PERFORMANCE OF ANAL CYTOLOGY COMPARED WITH HIGH-RESOLUTION ANOSCOPY AND HISTOLOGY IN WOMEN WITH LOWER ANOGENITAL TRACT NEOPLASIA

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Short title: Anal cytology in high-risk women.

Summary:

A history of vulvar high-grade lesions (HSIL)/cancer, immunosuppression and concomitant genital HSIL/cancer were associated with abnormal anal cytology in women. Sensitivity of anal cytology for anal HSIL/cancer detection was significantly higher in those immunosuppressed and with more extensive disease.

ABSTRACT

BACKGROUND: Information on the performance of anal cytology in women who are high-risk for human papillomavirus-related lesions and the factors that might influence it are largely lacking.

AIMS: Evaluate the performance of anal cytology in women with lower anogenital tract neoplasia.

METHODS: retrospective study including all new referrals of women with a previous history of anogenital neoplasia, from January 2012 to July 2017, with concomitant anal cytology and high-resolution anoscopy with or without biopsies.

RESULTS: 636 anal cytology samples and 323 biopsies were obtained from 278 women. Overall sensitivity and specificity of 'any abnormality' on anal cytology to predict 'any abnormality' in histology was 47% (95% CI 41–54%) and 84% (95% CI 73–91%), respectively. For detecting high-grade squamous intraepithelial lesions (HSIL)/cancer, sensitivity was 71% (95% CI 61–79%) and specificity was 73% (95% CI 66–79%). There was a poor concordance between cytological and histological grades (κ=0.147). Cytology had a higher sensitivity to predict HSIL/cancer in immunosuppressed *vs.* non-immunosuppressed patients (92% *vs.* 60%, *P*=0.002). The sensitivity for HSIL detection was higher when two or more quadrants were affected in comparison with only one (86% *vs.* 57%, *P*=0.006). A previous history of vulvar HSIL/cancer (OR 1.71, 1.08–2.73; *P*=0.023), immunosuppression (OR 1.88, 1.17–3.03; *P*=0.009) and concomitant genital HSIL/cancer (OR 2.51, 1.47–4.29; *P*=0.001) were risk factors for abnormal cytology.

CONCLUSIONS: Patient characteristics can influence the performance of anal cytology in women. The sensitivity for detecting anal HSIL/cancer was higher in those immunosuppressed and with more extensive disease.

KEYWORDS: anal cytology; high-resolution anoscopy; anal histology; women; lower anogenital tract neoplasia.

INTRODUCTION

Women with a previous history of human papillomavirus (HPV) related lower genital tract high-grade squamous intraepithelial lesions (HSIL) or cancer are a high-risk group for anal cancer [1, 2, 3]. A 13-fold increase in risk has been described, with a higher risk in those with previous vulvar lesions [3]. It has been reported that women diagnosed with anal cancer are 10 times more likely to have a history of HPV-related gynecological cancer as compared to matched controls. Several studies have also found a higher risk of anal squamous intraepithelial lesions (ASIL)/anal intraepithelial neoplasia (AIN) in patients with lower genital tract neoplasia (LGTN) [4, 5, 6].

A reduction in cervical cancer rates and mortality has been achieved through cervical cancer screening based on cervical cytology [7]. Following the parallels recognized between anal and cervical carcinogenesis, a similar strategy based on cytology, with referral of those with abnormalities to high-resolution anoscopy (HRA) has been advocated (by some) for high-risk groups [8]. In high-risk women (e.g. HIV-positive) anal cytology screening has been recommended by the Infectious Disease Society of America [9] and is routinely performed in some institutions [10].

Most of the studies that have evaluated the performance of anal cytology in comparison to HRA and histology were performed in HIV-positive men and/or men-who-have sex with men (MSM) [11-16], with few that exclusively focus on high-risk women. This is a less well-studied group and information on the sensitivity and specificity of anal cytology relative to HRA and histology is scant. Besides the limited number of published studies, in most cases with small samples of cytology/histology for comparison and low rates of abnormal results, there is also significant variation in the reported sensitivity for detecting histological abnormalities, ranging from 8% [17] to 70% [18]. There is a need for data from larger patient cohorts and for evaluation of the correlation between cytology and HRA/histology in different settings and in relation to patient and lesion characteristics. This information is required to identify the optimal screening strategy for any given population.

