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ORIGINAL PAPERS

Preliminary results of a screening program for anal cancer and its precursors for HIV-infected men who have sex with men in Vigo-Spain

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ABSTRACT

Background and aims: Men who have sex with men (MSM) infected with human immunodeficiency virus (HIV) have the highest risk of developing anal cancer (AC). The objective of this study was to describe our screening implementation program in this population, and report the prevalence of human papillomavirus (HPV) anal infection, and cytological and histological findings in a Spanish medium-size community (Vigo, Spain).

Method: Prospective cohort analysis of 240 HIV-infected MSM. Cellular anal sample and high risk HPV (HR-HPV)-tests were performed to study cytological changes and HPV genotyping. High resolution anoscopy (HRA) was performed in 209 patients. Results were analyzed with respect to epidemiological, clinical and analytical factors.

Results: Of 209 patients selected for HRA, the prevalence of HR-HPV anal infection, cytological and histological alterations was 85.6%, 47.5%, and 39.8%, respectively. Sensitivity and specificity for ≥ ASCUS (atypia of squamous cells of undetermined significance) cytology in relation to histological alterations were 61% and 85%, (OR: 8.7; IC 95%: 4.4-17.2), respectively. Observed concordance between high-grade squamous intraepithelial lesion (HSIL) cytology and HSIL anal intraepithelial neoplasia types 2 and 3 (AIN-2/3) histology was 64% (OR: 11.4; IC 95%: 3.6-36.7). One patient with HSIL cytology presented a prevalent anal squamous carcinoma.

Conclusions: HRA was feasible with similar results to relevant groups. There was a high prevalence of anal HR-HPV infection, and cytological and histological alterations.

Key words: Men who have sex with men. HIV. High risk HPV. Cytology. Anoscopy. Histology. Anal intraepithelial neoplasia. Anal cancer. Screening.

INTRODUCTION

Persistent infection with high-risk human papillomavirus (HR-HPV) is a necessary condition to develop anal cancer. Its precursor lesion is high-grade anal intraepithelial neoplasia (1-5), which includes HSIL (AIN-2) and HSIL (AIN-3). Although infrequent in the general population, the overall incidence of anal cancer has slowly increased in the last two decades, approaching 2 per 100,000 person-years (6-8), albeit with few references documented in Spain (9,10). In contrast, the incidence of anal cancer is up to 144 per 100,000 person-years in HIV-infected men who have sex with men (MSM) (11,13). In response, a growing number of HIV clinics are implementing screening programs for anal cancer and its precursors modeled on procedures used in cervical cancer screening (14-18).

The 2009 European AIDS Clinical Society guide recommended anal screening using anal cytology in some HIV patients (19), and performing HRA in patients with altered anal cytology, to identify possible histological lesions. However, their implementation varies according to national recommendations for screening and financial resources. So, at the end of 2015, there were few programs for AC screening in our country, and the majority of them were in large cities. In Spain, the autonomy of the health care systems and the geographic distances may add logistical barriers, thus discouraging risk patients from accessing a screening program for anal lesions.

The argument that the prevalence of AC precursor lesions could be lower in medium-sized cities than in big cities with larger populations of HIV-MSM (20) favors the reduced availability of anal screening programs.

Vigo is a city with the largest population in the Autonomous Community of Galicia (300,000 inhabitants), and our center attends approximately 1,400 HIV-infected patients. Due to the increase in the incidence of non-AIDS-defining cancers, notably AC, in 2012 we decided to create a multidisciplinary team dedicated to screening AC and its precursor lesions. The team consisted of a doctor who specializes in HIV, a surgeon, a pathologist, a gynecologist and two nurses. To consider evidence which supports implementing such programs in Spain, it is critical to assess their

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feasibility in routine clinical practice. Therefore, we conducted this study to: a) describe our implementation of the screening program; and b) report the prevalence of HSIL (AIN-2/3) in an average-sized Spanish city.

