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Routine Screening of Anal Cytology in Persons With Human Immunodeficiency Virus and the Impact on Invasive Anal Cancer: A Prospective Cohort Study

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Background. The efficacy of screening programs to prevent anal cancer in persons with human immunodeficiency virus 1 (HIV-1) is unclear.

Methods. To examine the impact of a screening program to detect anal cancer precursors on the incidence of cases of invasive anal squamous-cell carcinoma (IASCC) in persons with HIV-1, we performed a single-center, retrospective analysis of a prospective cohort of outpatients with HIV-1 attending a reference HIV unit from January 2005 onward. All participants were invited to participate in a continued structured screening program for anal cancer prevention. We estimated the incidence of IASCC and performed a comparative analysis between subjects enrolled in the screening program (screening group) and those who declined to participate (nonscreening group). To reduce any selection bias, a propensity score analysis was applied.

Results. We included 3111 persons with HIV-1 (1596 men-who-have-sex-with-men [MSM], 888 men-who-have-sex-with-women [MSW], 627 women; mean age, 41 years), with a median follow-up of 4.7 years (14 595 patient-years of follow-up); 1691 (54%) participated in the screening program. Ten patients were diagnosed with IASCC: 2 (MSM) in the screening group and 8 (4 MSM, 2 MSW, and 2 women) in the nonscreening group. The incidence rates of IASCC were 21.9 (95% confidence interval [CI], 2.7–70.3) and 107.0 (95% CI, 46.2–202.0) per 100 000 person-years, respectively. After a propensity score adjustment, the difference was significant in favor of the screening group (hazard ratio, 0.17; 95% CI, .03–.86).

Conclusions. The number of cases of IASCC was significantly lower in persons with HIV engaged in an anal cytology screening program. These results should be validated in a randomized clinical trial.

Keywords. people living with HIV (PLW HIV); anal cancer; anal screening program; anal cytology; human papilloma virus.

Cancer remains an important cause of morbidity and mortality in people living with human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) (PLHA) [1]. Coinfection with oncogenic viruses (eg, human papillomavirus [HPV]) increases the risk of some types of cancers, such as invasive anal squamous-cell carcinoma (IASCC) [2–4]. This is especially true in men with HIV-1 who have sex with men (MSM), who have an estimated incidence rate of 131 (95% confidence interval [CI], 109–157) per 100 000 person-years [5], while the incidence rates of anal cancer in men with HIV-1 who have sex with women (MSW) and in women with HIV-1 are 46 (95% CI, 25–77) and 30 (95% CI, 17–50) per 100 000 person-years, respectively [5]. These incidence rates of IASCC in PLHA are much higher than those seen in the general population (<2 new cases per 100 000 person-years) [6–15].

Although there are substantial differences between the natural histories of anal and cervical HPV infection [16], including slower anal HPV clearance in men and persisting rates of anal HPV infection and dysplasia with age, it is believed that, as with cervical cancer, IASCC could potentially be prevented through screening programs. For anal cancer, screening by means of anal cytology with follow-up high-resolution anoscopy (HRA) when needed should facilitate the detection and subsequent treatment of high-grade squamous intraepithelial lesions (HSILs). However, there is as yet no full consensus on the best strategy for detecting HSILs in such screening programs. A US National Cancer Institute–sponsored multicenter randomized trial (Anal Cancer HSIL Outcomes Research [ANCHOR] Study) is expected to shed light on whether these screening programs

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prevent the development of IASCC [17]. However, definitive results are not expected until 2022. Although the approach is not uncontroversial, it has also been suggested that screening programs for anal cancer prevention should be implemented in HIV-negative women with previous cervical HPV-related disease [18], immune-suppressed transplant recipients, and all PLHA [19, 20].

In 2005, our HIV unit implemented a continued screening program for anal cancer prevention, based on cytological detection of HPV-related abnormalities at yearly check-ups, followed by histological confirmation of the presumed cancer-precursor lesion and treatment of HSILs [21]. Our hypothesis was that such a screening program would reduce the incidence of IASCC in PLHA. After 12 years of operation, it was decided to gather data that might tentatively confirm or refute the initial hypothesis. We therefore examined the incidence of IASCC among those that had passed through a structured screening program and compared it with those who had not. The results of this exploratory analysis would endorse whether it was worth continuing the program or not.

METHODS

Study Design

This was a single-center, retrospective analysis of a prospective cohort of PLHA who attended the reference HIV unit of the Hospital Germans Trias i Pujol, Badalona, Catalonia, Spain, a public university teaching hospital. The study was carried out in accordance with the stipulations of the Declaration of Helsinki and the protocol was approved by the local institutional review board. All patients gave written informed consent for their medical information to be used for purposes of scientific research. The study covers the first 12 years of our Clinical Proctology HIV Section between 1 January 2005 and 31 December 2016.