METHODS

Study design, inclusion and exclusion criteria

This was a retrospective study including all new referrals of women with a previous history of anogenital neoplasia to the Homerton Anal Neoplasia Service (HANS), from January 2012 to July 2017. Although, this was a retrospective analysis, patient information was available from prospectively collected data, filled out at the time of the patient consultation. HANS is a tertiary reference centre in London/UK, dedicated to anal neoplasia diagnosis and treatment. Inclusion criteria were 1) women with a previous history of anogenital neoplasia of low-grade (including condyloma), high-grade or cancer; 2) anal cytology and HRA with or without biopsies performed at the same sitting and 3) new referral between January 2012 and July 2017. Paucicellular cytological samples and incomplete/unsatisfactory HRA were excluded. The Health Research Authority approved this research (IRAS 232985).

Outcomes

Our primary outcome was to evaluate the sensitivity, specificity, and positive and negative predictive values (PPV and NPV) of 'any abnormality' on anal cytology (all abnormal results, excluding any negative cytology) to predict findings on HRA and histology in women with history of lower anogenital tract neoplasia. As described in a previous study by our group [16], for comparison with histology, two different outcomes were considered: the presence of any histological abnormality (low-grade, high-grade or cancer) and the presence of HSIL/cancer.

Our secondary outcomes were to evaluate factors that can influence the performance of anal cytology in this population and the concordance between anal cytology and histological grades.

Anal cytology

Anal cytology was always performed before HRA using a sterile flocked swab (MWE, Corsham, UK), with patients in the lithotomy position. The swab was introduced blindly into the anal canal and gradually withdrawn with rotational movement and the application of gentle pressure for 30 seconds. Samples were placed into PreservCyt ThinPrep® solution (Hologic UK, Crawley, UK), stirring for 20 seconds. Experienced operators all trained by M.N. collected the samples.

Anal cytology was read by a limited number of experienced Cytopathologists, all practising at the same institution. Classification of cytology was carried out according to the guidelines for reporting cervical cytopathology in the UK. The most recent National Health Service (NHS) Cervical Screening Programme recommendations (3rd ed.) were published in 2013 [19]. This study enrolled patients from 2012 onward, so the previous classification was also used. The reports of anal cytology (based on cervical cytology) included: negative, borderline changes, borderline changes with koilocytosis, mild/low-grade dyskaryosis, moderate dyskaryosis, severe dyskaryosis and possible invasive squamous invasive carcinoma. Moderate and severe dyskaryosis were considered together as high-grade cytology, whereas all other categories, except negative cytology and invasive, were included in the low-grade category. Four possible cytological categories were therefore used in this study: negative, low-grade, high-grade and squamous cell carcinoma.

High-resolution anoscopy and histology

HRA was performed using an Olympus® colposcope (Tokyo, Japan). Patients were observed in the lithotomy position with a disposable anoscope inserted and a colposcope used to examine the squamocolumnar junction, the anal canal and the perianus after the topical application of 5% acetic acid. Biopsies were obtained using a Tischler punch-biopsy forceps in areas of suspicion for HSIL and/or cancer. After the examination, a diagrammatic representation of the areas of disease was

drawn, with reference to the location, the number of quadrants involved and the diagnostic impression of a normal examination, LSIL, HSIL and/or cancer. Anal and perianal HSIL circumferential extension was described using four possible categories: ≤ 25% (one quadrant), > 25% and ≤ 50% (two quadrants), > 50% and ≤ 75% (three quadrants) and > 75 % (four quadrants).