METHODS

A cross-sectional study of HIV-MSM that were selected for a prevalence study of HPV infection and anal cytological alteration. We performed HRA to study the prevalence of AIN and AC in a sub-selection of patients. The study is part of a prospective observational study of diagnostic and therapeutic screening of precursor lesions of AC in HIV-MSM, attending the University Hospital Complex of Vigo from March 1st 2012 to March 31st 2015. The study was approved by the ethical Committee of Galicia (registration no. 2013/059 code) and the appropriate informed consent was obtained.

Prevalence study of cytological abnormalities and HR-HPV infection

We selected 264 HIV-MSM who met the following inclusion criteria: HIV-MSM, age > 22 years, and the absence of a previous history of AIN or invasive AC. The criteria for exclusion were: heterosexuality and female gender.

The selected patients received oral and written information, as well as the offer of simple anal screening and possible follow-up with complex anal screening (Fig. 1), using HRA for diagnosis, treatment and follow-up of high-grade histological lesions. The current couple was offered simple screening and participation in the study.

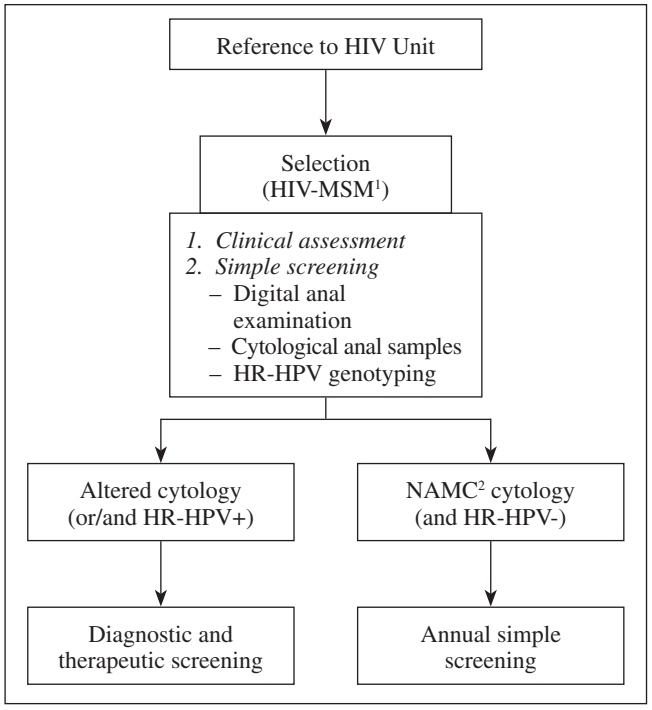

Fig. 1. First decision algorithm in patients referred for assessment of anal screening. [1]HIV-MSM: HIV-infected men who have sex with men. [2]NAMC: No atypical or malignant cells (negative cytology).

Simple screening included epidemiological, clinical data collection, digital ano-rectal examination, anal cell sample in liquid medium for cytological study, and HR-HPV genotyping.

Anal cytology samples were taken using the ThinPrep liquid means of transport (Hologic Inc., Bedford, USA). We used a Carl Zeiss 150FC (Carl Zeiss, Oberkochen, Germany) colposcope for HRA. HR-HPV genotyping was carried out by polymerase chain reaction amplification according to the Cobas 4800 HPV system test (Hoffmann - La Roche Ltd. Rotkreuz, Switzerland), with three TaqMan probes: two specific for the HPV 16 and HPV 18, and another for types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.

Cytological findings were interpreted using the terminology of the 2001 Bethesda system: no atypical or malignant cells (NAMC); atypical squamous cells of undetermined significance (ASCUS); low-grade squamous intraepithelial lesion (LSIL); high-grade squamous intraepithelial lesion (HSIL). The cytological and histological studies were performed by the same pathologist (JGCF). The cytological sample was considered as invalid if there were no anal squamous epithelial cells or there was predominance of fecal or hematic waste. The HR-HPV test was carried out using the same sample as for the cytological test.

Study of prevalence of AIN and AC

We selected 235 patients with cytological alterations and/or positive HR-HPV tests from the 264 patients. From this group we performed HRA in 209 patients. We analyzed the histological results with the different variables considered in the study (Table I). Study of the sensitivity and specificity of the cytology with respect to the histological result was performed in all patients. We specifically analyzed ≥ ASCUS cytology with respect to the histological result.

