Human papillomavirus (HPV) serology among women living with HIV: Type-specific seroprevalence, seroconversion and risk of cervical re-infection

Running title: HPV serology in women living with HIV-1

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Word count full manuscript=3500; abstract=200; figures=2; tables=4

ABSTRACT

Background: Human papillomavirus serodynamics following infection has never been evaluated prospectively among women living with HIV (WLHIV). We determined HPV seroprevalence, seroconversion and cervical HPV-DNA acquisition among WLHIV.

Methods: Prospective study of 604 WLHIV in Johannesburg, South Africa aged 25-50 years. At baseline and 16 months later (endline), type-specific antibodies to HPV-types (HPV6/11/16/18/31/33/35/39/45/52/56/58/59/68/73) were measured using HPV pseudovirions and corresponding cervical HPV-DNA genotypes detected using INNO-LiPA.

Results: Seroprevalence of any-HPV type was 93.2% and simultaneous seropositivity for HPV-types of the bivalent (HPV16/18), quadrivalent (HPV6/11/16/18) and nonavalent (HPV6/11/16/1831/33/45/52/58) vaccines were 21.4%, 10.9% and 2.8%. Among 219 women with cervical HPV-DNA but seronegative for the same-type and without high-grade cervical intraepithelial neoplasia at baseline, 51 (23.3%) had type-specific seroconversion at endline. Among these women, the risk of type-specific seroconversion was higher among recent antiretroviral therapy users (ART≤2 years vs. ART-naive: adjusted OR=2.39, 95%CI:1.02-5.62), and lower among women with low CD4+ at endline (≤350 vs. >350 cells/mm^3: aOR=0.51, 95%CI:0.24-1.07). Risk of cervical HPV DNA acquisition was significantly lower in women who were seropositive for HPV18, 35 and 58 at baseline.

Conclusion: WLHIV have evidence of seroconversion in response to baseline HPV-DNA, dependent on CD4+ count and ART. Baseline HPV seropositivity confers limited protection against some HPV types.

Key words: human papillomavirus (HPV); serology; antibodies; HIV; Africa

INTRODUCTION

As women living with HIV (WLHIV) are experiencing longer life expectancy due to increased availability of antiretroviral therapy (ART), many remain at high risk of infection with HPV, acquiring anogenital warts (AGW), and progressing to cervical and other genital cancers. Primary prevention of HPV through vaccination can reduce the burden of disease and on screening and treatment services. The bivalent and quadrivalent HPV vaccines target two high-risk (HR)-HPV types (HPV16 and 18) responsible for about 70% of cervical cancers[1], whereas the nonavalent vaccine protects against a wider range of HR-HPV types (HPV16/18/31/33/45/52/58), as well as types causing AGW (HPV6/11) and is estimated to prevent up to 90% of cervical cancers in women from the general population[1-3].

There is limited evidence of the serological response to HPV infection among WLHIV. The evidence regarding protection against re-infection for the same HPV type induced after natural infection is also unclear. A recent meta-analysis of 14 studies among 24,000 individuals (90% women) from Europe, North America, Latin America, Asia and Australia investigating the potential protective effect of naturally acquired type-specific antibodies[4] reported 30-35% protection against subsequent infection with HPV16 (pooled relative risk [RR]=0.65, 95% confidence interval [CI]:0.50-0.80) and HPV18 (RR=0.70, 95%CI:0.43-0.98) among women in the general population. However, seropositivity to a wider range of HPV types and possible seroprotection following natural infection have not been evaluated among WLHIV. An improved understanding of HPV type-specific serological responses in such high-risk populations and their risk of both new infection and reinfection is needed to guide targeted HPV control efforts, including possible use of HPV vaccination.

We conducted a large prospective study of cervical cancer screening in a cohort of WLHIV in Burkina Faso and South Africa (*HARP, HPV in Africa Research Partnership*)[5]. In this paper, among women enrolled in the *HARP* study in South Africa, we evaluated HPV seroprevalence and concordance of HPV DNA with same-type seropositivity at baseline for 15 HPV genotypes. Second, among women without high-grade cervical intraepithelial neoplasia (CIN2+) at baseline, we evaluated the factors associated with HPV seroconversion following baseline HPV-DNA infection, and risk of incident HPV-DNA detection among women seropositive for same-type HPV at baseline.

METHODOLOGY

Study population and specimen collection

The *HARP* cohort study has been described in detail elsewhere[5]. In South Africa, women were recruited from HIV treatment centres and surrounding communities in Johannesburg from December 2011 to October 2012. Inclusion criteria were being HIV-1 seropositive, aged 25-50 years and resident in the city. Exclusion criteria were history of prior treatment for cervical cancer, previous hysterectomy, and being pregnant or less than 8 weeks postpartum. Eligible participants provided signed informed consent. Enrolment was stratified in a 2:1 ratio of ART-users:ART-naïve. Participants were followed-up every 6 months for CD4+ T-lymphocytes count and up to scheduled month 18 visit (endline) when procedures similar to baseline were repeated. Ethical approval was granted by the University of Witwatersrand, Johannesburg, and the London School of Hygiene and Tropical Medicine.

Laboratory testing

HPV-DNA genotyping was performed at baseline and endline using the INNO-LiPA HPV genotyping Extra® assay (Fujirebio, Courtaboeuf, France). Analysis of HPV DNA positivity was restricted to the 15 types covered by the serology assay (HPV6/11/16/18/31/33/35/39/45/52/56/58/59/68/73) with HR-HPV being similarly defined with the following 12 types: HPV16/18/31/33/35/39/45/52/56/58/59/68[6]. HPV antibodies were detected using a multiplexed binding assay, which uses pseudovirions (PsV) as antigens and detects HPV type specific IgG antibodies (PsV-Luminex). Serology was performed for 'carcinogenic/probable carcinogenic' types HPV16/18/31/33/35/39/45/52/56/58/59/68 (except the HR-HPV type 51) and the low-risk (LR) types HPV6/11/73 at Karolinska Institute, Stockholm, Sweden[7, 8]. Serum samples were analysed in a 1:50 and 1:150 dilutions. Cut-off values to define seropositivity were calculated independently for each HPV type by analysing the mean fluorescence intensity unit (MFI) values obtained from a panel of 100 Swedish children's sera (≤12 years old). The cut-off algorithm was as recommended by the global HPV LabNet (mean MFI value of a negative control serum panel plus 3 standard deviations)[9]. If this cut-off value was unreasonably low (less than 400 MFI), 400 MFI was used as cut-off to have a sensitivity and specificity similar to classical HPV ELISAs[10].

