



Human papillomavirus seroprevalence and seroconversion following baseline detection of nine human papillomavirus types in young women

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ABSTRACT

Background: Estimates of the humoral immune response to incident human papillomavirus (HPV) infections are limited.

Methods: In this post hoc analysis of 3875 women aged 16–23 years from a 4-valent HPV vaccine trial (NCT00092482), HPV seroprevalence on day 1 was measured with a 9-valent HPV (HPV 6/11/16/18/31/33/45/52/58) competitive Luminex immunoassay and compared with cervical/external genital HPV detection by polymerase chain reaction. In the control group, among women who were HPV DNA-negative on day 1, seroconversion following initial HPV detection was estimated using Kaplan-Meier methods.

Results: Type-specific HPV seropositivity among women with no day 1 cervical/external genital HPV detection was 0.6%–3.6%. Women with any 9-valent HPV (9vHPV) cervical/external genital detection (796/3875; 20.5%) had concordant seropositivity ranging from 13.4% (HPV 45) to 38.5% (HPV 6). Among women in the control group who were negative for all HPV types on day 1, seroconversion by month 30 after initial detection ranged from 29% (HPV 45) to 75% (HPV 16).

Conclusions: Humoral immune response to HPV is variable and dynamic, depending on type-specific exposure. This longitudinal analysis provides insight into the relationship between incident infection and seropositivity. ClinicalTrials.gov; NCT00092482 <https://clinicaltrials.gov/ct2/show/NCT00092482>.

Abbreviations: 4vHPV, quadrivalent HPV vaccine; 9vHPV, 9-valent HPV vaccine; ASC-H, atypical squamous cells—cannot rule out high-grade squamous intraepithelial lesion; ASC-US, atypical squamous cells—undetermined significance; CI, confidence interval; cLIA, competitive Luminex immunoassay; HPV, human papillomavirus; HR, high-risk; HSIL, high-grade squamous intraepithelial lesion; IQR, interquartile range; LSIL, low-grade squamous intraepithelial lesion; mMU/mL, milli-Merck Units per mL; OR, odds ratio; Pap, Papanicolaou; PCR, polymerase chain reaction.

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1. Introduction

Development of antibodies in response to anogenital human papillomavirus (HPV) infection is type-specific and can be variable [1–3]. In a cohort of women aged 18–20 years, seroconversion within 18 months after an incident infection with HPV 16, HPV 18, and HPV 6 (as measured by a capture immunoglobulin G antibody test) was 59.5%, 54.1%, and 68.8%, respectively [3]. Because the natural, specific immune responses are slow to mount and can vary following start of infection, HPV seropositivity is not a strong indicator of the presence of current HPV infection [3]. However, measures of HPV seroprevalence can be useful in estimating cumulative HPV exposure. These measures are likely an underestimate, because not all women seroconvert following HPV exposure and seropositivity may wane and become undetectable over time [4,5].

Natural history research into immune responses to HPV infection is relatively limited. Although some population-based studies have reported seroprevalence of high-risk (HR) HPV types [4,6–13], fewer have examined the relationship between HPV seropositivity and anogenital HPV infection for HR types other than 16 and 18, or with seropositivity and infection for low-risk HPV types [1,4,14–16]. In addition, few longitudinal studies have addressed temporal patterns of seropositivity and seroconversion after start of an incident anogenital infection. These measures can enhance our understanding of natural immune response to HPV infection and overall HPV natural history [17,18].

In this study, HPV-related serologic responses were measured in women aged 16–23 years who participated in a multinational clinical trial of the 4-valent HPV (4vHPV) vaccine. Stored sera samples were analyzed for antibodies against the L1 major capsid proteins of 4vHPV vaccine types (6/11/16/18) and for 5 additional HR types (31/33/45/52/58) targeted by the 9-valent HPV (9vHPV) vaccine. The HPV-related serologic responses were then compared with cervical/external genital HPV detection by polymerase chain reaction (PCR).

2. Methods

2.1. Study design and population

Data from a randomized, placebo-controlled clinical trial that aimed to establish an immunogenicity bridge between the monovalent HPV16 and 4vHPV vaccines in 3882 women aged 16–23 years (4vHPV vaccine group, $n = 1784$; monovalent vaccine group, $n = 304$; placebo group, $n = 1794$) enrolled between May 2002 and June 2004, of whom 3875 received vaccine or placebo (4vHPV vaccine group, $n = 1783$; monovalent vaccine group, $n = 304$; placebo group, $n = 1788$) were included in this analysis (Protocol V501-012; NCT00092482) [19]. The trial was conducted in Asia (Hong Kong and Thailand), Europe (Austria, Czech Republic, Germany, Italy, Russian Federation, and the United Kingdom), Latin America (Brazil, Colombia, Mexico, and Puerto Rico), North America (Canada and the United States), and Oceania (Australia and New Zealand) [20]. Key inclusion criteria were immunocompetent females with 0–4 lifetime sex partners and no prior history of cervical intraepithelial neoplasia, genital warts, or abnormalities detected with Papanicolaou [Pap] testing [19]. Participants were enrolled regardless of HPV status or Pap test result on day 1 [19].

Details of the study design, protocols, and primary results have been previously reported [19]. The study was sponsored by Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA and was conducted in accordance with principles of Good Clinical Practice, the Declaration of Helsinki, and all applicable regulatory requirements. The protocol was approved by the appropriate institutional review board and regulatory agency governing each study site. All patients involved in the study had previously given written informed consent.

