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Delayed dosing intervals for quadrivalent human papillomavirus vaccine do not reduce antibody avidity

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ABSTRACT

The quadrivalent HPV vaccine (4vHPV) was originally recommended as a three-dose series (0/2/6 months), though delays in completing the series frequently occur. We previously found delayed dosing in girls resulted in similar or higher antibody titers compared to on-time dosing. Archived sera from 262 healthy females aged 9–18 recruited from pediatric clinics were tested to determine if delayed dosing intervals affected antibody avidity. Avidity index (AI; ratio of IgG Ab bound in the treated and untreated sample) was determined pre- and post-dose 3 4vHPV for each participant using a modified multiplex ELISA. Data were grouped by dosing intervals: (1) on-time dose 2 and 3, (2) delayed dose 2 and on-time dose 3, (3) on-time dose 2 and delayed dose 3, (4) delayed dose 2 and 3. Overall, mean AI was highest for HPV16 and lowest for HPV6. As expected, AI did not differ between groups 1 & 3 or groups 2 & 4 pre-dose 3, however, for most types mean AI was significantly higher both pre- and post-dose 3 for groups with delayed dose 2. For all types, mean AI was higher post-dose 3 in all delayed dosing groups compared to group 1. One month post-dose 3, there was a positive but weak correlation between AIs and antibody titer for HPV 6 ($\rho = 0.25$, $p = .0001$), HPV 11 ($\rho = 0.14$, $p = .0370$), HPV 16 ($\rho = 0.11$, $p = .0934$), and HPV 18 ($\rho = 0.37$, $p < .0001$). Our findings suggest longer intervals between doses result in higher antibody avidity, providing further evidence that delayed dosing of 4vHPV does not hinder the immune response.

Introduction

In the United States, routine prophylactic human papillomavirus (HPV) vaccination is recommended for males and females. The Advisory Committee on Immunization Practices (ACIP) provided new recommendations for the dosing schedule for all HPV vaccines in December 2016, reducing the number of doses to two at 0 and 6–12 months for boys or girls who begin the series prior to their 15th birthday. A 3-dose schedule is still recommended for persons starting the series after their 15th birthday or for those who are immunocompromised.[1] At the time of enrollment for this study, the recommended dosing schedule was three doses of bivalent vaccine (2vHPV, targeting types 16 and 18) or quadrivalent vaccine (4vHPV, targeting types 6, 11, 16, and 18; used in this study) at 0, 1–2, and 6 months; the nonavalent vaccine (9vHPV, targeting types 6, 11, 16, 18, 31, 33, 45, 52, and 58) had not yet been licensed.

Prior to the change in dosing schedule for adolescents younger than 15 years, several studies reported that delays in HPV vaccine series completion frequently occurred.[2-4] However, studies examining delayed dosing have not found a negative impact on antibody titers[4-9] including the parent study to this manuscript that evaluated the antibody titers of girls between 9 and 18 years of age receiving 4vHPV at standard

and nonstandard dosing intervals (delay of the second dose, delay of the third dose, or delay of both second and third dose).[10]

No minimum level of antibody required for protection has been identified, though antibody levels produced in response to vaccination are sufficient for protection.[11,12] Some have suggested further study of antibody avidity as a possible surrogate for protection.[13,14] Avidity is a measure of how tightly an antibody binds its cognate antigen and is an indication of a primed memory immune response.[15] A few studies have described avidity responses to 2vHPV vaccine given at two doses and over time.[13,14,16] Because antibody avidity may provide further insight into the robustness of the antibody response to 4vHPV and there are few data on antibody avidity of those receiving nonstandard 4vHPV dosing intervals,[17] we aimed to measure and compare avidity in this same cohort from the Alternate Dosing Schedules Study for HPV Vaccine described in Russell et al.[10] utilizing residual serum samples of consenting participants.

