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Precis: Cytological reporting of anal high-grade squamous intraepithelial lesion (HSIL) predicts histological HSIL in 92.6% of men who have sex with men within 12 months, but this often requires several high-resolution anoscopic examinations.

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Abstract:

Background: Longitudinal studies of histological outcomes after anal cytological screening in men who have sex with men (MSM) are rare. We measured the positive predictive values (PPV) of each level of baseline cytological abnormality in MSM in Sydney, Australia, over a 12 month period.

Methods: The Study of the Prevention of Anal Cancer (SPANC) is a 3-year prospective study of the natural history of anal human papillomavirus infection in MSM aged >35 years. For each participant with baseline cytological abnormality, the worst histology was recorded at baseline high-resolution anoscopy (HRA), and at 6 and 12 months. PPV for a histological HSIL diagnosis were calculated for each level of baseline cytological abnormality at each time-point.

Results: Among 424 men who completed 3 visits, PPV of cytological HSIL increased from 71.6% at baseline to 86.4% at 6 months and 92.6% at 12 months (p<0.001). For cytological atypical squamous cells, cannot exclude HSIL (ASC-H), PPV increased from 51.5% at baseline to 69.7% at 6 months and 75.8% at 12 months (p=0.004). At each time point, the PPV of cytological HSIL was significantly higher than PPV of ASC-H. PPV of 'low-grade' cytology reports were significantly lower than PPV of ASC-H at each timepoint.

Conclusions: In a cohort of MSM, baseline histological HSIL diagnosis after a cytoprediction of HSIL is high and increases with further examinations over 12 months. Lower levels of cytological abnormality have significantly lower PPVs. These data can inform patient management and quality assessment of each aspect of the screening pathway.

Key words: anal cancer; anal cytology; human papillomavirus; high-grade squamous intraepithelial lesion; positive predictive value; high resolution anoscopy.

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Introduction:

Anal canal squamous cell carcinoma is increasing in incidence in many developed countries, particularly in several 'high-risk' groups. These groups include immunosuppressed people (such as those with HIV infection and solid organ transplant recipients), men who have sex with men (MSM) and women with previous human papillomavirus (HPV)-related lower genital tract neoplasia [1-5]. As with cervical squamous cell carcinoma (SCC), anal canal SCC is caused by high-risk mucosal HPV types, particularly HPV16 [6, 7]. The neoplastic 'pathway', from HPV infection of the transformation zone to high-grade squamous intraepithelial lesion (HSIL) to carcinoma, is also believed to be similar to that seen in the cervix [8, 9]. Because of these similarities between the two anatomical sites and the success of cervical screening programs, there have been proposals in recent years to establish a cytology-based anal screening program among high-risk groups, adapting the cervical screening paradigm to the anal canal [10].

The Study of the Prevention of Anal Cancer (SPANC) is an ongoing longitudinal study of the natural history of anal HPV infection and its associated anal squamous cellular abnormalities. The study population is a cohort of largely community-recruited MSM in Sydney, Australia. A key feature of SPANC is that all men have anal liquid-based cytology (LBC) and high resolution anoscopy (HRA), with directed biopsy of abnormalities, on each of five visits over three years. Based on the baseline visit results, we have been able to assess the overall performance of anal LBC in the detection of histological HSILs within the cohort [11].

Unlike cervical screening programs, current anal cytological investigation protocols generally recommend referral for HRA in the presence of any non-negative result, as anal cytology often under-represents the severity of the abnormality [9, 12, 13]. As HRA is an invasive and expensive procedure that requires a high level of expertise, it is important to measure the 'accuracy' of individual cytological findings, in particular for predicting the histological presence of HSIL, the presumed cancer precursor lesion. In this study, we used the first 12-month follow-up data from SPANC to measure the positive predictive value (PPV) of cytological HSIL (cytoHSIL) for histological HSIL. We then compared this with PPVs (also for histological HSIL) with the 'lower' risk cytology reports of 'Atypical squamous cells, can't exclude HSIL' (ASC-H), 'Low-grade squamous intraepithelial lesion' (LSIL) and 'Atypical squamous cells of undetermined significance' (ASC-US). Typical cytological appearances for each category are illustrated in Figures 1-4.