2.2. Trial visits and specimen assays

Participants underwent gynecologic examinations on day 1 and at months 7, 12, 24, 36, and 48, during which an endocervical and ectocervical swab (considered 1 specimen), a combined labial-vulvar-perineal swab, and a perianal swab (pooled as 1 specimen) were collected [20]. Swab samples were tested for cervical/external genital HPV using a PCR assay for 14 HPV types—the HPV types represented in the 9vHPV vaccine (6/11/16/18/31/33/45/52/58) and 5 additional HR-HPV types (35/39/51/56/59). However, assays for HPV 6/11/39/51/56 were performed only through month 7 [21,22]. Cervical cytology samples were collected on day 1 for Pap testing (ThinPrep; CYTYC Corporation, Boxborough, MA, USA), and results were classified based on the 2001 Bethesda system: ASC-US (atypical squamous cells—undetermined significance), LSIL (low-grade squamous intraepithelial lesion), ASC-H (atypical squamous cells—cannot rule out high-grade squamous intraepithelial lesion), HSIL (high-grade squamous intraepithelial lesion), and atypical glandular cells or worse [20]. Serologic samples were obtained on day 1 and at months 7, 12, 24, and 48 and analyzed for antibodies to the L1 major capsid protein of HPV 6/11/16/18 using a 4vHPV competitive Luminex immunoassay (cLIA).

For this analysis, 2791 serologic samples stored from this trial were retrieved and analyzed using the 9vHPV cLIA (HPV 6/11/16/18/31/33/45/52/58) [23]. Serostatus cut-offs in milli-Merck Units per mL (mMU/mL) were: HPV 6, 30; HPV 11, 16; HPV 16, 20 (2.74 IU); HPV 18, 24 (4.51 IU); HPV 31, 10; HPV 33, 8; HPV 45, 8; HPV 52, 8; and HPV 58, 8. The International Units for HPV 16 and 18 are based on the quadrivalent cLIA [24]; the values in this paper were assayed in the 9-valent cLIA, which has similar cutoff values. IU conversion values for other HPV types are not yet available.

2.3. Sample selection and statistical analyses

Serologic samples included in the present analysis were identified based on cervical/external genital HPV DNA detection on day 1. These samples were categorized into 4 non-exclusive groups, each with separate statistical analyses, as described below (Fig. 1).

Group 1 included a random sample of 697 women (from the vaccine and control groups) who had no cervical/external genital detection of 14 HPV types included in the MSD Duplex/Multiplex PCR assay (9vHPV types 6/11/16/18/31/33/45/52/58 and HR-HPV types 35/39/51/56/59) on day 1. The sample was stratified by number of lifetime sex partners (1, 2–3, 4+, or “missing”), the geographic region from which the participant was enrolled, and age (16–20 years or 21–23 years). Seroprevalence on day 1 was analyzed for this group.

Group 2 included all 796 women (from the vaccine and control groups) with cervical/external genital detection by PCR for ≥ 1 9vHPV vaccine types (HPV types 6/11/16/18/31/33/45/52/58) on day 1. HPV type-specific seroprevalence in women with concordant cervical/external genital HPV detection was analyzed for this group.

Group 3 comprised 720 women from the vaccine and control groups who had day 1 cervical/external genital detection of any of HR-HPV types targeted by the 9vHPV vaccine (HPV 16/18/31/33/45/52/58). Among these women, 335 were seropositive for ≥ 1 of the 7 HR-HPV types on day 1. The association between baseline characteristics and seropositivity among women with any cervical/external genital detection of the 7 HR-HPV types was assessed via age-adjusted odds ratios (ORs) and 95% confidence intervals (CIs). A multivariate OR model was also developed with adjustment for age, smoking status, number of lifetime sex partners, and type of contraceptive. Age-adjusted results are presented herein; both models yielded similar results, and the model with fewer adjustment parameters was more robust.

Group 4 included all 354 women in the control group who had no evidence of HPV on day 1 (i.e., negative PCR for 14 HPV types [9vHPV types 6/11/16/18/31/33/45/52/58 and HR-HPV types 35/39/51/56/59], seronegative results for all 9vHPV types, and normal Pap findings),

