

# Incidence and clearance of anal high-risk human papillomavirus in HIV-positive men who have sex with men: estimates and risk factors

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**Background:** To estimate incidence and clearance of high-risk human papillomavirus (HR-HPV), and their risk factors, in men who have sex with men (MSM) recently infected by HIV in Spain; 2007–2013.

**Methods:** Multicenter cohort. HR-HPV infection was determined and genotyped with linear array. Two-state Markov models and Poisson regression were used.

**Results:** We analysed 1570 HR-HPV measurements of 612 MSM over 13 608 person-months (p-m) of follow-up. Median (mean) number of measurements was 2 (2.6), median time interval between measurements was 1.1 years (interquartile range: 0.89–1.4). Incidence ranged from 9.0 [95% confidence interval (CI) 6.8–11.8] per 1000 p-m for HPV59 to 15.9 (11.7–21.8) per 1000 p-m for HPV51. HPV16 and HPV18 had slightly above average incidence: 11.9/1000 p-m and 12.8/1000 p-m. HPV16 showed the lowest clearance for both ‘prevalent positive’ (15.7/1000 p-m; 95% CI 12.0–20.5) and ‘incident positive’ infections (22.1/1000 p-m; 95% CI 11.8–41.1). More sexual partners increased HR-HPV incidence, although it was not statistically significant. Age had a strong effect on clearance ( $P$ -value < 0.001) due to the elevated rate in MSM under age 25; the effect of HIV-RNA viral load was more gradual, with clearance rate decreasing at higher HIV-RNA viral load ( $P$ -value 0.008).

**Conclusion:** No large variation in incidence by HR-HPV type was seen. The most common incident types were HPV51, HPV52, HPV31, HPV18 and HPV16. No major variation in clearance by type was observed, with the exception of HPV16 which had the highest persistence and potentially, the strongest oncogenic capacity. Those aged below 25 or with low HIV-RNA- viral load had the highest clearance.

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## Introduction

The epidemiology of high risk human papillomavirus (HR-HPV) infection and associated pathologies in HIV-positive men who have sex with men (MSM) has changed profoundly in the last decade [1–3]. The survival gains caused by combination antiretroviral therapy (cART) have been associated with an increase in anal intraepithelial neoplasia (AIN) and anal cancer in MSM, which would have gone unobserved due to competing mortality in the preART era [1–5]. Anal HR-HPV prevalence depends on incidence and persistence of infections. Because HR-HPV persistence increases the risk of AIN [6], understanding its magnitude and risk factors is necessary to develop preventive interventions [5,7–9].

Critchlow *et al.* reported that HPV incidence was higher in HIV-positive MSM than in HIV-negative ones in Seattle, USA. Unprotected receptive anal intercourse was associated with higher HPV incidence. HPV clearance was lower in HIV-positive MSM and HPV16 and 18 showed higher persistence than HPV31, 33, 35 and 39 [7]. Pokomandy *et al.* described that, among HIV-positive MSM with long-standing HIV infection (93% on cART) in Montreal, Canada, HPV16 and HPV52 had the highest incidence [10.8 per 1000 person-months (p-m)] whereas HPV16 had the lowest clearance rate (12.2/1000 p-m) [9]. Darwich *et al.* reported that HPV16 had one of the highest incidences (5.9/1000 p-m) and the lowest clearance rate (18.7/1000 p-m) in HIV-positive MSM in Barcelona, Spain [8]. Phanuphak *et al.* studied HIV-positive and HIV-negative MSM in Bangkok, Thailand, and found HPV16 to have the highest incidence (16.1/1000 p-m) and lower clearance (52/1000 p-m); smoking was associated with increased HR-HPV persistence among HIV-positive MSM [10]. Hernandez *et al.* described that in HIV-positive MSM enrolled in San Francisco, USA, from 1998–2000, HPV18 and HPV16 had the highest incidence (3.1/1000 p-m and 2.9/1000 p-m) and recent receptive anal intercourse was an important risk factor for incident infection [11].

None of these studies, though, took into account the interval censored nature of the data, that is, that the events of interest (incidence or clearance) are not directly observed. Estimates may be biased downwards if more than one infection and/or clearance episodes had happened between negative and positive test results. Furthermore, these studies (except Hernandez *et al.* assumed infection and clearance to have happened at the date of sampling [11].