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Methods:

The methodology of SPANC has been described in detail elsewhere[14]. In brief, 617 men aged at least 35 years of age, who reported having sex with another man in their lifetime, were recruited mainly from community-based settings. Those who reported a previous HRA or a history of anal cancer were excluded. Signed informed consent was provided by all participants. Ethics approval was granted by the Human Research Ethics Committees at the St. Vincent's Hospital in Sydney and the University of New South Wales. There are five study visits over a 3-year period. Recruitment was completed between September 2010 and August 2015. At each visit, men underwent a digital anorectal examination, an anal swab test for LBC (ThinPrep, Hologic Inc., Marlborough, Mass) and HPV DNA detection and genotyping, followed by HRA during which lesions suspected of being HPV-related were biopsied. All cytological and histological specimens were processed and reported at Douglass Hanly Moir Pathology in Sydney, Australia, by one of three specialist anogenital pathologists.

Anal cytology was reported using The Bethesda System (TBS) 2001 criteria and terms [15]. A "satisfactory" slide was defined (as in TBS) as having at least 2000 nucleated squamous cells or any abnormal cells. Repeat collection was always attempted for participants with initial unsatisfactory LBC and if the repeat collection was satisfactory, this result was used as the baseline cytology result. If a repeat LBC was also unsatisfactory, this was recorded as the baseline cytology result. The cytological results for satisfactory slides were classified as negative, ASC-US, LSIL, ASC-H or HSIL.

Reporting of the biopsies was performed blinded to the cytology result and in accordance with criteria, terminology, and recommendations of the Lower Anogenital Squamous Terminology (LAST) Standardization Project [16] Histological results were reported as negative for squamous intraepithelial lesion (SIL), exophytic LSIL, flat LSIL, HSIL-anal intraepithelial neoplasia grade 2 (AIN2), HSIL-anal intraepithelial neoplasia grade 3 (AIN3), or squamous cell carcinoma. As recommended by LAST, p16INK4a (p16) immunostaining was used to confirm all HSIL-AIN2 diagnoses. We have previously demonstrated very high inter- and intraobserver repeatability amongst the three pathologists reporting these biopsies [17]. When multiple biopsies were obtained, the biopsy with the highest degree of abnormality was used as the histological diagnosis in the analysis. Biopsies were not obtained from participants who were assessed as having no abnormalities suggestive of SIL on HRA examination. These men were classified as being negative for SILs, as were those in whom biopsies showed no HPV-associated abnormalities.

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PPVs were calculated by measuring the proportion of participants in each category of baseline cytoprediction, (including negative and unsatisfactory results, even though the calculation in these cases cannot truly be termed a PPV) who had histological HSIL at baseline, 6 months (i.e. at baseline and/or 6 month visit) and 12 months (i.e. at baseline and/or 6 month and/or 12 month visit). In each of the baseline cytological category, we used generalised linear method to examine the trends in PPV of histologically diagnosed HSIL over time, i.e. at baseline, 6-month, and 12-month, and p values for trend were presented. The same analysis was also stratified by participants' HIV status.

Results:

A total of 617 men were recruited into SPANC. The median age of participants was 49 years (range, 35-79 years). Nearly all men (95.3%) identified as gay or homosexual. Greater than one-third (35.7%) of the men were HIV positive. The great majority of the HIV-positive men (93.6%) were currently receiving antiretroviral treatment, reported an undetectable viral load (89.5%), and had a CD4 count of > 350 cells/L (88.0%).

Of the 617, 424 participants (68.7%) who had completed all of the first 3 study visits (baseline, 6-, and 12-months) by 31st December 2016 and had satisfactory baseline cytology, were included in the analysis. 15 further men had two unsatisfactory anal cytology specimens at baseline.

Overall, a baseline HSIL cytoprediction was associated with histological HSIL at baseline biopsy in 71.6% of participants. At the six month visit, the PPV had risen by 14.8% to 86.4% and by a further 6.2% to 92.6% by 12 months. This trend was statistically significant (p <0.001) (Table 1). As can be seen in Tables 2 and 3, the increase in histological confirmation of HSIL with subsequent HRAs was seen among both HIV-negative (65.4% at baseline, 82.7% at 6 months and 88.5% at 12 months) and HIV-positive men (82.8% at baseline, 93.1% at 6 months and 100% at 12 months). The increase in PPV was statistically significant in each case (HIV-negative p= 0.006 and HIV-positive p=0.032). PPVs were higher for HIV-positive men, but the differences between HIV-positive and HIV-negative did not reach statistical significance.

