



# Liquid-based Anal Cytology as a Screening Tool for Prevention of Anal Cancer in at-risk Populations: A Single-Center Retrospective Analysis on 111 Patients

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## Abstract

**Objective** Squamous cell carcinoma of the anus (SCCA) is associated with human papillomavirus (HPV) infection in almost 90% of the cases. Its incidence is alarmingly high among men who have sex with men (MSM) and continues to increase at an average rate of 2% per year. The objective of the present study is to evaluate the usefulness and performance of liquid-based anal cytology as a screening tool for prevention and early detection of SCCA in a cohort of at-risk men.

**Method** We conducted a retrospective study including 111 MSM, aged between 22 and 62 years old, who underwent anal cytological screening with a liquid-based Pap test at our sexually transmitted diseases (STDs) clinic from January 2015 to March 2017.

**Results** Out of 111 anal smears, 57 (51,4%) resulted negative, 42 (37,8%) abnormal, and 12 (10,8%) unsatisfactory for the cytological evaluation. Only patients with an abnormal cytology underwent anoscopy and subsequent biopsy. The histological results were as follows: negative for squamous intraepithelial lesion (SIL) in 5 cases, low-grade SIL (L-SIL) in 21, high-grade SIL (H-SIL) in 5, SCCA in 1. Five patients had a normal anoscopy and biopsy was not taken.

**Conclusion** Liquid-based cytology, reducing the “darkening factors” typical for the conventional smears, has a higher positive predictive value than the traditional technique. Moreover, a cytological diagnosis of atypical squamous cells of undetermined significance (ASC-US) or L-SIL may hide a severe dysplasia or even a carcinoma. Thus, all patients with an abnormal anal cytology at any grade should be considered for anoscopy.

## Keywords

- ▶ anal cancer
- ▶ anal cytology
- ▶ liquid-based cytology
- ▶ pap test
- ▶ screening

## Introduction

Worldwide, as many as 4.5% of new cancer cases are associated with human papillomavirus (HPV) infection.<sup>1</sup>

Squamous cell carcinoma of the anus (SCCA) is the second most commonly HPV-related cancer, immediately after cervical cancer. Nearly 90% of anal cancers are attributable to HPV.<sup>2</sup>

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Based on data reported by the Surveillance, Epidemiology, and End Results Program (SEER),<sup>3</sup> the number of new cases of anal cancer is 1.8 per 100,000 men and women per year. However, SCCA is one of the most frequent non-AIDS-defining neoplasms in HIV-positive patients, especially in HIV-positive men who have sex with men (MSM), with a very high incidence of up to 144 cases per 100,000 individuals per year.<sup>4-6</sup> In the population of MSM, both HIV-positive and HIV-negative men, the incidence is alarmingly high and continues to increase at a gross rate of 2% with each passing year.<sup>7,8</sup>

In economically developed countries, cervical cancer in women has plummeted to a fifth of its initial incidence, as a result of cervical screening programs with the Pap smear.<sup>7</sup> Based on the great success of cervical cytological screening, some authors<sup>9-13</sup> have proposed a similar screening for the prevention of anal cancer in the at-risk male population. Cervical cancer and anal cancer share several similar characteristics: both cancers are strictly associated with a persistent infection of epithelial cells by oncogenic HPV types, and both are preceded by preinvasive neoplastic lesions (cervical intraepithelial neoplasia [CIN], in the cervix, anal intraepithelial neoplasia [AIN], in the anus), that may be identified with Pap smear.<sup>11-13</sup>

A two-tiered classification for intraepithelial lesions has been recently proposed by the Lower Anogenital Squamous Terminology Standardization (LAST) Project<sup>14</sup>: low-grade squamous intraepithelial lesions (L-SIL) and high-grade squamous intraepithelial lesions (H-SIL).<sup>15,16</sup> However, it has been found that anal condyloma, considered a variant of L-SIL, may also harbor foci of H-SIL or even invasive squamous cell carcinoma. The coexistence of different lesions in the same patient is due to the frequent detection of multiple HPVs, both high- and low-risk, in MSM, especially HIV-infected patients.<sup>17-20</sup>

A cytological screening with anal Pap test is usually performed in all MSM at the Sexually Transmitted Diseases (STDs) Center of the Dermatological Clinic of University of Florence. From 2013 to 2014, conventional anal Pap smears were performed, but since 2015 the STD Center of Florence has started to use liquid-based smears for anal cytology, in close analogy with what happened for cervical cytology.

