Research Article

Cancer Epidemiology, Biomarkers & Prevention

Attribution of 12 High-Risk Human Papillomavirus Genotypes to Infection and Cervical Disease

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Abstract

Background: We estimated the prevalence and incidence of 14 HPV types (6/11/16/18/31/33/35/39/45/51/52/56/58/59) in cervicovaginal swabs, and the attribution of these human papilloma virus (HPV) types in cervical intraepithelial neoplasia (CIN), and adenocarcinoma *in situ* (AIS), using predefined algorithms that adjusted for multiple-type infected lesions.

Methods: A total of 10,656 women ages 15 to 26 years and 1,858 women ages 24 to 45 years were enrolled in the placebo arms of one of three clinical trials of a quadrivalent HPV vaccine. We estimated the cumulative incidence of persistent infection and the proportion of CIN/AIS attributable to individual carcinogenic HPV genotypes, as well as the proportion of CIN/AIS lesions potentially preventable by a prophylactic 9-valent HPV6/11/16/18/31/33/45/52/58 vaccine.

Results: The cumulative incidence of persistent infection with ≥1 of the seven high-risk types included in the 9-valent vaccine was 29%, 12%, and 6% for women ages 15 to 26, 24 to 34, and 35 to 45 years, respectively. A total of 2,507 lesions were diagnosed as CIN or AIS by an expert pathology panel. After adjusting for multiple-type infected lesions, among women ages 15 to 45 years, these seven high-risk types were attributed to 43% to 55% of CIN1, 70% to 78% of CIN2, 85% to 91% of CIN3, and 95% to 100% of AIS lesions, respectively. The other tested types (HPV35/39/51/56/59) were attributed to 23% to 30% of CIN1, 7% to 14% of CIN2, 3% to 4% of CIN3, and 0% of AIS lesions, respectively.

Conclusions: Approximately, 85% or more of CIN3/AIS, >70% CIN2, and approximately 50% of CIN1 lesions worldwide are attributed to HPV6/11/16/18/31/33/45/52/58.

Impact: If 9-valent HPV vaccination programs are effectively implemented, the majority of CIN2 and CIN3 lesions worldwide could be prevented, in addition to approximately one-half of CIN1. *Cancer Epidemiol Biomarkers Prev; 1–12. ©2014 AACR.*

Introduction

Following the completion of 3 worldwide clinical studies conducted in more than 20,500 women ages 16 to 26 years (1–3), a quadrivalent HPV6/11/16/18 L1 virus–like particle (qHPV) vaccine was approved by the U.S. Food and Drug Administration (FDA) in 2006 for the

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prevention of HPV6/11-related genital warts, and HPV16/18-related cervical cancers and precancers. It was subsequently approved in 2008 for preventing vulvar and vaginal cancers and precancers (4). The indication was then further expanded to males ages 9 to 26 years for the prevention of genital warts and to males and females ages 9 to 26 years for the prevention of anal cancers and precancers attributed to HPV6/11/16/18 (5, 6). In addition, the qHPV vaccine has been shown to be safe and highly efficacious in preventing anogenital infection and neoplasia in women ages 24 to 45 years (7). The vaccine is approved in 132 countries, including Australia, Canada, and a number of European, African, Latin American, and Asian countries.

Recent data from the Centers for Disease Control and Prevention (CDC) have shown a 56% decline in HPV vaccine–type infections in teenage girls since HPV vaccine licensure (8). In Australia, the qHPV vaccination program has led to a significant reduction in HPV prevalence and genital warts, as well as high-grade cervical lesions in young women (9–13). Marked reductions in HPV prevalence and/or genital warts have also been observed in the United States, Sweden, and Denmark (8, 14–17). Six years after licensure of the qHPV vaccine in Denmark, a reduced risk of cervical lesions has also been observed at the population level (18).

In 1995, the International Agency for Research on Cancer classified HPV16/18 as cervical carcinogens, and in 2011, the group was expanded to include HPV31/33/35/39/45/51/52/56/58/59 (19). After HPV16/18, HPV31/33/35/45/52/58 are the most frequent types detected in invasive cervical cancer worldwide (19). Several previous studies have since estimated the prevalence of 7 high-risk HPV types that are targeted by an investigational 9-valent HPV (9vHPV) vaccine (i.e., HPV16/18/31/33/45/52/58) in invasive cervical cancer and precancers (20). Using a proportional attribution method to adjust for lesions with multiple HPV types detected (21), a recent study of 8,977 HPV-positive cervical cancer specimens found the relative contribution of the 7 types worldwide to be 89%, with good consistency across regions (20). Although there is consistency for the overall attribution of the 7 types in invasive cervical cancer, the estimates in the literature for precancerous cervical lesions or high-grade abnormal cytology [cervical intraepithelial neoplasia (CIN) grades 2/3 and high-grade squamous intraepithelial lesions (HSIL)] with no adjustment for coinfected lesions (i.e., without use of the proportional or hierarchical attribution methods to adjust for lesions that contain more than one HPV type), ranged widely from 59% to 94% (20). To further our understanding of the attributable fraction of the high-risk 9vHPV vaccine types (HPV16/18/31/33/45/52/58) to cervical disease, we performed a longitudinal evaluation of HPV infection and HPV genotype distribution using four distinct mathematical approaches among 10,656 women enrolled in the placebo arms of three phase III clinical trials of the qHPV vaccine (1, 2, 7). We also evaluated the attribution of the 14 HPV types, which were tested for in the clinical trials (HPV6/11/16/18/31/33/35/39/45/51/52/56/58/59), the high-risk types that are present in a qHPV vaccine (HPV16/18), as well as those which are not present in any HPV vaccine (HPV35/39/51/56/59).

Materials and Methods

Study designs and population

Between December 2001 and May 2003, 17,622 women ages 15 to 26 years were enrolled in 1 of 2 randomized, double-blind, placebo-controlled trials of the qHPV vaccine (protocols 013 and 015; refs. 1 and 2). Between June 2004 and April 2005, 3,819 women ages 24 to 45 years were enrolled in a third double-blind, placebo-controlled trial of the same vaccine (protocol 019; ref. 7). As protocols 013 and 015 enrolled only a small percentage of women above the age of 23 years, the age range for protocol 019 was chosen to overlap with the previous phase III studies. The study designs, protocols, and results of the primary hypotheses for each of the studies have been previously described and are summarized in Supplementary Table S1 (1, 2, 7). The studies were conducted in accordance with principles of Good Clinical Practice and were approved by the appropriate institutional review boards and regulatory agencies.

Analyses for infection

The following genital swab specimens were obtained from all subjects across all trials: an endo/ectocervical swab (one specimen) and a combined labial/vulvar/perianal swab. For all 3 studies, prevalence of HPV infection at day 1 was assessed in the vaccine and placebo arms combined to increase precision (given the randomized nature of the trials, it is expected that the placebo arm infection prevalence will be similar to the prevalence in the combined trial arms). In each study, day 1 swabs were tested for 14 HPV types (6/11/16/18/31/33/35/39/45/51/52/56/58/59) using a PCR-based assay as described in the Supplementary Material (22–24). In a subset of subjects in protocol 013, swabs collected at months 3 and 7 were tested for 11 HPV types (6/11/16/18/31/33/35/45/52/58/59), and those collected at months 12-18-24-30-36-42-48 were tested for 9 HPV types (16/18/31/33/35/45/52/58/59). In protocol 019, all swabs obtained at day 1 and months 7-12-18-24-30-36-42-48 were tested for all 14 HPV types.

Disease attribution

All biopsies and excisional procedure specimens were tested for the 14 HPV types as described in the Supplementary Material. All specimens were processed and adjacent histologic sections of each specimen were first read for clinical management by pathologists at a central laboratory (Diagnostic Cytology Laboratories) and then read for endpoint determination by a panel of up to 4 pathologists who were blinded to central laboratory and clinical diagnoses, treatment group, and HPV status. The

following histologic endpoints were included in the analyses reported here: CIN grade 1 to 3, and/or *adenocarcinoma in-situ* (AIS). There were no cases of cervical cancer.

