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HPV infections and cytologic abnormalities in vaccinated women 21–34 years of age: Results from the baseline phase of the Onclarity trial

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HIGHLIGHTS

- The impact of HPV vaccination was determined in a cervical cancer screening population from the USA-based, Onclarity Trial.
- HPV and cytology testing were determined in 14,153 women, 21–34 years; and compared by vaccination status.
- The prevalence of overall HPV, and genotypes 16, 18, 31, and 33/58 were significantly lower in vaccinated women.
- Prevalence of ≥LSIL cytology (for any HPV result), and ≥CIN2 (only for HPV 16+ or 18+ cases), was lower in vaccinated women.
- Data in this article suggest that "catch-up" vaccination provides benefit for adolescents and young adults.

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ABSTRACT

Objectives. Countries with school-based human papillomavirus (HPV) vaccination have seen significant reductions in vaccine-targeted HPV infections, cytologic abnormalities, and high-grade cervical intraepithelial neoplasia (≥CIN2). However, the impact of HPV vaccination in the United States (where vaccination is largely opportunistic) may be less due to lower coverage rates and vaccination in patients at ages beyond the recommended routine vaccination age.

Methods. The Onclarity trial enrolled 33,858 subjects ≥21 years who were screened with cytology and the BD Onclarity HPV Assay. HPV positive women or those with cytologic abnormalities underwent colposcopy and biopsy. The prevalence of HPV, cytologic abnormalities, and ≥CIN2 was compared in a subset of 14,153, vaccinated and unvaccinated women, 21–34 years. Results were compared by vaccination status; Mantel-Haenszel analysis was performed to determine the association between vaccination status and prevalence, adjusting for age.

Results. The prevalence of overall HPV, HPV16, 18, 31, and 33/58 were all lower in vaccinated women for each age group; a significant difference ($p < 0.001$) was observed in vaccinated women for all ages combined. Cytologic low-grade squamous intraepithelial lesion (LSIL) or worse was lower in vaccinated women ($p = 0.021$), as was ≥CIN2 prevalence associated with HPV 16 or 18 ($p = 0.011$).

Conclusions. Women with a prior history of HPV vaccination have a lower prevalence of any high-risk HPV, HPV 16, 18, 31, and 33/58; a cytology result of ≥LSIL, and ≥CIN2 associated with HPV 16/18 compared to unvaccinated women. A lower HPV prevalence in older, vaccinated women suggests that "catch-up" vaccination provides benefit.

1. Introduction

The quadrivalent human papillomavirus (HPV) vaccine (4vHPV) (Gardasil; Merck & Co.) that protects against HPV 6, 11, 16, and 18 was approved for use in the United States (U.S.) in 2006 [1]. In late 2006, the Advisory Committee for Immunization Practice (ACIP) recommended its routine use in 11–12 year-old girls and as a "catch-up" vaccination for 13–26 year old females who had not yet been vaccinated [2]. Unlike countries such as the United Kingdom and Australia that have school-based vaccination programs, HPV vaccination rates in the U.S. were initially modest, but by 2017 HPV vaccination rates in girls 13–17 years of age had increased to 68.6% and 53.1% for a single dose and completion of vaccination, respectively [3–5]. In contrast,

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vaccination coverage of younger cohorts has reached nearly 90% in other, developed countries [6]. Compared to countries that have implemented school-based programs, larger proportions of vaccine recipients in the U.S. have been older than the recommended routine age of vaccination. [7] This may lead to a reduction in vaccine efficacy in the U.S. since Australian registry data has documented significant reductions in vaccine efficacy in reducing cervical disease with increasing age at vaccination (comparing vaccination at ≤14 yrs. of age with up to 17 years of age) and quantitative antibody responses to HPV vaccination are generally higher for children compared to adults [8–10].

Using data from the baseline phase of the Onclarity trial, we have compared the prevalence of genotype-specific HPV infection, cytologic results, and biopsy-confirmed cervical intraepithelial neoplasia, grade 2 or worse (≥CIN2) in vaccinated and unvaccinated subjects. This trial included 14,153 women 21–34 years of age who were undergoing routine cervical cancer screening in the U.S. There were very few vaccinated subjects older than 34 years. Liquid-based cervical cytology specimens were tested using the Onclarity HPV assay and participants who were HPV positive or had an abnormal cervical cytology result underwent colposcopy with cervical biopsies that were adjudicated by a panel of pathologists. This study design resulted in a unique opportunity to compare the prevalence of specific HPV genotypes, cytologic abnormalities, and biopsy-confirmed CIN in vaccinated compared to unvaccinated women in a large U.S. study population.

