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Human papillomavirus antibody response following highly active antiretroviral therapy initiation among men who have sex with men

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Abstract: Objective: To describe effects of highly active antiretroviral therapy (HAART) on high-risk (HR) human papillomavirus (HPV) antibody response in HIV-positive men who have sex with men (MSM), and the meaning of this response for subsequent HPV-related cancer risk. Design: Prospective seroepidemiological study of 281 HIV-positive MSM initiating HAART between 1995 and 2004 in the Swiss HIV Cohort Study. Methods: For each individual, two serum samples, one at HAART initiation (pre-HAART) and another 24 months later (post-HAART), were tested for L1 antibodies to HPV6, 11, 16, 18, 31, 33, 35, 45, 52 and 58, as well as HPV16-E6 antibodies, using a multiplex serology assay. Identification of HPV-related cancer included data linkage with Swiss cancer registries. Results: Pre-HAART, 45.2% were seropositive for any high-risk (HR)-HPV-L1 and 32.4% for HPV16-L1. Sexual intercourse during the last six months was the only evaluated factor associated with L1 seropositivity pre-HAART. Seropositivity increased post-HAART to 60.5% for any HR-HPV-L1 (prevalence ratio versus pre-HAART = 1.34, 95% confidence interval (CI) 1.14-1.57), and 48.0% for HPV16-L1 (prevalence ratio versus pre-HAART = 1.48, 95% CI 1.20-1.83), and seroconversion was significantly associated with both lower CD4+ count and CD4+/CD8+ ratio ($p < 0.01$). Only one individual was HPV16-E6 seropositive pre-HAART, but two more seroconverted post-HAART. Anal cancer incidence among the three HPV16-E6-positives post-HAART was significantly increased compared to HPV16-E6-negatives (incidence rate ratio = 63.1, 95% CI 1.1-1211). Conclusion: HAART-related immune reconstitution increases HPV-specific antibody responses, which may discriminate future anal cancer risk in this high-risk population.

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Human papillomavirus antibody response following HAART initiation among MSM

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Objective: To describe effects of HAART on high-risk human papillomavirus (HPV) antibody response in HIV-positive MSM and the meaning of this response for subsequent HPV-related cancer risk.

Design: Prospective seroepidemiological study of 281 HIV-positive MSM initiating HAART between 1995 and 2004 in the Swiss HIV Cohort Study.

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Results: Pre-HAART, 45.2% were seropositive for any high-risk HPV-L1 and 32.4% for HPV16-L1. Sexual intercourse during the last 6 months was the only evaluated factor associated with L1 seropositivity pre-HAART. Seropositivity increased post-HAART to 60.5% for any high-risk HPV-L1 [prevalence ratio versus pre-HAART = 1.34, 95% confidence interval (CI) 1.14–1.57] and 48.0% for HPV16-L1 (prevalence ratio versus pre-HAART = 1.48, 95% CI 1.20–1.83), and seroconversion was significantly associated with both lower CD4⁺ cell count and CD4⁺/CD8⁺ ratio ($P < 0.01$). Only one individual was HPV16-E6-seropositive pre-HAART, but two more seroconverted post-HAART. Anal cancer incidence among the three HPV16-E6-positives post-HAART was significantly increased compared with HPV16-E6-negatives (incidence rate ratio = 63.1, 95% CI 1.1–1211).

Conclusion: HAART-related immune reconstitution increases HPV-specific antibody responses, which may discriminate future anal cancer risk in this high-risk population.

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Introduction

HIV-positive MSM have a high burden of anal high-risk human papillomavirus (HPV) infection [1]. Consequently, they also suffer a high incidence of anal cancer [1], for which persistent anal high-risk HPV infection, predominantly HPV16 [2], is the major cause. In the era of prolonged survival due to HAART, anal cancer incidence in HIV-positive MSM has reached more than 100 cases per 100 000 person-years [3,4].

Antibodies to high-risk HPV L1 protein are considered as markers of cumulative HPV exposure. However, L1 seroconversion does not always occur following natural HPV infection, and seroconversion rates in men may differ by the anatomical site of HPV infection [5,6]. There have been relatively few studies that have evaluated HPV L1 seropositivity in HIV-positive MSM [6–9], and the association between anal high-risk HPV infection and the detection of HPV-L1 antibodies is not clear in this population [6].