Statistical analysis

Cervical HPV DNA status

Women were considered "HPV-DNA positive" if positive by INNO-LiPA for any of the HPV types included in the serology assay, and "HPV-DNA negative" if negative for all of these types. HPV-DNA genotype-specific persistence was defined as being positive for the same type at baseline and endline visits. Type-specific clearance was defined as being positive for a specific type at enrolment and negative for that type at endline visit. Given that no woman was simultaneously infected by all 15 HPV types, all women were at risk of acquiring at least one HPV infection and all were included in the analysis of associations of baseline seropositivity with same-type HPV DNA incidence.

HPV serology status

HPV serology results are presented as binary results (positive and negative for a given type) based on the pre-assigned cut-off. Overall and type-specific or group (vaccine targets) HPV seroprevalence was defined as being seropositive for any/type-specific/grouped types respectively, among women with serology data at baseline. HPV type-specific seroconversion was defined as being HPV-DNA positive (i.e., recently exposed) and same-type seronegative at baseline that became same type seropositive at endline, irrespective of the DNA status at endline. We also analysed type-specific seropersistence (having same-type detectable antibody at baseline and endline) and seroreversion (seropositive for a specific HPV type at baseline and seronegative for the same type at endline) among all women who were seropositive for any HPV type at baseline, irrespective of their cervical HPV-DNA status.

Longitudinal analyses were restricted to women without prevalent CIN2+ at baseline, as the natural history of HPV infection and serology dynamics in women with CIN2+ would be difficult to interpret since many women had received ablative therapy.

For comparison of HPV seropositivity among type-specific HPV DNA positive and DNA negative WLHIV at baseline, prevalence ratios (PRs) were obtained from logistic regression using marginal standardization to estimate PRs, and the delta method to estimate 95% confidence intervals (CI)[11]. Associations between HPV seroconversion and exposure variables were estimated with generalised estimating equations (GEE) to account for seropositivity by multiple HPV types within women[12]. To explore associations of HPV seroconversion with HIV-related factors, pre-specified analyses included stratification by ART duration (≤2 or >2 years) and CD4+ cell counts (≤350 or >350 cells/mm^3) at baseline and endline, and HIV-1 viral suppression (plasma HIV-1 RNA <1000 or ≥1000 copies/ml) at baseline. Stable high CD4+ count was defined as having CD4+ counts >500 cells/mm^3 at baseline, month 12 (intermediate) and endline visits. Multivariable analyses were adjusted for socio-demographic and behavioral factors which were independently associated with HPV seroconversion. For associations of baseline seropositivity with HPV-DNA incidence, logistic regression was used to estimate odds ratios (OR) and 95%CI. Data were analysed using Stata version 14 (Stata Statistical Software, College Station. TX: Stata Corporation).

RESULTS

Study population

A full description of *HARP* study participants has been published elsewhere[5]. Of the 623 WLHIV enrolled in South Africa, the median age at enrolment was 34 years (interquartile range [IQR]: 30-40), and median time in follow-up was 16 months (IQR: 15.6-16.8). In total, 604 (97.0%) WLHIV had valid results for both HPV serology and genotyping at baseline (**Figure 1**). Of these, 390 (64.6%) reported taking ART throughout the study period with a median duration of 3.4 years (IQR:1.8-5.3) during follow-up, 42 (7.0%) initiated ART during follow-up and 172 (28.5%) remained ART-naive throughout the study. The median baseline CD4+ cell counts of ART users, ART initiators and ART-naive were 421 cells/mm^3 (IQR:285-580), 333 cells/mm^3 (IQR:260-403) and 475 cells/mm^3 (IQR:366-625), respectively. CD4+ cell counts change per year were +7 cells/mm^3 (IQR: -55 to 89), +83 cells/mm^3 (IQR: -33 to 205) and -36 cells/mm^3 (IQR: -119 to 24) in the three groups, respectively.

HPV seroprevalence at baseline

Seroprevalence of any of 15 HPV types was 93.2% (95%CI:90.9-95.1%), of whom 89.9% (506/563) were seropositive for multiple types (**Figure 2**). The seroprevalence of any 12 HR-HPV type was 90.7% (95%CI:88.1-92.9%). Overall, almost all women (n=591; 97.8%) were positive by either serology or DNA for any HPV, and 583 (96.5%) for any HR-HPV types.

Seroprevalence was highest for HPV31 (59.6%), followed by HPV58 (54.8%) and HPV16 (43.1%) (**Figure 2**). The seroprevalence of any HPV genotypes included in the bivalent (HPV16/18), quadrivalent (HPV6/11/16/18) or nonavalent (HPV6/11/16/18/31/33/45/52/58) vaccines were 59.3%, 73.5% and 87.6%, respectively. Simultaneous seropositivity for all HPV types included in the bivalent, quadrivalent and nonavalent vaccines was 21.4%, 10.9% and 2.8%, respectively.

Correlation of HPV-DNA and antibody-specific prevalence

Of 472 women DNA positive for any of the 15 HPV types at baseline, 279 (59.1%) were seropositive for the same HPV type (range by type: 25.5% for HPV45 to 68.9% for HPV31; **Table 1**). The type-specific HPV seroprevalence was significantly higher among those with same-type DNA positive compared to same-type DNA negative for HPV31, HPV35, HPV39, HPV52, HPV58 and low-risk type HPV11.

HPV seroconversion and association with DNA persistence and clearance

Of all 451 women without CIN2+ at enrolment who were followed-up until endline, genotyping and serology data at baseline and endline were available for 433 (96.0%; **Figure 1**). There were 219 women who were HPV-DNA positive and same-type seronegative at baseline. Same-type seroconversion was observed for 23.3% (51/219), irrespective of DNA status during follow-up.