Our aim was to estimate the incidence and clearance rates for type-specific HR-HPV infections as well as type-specific incidence/clearance ratios in MSM recently infected by HIV in a nation-wide study in Spain. Also, we wanted to study risk factors for incidence and clearance for all 12 HR-HPV types.

## Methodology

### Volunteers and methods

CoRIS-HPV, which has been previously described [12], is a cohort of HIV-positive patients which was set up in 2007 to study HR-HPV infection within CoRIS (Cohort of the Spanish Network of Excellence on HIV/AIDS Research, RIS in Spanish). CoRIS is a multicenter cohort established in January 2004 of individuals with HIV infection and cART naive at entry [13]. Patients in CoRIS are followed periodically according to routine clinical practice, usually every 4 months. Ethics approval has been obtained and participants sign an informed consent for each study.

Within CoRIS-HPV, MSM undergo anal HPV detection and anal cytological examination on a yearly basis, and high-resolution anoscopy when clinically indicated [14]. In addition to socio-demographic, clinical and immunovirological variables routinely collected at CoRIS [13], study participants also answer a questionnaire on sexual behaviour, history of genital warts and tobacco use. For these analyses, MSM with at least one follow-up HPV test were eligible. Follow-up ended at the last clinic visit and the administrative censoring date was May 2013. Only one follow-up HPV test was required in our definition of infection and clearance.

### Human papillomavirus DNA detection and genotyping

Samples for HPV detection were collected from the anal canal with a cytobrush and placed in 1 ml of Specimen Transport Medium (Qiagen, Hilden, Germany), stored at  $-20^{\circ}\text{C}$ , and shipped to the Retroviruses and Papillomavirus Unit of the National Centre for Microbiology in Madrid. DNA was extracted from a 200- $\mu\text{l}$  aliquot of the original anal sample using an automatic DNA extractor (Biorobot M48 Robotic Workstation; Qiagen, Valencia, California, USA). Anal HPV infection and genotyping was determined through the Linear Array HPV Genotyping test (Roche Molecular Systems, Inc., Pleasanton, California, USA) that detects 37 HPV types. Human  $\beta$ -globin gene fragment detection was used as internal control. The results were considered satisfactory if low and high  $\beta$ -globin levels, or at least one HPV type, were detected. As the linear Array HPV52 probe cross-reacts with HPV33, HPV35 and HPV58, in samples in which either of these HPV types were detected a specific HPV52 PCR system designed in E6 gene was performed [15]. A more detailed description of virological methods has been published [16]. Twelve HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59) were considered as HR-HPV types following the classification by Muñoz *et al.* [17].

### Statistical analysis

Because the information on HPV status that is collected at the visits may not be sufficient to describe the dynamics of

infection and clearance, particularly when test interval is 12 months such as in our study, we estimated incidence and clearance rates using a two-stage Markov model that allowed for interval censored event data. In the Markov model individuals can switch between states ‘negative’ and ‘positive’ at rates  $\lambda_{\text{inf}}$  (negative to positive) for incident infection and  $\lambda_{\text{clear}}$  (positive to negative) for clearance. The Markov assumption entails that the rate only depends on the current state; history of past HPV infection is not taken into account. Markov models can be defined by means of transition probabilities; an equivalent infinitesimal definition is based on the transition hazards, which is what we used. Both incidence and clearance rates were estimated jointly by maximizing the likelihood of the observed data, taking into account its interval censored nature. This likelihood takes the data as they are, that is, it only uses the information on HR-HPV infection status at the times of sample collection, but individuals can switch between both states at any time point, not just at the time of testing. As a consequence, we allow several infections and clearances to occur between two samples. Fitting Markov models with interval censored data has been described in detail [18].

We estimated incidence and clearance rate for each HPV type separately, assuming a constant rate over time. Incidence and clearance rates are expressed as events per 1000 person-months (p-m). Ratios of infection and clearance were estimated as well. Sampling uncertainty is expressed via 95% confidence intervals (CI).

