MAJOR ARTICLE

Risk of Newly Detected Infections and Cervical Abnormalities in Women Seropositive for Naturally Acquired Human Papillomavirus Type 16/18 Antibodies: Analysis of the Control Arm of PATRICIA

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(See the editorial commentary by Franceschi and Baussano on pages 507–9.)

Background. We examined risk of newly detected human papillomavirus (HPV) infection and cervical abnormalities in relation to HPV type 16/18 antibody levels at enrollment in PATRICIA (Papilloma Trial Against Cancer in Young Adults; NCT00122681).

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Methods. Using Poisson regression, we compared risk of newly detected infection and cervical abnormalities associated with HPV-16/18 between seronegative vs seropositive women (15–25 years) in the control arm (DNA negative at baseline for the corresponding HPV type [HPV-16: n = 8193; HPV-18: n = 8463]).

Results. High titers of naturally acquired HPV-16 antibodies and/or linear trend for increasing antibody levels were significantly associated with lower risk of incident and persistent infection, atypical squamous cells of undetermined significance or greater (ASCUS+), and cervical intraepithelial neoplasia grades 1/2 or greater (CIN1+, CIN2+). For HPV-18, although seropositivity was associated with lower risk of ASCUS+ and CIN1+, no association between naturally acquired antibodies and infection was demonstrated. Naturally acquired HPV-16 antibody levels of 371 (95% confidence interval [CI], 242–794), 204 (95% CI, 129–480), and 480 (95% CI, 250–5756) EU/mL were associated with 90% reduction of incident infection, 6-month persistent infection, and ASCUS+, respectively.

Conclusions. Naturally acquired antibodies to HPV-16, and to a lesser extent HPV-18, are associated with some reduced risk of subsequent infection and cervical abnormalities associated with the same HPV type.

Keywords. HPV; naturally acquired antibodies; infection; cervical abnormality; risk reduction.

Human papillomavirus (HPV) types 16 and 18 cause approximately 70% of invasive cervical cancer worldwide [1]. Two prophylactic vaccines against HPV infection are available and have been shown to prevent persistent HPV infection and precancerous cervical abnormalities associated with HPV-16/18 [2–6].

Approximately 50%–70% of women develop serum antibodies after naturally acquired infection with HPV-16 or HPV-18 [7–13]. Naturally acquired antibodies can remain detectable for at least 4–5 years, albeit at much lower levels than those induced by vaccination [14]. Whereas some studies have not shown an immune protection role for naturally acquired antibodies [15–18], others have shown that they may provide protection against future infection [19–21]. Underpowering of studies and differences in methodology may explain these discrepancies. In addition, the levels of naturally acquired antibodies that may provide protection have not yet been established.

Vaccine efficacy data from the Papilloma Trial Against Cancer in Young Adults (PATRICIA) of the HPV-16/18 AS04-adjuvanted vaccine (Cervarix®) have been reported previously [3, 22–24]. The intensive follow-up of the control arm of large vaccine trials provides an opportunity to evaluate the natural history of HPV infection, including the impact of naturally induced antibodies on infection and cervical abnormalities. The present paper describes an analysis of the risk of newly detected HPV infection and development of cervical abnormalities according to the baseline level of naturally acquired antibodies to HPV-16 or HPV-18 in women from the control group of PATRICIA over a 4-year follow-up.

METHODS

This analysis was based on data obtained from the control arm of the multinational (14 countries), double-blind, randomized , controlled PATRICIA trial. The objective was to investigate whether naturally acquired antibodies to HPV-16 or HPV-18 reduced the risk of newly detected infection and/or associated cervical abnormalities with the same HPV type.

Study Population and Procedures

The clinical trial methodology has been described in detail [3, 22]. Women aged 15–25 years with no more than 6 lifetime sexual partners were enrolled and randomized to the HPV-16/18 AS04-adjuvanted vaccine or control hepatitis A vaccine (both supplied by GlaxoSmithKline Vaccines, Rixensart, Belgium). Data from the control arm are reported here. Cervical liquid-based cytology samples were collected at baseline and every 6 months and used to perform HPV DNA typing every 6 months and for cytopathological examination (Bethesda system) every 12 months. A prespecified algorithm for abnormal cytology and colposcopy referral was used [23]. Cervical samples and biopsy material were tested for HPV DNA as previously described [25]. Serum antibodies to HPV-16 and HPV-18 were determined at baseline by enzyme-linked immunosorbent assay (ELISA) targeting L1-based virus-like particles (VLPs) [26]. Seropositivity was defined as an antibody level greater than or equal to the assay threshold: 8 ELISA units (EU)/mL for HPV-16 and 7 EU/mL for HPV-18 [26].

Endpoints were incident infection (which may include newly acquired infections and recurrent infections present below detection levels at baseline), 6- and 12-month persistent infection, atypical squamous cell of undetermined significance or greater (ASCUS+), cervical intraepithelial neoplasia (CIN) grade 1 or greater (CIN1+), and CIN grade 2 or greater (CIN2+) associated with HPV-16 or HPV-18. All CIN cases were reviewed by an independent endpoint committee [22]. Women completed a behavioral questionnaire, which asked about experience with sexual intercourse (age at first sexual intercourse and number of partners over the past 12 months) and lifetime tobacco exposure at the second study visit, 1 month after the first vaccination, and yearly thereafter. The term sexual intercourse included penetrative, genital-to-genital, or oral–genital sexual contact.

Written informed consent or assent was obtained and the protocol and other materials were approved by local independent ethics committees or institutional review boards. The trial was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and