Antibodies against HPV E6 protein, on the other hand, have been shown to be highly specific markers for HPV-related cancer [10–16]. However, although HPV16-E6 is a highly sensitive marker for oropharyngeal cancer and can be consistently detected many years before cancer diagnosis [14,16], the picture is less clear for anal and cervical cancer [11,13,15] and even less so in HIV-positive persons [10,12].

To our knowledge, there are no studies evaluating the specific effects of HAART immune reconstitution on HPV antibody responses, nor the meaning of these responses for long-term HPV-related cancer risk, which is important to prevent in this high-risk population. Hence, this study used a multiplex HPV serology method based on a glutathione *S*-transferase (GST) capture immunosorbent assay [17] to describe HPV-L1 and HPV-E6 antibodies pre and post-HAART among a large sample of HIV-positive MSM [18], who were also followed up for cancer [3], within the framework of the Swiss HIV Cohort Study (SHCS).

Methods

Patients were participants in the SHCS, an ongoing study that has been enrolling persons infected with HIV since 1988 from five large university hospitals, two large cantonal hospitals, affiliated regional hospitals and private practitioners in Switzerland (www.shcs.ch). Detailed information on disease, laboratory tests and HIV-related treatments were collected at enrolment and at each 6-month follow-up visit [19].

A total of 1624 MSM initiated HAART between November 1995 and June 2004 while under active

follow-up in the SHCS. We excluded 878 patients with prior use of any antiretroviral therapy. From this group, we identified 281 MSM who had serum samples available within 1 month before (pre-HAART) and 21–27 months after (post-HAART) HAART initiation [18].

Markers of immunodeficiency (CD4⁺ and CD8⁺ cell counts, CD4⁺/CD8⁺ ratio, HIV viral load), AIDS status and tobacco consumption were extracted from the SHCS database. Questions on sexual intercourse during the last 6 months were asked at follow-up visits after April 2000 only. Individuals diagnosed with HPV-related cancer cases from HAART initiation until May 2016 were identified in the SHCS database and/or through record linkage with eight Swiss cancer registries [3,20].

Antibodies were tested at the German Cancer Research Center, Heidelberg, Germany, using a multiplex HPV serology assay based on a GST capture ELISA [21,22] combined with fluorescence-labeled polystyrene beads (SeroMAP Microspheres; Luminex Corp., Austin, Texas, USA) [17,23]. Viral antigens were expressed in *Escherichia coli* as double fusion proteins with N-terminal GST and a C-terminal peptide (tag) derived from the large T-antigen of simian virus 40. Glutathione-casein was coupled to internally fluorescence-labeled polystyrene beads, and GST-fusion proteins were affinity purified on the beads directly in a one-step in-situ procedure. The differently labeled beads carrying different antigens were then mixed and incubated with human serum that had been diluted 1 : 100 in blocking buffer. Antibodies bound to the beads were then stained with biotinylated antihuman immunoglobulin followed by the fluorescent reporter conjugate streptavidin-R-phycoerythrin. A Luminex analyzer was used to quantify the antibodies bound to antigens on beads and to identify the antigen by bead color. Antibody quantity was determined as the median R-phycoerythrin fluorescence intensity (MFI) from at least 100 beads of the same internal color.

MFI values were dichotomized as antibody positive or negative. Cutoff values for HPV16, 18, 31, 33, 35, 45, 53 and 58 L1 were predetermined independently for each antibody by analyzing MFI values obtained from Korean HPV-DNA-negative virgin women using the algorithm of five SDs after exclusion of outliers [24]. Cutoff MFI values were defined at 1000 for HPV16-E6 [16,25], and 800 and 400 for HPV6 and HPV11 L1 [26]. For any high-risk HPV-L1, seropositivity was defined as positive for at least one HPV type among HPV16, 18, 31, 33, 35, 45, 53 and 58.

Seroprevalence of HPV antibodies was calculated for strata of age, HIV viral load, CD4⁺ and CD8⁺ cell counts, and CD4⁺/CD8⁺ ratio at HAART initiation. Differences between strata were compared by χ^2 test or Cochran–Armitage trend test. At 24-month follow-up, changes in seropositivity for HPV antibodies were

evaluated by crude prevalence ratios and corresponding 95% confidence intervals (CIs).