HRA was performed using a colposcope, whose powerful beam of light allows the magnified and sharp observation of the anal mucosa. In all cases we performed double impregnation, first with acetic acid (identifying acetowhite lesions), and secondly with Lugol's solution, identifying acetowhite lesions that do not uptake Lugol's iodine (Lugol-negative acetowhite lesions). These are the only cases where we recommend biopsy (22-24).

In order to describe anal findings, the anal canal was divided into four quadrants, where the anterior and posterior boundaries are the pubis and coccyx, respectively. This approach is already referred to in the literature (22). The HRA was carried out by the same operator (MID) using the technique of acetic acid and Lugol based on the description of UCSF (23,24).

We used the terminology recommendations from the Lower Anogenital Squamous Terminology Standardization Project (LAST Project 2012) (25). The recommended terminology is: anal intraepithelial neoplasia type 1, LSIL (AIN-1); anal intraepithelial neoplasia type 2, HSIL (AIN-2); and anal intraepithelial neoplasia type 3 HSIL (AIN-3). We considered the biopsy as being valid when it revealed the squamous epithelium and invalid when it revealed glandular epithelium. All histological specimens considered as HSIL (AIN-2) underwent immuno-staining for p16 protein.

If the result of the histological study was negative, LSIL (AIN-1) or HRA were negative (lack of suspicious Lugol negative lesions), HRA annual monitoring was indicated. In the case of discordant

Table I. Different variables analyzed in the study

Study variables (epidemiological, clinical, analytical, cytological, histological, endoscopical)	
	264 patients
Age, years: (mean ± SD)	43 ± 11.5
Nationality (Spanish and European): n (%)	235 (89%)
Tobacco use: n (%)	126 (47.8%)
Sexual partners last year: median (IQR)	2 (1-8)
Condom last year: n (%)	137 (51.9%)
Type of sexual relationship: n (%)	Passive: 39 (14.8%) Active: 26 (9.8%) Both: 199 (75.4%)
A history of STD: n (%)	Syphilis: 92 (35%) *Chlamydia:* 5 (2%) Gonorrhea: 30 (11.4%)
Hepatitis B: n (%)	VHB+: 92 (35%) HBAg+: 17 (6.5%)
Hepatitis C: n (%)	HCV: 13 (4.9%)
Time of HIV infection: median years (IQR)	7 (4-13)
Count CD4 (cell/µl) initial: (mean ± SD)	684, 6 ± 314
Nadir CD4 (cell/µl): (mean ± SD)	365, 3 ± 231
HIV stage: n (%)	A: 167 (63.3%) B: 50 (18.9%) C: 47 (17.8%)
Antiretroviral therapy (ART): n (%)	199 (75.4%)
Undetectable viral load: n (%)	208 (78.9%)
Detection of HPV: n (%)	226 (85.6%)
VPH16: n (%)	105 (40%)
VPH18: n (%)	44 (16.8%)
HR-HPV (not HPV-16 not HPV-18): n (%)	211 (79.8%)
Cytological results: n (%)	264 (100%)
NAMC	139 (52.4%)
ASCUS	52 (19.7%)
LSIL	58 (22.0%)
HSIL	14 (5.6%)
Results invalid	1 (0.4%)
Histological result (n, [%])	209 (79%)
Negative	123 (58.8%)
LSIL (AIN-1)	47 (22.5%)
HSIL (AIN-2)	24 (11.5%)
HSIL (AIN-3)	10 (4.8%)
Squamous AC	1 (0.5%)
Anoscopy findings	
Number of biopsies: median, range	1 (0-4)
Number affected quadrants: median, range	1 (0-4)

LSIL: Low-grade squamous intraepithelial lesion; HSIL: High-grade squamous intraepithelial lesion; LSIL (AIN-1): Low-grade squamous intraepithelial lesion (anal intraepithelial neoplasia type 1); HSIL (AIN-2): High-grade squamous intraepithelial lesion (anal intraepithelial neoplasia type 2); HSIL (AIN-3): High-grade squamous intraepithelial lesion (anal intraepithelial neoplasia type 3).

results (HSIL cytology without finding suspicious lesions in the HRA), a HRA was repeated at three months to rule out omitted diagnosis of HSIL (AIN-2/3).

