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# Incidence, Persistence, Clearance, and Correlates of Genital Human Papillomavirus Infection and Anogenital Warts in a Cohort of Men Living With Human Immunodeficiency Virus in South Africa

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**Objective:** To estimate the incidence; persistence and correlates of human papillomavirus (HPV) infection and anogenital warts (AGW) among men living with human immunodeficiency virus (MLHIV).

**Methods:** Overall, 304 MLHIV 18 years or older were enrolled and attended follow-up visits at 6, 12, and 18 months. Clinicians examined for AGW, collected blood, and penile swabs for HPV testing (Roche Linear Array) at each visit. Time to AGW incidence or clearance was estimated by Kaplan-Meier method. Factors associated with persistent HPV infection and AGW clearance were evaluated with generalized estimating equations and Cox regression, respectively.

**Results:** Mean age of participants was 38 years (standard deviation, 8 years); 25% reported more than 1 sexual partner in the past 3 months. Most (65%) participants were on antiretroviral treatment (ART) with a median CD4<sup>+</sup> count of 445 cells/μL (interquartile range, 328–567). Prevalence of HPV infection and AGW at enrolment were 79% (224 of 283) and 12% (36 of 304), respectively. Two hundred fifty-nine men were followed up for a median (interquartile range) 1.4 years (0.5–1.7 years). Incidence of any-genital HPV infection was 2.9 (95% confidence interval, 1.5–5.5) per 100 person-years. Persistence of any-genital HPV infection was 35% (68 of 192) and was higher among MLHIV with low CD4<sup>+</sup> count (adjusted odds ratio, 3.54; 95% confidence interval, 2.07–6.05). Incidence of AGW was 1.4 per 100 person-years. Men living with human immunodeficiency virus with high CD4<sup>+</sup> count were more likely to clear AGW than those with low CD4 count (adjusted hazard ratio, 3.69; 95% confidence interval, 1.44–9.47). No associations were observed between persistent genital HPV infection, AGW clearance with enrolment ART status or duration.

**Conclusions:** Human immunodeficiency virus–positive men have a high burden of genital HPV infection and AGW. The ART and HPV vaccine could reduce this burden.

Human papillomavirus (HPV) is the most common sexually transmitted infection worldwide.<sup>1</sup> Genital HPV causes a substantial burden of disease burden in men. High-risk (HR) HPV types cause about 50% of the estimated 26,000 annual incident penile cancer cases that occur worldwide.<sup>2</sup> Low-risk (LR) HPV types 6 and 11 are responsible for 90% of anogenital warts (AGW).<sup>3</sup>

Persistence of genital HPV infection is important for progression to disease.<sup>4</sup> Human immunodeficiency virus (HIV) infection is associated with an increased risk of persistence and progression to disease.<sup>5,6</sup> People living with HIV have a higher prevalence of genital HPV infection and are more likely to be infected with multiple HPV types compared with HIV-negative individuals.<sup>7</sup> In people living with HIV, AGW tend to be florid and are often difficult to treat, particularly in resource-constrained settings.<sup>8</sup> These poor clinical outcomes are due to immunosuppression which impairs the clearance of HPV infections.<sup>9</sup> Although the natural history of cervical cancer and progression from HPV infection to development of invasive cervical disease has been well documented,<sup>10–12</sup> relatively little is known about the natural history of genital HPV infection and related diseases in men,<sup>13,14</sup> especially among predominantly heterosexual men living with HIV (MLHIV). Moreover, although HPV viral load (VL) has been

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shown to be a strong predictor of persistent cervical HPV infection, its role in predicting persistent genital HPV infection in men is not clear.<sup>15</sup>

The development and roll out of highly efficacious HPV vaccines is arguably one of the biggest public health innovations of the 21st century. In 2014, the South African government introduced HPV vaccination into the national immunization schedule, and the vaccine is currently only being delivered to girls with a goal to prevent cervical cancer which is a leading cause of death in women. Results from high-income countries, such as Australia, show significant declines in the cases of AGW among vaccinated women and a herd-immunity effect in unvaccinated heterosexual men.<sup>16</sup> In settings with a high HIV prevalence, the impact of a girls-only program on herd immunity in men may be reduced.<sup>17</sup> Data from Australia have also shown that men who have sex with men will not be protected by herd immunity from a girls-only program.<sup>16</sup>