Materials and methods

Study Design

This study utilized residual specimens from consenting/ assenting participants of the Alternate Dosing Schedules

Study for HPV Vaccine (ADS; the ADS study was registered with ClinicalTrials.gov: NCT02280642 and approved by the Duke University Health System IRB protocol no. 00014388). Study details have been previously published.[10] Briefly, participants were healthy females from 9 to 18 years of age recruited between June 2009 and March 2012 from three pediatric practices in North Carolina. Participants were recruited at time of receipt of either dose 2 or dose 3 of 4vHPV. As in the prior ADS report, participants were grouped according to timing of receipt of dose two and three of 4vHPV: (1) both doses on-time, (2) dose two delayed, (3) dose three delayed, and (4) both doses delayed. Thus, group assignment was not randomized. On-time dose two was defined as 30 to 90 days after dose one. On-time dose three was defined as 60 to 180 days after dose two. Delayed dose two was defined as greater than 90 days following dose one. Delayed dose three was defined as greater than 180 days following dose two. Serum samples were collected from participants immediately prior to administration of dose three and then again at a follow up study visit 25 to 42 days following receipt of dose three (see Figure 1). Of the original 331 eligible ADS patients, 282 consented to future use of study samples and had residual sera. Of these, 20 participants were excluded because serum samples were collected outside of the 25–42-day collection window post-dose 3, leaving 262 participants contributing samples for this analysis. Two of these participants were missing samples pre-dose 3 and 14 participants were missing samples post-dose 3.

Multiplex IgG avidity ELISA (M4-AvELISA)

We used the previously described multiplex avidity ELISA (M4-AvELISA).[18] HPV L1/L2 VLPs (types 6, 11, 16, and 18) were prepared and coated on seven-spot Meso Scale Discovery (MSD) standard plates as previously described.[19] Briefly, the assay uses the multiplex M4ELISA format, binding blocking and washing conditions. Each sample is tested in four wells. After binding and prior to detection, two wells are treated with guanidine hydrochloride (GuHCl, Sigma Aldrich, G4505) reconstituted to 1 M in phosphate buffered saline with 0.1% Tween 20 (PBST) containing 1 mg/mL bovine serum albumin (Sigma Aldrich, A4503) 1 h at 37°C, the other two untreated. Detection uses biotinylated anti-human IgG antibody (Fc specific) (Biotrend Chemicals LLC, I-1119-250) followed by sulfo-tagged streptavidin (MSD, R32AD-1) and is read on the Sector Imager 6000 (MSD).

Serum dilutions were selected to yield relative light units (RLUs) within the detectable range of the assay (between 1,000 to 1,000,000 RLUs) for all types. If the RLUs of the duplicates (either treated or untreated) had a coefficient of variation (% CV) greater than 20%, samples were considered failed for a particular type and subsequently retested for a final result. Reference serum was serially diluted 3.16-fold from 1:31.6 to 1:1,000,000 in 1% ECL and used to generate a standard curve. Additional two wells per plate were used for avidity control sera at 1:1000 dilution. The concentration of antibody bound with and without dissociation treatment was determined based on the standard curve fitted with a weighted $(1/y^2)$ five-paramater line using GraphPad Prism version 5 (GraphPad Software). The avidity index (AI) was calculated as a ratio of the treated and untreated concentrations. Thus, AIs near 1 correlate to high avidity and AIs near 0 correlate to low avidity. If more than one dilution for a sample was tested to ensure that RLUs for each type fell within the detectable range, than avidity index was calculated based on the lowest dilution that yielded RLUs within the detectable range. Quality control repeats were tested on 16-17% of samples, depending on the HPV type. The median coefficient of variation was 4-5% for each type (data not shown) (data not shown).

