MAJOR ARTICLE

Seroprevalence of 8 Oncogenic Human Papillomavirus Genotypes and Acquired Immunity Against Reinfection

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Background. Natural human papillomavirus (HPV) antibody titers have shown protection against subsequent HPV infection, but previous studies were restricted to few HPV genotypes. We examined the association of naturally occurring antibodies against 8 carcinogenic HPV types with subsequent infections.

Methods. A total of 2302 women enrolled in the Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesion Triage Study provided blood samples at baseline. Serum samples were tested for antibodies against 8 carcinogenic HPV genotypes (16, 18, 31, 33, 35, 45, 52, and 58) using a multiplex serology assay. We analyzed the relationship between HPV antibodies and HPV infection during 2 years of follow-up among women negative for the specific HPV type at baseline.

Results. Baseline seroprevalence for HPV16 L1 was associated with decreased risk of DNA positivity for HPV16 (odds ratio, 0.39 [95% confidence interval, .18–.86]) at ≥2 follow-up visits. We observed similar but nonsignificant decreased risks for HPV18 and 31. These findings were restricted to women reporting a new sex partner during follow-up. There was no association between baseline seroprevalence and detection of precancer during follow-up.

Conclusions. Seroprevalence conferred protection against subsequent HPV infection for HPV16 and indicated possible protection for 2 other genotypes, suggesting that this effect is common to several HPV genotypes.

Keywords. Human papillomavirus; natural immunity; serology.

Sexually transmitted infections with human papillomaviruses (HPVs) are highly prevalent among women and men worldwide [1]. HPV prevalence typically peaks soon after the age of sexual initiation for a population, but most HPV infections will become undetectable within 2 years [2, 3]. Some infections present with minor cervical abnormalities, such as cytologic atypical squamous cells of undetermined significance (ASC-US) or low-grade squamous intraepithelial lesions (LSILs). Persistently detectable infections have an increased risk of progression to precancerous lesions (defined here broadly as cervical intraepithelial neoplasia grade 2+ [CIN2+]), which have a substantial risk of progressing to cervical cancer if left untreated [4]. Some studies have suggested that approximately 60% of HPV infections result in detectable antibodies against HPV L1, and there is evidence that a longer duration of infection is associated with higher seroconversion rates [5, 6].

Prophylactic vaccination with bivalent (HPV16 and 18) and quadrivalent (HPV6, 11, 16, and 18) virus-like particle (VLP) vaccines has a high efficacy to protect against infection with those genotypes and associated clinical end points [7–9]. It is less clear to what extent naturally acquired antibody titers confer protection

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against reinfection with the same HPV genotype. Studies of protection against reinfection resulting from natural infection with HPV have been performed using various serologic assays and have shown heterogeneous results. VLP-based immunoassays (eg, enzyme-linked immunosorbent assays [ELISAs]) measure the total concentration of serum antibody that bind to VLPs, but establishing VLP assays for a wide range of genotypes is challenging. Existing VLP assays for multiple oncogenic genotypes [10] are expensive because of synthesis of the VLPs, making their use in large epidemiologic studies difficult. Multiplex serology assays that measure seroreactivity to many antigens by use of recombinant proteins have recently been developed, which could allow for easier testing of seroreactivity to multiple HPV genotypes [11, 12]. The goal of this analysis was to use a multiplex serology assay for 8 oncogenic HPV genotypes (HPV16, 18, 31, 33, 35, 45, 52, and 58) to examine whether there was an association between enrollment HPV serostatus as measured by this assay and risk of type-specific HPV reinfection and incident CIN2+ over a 2-year follow-up period in women free of CIN2+ at baseline in the ASCUS-LSIL Triage Study (ALTS).

METHODS

The ASCUS-LSIL Triage Study

The ALTS was a multicenter, randomized clinical trial conducted by the National Cancer Institute to evaluate different methods of clinical follow-up for equivocal (ASCUS) and low-grade (LSIL) cervical cytology abnormalities [13]. The trial enrolled 3488 women with a diagnosis of ASCUS and 1572 women with a diagnosis of LSIL from 4 participating clinical centers in the United States. The enrollment and semiannual follow-up visits included a pelvic examination; collection of cervical specimens for cytological evaluation and HPV DNA testing; a questionnaire on demographic, behavioral, and health-related information; and cervicography. Blood was drawn only at enrollment among women who agreed separately to this additional procedure. Patients were randomized to one of 3 management strategies: (1) immediate colposcopy at enrollment, (2) follow-up with cytology only, and (3) use of HPV DNA results and cytology to triage colposcopy follow-up [13].

