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ORIGINAL RESEACRH

Screening for Squamous Cell Anal Cancer in HIV Positive Patients: A Five-Year Experience

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ABSTRACT

Aim: Potential screening modalities for early diagnosis of squamous cell anal cancer (SCC) in HIV patients include digital anorectal examination (DARE), anal Papanicolaou testing (Pap test), human papilloma virus (HPV) co-testing, and high-resolution anoscopy. The aim of this study was to demonstrate the results of a five-year screening program for SCC in HIV patients. *Materials and Methods*: We conducted a retrospective study on 204 HIV patients who underwent a screening program for SCC from October 2010 to January 2015. All patients were screened by DARE, anal Pap test, including HPV test and cytology, and high-resolution video-proctoscopy (HR-VPS) with and without acetic acid 3%. Depending on macroscopic appearance and biopsies, patients underwent observation or treatment. Median follow-up was 36 months. *Results*: Cytologic abnormalities (Cyt+) for high-risk HPV genotypes were recorded in 34% of patients. HR-VPS was positive in 59 patients (29%), of whom 13 patients (22%) were positive for warts; the rest have typical features of anal intraepithelial neoplasia (AIN). Sixteen (8%) patients had AIN (AIN I–III) and underwent wide local excision, ablation, or imiquimod. Absence of progression was recorded. Fourteen patients (7%) had SCC: eight (57%) with no evidence of recurrence, two (14%) had recurrence, and four (29%) died from metastatic disease. *Conclusions*: Our data demonstrated a successful screening program in preventing SCC in HIV patients. We demonstrate the advantages of progression towards SCC. Moreover, we used a new screening tool, the HR-VPS, a low-cost and manageable instrument to collect patients' long-term data.

Keywords: HIV; HPV; anal dysplasia; high-resolution video-proctoscopy; screening; anal cancer

INTRODUCTION

The incidence of squamous cell carcinoma (SCC) of the anus, which is responsible for 80% of all anal cancers, has been increasing in the past decade [1]. Annual anal cancer incidence is about 1–3 new cases per 100,000 people in Europe [2], and the number of new cases of cancer has significantly increased in distinct groups of the population, defined as risk groups, in developing the malignancy [3].

The risk groups are composed of chronic human immunodeficiency virus (HIV)-infected patients or individuals engaged in recognized risk activities, namely participation in anal receptive intercourse (especially males) and men who have sex with men (MSM) living with HIV, immunosuppressed transplanted patients, individuals with a history of sexually transmitted diseases (STD), women presenting with cervical cancer or a history of cervical cancer, vulvar or vaginal squamous intraepithelial lesions (SILs), individuals with chronic anal inflammation due to fistulas, fissures, and haemorrhoids, tobacco smokers, and individuals with anal cancer due to genetic factors [4]. Among STD, the human papilloma virus (HPV) is well known for causing epithelial proliferative lesions and, due to its oncogenic potential, evolving into benign warts or high-grade intraepithelial lesion (HSIL) that can turn to skin and mucous malignant tumors [5].

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Persistent infection of the anal canal by high-risk strains of HPV, types 16 and 18, is the most common cause of anal cancer, and individuals infected with HIV are at increased risk of persistent HPV infection [5, 6]. This population is 52 times more likely to develop anal cancer [6–8].

In this scenario, a number of risk stratification strategies as well as screening techniques have been suggested, and currently little consensus exists among national societies. Even randomized controlled trials confirm that screening and treatment outcomes are lacking [9, 10]. The most widely disseminated screening algorithm was popularized by researchers at the University of California, San Francisco: The basis of the algorithm being that all high-risk patients should get screened annually with an anal Papanicolaou testing (Pap test), all atypical cytology results should be referred for high-resolution anoscopy (HRA), and anal intra-epithelial neoplasia (AIN) I should be followed yearly with HRA, while AIN II or AIN III should be treated surgically [11]. In spite of many algorithms suggested over the last decades, national screening guidelines for anal cancer are nonexistent, and the AIDS advocacy groups note that the increased risk of anal cancer in the HIV positive population differs in their recommended approaches [9–15].