We undertook a retrospective study on patients who underwent anal cytological screening with a liquid-based Pap test between 2015 and 2017. The aim of the present study was to evaluate the performance of liquid-based anal cytology in terms of adequacy and diagnostic accuracy. Moreover, our results were retrospectively compared with those obtained in a previous study<sup>9</sup> from slides prepared by a conventional technique, to detect the best strategy in diagnosing anal intraepithelial neoplasia in HIV-infected and -uninfected MSM. A prospective study aimed to directly compare the two methods needs to be conducted in the near future.

## Method

Data for the present retrospective study were collected at the STD Center of the Dermatological Clinic of University of Florence between January 2015 and March 2017.

All participants gave informed consent. After a clinical evaluation, they were subjected to an anal sampling for cytology. A nonsterile disposable cytobrush, ordinarily used for cervical Pap smear in females, was gently inserted between ~ 3 and 5 cm into the anal canal to obtain cells from the transformation zone. Once fully inserted, the endobrush was firmly rotated three times clockwise and three times anticlockwise, applying a vigorous lateral pressure on the walls of the anal canal, to maximize cellular yield. The brush was then removed, put in a liquid-based cytology fluid (ThinPrep) and rotated for ~ 10 seconds to facilitate cell detachment.

All samples were sent to the Cancer Prevention Laboratory of the Institute for the Study and Prevention of Cancer (ISPRO) of Florence. Liquid-based slides were prepared by the same processor used for cervicovaginal cytology. The processor mechanically disperses the cells, which are then drawn on a filter by negative pressure and transferred on a glass slide; at the end, the slides are stained with Papanicolaou stain, exactly as conventional smears. Each sample was examined simultaneously by four cytopathologists.

Several advantages of liquid-based cytology over conventional smears are documented, such as absence of obscuring material (blood, inflammatory cells, and mucus), lack of air-drying artifacts, and the presence of consistently well-preserved cells and a monolayer cellular surface that is easier to screen.<sup>21-23</sup>

Samples with scant cellularity or presence of numerous anucleated squamous cells were classified as "inadequate/inconclusive" for the cytological interpretation. The adequate cellularity of an anorectal sampling is similar to a cervical one: a slide with between 2,000 and 3,000 nucleated squamous cells is considered adequate. For thin-layer preparations, this is equivalent to 1 to 2 squamous nucleated cells/HPF for ThinPrep (diameter of 20mm).

The presence of rectal columnar cells and/or of metaplastic squamous cells is not a requirement for adequacy, but must still be reported, because it is an indication that the transformation zone has been sampled.

Anal cytological results were classified using the 2001 Bethesda System<sup>24</sup> as: negative for intraepithelial lesion or malignancy (NILM), atypical squamous cells of undetermined significance (ASC-US), atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H), L-SIL, H-SIL, and carcinoma.

All patients who had an abnormal anal cytological result of ASC-US and above underwent anoscopy, always with application of 5% acetic acid solution, used to aid visualization of abnormal anal tissue. Anoscopy was performed with the patient in left lateral decubitus; an anoscope was placed into the anus with lidocaine lubrication, and then a gauze soaked in 5% acetic acid solution was inserted into the anal canal while the anoscope was gently removed. The gauze was removed after a few minutes to avoid a widespread "false positive" reaction. After application of the acetic acid solution, the anoscope was inserted again and the mucosa was carefully examined with the aid of a colposcope for lighting and magnification. When the anoscopy showed the presence of "aceto-white changes" characteristic of AIN, including flat