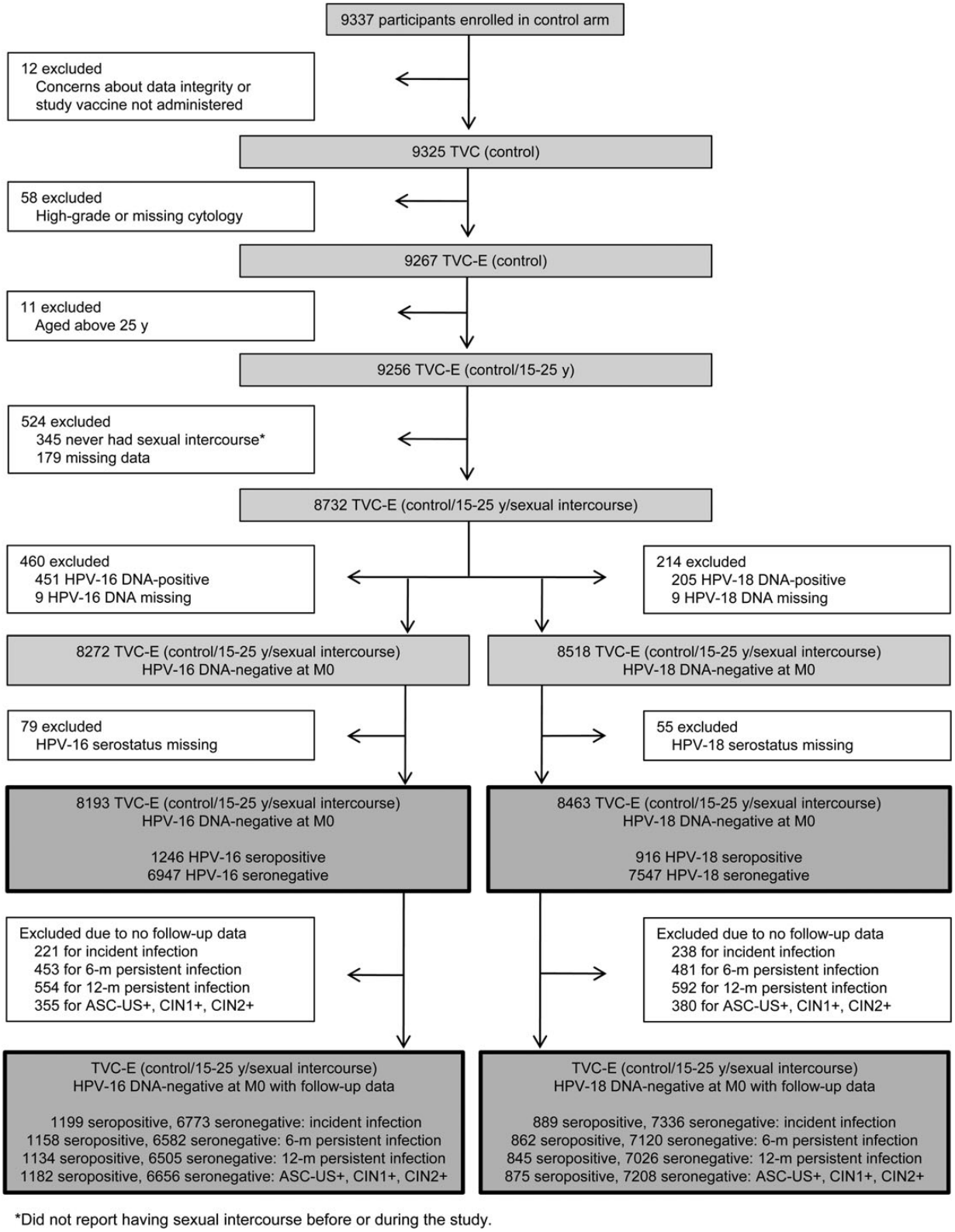


Figure 1. Participant disposition. Abbreviations: ASCUS, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; M0, Month 0; TVC-E, total vaccinated cohort for efficacy.

is registered at ClinicalTrials.gov under registration number NCT00122681.

Statistical Analysis

Analysis Cohort

The analysis was performed in the control group of the total vaccinated cohort for efficacy, including women who received at least 1 dose of control vaccine and had normal or low-grade cytology at baseline. The analysis included only women who had potentially been exposed to HPV infection via sexual intercourse and those without current HPV infections at baseline (Figure 1). Thus, only newly detected infections (new infections or possibly recurrent infections present below detectable levels at baseline) were accounted for in the analysis.

Exposure Variables

The main exposure variables were HPV-16 and HPV-18 serostatus at baseline. Serostatus was analyzed as (1) a binary variable (seropositive or seronegative) according to ELISA assay threshold, and (2) according to quartiles in seropositive women (8–12, >12–21, >21–59, and >59–2805 EU/mL for HPV-16; 7–10, >10–17, >17–43, and >43–1086 EU/mL for HPV-18). We report both univariate and multivariable analyses. Based on established risk factors known to influence the risk of HPV infection, and a previous analysis of risk factors for HPV infection and cervical abnormalities in the PATRICIA study [27], the following covariates were taken into account in the multivariable analyses: marital status, tobacco exposure (number of pack-years), age at first sexual intercourse, number of sexual partners, history of sexually transmitted infection (STI), at least 1 previous pregnancy, and region. Other covariates known to influence the risk of HPV infection were not included in the multivariable analyses because of strong correlations with the above covariates: condom use was correlated with STI, age at first sexual intercourse was correlated with age at baseline, and at least 1 previous pregnancy was correlated with at least 1 delivery. The hazard ratio estimates were found to be very similar using both approaches, and therefore discussion of the results focuses on the multivariable analyses.

General Statistical Considerations and Multivariable Regression Analyses

All analyses were performed using SAS version 9.2. The incidence rate was calculated as the number of incident events divided by the total person-time. Person-years were calculated as the sum of the follow-up for each participant expressed in years. The follow-up period started on the day after first vaccination and ended on the first occurrence of the endpoint or the last visit (whichever occurred first).

The relationship between the exposure variables and the risk of newly detected infection or cervical abnormalities was assessed using Poisson regression analyses. First, the effect of the exposure variables on the endpoints was evaluated based on the relative incidence rates (rate ratios) and their 95% confidence intervals (CIs) obtained by Poisson regression. The final multivariable analysis allowed estimation of the relative role of initial HPV-16 or HPV-18 serostatus while adjusting for the simultaneous effects of the 7 covariates selected as potential confounders. Only subjects with no missing data were included in the multivariable analyses; however, the analyses were also performed taking into account missing data as a specific category for each covariate. The results were very similar (data not shown).

Second, the relationship between the risk of newly detected infection or cervical abnormalities and the baseline antibody level was analyzed using Poisson regression including antibody titer as a continuous variable. Seronegative women were assigned a value of half the ELISA assay cutoff level. Predicted antibody titers corresponding to a 50%, 70%, and 90% risk reduction were derived. Age at first sexual intercourse and smoking were included as covariates in a sensitivity analysis. Because of the large proportion (85%) of seronegative subjects at baseline, sensitivity analyses including a subset of 100 randomly selected seronegative subjects were also carried out.

RESULTS

Participant Characteristics at Study Entry

A total of 8193 (1246 HPV-16 seropositive [15.2%] and 6947 HPV-16 seronegative [84.8%]) and 8463 (916 HPV-18 seropositive [10.8%] and 7547 HPV-18 seronegative [89.2%]) women were included in the analysis of HPV-16– and HPV-18–related endpoints, respectively (Figure 1; Table 1).

Risk of Newly Detected HPV-16/18 Infection and Associated Cervical Abnormalities According to Levels of Naturally Acquired Antibodies

The multivariable analysis showed that the presence of naturally acquired antibodies to HPV-16 at baseline was associated with a lower risk of newly detected incident infection, 6- and 12-month persistent infection, and ASCUS+ associated with HPV-16 (Table 2; Figure 2*A*–*C*). The risk was gradually reduced as antibody levels rose. Although HPV-16 serostatus (positive vs negative) did not show a significant association with CIN1+, participants with an antibody level in the highest quartile at baseline did have a significantly reduced risk of developing CIN1+ compared with seronegative subjects, and the linear trend by quartile was statistically significant ($P = .0006$; Table 2). The linear trend by antibody quartile was also significant for CIN2+ ($P = .018$), although none of the individual quartile groups showed a significant reduction in risk (Table 2).

Seropositivity to HPV-18 at baseline was not significantly associated with a lower risk of newly detected incident infection or persistent infection (Table 3; Figure 3*A* and 3*B*). However,

Table 1. Frequency Distribution of Exposure Variables, Age Group, and Country According to Initial Serostatus in Women Who Were DNA Negative for the Corresponding Human Papillomavirus Type at Baseline

