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The performance of anal cytology as a screening test for anal high-grade squamous intraepithelial lesions in homosexual men

Running title: anal cytology performance in homosexual men

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Abstract

Background: Studies on the performance of anal cytology in which both the screening test (cytology) and the diagnostic test (high-resolution anoscopy, HRA) are performed in all members of a screening population are rare. We evaluated the performance of liquid-based anal cytology in a cohort of homosexual men in Sydney.

Methods: The Study of the Prevention of Anal Cancer (SPANC) is a three-year prospective study of the natural history of anal human papillomavirus infection in homosexual men aged ≥ 35 years. At baseline, all participants underwent a liquid-based anal cytology test and HRA at the same clinical visit. Biopsies were taken for histological assessment if lesions suspicious of human papillomavirus infection were visible during HRA. Using any cytological abnormality as the threshold, sensitivity, specificity, positive and negative predictive values (PPV and NPV) were calculated against histologically diagnosed high-grade squamous intraepithelial lesions (HSIL).

Results: Among 617 men recruited, the median age was 49 years (range: 35-79) and 35.7% were HIV-positive. Overall, the sensitivity of cytology was 83.2%, specificity was 52.6%, PPV was 45.8% and NPV was 86.7%. Specificity improved with increasing age (p trend=0.041). Sensitivity was significantly higher in men with more than one anal octant of biopsy-confirmed HSIL (92.9% vs 77.7%, p=0.010), and in those who had 10 or more metaplastic cells present on their cytology slides (87.5% vs 70.2%, p=0.007).

Conclusion: Anal cytology had a higher specificity in older men while maintaining sensitivity. Sensitivity was higher among those with more extensive HSIL and those with metaplastic cells present on cytology.

Keywords: anal cytology; homosexuality, male; anal cancer; human papillomavirus; Australia.

Introduction

Anal cancer incidence has increased in developed nations over the past three decades, but remains uncommon in the general population [1-5]. Certain populations have a greatly increased risk, including homosexual men, people with HIV, and women with previous genital human papillomavirus (HPV)-related disease [6-11].

Anal squamous cell carcinoma shares a number of similarities with its cervical counterpart. Both have a causal association with high-risk human papillomavirus (HR-HPV), presumed origin at a mucosal transformation zone, and have a stage of intraepithelial cancer precursor – high-grade squamous intraepithelial lesion (HSIL) – that can be detected by exfoliative cytology [12]. Well-organised cervical cancer screening programs have led to marked reductions in the incidence of cervical cancer [3, 13, 14]. It has been proposed that cytology-based anal cancer screening programs may similarly lead to a reduction in cancer incidence. Such programs have been implemented in a limited number of settings, mostly in clinical settings providing care to homosexual men with HIV [12, 15]. As with cervical screening, people who have a degree of anal cytological abnormality above a threshold are referred for a diagnostic test, in this case high-resolution anoscopy (HRA)-guided biopsy, for histological assessment.

There have been relatively few studies of anal cytology test performance for the detection of histologically confirmed HSIL. Recent reviews have highlighted the wide variations in reported sensitivity and specificity of cytology in the detection of histologically confirmed anal HSIL [16-18]. Most of these studies are based on small clinic-based samples of homosexual men with HIV, and the largest previous study included only 401 patients [19]. Analysis of screening test sensitivity requires that the diagnostic test (HRA) is performed on all study participants, not just those who screen positive, in order to identify false-negative

cases. However, such studies are uncommon probably because of the researchers' reluctance to subject study volunteers to this relatively invasive diagnostic test. To our knowledge, only six studies that have performed liquid-based anal cytology and HRA at the same visit and recruited more than 100 participants have been reported (Table 1 [19-24]).

In this study, we reported the performance of liquid-based anal cytology in the detection of histological HSIL in the baseline visit of a cohort of HIV-negative and –positive homosexual men in Sydney, Australia. All men underwent the screening anal cytology test and the diagnostic HRA at the same study visit.