The obvious goal of anal cancer screening is to identify and treat both early invasive anal SCC (ASCC) and high-grade (HGAIN). Potential screening modalities for anal cancer include digital anorectal examination (DARE), Pap test, HPV testing (polymerase chain reaction HPV DNA), and HRA [10].

Researchers popularized the most widely disseminated screening algorithm. The basis of the algorithm being that all high-risk patients get screened annually with an anal Pap test, all atypical cytology results are referred for HRA, and AIN I is followed yearly with HRA, while AIN II or AIN III is removed surgically [16, 17].

The results of our five-year experience in screening for SCC in HIV patients are fully described in the following paragraphs.

MATERIALS AND METHODS

We conducted a retrospective study of HIV positive patients who underwent anal screening between October 2010 and January 2015 at the Infective Disease Unit of the University of Perugia Medical Centre. We included HIV positive/AIDS patients, immunosuppressed transplanted patients, patients with previous treatment for leukemia/lymphoma, individuals with a history of STD and previous anal neoplasia, women presenting with cervical, vulvar, or vaginal SILs or a history of cervical cancer. Patients were interviewed for the presence of anal cancer additional risk factors, such as smoking and previously diagnosed anal, vaginal, or oral high-risk HPV. Anal cytology was classified on the basis of the 2001 revised Bethesda System of Cervical Cytology Classification [18]; therefore, we detected anal HPV (high-risk [HR] or low-risk [LR]), low-grade intraepithelial lesion (LSIL), HSIL, atypical squamous cells of undetermined significance (ASCUS), AIN I/II/III, and micro-invasive and invasive SCC. Each patient underwent anal Pap test, HPV test, polymerized chain reaction (PCR), HPV DNA, and cytology by thin prep, high-resolution video-proctoscopy (HR-VPS) with and without acetic acid 3%. We proceeded by introducing a 1-mL/3-mL acetic acid-soaked swab in the anal canal for a minute followed by examination with an anoscope.

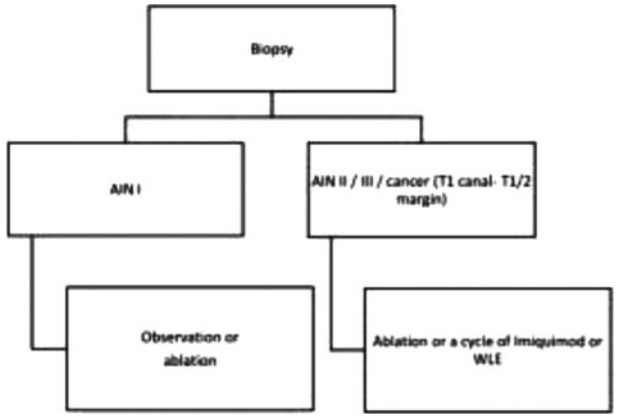

FIGURE 1 Our anal screening protocol for AIN I/II/III after biopsy.

The treatments for AIN I/II/III consist of wide local excision (WLE), use of trichloroacetic acid 85%, laser therapy, infrared coagulation, diathermy ablation, and use of imiquimod, 5-fluorouracil (5 FU), vaccination against HPV. According to our anal screening operative protocol (Figures 1 and 2), most of the patients with positivity for AIN I underwent observation and those with a significant number of warts and positivity for AIN I underwent treatment, while patients with positivity for AIN II/III underwent surgical or chemical

FIGURE 2 Treatment of anal carcinoma after wide local excision (WLE).

treatment decided by the surgeon. Patients with carcinoma in situ of the anal canal/margin were treated by WLE. Those with micro invasive carcinoma of the anal canal/margin (T1/T2 of the anal margin or T1 of the anal canal with adequate lateral and deep margins of resection) underwent follow-up (anoscopy at three months and HR-VPS with biopsy within six to eight months). The others, with micro invasive carcinoma of the anal canal/margin or T2 invasive carcinoma of the margin, or T1 invasive carcinoma of the anal canal with lateral and deep margins infiltrated by SCC/severe dysplasia (AIN III), underwent widening of margins or other strategies. Patients with invasive carcinoma further T2 of the anal margin or further T1 of the anal canal or those with margins infiltrated by SCC not surgically removable underwent the Nigro protocol [19]. The data were collected in Excel database and included gender, age, nationality, patient's medical history, date of first examination, CD4 count, Pap tests, HPV, VPS, and biopsies' results, treatments, and follow-up. According to histopathologic results of biopsies, patients underwent observation, topic imiquimod, surgical ablation, or combination of the two techniques. The median follow-up was 36 months (range 12–60 months).