2. Materials and methods

2.1. Study design

The BD Onclarity HPV Trial is an ongoing three-year longitudinal study of the performance of a new HPV Assay for cervical cancer screening. This report includes data collected during the enrollment period (baseline phase). The design, screening procedures, inclusion/exclusion criteria, and description of cytology and HPV testing methodology has been previously described in detail [11]. Women were recruited consecutively during routine cervical cancer screening at 31 clinical sites across 17 states between August 26, 2013 and June 12, 2015. Overall, 33,858 subjects ≥21 years were recruited, of whom, 33,491 had HPV genotyping data. There was a pre-specified enrollment cap (10%) on the number of women self-reporting HPV vaccination history. The current analysis is restricted to the 14,153 subjects between the ages of 21–34 years of age who had cytology and HPV testing results. Detailed inclusion/exclusion criteria as well as the Consort Diagram have been previously described [11].

All sites obtained institutional review board approval and written informed consent from all subjects prior to any trial-related procedures. This study was conducted according to those principles outlined by the Declaration of Helsinki and by Good Clinical Practice.

2.2. Enrollment visit, cytology and HPV testing

During enrollment, women provided demographic information, and medical history including self-reported HPV vaccination history. Liquid-based cervical cytology samples were collected from each participant using BD SurePath™ media (TriPath Imaging, Inc., Burlington, NC) and was used for both cervical cytology and HPV testing. Cytological evaluation and results were reported according to the 2001 Bethesda System [12]. HPV testing was performed using the BD Onclarity™ HPV Assay (Becton, Dickinson and Company, BD Life Sciences – Diagnostic Systems, Sparks, MD) which is a multiplex real-time PCR assay that detects 14 high-risk HPV genotypes [13]. The assay results include individual genotyping for types 16, 18, 31, 45, 51, and 52. The remaining HPV types are aggregated in three groups: 33/58, 35/39/68 and 56/59/66. The specific details of the assay have been published previously [14].

2.3. Colposcopy and central pathology review

Subjects were referred to colposcopy during the trial as previously described [11]. This included subjects ≥21 years with an abnormal cytology result; subjects ≥25 years old who were HPV positive; subjects with unsatisfactory cytology; and approximately 5% of subjects (selected at random) ≥25 years who were both HPV and cytology negative. At the time of colposcopy, both study participants and colposcopists were blinded to cytology and HPV test results. A standardized colposcopy and biopsy protocols were followed.

At least two gynecological pathologists (TCW and MHS), blinded to all information except subject age, independently evaluated all biopsies. Results were reported using the three-tiered cervical intraepithelial neoplasia (CIN) terminology. If the initial two pathologists were in disagreement regarding a diagnosis, a third pathologist reviewed the slides. Consensus occurred when two of the three pathologists agreed on a diagnosis. If all three diagnoses were discordant, the specimen (s) in question were reviewed, together, by all three pathologists to achieve a consensus pathology diagnosis. When one reviewer rated a specimen as ≥CIN2, with a second reviewer scoring the same sample as <CIN2, or when at least reviewer identified a specimen as CIN2, $p16^{INK4A}$ was utilized in adjudicating a final diagnosis.

2.4. Statistical analyses

The primary outcome measures were the prevalence of HPV genotypes, cytological categories, histopathology; all stratified by the vaccination status (vaccinated or unvaccinated) and age group (21–24 years, 25–29 years, 30–34 years). Prevalence between groups were compared using a Mantel-Haenszel test and odds ratios (OR) were calculated based on the three age strata. Prevalence of HPV genotypes include mixed genotype values.

