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Catharina J. Alberts,[1] Isabelle Heard,[2,3] Ana Canestri,[4] Lucie Marchand,[5] Jean-François Fléjou,[6,7] Lionel Piroth,[8,9] Tristan Ferry,[10] Jean-Michel Didelot,[11] Laurent Siproudhis,[12,13,14] Sébastien Henno,[15] Isabelle Poizot-Martin,[16,17] Teresa M. Darragh,[18] Gary M. Clifford,[1*] Jean-Damien Combes,[1] Isabelle Etienney[19]; for the ANRS EP57 APACHES Study group.

[1]International Agency for Research on Cancer, Lyon,

[2]Centre National de Référence des HPV, Institut Pasteur, Paris,

[3]Hôpital Tenon, AP-HP, Paris,

[4]Service des Maladies Infectieuses et Tropicales, Hôpital Tenon, AP-HP, Paris

[5]ANRS (France Recherche Nord et Sud Sida-HIV et Hépatites), Paris,

[6]Service d'Anatomie et Cytologie Pathologiques, Hôpital Saint-Antoine, GH HUEP, AP-HP, Paris,

[7]Faculté de Médecine Sorbonne Université, Paris,

[8]Département d'Infectiologie, CHU de Dijon, Dijon,

[9]INSERM CIC 1432, Université de Bourgogne, Dijon,

[10]Département d'Infectiologie, Hôpital de la Croix-Rousse, Hospices Civils de Lyon, Lyon,

[11]Département d'Hépato-Gastroentérologie, CHU Saint Eloi et Université de Montpellier, Montpellier,

[12]Department of Gastroenterology, University Hospital of Rennes, Pontchaillou,

[13]INSERM U1241, University of Rennes 1, Rennes,

[14]INPHY CIC 1414 University Hospital of Rennes, Pontchaillou,

[15]Service d'Anatomie et Cytologie Pathologiques, CHU Pontchaillou, Rennes,

[16]Service d'Immuno-Hématologie Clinique, Université Aix Marseille, APHM Sainte-Marguerite, Marseille,

[17]Inserm U912 (SESSTIM), Marseille, France

[18]Department of Pathology, University of California San Francisco, San Francisco, CA, USA

[19]Service de Proctologie Médico-Interventionnelle, Groupe Hospitalier Diaconesses Croix-Saint-Simon, Paris, France

Corresponding author. Dr Gary M. Clifford, International Agency for Research on Cancer, 150 cours Albert Thomas, 69372 Lyon Cedex 08, France. Tel.: +33-472738425; Fax: +33-472738345; E-mail: CliffordG@iarc.fr

Short summary (max 40 words):

We analyzed incidence and clearance of HPV-16 and HPV-18 in a French cohort of HIV-infected MSM. HPV-16 and HPV-18 incidence were similar, while HPV-16 clearance was slower than HPV-18. Finally, HPV-16 incidence correlated with a prior detection of high-grade lesions.

Abstract:

Prospective data on the natural history of anal HPV infection are scarce in HIV-infected men who have sex with men (MSM). We analyzed incidence and clearance of HPV-16 and HPV-18 in a French cohort of HIV-infected MSM, aged ≥35 years, followed-up annually (n=438, 2014-2018). HPV-16 and HPV-18 incidence were similar (~10% incident infections at 24 months). HPV-16 incidence was higher among high-grade versus no lesion at baseline (aIRR=3.0;95%CI=1.07-8.18). HPV-16 cleared significantly slower than HPV-18 (32% versus 54% by 24 months). In conclusion, anal HPV-16 is more persistent than HPV-18, and its incidence correlates with a prior detection of high-grade lesions.

Key words. incidence; clearance; anal HPV infection; men who have sex with men; human immunodeficiency virus; France.

Background

The incidence of anal cancer has increased in the past decades [1], for which the main risk-factor is anal exposure to high-risk human papillomavirus (hrHPV) [2] especially HPV-16 [3, 4]. The high burden of anal cancer among men who have sex with men (MSM), especially among HIV-infected MSM, is well characterized [3-5]. However, data on the natural history of anal HPV infections among HIV-infected MSM are scarce.