For clearance rates, one constant value would not be appropriate if there were a group with high clearance and another group with low clearance rates. Therefore, we included a covariable that makes a distinction between clearance after an HPV positive visit at entry (prevalent positive cases) and clearance after an observed new infection (incident positive cases). This covariable was modelled as time-dependent: once a ‘prevalent positive’ person had cleared HPV and got re-infected, he contributed to the estimate of the ‘incident positive’ group after reinfection. These data, however, do not allow distinguishing reinfection from reactivation.

In risk factor analyses, we allowed age,  $\text{CD4}^+$  T-cell counts, HIV-RNA viral load and cART to affect both transition rates. The incidence rate was additionally allowed to depend on number of male sexual partners, consistent condom use with anal sex in the last 12 months and country of origin. Clearance was allowed to depend on variables that were different to those for incidence, as conceptualized from the literature: current smoking status, educational level and Centers for Disease Control and Prevention (CDC) stage [19]. First, we did the analysis per HR-HPV type, in which we assumed effects on the logarithm of the rate to be linear for age, square-root of  $\text{CD4}^+$  cell count, the base-10 logarithm of HIV-RNA viral load and the base-2 logarithm of number of

sexual partners. We quantified their effects using the same Markov model that we used for the overall incidence calculations. For the reasons given below, we used and compared results with a Poisson regression model, again assuming a constant rate over time. In this model, we followed individuals until the first event and assumed that there were no unobserved events before. Since detection times cannot be equated to incidence or clearance dates, we took the midpoint of the time between two measurements to define incidence or clearance.

We informally compared results from both regression models in order to see whether we could use the Poisson model when fitting a model for all types jointly. In this model, the effect of the risk factors was assumed equal for all types, but we allowed overall rates (the intercepts) to differ by HPV type [20]. For this model, we did not have software that both allows for interval censored transitions in a Markov model and correlation induced by considering all HR-HPV types in one model. In this combined Poisson model, we used generalized estimating equations to correct for multiple HPV types, assuming an exchangeable correlation structure. By assuming effects to be equal, we gained enough power to increase flexibility and allow the effects of continuous variables to be nonlinear; we used restricted cubic splines (four knots for age and three knots for the other variables).

Missing data were imputed using MICE (multiple imputation using chained equations) [21]. The proportion of missing data was low (Table 1). Ten imputed datasets were generated. The time-varying variables  $\text{CD4}^+$  cell count (on square root scale) and HIV-RNA viral load (on fourth root scale) were imputed based on a longitudinal mixed effects model for the development of the respective markers. The same was done for the base-2 logarithm of the number of partners over the last 12 months and condom use during anal sex. After the imputation procedure, all values beyond the range were cut off to the smallest and the largest value. We compared results with and without MICE.

Analyses were conducted in R version 3.0.2 [22].

## Results

Of 1476 HIV-positive MSM within CoRIS-HPV, 612 had at least two visits; characteristics of these 612 men did not differ substantially from the other 864 (data not shown). Of the 603 men with information on HPV status at study entry, 500 (83%) were infected with at least one HR-HPV type. There were 1570 measurements over 13 608 p-m follow-up. The median (mean) number of measurements per individual was two (2.6) and the median time interval between measurements was 1.1 years [interquartile range (IQR): 0.89–1.4]. Median age

**Table 1. Baseline characteristics of 612 HIV-positive MSM from CoRIS-HPV cohort study.**

	No. (%)
Age	
Median (IQR)	33.0 (28.1–39.1)
Country of origin	
Europe	459 (75)
Other	153 (25)
Educational level	
Less than or equal to secondary school	331 (54.1)
University	280 (45.8)
Missing	1 (0.1)
Tobacco use	
Current smoker	270 (44.1)
No smoker	325 (53.1)
Missing	17 (2.8)
Number of sexual partners in last 12 months	
Median (IQR)	6 (2–20)
Missing	59 (9.6)
Unprotected anal intercourse in the last 12 months	
No	196 (32.2)
Yes	399 (64.7)
Missing	17 (3.1)
CDC stage at baseline	
Primary infections/asymptomatic	570 (93.1)
Symptomatic/AIDS	42 (6.9)
cART at baseline	
No	374 (61)
Yes	238 (39)
CD4 <sup>+</sup> cell count at baseline (cells/ $\mu$ l)	
Median (IQR)	569 (444–741)
Missing	3 (0.5)
HIV RNA Viral Load at baseline (log <sub>10</sub> copies/ml)	
Median (IQR)	4.1 (3.1–4.6)
Missing	3 (0.5)

cART, combination antiretroviral therapy; IQR, Interquartile range.

