Anal squamous intraepithelial lesions are frequent among young HIV-infected men who have sex with men followed at the Spanish AIDS Research Network Cohort (CoRIS-HPV).

Cristina González, Montserrat Torres, Amparo Benito, Jorge del Romero, Carmen Rodríguez, María Fontillón, Mónica Trastoy, Pompeyo Viciana, Julia del Amo, Marta Ortiz and Beatriz Hernández-Novoa; On Behalf of CoRIS-HPV Study Group.

Short title: "Anal lesions in Spanish men who have sex with men".

Corresponding author:

Beatriz Hernández-Novoa, PharmD, PhD

Enfermedades Infecciosas. 4ª Planta Ctro. Control A. Laboratorio

Hospital Ramón y Cajal. Ctra. de Colmenar Km 9,100. 28034 Madrid. Spain

Phone: +34 91 3368711. Fax: +34 91 3368792

bhernandez.hrc@salud.madrid.org

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Abbreviations used:

SIL: squamous intraepithelial lesions

MSM: men who have sex with men

HR-HPV: high risk human papillomavirus

AIN: anal intraepithelial neoplasia

ASCUS: atypical squamous cells of uncertain significance

LSIL: low grade squamous intraepithelial lesion

HSIL: high grade squamous intraepithelial lesion

cART: combined antiretroviral treatment

IQR: interquartile range

OR: odds ratios

CI: confidence intervals

HRA: high resolution anoscopy

Brief description (max 75 words) on the novelty and impact of the work: We report the baseline characteristics of a Spanish ongoing multicenter cohort of young HIV-infected MSM showing how the risk of anal squamous intraepithelial lesions (SIL) increases with the number of detected high risk (HR) human papillomavirus (HPV) genotypes. Our results describe the burden of anal lesions with multiple HR-HPV infections and contribute to the understanding of the natural history of anal SIL in our setting.

Abstract

The aim of this study was to determine the baseline prevalence of anal squamous intraepithelial lesions (SIL) and associated risk factors in HIV-infected men who have sex with men (MSM) in a Spanish ongoing multicenter cohort. CoRIS-HPV started in 2007, nested in the Spanish AIDS Research Network Cohort (CoRIS). Anal liquid cytology testing was performed. High risk (HR) HPV infection was determined and positive samples were genotyped. We analyzed all subjects up to April 2011. Multivariate logistic regression analyses were performed. 551 subjects with baseline anal liquid cytologies were analyzed; 37.0% negative for intraepithelial lesion, 9.0% ASCUS, 41.0% LSIL, 4.0% HSIL and 9.0% inadequate. Prevalence of anal SIL (excluding ASCUS) in valid samples (n=450) was 54.7% (95% CI, 49.9-59.3). Globally HR-HPV prevalence was 81.7% (95% CI, 78.0 to 85.2). Multiple infections (≥ 2 HR-HPV genotypes) were documented in 77.7% (95% CI, 73.1 to 82.0). The only risk factor associated with anal SIL was the number of HR-HPV types; MSM with five or more HR-HPV genotypes had an odds of anal SIL seven times greater (OR 7.4; 95% CI, 2.8 to 19.6) than those with one HR-HPV genotype. No associations were found for age, educational level, smoking, geographical origin, CD4 T cell count, antiretroviral treatment or number of sexual partners. The prevalence of anal SIL in young HIV-positive MSM is high and the main risk factor is multiple infections with HR-HPV types.

INTRODUCTION

High risk human papillomavirus (HR-HPV) is an etiologic agent of anal cancer [1]. Percentages of HPV coinfection are very high among HIV-infected patients, especially in men who have sex with men (MSM) [2-5].

In the past years, most studies carried out in HIV-infected MSM in the USA and Europe have documented HPV prevalences around 85.0%-95.0% [5-7]. In Spain, few data are available on anal HPV prevalence; Sirera *et al.* reported in 2006 a HR-HPV prevalence of 83.0% in 52 MSM. This same group in a recent study of anal condylomata in 640 HIV-infected men (473 MSM) reports an overall prevalence of anal HPV of 73% [8]. In 2011, Ortiz *et al.* [9] communicated in 760 MSM from the Spanish AIDS Research Network (RIS) HPV-Cohort (HPV-CoRIS), a HR-HPV prevalence of 85.6%; 61.6% of these patients were infected by more than two different HR-HPV genotypes.

