

# Risk of HPV-16/18 Infections and Associated Cervical Abnormalities in Women Seropositive for Naturally Acquired Antibodies: Pooled Analysis Based on Control Arms of Two Large Clinical Trials

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**Background.** Studies on the role of antibodies produced after infection with human papillomavirus 18 (HPV-18) and subsequent protection from HPV-18 infection have been conflicting, mainly due to inadequate sample size.

**Methods.** We pooled data from the control arms of the Costa Rica Vaccine Trial and the PATRICIA trial. Using Poisson regression we compared the risk of newly detected 1-time HPV-18 infection, HPV-18 1-year persistent infection (12MPI), and HPV-18-associated atypical squamous cells of undetermined significance or greater (ASC-US+) lesions between HPV-18 seropositive and seronegative women.

**Results.** High HPV-18 antibodies at enrollment was associated with reduced subsequent HPV-18 detection ( $P$  trend = 0.001; relative rate [RR] = 0.69; 95% confidence interval [CI], 0.47–1.01 for the third quartile; RR = 0.63; 95% CI, 0.43–0.94 for the fourth quartile, compared to seronegative). The risk of 12MPI showed a decreasing trend with increasing antibodies ( $P$  trend = 0.06; RR = 0.72; 95% CI, 0.29–1.77; RR = 0.42; 95% CI, 0.13–1.32 for the third and fourth quartiles, respectively). Lastly, we observed a significant decreased risk of HPV-18 ASC-US+ with increasing antibody ( $P$  trend = 0.01; RR = 0.46; 95% CI, 0.21–0.97 for the fourth quartile). We also observed a significant decreased risk of HPV-16 infection, 12MPI, and ASC-US+ with increasing HPV-16 antibody level.

**Conclusions.** High HPV-18 naturally acquired antibodies were associated with partial protection from future HPV-18 infections and associated lesions.

**Clinical Trials Registration.** NCT00128661 and NCT001226810.

**Keywords.** human papillomavirus; HPV; immunity; naturally acquired antibodies.

Persistent infection with human papillomavirus (HPV) is a necessary cause of cervical precancers and cancer [1–3]. Approximately 13 HPV types have been classified as oncogenic, (genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) and an additional genotype (66) is classified as “limited evidence in humans” [4]. HPV-16 and HPV-18 cause approximately 70% of invasive cervical cancers worldwide [5, 6].

Most HPV infections clear naturally within approximately 2 years of acquisition [7–11]. Innate and cell-mediated immunity

are likely responsible for clearance, defined as lack of detectability using standard polymerase chain reaction (PCR) assays, although the exact mechanism and meaning (ie, whether the virus is truly gone or only controlled) is poorly understood [9, 10].

From a natural history perspective, serologic measures of HPV antibodies can reflect past exposure [12], while DNA-based detection reflect infection at the time of testing. Only a fraction (50%–70%) of women develop antibodies after natural infection [13–15], at much lower levels than induced by vaccination [16].

Earlier reports investigating protection by naturally acquired antibodies have been conflicting, partially attributed to heterogeneity of both antibodies and DNA assays [17–20], while recent studies have consistently shown partial protection from incident HPV detection (defined as new HPV DNA or RNA detection after testing negative) in women who were previously exposed to HPV and mounted an immune response as measured by HPV-16/18-specific antibodies [21–23]. Due to limited sample size, these studies mainly investigated the effect of antibodies on 1-time HPV infection, which in most cases clears within 2 years [9]. It is important to establish whether protection

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is observed for persistent infections and precancerous lesions that have a higher risk of progressing to cancer [9, 10].

The PApilloma TRIal against Cancer In young Adults (PATRICIA) and Costa Rica HPV-16/18 Vaccine Trial (CVT) are phase III trials of the HPV-16/18 AS04-adjuvanted vaccine cumulatively conducted in over 25 000 women. Because of their similar prospective design, procedures, and follow-up, analyses of participants in the control arms of these randomized trials provided a unique opportunity to evaluate the impact of naturally induced antibodies detected at baseline on subsequent HPV detection and related cervical abnormalities in young women.

Separate analysis within CVT and PATRICIA trials independently reported the protective role of naturally acquired HPV-16 antibodies in future HPV-16 detection [21, 22]. A protective role for naturally acquired HPV-18 antibodies in future HPV-18 detection was shown in CVT [21], but it was not clearly established in PATRICIA, due to limited power (lower numbers of incidentally detected HPV-18 infections in PATRICIA than CVT). Thus, using pooled data from these trials, the primary objectives of this analysis was to assess the relationship between baseline (study entry) naturally acquired HPV-18 antibodies and (1) newly detected HPV-18 infection, (2) newly detected HPV-18 infections that persisted over 12 months, and (3) newly detected HPV-18 infections with concurrent cytological abnormalities (defined in the Methods section). As secondary objectives, we report findings for HPV-16 incident and persistent detections as well as HPV-16-related lesions. Lastly, we estimated levels of type-specific antibodies required for 50%, 70%, and 90% reduction in incidence of future HPV-18 and HPV-16 detection. In exploratory analyses, we further assessed the relationship between baseline naturally-acquired type-specific antibodies histological outcomes: (1) incidence of new HPV-18 cervical intraepithelial neoplasia (CIN) grade 1 or 2, or 3, squamous cell carcinoma (SCC), adenocarcinoma in situ (AIS), adenocarcinoma (ADC; CIN1+); (2) CIN 2; or (3) SCC, AIS, ADC (CIN2+); and separately HPV-16 CIN1+ and CIN2+. Further analyses were performed to assess whether HPV-18 antibodies are protective against newly detected HPV-45, a type phylogenetically related to HPV-18; and the association of HPV-16 antibodies and newly detected HPV-31 and HPV-33, types phylogenetically related to HPV-16.