		HPV-16 Initial Serostatus, No. (%)		HPV-18 Initial Serostatus, No. (%)	
Exposure Variable[a]	Categories	Seronegative (n = 6947)	Seropositive (n = 1246)	Seronegative (n = 7547)	Seropositive (n = 916)
Initial antibody titer (seropositive only)	Geometric mean titer (95% CI)		28.9 (27.2–30·7)		23.0 (21.5–24.6)
	Median (range)		21.0 (8–2805)		17.0 (7–1086)
	Quartile (Q1-Q3)		12.0–59.0		10.0–43.0
Age at baseline	15–17 y	2349 (33.8)	259 (20.8)	2516 (33.3)	185 (20.2)
	18–25 y	4598 (66.2)	987 (79.2)	5031 (66.7)	731 (79.8)
Country	Finland	1925 (27.7)	203 (16.3)	2060 (27.3)	135 (14.7)
	Philippines	956 (13.8)	156 (12.5)	1004 (13.3)	119 (13.0)
	United States	832 (12.0)	233 (18.7)	979 (13.0)	141 (15.4)
	Thailand	726 (10.5)	117 (9.4)	725 (9.6)	121 (13.2)
	Brazil	601 (8.7)	206 (16.5)	675 (8.9)	167 (18.2)
	Taiwan	557 (8.0)	70 (5.6)	586 (7.8)	50 (5.5)
	Mexico	354 (5.1)	81 (6.5)	393 (5.2)	53 (5.8)
	Germany	286 (4.1)	47 (3.8)	324 (4.3)	37 (4.0)
	Australia	201 (2.9)	33 (2.7)	229 (3.0)	21 (2.3)
	Canada	168 (2.4)	44 (3.5)	196 (2.6)	38 (4.2)
	Spain	165 (2.4)	15 (1.2)	180 (2.4)	8 (0.9)
	United Kingdom	90 (1.3)	30 (2.4)	105 (1.4)	22 (2.4)
	Belgium	71 (1.0)	9 (0.7)	74 (1.0)	4 (0.4)
	Italy	15 (0.2)	2 (0.2)	17 (0.2)	0
Marital status	Living or lived with partner	2166 (31.2)	461 (37.0)	2315 (30.7)	358 (39.1)
	Single[b]	4677 (67.3)	761 (61.1)	5118 (67.8)	539 (58.8)
	Missing	104 (1.5)	24 (1.9)	114 (1.5)	19 (2.1)
Tobacco exposure, No. of pack-years	None or <6 mo (<0.5)	4942 (71.1)	785 (63.0)	5243 (69.5)	597 (65.2)
	At least 6 mo (≥0.5)	1937 (27.9)	447 (35.9)	2231 (29.6)	308 (33.6)
	Missing	68 (1.0)	14 (1.1)	73 (1.0)	11 (1.2)
Age at first sexual intercourse	≥18 y	2844 (40.9)	434 (34.8)	3033 (40.2)	314 (34.3)
	15–17 y	3321 (47.8)	615 (49.4)	3589 (47.6)	479 (52.3)
	<15 y	769 (11.1)	192 (15.4)	909 (12.0)	122 (13.3)
	Missing	13 (0.2)	5 (0.4)	16 (0.2)	1 (0.1)
No. of sexual partners prior to the past 12 mo[c]	0	1664 (24.0)	132 (10.6)	1700 (22.5)	117 (12.8)
	1	3396 (48.9)	542 (43.5)	3608 (47.8)	407 (44.4)
	2–3	1347 (19.4)	358 (28.7)	1572 (20.8)	248 (27.1)
	≥4	499 (7.2)	204 (16.4)	619 (8.2)	139 (15.2)
	Missing	41 (0.6)	10 (0.8)	48 (0.6)	5 (0.6)
No. of sexual partners during the past 12 mo[c]	0	1067 (15.4)	88 (7.1)	1095 (14.5)	67 (7.3)
	1	4605 (66.3)	888 (71.3)	4971 (65.9)	656 (71.6)
	2–3	1081 (15.6)	216 (17.3)	1243 (16.5)	161 (17.6)
	≥4	171 (2.5)	47 (3.8)	212 (2.8)	27 (3.0)
	Missing	23 (0.3)	7 (0.6)	26 (0.3)	5 (0.6)
Condom use prior to the past 12 mo[c]	No partner	1694 (24.4)	140 (11.2)	1737 (23.0)	119 (13.0)
	Yes	1682 (24.2)	366 (29.4)	1839 (24.4)	264 (28.8)
	No	3448 (49.6)	719 (57.7)	3846 (51.0)	511 (55.8)
	Missing	123 (1.8)	21 (1.7)	125 (1.7)	22 (2.4)
Condom use during the past 12 mo[c]	No partner	1049 (15.1)	87 (7.0)	1076 (14.3)	67 (7.3)
	Yes	2151 (31.0)	451 (36.2)	2357 (31.2)	336 (36.7)
	No	3642 (52.4)	690 (55.4)	4003 (53.0)	498 (54.4)
	Missing	105 (1.5)	18 (1.4)	111 (1.5)	15 (1.6)

Table 1 continued.

Exposure Variable[a]	Categories	HPV-16 Initial Serostatus, No. (%)		HPV-18 Initial Serostatus, No. (%)	
		Seronegative (n = 6947)	Seropositive (n = 1246)	Seronegative (n = 7547)	Seropositive (n = 916)
At least 1 previous pregnancy	Yes	2139 (30.8)	515 (41.3)	2312 (30.6)	391 (42.7)
	No	4778 (68.8)	724 (58.1)	5202 (68.9)	520 (56.8)
	Missing	30 (0.4)	7 (0.6)	33 (0.4)	5 (0.6)
At least 1 delivery	Yes	1481 (21.3)	332 (26.7)	1576 (20.9)	267 (29.2)
	No	5430 (78.2)	907 (72.8)	5933 (78.6)	643 (70.2)
	Missing	36 (0.5)	7 (0.6)	38 (0.5)	6 (0.7)
STI history	No	6633 (95.5)	1099 (88.2)	7156 (94.8)	814 (88.9)
	Yes–*Chlamydia trachomatis*	93 (1.3)	61 (4.9)	123 (1.6)	41 (4.5)
	Yes–other	210 (3.0)	84 (6.7)	257 (3.4)	58 (6.3)
	Missing	11 (0.2)	2 (0.2)	11 (0.2)	3 (0.3)
Contraceptive use[d]	No contraception	2526 (36.4)	314 (25.2)	2660 (35.3)	229 (25.0)
	Hormonal	4188 (60.3)	874 (70.1)	4639 (61.5)	638 (69.7)
	Intrauterine device	327 (4.7)	92 (7.4)	367 (4.9)	65 (7.1)
	Sterilized	63 (0.9)	20 (1.6)	67 (0.9)	15 (1.6)

Information on tobacco exposure, age at first sexual intercourse, number of sexual partners, pregnancy, and condom use was obtained from the behavioral questionnaire at baseline. Data from the behavioral questionnaires administered yearly during the follow-up period were used to estimate age at first sexual intercourse when sexual activity began during follow-up and to calculate the number of pregnancies during follow-up. Information on age at baseline (grouped as 15–17 and 18–25 years), marital status, country, contraceptive use (hormonal, intrauterine device, and sterilization), and STIs was obtained from case record forms. Countries were grouped by geographic region (Europe, Asia Pacific, Latin America, and North America).

Abbreviations: CI, confidence interval; HPV, human papillomavirus; STI, sexually transmitted infection.

[a] Only the following were considered as potential confounders in the multivariable analyses (as shown in Tables 2 and 3): marital status, tobacco exposure (number of pack-years), age at first sexual intercourse, number of sexual partners, STI history, at least 1 previous pregnancy, and region. Data on other variables are descriptive only; these variables were not included in the multivariable analysis because of strong correlations with other variables: condom use was correlated with STI; age at first sexual intercourse with age at baseline; at least 1 previous pregnancy with at least 1 delivery. Therefore, only STI, age at first sexual intercourse, and at least 1 previous pregnancy were kept in the multivariable analysis.

[b] Not married, widowed, divorced, separated, living with or had lived with partner.

[c] For analysis of HPV infection, the number of sexual partners during the past 12 months was considered; for analysis of atypical squamous cells of undetermined significance or greater, cervical intraepithelial neoplasia grade 1/2 or greater, the lifetime number of sexual partners prior to the past 12 months was considered.

[d] Women could be using >1 method of contraception.

baseline HPV-18 serostatus (positive vs negative) showed a significant association with ASCUS+ and CIN1+ (Table 3; Figure 3*C*). There was no apparent effect of naturally acquired antibodies according to quartile (Table 3).

Several covariates were shown to be significantly associated with new HPV-16 and HPV-18 incident infections in the multivariable analysis (Supplementary Tables 1 and 2).

Figure 4*A* and 4*B* show the relationship between HPV-16 antibody level and 6-month persistent infection or ASCUS+ associated with HPV-16. The frequency of 6-month persistent HPV-16 infection declined as antibodies rose. An HPV-16 antibody level of 204 (95% CI, 129–480) EU/mL yielded a 90% reduction in 6-month persistent infection; values for a 70% and 50% reduction were 107 (95% CI, 68–251) and 61 (95% CI, 39–144) EU/mL, respectively (Figure 4*A*). A corresponding analysis for ASCUS+ showed that an HPV-16 antibody level of 480 (95% CI, 250–5756), 251 (95% CI, 131–3010) and 144 (95% CI, 75–1733) EU/mL yielded 90%, 70%, and 50% reductions, respectively (Figure 4*B*). With regard to incident HPV infection, an HPV-16 antibody level of 371 (95% CI, 242–794) EU/mL yielded a 90% reduction in incident infection; values for a 70% and 50% reduction were 194 (95% CI, 118–415) and 112 (95% CI, 73–415) EU/mL, respectively. Sensitivity analyses produced similar results.

