was not designed or powered to evaluate this question, a previous study reported that potential reactivated infections were associated with similar risks of CIN2+ compared to newly acquired infections [26]. A recent study from a large US health system showed a substantial number of women with HPV testing patterns reflective of newly detected and reappearing infection [27]. These data show that an HPV-positive test result is strongly predictive of CIN3+ diagnosis whether detected as a baseline screening test or preceded by positive or negative prior tests, with incidence rates of CIN3+

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ranging from 792/100,000 in women with 3 prior negative tests to 2449/100,000 in women with 3 prior positive tests, and 1223/100,000 in women with intermittent HPV detection (compared to 18/100,000 in women with 4 consecutive negative HPV tests). In light of these results, it will be important to understand whether the higher cumulative exposure to HPV in post-sexual revolution birth cohorts will translate to an increased risk of positive screening tests in more recent birth cohorts currently entering menopause [28-30]. A proportionate increase in postmenopausal HPV-infected women is concerning given the well-known limitations of morphologic screening and diagnosis after menopause [31, 32].

The impact of cohort effects and latency on cancer risk throughout the lifespan are currently not possible to estimate with empirical data. Many screening and vaccination recommendations are thus based on expert opinion and health decision models. However, most health decision models do not account explicitly for controlled (latent) HPV infection [33] due to limited data on 1) the proportion of infections that become undetectable that are in fact in a latent state; 2) the transition risk for redetection of latent infections as a function of age; and 3) the relative risk of progression to precancer among redetected versus newly acquired infections. These unknowns may vary between populations, depending on differences in cell-mediated immunity. Recent modeling efforts have evaluated the impact of including a "latent-reactivated transition" in the lifetime natural history in men and women, and largely conclude that inclusion of this transition improved model fit [12, 30]. Given the evidence that latency is part of the natural history of HPV infection [5, 6, 8, 19], and the present finding that there is likely to be heterogeneity in the risk of redetection in a population according to cumulative lifetime HPV exposure, it would be of great interest for health decision models to assess the

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potential impact of incorporating latency and redetection on policy decisions. The extent to which changes in model structure will be necessary depends in part upon whether HPV incidence is directly estimated from current sexual behavior data or whether the models are agnostic regarding the source of newly detectable infections in older women (i.e., whether acquired through recent, as opposed to past, sexual behavior). It would be worth investigating whether models fully capture population heterogeneity in cumulative HPV exposure over the lifespan, and, if not, whether such heterogeneity might impact model predictions when evaluating interventions such as adult HPV vaccination and age to end screening. The impact of including latency and redetection in a model is likely to be most relevant for HPV transmission models that are used to evaluate HPV vaccination strategies involving mid-adult women. To evaluate proposed vaccination strategies involving mid-adult and older women, transmission models should consider whether distinguishing the new acquisition of HPV by age from reactivated infections by age leads to different policy conclusions. The overall impact of this distinction will also depend on the level of effectiveness of vaccination against reactivated infections, which remains uncertain. Given that most model-based analyses of vaccination have attributed detected HPV in older women to new infections, the results have been biased in favor of vaccinating mid-adult women[34-36]. Thereby, to the extent that HPV vaccines may not be efficacious against reactivated infections, results from current model-based analyses may have over-estimated the benefit and cost-effectiveness of vaccinating mid-adult and older women.

We acknowledge some important limitations to this analysis. First, the HPV detection assay used is not a clinical assay which may have resulted in slightly higher detection rates compared with FDA-

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approved HPV screening tests in which the sensitivity of the assay is attenuated to maximize the sensitivity and specificity of the test for detection of CIN2+. Second, as our results are based on a low-risk, well-screened population, new detection and loss of detection rates may not be representative of higher risk populations. Third, we cannot exclude recall bias or social desirability bias in that women who reported no new sex partner may have had a new partner, which may have resulted in an underestimation of new detection rates among women who reported having a new sexual partner and an overestimation of rates among women reporting no new partner. Additionally, we cannot rule out an underreporting of LTSP, which may have resulted in an underestimation of rates among women with ≥5 LTSP and an overestimation of women with <5 LTSP. However, these results have been consistent across other populations of older, US women, suggesting that these biases are unlikely to completely explain our observations [17, 37].