If the histological lesion is a HSIL (AIN-2/3) we recommend its treatment by infrared electroablation or electrofulguration. Patients undergoing this procedure have a control HRA at three months, and then every six months thereafter (Fig. 2).

The ablative treatment is carried out using an infrared coagulator with a handle of 210 mm (Lumatec, Deisenhofen, Germany) and electrocoagulater (Valley Lab, Boulder, USA).

Statistical study

The variables considered in the study are summarized in table I. They were compiled from the medical records of each participant. Descriptive statistics are presented using the t-test, mean and standard deviation (SD) for continuous variables and the Kolmogorov-Smirnov test for non-parametric continuous variables. Comparison between quantitative variables with normal distribution was carried out using the ANOVA and Mann-Whitney test for non-parametric continuous variables. The qualitative variables are expressed in absolute frequencies and percentages. Comparison of proportions was performed with the χ-square test, with the correction for continuity of Yates, or Fisher's exact test when any of the values observed in the contingency table were less than five. The McNemar's test was used for comparison of proportions of paired data. The odds ratio (OR) and the confidence intervals of 95% (95% CI) were calculated by univariate analysis. The level of statistical significance of the various tests used was set at $p < 0.05$. The results were analyzed with the statistical program SPSS version 21 (SPSS Inc., Chicago, USA).

Sample size

We used the same version of the SPSS program. A sample size of the population of HIV-MSM in Vigo, with an error margin of 5% and a 95% confidence interval, would require a sample of 208 patients; but using a model of expected proportion of losses of 10%, it would need a sample of no less than 229 patients.

RESULTS

Clinical and epidemiological findings

We selected 264 HIV-MSM who lived in Vigo or its reference area for inclusion in our AC (Fig. 1) screening program. Of these, 89% were European and 11% were Latin American. The average age of the participants in the study was 43 years old (range: 22-79). The percentage of patients undergoing antiretroviral treatment (ART) was 75.4%, with an average of 59.4 months of treatment (range: 1-283), 78.9% of them with viral load < 50 copies/ml. The nadir of CD4 count and the current median was 348 (2-1,628) and 669 (21-1,805) cells/µl, respectively. The median number of different sexual partners in the year before screening was two (0-300 couples). Nearly half of