DISCUSSION

This study focused on the role of naturally acquired antibodies in prevention of newly detected HPV infection and associated cervical abnormalities in sexually active women who had no severe cervical abnormalities and no evidence of an active HPV infection (ie, were DNA negative for the type under consideration) at study entry. The results show that women with higher levels of naturally acquired antibodies to HPV-16 detected using an L1-based VLP assay had a lower risk of a subsequent newly detected infection or cervical abnormality associated with HPV-16. There was a similar effect for HPV-18, although the association was weaker and the relationship between the antibody

Table 2. Risk of Newly Detected Human Papillomavirus Type 16 (HPV-16) Infections or Cervical Abnormalities Associated With HPV-16 According to Initial Serostatus (Univariate and Multivariable Regression Analyses) in Women Who Were HPV-16 DNA Negative at Baseline

Endpoint	Serostatus at Baseline	No. Cases With New Endpoint	No. Cases Without New Endpoint	PY	Incidence per 100 PY	Univariate Analysis Rate Ratio (95% CI)	Final Multivariable Analysis Adjusted on Confounders Retained	
							Rate Ratio (95% CI)	*P* Value
New HPV-16 incident infection (cases = 1185, noncases = 6787)[a]	Seronegative	1059	5714	23 299	4.55	1.00	1.00	
	Seropositive	126	1073	4114	3.06	0.67 (.56–.81)	0.64 (.53–.78)	<.0001
	Seropositive, quartiles, EU/mL							
	8–12	43	289	1125	3.82	0.84 (.62–1.14)	0.89 (.65–1.21)	.45
	>12–21	36	235	923	3.90	0.86 (.62–1.20)	0.80 (.57–1.11)	.18
	>21–59	29	271	1016	2.85	0.63 (.43–.91)	0.58 (.40–.85)	.0057
	>59–2805	18	278	1050	1.71	0.38 (.24–.60)	0.34 (.21–.54)	<.0001
							P value for linear trend	< .0001
New HPV-16 six-mo persistent infection (cases = 626, noncases = 7114)[a]	Seronegative	560	6022	23 787	2.35	1.00	1.00	
	Seropositive	66	1092	4195	1.57	0.67 (.52–.86)	0.67 (.52–.87)	.0025
	Seropositive, quartiles, EU/mL							
	8–12	27	291	1146	2.36	1.00 (.68–1.47)	1.09 (.74–1.61)	.67
	>12–21	17	247	947	1.79	0.76 (.47–1.23)	0.71 (.44–1.16)	.17
	>21–59	13	273	1037	1.25	0.53 (.31–.92)	0.55 (.31–.95)	.033
	>59–2805	9	281	1065	0.85	0.36 (.19–.69)	0.34 (.17–.65)	.0013
							P value for linear trend	< .0001
New HPV-16 twelve-mo persistent infection (cases = 405, noncases = 7234)[a]	Seronegative	362	6143	24 113	1.50	1.00	1.00	
	Seropositive	43	1091	4213	1.02	0.68 (.50–.93)	0.68 (.49–.94)	.019
	Seropositive, quartiles, EU/mL							
	8–12	21	293	1152	1.82	1.21 (.78–1.89)	1.31 (.84–2.04)	.23
	>12–21	10	248	955	1.05	0.70 (.37–1.31)	0.65 (.35–1.23)	.19
	>21–59	9	269	1034	0.87	0.58 (.30–1.12)	0.59 (.30–1.15)	.12
	>59–2805	3	281	1073	0.28	0.19 (.06–.58)	0.17 (.06–.55)	.0027
							P value for linear trend	< .0001
New ASCUS+ associated with HPV-16 (cases = 536, noncases = 7302)[a]	Seronegative	484	6172	24 347	1.99	1.00	1.00	
	Seropositive	52	1130	4251	1.22	0.62 (.46–.82)	0.60 (.45–.80)	.0006
	Seropositive, quartiles, EU/mL							
	8–12	20	309	1176	1.70	0.86 (.55–1.34)	0.91 (.58–1.42)	.67
	>12–21	13	253	959	1.36	0.68 (.39–1.18)	0.66 (.38–1.14)	.14
	>21–59	11	281	1049	1.05	0.53 (.29–.96)	0.51 (.28–.93)	.028
	>59–2805	8	287	1067	0.75	0.38 (.19–.76)	0.33 (.16–.67)	.0022
							P value for linear trend	< .0001

Table 2 continued.

Endpoint	Serostatus at Baseline	No. Cases With New Endpoint	No. Cases Without New Endpoint	PY	Incidence per 100 PY	Univariate Analysis Rate Ratio (95% CI)	Final Multivariable Analysis Adjusted on Confounders Retained	
							Rate Ratio (95% CI)	*P* Value
New CIN1+ associated with HPV-16 (cases = 177, noncases = 7661)[a]	Seronegative	157	6499	24 939	0.63	1.00	1.00	
	Seropositive	20	1162	4326	0.46	0.73 (.46–1.17)	0.70 (.44–1.14)	.15
	Seropositive, quartiles, EU/mL							
	8–12	10	319	1204	0.83	1.32 (.70–2.50)	1.38 (.72–2.64)	.33
	>12–21	5	261	973	0.51	0.82 (.34–1.99)	0.78 (.32–1.90)	.58
	>21–59	4	288	1063	0.38	0.60 (.22–1.61)	0.56 (.21–1.52)	.26
	>59–2805	1	294	1086	0.09	0.15 (.02–1.05)	0.13 (.02–.90)	.039
							P value for linear trend = .0006	
New CIN2+ associated with HPV-16 (cases = 121, noncases = 7717)[a]	Seronegative	109	6547	24 984	0.44	1.00	1.00	
	Seropositive	12	1170	4339	0.28	0.63 (.35–1.15)	0.62 (.34–1.15)	.13
	Seropositive, quartiles, EU/mL							
	8–12	6	323	1208	0.50	1.14 (.50–2.59)	1.21 (.53–2.76)	.66
	>12–21	2	264	980	0.20	0.47 (.12–1.89)	0.45 (.11–1.83)	.26
	>21–59	3	289	1065	0.28	0.65 (.21–2.03)	0.63 (.20–1.99)	.43
	>59–2805	1	294	1086	0.09	0.21 (.03–1.51)	0.19 (.03–1.38)	.10
							P value for linear trend = .018	

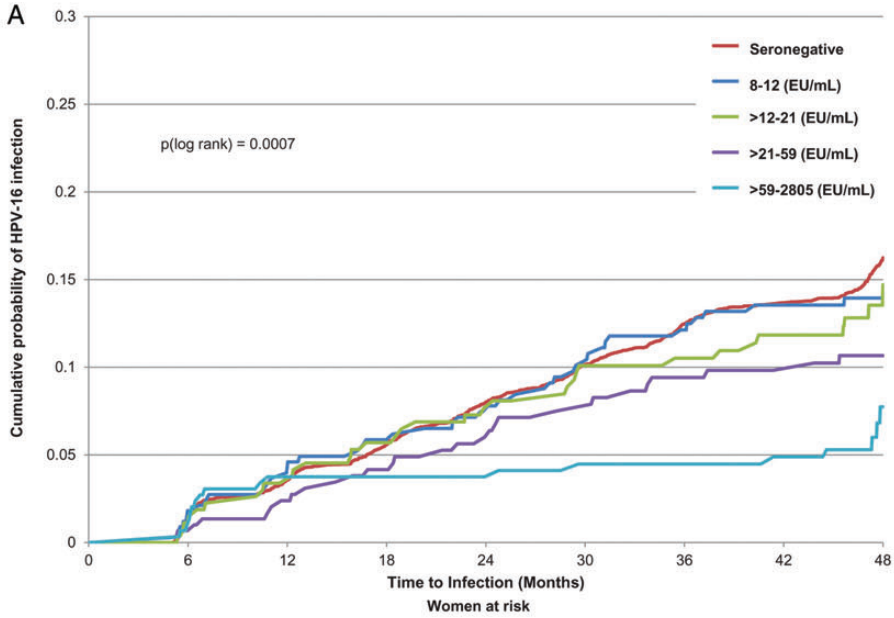
Incident infection was defined as a new detection of the HPV type at any time during the follow-up period; 6- and 12-month persistent infection were defined as detection of the same HPV type in 2 consecutive samples over a minimum of 150 days and 300 days, respectively; ASCUS+ was defined as ASCUS, low-grade squamous intraepithelial lesion (LSIL), atypical squamous cells/high-grade ASCUS, does not exclude high-grade squamous intraepithelial lesion (HSIL), atypical glandular cells of undetermined significance (AGUS), or HSIL. CIN1+ was defined as CIN1, CIN2, CIN3, adenocarcinoma in situ, or invasive cervical cancer; CIN2+ excluded CIN1.