Statistical analysis

As one objective of this study was to estimate the prevalence of 12 high-risk HPV types in cervical disease by age, protocols 013 and 015 were combined for the younger age cohort (i.e., women ages 15–26 years). Although 14% of the women enrolled in protocol 019 were ages 24 to 26 years, they were not included in the younger cohort because of the differing inclusion criteria (i.e., no limit on lifetime number of sexual partners and the allowance of a history of a past cervical biopsy).

The cumulative probability of acquiring an incident HPV infection was estimated by using the Kaplan-Meier method. Analyses for incident infection were restricted to placebo recipients who were seronegative to HPV6/11/16/18, DNA negative for the 14 tested HPV types, and had a normal Pap test result at day 1. For each subject, an incident HPV infection was defined as the first positive result after having had only negative results, with time to acquisition being the date of the first positive visit. A persistent incident infection was defined as detection of a new HPV infection in genital swabs collected on at least 2 consecutive visits spaced at least 6 months apart (±4 week window), with time to acquisition defined as the date of the second consecutive positive visit. If a woman were positive at only the last visit, it was counted as an incident infection.

Analyses for the prevalence of the 14 tested HPV types in cervical lesions (defined as a biopsy or surgical excisional specimen) was performed in 10,656 women randomized to the placebo arms of the 3 studies [representing 99% of the total number randomized ($N = 10{,}720$) to the placebo arms]. Because a woman may develop more than one lesion during the studies, an individual can be counted multiple times in the tables and figures.

We used 4 approaches to estimate the attribution of individual HPV types to cervical lesions, with 2 approaches to adjust for lesions with more than 1 HPV type detected. For each of the 4 analyses, all lesions (i.e., both HPV positive and HPV negative) were included in the denominator, as the HPV-negative lesions may have been caused by a nontested type.

Minimum (Min) estimate. The minimum estimate of attribution was calculated by including in the numerator only those lesions where a respective HPV type was present as a single infection. In a separate analysis, only lesions with a single type detected were included in *both* the numerator and denominator (Fig. 1).

Proportional (Prop) attribution estimate. Consistent with the literature (25, 26), this estimate was calculated following the method of Insinga and colleagues, (21), whereby a fractional allocation for each individual HPV type was used when evaluating multitype infected lesions. This was based on the relative number of instances in which each HPV type was observed as a single infection in a lesion of a given grade. For example, if one were to derive an apportionment for 2 CIN3 lesions found to test positive for HPV16 and 51, and if there were 9 CIN3 lesions with HPV16 only and a single CIN3 lesion with HPV51 only, then $[2 \times 9/(9 + 1)]$ or 1.8 of these 2 multitype infected lesions would be attributed to HPV16 and $[2 \times 1/(9 + 1)]$ or 0.2 attributed to HPV51.

Hierarchical. A modified version of the hierarchical attribution estimate of Wentzensen and colleagues was also performed for lesions with more than one HPV type detected (27). We attributed the cervical lesion to the HPV type that is most commonly detected in invasive cervical cancer (28). For example, a lesion with HPV16 and 59 would be attributed to HPV16. A lesion was attributed to HPV31/33/45/52 or 58 (i.e., the additional high-risk HPV types included in the investigational 9vHPV vaccine), only if there were no coinfection with HPV16 and/or HPV18; and to HPV35/39/51/56/59 (i.e., the other high-risk HPV types tested, which are less commonly detected in invasive cervical cancers; ref. 28) only if there were no coinfection with HPV16/18/31/33/45/52 and/or HPV58.

Any type estimate. This estimate was calculated by including in the numerator any lesion in which a respective HPV type was present, regardless of coinfection with other types.

Results

Baseline characteristics

The baseline characteristics of the study participants have been previously described (1, 2, 7). Briefly, the mean age at enrollment was 20.0 and 34.3 years in protocols 013/015 and 019, respectively. Protocol 019 enrolled a higher proportion of subjects from Asia-Pacific and Latin America (31% and 42%) than protocols 013/015 (4% and 31%). Although protocol 019 did not have a limit on the lifetime number of sexual partners at enrollment, the mean number (2) was the same as the studies in younger women whereby the lifetime number was limited to 4.

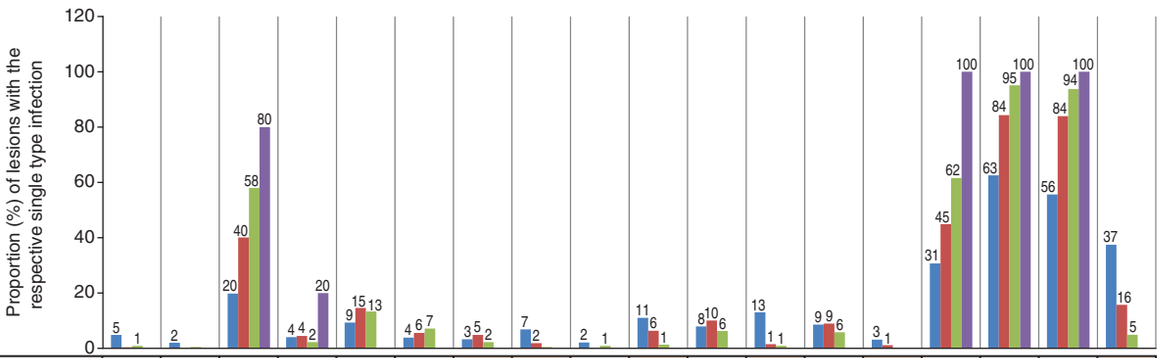
Incidence and prevalence of HPV infection

The overall prevalence of any HPV infection at day 1 (vaccine and placebo arms combined) was higher for the women ages 15 to 26 years (33%) than the groups ages 24 to 34 (30%) years and 35 to 45 years (20%; Table 1). For the HPV types contained in the 9vHPV vaccine, the overall prevalence was 25%, 20%, and 13% in these 3 age groups. The nonvaccine types (HPV35/39/51/56/59) comprised 19%, 17%, and 11% of all HPV infections detected at day 1 in these age groups, respectively.

When considering women who were randomized to the placebo arms and who were negative to the 14 types at day 1, the overall cumulative incidence of any infection with ≥1 of the high-risk types contained in the 9vHPV vaccine was 42%, 20%, and 14% for women ages 15 to 26, 24 to 34, and 35 to 45 years, respectively (Table 2). The cumulative incidence of persistent infection with ≥1 of the high-risk

A

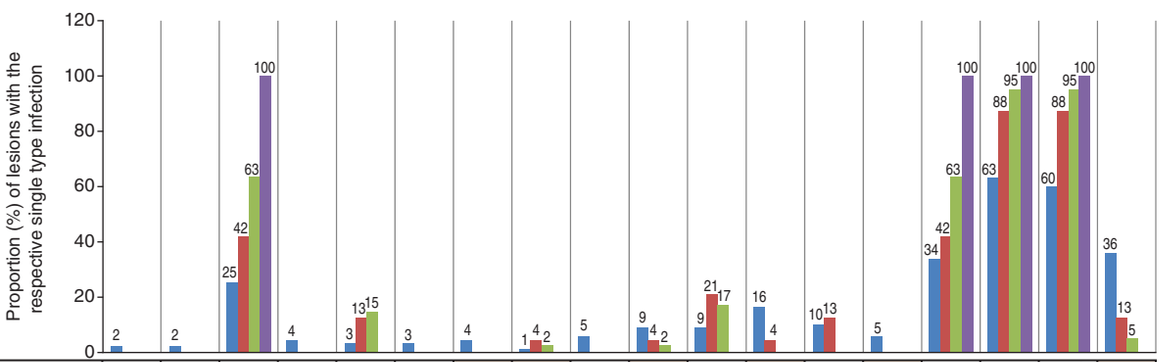
Single infections in women ages 15–26 (protocols 013 and 015). Only lesions with a single type infection are included in both the numerator and the denominator.



