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Antibody responses to Quadrivalent HPV Vaccination in HIV-Infected Young Women as Measured by Total IgG and Competitive Luminex Immunoassay

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Abstract

We compared antibody responses of HIV-infected young women to the HPV6, 11, 16, 18 vaccine using total IgG LIA and cLIA assays. HPV18 seropositivity after HPV vaccination as measured with IgG LIA remained high (98%) 48 weeks after vaccination, in contrast with seropositivity as measured with cLIA (73%). Seropositivity rates at week 48 as measured by both IgG LIA and cLIA remained high for HPV6, 11 and 16 (93.5–100%). These results suggest that the lower rate of seropositivity to HPV18 when cLIA vs. IgG LIA is used is a function of the assay, and does not imply lower vaccine immunogenicity.

Keywords

HPV Vaccines; Antibody Response; Immunoglobulin G; HIV

Introduction

The quadrivalent human papillomavirus (HPV) vaccine, which is comprised of virus-like particles (VLPs) made from the L1 major capsid protein of HPV6, 11, 16 and 18, induces a complex polyclonal antibody response directed against specific conformational and linear epitopes on the VLP.^{1,2} A number of studies have demonstrated the importance of

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Potential conflicts of interest

Dr. Kahn is the co-chair of another HPV vaccine clinical trial in HIV-positive individuals, for which Merck & Co., Inc., provided vaccine and immunogenicity titers. Dr. Kahn chaired a grant review committee for the Society for Adolescent Health and Medicine evaluating public health demonstration project proposals to improve adolescent vaccination; grant funding for this program was from Merck, Inc.

neutralizing antibodies in protection from infection.²⁻⁵ Although the antibody threshold that correlates with protection against HPV infection or disease is unknown, clinical trials have demonstrated that vaccination induces robust immune responses and is highly effective in preventing vaccine-type anogenital precancers.^{6,7}

HIV-infected women are at significantly higher risk than HIV-uninfected women for persistent HPV infection and progression to cervical precancers and invasive cervical cancer; in addition, the incidence of cervical cancer increases with more severe immunosuppression.^{8,9} HPV vaccine trials among HIV-infected young women and men demonstrated that HPV type-specific immune responses to vaccination are generally robust.¹⁰⁻¹⁶ However, studies have demonstrated substantially lower seropositivity rates for HPV18 compared to HPV6, 11, and 16. In one study, 32.4% of those not on antiretroviral therapy (ART) and 13.3% of those on ART became (or remained) seronegative for HPV18 at 48 weeks after vaccination.¹² In addition, Kojic et al. reported lower seropositivity rates in women with higher HIV viral load and/or a lower CD4 count,¹⁴ while Kahn et al. reported lower seropositivity rates among those not on ART vs. those on ART.¹²

The finding of a decline over time in HPV18 seropositivity in HIV-infected young women raises concern about the long-term efficacy of vaccination and breakthrough cases of HPV18-related cancers. However, trials of the quadrivalent vaccine in HIV-uninfected women demonstrated that efficacy was sustained despite a decrease in HPV18 immune titers, raising the possibility that an explanation for waning immunity is low sensitivity of the assay for detecting antibodies to HPV18 important for protection against infection.¹⁷⁻¹⁹ In clinical trials of the quadrivalent vaccine, antibodies to the HPV L1 VLPs were measured using a competitive Luminex immunoassay (cLIA).²⁰ This type-specific assay simultaneously evaluates the antibody response to a unique conformational, neutralizing epitope on each of the four HPV types that comprise the vaccine, measuring a restricted anti-VLP HPV neutralizing response which is one component of the total immune response to vaccination.²¹ The assay is highly type-specific, allows for immune responses to multiple VLPs to be evaluated at the same time, and enables high throughput processing.²² However, it measures only a subset of neutralizing antibodies that compete with the specific monoclonal antibody for VLP surface binding. It therefore underrepresents the complete VLP-induced protective antibody response elicited by vaccination with L1 VLPs: it is likely that many other antibodies, some neutralizing, are produced by vaccination but not measured by the cLIA.^{21,22} Furthermore, vaccination with L1 VLPs may not elicit identical immune responses in all individuals: some women may make efficient, neutralizing antibodies to epitopes on HPV 18 VLPs that differ from the HPV18 type-specific epitope measured in the HPV-18 cLIA. This may explain the decline in HPV18 antibodies in clinical trials.²¹ It is difficult to determine a cLIA level that correlates with vaccine efficacy, because despite the decrease in antibody titers post-vaccination, the efficacy of the quadrivalent HPV vaccine remains high with very few breakthrough cases of HPV6, 11, 16 or 18-associated disease.²³ Thus, the ability of the HPV cLIA to measure a long-term protective antibody response in women may be limited.