Data analysis

All statistical analyses were performed using JMP version 11 (SAS). Descriptive statistics were used to summarize baseline demographics as well as the intervals between dosing and blood draws. Chi-square tests were used to assess distributional differences in categorical variables among the four schedule groups. Nonparametric tests were used for comparisons as the AIs were not normally distributed. Mean AIs for each HPV type were compared for each group to the reference group following dose two and three using the Dunn Method for Joint Ranking nonparametric comparison with reference.
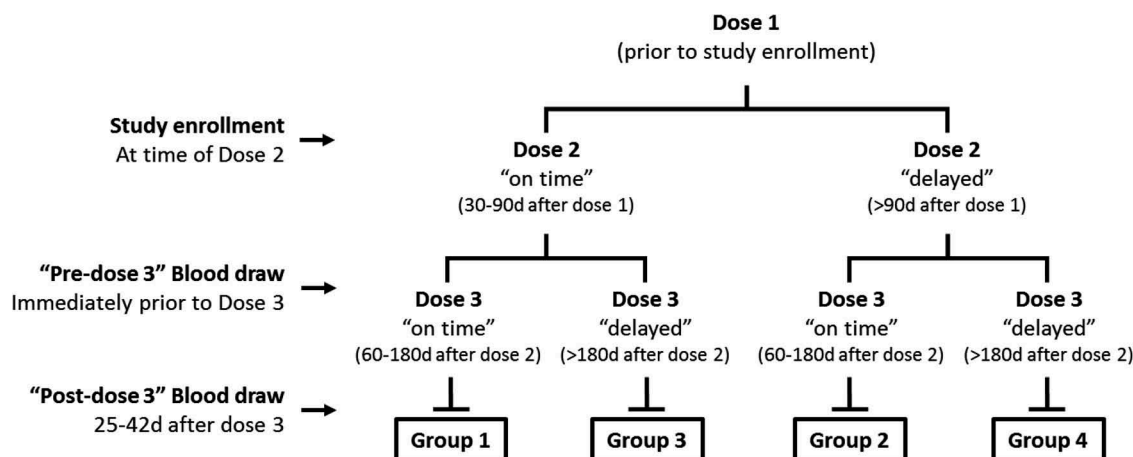

Figure 1. Flow diagram of vaccine dose and blood draw timing for the different dosing groups.

Group 1 (both dose two and three on-time) was considered the reference group in these comparisons. Reported p-values using Dunn Method for Joint Ranking reflect a Bonferroni correction. The correlation between AI and antibody titer was compared using Spearman's correlation. Antibody titers used for correlation analysis were previously published.[10]

Results

Participants

There was no significant difference in race or ethnicity across the four study groups, but there was a statistically significant difference in age distribution ($p < .001$; Table 1). Participant characteristics did not differ from those presented in Russell et al.[10] The mean age for both doses on time and delayed dose 2 groups was 13 years, while the mean age for the delayed dose 3 and both doses delayed groups was 14 and 15 years, respectively. Table 2 describes the mean and median time intervals and ranges between 4vHPV doses and final blood draw for each study group, including those samples collected outside of the 25–42-day collection window post-dose 3.

Antibody avidity

Overall, the mean AIs increased from pre- to post-dose 3 for all HPV types (Table 3). When comparing avidity across HPV types, the mean AI was highest for HPV 16 and lowest for HPV 6 for all dosing groups pre- and post-dose 3 (Table 3). Pre-dose 3 of 4vHPV, both group 2 (delayed dose 2) and group 4 (both doses delayed) had a significantly higher mean AI compared to group 1 (on-time group) for all HPV types (Figure 2A), except for HPV 6 in group 4. The mean AIs were significantly higher post-dose 3 in all delayed dosing

groups compared with on-time group (Figure 2B), for all HPV types except for HPV 6 in group 3. No difference in mean AIs was observed pre-dose 3 in either group with delayed dose 2 (groups 2 and 4) compared to post-dose 3 in the on-time dosing group (group 1; see Table 3). There were no statistically significant differences in mean AIs pre- or post-dose 3 when comparing across age groups (9–13 and 14–18 years of age) for any HPV type and adjusting for age did not affect mean AIs for each group pre- or post-dose 3 for any HPV type (data not shown).

There was positive but weak correlation between AIs (all groups, post-dose 3) and antibody titer for HPV 6 ($\rho = 0.25$, $p = .0001$; Figure 3A), HPV 11 ($\rho = 0.14$, $p = .0370$; Figure 3B), HPV 16 ($\rho = 0.11$, $p = .0934$; Figure 3C), and HPV 18 ($\rho = 0.37$, $p < .0001$; Figure 3D).

Discussion

Our findings suggest that delays in dose 2, dose 3, or both doses of 4vHPV do not result in reduced antibody avidity at 1 month following receipt of dose 3. In fact, dosing delays resulted in significantly higher mean AI for all four HPV types following dose 3, except for HPV 6 in the delayed dose 3 group. Previous studies have observed no correlation between antibody titer, as measured either by pseudovirion-based neutralization assay or ELISA, and avidity following vaccination with 2vHPV[13,16,20] or 4vHPV.[20] Similarly, we observed weak correlation between AI and Ab titer 1 month following dose 3 of 4vHPV. Together these data suggest that antibody titer does not necessarily indicate the quality of immune response (as indicated by antibody avidity).