## METHODS

### Study Population

Study participants were women randomized to the control arms of the PATRICIA trial (NCT00122681; 9337 women aged 15–25 years) or CVT trial (NCT00128661; 3736 women aged 18–25 years; 1 participant was 17 years old at enrollment) (Figure 1). The clinical trial methodology, inclusion and exclusion criteria, trial locations, and dates have been described elsewhere [16, 24]. Written informed consents and/or assents were

obtained from all participants or their parents, and the protocol and other materials were approved by independent ethics committees or institutional review boards.

Briefly, women were randomized to HPV-16/18 AS04-adjuvanted vaccine or control hepatitis-A vaccine and followed for 4 years. Gynecological examinations were performed every 6 (PATRICIA) or 12 months (CVT) on sexually experienced participants. At these visits, cervical liquid-based cytology samples were collected and used for HPV DNA-typing and cytopathological examination using the Bethesda system. Repeated cytology and/or colposcopy referral were carried out according to predefined clinical management algorithms, which were the same for CVT and PATRICIA trials [24, 25]. Participants completed a demographic and behavioral questionnaire, either at enrollment (CVT) or at the second study visit, 1 month after the first vaccination (PATRICIA), and yearly thereafter during the follow-up period for both trials.

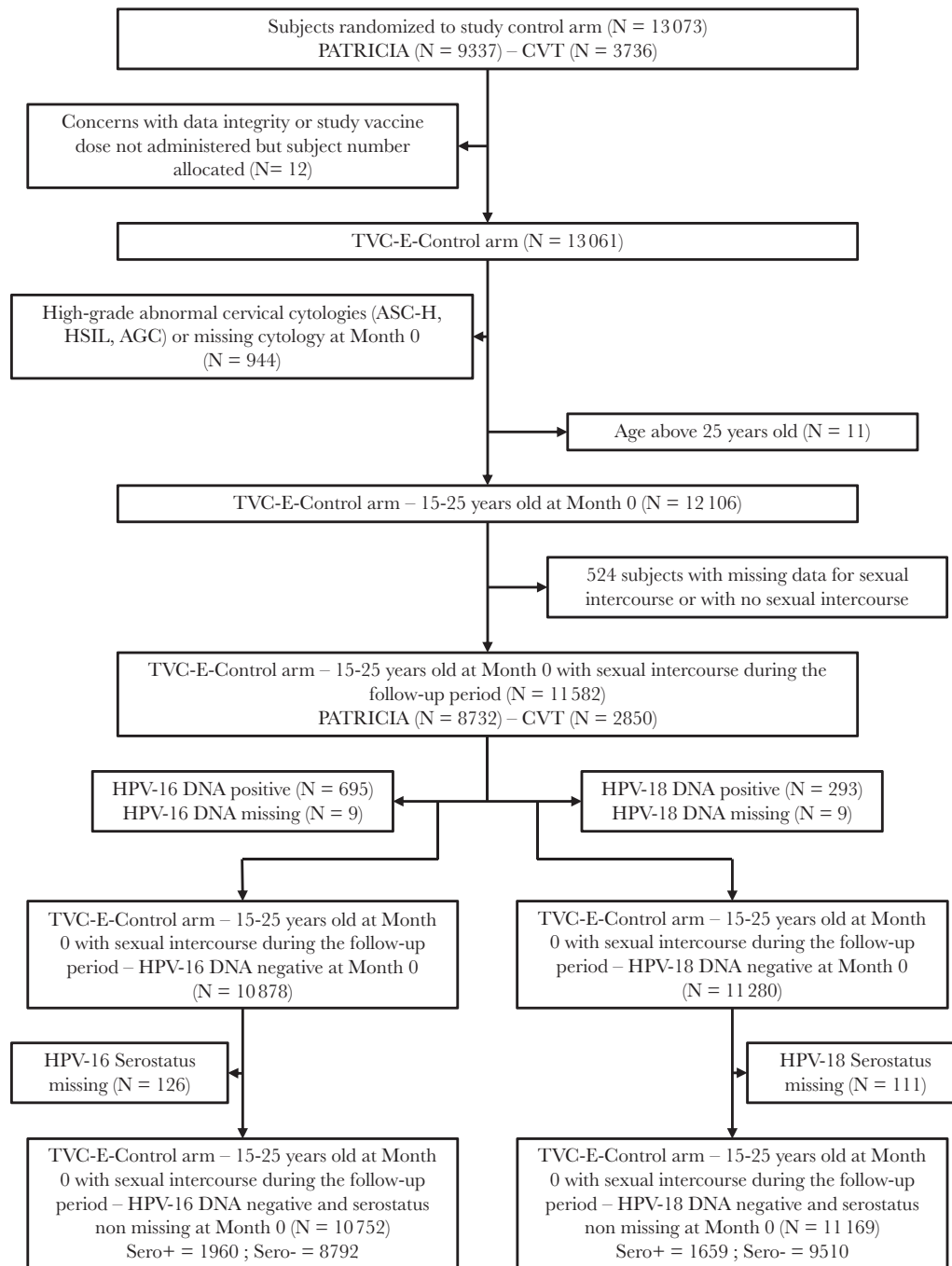
Both trials used the broad spectrum PCR SPF10-LiPA25 (version 1) and PCR TS16/TS18 DEIA DNA-based assays to test cervical samples and biopsy material (PATRICIA) for 14 oncogenic HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68/73) and 11 nononcogenic HPV types (6, 11, 34, 40, 42, 43, 44, 53, 54, 70, and 74). These assays were performed at GlaxoSmithKline (GSK) laboratories for PATRICIA, and Delft Diagnostic Laboratory (DDL, under contract from GSK) for CVT; here these are considered to be the same laboratory (“GSK laboratories or GSK-designated laboratory”). Cytology was performed by different laboratories (Quest for PATRICIA and National Cancer Institute [NCI] designated laboratory for CVT).