For this study the Lower Anogenital Squamous Terminology (LAST), including the terms low-grade squamous intraepithelial lesions (LSIL) and high-grade squamous intraepithelial lesions (HSIL), was routinely used [20]. p16 positive AIN2 lesions were considered HSIL and AIN2 p16 negative lesions as LSIL. The presence of a cytopathic effect of anal HPV (koilocytosis) and condyloma were considered LSIL as defined by LAST. Four possible histological grades were used in this analysis: normal/negative, LSIL, HSIL and squamous cell carcinoma. Several experienced Pathologists, all from the same institution, read the anal histology slides, with consensus discussion of all difficult or equivocal cases. When several biopsies, with different levels of abnormality, were taken from the same patient, the most severe histological grade was used for the analysis.

Statistical analysis

Sensitivity, specificity, NPV and PPV were calculated with associated 95% confidence intervals (CI). In most of the analyses presented, repeat observations were included for some patients and so estimates of diagnostic indices were adjusted for within-patient clustering using generalized estimating equations (GEE) logistic regression [21, 22] with an 'exchangeable' correlation structure for each individual and robust variance estimation for the parameters. Differences in sensitivity according to factors of interest were also evaluated within the GEE logistic regression framework.

Multivariable GEE logistic regression including all potential predictive variables was also conducted for the outcome of 'any abnormality' on anal cytology without considering the histology results (i.e. to identify variables associated with the presence of abnormal cytology unrelated to its sensitivity for predicting histology). Backward stepwise variable selection was implemented using factors that were significant on univariable analysis.

The concordance between cytological grades and histological grades was assessed using Cohen's kappa coefficient (κ). There was only a small number of women with missing data for the variables potentially associated with the sensitivity of anal cytology and so complete case analyses were conducted.

Statistical analysis was performed using Stata, version 14.1 (StataCorp, College Station, TX, USA).

RESULTS

During the study period, there were 334 new referrals, of which 279 women had a previous history of anogenital neoplasia and concomitant anal cytology and HRA performed. This corresponded to 670 appointments with anal cytology and satisfactory HRA in the same sitting, but 34 anal cytology samples were paucicellular (5%) and were excluded. The final analysis included 278 women from whom 636 anal cytological samples (with the same number of matching HRA) and 323 biopsies were obtained.

The mean age of the women included (age at first visit) was 46±15 years, 34 patients (12%) were HIV-positive, 38 patients (14%) were on chronic immunomodulators/ immunosuppressive medications and 137 of 250 (55%), from whom data were available, were current or previous smokers. A history of anogenital HSIL or cancer was present in 199/270 (74%) women, with the following breakdown of affected sites: 94/268 (35%) cervical, 94/270 (35%) vulvar, 14/270 vaginal (5%), 65/270 (24%) anal and 31/270 (11%) perianal. In some cases (missing cases) a clear distinction between a previous history of low and high-grade was not recorded.

Two-hundred and five (32%) anal cytology samples and 356 HRA (56%) were described as having abnormal results (any abnormality). From the collected 323 biopsies, 260 (80%) were diagnosed as having ASIL (any abnormality), with 105 (33%) biopsies classified as HSIL/cancer.

Overall sensitivity of 'any abnormality' on anal cytology to predict any abnormality in HRA was 44% (95% CI 38–50%) and specificity was 85% (95% CI 79–89%). Overall sensitivity of 'any abnormality' on anal cytology to predict 'any abnormality' in histology was 47% (95% CI 41–54%), specificity was 84% (95% CI 73–91%) and overall sensitivity of 'any abnormality' on anal cytology to predict HSIL or cancer in histology was 71% (95% CI 61–79%), with a specificity of 73% (95% CI 66–79%), Table 1. There was a poor concordance between anal cytological grades and histological grades (κ=0.147), Table 2.

Immunosuppression due to HIV or chronic immunomodulators/immunosuppressive medications was associated with a higher sensitivity of anal cytology to predict any abnormality in histology (63% *vs.* 40%, *P*=0.002) and also for HSIL/cancer (92% *vs.* 60%, *P*=0.002). In those immunosuppressed, the NPV of a negative anal cytology for the absence of HSIL/cancer was 93% (95% CI 79–98%), Table 3.