Methods

Participants

Participants were men in the Study of the Prevention of Anal Cancer (SPANC). The methods of the study have been described in detail elsewhere [25]. Briefly, the SPANC study is an ongoing longitudinal study of the natural history of anal HPV infection and its associated anal squamous cellular abnormalities. Men who were aged 35 years and above and reported having sex with another man in their lifetime were eligible. Those who had a previous HRA or a history of anal cancer were excluded. Signed informed consent was obtained from all participants. Ethics approval was granted by the Human Research Ethics Committees at the St Vincent's Hospital, Sydney and the University of New South Wales.

Participants were recruited mainly from community-based settings in Sydney, including gay-community social events and organisations, referrals from other participants. In addition, about 35% of HIV-positive and 5% of HIV-negative men were recruited through clinics.

Anal cytology

A Dacron® swab moistened with tap water was used to sample the anal canal without the aid of an anoscope by the study clinician. Immediately after sampling, the swab was rinsed in a vial containing 20 ml of PreservCyt (Hologic, Inc., Marlborough, MA, United States) fixative medium.

At a specialist anogenital pathology laboratory, a ThinPrep slide (Hologic Inc., Marlborough, MA, United States) was produced and manually screened by an experienced study cytologist. One of the three study pathologists was responsible for final reporting using the Bethesda System (TBS) 2001 criteria and terms [26]. A 'satisfactory' slide was defined as having at least 2,000 nucleated squamous cells [27]. A repeat anal cytology test was performed no less

than two weeks later in participants with an initial unsatisfactory cytological assessment (n=61, 9.9%). Participants with two consecutive unsatisfactory anal cytology tests were excluded from the analyses. The presence or absence of a transformation zone component on cytology (10 or more rectal columnar cells and/or 10 or more squamous metaplastic cells, as defined by the Bethesda System [26]) was recorded but was not included in the definition of satisfactory status. The cytological results for satisfactory slides were classified as negative, atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesion (LSIL), atypical squamous cells, cannot exclude HSIL (ASC-H), or HSIL.

High-resolution anoscopy and histology

High-resolution anoscopy was performed immediately after the anal cytology collection. Biopsies of visual abnormalities suspicious of HPV-related lesions were taken and formalin-fixed for processing and histopathological assessment.

At the same laboratory where cytological assessments were conducted, Haematoxylin and Eosin (H&E) stained slides of biopsy specimens were prepared and examined by one of the three experienced pathologists. Reporting of the biopsies was performed blinded to cytology result and in accordance with criteria, terminology and recommendations of the Lower Anogenital Squamous Terminology (LAST) Project [28, 29]. Results were reported as negative, exophytic LSIL, flat LSIL, HSIL-AIN2, HSIL-AIN3, or squamous cell carcinoma. As recommended by LAST, p16INK4a (p16) immunostain was used to confirm all HSIL-AIN2 diagnoses. When H&E stained biopsies were considered to represent HSIL-AIN2, p16 immunostaining was performed on an unstained spare slide. Biopsies which were p16 positive were then finally confirmed as HSIL-AIN2, and p16-negative biopsies were then reported as LSIL or negative, depending on other criteria. When multiple biopsies were

taken, the most severe result was used as the histological diagnosis in the analysis. Biopsies were not taken in participants who were assessed to have no visual abnormalities suggestive of SIL on HRA examination. These men were classified as being negative for squamous intraepithelial lesions (SIL).

Statistical analyses

Statistical analyses were performed using STATA 14.0 (STATA Corporation, College Station, TX). Existing guidelines suggest that the cytological threshold for HRA referral should be any cytological abnormality, including ASC-US, LSIL, ASC-H and HSIL [30]. The referral rate was defined as the proportion who had any cytological abnormality, as these men would be referred to diagnostic HRA in a screening setting. We examined the performance characteristics of ASC-US + cytology in the detection of histologically diagnosed HSIL. In addition, we compared the performance characteristics in the detection of histological HSIL-AIN2 and HSIL-AIN3. The following test performance characteristics were calculated:

Sensitivity: the proportion of participants with histologically diagnosed HSIL, who had any cytological abnormality;

Specificity: the proportion of participants with no histologically diagnosed HSIL who had negative cytology;

Positive predictive value (PPV): the proportion of participants with cytological abnormality who had histologically diagnosed HSIL;

Negative predictive value (NPV): the proportion of participants who had negative anal cytology who had no histologically diagnosed HSIL.