Serum antibody to HPV-16 and HPV-18 at baseline were determined by enzyme-linked immunosorbent assay (ELISA), performed by GSK laboratories for both PATRICIA and CVT. Seropositivity was defined as an antibody titer greater than or equal to the assay threshold: 7 ELISA units (EU)/mL for HPV-18 and 8 EU/mL for HPV-16 [12, 21, 22].

### Statistical Analysis

#### Analysis Cohort

The analysis was performed on the total vaccinated cohort for efficacy (TVC-E) in the control arm of both trials. The TVC-E included all women who received at least 1 dose of the control vaccine and who had normal or low-grade cytology at baseline. Women who did not have a follow-up visit were excluded from the analysis (Figure 1). Further, women were excluded if they were: (1) over 25 years old at enrollment; (2) self-reported virgins at enrollment and did not begin sexual activity during the follow-up period, or had missing data for this variable (thus included women who had potentially been exposed to HPV infection via sexual intercourse); (3) DNA positive at baseline for the corresponding HPV type considered in the analysis or had missing HPV DNA results for that type (ie, women who were not known to be DNA negative for HPV-18, or HPV-16, were excluded from



**Figure 1.** Consort diagram of the study population. Abbreviations: AGC, atypical glandular cells; ASC-H, atypical squamous cells-cannot exclude HSIL; CVT, Costa Rica vaccine trial; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; N, number of subjects; PATRICIA, Papilloma Trial Against Cancer in Young Adults; Sero+, seropositive; Sero-, seronegative; TVC-E, total vaccinated cohort for efficacy.

the respective analyses) (Figure 1). Thus, only newly detected infections over the follow-up were accounted for in the analysis.

#### Outcome Variables

Endpoints evaluated were newly detected infection with HPV-18 or HPV-16, 12-month persistent HPV-18 or HPV-16 infection (12MPI), and any ASC-US+ associated with HPV-18 or HPV-16 defined as:

- Incident infection: new detection of the HPV type at any time during the follow-up period among women negative by DNA testing for that type until that point.
- 12MPI: detection of the same HPV type in at least 2 samples not interrupted by negative samples over a minimum of 10 months.
- ASC-US+: atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesion

(LSIL), high-grade squamous intraepithelial lesion (HSIL), atypical squamous cells-cannot exclude HSIL (ASC-H), or atypical glandular cells (AGC) associated with HPV-18 or HPV-16.

In exploratory analyses, we assessed the relationship between baseline type-specific antibodies and histological outcomes of CIN1+, and CIN2+ (Supplementary Tables), and phylogenetically related HPV-45, and HPV-31, and HPV-33 (results not shown).

#### Exposure Variables

The main exposure variables were HPV-18 and HPV-16 serostatus at study enrollment. Serostatus was expressed as: (1) binary variable (seropositive or seronegative) according to the ELISA assay threshold; (2) quartiles ( $\geq 7-10$ ,  $>10-16$ ,  $>16-40$ , and  $>40-2541$  EU/mL for HPV-18, and  $\geq 8-13$ ,  $>13-24$ ,  $>24-64$ , and  $>64-3202$  EU/mL for HPV-16); or (3) a continuous variable (antibody level).

Additional covariates known to be associated with risk of acquiring HPV were also taken into account in the analyses, and defined as shown in Supplementary Table 1. In summary, 8 potential confounders (covariates) were included in the models: study (CVT, PATRICIA), geographical region, marital status, cigarette smoking (packs per year), lifetime number of sexual partners, *Chlamydia trachomatis* infection at enrollment, age at first sexual intercourse, and previous pregnancy.

All analyses were performed using SAS version 9.2.

#### Poisson Regression Analysis

Incidence rate was calculated as the number of incidently detected events divided by the total person-time. Person-time was 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 with the control vaccine, and ended on the date of the first occurrence of the endpoint or on the date of the last visit (whichever occurred first).

We used Poisson regression to evaluate the univariate and multivariable effect of the exposure variables on the endpoints and obtain relative incidence rates (relative rates [RR]) and 95% confidence intervals (CI). The final multivariable analysis allowed an estimation of the relative contribution of initial HPV-18 or HPV-16 serostatus while adjusting for the simultaneous effects of the 8 covariates selected as potential confounders. Only subjects with no missing data for the different variables were included in the multivariate analyses.

The relationship between the risk of newly detected infection and the baseline antibody was analyzed using Poisson regression, where we included the antibody titer as a continuous variable. Seronegative women were assigned a value of half the ELISA assay cut-off level. Age at first sexual intercourse, lifetime number of sexual partners, and smoking status were included as covariates. Interaction between antibody level and

study (PATRICIA, CVT) was also tested but removed from the final model because it was not statistically significant ( $P > .10$ ). Predicted antibody titers corresponding to a 50%, 70%, and 90% reduction in incidence were derived from this model.

## RESULTS

#### Participant Characteristics at Study Entry

Mean age at study entry was 20.4 years (standard deviation 3.0). Twenty-four percent (2776/11 582) were between 15 and 17 years old and 76% between 17 and 25 years old. The distributions of age and selected exposure variables according to initial serostatus are shown in Supplementary Table 1.

Participant characteristics are shown in Figure 1. A total of 11 169 women were included in the HPV-18-related analyses, of whom 1659 (15%) were HPV-18 seropositive at baseline (geometric mean titer 22.4 EU/mL). A total of 10 752 women were included in the HPV-16-related analyses, of whom 1960 (18%) were HPV-16 seropositive at baseline (geometric mean titer 31.2 EU/mL) (Figure 1).