Considering the outcome of 'any HSIL on histology', the sensitivity of 'any abnormality' on anal cytology to detect HSIL was higher when two or more quadrants were affected by comparison with only one quadrant (86% *vs.* 57%; *P*=0.006). No further improvement was seen when HSIL was present in three or more (sensitivity 83%, *P*= 0.896) or four quadrants (sensitivity 82%, *P*=0.828). A significant positive association remained between immunosuppressed status (OR 5.62, 95%CI 1.50–21.1, *P*=0.01) and the sensitivity of 'any abnormality on anal cytology' to detect HSIL, following adjustment for the presence of HSIL in two or more quadrants.

The presence of a previous vulvar HSIL or cancer was associated with higher sensitivity of 'any abnormality' on anal cytology to predict any abnormality on histology (57% *vs.* 41%, *P*=0.028, Table 4), but a significant difference was not found in the sensitivity for HSIL or cancer on histology (74% *vs.* 67%, *P*=0.469). Patients with a concomitant histological proven genital HSIL/cancer (in the same sitting as anal cytology) had a higher sensitivity of anal cytology to predict any abnormality in anal histology than those without (80% *vs.* 42%, *P*<0.001). The difference in sensitivity to predict

HSIL/cancer between the two groups was not statistically significant (*P*=0.053), although it was higher in those with concomitant genital HSIL/cancer (88% *vs.* 66%), Table 5.

No significant difference in the sensitivity for either any abnormality in histology or HSIL/cancer on histology, respectively, was observed for patient age ≥50 years (*P*=0.615; *P*=0.275), smoking history (*P*=0.452; *P*=0.379) and for new *vs.* follow-up patients (*P*=0.390; *P*=0.393, Table 6). There was also no significant difference in anal cytology sensitivity in patients with a previous cervical HSIL/cancer *vs.* none (53% *vs.* 45% *P*=0.243 for any histological abnormality; 77% *vs.* 67% *P*=0.282 for HSIL/cancer) or for a previous vaginal HSIL/cancer history *vs.* none (52% *vs.* 47% *P*=0.806 for any histological abnormality; 83% *vs.* 70% *P*=0.533 for HSIL/cancer). A table with the performance of anal cytology in women with a previous history of LGTN (excluding anal and/or perianal HSIL/cancer history) and a summary table are presented as a supplementary data (Table S1 and S2, respectively).

Entering all variables that showed a significant association with the sensitivity of 'any abnormality' on anal cytology to predict any abnormality on histology into a multivariable GEE logistic regression analysis, having a history of previous vulvar HSIL/cancer was no longer statistically significant (*P*= 0.097), although a positive association was nonetheless still estimated (OR 1.69, 95%CI 0.91–3.13). When this variable was removed from the model, immunosuppressed status (OR 2.07, 95%CI 1.13–3.79, *P*=0.019) and concomitant genital HSIL/cancer (OR 3.97, 95%CI 1.75–9.98, *P*=0.001) remained independent predictors of the sensitivity of cytology for detecting any abnormality in histology. Multivariable regression for the outcome of 'any abnormality' on anal cytology without considering the histology results led to a final model that included positive associations with a previous history vulvar HSIL/cancer (OR 1.71, 1.08–2.73; *P*=0.023), immunosuppressed status (OR 1.88, 1.17–3.03; *P*=0.009) and concomitant genital HSIL/cancer (OR 2.51, 1.47–4.29; *P*=0.001).

DISCUSSION

There have been few studies specifically analyzing the performance of anal cytology in comparison with HRA/anal histology in high-risk women. The results have been conflicting, although most studies reported a very low sensitivity [17, 23, 24], with a small number of anal histological samples for comparison and, in most cases, a low rate of ASIL/AIN diagnosed. Information on features that might influence anal cytology has also been lacking [18]. In a study [18], with a larger number of abnormal anal samples (21% of cytology and 77% of the biopsies), a much higher sensitivity was found (70%, and for detecting anal HSIL 84%), with a specificity of 93%. In our cohort there was a higher rate of abnormal results, including HSIL/cancer. Seventy-four percent of the women included had a previous history of anogenital HSIL/cancer, which might have influenced this rate of abnormal results and a better performance of anal cytology. The total number of biopsies was much higher in our study than in any of the previous studies, allowing us to evaluate correlations with features that might influence the performance of anal cytology. The concordance between anal cytology grades and anal histology was poor, as has previously been described [23].