Further analyses were carried out to examine whether the performance of anal cytology differed in different sub-groups as determined by 1) participants' characteristics, including

age and HIV status; 2) the nature of the lesion, including the extent of histological HSIL and the number of biopsies taken; and 3) sample cellular composition, including the presence of rectal columnar cells and/or squamous metaplastic cells. For age associated performance, participants were regrouped into 35-44 years, 45-49 years, 50-54 years and 55 years and above by roughly equal numbers in each age group. The extent of HSIL disease was measured by the number of octants of the anal mucosa where HSIL was detected on biopsy. The octants were described as anterior, posterior, left and right anterior, left and right posterior, and left and right lateral. Performance was compared between those who had HSIL affecting a single octant and those who had HSIL affecting multiple octants.

Results

The median age of the 617 SPANC participants was 49 years (range: 35-79). Nearly all men (95.3%) identified as gay or homosexual. More than a third (35.7%) of men were HIV-positive. The great majority of these (93.6%) was currently on anti-retroviral treatment, reported an undetectable viral load (89.5%) and a CD4 count of more than 350 cells/µl (88.0%).

A total of 588 participants had both cytological and histological assessment results available. Twenty-nine men (4.7%) whose first and repeat cytology tests were unsatisfactory were excluded, including one man (0.2%) who was unable to tolerate HRA. In the remaining 588 men, anal cytology results were as follows: negative 241(41.0%), ASC-US 103 (17.5%), LSIL 47 (8.0%), ASC-H 88 (15.0%), and HSIL 109 (18.5%, Table 2). Hence, using any cytological abnormality as the threshold, the referral rate was 59%.

Based on HRA guided biopsy in the 588 men with a satisfactory cytology result, the histological results were as follows: negative for SIL 136 (23.1%), LSIL 146 (24.8%), HSIL-AIN2 49 (8.3%), and HSIL-AIN3 or worse 142 (24.2%, including one participant diagnosed with squamous cell carcinoma). In addition, 115 (19.6%) participants had a visually normal HRA and therefore did not have a biopsy taken. The correlation between cytological and histological results is shown in Table 2.

Anal cytology performance

Predicting histological HSIL

Performance characteristics at threshold of any anal cytological abnormality in detection of histological HSIL were as follows: sensitivity 83.2% (95% CI 77.2-88.2), specificity 52.6%

(95% CI 47.6-57.6), PPV 45.8% (95% CI 40.5-51.2), and NPV 86.7% (95% CI 81.8-90.7, Table 3).

Performance in relation to participants

There were no differences in test sensitivity (p=0.924) and specificity (p=0.116) between HIV-positive and HIV-negative men, but because HIV-positive men had more cytological abnormalities, they had a significantly higher referral rate (66.2% vs 54.9%, p=0.007), a higher PPV (p=0.013) and a lower NPV (p=0.027), than HIV-negative men.

In relation to participants' age, there was a trend towards decreased referral rate in older men, but this did not reach the significant level (61.6% in those aged under 45 years and 56.1% in those aged above 55 years, p trend=0.131). There were trends towards increased sensitivity, specificity, PPV and NPV with increasing age and this was statistically significant for specificity (p trend=0.041).

Performance in relation to lesion

Sensitivity was higher in men who had histological HSIL affecting more than one octant compared with those with HSIL present in one octant only (92.9% vs 77.7%, p=0.010). Sensitivity was also higher in men who had more biopsies taken (p trend=0.077), but this was accompanied by a significant decrease in specificity (p trend<0.001, Table 3).

Performance in relation to sample cellular composition

Cytological slides with the presence of 10 or more metaplastic cells had significantly higher sensitivity (87.5% vs 70.2%, p=0.007), but lower specificity (48.3% vs 64.2%, p=0.005) than those had fewer than 10 metaplastic cells present. In contrast, the presence of columnar cells

was not associated with performance (p=0.583 for sensitivity and p=0.490 for specificity comparison, Table 3).