In contrast to the cLIA, the total IgG LIA measures total IgG antibody binding to HPV VLPs and provides a more general measure of the humoral immune response to

vaccination.²⁴ Compared to the cLIA, the total IgG is a more sensitive assay that measures a broader subset of the total immune response to vaccination. This assay captures VLP binding antibodies to HPV type-common epitopes, HPV type-specific epitopes, neutralizing epitopes, non-neutralizing epitopes, linear epitopes and conformational epitopes.²¹

Data comparing the changes in seropositivity using the HPV18 cLIA and the total IgG LIA in HIV-infected women will be helpful in increasing our understanding of the immune response to HPV vaccines in HIV-infected individuals, clarifying the extent and duration of the immune response to vaccination, guiding clinical practice (e.g. whether a fourth vaccine dose is needed in HIV-infected individuals), understanding the limitations and benefits of different immunoassays, and understanding the ability of assays to define an immune correlate of protection if breakthrough cases occur after vaccination.^{21,22} Therefore, the aim of this study was to compare the changes in antibody response over time of HIV-infected young women to the quadrivalent HPV6, 11, 16 and 18 L1 VLP vaccine using total IgG LIA and cLIA assays, 4 and 24 weeks after the third vaccine dose. Antibody responses were measured using geometric mean titers (GMTs) and seropositivity rates. Our hypothesis was that total IgG responses would remain high, with a seropositivity rate > 90%, 24 weeks after the third vaccine dose among HIV-infected young women who received all three vaccine doses within the recommended timeframe.

Methods

Participants were HIV-infected young women 18–23 years of age who had participated in a clinical trial of the quadrivalent HPV vaccine. Details of study recruitment, procedures, and methodology are available in a previously published manuscript.¹² Stored sera from women enrolled in the original study and already tested for cLIA were retested for total IgG LIA at 4 and 24 weeks after the third vaccination, corresponding to study weeks 28 and 48, respectively. Immunogenicity analyses were performed for subjects (N=76) who received 3 doses of the quadrivalent vaccine within the study windows and had sera available at 28 and 48 weeks, and who were seronegative and PCR negative at baseline and PCR negative through month 7 to the appropriate HPV type(s).

We determined the proportion of participants seropositive for HPV6, 11, 16 and 18 as measured by total cLIA and total IgG LIA at week 28 (4 weeks after the third vaccine dose) and week 48 (24 weeks after the third vaccine dose). The assay has been standardized and validated for measurements of HPV antibody responses.²¹ Serostatus cut-offs for cLIA were HPV6 (20 mMU/mL), HPV11 (16 mMU/mL), HPV16 (20 mMU/mL), and HPV18 (24 mMU/mL), and for total IgG were HPV6 (15 mMU/mL), HPV11 (15 mMU/mL), HPV16 (7 mMU/mL), and HPV18 (10 mMU/mL). We also determined the GMTs for total IgG and cLIA GMTs at weeks 28 and 48. Stratified analyses were conducted by ART status. Of note, GMTs cannot be compared between HPV types because they are standardized differently.

A McNemar's test for paired data was used to compare seropositivity as measured by total IgG LIA and cLIA for each HPV type at weeks 28 and 48. Analyses were conducted for all participants and stratified by ART status. Because GMTs are not comparable for total IgG and cLIA, as one assay measures general antibody binding to the VLP and the other

measures competitive antibody binding to a specific neutralizing epitope,²¹ univariable analyses comparing GMTs would not be meaningful and were not conducted.