HPV DNA Genotyping

Cervical specimens preserved in specimen transport medium were genotyped for HPV DNA by using the Line Blot Assay as previously described [14–18]. Extracted DNA from the cervical specimens was amplified using a PGMY09/11 L1 consensus primer system [14, 18, 19], and the amplicon was tested using reverse line blot hybridization for the presence of 27 human papillomavirus genotypes (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51 to 59, 66, 68, 73 [PAP238a], 82 [W13b], 83 [Pap291], and 84 [PAP155]). HPV genotyping was performed on cervical specimens from the enrollment visit and on specimens from 4 trial follow-up visits (6, 12, 18, and 24 months after enrollment).

HPV L1 Serology Testing

We used a Luminex-based multiplex serology assay to test serum samples from the enrollment visit of ALTS (n = 2462) for seroreactivity to the HPV major capsid (L1) protein for 8 carcinogenic HPV genotypes (16, 18, 31, 33, 35, 45, 52, and 58). The multiplex serology assay uses glutathione *S*-transferase fusion proteins with the L1 protein targets expressed as previously described [11, 20], which are bound to fluorescence-labeled polystyrene beads (SeroMap, Luminex, Austin, TX). Each antigen type was loaded onto a different-colored bead set, and 3000 beads per set were loaded into each well of 96-well plates and incubated with serum and reporter antibodies. Bead mixture reporter fluorescence was analyzed in a Luminex 100, which quantifies the fluorescence intensity associated with the antibodies bound to the viral antigens by bead color. Results were reported as the median fluorescence intensity (MFI) of a minimum of 100 beads analyzed per bead set/antigen. The background fluorescence level was set using the MFI of GST-tag-loaded beads without the fusion proteins loaded, and specific reactivity (net MFI) for a given HPV protein was calculated by subtracting the background level from the MFI of the antigen-loaded beads [11]. Seropositivity thresholds were defined on the basis of a prior study of 125 South Korean women who reported no lifetime sex partners. The positivity threshold was set at 3 SDs above the mean level of MFI reactivity in these women, excluding positive outliers [21].

Statistical Analysis

Serological testing was performed on 2462 women drawn from the 5060 women included in ALTS who had an available serum sample from the enrollment visit for serologic testing and who had a CIN diagnosis of less than grade 2 (<CIN2). Women (n = 161) were also excluded if they had no HPV DNA data for their postenrollment visits. Thus, the final analytic population was 2302. Serum sample availability appeared to be randomly distributed in our population and showed no association with baseline histological diagnosis, with worst histological diagnosis over the course of the trial, or with multiple baseline demographic characteristics (data not shown).

To study the association between seropositivity and subsequent risk of HPV infection, we used logistic regression methods to calculate odds ratios (ORs) for the association between baseline binary seropositivity and new DNA detection or incident precancer in women who were DNA negative at baseline for the HPV genotype being examined, with adjustment for whether a woman reported a new sex partner over the course of the study. In women who are seronegative for HPV, detection of HPV DNA during follow-up may signify a first-time infection or a reinfection with a previously acquired type. In

Table 1. Frequency of Human Papillomavirus (HPV) DNA Detection During Follow-up Among Women Who Were DNA Negative and Either L1 Seropositive or Seronegative for the Same HPV Type at Baseline