Among MSM, anal incidence rates of HPV-16 can be 2 times higher in HIV-positive MSM compared to HIV-negative MSM, while no significant difference in clearance has been found[6, 7]. Among HIV-infected MSM, sexual risk behavior is an important predictor of HPV incidence [7, 8], while smoking has been found to be a predictor of HPV persistence [7]. It is unclear whether HIV-related factors are predictors of HPV incidence and/or clearance [6, 9].

In a prospective multicenter study of HIV-infected MSM in France, in which we have previously shown hrHPV and HPV-16 to be highly prevalent at baseline [10], we explore the incidence and clearance of HPV-16 and HPV-18 infection as well as the determinants thereof.

Methods

Study participants

As described previously [10, 11], the ANRS EP57 APACHES study concerns an HIV-infected MSM population aged ≥35 years followed up in infectious disease units in six hospitals across France. Participants were included between December 2014 and June 2016, with the last visit date in June 2018. Here, we analyzed annual follow-up visits [10]. Exclusion criteria were anal cancer or histologic high-grade anal intraepithelial lesions (hHSIL) treated in the preceding 12 months. The study was approved by the International Agency for Research on Cancer (IARC) Ethics Committee and the Comité de Protection des Personnes de Paris Ile de France VI in accordance with the Declaration of Helsinki.

Data collection

All participants completed a questionnaire on tobacco use and sexual behavior. Participants' medical records were used to retrieve HIV-related data. The number of new recent sexual partners was asked at every subsequent visit.

Biological samples

Two anal swabs (Anex brush) were taken at each visit by a proctologist. Swabs were transferred to PreservCyt medium (Hologic, Boxbourough, Massachusetts, USA) to be used for cytology and for HPV DNA testing. After specimen collection, patients received a standard proctologic examination (including digital anal-rectal examination [DARE]) and high-resolution anoscopy as described before [10, 11]. Lesions were classified based on the highest diagnostic category found using either cytology or histology [10]. Currently, (consensus) cytology and histology is only available for the baseline visits. Anal swabs were tested for HPV DNA using the Cobas 4800 system (Roche Molecular Systems, Alameda). This assay provides partial genotyping: HPV-16, HPV-18 and other hrHPV aggregated (-31,-33,-35,-39,-45,-51,-52,-56,-58,-59,-66 and -68).

Statistical analyses

Incidence and clearance rates were estimated for HPV-16 and HPV-18 using Poisson regression analyses, assuming a constant rate over time. Cumulative hazard estimates for incidence and clearance were deducted from Kaplan-Meier survival analysis. We included all participants completing ≥2 visits.

Incidence was defined as a positive HPV test result preceded by a negative HPV test. Time at risk for incidence started from the first HPV negative visit of the HPV type under consideration, and ended, either at the midpoint between the last negative test and the first positive test, or at the date of the last HPV negative sample (if the HPV outcome was not

detected). Incident infection with the same HPV type, after clearance of a first infection, was ignored in this analysis.

Clearance was defined as one negative test result that was preceded by a positive test result. People entered risk-set for clearance from the first HPV positive visit and they left the risk-set either at the midpoint between the last positive test and the first negative test, or at the date of the last HPV positive sample (if the HPV infection had not cleared).

The incidence and clearance rate and rate ratios were estimated using Poisson regression. Poisson regression was also used to estimate the incidence/clearance-ratio within one HPV type.

Results

Description of study population

In this study, 438 HIV-infected MSM who had ≥2 visits were included. Baseline HPV-16, HPV-18, other hrHPV, and any hrHPV prevalence were 29%, 12%, 64%, and 71%, respectively; this was similar to the full cohort of participants (n=490) [10]. The median number of visits was 3 (interquartile range, [IQR]:2-4), the median time interval between two visits was 12 (IQR:12-13) months. Median age at entry was 51 years (IQR:46-57) and median baseline CD4+ cell count was 682 cells/µl (IQR:499-884). Most individuals were on cART (95%) and had an undetectable HIV viral-load (93%). 18% had a high-grade lesion detected at baseline (Supplementary Table 1).