Results

Baseline demographic characteristics of participants were reported in detail in a previous manuscript;¹² briefly, the mean age of participants was 21.5 years (standard deviation 2.1 years), 76.3% were Black/African-American, 18.4% were Hispanic, 30% were taking antiretrovirals, 98.7% had a CD4+ T-cell count ≥ 350 cells/mm³, and 38.2% had an HIV viral load < 400 copies/mL.

Seropositivity rates and GMTs obtained by IgG LIA and cLIA testing are shown in the Table. Seropositivity rates across all HPV serotypes at 28 and 48 weeks ranged from 88.2% to 100% using the total IgG LIA and from 72.7% to 100% using the cLIA assay. Seropositivity rates for all four serotypes at 28 weeks and for all serotypes except HPV18 at 48 weeks were similar when measured using the total IgG LIA vs. the cLIA assay. Seropositivity rates for HPV18 were substantially higher at 48 weeks when measured using IgG vs. cLIA for all participants (98.1% vs. 73.1%), those on ART (94.7% vs. 73.7%), and those not on ART (100% vs. 72.7%). However, the only statistically significant difference was for the group that included all participants at 48 weeks ($p=.0002$). Seropositivity rates within a given assay were similar in those taking vs. not taking ART.

GMTs were robust when measured by total IgG and cLIA; as the assays represent distinct measurements of HPV antibody responses, comparisons between the assays were not performed. GMTs were higher for those taking vs. not taking ART as previously reported.¹²

Discussion

In this study, we found that among HIV-infected young women, HPV18 seropositivity rates after HPV vaccination as measured with IgG LIA remained high (98%) 24 weeks after the third vaccine dose, in contrast with seropositivity rates as measured with cLIA (73%). These results are similar to those reported in healthy women, in whom seropositivity to HPV18 measured by total IgG LIA was approximately 97% at month 48 after vaccination.²¹ Nygard et al. examined seropositivity rates in healthy women 9 years after vaccination and found that 91.4% remained seropositive to HPV18 when measured by total IgG LIA.²⁵ However, seropositivity rates were lower for all four HPV types as measured with cLIA among women with advanced compared to less advanced HIV disease, suggesting the importance of HPV vaccination prior to CD4 T-cell decline.¹⁴

Given that HIV-infected women are at substantially increased risk of HPV-related cancers,^{8,9} it is encouraging that seropositivity rates in this study, as measured by total IgG LIA, are similar to those in HIV-uninfected women in the same age group. Our results and those of others suggest that the quadrivalent HPV vaccine elicits a robust and sustainable immunologic response, and are consistent with observations that the vaccine provides sustained protection against precancers and cancers. In healthy women, protection has been demonstrated through at least eight years following vaccination.¹⁷

Data comparing the HPV18 cLIA and total IgG LIA in HIV-infected women are helpful in understanding the limitations and benefits of different immunoassays to measure vaccine response.²² As noted by Nygard et al., if the specific neutralizing epitope used by the cLIA is highly immunodominant, then antibody responses are long-lasting; however, if the specific neutralizing epitope is less immunodominant, then the loss of antibodies over time does not reflect loss of protection. This is supported by a study conducted by Brown et al., in which the investigators compared serologic responses to the quadrivalent HPV vaccine using three assays: cLIA, total IgG LIA, and a pseudovirion based neutralization assay (PBNA). The investigators found that for both HPV16 and HPV18, the cLIA, IgG, and PBNA assays were highly correlated. The positivity rates and correlation between the IgG and PBNA in sera that were negative by cLIA confirmed the presence of neutralizing antibody in the cLIA HPV18-negative samples. The authors concluded that both the cLIA and IgG accurately measure neutralizing antibody against HPV16 and HPV18 L1 VLPs.²⁶