Type, Baseline Serostatus	Baseline Type-Specific DNA–Negative Test Result	Follow-up Visits With Type-Specific DNA–Positive Test Result		
		≥1	≥2	≥2 Sequential
HPV16				
Seronegative	1507 (76.5)	134 (8.9)	57 (3.8)	50 (3.3)
Seropositive	463 (23.5)	32 (6.9)	7 (1.5)	5 (1.1)
Total	1970	166	64	55
HPV18				
Seronegative	1687 (80.1)	64 (3.8)	27 (1.6)	23 (1.4)
Seropositive	420 (19.9)	13 (3.1)	3 (0.7)	3 (0.7)
Total	2107	77	30	26
HPV31				
Seronegative	1672 (79.9)	77 (4.6)	28 (1.7)	27 (1.6)
Seropositive	421 (20.1)	15 (3.6)	4 (1.0)	4 (1.0)
Total	2093	92	32	31
HPV33				
Seronegative	2000 (91.9)	49 (2.45)	16 (0.01)	14 (0.01)
Seropositive	176 (8.1)	3 (1.7)	0	0
Total	2176	52	16	14
HPV35				
Seronegative	1807 (84.6)	82 (4.5)	29 (1.6)	27 (1.5)
Seropositive	328 (15.4)	17 (5.2)	7 (2.1)	7 (2.1)
Total	2135	99	36	34
HPV45				
Seronegative	1694 (79.0)	70 (4.1)	22 (1.3)	16 (0.9)
Seropositive	451 (21.0)	27 (6.0)	7 (1.6)	7 (0.6)
Total	2145	97	29	23
HPV52				
Seronegative	1810 (88.5)	145 (0.1)	64 (0.03)	56 (0.03)
Seropositive	235 (11.5)	21 (0.1)	7 (0.03)	7 (0.03)
Total	2045	166	71	63
HPV58				
Seronegative	1947 (91.9)	75 (0.04)	25 (0.01)	23 (0.01)
Seropositive	171 (8.1)	7 (0.04)	3 (0.02)	3 (0.02)
Total	2118	82	28	26

Data are no. or no. (%) of women.

seropositive women, detection of DNA from the same HPV type is likely reinfection. HPV (re-)infection was defined in 3 ways, to distinguish transient from more-persistent HPV infections: (1) HPV DNA detected at any of the 4 follow-up visits, (2) HPV DNA detected at any 2 follow-up visits, and (3) HPV DNA detected at 2 sequential follow-up visits. This analysis was also performed to evaluate the association between baseline seropositivity and detection of DNA from a new yet related HPV genotype, to evaluate cross-protective effects. To estimate protection against reinfection across all genotypes, we computed a pooled summary estimate of the OR across all 8 tested types, assuming a fixed-effects model, by use of the Mantel-Haenszel method, as well as a pooled estimate for HPV16 and HPV18 only, to allow for comparison to other studies in the literature that used this metric. We repeated all protection analyses with age at enrollment and reported lifetime number of sex partners included in the logistic regression model. We also repeated our analysis after excluding all women who did not report any sexual intercourse over the course of the study, to eliminate those unlikely to acquire new HPV infections during follow-up.

Each calculation included women if they had a DNA-negative test result for that genotype at the enrollment visit, which included some women who did not have follow-up HPV DNA data from all 4 trial visits. To account for this, we repeated all analyses but restricted inclusion to women who had full HPV DNA data for all 4 follow-up visits (n = 1277). All statistical analyses were performed using Stata, version 11, and R.

RESULTS

Seropositivity rates and cumulative incidence of HPV DNA detection during the 2 years of follow-up among women with at least 1 follow-up visit are presented in Table 1. Of the 1970 women in the analytic data set who were HPV16 DNA negative at baseline, 23.5% were seropositive for HPV16 L1. Seropositivity was about 20% for HPV18 (n = 2107 DNA negative), HPV31 (n = 2093 DNA negative), and HPV45 (n = 2145 DNA negative) and lower for the remaining types analyzed in women who were DNA negative for that genotype at baseline. Risk factors for HPV infection and cervical precancer were similar in the full data set, in women with at least 1 follow-up visit who were included in the analytic data set, and in women with full HPV follow-up data (Supplementary Table 1). Across all 8 genotypes, only 20%–50% of the incident DNA detections observed in the first year of follow-up were detectable in the second year as well (Supplementary Table 2).

We used multivariate logistic regression analysis to examine the association between baseline HPV L1 seropositivity (yes/no) and type-specific HPV DNA detection at follow-up trial visits. We calculated crude ORs and ORs adjusted for report of a new sex partner over the course of the trial (Table 2). Seropositivity was associated with an increased odds of having a new sex partner during the trial period for all HPV genotypes, excluding HPV18 and HPV33. Our estimates of protection against reinfection associated with baseline seroprevalence did not change when reported lifetime number of sex partners and age were also included in the logistic regression models, so these variables were not included in the final model.