3. Results

Table 1 presents the demographics of the 14,153 women, 21–34 years of age, enrolled in the study. There were similar numbers of enrollees in each of the three age groups (21–24 years, 25–29 years, and 30–34 years). The majority of known vaccinated women (89.2%) received the 4vHPV vaccine, which targets HPV genotypes 6, 11, 16, and 18. The proportion of women that had received one or more doses of the HPV vaccine decreased with increasing age. In the 21–24 age group, 37.4% had been vaccinated whereas in the 30–34 age group only 7.3% had been vaccinated. The majority (76.3%) of vaccinated women had received all three doses of vaccine and the rate of completion of the vaccination series was similar across all age groups. Vaccination rates were lower among Black subjects than in White subjects. Black subjects accounted for 23.9% of unvaccinated subjects, but only 15.3% of vaccinated subjects. Similarly, Hispanic subjects accounted for 20.0% of unvaccinated subjects and only 12.7% of vaccinated subjects.

3.1. Prevalences of HPV results

The prevalence of any HPV genotype was significantly lower ($p < 0.001$) in vaccinated compared to unvaccinated women (Table 2). The biggest differences were for vaccine-targeted genotypes, HPV 16 and 18. The prevalence of HPV 16 in vaccinated women was lower by 83%, 58%, and 31% for subjects aged 21–24, 25–29, and 30–34 years, respectively; (OR of 0.3; $p < 0.001$ for all ages combined). The prevalence of HPV 18 was even lower in vaccinated compared to unvaccinated women across the three age groups, 89%, 79%, and 73%, respectively; (OR of 0.2; $p < 0.001$ for all ages). A lower prevalence of HPV genotypes in vaccinated subjects was not restricted to vaccine targeted genotypes but was also observed with HPV 31, as well as HPV 33/58 (pooled result). For all subjects, the OR of detecting HPV 31 in vaccinated versus unvaccinated was 0.6 ($p < 0.001$), however, the differences were

Table 1
Baseline demographics among vaccinated and unvaccinated subjects.

Age group, years	21–24		25–29		30–34		All age groups		
	VACC n = 1424	UNVACC n = 2380	VACC n = 1187	UNVACC n = 4118	VACC n = 366	UNVACC n = 4678	VACC n = 2977	UNVACC n = 11,176	Total subjects n = 14,153
Age, years									
Mean (SD)	22.5 (1.1)	22.7 (1.1)	26.6 (1.4)	27 (1.4)	31.5 (1.3)	32 (1.4)	25.3 (3.3)	28.2 (3.8)	27.6 (3.9)
Race, % (n)									
White	79.3% (1129)	66.7% (1588)	84.3% (1001)	71.9% (2959)	82.2% (301)	76.5% (3581)	81.7% (2431)	72.7% (8128)	74.6% (10,559)
Black	17.7% (252)	30.1% (717)	12.9% (153)	24.6% (1015)	13.4% (49)	20.0% (936)	15.3% (454)	23.9% (2668)	22.1% (3122)
Asian	1.2% (17)	1.1% (27)	0.9% (11)	1.2% (49)	2.7% (10)	1.5% (68)	1.3% (38)	1.3% (144)	1.3% (182)
Other[a]	1.8% (26)	2.0% (48)	1.9% (22)	2.3% (95)	1.6% (6)	2.0% (93)	1.8% (54)	2.1% (236)	2.0% (290)
Ethnicity, % (n)									
Not Hispanic or Latino	88.1% (1255)	80.6% (1918)	86.9% (1032)	80.1% (3298)	85.5% (313)	79.7% (3727)	87.3% (2600)	80.0% (8943)	81.6% (11,543)
Hispanic or Latino	11.9% (169)	19.4% (462)	13.1% (155)	19.9% (820)	14.5% (53)	20.3% (951)	12.7% (377)	20.0% (2233)	18.4% (2610)
# Vaccine doses, % (n)									
One dose	7.7% (109)		9.9% (118)		8.2% (30)		8.6% (257)		
Two doses	8.1% (115)		10.7% (127)		9.6% (35)		9.3% (277)		
Three doses	78.2% (1113)		74.5% (884)		74.9% (274)		76.3% (2271)		
Unknown	6.1% (87)		4.9% (58)		7.4% (27)		5.8% (172)		

Abbreviations: VACC, vaccinated; UNVACC, unvaccinated; SD, standard deviation.
[a] Includes American Indian, Alaska Native, Native Hawaiian, or other Pacific Islander.