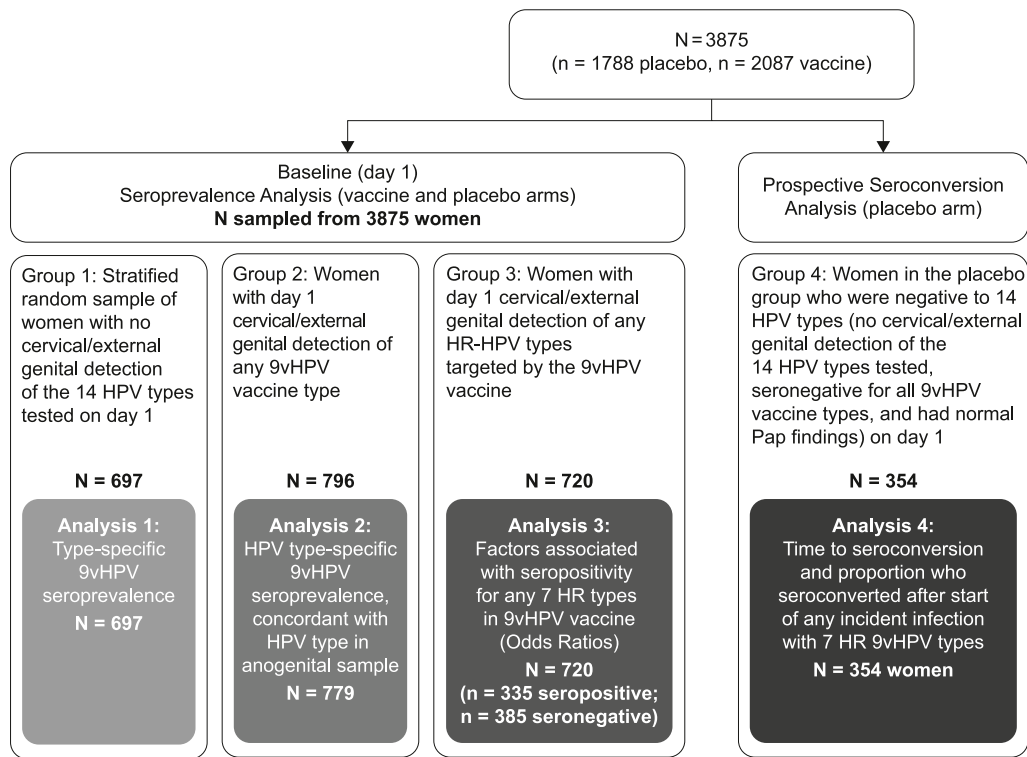


Fig. 1. Serologic sample selection and analyses based on cervical/external genital HPV DNA detection on day 1. Of 3882 subjects enrolled, 3875 subjects received either vaccine or placebo and were included in this analysis. 9vHPV = 9-valent HPV, HPV = human papillomavirus, HR = high-risk, Pap = Papanicolaou.

and then had ≥ 1 HPV type detected by PCR in a cervical/external genital sample at a subsequent trial visit. Among this group, serology was tested by HPV-9 cLIA for the day 1 visit, as well as the trial visit with incident cervical/external genital detection of a 9vHPV vaccine HR-HPV type, and all subsequent visits. Among these 354 women, the incidence of type-specific seroconversion after the date of the first positive cervical/external genital HPV detection was estimated, including incident-

persistent infection, defined as the detection of the same HPV type in cervical/external genital swabs collected on ≥ 2 consecutive visits spaced ≥ 6 months apart (± 4 weeks). Seroconversion data for each HPV type were truncated at 30 months after the first cervical/external genital detection, because by this time, most women had completed their trial follow-up visits. Kaplan-Meier methods were used in this analysis.

All analyses were conducted using SAS version 9.3.

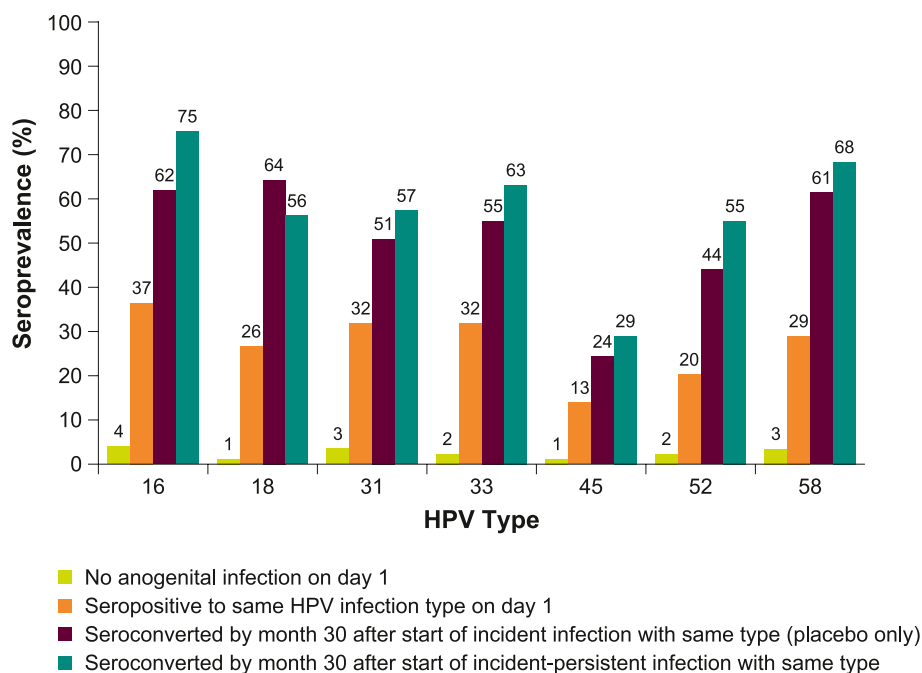


Fig. 2. Type-specific HPV seropositivity by cervical/external genital infection status on day 1 among 3875 women 16–23 years old (1788 in placebo group). HPV = human papillomavirus.

3. RESULTS

Of the 697 young women with no cervical/external genital HPV detection on day 1 (Group 1, 18.0% of all trial participants), 96.4%–99.4% were also seronegative (Fig. 2; Supplementary Table S1). The highest seropositivity in this group was for HPV 16 (3.6%), and the lowest seropositivity was for HPV 45 (0.7%) (Fig. 2; Supplementary Table S1).