#### Poisson Regression Analyses

##### HPV-18

Overall, there were 1079 incidently detected HPV-18 infections, 190 12MPI, 426 ASC-US+, 78 CIN1+, and 39 CIN2+ detected during the 4-year follow-up period. Results from the univariate and multivariable Poisson regression models are presented in Table 1. In multivariable models, we observed a 17% decreased risk of newly detected HPV-18 infection for seropositives ( $P = .06$ ). The risk of infection decreased with increasing quartiles of antibodies ( $P$  trend = 0.001; RR = 0.69; 95% CI, 0.47–1.01 for the third quartile and RR = 0.63; 95% CI, 0.43–0.94 for the highest quartile, compared to seronegatives). We did not observe statistically significant associations between enrollment seropositivity and HPV-18 12MPI; however, the risk of 12MPI was lower in the third and fourth quartiles of antibody level (RR = 0.72; 95% CI, 0.29–1.77 and RR = 0.42; 95% CI, 0.13–1.32, respectively) with borderline statistically significant trend ( $P$  trend = 0.06). Lastly, we observed a significantly decreased risk of HPV-18 ASC-US+ with increasing quartiles of antibodies ( $P$  trend = 0.01; RR = 0.46; 95% CI, 0.21–0.97 for the last quartile compared to seronegatives) but were underpowered to observe associations with HPV-18 CIN1+ or CIN2+ (CIN1+ RR = 0.32; 95% CI, 0.04–2.34 for the last quartile compared to seronegatives; Supplementary Table 2). Results for the full models are presented in Supplementary Table 2.

Figure 2A and 2B illustrate the quantitative model that shows a decreased risk of HPV-18 incidently detected infection/ASC-US+ with increasing antibodies levels. The adjusted model-predicted HPV-18 antibody titers associated with a 90% risk reduction of incidently detected infections were 622 EU/mL (95% CI, 334–3838), and antibody titers associated with a 90% risk reduction of HPV-18/ASC-US+ were 480 EU/mL (95% CI,

**Table 1. Univariate and Multivariable Poisson Regression for Incident Infections, 12-Month Persistent Infections, and ASC-US+ Cervical Cytology Associated With HPV-18: TVC-E Control Arm, 15–25 Years Old at Enrollment Among Participants Reporting Ever had Sexual Intercourse**

Exposure Variable	Categories	No. of Subjects	No. of Events	Incidence per 100/per Year	Univariate Analysis		Multivariable Analysis	
					Relative Rate (95% CI)	P value	Relative Rate (95% CI)	P value
<b>Endpoint: HPV-18 incident infection</b>								
		(N = 10 846)	(N = 1079)		(N = 10 846)		(N = 10 046)	
HPV-18 serostatus	Negative	9242	945	2.78	1.00	...	1.00	...
	Positive(≥7 EU/mL)	1604	134	2.17	0.78 (0.65–0.94)	.008	0.83 (0.69–1.01)	.06
HPV-18 titers	Negative (<7 EU/mL)	9242	945	2.78	1.00	...	1.00	...
	Q1 <sup>a</sup> (7–10 EU/mL)	457	47	2.72	0.98 (0.73–1.31)	.89	1.14 (0.85–1.54)	.39
	Q2 (10–16 EU/mL)	355	30	2.17	0.78 (0.54–1.13)	.19	0.88 (0.6–1.27)	.49
	Q3 (16–40 EU/mL)	394	31	2.02	0.73 (0.51–1.04)	.08	0.69 (0.47–1.01)	.06
	Q4 (40–2541 EU/mL)	398	26	1.71	0.62 (0.42–0.91)	.01	0.63 (0.43–0.94)	.02
								P trend .001
<b>Endpoint: 12-month persistent HPV-18 infection</b>								
		(N = 10 461)	(N = 190)		(N = 10 461)		(N = 9715)	
HPV-18 serostatus	Negative	8915	166	0.48	1.00	...	1.00	...
	Positive(≥7 EU/mL)	1546	24	0.38	0.80 (0.52–1.23)	.31	0.85 (0.54–1.35)	.50
HPV-18 titers	Negative (<7 EU/mL)	8915	166	0.48	1.00	...	1.00	...
	Q1 <sup>a</sup> (7–10 EU/mL)	441	8	0.45	0.94 (0.46–1.91)	.86	1.13 (0.55–2.33)	.73
	Q2 (10–16 EU/mL)	342	7	0.50	1.05 (0.49–2.23)	.90	1.20 (0.56–2.58)	.64
	Q3 (16–40 EU/mL)	385	6	0.38	0.80 (0.35–1.81)	.59	0.72 (0.29–1.77)	.47
	Q4 (40–2541 EU/mL)	378	3	0.19	0.41 (0.13–1.28)	.12	0.42 (0.13–1.32)	.14
								P trend .06
<b>Endpoint: HPV-18 ASC-US+</b>								
		(N = 10 679)	(N = 426)		(N = 10 679)		(N = 9895)	
HPV-18 serostatus	Negative	9100	376	1.08	1.00	...	1.00	...
	Positive(≥7 EU/mL)	1579	50	0.79	0.74 (0.55–0.99)	.04	0.84 (0.61–1.15)	.27
HPV-18 titers	Negative (<7 EU/mL)	9100	376	1.08	1.00	...	1.00	...
	Q1 <sup>a</sup> (7–10 EU/mL)	448	18	1.01	0.94 (0.58–1.5)	.79	1.12 (0.69–1.84)	.64
	Q2 (10–16 EU/mL)	348	13	0.94	0.87 (0.5–1.51)	.62	1.06 (0.6–1.84)	.85
	Q3 (16–40 EU/mL)	390	12	0.77	0.71 (0.4–1.26)	.24	0.75 (0.41–1.38)	.36
	Q4 (40–2541 EU/mL)	393	7	0.45	0.42 (0.2–0.88)	.02	0.46 (0.21–0.97)	.04
								P trend 0.01