When considering the total number of baseline infections as denominator (n=326), there were 56 (17.2%) HPV seroconversion events (**Table 2**). Risk of type-specific seroconversion was highest for HPV31 (53.9%) and HPV33 (33.3%) and lowest for HPV18 (17.1%) and HPV16 (2.4%). Overall, risk of type-specific seroconversion was greater with same-type persistent DNA infection compared to cleared infection; irrespective of newly detected DNA during follow-up (**Table 2**: any HPV type seroconversion: 23.0% vs. 15.1%; crude OR=1.75, 95%CI:0.95-3.24; aOR=1.56, 95%CI:0.78-3.13, adjusted for injectable contraception and CD4+ cell count at endline).

HIV-related factors associated with HPV seroconversion

When considering the number of baseline infections as denominator, the risk of HPV seroconversion was similar by baseline CD4+ count (**Table 3**) but lower with low endline CD4+ (≤350 vs. >350 cells/mm^3:10.8% vs. 19.4%; aOR=0.51, 95%CI:0.24-1.07, adjusted for injectable contraceptive use), and this association was significant among ART users only (≤350 vs. >350 cells/mm^3: 8.0% vs. 26.3%; aOR=0.25, 95%CI: 0.08-0.75).

The highest risk of seroconversion was among short-duration ART users (≤2 years ART vs. ART-naïve at endline: 24.6% vs. 14.2%; aOR=2.39, 95%CI:1.02-5.62, adjusted for injectable contraceptive use and endline CD4+ cell count). HPV seroconversion was also more likely among women who reported high adherence to ART at baseline (60-90% vs. <60% adherence: 22.4% vs. 7.1%; aOR=8.93, 95%CI:1.13-70.36).

Newly detected HPV DNA over 16 months according to type-specific seropositivity at baseline

Among the 433 women with genotyping and serology data at both visits (**Figure 1**), 221 (51.0%) women had newly detected HPV-DNA at endline, with a total of 327 incident infections (**Table 4**). The risk of incident infection was lower among women who were same-type seropositive compared to seronegative at baseline for HPV18 (1.5% vs. 6.4%; aOR=0.14, 95%CI:0.02-0.80, adjusted for baseline CD4+ cell count, ART status and seropositivity for any type from same HPV phylogenetic family**)**; HPV35 (4.1% vs 11.9%; aOR=0.26, 95%CI:0.10-0.68) and HPV58 (1.8% vs. 5.4%; aOR=0.19, 95%CI:0.04-0.89). Conversely, the risk was higher among those with same-type baseline seropositivity for HPV45 (10.5% vs. 4.0%) but the association did not persist following adjustment for seropositivity from same HPV phylogenetic family (aOR=2.81, 95%CI:0.87-9.04), although numbers were small.

Among 403 women with detectable antibodies at baseline, 384 (95.3%) had the same type detected again at endline. HPV-DNA incidence was similar among women with seropersistence and seroreversion (**Table 4**).

DISCUSSION

This prospective study of serological dynamics for 15 HPV types among WLHIV found a very high seroprevalence at baseline, new-type seroconversion and type-specific seropersistence 16 months later. In keeping with these findings, these women also had high prevalence, incidence and persistence of HR-HPV-DNA, including multiple type infection[5]. To our knowledge, this is the first study to report HPV seroconversion in conjunction with HPV-DNA over time among WLHIV.

We report a similar seroprevalence for types HPV6, 11 and 16 as reported elsewhere among WLHIV in South Africa[13] and the USA[14]. We found that the proportion of women who were seropositive for multiple HPV types was higher than the prevalence of multiple HPV-DNA infections at baseline, suggesting prior exposure to these types. Although the prevalence of antibodies to HPV vaccine types was high, the simultaneous seropositivity for all 9vHPV vaccine types was observed in a minority of women.

After 16 months of follow-up, we report evidence of same-type seroconversion, which was higher among women with same-type persistence compared to cleared infection at endline. Seroconversion rates reported in this study are lower than those reported among HIV-negative women. A study among 588 HIV-negative HPV-unvaccinated women aged 18-20 years[15] reported seroconversion rates of 59.5%, 54.1% and 68.8% for HPV16, HPV18 and HPV6, respectively within 18 months of detection of same-type incident infection, and was more likely among women with same-type persistent infection, as found in our study. Similarly, among 6,528 women in Costa Rica[16] 21.7% of women with HPV-DNA at baseline had seroconversion for HPV16 over a median 6.4 years, and was higher among women with persistent compared to cleared infection. While there are no studies among WLHIV to make any comparison, seroconversion among 245 HIV-infected men who have sex with men (MSM) in the Netherlands following anal or penile HPV infection was 23% over 12 months[17] and 42% over 24 months among 281 HIV-infected MSM initiating ART in Switzerland[18].

Seroconversion in our study varied by HPV type, and was highest for HPV31 and lowest for HPV16. The type-specific seroconversion rates reflected the type-specific infection states at follow-up. The overall incident and persistent HPV-DNA was higher for HPV16 than for other types[19]. The lower seroconversion and subsequent increased risk of HPV16 incidence and persistence could be a result of its immune evasion mechanisms[20].

Seroconversion was more frequent among women with higher CD4+ cell count (>350 cells/mm^3) at endline and this association was observed in the short-duration (≤2 years) ART users only. A study among 281 MSM initiating ART and followed for a median of 2 years[18] reported that those with lower nadir CD4+ cell count (<200 cells/mm^3) at ART initiation had the highest seroconversion rates, compared to MSM initiating ART at higher CD4+ cell count (≥350 cells/mm^3). It is possible that ART-related immune reconstitution during follow-up may have promoted seroconversion, and this was highest among men with the lowest CD4+ cell count, possibly because they had the greatest CD4+ cell count recovery. Although nadir CD4+ cell count was not available in our study, short-duration ART users at endline had a lower baseline CD4+ cell count compared to both the long-duration ART or ART-naïve women (median CD4+ count of 324, 461 and 497 cells/mm^3, respectively[5]). Although baseline CD4+ cell count was not associated with seroconversion, the subsequent increase in CD4+ cell count at endline through ART-initiated immune reconstitution (median CD4+ cell count among short duration ART users increased by +103 cells/mm^3 [IQR: -13 to 196]) may have stimulated seroconversion among the recent ART users in response to the high rate of HPV-DNA persistence reported in this cohort[5]. This is also supported by our findings of higher seroconversion rates among women with better ART adherence.