If single-time-point DNA detection was included in the analysis, HPV genotype seropositivity did not show a statistically significant protective effect against type-specific DNA detection (Table 2). The majority of DNA detections observed during ALTS follow-up were detected at a single visit; only 38.5% of HPV16 DNA detections were observed at ≥2 follow-up visits. When incident HPV outcomes were restricted to women who had a type-specific DNA detection at ≥2 follow-up visits, HPV16 L1 seropositivity was associated with a reduced odds of persistent HPV detection (OR, 0.38 [95% CI, .17–.83]), with adjustment for a new sex partner in the trial period. Crude estimates without adjustment for a new sex partner were similar. A similar point estimate of protection against incident detection at ≥2 follow-up visits was observed for HPV18 (OR, 0.44) and HPV31 (OR, 0.56), but the results were not statistically significant. We also examined protection against HPV DNA–positive results at 2 sequentially occurring visits; this produced little change in our point estimates, compared with HPV DNA detection at ≥2 follow-up visits (data not shown). When analysis was limited to women who had full HPV DNA data for all 4 follow-up visits (n = 1100), the association between HPV16 seroprevalence and protection against incident HPV DNA

Table 2. Multivariate Logistic Regression Analysis of the Association Between Baseline Human Papillomavirus (HPV) L1 Seropositivity, Reported New Sex Partner During the Trial Period, and Type-Specific HPV DNA Detection During Follow-up

		Type-Specific DNA–Positive Test Result During Any Follow-up Visit				Type-Specific DNA–Positive Test Result During ≥2 Follow-up Visits			
Type	New Sex Partner, Women, No.	HPV Detection, Women, No.	L1 Seropositivity, Unadjusted OR (95% CI)	L1 Seropositivity, Adjusted OR (95% CI)	New Sex Partner, OR (95% CI)	HPV Detection, Women, No.	L1 Seropositivity, Unadjusted OR (95% CI)	L1 Seropositivity, Adjusted OR (95% CI)	New Sex Partner, OR (95% CI)
HPV16	604	166	0.76 (.51–1.13)	0.74 (.50–1.11)	**2.23 (1.61–3.07)**	64	**0.39 (.18–.86)**	**0.38 (.17–.83)**	**3.08 (1.86–5.11)**
HPV18	654	77	0.81 (.44–1.48)	0.8 (.44–1.47)	1.29 (.80–2.07)	30	0.44 (.13–1.46)	0.44 (.13–1.47)	0.97 (.44–2.13)
HPV31	659	92	0.77 (.44–1.34)	0.75 (.43–1.32)	**1.81 (1.19–2.76)**	32	0.56 (.19–1.61)	0.54 (.19–1.56)	**2.54 (1.26–5.11)**
HPV33	681	52	0.69 (.21–2.23)	0.69 (.21–2.23)	1.27 (.72–2.26)	16	Omitted	Omitted	1.72 (.64–4.65)
HPV35	673	99	1.15 (.67–1.96)	1.14 (.67–1.96)	**1.87 (1.24–2.80)**	36	1.34 (.58–3.08)	1.33 (.58–3.07)	1.39 (.71–2.73)
HPV45	673	97	1.48 (.94–2.33)	1.46 (.92–2.30)	**1.63 (1.07–2.46)**	29	1.2 (.51–2.82)	1.18 (.50–2.79)	1.55 (.73–3.26)
HPV52	634	166	1.13 (.70–1.82)	1.09 (.67–1.76)	**2.41 (1.75–3.31)**	71	0.84 (.38–1.85)	0.79 (.36–1.76)	**3.2 (1.98–5.17)**
HPV58	670	82	1.06 (.48–2.35)	1.11 (.50–2.46)	**1.65 1.05–2.58**	28	1.37 (.41–4.59)	1.47 (.44–4.95)	**2.21 (1.05–4.67)**

Analyses include women who had a cervical intraepithelial neoplasia diagnosis of less than grade 2 and were type-specific HPV negative at baseline (range, 1970–2200 women, depending on genotype). ORs for seropositivity and report of a new sex partner are mutually adjusted.

Abbreviations: CI, confidence interval; OR, odds ratio.

