Association of HPV types 6, 11, 16, and 18 DNA detection and serological response in unvaccinated adolescent women

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Abstract

Antibodies directed against the HPV L1 protein are detected in approximately 70% of individuals with HPV infections. The factors associated with a serological response are not well characterized. Adolescent women (n=117), ages 15–17 at enrollment were followed for a mean of 6.2 years. Quarterly vaginal swabs (mean 22 per participant) were used to identify HPV 6, 11, 16, or 18 DNA using the Roche PCR/Linear Array. Sera collected at a mean of 4.7 years from enrollment were tested by cLIA. Chi-square tests were used to test the associations of HPV DNA and seropositivity for any of HPV types 6, 11, 16, or 18, and each of the four specific types separately. Regression models were fit to assess associations between strength of HPV DNA signal (as represented by mean and cumulative strength of signal), duration of HPV detection, seropositivity, and serotiter. Detection of HPV DNA was associated with seropositivity for four types combined and for HPV types 6, 16, and 18. A significant association of mean or cumulative strength of HPV DNA signal with seropositivity, and with serotiter was found for low-risk (LR) types (HPV 6 and 11), but not for high-risk (HR) types (HPV 16 and 18). Duration of infection had no association with serologic response for either LR or HR types. Therefore, there may be important differences in the way that anti-L1 antibodies are elicited to LR and HR-HPV types.

Keywords

Human Papillomavirus; antibody; adolescent women

INTRODUCTION

The factors associated with the immune response to human papillomavirus (HPV) infection are not fully understood [Einstein et al., 2009; Stanley, 2005]. HPV infects young women soon after the initiation of sexual activity [Winer et al., 2009; Winer et al., 2011]. HPV DNA can be detected in cervical or cervicovaginal specimens by polymerase chain reaction (PCR)

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or other sensitive measures in a high percentage of young, sexually active women [Brown et al., 2005; Moscicki et al., 2000; Tarkowski et al., 2004; Weaver et al., 2011]. Episodes of type-specific HPV DNA detection (incident infections) generally last between three and twelve months, then become undetectable in approximately 90% of cases [Ho et al., 1998; Trottier and Franco, 2006; Woodman et al., 2001].

In young women, type-specific antibodies directed against viral capsid proteins can be detected in approximately 50 to 70% of women with incident HPV infections [Castle et al., 2002; Ho et al., 2004; Nonnenmacher et al., 1996; Wideroff et al., 1996]. Such antibodies are first detected approximately six to twelve months after infection, and persist at relatively constant levels for many years [Ho et al., 2004]. In contrast, the measurable abundance of HPV DNA often waxes and wanes over time [Brown et al., 2005; Weaver et al., 2011]. Therefore, serum antibodies represent a more suitable method to gauge lifetime exposure to HPV.

Several studies have focused on associations of seropositivity and certain behaviors such as number of lifetime sexual partners and use of oral contraceptives. For example, Silins et al., examined determinants of seropositivity against HPV in a group of 275 women. In multivariate analysis, the number of lifetime sexual partners was associated with seropositivity to both oncogenic and non-oncogenic HPV types [Silins et al., 2000]. Clifford et al., examined seroprevalence and determinants of seropositivity in a cross-sectional study of 817 female university students [Clifford et al., 2007]. HPV seropositivity was higher among sexually active women, and higher among HPV DNA positive women. However, there are numerous unanswered questions about the natural history of this common infection [Gravitt, 2011].

For example, very few studies have analyzed associations of seropositivity in natural genital tract HPV infection and the associations of specific parameters of the infection, such as duration and magnitude ("viral load"). Intuitively, both the duration and the magnitude of an incident HPV infection would influence the elicited antibody response elicited, but this has not been critically examined in longitudinal studies. A study was therefore conducted to examine associations between the duration of incident HPV types 6, 11, 16, and 18 infections, the approximate viral load of these four types, and combinations of these parameters of infection with type-specific seropositivity and the magnitude of the serologic response. The study was conducted using specimens from a cohort of closely followed adolescent women.