Relative rates are presented for the multivariable model including serostatus as binary and separately for models that include serostatus as categorical variables. Full models including titers are available in [Supplementary Table 1](#). Models coadjusted for: study (CVT and PATRICIA), study region, marital status, age at first sexual intercourse, pack-years smoked (0–0.5 vs 0.5+), lifetime sexual partners, previous pregnancy, *Chlamydia* test results. Abbreviations: ASC-US+, atypical squamous cells of undetermined significance or greater; CI, confidence interval; CVT, Costa Rica vaccine trial; HPV, human papillomavirus; N, number of subjects with available data; PATRICIA, the Papilloma Trial Against Cancer in Young Adults; TVC-E, total vaccinated cohort for efficacy. <sup>a</sup>Q1–Q4: Quartiles 1 to 4.

217—not determined). The corresponding values for 50% and 70% risk reduction were 187 EU/mL (95% CI, 100–1155) and 325 EU/mL (95% CI, 174–2007) for incident detection, and 144 EU/mL (95% CI, 65—not determined) and 251 EU/mL (95% CI, 114—not determined) for ASC-US+.

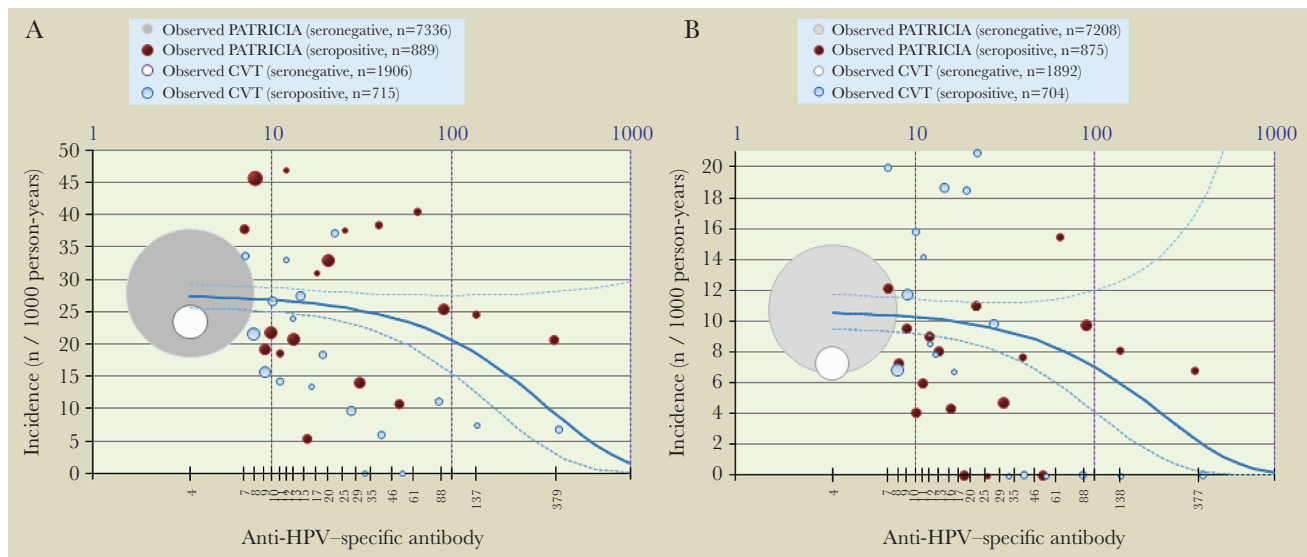
**HPV-16–Related Endpoints**

Overall, there were 1534 incidently detected HPV-16 infections, 517 12MPI, 719 ASC-US+, 222 CIN1+, and 143 CIN2+ detected during the 4-year follow-up period. Newly detected HPV-16 infections were reduced by 37% (RR = 0.63; 95% CI, 0.53–0.73), 12MPI by 30% (RR = 0.70; 95% CI, 0.54–0.92), and ASC-US+ by 43% (RR = 0.57; 95% CI, 0.45–0.73) in HPV-16-seropositive

women, and decreased significantly with increasing HPV-16 antibody levels ([Table 2](#)); all associations were statistically significant. HPV-16–associated CIN1+ and CIN2+ were also statistically significantly reduced with increasing HPV-16 antibody levels (CIN1+ RR = 0.09; 95% CI, 0.01–0.68 for the last quartile compared to seronegatives and CIN2+ RR = 0.15; 95% CI, 0.02–1.08 for the last quartile compared to seronegatives). Univariate and multivariate models with all covariates assessed, and the different endpoints are presented in [Supplementary Table 3](#).

The quantitative model showed a decreased risk of incident HPV-16 infection/ASC-US+ with increasing antibody levels ([Figure 3](#)). The adjusted model-predicted HPV-16 antibody





**Figure 2.** Quantitative Poisson regression model showing relationship between initial HPV-18 antibody level and incident HPV-18 infection (A) and HPV-18-associated ASC-US+ (B). TVC-E control arm: 15–25 years old, who reported ever having had sexual intercourse. Infection: the adjusted model-predicted HPV-18 antibody titers associated with a 90% risk reduction of incident infection were 622 EU/mL (95% CI, 334–3838). ASU-CS+: the adjusted model-predicted HPV-18 antibody titers associated with a 90% risk reduction of ASC-US+ were 480 EU/mL (95% CI, 217–not determined). The dot size is proportional to the number of subjects; gray/white dot represents all seronegative subjects, and red/blue 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). Abbreviations: ASC-US+, atypical squamous cells of undetermined significance or greater; CI, confidence interval; CVT, Costa Rica Vaccine Trial; HPV, human papillomavirus; n, number of subjects in a given category; PATRICIA, The PAPilloma TRIal against Cancer In young Adults; TVC-E, total vaccinated cohort for efficacy.

