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Anal Cancer Screening for Diagnosis and Treatment of Anal Squamous Intaepithelial Lesions in HIV-positive MSM Patients

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Abstract

Incidence of anal cancer is increasing in HIV+ patients. We have structured a control program in HIV-positive MSM with anal PAP-test and HPV genotyping based on the outcome of anal cytology. Follow-up has been listed according to the HPV genotype infection. Traditional anoscopy and histological exam (also if no visible mucosal lesions, by quadrant random biopsies) was offered to every patient with every abnormal cytology for detection and treatment of squamous anal lesions. Of 87 subjects enrolled, 22 patients (25%) had pathological cytology and 65 patients had PAP-smear negative. No differences in demographic and immuo-virological profile were found in the two groups. Precancerous anal lesions and cancer (1 AIN2, 2 AIN3 and 1 SCC) were treated surgically if advanced otherwise with imiquimod cream into the anal canal; squamous cell carcinoma was referred to oncologist. High prevalence of HPV infection and anal intraepithelial neoplasia are important risk factors for anal cancer: anal precancerous lesions can be detected early and treated in at-risk populations.

Keywords: Anal; Cancer; HPV; AIN; Screening; HIV; MSM

Introduction

Anal cancer incidence has increased in the past few years, and nowadays it is one of the most frequent cancers diagnosed in people with HIV [1].

A North American cohort study lasted from 1996 to 2007 showed that unadjusted anal cancer incidence rates per 100,000 were 131 person-years for HIV positive MSM, 46 for HIV-positive heterosexual men and 2 for HIV uninfected general male population [2]. HIV positive MSM have a doubled estimated risk compared to seronegative MSM and sixty times greater than general population [3]. Even HIV positive women and heterosexuals men have an increased risk of developing anal cancer [4].

Precancerous lesions (low and high grade dysplasia) are related to infection with high risk HPV genotypes (HR-HPV). Near 100% of HIV positive patients with anal intercourse history are HPVs carriers. Moreover in HIV-positive population an increased risk of progression from low-grade to high-grade dysplasia is also described.

Unlike AIDS-defining malignancies, it seems that introduction of HAART did not reduce anal cancer incidence and precancerous lesions prevalence [5] and therapy would not prevent tumor development [6]. On the other hand, HIV treatment is associated with reduction of patient mortality, increased life expectancy and prolonged exposure to oncogenic viruses as HR-HPV. HIV-induced immunosuppression can favor HPV persistence in anal canal and thus precancerous lesions development. From this point of view HAART would increase length of HPV infection and precancerous lesions co-presence, favoring evolution towards anal cancer [7].

Currently, there are no techniques already validated for this malignancy's screening and no guidelines are available for the treatment.

Good PAP-test's sensitivity seems to be proven, but there is some evidence that the degree of cytologic atypia is not always correlated with the epithelial lesions' histological grade [8,9]; therefore cytological abnormalities of any grade needs to be assessed by anoscopy and histological check. High resolution anoscopy (HRA) is also recommended for early detection of anal precancerous lesions. Some authors recommend HRA as the main screening tool for higher cost-effectiveness ratio when compared to anal cytology [10].

Limit of HRA are the lack of equipment in many centers of our country and well-experienced proctologists in the execution of the exam. The Italian Guide-lines for treatment and management of patients with HIV infection recommend annually execution of anal PAP test in HIV MSM male and in HIV male and female with history of HPV disease; secondary HRA for all patients with abnormal cytology is recommended. Following this advice, we have structured a control program based on anal cytology and follow up listed according to the HPV genotype infection. We used traditional anoscopy performed by an experienced proctologist followed by histological examination by quadrant random biopsies in any anoscopy resulted negative at first control.

Methods

Anal cytobrush and anal swab for HPV testing was offered to every consecutive HIV positive MSM patient attending to the Clinic from October 2011 to October 2012.

Anal cytology was performed by Cytobrush Plus GT spatula and preserved in THIN-PREP liquid medium. Anal cytology findings were graded according to Bethesd 2001 system criteria nomenclature. We used Eswab-Copan Diagnostic smear and we detected HPV-DNA by PCR-genotyping (Tac Gold PCR and Innolipa Genotyping Extra Assay, Innogenetics N.V. (Gent, Belgium)).

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At first anal brushing results were evaluated. Every subject with negative cytology (NIL – negative for intraepithelial lesion) were scheduled for a follow-up based on HPV genotype infection; patients with high risk HPV viruses (HR-HPV) were reassessed every six months, whereas patients with low risk HPV viruses or without HPV infection were scheduled to be seen yearly. Genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66 were considered at high risk [11].