In conclusion, the within-woman natural history of HPV infection appears to include dynamic transitions between detection and non-detection of immunologically controlled infections. Women with a higher risk of harboring latent infection (i.e., those reporting a higher number of LTSP) will have a higher risk of new detection in screening. Given that other studies suggest that risk of cervical precancer from recurrent HPV detection is similar to that from presumed newly acquired infection, sexual history may be an important consideration in deciding when to exit screening. Studies are inconclusive regarding the benefit of HPV vaccination in preventing recurrent detection, and randomized trials are needed to more directly estimate the impact of prophylactic vaccination on control of latent infections and reduced risk of persistence and progression to cervical precancer.

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Acknowledgements

Funding

This work was supported by the National Institutes of Health R01CA123467. Anne Hammer was funded by a grant from the Danish Cancer Society.

Conflicts of interest

Potential conflicts of interest: AH has received speaker's fee from Astra Zeneca, Denmark, outside of the submitted work. PP, AFR, NC, AB, RV, MIS, AOY, PG: no conflicts of interest.

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Tables

Table 1. Basic characteristics of the study cohort

Variables	**All ages** n=731 n (%)	**Age 35-49** n=466 n (%)	**Age 50-60** n=265 n (%)
Race			
White	548 (75.0)	342 (73.4)	206 (77.7)
Black	132 (18.1)	88 (18.9)	44 (16.6)
Asian/Hawaiian/Pacific Islander	25 (3.4)	19 (4.1)	6 (2.3)
American Indian	2 (0.3)	1 (0.2)	1 (0.4)
Not reported	24 (3.3)	16 (3.4)	8 (3.0)
Highest education completed			
High school	117 (16.0)	78 (16.7)	39 (14.7)
Post high school	164 (22.4)	105 (22.5)	59 (22.3)
College/Post graduate	450 (61.6)	283 (60.7)	167 (63.0)
Yearly income (USD)			
<40,000	55 (7.5)	34 (7.3)	21 (7.9)
40-80,000	196 (26.8)	131 (28.1)	65 (24.5)
80-120,000	173 (23.7)	116 (24.9)	57 (21.5)
>120,000	244 (33.4)	151 (32.4)	93 (35.1)
Unknown	63 (8.6)	34 (7.3)	29 (10.9)
Smoking history			
Never	476 (65.1)	307 (65.9)	169 (63.8)
Former	182 (24.9)	106 (22.8)	76 (28.7)
Current	73 (10.0)	53 (11.4)	20 (7.6)
Menopausal status			
Premenopausal	467 (63.9)	301 (64.6)	166 (62.6)
Postmenopausal	231 (31.6)	146 (31.3)	85 (32.1)
Missing	33 (4.5)	19 (4.1)	14 (5.3)
Marital status			
Married	464 (63.5)	291 (62.5)	173 (65.3)
Divorced/Separate/Widowed	133 (18.2)	76 (16.3)	57 (21.5)
Never married	134 (18.3)	99 (21.2)	35 (13.2)

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Variables	All ages n=731 n (%)	Age 35-49 n=466 n (%)	Age 50-60 n=265 n (%)
Lifetime sexual partners			
<5 partners	277 (37.9)	162 (34.8)	115 (43.4)
≥5 partners	453 (62.0)	303 (65.0)	150 (56.6)
Missing	1 (0.1)	1 (0.2)	0 (0.0)
Sexual behavior			
No recent sex	112 (15.3)	57 (12.2)	55 (20.8)
Recent sex, same partner	534 (73.1)	349 (74.9)	185 (69.8)
Recent sex, new partner	85 (11.6)	60 (12.9)	25 (9.4)
Current hormone use			
No	554 (75.8)	316 (67.8)	238 (89.8)
Yes	177 (24.2)	150 (32.2)	27 (10.2)
Ever sexually transmitted infection, self-report*			
No	412 (56.4)	260 (55.8)	152 (57.4)
Yes	319 (43.6)	206 (44.2)	113 (42.6)
Ever abnormal Pap, self-report			
No	387 (52.9)	242 (51.9)	145 (54.7)
Yes	336 (46.0)	219 (47.0)	117 (44.2)
Missing	8 (1.1)	5 (1.1)	3 (1.1)

*reported ever having been diagnosed with chlamydia, gonorrhea, herpes, trichomonas, syphilis, chancroid, bacterial vaginosis, or genital warts

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Table 2. Prevalence of HPV at baseline and rates of new HPV detection among middle-aged women, overall and stratified by age at baseline.