Study Population

The study included all adult (age ≥18 years) PLHA who completed at least 1 year of follow-up, including routine check-ups for their HIV infection. Although all participants had been invited to take part in the screening program, not all did so. Therefore, this study included both PLHA who agreed to participate in the screening program as well as those who refused.

The following baseline data were gathered for all PLHA included in this study: date of birth, date of HIV diagnosis, HIV-1 plasma RNA, $CD4^+$ T-cell count, and nadir $CD4^+$ counts (the lowest CD4 value of each subject). Further data were gathered specifically for the group who participated in the screening program: anal cytology results (normal, atypical squamous cells of unknown significance [ASCUS], low-grade squamous intraepithelial lesions [LSILs], or HSILs using the Papanicolaou test) and anal histology results (normal, anal intraepithelial neoplasia [AIN; low grade: AIN-1; high grade: AIN-2/3]) from directed biopsies using HRA.

The follow-up period was defined as the time between baseline (first visit after 1 January 2005) and the last visit to the HIV unit.

Protocol of Screening Program for Anal Cancer Prevention

The screening program was drawn up in 2004 and is shown in Figure 1. When this preventive protocol was designed, our goal was to cover the maximum number of PLHA who attended our HIV unit.

At the baseline visit a clinical examination was carried out, including a digital rectal examination and collection of a sample from the anal canal for cytological examination. If the result was normal, the participant was visited again on a yearly basis. However, if the cytology result was abnormal (ASCUS, LSILs, or HSILs), an HRA was carried out within the next 3 months. If lesions were not visualized on HRA, no biopsy was done, and a new cytology was scheduled for 6–12 months later. If lesions were visualized with HRA, a directed biopsy was performed. If this was normal or AIN-1, a new visit including cytology was scheduled 6 months later. If the result was AIN2/AIN3, the subject was treated with infrared coagulation or surgery as soon as possible. After treatment, the subject was visited (including cytological examination) again after 3 to 6 months. If the cytology was normal, the patient was visited again at 6–12 months, including a new digital rectal examination; however, if it was abnormal (ASCUS, LSILs, or HSILs) a new HRA was performed as soon as possible. Any subject with anal symptoms during follow-up was referred to the proctology section and a complete examination was done.

Anal Canal Cytological Procedure

An anal canal sample for cytological examination was obtained by introducing a cytobrush (Eurogine SL, Spain) 3 cm into the anal canal and gently rotating for 30–45 seconds. The cytobrush was introduced into 20 mL of PreservCyt/ThinPrep Pap test solution (Cytyc Iberia SL, Spain) and shaken for 30 seconds. Cytological changes were classified according to the Bethesda System. Generally, samples were independently assessed by 2 expert cytopathologists.

High-resolution Anoscopy Procedure

High-resolution anoscopy was performed as described elsewhere [22]. Anoscopies were performed according to the Consensus Recommendations of the International Guidelines for Practice Standards in the detection of Anal Cancer Precursors [23]. One of 3 anoscopists (BR, MP, FG-C) performed the HRA and took the biopsy samples. Histological changes were classified as AIN-1, AIN-2, or AIN-3. Again, samples were assessed by 2 expert pathologists.