Among the 796 women with any 9vHPV cervical/external genital HPV detection on day 1 (Group 2, 20.5% of all trial participants), seropositivity concordant with the same type measured in cervical/external genital samples on day 1 was highest for HPV 6 (38.5%), HPV 16 (36.5%), and HPV 31 (32.4%), and lowest for HPV 45 (13.4%) (Fig. 2; Supplementary Table S1).

The association between baseline characteristics and seropositivity was assessed for 720 women who had cervical/external genital detection of any HR 9vHPV vaccine type on day 1 (Group 3). Women aged 21–23 years were 48% more likely than women aged 16–20 years to be seropositive for a HR 9vHPV vaccine type on day 1 (OR [95% CI], 1.48 [1.10–1.99]) (Table 1). Women from North America were also generally more likely to be seropositive relative to women from other regions. Younger age at first intercourse and a higher number of lifetime sex partners were associated with seropositivity (Table 1). Relative to women with normal cervical cytology on day 1, women with ASC-US or LSIL were more likely to be seropositive, with ORs (95% CI) of 2.73 (1.55–4.81) and 1.88 (1.25–2.82), respectively. The sample size was small for women with ASC-H/HSIL, and findings were not significantly associated with seropositivity (OR [95% CIs], 1.32 [0.50–3.50]) (Table 1).

Among the 354 women in the placebo group whose results were negative for 14 HPV types on day 1 and then developed an incident (new) cervical/external genital infection with any HR 9vHPV vaccine type (Group 4, 19.8% of women in the control group), HPV 16, 52, and 31 were the most commonly detected types in the cervical/external genital samples (Table 2). Most infections persisted, ranging from 60% of HPV 58 infections to 76% of HPV 33 infections (Supplementary Table S2). Median time to seroconversion varied by HPV type, but generally ranged from 12 to 18 months after start of incident or incident-persistent infection (Table 2).

The cumulative incidence of seroconversion of any HR 9vHPV vaccine type (16/18/31/33/45/52/58) within 30 months after start of an incident or incident-persistent cervical/external genital infection with the same type generally exceeded 50%, except for HPV 45 (seroconversion 24% and 29% after start of incident and incident-persistent infection, respectively) and HPV 52 (44% seroconversion after start of incident infection) (Table 2; Fig. 3A and B; Supplementary Table S2). Seroconversion rates were highest for women with incident HPV 16 infections (62% after start of incident infection; 75% after start of incident-persistent infection). Point estimates for seroconversion rates were generally higher among women who developed incident-persistent infections than among those who developed any infection (persistent or not); however, CIs overlapped. CIs also overlapped among all HPV types, except for HPV 45 and 16, and for HPV 45 and 18 (Table 2; Fig. 3A–D; Supplementary Table 2).

4. Discussion

In this cohort of 3875 women aged 16–23 with ≤ 4 lifetime sex partners at day 1, approximately 20.5% had prevalent cervical/external genital HPV detection containing ≥ 1 9vHPV vaccine type, of whom approximately 20%–40% were also seropositive to that same HPV type on day 1 (except for HPV 45, with 13% seropositive). Most women had no cervical/external genital HPV detection on day 1; among a sample of these women who also had normal cervical cytology results at day 1, almost all were seronegative ($\leq 3.6\%$ of women were seropositive for any given 9vHPV type). Therefore, in this population of younger

women, most were likely HPV naïve at baseline. Approximately 19.5% of women in the placebo group who had negative results for 14 HPV types and had normal cervical cytology results on day 1 developed an incident cervical/external genital infection during the trial. In general, more than 50% of women seroconverted to the same genotype in their swabs within 30 months after start of infection. Median seroconversion times generally ranged between 12 and 18 months. The highest seroconversion rate was for HPV 16 (75% seroconversion after start of incident-persistent infection) and the lowest was for incident HPV 45 infections (24% seropositive by month 30). CIs for seroconversion rates overlapped except for HPV 45, suggesting no significant differences between HPV types.

Our results are consistent with those from cross-sectional studies conducted in the Netherlands and China, which also demonstrated significant type-specific associations between cervical HPV DNA detection and seropositivity to HPV types 16/18/31/33/45/52/58 [14,15,25]. An analysis of a large, multiethnic cohort of women in the Netherlands (HELIUS study; median subject age 27 years) also identified significant type-specific associations between cervico-vaginal HR-HPV DNA detection and seropositivity for the 7 HR-HPV types tested [15]. Seropositivity among women in our study with no cervical/external genital HPV DNA was lower compared with seropositivity among women in the HELIUS study who had no vaginal HR-HPV DNA (e.g., HPV 16 seroprevalence, 3.6% vs 13%). In contrast, seropositivity among women with cervical/external genital HPV DNA detection was higher in our study compared with the HELIUS study (36.5% vs 19%). Differences in ages, risk factors, and analysis methods may account for these findings.

Strong correlations between prevalent HPV infection and seropositivity were also reported in adult women (18–64 years) from the United States, Costa Rica, and Slovenia [3,16,26]. The population-based cohort of 10,049 adult women in Costa Rica demonstrated that prevalent cervical HPV 16 DNA positivity was associated with a 10-fold increase in the risk of HPV 16 seroconversion [26]. More recently, a study of adult women aged 20–64 years participating in Slovenia's national cervical cancer screening program reported statistically significant associations between cervical HPV detection at baseline and seropositivity for HPV types 16/31/39/45/52/56/58/59 3 years later [16]. Women who were HPV DNA-positive and seronegative for these types were 3.9–46.2 times more likely to have seroconverted compared with women who were HPV DNA-negative and seronegative [16].