Every patient with abnormal cytology such as atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesions (LSIL), atypical squamous cells of undetermined significance cannot exclude high grade squamous intraepithelial lesions (ASC-H), and high grade squamous intraepithelial lesions (HSIL), underwent a traditional anoscopy; in fact, until now, in our centre is not possible to refer patients to high resolution anoscopy (HRA).

The proctologist tested our patients with traditional anoscopy, five minutes after application of 3% acetic acid solution on the perianal area and into the anal canal.

In the presence of intra-anal white lesions, plaques, condylomata or other exophytic lesions histological exams was made for a definitive histological diagnosis.

Furthermore, patients with abnormal cytology and without clear lesions were asked to perform random quadrant biopsies. Proctologist divided anal canal in four virtual quadrants and collected two or three specimens for each of the labeled quadrant.

Results

Eighty-seven consecutive MSM HIV-infected patients were screened for anal cytology. Only one patient refused to perform the exam. Patient's demographic and clinical characteristics are described in Table 1.

Statistical methods

Continuous variables were compared with t test; categorical variables were compared with Chi Square test.

Among the 87 HIV-positive patients enrolled, 22 (25%) had abnormal cytology and 65 patients had negative PAP-smear for intraepithelial lesions. Median age was 47 years. No statistically significant differences in ethnicity, age, mean of nadir and latest CD4 cell count were observed between the two groups. Sexual transmitted diseases (STD) were more frequent (p=0.098) in patients with normal cytology; mean number of years after HIV diagnosis was greater (p=0.07) in patients with abnormal anal cytology; the mean duration of therapy (80/87 patients on antiretroviral drugs) was 2.5 years higher for subjects with normal cytology (p=0.05).

There were no differences in HIV-RNA copies and AIDS diagnosis between those with abnormal *versus* normal Pap cytology (p=1 and p=0.09, respectively).

As for as HPV anal infection, only one patient had negative smear and three cases were undetermined (PCR unamplified): cytological exam was negative for all these patients. There were no differences between abnormal and normal cytology groups in distribution of high-risk genotype infection (p=0.21). We found multiple HR-HPV infection in 58/83 patients (18/22 in abnormal cytology group) and those were included as HR samples. The most frequent HR-HPV genotypes founded on anal swabs were 16, 18, 31, 33. Correlation between genotyping and cytology/histology in screened patients in shown in Table 2.

Variables		All No.=87	Normal cytology No.=65	Abnormal cytology No.=22	p-value
Age AT enrollement years (min-max)		46.00 (23-78)	45.58 (23-78)	46.41 (27-64)	0.711
Etnicity	Italian	65 (74.7%)	47 (72.3%)	18 (81.9%)	0.546
	Other	22 (25.3%)	18 (27.7%)	4 (18.1%)	
History OF STD (except HIV)	Yes	63 (72.4%)	45 (69.2%)	18 (81.8%)	0.098
	No	24 (27.6%)	20 (30.8%)	4 (18.2%)	
Time after HIV diagnosis years mean (min-max)		7.19 (0-25)	7.86 (0-25)	5.54 (0-15)	0.077
Antiretroviral therapy	Yes	80 (92%)	62 (95.4%)	18 (81.8%)	
	No	7 (8%)	3 (4.6%)	4 (18.2%)	
Time after ARV beginning years (min-max)		6.23 (0-25)	6.83 (0-25)	4.38 (0-13)	0.055
HIV-RNA<50 copies/mL		54	42	12	1
NADIR CD4 cell count mean cell/μL (min-max)		279.51 (6-771)	287.68 (7-740)	255.41 (6-771)	0.535
LATEST CD4 cell count mean cell/μL (min-max)		911.5 (95-1388)	705.54 (95-1592)	560.86 (173-1388)	0.060
AIDS defining condition		19/87 (21.8%)	12/65 (18.4%)	7/22 (31.8%)	0.095
HR-HPV infection		67/83	47 (56%)	20 (24%)	0.21

Table 1: demographic and immune-virological characteristics of two groups: PAP-test negative and abnormal PAP-test (including all grade of cytological atypia).