Incidence and clearance of HPV-16 and HPV-18

Anal incidence rates (IRs) and clearance rates (CRs) are presented in Table 1. IRs of HPV-16 and HPV-18 were 4.4 (95%C=3.15-6.17) and 4.3 (95%C=3.12-5.81) per 1,000 person-months, respectively. The incidence of HPV-16 and HPV-18 was similar (the incidence rate ratio of HPV-18 versus HPV-16 [IRR] was 1.0 (95%C=0.61-1.52)). CRs of HPV-16 and HPV-18 were 16.0

(95%CI: 12.04-21.19) and 31.4 (95%CI: 23.14-42.68) per 1,000 person-months, respectively. HPV-16 cleared 2.0 (95%CI: 1.30-2.99) times slower than HPV-18. The incidence/clearance ratio was 0.3 (95%CI=0.18-0.43) for HPV-16 and 0.1 (95%CI=0.09-0.21) for HPV-18.

Determinants of HPV-16 and HPV-18 incidence

In Table 2, determinants of anal HPV-16 incidence are presented. Significant determinants of HPV-16 incidence were 'current' versus 'never' tobacco use (adjusted-IRR [aIRR]=3.2; 95%CI=1.14-8.73), having a new receptive ano-genital partner (aIRR=2.3; 95%CI=1.06-4.85), and having a high-grade lesion detected at baseline (aIRR=3.0; 95%CI=1.07-8.18). Of the 75 individuals with a high-grade lesion, 57% (n=43) were already HPV-16 positive at baseline, and among the 32 who were HPV-16 negative at baseline, 7 were HPV16-positive at their next 12 month visit and 1 at his 36 month visit (Table 2). Of note, the 8 individuals with an incident HPV-16 event also tested HPV16-negative by PapilloCheck at baseline.

Anal HPV-16 incidence was not associated with age, nor with most HIV-related variables (cART use, AIDS-defining condition, HIV-viral load at baseline, or CD4+ at baseline and nadir). However, participants with $CD4^+/CD8^+$-ratio ≥1 had lower incidence than those with $CD4^+/CD8^+$-ratio <0.5 (aIRR=0.3; 95%CI:0.10-0.87).

The only significant determinant for anal HPV-18 incidence, in either bivariable or multivariable analyses, was number of lifetime receptive-anal sexual partners (for 10-39 versus 0-9, aIRR=5.2; 95%CI=1.19-22.85) (Supplementary Table 2).

Determinants of HPV-16 and HPV-18 clearance

Determinants of anal HPV-16 and HPV-18 clearance are presented in Supplementary Tables 3 and 4, respectively. Clearance of HPV-16 happened less often after a persistent infection compared to an incident infection (aIRR=0.3; 95%CI=0.11-1.01). This association was significant for HPV-18 (aIRR=0.3; 95%CI=0.10-0.67). Participants with a high-grade lesion

cleared HPV-18 infection less often than participants without a lesion (aIRR=0.2; 95%CI=0.06-0.60), but a similar significant effect was not seen for HPV16 (aIRR=0.7; 95%CI=0.31-1.60).

Discussion

Given the limited prospective data on the natural history of anal HPV infections among HIV-infected MSM, we provide a detailed analysis of the incidence and clearance of anal HPV-16 and HPV-18. Previously, we showed that one third of the APACHES study population were HPV-16 positive and 12% HPV18-positive at baseline [10]. Here we show that after 24 months, about 10% of all HIV-infected MSM have new HPV-16 or HPV-18 infection detected, and that 32% versus 54% subsequently cleared their HPV-16 or HPV-18 infection, respectively. Furthermore, we found that HPV-16 incidence was significantly higher among individuals with a high-grade lesion at baseline, hinting at the possibility of a small number of HPV-16 infections missed in baseline anal swabs. Furthermore, HIV-related parameters did not show associations with anal HPV clearance, at least not among this contemporary virally-controlled population of HIV-infected MSM.

IRs and CRs of this study are within the range of previous studies [6-9]. Note that studies use different sampling and laboratory techniques, and different definitions of incidence and clearance, hampering direct comparisons between studies. HPV-16 is found to have the highest incidence in most studies and to have the lowest clearance in all studies [6-9]. Only a few studies estimate the incidence/clearance-ratio of individual HPV types [9, 12], showing -- in agreement with our study -- the highest incidence/clearance-ratio for HPV-16, highlighting the unique carcinogenic potential of HPV-16 [4].