at entry was 33 years (IQR: 28–39), 75% were European (95% of them Spanish) and of the remaining 153, 91% were Latin-Americans. Median number of sexual partners in the preceding 12 months was six (IQR: 2–20) and 65% reported unprotected anal intercourse in the previous year. Median baseline CD4<sup>+</sup> cell count was 569 cells/ $\mu$ l (IQR: 444–741), median log<sub>10</sub> HIV-RNA viral load was 4.1 (IQR: 3.1–4.6) and 61% were cART naïve at baseline; four started cART during follow-up (Table 1).

### Incidence, clearance and incidence/clearance ratios for individual HR-HPV types

Incidence and clearance rates for each HR-HPV type are plotted in Fig. 1. Incidence as estimated by the Markov model ranged from 9.0/1000 p-m (95% CI 6.8–11.8) for HPV59 to 15.9/1000 p-m (95% CI 11.7–21.8) for HPV51. HPV16 and HPV18 had an average and slightly above average incidence respectively [11.9/1000 p-m (95% CI 9.20–15.3) and 12.8/1000 p-m (95% CI 8.6–18.9)] (Fig. 1). Clearance rates showed more variability; HPV16 showed the lowest clearance for both ‘prevalent positive’ (15.7/1000 p-m 95% CI 12.0–20.5) and ‘incident positive’ infections (22.1/1000 p-m 95% CI 11.8–41.1) (Fig. 1). For most HR-HPV types, ‘incident positive’ infections had higher clearance than ‘prevalent positive’ infections. The relatively high incidence and the

low clearance rate together made HPV16 stand out as the genotype with the highest incidence/clearance ratio, both when using clearance from ‘prevalent positive’ infections [0.76; (95% CI 0.53–1.08)] and ‘incident positive’ infections [0.54; 95% CI 0.31–0.93] (Fig. 1).

### Risk factors for incidence and clearance of HR-HPV

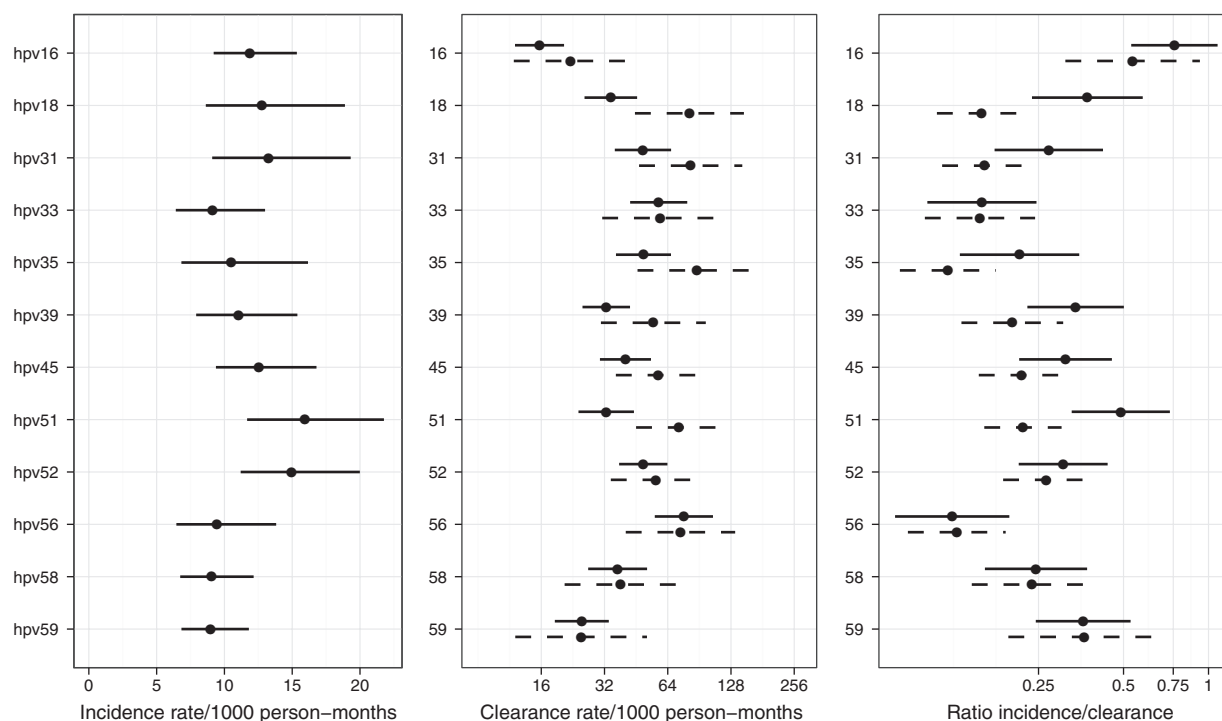
In the regression models per HR-HPV type, there were few statistically significant effects, neither on the incidence, nor on the clearance rate (Appendix Figure 1, <http://links.lww.com/QAD/A772>). Effects were fairly consistent over the HR-HPV types. Furthermore, the Markov and Poisson models showed comparable results, which justified the use of the Poisson model as an approximation in the combined analyses over all HR-HPV types.