Factors associated with seroconversion were investigated in patients seronegative at HAART initiation, by calculating crude odds ratio (OR) of seroconversion and corresponding 95% CI according to baseline characteristics. For any high-risk HPV-L1, seronegativity at HAART initiation was defined as negative for at least one HPV type among HPV16, 18, 31, 33, 35, 45, 53 and 58.

Type-specific mean MFI values at HAART initiation and at 24-month follow-up were compared among all 281 MSM (irrespective of being seronegative/positive) using Wilcoxon signed-rank sum test and were additionally compared across strata of CD4⁺ at HAART initiation.

For anal cancer incidence rate analyses according to post-HAART serology result, calculation of person-years at risk began at the date of post-HAART serology and ended on the date of last SHCS follow-up, anal cancer or death, whichever was earliest. Incidence rate ratios and corresponding 95% CI were calculated using exact estimates.

Results

Patient characteristics

Of the 281 included MSM, mean age was 40.8 years (range 19.6–73.4) and 56 (19.9%) had history of AIDS at HAART initiation (Table 1). At HAART initiation, mean CD4⁺ cell count was 240 cells/ μ l (median 194, range 2–1037) and mean viral load 390 000 copies/ml (median 100 000, range 0–8750 000). Post-HAART, mean CD4⁺ was 504 cells/ μ l (median 444, range 28–1829) and mean viral load 20 000 copies/ml (median 0, range 0–2300 000). Table 1 also shows associations of selected characteristics with HPV16-L1 and high-risk HPV-L1 seropositivity pre-HAART, of which only sexual intercourse during the last 6 months was statistically significant ($P=0.01$ and 0.04 , respectively).

Human papillomavirus antibody seropositivity at date of HAART initiation (pre-HAART)

Pre-HAART, 91 (32.4%) participants were HPV16-L1-seropositive (Table 2). Seropositivity for other high-risk HPV types ranged from 7.8% (HPV58) to 17.8% (HPV31), and 127 (45.2%) were positive for at least one high-risk HPV type among HPV16, 18, 31, 33, 35, 45, 52 and 58 (Table 2). Seropositivity of HPV6 and HPV11 L1 were 40.2 and 29.2%, respectively (Table 2). Only one participant (0.4%) was positive for HPV16-E6 antibody pre-HAART.

HAART-related changes in human papillomavirus antibody seropositivity

Post-HAART, seropositivity of all evaluated HPV antibodies increased (Table 2). A total of 135 (48.0%)

participants were HPV16-L1-seropositive, corresponding to a prevalence ratio of 1.48 (95% CI 1.20–1.83) compared with pre-HAART. Post-HAART seropositivity for other high-risk HPV types ranged from 14.2% (HPV58) to 34.5% (HPV31), and 170 (60.5%) were seropositive for at least one high-risk HPV type. Prevalence ratios for high-risk HPV-L1 seropositivity post-HAART versus pre-HAART ranged from 1.64 (1.17–2.29) for HPV35 to 2.17 (1.51–3.13) for HPV18. Seroprevalence was 53.0% for HPV6 [prevalence ratio = 1.32 (1.10–1.58)] and 45.6% for HPV11 [prevalence ratio = 1.32 (1.10–1.58)].

The one HPV16-E6-seropositive patient pre-HAART remained seropositive post-HAART, and two more individuals seroconverted, resulting in a total of three seropositive (1.1%) post-HAART [prevalence ratio = 3.00 (0.31–28.7)].

Factors associated with seroconversion are described in Table 3. Among participants HPV16-seronegative pre-HAART, seroconversion for HPV16-L1 was associated with low CD4⁺ cell count [OR = 5.28 (1.88–14.8) for CD4⁺ <50 versus \geq 350 cells/ μ l] and low CD4⁺/CD8⁺ ratio [OR = 6.56 (1.98–21.71) for CD4⁺/CD8⁺ ratio <0.10 versus \geq 0.5] at HAART initiation. Results were consistent for seroconversion for any high-risk HPV-L1 (Table 3) and were statistically significant for certain individual types: low CD4⁺ cell count with seroconversion of HPV31, 33 and 52 and low CD4⁺/CD8⁺ ratio with seroconversion of HPV31, 33, 35, 45 and 52 (data not shown).

Changes in human papillomavirus antibody titers

Between pre-HAART and post-HAART, mean MFI values (a semiquantitative measure of antibody titers) increased for all evaluated HPV-L1 antibodies (Fig. 1a) and were statistically significant for all high-risk HPV types. Mean MFI values for HPV16-L1 increased from 600 to 950, and increases were greatest for participants with CD4⁺ cell count less than 50 cells/ μ l (Fig. 1b).