High-Resolution Video Proctoscope

The anoscopy was performed using a high-resolution video proctoscope. The HR-VPS consists of a Proctostation THD© device (THD SPA, 2016; Correggio RE, Italy), which is a portable touch screen 16–9 connected to a high-resolution camera (Figure 3). The camera is wrapped in a disposable cover and connected with a disposable self-illuminating anoscope (Figure 4), which is equipped with side windows to perform biopsies and ablative treatments under visual control. The "patient management" software allows to include epidemiologic and clinical data in the patients' personal folder, which can be updated anytime. Each examination is recorded as a complete video associated with audio; the operator can also take pictures at all times. Contrary to what happens when using a colposcope adjusted for anoscopy, VPS with proctostation is performed in the Sims position as an ordinary anal examination, therefore patients need no bowel preparation. The operator inserts the anoscope with the aid of an introducer, which is then replaced by the camera. The operator then uses the tool with a single hand, observing the examination on the monitor (Figures 5 and 6). A pedal allows the operator to turn the recording on/off and to take pictures of areas of interest. Afterwards, acetic acid is introduced. If necessary, biopsies can be performed under visual control by introducing forceps through side windows without changing the position of or switching off the instrument. Once the examination is concluded, VPS is extracted and the disposable part is thrown away en bloc.

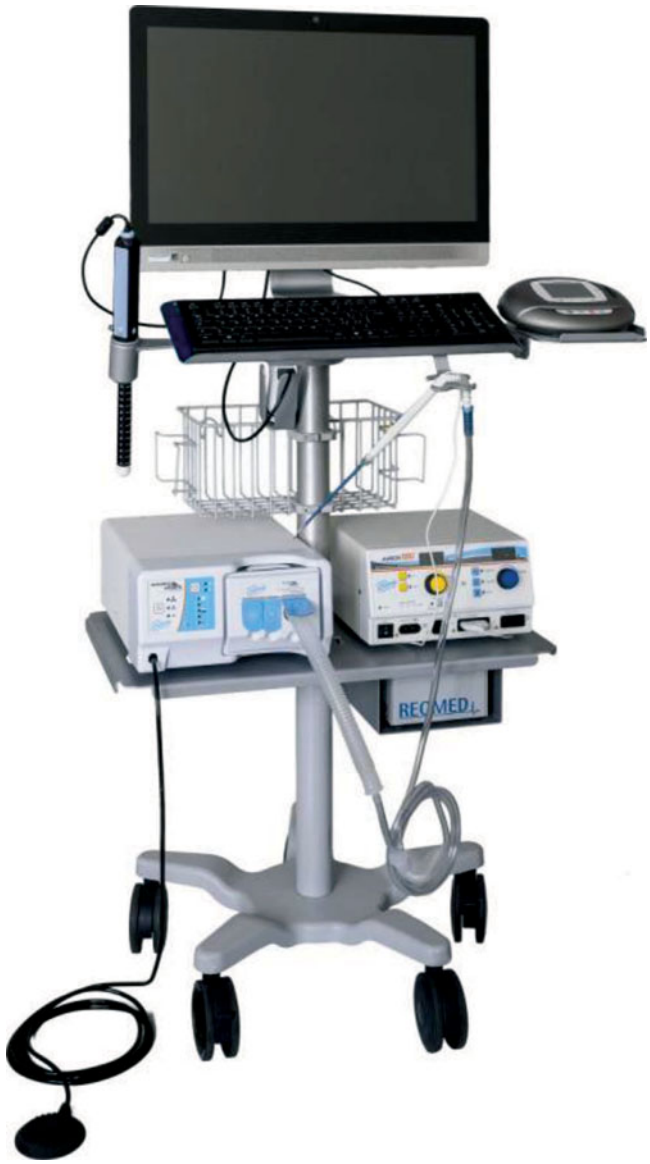

FIGURE 3 The HR-VPS, Proctostation THD.©

After performing the endoscopic procedures, we classified the anoscopic findings on the basis of the 2002 revised Barcelona Classification [20]. Every HR-VPS positivity was biopsied and histopathologic analysis was performed on all specimens.