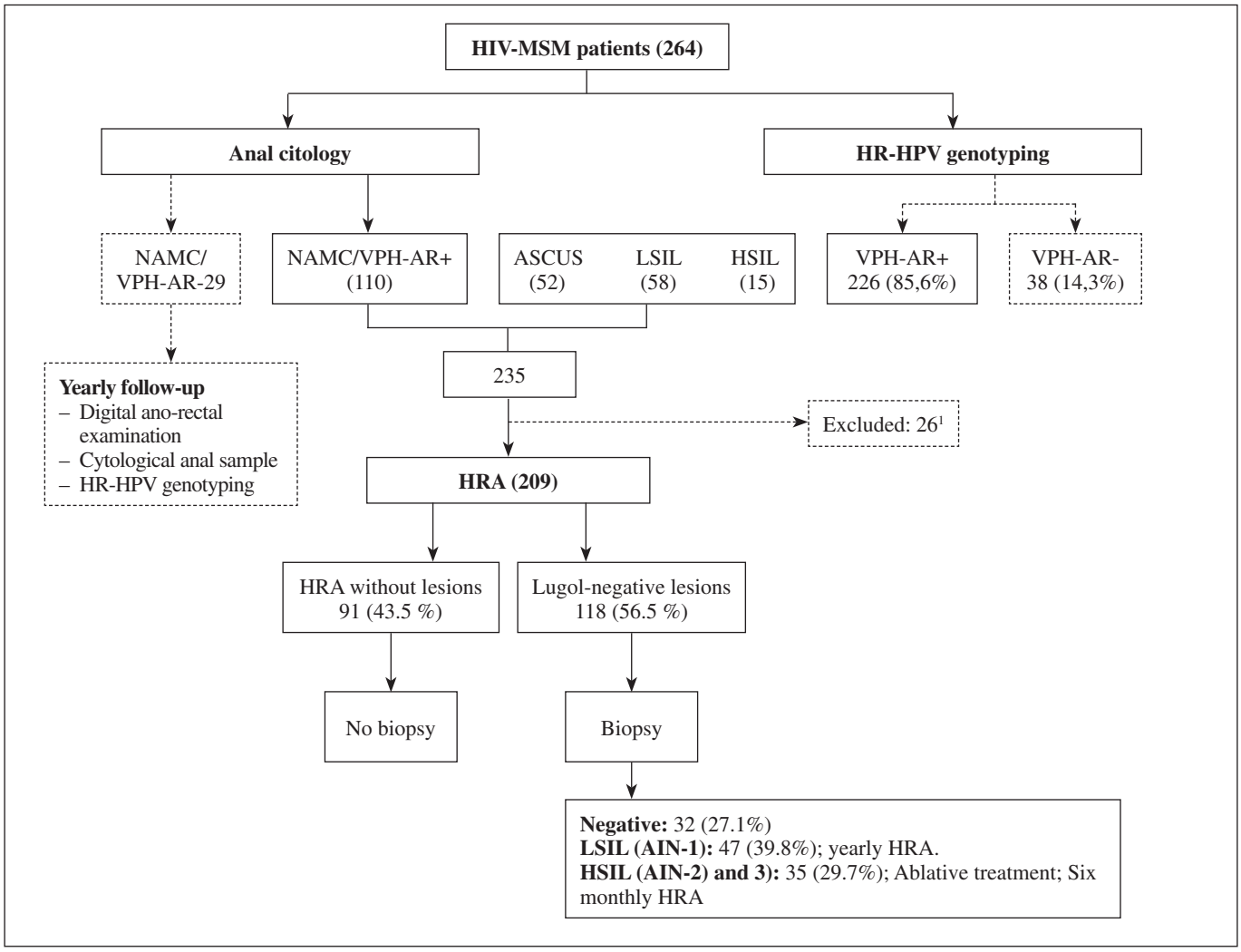

Fig. 2. Algorithm for screening and management of precursor lesions of AC in the Complejo Hospitalario Universitario de Vigo. NAMC: Not atypical or malignant cells (negative cytology); ASCUS: Atypia of squamous cells of undetermined significance; LSIL: Low-grade squamous intraepithelial lesion; HSIL: High-grade squamous intraepithelial lesion; LSIL (AIN-1): Anal intraepithelial neoplasia type 1; HSIL (AIN-2/3): Anal intraepithelial neoplasia type 2 and 3. [1]Change of residence: six; death: one; rejects study: four; HRA slope: 15. [2]Histology HSIL (AIN-2/3) includes one prevalent AC.

the patients (48.1%) reported not having used condoms in their sexual relations in the prior year.

History of syphilis was present in 34.9%, *Neisseria gonorrhoeae* in 11.4%, *Chlamydia* in 2%; history of hepatitis B in 35%, positive HBAg in 6.5%, and active chronic hepatitis C infection in 4.9% of the patients.

Virological findings

We detected HR-HPV in 85.6% of the cases, of which 109 (48.4%) had normal cytology results. HPV 16 was detected in 105 (40%) cases, HPV 18 in 44 (16.8%), and other types of HPV (neither HPV 16 nor HPV 18) in 210 (79.8%). The median number of couples throughout life in HPV infected patients was 57.5 *vs* 20 for HPV-negative, p = 0.048. We observed that 50.5% of the HPV-positive cases were active smokers *vs* 31% of the HPV-negative, p = 0.036.