Incidence of human papillomavirus-related cancer

The 281 included MSM were actively followed up in the SHCS for a total of 3771 person-years following HAART initiation, including 558 person-years before and 3213 person-years after the date of post-HAART serology. Five incident anal (and no oropharyngeal or penile) cancers were diagnosed during this time, corresponding to an overall anal cancer incidence rate of 132/100 000 person-years, of which two were diagnosed before and two after the date of post-HAART serology.

Associations between HPV16 serology status post-HAART (by which time many participants had seroconverted) and anal cancer incidence are shown in Table 4. Anal cancer incidence was 0/100 000 person-

Table 1. Human papillomavirus antibody seropositivity among 281 MSM, overall and by selected characteristics, at HAART initiation.

	<i>n</i>	HPV16-L1		Any HR-HPV-L1 ^a	
		<i>n</i> (%)	<i>p</i> ^b	<i>n</i> (%)	<i>p</i> ^b
Age (years)					
≥45	81	28 (34.6)		40 (49.4)	
35–44	104	38 (36.5)		52 (50.0)	
<35	95	25 (26.3)	0.22	35 (36.8)	0.09
Tobacco					
Ever	159	46 (28.9)		65 (40.9)	
Never	118	43 (36.4)	0.19	60 (50.9)	0.10
History of AIDS					
Yes	225	76 (33.8)		104 (46.2)	
No	56	15 (26.8)	0.32	23 (41.1)	0.49
Sexual intercourse in the last 6 months ^c					
None	21	5 (23.8)		8 (38.1)	
Steady partner only	20	5 (25.0)		10 (50.0)	
Occasional partner(s) only	21	9 (42.9)		13 (61.9)	
Steady and occasional partner(s)	14	9 (64.3)	0.01	10 (71.4)	0.04
HIV viral load (copies/ml)					
<10 000	38	13 (34.2)		14 (36.9)	
10 000–99 999	99	28 (28.3)		44 (44.4)	
100 000–499 999	90	29 (32.2)		44 (48.9)	
≥500 000	46	20 (43.5)	0.24	23 (50.0)	0.19
CD4 ⁺ cell count (cells/μl)					
≥350	68	22 (32.4)		27 (39.7)	
200–350	71	24 (33.8)		34 (47.9)	
50–199	93	33 (35.9)		48 (51.6)	
<50	49	12 (24.5)	0.55	18 (36.7)	0.87
CD8 ⁺ cell count (cells/μl)					
≥1000	89	35 (39.3)		45 (50.6)	
750–999	58	14 (24.1)		18 (31.0)	
500–749	66	21 (31.8)		34 (51.5)	
<500	68	21 (30.9)	0.35	30 (44.1)	0.83
CD4 ⁺ /CD8 ⁺ ratio					
≥0.5	47	13 (27.7)		17 (36.2)	
0.25–0.49	80	28 (35.0)		39 (48.8)	
0.10–0.25	89	34 (38.2)		46 (51.7)	
<0.10	61	16 (26.2)	0.9	25 (41.0)	0.65

HPV, human papillomavirus; HR, high risk; SHCS, Swiss HIV Cohort Study.

^aAny HPV-L1: seropositivity of at least one HPV type among HPV16, 18, 31, 33, 35, 45, 52 and 58.

^bCochran–Armitage trend test used for ordinal categories, χ^2 test for dichotomous categories.

^cAsked to SHCS participants at follow-up visits after April 2000 only ($n = 76$).

years for post-HAART HPV16-L1 seronegatives versus 196/100 000 person-years for seropositives, corresponding to a nonsignificant incidence rate ratio of ∞ (95% CI 0.5– ∞) and 63/100 000 person-years for HPV16-E6 seronegatives versus 3956/100 000 person-years in seropositives, corresponding to a statistically significant incidence rate ratio of 63.1 (95% CI 1.1–1211) (Table 4).

The participant who was HPV16-E6-seropositive both pre-HAART (MFI = 1408) and post-HAART (MFI = 1916) had not developed anal cancer by 2016, 15 years after post-HAART serology date. Of the two participants that seroconverted for HPV16-E6 post-HAART, one died 7 months later following a diagnosis of lymphoma (pre-HAART MFI = 63; post-HAART MFI = 10 909), and the other developed anal cancer 9 years later (pre-HAART MFI = 10; post-HAART MFI = 2382).