PPVs for the cytological category of ASC-H were lower than those for cytoHSIL at each of the three HRA examinations for both HIV positive and negative men. The PPV of cytoHSIL at baseline (71.6%) was significantly greater than that of ASC-H (51.5%, p = 0.012). The difference in PPV values was also significant at 6 months (cytoHSIL 86.4%, ASC-H 69.7%, p=0.013) and at 12 months (cytoHSIL 92.6%, ASC-H 75.8%, p=0.004). For the HIV-negative group, the PPV of cytoHSIL was significantly higher than that of ASC-H at baseline (p=0.038) and

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at 12 months (p=0.032) but not at 6 months (p=0.068). For the HIV-positive group, the PPV of cytoHSIL was not significantly higher than that of ASC-H at baseline (p=0.314), at 6 months (p=0.115) or at 12 months (p=0.068). PPVs were always higher for HIV-positive than for HIV-negative men but they did not reach statistical significance.

For the two 'low-grade' cytological categories (LSIL and ASC-US, which were considered together), the PPVs for histological HSIL were significantly lower than for ASC-H for each HRA visit (baseline p=0.003, 6 months p<0.001 and 12 months p<0.001). This was true among both HIV-positive (baseline p=0.033, 6 months p=0.008 and 12 months p=0.003) and HIV-negative men (baseline p=0.020, 6 month p=0.002 and 12 months p= 0.025). For negative cytology reports at baseline, an increasing proportion of men had histological HSIL found at each subsequent HRA but this increase was not statistically significant. For the men with an unsatisfactory report, histological HSIL was identified in only a small number of participants at baseline and 6 month visits, with no further histological HSIL found at 12 months.

Several recent studies with similar design to SPANC have reported performance characteristics of anal cytology and these are presented in Table 4[18-26]. None of these report change in PPV over time.

Discussion:

In a natural history study of anal HPV infection and HPV-related anal lesions, we have demonstrated that the proportion of MSM with histological HSIL after a cytoprediction of HSIL is high and increases with further examinations over a 12 month period. This suggests that cytoHSIL is a reliable predictor of histological HSIL. ASC-H is also associated with a high proportion of histological HSIL, but warrants a separate category due to the significant difference in PPVs between ASC-H and cytoHSIL. Low-grade cytocategories of LSIL and ASC-US have significantly lower PPVs than the high grade categories.

As PPV is affected by changes in prevalence of disease in a population [8], it would be helpful to perform similar analyses in other at-risk populations, which have lower disease prevalence.

We have previously demonstrated that the overall test performance of anal cytology in SPANC is comparable to that reported in several recent studies [11, 18-21, 25]. While knowledge of sensitivity and specificity are

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important for understanding how a screening test performs in populations, PPV can be utilised in making clinical decisions for individual participants being screened. Knowledge of likely outcomes for each cytological report will enable the most appropriate management decisions to be made for each participant. Table 4 compares PPVs in studies of similar design to ours, in which cytology and histology are both performed at the same visit [18-26] . The SPANC study is the largest such study to date, and has longitudinal data (not included in table), which may increase its relevance to clinical management decisions. Only two of the studies utilised the complete Bethesda system [18, 21]and none reported longitudinal data. There is a wide variation in PPVs in each category e.g. PPV of cytoHSIL ranges from 0-88%. This variation may reflect differences in level of experience and accuracy in both the clinical skill of HRA and in the pathological skills of cytology and histopathology interpretation. Differences in prevalence of HSIL in the study populations would also contribute.

Failure to identify all histological HSIL at the baseline visit may occur as a result of several factors. Firstly, HRA is a technically difficult examination, due to the nature of the anal canal as a potential space with its many mucosal folds [27]. Secondly, anoscopists were unaware of the baseline cytology result at the initial HRA, as it was performed as the same visit, whereas at subsequent visits they were aware of earlier cytology and HRA findings. Thus anoscopists may have searched for a HSIL lesion more thoroughly in men with a previous HSIL cytoprediction. Thirdly, a small lesion not visualised at the baseline HRA may become larger over time, and thus more readily visible at subsequent examinations. Finally, there is a documented ’learning curve’ for anoscopists [28-30]. Using histology alone may underestimate the true burden of HSIL present in a population and this supports our earlier publication recommending the use of a ‘composite’ HSIL result, using the presence of cytoHSIL and/or histological HSIL [31].

