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Short communication

The immune response to a two-dose schedule of quadrivalent HPV vaccine in 9–13 year-old girls: Is it influenced by age, menarche status or body mass index?

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ABSTRACT

HPV vaccines are highly immunogenic. A two-dose schedule for 9–14 year-old is recommended. However, no data exist regarding the impact of age, menarche status and body mass index (BMI) on the immune response to a two-dose schedule. In this post-hoc analysis, we present antibody titers to HPV6/11/16/18 in 9–13 year-old girls participating in a randomized clinical trial and assigned to receive two doses of quadrivalent HPV vaccine at 6 months interval (NCT00501137). Antibody titers were measured at month 7 and 24 of the study by using a competitive Luminex immunoassay (cLIA). Both, at Month 7 and 24 the GMTs for four HPV genotypes were similar across the age bands, and did not vary significantly by menarche status. Overweight and obese girls had lower GMTs. More than 99% of girls remained seropositive for HPV 6/11/16 and 89% for HPV18 at month 24. Comprehensive data in overweight and obese vaccines are warranted.

1. Introduction

Commercially available HPV vaccines are recommended by the World Health Organization, the European Medicines Agency and the Advisory Committee on Immunization Practices for use in a two-dose schedule in 9 to 13/14 year-old girls or boys and girls [1–4]. The 2-dose HPV vaccination schedule is presently widely used in preadolescents and adolescents [5,6]. However, little is known about the potential impact of age, menarche status and body mass index (BMI) on the magnitude of the immune response to two doses of HPV vaccines.

The maximum age to which the two-dose schedule might be applied remains unknown. Recently it was argued, that the current two-dose recommendation might be extended to 15–18 year-old vaccines [7]. However, for the three-dose schedule, a lower magnitude of the immune response was reported in those vaccinated at the age of 13–15 years compared to those vaccinated with the first dose at 9–12 years [8,9]. It remains unclear if the observed differences in the magnitude of the immune response are due to physiological changes related to puberty or body mass. This analysis aims to assess the potential impact of age, menarche status and body mass index (BMI) at the time of vaccination on the magnitude of the immune response and its persistence when administering two doses (0, 6 month) of the quadrivalent HPV vaccine (4vHPV) to 9–13 year-old girls. Such data might be helpful when deciding about optimal vaccine dosing in different sub-groups of adolescents, and to give some inputs regarding the potential use of the two-dose schedule in older age groups.

2. Methods

The immune response observed in 9–13 year-old girls who were randomly assigned to the two-dose group (0, 6 month schedule) of the Canadian BCGOV01 clinical trial (NCT 00501137) were

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included in this post-hoc analysis. Details of recruitment and source study design have been presented elsewhere [10]. Briefly, after informed consent obtained, participants were randomized in three groups: (I) 9–13 year-old girls receiving 2 doses 6 months apart, (II) 9–13 year-old girls receiving 3 doses (0, 2, 6 months), and (III) 16–24 year-old women receiving 3 doses (0, 2, 6 months) of quadrivalent vaccine (4vHPV). Blood samples were collected and tested for antibodies at month 0, 7 and 24 of the study. At month 0, all 9–13 year-old participants included in this analysis were seronegative for HPV11, 16 and 18 and all but two (0.8%) were seronegative for HPV6. The results at month 0 were judged non-pertinent for this analysis and not included. Blood samples were also collected at month 18 and 36 of the study but only from half of participants. These two samples were also not included in this post-hoc analysis because of relatively small number of participants by age band, menarche status and BMI. HPV antibodies were measured by Merck competitive Luminex Immuno Assay (cLIA) [10,11]. The source study was approved by Health Canada and ethics review boards at each of the 3 provincial centers involved.

Age, menarche status and BMI were assessed and documented by a research nurse at the time of first dose administration. BMI was categorized based on age-specific cut-offs as underweight, normal, overweight or obese [12]. The potential impact of age, by annual age band (9, 10, 11, 12 and 13 y-o, as well as 9–10 vs 11–13 y-o), menarche status and BMI on anti-HPV geometrical mean titers (GMTs) was assessed using Generalised linear models (GLM) on log-transformed titers. Adjustment for multiple comparisons was taken into account using "Tukey-Kramer" method. Analyses were performed using SAS version 9.4 (Inc., Cary, N.C., USA). A 2-sided $p < 0.05$ was considered as significant.

3. Results

Immunogenicity results by cLIA were available and included in this analysis for 253 and 201 girls at months 7 and 24, respectively.

At the time of first dose administration the mean age (±standard deviation) of participants was 12.3 ± 1.4 years, the mean BMI was 19.6 ± 3.5 (range 13.7–38.7), and no participants reported being sexually active. Menarche was reported by 113 participants (44.7%).

At month 7 and 24 of the study, 99–100% girls were seropositive for HPV6/11/16 and 18 (except at month 24 for HPV18 (89%)) (data not shown). The GMTs for all four HPV genotypes (HPV6/11/16/18), were similar across annual age bands. The GMT ratios for 9–10 years of age compared with 11–13 years of age varied between 0.94 and 0.98 for all four HPV types. All 95%CI included unity (Table 1).