We established a cohort study of predominately heterosexual MLHIV to learn about the epidemiology of HPV in this population and help inform future HPV prevention policies among men in South Africa. In this analysis, we estimated the prevalence, incidence, persistence and clearance of genital HPV infections and AGW, and the correlates of these outcomes. Based on this evidence, we postulate what benefits might accrue from HPV vaccination in males.

## METHODS

### Study Design, Population, and Data Collection

The design of the cohort study has been published previously.<sup>18</sup> In brief, 304 HIV seropositive men 18 years or older who reported sexual activity in the 3 months before enrolment were recruited from antiretroviral treatment (ART) clinics in inner-city Johannesburg. Participants were enrolled irrespective of sexual orientation and followed up every 6 months for up to 18 months. Data on sociodemographic, behavioral and clinical characteristics were collected by an interviewer-administered questionnaire at each visit. In addition, participants completed sensitive questions on sexual behavior using a computer-assisted self-interview, to improve privacy. The presence of AGW was assessed at each visit by a trained clinician during a standardized genital examination. According to National Sexually Transmitted Infection Management Guidelines, men with AGW at any visit were treated with Podophyllin 20% solution at weekly intervals until lesions disappear.<sup>19</sup> Cure of AGW was established by genital examination before each study visit.

### Sample Collection

Venous blood was taken at each visit to test for CD4<sup>+</sup> cell count (FACScount, BD; BD Biosciences, San Jose, CA) and HIV-1 plasma VL (PVL) using Roche Taqman (Roche Diagnostics, Mannheim, Germany). A genital sample for HPV DNA testing was collected by rubbing a cotton swab around the glans penis, coronal sulcus, and ventral surface of the penis as previously described.<sup>12,18</sup> Swabs were stored at -70°C before HPV DNA testing.

### Laboratory Methods

The CD4<sup>+</sup> counts and HIV-1 PVL were measured using FACScount, BD (BD Biosciences) and Roche Taqman (Roche Diagnostics), respectively. Participants who had a CD4<sup>+</sup> count less than 350 cells/ $\mu$ L were referred to an HIV clinic for ART initiation, in accordance with the national guidelines at the time.<sup>20</sup>

The HPV detection and genotyping was performed using an identical method at enrolment and at last follow-up visit, testing was done only for these 2 visits due to cost constraints. The

MagNA Pure LC DNA Isolation Kit I (Roche Diagnostics) was used to extract HPV DNA from the swabs. The HPV genotype distributions were then assessed by the Roche Linear Array assay (RLA; Roche Diagnostics). The HPV 16 and HPV 18 VLs (copies per million human cells) were quantified at enrolment on samples that were positive for these types using quantitative duplex real-time polymerase chain reaction method.<sup>21</sup> This method allows the HPV 16, HPV 18, and albumin gene copy number to be quantified in the same assay. The human  $\beta$ -globin gene served as an internal control for cellular adequacy and extraction efficiency. Results for samples with an inadequate control were reported as invalid and excluded from analysis. Of the 272 available for HPV testing, 13 (5%) had inadequate control.