Although the relationship between incident HPV infection and seropositivity has also been previously assessed, most studies focused only on HPV 6/11/16/18 [3,27,28]. In studies of adolescent and young women from the United States, incident HPV 6/16/18 infection was associated with seroconversion rates of 54%–69% after 12–18 months of follow-up [3,27]. Higher seroconversion rates after incident infections with HPV 16 (74.4%) or 18 (68.4%) were reported among females aged 15–17 years [28]. Few studies have investigated the temporal pattern of antibody response for other HPV types. We found that seroconversion by month 30 was highest for HPV 16/18/58, followed by 33/31/52, whereas seroconversion after incident HPV 45 infection was much lower (Fig. 2; Supplementary Table S1). The explanation for why there is a lower degree of seroconversion to HPV 45 is not clear but may be related to the specific antigen targeted by the antibody used in the cLIA assay, or due to a true lower immunogenicity of the HPV 45 virion. Differences in the temporal patterns of the antibody response were also observed across types. Incident-persistent HPV infection led to higher seroconversion rates, consistent with the hypothesis that type-specific HPV seropositivity may act as a marker of cumulative HPV exposure, although it appears to underestimate the true cumulative burden of infection [4,5].

Understanding the potential protective effect of naturally acquired HPV antibodies on subsequent infections is useful. Young women (15–25 years) enrolled in the control arms of the Costa Rica Vaccine Trial and the PATRICIA Trial who had naturally acquired HPV 16 or HPV 18 seropositivity at baseline had partial protection against

Table 1
Association between selected baseline characteristics and HPV seropositivity at enrollment among 720 women with prevalent cervical/external genital HPV detection with any 7 HR 9vHPV vaccine types (group 3), placebo, and vaccine arms combined.

Baseline Characteristics (m = number in row)	Seronegative (%) for All HR Types n = 385	Seropositive (%) ^a			Age-adjusted OR for HPV Seropositivity (95% CI)		
		16/18 n = 194	31/33/45/52/58 n = 207	16/18/31/33/45/52/58 n = 335	16/18	31/33/45/52/58	16/18/31/33/45/52/58
Age (mean/SD)	20.2 (1.74)	20.5 (1.82)	20.7 (1.79)	20.5 (1.80)	–	–	–
Age, years (5-year categories)							
16–20 (m = 375)	217 (57.9)	94 (25.1)	88 (23.5)	156 (41.6)	1.00	1.00	1.00
21–23 (m = 351)	168 (47.9)	100 (28.5)	119 (33.9)	179 (51.0)	1.37 (0.97–1.94)	1.75 (1.24–2.46)	1.48 (1.10–1.99)
Region							
North America (m = 231)	118 (51.1)	77 (33.3)	58 (25.1)	111 (48.1)	1.00	1.00	1.00
Asia (m = 18)	13 (72.2)	1 (5.6)	4 (22.2)	5 (27.8)	0.11 (0.01–0.87)	0.57 (0.18–1.84)	0.38 (0.13–1.09)
Europe (m = 150)	64 (42.7)	54 (36.0)	47 (31.3)	84 (56.0)	1.25 (0.78–1.98)	1.43 (0.87–2.35)	1.35 (0.89–2.05)
Latin America (m = 267)	147 (55.1)	52 (19.5)	86 (32.2)	118 (44.2)	0.52 (0.34–0.81)	1.16 (0.77–1.76)	0.83 (0.58–1.19)
Oceania (m = 60)	43 (71.7)	10 (16.7)	12 (20.0)	17 (28.3)	0.37 (0.17–0.78)	0.59 (0.29–1.21)	0.43 (0.23–0.80)
Smoking status							
Never (m = 403)	207 (51.4)	109 (27.0)	121 (30.0)	193 (47.9)	1.00	1.00	1.00
Current (m = 254)	145 (57.1)	66 (26.0)	61 (24.0)	107 (42.1)	0.86 (0.59–1.25)	0.72 (0.50–1.06)	0.80 (0.58–1.10)
Former (m = 69)	33 (47.8)	19 (27.5)	25 (36.2)	35 (50.7)	1.07 (0.58–1.97)	1.24 (0.70–2.19)	1.11 (0.66–1.86)
Age at first intercourse, years							
≤17 (m = 459)	232 (50.5)	130 (28.3)	136 (29.6)	221 (48.1)	1.00	1.00	1.00
18–19 (m = 201)	119 (59.2)	53 (26.4)	48 (23.9)	82 (40.8)	0.69 (0.46–1.03)	0.57 (0.38–0.86)	0.62 (0.44–0.89)
≥20 (m = 61)	30 (49.2)	10 (16.4)	22 (36.1)	31 (50.8)	0.47 (0.22–1.01)	0.90 (0.48–1.67)	0.84 (0.48–1.47)
P trend					0.0159	0.1182	0.0728
Type of contraceptive							
Any contraceptive except condom (m = 458)	239 (52.2)	123 (26.9)	132 (28.8)	216 (47.2)	1.00	1.00	1.00
Male/female condom use only (m = 181)	99 (54.7)	43 (23.8)	55 (30.4)	80 (44.2)	0.84 (0.55–1.27)	1.00 (0.67–1.48)	0.89 (0.63–1.26)
Condom plus other types (m = 84)	45 (53.6)	27 (32.1)	20 (23.8)	38 (45.2)	1.19 (0.71–2.02)	0.82 (0.46–1.46)	0.97 (0.60–1.55)
Number of lifetime sex partners							
1 (m = 124)	80 (64.5)	22 (17.7)	25 (20.2)	42 (33.9)	1.00	1.00	1.00
2–3 (m = 426)	210 (49.3)	123 (28.9)	135 (31.7)	214 (50.2)	2.05 (1.21–3.46)	1.94 (1.17–3.20)	1.87 (1.22–2.85)
≥4 (m = 171)	91 (53.2)	48 (28.1)	46 (26.9)	78 (45.6)	1.83 (1.01–3.31)	1.48 (0.83–2.64)	1.55 (0.96–2.52)
P trend					0.0936	0.3226	0.1496
Number of new sex partners in last 6 months							
0 (m = 433)	221 (51.0)	117 (27.0)	137 (31.6)	208 (48.0)	1.00	1.00	1.00
1 (m = 256)	137 (53.5)	70 (27.3)	65 (25.4)	117 (45.7)	0.98 (0.68–1.41)	0.78 (0.54–1.12)	0.92 (0.67–1.26)
≥2 (m = 31)	22 (71.0)	6 (19.4)	4 (12.9)	9 (29.0)	0.57 (0.22–1.47)	0.34 (0.11–1.02)	0.49 (0.22–1.09)
P trend					0.4371	0.0310	0.1608
Prior pregnancies							
None (m = 548)	300 (54.7)	148 (27.0)	146 (26.6)	242 (44.2)	1.00	1.00	1.00
Any (m = 178)	85 (47.8)	46 (25.8)	61 (34.3)	93 (52.2)	1.05 (0.69–1.58)	1.40 (0.95–2.06)	1.30 (0.93–1.83)
Papanicolaou result							
Negative (m = 499)	289 (57.9)	112 (22.4)	134 (26.9)	207 (41.5)	1.00	1.00	1.00
ASC-US (m = 60)	21 (35.0)	26 (43.3)	20 (33.3)	39 (65.0)	3.36 (1.81–6.25)	2.18 (1.13–4.19)	2.73 (1.55–4.81)
LSIL (m = 123)	53 (43.1)	44 (35.8)	43 (35.0)	68 (55.3)	2.25 (1.42–3.57)	1.85 (1.17–2.92)	1.88 (1.25–2.82)
ASC-H/HSIL (m = 17)	9 (52.9)	6 (35.3)	2 (11.8)	8 (47.1)	1.79 (0.62–5.18)	0.51 (0.11–2.42)	1.32 (0.50–3.50)