The interpretation of anal cytology has been considered as a more laborious and difficult task than for the cervix [18]. Our results are in a similar range to that described for cervical cytology (liquid-based cytology). For the detection of cervical intraepithelial neoplasia-CIN 2+, a sensitivity ranging from 52% to 94% and a specificity ranging from 73–97% for 'any abnormality' in cervical cytology has been reported [25].

Immunosuppressed women were the group with highest sensitivity (92%) of cytology for detecting anal HSIL/cancer. Previous studies, mostly including a male population (HIV-positive MSM) have also showed a better sensitivity in immunosuppressed *vs.* non-immunosuppressed [11, 15, 16] and in those with more extensive disease [16]. In a systematic review, including studies on HIV-positive patients (mostly male), overall sensitivity of anal cytology ranged from 69% to 93% and specificity from 32% to 59% [26]. As far as we know, the performance of anal cytology in women related to a previous history of and/or concomitant genital HSIL/cancer has not been described. A previous

history of genital HSIL/cancer (*vs.* no history) was associated with a higher sensitivity of cytology for predicting anal HSIL/cancer whichever site was considered (cervix, vagina or vulva), although these differences were not statistically significant.

The major strengths of this study relate to the size of the sample included, the largest reported in women, and the fact that a number of patient characteristics could be assessed as possible modifiers of the performance of anal cytology. All women with anal cytology also underwent HRA and this reduced the probability of overestimation of sensitivity (if HRA was not used on those with negative cytology). All of the procedures were conducted in a service with a substantial experience in managing these cases and by a team using the same protocol. This is relevant for obtaining better results and needs to be considered when discussing an anal screening program among high-risk populations. This also might have influenced the overall better performance of anal cytology in this study.

There are some limitations to be considered. Because of the retrospective nature of the study, there are some missing data (smoking, previous history of HSIL/cancer). Information was not recorded in some HIV-positive patients for CD4 nadir, so no analysis was done in relation to this. Several Cytopathologists/Pathologists interpreted the samples, which can make the results more heterogeneous due to interobserver variation. Almost all cases of concomitant genital HSIL/cancer were vulvar, so no assessment per genital site was done. Seventy-four percent of the women had a previous history of HSIL/cancer and this may limit the generalization of these conclusions to other populations and practices. The UK reporting system for cervical cytology (and anal) is different from the Bethesda system [27]. For analysis purposes, only four cytological categories were used in this study (normal, low-grade, high-grade and cancer), so correlations with Bethesda system can be made: atypical squamous cells of undetermined significance (ASC-US) and LSIL with low-grade, and atypical squamous cells which cannot exclude high-grade squamous intraepithelial lesions (ASC-H) and HSIL with high-grade. Other studies have used a similar correlation for cervical cytology [25]. HPV testing was not routinely performed with anal cytology, and there is no analysis related to the performance of HPV testing with or without anal cytology compared with HRA/histology.

In summary, the performance of anal cytology in this group of women was better than that described in most of the (few) previous studies. As with cervical cytology, anal cytology, has some important limitations, including poor correlation with the histological grades, limited sensitivity and the possibility of false negative results [25]. Despite this, compared with HRA/colposcopy, cytology is a less expensive method, easier to perform and less invasive, therefore potentially suitable as a screening method in at risk populations. A history of vulvar HSIL/cancer, immunosuppression and concomitant genital HSIL/cancer were associated with a higher risk of abnormal anal cytology. The sensitivity for detecting anal HSIL/cancer was higher in immunosuppressed women and those with two or more quadrants of high-grade disease. In immunosuppressed women, given the overall performance to predict HSIL/cancer, anal cytology should be considered the initial screening technique.