MATERIALS AND METHODS

Study participants

Participants for this analysis included adolescent females enrolled in a cohort recruited for a longitudinal study assessing risk and protective behaviors for sexually transmitted infections. This project is known as the Young Women Project [Katz et al., 2001]. The study was approved by the Institutional Review Board at the Indiana University School of Medicine. Recruitment began in the fall of 1998 and the last observation was in 2008. This current analysis consists of 117 adolescent women from this cohort who had an available serum

sample, which was collected at a mean of 4.7 years from enrollment. These participants, ages 15 to 17 at enrollment, were followed for 6.2 years. None of the women received HPV vaccination because it was not available in the clinics until 2007, and was limited at that time to women less than 19 years of age.

Adolescent women attending one of three primary care clinics in Indianapolis, Indiana, U.S.A., were eligible for enrollment. Inclusion criteria for the study were as follows: age of 14 to 17 years, able to understand English and provide written consent, no serious psychiatric problems or mental deficiencies, and have parental permission for participation in the study. Adolescents could be enrolled regardless of past sexual experience although pregnant women were not enrolled. Informed consent and parental permission were obtained at enrollment. All participants received financial compensation for their time and effort.

Participants provided self-obtained vaginal samples approximately every three months at their quarterly interview that were tested for HPV. Quarterly self-obtained vaginal swabs were used to identify HPV 6, 11, 16, or 18 DNA. A serum sample was collected for each participant near the end of the study. Sera were tested for antibodies against the L1 major capsid protein of HPV types 6, 11, 16 and 18, as described below.

DNA isolation and HPV testing

DNA was extracted from self-obtained vaginal cotton swabs using QIAamp MinElute Media Kit (Qiagen, Valencia, California) as previously described [11]. The Linear Array HPV Genotyping Test (Roche Molecular Diagnostics, Indianapolis, Indiana) (LA-HPV) was used for HPV detection and genotyping [Brown et al., 2005; Castle et al.; Gage et al.]. This assay detects 37 HPV types using non-degenerate, 5′ biotin-labeled primer pools for PCR amplification within the L1 region of the HPV genome. Reactions were amplified in a PerkinElmer TC9600 Thermal Cycler (PerkinElmer) as previously described [Brown et al., 2009; Fife et al., 2009]. A positive (sample provided by Roche Molecular Diagnostics) and negative control reactions (no DNA) were performed with each assay. The GH20/PC04 human β-globin target was co-amplified to determine sample adequacy. Detection of specific HPV types was performed as previously described [Brown et al., 2005; Shew et al., 2006]. The 37 individual HPV types detected in the LA-HPV are comprised of HR-HPV types (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73, 82, and IS39) and low-risk (LR-HPV) HPV types (6, 11, 40, 42, 54, 55, 61, 62, 64, 72, 81, 83, 84, and CP6108).

A semi-quantitative scoring system was developed to estimate HPV viral load in samples from the adolescent women. PCR was performed (100 uL each) containing 0, 1, 10, 50, 100. 150, 200, 250, 500, and 1000 copies of cloned HPV 16 per reaction. In addition, 1000 copies of human DNA were added per reaction, and the GH20/PC04 human β-globin target was co-amplified with the HPV 16 sequence. All reactions were performed using the Roche Linear Assay (Roche Diagnostics, Indianapolis, Indiana). The low positive beta-globin band was assigned a value of 2, and the high positive band was assigned a value of 4. The intensity of HPV16 bands on assay strips was compared to the low and high beta-globin bands and scored relative to these bands. HPV 16 bands were scored as follows: No signal on the strip: 0+, HPV band visible but intensity lower than the low beta-globin band: 1+, HPV band

intensity equal to the low beta-globin band: 2+, HPV band intensity lower than the high beta-globin band but higher than low beta-globin band: 3+, HPV band intensity equal to the high beta-globin band: 4+, and HPV band intensity higher than the high beta-globin band: 5+. The same individual (B.Q.) interpreted all strips in a blinded manner to serologic results.