In the combined analysis, none of the variables had a statistically significant effect on HR-HPV incidence; *P*-values for the effect of age, CD4<sup>+</sup> cell count and HIV-RNA viral load were 0.17, 0.49 and 0.55, respectively (Fig. 2). Although not significant (*P*=0.31), increasing HR-HPV incidence was observed with increasing numbers of sexual partners in the preceding year. MSM with 10 sexual partners in the last year had an incidence that was 1.22 (95% CI 0.88–1.69) times higher than those who had one partner; beyond 15 partners the effect levelled off. The relative incidence of the other three covariables were: UAI 1.17 (95% CI 0.92–1.48; *P*=0.18); non-European country of origin 1.03 (95% CI 0.81–1.30; *P*=0.91); cART naïve 1.01 (95% CI 0.79–1.29; *P*=0.90). Figure 2 provides the incidence ratios for the effect of age (relative to 35 years), CD4<sup>+</sup> cell count (relative to 625 cells/ $\mu$ l), HIV RNA viral load (relative to 1000 copies/ml) and number of sexual partners in the last 12 months (relative to one partner).

Age and HIV-RNA viral load had a statistically significant effect on HR-HPV clearance (*P*<0.001 and 0.008, respectively). Age showed a distinctive nonlinear effect (Fig. 3); the highest clearance rate was found in MSM under age 25, whereas MSM around 30 seemed to have the lowest clearance rate. For HIV-RNA viral load there was a trend with lower HR-HPV clearance rates for those with higher HIV-RNA viral load. No statistically significant effect was observed for CD4<sup>+</sup> cell count (*P*=0.42). University education, asymptomatic stage and not using cART increased clearance rates by 1.15 (95% CI 0.96–1.34; *P*=0.13), 1.06 (95% CI: 0.70–1.59; *P*=0.78) and 1.23 (95% CI: 0.98–1.55; *P*=0.07).

### Discussion

In MSM recently infected by HIV in a contemporary multicenter cohort in Spain, the highest HR-HPV incidence was observed for HPV51 and 52 followed by



**Fig. 1. Incidence rates, clearance rates and incidence/clearance ratios with 95% CI, based on the two-state Markov model.** Dashed lines in clearance rate and ratio are based on the 'incident HR-HPV infections', solid lines on 'prevalent HR-HPV infections'.