There were no significant differences with regard to age, nationality, duration of HIV infection, HIV stage, condom use in the previous year, tobacco use, STD, current or nadir CD4 count, ART or type of treatment, time with viral load deleted, time with CD4 lymphocyte count above or below 500 or 200 respectively, and hepatitis C co-infection (Table I).

Cytological findings

The prevalence of cytologic alterations in the 264 HIV-MSM selected was 47.5%: ASCUS 52 (19.8%),

LSIL 58 (22.1%), HSIL 15 (5.7%), NAMC cytology 138 (52.5%), one invalid sample. The presence of cytological alterations was associated with the following variables:

Younger age (average age 38 *vs* 46 years) p < 0.001; HR-HPV+, p = 0.01; infection only by HPV 16 and/or HPV 18, p < 0.001; non-use of condom in the past year (53.6% of the 125 that did not use condom *vs* 41% of the 134 who used it), p = 0.043; suspicious digital ano-rectal examination (presence of cytological alterations and HSIL, p = 0.017 and p = 0.008, respectively). We only saw an inverse tendency between cytological alterations and a CD4 count lower than 200 CD4/µl, p = 0.098. In contrast, the median and mean time with undetectable viral load was associated with a smaller number of cytological alterations: 11 and 30 months without cytological alterations *vs* 0 and 20 months with cytological alterations, respectively.

When comparing patients with basal HSIL and not HSIL cytology, we saw a greater percentage of HSIL in patients with a history of hepatitis C, 3/15 (20%) *vs* 9/230 (3.9%), p = 0.005. When analyzing the numerical variable, we only found statistically significant differences with the time of the CD4 count < 200 CD4 (median and mean of 6 and 25 *vs* 1.9 and 25 months, respectively), p = 0. 01, and with the maximum historical count of the load viral (median of 3,000 *vs* 100,000 copies/ml), p = 0.038.

Histological findings

HRA was performed in 209 patients with any baseline anal cytology abnormalities or positive HR-HPV genotyping. One hundred and twenty three patients (58.8%) had no suspicious lesions at HRA (91-43.5%), or negative biopsy results (32-15.3%). Biopsies demonstrated altered histology in 39.8% (LSIL [AIN-1] 47 [22.5%], HSIL [AIN-2/3] 34 [15.8%], and invasive AC 1 [0.5%]) (Table II). If we only include VPH-AR+ patients with altered cytology, this prevalence increases to 27.8%. There were four (1.9%) invalid biopsies. The median number of biopsies at HRA was 1 (range 0-4). We did not perform blind biopsies. Patients with two or more anal quadrants involved had a higher proportion of HSIL (AIN 2-3) lesions than those with only one quadrant involved (37% *vs* 17.6%, p = 0.014). Specifically, for the 33 patients with HSIL biopsy results, the number of quadrants involved in the anal canal were one (39.4%), two (45.5%), three (3%), and four (12.1%).

Patients with altered histology were younger (40 ± 10.9 *vs* 44 ± 11.8 years, p = 0.020). Patients with HSIL (AIN-2/3) presented a longer time with CD4 lymphocytes < 200/ul than patients with HSIL (AIN-2/3) (mean time of 14 months [IC 95%: 0.6-27.1] *vs* 1.3 months [IC 95%: 0.2-2.5], p = 0.024). On comparing patients with AIN2-3 histology *vs* not AIN 2/3, we saw significant differences in patients with hepatitis C (OR: 10.9; 95% CI: 2.6-46.1). Patients with HSIL cytology were more likely to have an altered biopsy (OR: 6; 95% CI: 1.7-16) and HSIL (AIN-2/3) (OR: 11.4; 95% CI: 3.6-36.8). This finding was similar to the altered *vs* not altered cytology (OR: 8.7; 95% CI: 4.4-7.3) and altered cytology *vs* HSIL (AIN2-3) histology (OR: 12; 95% CI: 3.5-40.7). Regarding HPV, only the detection of HPV 16 showed this association (OR: 1.96; 95% CI: 1.1-3.5 and OR: 2.41; 95% CI: 1.1-5.1, respectively). There were no significant differences between the other variables included in the study.