RESULTS

The group of 204 HIV patients included 163 males (80%) and 41 females (20%), with the median age of 46 and 42 years respectively.

Among the male group, 81% were MSM. A diagnosis of AIDS was made in 26% of the patients and a substantial number of them (43%) had a CD4+ <200 cell/mmc. Among the female group, HIV infection was contracted via sexual route in 83% of the cases. Twenty-nine patients had a past medical history of cervical

FIGURE 4 A disposable self-illuminating anoscope which has a side windows to perform biopsies and ablative treatments under visual control.

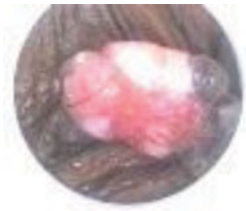

FIGURE 5 An endoanal lesion visualised with the HR-VPS.

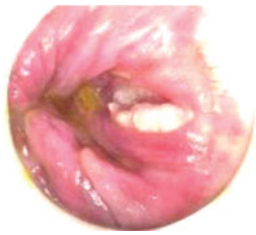

FIGURE 6 A second example of a lesion seen in HR.

intraepithelial neoplasia (CIN), vaginal intraepithelial neoplasia (VaIN), or vulvar intraepithelial neoplasia (VIN), while two patients experienced cervical and vaginal cancers respectively. AIDS diagnosis was made in 26% of all the patients and a low viral-load (<50 copies) was detected in 69% of both populations. Most of the patients (90%) were on antiretroviral therapy.

Cytologic abnormalities (Cyt+) were recorded in 31% of male patients; the most common cytologic alteration being LSIL (65%), then ASCUS (27%), and HSIL (4%). Cytology had no value in five patients (3%). We noticed a contrast result in timing between the detection of HIV diagnosis and the Pap test performance: patients with Cyt+ seem to have been diagnosed with seropositivity for less years than those with Cyt– with a statistically significant difference ($p = 02$).

Patients with negative anal Pap test had a low viral load (74% with HIV-RNA <50 copies/mL), and a greater number (99%) of them were on antiretroviral therapy, statistically significant data ($p = .03$ and .0001 respectively). All patients negative for HPV infection were also Cyt–. Genotype 16 and 18 were recorded in 61% of the patients. HR-VPS was positive for acetic acid reactive lesions in 20% of the patients with a negative Pap test, confirming the higher sensitivity of visual modality compared to anal Pap test. This result was significant, especially for the discovery of AINs and cancers. Therefore HR-VPS was positive in 59 patients with acetic acid positive lesions, of whom 13 cases were with multiple warts and others were with mosaicisms, vascular punctations, leusocplakias, and protruding lesions. All the lesions found during anoscopy were biopsed.

We diagnosed 16 patients affected with AIN (range AIN I–III), and the Pap test was positive in 14 of them (1 ASCUS, 8 LSIL, 2 ASC-H, and 3 HSIL). We diagnosed three patients with AIN I, four patients with AIN II, and nine patients with AIN III. In each case of AIN, the anoscopy showed different features (acetic acid positive lesion, condiloma, leukoplakia, and warts). According to the protocol, the easily removable lesions were excised surgically or ablated with electrocautery, those not easily removable were treated by topic imiquimod for a period of three months and following surgery. Two AIN I patients (67%), AIN II patients (100%), and four AIN III patients (44%) had a complete response to the treatment. The rest of the patients with partial response underwent repeated cycle of therapy and surgical treatments. No one of them developed anal cancer (Table 1).