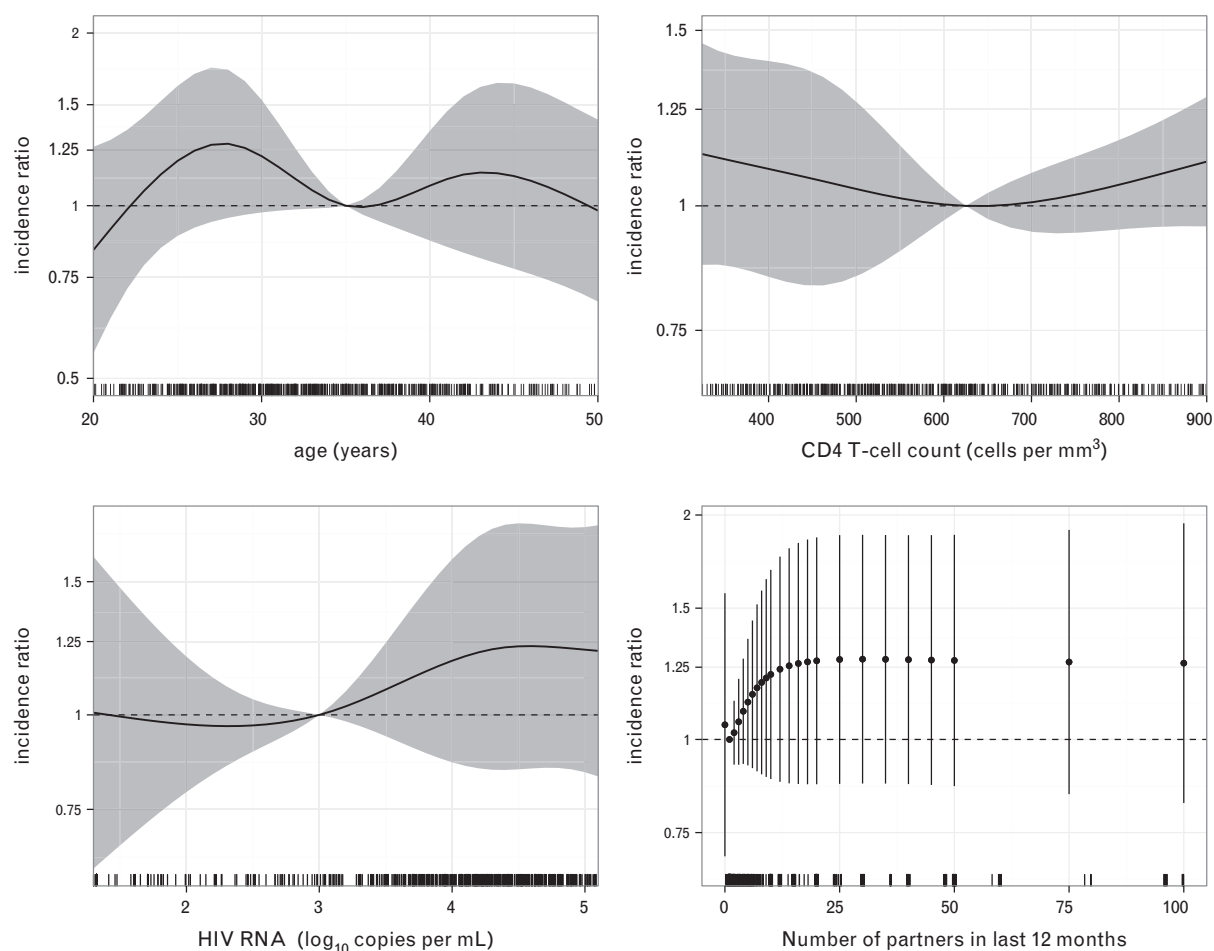
HPV31, HPV18, HPV45 and HPV16. HPV16 had the lowest clearance rate and the largest incidence/clearance ratio, especially in the individuals that were already HR-HPV-infected at entry, and thus potentially the highest oncogenic capacity. In the analyses of effects of covariables on incidence and clearance of the 12 HR-HPV types together, none of the risk factors for HR-HPV incidence yielded statistically significant results. For HR-HPV clearance, age and plasma HIV-RNA viral load revealed significant effects; a strongly nonlinear effect of age was observed as HIV-positive MSM younger than 25 years had up to twice the clearance of older ones. Men with higher plasma HIV-RNA viral load had lower HR-HPV clearance.