Anal cancer is infrequent in general population, but has become one of the most common emerging non AIDS-defining cancers in western countries [10-13], as HIV-infected MSM are at higher risk [11]. Several studies have shown an increase in the incidence of both anal intraepithelial neoplasia (AIN) and in invasive anal cancer in HIV-infected men [12, 14-19].

Even with the advent of HAART, anal cancer incidence in HIV-infected subjects has not been reduced in the USA [11, 12, 20-23], Australia [24] or in European countries, such as France [13] or England [10]. Moreover, most studies show an increase in the incidence of anal cancer when comparing post-HAART to pre-HAART era [10-12, 20, 22-25]. There is no apparent correlation among the risk of developing invasive anal cancer and the CD4 T cell count [16, 26].

Anal squamous intraepithelial lesions (SIL), particularly high grade lesions, are thought to be the precursors of anal cancer, either determined by cytology (anal SIL) or by biopsy (AIN) and, several reports have shown that its prevalence is high in HIV-positive MSM [3, 6, 7, 27-34]. In our country, only one group has published data on this field. In 1996 they reported 48.0% of overall prevalence of anal cytology abnormalities in HIV-infected MSM (n=52) [4] and in a recent study of condylomata in their HIV-infected cohort they also shows high prevalences of anal SIL in MSM [8].

In Spain, as in most parts of the world, currently no protocols or guidelines exist regarding screening, follow up and treatment of HPV-associated anal SIL in

HIV-infected MSM. The present study aims to determine the prevalence of anal SIL, by liquid cytology, and associated risk factors in a large ongoing multicenter cohort of HIV-infected MSM in Spain, CoRIS-HPV.

Methodology

Subjects and methods

CoRIS-HPV is a cohort study within CoRIS, an open, prospective, multicenter cohort of adult patients with confirmed HIV infection and naïve to antiretroviral treatment at study entry, established in January 2004 in 30 sites from 13 of Spain's 17 Autonomous Communities. CoRIS is the cohort of the Spanish Network of Excellence on HIV/AIDS Research (RIS in Spanish) and collects baseline and follow-up socio-demographic (age, sex, category of transmission of HIV, educational level, geographical origin), clinical (AIDS and non AIDS-defining conditions), immunological (CD4 T cell counts), virological (HIV Viral Load), antiretroviral treatment and vital status (including cause of death) data. CoRIS is linked to a BioBank. Patients are followed periodically in accordance with routine clinical practice, usually every four months and data are subjected to internal quality controls. An external audit of 10.0% of subjects is conducted bi-annually. Ethics approval by the coordinating center (Instituto de Salud Carlos III) and the corresponding hospitals and signed informed consent have been obtained.

CoRIS-HPV was set up in 2007 with the objective to study the epidemiology of HR-HPV infection in MSM. Of the 30 sites which contribute data to CoRIS twelve are located in seven different Autonomous Communities and are also part of CoRIS-HPV. Participants are informed about the nature of the study and are required to sign an *ad-hoc* informed consent. Specific ethics approval for this study has also been obtained. As well as the variables collected in CoRIS, subjects in CoRIS-HPV are requested to answer a questionnaire on sexual behavior (age of first sexual intercourse, number of life-time sexual partners, number of sexual partners in the preceding 12 months, frequency of unprotected intercourse in the preceding 12 months), history of genital warts and tobacco use. Subjects are informed that the data contained in this questionnaire will not be passed on to their clinical chart or to their regular physician. Baseline and follow-up anal samples are collected annually (or more often if clinically indicated) and processed for HR-HPV DNA detection; liquid cytologies are performed at the same time points.