Confounders (exposure variables) retained for the multivariable analysis were marital status, tobacco exposure (number of pack-years), age at first sexual intercourse, number of sexual partners prior to the past 12 months (ASCUS+, CIN1+, CIN2+), number of sexual partners during the past 12 months (incident and persistent infections), at least 1 previous pregnancy, sexually transmitted infection history, and region.

Linear trend was evaluated across 5 classes: (1) seronegative; (2) first quartile; (3) second quartile; (4) third quartile; (5) fourth quartile.

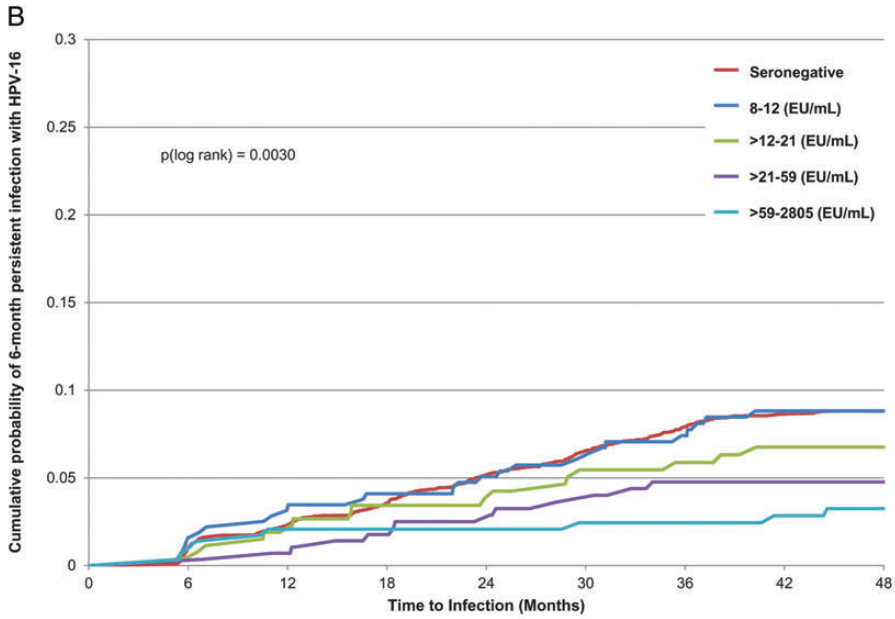
Abbreviations: ASCUS, atypical squamous cell of undetermined significance; CI, confidence interval; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; PY, patient-years.

[a] No. of cases and noncases and patient-years reported for the univariate analysis.



Women at risk

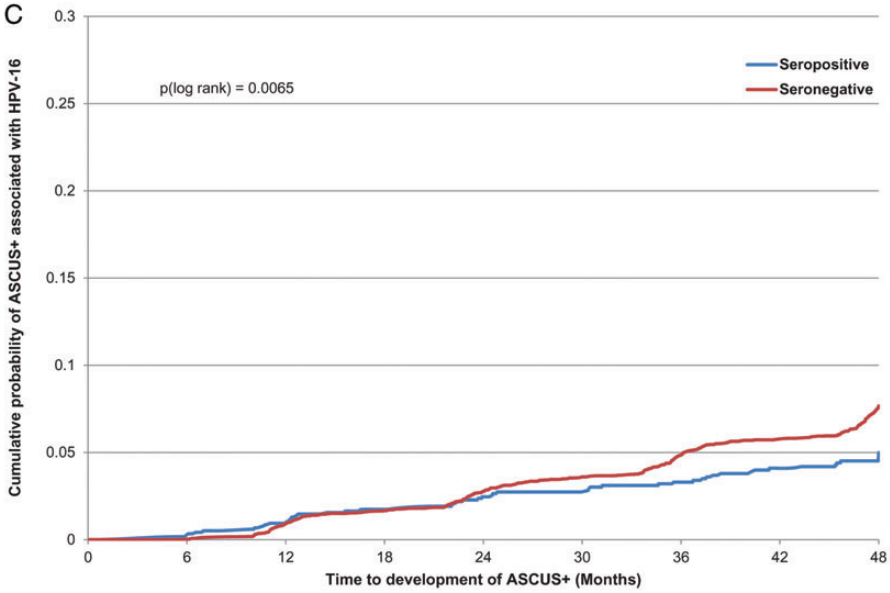
Seropositive, quartiles (EU/mL)	Baseline	M6	M12	M18	M24	M30	M36	M42	M48
8-12	332	322	309	294	284	269	252	239	108
>12-21	271	263	253	242	231	221	208	198	74
>21-59	300	292	278	263	252	245	227	218	97
>59-2805	296	292	278	272	264	259	247	234	97
Seronegative	6773	6636	6362	6130	5877	5640	5241	4972	2153



Women at risk

Seropositive, quartiles (EU/mL)	Baseline	M6	M12	M18	M24	M30	M36	M42	M48
8-12	318	313	308	299	291	281	266	253	115
>12-21	264	263	257	247	239	231	218	209	78
>21-59	286	286	282	269	261	255	239	231	101
>59-2805	290	288	282	277	269	265	253	240	99
Seronegative	6582	6518	6415	6243	6039	5850	5499	5246	2283

Figure 2. Cumulative probability of detecting an incident or 6-month persistent infection or developing atypical squamous cells of undetermined significance or greater (ASCUS+) associated with human papillomavirus type 16 (HPV-16). *A*, Incident HPV-16 infection. *B*, HPV-16 six-month persistent infection. *C*, ASCUS+ associated with HPV-16.



Women at risk

	Baseline	M6	M12	M18	M24	M30	M36	M42	M48
Seropositive	1182	1173	1137	1094	1063	1044	997	954	401
Seronegative	6656	6642	6494	6333	6170	6011	5703	5436	2393

Figure 2 continued.

level against HPV-18 L1-based VLPs and newly detected infection and associated abnormalities was not significant.

There was a clear pattern of reduced risk of a newly detected incident infection, persistent infection, ASCUS+, or CIN1+ associated with HPV-16 as baseline antibody levels rose, and the linear trend by antibody quartile was significant for all endpoints. Only the highest level of natural antibodies reduced new CIN1+ associated with HPV-16, whereas natural antibodies above the median (21 EU/mL) reduced the occurrence of ASCUS+. The evidence was less clear for CIN2+, due to fewer cases, but the linear trend by antibody quartile was also significant.

The present results did not show a reduced risk of infection or disease with higher levels of HPV-18 antibodies. However, the analysis of HPV-18 serostatus (positive vs negative) showed some evidence for a reduced incidence of ASCUS+ and, with a lower number of cases, of CIN1+ among seropositive women, suggesting that naturally acquired HPV-18 antibodies might play some protective role in the prevention of associated abnormalities. The same analyses for HPV-31, HPV-33, and HPV-45, which are closely related to HPV-16 or HPV-18, did not show a significant association (data not shown); these analyses were, however, severely limited by the low number of related cases.

It was interesting to note the very similar results obtained with the univariate and multivariable models. A number of covariates were taken into account in the multivariable analyses, based on established risk factors known to influence the risk of HPV infection, but results were independent of potential confounders accounted for. The similarity of the two approaches reinforces the robustness of the results. As expected, no cases of cervical cancer were seen, due to the young age of women in the study and the intensive follow-up for CIN2 detection.