At Month 7 and month 24 the GMTs ratios in pre-menarche and post-menarche girls varied from 0.98 to 1.04, depending on HPV genotype and study time point. All 95%CI also included unity (Table 2).

At the time of the first dose administration, 77.8% of participants included in month 7 analysis had a normal BMI and 20.6% were classified either as overweight or obese. Of participants included in month 24 analysis, 74.6% had a normal BMI and 23.4% were overweight or obese.

The proportion of participants classified as underweight was below 2%. At month 7, significantly lower antibody responses for HPV11 and HPV18 was observed in obese girls compared to those with a normal BMI. At month 24, the antibody GMTs for HPV11, 16 and 18 types were significantly lower in overweight girls compared to girls with normal BMI. At the same time point the antibody GMTs in obese girls were statistically lower for HPV6, 11 and 18. The HPV type specific GMTs, their 95%CI, and the GMTs ratios between different study groups observed at two study time points are presented in Table 3.

4. Discussion

Previously, it was reported that age at first HPV vaccine dose administration influences the magnitude of the antibody response to a three-dose vaccination schedule [9,13]. We did not observe difference in the magnitude and the persistence of antibodies among different age bands included in our analysis, either when stratified by year or when 9–10 year-old where compared with 11–13 year-old. This discrepancy might be explained by different age cohorts included in the two analyses. In our analysis we included 9–13 year-old subjects and in the previous analysis 9–26 year-old subjects were included. In the previous analysis, the largest differences were reported when comparing the GMTs observed in 9–14 year-old subjects with those aged 15 years and above [9].

Sex hormones are known to have multiple effects on the immune response to other vaccines [14]. We did not observe a difference in anti-HPV GMTs between girls who have had their menarche compared to those pre-menarche. Additional studies which include more subjects of both genders and wider age groups are warranted before concluding on the impact of sex hormones on the immune response to HPV vaccines.

It was previously reported that obesity increased the likelihood of a poor vaccine-induced immune response [15]. For those overweight, intramuscular injections often end up being given subcutaneously, especially if the needle used is not of appropriate length [8]. Subcutaneous injections are thought to be less effective than intramuscular injections, due to a lower number of patrolling dendritic cells in adipose tissue compared to muscles but also to the possible effect of an altered immune response in the recipients with high BMI [16]. Our results also indicate a clear trend to lower antibody titers in overweight and obese girls with GMTs statistically significant lower for three out of four HPV types by month 24 of the study. These results are congruent with those reported by a previous study which showed lower antibody response for HPV16 and 18 in those with a higher BMI ($\geq$28 kg/m^2) [16]. The HPV vaccine monograph recommends the use of 22–25 gauge needle (2.5–3.8 cm) [3]. In our source study, conducted in a highly controlled setting of a randomized controlled clinical trial, a 22 gauge needle was used. The vaccine was administered in the deltoid region of the upper arm, which makes unlikely the subcutaneous administration. Thus, the observed lower magnitude of the antibody response to HPV vaccination in overweight and obese adolescent girls most probably is due to some immunologic alterations.

Absence of difference in the magnitude of the immune response by age and menarche status in this post-hoc analysis might be due to the recruitment of the younger age groups, a relatively small number of age bands and the relatively limited number of subjects per group. Despite these limitations, the observed similarity in the magnitude of the immune response in different study groups allow to suppose that similar protection is insured when vaccinating healthy 9–13 year-old girls with two doses of 4vHPV vaccine given at six months interval. However, additional post-hoc analyses and a priory designed for this objective clinical trials including larger samples sizes allowing for multiple comparisons and wider age groups would be helpful to better understand the role of age-related physiological processes and individual characteristics on the magnitude of the immune response to HPV vaccines.

The clinical importance of the magnitude of the immune response remains not well understood and the absence of breakthrough cases in vaccinated individuals with undetectable antibodies [17] may indicate that the threshold of the presently used assays are above the protective threshold or that the cellular immunity is present despite antibody lost. Thus, the clinical

Table 1
Anti-HPV6/11/16/18 GMTs and their 95% Confidence Intervals (95%CI) at Month 7 and Month 24 of the study.