A second possibility for the increase in PPV of HSIL with time is that new lesions have developed between visits. Incident anal HPV infections are common in this population [32] so it is likely that the appearance of new lesions does account for at least some of the increase in PPV. However, if this were the sole reason, we would expect a uniform increase in PPV, such that the increase in PPV from baseline to 6 months and from 6 months to 12 months would be approximately equal. This is not the case. The PPV increase from baseline to 6 months is more than twice the PPV increase from 6 months to 12 months (14.8% vs 6.2%), suggesting that the other factors described above have greater impact on the PPV of anal LBC than does the development of new lesions.

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Although we have shown that cytoHSIL is a powerful predictor of histological HSIL, a PPV of 100% is not a realistic expectation for two reasons. Firstly, there may be cytological 'overcalls' related to the subjectivity of cytological interpretation [33, 34]. Secondly, some HSIL lesions may be transient [32] and may have resolved by the time of a subsequent HRA examination.

The category of ASC-H is used when changes suggestive of HSIL are seen on the cytology slide, but a confident prediction is not possible. This may reflect subtle variability in immature metaplastic cells, the presence of very few atypical cells or the presence of cells generally predictive of LSIL, but with a higher than expected nuclear:cytoplasmic ratio. Our results demonstrate that the PPV remains sufficiently high to warrant a thorough search for lesions at HRA. The lack of a significant difference between cytoHSIL and ASC-H in the HIV positive participants is probably a result of the small number of participants in this category. The PPV increases with further HRA examinations (although non-significantly among HIV positive men), suggesting that histological HSIL may have been missed on earlier examinations. Again, the possibility of new infections may contribute, but as with cytoHSIL, the bulk of the increase in PPV occurs from baseline to 6 months (18.2% vs 6.1% from 6 months to 12 months), suggesting that new lesions play a smaller role than other factors.

The 'low-grade' categories of LSIL and ASC-US can be considered together, as both are likely to represent evidence of productive viral infection and the outcomes are similar. These categories are associated with significantly lower PPVs for histological HSIL than the high-grade cytological categories described above. However, as has been previously reported by our group [11] and others [18-22, 24-26], the proportion of men with low-grade cytology and high-grade histology is still high, with almost 50% having HSIL found after three HRAs in our study. This apparent cytological-histological discrepancy likely reflects the multiplicity of HPV types and lesions found in the anal canal, with the cytology result reflecting sampling of an LSIL and the histology result indicating biopsy of a different lesion which was HSIL.

The current accepted diagnostic algorithm recommends HRA after any level of abnormal cytology [12, 13]. Our findings of high PPV for histological HSIL 12 months after cytoHSIL (92.6%), ASC-H (75.8%) and LSIL/ASC-US combined (nearly 50%) support this protocol. However, although current practice is to perform HRA for each cytological category, the PPVs are significantly different. Just as 'risk stratification' now underlies management

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of women in the cervical screening pathway [35], so can this stratification principle be applied to the anal screening pathway. Factors such as persistence of cytological abnormality over time and presence of specific HPV genotypes may enable us to further stratify risk within particularly the low-grade and ASC-H cytocategories. We also have baseline data for mRNA activity, viral load and p16/Ki-67 immunostaining [36]. The focus of our future research will be to measure the usefulness of all of these factors in further refining a risk assessment paradigm to guide clinical management of individual patients.

The observation that 12.6% of men with negative cytology at baseline had histological HSIL identified at that particular visit has been previously reported by our group [11]. Others have reported 'false negative rates' ranging from 11-81% [18-22, 24, 25]. The high rate of histological HSIL in the anus with negative cytology may relate to the anatomical complexity of the anal canal, the size of the area sampled, and the "blind" sampling technique used, compared with the sampling of the cervix under direct vision. These factors suggest that, in screening programs targeting high risk populations, repeat cytology at regular intervals is likely to improve detection of histological HSIL, as has been shown in cytology-based cervical screening programs [9].

Histological HSIL outcomes also occurred in men with two consecutive unsatisfactory cytology reports. We have previously demonstrated a number of associations with unsatisfactory anal cytology specimens [37]. Although we found that unsatisfactory cytology was less common in men with histological HSIL [37], there is still a small risk of high-grade disease.

Our data may be useful in a screening program especially in instances of cytological-histological discrepancy. The PPVs of cytoHSIL and ASC-H could be even higher in a screening program, as the HRA would occur after the anal cytology result is available.