Table 3. Association Between Human Papillomavirus (HPV) L1 Seropositivity at Baseline and DNA Positivity for the Same HPV Type During Follow-up, Stratified by Report of a New Sex Partner During Follow-up

	Odds Ratio (95% Confidence Interval)	
Type	No New Sex Partner (n = 1574)	New Sex Partner (n = 729)
HPV16	1.08 (.65–1.79)	**0.45 (.23–.87)**
HPV18	0.70 (.31–1.58)	0.96 (.38–2.43)
HPV31	1.15 (.58–2.27)	0.37 (.13–1.06)
HPV33	1.16 (.35–3.86)	No detections
HPV35	1.45 (.74–2.86)	0.81 (.34–1.97)
HPV45	1.19 (.63–2.25)	1.86 (.95–3.64)
HPV52	1.13 (.57–2.23)	1.05 (.53–2.08)
HPV58	1.23 (.48–3.16)	0.90 (.21–3.89)

detection at ≥2 follow-up visits remained statistically significant (Supplementary Table 3).

When women were stratified by report of a new sex partner during the follow-up period, we found that HPV16 seropositivity at baseline was protective against HPV16 DNA detection during the follow-up period among the 729 women with a new sex partner (OR, 0.45 [95% CI, .23–.87]) but had no effect on HPV16 DNA detection among women without a new sex partner (Table 3). When this analysis was limited to women with full follow-up data, the observed associations were similar but no longer statistically significant (Supplementary Table 3).

No association was observed between baseline binary L1 seropositivity and incident CIN2+ detection with type-concordant incident HPV DNA detection during the follow-up period for any genotype (Table 4).

We constructed a pooled summary OR for protection for HPV16 and HPV18 only and a pooled summary OR across all 8 HPV genotypes, using the Mantel-Haenszel method (Table 5). Pooled estimates that included HPV16 and HPV18 only showed a protective effect of seroprevalence against 2 sequential DNA detections (OR, 0.38 [95% CI, .18–.78]). No statistically significant protective effect for either sequentially detected infections or detections at any visit was observed when all 8 genotypes were included.

Table 6 presents the risk of postenrollment infection with closely related HPV types among women who were seropositive for HPV16 or HPV18 and DNA negative for a related genotype at study enrollment. Women who were HPV16 seropositive at baseline had a reduced odds of incident infection with HPV31 (OR, 0.62 for detection at 2 sequential visits) and HPV33 (OR, 0.52 for detection at 2 sequential visits), compared with seronegative women. This indicates possible cross-protective effects of baseline seropositivity for HPV16, but the results were not statistically significant. HPV18 did not show any evidence of cross-protection against incident infection with HPV45, a closely related genotype. We also performed this analysis for seropositivity for the related genotypes and its association with incident HPV16 or HPV18 DNA detection and saw no statistically significant protective effect.

DISCUSSION

Although high antibody titers following HPV VLP vaccination are associated with preventing infection with the HPV genotypes covered by the vaccine, it is unclear to what extent naturally occurring antibody levels resulting from an HPV infection provide protection against future reinfection with the virus.

Table 4. Logistic Regression Analysis of the Association Between Human Papillomavirus (HPV) L1 Seropositivity at Baseline, Reported New Sex Partner During the Trial Period, and Cervical Intraepithelial Neoplasia Grade ≥2 (CIN2+) With Type-Specific HPV DNA Detection During Follow-up

	CIN2+ and DNA–Positive Test Result During Any Follow-up Visit			CIN2+ and Type-Specific DNA–Positive Test Result During ≥2 Follow-up Visits		
Type	Women, No.	L1 Seropositivity, OR (95% CI)	New Sex Partner, OR (95% CI)	Women, No.	L1 Seropositivity, OR (95% CI)	New Sex Partner, OR (95% CI)
HPV16	35	1.11 (.52–2.39)	1.93 (.98–3.77)	25	0.60 (.21–1.77)	2.12 (.96–4.69)
HPV18	18	0.48 (.11–2.10)	2.28 (.90–5.77)	13	0.70 (.15–3.17)	2.63 (.88–7.88)
HPV31	26	0.51 (.15–1.69)	1.62 (.74–3.56)	16	0.56 (.13–2.48)	1.32 (.48–3.66)
HPV33	10	Omitted	2.21 (.64–7.68)	9	Omitted	1.77 (.47–6.61)
HPV35	16	1.83 (.58–5.71)	**3.64 (1.32–10.08)**	10	2.35 (.60–9.15)	3.26 (.92–11.60)
HPV45	12	0.35 (.04–2.66)	0.74 (.20–2.75)	7	0.63 (.07–5.23)	0.88 (.17–4.56)
HPV52	35	1.23 (.47–3.21)	**3.02 (1.53–5.94)**	23	0.69 (.16–2.95)	**4.27 (1.80–10.1)**
HPV58	12	1.14 (.15–8.96)	3.05 (.97–9.70)	6	Omitted	**10.57 (1.23–90.73)**

Analyses include women who had a cervical intraepithelial neoplasia diagnosis of less than grade 2 and were type-specific HPV negative at baseline (range, 1970–2200 women, depending on genotype).