On using anal biopsy as a reference standard, the sensitivity and specificity of altered cytology ≥ ASCUS with

Table II. Basal cytological findings compared with histology

Histology	*Cytological findings*				
	ASCUS (n/%)	*LSIL (n/%)*	*HSIL (n/%)*	*NAMC (n/%)*	*TOTAL (n/%)*
LSIL (AIN-1) (% of total)	12 5.7%	22 10.5%	2 1.0%	11 5.3%	47 (22.5%)
HSIL (AIN-2/3) (% of total)	11 5.2%	12 5.7%	8[1] 3.8%	3 1.5%	34 (16.3%)[2]
Anal cancer (% of total)	0 0.0%	0 0.0%	1 0.5%	0 0.0%	1 (0.5%)
Negative biopsy or not indicated[3]	23 11.0%	18 8.6%	3 1.4%	79 37.8%	123 (58.8%)
Invalid (% del total)	2 1.0%	1 0.5%	0 0.0%	1 0.5%	4 (1.9%)
Total (% del total)	48 23.0%	53 25.4%	14 6.7%	94 45.0%	209 (100%)[1]

[1]HRA was performed in all patients with cytology of any type and/or HR-HPV infection. [2]Prevalence increases to 16.8% if we include one prevalent AC. [3]Biopsy negative or not indicated (HRA suspicious findings). NAMC: Not atypical or malignant cells (negative cytology); ASCUS: Atypia of squamous cells of undetermined significance; LSIL: Low-grade squamous intraepithelial lesion; HSIL: High-grade squamous intraepithelial lesion; LSIL (AIN-1): Anal intraepithelial neoplasia type 1; HSIL (AIN-2/3): Anal intraepithelial neoplasia type 2 and 3.

respect to histology were 61% and 85% (PPV: 83%, NPV: 64%), respectively (Table III). The concordance between our cytological and histological findings for HSIL was 64.3%. The proportion of patients with HSIL histological lesions associated with cytologic NAMC, ASCUS and LSIL findings was 1.5%, 5.2% and 5.7%, respectively. P16 in the HSIL (AIN-2) was positive in 77.3% of the cases.

DISCUSSION

Our experience implementing a screening program for AC and its precursor lesions in an average-sized Spanish city shows: a) a high prevalence of anal HPV infection in our population of HIV-MSM; b) significant numbers of patients with HSIL (AIN-2/3); and c) acceptable diagnostic operation with the methodology used.

The prevalence of HPV in HIV-MSM observed in our study was high (85.6%). This was similar to reports from North America (1,2,5) and the Spanish multicenter group CoRIS-HPV (85.6%) (26). Most of our patients (65.3%) had multiple HR-HPV infections, with a similar frequency to the CoRIS-HPV group (63.7%). The association of HR-HPV infection with some habits (tobacco addiction) and promiscuity (greater number of partners throughout life) highlights the value of preventive measures and sexual education in the general and risk populations. The effect of tobacco has been studied in ano-genital cancer and, although its oncogenic mechanism is unknown, it has been linked with an increased incidence of infection and cancer in this location (27). On the other hand, repeated exposure to HR-HPV favors reinfection by the virus. This is more frequent when the immunosuppression is not so advanced, and there is even the ability to spontaneously clear HPV, prior to establishing chronic HPV infection. This would explain why only three (1.5%) of our patients with NAMC cytology and concurrent HR-HPV infection presented a histological HSIL lesion, illustrating the limited positive predictive value that the presence of HPV has for identifying HSIL lesions. This has already been reported in the literature (25).

The global distribution of cytologic results is similar to other cohorts of HIV-MSM, with 5.6% HSIL (Table II) and 19.7% ASCUS (48% of these associated with atypical histology). The discordancy between cytology and histology has been referred to by many authors in different locations and by different anatomical pathologists and surgeons, as well as the recognized inter-observer variability of anal cytology (22,28-30). However, Cachay et al. state that anal and cervical cytology may have a similar use (15). The diagnosis of HSIL (AIN-2) was apparently correct, if we consider the high percentage of p16+ detected in these lesions, although it is the follow-up study that confirms that its behavior is similar to HSIL (AIN-3).