Serologic Testing

Sera were tested for the presence of neutralizing antibodies to HPV 6, 11, 16 and 18 using the Competitive Luminex Immunoassay (cLIA), which measures antibody binding to a single neutralizing epitope for each HPV-type L1 virus-like particle. The cLIA does not measure complete antibody binding, but instead measures a type-specific, conformational, neutralizing response that is a subset of the total immune response [Dias et al., 2005; Opalka et al., 2003]. As a unique reference standard curve is generated for each HPV type, and because each HPV type employs a type-specific monoclonal antibody with a unique binding affinity, the recorded titers cannot be directly compared between HPV types. The serostatus cutoffs employed for this study were those used for the cLIA in the quadrivalent HPV vaccine clinical trials [Villa et al., 2005; Villa et al., 2006].

Statistical Analysis

Descriptive statistics were used to describe the sample characteristics. Type-specific HPV DNA positivity was defined as detection of HPV 6, 11, 16, or 18 DNA in two or more vaginal swabs during the time from study enrollment to the serum sample collection. Duration of a type-specific HPV detection period was defined as the time between the initial HPV DNA detection to the last detection of that type before the serum sample collection. Additionally, the time from the last type-specific HPV DNA detection to the serum sample collection was determined. Magnitude of HPV 6, 11, 16, or 18 DNA detection was quantified by the mean signal strength and accumulative signal strength in the Roche assay. The mean signal strength was defined as the average score of signals for type specific HPV 6, 11, 16, or 18 DNA detections during the time from study enrollment to the serum sample collection. The accumulative signal strength was defined as the accumulative score of signals for HPV 6, 11, 16, or 18 detections. The combination of HPV 6, 11, 16, or 18 DNA duration and magnitude was quantified by the average signal strength per year during the entire HPV DNA detection period. It was defined as the accumulative signal strength divided by duration of HPV 6, 11, 16, or 18 DNA detection period.

To measure associations of HPV DNA detection and seropositivity, four kinds of episodes of HPV DNA detection and serologic combinations were defined. These were 1) type-specific DNA positivity and seropositivity, 2) type-specific DNA positivity and seronegativity, 3) type-specific DNA negativity and seropositivity, and 4) type-specific DNA negativity and seronegativity. Chi-square tests were used to test the associations of HPV DNA positivity/ negativity and seropositivity/negativity for HPV types 6, 11, 16, or 18 combined, and each of the four specific types separately. Data from all 117 participants were included in the analysis. Single positive HPV DNA detections (HPV DNA detected in only one vaginal swab sample during the time from enrollment to serum sample collection for a type specific HPV) were excluded from this analysis.

Association of serological response with duration, magnitude, and combination of duration and magnitude of HPV infection were determined. Participants who were HPV DNA-positive for any of HPV types 6, 11, 16, or 18 were included in this analysis. Each participant contributed up to four possible observations of HPV detection and serological responses. Univariate logistic regression models using generalized estimating equation (GEE) were fit to examine the associations of seropositivity with each variable of duration, magnitude, or combination of duration and magnitude of HPV infection. Models for any of the HPV 6, 11, 16, or 18 combined, and high-risk types (HPV 16 or 18) and low-risk types (HPV 6 or 11) were fit separately. GEE was used in logistic regression models to adjust for the correlations between multiple HPV types from individual participants.

The value of a serological titer was appointed as 0 if seronegativity occurred for a type-specific HPV DNA detection. Given the skewed distribution of serological titers, natural log transformation was used to transform the variable to a more symmetric distribution. Univariate regression models with random effect of HPV types were fit to examine the associations of the serological titer with each variable of duration, magnitude, or combination of duration and magnitude of HPV detection. Four types combined model, high-risk type models, and low-risk type models were fit separately. Random effect of HPV types was used in regression models to adjust for the correlations between multiple HPV types from individual participants.

RESULTS

Participant characteristics

The average age of the 117 study participants at enrollment was 15.4 years (SD +/− 1.0 years). One hundred and nine participants were African-American (93.2%) and 8 (8.8%) were white. The mean duration of follow-up was 6.04 years (SD +/− 1.46 years). The man number of vaginal swabs collected and analyzed per participants was 21.4 (SD +/− 4.5; range 8 – 31). The mean time from enrollment in the study to collection of the serum sample collection for HPV antibody testing was 4.7 years (SD +/− 2.2 years).