HPV type(s)	6	11	16	18	31	33	35	39	45	51	52	56	58	59	q HPV*	9v HPV†	HR‡	Non§
No. of lesions CIN1	34	14	138	28	65	27	23	48	15	77	55	91	60	22	214	436	388	261
No. of lesions CIN2	1	0	107	12	39	15	13	5	0	17	27	4	24	3	120	225	224	42
No. of lesions CIN3	2	1	130	5	30	16	5	1	2	3	14	2	13	0	138	213	210	11
No. of lesions AIS	0	0	8	2	0	0	0	0	0	0	0	0	0	0	10	10	10	0

B

Single infections in women ages 24–45 in protocol 019. Only lesions with a single type infection are included in both the numerator and the denominator.



HPV type(s)	6	11	16	18	31	33	35	39	45	51	52	56	58	59	q HPV*	9v HPV†	HR‡	Non§
No. of lesions CIN1	2	2	23	4	3	3	4	1	5	8	8	15	9	5	31	58	55	33
No. of lesions CIN2	0	0	10	0	3	0	0	1	0	1	5	1	3	0	10	21	21	3
No. of lesions CIN3	0	0	26	0	6	0	0	1	0	1	7	0	0	0	26	39	39	2
No. of lesions AIS	0	0	3	0	0	0	0	0	0	0	0	0	0	0	3	3	3	0

Figure 1. Prevalence of HPV infections in HPV-positive cervical lesions containing only single HPV type infections among women enrolled in protocols 013 and 15 (i.e., ages 15–26 years; A) and women enrolled in protocol 019 (i.e., ages 24–45 years; B). *qHPV, positive for an qHPV vaccine type (6/11/16/18); †9vHPV, positive for an 9vHPV vaccine type (6/11/16/18/31/33/45/52/58); ‡HR, positive for a high-risk 9vHPV vaccine type (16/18/31/33/45/52/58); §Non, positive for a nonvaccine type (35/39/51/56/59).

types contained in the 9vHPV vaccine among these 3 age cohorts was 29% and 12%, and 6% respectively. Of note, for the 15- to 26-year-old cohort, the follow-up time for incident HPV6/11 infection was only 18 months (the swabs in protocols 013 and 015 were not generally tested for these low-risk types after month 7), whereas in the

Table 1. Prevalence of HPV infection at baseline, irrespective of coinfections, vaccine and placebo arms combined

HPV type	Women ages 15 to 26 years[a] *N* = 17,590 *n* (%)	Women ages 24 to 34 years[a] *N* = 1,911 *n* (%)	Women ages 35 to 45 years[a] *N* = 1,908 *n* (%)
Any	5,590 (33)	564 (30)	379 (20)
6	716 (4)	45 (2)	26 (1)
11	119 (1)	7 (0.4)	2 (0.1)
16	1,552 (9)	116 (6)	54 (3)
18	642 (4)	48 (3)	31 (2)
31	781 (4)	59 (3)	45 (2)
33	358 (2)	14 (1)	13 (1)
35	270 (2)	30 (2)	19 (1)
39	798 (9)	68 (4)	44 (2)
45	418 (2)	40 (2)	25 (1)
51	1,268 (7)	90 (5)	51 (3)
52	984 (6)	113 (6)	57 (3)
56	1,336 (8)	147 (8)	92 (5)
58	642 (4)	61 (3)	39 (2)
59	641 (4)	56 (3)	28 (2)
Any of the nonvaccine high-risk types (35/39/51/56/59)			
	3,316 (19)	318 (17)	207 (11)
Any of the qHPV vaccine types (6/11/16/18)			
	2,59 (15)	192 (10)	106 (6)
Any of the 9vHPV vaccine types (6/11/16/18/31/33/45/52/58)			
	4,245 (25)	375 (20)	238 (13)

[a]The denominator for each type analysis excludes subjects with missing data for the respective HPV type(s). The categories are not mutually exclusive.

older cohort (the swabs in protocol 019 were tested for HPV6/11 at all time points) the follow-up time was 48 months. Despite this shorter follow-up time, the cumulative incidence of persistent HPV6/11 infection in the younger women consistently exceeded that of the older women, reflecting the increased risk of exposure to and infection with HPV6/11 in this age group.

Baseline characteristics of women who developed cervical disease

Among the 8,798 women ages 15 to 26 years who were enrolled in the placebo arms of protocol 013 and 015, a total of 1,366 CIN1, 456 CIN2, 393 CIN3, and 19 AIS lesions were diagnosed during the approximately 4 years of follow-up (Table 3A). Among the 1,858 women ages 24 to 45 years who were enrolled in the placebo arm of protocol 019, a total of 172 CIN1, 41 CIN2, 55 CIN3, and 5 AIS lesions were diagnosed during follow-up. More than half of the women who developed CIN2 or worse had a normal Pap test result at study entry [336/526 (64%) ages 15–26 years and 30/54 (56%) ages 24–45 years; Supplementary Table S2]. For those who developed CIN3, 63% and 86% of the younger and older women were positive to at least 1 of the 9vHPV vaccine types at study entry (HPV6/11/16/18/31/33/45/52/58), and 19% and 34% of the younger and older women were positive to at least 1 of HPV31/33/45/52/58 with no HPV6/11/16/18 detected at study entry (Supplementary Table S2).

HPV prevalence in cervical lesions—women ages 15 to 26 years

Of the total 2,234 lesions diagnosed in women ages 15 to 26 years, 1,198 (54%) had a single HPV type detected, 712 (32%) had >1 HPV type detected, 313 (14%) were negative to each of the 14 tested types, and 11 (0.5%) had missing data. Table 3 presents the minimum and proportional attribution estimates, as well as the prevalence of any of the individual 14 tested HPV genotypes by cervical disease categories. Regardless of the attribution method or lesion grade, the most frequent type detected was HPV16.

More than 1 HPV type was detected in 30% of CIN1 and CIN2 lesions, and 38% and 42% of CIN3 and AIS lesions, respectively. As approximately 95% of HPV-positive invasive cervical cancers have only 1 HPV type detected (28), we also considered the distribution of HPV types among lesions where only a single HPV type was detected by whole tissue section PCR (Fig. 1A). Among the carcinogenic HPV types, HPV16/18/31/33 showed a trend of increasing prevalence from CIN1 to AIS. When comparing the qHPV vaccine types to the 9vHPV vaccine types, an additional 32%, 39%, 33%, and 0% of CIN1, CIN2, CIN3, and AIS lesions were attributed to the 5 additional HPV types contained in the 9vHPV vaccine (100% attribution to AIS was observed for both vaccines). For the nonvaccine types (i.e., HPV35/39/51/56/59), there was a trend of decreasing prevalence from CIN1 to AIS (37%, 16%, 5%, and 0% for CIN1, CIN2, CIN3, and AIS, respectively). HPV6/11 were primarily detected as a single infection in CIN1.

Figures 2 and 3 compare the various attribution methods for the high-risk HPV types by cervical disease category. For HPV16/18, the proportional, hierarchical, and any estimates converged (defined as a $\leq$5% difference) for all disease categories except CIN1, where the proportional method gave 20% attribution for HPV16/18 to CIN1, compared with 28% for the other 2 methods. This is because HPV16/18 were present as a single infection in a lower proportion (12%) of the CIN1 lesions, compared with HPV31/33/45/52/58 (16%) and HPV35/39/51/56/59 (19%). Therefore, the fractional allocation for HPV16/18 is decreased for CIN1 relative to the hierarchical and any estimates. In the hierarchical method, the contribution of the additional high-risk HPV types contained in the 9vHPV vaccine (i.e., HPV31/33/45/52/58) was determined by only considering those lesions where there was no coinfection with HPV16 and 18. The percent of disease, which was thus attributable to the high-risk 9vHPV vaccine types was estimated to be 55%, 78%, 91%, and 95% for CIN1, CIN2, CIN3, and AIS, respectively. When HPV6/11 were included in the hierarchical method, the percent of disease attributable to the 9vHPV vaccine types increased to 59% for CIN1, with no change in the estimates for the

Table 2. Cumulative proportion of new incident and persistent HPV infections by age[a]