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Table 1: Overall diagnostic performance of 'any abnormality' on anal cytology to predict findings on high-resolution anoscopy (n=636) and anal histology (n=323)

	ANAL CYTOLOGY			
	SENSITIVITY % (95% CI)	SPECIFICITY % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
HRA ASSESSEMENT (ANY ABNORMALITY)	44 (38-50); 160/356	85 (79-89); 235/280	79 (72-85); 160/205	54 (49-60); 235/431
HISTOLOGY (ANY ABNORMALITY)	47 (41-54); 125/260	84 (73-91); 53/63	93 (87-96); 125/135	28 (22-35); 53/188
HISTOLOGY (HIGH-GRADE/CANCER)	71 (61-79); 75/105	73 (66-79); 158/218	55 (46-64); 75/135	84 (78-89); 158/188

CI: confidence interval; HRA: high-resolution anoscopy, NPV: negative predictive value; PPV: positive predictive value

Overall *n/n* values are shown but do not correspond exactly to point estimates of indices owing to statistical correction for repeat observations.

Table 2: Comparison between anal cytology and anal histology grades (n=323).

		CYTOLOGY GRADES			
		NEGATIVE n (%)	LOW-GRADE n (%)	HIGH-GRADE n (%)	CANCER n (%)
HISTOLOGY GRADES	NEGATIVE n (%)	53 (28)	9 (9)	1 (3)	0
	LOW-GRADE n (%)	106 (56)	45 (46)	4 (12)	0
	HIGH-GRADE n (%)	28 (15)	43 (44)	28 (82)	1 (33)
	CANCER n (%)	1 (1)	1 (1)	1 (3)	2 (67)

K= 0.147 (poor concordance) between cytology and histology grades. A very similar result is obtained if only the first observation per patient is used (K=0.146).

Table 3: Diagnostic performance of 'any abnormality' on anal cytology in immunosuppressed (HIV and or drugs) and non-immunosuppressed patients to predict findings on histology.

	IMMUNOSUPPRESSED				NON-IMMUNOSUPPRESSED			
	SENSITIVITY % (95% CI)	SPECIFICITY % (95%CI)	PPV % (95%CI)	NPV % (95% CI)	SENSITIVITY % (95% CI)	SPECIFICITY % (95%CI)	PPV %(95%CI)	NPV % (95% CI)
HISTOLOGY (ANY ABNORMALITY)	63 (51-73); 48/77	84 (51-96); 11/13	96 (85-99); 48/50	28 (16-43); 11/40	40 (32-48); 77/183	83 (71-91); 42/50	91 (83-95); 77/85	28 (22-36); 42/148
HISTOLOGY (HIGH-GRADE/CANCER)	92 (78-97); 34/37	68 (54-80); 37/53	68 (53-79); 34/50	93 (79-98); 37/40	60 (48-71); 41/68	74 (67-81); 121/165	48 (36-59); 41/85	82 (74-87); 121/148

CI: confidence interval; NPV: negative predictive value; PPV: positive predictive value

Overall *n/n* values are shown but do not correspond exactly to point estimates of indices owing to statistical correction for repeat observations. Comparison of sensitivities for immunosuppressed and non-immunosuppressed using generalized estimating equations logistic regression: any abnormality in histology **p= 0.002** and for high-grade histology **p = 0.002.**

Table 4: Diagnostic performance of 'any abnormality' on anal cytology in patients with and without previous vulvar HSIL to predict findings on histology.