There is an effort to increase the implementation of screening programs for AC in different countries, albeit with considerable variations in the methods used. Thus, some groups use anal cytology as a benchmark for the realization of HRA, and others do not (31,32). This highlights the need for objective parameters that ensure the quality of the HRA. We believe that the global implementation of our program for AC screening was acceptable. This was demonstrated by the low number of patients with invalid results from anal cytology (0.5%), as well as the discordant cytological and histological findings (HSIL cytology and absence of histological abnormalities), three (1.4%) (33). Our rate of identification of high-grade histological lesions was similar to that of some other relevant groups (34) and two important studies performed in our country (35,36), the first of which was carried out in one of the largest cities in Spain. The concordance between HSIL cytology and the histological HSIL result has been high, denoting the diagnostic value of this cytological grade (22,37,38). Regarding analysis of sensitivity and specificity (Table III), altered cytology presents a low sensitivity (64%) and a higher specificity (85%). We partially relate this with the variability of results and the systematic use of double impregnation with acetic acid and Lugol's solu-

Table III. Sensitivity and specificity for the presence of HR-HPV and altered cytology compared with altered histological findings

	Chi-squared	*OR*	*IC (95%)*	*Sensitivity OR (%) (95% CI)*	*Specificity OR (%) (95% CI)*	*VPP (%)*	*VPN (%)*
HR-HPV-positive *vs* altered histology[1]	0.78	0.826	(0.2-3.17)	40%	56%	95%	4%
VHP16 *vs* altered histology[1]	0.019	1.96	(1.1-3.5)	49%	67%	55%	62%
VHP16 *vs* histology HSIL (AIN2-3)[2]	0.019	2.4	(1.1-5.1)	24%	88%	63%	59%
Altered cytology *vs* altered histology[1]	0.000001	8.7	(4.4-17.2)	61 %	85%	83%	64%
Altered cytology[3] *vs* histology HSIL (AIN2-3)[2]	0.000001	12	(3.5-40.7)	29%	97%	91%	53%
HSIL cytology *vs* altered histology[1]	0.002	6.2	(1.6-23.0)	79%	63%	13%	98%
HSIL cytology *vs* HSIL (AIN-2/3) histology[2]	0.000001	11.4	(3.5-36. 7)	64%	86%	26%	97%

[1]Altered histology: LSIL (AIN-1), HSIL (AIN-2), HSIL (AIN-3). [2]Histology HSIL (AIN-2-3) includes one prevalent AC. [3]Altered cytology: includes ASCUS, LSIL, HSIL.

tion, an aspect already referred to by other authors (39). Altered cytology *vs* HSIL histology, and HSIL cytology *vs* HSIL histology have a greater CI, therefore with a lower probability of error but less precision. The number of quadrants with suspicious lesions for HRA is associated with a greater number of biopsies performed and a higher rate of HSIL (AIN-2/3). Clinically, we have been able to diagnose prevalent invasive AC in its early stages, which has allowed rapid treatment.

The main limitation of our study is its focus on HIV-MSM; it does not include other individuals with known risk. Our study in a particular group of patients (HIV-MSM) does not allow us to extrapolate the results to other risk groups, for example, to compare with MSM-HIV-negative, HIV patients not MSM, or women with CIN-2/3, although in our practice we are gradually incorporating them to the screening program. Until the end of the follow-up study we will not have a structured assessment of tolerability of procedures (pain and bleeding post-procedures), but loss of patients after being included in the screening program is low, which indirectly validates the acceptability of HRA. HRA is not a difficult technique, but it requires training and continuous improvement (40,41).

Our findings question the preconception that the risk of HR-HPV infection and AC is limited to larger cities.

In short, we found a high prevalence of anal infection by HR-HPV and AIN (2/3) in our HIV-MSM. Implementing a screening program is possible for multidisciplinary management. The data provided can contribute to the information for those responsible for health strategies related to AC screening and their precursors in average-sized communities with populations such as ours.

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