	Prevalence at enrollment (%)	Woman-months of follow-up	Number of incident infections	Cumulative new detection (%)		New detection rate per 1000 woman-months (95% CI)
Any HPV type						
All ages	18.6	11,457	110	12.1	20.9	9.6 (8.0, 11.6)
Age 35-49	20.4	7,062	79	14.4	23.9	11.2 (9.0, 14.0)
Age 50-60	15.5	4,389	31	8.3	16.0	7.1 (5.0, 10.0)
Any HR HPV type						
All ages	8.5	13,745	68	5.4	11.8	5.0 (3.9, 6.3)
Age 35-49	10.5	8,539	47	6.4	13.2	5.5 (4.1, 7.3)
Age 50-60	4.9	5,206	21	3.6	9.5	4.0 (2.6, 6.2)
Any nonavalent HPV type						
All ages	5.8	14,311	54	3.9	9.1	3.8 (2.9, 4.9)
Age 35-49	6.9	9,007	37	4.3	10.2	4.1 (3.0, 5.7)
Age 50-60	3.8	5,304	17	3.2	7.3	3.2 (2.0, 5.2)
Type-specific						
HPV 6	0.6	15,723	4	0.1	0.7	0.3 (0.1, 0.7)

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	Prevalence at enrollment (%)	Woman-months of follow-up	Number of incident infections	Cumulative new detection (%)		New detection rate per 1000 woman-months (95% CI)
HPV 11	0.1	15,819	0	NA	NA	NA
HPV 16	1.6	15,353	15	1.4	2.4	1.0 (0.6, 1.6)
HPV 18	1.0	15,555	10	0.8	1.6	0.6 (0.4, 1.2)
HPV 31	0.4	15,699	7	0.4	1.0	0.4 (0.2, 0.9)
HPV 33	0.6	15,697	3	0.4	0.4	0.2 (0.1, 0.6)
HPV 45	0.6	15,637	11	0.3	1.9	0.7 (0.4, 1.3)
HPV 52	1.0	15,566	14	0.7	2.5	0.9 (0.5, 1.5)
HPV 58	0.6	15,710	4	0.3	0.7	0.3 (0.1, 0.7)

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Table 3. Rates of any new HR HPV detection among middle-aged women, stratified by recent and previous sexual behavior[a] and age.

Variable	Woman-months of follow-up	Number of new detection events	New detection rate per 1000 woman-months (95% CI)	Unadjusted Hazard ratio (95% CI)	Adjusted Hazard ratio[b] (95% CI)
Age 35-49					
< 5 LTSP (same partner and no sex)	3,094	8	2.6 (1.3, 5.2)	1.0 (ref)	1.0 (ref)
≥ 5 LTSP (same partner and no sex)	4,943	31	6.3 (4.4, 8.9)	2.4 (1.1, 5.2)	2.2 (1.0, 4.9)
New partner (< 5 LTSP and ≥ 5 LTSP)	334	7	21.0 (10.0, 44.0)	8.1 (2.9, 22.2)	5.6 (2.0, 16.3)
Age 50-60					
< 5 LTSP (same partner and no sex)	2,356	4	1.7 (0.6, 4.5)	1.0 (ref)	1.0 (ref)
≥ 5 LTSP (same partner and no sex)	2,639	11	4.2 (2.3, 7.5)	2.4 (0.8, 7.5)	1.9 (0.6, 6.3)
New partner (< 5 LTSP and ≥ 5 LTSP)	149	6	40.3 (18.1, 89.7)	27.2 (7.5, 98.4)	14.11 (3.4, 59.1)
All ages					
< 5 LTSP (same partner and no sex)	5,450	12	2.2 (1.3, 3.9)	1.0 (ref)	1.0 (ref)
≥ 5 LTSP	7,582	42	5.5 (4.1, 7.5)	2.5 (1.3, 4.7)	2.2 (1.2, 4.2)

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(same partner and no sex)					
New partner (< 5 LTSP and ≥ 5 LTSP)	483	13	26.9 (15.6, 46.4)	12.6 (5.8, 27.7)	8.1 (3.5, 18.6)

LTSP: life time number of sexual partners. [a]Women reporting the same partner (i.e., group B as defined in Supplementary Figure 1) and women reporting no sex (i.e., group A as defined in Supplementary Figure 1) were combined as we found no significant difference in rates of new HPV detection between the groups. [b]Adjusted for marital status.