Several studies have been unable to draw clear conclusions regarding the role of naturally acquired antibodies in preventing subsequent HPV infection [15–19, 28]. However, the findings of the Costa Rica Vaccine Trial indicated that women with antibody titers in the highest tertile for HPV-16 and HPV-18 (≥60 and ≥28 EU/mL, respectively) had a significantly lower risk of an incident cervical infection with the same HPV type [20]. Although the analyses of the Costa Rica Vaccine Trial and our study were conducted in a similar way, used the same ELISA technique, and controlled for similar demographic and behavioral characteristics, there are some differences, including subjects' age (18–25 years in the Costa Rica Vaccine Trial), and a wider geographic distribution and higher number of subjects in our study, which gave our study a greater power to identify significant effects. In addition, for the women included in the control arms, HPV-16 and HPV-18 DNA was detected in 8.6% and 3.1%, respectively, of women in the Costa Rica Vaccine Trial, compared with 5.2% and 2.3% in our study. Similarly, HPV-16 and HPV-18 seroprevalence was lower in our study (15.2% and 10.8%, respectively) than in the Costa Rica Vaccine

Table 3. Risk of Newly Detected Human Papillomavirus Type 18 (HPV-18) Infections or Cervical Abnormalities Associated With HPV-18 According to Initial Serostatus (Univariate and Multivariable Regression Analyses) in Women Who Were HPV-18 DNA Negative at Baseline

Endpoint	Serostatus at Baseline	No. Cases With New Endpoint	No. Cases Without New Endpoint	PY	Incidence Per 100 PY	Univariate Analysis Rate Ratio (95% CI)	Final Multivariable Analysis Adjusted on Confounders Retained	
							Rate Ratio (95% CI)	*P* Value
New HPV-18 incident infection (cases = 837, noncases = 7388)[a]	Seronegative	755	6581	25 917	2.91	1.00	1.00	
	Seropositive	82	807	3121	2.63	0.90 (.72–1.13)	0.94 (.75–1.19)	.61
	Seropositive, quartiles, EU/mL							
	7–10	27	223	859	3.14	1.08 (.74–1.58)	1.24 (.84–1.82)	.28
	>10–17	16	188	739	2.16	0.74 (.45–1.22)	0.75 (.46–1.23)	.26
	>17–43	21	194	761	2.76	0.95 (.61–1.46)	0.96 (.62–1.49)	.86
	>43–1086	18	202	762	2.36	0.81 (.51–1.29)	0.81 (.50–1.30)	.38
						P value for linear trend = .22		
New HPV-18 six-mo persistent infection (cases = 297, noncases = 7685)[a]	Seronegative	272	6848	26 426	1.03	1.00	1.00	
	Seropositive	25	837	3202	0.78	0.76 (.50–1.14)	0.86 (.56–1.30)	.46
	Seropositive, quartiles, EU/mL							
	7–10	8	235	887	0.90	0.88 (.43–1.77)	1.09 (.54–2.21)	.80
	>10–17	7	192	756	0.93	0.90 (.43–1.91)	0.94 (.44–2.01)	.88
	>17–43	6	204	780	0.77	0.75 (.33–1.68)	0.81 (.36–1.83)	.61
	>43–1086	4	206	779	0.51	0.50 (.19–1.34)	0.56 (.21–1.51)	.25
						P value for linear trend = .17		
New HPV-18 twelve-mo persistent infection (cases = 147, noncases = 7724)[a]	Seronegative	137	6889	26 592	0.52	1.00	1.00	
	Seropositive	10	835	3210	0.31	0.60 (.32–1.15)	0.66 (.34–1.26)	.21
	Seropositive, quartiles, EU/mL							
	7–10	2	235	894	0.22	0.43 (.11–1.75)	0.56 (.14–2.26)	.41
	>10–17	3	193	756	0.40	0.77 (.25–2.42)	0.79 (.25–2.49)	.69
	>17–43	2	206	786	0.25	0.49 (.12–2.00)	0.52 (.13–2.13)	.37
	>43–1086	3	201	774	0.39	0.75 (.24–2.36)	0.75 (.23–2.39)	.63
						P value for linear trend = .68		
New ASCUS+ associated with HPV-18 (cases = 338, noncases = 7745)[a]	Seronegative	316	6892	26 571	1.19	1.00	1.00	
	Seropositive	22	853	3222	0.68	0.57 (.37–.88)	0.64 (.41–.99)	.043
	Seropositive, quartiles, EU/mL							
	7–10	7	237	892	0.79	0.66 (.31–1.40)	0.81 (.38–1.72)	.58
	>10–17	5	195	756	0.66	0.56 (.23–1.34)	0.60 (.25–1.46)	.26
	>17–43	4	209	789	0.51	0.43 (.16–1.14)	0.46 (.17–1.25)	.13
	>43–1086	6	212	786	0.76	0.64 (.29–1.44)	0.67 (.29–1.51)	.33
						P value for linear trend = .17		

Table 3 continued.

Endpoint	Serostatus at Baseline	No. Cases With New Endpoint	No. Cases Without New Endpoint	PY	Incidence Per 100 PY	Univariate Analysis Rate Ratio (95% CI)	Final Multivariable Analysis Adjusted on Confounders Retained	
							Rate Ratio (95% CI)	*P* Value
New CIN1+ associated with HPV-18 (cases = 67, noncases = 8016)[a]	Seronegative	66	7142	27 026	0.24	1.00	1.00	
	Seropositive	1	874	3266	0.03	0.13 (.02–.90)	0.13 (.02–.94)	.043
	Seropositive, quartiles, EU/mL							
	7–10	0	244	904	0.00	ND	ND	ND
	>10–17	0	200	769	0.00	ND	ND	ND
	>17–43	0	213	796	0.00	ND	ND	ND
	>43–1086	1	217	797	0.13	0.51 (.07–3.70)	ND	ND
New CIN2+ associated with HPV-18 (cases = 34, noncases = 8049)[a]	Seronegative	34	7174	27 062	0.13	1.00	ND	ND
	Seropositive	0	875	3267	0.00	ND	ND	ND
	Seropositive, quartiles, EU/mL							
	7–10	0	244	904	0.00	ND	ND	ND
	>10–17	0	200	769	0.00	ND	ND	ND
	>17–43	0	213	796	0.00	ND	ND	ND
	>43–1086	0	218	798	0.00	ND	ND	ND

Incident infection was defined as a new detection of the HPV type at any time during the follow-up period; 6- and 12-month persistent infection were defined as detection of the same HPV type in 2 consecutive samples over a minimum of 150 days and 300 days, respectively; ASCUS+ was defined as ASCUS, low-grade squamous intraepithelial lesion (LSIL), atypical squamous cells/high-grade ASCUS, does not exclude high-grade squamous intraepithelial lesion (HSIL), atypical glandular cells of undetermined significance (AGUS), or HSIL. CIN1+ was defined as CIN1, CIN2, CIN3, adenocarcinoma in situ, or invasive cervical cancer; CIN2+ excluded CIN1.

Confounders (exposure variables) retained for the multivariable analysis were marital status, tobacco exposure (number of pack-years), age at first sexual intercourse, number of sexual partners prior to the past 12 months (ASCUS+, CIN1+, CIN2+), number of sexual partners during the past 12 months (incident and persistent infections), at least 1 previous pregnancy, sexually transmitted infection history, and region.

Linear trend was evaluated across 5 classes: (1) seronegative; (2) first quartile; (3) second quartile; (4) third quartile; (5) fourth quartile.

Abbreviations: ASCUS, atypical squamous cell of undetermined significance; CI, confidence interval; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; ND, analysis not performed because of too few cases for inferential analysis; PY, patient years.

[a] No. of cases and noncases and patient-years reported for the univariate analysis.

Trial (25% for both HPV-16 and HPV-18). These differences may indicate that women in the Costa Rica Vaccine Trial might have been more likely to have had a past exposure to HPV and thus more time to mount a natural antibody response. Our results extend those of the Costa Rica Vaccine trial to persistent infection, ASCUS+, CIN1+, and CIN2+.