It should be noted that the total IgG immunoassay is not a functional assay in that it measures all IgG binding to the VLP regardless of the protective potential of that antibody. The total IgG provides a broader measure of the immune response and is more sensitive, but less specific.²¹ Therefore, the IgG LIA may overestimate the level of protective antibodies generated by vaccination. Some have proposed that the cLIA, with its high specificity, may be most appropriate for assessing baseline immune status, while the total IgG, with its high sensitivity, may be most appropriate for assessing the vaccine-induced immune response over time.²¹ In addition, it is possible that the higher seropositivity rates for HPV18 when using the LIA were due to HPV16 specific antibodies. However, the correlated neutralization assay results have demonstrated that the LIA antibodies include HPV type-specific neutralizing antibodies.²⁶

In conclusion, the results of this study suggest that the lower rate of seropositivity to HPV18 when cLIA vs. IgG LIA is used is a function of the assay, and does not imply lower inherent immunogenicity of the vaccine against HPV18 or lack of protection against infection in HIV-infected young women. Long-term clinical observation after HPV vaccination will be important to monitor for breakthrough cases of HPV-related cancers.

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References

- Christensen ND, Dillner J, Eklund C, et al. Surface conformational and linear epitopes on HPV-16 and HPV-18 L1 virus-like particles as defined by monoclonal antibodies. *Virology*. 1996; 223(1): 174–184. [PubMed: 8806551]
- Brown DR, Bryan JT, Schroeder JM, et al. Neutralization of human papillomavirus type 11 (HPV-11) by serum from women vaccinated with yeast-derived HPV-11 L1 virus-like particles: correlation with competitive radioimmunoassay titer. *J Infect Dis*. 2001; 184(9):1183–1186. [PubMed: 11598842]
- Christensen ND, Hopfl R, DiAngelo SL, et al. Assembled baculovirus-expressed human papillomavirus type 11 L1 capsid protein virus-like particles are recognized by neutralizing monoclonal antibodies and induce high titres of neutralizing antibodies. *J Gen Virol*. 1994; 75(Pt 9): 2271–2276. [PubMed: 7521393]
- Kirnbauer R, Chandrachud LM, O'Neil BW, et al. Virus-like particles of bovine papillomavirus type 4 in prophylactic and therapeutic immunization. *Virology*. 1996; 219(1):37–44. [PubMed: 8623552]
- Koutsky LA, Ault KA, Wheeler CM, et al. A controlled trial of a human papillomavirus type 16 vaccine. *N Engl J Med*. 2002; 347(21):1645–1651. [PubMed: 12444178]
- Garland SM, Hernandez-Avila M, Wheeler CM, et al. Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases. *N Engl J Med*. 2007; 356(19):1928–1943. [PubMed: 17494926]
- Kjaer SK, Sigurdsson K, Iversen OE, et al. A pooled analysis of continued prophylactic efficacy of quadrivalent human papillomavirus (Types 6/11/16/18) vaccine against high-grade cervical and external genital lesions. *Cancer Prev Res (Phila)*. 2009; 2(10):868–878. [PubMed: 19789295]
- Ellerbrock TV, Chiasson MA, Bush TJ, et al. Incidence of cervical squamous intraepithelial lesions in HIV-infected women. *JAMA*. 2000; 283(8):1031–1037. [PubMed: 10697063]
- Abraham AG, Strickler HD, Jing Y, et al. Invasive cervical cancer risk among HIV-infected women: A North American multi-cohort collaboration prospective study. *J Acquir Immune Defic Syndr*. 2012
- Levin MJ, Moscicki AB, Song LY, et al. Safety and immunogenicity of a quadrivalent human papillomavirus (types 6, 11, 16, and 18) vaccine in HIV-infected children 7 to 12 years old. *J Acquir Immune Defic Syndr*. 2010; 55(2):197–204. [PubMed: 20574412]
- Weinberg A, Song LY, Saah A, et al. Humoral, mucosal, and cell-mediated immunity against vaccine and nonvaccine genotypes after administration of quadrivalent human papillomavirus vaccine to HIV-infected children. *J Infect Dis*. 2012; 206(8):1309–1318. [PubMed: 22859825]