Discussion

In a cohort of HIV-infected MSM, we were able to describe, for the first time to our knowledge, that HAART is associated with seroconversion and increasing titers of HPV antibodies. In this high-risk population, for whom approximately half were already seropositive for at least one high-risk HPV type before HAART, another fifth seroconverted to high-risk HPV-L1 in the 2 years following HAART initiation. Those with the lowest CD4⁺ cell count and CD4⁺/CD8⁺ ratio at HAART initiation were the most likely to seroconvert, suggesting that HAART-related immune reconstitution contributes to revealing a detectable immune response to HPV. This was apparent even for HPV16-E6 antibodies, for which two out of three seropositive cases were detected only post-HAART. Furthermore, although numbers of cancer cases were small, HAART-related seroconversion seemed

Table 2. Changes in human papillomavirus antibody seropositivity at HAART initiation and 24-month follow-up in 281 MSM.

	Pos at HAART initiation n/total (%)	Pos at 24-month follow-up n/total (%)	Prevalence ratio (95% CI)	Status at both time points			
				+/+	+/-	-/+	-/-
HPV16-L1	91/281 (32.4)	135/281 (48.0)	1.48 (1.20–1.83)	80 (28.5)	11 (3.9)	55 (19.6)	135 (48.0)
HPV18 L1	35/281 (12.5)	76/281 (27.1)	2.17 (1.51–3.13)	28 (10.0)	7 (2.5)	48 (17.1)	198 (70.5)
HPV31 L1	50/281 (17.8)	97/281 (34.5)	1.94 (1.44–2.61)	45 (16.0)	5 (1.8)	52 (18.5)	179 (63.7)
HPV33 L1	27/281 (9.6)	55/281 (19.6)	2.04 (1.33–3.13)	24 (8.5)	3 (1.1)	31 (11.0)	223 (79.4)
HPV35 L1	44/281 (15.7)	72/281 (25.6)	1.64 (1.17–2.29)	41 (14.6)	3 (1.1)	31 (11.0)	206 (73.3)
HPV45 L1	31/281 (11.0)	83/281 (29.5)	2.68 (1.83–3.91)	25 (8.9)	6 (2.1)	58 (20.6)	192 (68.3)
HPV52 L1	36/281 (12.8)	64/281 (22.8)	1.78 (1.22–2.58)	31 (11.0)	5 (1.8)	33 (11.7)	212 (75.4)
HPV58 L1	22/281 (7.8)	40/281 (14.2)	1.82 (1.11–2.98)	19 (6.8)	3 (1.1)	21 (7.5)	238 (84.7)
Any HR-HPV-L1 ^a	127/281 (45.2)	170/281 (60.5)	1.34 (1.14–1.57)	116 (41.3)	11 (3.9)	54 (19.2)	100 (35.6)
HPV6 L1	113/281 (40.2)	149/281 (53.0)	1.32 (1.10–1.58)	96 (34.2)	17 (6.1)	53 (18.9)	115 (40.9)
HPV11 L1	82/281 (29.2)	128/281 (45.6)	1.56 (1.25–1.95)	73 (26.0)	9 (3.2)	55 (19.6)	144 (51.3)
HPV16-E6	1/281 (0.4)	3/281 (1.1)	3.00 (0.31–28.7)	1 (0.4)	0 (0.0)	2 (0.7)	278 (98.9)

CI, confidence interval; HPV, human papillomavirus.

^aAny HPV-L1: seropositivity of at least one HPV type among HPV16, 18, 31, 33, 35, 45, 52 and 58.

to improve the ability of HPV16-L1 and E6 antibody status to discriminate future anal cancer risk.

Increases in seroprevalence and mean MFI values were observed for all eight evaluated high-risk HPV-L1 antibodies. Seroconversion rates were far above those of seroreversion and so could not be explained by random

fluctuation of test results around the cutoffs of the serology assay, nor by high natural turnover of HPV antibody responses. Furthermore, although we confirmed an association of recent sexual intercourse with HPV seropositivity [6,9,26,27], HAART initiation did not appear to increase risky sexual behavior (46.1% declaring occasional partners at HAART initiation versus

Table 3. Factors associated with seroconversion^a after 24 months among patients human papillomavirus-L1-seronegative at HAART initiation.