titers associated with a 90% risk reduction of incident infections were 371 EU/mL (95% CI, 268–606). The corresponding values for 12MPI and for ASC-US+ were 299 EU/mL (95% CI, 184–822) and 384 EU/mL (95% CI, 245–886). The corresponding values for 50% and 70% risk reduction were 112 EU/mL (95% CI, 81–182) and 194 EU/mL (95% CI, 140–317) for incident infection, and 116 EU/mL (95% CI, 74–267) and 201 EU/mL (95% CI, 128–463) for ASC-US+.

## DISCUSSION

In this large study of over 10 000 women, we demonstrated that women with naturally acquired antibodies to HPV-18 had a lower risk of newly detected HPV-18 infection or associated cervical lesions over 4 years. Risk decreased as antibody levels increased and higher antibody titers were associated with lower risk of most endpoints evaluated. There was also a clear pattern of reduced risk of an incidently detected or persistent HPV-18 infection, associated ASC-US+, or CIN1+ with increasing quartiles of HPV-18 antibody titers. Results were independent of the behavioral and other risk factors accounted for in the analysis. Too few cases of CIN2+ were observed to perform an inferential analysis; as expected in women of this age group (15–25 years old at study entry), there were no cases of cervical cancer in these randomized trials where women were closely followed up. Thus, the results of this study firmly demonstrate for the first time that naturally acquired HPV-18 antibodies also play

a protective role in the prevention of subsequent new detection and associated abnormalities over the 4 years' follow-up.

Furthermore, our analyses confirmed the relationship between HPV-16 antibody titer and detection of new HPV-16 infection and associated abnormalities, similar to previous observations in CVT [21] and PATRICIA [22]. In addition to confirmation of past findings, this analysis also served as a check of the validity of our HPV-18 findings.

We did not observe any association between HPV-18 antibodies and newly detected phylogenetically closely related genotype HPV-45, nor between HPV-16 antibodies and the new detection of phylogenetically closely related genotypes HPV-31 and HPV-33 (data not shown), indicating that the lower antibody levels following natural infection may be too low to provide immune crossprotection for phylogenetically related HPV types.

This study is among the first to demonstrate a statistically significant quantitative relationship between naturally acquired antibodies and protection from future new HPV-18 detection, persistent HPV-18 infections, and HPV-18-associated ASC-US+. These models allowed us to estimate antibody titers associated with a certain level of risk reduction. We estimated that a naturally acquired antibody titer of approximately 622 EU/mL was associated with a 90% reduction of HPV-18 infection detection, whilst a titer of 480 EU/mL was associated with a 90% reduction in risk of HPV-18-related ASC-US+. The results of analyses of the quantitative relationship between