As well as influencing individual patient management, PPVs could be used to monitor individual anoscopists' and cytopathologists' diagnostic performance over time. Guidelines for HRA quality have recently been published [38] and include a number of basic metrics. Importantly, there is a recommendation that all patients with cytoHSIL, but no histological HSIL have an HRA within 6 months. Future versions of these guidelines could potentially include minimum standards for PPV of cytoHSIL. Determining an appropriate threshold for such a

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value would require a comprehensive benchmarking process across different institutions and at-risk populations.

In conclusion, cytoHSIL is a meaningful prediction, with histological HSIL confirmed in more than 90% of cases after three HRAs over a 12 month period. HRA is a technically difficult procedure and if cytology suggests HSIL but histological HSIL is not found at the first examination, we recommend that HRA should be repeated. HRA examination is warranted in MSM with lower grade cytological abnormalities, but follow-up and repeat HRA need not be as intensive as for men with cytoHSIL and ASC-H reports.

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Table 1: PPVs for each category of baseline cytoprediction, calculated based on histologically confirmed HSIL at baseline, by 6 months and by 12 months.

	HSIL histology (histoHSIL)					
	Baseline cytoprediction	**number**	**histoHSIL at baseline No (%)**	**histoHSIL by 6 months No (%)**	**histoHSIL by 12 months No (%)**	**P value# for trend over time**
1	HSIL	81†	58 (71.6)	70 (86.4)	75 (92.6)	<0.001
2	ASC-H	66	34 (51.5)	46 (69.7)	50 (75.8)	0.004
3	LSIL	32	10 (31.3)	13 (40.6)	16 (50.0)	0.129
4	ASC-US	70	19 (27.1)	25 (35.7)	32 (45.7)	0.023
5	Negative	175	22 (12.6)	25 (14.4)	34 (19.5)	0.076
6	TOTAL	424	143 (33.7)	179 (42.2)	207 (48.8)	<0.001
7	Unsatisfactory	15	2 (13.3)	6 (40.0)	6 (40.0)	0.137
	P values‡ between baseline cytopredictions 1 and 2		0.012	0.013	0.004	
	P values§ between cytopredictions 2 and 3&4 (combined)		0.003	<0.001	<0.001	

Legend

PPV=positive predictive value

HSIL – High-grade squamous intraepithelial lesion

ASC-H - Atypical squamous cells, can't exclude HSIL

LSIL – Low-grade squamous intraepithelial lesion

ASC-US - Atypical squamous cells of undetermined significance

†1 patient with HSIL also had a superficially invasive SCC in one of 3 biopsies.

‡P value refers to the difference in the proportion with histoHSIL in the three time periods for each baseline cytoprediction.

§P value refers to the difference in the proportion with histoHSIL in the three time periods for each baseline cytoprediction.

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Table 2: HIV negative men: PPVs for each category of baseline cytoprediction, calculated based on histologically confirmed HSIL at baseline, by 6 months and by 12 months.

	HSIL histology (HHSIL)					
	Baseline cytoprediction	**number**	**histoHSIL at baseline No (%)**	**histoHSIL by 6 months No (%)**	**histoHSIL by 12 months No (%)**	**P value for trend overtime**
1	HSIL	52	34 (65.4)	43 (82.7)	46 (88.5)	0.006
2	ASC-H	45	20 (44.4)	30 (66.7)	32 (71.1)	0.013
3	LSIL	16	3 (18.8)	5 (31.3)	8 (50.0)	0.067
4	ASC-US	41	10 (24.4)	15 (36.6)	20 (48.8)	0.024
5	Negative	125	12 (9.6)	13 (10.4)	20 (16.0)	0.115
6	TOTAL	279	79 (28.3)	106 (38.0)	126 (45.2)	<0.001
7	Unsatisfactory	12	2 (16.7)	5 (41.7)	5 (41.7)	0.217
	P values †between baseline cytopredictions 1 and 2		0.038	0.068	0.032	
	P values ‡between baseline cytopredictions 2 and 3 & 4 (combined)		0.020	0.002	0.025	

Legend:

PPV=positive predictive value

HSIL – High-grade squamous intraepithelial lesion

ASC-H - Atypical squamous cells, can't exclude HSIL

LSIL – Low-grade squamous intraepithelial lesion

ASC-US - Atypical squamous cells of undetermined significance

†P value refers to the difference in the proportion with histoHSIL in the three time periods for each baseline cytoprediction.