### Definition of Genital HPV Infection and AGW Outcomes

Groups of HPV genotypes were categorized as follows: (i) *any HPV infection*, that is, detection of at least 1 of the 37 HPV genotypes that can be isolated by the RLA; (ii) *any HR-HPV infection*, that is, detection of at least 1 of the following HPV genotypes: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 or 68; (iii) *any LR-HPV infection*, that is, detection of at least 1 of the following HPV genotypes: 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 66, 69, 70, 71, 72, 73, 81, 83, 84, IS39 or CP6108; (iv) *any alpha-7 infection*, that is, detection of at least 1 of the following HR-HPV genotypes: 18, 45; 39 or 59; and (v) *any alpha-9 infection*, that is, detection of at least 1 of the following HR-HPV genotypes: 16, 31, 33, 35, 52 or 58. The HPV 16 or 18 VL were log transformed to base 10, to normalize distribution, as previously described.<sup>21</sup> Prevalent genital HPV infection was defined as detection of HPV DNA at enrolment. The HPV DNA infection outcomes at final visit were defined as (i) *incident infection*, that is, detection of any HPV DNA type that was not detected at enrolment; (ii) *persistence*, that is, detection of the same HPV DNA type that was detected at enrolment; and (iii) *clearance*, that is, absence of detection of an HPV DNA type that was detected at enrolment.

Prevalent AGW was defined as detection of genital warts during clinical examination at enrolment. AGW outcomes at each follow-up visit were defined as (i) *incident*, that is, detection of AGW in a participant that had no AGW at enrolment; (ii) *persistent*, that is, detection of AGW at all follow-up visits in a participant who had AGW at enrolment; and (iii) *clearance*, that is, the absence of detection of AGW at all follow-up visits in a participant who had AGW at enrolment.

Eight variables measuring the HIV disease of participants were generated, including ART status, CD4<sup>+</sup> count, and PVL at enrolment. Controlled HIV disease at enrolment was defined as being on ART for at least 6 months, with CD4<sup>+</sup> count of 350 cells/ $\mu$ L or higher and undetectable HIV-1 PVL (ie, <40 copies/mL). High stable CD4<sup>+</sup> count was defined as a CD4<sup>+</sup> count greater than 500 cells at all follow-up visits. Sustained HIV virological control was defined HIV-1 PVL less than 40 copies/mL at all follow-up visits.

### Statistical Analysis

Descriptive statistics were used to summarize the prevalence of genital HPV infection and AGW. The incidence rate and 95% confidence interval (95% CI) of genital HPV infection were estimated by Kaplan-Meier method. Person time was calculated as the time from the date of sample collection at enrolment to the date of sample collection at the final follow-up visit. Persistence of HPV infection was computed by expressing the number of persistent infections as a proportion of prevalent infections at enrolment.

Associations between any persistent genital HPV infection and exposure variables were evaluated using generalized estimating

equations (GEE) with robust standard errors (vce) to account for multiple HPV genotypes and multiple infection states (persistence and clearance) that could occur within each participant.<sup>22</sup> The unit of analysis was infections, not men, as the outcome measured was persistence of an HPV infection in follow up (men could have more than 1 infection). The exchangeable correlation option was used to account for within-participant correlation of the different HPV genotypes. Separate GEE models were run for each of the 8 HIV-related variables and for HPV VL at enrolment. These models identified independent predictors for persistent infection after adjusting for potential confounders (ie, age, citizenship, employment status, consistent condom use, circumcision, and AGW) associated with the outcome in bivariate analysis at *P* less than 0.10.<sup>23</sup>

The median time to AGW incidence or clearance was estimated using the KM method, and differences were assessed for significance using the Mantel-Haenszel log rank test. Associations between AGW clearance and exposure variables were assessed using Cox proportional hazards regression. Adjusted Cox regression models were run to identify independent risk factors using the same model-building approach as described above. Risk factor analysis for incident AGW was not run due to low incidence rates. All data were analyzed using Stata version 13 (Stata Statistical Software; Stata Corporation, College Station, TX).

## Ethics Statement

Ethical approval for the study was obtained from the Wits Human Research Ethics Committee (reference numbers: M111191 and M160859). All study participants provided written, informed consent after full explanation of the study objectives and testing procedures.