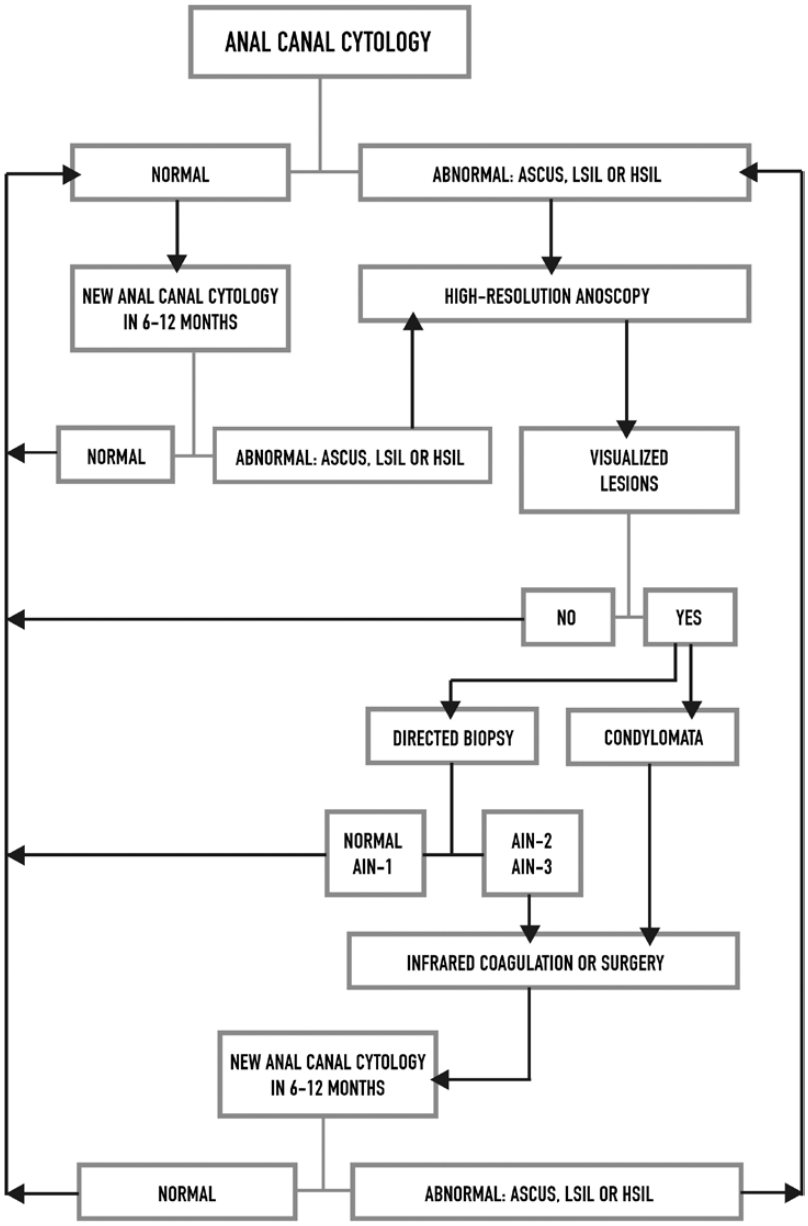

Figure 1. Flow chart of the screening program for anal cancer prevention in people with human immunodeficiency virus infection. Abbreviations: AIN, anal intraepithelial neoplasia; ASCUS, atypical squamous cells of unknown significance; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.

If anal canal condylomata were diagnosed during the clinical examination, either by digital rectal examination or HRA or histological findings, the subject was treated by infrared coagulation or surgery.

Incidence of Invasive Anal Squamous-cell Carcinoma

In order to identify all the participants diagnosed with IASCC over the 12 study years, searches were carried out through 3 different electronic medical database from our hospital: (1) the HIV unit medical files, (2) the general medical files (which include data from the surgery and oncology departments), and (3) the specific cancer diagnosis files of the pathology department. The results of these 3 searches were cross-checked and linked.

Statistical Analysis

Sample Size

Due to the exploratory nature of our aim, no formal calculation of sample size was performed. The final sample size was defined simply as the number of PLHA from our HIV unit who fulfilled the inclusion criteria.

Statistical Procedures

The incidence of IASCC was determined using the Kaplan-Meier method. Incidence rates were also calculated based on a person-time denominator (100 000 person-years). Bivariate and multivariate Cox proportional hazard regression models were performed, when appropriate, to determine potential

factors associated with the incidence of IASCC, and hazard ratios (HRs) for incidence rates were estimated.

To minimize the selection bias effect of potential confounders, propensity scores were generated by using multiple logistic regression to estimate the probability of whether or not the subjects were enrolled in the screening program. The covariates entered into the propensity score were sexual behavior, $CD4^+$ nadir cell count, $CD4^+$ count at baseline visit, HIV-1 plasma RNA, and time of diagnosis of HIV infection. Cox regression to compare HRs between enrolled and nonenrolled cohorts was repeated adjusting for propensity score. The proportionality of risks in the Cox models was verified using Schoenfeld residuals.

Data analysis was carried out using the R statistical programming environment (version 3.5, R Core Team, 2015).

RESULTS

Patient Characteristics

A total of 3111 PLHA (1596 MSM, 888 MSW, and 627 women) fulfilled the inclusion criteria. Of them, 1691 (54%) were enrolled and followed up in the screening group (1095 MSM, 257 MSW, and 339 women). The median length of follow-up was 4.6 years within the screening group and 4.8 years in the nonscreening group. Therefore, the study analyzed 7779 and 6816 patient-years of follow-up, respectively.

Figure 2 shows the study flow chart. It is noteworthy that an additional 492 (16%) subjects were enrolled in the screening program but dropped out after the baseline visit. These subjects therefore failed to meet the inclusion criterion of having been in the screening program for at least 6 months and were added to the nonscreening program, yielding a total of 1420 participants (501 MSM, 631 MSW, and 288 women) in this group. Table 1 shows the baseline characteristics. There were significant differences between groups for the percentage of MSW and MSM, $CD4^+$ cell count, $CD4^+$ nadir count, and percentage with suppressed plasma HIV-1 RNA.