We found a higher incidence of anal HPV-16 among individuals with high-grade lesions at baseline. This association remained strong even after adjustment for sexual activity, and given that 22% (7/32) of these incident infections were detected at the next visit, this suggests

a small number of missed HPV-16 infections at baseline. Hence, despite the already high correlation found between HPV-16 and high-grade lesions [10], our results suggest that HPV-16 positivity in high-grade lesions might be even higher than that estimated based on a single concurrent anal swab. Although we excluded a small proportion of swabs with inadequate human DNA, sampling procedures of anal swab may need further standardization to ensure quality control given the complexity of sampling the entire anal canal.

Tobacco use was a borderline significant predictor for HPV-16 incidence and showed a positive trend with HPV-18 incidence. This association might be due to residual confounding, but it has also been shown that components of tobacco smoke inflict genotoxic damage in the anal epithelium of smokers [13].

Despite the fact that prevalence/incidence and the sequelae related to HPV infection are more often present among HIV-infected MSM [3, 5, 6], our study, in agreement with others [6, 9], supports the finding that HIV-related parameters are not strongly associated with anal HPV incidence/clearance. Further research needs to clarify whether this is because studies are underpowered and/or other unmeasured factors play a role among HIV-infected MSM. Higher $CD4^+/CD8^+$-ratio was associated with lower HPV-16 incidence; given that it was not consistent with other HIV-related variables, nor with the finding for HPV18, we do not have an explanation for this.

One of the study's limitations is the number of events, which should be kept in mind when interpreting the analysis of the determinants of HPV incidence and clearance. The data did not enable stricter definitions to be applied for incidence (e.g., a positive visit preceded by two negative visits [0-0-1]) and clearance (e.g., a negative visit preceded by two positive visits [1-1-0]). Stricter definitions might avoid counting a deposition as an incident infection, rather than an active infection of the anal epithelium [6, 12, 14]. Furthermore, only

aggregated data were available for 'other hrHPV', hampering a more detailed analysis of hrHPV types beyond HPV-16 and -18. Approximately 70% of this population were hrHPV positive at baseline [10]. Using the aggregated hrHPV-positivity, we observe that after 24 months, approximately half of the 30% hrHPV negative acquire a new hrHPV infection, and that one fifth of the hrHPV positive individuals become hrHPV negative. This high prevalence and turnover highlights how aggregated hrHPV testing (i.e. without genotyping) is not a useful marker for triage in this high-risk population.

Although MSM are clearly an important target group for primary prevention, caution is needed when translating epidemiological data on HPV among older MSM to implications for HPV vaccination. Around 30% of the men in this population were already HPV-16 positive [11], 68% still had their HPV-16 infection after 24 months, and 10% acquired a new HPV-16 infection within 24 months. Furthermore, a recent study showed that incident infections may be caused by reactivated latent HPV infections rather than new incident infections [14], suggesting that HPV-infection was already present. We might expect that a large proportion of any future anal cancer in this MSM population arise from pre-existing infections.

In conclusion, incidence of HPV-16 and HPV-18 were similar, HPV-16 clearance was significantly lower, correlating with its known higher carcinogenic potential. In addition to the possibility of high-grade lesions going undetected in HPV-16 positive individuals through the limitations of high-resolution anoscopy [11], our data suggest that the reverse might also be possible, i.e. that a small proportion of HPV-16 infections in high-grade lesions may go undetected in a single anal swab. APACHES is currently finalizing study exit outcomes of cytology and histology diagnoses, future analyses of which, will provide more detailed insights into how HPV(-16) incidence and persistence predict the prospective risk for high-grade anal lesions.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

ANRS EP57 APACHES Study group: C. Arvieux, A. Canestri, G.M. Clifford, J-D. Combes, V. Costes-Martineau, J. Del Grande, J-M. Didelot, I. Etienney, M. Falguières, T. Ferry, H. Fischer, J-F. Flejou, I. Heard, S. Henno, N. Hoyau, M. Landon, H. Laude, F. Le Breton, A-C. Lesage, A. Lion, G. Maincent, L. Marchand, H. Njatonirina, O. Patey, V. Petrov Sanchez, V. Pialoux, L. Piroth, I. Poizot-Martin, O. Zaegel-Faucher, E. Ressiot, S. Radenne, J. Reynes, L. Siproudhis, C. Tamalet, and P. Tattevin.

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