## RESULTS

### Study Population

The study population has been described elsewhere.<sup>18</sup> A total of 304 MLHIV were enrolled between March 2011 and October 2012. Participants had mean age of 38 years (standard deviation, 8 years); 25% reported more than 1 sexual partner in the past 3 months; and only 5% (*n* = 15) reported ever having sex with other men. At enrolment, the majority were already taking ART (65%, *n* = 197), for a median duration of 33 months (interquartile range [IQR], 15–58), of whom about half (*n* = 106, 54%) were virologically suppressed with median CD4<sup>+</sup> count of 445 cells/μL (IQR, 328–567). Almost one fifth (*n* = 55, 18%) of MLHIV were classified as having controlled HIV disease at enrolment and 28, 11% had HIV virological control over all study follow-up visits.

The prevalence of any-genital HPV infection at enrolment was 79% (224 of 283), and the prevalence of AGW was 12% (36 of 304). The median HPV 16 VL was 6.32 log<sub>10</sub>/10<sup>6</sup> cells (IQR, 5.81–7.62) among the 15 men with sufficient sample for this measure (testing was not possible on 23 HPV 16-positive men). The median HPV 18 VL was 6.01 log<sub>10</sub>/10<sup>6</sup> cells (IQR, 5.11–6.33) for 5 of the 21 men positive for this type. Overall, 287 (95%) of the men attended at least 1 follow-up visit.

### Incidence and Persistence of Genital HPV Infection

A total of 259 men (90% of participants with a follow-up visit) had genital HPV DNA results at both enrolment and final visits as some of the HPV results were invalid or missing. The incidence of any-genital HPV infection was 2.9 (95% CI, 1.5–5.5) per 100 person-years. The proportion of any LR-HPV incident infection (13%) was higher than that for any HR-HPV types (6%,

**TABLE 1.** Persistence of Genital HPV Infection at 18 Months Follow-up

	Positive at Enrolment	Persistence*	Clearance†
	N	n (%)	n (%)
Any HPV	192	68 (35)	124 (65)
HR HPV			
Any HR types‡	126	28 (12)	98 (88)
Alpha 7†	59	10 (17)	49 (83)
Alpha 9§	86	18 (21)	68 (79)
HPV 16	29	10 (35)	19 (65)
HPV 18	20	0 (0)	20 (100)
HPV 31	6	0 (0)	6 (100)
HPV 33	11	1 (9)	10 (91)
HPV 35	32	1 (3)	31 (97)
HPV 39	12	2 (17)	10 (83)
HPV 45	20	2 (10)	18 (90)
HPV 51	24	1 (4)	23 (96)
HPV 52	17	3 (18)	14 (82)
HPV 56	11	2 (18)	9 (82)
HPV 58	20	0 (0)	20 (100)
HPV 59	22	5 (23)	17 (87)
HPV 68	20	2 (10)	18 (90)
LR HPV			
Any LR types¶	166	55 (33)	111 (67)
HPV 6	35	7 (20)	28 (80)
HPV 11	18	3 (17)	15 (83)

\* Detection of the same HPV DNA type that was detected at enrolment.

† Alpha-7 types include: HPV 18, 39, 45, and 59.

‡ HR-HPV types include: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68.

§ Alpha-9 types include: HPV 16, 31, 33, 35, 52, and 58.

¶ LR-HPV types include: HPV 6, 11, 40, 42, 54, 55, 61, 62, 66, 67, 69, 70, 71, 72, 73, 81, 83, 84, IS39, and CP6108.

$P = 0.04$ , data not shown). The persistence of any HPV infection was 35% (68 of 192). HPV 16 had comparatively higher persistence compared with HPV 18 (35% vs. 0%), whereas the corresponding figures for HPV 6 and HPV 11 types were 20% and 17%, respectively (Table 1).

### Factors Associated With Persistent Genital HPV Infection

Persistent genital HPV infection was associated with CD4<sup>+</sup> count with a stepwise increase in odds with each decrease in CD4 count category (Table 2). Persistence was higher among MLHIV with low CD4<sup>+</sup> count (<200 vs. >500 cells/μL; adjusted odds ratio [aOR], 3.54; 95% CI, 2.07–6.05;  $P < 0.001$ ). Men with detectable HIV-1 PVL at enrolment were more likely to have persistent genital HPV infection (aOR, 1.60; 95% CI, 1.01–2.56;  $P = 0.05$ ). Furthermore, persistence of genital HPV infection was more likely among MLHIV with higher HPV 16 VL at enrolment (aOR, 3.67 per log<sub>10</sub> copies/10<sup>6</sup> cells increase, 95% CI, 1.77–7.61). The ART status or duration on ART at enrolment was not independently associated with persistent anal HPV infection.