Figure 3 depicts the results of anal cytology screenings performed at baseline from 2183 participants (1691 subjects enrolled in the screening program and 492 with only data from the baseline visit). At baseline, 965 (44%) subjects had a normal anal cytology and 144 (7%) had an HSIL. During follow-up, 49 (17%) subjects developed an HSIL and 151 (54%) an LSIL. We performed 1288 HRAs and obtained 744 biopsies, with 104

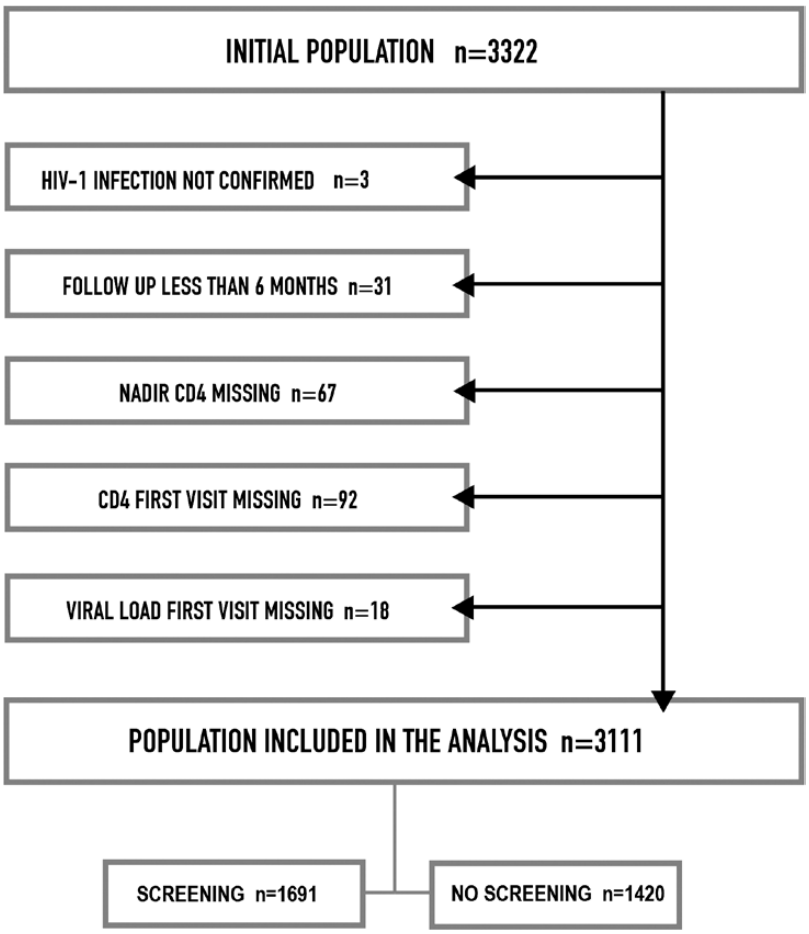

Figure 2. Study flow chart. Abbreviation: HIV-1, human immunodeficiency virus 1.

Table 1. Baseline Characteristics of the Study Population

Baseline Characteristics	Enrolled in the Screening Program (n = 1691)	Not Enrolled in the Screening Program (n = 1420)	*P* Value
Mean (SD) age, years	41.5 (9.7)	41.6 (8.9)	.366
Gender, n (%)			
Women	339 (20.1)	288 (20.3)	.507
MSW	257 (15.2)	631 (44.4)	<.001
MSM	1095 (64,8)	501 (35.3)	<.001
Median (IQR) time of known HIV infection, years	7.9 (0.9–16.1)	10.1 (3.4–15.2)	.134
Mean (SD) $CD4^+$ cells/μL at baseline	572 (283)	443 (403)	<.001
Mean (SD) $CD4^+$ nadir cells/μL	276 (179)	215 (167)	<.001
$CD4^+$ nadir: <200 cells/μL, n (%)	611 (36)	728 (51)	<.001
HIV-1 plasma RNA: <50 copies/mL, n (%)	1020 (60)	656 (46)	<.001

Abbreviations: HIV, human immunodeficiency virus; IQR, interquartile range; MSM, men who have sex with men; MSW, men who have sex with women; SD, standard deviation.

cases of AIN-2 and 37 cases of AIN-3. Table 2 presents the cytological findings stratified for women, MSW, and MSM. There was a higher rate of subjects with LSILs in MSM than in MSW or women (37% vs 12% and 16%, respectively; $P < .001$).