Associations of HPV DNA detection and seropositivity

Overall, for any of HPV types 6, 11, 16, or 18, 97 infections (new type-specific HPV DNA detection on at least two quarterly visits) occurred among the 117 young women. Of 97 infections with HPV types 6, 11, 16, or 18 (excluding 13 single positive DNA detections), 75 (77.3%) were associated with seropositivity against the specific HPV type, and 22 cases of type-specific HPV DNA detection were associated with type-specific seronegativity (22.7%) (Table 1). Overall detection of HPV types 6, 11, 16, or 18 DNA was highly associated with type-specific seropositivity (P<.0001). During the entire study period, there were 427 possible episodes for these four HPV types; 221 of these (51.8%) were negative for type-specific HPV DNA and were seronegative; 75 of 427 (17.6%) were positive for type-specific HPV DNA and were seropositive; 22 of 427 (5.2%) were positive for type-specific HPV DNA and were seronegative; and 109 of 427 (25.5%) were negative for type-specific HPV DNA and were seropositive.

Of 36 incident HPV 6 infections occurring among the 117 participants with adequate end-of-study serum for analysis, 30 (83.3%) were associated with seropositivity against HPV 6 (Table 1). Overall detection of HPV 6 DNA was associated with HPV 6 seropositivity (P=.0009). During the entire study period, there were 113 possible episodes for HPV 6; 38 episodes (33.6%) were negative for HPV 6 DNA and were seronegative; 30 of 113 (26.6%) were positive for HPV 6 DNA and were seropositive; 6 of 113 (5.3%) were positive HPV 6 DNA and were seronegative; and 39 of 113 (34.5%) were negative for HPV 6 DNA and were seropositive.

Overall detection of HPV 11 was not associated with type-specific seropositivity (P=.058). However, only three cases of HPV 11 infection occurred among the participants who had adequate serum for analysis.

Of 39 incident HPV 16 infections occurring among the 117 participants with adequate end-of-study serum for analysis, 29 (74.4%) were associated with seropositivity against HPV 16 (Table 1). Overall detection of HPV 16 DNA was associated with HPV 16 seropositivity (P<.0001). During the entire study period, there were 107 possible episodes of HPV 16; 55 (51.4%) were negative for HPV 16 DNA and seronegative, 29 of 107 (27.1%) were positive for HPV 16 DNA and were seropositive; 10 of 107 (9.4%) were positive HPV 16 DNA and were seronegative; and 13 of 107 (12.2%) were negative for HPV 16 DNA and were seropositive.

Of 19 incident infections with HPV 18 occurring among the participants with adequate end-of-study serum for analysis 13 (68.4%) were associated with HPV 18 seropositivity (Table 1). Overall detection of HPV 18 DNA was associated with type-specific seropositivity (P=.0002). During the entire study period, there were 113 possible episodes for HPV 18; 71 (62.8%) were negative for HPV 18 DNA and seronegative, 6 of 113 (5.3%) were positive for HPV 18 DNA and were seropositive; 10 of 113 (8.8%) were positive HPV 18 DNA and were seronegative, and 23 of 113 (20.4%) were negative for HPV 18 DNA and were seropositive.

Associations of duration and magnitude of HPV DNA positivity and seropositivity

This analysis examined associations of the duration of type-specific HPV DNA detection, the mean signal strength of type-specific HPV DNA detection in the Roche Linear Array assay (an estimate of HPV viral load), and the combination of duration and signal strength with type-specific seropositivity to the HPV L1 protein. For HPV types 6, 11, 16, and 18 combined, the mean signal strength for type-specific HPV DNA detection was associated with seropositivity (P=.013, OR=1.70, 95% confidence interval 1.12 - 2.57). Duration of type-specific HPV DNA detection, cumulative signal strength, and time from the last HPV DNA detection to acquisition of the serum sample were not associated with seropositivity (Table 2).

As above, a semi-quantitative scoring system was developed to estimate HPV viral load in samples from the adolescent women. The signal strength in the Roche Linear Array assay indicated that 50 copies of cloned HPV 16 per PCR yielded 1+ signal strength, 150 copies

yielded 2+ signal strength, 200 copies yielded 3+ signal strength, 500 copies yielded 4+ signal strength, and 100 copies resulted in 5+ signal strength.