### Incidence and Clearance of AGW

Of the 304 enrolled MLHIV, 250 (82%) had follow-up AGW data available for incidence analysis. A total of 54 men were excluded from incident analysis (36 had prevalent AGW and 18

did not have at least 1 follow-up visit). These 250 participants had a total follow-up time of 345.7 person-years, with a median follow-up time of 1.5 years (range, 0.5–1.9). Only 5 incident cases of AGW were recorded, translating to an overall AGW incidence rate of 1.4 per 100 person-years (95% CI, 0.6–3.5).

Of the 36 MLHIV who had AGW at enrolment, 33 (91%) had data available for analysis of time to AGW clearance. Three men did not have at least 1 follow-up visit. These 33 men were followed for a total of 27.2 person-years, with a median duration of follow-up of 0.6 years (range, 0.4–1.5). A total of 29 men (29 events) had cleared AGW by month 18, giving a clearance rate of 107.0 events per 100 person-years. The median time to AGW clearance was 0.7 years and only 20% had cleared AGW by month 6 (IQR, 0.5–1.1; Fig. 1A).

### Factors Associated With Clearance of AGW

There was a notable inverse dose relationship between clearance of AGW and CD4<sup>+</sup> at enrolment. MLHIV with CD4<sup>+</sup> count of at least 350 cells/μL at enrolment were almost 3.7 times more likely (95% CI, 1.44–9.47,  $P = 0.007$ ) to clear AGW compared to those with a CD4<sup>+</sup> less than 350 cells/μL (Table 3 and Fig. 1B). Men with well controlled HIV disease at enrolment were almost 7.5 times more likely to clear AGW compared to those with poor control (aOR, 7.47; 95% CI, 1.56–35.80,  $P = 0.01$ ). No associations were noted between AGW clearance,

**TABLE 2.** Associations Between HIV-related Factors and Enrolment HPV VL, and Persistent Genital HPV Infection, Using Infections as Unit of Measure\*

	N = 89 n (%) or median (IQR)	Crude		Adjusted†	
		aOR (95% CI)	P	aOR (95% CI)	P
ART status at enrolment					
No ART	24 (10.1)	1		1	
ART	65 (13.6)	1.35 (0.92–1.98)	0.13	1.40 (0.84–2.33)	0.20
Duration on ART at enrolment, mo					
>12	35 (11.6)	1		1	
6–12	16 (14.8)	1.32 (0.71–2.46)	0.39	1.22 (0.67–2.24)	0.53
<6	12 (25.0)	2.53 (1.42–4.52)	0.002	1.57 (0.82–3.02)	0.17
Enrolment CD4 <sup>+</sup> count, cells/μL					
>500	15 (7.0)	1		1	
351–500	23 (10.9)	1.59 (0.84–3.00)	0.15	1.45 (0.68–3.09)	0.34
201–350	19 (15.8)	2.41 (1.09–5.29)	0.03	1.97 (0.82–4.74)	0.13
<200	29 (22.0)	3.65 (2.26–5.90)	<0.001	3.54 (2.07–6.05)	<0.001
Enrolment CD4 <sup>+</sup> count, cells/μL					
≤350	48 (19.1)	1		1	
>350	38 (8.9)	0.42 (0.28–0.65)	<0.001	0.45 (0.28–0.74)	<0.001
High stable CD4 <sup>+</sup> count‡	12 (13.5)	0.64 (0.36–1.15)	0.14	0.64 (0.35–1.16)	0.14
Detectable HIV-1 PVL at enrolment (>40 copies/mL)	62 (15.0)	1.84 (1.19–2.85)	0.01	1.60 (1.01–2.56)	0.05
Sustained HIV virological control§	6 (6.7)	0.58 (0.26–1.34)	0.20	0.66 (0.29–1.49)	0.32
Disease control status at enrolment¶					
Poorly controlled	52 (16.5)	1		1	
ART naive	24 (10.1)	0.60 (0.41–0.87)	0.007	0.65 (0.40–1.07)	0.09
Well controlled	11 (7.5)	0.41 (0.23–0.75)	0.004	0.50 (0.24–1.05)	0.06
HPV VL at enrolment					
Log <sub>10</sub> HPV 16 copies/10 <sup>6</sup> cells	6.32 (5.81–7.62)	1.49 (1.09–2.05)	0.01	3.67 (1.77–7.61)	<0.001
Log <sub>10</sub> HPV 18 copies/10 <sup>6</sup> cells	6.01 (5.11–6.33)	0.67 (0.44–1.01)	0.06	0.58 (0.43–1.03)	0.07