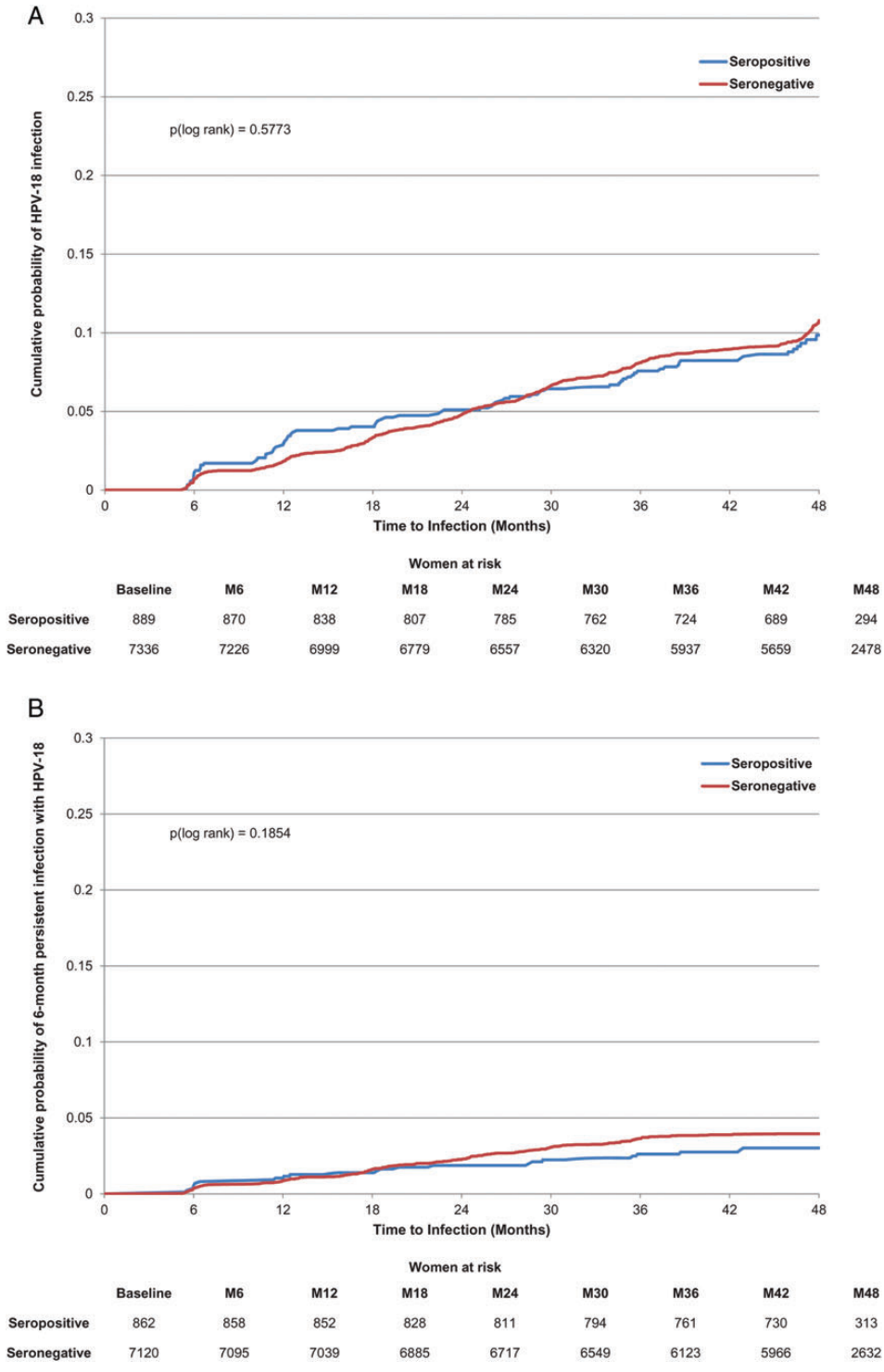


Figure 3. Cumulative probability of detecting an incident or 6-month persistent infection or developing atypical squamous cells of undetermined significance or greater (ASCUS+) associated with human papillomavirus type 18 (HPV-18). *A*, Incident HPV-18 infection. *B*, HPV-18 six-month persistent infection. *C*, ASCUS+ associated with HPV-18.

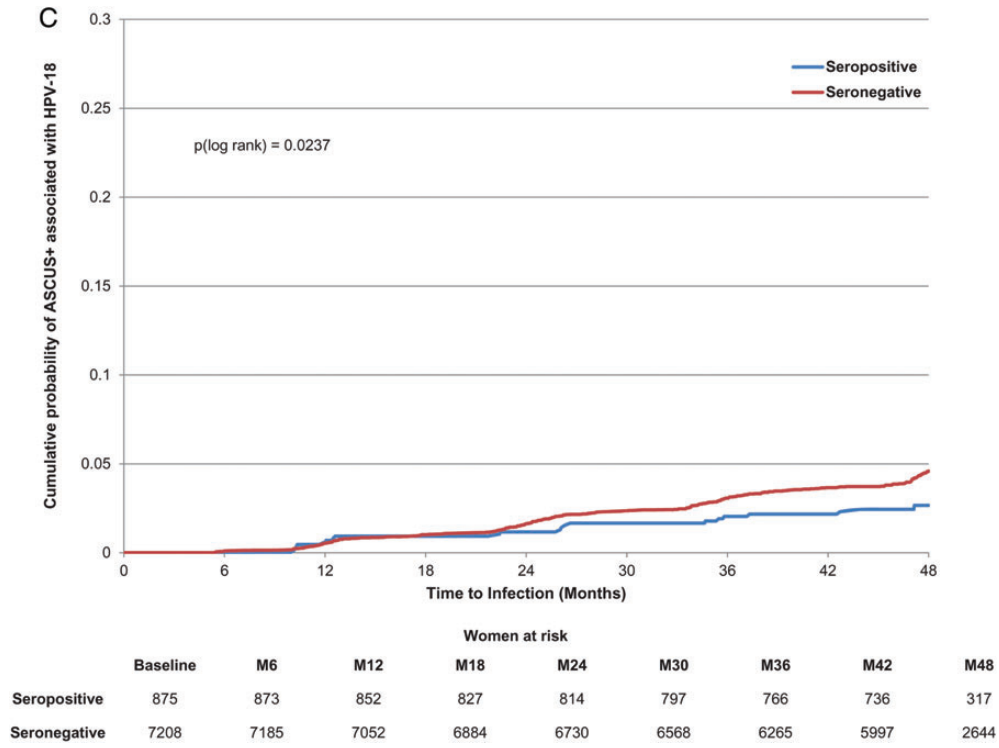


Figure 3 continued.

The present study is the first to demonstrate a statistically significant quantitative relationship between titers of naturally acquired antibodies to HPV-16 and the incidence of newly detected and 6-month persistent HPV-16 infection and HPV-16-associated ASCUS+. Antibody titers of approximately 370, 204, and 480 EU/mL were associated with a 90% risk reduction of incident infection, 6-month persistent infection, and ASCUS+, respectively. However, these values do not represent correlates of protection with regard to antibody levels induced by vaccination, because there are certainly some differences between naturally acquired vs vaccine-induced antibody production. For example, the mechanism of exposure of the immune system to HPV antigens via vaccination allows induction of a higher antibody level. In PATRICIA, antibody titer 1 month after the full vaccination course was 9341.5 (95% CI, 8760.4–9961.1) and 4769.6 (95% CI, 4491.2–5065.3) EU/mL for HPV-16 and HPV-18 antibodies, respectively [22]. Antibody properties such as affinity, avidity, and specificity may also be different. In addition, naturally acquired HPV infections potentially enable broad exposure of many HPV-specific proteins during the virus life cycle, unlike HPV vaccines based on L1 VLPs. Thus, natural infections are likely not restricted to the generation of L1 antibody responses but would be expected to include a spectrum of HPV-specific cell-mediated and humoral immune responses that could contribute to reduction in new infection.

In the Costa Rica vaccine study, the HPV-16 antibody titer at 48 months after 1 dose was 137 EU/mL; the 90th percentile was 703 EU/mL [29]. In the present study, the HPV-16 antibody levels producing a 90% reduction in incident infection, 6-month persistent infection, and ASCUS+ were, respectively, 371 EU/mL, 204 EU/mL, and 480 EU/mL. Corresponding values for a 70% reduction were 194 EU/mL, 107 EU/mL, and 251 EU/mL. These estimates might be useful as we move toward understanding minimal levels of HPV protective antibodies.