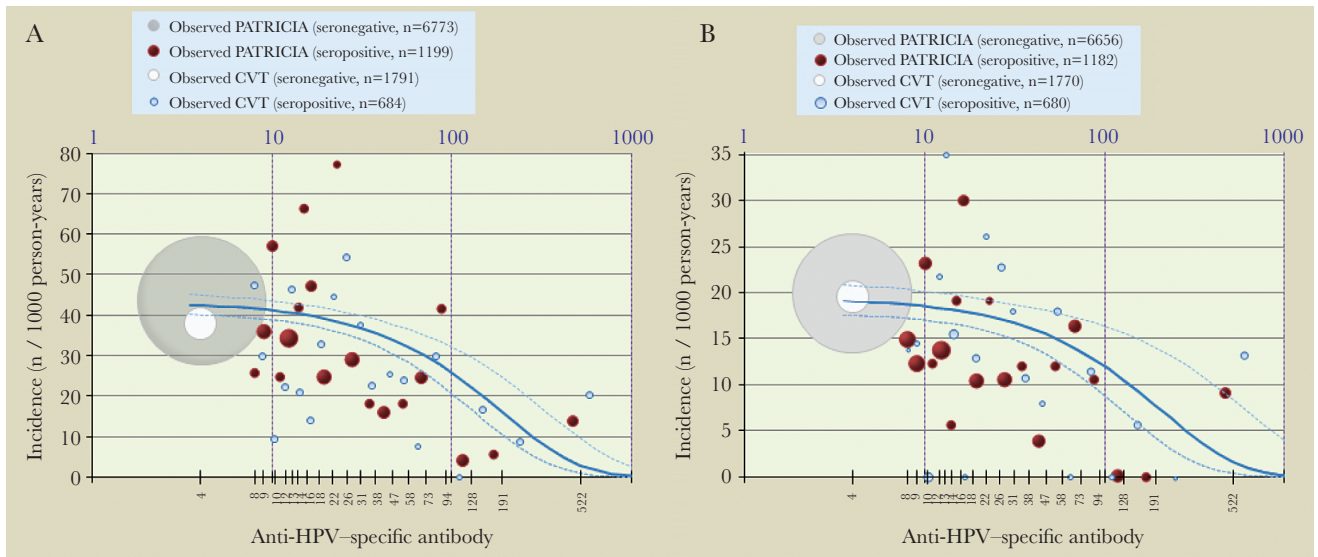
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Exposure Variable	Categories	No. of Subjects	No. of Events	Incidence per 100/per Year	Univariate Analysis		Multivariable Analysis	
					Relative Rate (95% CI)	PValue	Relative Rate (95% CI)	PValue
<b>Endpoint: HPV-16 incident infection</b>								
		(N = 10 447)	(N = 1534)		(N = 10 447)		(N = 10 046)	
HPV-16 serostatus	Negative	8564	1338	4.37	1.00	...	1.00	...
	Positive (≥8 EU/mL)	1883	196	2.81	0.64 (0.55–0.75)	<.0001	0.63 (0.53–0.73)	<.0001
HPV-16 titers	Negative (<8 EU/mL)	8564	1338	4.37	1.00	...	1.00	...
	Q1 <sup>a</sup> (8–13 EU/mL)	530	63	3.28	0.75 (0.58–0.97)	.03	0.76 (0.59–0.99)	.04
	Q2 (13–24 EU/mL)	428	61	3.88	0.89 (0.69–1.15)	.37	0.86 (0.66–1.12)	.27
	Q3 (24–64 EU/mL)	467	44	2.56	0.59 (0.43–0.79)	.0005	0.58 (0.43–0.8)	.0007
	Q4 (64–3202 EU/mL)	458	28	1.59	0.36 (0.25–0.53)	<.0001	0.32 (0.22–0.48)	<.0001
								P trend <.0001
<b>Endpoint: 12-month persistent infection</b>								
		(N = 10 082)	(N = 517)		(N = 10 082)		(N = 9344)	
HPV-16 serostatus	Negative	8268	448	1.41	1.00	...	1.00	...
	Positive (≥8 EU/mL)	1814	69	0.97	0.68 (0.53–0.88)	.003	0.70 (0.54–0.92)	.01
HPV-16 titers	Negative (<8 EU/mL)	8268	448	1.41	1.00	...	1.00	...
	Q1 <sup>a</sup> (8–13 EU/mL)	510	26	1.31	0.93 (0.63–1.38)	.72	0.97 (0.64–1.47)	.89
	Q2 (13–24 EU/mL)	414	16	0.98	0.70 (0.42–1.15)	.16	0.74 (0.45–1.22)	.24
	Q3 (24–64 EU/mL)	443	19	1.08	0.77 (0.48–1.21)	.25	0.81 (0.5–1.3)	.38
	Q4 (64–3202 EU/mL)	447	8	0.45	0.32 (0.16–0.64)	.001	0.29 (0.14–0.61)	.001
								P trend 0.0002
<b>Endpoint: HPV-16 ASC-US+</b>								
		(N = 10 288)	(N = 719)		(N = 10 288)		(N = 9516)	
HPV-16 serostatus	Negative	8426	632	1.98	1.00	...	1.00	...
	Positive (≥8 EU/mL)	1862	87	1.21	0.61 (0.49–0.77)	<.0001	0.57 (0.45–0.73)	<.0001
HPV-16 titers	Negative (<8 EU/mL)	8426	632	1.98	1.00	...	1.00	...
	Q1 <sup>a</sup> (8–13 EU/mL)	526	30	1.50	0.76 (0.53–1.09)	.14	0.78 (0.54–1.13)	.19
	Q2 (13–24 EU/mL)	422	24	1.49	0.75 (0.5–1.13)	.17	0.73 (0.48–1.1)	.13
	Q3 (24–64 EU/mL)	458	23	1.30	0.66 (0.43–0.99)	.05	0.60 (0.38–0.93)	.02
	Q4 (64–3202 EU/mL)	456	10	0.56	0.28 (0.15–0.52)	<.0001	0.21 (0.1–0.42)	<.0001
								P trend <.0001

Relative rates are presented for the multivariable model including serostatus as binary and separately for models that include serostatus as categorical variables. Full models including titers are available in Supplemental Table 2. Models coadjusted for: study (CVT and PATRICIA), study region, marital status, age at first sexual intercourse, pack-years smoked (0–0.5 vs 0.5+), lifetime sexual partners, previous pregnancy, *Chlamydia* test results. Abbreviations: ASC-US+, atypical squamous cells of undetermined significance or greater; CI, confidence interval; CVT, Costa Rica vaccine trial; HPV, human papillomavirus; N, number of subjects with available data; PATRICIA, Papilloma Trial Against Cancer in Young Adults; TVC-E, total vaccinated cohort for efficacy. <sup>a</sup>Q1–Q4: quartiles 1 to 4.

antibodies and various outcomes are interesting as they consistently showed that higher antibody levels are needed for protection, and not that ASC-US+ lesions may need a higher or lower absolute level. Moreover, the apparently higher titer needed for protection from newly detected infection could be due to misclassifications of titers, especially at the lower end of the assay cut-off. The corresponding values for HPV-16 were 371 EU/mL and 384 EU/mL. However, we note that absolute values are assay and laboratory specific, and not transferable between assays and laboratories. Furthermore, these values should not be interpreted as correlates of protection with regard to vaccination, as there are key differences between naturally acquired

versus vaccine-induced antibody production that might affect the values conferring protection [26]. However, while both natural and vaccine-induced antibody levels are lower for HPV-18 than HPV-16, the long-term vaccine-induced antibody level is clearly higher than our 90% reduction model-derived thresholds for both HPV-18 and HPV-16, particularly with the HPV-16/18 AS04-adjuvanted vaccine [27]. Considering the mentioned limitations of difficulty in comparing values across assays, we note that a higher antibody level seems to be necessary to prevent HPV-18 detection and associated ASC-US+ lesions than HPV-16. This may be related to misclassification of both the DNA and the serology assays, or some of these



**Figure 3.** Quantitative Poisson regression model showing relationship between initial HPV-16 antibody level and incident HPV-16 infection (A) and ASC-US+ (B). TVC-E control arm: 15–25 years old, who reported ever having had sexual intercourse. Infection: the adjusted model-predicted HPV-16 antibody titers associated with a 90% risk reduction of incident infection were 371 EU/mL (95% CI, 268–606). ASC-US+: the adjusted model-predicted HPV-16 antibody titers associated with a 90% risk reduction of ASC-US+ were 384 EU/mL (95% CI, 245–886). The dot size is proportional to the number of subjects; the gray/white dot represents all seronegative subjects, and red/blue 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). Abbreviations: ASC-US+, atypical squamous cells of undetermined significance or greater; CI, confidence interval; CVT, Costa Rica Vaccine Trial; HPV, human papillomavirus; n, number of subjects in a given category; PATRICIA, The PApilloma TRIal against Cancer In young Adults; TVC-E, total vaccinated cohort for efficacy.

infections may be depositions and not real infections, or may be random occurrence.