For the two low-risk HPV types combined (HPV 6 and HPV 11), the mean signal strength was associated with seropositivity (P=.034, OR=2.7, 95% CI=1.08 - 6.78) (Table 2). In addition, for the low-risk HPV types combined, the cumulative signal strength was associated with seropositivity (P=.027, OR=1.39, 95% CI=1.04 - 1.85). No other predictor was associated with seropositivity. For the two high-risk HPV types combined (HPV 16 and HPV 18), no predictor was associated with seropositivity (Table 2).

Association of serologic titer and duration, magnitude, and combination of duration and magnitude of infection

Determinations were performed to measure associations of duration of type-specific HPV DNA detection, mean signal strength of HPV DNA, and combinations of duration and strength of signal with the serologic titer for HPV types 6, 11, 16, and 18 (Table 3). For all four HPV types combined, a higher mean signal strength was associated with a higher serologic tighter (P=.018, estimate 1.30, 95% CI=0.23 - 2.38). No other predictors, including duration of HPV DNA detection were associated with a higher serologic titer.

For the two LR-HPV types combined, a higher mean strength of signal was associated with a higher mean serologic titer (P=.015, estimate 1.73, 95% CI=0.36 - 3.10) (Table 3). The cumulative signal strength for LR-HPV types was also associated with a higher mean serologic titer. For the two LR-HPV types, no other predictors were associated with a higher serologic titer. For the two HR-HPV types combined, no predictors were associated with a higher serologic titer (Table 3).

DISCUSSION

In this study of closely followed adolescent women at high risk for HPV infections, the presence of neutralizing antibodies directed against the L1 major capsid protein of HPV types 6, 11, 16, or 18 was highly associated with type-specific HPV DNA detection. The strength of signal of HPV infection in the Roche Linear Array assay, an estimator of viral load, was associated with type-specific antibody development for HPV types 6, 11, 16, or 18 combined, and for LR-HPV types, but not for HR-HPV types. The duration of HPV infection was not associated with antibody development for all four types combined, or for LR and HR-HPV types.

A better understanding of the natural history of HPV infections is necessary for several reasons. First, development of preventative strategies is dependent on a comprehensive knowledge of factors (behavioral and molecular) associated with HPV infection. Second, it is clear that examining clinical specimens for HPV DNA in cross-sectional studies under-represents the burden of infection when compared to studies of antibodies to HPV. Seropositivity against the HPV L1 major capsid protein can be utilized in natural history studies is an indicator of lifetime HPV exposure. This is important because incident HPV infections generally become undetectable by sensitive methods such as PCR after a few months in nearly 90% of cases [Ho et al., 2004]. Third, a more complete understanding of

the immune response to natural infection is desirable, especially now that safe and effective HPV vaccines are available.

Previous studies of the natural history of HPV infection in young women indicate that a serologic response to the HPV L1 major protein occurs in approximately 50 to 70% of individuals with incident HPV infections of the genital tract [Castle et al., 2002; Ho et al., 2004; Nonnenmacher et al., 1996; Wideroff et al., 1996]. In our study of a closely followed cohort of adolescent women, at very high risk for HPV infection, 77.3% of incident infections with any of HPV types 6, 11, 16, or 18, were associated with seropositivity at the end of the study, as measured by the competitive Luminex-based immunoassay. This assay is highly type-specific but underestimates the total antibody response to HPV This assay utilizes a competitive strategy with a single, neutralizing, type-specific monoclonal antibody each of HPV types 6, 11, 16, and 18 [Dias et al., 2005; Opalka et al., 2003]. Thus, an antibody assay that captures all antibodies elicited by natural infection may indicate a higher rate of seropositivity than was found in the current study.