**Table 1** Liquid-based anal Pap test results based on HIV status

	HIV-POSITIVE	HIV-NEGATIVE	TOTAL
UNSATISFACTORY	0	12 (16%)	12
NILM	19 (52.8%)	38 (50.7%)	57
ASC-US	6 (16.7%)	8 (10.6%)	14
ASC-H	0	3 (4%)	3
L-SIL	8 (22.2%)	12 (16%)	20
H-SIL	3 (8.3%)	2 (2.7%)	5
ANY ABNORMALITY (ASC-US, ASC-H, L-SIL, H-SIL)	17 (47.2%)	25 (33.3%)	42
TOTAL	36 (32.4%)	75 (67.6%)	111

Abbreviations: ASC-H, atypical squamous cells, which cannot exclude a high-grade lesion; ASC-US, atypical squamous cells of undetermined significance; H-SIL, high-grade squamous intraepithelial lesion; L-SIL, low-grade squamous intraepithelial lesion; NILM, negative for intraepithelial lesion or malignancy.

or slightly raised areas of thickened mucosa with or without vascular pattern abnormalities, a biopsy was performed and sent to the Pathologic Anatomy Department of Careggi for the definitive histopathological diagnosis.

## Results

In the selected period, we screened for anal cancer 111 male patients aged between 22 and 62 years old (median age: 38 years old). All included patients were homo- or bisexual; 36 (32.4%) were HIV-positive and 75 (67.6%) were HIV-negative. At least 1 previous or concurrent sexually transmitted infection was found in 86 (77.5%) patients: 62 (55.8%) patients had a history of anogenital warts, 43 (38.7%) patients had a history of syphilis, 35 (31.5%) had a previous diagnosis of gonorrhea, 18 (16.2%) had a previous anogenital infection by Chlamydia trachomatis, 12 (10.8%) had a history of hepatitis B virus (HBV) infection, and 4 (3.6%) had a concurrent hepatitis C virus (HCV) infection. All 36 HIV-positive patients included in the study were under treatment with Highly Active Anti-Retroviral Therapy (HAART), with undetectable plasma viral load and a good immunological status.

Out of 111 anal Pap smears prepared using liquid-based slides (ThinPrep), 57 (51.4%) resulted negative, 12 (10.8%) unsatisfactory for the cytological evaluation, and 42 (37.8%) abnormal.

Among abnormal anal Pap tests, 14 cases of ASCUS (12.6%), 3 cases of ASC-H (2.7%), 20 cases of LSIL (18%), and 5 cases of HSIL (4.5%) were identified.

Among the 111 participants included in the analyses, 36 (32.4%) were HIV-infected MSM and 75 (67.6%) were HIV-uninfected MSM. In ►Table 1, we report the anal Pap test results based on HIV status.

In our study, only participants with an abnormal anal cytological result from ASC-US and above had anoscopy, performed after application of 5% acetic acid solution.

The correlation between the cytological and histological results is shown in ►Table 2.

Among the 5 patients who had H-SIL as cytologic diagnosis, 2 patients (40%) were lost to follow-up; in the 3 remaining patients, anoscopy always showed aceto-white lesions in the anal canal mucosa. Histological examination allowed to identify 2 cases of H-SIL (1 case of AIN II-III and 1 carcinoma in situ) and 1 case of infiltrating carcinoma.

**Table 2** Correlation between anal cytology and histology results in 99 patients with adequate cytology results

CYTOLOGY	HISTOLOGY						
	TOTAL	NO BIOPSY	NEGATIVE	INFLAMMATION	AIN I	AIN II-III, Cis	SCCA
NILM	57	57	—	—	—	—	—
ASC-US	14	0	5	4	4	1	0
ASC-H	3	0	0	0	2	1	0
L-SIL	20	3*	0	1	15	1	0
H-SIL	5	2*	0	0	0	2	1
TOTAL	99	62	5	5	21	5	1

Abbreviations: AIN, anal intraepithelial neoplasia; ASC-H, atypical squamous cells, which cannot exclude a high-grade lesion; ASC-US, atypical squamous cells of undetermined significance; Cis, carcinoma in situ; H-SIL, high-grade squamous intraepithelial lesion; L-SIL, low-grade squamous intraepithelial lesion; NILM, negative for intraepithelial lesion or malignancy; SCCA, squamous cell carcinoma of the anus.