Our estimates for HR-HPV incidence are larger than those reported by most other authors [8–11]. Clearance rates are also higher except for the study by Phanuphak *et al.* [10]. Direct comparisons are hampered by differences in sample characteristics (age distribution, maturity of HIV infection), width of follow-up intervals, and number of follow-up samples to define outcomes. Incidence and clearance rates were defined based on single HPV determinations in a follow-up visit. Some authors have used two successive six monthly HPV negative tests to define clearance in HIV-negative populations [11,23]. Given our study was designed to have yearly HPV determinations, requesting a second confirmatory test would have required to wait for as long as two years before establishing outcomes and could

therefore have introduced other sources of misclassification. Some of the differences between our results and other authors' may also be related to the incorporation of the interval censored information. Our two-state Markov model makes allowance for unobserved re-infections between visits. When we used standard person-years calculations for overall incidence and clearance rate and equated time of diagnosis to time of event occurrence, incidence estimates decreased by 22% for HPV16 to 53% for HPV56, and clearance decreased between 22% for HPV16 and 42% for HPV51.

HR-HPV persistence of 'prevalent cases', that is, individuals already HR-HPV positive at entry was higher than that of 'incident cases' suggesting prevalent cases partly encompass long-standing infections which are, most likely, the ones leading to aberrant epithelial transformations.

Our group has recently reported that the number of HR-HPV types in the anal canal in HIV-positive MSM is driven by recent sexual behaviour and age, with peak prevalence around the age of 35 [12]. In these new and longitudinal analyses, we provide more in-depth understanding of our previous findings as we have found that age had little to no relation to incidence but individuals of younger age had higher HR-HPV clearance. To our knowledge, no other study in HIV-positive MSM has identified this age effect. The direction of the association between age and clearance described by Giuliano *et al.* in



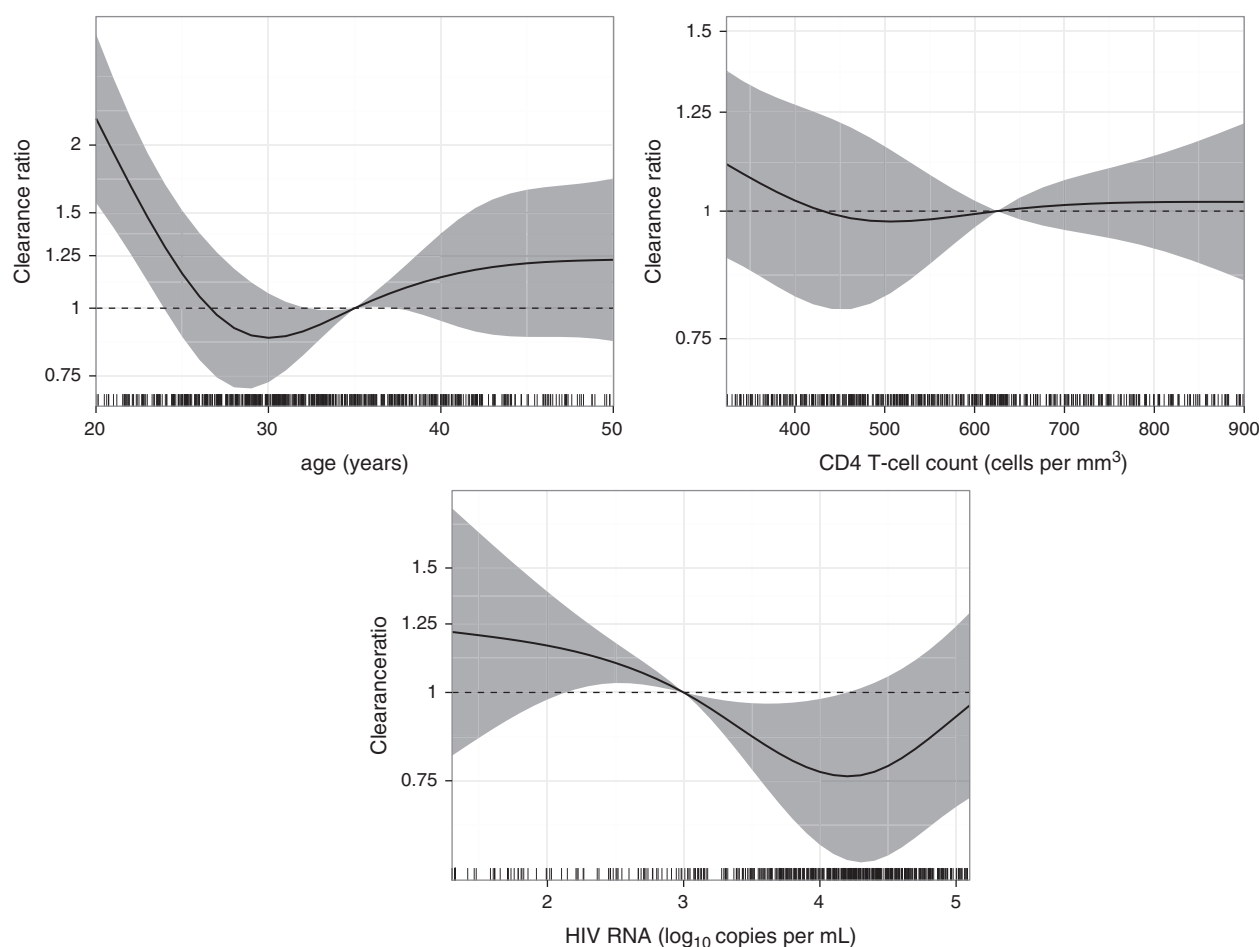
**Fig. 2.** Rate ratios (RRs) for the effect of age (relative to 35 years),  $CD4^+$  cell count (relative to 625 cells/ $\mu$ l), HIV RNA viral load (relative to 1000 copies/ml) and number of sexual partners in the last 12 months (relative to one partner) on HR-HPV incidence (all 12 types together).