	Cumulative proportion of new incident infections (95% CI)			Cumulative proportion of new persistent infections (95% CI)		
	Women ages 15 to 26 years (protocol 13) $N = 1{,}049$[a]	Women ages 24 to 34 years (protocol 019) $N = 473$[a]	Women ages 35 to 45 years (protocol 019) $N = 515$[a]	Women ages 15 to 26 years (protocol 13) $N = 1{,}049$[a]	Women ages 24 to 34 years (protocol 019) $N = 473$[a]	Women ages 35 to 45 years (protocol 019) $N = 515$[a]
HPV type						
6	4.5 (2.3–8.4)[b]	6.3 (4.3–9.1)	2.1 (1.1–4.0)	2.9 (0.5–14.8)[b]	2.4 (1.4–4.3)	0.9 (0.3–2.3)
11	0.9 (0.2–4.5)[b]	0.9 (0.3–2.3)	0.4 (0.1–1.7)	0.5 (0.07–3.6)[b]	0.4 (0.1–1.8)	0.0 (—)
16	23.8 (20.5–27.5)	8.7 (6.4–11.7)	3.6 (2.2–6.0)	15.1 (12.0–18.9)	6.35 (4.25–9.4)	1.8 (0.9–3.4)
18	9.0 (6.8–11.9)	2.40 (1.3–4.3)	3.0 (1.6–5.7)	4.2 (3.0–5.7)	1.99 (0.96–4.1)	0.8 (0.3–2.1)
31	10.7 (8.6–13.4)	2.60 (1.4–4.7)	1.4 (0.7–3.0)	7.1 (5.1–10.1)	1.9 (0.96–3.9)	0.5 (0.1–2.0)
33	3.4 (2.4–4.9)	1.8 (0.9–3.5)	0.6 (0.2–1.8)	2.1 (1.4–3.3)	0.66 (0.2–2.0)	0.0 (—)
35	5.0 (3.2–7.9)	2.2 (1.1–4.2)	1.6 (0.8–3.1)	2.1 (1.2–3.5)	1.1 (0.39–3.1)	0.4 (0.1–1.6)
39	NT[c]	4.0 (2.5–6.4)	4.1 (2.4–6.7)	NT[c]	1.8 (0.9–3.6)	1.6 (0.8–3.2)
45	5.1 (3.6–7.3)	2.9 (1.6–5.3)	1.9 (0.9–4.0)	2.6 (1.8–3.9)	0.7 (0.2–2.0)	0.6 (0.2–1.9)
51	NT[c]	8.4 (6.1–11.6)	4.5 (2.8–7.2)	NT[c]	4.9 (3.1–7.6)	1.5 (0.7–3.1)
52	14.7 (11.5–18.7)	3.8 (2.36–6.0)	3.8 (2.3–6.2)	7.4 (5.4–10.1)	1.8 (0.9–3.5)	1.6 (0.8–3.2)
56	NT[c]	11.1 (8.4–14.7)	7.1 (5.0–9.8)	NT[c]	4.3 (2.7–6.6)	2.5 (1.4–4.4)
58	7.8 (6.1–9.9)	3.7 (2.2–5.9)	2.5 (1.4–4.4)	5.8 (3.8–8.9)	1.1 (0.5–2.6)	1.3 (0.4–4.3)
59	13.3 (10.4–17.1)	2.9 (1.7–4.9)	2.5 (1.4–4.7)	5.3 (4.0–7.0)	1.6 (0.7–3.2)	1.0 (0.4–2.4)
Any of the nonvaccine types						
	35/59[c]	35/39/51/56/59	35/39/51/56/59	35/59[c]	35/39/51/56/59)	35/39/51/56/59
	17.2 (13.7–21.4)[c]	21.1 (17.4–25.5)	14.6 (11.7–18.2)	7.2 (5.6–9.3)[c]	11.0 (8.3–14.5)	6.2 (4.3–8.7)
Any of the qHPV vaccine types						
	16/18[b]	6/11/16/18	6/11/16/18	16/18[b]	6/11/16/18	6/11/16/18
	28.5 (24.9–32.5)[b]	15.9 (12.9–19.7)	8.2 (5.8–11.6)	18.3 (15.1–22.2)[b]	10.5 (7.8–14.1)	3.3 (2.0–5.3)
Any of the 9vHPV vaccine types						
	16/18/31/33/45/52/58[b]	6/11/16/18/31/33/45/52/58	6/11/16/18/31/33/45/52/58	16/18/31/33/45/52/58 [b]	6/11/16/18/31/33/45/52/58	6/11/16/18/31/33/45/52/58
	41.9 (38.2–45.8)[b]	24.1 (20.3–28.4)	15.3 (12.1–19.3)	29.0 (25.5–32.9)[b]	14.3 (11.2–18.2)	6.49 (4.44–9.44)
Any of the high-risk vaccine types (16/18/31/33/45/52/58)						
	41.9 (38.2–45.8)	19.9 (16.5–24.0)	13.9 (10.9–17.8)	29.1 (25.5–32.9)	12.1 (9.2–15.9)	6.07 (4.09–8.98)

[a]Analyses for incident and persistent infection was restricted to placebo recipients who were seronegative to HPV6/11/16/18, DNA negative for the 14 tested HPV types, and had a normal Pap test result at day 1, as denoted by the *N*'s in the header rows. Persistent infection was defined as detection of a new HPV genital infection in follow up genital swabs collected on at least 2 consecutive visits spaced at least 6 months apart.

[b]In protocol 013, swabs were not tested for HPV types 6 and 11 after month 7; therefore, the average follow-up for cumulative incidence of infection is 18 months for 6/11, vs 48 months for all other HPV types. HPV6/11 are not included in the cumulative incidence estimates for the qHPV vaccine types and the 9vHPV vaccine types.

[c]In protocol 013, swabs were not tested (NT) for HPV39/51/56 after day 1. HPV39/51/56 are not included in the cumulative incidence estimates for the nonvaccine types.

Table 3. Prevalence and distribution of HPV genotypes in cervical lesions, by age

A. Women ages 15 to 26 years (protocols 013 and 015), *N* = 8,798

HPV type	CIN1[a] (no. of lesions = 1,366), *n* (%)			CIN2[a] (no. of lesions = 456), *n* (%)			CIN3[a] (no. of lesions = 393), *n* (%)			AIS[a] (no. of lesions = 19), *n* (%)		
	Min.[b]	Prop.	Any[c]	Min.[b]	Prop.	Any[c]	Min.[b]	Prop.	Any[c]	Min.[b]	Prop.	Any[c]
6	34 (2)	45 (3)	77 (6)	1 (0.2)	1 (0.3)	12 (3)	2 (1)	3 (0.7)	16 (4)	0 (0)	1 (5)	0 (0)
11	14 (1)	16 (1)	30 (2)	0 (0)	0 (0)	5 (1)	1 (0.3)	1 (0.3)	4 (1)	0 (0)	0 (0)	0 (0)
16	138 (10)	230 (17)	294 (22)	107 (23)	154 (34)	168 (37)	130 (33)	216 (55)	225 (57)	8 (42)	14 (75)	15 (79)
18	28 (2)	48 (4)	110 (8)	12 (3)	20 (4)	44 (10)	5 (1)	8 (2)	29 (7)	2 (11)	4 (20)	7 (37)
31	65 (5)	106 (8)	166 (12)	39 (9)	58 (13)	76 (17)	30 (8)	47 (12)	67 (17)	0 (0)	0 (0)	0 (0)
33	27 (2)	36 (3)	78 (6)	15 (3)	23 (5)	36 (8)	16 (4)	28 (7)	47 (12)	0 (0)	0 (0)	1 (5)
35	23 (2)	30 (2)	51 (4)	13 (3)	16 (4)	23 (5)	5 (1)	6 (2)	10 (3)	0 (0)	0 (0)	0 (0)
39	48 (4)	67 (5)	107 (8)	5 (1)	8 (2)	22 (5)	1 (0.3)	2 (1)	17 (4)	0 (0)	0 (0)	1 (5)
45	15 (1)	20 (1)	53 (4)	0 (0)	0 (0)	16 (4)	2 (1)	0 (0)	21 (5)	0 (0)	0 (0)	1 (5)
51	77 (6)	132 (10)	195 (14)	17 (4)	29 (6)	54 (12)	3 (1)	5 (1)	29 (7)	0 (0)	0 (0)	2 (11)
52	55 (4)	91 (7)	156 (11)	27 (6)	48 (11)	73 (16)	14 (4)	29 (7)	59 (15)	0 (0)	0 (0)	4 (21)
56	91 (7)	155 (11)	217 (16)	4 (1)	5 (1)	27 (6)	2 (0.5)	3 (0.9)	31 (8)	0 (0)	0 (0)	1 (5)
58	60 (4)	89 (7)	135 (10)	24 (5)	34 (7)	49 (11)	13 (3)	22 (6)	36 (9)	0 (0)	0 (0)	1 (5)
59	22 (2)	30 (2)	61 (4)	3 (1)	4 (1)	15 (3)	0 (0)	0 (0)	9 (2)	0 (0)	0 (0)	0 (0)