An additional explanation for higher seroconversion among short-duration ART users is linked to the higher HPV-DNA prevalence, persistence and lack of viral clearance in this group compared to prolonged ART users in this cohort[5]. It can be speculated that among recent ART initiators, immune reconstitution may have been not entirely effective to prevent persistence in the shortterm[5]. Our finding of an association of seroconversion with higher endline (but not baseline) CD4+ count in this study, may reflect the delay required for seroconversion to occur after infection, which might also be influenced by the initial state of immunosuppression. As seen in young HIV-negative women, HPV16 seroconversion occurred between 6 to 12 months after DNA detection[15]. In our study, the duration of HPV-DNA infection at baseline could not be determined.

Women taking ART for a prolonged duration (>2 years) had similar low seroconversion rate as ART-naïve women. This may be because women on prolonged ART and strong immune recovery may have fewer prevalent and persistent infections[5], which would be required to trigger seroconversion. The more puzzling finding, however, is that ART-naïve women in this study had as high rates of persistent HR-HPV infection as short-duration ART users, and their CD4+ cell count at either timepoint was not associated with seroconversion. Increases in HPV seropositivity by ART status have been shown to be independent of CD4+ count in other studies among MSM[18] and WLHIV[14]. No study has yet compared seroconversion among ART users and ART-naïve women following natural infection, but vaccination studies among WLHIV have shown that, while seroconversion rates are similar among ART users and ART-naive women in the US and Puerto Rico[21], HPV16 and HPV18 antibody titres were lower among ART-naïve but comparable between HIV-negative and ART users[21]. These data suggest there may be some beneficial impact of ART in promoting seroconversion.

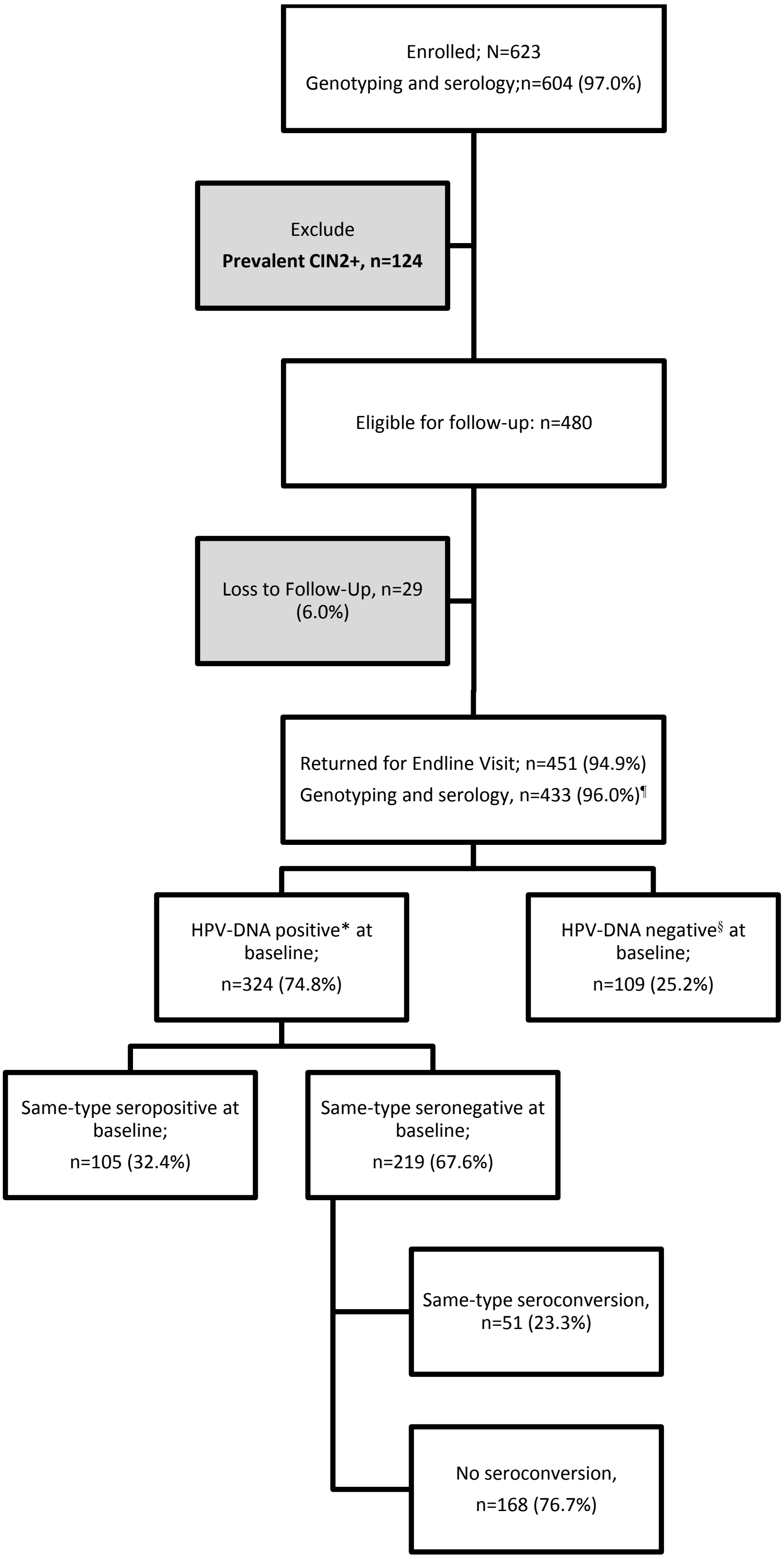
We found evidence that HPV antibodies detected at baseline provided protection against DNA detection only for HPV18, 35 and 58, for which infection rates were lower. A recent meta-analysis, which assessed whether naturally acquired immunity conferred protection against subsequent infection by the same type[4], reported that HIV-negative women with antibodies against HPV16 and HPV18 had a modest 35% and 30% lower risk of subsequent infection, respectively against the corresponding type. We found no protection conferred by HPV16 antibodies against HPV16 DNA detection in this study. Others have reported that HPV16 is better able to evade the host immune surveillance relative to other HPV types[22], which may explain its predominance in high-grade cervical lesions in HIV-positive and HIV-negative women[3]. A prospective study among 829 WLHIV risk-matched with 413 HIV-negative women in the US[23] reported no statistically significant difference in the risk of reinfection of any HR-HPV (HPV16/18/31/35/45) among women with same-type seropositivity compared to same-type seronegative at baseline over a median 4.5 years, with the exception of a reduced risk with HPV45.