We diagnosed 14 (7%) new cases of SCC in HIV patients, immediately after excision of condiloma and anal vegetative lesions. The patients' median age was 52 years (range 42–66 years), most being males (83%) and homosexual (90%), and the majority of the patients were European White (83%). The median age between the diagnosis of HIV and SCC was 14 years. One patient had a positive result for LSIL and three for HSIL on both Pap test and HR-VPS; the others having negative Pap tests, however, were found to have positive results for anal vegetative lesions and anal condiloma at HR-VPS. Nine patients (64%) were affected by infiltrating SCC of the anal canal. Eight of them underwent chemotherapy plus radiotherapy (CRT) according to the Nigro protocol; one of these patients also underwent palliative colostomy due to extensive anal and rectal infil-

TABLE 1 Resume of patients' outcome affected by AIN

	Patients number	Anoscopy findings	Treatment	Complete response	Repeated treatment	Cancer developed
AIN I	3 (19%)	+	Protocol	2 (67%)	1 (33%)	—
AIN II	4 (25%)	+	Protocol	4 (100%)	—	—
AIN III	9 (56%)	+	Protocol	4 (44%)	5 (56%)	—

TABLE 2 Outcome of patients affected by infiltrating SCC ad micro-invasive SCC

	Patients number	CTR	CTR + surgery	Surgery	Number of recurrence	Local recurrence	Deaths because of metastasis
Infiltrating SCC	9 (64%)	7 (78%)	1 (11%)	1 (11%)	3 (33.3%)	2 (22.2%)	4 (44.4%)
Micro-invasive SCC	5 (36%)	—	—	5 (100%)	5 (100%)	—	—

tration, and another one died of primary bronchial cancer. The ninth patient affected by infiltrating and locally advanced SCC underwent abdominal perineal resection (APR). In the remaining five patients (36%), a micro-invasive SCC was diagnosed after WLE (less than 3 mm of invasion in lesions <7 mm in size). We did not observe local relapses and deaths in this group of patients. Finally, eight patients (57%) were treated without evidence of recurrence, one patient affected by previous anal cancer and treated by WLE and another affected by T3N1MO margin and canal SCC treated by CRT alone had local recurrence of the disease (the last one died of primary bronchial cancer afterwards), and four patients (29%) died from metastatic disease (two cases of metastatic spread to the liver and two cases of local relapse). We also diagnosed two (14%) cancers of the anal canal, five (36%) of the anal margin, and four (29%) of the anal margin and anal canal together. For three patients (21%), the site of the tumor was not given in data collection. We also diagnosed 11 new cases (79%) of SCC and three (21%) cases of previous cancer (Table 2).

DISCUSSION

While the management of anal cancer screening today remains controversial, the way we approached anal cancer prevention gave us satisfying results.

Anal Screening Program

Nowadays, professional society recommendations for anal cancer screening differ from center to center; some of them support only DRE and HPV testing, others add anal Pap test and standard anoscopy, still others use high-resolution anoscopy instead of standard anoscopy. No national screening guidelines for anal cancer exist, and in spite of DRE, anal Pap test, HPV testing, and high-resolution anoscopy sensitivity are specificity acknowledged by the literature [4, 9]; some societies continue to perform only DRE.

In our experience, contrary to literature, no AINs progressed to malignancy in five-year experience of anal cancer surveillance in HIV population, and all patients with AINs undergoing the first treatment of imiquimod following surgery/ablation had a complete or partial regression of the disease. Our results show that we did not have any case of progression of AIN to anal cancer.

The prevalence of AIN and anal cancer is unacceptably high in the population at the highest risk and in the greatest need for screening [9, 10]. The estimated progression rate of AIN to squamous cell cancer is about 11% over a period of 42 months of follow-up. Although AIN I has a low risk of progression to malignancy, it has been observed that around 62% of HIV positive men with baseline AIN I progress to AIN II/III over a two-year follow-up period [21]. There is also a low percentage (23.5%) of HSILs in men spontaneously regressed per year, mostly to LSILs [21].