B. Women ages 24 to 45 years (protocol 019) N = 1,858

HPV type	CIN1[d] (no. of lesions = 172), *n* (%)			CIN2[d] (no. of lesions = 41), *n* (%)			CIN3[d] (no. of lesions = 55), *n* (%)			AIS[d] (no. of lesions = 5), *n* (%)		
	Min.[b]	Prop.	Any[c]	Min.[b]	Prop.	Any[c]	Min.[b]	Prop.	Any[c]	Min.[b]	Prop.	Any[c]
6	2 (1)	3 (2)	7 (4)	0 (0)	0 (0)	3 (7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
11	2 (1)	3 (2)	4 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
16	23 (13)	30 (17)	34 (20)	10 (24)	16 (39)	17 (41)	26 (47)	32 (58)	33 (60)	3 (60)	5 (100)	5 (100)
18	4 (2)	4 (2)	4 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
31	3 (2)	4 (2)	5 (3)	3 (7)	3 (8)	4 (10)	6 (11)	7 (12)	8 (15)	0 (0)	0 (0)	1 (20)
33	3 (2)	4 (2)	5 (3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
35	4 (2)	4 (2)	5 (3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)
39	1 (1)	1 (0.8)	5 (3)	1 (2)	1 (2)	1 (2)	1 (2)	1 (2)	1 (2)	0 (0)	0 (0)	0 (0)
45	5 (3)	7 (4)	9 (5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
51	8 (5)	13 (8)	19 (11)	1 (2)	1 (3)	2 (5)	1 (2)	1 (2)	3 (5)	0 (0)	0 (0)	0 (0)
52	8 (5)	14 (8)	20 (12)	5 (12)	6 (14)	7 (17)	7 (13)	8 (15)	11 (20)	0 (0)	0 (0)	1 (20)
56	15 (9)	20 (12)	24 (14)	1 (2)	1 (3)	4 (10)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)
58	9 (5)	12 (7)	16 (9)	3 (7)	4 (10)	4 (10)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
59	9 (5)	6 (3)	9 (5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

NOTE: A subject is counted only once within each applicable row, but may appear in more than one row.

[a]Of the 1,366 CIN1 lesions, 697 had a single type infection, 416 had >1 HPV type, 246 were negative to all 14 tested types, and 7 had missing data. Of the 456 CIN2 lesions, 267 had a single type infection, 139 had >1 HPV type, 47 were negative to all 14 tested types, and 3 had missing data. Of the 393 CIN3 lesions, 224 had a single-type infection, 149 had >1 HPV type, 19 were negative to all 14 tested types, and 1 had missing data. Of the 19 AIS lesions, 10 had a single type infection, 8 had >1 HPV type, and 1 was negative to all 14 tested types.

[b]Minimum estimate of attribution. Each lesion is counted only once within the row.

[c]Attribution irrespective of coinfection. Each lesion is counted only once within the row.

[d]Of the 172 CIN1 lesions, 92 had a single-type infection, 33 had >1 HPV type, 45 were negative to all 14 tested types, and 2 had missing data. Of the 41 CIN2 lesions, 24 had a single-type infection, 8 had >1 HPV type, 7 were negative to all 14 tested types, and 2 had missing data. Of the 55 CIN3 lesions, 41 had a single-type infection, 8 had >1 HPV type, 5 were negative to all 14 tested types, and 1 had missing data. Of the 5 AIS lesions, 3 had a single type infection, 2 had >1 HPV type, and 0 were negative to all 14 tested types.

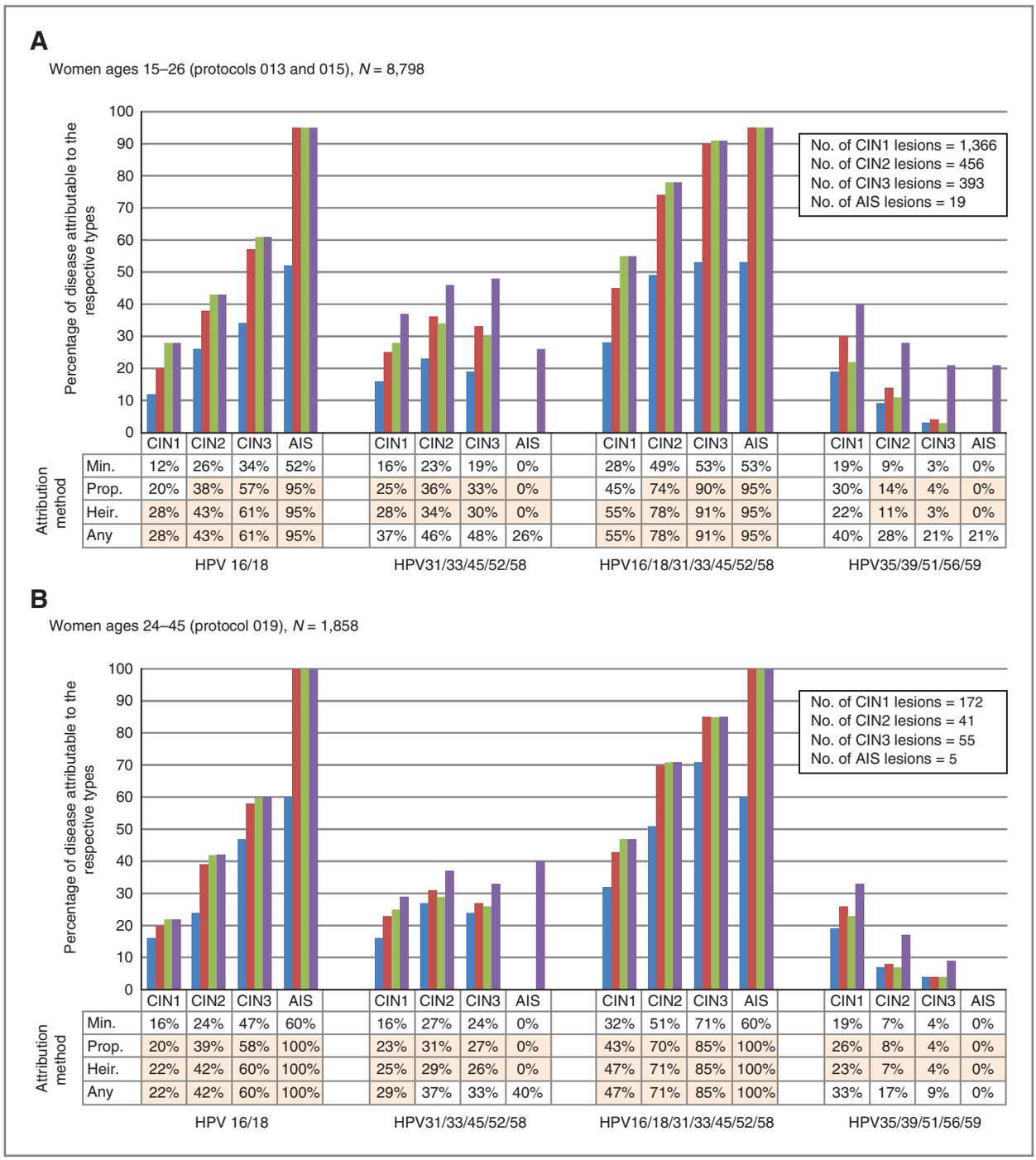


Figure 2. Percent of disease attributed to the respective HPV types for women ages 15 to 26 years (A) and women ages 24 to 45 years (B), using 4 attribution methods. Minimum (Min): numerator includes only those lesions where a respective HPV type was present as a single infection. Proportional (Prop): fractional allocation for each individual HPV type based on the relative number of instances in which each HPV type was observed as a single infection in a lesion of a given grade. Hierarchical (Heir): a lesion is attributed to HPV31/33/45/52/58, only if there was no coinfection with HPV16 and 18; and to HPV35/39/51/56/59 only if there was no coinfection with HPV16/18/31/33/45/52 and 58. Any: Numerator includes any lesion in which a respective HPV type was present, regardless of coinfection with other types. For each method, all lesions (HPV positive and negative) are included in the denominator.