Incidence of Invasive Anal Squamous-cell Carcinoma

Invasive anal squamous-cell carcinoma was diagnosed in a total of 10 subjects in the 12 years covered by the study, of whom 2 (both MSM) had participated in the screening program and 8 (4 MSM, 2 MSW, 2 women) had not (Table 3). All of them had a nadir $CD4^+$ cell count of less than 150 cells. However, at the time of IASCC diagnosis half of them had 350 or more CD4 cells.

The cumulative incidence of IASCC was 0.1% (95% CI, .03–.4%) for the screening group and 0.6% (95% CI, .3–1.1%) for the nonscreening group (chi-square test, $P = .051$). Curves of time to anal cancer diagnosis are shown in Figure 4. The incidence rate of IASCC was lower in the screening group (21.9; 95% CI, 2.7–70.3 per 100 000 person/years) versus the nonscreening group (107.0; 95% CI, 46.2–202.0) (log-rank test, $P = .027$). The Cox regression model showed a statistically significant protective effect of being enrolled in the screening program (HR, 0.20; 95% CI, .04–.97). In other words, not being enrolled in the screening program was a risk factor for IASCC development (HR, 4.84; 95% CI, 1.03–22.82). The only risk factor associated with IASCC in multivariate analysis was "time from known HIV infection" (the longer the time, the greater the risk; HR, 1.14; 95% CI, 1.02–1.26). Sexual behavior, $CD4^+$ nadir cell count, baseline $CD4^+$ count, or HIV-1 plasma RNA were not identified as risk factors.

We initially utilized a multiple logistic regression to estimate the probability of being enrolled or not in the screening program. Propensity score means were 0.70 (standard deviation [SD], ±0.18) in the screening group and 0.48 (SD, ±0.23) in the nonscreening group (Supplementary Figure 1). Propensity score matching left out of the analysis 1059 controls and 109 patients and did not properly adjust the variables. Standardized mean differences by baseline CD4 and age remained above 0.1 (Supplementary Figure 2) and therefore adjustment by propensity scoring for these 2 variables was not satisfactory.

After adjusting for propensity score, the Cox model also yielded a significantly protective effect in favor of being enrolled in the screening program (HR, 0.17; 95% CI, .03–.86).

In the screening group, the cumulative incidence of IASCC in MSM was 0.2% (2 out of 1095; 95% CI, .1–.7%), with no cases among MSW (0%; 0 out of 257; 95% CI, .0–1.5%) or women (0%; 0 out of 339; 95% CI, 0.0–1.1). The incidence rates were 34 (95% CI, 4–108), 0 (95% CI, 0–186), and 0 (95% CI, 0–137) per 100 000 person-years, respectively. In the nonscreening group, the cumulative incidence of IASCC was 0.8% in MSM (4 out of 501; 95% CI, .3–2.0%), 0.3% in MSW (2 out of 631; 95% CI, .1–1.2%), and 0.7% in women (2 out of 288; 95% CI, .2–2.5%). The corresponding incidence rates were 159 (95% CI, 43–379), 58 (95% CI, 7–186), and 132 (95% CI, 6–422) per 100 000 person-years, respectively.

In the screening group, 2 subjects with IASCC have survived. In the nonscreening group, 5 out of 8 subjects with IASCC have died.

DISCUSSION

In a prospective cohort including 14 595 patient-years of follow-up, PLHA enrolled in a structured anal screening program had a significantly lower rate of IASCC in a multiple logistic regression analysis adjusted for propensity score. To the best of our knowledge this is the first cohort analysis that demonstrates an impact on the incidence of IASCC of such a screening strategy. On the other hand, although among persons with HIV-1 the greater risk of IASCC (6 out of 10 IASCC cases) was in MSM, MSW and women must also be taken into account given that these 2 groups present a higher risk of IASCC in comparison to the general population [5].

In the absence of fully powered randomized clinical trials that conclusively establish a cause–effect relationship between enrollment in a screening program and reduced incidence of IASCC, data from prospective cohorts with multivariable-adjusted analyses offer the highest level of evidence available. These cohort data are currently lacking, and thus universal anal screening programs in PLHA were only based on expert consensus recommendations because of the similarities between cervical and anal cancer with HPV infection [24, 25]. The present analysis was only intended to gather data that would allow us to make a reasonably well-informed decision about whether or not to discontinue the screening program. The ANCHOR study is an ongoing National Cancer Institute–sponsored randomized trial aimed at determining whether anal screening prevents the development