\*N = 89, the total number of infections (68 men had ≥ 1 infection).

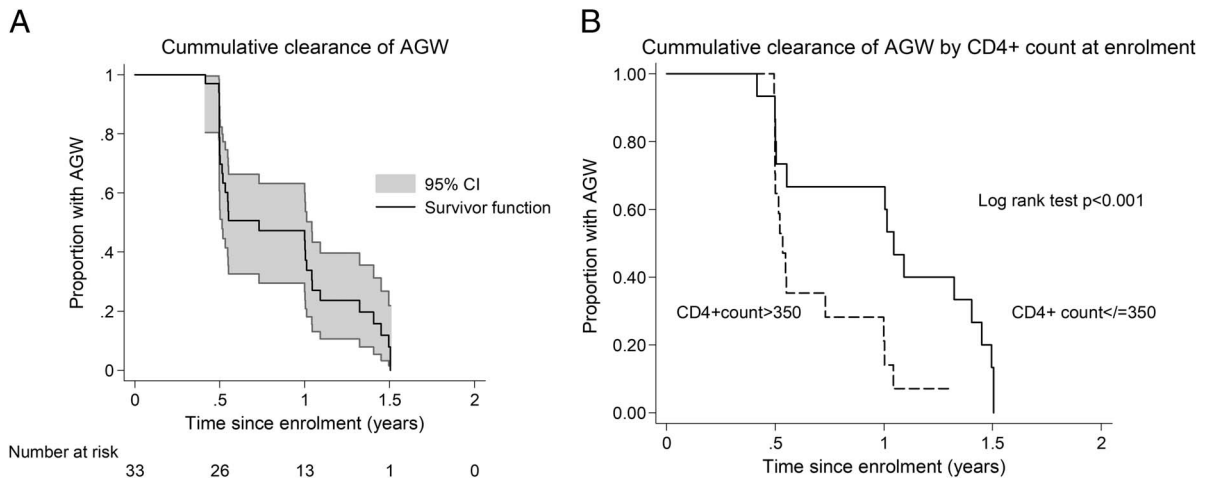
† Nine separate GEE models for each HIV-related factor were adjusted for age, citizenship, employment status, consistent condom use, circumcision and presence of AGW.

‡ CD4<sup>+</sup> count >500 cells/μL at all follow-up visits.

§ HIV PVL <40 copies/mL at all follow-up visits.

¶ Well-controlled disease defined as on ART for >6 months, CD4<sup>+</sup> >350 cells/μL and undetectable PVL.

|| As a continuous variable rounded to the next integer.



**Figure 1.** Cumulative clearance of AGWs (A) overall and (B) stratified by CD4+ count at enrolment.

and enrolment ART status or duration, or prevalent or persistent HPV infection (Table 3).