Comparison of test performance in the detection of HSIL-AIN2 and HSIL-AIN3

A comparison of performance characteristics in the detection of HSIL-AIN2 and HSIL-AIN3 is shown in Table 4. Anal cytology had significantly higher sensitivity (p=0.015) and PPV (p<0.001) in detecting AIN3 than in detecting AIN2. In relation to HIV status, the NPV was significantly lower in HIV-positive than in HIV-negative participants in the detection of AIN2 (p=0.024), and the PPV was significantly higher in HIV-positive and in HIV-negative participants in the detection of AIN3 (p=0.004).

Discussion

In homosexual men mainly recruited from community-based settings in Sydney, 59% had abnormal cytology and 35% had histological HSIL. The overall sensitivity of liquid-based anal cytology in the detection of histological HSIL was just above 80%, and the specificity was 53%. The sensitivity and specificity did not differ between HIV-positive and HIV-negative men, although PPV was significantly higher (53.9% vs 40.3%) and NPV was lower (79.2% vs 89.9%) in the HIV-positive. The specificity of anal cytology increased with age. Sensitivity increased significantly in those with HSIL affecting more octants and those with more biopsies taken.

The performance of anal cytology is subject to operational and methodological variations [16-18, 31]. In interpreting the findings of the test performance of anal and cervical cytology, the histological diagnoses arising from HRA- and colposcopy- guided biopsies are traditionally accepted as the "gold" standard [31-34]. However, there is evidence that HRA is technically more difficult than colposcopy. There are convoluted mucosal folds of the anal canal, and performance characteristics of HRA also vary with the training and experience of the anoscopist [20, 35, 36]. For these reasons, it is accepted that HRA-guided biopsy is more likely to miss an existing HSIL than is cervical colposcopy [37]. Thus, some positive cytological findings in which no histological HSIL is found may represent a false negative HRA rather than a false positive cytology. If this is the case, studies such as ours may under-estimate true specificity. In the present study, around one-third of cytological HSIL were not confirmed by HRA-guided biopsy at baseline. Future analyses have been planned to determine how this varied by anoscopists' experience, and whether histological HSIL was diagnosed at the next study visit in these men.

Recent studies in which liquid-based screening cytology and diagnostic HRA were performed on all participants generally reported sensitivities comparable to the present study, but lower specificities [19-24]. In three clinic-based studies conducted in the UK, US and Canada, the reported sensitivity ranged from 81% to 89% and specificity ranged from 37% to 39% [19, 21, 22]. Similarly, a recent Spanish clinic-based study of 101 patients reported a sensitivity of 83% and a specificity of 41% [24]. Conversely, a Thai clinic-based study of 246 patients reported a substantially lower sensitivity of 19% and a much higher specificity of 88% [23]. Participants in the Thai study were much younger with a mean age of 29 years, while the other studies involved older participants with a mean/median age from the late 30s to the early 50s. The difference in participant age composition and variations in clinical experience in anal cytology and HRA may have contributed to the differences in reported sensitivities and specificities.

The sensitivity of liquid-based anal cytology in detection of HSIL is similar to that of cervical cytology at round 80%, but the specificity is much lower compared with the latter, which is usually close to 90% [38]. However, it is important to note that cervical cytology has a higher threshold for immediate referral in routine practice in most countries with organised screening programs including Australia. This threshold is usually ASC-H. Although histological HSIL was most common in those with cytological HSIL (67%), between 28% (ASC-US) and 48% (ASC-H) of those with less severe cytological abnormalities had histologically confirmed HSIL in the present study. This suggests that increasing referral threshold in anal cytology is imprudent when the population at risk is homosexual men.

The relationship between HIV status and anal cytology performance is inconsistent across studies. The present study found almost identical sensitivity between HIV-positive and HIV-negative participants while the specificity was non-significantly higher in the latter. A study

of 125 homosexual men identified through random digit dialing in San Francisco found the specificity was significantly higher in HIV-negative than in -positive men (76% vs 47%, p=0.02) [20]. Interestingly, the aforementioned UK study found no association between sensitivity and HIV status. Rather, a CD4 count of lower than 400 cells/µl was identified as a strong predictor of higher sensitivity [21]. Since only 12% (n=25) of HIV-positive men in our study had a recent CD4 count of <350 cells/µl, we were unable to perform a meaningful analysis of the effect of CD4 count on test performance.