Other studies have evaluated whether HPV antibodies detected at two or more time points were associated with subsequent detection of same-type HPV-DNA among HIV-negative women. A prospective study with longitudinal serology measurements among 608 HIV-negative women seen at 6-month intervals for 3 years in the US[24] reported a reduction in risk of subsequent HPV16 detection among women with a sustained high level of HPV16 antibody (seropositivity at two or more time points) and its phylogenetically related types (HPV31/33/58). HPV seropersistence in our study over 16 months was very high, however there was limited evidence that WLHIV who had type-specific seropersistence had lower HPV-DNA detection compared to women with seroreversion at endline. This finding suggests that seroprevalence, seropersistence and seroreversion are not good markers of protection against subsequent DNA detection. Given the limited evidence that natural immunity can protect against subsequent infection[4], a multivalent vaccine such as the nonavalent vaccine would be beneficial given that 55% of WLHIV in this study had multiple HR-HPV infection at baseline, 18% had multiple persistent types at endline[19] and few women were simultaneously infected by all vaccine-types.

This study was constrained by a limited number of visits between baseline and endline and the overall relatively short follow-up duration. Therefore, we cannot establish whether the seroconversion event occurred in response to either an infection which persisted during follow-up or a baseline infection which cleared followed by new DNA detection of the same type. Moreover, when evaluating the risk of new DNA detection according to same-type seropositivity at baseline, we cannot exclude the possibility that the infection detected at 16-months was a reinfection or a recurrence of a latent undetected infection. However, infrequent condom use, presence of other sexually transmitted infection or reproductive tract infections (including *Chlamydia trachomatis, Trichomonas vaginalis*, and bacterial vaginosis), vaginal cleansing and low CD4+ counts at enrolment were associated with new DNA detection (data not shown). These factors may point to behaviours or factors enhancing the risk of a new sexually acquired infection, or modifiers of vaginal biome or mucosal immunity which may enhance the possibility of reactivation of a latent HPV infection. Analysis of new DNA detection over 16 months according to type-specific positivity was constrained by the small numbers of individual genotype positivity. This study had several important strengths, including its longitudinal design, the availability of serology and genotyping data at both time points. It is the first study to measure HPV serodynamics longitudinally among WLHIV.

In conclusion, this study shows that WLHIV have high HPV seroprevalence and sero-persistence of type-specific antibodies. We found evidence of seroconversion over 16 months that was dependent on CD4+ cell count at endline. The high HPV incidence and the limited evidence that naturally acquired antibodies protect against new DNA detection combined with the fact that even though WLHIV have multiple infections, few have been infected with all preventable infections, suggest that WLHIV could benefit from highly multivalent HPV vaccination. Studies, including mathematical modelling studies, assessing the efficacy and cost-effectiveness of nonavalent HPV vaccine are warranted in this population.

Figure 1 Study flowchart

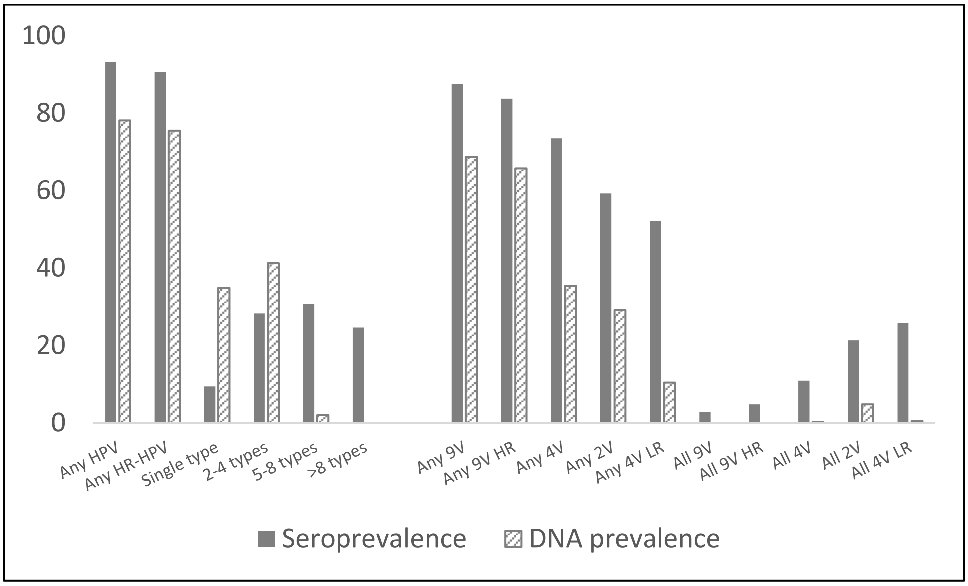


Legend:

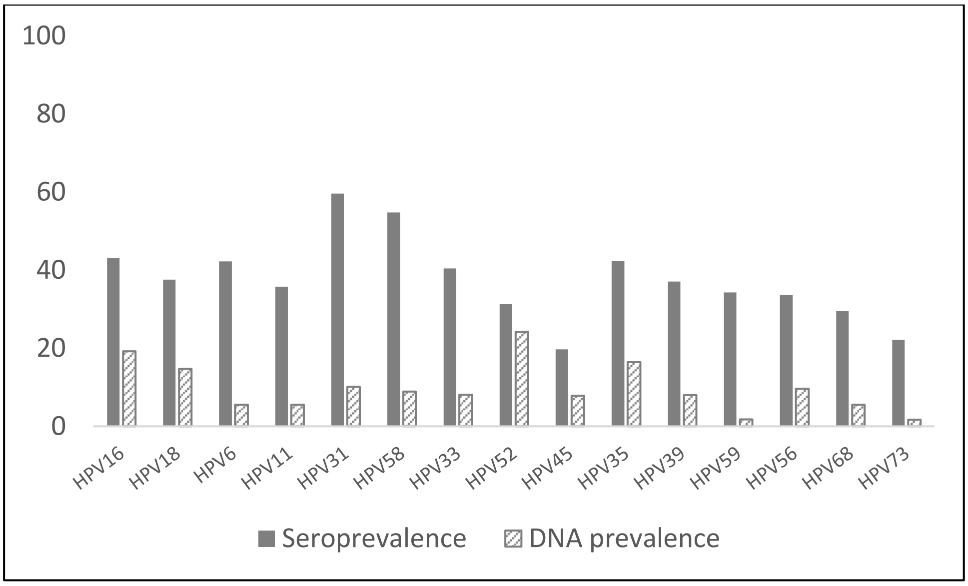
¶given that no woman was infected by all 15 HPV ypes, all women were at risk of acquiring at least one-HPV; *DNA positive for any 15 type (HPV6/11/16/18/31/33/35/39/45/52/56/58/59/68/73); §DNA negative for all 15 type (HPV6/11/16/18/31/33/35/39/45/52/56/58/59/68/73)

Figure 2. HPV type seroprevalence and DNA prevalence among 604 women living with HIV in Johannesburg, South Africa

Panel A



Panel B



Legend:

Panel A: Prevalence of combination of HPV types: * Any HPV type prevalence defined as positive for at least one HPV type (6/11/16/18/31/33/35/39/45/52/56/58/59/68/73) at baseline; Any HR-HPV type prevalence defined as positive for at least one HR type (16/18/31/33/35/39/45/52/56/58/59/68) at baseline; Any 9V=Positive for any of HPV6/11/16/18/31/33/45/52/58; Any 9V-HR=Positive for any of

HPV16/18/31/33/45/52/58; Any 4V= Positive for any of HPV6/11/16/18; Any 4V-LR= Positive for any of HPV6/11; Any 2V= Positive for any of HPV16/18; All 9V=Positive for ALL of HPV6,11,16,18,31,33,45,52 and 58; All 9V-HR=Positive for ALL of HPV16,18,31,33,45,52 and 58; All 4V=Positive for ALL of HPV6,11,16 and 18; All 2V=Positive for BOTH HPV16 and 18; All 4V-LR=Positive for BOTH HPV6 and 11. Panel B: Prevalence of individual HPV types.

Table 1. Comparison of HPV seropositivity among type-specific HPV DNA positive and DNA negative women living with HIV (N=604) at baseline

	DNA positive (N)	Seropositive (n)	Seropositive/ DNA positive (%)	DNA negative (N)	Seropositive (n)	Seropositive/ DNA negative (%)	**aPR (95%CI)**[1]
Bi-/Quadrivalent types							
HPV6	33	14	**42.4**	571	241	**42.2**	0.94 (0.60-1.49)
HPV11	33	19	**57.6**	571	197	**34.5**	**1.71 (1.24-2.35)**
HPV16	115	50	**43.5**	489	210	**42.9**	1.02 (0.80-1.31)
HPV18	89	34	**38.2**	515	193	**37.5**	1.10 (0.83-1.47)
Additional Nonavalent types							
HPV31	61	42	**68.9**	543	318	**58.6**	1.28 (1.08-1.52)
HPV33	48	25	**52.1**	556	219	**39.4**	1.25 (0.91-1.71)
HPV45	47	12	**25.5**	557	107	**19.2**	1.42 (0.84-2.42)
HPV52	146	60	**41.1**	458	129	**28.2**	**1.54 (1.19-1.98)**
HPV58	54	36	**66.7**	550	295	**53.6**	**1.27 (1.03-1.55)**
Non-vaccine types							
HPV35	99	59	**59.6**	505	197	**39.0**	1.52 (1.23-1.88)
HPV39	48	25	**52.1**	556	199	**35.8**	**1.46 (1.08-1.98)**
HPV56	58	20	**34.5**	546	183	**33.5**	1.01 (0.68-1.49)
HPV59	11	6	**54.5**	593	201	**33.9**	1.59 (0.90-2.79)
HPV68	33	12	**36.4**	571	166	**29.1**	1.33 (0.82-2.14)
HPV73	10	4	**40.0**	594	130	**21.9**	1.15 (0.35-3.74)
Any HPV [a,b]	472	279	**59.1** [a]	132	119	**90.2** [b]	
Any HR-HPV [a,b]	456	267	**58.6** [a]	148	127	**85.8** [b]	

[a]HPV type seropositive among DNA positive for the same type; [b] Any type HPV seropositive among DNA negative for all types; [1]adjusted Prevalence Ratio [PR] for type-specific seroprevalence if same-type DNA positive compared to DNA negative, adjusted for injectable contraception, HIV viral suppression (plasma HIV-1 RNA <1000 copies/ml) found to be associated with any HPV seroprevalence at baseline, and CIN2+ status.