Abbreviations: ASC-H = atypical squamous cells—cannot rule out HSIL, ASC-US = atypical squamous cells—undetermined significance, HPV = human papillomavirus, HR = high-risk, HSIL = high-grade squamous intraepithelial lesion, LSIL = low-grade squamous intraepithelial lesion, OR = odds ratio; SD = standard deviation.

^a A total of 335 women were seropositive for any 7 HR HPV types; women could be seropositive to more than 1 HPV type.

Table 2

Group 4: number and percentage of women who develop incident HPV infections and median time (months) to seroconversion after start of incident cervical/external genital HPV infection with the same HPV type.^a

HPV Type	# of Women With New Infections		Time to Seroconversion After Start of Infection ^c	
	Incident infection, n (%) ^b	Incident-persistent infection, n (%) ^b	Incident infection, median (IQR), months ^c	Incident-persistent infection, median (IQR), months ^c
16	180 (50.8)	110 (61.1)	11.2 (6.3–17.0)	11.7 (7.4–17.9)
18	70 (19.8)	40 (57.1)	14.5 (10.9–18.1)	17.4 (10.9–20.1)
31	87 (24.6)	52 (59.8)	11.8 (6.8–16.3)	13.4 (9.3–19.1)
33	31 (8.8)	21 (67.7)	12.1 (5.9–13.5)	12.3 (6.6–15.3)
45	36 (10.2)	20 (55.6)	14.6 (12.9–18.2)	14.6 (13.8–17.3)
52	105 (29.7)	55 (52.4)	13.0 (7.1–21.3)	17.5 (7.7–23.3)
58	62 (17.5)	32 (51.6)	11.4 (9.3–20.8)	11.4 (9.9–21.5)

HPV = human papillomavirus, IQR = interquartile range, PCR = polymerase chain reaction.

^a Among the 354 women (denominator) in the placebo group who had normal Papanicolaou test results and negative results for all tested HPV types by PCR and serology at enrollment.

^b Number (%) of women who developed infection irrespective of serology status on date of infection, out of the 354 HPV-naive women in the placebo group at enrollment.