Due to recent changes to HPV vaccine dosing schedule in the US[1] from three doses to two in persons who start the

**Table 1.** Participant characteristics (N = 262).

	Group					
	Both doses on-time (ref) (N = 32)	Dose 2 delayed (N = 117)	Dose 3 delayed (N = 39)	Both doses delayed (N = 74)	All (N = 262)	
	N (%)	N (%)	N (%)	N (%)	N (%)	p-value
Age (years)						<.0001
9-13	19 (59)	67 (57)	18 (46)	15 (20)	119 (45)	
14-18	13 (41)	50 (43)	21 (54)	59 (80)	143 (55)	
Ethnicity						.4106
Non-Hispanic	32 (100)	115 (98)	37 (95)	73 (99)	257 (98)	
Hispanic	0 (0)	2 (2)	2 (5)	1 (1)	5 (2)	
Race						.9079
White	17 (53)	52 (44)	21 (54)	37 (50)	127 (48)	
Black	12 (38)	51 (44)	13 (33)	30 (41)	106 (41)	
Other	3 (9)	14 (12)	5 (13)	7 (9)	29 (11)	

Table 2. Time intervals between vaccine doses and final blood draw by study group.

	Group				
	Both doses on-time (ref)	Dose 2 delayed	Dose 3 delayed	Both doses delayed	All
Interval between	Mean/median number of days (range)				
Dose 1 and 2	66/63 (38–89)	391/366 (91–1392)	67/64 (40–90)	369/370 (91–795)	297/261 (38–1392)
Dose 2 and 3	126/124 (90–160)	118/119 (68–176)	397/322 (182–1533)	415/377 (185–893)	244/142 (68–1533)
Dose 3 and final blood draw	31/30 (27–42)	31/29 (26–42)	31/29 (27–41)	31/29 (25–41)	31/29 (25–42)

Table 3. Comparison of pre-dose 3 and post-dose 3 mean avidity indices by HPV type and group.