samples from the coronal sulcus, glans penis, shaft, and scrotum from HIV-negative men was in opposite direction; HR-HPV clearance increased with age [23]. They attribute this pattern to the higher prevalence of HPV antibodies in older men and thus antibody-mediated acquired immunity. Nyitray *et al.* did not find an association between HPV clearance and age in HIV-negative MSM, whereas they report decreasing HPV clearance with increasing age in men who have sex with women [24]. A larger number of sexual partners in the preceding 12 months showed a suggestive but not statistically significant trend towards higher HR-HPV incidence, consistent with what has been described for HIV-negative men [23].

We did not find an effect of  $CD4^+$  cell count on HR-HPV clearance. However, individuals with high HIV-RNA viral load had lower HR-HPV clearance. Uncontrolled HIV infection may increase HR-HPV persistence through altered cell-mediated immunity, local molecular interactions and reduced tight junction function [25–27]. This effect has not been previously described in HIV positive MSM. Kang *et al.* described

lower HR-HPV clearance in HIV-positive women with high HIV-RNA viral load. In-vitro studies show increased expression of HPV E1 and L1 viral genes in the presence of HIV transactivator of transcription (tat) proteins [28]. During HPV replication, HIV-tat protein was shown to enhance HPV transcription [29–31]. The immune response to chronic viral infections is polyfunctional type 1 of both  $CD4^+$  helper T cells and  $CD8^+$  cytotoxic cells [32]. The lack of a clear relationship between  $CD4^+$  cell counts and HR-HPV clearance in HIV-positive MSM has been reported by Phanuphak *et al.* [10]. Critchlow *et al.* reported weak associations between  $CD4^+$  cell count and HR-HPV persistence [7] but none was found by Pokomandy and Darwich [8,9]. Studies conducted in other HIV-positive populations besides MSM did neither show an association between HPV clearance and  $CD4^+$  cell count [33].

Our results contribute to the scarce body of literature on natural history of anal HR-HPV infection in HIV-positive MSM, particularly in those recently infected by HIV, by providing estimates on incidence and persistence of HR-HPV infection, as well as risk factors.



**Fig. 3.** Rate ratios (RRs) for the effect of age (relative to 35 years), CD4<sup>+</sup> cell count (relative to 625 cells/ $\mu$ l), HIV RNA viral load (relative to 1000 copies/ml) on HR-HPV clearance (all 12 HR-HPV types together).

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Meetings and Conferences: No presentations.

CoRIS-HPV Study Group

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### Conflicts of interest

There are no conflicts of interest.

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