HPV DNA detection and genotyping

Samples were collected with a cytobrush and placed in 1 ml of Specimen Transport Medium (Digene Corporation, Gaithersburg, Maryland, USA) and sent to the Retroviruses and Papillomavirus Unit of the National Centre for Microbiology in Madrid, and stored at -20ºC until required for testing. DNA was extracted from a 200 μl aliquot of the original anal sample using an automatic DNA extractor (Biorobot M48 Robotic Workstation; Qiagen, Valencia, CA, USA). For quality control, a negative control (PCR quality water) and a positive control (SiHa cells infected with HPV 16) were included in each DNA extraction run (ten sample batch). Anal HR-HPV infection was determined with "Amplicor HPV DNA Test" (Roche Molecular Systems, Inc., Branchburg, NJ, USA) which detects 13 HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) and an additional primer set for the human β-globin gene was used to provide a control for cell adequacy, extraction, and amplification. The results were considered satisfactory if the β-globin or HPV amplification was detectable and controls' results were valid. All positive samples were genotyped using PCR amplification followed by reverse line blot hybridization using Linear Array® HPV Genotyping test (Roche Molecular Systems, Inc.).

Liquid cytologies

Anal cytology samples were obtained in a blinded fashion. We used an endocervical brush device for greater yield of cell collection (Cytobrush®, Hologic Inc., Bedford, MA, USA). The cytobrush was inserted into the anal canal until it bypassed the internal sphincter and met the distal rectal wall. It was rotated in a corkscrew fashion as it was withdrawn in order to sample cells from all areas of the anal canal, bending it slightly with gentle pressure to allow for adequate collection of cells. Once removed, cells were completely resuspended in the Thinprep® vial in PreservCyt® solution (Hologic Inc.) and stored according to the manufacturer's recommendations. Cellularity was evaluated together with the presence of glandular cells or cells from the transformation zone, as well as morphologic alterations of the squamous cells. Results were given according to the cervical cytology Bethesda 2001 classification adapted to anal samples. The following categories were established: inadequate sample (not enough cells for diagnosis present), negative for intraepithelial lesion, atypical squamous cells of uncertain significance (ASCUS), low grade squamous intraepithelial lesion (LSIL) or high grade squamous intraepithelial lesion (HSIL). As no cases of squamous cell carcinoma were diagnosed this category was dismissed. All samples were read in a blinded fashion (with no access to the HR-HPV results or the rest of the study variables); most of them (83.0%) were read by two independent cytologists and those with discordant results were re-evaluated to achieve a consensus diagnosis. Also, an effort was done in order to avoid the over diagnosis of ASCUS, i.e. as there is scarce cellularity, slides are carefully examined to shift the diagnosis to “negative”, “LSIL” or “HSIL” when possible.

Definition of variables

The outcome variable for these analyses, anal cytology results, was classified as a dichotomous variable in which abnormal results -excluding ASCUS- “LSIL and HSIL” were joined and compared with negative cytologies. As ASCUS can derive to the absence of lesion or to the complete spectrum of cellular alterations, we considered that categorizing them as abnormal results would lead to overdiagnosis of anal SIL. Sensitivity analyses including ASCUS were also performed. The independent variables tested in the analyses were age (categorized by quartiles as ≤28 years, 29-33 years, 34-39 years and ≥40 years), number of lifetime sexual partners and also in the last 12 months (categorized by quartiles), age at first sexual intercourse (categorized by median age), educational level (categorized as none/primary, secondary and university), geographical origin (categorized as Spanish, Latin-American and Others), CD4 T cell count closest (within ± 6 months) to cytology sampling (treated as continuous variable and categorized, according to most HIV treatment initiation recommendations, as ≤ 350 cells/mm^3, 351-500 cells/mm^3 and ≥501 cell/mm^3), baseline Viral Load copies/ml (categorized as > 100000 and ≤ 100000), combined antiretroviral treatment (cART) use at CoRIS-HPV cohort entry and number of HR-HPV types.

Statistical analysis

We analyzed subjects up to April 2011. Descriptive analyses of the subjects’ characteristics were performed. Frequency distributions are presented for qualitative variables; means and their standard deviation are presented for quantitative variables with symmetrical distributions and the median and interquartile range (IQR) when the distribution is asymmetric. We used the chi-square test for the comparison of qualitative variables, analysis of variance for the comparison of means, and non-parametric tests for the comparison of medians.