other grade lesions. Overall HPV6/11 were detected in 8% the CIN1 lesions with no adjustment for multitype infected lesions.

As seen with HPV16/18, the hierarchical method yielded a higher attribution of the high-risk 9vHPV vaccine types to CIN1, compared with the proportional

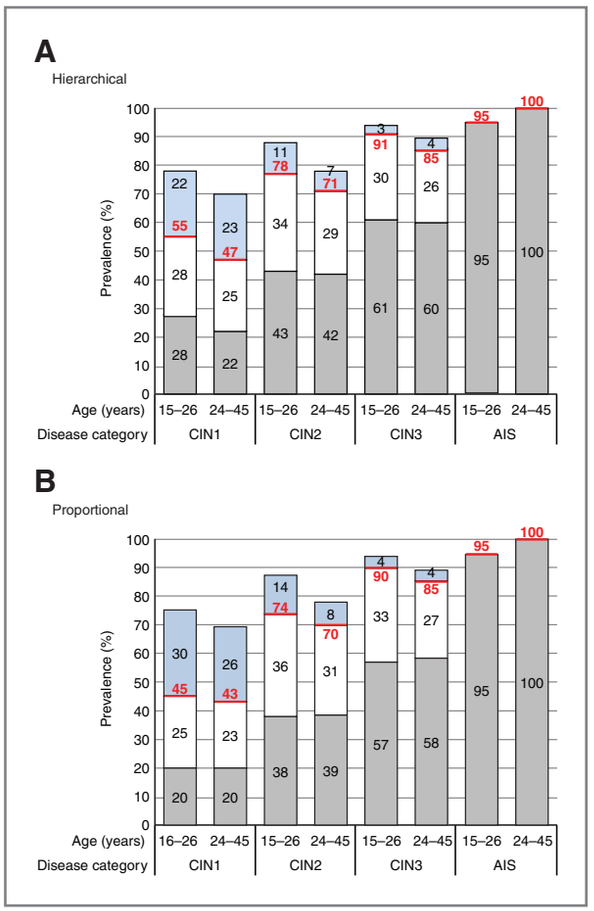


Figure 3. Prevalence of HPV types in cervical lesions among women enrolled in protocols 013 and 15 (i.e., ages 15–26 years) and women enrolled in protocol 019 (i.e., ages 24–45 years) using hierarchical attribution method (A) and proportional attribution method (B).

attribution method. This is because the hierarchical method attributes these types to the CIN1 lesions, without considering the contribution of the nonvaccine types to CIN1 lesions. For example, approximately 32% of these CIN1 lesions were coinfected with at least one high-risk 9vHPV vaccine *and* at least one of HPV35/39/51/56 or HPV59. HPV35/39/51/56 or HPV59 were detected in 40% of all CIN1 lesions, with 19% as single infections (Fig. 1B). In the hierarchical method, if one were to attribute the CIN1 cases to HPV31/33/45/52/58 only if there was no coinfection with HPV16/18/35/39/51/56/59, the hierarchical estimation for the high-risk 9vHPV vaccine types for CIN1 would be 46%, similar to the proportional method of 45%. Applying the same definition for the higher grade lesions resulted in a hierarchical estimate of 70%, 84%, and 95% for the high-risk 9vHPV vaccine types for CIN2, CIN3, and AIS, respectively.

For each of the 4 methods, the prevalence of the nonvaccine types decreased as lesion grade increased (Fig. 2). For these nonvaccine types, the hierarchical and proportional methods converged for CIN2, CIN3, and AIS. Unlike the other type combinations, the proportional method gave a higher attribution estimate (30%) than the hierarchical method (22%) for CIN1, as these HPV types received a higher fractional allocation because they were more often detected in CIN1 as a single infection.

HPV prevalence in cervical lesions—women ages 24 to 45 years

Among women ages 24 to 45 years, there was a similar proportion of lesions with a single HPV type detected compared with the younger cohort (59% vs. 54%); however, a lower proportion of the older women had >1 HPV type detected (19% vs. 32%) and a higher proportion of the older women were negative to all of the 14 tested HPV types (21% vs. 14%). Regardless of the attribution method or lesion grade, the most frequent type detected was HPV16 (Table 3). For CIN2 and CIN3, the second most frequent type detected regardless of attribution method was HPV52. For AIS, the HPV types detected in the older women were HPV16 (single infection in 3 lesions), HPV16/31 (1 lesion), and HPV16/56 (1 lesion). When considering only those lesions where a single HPV type was detected (Fig. 1B), the 9vHPV vaccine types were attributed to 63%, 88%, 95%, and 100% of CIN1, CIN2, CIN3, and AIS, representing a similar percent increase in attribution over the qHPV vaccine as that seen in the younger women.

For HPV16/18, the proportional, hierarchical, and any estimates converged for all disease categories (Fig. 2B). Unlike the younger women, for CIN1, HPV16/18 were detected as a single infection in a similar proportion (16%) to that of HPV31/33/45/52/58 (16%) and HPV35/39/51/56/59 (19%). Using the hierarchical method, the percentage of disease that was attributable to the high-risk 9vHPV vaccine types was slightly lower than that observed for the younger women, with estimates of 47%, 71%, and 85% for CIN1, CIN2, and CIN3, respectively; but the estimate for AIS was higher at 100%. However, a robust comparison of the age groups is limited, because of the total small number of AIS lesions detected in the older cohort ($n = 5$). Using the proportional method of attribution, there was a $\leq$5% difference in the attribution of the high-risk vaccine types between the younger and older cohorts (Fig. 3B). When HPV6/11 are included in the hierarchical and proportional estimates, the attribution of the 9vHPV vaccines types to CIN1 increased by 3% to 4%, with no change in the estimates for the higher grade lesions.

Discussion

This study is one of the first to utilize 4 distinct methods to determine the attribution of 14 HPV types in cervical precancers. In the absence of tissue-based genotyping evidence such as laser-capture microdissection, the existence of multiple HPV infections in a single lesion complicates the estimation of the attribution of cases to specific HPV types. Clarifying the contribution of individual genotypes allows an estimation of the potential impact of

prophylactic vaccines on cervical cancers and precancers and screening paradigms. Considering just the 2 more conservative attribution methods that adjusted for multiple-type infected lesions (proportional and hierarchical), among women ages 15 to 45 years, our results indicate that the 7 high-risk types included in a broad spectrum vaccine currently under development would prevent 43% to 55% of CIN1, 70% to 78% of CIN2, 85% to 91% of CIN3, and 95% to 100% of AIS. The relative contribution of the 7 types to invasive cervical cancer worldwide is estimated to be approximately 90%, with adjustment for multiple-type–infected lesions (20).