Abbreviations: CI, confidence interval; OR, odds ratio.

Table 5. Pooled Summary Odds Estimates for Type-Specific Protection Associated With Baseline L1 Seropositivity for All 8 Human Papillomavirus (HPV) Types Examined

Outcome Definition	OR[a] (95% CI)
All 8 genotypes	
HPV DNA positive at any 2 follow-up visits	0.97 (.80–1.17)
HPV DNA positive at 2 sequential follow-up visits	0.77 (.54–1.09)
HPV16 and HPV18 combined	
HPV DNA positive at any 2 follow-up visits	0.78 (.56–1.08)
HPV DNA positive at 2 sequential follow-up visits	**0.38 (.18–.78)**

Data are adjusted for report of a new sex partner during the trial period.
Abbreviations: CI, confidence interval; OR, odds ratio.
[a] For L1 seropositivity.

This article presents an analysis of short-term protection against reinfection associated with naturally occurring HPV seropositivity for 8 oncogenic HPV genotypes, as measured by a multiplex serology assay, among women referred for equivocal or mildly abnormal cytology findings.

The majority of newly detected HPV types observed during the 2-year follow-up period for the 8 genotypes analyzed in our study were only transiently detectable (Supplementary Table 2). Previous studies indicate that the majority of prevalently and incidently detected HPV infections in young women will clear within 24–36 months, although infections that persist are associated with a high risk of progression to precancer [22, 23]. In our study, the majority of detected infections in the first year did not persist beyond 12 months.

We observed a protective effect against incident DNA detection at ≥2 follow-up visits associated with baseline seropositivity for HPV16 and a suggested but non–statistically significant protective effect for seropositivity to HPV18 and HPV31 (Table 2). We found that women who reported a new sex partner during the trial period showed a protective association between HPV16 seropositivity and detection of HPV16 at any follow-up visit (Table 3). However, among women who reported no new sex partners in the follow-up period, the association was null. This suggests that the effect we observed is due to protection against reaquisition of HPV, as women who are at lower risk of reexposure to HPV16 do not show an association between seropositivity and HPV16 detection.

Protection against incident type-specific infection among individuals seropositive for HPV16 has previously been observed over a 7-year follow-up period, using serologic measurements from both a VLP ELISA assay and a competitive Luminex immunosorbent assay (cLIA). [24] The point estimates for protection associated with naturally occurring antibodies from that study (HPV16 cLIA: OR, 0.44 [95% CI, .21–.93]; HPV16 VLP ELISA: OR, 0.56 [95% CI, .33–.93]) were similar to our estimates of protection associated with HPV16 L1 seropositivity, as was their summary estimate of protection for HPV16 and HPV18. Other studies of prevalent seropositivity in young women observed similar protective effects for HPV16 and HPV18 [25–27], but some population-based studies with wider age ranges have not observed natural immunity to HPV16 [28]. Results from the mid-adult Gardasil HPV vaccine trial suggest that seropositivity was protective against vaccine HPV types in women aged 24–34 years but showed no protective effect among women aged 35–45 years [29]. The women in our study covered a wide age range, but there was a majority of younger women, with approximately 50% of participants between the ages of 18 and 25 years and 35% aged >30 years.

Our results indicate that there may be partial protection afforded against establishment of the virus in the cervical epithelium from naturally occurring seropositivity for HPV16 and, potentially, for other HPV genotypes, including HPV18 and HPV31. However, we did not observe a protective effect for the other HPV genotypes included in the assay. Differences between genotypes may stem from misclassification by the assay and/or the outcome definition of HPV acquisition. The

Table 6. Risk of Infection With Closely Related Human Papillomavirus (HPV) Types During Follow-up Among Women Seropositive Versus Those Seronegative for HPV16 or HPV18 and DNA Negative for the Related Genotype at Baseline