The association between abnormal cytological results and the exposure variables was analyzed using multivariate logistic regression models. We also performed separate analyses for LSIL and HSIL. Crude and adjusted odds ratios (OR) with their 95% confidence intervals (CI) were obtained as the measure of association. We used likelihood ratio tests and Wald tests to derive p values. A significance level of <0.1 was chosen to select the variables entering the multivariate logistic regression model. A backward approach was chosen and the variables included in the final regression models were those which maximized the likelihood values. Only variables that retained statistically significant associations with the outcome variable were left in multivariate analyses. For all of the above described models, robust methods were used to estimate the confidence intervals, assuming correlation among subjects recruited within each centre and independence among subjects from different centers. The analyses were conducted using Stata 11 (StataCorp LP, College Station, TX, USA).

RESULTS

As of April 2011 CoRIS-HPV comprised 551 MSM with a baseline liquid cytology obtained at the first visit. We analyzed 450 MSM with a valid baseline liquid cytology result, i.e. negative for intraepithelial lesion, LSIL or HSIL; excluding inadequate samples and ASCUS (figure 1). The excluded 101 patients did not differ in terms of clinical or demographic characteristics.

Most patients (53.2%) were younger than 34 years (Median age 33 years, IQR 28-39 years), 62.0% were Spanish, 80.3% had undergone secondary or university studies and 44.0% were active smokers (table 1). Seventy-eight percent of the subjects had more than 350 CD4 T cells/mm^3 at study entry (table 1), and overall, 16.0% had started cART at CoRIS-HPV cohort entry. Overall, 38.0% had positive syphilis serology (by treponemic tests) and 26.4%

recalled having had condylomas at some point in time, in 81.4% of the cases the location was perianal (data not shown).

As to the number of sexual partners, 65.0% had had more than 41 partners along their lifetime and 47.7% had between one and ten companions in the last year. Fifty-nine percent of the MSM had their first sexual intercourse before the age of 17. Up to three quarters of the patients reported having unprotected sex in the preceding year. Among those who acknowledged having high risk sexual practices, 61.0% of them admitted to having had protected sex sporadically and in 30.7% of the cases never using condoms.

Overall HR-HPV prevalence was 81.7% (95% CI, 78.0 to 85.2); among patients positive for HR-HPV detection 22.3% had only one HR-HPV type, 29.6% two types, 23.0% three types, 14.1% four types and 11.0% five or more types. Multiple infections, defined as bearing two or more HR-HPV genotypes, was detected in 77.7% of the cases.

Baseline liquid cytology results in 551 MSM were: 37.0% negative for intraepithelial lesion, 9.0% ASCUS, 41.0% LSIL, 4.0% HSIL and 9.0% were inadequate samples (figure 2). All samples bearing HSIL were positive for HR-HPV and for those with available genotype results, all had more than 2 types. As for LSIL samples, 89.7% were positive for HR-HPV and for those with available genotype results, 82.0% had more than 2 HR-HPV types. Finally, among the negative cytologies, 76.5% had HR-HPV and for those with available genotype results, 69.1% had more than 2 types. Overall prevalence of anal SIL (comprising LSIL and HSIL and excluding ASCUS) was 54.7% (95% CI, 50.0 to 59.3).

The results of the univariate analysis of the 450 valid baseline anal liquid cytologies from patients with available data for all study variables are shown in table 2. We observed an increase in the risk of anal SIL according to the number of genotypes detected, in a gradual fashion (p trend <0.01). Patients with five or more HR-HPV genotypes had a risk of anal SIL seven times greater than those infected by just one HR-HPV genotype (OR: 7.2; 95% CI, 2.7 to 19.1). Although the overall p value for the variable age was not significantly associated to the presence of anal SIL, men older than 40 years had a statistically significant lower risk of anal SIL (40.0%) than those 28 years old or younger (OR: 0.6; 95% CI, 0.4 to 0.9). No statistically significant differences were observed according to geographical origin (p=0.42), educational level (p=0.79), smoking habit (p=0.79), nor with other sexual behavior related variables such as number of partners in the last 12 months (p=0.84) or having had anal intercourse without condom in the last 12 months (p=0.93) (table 2). With regards to CD4 T cell count analyzed as a continuous variable no statistically significant differences were observed when comparing the median CD4 count in MSM with or without anal SIL. When treated as categorical no associations were observed with the CD4 count defined cut-off values (p=0.32). Sensitivity tests with different cut-off values were performed and results remained unchanged (data not shown).