As the grade of CIN increased, the mathematical approaches (proportional, hierarchical, and any) for assessing HPV-type attribution converged for the high-risk vaccine types, regardless of age. For CIN2 and CIN3, the estimates with these 3 approaches for the 2 age cohorts were 74% to 78% and 70% to 71% (CIN2), and 90% to 91% and 85% (CIN3). For AIS, all estimates gave 95% and 100% attribution for the younger and older aged cohorts. This could be because of the fact that HPV16 was uniformly the most common HPV type in each of the histologic grades, and was more commonly detected as a single infection in the higher grade lesions. Thus, the proportional, hierarchical, and maximum estimates converge as a result of the high prevalence and well-established oncogenic potential of HPV16 (28). In both age cohorts, the nonvaccine types were most commonly detected in CIN1, and in the older cohort, they were detected as single infections in CIN1 in a similar proportion to HPV16/18 or HPV31/33/45/52/58 (19% vs. 16%).

In this study, the overall prevalence of any HPV infection at day 1 was higher for the younger women compared with the older women (33% vs. 25%) and was higher when considering each individual HPV type. A previous study in the same clinical trial population of women ages 15 to 26 years described the transition probabilities for incident HPV16/18/31/33/35/45/52/58/59 infections and CIN lesions (26). Nearly all incident HPV16/18/31/33/35/45/52/58/59 infections either manifested as detectable CIN lesions or went below the limit of PCR detection within 36 months (26). Our data confirm the wide body of literature that not all oncogenic HPV types persist and manifest as high-grade lesions. This is most apparent in the analysis where only lesions with a single HPV-type detected were considered in both the numerator and denominator (Fig. 1). Regardless of age, we observed a clear trend of increasing HPV16/18/31/33/45/52/58 prevalence across the lesions grades; 56% to 60% for CIN1, 84% to 88% for CIN2, 94% to 95% for CIN3, and 100% for AIS. In contrast, there was a decreasing trend for HPV35/39/51/56/59, with a range of 36% to 37% for CIN1, 13% to 16% for CIN2, 5% for CIN3, and 0% for AIS. Of note, in this analysis where only lesions with a single HPV-type detected were considered in both the numerator and denominator, we observed the lowest overall differential in the attribution estimates between the 2 age cohorts for both the vaccine and nonvaccine types.

The 5 most common HPV types detected at day 1 in younger women were HPV16/39/51/52/56, whereas the 5 most common HPV types detected in older women were HPV16/31/51/52/56 (Table 1). Although HPV39, a type not included in the 9vHPV vaccine, was the second most common type detected at day 1 in the younger women (9%), it was most commonly detected as a single infection in CIN1 lesions. HPV6/11 were also most commonly detected as a single infection in CIN1. Overall, HPV6/11 were detected in 8% and 6% of the CIN1 lesions in the younger and older aged cohorts with no adjustment for multiply infected lesions, similar to previous studies where HPV6/11 were detected in approximately 9% of low-grade cervical lesions (29, 30).

As noted by the American College of Obstetrics and Gynecology, both cervical cytology and high-risk HPV DNA testing can detect cervical cancer and its precursors, but each also detect abnormalities that will not go on to become cancer (31). Although annual screening with cytology alone has saved many lives, it has also been shown to increase the number of unnecessary procedures and treatments (31). Recently, the U.S. FDA Medical Devices Advisory Committee recommended HPV testing as the primary screening tool in women 25 years and older to assess risk of cervical cancer (32). Our study enhances the body of evidence used to inform screening recommendations and treatment. More than half of the women who developed CIN2/3 or AIS had a normal Pap test result at study entry and for those who developed CIN3, 63% and 86% of the younger and older women were positive to at least one of the 9vHPV vaccine types. As approximately one half of the CIN1 lesions detected in our study were associated with the types in the 9vHPV vaccine, the ability to prevent such lesions should result in a substantial reduction in both the risk and costs associated in evaluating these lesions.

The study has some limitations. The clinical trial populations are not entirely representative of the general population of women ages 15 to 45 years because of the exclusion/inclusion criteria of the trials. However, the estimates of the contribution of the HPV types to high-grade disease are consistent with prior published meta-analyses (20). We also had a small total number of AIS cases, with HPV18 or 45 detected in 7/24 (29%) and 1/24 (4%) of these cases. In a study of 470 adenocarcinomas, HPV18 and 45 were detected in 32% and 12% (28), thus our data may underestimate the potential impact of the 9vHPV vaccine in preventing adenocarcinoma.

Vaccination against HPV offers the opportunity to effectively prevent infection and disease caused by HPV. A 9vHPV vaccine has recently been shown to be highly safe and efficacious against the original 4 HPV types in the qHPV as well as the additional 5 types (33). If vaccination programs with this new generation vaccine are effectively implemented (20), approximately 90% of invasive cervical cancer cases worldwide could be prevented, in addition to the majority of precancerous lesions. However, despite the clear safety profile of the currently disseminated HPV

vaccines, uptake in the United States and other resource-rich countries has been inadequate (34). To achieve the population level potential of the HPV vaccine to reduce cancer, vaccine uptake must increase.

Disclosure of Potential Conflicts of Interest

E.A. Joura reports receiving a commercial research grant from Merck and GlaxoSmithKline, has received speakers' bureau honoraria from Merck, SPMSD, and GlaxoSmithKline, and is a consultant/advisory board member for Merck and SPMSD. K. Ault has provided expert testimony for Clinical trial with Merck and NIH. F. Xavier Bosch has received speakers' bureau honoraria from GlaxoSmithKline and MSD/SPMSD. D. Brown has received speakers' bureau honoraria from Honoraria, has ownership interest in a patent, and is a consultant/advisory board member for Consultant. J. Cuzick is a consultant/advisory board member for Genera, Qiagen, Abbott, BD, Hologic, Merck, and OncoHealth, and has received speakers' bureau honoraria from Abbott, BD, and Hologic. . D. Ferris reports receiving a commercial research grant from, has received speakers' bureau honoraria from, and is a consultant/advisory board member for Merck. S.M. Garland reports receiving a commercial research grant from GlaxoSmithKline HPV vaccine phase III trials, CSLBio, and Merck Investigator Initiated grants, has received speakers' bureau honoraria from Sanofi Pasteur and Merck, and is a consultant/advisory board member for Merck. W. Huh is a consultant/advisory board member for Merck. O.-E. Iversen is a consultant/advisory board member for Scientific Advisory Board for second generation HPV vaccines, Merck. S.K. Kjaer reports receiving a commercial research grant from and is a consultant/advisory board member for Merck, and has received speakers' bureau honoraria from Merck and Sanofi Pasteur MSD. N. Munoz is a consultant/advisory board member for Merck. E. Myers reports receiving a commercial research grant from GlaxoSmithKline and GenProbe/Hologic, Inc. and is a consultant/advisory board member for Merck. M. Steben reports receiving a commercial research grant from Merck and has received speakers' bureau honoraria from Merck and Valeant. M. Steben is a consultant/advisory board member for Merck. M. Steben has provided expert testimony for Valeant. Cosette M. Wheeler reports receiving a commercial research grant from Roche Molecular Systems. A. Saah is a Director at Merck and has ownership interest (including patents) in Merck. H.L. Sings has ownership interest (including patents) in Merck. No potential conflicts of interest were disclosed by the other authors.