restricted to the two younger age groups. Similarly, the OR of detecting HPV 33/58 in vaccinated versus unvaccinated was 0.7 ($p = 0.008$) with the largest differences seen in the two older age groups. In contrast, the prevalence of HPV 56/59/66 (pooled result) was significantly higher in vaccinated versus unvaccinated subjects; OR of 1.2 ($p = 0.019$). We also evaluated the impact of number of vaccine doses on HPV prevalence. The prevalence of HPV 16, 18, 31 and 33/58 combined was 5.0% in vaccinated subjects who had received all three doses of vaccine and 5.4% in subjects who had only received one or two doses (OR of 0.9; $p = 0.766$) (Table S1). We also compared the prevalence of ≥CIN2 and ≥CIN3 in subjects who received one or two doses and those who received three doses (Table S1). The prevalence of ≥CIN2 was significantly lower ($p = 0.011$) in subjects who received three doses as opposed to those who received one or two doses. Although a similar difference was present for ≥CIN3, the difference was not significant due to smaller number of cases.

We analyzed differences in genotype prevalence after vaccination by ethnic/racial groupings (Table S2). A lower prevalence of HPV 16 in vaccinated women was found for Hispanic, non-Hispanic, and White subjects, but not for Black subjects. Similarly, a lower prevalence of HPV 31 was not found in vaccinated Black subjects. A lower prevalence of HPV 18 was found in vaccinated women in all ethnic/racial groupings. It should be noted that the number of vaccinated Hispanics and Blacks is relatively small, only 377 and 454 subjects, respectively.

3.2. Prevalence of biopsy-confirmed cervical disease

The prevalence of biopsy-confirmed ≥CIN2 was lower in vaccinated compared to unvaccinated subjects, but the difference was not statistically significant (OR of 0.8; $p = 0.2$ for all ages) (Table 3). However, the prevalence of ≥CIN2 lesions associated with HPV 16/18 was significantly lower in vaccinated subjects (OR of 0.5; $p = 0.011$ for all ages). Biopsy-confirmed ≥CIN3 lesions, either overall or associated with HPV 16/18, were not significantly lower in vaccinated subjects.

3.3. Prevalence of cytologic abnormalities

We detected only a modest impact of vaccination status on the prevalence of cytologic abnormalities (Table 4). The prevalence of a negative for intraepithelial lesion or malignancy (NILM) result was significantly higher in vaccinated subjects ($p = 0.021$) but the difference in overall prevalence values for unvaccinated versus vaccinated subjects with NILM cytology was small (<1%). The prevalence of the two atypical squamous cell subcategories was similar in vaccinated and unvaccinated subjects. The prevalence of a cytologic result of ≥low-grade squamous intraepithelial lesion (LSIL) was significantly lower among vaccinated subjects in all age groups ($p = 0.022$), but the differences were relatively small, except in the 30–34 age group. The prevalence of ≥high-grade squamous Intraepithelial Lesion (HSIL) was slightly lower in vaccinated subjects in all age groups, but the overall difference was not significant ($p = 0.189$).

4. Discussion

The results from the Onclarity HPV trial confirm findings from multiple countries including the U.S. that have reported a lower prevalence of vaccine-targeted HPV genotypes after the introduction of the 4vHPV vaccine [15]. We directly compared the prevalence of 14 HPV genotypes in vaccinated versus unvaccinated women. The prevalence of all HPV genotypes combined was slightly lower in vaccinated women in the three age groups that were examined; 21–24 year, 25–29 years; and 30–34 years. A much greater difference in prevalence was found in all three age groups for the two vaccine-targeted genotypes, HPV 16/18. Although we did not determine the age at which the subjects were vaccinated, based on the U.S. approval date of the 4vHPV vaccine and the subjects' age at enrollment, the youngest that subjects in the three age groups could have been at the time of vaccination is 11–16 years, 15–22 years, and 21–27 years, respectively. The finding that reductions in the prevalence of HPV 16/18 in vaccinated subjects were less in the older age groups is consistent with the results of other studies and reflects that there is a lower likelihood of a prevalent HPV infection when younger women are vaccinated [15]. The finding of a reduction in HPV 16/18 in vaccinated subjects in the oldest age group, especially for HPV 18, supports current U.S. recommendations for catch-up vaccination through 26 years [16]. The reduction in the prevalence of HPV 16 in vaccinated compared to unvaccinated subjects varied somewhat by race/ethnicity. The reasons for this variability are unclear but it may reflect differences in age at vaccination. A similar finding has been reported from the National Health and Nutrition Examination Survey (NHANES) [17].