Although a protective level of antibody for any HPV type has not been established, it is possible that such antibodies may protect against re-infection. It is possible that such protection may be dependent on antibody titers. Several studies show such protection against re-infection. Malik et al., examined a cohort of 508 college-age women for three years to determine whether persistent HPV antibodies following natural infection were protective against subsequent infection [Malik et al., 2009]. Protection from incident infection with any HPV type was associated with seropositivity to any of seven HPV types including HPV 16. In addition, the risk of type-specific HPV infection was decreased in women with persistent antibodies lasting at least two years to the specific HPV [Malik et al., 2009]. In another study, Wentzensen et al., measured antibodies to HPV types 6, 11, 16, or 18 by cLIA and VLP ELISA in 974 young women to study associations between seropositivity and protection from subsequent infection with the same HPV types [Wentzensen et al., 2011]. Seropositivity in either assay predicted protection from subsequent type-specific HPV infection. Safaen et al., performed a study in young women to determine if antibodies elicited by HPV 16 or 18 infection would protect women against subsequent infection by the same type, compared to antibody negative women [Safaeian et al., 2010]. HPV 16 or 18 seropositivity was associated with a statistically significant reduced risk of subsequent HPV 16 or 18 infection. In contrast, Viscidi et al., examined the association of baseline seropositivity to HPV types 16, 18, or 31 L1 VLPs in 7,046 women, and the risk of subsequent HPV infection five to seven years later [Viscidi et al., 2004]. Seropositivity was not associated with a significant decrease risk of infection with homologous HPV types compared to women who were seronegative at baseline. However, only a single test HPV test was performed at the end of the study.

Data from the current analysis indicate that the magnitude of an incident HPV type 6, 11, 16, and 18 infection, as reflected by signal strength in the Roche Linear Array assay is associated with seropositivity in the cLIA assay. Interestingly, this association was found for HPV types 6 and 11 but not for HPV 16 and 18, although there was a trend towards significance of this association for these HR-HPV types. These results suggest that there may be differences in the way that anti-L1 antibodies are elicited.

Interestingly, the duration of HPV infection was not associated with seropositivity or a higher serologic titer for either low risk or high-risk types. HPV does not induce cell lysis and has no viremic phase [Stanley, 2012]. Thus, the virus may not be readily recognized by the immune system in low abundance infections, even those of long duration. In contrast, infections of a high viral load may be associated with seropositivity preferentially.

Limitations of this analysis include the small number of participants. However, the young women in our study were followed very closely for approximately six years with frequent sampling, thus allowing a detailed analysis of the associations of HPV incident infections and the serologic response to infection. Second, the cLIA assay likely underestimates the total antibody response to HPV [Brown et al., 2011]. However the cLIA assay is very type-specific, allowing near certainty that HPV DNA and serologic associations were also type-specific in this analysis. Third, the number of HPV 11 infections was too small to permit a type-specific analysis of the serologic response. Lastly, the use of the signal strength in the Roche Linear Array assay is an estimator of viral load, not an exact determination. Other groups have used similar approaches to estimate viral load, such as the intensity of PCR amplicons on agarose gels or signal strength in Hybrid Capture assays [Porras et al., 2010; Wang et al., 2004]. Thus, while the scoring system utilized for the estimate of viral load is somewhat subjective, the same investigator scored every assay. In addition, an experiment was performed using specific numbers of viral copies per PCR was performed, indicating an approximately linear relationship from 50 copies to 500 copies of HPV 16 DNA per reaction with the scoring system created for this analysis.

In summary, in adolescent women, development of type-specific antibody was associated with DNA positivity for HPV types 6, 16, 18. The strength of signal of HPV infection was associated with type-specific antibody development for LR-HPV types (mainly HPV 6), but not for HR-HPV types. Duration of HPV infection was not associated with antibody development. There may be differences in the way that anti-L1 antibodies are elicited to LR and HR-HPV types.

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Table 1

HPV DNA detection and seropositivity

	Seropositivity N(%)	Seronegativity N(%)	P
All HPV 6, 11, 16, or 18[1]			
DNA positivity	75 (17.6)	22 (5.2)	<0.001[2]
DNA negativity	109 (25.5)	221 (51.8)	
HPV 6			<0.001[2]
DNA positivity	30 (26.6)	6 (5.3)	
DNA negativity	39 (34.5)	38 (33.6)	
HPV 11			
DNA positivity	3 (3.2)	0	0.058[3]
DNA negativity	34 (36.2)	57 (60.6)	
HPV 16			
DNA positivity	29 (27.1)	10 (9.4)	<0.001[2]
DNA negativity	13 (12.2)	55 (51.4)	
HPV 18			
DNA positivity	13 (11.5)	6 (5.3)	<0.001[2]
DNA negativity	23 (20.4)	71 (62.8)	

[1] 117 participants were included in this analysis (427 potential HPV events)