^c Median (IQR) number of months to HPV seropositivity after start of incident cervical/external genital infection among women remaining at risk (i.e., seronegative on start date of infection with same HPV type; start date is trial visit at which cervical/external genital HPV was first detected; time 0 on Fig. 3).

subsequent HPV 16 or HPV 18 incident HPV DNA detection, and ASC-US or worse Pap results, compared with women who were seronegative [29]. Adult women in the control group of the VIVIANE Trial who were seropositive for HPV 16 at baseline also had evidence of partial protection; however, little-to-no protection was observed among those who were seropositive for HPV 18 at baseline [30]. In an analysis of young women (16–26 years) from three 4vHPV vaccine clinical trials who were HPV DNA-negative but seropositive to a specific HPV type (6/11/16/18) at baseline, subsequent cervical or external genital disease related to one of the 4vHPV types during follow-up (approximately 40 months) was observed in some women in the placebo arm, whereas no cases of subsequent disease were observed in vaccinated women [31]. This finding suggests that naturally acquired HPV antibodies may not provide complete protection against subsequent HPV infection/activation, whereas 4vHPV vaccination appears to prevent reinfection or reactivation of disease associated with the 4vHPV types [31]. Similar findings were also observed in a randomized, placebo-controlled 4vHPV vaccine clinical trial of adult women (24–45 years), which reported more cases of HPV 6/11/16/18-related persistent infections in the placebo arm compared with the vaccinated arm (15 vs 5) among women with evidence of previous infections or exposure to HPV (i.e., seropositive but DNA negative) [32]. Taken together, these studies suggest that antibody responses elicited by natural HPV infection provide only partial protection, although reactivation of latent infection may possibly explain some repeated type-specific HPV DNA detections.

The factors associated with antibody response to HPV infection are not fully understood [33,34]. As in other studies, we found an association between HPV seropositivity and younger age of sexual debut and number of lifetime sex partners in women with HPV cervical/external genital detection on day 1 [1,4,35–41]. These factors may be correlated with earlier and more repeated exposure to HPV infection, which serve to increase the probability of seropositivity as a result of immune boosting from repeated infections [36]. There was also an unexpected trend between a higher number of recent sex partners and lower seropositivity. However, the risk estimate is imprecise due to the small number of seropositive patients with ≥ 2 recent sex partners (Table 1). The observation that a higher number of recent sex partners did not

increase the risk of seropositivity could be related to the temporal dynamics of seroconversion, because repeated exposure to the same HPV type (e.g., through the same partner) is more likely to stimulate an immune response. Alternatively, it is possible that seroconversion had not yet occurred in some of the women who had a cervical/external genital infection on day 1 because the median time to seroconversion was 12–18 months after start of infection (Table 2).

Women with HR-HPV DNA detection and abnormal cervical cytology (ASC-US or LSIL) on day 1 were approximately 2–3 times as likely to also be seropositive for any HR 9vHPV vaccine types, compared with women with normal cervical cytology findings. It is possible that type-specific antibodies are more easily generated under conditions of higher viral load, which may occur in women with ASC-US or LSIL.

Our findings were consistent with those of previous studies evaluating prevaccination HPV seroprevalence in adult women [7,38]. In a study of 3259 adult women (aged 20–64 years) participating in the Slovenian cervical screening program, seropositivity for any of 11 HR types (HPV 16/18/31/33/35/39/45/52/56/58/59) was 2.2 times more likely in women with ASC-US and 2.9 times more likely in women with LSIL, compared with women who had normal cervical cytology [7]. Similar findings were also reported in a pooled analysis of data from the enrollment visit of the Costa Rica HPV Vaccine Trial (n = 646), in which young adult women (aged 18–25) with abnormal cervical cytology (LSIL) were 1.63–2.12 times more likely to be HPV 16-seropositive. Although several studies reported no significant association between high-risk HPV seropositivity and abnormal cervical cytology, these studies had small sample sizes and the study populations were carefully selected (e.g., limited to pregnant women in the third trimester) [35,36].

This analysis has several notable strengths. Data were drawn from a large cohort of women from five continents who were followed closely for 48 months with repeated assessments. This design allowed investigation of serologic status in a diverse population of young women and provided estimates of seroconversion rates following infections with HR-HPV types other than HPV 16/18. The assay utilized in our study (HPV-9 cLIA) has high specificity and low cross-reactivity among the 9vHPV types [23], allowing with near certainty that HPV DNA and serologic associations were also type-specific in this analysis.

One limitation of the study is likely underestimation of the total antibody response to HPV by the cLIA, which uses a competitive strategy with a single, neutralizing, type-specific monoclonal antibody against each HPV type in the assay [42,43]. A higher seroprevalence might be reported with an assay that captures the entire range of antibodies elicited by infection [44]. These factors may contribute to differences among published studies. Also, subsequent incident infections or co-infections in our study population could have influenced seroconversion rates. In addition, because serologic samples were collected annually, it is possible that some women who developed an infection seroconverted and then reverted to seronegativity between sampling time frames, in which case, seroconversions would be missed. Further, the trial population comprised a selected group of young women, generally with a small number of lifetime sex partners and access to primary care, which may limit generalization to other populations. For example, given that a direct linear correlation has been previously demonstrated between HPV 16, 33, or 18 seropositivity and number of sex partners [45], censoring of the number of lifetime sex partners (0–4) in the eligibility criteria for this study is likely to impact on the generalizability of the results. Low patient numbers in some of the Pap result categories may also have limited analysis of the relationship between Pap findings and seropositivity at baseline. However, the observed association between abnormal cervical cytology and concurrent seropositivity on day 1 is as expected, supporting the notion that seroconversion in women with cytological abnormalities signals persistent HPV infection. Finally, some estimates of seroconversion are based on small sample sizes, thereby limiting their precision.