We observed a lower prevalence of HPV 31 ($p < 0.001$) as well as of HPV 33/58 ($p = 0.008$) in vaccinated subjects. The difference in prevalence of HPV 31 was restricted to vaccinated subjects <30 years. By self-report, approximately 90% of vaccinated subjects in the current study received the 4vHPV vaccine. A 2014 WHO position paper on HPV vaccines concluded that the 4vHPV vaccine induces neutralizing antibodies to HPV 31, 33, and 52 but noted that the clinical significance and longevity of this cross-protection was unclear. A recent meta-analysis found consistent evidence of cross-protection for HPV 31 among girls ≤19 yrs. of age, but little evidence for reductions of HPV 33 and HPV

Table 2
Prevalence and OR of high-risk HPV genotypes by age groups and vaccine status[a].

Age group, years	21–24 years		25–29 years		30–34 years		All Ages	
Total subjects N = 14, 153	VACC n = 1424	UNVACC n = 2380	VACC n = 1187	UNVACC n = 4118	VACC n = 366	UNVACC n = 4678	OR (95% CI)	p-Value
HPV genotype								
Any type, (n = 3124) 22.1%	27.0%	31.7%	19.9%	23.1%	12.6%	16.0%	0.8 (0.7, 0.9)	<0.001
HPV 16, (n = 564) 4.0%	1.1%	6.3%	2.2%	5.2%	2.2%	3.2%	0.3 (0.2, 0.4)	<0.001
HPV 18, (n = 160) 1.1%	0.2%	1.8%	0.3%	1.4%	0.3%	1.1%	0.2 (0.1, 0.4)	<0.001
HPV 31, (n = 304) 2.1%	1.5%	2.6%	1.0%	2.4%	1.9%	2.1%	0.6 (0.4, 0.8)	<0.001
HPV 33/58, (n = 328) 2.3%	3.0%	3.4%	1.5%	2.6%	0.0%	1.7%	0.7 (0.5, 0.9)	0.008
HPV 45, (n = 220) 1.6%	1.6%	1.9%	1.6%	1.7%	0.5%	1.3%	0.8 (0.6 1.2)	0.353
HPV 51, (n = 356) 2.5%	4.4%	4.0%	2.2%	2.7%	0.8%	1.2%	1.0 (0.7, 1.2)	0.801
HPV 52, (n = 529) 3.7%	5.1%	6.3%	3.7%	3.7%	1.6%	2.2%	0.9 (0.7, 1.1)	0.169
HPV 35/39/68, (n = 754) 5.3%	7.8%	7.9%	5.5%	5.2%	3.6%	3.5%	1.0 (0.8, 1.2)	0.892
HPV 56/59/66, (n = 846) 6.0%	10.8%	9.0%	6.1%	5.2%	4.6%	3.7%	1.2 (1.0, 1.4)	0.019
HPV 16/18[b], (n = 700) 4.9%	1.3%	7.7%	2.5%	6.4%	2.5%	4.2%	0.3 (0.2, 0.4)	<0.001
Other 12 HPV types[c], (n = 2424) 17.1%	25.7%	24.0%	17.4%	16.7%	10.1%	11.9%	1.1 (0.9, 1.2)	0.425
HPV 16, 18, 31, 33/58[d], (n = 1250) 8.8%	5.4%	12.6%	5.1%	10.7%	4.4%	7.6%	0.4 (0.4, 0.5)	<0.001
Other nine HPV types[e], (n = 1874) 13.2%	21.6%	19.1%	14.8%	12.4%	8.2%	8.4%	1.2 (1.0, 1.3)	0.009

Abbreviations: OR, odds ratio, HPV, human papillomavirus; VACC, vaccinated; UNVACC, unvaccinated; CI, confidence interval.
[a] Percent values represent genotype prevalence by vaccination status within columns.
[b] Some HPV 16 or 18 results include mixed results with one or more of genotypes 31, 33/58, 45, 51, 52, 35/39/68, and 56/59/66.
[c] Only includes genotypes 31, 33/58, 45, 51, 52, 35/39/68, and 56/59/66.
[d] Some HPV 16 18, 31, or 33/58 results include mixed results with one or more of genotypes 45, 51, 52, 35/39/68, and 56/59/66.
[e] Only includes genotypes 45, 51, 52, 35/39/68, and 56/59/66.