Patients	Age	CDC	HPV types	HR-HPV types	Cytology	Cancer and precancerous lesions
No.1	53	A2	16	YES	AIN-HSIL	AIN 3
No.2	47	B3	31	YES	ASCUS	
No.3	35	A2	16, AFRICA TYPE 2, 58	YES	LSIL	
No.4	40	A2	16, AFRICA TYPE 2	YES	LSIL	AIN 3
No.5	49	B3	11	NO	ASCUS	
No.6	48	A1	18,33	YES	LSIL	
No.7	55	C3	11, 31, 33, 40, 52, 53, 58	YES	LSIL	

No.8	43	A2	6, 16, 31, 33, 40, 45, 51, 52, 53, 58, 68	YES	ASCUS	
No.9	41	A2	6	NO	LSIL	AIN 2
No.10	38	B1	16, 31, 33, 43, 44, 52, 53, 58, 66	YES	LSIL	
No.11	50	C3	6, 11, 16, 31, 33, 40, 52, 53, 58	YES	LSIL	
No.12	41	C3	31, 33, 40, 44, 45, 52, 53, 56, 58, 68, 70, 73	YES	ASCUS	
No.13	27	A2	6, 11, 16, 18, 26, 31, 33, 39, 44, 45, 51, 68, 70, 73	YES	ASCUS	
No.14	50	C3	6, 11, 16, 35, 51, 53,	YES	LSIL	
No.15	44	C3	11, 18, 31, 33, 39, 51, 53, 68, 73	YES	LSIL	
No.16	64	A2	16, 66	YES	LSIL	
No.17	48	C2	6, 11, 18, 31, 33, 39, 40, 43, 52, 53, 54, 58, 68, 73	YES	ASCUS	
No.18	43	C3	11, 31, 33, 40, 52, 53, 54, 58, 69, 71	YES	LSIL	
No.19	44	B3	6, 18, 39, 68, 73	YES	LSIL	
No.20	55	A1	11, 35, 52	YES	LSIL	
No.21	48	A3	16, 51	YES	LSIL	
No.22	58	C3	16, 51	YES	HSIL	Squamous anal cancer

Table 2: Correlation between HPV genotyping and cytology/histology findings.

Anscopy		Histology		Histology results	Treatment
	No.	YES (Patients No.)	No (Patients No.)	(Patients No.)	(Patients No.)
Negative	7	3	4	No. 1 AIN 3 No. 1 Koilocytosis No. 1 Normal	No. 1 (AIN3): Imiquimod
Condilomatosis diffusal	7	5	2	No. 4 Condyloma No. 1 AIN2	No. 5: Surgical treatment
Plaque acetic-white	3	1	2	No.1 AIN3	No.1 (AIN3): Imiquimod
Ulcerate-vegetant lesion	1	1	-	No. 1 Squamous cell carcinoma	No. 1 Radiotherapy

Table 3: Anoscopy, histology results and treatment of lesions.

Among 22 patients with abnormal anal cytology, 18 patients underwent anoscopy (see Table 3); three patients refused to continue the screening program and one moved to another center after initial PAP-test screening. Of 18 patients who received anoscopy, 10 patients also received histological examination. We proposed biopsy to every patient: 4 patients with negative anoscopy have preferred to continue follow-up every six months with pap tests and anoscopy and the other 4 patients refused to underwent in day surgery unit for biopsy.

The proctologist found diffuse condylomatosis with multiple warts, covering the entire perimeter of anal canal on seven anoscopies; of these five subjects were treated surgically after histological exam. Two patients refused to receive surgical removal of lesions. Excisional treatment required at least two sessions for each patient and our pathologist analyzed every surgical specimen: one of these resulted positive for AIN2. After surgery, control with anoscopy every four months was scheduled.

An exophytic ulcerated and bleeding lesion of three centimeters with inflammation of the surrounding mucosa inside the anal canal was detected during the examination in one patient. The biopsy resulted in squamous cell carcinoma of the anus infiltrating the sub-mucosa. We referred this patient to the oncologist for staging and treatment.

White-acetic-acid areas, in absence of exophytic lesions, were found in three patients: two patients were lost at follow up, while one patient, with preoperative cytology positive for LSIL, underwent to anal biopsy, resulted positive for AIN3. The patient was treated with anal imiquimod application (imiquimod cream 5% three times a week for three months) according to previous trials showing the ability of imiquimod to control and downstage AIN lesions in HIV patients [12].

We obtained quadrant random biopsies from three of the seven patients without anal canal lesions: one patient had AIN3 (result of at least two of the eight fragments taken from anal canal) and the other two were negative for histological lesions.

In absence of macroscopic mucosal lesions, we treated also this AIN3 lesion with self-application of imiquimod cream into the anal canal for three months; this patient had cytological diagnosis of HSIL.

Therapy response of two patients affected by AIN3 and treated with imiquimod was assessed by PAP-test and histology (three months after suspension to allow AIN clearance). The first patient, who had HSIL on cytology and AIN3 on histology, at end-of-treatment had normal PAP-test (NIL) and normal histology; the second one did not respond to imiquimod therapy (the diagnosis of LSIL and AIN3 on PAP-test and biopsy was confirmed and we scheduled a follow-up every three months with anoscopy). This therapy was well tolerated by both, without the occurrence of adverse events. Random biopsies following imiquimod therapy were obtained to assess any presence of anal dysplasia.