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Table 4. Loss of detection of any HR HPV among middle-aged women, overall and stratified by age at baseline.

	Woman-months of follow-up	Number of loss of detection events	Cumulative loss of detection (%)		Loss of detection rate per 1000 woman-months (95% CI)
			12-month	24-month	
Any HR HPV type					
All ages	1,315	58	46.9	64.6	44.1 (34.1, 57.1)
Age 35-49	951	45	50.3	68.2	47.3 (35.3, 63.4)
Age 50-60	364	13	38.4	54.4	35.7 (20.7, 61.4)
Prevalent	961	32	37.4	58.1	33.3 (23.6, 47.1)
New detection	354	26	61.8	NA*	73.4 (50.0, 107.8)
Type-specific					
HPV 6	45	3	50.0	50.0	67.2 (21.7, 208.23)
HPV 11	18	0	0	0	0
HPV 16	365	5	14.4	27.6	13.7 (5.7, 32.9)
HPV 18	184	5	39.2	38.2	27.2 (11.3, 65.4)
HPV 31	65	3	65.7	NA*	45.8 (14.8, 142.1)
HPV 33	55	4	50.0	NA*	72.4 (27.2, 192.9)
HPV 45	136	1	10.0	10.0	7.3 (1.0, 52.1)
HPV 52	149	4	37.8	37.8	26.8 (10.0, 71.3)
HPV 58	53	4	66.7	66.7	75.8 (28.4, 201.9)

*We were unable to calculate these estimates as we didn't have enough follow-up time to look at 24-month cumulative loss of detection

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Figure legends

Figure 1. Number of HPV serotypes detected among women aged 35-60 in Baltimore, Maryland, stratified by lifetime number of sexual partners

Figure 2. Conceptual model for new HPV detection occurring because of new acquisition vs. recurrent detection of previously acquired infection.

Figure 3
Rates of new HPV detection by recent and past sexual behavior among women aged 35-60 in Baltimore, Maryland.
Kaplan Meier curves illustrating new detection rates for women who reported having sex with the same partner or no sex, stratified by lifetime number of sexual partners (LTSP).