12. Kahn JA, Xu J, Kapogiannis BG, et al. Immunogenicity and Safety of the Human Papillomavirus 6, 11, 16, 18 Vaccine in HIV-Infected Young Women. *Clin Infect Dis*. 2013; 57(5):735–744. [PubMed: 23667266]
13. Denny L, Hendricks B, Gordon C, et al. Safety and immunogenicity of the HPV-16/18 AS04-adjuvanted vaccine in HIV-positive women in South Africa: a partially-blind randomised placebo-controlled study. *Vaccine*. 2013; 31(48):5745–5753. [PubMed: 24091311]
14. Kojic EM, Kang M, Cespedes MS, et al. Immunogenicity and safety of the quadrivalent human papillomavirus vaccine in HIV-1-infected women. *Clin Infect Dis*. 2014; 59(1):127–135. [PubMed: 24723284]
15. Giacomet V, Penagini F, Trabattoni D, et al. Safety and immunogenicity of a quadrivalent human papillomavirus vaccine in HIV-infected and HIV-negative adolescents and young adults. *Vaccine*. 2014; 32(43):5657–5661. [PubMed: 25149430]
16. Toft L, Tolstrup M, Storgaard M, Ostergaard L, Sogaard OS. Vaccination against oncogenic human papillomavirus infection in HIV-infected populations: review of current status and future perspectives. *Sexual health*. 2014; 11(6):511–523. [PubMed: 25218800]
17. De Vincenzo R, Conte C, Ricci C, Scambia G, Capelli G. Long-term efficacy and safety of human papillomavirus vaccination. *International journal of women's health*. 2014; 6:999–1010.
18. Luna J, Plata M, Gonzalez M, et al. Long-term follow-up observation of the safety, immunogenicity, and effectiveness of Gardasil in adult women. *PloS one*. 2013; 8(12):e83431. [PubMed: 24391768]
19. Rana MM, Huhtala H, Apter D, et al. Understanding long-term protection of human papillomavirus vaccination against cervical carcinoma: Cancer registry-based follow-up. *Int J Cancer*. 2013; 132(12):2833–2838. [PubMed: 23180157]
20. Dias D, Van Doren J, Schlottmann S, et al. Optimization and validation of a multiplexed luminex assay to quantify antibodies to neutralizing epitopes on human papillomaviruses 6, 11, 16, and 18. *Clin Diagn Lab Immunol*. 2005; 12(8):959–969. [PubMed: 16085914]
21. Brown DR, Garland SM, Ferris DG, et al. The humoral response to Gardasil over four years as defined by total IgG and competitive Luminex immunoassay. *Hum Vaccin*. 2011; 7(2):230–238.
22. Schiller JT, Lowy DR. Immunogenicity testing in human papillomavirus virus-like-particle vaccine trials. *J Infect Dis*. 2009; 200(2):166–171. [PubMed: 19519255]
23. Olsson SE, Villa LL, Costa RL, et al. Induction of immune memory following administration of a prophylactic quadrivalent human papillomavirus (HPV) types 6/11/16/18 L1 virus-like particle (VLP) vaccine. *Vaccine*. 2007; 25(26):4931–4939. [PubMed: 17499406]
24. Opalka D, Matys K, Bojczuk P, et al. Multiplexed serologic assay for nine anogenital human papillomavirus types. *Clin Vaccine Immunol*. 2010; 17(5):818–827. [PubMed: 20237197]
25. Nygard M, Saah A, Munk C, et al. Evaluation of the Long-Term Anti-Human Papillomavirus 6 (HPV6), 11, 16, and 18 Immune Responses Generated by the Quadrivalent HPV Vaccine. *Clin Vaccine Immunol*. 2015; 22(8):943–948. [PubMed: 26084514]
26. Brown D, Muller M, Sehr P, et al. Concordance assessment between a multiplexed competitive Luminex immunoassay, a multiplexed IgG Luminex immunoassay, and a pseudovirion-based neutralization assay for detection of human papillomaviruse types 16 and 18. *Vaccine*. 2014; 32(44):5880–5887. [PubMed: 25148777]