[2] Chi-square test

[3] Fishers exact test

Table 2

Logistic regression models[1] of seropositivity with duration, magnitude, or combination of duration and magnitude of HPV infection

Predictor	Mean (std) Seropositivity	Mean (std) Seronegativity	OR	95% CI	P
Any of HPV 6, 11, 16, or 18					
Duration of DNA detection period (years)	1.19 (1.08)	1.17 (1.15)	1.01	0.65 –1.58	0.948
Duration of last DNA detection to serum sample collection (years)	2.15 (2.10)	1.90 (2.05)	1.06	0.85 –1.32	0.589
Mean DNA signal strength	4.18 (0.93)	3.51 (1.43)	1.70	1.12 –2.57	0.013
Cumulative DNA signal strength	16.88 (9.88)	13.41 (10.27)	1.04	0.97 –1.12	0.226
Mean DNA signal strength per year during DNA detection period	20.99 (11.87)	19.97 (14.00)	1.00	0.97 –1.05	0.732
High-risk HPV types (HPV 16 or 18)					
Duration of DNA detection period (years)	1.25 (1.10)	1.28 (1.20)	0.97	0.58 –1.62	0.914
Duration of last DNA detection to serum sample collection (years)	2.45 (2.08)	1.91 (2.27)	1.14	0.83 –1.55	0.417
Mean DNA signal strength	3.77 (1.43)	3.77 (1.43)	1.54	0.93 –2.55	0.095
Cumulative DNA signal strength	15.94 (10.87)	15.94 (10.87)	1.02	0.96 –1.10	0.437
Mean DNA signal strength per year during DNA detection period	20.16 (13.37)	10.16 (13.37)	1.01	0.96 –1.06	0.754
Low-risk HPV types (HPV 6 or 11)					
Duration of DNA detection period (years)	1.12 (1.07)	0.88 (1.06)	1.29	0.42 –3.96	0.652
Duration of last DNA detection to serum sample collection (years)	1.76 (2.10)	1.85 (1.50)	0.98	0.71 –1.35	0.885
Mean DNA signal strength	4.08 (1.03)	2.83 (1.29)	2.70	1.08 –6.78	0.034
Cumulative DNA signal strength	15.03 (10.32)	6.67 (3.67)	1.39	1.04 –1.85	0.027
Mean DNA signal strength per year during DNA detection period	20.60 (12.28)	19.44 (16.92)	1.01	0.93 –1.09	0.863

[1]97 HPV DNA detection and serum episodes from 68 participants included in this analysis

Table 3

Regression models[1] of serological titer with duration, magnitude, or combination of duration and magnitude of HPV infection

Predictor	Estimate	95% CI	P
Any of HPV 6, 11, 16, or 18			
Duration of DNA detection period (years)	−0.07	−1.17 – 1.04	0.907
Duration of last DNA detection to serum sample collection (years)	0.11	−0.47 – 0.69	0.709
Mean DNA signal strength	1.30	0.23 – 2.38	0.018
Cumulative DNA signal strength	0.09	−0.03 – 0.21	0.140
Mean DNA signal strength per year during DNA detection period	0.02	−0.08 – 0.12	0.705
High-risk HPV types (HPV 16 or 18)			
Duration of DNA detection period (years)	−0.11	−1.64 – 1.42	0.884
Duration of last DNA detection to serum sample collection (years)	0.22	−0.58 – 1.02	0.580
Mean DNA signal strength	1.13	−0.47 – 2.73	0.163
Cumulative DNA signal strength	0.07	−0.11 – 0.24	0.448
Mean DNA signal strength per year during DNA detection period	0.01	−0.13 – 0.15	0.891
Low-risk HPV types (HPV 6 or 11)			
Duration of DNA detection period (years)	0.16	−1.45 – 1.78	0.838
Duration of last DNA detection to serum sample collection (years)	0.05	−0.80 – 0.91	0.905
Mean DNA signal strength	1.73	0.36 – 3.10	0.015
Cumulative DNA signal strength	0.17	0.01 – 0.33	0.040
Mean DNA signal strength per year during DNA detection period	0.03	−0.10 – 0.17	0.609

[1]97 HPV DNA detection and serum episodes from 68 participants were included in this analysis.