	HPV16-L1			Any HR-HPV-L1 ^b		
	n (%)	OR (95% CI)	P trend	n (%)	OR (95% CI)	P trend
Age (years)						
≥45	15/53 (28.3)	1		29/78 (37.2)	1	
35–44	21/66 (31.8)	1.16 (0.53–2.55)		45/100 (45.0)	1.38 (0.75–2.53)	
<35	19/70 (27.1)	0.94 (0.43–2.09)	0.85	41/95 (43.2)	1.28 (0.70–2.37)	0.46
Tobacco						
Ever	27/113 (23.9)	1		58/157 (36.9)	1	
Never	27/75 (36.0)	1.79 (0.95–3.40)	–	54/112 (48.2)	1.59 (0.97–2.60)	–
History of AIDS						
No	41/149 (27.5)	1		87/217 (40.1)	1	
Yes	14/41 (34.2)	1.24 (0.75–2.04)	–	28/56 (50.0)	1.49 (0.83–2.70)	–
HIV viral load (copies/ml)						
<10 000	6/25 (24.0)	1		11/36 (30.6)	1	
10 000–99 999	23/71 (32.4)	1.52 (0.53–4.31)		46/96 (47.9)	2.09 (0.93–4.72)	
100 000–499 999	15/61 (24.6)	1.03 (0.35–3.06)		33/88 (37.5)	1.36 (0.59–3.13)	
≥500 000	9/26 (34.6)	1.68 (0.49–5.69)	0.78	22/45 (48.9)	2.17 (0.87–5.45)	0.42
CD4 ⁺ cell count (cells/μl)						
≥350	7/46 (15.2)	1		19/65 (29.2)	1	
200–350	13/47 (27.7)	2.13 (0.76–5.95)		26/70 (37.1)	1.43 (0.70–2.94)	
50–199	17/60 (28.3)	2.20 (0.83–5.88)		41/89 (46.1)	2.07 (1.05–4.07)	
<50	18/37 (48.7)	5.28 (1.88–14.8)	<0.01	29/49 (59.2)	3.51 (1.61–7.67)	<0.01
CD8 ⁺ cell count (cells/μl)						
≥1000	14/54 (25.9)	1		36/85 (42.4)	1	
750–999	16/44 (36.4)	1.63 (0.69–3.88)		28/58 (48.3)	1.27 (0.65–2.49)	
500–749	10/45 (22.2)	0.82 (0.32–2.07)		22/64 (34.4)	0.71 (0.36–1.40)	
<500	15/47 (31.9)	1.34 (0.56–3.18)	0.85	29/66 (43.9)	1.07 (0.56–2.04)	0.77
CD4 ⁺ /CD8 ⁺ ratio						
≥0.5	4/34 (11.8)	1		12/45 (26.7)	1	
0.25–0.49	13/52 (25.0)	2.50 (0.74–8.45)		31/79 (39.2)	1.78 (0.80–3.95)	
0.10–0.24	16/55 (29.1)	3.08 (0.93–10.16)		37/84 (44.1)	2.16 (0.98–4.76)	
<0.10	21/45 (46.7)	6.56 (1.98–21.71)	<0.01	34/61 (55.7)	3.46 (1.51–7.96)	<0.01

CI, confidence interval; HPV, human papillomavirus; HR, high risk; OR, odds ratio.

^aSeroconversion = seroconversion of at least one HPV type.

^bFor any HPV-L1: seronegative = seronegative for at least one HPV type among HPV16, 18, 31, 33, 35, 45, 52 and 58.