45 in this age group [18]. The results of the meta-analysis were heterogeneous for reductions in HPV 31, 33, and 45 in women 20–24 years. Recent data from NHANES found no vaccine effectiveness against HPV 31, 33, and 45 (combined) in females aged 14–24 years [19]. Conflicting results with regards to cross-protection for genotypes other than 16/18 may be due in part to technicalities in the HPV detection methodologies utilized [20]. When multiple genotypes are present in a sample, competition can occur during PCR and genotypes present in low copy numbers may go undetected due to out-competition by those at high copy numbers [21]. Because of reductions of HPV 16/18 after vaccination, it is possible that with some PCR assays, genotypes such as HPV 31 and 33/58 might be preferentially detected in vaccinated subjects, making it difficult to assess comparative reductions after vaccination. The HPV PCR assay used in this study (Onclarity) utilizes sequence-specific (non-consensus) E6/E7 DNA amplification and HPV 16, 18, and 45 are amplified separately from other HPV genotypes. In addition, the accuracy of the

Table 3
Prevalence and OR of biopsy-confirmed CIN by age group and vaccine status[a].

			21–24 years			25–29 years			30–34 years			All ages
Total subjects n = 14,153			VACC (n = 1424)	UNVACC (n = 2380)	OR (95% CI)	VACC (n = 1187)	UNVACC (n = 4118)	OR (95% CI)	VACC (n = 366)	UNVACC (n = 4678)	OR (95% CI)	Overall OR (95% CI)
Histopathology	%	(n)										
≥CIN2	2.0%	(279)	1.3% (18)	1.7% (41)	0.7 (0.8, 2.4)	2.1% (25)	2.1% (88)	1.0 (0.7, 1.6)	1.1% (4)	2.2% (103)	0.5 (0.8, 5.6)	0.8[b] (0.6, 1.1)
With HPV16/18	0.8%	(110)	0.2% (3)	0.7% (16)	0.3 (0.9, 11.0)	0.6% (7)	1.0% (40)	0.6 (0.7, 3.7)	0.3% (1)	0.9% (43)	0.3 (0.5, 24.7)	0.5[c] (0.2, 0.9)
W/o HPV16/18	1.2%	(169)	1.1% (15)	1.1% (25)	1.0 (0.5, 1.9)	1.5% (18)	1.2% (48)	1.3 (0.4, 1.3)	0.8% (3)	1.3% (60)	0.6 (0.5, 5.0)	1.1 (0.7, 1.6)
≥CIN3	0.8%	(119)	0.4% (6)	0.3% (8)	1.3 (0.3, 2.3)	1.1% (13)	0.9% (38)	1.2 (0.5, 1.6)	0.5% (2)	1.1% (52)	0.5 (0.5, 8.4)	1.0 (0.6, 1.7)
With HPV16/18	0.5%	(65)	0.1% (2)	0.3% (6)	0.6 (0.4, 8.9)	0.5% (6)	0.5% (22)	1.0 (0.4, 2.6)	0.0% (0)	0.6% (29)	0.0 (N/A)	0.6 (0.3, 1.4)
W/o HPV 16/18	0.4%	(54)	0.3% (4)	0.1% (2)	3.4 (0.1, 1.6)	0.6% (7)	0.4% (16)	1.5 (0.3, 1.6)	0.5% (2)	0.5% (23)	1.1 (0.2, 3.8)	1.6 (0.8, 3.2)

Abbreviations: OR, odds ratio; CIN; cervical intraepithelial neoplasia; VACC, vaccinated; UNVACC, unvaccinated; CI, confidence interval; HPV, human papillomavirus; w/o, without; N/A not applicable.
[a] Percent values represent genotype prevalence by vaccination status within columns.
[b] p-Value =0.2.
[c] p-Value =0.011.

Table 4
Prevalence and OR of cytologic results by age group and vaccine status[a].