**DISCUSSION**

In this analysis, we found that 35% of genital HPV infections persisted for 12 months or more. The incidence rate of

genital infections was 2.9 per 100 person-years. The high proportion of persistent genital HPV infection that we found in our study is similar to 32% that has been reported among heterosexual MLHIV in a study in Italy.<sup>24</sup> These data show that about a third of genital HPV infections persist after 12 months and thus could potentially progress to HPV-related diseases.<sup>4</sup> This also suggests

**TABLE 3.** Factors Associated With Clearance of AGW During Follow-up

	No. Events	Rate per 100	Crude		Adjusted*	
	person-years	person-years	HR (95% CI)	P	aHR (95% CI)	P
ART status at enrolment						
No ART	8/7.8	101.50	1			
ART	21/19.3	108.81	1.16 (0.50–2.76)	0.72	0.97 (0.39–2.39)	0.94
Duration on ART at enrolment, mo						
>12	11/9.4	117.27	1		1	
6–12	8/6.8	117.11	1.05 (0.42–2.64)	0.92	0.98 (0.38–2.51)	0.97
<6	2/3.1	64.80	0.41 (0.09–1.87)	0.25	0.28 (0.05–1.42)	0.12
Enrolment CD4 <sup>+</sup> count (cells/μL)						
>500	7/4.1	170.45	1		1	
351–500	8/7.3	110.18	0.47 (0.16–1.33)	0.15	0.51 (0.17–1.52)	0.23
201–350	9/10.7	83.73	0.16 (0.05–0.55)	0.01	0.13 (0.04–0.45)	0.01
<200	5/4.6	109.75	0.31 (0.09–1.06)	0.06	0.28 (0.08–0.98)	0.05
Enrolment CD4 <sup>+</sup> count (cells/μL)						
≤350	14/15.3	91.48	1		1	
>350	15/11.4	131.95	2.89 (1.20–6.96)	0.02	3.69 (1.44–9.46)	0.007
High stable CD4 <sup>+</sup> count†	5/3.6	139.94	2.27 (0.81–6.42)	0.12	2.43 (0.85–6.97)	0.09
Undetectable HIV-1 PVL (<40 copies/mL)	19/17.7	106.83	0.97 (0.445–2.12)	0.94	0.80 (0.34–1.84)	0.59
Sustained HIV virological control‡	5/4.5	111.97	1.05 (0.39–2.82)	0.92	1.01 (0.20–2.16)	0.48
Disease control status at enrolment§						
Poorly controlled	18/17.7	101.47	1		1	
ART naïve	8/7.8	101.49	0.96 (0.40–2.31)	0.93	1.32 (0.51–3.43)	0.09
Well controlled	3/1.5	192.23	3.33 (0.91–12.23)	0.07	7.47 (1.56–35.80)	0.01
Prevalent HPV						
Any LR-HPV	26/23.9	108.46	0.75 (0.10–5.71)	0.78	0.63 (0.08–4.93)	0.66
HPV 6	15/14.9	100.62	0.65 (0.29–1.46)	0.30	0.66 (0.29–1.49)	0.31
HPV 11	10/7.8	128.65	1.69 (0.75–3.82)	0.21	1.78 (0.77–4.12)	0.18
Persistent HPV infection						
Any LR-HPV	12/11.4	105.06	0.82 (0.37–1.79)	0.61	0.97 (0.42–2.27)	0.94
HPV 6	4/4.5	88.33	0.77 (0.26–2.25)	0.64	0.95 (0.30–3.03)	0.93
HPV 11	2/1.9	106.64	0.93 (0.22–3.97)	0.92	0.76 (0.17–3.32)	0.71

\* aHR: associations were adjusted for age, marital status and age at sexual debut.

† CD4<sup>+</sup> count was >500 cells/μL at all follow-up visits.

‡ HIV-1 PVL <40 copies/mL at all follow-up visits.

§ Well-controlled disease defined as on ART for >6 months, CD4<sup>+</sup> >350 cells/μL and undetectable HIV-1 PVL.

that a significant proportion of men infected with HPV will harbor the virus and transmit it to their sexual partners.