In the multivariate analyses only age and number of HR-HPV types entered the models and only the number of HR-HPV genotypes was significantly associated with the risk of anal SIL. The higher the number of HR-HPV types, the greater the odds of anal SIL; MSM with five or more HR-HPV genotypes had an odds seven times higher of anal SIL (OR: 7.4; 95% CI, 2.8 to 19.6) than those with one HR-HPV genotype.

Sensitivity analyses were performed including ASCUS in the anal SIL category and results were unchanged (data not shown).

We conducted additional analyses to model predictors of HSIL compared to MSM with normal cytologies. Although numbers are small, the only risk factor associated with HSIL was, too, the number of HR-HPV types (data not shown).

DISCUSSION

Half of the HIV-positive MSM in our cohort had baseline anal SIL, the majority were low grade lesions and the only risk factor associated was the number of HR-HPV types. Similar to that of previously published studies, the prevalence of anal SIL detected in HIV-positive MSM in our cohort was 54.7% [7, 30, 35-37]. We only detected a 4% of HSIL, in the range of that described by other authors [30, 35, 37]. In contrast, some studies report lower levels of LSIL from 23.0 to 26.0% [30, 35, 37] at the expense of high rates of ASCUS, from 15.0% [30] to 33.0% [35, 37]. In this sense, the ASCUS rate has been defined as a cytology quality control tool, and the rate of ASCUS we have found is in the range of acceptability.

The only risk factor associated to the presence of anal SIL in our population was infection by multiple HR-HPV types. HIV-positive MSM co-infected with five or more HR-HPV genotypes had seven times higher risk of anal SIL than those with only one genotype. There is little data relative to the risk of detecting anal SIL in HIV-infected MSM according to the presence of multiple HR-HPV coinfections.

Concomitant infection by more than one HR-HPV genotypes has been associated to the presence [6, 38, 39] or progression of AIN [35]. Early reports by Palefsky *et al.* showed the association between the presence of more than one HPV genotypes and the progression [40] or incidence [41] of anal SIL. A report by Conley *et al.* has shown association between the higher number of HR-HPV genotypes and the presence of anal SIL, but the analysis was globally done and comprised MSM, heterosexual men and women [30]. The only previous study assessing the relationship between the presence of anal SIL and multiple HPV infection in HIV-infected MSM we are aware of reported that the risk of having anal SIL doubled when ≥3 HPV genotypes were present compared to single infection, although both low risk and HR-HPV types were analyzed together [31].

In the univariate analysis we found that MSM aged 33 or younger were more likely to have anal SIL but this association was not found after the multivariate analysis as it was confounded by the higher prevalence of multiple HPV coinfections in the younger group.

We explored the relationship between CD4 T cell counts and the presence of anal SIL and we did not find any association. This fact has also been documented in previous studies where no association between CD4 T cell counts and anal SIL was found in HIV-positive MSM [7, 32, 34, 42] although the study of Wilkin *et. al* included also heterosexual men in the analysis [34] . On the contrary, other studies have reported that a low CD4 T cell count has been associated with the development of anal SIL [28, 34] . In relation to this, the fact that in our cohort more than 78.0% of the subjects had more than 350 CD4 T cells/mm^3 could account for the lack of association with anal SIL.

Some studies have also investigated the association between AIN or anal SIL and nadir CD4 T cell count, and all have shown that HIV-positive MSM with a low nadir CD4 T cell count had higher risk of anal SIL or AIN [30, 34, 43]. Given our study was nested in a young cohort of naïve patients where only 16.0% of MSM had already started cART, we have not been able to explore the role of nadir CD4 T cell count as yet; further follow-up of the cohort will allow us to do so.