Total subjects (N = 14,153)			21–24 years		25–29 years		30–34 years		All ages
			VACC (n = 1424)	UNVACC (n = 2380)	VACC (n = 1187)	UNVACC (n = 4118)	VACC (n = 366)	UNVACC (n = 4678)	Overall OR (95% CI)
Cytology,	(n)	%							
NILM	(n = 12,448)	88.0%	86.1%	84.0%	88.6%	87.3%	92.1%	90.6%	1.2[b] (1.0, 1.3)
ASC-US	(n = 986)	7.0%	7.5%	8.9%	7.1%	7.2%	6.3%	5.6%	0.9 (0.8, 1.1)
≥LSIL	(n = 719)	5.1%	6.4%	7.1%	4.3%	5.5%	1.6%	3.8%	0.8[c] (0.7, 1.0)
ASC-H	(n = 63)	0.4%	0.0%	0.5%	0.6%	0.6%	0.3%	0.4%	0.6 (0.2, 1.2)
≥HSIL	(n = 72)	0.5%	0.2%	0.3%	0.3%	0.5%	0.3%	0.7%	0.6[d] (0.2, 1.3)

Abbreviations: OR, odds ratio; VACC, vaccinated; UNVACC, unvaccinated; CI, confidence interval; NILM, negative for intraepithelial lesions or malignancies; ASC-US, atypical squamous cells-undetermined significance; LSIL, low-grade squamous intraepithelial lesions; ASC-H, atypical squamous cells-cannot exclude high grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion.
[a] Percent values represent genotype prevalence by vaccination status within columns.
[b] p-Value = 0.021.
[c] p-Value = 0.022.
[d] p-Value = 0.189.

assay has been validated using the WHO LabNet panel and the assay was found to be 100% proficient for detection of single and multiple HPV infections over a range of copy numbers [22]. The elimination of the potential for genotype unmasking with the HPV assay used in this study could explain why a reduction in HPV 31 and HPV 33/58 was observed here and not in previous studies.

We also observed a significant increase in prevalence of the group of nine genotypes, (after excluding HPV 16, 18, 31, and 33/58), in vaccinated versus unvaccinated subjects. This was due in part to an increased prevalence of HPV 56/59/66 (pooled). Increases in non-vaccine-targeted high-risk HPV have been reported in several other studies. A recent meta-analysis of the population-level effects of HPV vaccination documented slight increases in HPV 39 and 52 in vaccinated girls ≤19 years [18]. A longitudinal pre- and post-vaccination study of 13–26 year-old women receiving the 4vHPV vaccine found a significant increase in the prevalence of non-vaccine-targeted HPV in vaccine recipients (37.8% higher) but not among unvaccinated women. [23] Similar results were reported in 16–24 year-old women in an English study [24]. The explanation for the increase in non-vaccine-targeted high-risk HPV in vaccinated women in those studies is unclear, but again, technicalities in PCR assays could potentially explain selective increases of non-16/18 genotypes. Although it is possible that replacement of targeted HPV genotypes is occurring to some extent following vaccination, the OR associated with HPV 56/59/66, while significant, was small (OR = 1.2). Further, confounding factors, including race/ethnicity, preclude any definitive conclusion that replacement by non-vaccine-targeted genotypes, such as 56/59/66, is occurring in response to a decrease in HPV 16/18 (and/or 31, 33/58).

In countries with school-based vaccination programs there have been dramatic reductions in ≥CIN2 in younger women. Within three years after vaccination was begun in Australia a reduction in the incidence of high-grade cervical lesions in girls <18 years was found [25]. A subsequent linkage study between vaccination and cytology registries confirmed that the reductions in CIN were higher in vaccinated compared to unvaccinated women [10]. Similar studies from both Denmark and Sweden have documented reductions in ≥CIN2 shortly after the introduction of the HPV vaccine in young age groups with high vaccination coverage [26,27]. In all of these studies the reductions in ≥CIN2 were greater in females who were vaccinated at a younger age. U.S. population-based registries from both Connecticut and New Mexico have documented reductions of CIN in young women after the introduction of vaccination [28,29]. In Connecticut the prevalence of ≥CIN2 declined by 30–74% in 21–26 year old females with the greatest declines occurring in the youngest women [28]. In New Mexico, a significant population-level decrease in the incidence rate of all grades of CIN was found in 15–19 year old females. A reduction in CIN2 incidence was also found in 20–24 year old females, but in 25–29 year old females the incidence of CIN2 increased [29]. In New York state, the incidence of ≥CIN2 in women 21–29 years decreased from 770 per 100,000 women to 465 after the introduction of the HPV vaccine [30]. However, these studies also noted significant reductions in cervical cancer screening across the same time periods and some of the U.S. studies have concluded that declines in ≥CIN2 were more likely due to reduced screening than to vaccination [31]. A recent case-control study of women enrolled in Kaiser Permanente Northern California who were ≤26 years in 2006 when the HPV vaccine was introduced found that one or more HPV vaccine doses confers protection against ≥CIN2. The strongest protection was found in those who received at least 3 doses and had their first dose when 14–17 years old. No significant protection against ≥CIN2 was found in those ≥21 years at the time of the first dose [32].