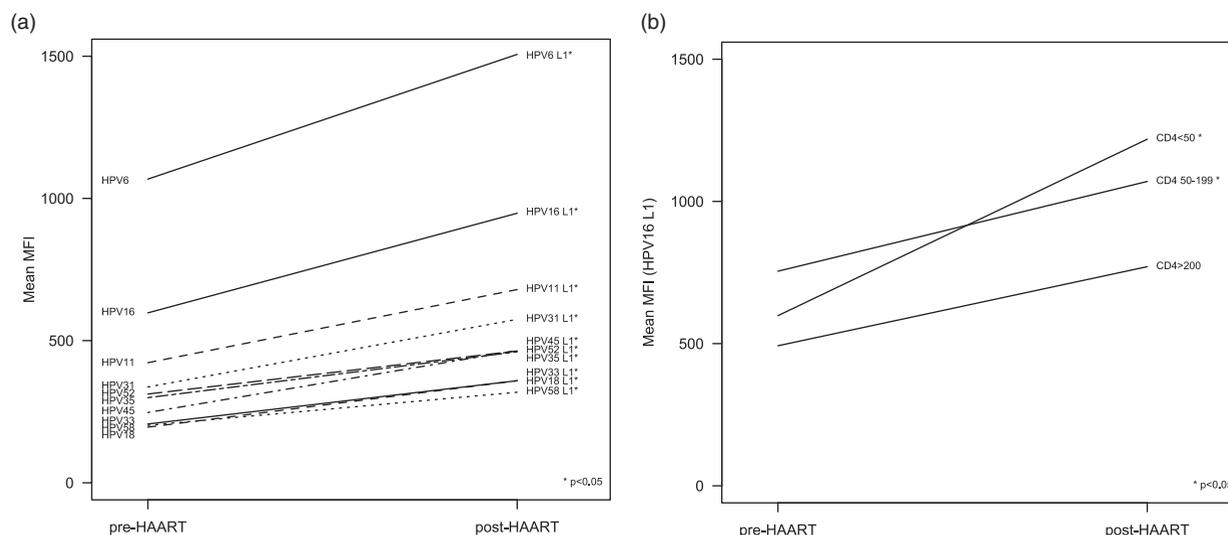


Fig. 1. Mean change in human papillomavirus–L1 antibody response stratified by human papillomavirus type (a) and in human papillomavirus 16-L1 by CD4⁺ cell count (b).

38.4% at 24-month follow-up; data not shown) and hence should also not explain high seroconversion rates. The fact that seroconversion and MFI increases were associated with lower CD4⁺ cell count and/or CD4⁺/CD8⁺ ratio pre-HAART would suggest that increases in measurable HPV antibodies are principally due to immune reconstitution, rather than exposure to new HPV infections.

In contrast to the effect of low CD4⁺ cell count on seroconversion and increases in L1 antibody titers, CD4⁺ cell counts were not associated with cross-sectional seroprevalence. This corroborates lack of association of CD4⁺ cell counts with HPV seroprevalence in other HIV-positive MSM [6,7,9] and HIV-positive women [28] and suggests that HAART-related increases in detectable HPV immune responses are not mediated solely via CD4⁺ cell counts. Indeed, we have described a similar phenomenon for Kaposi's sarcoma-associated herpesvirus antibodies in these samples [18].

High type-specific seroprevalences observed in the SHCS are consistent with those reported in other HIV-positive MSM [6,7,9,27], albeit with different serological assays. Based on the same assay and cutoff definition, HPV6 (53%) and 11 (46%) seroprevalence in the SHCS was similar to that reported in 245 HIV-positive MSM in

Australia (53 and 48%) [8]. HPV6 and 11 seroconversion, on the other hand, was much higher in the current study (12 and 11 per 100 person-years, calculated using comparable criteria, versus 2.4 and 1.5 per 100 person-years) [8], supporting the theory that HAART initiation is a moment particularly disposed to HPV seroconversion.

HPV-L1 seroprevalence is higher in HIV-positive than HIV-negative MSM [6,27]. This is expected to be related to their higher prevalence of anal HPV infection [1,29], known to be an important determinant of seropositivity in MSM [9,29]. Our data, however, confirm that anal HPV exposure is not sufficient for seroconversion in HIV-positive MSM and could explain why anal HPV infection was a significant determinant of HPV seroconversion in HIV-negative, but not HIV-positive, MSM [6] in a cross-sectional comparison that did not take HAART into account.

HPV-L1 antibodies acquired through natural infection have been shown to provide modest protection against subsequent HPV infection in female patients, but not in men [30]. This question requires data from large prospective studies with multiple visits, because detectable L1 antibodies are also a marker of exposure and are positively associated with HPV-related cancer [10,11,13–16]. Indeed, in the present study, all anal cancers occurred

Table 4. Anal cancer incidence according to human papillomavirus 16-L1 and E6 serology status post-HAART.