Table 2. Type-specific seroconversion at 16 months among 219 women living with HIV without CIN2+ at baseline

	All participants[a]		Participants with HPV DNA persistence[b]		Participants with HPV DNA clearance[c]	
	DNA+ & sero- at baseline	Seroconversion events	DNA+ & sero- at baseline	Seroconversion events	DNA+ & sero- at baseline	Seroconversion events
	N infections	n (%)[a]	N infections	n (%)[b]	N infections	n (%)[c]
HPV16	**41**	1 (2.4)	11	1 (9.1)	30	0 (0.0)
HPV18	**41**	7 (17.1)	14	3 (21.4)	27	4 (14.8)
HPV6	**13**	3 (23.1)	0	0 (0.0)	13	3 (23.1)
HPV11	**13**	2 (15.4)	3	1 (33.3)	10	1 (10.0)
HPV31	**13**	7 (53.9)	2	2 (100.0)	11	5 (45.5)
HPV33	**15**	5 (33.3)	1	1 (100.0)	14	4 (28.6)
HPV45	**25**	4 (16.0)	8	1 (12.5)	17	3 (17.6)
HPV52	**63**	12 (19.1)	21	6 (28.6)	42	6 (14.3)
HPV58	**12**	3 (25.0)	4	1 (25.0)	8	2 (25.0)
HPV35	**26**	6 (23.1)	11	3 (27.3)	15	3 (20.0)
HPV39	**19**	0 (0.0)	2	0 (0.0)	17	0 (0.0)
HPV56	**26**	4 (15.4)	4	1 (25.0)	22	3 (13.6)
HPV59	**4**	1 (25.0)	1	0 (0.0)	3	1 (33.3)
HPV68	**13**	1 (7.7)	3	0 (0.0)	10	1 (10.0)
HPV73	**2**	0 (0.0)	2	0 (0.0)	0	0 (0.0)
Any HPV	**326**	56 (17.2)	**87**	20 (23.0)	**239**	36 (15.1)
Any HR-HPV	**298**	51 (17.1)	**82**	19 (23.2)	**216**	32 (14.8)

[a]Seroconversion calculated among 219 women with DNA+/sero- status at baseline representing 326 infections; [b]Seroconversion calculated among DNA+/sero- at baseline and with type-specific persistence at endline; [c]Seroconversion calculated among DNA+/sero- at baseline and type-specific clearance at endline

Table 3. HIV-related factors associated with HPV seroconversion at 16 months using 319 events of DNA positive/same type seronegative at baseline (among 219 women living with HIV)

	N	n (%)	aOR (95% CI) [a]
All women			
Baseline CD4+ count (cells/mm^3)[b]			
≤350	**96**	19 (19.8)	1.19 (0.64-2.21)
>350	**221**	37 (16.7)	1.00
Endline CD4+ count (cells/mm^3)[b]			
≤350	**93**	10 (10.8)	0.51 (0.24-1.07)
>350	**191**	37 (19.4)	1.00
ART status at endline			
ART >2 years	**124**	22 (17.7)	1.77 (0.85-3.69)
ART ≤2 years	**61**	15 (24.6)	**2.39 (1.02-5.62)**
ART-naïve	**134**	19 (14.2)	1.00
Baseline ART users			
HIV-1 viral suppression at baseline			
<1000 copies/ml	146	27 (18.5)	1.00
≥1000 copies/ml	26	7 (26.9)	1.91 (0.67-5.38)
ART adherence at baseline[c]			
Low adherence (<60%)	**28**	2 (7.1)	1.00
Moderate adherence (60-90%)	**143**	32 (22.4)	**8.93 (1.13-70.36)**
Baseline CD4+ count (cells/mm^3) [d]			
≤350	**64**	15 (23.4)	1.38 (0.64-2.96)
>350	**106**	19 (17.9)	1.00
Endline CD4+ count (cells/mm^3) [e]			
≤350	**50**	4 (8.0)	**0.25 (0.08-0.75)**
>350	**99**	26 (26.3)	1.00
Stable high CD4+ count[f]			
Yes	**27**	4 (14.8)	1.00
No	**144**	30 (20.8)	1.52 (0.49-4.74)
ART-naïve women			
Baseline CD4+ count (cells/mm^3) [g]			
≤350	**32**	4 (12.5)	0.71 (0.22-2.31)
>350	**115**	18 (15.7)	1.00
Endline CD4+ count (cells/mm^3) [h]			
≤350	**40**	6 (15.0)	1.40 (0.46-4.30)
>350	**81**	9 (11.1)	1.00
Stable high CD4+ count [f]			
Yes	**27**	1 (3.7)	1.00
No	**121**	21 (17.4)	5.18 (0.66-40.85)

Adjusted Odds Ratio (aOR) using generalised estimating equation; [a]Associations with HPV seroconversion were adjusted for injectable contraception use and CD4+ count at endline which were found to be associated with any HPV type seropositivity in multivariate analysis (with exception of associations with ART at baseline when baseline CD4+ was used for adjustment); [b]CD4+ count at baseline available for 317; CD4+ count at endline available for 284; [c]ART adherence measure available for 171 ART users at baseline; [d] Baseline CD4+ count among ART users at baseline; [e] Endline CD4+ count among ART users throughout follow-up; [f] Stable high CD4+ count was defined as having CD4+ counts >500 cells/mm^3 at baseline, month 12 (intermediate) and endline visits; [g]Baseline CD4+ among participants who were ART-naïve at baseline; [h] Endline CD4+ count among participants who were ART-naïve throughout follow-up.

Table 4. Newly detected HPV DNA among 433 women living with HIV without CIN2+, measured over 16 months follow-up, stratified by same type seropositivity at baseline