The incidence rate of AGW, 1.4 per 100 person-years, was the same as reported from a systematic review of studies from sub-Saharan Africa.<sup>3</sup> The long time to AGW clearance (median, 8.4 months), even when treated with Podophyllin 20% solution, is concerning. This finding confirms data from a recent systematic review of the current methods for AGW treatment, which highlighted the need for new and more effective treatment methods, especially for resource-limited settings.<sup>8</sup> It also emphasized that, even though AGW are benign, they are responsible for considerable morbidity due to psychosocial distress among affected patients and significant cost implications from several visits to health care facilities for repeated treatment.<sup>25</sup>

Similar to previous studies, we found that CD4<sup>+</sup> count was a strong predictor of clearance of genital HPV infection and AGW.<sup>26,27</sup> This relates to the role of cell-mediated immunity in the clearance of HPV infection.<sup>9</sup> However, ART status and duration on ART at enrolment were not significant predictors, and this is in keeping with other previous reports.<sup>27,28</sup> This suggests that it is not the ART status per se that is important, but factors, such as timing of ART initiation, nadir CD4<sup>+</sup> count, virological control, and HIV disease status that influence the natural history of genital HPV infection. This aligns with our observation in this study that men with detectable HIV-1 PVL and higher HPV VL were more likely to have persistent genital HPV infections. Therefore, the current initiatives to improve ART coverage by increasing population HIV testing and immediate treatment initiation may provide benefit not only for HIV but also for control of HPV infection and AGW.<sup>29</sup>

The high proportion of persistent genital HPV infections suggests that HPV vaccination of men could be an effective public health measure in preventing HPV infection in men as a reservoir of infection.<sup>24</sup> In our cohort, there was only 20% clearance of AGW at 6-month follow-up. This is less than 44% clearance at 3 month follow-up among women (not living with HIV) reported in a study in Cape Town.<sup>30</sup> The long time to AGW clearance among MLHIV supports the idea of introducing quadrivalent HPV vaccines which also prevent AGW. Cost implications of extending the vaccine to boys might generate reluctance by national Ministries of Health.<sup>31</sup> Evidence from observational studies suggests that single-dose HPV vaccination generates sufficient immune responses for protection against HPV.<sup>32</sup> Randomized control trials to assess one-dose efficacy are currently underway.<sup>33</sup> If these trials show that 1 dose has high levels of durable protection, this could make it more affordable and feasible for the HPV vaccine program to reach more people, including boys and men.

The study had some limitations. Persistence of genital HPV infections may have been overestimated by assessment of infection at only 2 timepoints as we cannot rule out clearance of an HPV type and the reinfection with that same type between the visits. Likewise, the assessment interval might have been too long to determine incidence rates because some incident infections could have already been cleared by the time of the next assessment, leading to underestimation of true incidence rates. Visual inspection for AGW detection was done without histological confirmation, making it possible that other conditions (eg, condylo-mata lata, pearly penile papules, and penile intraepithelial neoplasia) could have been misclassified as warts.<sup>34</sup> The sample size for HPV VL was small; therefore, the results on the predictive value of HPV VL on persistent HPV infection should be interpreted with caution. In addition, the high proportion of paucicellular specimens suggests that the method of HPV VL testing might present operational feasibility challenges if it were to be scaled up in the future. It is also important to note that these data were collected about 4 years ago, and it is possible that situation might have changed with the maturing of

the HIV program in South Africa. More cohort studies are required to continue monitoring if some of the findings would change as the HIV program evolves. However, the guidelines have changed to immediate ART initiation which will provide some benefit as men on ART are less likely to shed virus. This implies that transmission may be reduced but not eliminated, and thus, the HPV vaccines might still be required for primary prevention. Despite these limitations, our study provides a significant contribution to the scarce data on genital HPV infection and AGW epidemiology among MLHIV in Africa.

Men living with HIV face a considerable burden of genital HPV infection and AGWs. The long time to AGW clearance highlights the need for more effective treatment for AGW. Effective, and possibly early, use of ART with immunological reconstitution and HIV virological control may contribute to the control of the HPV-associated burden. Human papillomavirus vaccination, if extended to boys, could also reduce this burden among men and their partners in the future.

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