	DNA-Positive Test Result at Any Follow-up Visit	DNA-Positive Test Result at Any 2 Follow-up Visits	DNA-Positive Test Result at Any 2 Sequential Follow-up Visits
Variable	OR (95% CI)	OR (95% CI)	OR (95% CI)
HPV16 seropositive (vs seronegative)			
HPV31	0.66 (.38–1.15)	0.59 (.22–1.54)	0.62 (.23–1.61)
HPV33	0.72 (.20–2.53)	0.72 (.20–2.53)	0.52 (.11–2.32)
HPV52	1.12 (.78–1.62)	0.95 (.54–1.68)	1.03 (.57–1.85)
HPV58	1.20 (.72–1.96)	0.70 (.26–1.84)	0.77 (.29–2.04)
HPV18 seropositive (vs seronegative)			
HPV45	1.19 (.74–1.92)	0.99 (.40–2.45)	1.06 (.39–2.87)

Abbreviations: CI, confidence interval; OR, odds ratio.

serologic assay we used yielded weaker associations between seropositivity and prevalent HPV DNA detection than has been previously observed using other assays [30], which suggests potential misclassification of seropositivity for some of the included HPV genotypes. It is also possible that the protection offered by naturally occurring antibodies may be different across HPV genotypes. In addition, a fraction of the apparently incident outcomes may not reflect recent acquisitions, but rather reactivations of latent infection. Because reactivations will likely occur more frequently among women seropositive for that genotype (since reactivation would be conditioned on prior infection), bias may have been introduced by the inability to differentiate new from recurrent or reactivated infections [31–33].

Recurrent or reactivated infection is likely not uncommon, as a recent study of reappearance of cervical HPV infections after a period of nondetection in young women suggests that approximately 8% of infections with HPV16 that became undetectable would reappear within 3 years [34]. Because we lack data on the history of HPV detection in women before study entry, even infections that appeared to be first detected during our observational study may have been recurrences or reactivations. Transient reactivation of latent HPV infections may explain why we only observed a protective effect for infections that were detectable at multiple visits. Naturally occurring HPV antibodies would likely not provide protection against an infection that had already been established in the cervical epithelium and that was reactivated intermittently.

No association was observed for baseline seropositivity to an HPV genotype and detection of incident CIN2+ during follow-up. While some other studies with longer follow-up periods have detected a protective effect for natural immunity against incident precancer [24], it is not surprising that no effect was observed within 2 years, as the development of discernible cervical precancer following an incident infection likely takes much longer [35]. Colposcopy-directed biopsy has been shown to miss CIN2+ lesions on the cervix, resulting in substantial disease misclassification [36, 37]. Most CIN2+ cases in ALTS were diagnosed at baseline, and many of the seemingly incident precancers detected over the course of our study were most likely a result of preexisting infections or early lesions that were missed at the enrollment study visit [38].

Limitations of our study included our single-time-point measurement of seropositivity. There are also uncertainties when comparing results of different serologic assays: VLP ELISAs measure total antibody concentration and do not discriminate between neutralizing and nonneutralizing antibody, while pseudovirion-based neutralization assays and competitive Luminex assays detect neutralizing antibodies only [24, 39]. GST-L1 fusion proteins, used in our study, have shown reactivity with a large number of monoclonal antibodies that were raised against HPV VLPs when used in ELISAs, indicating that these fusion proteins display nearly all of the linear, conformational, and neutralizing epitopes that have been previously defined for VLPs [40]. HPV16 L1 seropositivity, as measured by the GST-L1 fusion protein multiplex assay, appears to provide partial protection against incident HPV16 detection, as seen in other studies conducted with HPV VLP ELISAs and cLIAs [24, 25], but this multiplex serology assay and the VLP ELISAs measure total antibody concentration, which may not be as relevant to protection against HPV infection as an assay that measures only neutralizing epitopes.

In conclusion, in an analysis of 2302 women referred for mildly abnormal cervical cytology findings who were free of precancer at study baseline, we observed a protective effect for HPV16 L1 seropositivity, as measured by the multiplex serology assay, and HPV16 DNA detection among women who were negative for HPV16 DNA at trial baseline and reported a new sex partner over the course of the study. Our point estimate of protection is similar to those estimated with other assays of HPV seropositivity. Larger studies and longer follow-up periods will be necessary to examine protection from natural immunity for all genotypes against disease end points such as incident cervical precancer.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

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