Although it could be expected that variables reflecting sexual behavior would be related to the presence of anal SIL, we did not observe any association with the number of sexual partners, unprotected sex or the age of first sexual intercourse. Previous analysis of the same cohort has shown that the only risk factor associated with HR-HPV infection was the number of lifetime sexual partners [44], as reported by other studies [45-47].

Cigarette smoking plays a role in the etiology of anogenital cancers [48, 49] although other studies found no association with the presence of HR-HPV infection or the incidence of HSIL [33]. Likewise, in our study we did not observe an association with smoking habits and the presence of anal SIL.

Although we do not have high resolution anoscopy (HRA) guided biopsy confirmation for the great majority of the anal SIL as yet, these are underway prioritizing HSIL, LSIL and ASCUS. To date, all patients in this study are followed up annually, and only HR-HPV detection and liquid cytology are performed. We are aware that liquid cytology is far from perfect, but it may be used as a screening tool in high risk populations like HIV-infected MSM, as recently assessed [50]. No anal screening program has been established in Spain yet and few centers have the resources to implement HRA.

In summary, these are the baseline data from a large ongoing multicenter cohort of young HIV-positive mainly Spanish and highly educated (80.3% had undergone secondary or university studies) MSM followed up in Spain. Our data show that the prevalence of anal SIL is high and that its main risk factor is multiple infection with HR-HPV types; condition that is indeed very frequent in HIV-positive MSM, the most vulnerable population at risk of anal cancer. The natural history of anal SIL is yet to be determined, so studies such as this one are necessary to uncover the baseline burden of HR-HPV-associated lesions and to advance in the generation of evidence as to assess the need of establishing anal SIL screening programs.

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CoRIS-HPV group participating centres and members:

Hospital Universitario San Cecilio de Granada: Alejandro Peña, Federico García; **Centro Nacional de Microbiología (ISCIII). Madrid**: Marta Ortiz, Montse Torres; **Hospital Xeral de Vigo:** Antonio Ocampo, Alfredo Rodriguez Da Silva, Celia Miralles, Gustavo Mauricio Iribarren, Joaquín González-Carreró; **Hospital Universitario Ramón y Cajal-IRYCIS Madrid:** Beatriz Hernández-Novoa, Nadia Madrid, Amparo Benito, Itziar Sanz; **Centro Sanitario Sandoval. Madrid:** Jorge del Romero, Mar Vera, Carmen Rodríguez, Carmen Martín Alegre, Juan Carlos Carrió, Montse Raposo; **Hospital Universitario Virgen del Rocío. Sevilla:** Pompeyo Viciana, Mónica Trastoy, Maria Fontillón; **Hospital Universitario de Elche. Alicante:** Mar Masiá, Cati Robledano, Félix Gutierrez, Sergio Padilla, Encarna Andrada; **Hospital Universitario Severo Ochoa. Madrid:** Miguel Cervero; **Hospital San Pedro-CIBIR. La Rioja:** José Ramón Blanco, Laura Pérez; **Hospital General Universitario de Alicante. Alicante:** Joaquín Portilla, Irene Portilla; **Hospital Universitario Donostia. San Sebastián:** Miguel Ángel Vonwichmann, Jose Antonio Iribarren, Xabier Camino; **Hospital Universitario La Paz-IdiPAZ. Madrid:** Elena Sendagorta, Pedro Herranz; **Hospital Universitario de Canarias. Santa Cruz de Tenerife:** Patricia Rodríguez, Juan Luis Gómez, Dacil Rosado; **Centro Nacional de Epidemiología (ISCIII). Madrid:** Julia del Amo, Cristina González, Mª Angeles Rodríguez. **Institut Català d'Oncologia. L'Hospitalet de Llobregat.** Silvia de Sanjosé.