\*Patients who did not return to visit or refused to undergo anoscopy.

Among the 20 patients with cytological report of L-SIL, 3 patients (15%) refused to undergo anoscopy; in the other patients, anoscopy with application of 5% acetic acid solution and subsequent biopsy led to the histological diagnosis of L-SIL (AIN I) in 15 (88.2%) cases and of H-SIL (AIN II-III) in 1 (5.9%) case. One patient received a diagnosis of infectious proctitis caused by the simultaneous presence of *C. trachomatis*, *Mycoplasma genitalium*, *Mycoplasma hominis* and *Ureaplasma urealyticum*.

All 3 patients who had ASC-H on anal Pap test underwent anoscopy with biopsy: 2 (66.7%) of them had a histological diagnosis of AIN I; only 1 (33.3%) patient resulted in AIN II-III.

Among the 14 patients with cytological report of ASC-US, anoscopy was negative in 5 cases (35.7%). In 4 patients (28.5%), the histological examination identified a nonspecific inflammation: in 1 patient, the cytological examination also showed eggs of *Enterobius vermicularis*, for which the specific antihelminthic therapy was undertaken.

In the remaining 5 patients with ASC-US on Pap test, the anoscopy with subsequent biopsy and histological examination of the suspected aceto-white lesions led to the diagnosis of AIN I in 4 cases (28.6%) and of AIN II-III in 1 case (7.1% of the total ASCUS).

The positive predictive value (PPV) of anal cytology was 64.3% in predicting histologic AIN I+ and 14.3% in predicting histologic AIN II+.

## Discussion

Comparing data from HIV-infected and -uninfected homosexual males, our study revealed a significantly higher rate of anal cytological abnormalities in HIV-positive patients (47.2% versus 33.3%;  $p = 0.018$ ).

We found more L-SIL and H-SIL cases in HIV-positive MSM than in HIV-negative MSM ( $p = 0.04$ ).

Comparing the results of the present study with data obtained from a previous cohort of patients subjected to conventional anal Pap smear,<sup>9</sup> we observed a higher detection rate of anal cytological abnormalities with the use of liquid-based technique, probably due to the reduction of the “darkening factors” that were typical of conventional method: fecal and bacterial contamination, inflammation, and air-drying artifacts.<sup>21–23</sup>

While the detection rate of ASC-US and ASC-H did not undergo significant changes using the 2 different techniques, the number of L-SIL (18 versus 5.7%) and H-SIL (4.5% versus 0) cases showed a substantial increase. The difference between conventional and liquid-based anal cytology for detection of L-SIL and H-SIL was statistically significant ( $p = 0.022$ ); in contrast, the difference was not statistically significant if all cytological abnormalities were considered ( $p = 0.19$ ).

We found a rate of unsatisfactory samples of 10.8% using the liquid-based cytological technique, while in the previous study,<sup>9</sup> anal Pap test was considered inconclusive/inadequate in 16.1% of the group tested with the conventional method.

Literature data did not show significant differences in sensitivity and specificity of cervical cytology to detect abnormal histologic diagnoses between conventional and

liquid-based techniques.<sup>25</sup> Studies of comparison between these two cytological techniques in anal cytology are more limited.<sup>21–23,26</sup>

In our retrospective analysis, although limited by a small number of patients studied with various characteristics, the use of the liquid-based cytological technique has shown to be able to accurately read a greater number of anal cytological abnormalities compared with conventional Pap smears.