As in our study, an association between improved specificity and increasing age has been observed in cervical cytology [39], but it has not been previously documented in anal cytology. In men with more frequent acute or transient HPV infections which produce LSIL lesions, specificity may be expected to be lower than those with fewer such LSIL lesions. This is because cytological abnormality can reflect the presence of either histological LSIL or HSIL. When cytology is abnormal due to histological LSIL, this result is regarded as being false positive because no histological HSIL is found and thus the specificity lowers. We postulate that in older homosexual men, a higher proportion of HPV infections may be chronic, due to their longer lifetime history of exposure. Therefore, in these men, abnormal cytological changes are more likely to be a true marker of histological HSIL, resulting in higher specificity. It is reassuring that anal cytology is capable of detecting anal cancer precursor with higher accuracy in older men in whom interventions to prevent cancer development may be more urgent.

Similar to a previous study [21], we also found an increased sensitivity of anal cytology in detection of HSIL in those in whom the disease affects a larger area. It is likely that abnormal squamous cells are more easily exfoliated from a larger HSIL surface area during anal sampling. An increasing number of biopsies may also be a marker of higher burden of

disease. Taking more biopsies may also contribute to a lower likelihood of missing high-grade lesions, therefore improving agreement between anal cytology and HRA. This has been shown to be true in the cervix [40]. If taking more biopsies is also a marker of increased likelihood of any anal lesion being present and the lesion is LSIL, the cytological abnormality detected will be regarded as being false-positive, as previously discussed, thus lowering specificity.

Cell composition of the anal sample is another determinant of anal cytology performance. We found that the presence of 10 or more metaplastic cells was associated with higher sensitivity. Metaplastic cells originate from the transformation zone, the area in which most anal HSIL arises, so it is not surprising that the presence of metaplastic cells predicts a higher sensitivity. On the other hand, and consistent with another study [12], the presence of columnar cells (derived from just superior to the transformation zone), was not associated with anal cytology performance. As the presence of metaplastic cells and columnar cells are not independent of each other, we plan to further investigate the importance of these two cell types.

As the largest study so far that is capable of assessing the performance of anal cytology, one of the strengths of SPANC is that the majority of participants were recruited from community-based settings. Thus, performance characteristics, particularly PPV and NPV that are associated with disease prevalence, are more likely to be representative of homosexual men in general. Clinic-based cohorts tend to focus on HIV-positive men who have a higher prevalence of HSIL, which is associated with a higher PPV of the screening test [41]. Also, p16 staining was universally applied to all lesions suspicious of histological HSIL-AIN2, a diagnosis which is prone to subjective operator bias and traditionally has poor reproducibility [28, 29]. In a SPANC sub-study, a very high degree of inter- and intra-

pathologist diagnostic reproducibility was found partially due to the use of p16 staining [42]. To our knowledge, this is the first published study assessing the performance of anal cytology following the LAST recommendations. The performance characteristics in the detection of HSIL-AIN2 are presented separately to HSIL-AIN3 for ease of cross-study comparison. The other strength worth noting is that both anoscopists and pathologists were blind to participants' medical history (except HIV status for the anoscopists) at baseline. This is as would be the case when a screening program is implemented in a community-based setting. One of the limitations of the study is that most of anoscopists were newly trained by the lead anoscopist (RJH) during the study. However, stringent training procedures (observing 50 HRA procedures then another 50 HRA procedures under supervision before contributing study data) ensured that all anoscopists were skilled with all necessary techniques in a standardised fashion to minimise operator bias. Furthermore, all anoscopists had regular reviews for quality assurance. As a sensitivity analysis, the performance characteristics were compared between the more experienced anoscopists (those who performed more than 200 HRAs) and those less experienced, and there were no significant differences between the two groups (data not shown).