Until now twenty patients, with negative cytology at the first control, performed a control anal PAP-test (four patients with HR-HPV infection after six months and 16 after one year). Four new pathological cytology (1 ASCUS, 1 LSIL and 2 HSIL) were found and patients are proceeding to anoscopy and biopsy.

Discussion

We aim to propose a practical clinical protocol for centers

where is not jet available high-resolution anoscopy, developing and implementing the indications of Italian guidelines. A multidisciplinary approach involving Infectious Diseases, Proctology and Pathology Specialists team was adopted. At least two pathologists analyzed independently the cytological and histological sample from single patients; same approach was used to examine controls samples after 6 and 12 months of negative PAP-smear. We have evaluated cytology and HPV typing respectively in PAP smears and anal swab of HIV positive homo/bisexuals men attending our centre and analyzed correlation between PAP smears' results and traditional anoscopy with biopsy and histological examination. We found abnormal anal Pap cytology in 25% of examined patients.

No statistically significant differences were found between patients with abnormal or normal anal PAP-test regarding age, mean T CD4+ cell count, years after HIV diagnosis, mean duration of therapy and in HIV-RNA copies/ml in patients on ARV, although nadir of CD4 tended to be lower and other sexual transmitted diseases (STDs) were more frequent in patients with abnormal anal cytology.

We found high prevalence of HPV infection in our series, in agreement with literature data on HIV.

Limits of our sceening program are the limited number of patients enrolled and obviously the unavailability of HRA that allows magnifying the image of the anal canal and is actually the most sensitive test helpful for high-grade AIN diagnosis.

Moreover, the limited availability of experts performing HRA make this exam difficult to obtain. Traditional anoscopy is obviously less sensitive but more available, therefore we proposed histological examination to patients with abnormal pap tests and negative anoscopy. We diagnosed AIN3 from random biopsies in one patient with no apparent anal canal lesions in traditional anoscopy. A screening algorithm has been prepared as shown in Figure 1.

We tested histological response to local therapy after 12 weeks from the treatment end; this is justified by literature data showing that the clearance of anal dysplasia after imiquimod topical treatment may also occur after several weeks of discontinuation of the drug [13].

Patients should be informed about the meaning of the outcome of a pathological cytology and about the possible need for other tests (anoscopy and biopsy). Patients can develop anxiety related to positive cytology and to the discomfort originating from invasive techniques. In our experience, patients have generally well accepted the screening.

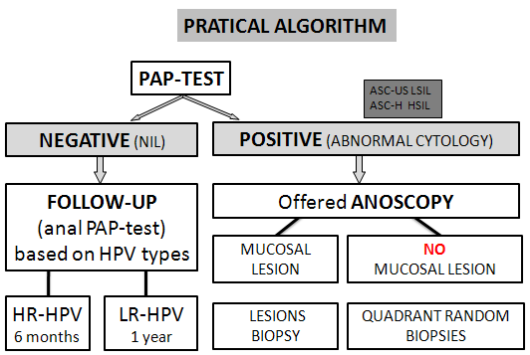

Figure 1: Screening program algorithm. First evaluated PAP-test results. Every NIL scheduled follow-up with PAP-test timely based on HPV-genotypes (every 6 months for HR-HPV infection and annually if no HR-HPV infection). All grade of abnormal cytologies recommended to anoscopy and histology (also to normal anoscopy).

Basic structure of screening program provides continuous monitoring of patients at risk. The single anal PAP-test does not exclude the presence of precancerous lesions and their possible progression, as indicated by detection of pathological cytology in the follow-up of patients with previous negative cytology. This finding highlights the importance to make continuous monitoring over time: an isolated negative anal PAP-test has, in fact, a low negative predictive value.

Probably the HAART, has a role, increasing the life expectancy of patients, exposing them for longer periods to the effect of oncogenic viruses, such as HPV, and the effect of physiological aging of the organism. Therefore patients treated nowadays are paradoxically, more at risk than they were in the past.

Anal PAP-test and anoscopy (particularly if HRA) are inexpensive and moderately invasive tests for early detection and treatment of precancerous lesions.

High resolution anoscopy is moderately invasive and is not widely available in our Country. There is currently a universal approach despite the lack of comprehensive studies on the effectiveness and cost-effectiveness of the use of these tools. Risk of developing anal cancer should always be considered in clinical practice and we need to adopt tools and features to screen HIV in at risk population.

Despite the small number of patients enrolled, we believe that a similar surveillance program could be the basis for major clinical study.

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