Age (years)	N	HPV6	HPV11	HPV16	HPV18
Month 7		GMT cLIA (95% CI)			
9	18	1138(440–1664)	2943(1042–2660)	4214(1782–9964)	1301(758–2232)
10	34	2453(1523–3948)	2160(1698–2749)	6618(4077–10743)	944(635–1402)
11	42	2391(1681–3402)	2427(1944–3030)	8814(6137–12658)	1320(943–1847)
12	43	2321(1468–3670)	2828(2006–3988)	6775(4259–10777)	1319(962–1809)
13	116	2065(1567–2722)	2313(1920–2786)	7847(6060–10160)	1116(931–1337)
9–10	52	1880(1205–2934)	1974(1585–2458)	5661(3714–8629)	1055(773–1440)
11–13	201	2183(1791–2661)	2439(2128–2795)	7791(6433–9436)	1198(1041–1378)
Ratio: 9–10/11–13		0.98(0.91–1.05)	0.98(0.94–1.01)	0.96(0.91–1.01)	0.98(0.93–1.03)
Month 24					
9	15	197(124–312)	273(156–478)	1039(595–1812)	110(59–207)
10	27	230(162–325	343(266–443)	1191(757–1872)	99(57–171)
11	34	267(198–360)	338(256–446)	1327(918–1919)	136(81–230)
12	36	309(223–430)	451(344–591)	1533(1060–2216)	140(91–216)
13	89	303(244–376)	380(303–478)	1540(1253–1893)	140(106–184)
9–10	42	217(167–284)	316(247–405)	1134(808–1591)	103(69–154)
11–13	159	296(254–345)	386(332–448)	1490(1271–1748)	139(113–171)
Ratio: 9–10/11–13		0.95(0.89–1.01)	0.97(0.91–1.04)	0.96(0.91–1.01)	0.94(0.84–1.05)

Table 2
Anti-HPV6/11/16/18 GMTs and their 95% Confidence Intervals (95%CI) by menarche status.

Menarche Status	N	HPV6	HPV11	HPV16	HPV18
Month 7		GMT cLIA			
Pre	140	2280(1814–2865)	2403(2087–2767)	8272(65649–10447)	1274(1070–1518)
Post	113	1932(1442–2587)	2254(1848–2748)	6245(4797–8129)	1046(867–1263)
Ratio: Pre/Post		1.03(0.97–1.09)	1.01(0.98–1.04)	1.04(0.99–1.08)	1.03(0.99–1.07)
Month 24					
Pre	111	260(220–306)	368(314–430)	1376(1137–1666)	133(104–171)
Post	90	302(242–376)	373(300–464)	1447(1158–1808)	127(96–168)
Ratio : Pre/Post		0.98(0.93–1.03)	1.01(0.96–1.06)	0.99(0.95–1.04)	1.01(0.92–1.11)

Table 3
GMTs, GMTs ratios and 95% Confidence Intervals by BMI categories, Month 7 and Month 24 of the study.

BMI	N	HPV6		HPV11		HPV16		HPV18	
Month 7		GMT cLIA	GMT Ratio	GMT cLIA	GMT Ratio	GMT cLIA	GMT Ratio	GMT cLIA	GMT Ratio
Underweight	4	1275 (29–56626)	0.57 (0.13–2.41)	1987 (318–12402)	0.80 (0.32–2.04)	6174 (118–32189)	0.81 (0.20–3.25)	1474 (170–12764)	1.17 (0.43–3.18)
Normal	197	2252 (1819–2788)	Ref	2479 (2164–2840)	Ref	7647 (6261–9388)	Ref	1265 (1095–1462)	Ref
Overweight	32	2002 1350–2968)	0.89 (0.51–1.53)	2182 (11617–2944)	0.88 (0.62–1.25)	7487 (5315–10548)	0.98 (0.58–1.65)	1090 (819–1452)	0.86 (0.59–1.26)
Obese	20	1396 (819–2381)	0.62 (0.32–1.21)	1492 (10342154)	0.60 (0.39–0.93)	4440 (2234–8825)	0.58 (0.30–1.11)	559 (332–943)	0.44 (0.28–0.70)
Month 24									
Underweight	4	228 (84–621)	0.74 (0.29–1.89)	299 (132–676)	0.72 (0.29–1.78)	1053 (548–2023)	0.67 (0.24–1.84)	192 (67–550)	1.24 (0.34–4.51)
Normal	150	309 (262–364)	Ref	417 (356–4987)	Ref	1580 (1334–1872)	Ref	154 (125–191)	Ref
Overweight	29	215 (163–283)	0.70 (0.48–1.01)	261 (191–358)	0.63 (0.44–0.90)	931 (667–1300)	0.59 (0.39–0.89)	80 (49–132)	0.52 (0.31–0.87)
Obese	18	182 (136–242)	0.59 (0.37–0.94)	252 (185–345)	0.61 (0.39–0.95)	1113 (654–1894)	0.70 (0.43–1.16)	66 (37–121)	0.43 (0.23–0.81)

importance of the lower antibody titers observed in overweight and obese girls in our analyses remains unknown. We cannot exclude that despite lower antibody titers, these girls acquired long term protection against HPV infections and related abnormalities.

Ongoing efficacy studies with one, two and three doses of HPV vaccines in pre-adolescent and adolescent girls [18–20] will help to better understand potential differences, if any, between the protection insured by different vaccination schedules in different age groups.

In summary, two doses of 4vHPV vaccine given six months apart are highly immunogenic in 9–13 year-old girls irrespective of age and menarche status. Overweight and obese girls might have lower antibody titers with time passing since vaccination, though the clinical relevance of these observations remains to be understood.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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