	All participants		Seronegative at baseline		Seropositive at baseline		aOR (95%CI)[3]	Among seropositive at baseline				
								Seropersistence at endline		Seroreversion at endline		aOR (95%CI)[4]
	N[1]	n (%)[2]	N[1]	n (%)[2]	N[1]	n (%)[2]		N[1]	n (%)[2]	N[1]	n (%)[2]	
Any Alpha-9 HR-HPV types												
HPV16	366	48 (13.1)	203	25 (12.3)	163	23 (14.1)	1.48 (0.69-3.15)	135	20 (14.8)	28	3 (10.7)	1.50 (0.39-5.72)
HPV31	394	20 (5.1)	169	8 (4.7)	225	12 (5.3)	1.12 (0.36-3.45)	204	10 (4.9)	21	2 (9.5)	0.57 (0.11-2.92)
HPV33	408	17 (4.2)	248	11 (4.4)	160	6 (3.8)	0.70 (0.21-2.33)	143	5 (3.5)	17	1 (5.9)	0.59 (0.06-5.60)
HPV35	374	33 (8.8)	227	27 (11.9)	147	6 (4.1)	**0.26 (0.10-0.68)**	110	4 (3.6)	37	2 (5.4)	0.67 (0.11-4.13)
HPV52	327	55 (16.8)	233	41 (17.6)	94	14 (14.9)	0.77 (0.36-1.65)	82	12 (14.6)	12	2 (16.7)	0.78 (0.14-4.37)
HPV58	404	14 (3.5)	187	10 (5.4)	217	4 (1.8)	**0.19 (0.04-0.89)**	191	3 (1.5)	26	1 (3.9)	0.29 (0.02-3.48)
Any Alpha-7 HR-HPV types												
HPV18	367	17 (4.6)	235	15 (6.4)	132	2 (1.5)	**0.14 (0.02-0.80)**	84	2 (2.4)	48	0 (0.0)	
HPV39	397	24 (6.1)	251	14 (5.6)	146	10 (6.9)	1.69 (0.65-4.42)	83	5 (6.0)	63	5 (7.9)	0.78 (0.21-2.85)
HPV45	403	21 (5.2)	327	13 (4.0)	76	8 (10.5)	2.81 (0.87-9.04)	37	4 (10.8)	39	4 (10.3)	1.42 (0.27-7.66)
HPV59	424	5 (1.2)	280	4 (1.4)	144	1 (0.7)	0.41 (0.02-8.74)	107	1 (0.9)	37	0 (0.0)	
HPV68	412	26 (6.3)	299	13 (4.4)	113	13 (11.5)	**4.07 (1.52-10.90)**	89	11 (12.4)	24	2 (8.3)	1.30 (0.26-6.60)
Other HPV types												
HPV56	396	16 (4.0)	264	10 (3.8)	132	6 (4.6)	1.56 (0.51-4.76)	98	5 (5.1)	34	1 (2.9)	1.51 (0.17-13.76)
LR-HPV types												
HPV6	410	13 (3.2)	241	7 (2.9)	169	6 (3.6)	1.28 (0.41-4.03)	142	5 (3.5)	27	1 (3.7)	1.09 (0.12-10.26)
HPV11	408	15 (3.7)	269	10 (3.7)	139	5 (3.6)	1.16 (0.37-3.67)	119	4 (3.4)	20	1 (5.0)	0.73 (0.08-7.07)
HPV73	428	3 (0.7)	331	1 (0.3)	97	2 (2.1)	28.03 (0.98-798.1)	72	1 (1.4)	25	1 (4.0)	0.40 (0.02-6.73)
Any HPV	**433[a]**	**327**	**427[b]**	**209 (48.9)**	**400[c]**	**118 (29.5)**						

[1]Number of women negative for that type at baseline; [2]number of incident infections among women negative or that type at baseline; [3]adjusted Odds Ratio (OR) for DNA incidence among same-type seropositive vs. seronegative at baseline, adjusted for age, smoking, condom, vaginal washing, bacterial vaginosis, Clamydia trachomatis, Trichomonas vaginalis, CD4 count and ART status at baseline as reported in [5] and for seropositivity for HPV type from same family group, i.e. HPV16 DNA incidence adjusted seropositivity for types HPV31/33/35/52/58 (except for HPV56,6,11 and 73 due to small numbers); [4]adjusted OR for DNA incidence among seropersistent vs. seroreverted at endline, adjusted for CD4+ at baseline only due to small numbers; [a]All women at risk of acquiring a HPV infection (no woman infected by all types at baseline); [b]all women with any HPV type seronegative and same type DNA negative at baseline; [c]All women with any HPV type seropositive and same type DNA negative at baseline.

CONFLICTS OF INTEREST: The authors have no conflicts of interest to disclose.

FUNDING: This work was supported by funding from the European Commission (EC) 7th Framework Programme under grant agreement No. HEALTH-2010-F2-265396, and from the UK Medical Research Council (MRC) PHINDS scheme (PH01/14-39). The research findings were presented at the 31st International Papillomavirus Conference 28 February-4 March 2017, Cape Town, South Africa; abstract number 404.

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ACKNOWLEDGEMENTS

Contributing members of the HARP study group included: A. Chikandiwa, E. Cutler, S. Delany-Moretlwe, D. A. Lewis, M.P. Magooa, V. Maseko, P. Michelow, B. Muzah, T. Omar, A. Puren (Johannesburg, South Africa); F. Djigma, J. Drabo, O. Goumbri-Lompo, N. Meda, B. Sawadogo, J. Simporé, A. Yonli, S Zan (Ouagadougou, Burkina Faso); V. Costes, M.N. Didelot, S. Doutre, N. Leventoux, N. Nagot, J. Ngou, M. Segondy (Montpellier, France); and A. Devine, C. Gilham, L. Gibson, H. Kelly, R. Legood, P. Mayaud, H.A. Weiss (London, UK).

The HARP Study Group also wishes to thank its International Scientific Advisory Group (ISAG) constituting of Prof. C. Lacey (Chair, University of York, UK), Prof. Y. Qiao (Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China), Prof. M. Chirenje (University of Harare, Zimbabwe) and Prof. S. de Sanjosé (Institut Catala d'Oncologia, Barcelona, Spain); and the members of the HARP Endpoint Committee (EPC) including T. Omar (Johannesburg, South Africa), O. Goumbri-Lompo (Ouagadougou, Burkina Faso), S. Doutre, N. Leventoux, V. Costes (Montpellier, France) and O. Clavero (Institut Catala d'Oncologia, Barcelona, Spain).

AUTHOR CONTRIBUTIONS

Conceived and designed the study: PM, SD, HW, JD, HK; Coordinated the study: HK, AC, SD, PM; Participant recruitment and management: AC, SD; Performed the lab testing: HF, JN; Analysed the data: HK, HF; Wrote the first draft of the manuscript: HK; Contributed to the writing of the manuscript: All; Criteria for authorship read and met: All; Agree with manuscript results and conclusions: all.

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