		<i>n</i>	Person-years	Anal cancers	Incidence rate (/100 000)	Incidence rate ratio
HPV16-L1	Negative	146	1685	0	0	1
	Positive	133	1528	3	196 (63–609)	∞ (0.5–∞)
HPV16-E6	Negative	276	3187	2	63 (16–254)	1
	Positive	3	25	1	3956 (557–28 086)	63.1 (1.1–1211)

HPV, human papillomavirus.

in MSM who were HPV16-L1-seropositive post-HAART.

HPV vaccination results in far greater seroconversion rates and antibody titers than natural HPV infection. Interestingly, in two studies of quadrivalent HPV vaccine of immunogenicity among HIV-infected men [31] and women [32], no relationships between HPV antibody concentrations and CD4⁺ cell counts were observed, but higher antibody concentrations were reported in HAART users. This suggests that the CD4⁺-independent effect of HAART on immune response that we have described in the SHCS for natural HPV infection may also play a role in vaccine-induced HPV antibody response, even if the clinical significance of lower L1 antibody concentrations remains unknown because there is no established threshold correlating with protection.

HPV16-E6 antibodies have already been shown to be specific, but insensitive, markers of cervical [12] and anal [10] cancer in the SHCS, but the present study is the first to provide prospective data on the meaning of HPV16-E6 antibodies for future risk of HPV-related cancer in HIV-positive populations. In our study of HIV-positive MSM, only one patient (0.4%) was HPV16-E6-seropositive at HAART initiation and remained cancer-free during his entire 15-year follow-up. Two more HPV16-E6-seropositive cases were revealed post-HAART, however, of which one developed anal cancer 11 years later, so that HPV16-E6 positivity post-HAART was associated with a 63-fold significant increase in anal cancer incidence, albeit with very wide 95% CIs. Two out of the three anal cancers were E6-seronegative, however, confirming observations in the general (30% of anal cancers E6-seropositive [15]) and HIV-positive (22% [10]) population of a low sensitivity of HPV16-E6 antibody for predicting anal cancer.

The principal novelty of our study design is represented by samples selected at a 2-year interval pre-HAART and post-HAART in a large and well characterized group of HIV-positive MSM. Indeed, this set of samples has been previously used to describe the effect of HAART on Kaposi's sarcoma-associated herpesvirus viremia and antibodies [18]. In addition, however, 15-year follow-up, during which time the SHCS has been regularly linked with cancer registries [3,20], allowed an ad-hoc cohort analysis of HPV-related cancer risk. Despite small absolute numbers, high anal cancer incidence rates (132 per 100 000 person-years) were comparable with other estimates in HIV-positive MSM in the HAART era (131 per 100 000 person-years [4]) and enabled us to identify significant serological determinants (HPV16-E6) of anal cancer risk. Of note, the absence of other HPV-related cancer diagnoses is consistent with the estimation that anal cancer accounts for 85% of HPV-related cancers in HIV-infected populations [33]. Other study limitations include the lack of control group of HIV+ MSM against

which to compare 2-year HPV seroconversion (e.g. naive or long-term HAART users) and the unavailability of a measure of HPV DNA, particularly from the anus, that prevented us from distinguishing seropositivity related to past or present infections. Although we had L1 serological assays for only eight of the 12 HPV types that have been designated as high-risk based on their carcinogenicity at the cervix, and only HPV16-E6, HPV16 is the only HPV type established as a cause of anal cancer [34], and is by far the predominantly detected type in large cases series of anal cancer [2,35], including those diagnosed among HIV-positive persons [36,37]. In addition, there is evidence for some cross-reactivity of HPV E6, but not L1, assay antigens, for antibodies of closely phylogenetically related types [13].

With respect to estimating the fraction of HIV-positive MSM who may profit from primary anal cancer prevention by prophylactic HPV vaccination, we show that seropositivity against HPV16 and other high-risk HPV types is already very high in HAART-naive HIV-infected MSM and that HAART reveals previously undetectable antibody responses in an additional fraction. This adds to evidence that not all anal HPV infections result in detectable immune responses [6,7], so that a definition of HPV seronegativity overestimates the truly HPV-naive population. With respect to secondary anal cancer prevention, our results suggest that HPV16-E6 antibodies might have some predictive value for risk stratification of HIV-positive MSM, at least among those commencing HAART. However, these findings remain preliminary, and there remains little consensus about the utility and/or the appropriate algorithm for further diagnostic work-up and treatment.

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Conflicts of interest

There are no conflicts of interest.

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