In contrast to the studies described above, we found the overall prevalence of ≥CIN2 in vaccinated subjects was not lower in any of the three age groups. There was, however, a lower prevalence of ≥CIN2 associated with HPV16/18 in all three age groups. Our study design most likely impacted why we did not find a reduction in the overall prevalence of ≥CIN2 in vaccinated subjects. In this study all women were screened, precluding screening frequency as a confounding factor for the decrease in ≥CIN2. Moreover, all subjects with a cytologic abnormality (irrespective of their HPV status), all HPV positive subjects ≥25 years, and a random subset of HPV and cytology negative subjects, ≥25 years, were referred to colposcopy. Population-based registries and case-control studies only have access to ≥CIN2 detected among women who have been referred to colposcopy based on standard management guidelines. Although data is limited and conflicting on this issue, it is possible that HPV 16 associated ≥CIN2 is more likely to be referred for colposcopy using standard management guidelines than is ≥CIN2 associated with other HPV genotypes [33,34]. ≥CIN2 lesions with HPV 16/18 are more likely accompanied by high-grade cytologic findings than ≥CIN2 lesions associated with other genotypes [35]. Moreover, lesion size is a determinant of the performance of both colposcopy and cervical biopsy; and HPV 16 associated ≥CIN2 lesions develop more rapidly than ≥CIN2 lesions associated with other HPV types [36,37]. Collectively, these factors could result in the preferential detection HPV 16 associated ≥CIN2 lesions in population-based registries and case control studies [38].

This study has a number of strengths and limitations. Its strengths include its large size, the fact that a clinically validated HPV genotyping assay was used, that the samples were not archived, and that all cervical

biopsies underwent a blinded consensus pathology review. Another strength is that all HPV positive women, as well as a random subset of women negative by both cytology and HPV testing, underwent colposcopy using a standardized protocol. A limitation of the study is that, by design, the proportion of HPV vaccinated women was limited to 10% of total women. Therefore the rate of vaccination by age group among enrollees does not necessarily reflect the vaccination rate in women presenting for enrollment or in the U.S. population. If more HPV vaccinated subjects had been enrolled we may have been able to better assess reductions in CIN after vaccination. Analysis for some subgroups was limited due to small numbers of women in a few specific categories such as vaccinated women 30–34 years with ≥CIN2. As another limitation this study relied on self-reported vaccination history and the exact age of vaccination and the timing of doses is not known.

5. Conclusion

In agreement with previous findings, the greatest differences in prevalence in vaccinated women across all age groups, compared to unvaccinated women, were observed with the HPV 16 and 18 genotypes. Differences were also observed across all age groups in the prevalence for HPV 31 and 33/58. Although vaccination did not affect overall prevalence of ≥CIN2 or ≥CIN3 at baseline, it was associated with fewer ≥CIN2 cases that were HPV16/18 positive.

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Potential conflicts of interest

MHS serves as a consultant in clinical trial design and as an expert pathologist for HPV vaccine and/or diagnostic trials for Becton, Dickinson and Company, BD Life Sciences – Diagnostic Systems, Roche, Inovio Pharmaceuticals and Merck and as a speaker for Roche and Becton, Dickinson and Company, BD Life Sciences.

TCW serves as a consultant in clinical trial design and as an expert pathologist for HPV vaccine and/or diagnostic trials for Becton, Dickinson and Company, BD Life Sciences – Diagnostic Systems, Roche, and Inovio Pharmaceuticals and as a speaker for Roche and Becton, Dickinson and Company, BD Life Sciences – Diagnostic Systems.

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