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Figures

Figure 1

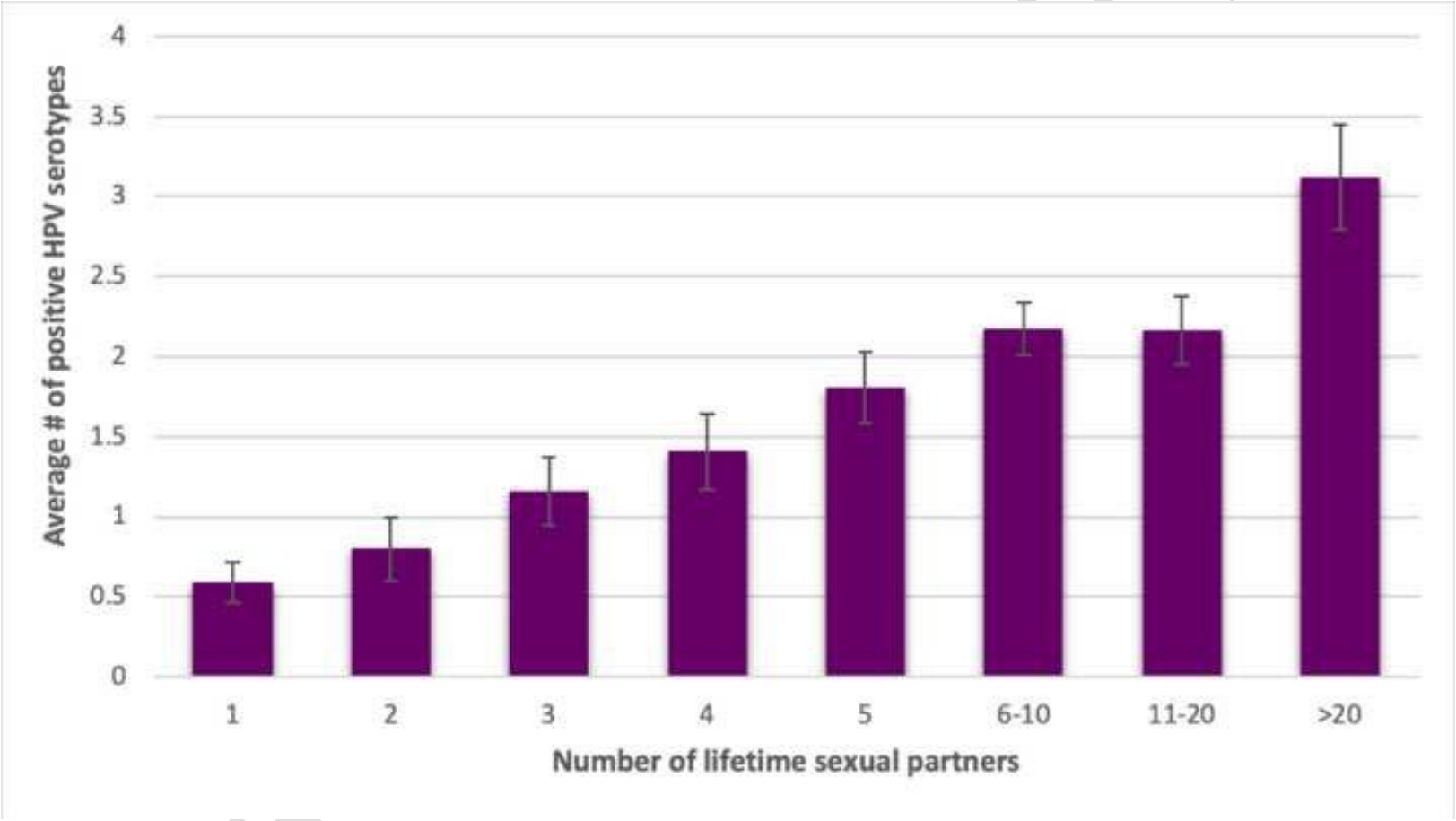


Figure 2.

Conceptual model for estimating proportion of new HPV detection attributed to new acquisition vs. recurrent detection of previously acquired infection (i.e., reactivation)