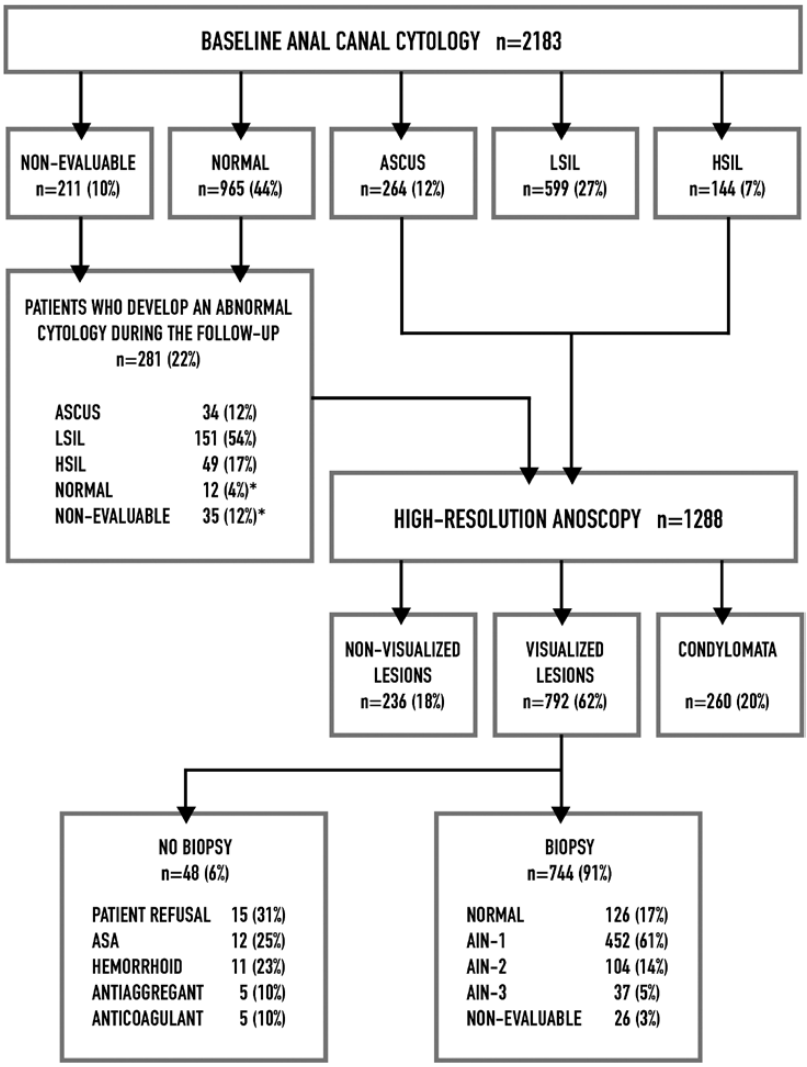

Figure 3. Results of anal canal cytologies, HRA, and directed biopsies with their histological diagnoses performed according to the flow chart of the screening program for anal cancer detection. Abbreviations: AIN, anal intraepithelial neoplasia; ASA, acetylsalicylic acid; ASCUS, atypical squamous cells of unknown significance; HRA, high-resolution anoscopy; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.

of IASCC, as compared with monitoring alone. Its results, expected by 2022, will put into context this issue.

There is no real consensus on the optimal design for a screening program to prevent anal cancer, with a variety of approaches currently in use. The approach taken in the present study is based on cytological detection of HPV-related abnormalities at each check-up, followed by histological confirmation of HSIL, and then treatment. Other more aggressive screening programs are based on histology as a first step and at each subsequent check-up (that is, both HRA and biopsy performed at

Table 2. Anal Canal Cytological Findings of Persons With HIV-1 (Women, Men Who Have Sex With Women, and Men Who Have Sex With Men) at First Visit of the Screening Program

		Cytology, n (%)				
	n (%)	Normal	ASCUS	LSIL	HSIL	Unsatisfactory
Women	514 (23)	280 (54)	61 (12)	81 (16)	37 (7)	55 (11)
MSW	369 (17)	218 (59)	47 (13)	43 (12)	13 (4)	48 (13)
MSM	1300 (60)	467 (36)	156 (12)	475 (37)	94 (7)	108 (8)
Total	2183	965 (44)	264 (12)	599 (27)	144 (7)	211 (10)

N = 2183.

Abbreviations: ASCUS, atypical squamous cells of unknown significance; HIV-1, human immunodeficiency virus 1; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; MSM, men who have sex with men; MSW, men who have sex with women.