Liquid-based anal cytology as an anal cancer screening test had a higher specificity in older men, who generally have higher cancer risk than their younger counterparts. However, the fact that close to 60% of men recruited largely from community-based settings have any cytology abnormality would result in a very high demand for referral HRA, should a screening program in its current form be implemented. Given the invasive nature of HRA, this might hinder screening uptake in this high-risk population. Further, the trained medical workforce may be unable to meet the demand for referral service even in resource-rich countries like Australia. The value of additional tests, such as HPV biomarkers, should be investigated as part of the development of an anal screening program.

Table 1. Studies[1] examining the performance of anal cytology in a population in which cytology and histology were performed on all participants

Studies	Year of publication	Setting	Number of participants	% HIV-positive	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Berry et al[20]	2009	Community-based	125	28	87 (HIV+) 55 (HIV-)	47 (HIV+) 76 (HIV-)	57(HIV+) 42(HIV-)	82(HIV+) 84(HIV-)
Nathan et al. [21]	2010	Clinic-based	395	54	81	37	30	85
Salit et al. [19]	2010	Clinic-based	401	100	84	39	31	88
Wentzensen, et al.[22]	2012	Clinic-based	363	100	89	39	31	92
Phanuphak, et al. [23]	2013	Clinic-based	246	50	19	88	20	87
Sendagorta, et al.[24]	2015	Clinic-based	101	100	83	41	55	73

[1]Including studies that recruited more than 100 participants and performed screening cytology and diagnostic high-resolution anoscopy on all participants.
PPV: positive predictive value; NPV: negative predictive value

Table 2. The correlation between anal cytology and histology results in 588 men who had adequate cytology results at the baseline visit in the Study of the Prevention of Anal Cancer

	Histology					
Cytology	**No biopsy**	**Negative**	**LSIL**	**AIN2**	**AIN3 or worse**	**Total**
Negative	72	97	40	14	18	241 (41.0%)
ASC-US	17	17	40	9	20	103 (17.5%)
LSIL	3	3	26	6	9	47 (8.0%)
ASC-H	26	9	21	9	33	88 (15.0%
HSIL	7	10	19	11	62	109 (18.5%)
Total	115 (19.6%)	136 (23.1%)	146 (24.8%)	49 (8.3%)	142 (24.2%)	588

ACS-US, atypical squamous cell of undetermined significance; ACS-H, atypical squamous cells – cannot exclude high-grade lesion; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; AIN2, anal intraepithelial neoplasia grade 2; AIN3, anal intraepithelial neoplasia grade 3.

Table 3. The performance of anal cytology in the detection of histological anal high-grade squamous intraepithelial lesions in the Study of the Prevention of Anal Cancer

	N	Sensitivity (%)	95% CI	Specificity (%)	95% CI	PPV (%)	95% CI	NPV (%)	95% CI
ASC-US or worse		83.2	77.2-88.2	52.6	47.6-57.6	45.8	40.5-51.2	86.7	81.8-90.7
Participants									
By HIV status									
HIV negative	375	83.0	74.2-89.8	55.3	49.2-61.2	*40.3*	33.5-47.3	*89.9*	84.4-94.0
HIV positive	213	83.5	74.3-90.5	46.7	37.6-56.0	*53.9*	45.3-62.3	*79.2*	68.0-87.8
By age				*					
<45	190	82.5	70.1-91.3	*47.4*	38.7-56.2	40.2	31.2-49.6	86.3	76.2-93.2
45-49	115	85.7	71.5-94.6	*46.6*	34.8-58.6	48.0	36.3-59.8	85.0	70.2-94.3
50-54	110	79.4	62.1-91.3	*59.2*	47.3-70.4	46.6	33.3-60.1	86.5	74.2-94.4
≥55	173	84.5	72.6-92.7	*58.3*	48.7-67.4	50.5	40.2-60.8	88.2	78.7-94.4
Lesion									
By HSIL affected area									
Single octant	121	*77.7*	69.2-84.8	52.6	47.6-57.6	33.3	27.9-39.2	88.6	83.8-92.3
Multiple octants	70	*92.9*	84.1-97.6	52.6	47.6-57.6	25.7	20.4-31.5	97.7	94.6-99.2
By Number of biopsies taken									
1	169	*75.4*	62.2-85.9	*67.9*	58.4-76.4	54.4	42.8-65.7	84.4	75.3-91.2
2	169	*85.5*	75.6-92.5	*46.2*	35.8-56.9	56.5	47.0-65.7	79.6	66.5-89.4
3 or more	135	*87.9*	76.7-95.0	*23.4*	14.5-34.4	46.4	36.8-56.1	72.0	50.6-87.9
Sampling									
Presence of metaplastic cells									
<10	156	*70.2*	55.1-82.7	*64.2*	54.5-73.2	45.8	34.0-58.0	83.3	73.6-90.6
≥10	432	*87.5*	81.0-92.4	*48.3*	42.4-54.2	45.8	39.8-51.9	88.5	82.5-93.1
Presence of rectal columnar cells									
<10	259	81.7	72.4-89.0	50.6	42.7-58.4	48.1	40.1-56.2	83.2	74.4-89.9
≥10	329	84.7	76.0-91.2	54.1	47.5-60.7	43.9	36.7-51.3	89.3	82.9-93.9