HPV is a mucosal infection, the virus remains in the cervical epithelium, and it is not exposed to the systemic immune system; thus, lower levels of antibodies are detected following natural infection than vaccination. In a vaccination setting, an individual is exposed to a large quantity of the virus-like particles systemically, and therefore the mechanism of exposure of the immune system to HPV antigens via vaccination leads to the induction of a higher antibody level. Properties of the antibodies (eg, affinity) may also be different.

Whether those with the highest naturally acquired antibodies level should be vaccinated is an interesting question, but it should be noted that, currently, the assays that measure HPV antibodies are variable, measuring qualitatively and quantitatively different subsets of the immune system [28–30]. As such, there is currently no utility in measuring antibodies in the context of natural infection for risk stratification purposes or to indicate vaccination, primarily due to the variability in the available assays.

The large sample size and well-characterized population from both vaccine trials, along with harmonized laboratory (both DNA and serological assessments) and study procedures are substantial strengths. There are some important differences, including populations (PATRICIA is a multinational study, whereas the population in CVT is more homogeneous); participants' age (15–25 years old in PATRICIA, 18–25 years old in CVT), and different cytology/histology laboratories (for

ASC-US+ and CIN1+ analyses); however, the studies had similar information on important behavioral risk factors known to be associated with incident HPV infections. The analysis was based on antibody levels measured at study baseline, and we did not determine how antibody titers changed over the course of the study. If naturally acquired antibody titers decline considerably over time, then it may be expected that the protection offered will wane. However, we could not determine this within the study.

Many women with HPV infection never develop detectable antibodies [12, 31], therefore some women found to be seronegative may have been previously exposed to infection. Two possible explanations are (1) that these women may have mounted an immune response against HPV other than antibodies, and/or (2) antibodies produced are lower than the assay's detection limit. Both possibilities may have resulted in an underestimate of the protective effect. However, this limitation would not be relevant to the observation of increasing protection with increasing titers.

Measurement of the antibody response is highly dependent upon the assay used. Neutralizing antibodies are likely to form the main basis for protection against HPV infection, but the assay used in our study measures total IgG response. Nevertheless, good correlation between this assay and an assay measuring only neutralizing anti-HPV antibodies has been shown previously [32].

It is not possible to distinguish between reinfection (a new HPV infection) and reactivation or redetection of an existing



infection that persisted at a low, undetectable level. It has been shown that women can experience reactivation or redetection of a type-specific infection after a period of nondetection [33]. The choice of the DNA assay is important in distinguishing between new versus reactivated/redetected infection; more sensitive whole-genome sequencing can be helpful in these efforts. Another study limitation is the 6-month (PATRICIA) or 1-year (CVT) interval between obtaining cervical samples. Transient infections arising and clearing between measurements might not have been detected.

In summary, our pooled analysis showed that naturally acquired HPV-18 antibodies provide immune protection from future newly detected HPV-18 infections and associated lesions over the 4-year follow-up, as previously observed for HPV-16 [21–23]. Although a correlate of vaccine protection cannot be inferred from natural history studies, it may help set a bench mark for protection, and may be useful in assessing the levels in 1-dose vaccinations. It is also reassuring that the antibody titer that appears to offer 90% protection against infection and ASC-US+ is several-fold lower than the titer observed after long-term follow-up of women who received the HPV-16/18 AS04-adjuvanted vaccine. This suggests that levels required for vaccine protection may be lower than those obtained with the current efficacious 3-dose regimen vaccines. Our data offer guidance in the continuing search for correlates of natural- and vaccine-induced immunity against genital HPV infections.

#### Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

#### Notes

**Contributions.** GlaxoSmithKline Biologicals SA designed the PATRICIA trial, in collaboration with investigators, and coordinated collection, analysis, and interpretation of data, and preparation of the manuscript. Investigators from the PATRICIA study group collected data for the trial and cared for the subjects. The CVT is a long-standing collaboration between investigators in Costa Rica and the NCI. The CVT was sponsored by the NCI. GlaxoSmithKline Biologicals SA provided vaccine and support for aspects of the trial associated with regulatory submission needs of the company. The NCI and Costa Rica investigators were responsible for the design and conduct of the study and collection, management, analysis, and interpretation of samples and data. All authors of this report had full access to all the trial data for the trial they participated in and access to summary-level trial data for both trials. All authors gave final approval of the manuscript.

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**Potential conflicts of interest.** M. S. is now employed by Roche. X. C. reports having received funding through his institution from the GSK group of companies for the purpose of conducting this study. X. C. also received grants from Merck & Co., Inc. outside the submitted work and occasional speaking honoraria from VIANEX and Merck Sharp & Dohme. M. H. S. reports having received HPV typing of specimens from Roche and BD at no cost for studies conducted by NCI. D. R. is an employee of the GSK group of companies and holds stocks and shares in the GSK group of companies. L. B. was employee of the GSK group of companies at the time of the study and holds shares in the GSK group of companies. M.-C. B. reports having received consulting fees from the GSK group of companies through her institution. All other authors from the core writing team report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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