In a recent study, imiquimod has been used in patients with HSILs with a complete or partial response observed in 66% patients after 16 weeks [22, 23]. In our study, we observed a complete or partial response achieved after 12 weeks of treatment. Even though some studies support the use of imiquimod [22–24], which appears to be effective in treating AIN in HIV positive patients, further studies are needed to document its utility in preventing high-grade dysplasia and/or anal cancer. There is still ambiguity in literature with regard to the optimal management of AINs. In spite of concordance regarding diagnosis, there is significant variation in the guidelines over recommendations on the treatment and surveillance of patients with HGAIN/AIN II/III [25]. Therefore, in spite of the fact that the best strategy to treat AINs has not yet been standardized in literature, we created our own local protocol (Figure 1), which allowed to achieve excellent results on the control of the disease. In our practice, we normally treat easily removable AIN I/II/III by surgery or ablation with electrocautery. We normally prefer wide surgical excisions, especially at the beginning of the treatment, believing that by having the opportunity to obtain satisfactory specimens, we will gather the most information possible regarding the early stage of the disease in order to be certainly more oriented to the best treatment for the patient.

Anal Cancer

New diagnoses of SCC were identified in the majority of patients (79%) with previous condiloma or anal vegetative lesions. In the literature, anal cytology and HPV detection had high sensitivity (69–93%) but low specificity (32–59%) for detecting HGAIN [9], particularly in patients with low virus load and those on anti-retroviral therapy. Therefore, these tests need to

be followed by anoscopy with directed biopsies [18]. In our experience, 29% of the patients underwent anal Pap test, which resulted positive for HPV and only one patient had positivity during the anoscopy; other patients had negative anal Pap test and positive HR-VPS.

In the literature, surgery is generally contraindicated as primary treatment option, especially for cancers localized in the anal canal [26]. Conversely, surgical excision alone for stage T1 anal verge cancer has successful results [27]. However, the current treatment for anal cancer is primary chemo-radiotherapy (CRT) with mitomycin C (MMC) and 5-fluorouracil (5-FU) [28]. Indeed, the development of combined CRT according to Nigro et al. [19], due to the successful oncologic treatment combined with a possible continence preservation, has had a big influence on the therapy concept of the anal canal carcinoma without resorting to radical surgery as the abdominal perineal resection (APR). Until the introduction of definitive CRT, APR was recommended for all tumors, except for those amenable to local excision. Contrarily, smaller lesions (<2 cm in diameter) involving the anal margin may be treated by primary surgery in the form of a local excision (Figure 2).

An Innovative Anal Screening Tool

The traditional video proctoscopy described for the first time in 2007 [29] presents some difficulties regarding the inability to release iconographic documentation of the examination as well as the inability to archive exams and to compare examinations at distance of time, paramount to evaluate the effectiveness of a therapy. On the contrary, the Proctostation THD©, enclosed in a single-device HD video images and patient data management system acquisition, has many advantages, being convenient and more accurate [30].

In conclusion, nearly all anal cancer guidelines avoid any direct recommendation regarding routine screening. With this study, we demonstrated a successful screening program in preventing SCC in HIV patients. Indeed, according to our local approved protocol, we have achieved our purpose: We have been able to control the evolution of all abnormalities identified during the anal cancer screening, prevented AIN to progress toward SCC, and reversed any form of AIN by surgery, ablation, or medical therapy. Finally, we were able to diagnose new cases of SCC and control them over the time with a high-rate successful treatment by performing surgical treatment alone in the majority of cases.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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RESEARCH ETHICS AND PATIENT CONSENT

We performed a retrospective local service evaluation. Our local institutional board approved the study. Patient consent was not required.

AUTHORS' CONTRIBUTIONS

CS and CAL have contributed to design of the study, acquisition of data, analysis and interpretation of data, and drafting/ revising the manuscript critically for important intellectual content. JDH, FC, and FB have contributed to the design and interpretation of data and revising the manuscript. EC has contributed to the conception and design, acquisition of data, analysis and interpretation of data, drafting/ revising the manuscript critically for important intellectual content. All authors contributed provided a final approval of the version to be published.

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