PPV: positive predictive value; NPV: negative predictive value; ACS-US, atypical squamous cell of undetermined significance; HSIL, high-grade squamous intraepithelial lesion.
Values in italic designate performances with statistically significant differences.

Table 4. **A comparison of anal cytology performance in the detection of histological anal high-grade squamous intraepithelial lesions between anal intraepithelial neoplasia grade 2 and grade 3 (AIN2 and AIN3) in the Study of the Prevention of Anal Cancer**

	n lesion present	n lesion absent	%	Sensitivity (%)	95% CI	Specificity (%)	95% CI	PPV (%)	95% CI	NPV (%)	95% CI
Histological HSIL-AIN2	50	397	11.2	*72.0*	57.5-83.8	52.6	47.6-57.6	*16.1*	11.5-21.5	93.7	89.7-96.5
Histological HSIL-AIN3	141	397	26.2	*87.2*	80.6-92.3	52.6	47.6-57.6	*39.5*	34.1-45.2	92.1	87.8-95.2
Histological HSIL-AIN2											
By HIV status											
HIV negative	29	275	9.5	79.3	60.3-92.0	55.3	49.2-61.2	15.8	10.3-22.7	*96.2*	91.9-98.6
HIV positive	21	122	14.7	61.9	38.4-81.9	46.7	37.6-56.0	16.7	9.2-26.8	*87.7*	77.2-94.5
By age											
<45	15	133	10.1	66.7	38.4-88.2	47.4	38.7-56.2	12.5	6.2-21.8	92.6	83.7-97.6
45-49	14	73	16.1	85.7	57.2-98.2	46.6	34.8-58.6	23.5	12.8-37.5	94.4	81.3-99.3
40-54	10	76	11.6	60.0	26.2-87.8	59.2	47.3-70.4	16.2	6.2-32.0	91.8	80.4-97.7
≥55	11	115	8.7	72.7	39.0-94.0	58.3	48.7-67.4	14.3	6.4-26.2	95.7	88.0-99.1
Histological HSIL-AIN3											
By HIV status											
HIV negative	71	275	20.5	84.5	74.0-92.0	55.3	49.2-61.2	*32.8*	26.0-40.1	93.3	88.2-96.6
HIV positive	70	122	36.5	90.0	80.5-95.9	46.7	37.6-56.0	*49.2*	40.3-58.2	89.1	78.8-95.5
By age											
<45	42	133	24.0	88.1	74.4-96.0	47.4	38.7-56.2	34.6	25.6-44.4	92.6	83.7-97.6
45-49	28	73	27.7	85.7	67.3-96.0	46.6	34.8-58.6	38.1	26.1-51.2	89.5	75.2-97.1
≥50	24	76	24.0	87.5	67.6-97.3	59.2	47.3-70.4	40.4	27.0-54.9	93.8	82.8-98.7
≥55	47	115	29.0	87.2	74.4-95.2	58.3	48.7-67.4	46.1	35.4-57.0	91.8	83.0-96.9

HSIL-AIN2, anal intraepithelial neoplasia grade 2; HSIL-AIN3, anal intraepithelial neoplasia grade 3. Values in italic designate performances with statistically significant differences.

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