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References

1. Garland SM, Hernandez-Avila M, Wheeler CM, Perez G, Harper DM, Leodolter S, et al. Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases. N Engl J Med 2007;356:1928–43.
2. FUTURE II Study Group. Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. N Engl J Med 2007;356:1915–27.
3. Block SL, Nolan T, Sattler C, Barr E, Giacoletti KE, Marchant CD, et al. Comparison of the immunogenicity and reactogenicity of a prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in male and female adolescents and young adult women. Pediatrics 2006;118:2135–45.
4. Joura EA, Leodolter S, Hernandez-Avila M, Wheeler CM, Perez G, Koutsky LA, et al. Efficacy of a quadrivalent prophylactic human papillomavirus (types 6, 11, 16 and 18) L1 virus-like-particle vaccine against high-grade vulval and vaginal lesions: a combined analysis of three clinical trials. Lancet 2007;369:1693–702.
5. Giuliano AR, Palefsky JM, Goldstone S, Moreira ED Jr., Penny ME, Aranda C, et al. Efficacy of quadrivalent HPV vaccine against HPV Infection and disease in males. N Engl J Med 2011;364:401–11.
6. Palefsky JM, Giuliano AR, Goldstone S, Moreira ED, Aranda C, Jessen H, et al. HPV vaccine against anal HPV infection and anal intraepithelial neoplasia. New Engl J of Med 2011;365:1576–85.
7. Munoz N, Manalastas R, Pitisuttihum P, Tresukosol D, Monsonego J, Ault K, et al. Safety, immunogenicity, and efficacy of quadrivalent HPV (types 6, 11, 16, 18) recombinant vaccine in adult women between 24 and 45 years of age: a randomized, double-blind trial. Lancet 2009;373:1949–57.
8. Markowitz LE, Hariri S, Lin C, Dunne EF, Steinau M, McQuillan G, et al. Reduction in human papillomavirus (HPV) prevalence among young women following HPV vaccine introduction in the United States, National Health and Nutrition Examination Surveys, 2003–2010. J Infect Dis 2013;208:385–93.
9. Tabrizi SN, Brotherton JM, Kaldor JM, Skinner SR, Cummins E, Liu B, et al. Fall in human papillomavirus prevalence following a national vaccination program. J Infect Dis 2012;206:1645–51.
10. Crowe E, Pandeya N, Brotherton JM, Dobson AJ, Kisely S, Lambert SB, et al. Effectiveness of quadrivalent human papillomavirus vaccine for the prevention of cervical abnormalities: case-control study nested within a population based screening programme in Australia. BMJ 2014;348:g1458.
11. Powell SE, Hariri S, Steinau M, Bauer HM, Bennett NM, Bloch KC, et al. Impact of human papillomavirus (HPV) vaccination on HPV 16/18-related prevalence in precancerous cervical lesions. Vaccine 2012;31:109–13.
12. Ali H, Donovan B, Wand H, Read TR, Regan DG, Grulich AE, et al. Genital warts in young Australians five years into national human papillomavirus vaccination programme: national surveillance data. BMJ 2013;346:f2032.
13. Gertig DM, Brotherton JM, Budd AC, Drennan K, Chappell G, Saville AM. Impact of a population-based HPV vaccination program on cervical abnormalities: a data linkage study. BMC Med 2013;11:227.

14. Bauer HM, Wright G, Chow J. Evidence of human papillomavirus vaccine effectiveness in reducing genital warts: an analysis of California public family planning administrative claims data, 2007–2010. Am J Public Health 2012;102:833–5.
15. Nsouli-Maktabi H, Ludwig SL, Yerubandi UD, Gaydos JC. Incidence of genital warts among U.S. service members before and after the introduction of the quadrivalent human papillomavirus vaccine. MSMR 2013;20:17–20.
16. Leval A, Herweijer E, Arnheim-Dahlstrom L, Walum H, Frans E, Sparen P, et al. Incidence of genital warts in Sweden before and after quadrivalent human papillomavirus vaccine availability. J Infect Dis 2012;206:860–6.
17. Baandrup L, Blomberg M, Dehlendorff C, Sand C, Andersen KK, Kjaer SK. Significant decrease in the incidence of genital warts in young Danish women after implementation of a national human papillomavirus vaccination program. Sex Transm Dis 2013;40:130–5.
18. Baldur-Felskov B, Dehlendorff C, munk C, Kjaer SK. Early impact of human papillomavirus vaccination on cervical neoplasia–nationwide follow-up of young Danish women. J Natl Cancer Inst 2014.
19. IARC monographs on the evaluation of carcinogenic risks to humans: Vol 100 B, a review of human carcinogens. Part B: biological agents. [Internet] Lyon, France: International Agency for Research on Cancer; 2012 [cited 2013 Nov 22]. Available from: http://monographs.iarc.fr/ENG/Monographs/vol100B/mono100B-1.pdf.
20. Serrano B, Alemany L, Tous S, Bruni L, Clifford GM, Weiss T, et al. Potential impact of a nine-valent vaccine in human papillomavirus related cervical disease. Infect Agent Cancer 2012;7:38.
21. Insinga RP, Liaw KL, Johnson LG, Madeleine MM. A systematic review of the prevalence and attribution of human papillomavirus types among cervical, vaginal, and vulvar precancers and cancers in the United States. Cancer Epidemiol Biomarkers Prev 2008;17:1611–22.
22. International patent numbers WO 2003/019143 A2, WO 2006/116276 A2, and WO 2006/116303 A2. [Internet] 2008 [cited 2011 Feb 21]. Available from: http://patentscope.wipo.int/search/en/result.jsf.
23. Villa LL, Costa RLR, Petta CA, Andrade RP, Ault KA, Giuliano AR, et al. Prophylactic quadrivalent human papillomavirus (types 6, 11, 16 and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial. Lancet Oncol 2005;6:271–8.
24. Mao C, Koutsky LA, Ault KA, Wheeler CM, Brown DR, Wiley DJ, et al. Efficacy of human papillomavirus-16 vaccine to prevent cervical intraepithelial neoplasia: a randomized controlled trial. Obstet Gynecol 2006;107:18–27.
25. Sahasrabuddhe VV, Castle PE, Follansbee S, Borgonovo S, Tokugawa D, Schwartz LM, et al. Human papillomavirus genotype attribution and estimation of preventable fraction of anal intraepithelial neoplasia cases among HIV-infected men who have sex with men. J Infect Dis 2013;207:392–401.
26. Insinga RP, Perez G, Wheeler CM, Koutsky LA, Garland SM, Leodolter S, et al. Incident cervical HPV infections in young women: transition probabilities for CIN and infection clearance. Cancer Epidemiol Biomarkers Prev 2011;20:287–96.
27. Wentzensen N, Schiffman M, Dunn T, Zuna RE, Gold MA, Allen RA, et al. Multiple human papillomavirus genotype infections in cervical cancer progression in the study to understand cervical cancer early endpoints and determinants. Int J Cancer 2009;125:2151–8.
28. de Sanjose S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, Lloveras B, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. Lancet Oncol 2010;11:1048–56.
29. Clifford GM, Rana RK, Franceschi S, Smith JS, Gough G, Pimenta JM. Human papillomavirus genotype distribution in low-grade cervical lesions: comparison by geographic region and with cervical cancer. Cancer Epidemiol Biomarkers Prev 2005;14:1157–64.
30. De Vuyst H, Clifford G, Li N, Franceschi S. HPV infection in Europe. Eur J Cancer 2009;45:2632–9.
31. The American Congress of Obstetricians and Gynecologists. New cervical cancer screening recommendations from the U.S. Preventive Services Task Force and the American Cancer Society/American Society for Colposcopy and Cervical Pathology/American Society for Clinical Pathology. [Internet] Washington, DC, [updated 2012 Mar 14; cited 2014 Jan13]. Available from: https://www.acog.org/About_ACOG/Announcements/New_Cervical_Cancer_Screening_Recommendations.
32. Roche [Internet] Switzerland: FDA Advisory committee unanimously recommends Roche's HPV test as primary screening tool for detection of women at high risk for cervical cancer. [updated 2014 Mar 13; cited 2014 Mar 27]. Available from: http://www.roche.com/media/media_releases/med-cor-2014-03-13.htm.
33. Merck [Internet] Whitehouse Station, NJ: Merck's investigational 9-valent HPV vaccine, V503, prevented 97 percent of cervical, vaginal and vulvar pre-cancers caused by five additional HPV types, in phase III study. [updated 2013 Nov 4; cited 18 Jun 2014]. Available from: http://www.mercknewsroom.com/news-release/research-and-development-news/mercks-investigational-9-valent-hpv-vaccine-v503-prevente.
34. Centers for Disease Control and Prevention [Internet] Atlant, GA: HPV vaccine: safe, effective, and grossly underutilized. [updated 2013 Jul 26; cited 2013 Aug 14]. Available from: http://www.cdc.gov/media/releases/2013/p0725-HPV-vaccine.html.

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