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Figure legends

Figure 1. Cytological samples included in the analyses

Figure 2. Cytological results of 551 MSM at baseline

Table 1: Descriptive analysis of 450 MSM with baseline liquid cytology

	Total N (%) 450 (100)
Age (years)	
≤ 28	113 (25.0)
29-33	127 (28.2)
34-39	109 (24.2)
≥ 40	101 (22.4)
Area of origin	
Spain	279 (62.0)
Latin-America	138 (30.7)
Others	28 (6.2)
Unknown	5 (1.0)
Educational level	
None/primary	77 (17.0)
Secondary	175 (39.0)
University	186 (41.3)
Unknown	12 (2.7)
Tobacco use	
Current smoker	197 (43.8)
Past smoker	16 (3.5)
Never smoker	222 (49.3)
Unknown	15 (3.3)
Unsafe sex in last 12 months	
No	101 (22.4)
Yes	344 (76.4)
Unknown	5 (1.2)
Unprotected anal intercourse	
Sometimes	206 (60.7)
Frequently	29 (8.5)
Always	104 (30.7)
Age at first sexual intercourse (years)	
≤ 17	265 (59.0)
≥ 18	170 (37.7)
Unknown	15 (3.3)
Number of lifetime sexual partners	
≤ 40	104 (23.0)
41-100	122 (27.0)
101-400	72 (16.0)
≥ 401	99 (22.0)
Unknown	53 (12.0)
Number of sexual partners in last 12 months	
≤ 3	122 (27.0)
4-10	93 (20.7)
11-36	98 (20.7)
≥ 37	124 (21.0)
Unknown	47 (10.4)
Baseline CD4 T cells/mm^3	
≤ 350	83 (18.4)
351-500	120 (26.7)
≥ 501	231 (51.3)
Unknown	16 (3.6)
Baseline Viral Load copies/ml	
> 100000	63 (14)
≤ 100000	368 (81.8)
Unknown	19 (4.2)
cART use at CoRIS-HPV cohort entry	
No	373 (82.9)
Yes	72 (16.0)
Unknown	5 (1.1)
Number of HR-HPV genotypes (n=368)	
1	82 (22.3)
2	109 (29.6)
3	85 (23.0)
4	52 (14.1)
≥ 5	40 (11.0)

MSM: men who have sex with men, HR-HPV: high risk human papillomavirus

Table 2. Univariate analysis of anal SIL in MSM

	Total N (%) 246/450 (54.7)	OR (95% CI)	p Value
Age (years)			
≤ 28	64/113 (56.6)	1	
29-33	79/127 (62.2)	1.3 (0.8-2.1)	0.08
34-39	57/109 (52.3)	0.8 (0.5-1.4)	**0.04***
≥ 40	46/101 (45.5)	0.6 (0.4-0.9)	
Area of origin			
Spain	156/279 (56.0)	1	
Latin-America	75/138 (54.4)	0.9 (0.6-1.4)	0.42
Others	12/28 (43.0)	0.6 (0.3-1.3)	
Educational level			
None/primary	44/77 (57.2)	1	
Secondary	92/175 (52.6)	0.8 (0.5-1.4)	0.79
University	102/186 (55.0)	0.9 (0.5-1.6)	
Tobacco use			
Current smoker	107/197 (54.3)	1	
Past smoker	10/16 (62.5)	1.4 (0.5-4.0)	0.79
Never smoker	119/222 (53.6)	1.0 (0.7-1.4)	
Risk sexual behaviours in last 12 months			
No	55/101 (54.5)	1	
Yes	189/344 (55.0)	1.0 (0.7-1.6)	0.93
Unprotected sexual intercourse			
Sometimes	111/207 (53.6)	1	
Frequently	13/29 (44.8)	0.7 (0.3-1.6)	0.26
Always	63/104 (60.6)	1.3 (0.8-2.2)	
Age at first sexual intercourse (years)			
≤ 17	148/265 (56.0)	1	
≥ 18	91/170 (53.5)	0.9 (0.6-1.3)	0.64
Number of lifetime sexual partners			
≤ 40	57/104 (55.0)	1	
41-100	64/122 (52.5)	0.9 (0.5-1.5)	0.46
101-400	39/72 (54.2)	1.0 (0.5-1.8)	
≥ 401	62/99 (62.6)	1.4 (0.8-2.4)	
Number of sexual partners in last 12 months			
≤ 3	69/122 (56.6)	1	
4-10	50/93 (53.8)	0.9 (0.5-1.5)	0.84
11-36	56/93 (60.2)	1.2 (0.7-2.0)	
≥ 37	53/95 (55.8)	1.0 (0.6-1.6)	
Baseline CD4 T cells/mm^3			
≤ 350	52/83 (62.7)	1	
351-500	63/120 (52.5)	0.7 (0.4-1.2)	0.32
≥ 501	126/231 (54.6)	0.7 (0.4-1.2)	
Number of HR-HPV genotypes			
1	36/82 (44.0)	1	
2	61/109 (56.0)	1.6 (0.9-2.9)	**<0.01**
3	50/85 (59.0)	1.8 (1.0-3.4)	**<0.01***
4	38/52 (73.0)	3.5 (1.6-7.4)	
≥ 5	34/40 (85.0)	7.2 (2.7-19.1)	