Immune responses to the quadrivalent HPV vaccine measured by cLIA and total IgG: seropositivity and GMTs (total N=76), 28 and 48 weeks after vaccination¹

Table 1

	ART Status	Participants (N)	IgG LIA seropositivity N (%)	cLIA seropositivity N (%)	Total IgG GMT (mMu/ml) Mean (Std. Dev.)	cLIA GMT (mMu/ml) Mean (Std. Dev.)
Anti-HPV6						
28 Weeks (n=30)	All	30	30 (100.0)	30 (100.0)	640.3 (780.7)	869.8 (1003.5)
	ART	10	10 (100.0)	10 (100.0)	946.3 (1051.4)	1294.4 (1342.2)
	No ART	20	20 (100.0)	20 (100.0)	487.2 (576.9)	657.5 (735.7)
48 Weeks (n=29)	All	29	28 (96.6)	29 (100.0)	231.0 (302.2)	341.1 (373.1)
	ART	9	9 (100.0)	9 (100.0)	334.6 (402.0)	491.9 (507.7)
	No ART	20	19 (95.0)	20 (100.0)	181.9 (239.3)	273.2 (284.51)
Anti-HPV11						
28 Weeks (n=50)	All	50	49 (98.0)	49 (98.0)	521.5 (644.0)	1001.6 (1244.0)
	ART	16	16 (100.0)	16 (100.0)	682.2 (818.1)	1521.9 (1868.9)
	No ART	34	33 (97.1)	33 (97.1)	443.7 (537.8)	749.2 (694.8)
48 Weeks (n=46)	All	46	43 (93.5)	45 (97.8)	129.9 (198.3)	319.0 (471.2)
	ART	17	15 (88.2)	16 (94.1)	219.5 (311.4)	521.6 (742.8)
	No ART	29	28 (96.6)	29 (100.0)	82.0 (63.6)	207.1 (130.3)
Anti-HPV16						
28 Weeks (n=41)	All	41	41 (100.0)	40 (97.6)	2790.3 (4234.8)	3314.8 (3675.2)
	ART	13	13 (100.0)	13 (100.0)	4223.9 (4985.5)	5046.2 (4482.3)
	No ART	28	28 (100.0)	27 (96.4)	2124.7 (3751.2)	2481.1 (2961.0)
48 Weeks (n=40)	All	40	40 (100.0)	39 (97.5)	753.1 (1336.0)	1044.5 (1594.6)
	ART	14	14 (100.0)	14 (100.0)	1301.2 (2015.0)	1822.2 (2431.4)
	No ART	26	26 (100.0)	25 (96.2)	458.0 (643.7)	609.0 (522.7)
Anti-HPV18						
28 Weeks (n=55)	All	55	55 (100.0)	52 (94.6)	552.0 (694.8)	647.4 (917.8)
	ART	16	16 (100.0)	16 (100.0)	759.1 (694.3)	978.7 (1270.0)
	No ART	39	39 (100.0)	36 (92.3)	467.0 (685.8)	500.1 (680.9)
48 Weeks (n=52)	All	52	51 (98.1) ²	38 (73.1) ²	125.8 (140.7)	224.2 (383.3)
	ART	19	18 (94.7)	14 (73.7)	166.5 (172.69)	308.7 (528.84)

ART Status	Participants (N)	IgG LIA seropositivity N (%)	cLIA seropositivity N (%)	Total IgG GMT (mMu/ml) Mean (Std. Dev.)	cLIA GMT (mMu/ml) Mean (Std. Dev.)
No ART	33	33 (100.0)	24 (72.7)	103.6 (116.88)	175.0 (267.29)

A McNemar's test for paired data was used to compare seropositivity as measured by total IgG LIA and cLIA for each HPV type at weeks 28 and 48. χ^2 $p=.0002$; otherwise no significant differences ($p < .05$) between IgG LIA and cLIA with respect to seropositivity; GMTs are not comparable across the two assays so were not compared.