Table 3. Characteristics of Persons With Human Immunodeficiency Virus-1 Participating in the Study Diagnosed With Invasive Anal Squamous-cell Carcinoma

	Enrolled in the Screening Program (n = 2)		Not Enrolled in the Screening Program (n = 8)							
Age at IASCC, years	48	50	41	43	46	51	58	46	41	50
Symptoms at IASCC diagnosis	Hemorrhoids, anal pain	Anorectal mass	Anal Pain	No data	Anal pain	Anal pain, rectal bleeding	Rectal bleeding	Anal pain, rectal bleeding	No data	Anal pain
Length of follow-up in the cohort at cancer diagnosis, years	8.1	4.5	1.4	3.4	4.2	4.5	2.1	7.0	2.3	8.4
TNM stage	T1-2NxM0	T2N1M0	T2NxM0	T2NxM0	T2N2M0	T4N2M0	T2NxM0	T3N0M0	T2-3NxM0	T2N0M0
Sexual practice	MSM	MSM	Woman, HTSX	Woman, HTSX	MSW	MSW	MSM	MSM	MSM	MSM
Time with HIV, years	24	27	16	20	15	17	7	25	14	29
CD4 nadir, cells/μL	17	137	21	11	44	6	No data	115	41	109
CD4 at IASCC, cells/μL	317	806	107	No data	44	10	1418	366	349	555
HIV-RNA at IASCC, copies/mL	84	<40	<40	No data	1400	<40	<40	<40	140	<40
Basal anal cytology (year)	Normal	Normal	Not done	Not done	Not done	HSIL	Not done	Not done	Not done	Not done
Anal cytologies performed,[a] n	9	4	0	0	0	0	0	0	0	0
Worst cytological diagnosis and HRA result	LSIL, normal	ASCUS, normal	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done
HPV genotypes at cytology sample	16, 33, 39	16, 59	Not done	Not done	Not done	Not done	Not done	Not done	Not done	Not done
At biopsy sample	Not done	Not done	39	Not done	Not done	Not done	16, 18, 56	Not done	Not done	Not done
Life status, final	Alive	Alive	Dead	Alive	Dead	Dead	Dead	Alive	Alive	Dead

Abbreviations: HIV, human immunodeficiency virus; HPV, human papillomavirus; HRA, high-resolution anoscopy; HTSX, heterosexual; IASCC, invasive anal squamous-cell carcinoma; MSM, men who have sex with men; MSW, men who have sex with women; TNM, tumor-node-metastasis.

[a]Number of anal canal cytologies performed between baseline and IASCC diagnosis.

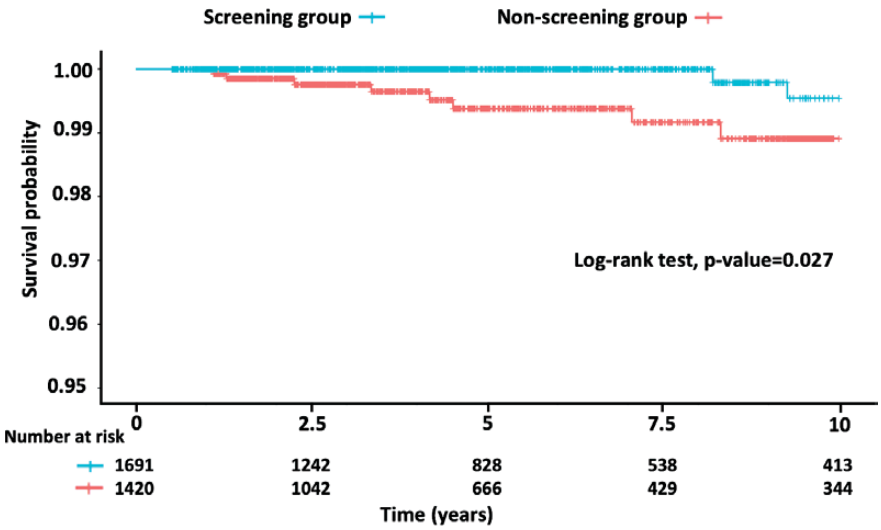

Figure 4. Actuarial probability (Kaplan–Meier curve) of remaining free of invasive anal squamous cell carcinoma for the 2 groups (enrolled in screening program, not enrolled in screening program) of people with human immunodeficiency virus infection who participated in the study.

each check-up). At present, diagnosis is dependent on histology, which generally hinges on HRA with biopsy [16]. Cytological results have been shown to have limited sensitivity in detecting histologically proven high-grade AIN [26] and the prevalence of high-grade AIN is strikingly lower when based on a cytological diagnosis than when based on histology [16]. Therefore, our findings may reflect an underdiagnosis of high-grade AIN. Nonetheless, despite this possible drawback, those patients who were enrolled in our screening program experienced a lower incidence of IASCC.