In conclusion, this longitudinal analysis of incident infection, as compared with a cross-sectional analysis of day 1 prevalent infections,

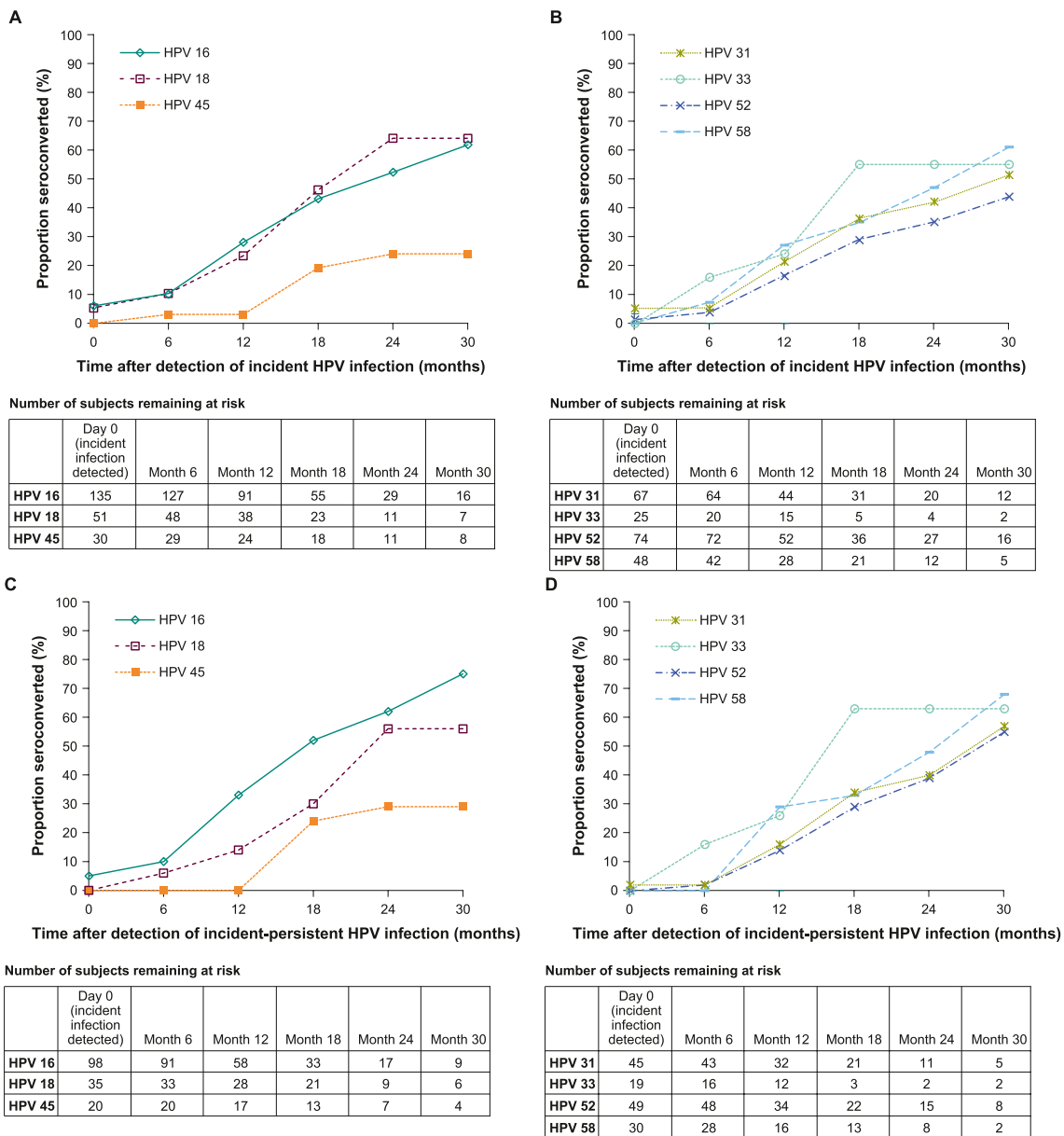


Fig. 3. Proportion of women who seroconverted over time after start of infection containing the same HPV type (placebo group, negative to all tested HPV types on day 1). Seroconversion after any incident infection with (A) HPV 16/18/45 and (B) HPV 31/33/52/58; seroconversion after any incident-persistent infection with (C) HPV 16/18/45 and (D) HPV 31/33/52/58. HPV = human papillomavirus.

provides more robust insight into the relationship between cervical/external genital infection and seropositivity.

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Author contributions

DRB, SMG, MS, CMW, and CV were involved in conception, design or planning of the study. DRB, DF, SMG, MS, CMW, and CV were involved in data acquisition. DRB, DF, MS, AS, SL, and CV were involved in analysis of the data. DRB, XC, WH, DF, SMG, MS, CMW, AS, AL, SL, and CV were involved in interpretation of the results. DRB, SMG, and CV were involved in drafting of the manuscript. All authors were involved in critically reviewing or revising the manuscript for important intellectual content and approved the final version for submission.

Data sharing statement

All relevant data is contained within the article. The datasets used and/or analyzed in the current study are available from the corresponding author on reasonable request.

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Appendix A. Supplementary data

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