Analyses of PATRICIA and the Costa Rica Vaccine Trial strongly suggest that higher levels of naturally acquired antibodies play a role in preventing newly detected HPV infections and associated abnormalities. Although a correlate of protection for vaccine-induced antibodies cannot firmly be inferred, as described above, it is worth noting that the levels that appear to be associated with some reduction of risk are considerably below the levels of vaccine-induced antibodies found in clinical trials of the HPV-16/18 vaccine in which protection against CIN2+, CIN3+, and adenocarcinoma in situ has been demonstrated [2, 3, 22, 23], and also considerably below the levels sustained up to 8.4 years after vaccination [30].

The analysis had some unavoidable limitations. We do not know when women were exposed to HPV infection prior to the study, so we could not distinguish whether the antibody level at baseline was the peak from a recent exposure, or had declined over time from a higher level. Many women with HPV

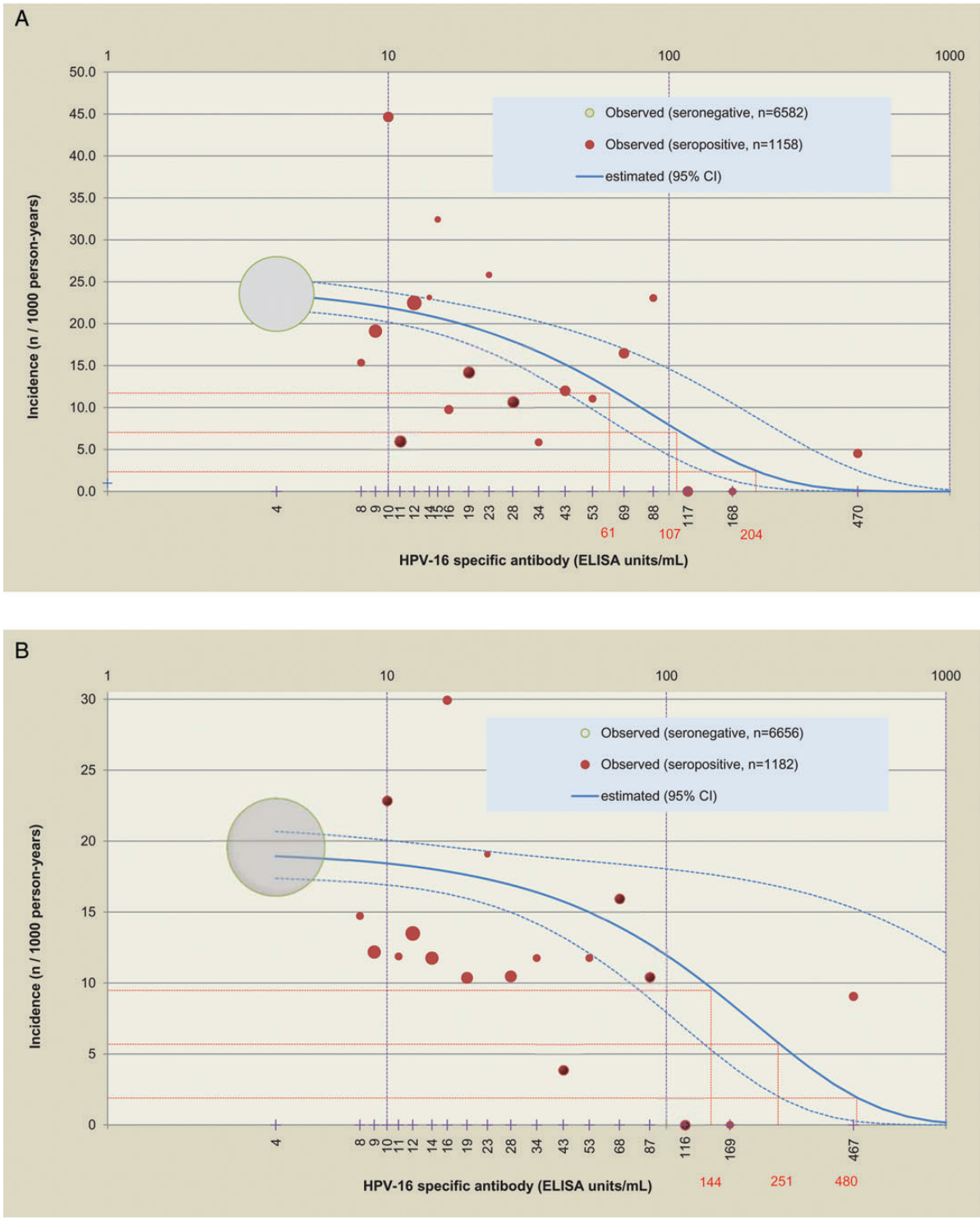


Figure 4. Relationship between initial antibody level and 6-month persistent infection or atypical squamous cells of undetermined significance or greater (ASCUS+) associated with human papillomavirus type 16. *A*, 6-month persistent infection. *B*, ASCUS+. The dot size is proportional to the number of subjects, the gray dot represents all seronegative subjects, and red dots represent approximately 5-percentile classes of seropositive subjects. The solid blue line corresponds to the Poisson regression model (the dotted lines are 95% confidence limits). The dotted red lines correspond to a 50%, 70%, and 90% reduction of the incidence of the endpoint (6-month persistent infection or ASCUS+), and the values in red are the corresponding threshold values of antibody titer. Sensitivity analyses including the covariates of age at first sexual intercourse and smoking history, or including only a subset of 100 seronegative subjects, produced similar results. For example, including the covariates of age at first sexual intercourse and smoking history for all seronegative subjects, the estimated antibody titers (with 95% confidence interval) yielding 90%, 70%, and 50% reductions in 6-month persistent infection were 180 (118–377), 94 (62–197), and 54 (36–114) EU/mL, respectively. Abbreviations: CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; HPV-16, human papillomavirus type 16.

infection never develop detectable antibodies [7–13, 31, 32], so some seronegative women may have been previously exposed to infection. These women may have mounted a cell-mediated immune response against HPV, which may have reduced the risk of CIN2+ development. This could have underestimated the protective effect. However, this limitation would not apply to the observation of increasing protection with titer. A further limitation is that it is not possible to distinguish between reinfection (a new HPV infection) and recurrence of an existing infection that was quiescent or had persisted at an undetectable level. It has been shown that women can experience recurrence of a type-specific infection after a period of nondetection [33]. Our findings suggest that the reduction of risk associated with naturally acquired antibodies might apply to both new and recurrent HPV infections. We have focused on ASCUS+ and CIN1+ as cervical abnormalities because few CIN2+ cases were detected. However, the limited utility of ASCUS+ and CIN1+ as disease-related endpoints should be noted.

The quality of the serologic assay is important in classifying women as previously exposed or unexposed, and measurement of the antibody response is dependent upon the assay, its specificity, and the cutoff value. There is variability in the measurement of the lower antibody titers as shown in the quantitative models (Figure 4*A* and 4*B*). Therefore, some women with antibody levels close to the threshold value could have been misclassified as seropositive or seronegative. The ELISA assay used in PATRICIA and the Costa Rica study has two disadvantages [34]: it potentially measures nonneutralizing antibodies (which would not be protective as neutralizing antibodies are likely to form the main basis for vaccine-induced protection against HPV infection), and it detects reactivity only to antibodies of the immunoglobulin G class (thus protection conferred by other antibody classes is not evaluated). These problems can be overcome by the pseudovirion-based neutralization assay (PBNA), which measures only potentially protective neutralizing antibodies of all immunoglobulin classes [26, 34]; however, this assay is currently too laborious for routine use in large clinical trials. Notably, a good correlation has been found between the PBNA and the ELISA assay for titers of vaccine-induced antibodies to HPV-16 and HPV-18 [26] and for titers of naturally acquired antibodies to HPV-16 [35].

In conclusion, this study confirms the utility of control arm data from vaccine efficacy trials in understanding acquisition and progression of HPV infections and related cervical abnormalities. The results suggest that naturally acquired antibodies to HPV-16, and to a lesser extent HPV-18, reduce the risk of subsequent infection and associated cervical abnormalities with the same HPV type.

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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