Among PLHA who did not engage in our screening program, the incidence rates for MSM (159 cases per 100 000 person-years) were similar to those previously reported by others (131 per 100 000 person-years), suggesting that this group really constitutes a correct control arm. With respect to MSW, the incidence rate (58 per 100 000 person-years) was comparable to that seen in other similar cohorts [5]. However, the incidence rate in women (132 per 100 000 person-years) was greater than what has been previously reported (30 per 100 000 person-years, respectively) [5]. This discrepancy may be at least partly explained by some of the baseline characteristics of this nonscreening cohort, with more than 50% having a CD4 nadir of less than 200 cells/μL compared with 36% in the screening group. In fact, immunological status plays a pivotal role in the natural history of HPV infection leading to anal SIL and anal cancer in persons with HIV [27–29]. We identified no subjects with IASCC among those with a $CD4^+$ nadir count greater than 150 cells among 14 595 person-years. These findings reinforce the concept that all persons with HIV-1 (MSM, MSW, and women) are a group at risk of IASCC in comparison to the general population and their inclusion in anal screening programs must be seriously evaluated.

We noted that 2 PLHA involved in the screening program nonetheless developed an IASCC. In both cases, the carcinoma was characterized by a fast, aggressive evolution. The 2 patients shared various characteristics, including hemorrhoids at exploration, CD4 nadir of less than 200 cells/μL, normal anal basal cytology, and persistent infection by multiple high-risk HPV including genotype 16; and both received regular follow-up examinations in the proctology unit. It is noteworthy that one of them had cytology results showing LSIL but a normal HRA 4 years previously and the other had cytology results showing ASCUS but a normal HRA 6 years previously. In both cases, the IASCC was diagnosed after surgical treatment for hemorrhoids, which probably hindered an earlier IASCC diagnosis. From a clinical practice standpoint, when faced with a patient with abnormal anal cytology and hemorrhoids, the approach tends to be more conservative to avoid biopsy-induced bleeding. Hence, based on our experience, our recommendation for PLHA with hemorrhoids is to perform a careful HRA to try to identify possible lesions and, if a lesion is observed, it should be treated with trichloroacetic acid or major ambulatory surgery, with a particularly close subsequent follow-up.

Our study is subject to several limitations. The sample size of our cohort was relatively small and subjects were not randomly assigned to the screening strategy. Subjects who decided to refuse the screening program had baseline characteristics that entailed a higher risk of IASCC development, including lower baseline $CD4^+$ counts, lower $CD4^+$ nadir cell counts, and a higher percentage of HIV-1 RNA greater than 50 copies/mL. The confounding introduced by these baseline characteristics could suggest a more health-oriented behavior in patients who voluntarily entered the program. However, the main risk factor identified in association with IASCC is the time from known HIV-1 infection (which was similar between groups) and a Cox regression to compare HRs

between groups adjusted for propensity score would appropriately correct the impact of these baseline variables into the model. It must be noted that propensity score adjustment cannot balance for unknown or known unmeasured confounding variables.

Likewise, due to the limited number of IASCC events, we cannot definitely exclude that other factors introduced into the analysis (sexual behavior, $CD4^+$ nadir or baseline $CD4^+$ count, or HIV-1 plasma RNA) would potentially be associated with IASCC development as well.

The population analyzed (all from a single geographical area) makes it risky to extrapolate our results beyond the population and conditions studied. Nevertheless, the incidence rates found among MSM and women not included in the screening program are within the reported ranges in other areas [30]. Similarly, the small number of cases of IASCC reported here and the limited follow-up period (just over a decade) might also be insufficient to estimate accurately the incidence of this cancer.

Despite all these limitations, the model was able to identify a significant protective benefit of the anal screening strategy. High-resolution anoscopy is currently the gold-standard procedure for detection of lesions in the anal canal, but it is subject to interobserver bias (despite thorough training).

In conclusion, in a prospective cohort analysis the number of cases of IASCC was significantly lower in PLHA (MSM, MSW, and women) who were enrolled in a preventative screening program compared with a similar group who were not. These results support the continued implementation of such programs, while results from randomized clinical trials and analyses involving larger cohorts are eagerly awaited to further clarify the efficacy of this strategy.

Supplementary Data

Supplementary materials are available at *Clinical 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

Authors' contributions. B. R., S. V., G. S., and B. C. designed and wrote the study protocol; B. R., G. S., M. P., F. G-C., and X. C. visited the patients and collected anal canal specimens; A. O., J. P., and S. V. managed the data and performed the statistical analysis. S. V., B. R., and J. M. L. wrote the manuscript. All of the authors have read and approved the final manuscript.

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Potential conflicts of interest. B. C. has received honoraria for speaking and participating in advisory boards from Abbott, Bristol-Myers Squibb, Boehringer-Ingelheim, Gilead Sciences, GlaxoSmithKline, Pfizer, Merck, and Janssen-Tibotec. J. M. L. has received honoraria for speaking and participating in advisory boards from Gilead Sciences, ViiV Healthcare, Merck Sharp & Dohme, Janssen-Cilag, and Thera Technologies. B.R. has received honoraria for speaking from Gilead Sciences and Janssen-Cilag and for participating in advisory boards from ViiV Healthcare. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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