The PPV of anal cytology was ~47.8% using a conventional slides preparation technique, as we had found 11 cases of AIN I+ among 23 abnormal Pap smears.<sup>9</sup> Instead, using a liquid-based technique, the PPV rose up to 64.3%: in particular, we identified 27 cases of histological abnormalities AIN I+ among 42 abnormal Pap tests ( $p = 0.02$ ).

Compared with the “end-point” of a histologically confirmed diagnosis of AIN II+, the PPV of anal cytology was 14.3% using liquid-based slides and 4.3% using conventional slides<sup>9</sup> ( $p = 0.019$ ).

From this indirect, retrospective comparison, we can deduce that liquid-based cytology, reducing the “darkening factors” typical of the conventional smears, is more diagnostic and allows us to have a higher PPV than the traditional technique. A prospective study that directly compares the two cytological techniques with the aim of confirming these observations needs to be conducted in the near future.

The greater diagnostic accuracy of liquid-based cytology can be also attributed to the different sampling technique: indeed, the cells obtained with this method, although in a smaller number, are not altered by rolling the swab directly on the glass slide, which is a traumatic and strongly operator-dependent maneuver.

Out of the 6 cases who had a histologically-confirmed diagnosis of AIN II and above in our study, if referral to anoscopy was triggered by H-SIL alone, only 3 cases (50%) would have been diagnosed on liquid-based cytology. This is in accordance with previous findings<sup>11,12,27</sup> that showed that anal cytology was a poor predictor of the histologic grade of severity of lesions identified after biopsy.

Weiss et al.<sup>28</sup> reported that even 67% of the cases of ASCUS corresponded to the presence of a high grade-AIN lesion on histological examination.

Compared with anal cytology, cervical cytology shows a greater degree of correlation with the histopathological findings<sup>29</sup>: in fact, a cytological report of ASC-US or L-SIL hides the presence of a high-grade-CIN in only between 5 and 17% of cases.<sup>30</sup> Instead, anal cytology has a high degree of correlation with histology only for the high-grade dysplasia (H-SIL), while there is a poor cytohistological agreement for the low-grade lesions (ASC-US / L-SIL).<sup>29</sup>

In our study in all three patients with H-SIL on Pap test who underwent anoscopy, we found a high-grade lesion (two cases of histologically confirmed AIN II-III/carcinoma in situ and one case of invasive carcinoma), but we also identified three cases of AIN II-III in patients with a cytological report of ASC-US, L-SIL and ASC-H, respectively.

Our findings are in accordance with literature data,<sup>9,11,12,17–19</sup> also confirming that a cytological diagnosis of low-grade lesion, such as L-SIL or ASC-US, may effectively

hide a severe dysplasia or even a carcinoma, which can be confirmed only with an accurate anoscopy and subsequent biopsy for histological examination. This suggests that all patients with an abnormal screening anal cytological result at any grade should be considered for anoscopy, preferably high-resolution anoscopy (HRA).<sup>26–29</sup>

## Conclusions

Anal cytology as a screening tool for the detection of HPV-related precancerous lesions and anal cancer is becoming more useful, especially in “high-risk” groups, such as HIV-positive patients and MSM.

Many factors, including air-drying and mechanical artifacts, bacteria, fecal material, inflammation, and scant cellularity, may compromise the adequacy of anal cytological smears prepared with the conventional technique. Liquid-based cytology, reducing these obscuring factors, allows us to obtain more satisfactory cytological samples with a higher number of diagnoses of SILs. Therefore, the use of the liquid-based technique may dramatically increase the utility of anal cytological screening.

However, even in well-prepared liquid-based slides, anal cytology continues to show a poor degree of correlation with histological findings; so, all patients with an abnormal anal Pap test, ASC-US or worse, should undergo anoscopy (preferably HRA) and eventually biopsy, which remains the “gold standard” for the diagnosis of AIN.

The lack of HRA investigation results in patients with negative cytology represents an important limitation of our work. A larger prospective study directly comparing liquid-based cytology with the conventional method and involving HRA and biopsy for all subjects undergoing anal cytological screening is necessary.

### Conflict of Interests

The authors have no conflict of interests to declare.

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