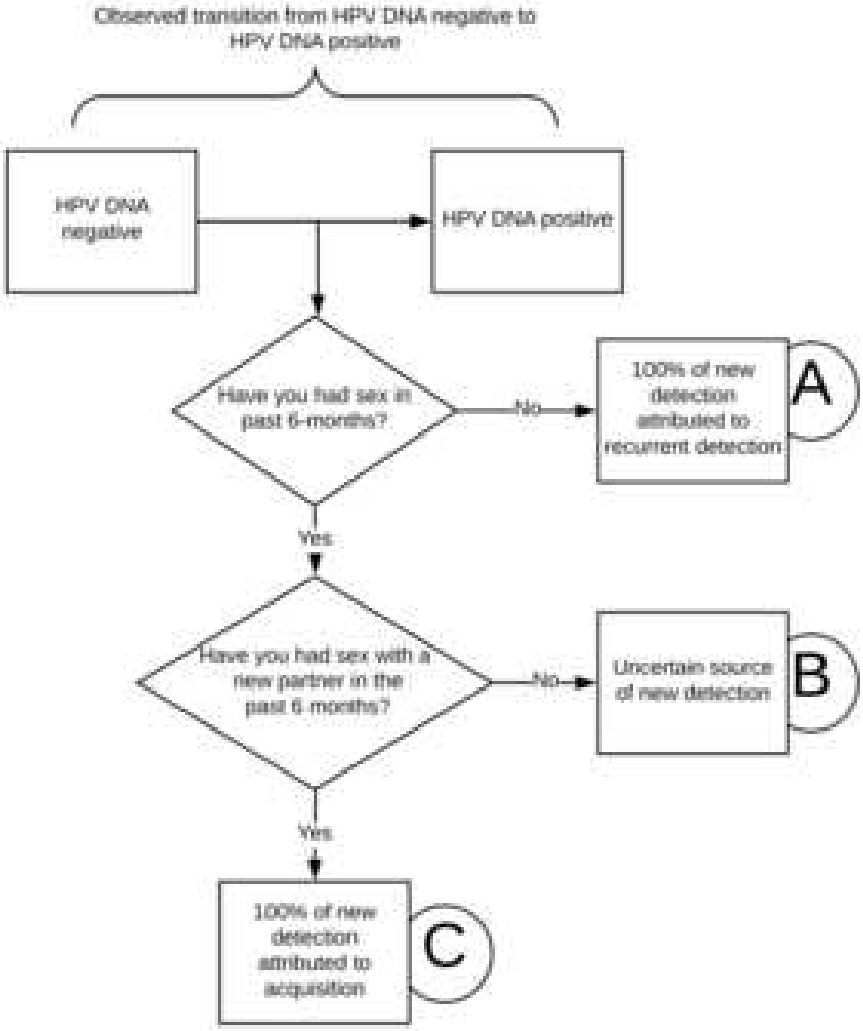
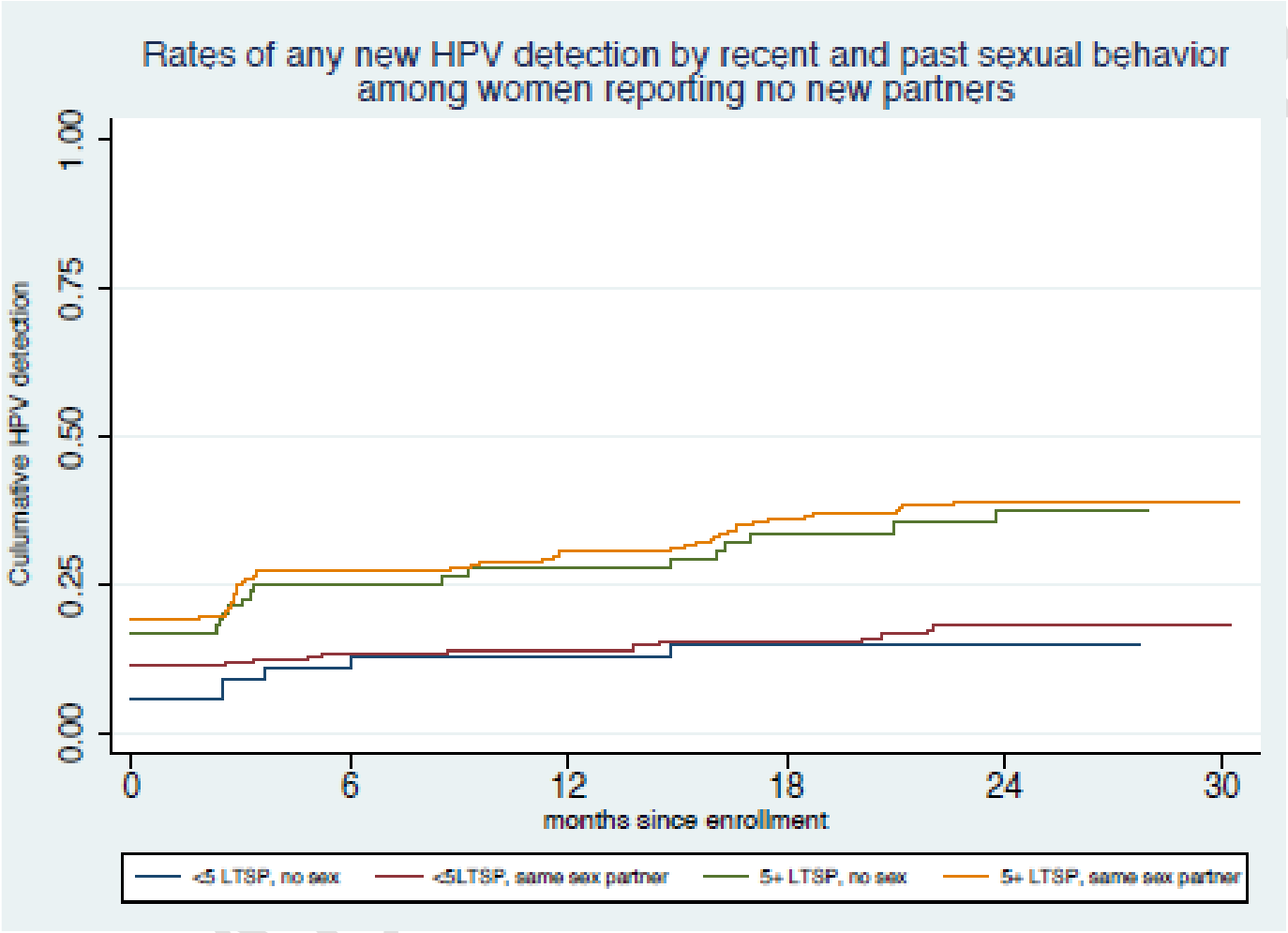