HPV Type	Dosing group	Avidity Index Mean (min, max)		
		Pre-dose 3	Post-dose 3	p-value
HPV6	On time dose 2 and 3	0.59 (0.38, 0.97)	0.64 (0.52, 0.74)	*.0175*
	Delayed dose 2	0.65 (0.25, 0.91)	0.69 (0.26, 0.98)	*.0159*
	Delayed dose 3	0.63 (0.22, 0.89)	0.69 (0.56, 0.88)	.1036
	Delayed dose 2 and 3	0.64 (0.08, 0.92)	0.68 (0.24, 0.86)	*.0461*
HPV11	On time dose 2 and 3	0.58 (0.42, 0.80)	0.69 (0.52, 0.83)	*.0001*
	Delayed dose 2	0.73 (0.40, 0.96)	0.75 (0.48, 0.95)	.2738
	Delayed dose 3	0.63 (0.37, 0.84)	0.76 (0.61, 0.95)	*<.0001*
	Delayed dose 2 and 3	0.73 (0.37, 0.91)	0.76 (0.56, 0.96)	.1143
HPV16	On time dose 2 and 3	0.70 (0.45, 0.92)	0.74 (0.42, 1.00)	.1082
	Delayed dose 2	0.80 (0.51, 0.98)	0.82 (0.59, 0.99)	.4095
	Delayed dose 3	0.72 (0.46, 0.85)	0.82 (0.69, 1.02)	*<.0001*
	Delayed dose 2 and 3	0.81 (0.51, 0.99)	0.85 (0.72, 0.95)	*.0161*
HPV18	On time dose 2 and 3	0.59 (0.27, 0.84)	0.67 (0.53, 0.81)	*.0064*
	Delayed dose 2	0.71 (0.43, 0.93)	0.74 (0.52, 0.90)	*.0258*
	Delayed dose 3	0.63 (0.36, 0.84)	0.74 (0.50, 0.92)	*.0009*
	Delayed dose 2 and 3	0.70 (0.36, 0.95)	0.78 (0.52, 0.94)	*.0002*
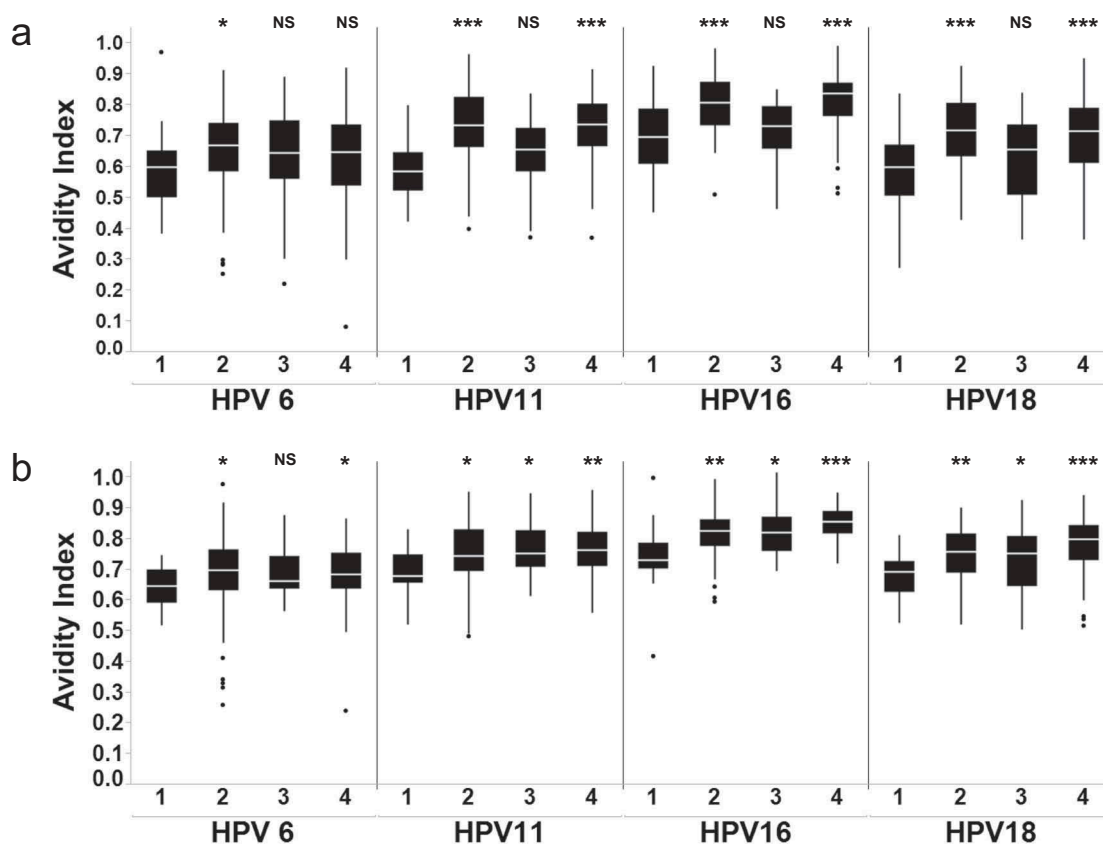
Figure 2. Mean avidity indices pre-dose 3 (a) and post-dose 3 (b) for delayed dose 2 (Group 2), delayed dose 3 (Group 3), and both doses delayed (Group 4) groups compared to both doses on-time (Group 1; *p < .05, **p < .001, and ***p < .0001) .

series before age 15 years, it is of interest to compare antibody avidity following dose 2 in groups that received delayed dosing versus following dose 3 in the on-time dosing group. We found no difference in mean AIs pre-dose 3 in participants with delayed dose 2 compared to post-dose 3 in on-time dosing participants, suggesting that the quality of the immune response is similar pre- and post-dose 3 of 4vHPV. Similar results were observed by Boxus *et al.*[16] in participants who received 2 doses of 2vHPV at 0 and 6 months compared to on-time 3 dose schedule group, as well as in the multicenter prospective cohort study in India, where no difference was

observed in antibody avidity in girls receiving two or three doses of 4vHPV.[17] In addition, we observed that mean AI was either no different or significantly higher pre-dose 3 in girls >15 years following delayed dosing schedules (groups 2, 4) compared to girls <15 years in the on-time group for all HPV types (data not shown).