*** p trend.**
MSM: men who have sex with men, SIL: squamous intraepithelial lesion, OR: odds ratio, HR-HPV: high risk human papillomavirus

Figure 1. Cytological samples included in the analyses

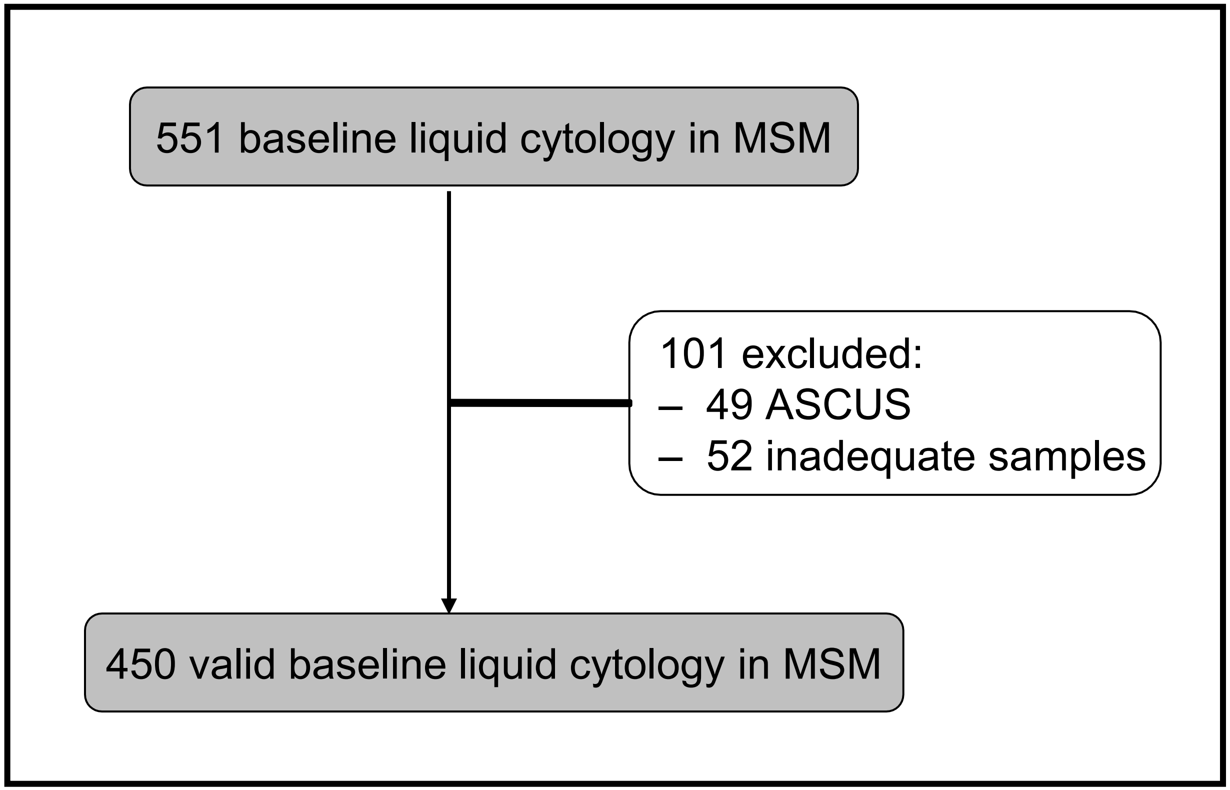

Figure 2. Cytological results of 551 MSM at baseline