Figure 3.


Rates of any new HPV detection by recent and past sexual behavior among women reporting no new partners
Cuiumative HPV detection
0.00
0.25
0.50
0.75
1.00
0
6
12
18
24
30
months since enrollment
<5 LTSP, no sex
<5LTSP, same sex partner
5+ LTSP, no sex
5+ LTSP, same sex partner

Supplementary Materials

Supplementary Table 1. New detection rates of HPV by recent and previous sexual behavior.

Sexual behavior category		**Woman-months of follow-up**	**Number of events**	**New detection rate per 1000 woman-months (95% CI)**
Any HPV				
< 5 LTSP	No sex	1,169	7	6.0 (2.9, 12.6)
	Same partner	3,829	12	3.1 (1.8, 5.5)
	New partner	44	2	45.4 (11.4, 181.6)
	All	5,091	21	4.1 (2.7, 6.3)
≥ 5 LTSP	No sex	1,426	17	11.9 (7.4, 19.2)
	Same partner	4,547	56	12.3 (9.5, 16.0)
	New partner	224	14	62.4 (37.0, 105.4)
	All	6,334	89	14.1 (11.4, 17.3)
All	No sex	2,621	24	9.2 (6.1, 13.7)
	Same partner	8,376	68	8.1 (6.4, 10.3)
	New partner	268	16	59.7 (36.5, 97.4)
Any HR HPV				
< 5 LTSP	No sex	1,293	2	1.5 (0.4, 6.2)
	Same partner	4,156	10	2.4 (1.3, 4.5)
	New partner	96	0	0.0
	All	5,595	12	2.1 (1.2, 3.8)

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Sexual behavior category		Woman-months of follow-up	Number of events	New detection rate per 1000 woman-months (95% CI)
≥ 5 LTSP	No sex	1,790	6	3.4 (1.5, 7.5)
	Same partner	5,792	36	6.2 (4.5, 8.6)
	New partner	387	13	33.6 (19.5, 57.8)
	All	8,124	56	6.9 (5.3, 9.0)
All	No sex	3,109	8	2.5 (1.3, 5.1)
	Same partner	9,948	46	4.6 (3.5, 6.2)
	New partner	483	13	26.9 (15.6, 46.4)

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Supplementary Table 2. Loss of detection by recent and previous sexual behavior and age.

Variable	Woman-months of follow-up	Number of loss of detection events	Loss of detection rate per 1000 woman-months (95% CI)
Age 35-49			
< 5 LTSP (Same partner and no sex)	195	11	56.5 (31.3, 102.0)
≥ 5 LTSP (Same partner and no sex)	644	33	51.3 (36.4, 72.1)
New partner (< 5 LTSP and ≥ 5 LTSP)	0	0	0.0
Age 50-60			
< 5 LTSP (Same partner and no sex)	34	1	29.5 (4.2, 209.7)
≥ 5 LTSP (Same partner and no sex)	312	12	38.5 (21.9, 67.8)
New partner (< 5 LTSP and ≥ 5 LTSP)	19	0	0.0
All ages			
< 5 LTSP (Same partner and no sex)	228	12	52.5 (29.8, 92.4)
≥ 5 LTSP (Same partner and no sex)	955	45	47.1 (35.2, 63.1)
New partner (< 5 LTSP and ≥ 5 LTSP)	128	0	0.0

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Supplemental Figure 1.