However, our conclusions are limited as this study was not designed to compare a 2-dose to 3-dose schedule and blood draw time point pre-dose 3 is not standardized. Moreover, we do not have long term follow up to assess the robustness of the immune response over time. The correlation analysis of
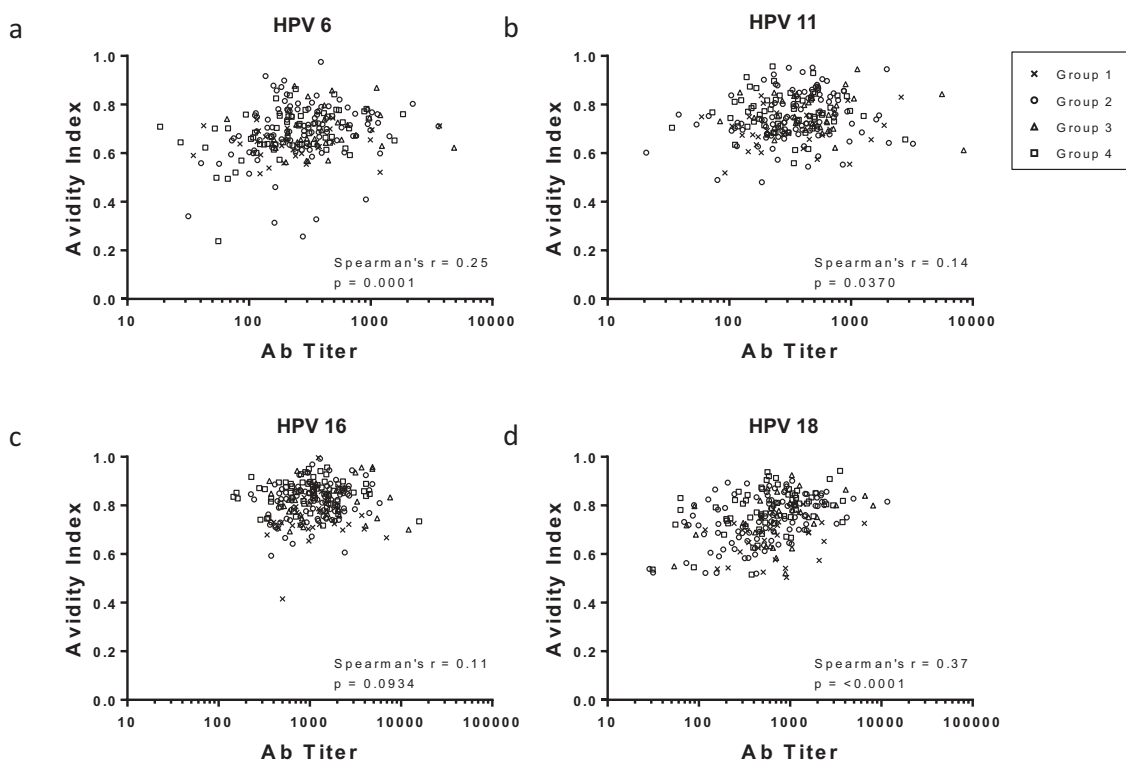
Figure 3. Overall Spearman correlation of log-transformed antibody titers to avidity indices for HPV 6 (a), HPV 11 (b), HPV 16 (c), and HPV 18 (d) one month post-dose 3 of 4vHPV.

antibody titer to avidity index may be biased by the pooling of all groups. Sample size was insufficient to perform separate spearman correlation analysis for each dosing group. Another limitation of this study is the possibility of confounding factors such as parental education, income level, and type of insurance which were unavailable for our analysis.

Although the role of antibody avidity in viral infections and the biological significance of AI as it relates to protection is not clearly understood, this comparative study provides further evidence that longer intervals between dose 1 and dose 2 and/or dose 3 of 4vHPV vaccine do not diminish the immune response and, in fact, antibody avidity was higher groups with delayed dosing compared to on-time. Moreover, these data add to the body of evidence that antibody titer alone may not be enough to establish a correlate of protection for HPV. More studies on long-term antibody avidity, especially in persons who receive off-schedule or reduced number of doses could provide further insight into the quality of immune response in these participants despite potentially low antibody titers.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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