	PREVIOUS VULVAR HSIL OR CANCER				NO PREVIOUS VULVAR HSIL OR CANCER			
	SENSITIVITY % (95% CI)	SPECIFICITY % (95%CI)	PPV % (95%CI)	NPV % (95% CI)	SENSITIVITY % (95% CI)	SPECIFICITY % (95%CI)	PPV %(95%CI)	NPV % (95% CI)
HISTOLOGY (ANY ABNORMALITY)	57 (46-67); 53/82	70 (42-88); 6/10	94 (85-98); 53/57	16 (8-30); 6/35	41 (34-50); 63/150	87 (75-94); 43/49	91 (82-96); 63/69	34 (27-41); 43/130
HISTOLOGY (HIGH-GRADE/CANCER)	74 (59-84); 38/45	63 (50-75); 28/47	64 (50-75); 38/57	75 (62-84); 28/35	67 (53-79); 33/49	76 (68-83); 114/150	47 (35-60); 33/69	88 (80-93); 114/130

CI: confidence interval; HSIL: high-grade squamous intraepithelial lesions; NPV: negative predictive value; PPV: positive predictive value

Overall *n/n* values are shown but do not correspond exactly to point estimates of indices owing to statistical correction for repeat observations. Comparison of sensitivities according to presence of previous vulvar HSIL or cancer using generalized estimating equations logistic regression: any abnormality in histology **p=0.028**; HSIL or cancer in histology **p=0.469**.

Table 5: Diagnostic performance of 'any abnormality' on anal cytology in patients with and without genital HSIL/cancer (histological proven) in the same sitting to predict findings on histology.

	GENITAL HSIL				WITHOUT GENITAL HSIL			
	SENSITIVITY % (95% CI)	SPECIFICITY % (95%CI)	PPV % (95%CI)	NPV % (95% CI)	SENSITIVITY % (95% CI)	SPECIFICITY % (95%CI)	PPV %(95%CI)	NPV % (95% CI)
HISTOLOGY (ANY ABNORMALITY)	80 (64-89); 31/39	100 (NA); 1/1	100 (NA); 31/31	11 (1-53); 1/9	42 (35-49); 94/221	84 (73-91); 52/62	90 (83-95); 94/104	29 (23-36); 52/179
HISTOLOGY (HIGH-GRADE/CANCER)	88 (68-96); 22/25	39 (18-65); 6/15	74 (55-87); 22/31	67 (31-90); 6/9	66 (54-76); 53/80	75 (68-81); 152/203	50 (39-60); 53/104	85 (79-90); 152/179

CI: confidence interval; HSIL: high-grade squamous intraepithelial lesions; NA: non-applicable; NPV: negative predictive value; PPV: positive predictive value

Overall *n*/*n* values are shown but do not correspond exactly to point estimates of indices owing to statistical correction for repeat observations. Comparison of sensitivities between patients with and without genital HSIL using generalized estimating equations logistic regression: any abnormality in histology **p<0.001** and for high-grade histology **p= 0.053.**

Table 6: Diagnostic performance of 'any abnormality' on anal cytology for new and follow-up patients to predict findings on histology.

	NEW PATIENTS				FOLLOW-UP PATIENTS			
	SENSITIVITY % (95% CI)	SPECIFICITY % (95%CI)	PPV % (95%CI)	NPV % (95% CI)	SENSITIVITY % (95% CI)	SPECIFICITY % (95%CI)	PPV %(95%CI)	NPV % (95% CI)
HISTOLOGY (ANY ABNORMALITY)	50 (40-59); 58/117	89 (67-99); 17/19	97 (88-99.6); 58/60	22 (14-33); 17/76	45 (36-54); 67/143	82 (68-91); 36/44	89 (79-94); 67/75	32 (24-42); 36/112
HISTOLOGY (HIGH-GRADE/CANCER)	67 (53-80); 35/52	70 (59-80); 59/84	58 (45-71); 35/60	78 (67-86); 59/76	75 (60-86); 40/53	74 (66-82); 99/134	51 (38-64); 40/75	89 (81-94); 99/112

CI: confidence interval; NPV: negative predictive value; PPV: positive predictive value

Overall *n/n* values are shown but do not correspond exactly to point estimates of indices in follow-up patients owing to statistical correction for repeat observations. Comparison of sensitivities for follow-up and new patients using generalized estimating equations logistic regression: any abnormality in histology **p= 0.390** and for high-grade histology **p= 0.393.**