‡P value refers to the difference in the proportion with histoHSIL in the three time periods for each baseline cytoprediction.

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Table 3: HIV positive men: PPVs for each category of baseline cytoprediction, calculated based on histologically confirmed HSIL at baseline, by 6 months and by 12 months.

	HSIL histology (histoHSIL)					
	Baseline cytoprediction	**number**	**histoHSIL at baseline**	**histoHSIL by 6 months**	**histoHSIL by 12 months**	**P value for trend overtime**
1	HSIL	29†	24 (82.8)	27 (93.1)	29 (100.0)	0.032
2	ASC-H	21	14 (66.7)	16 (76.2)	18 (85.7)	0.141
3	LSIL	16	7 (43.8)	8 (50.0)	8 (50.0)	0.726
4	ASC-US	29	9 (31.0)	10 (34.5)	12 (41.4)	0.410
5	Negative	50	10 (20.0)	12 (24.0)	14 (28.0)	0.382
6	TOTAL	145	64 (44.1)	73 (50.3)	81 (55.9)	0.042
7	Unsatisfactory	3	0 (0.0)	1 (33.3)	1 (33.3)	N/A
	P values ‡between baseline cytopredictions 1 and 2*		0.314	0.115	0.068	
	P values § between baseline cytopredictions 2 and 3&4 (combined)*		0.033	0.008	0.003	

*Fisher's exact p value.

Legend:

PPV=positive predictive value

HSIL – High-grade squamous intraepithelial lesion

ASC-H - Atypical squamous cells, can't exclude HSIL

LSIL – Low-grade squamous intraepithelial lesion

ASC-US - Atypical squamous cells of undetermined significance

†1 patient with HSIL also had a superficially invasive SCC in one of 3 biopsies.

‡P value refers to the difference in the proportion with histoHSIL in the three time periods for each baseline cytoprediction.

i§P value refers to the difference in the proportion with histoHSIL in the three time periods for each baseline cytoprediction.

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Table 4: Comparison of PPVs in studies in which cytology and histology performed on all participants at same visit and >100 participants recruited

Study	Cytoprediction (number)	PPV (%)
Berry 2009[18] n=125	HSIL (n=8)	88
	ASC-H (n=7)	57
	LSIL (n=21)	38
	ASC-US (n=16)	44
Nathan 2010[19] n=395	HSIL (n=22)	55
	'Low-grade' (n=162)	28
Salit 2010[20] n=401	HSIL (n=47)	45
	LSIL (n=172)	30
	ASC-US (n=49)	18
Wentzensen 2012[21] n=363	HSIL (n=60)	52
	ASC-H (n=27)	37
	LSIL (n=67)	25
	ASC-US (n=73)	15
Phanuphak 2013[22] n=246	HSIL (n=3)	0
	LSIL (n=9)	11
	ASC-US (n=18)	28
Wentzensen 2014[23] n=342	HSIL (n=68)	43
Dietrich 2015[24] n=123	HSIL (n=15)	67
	LSIL (n=18)	44
	ASC-US (n=7)	71
Sendagorta 2015[25] n=101	HSIL (n=12)	83
	LSIL (n=50)	52
	ASC-US (n=9)	33
Schofield 2016[26] n=284	'High-grade' (n=21)	43
	'Low-grade' (n=72)	49
Roberts 2017 n=424	HSIL (n=81)	72
	ASC-H (n=66)	52
	LSIL (n=32)	31
	ASC-US (n=70)	27

Legend:

PPV = positive predictive value

HSIL – High-grade squamous intraepithelial lesion

ASC-H - Atypical squamous cells, can't exclude HSIL

LSIL – Low-grade squamous intraepithelial lesion

ASC-US - Atypical squamous cells of undetermined significance

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Figure Legends:

Figure 1: High-grade squamous intraepithelial lesion. Crowded atypical cells with hyperchromatic nuclei, nuclear membrane irregularities and high nuclear:cytoplasmic ratio.

Figure 2: Atypical squamous cells, can't exclude HSIL. Sparsely cellular preparation with two concerning but somewhat degenerate cells.

Figure 3: Low-grade squamous intraepithelial lesion. Typical koilocytes.

Figure 4: Atypical squamous cells of undetermined significance. Group of cells with nuclear enlargement and mild hyperchromasia but a low nuclear:cytoplasmic ratio.

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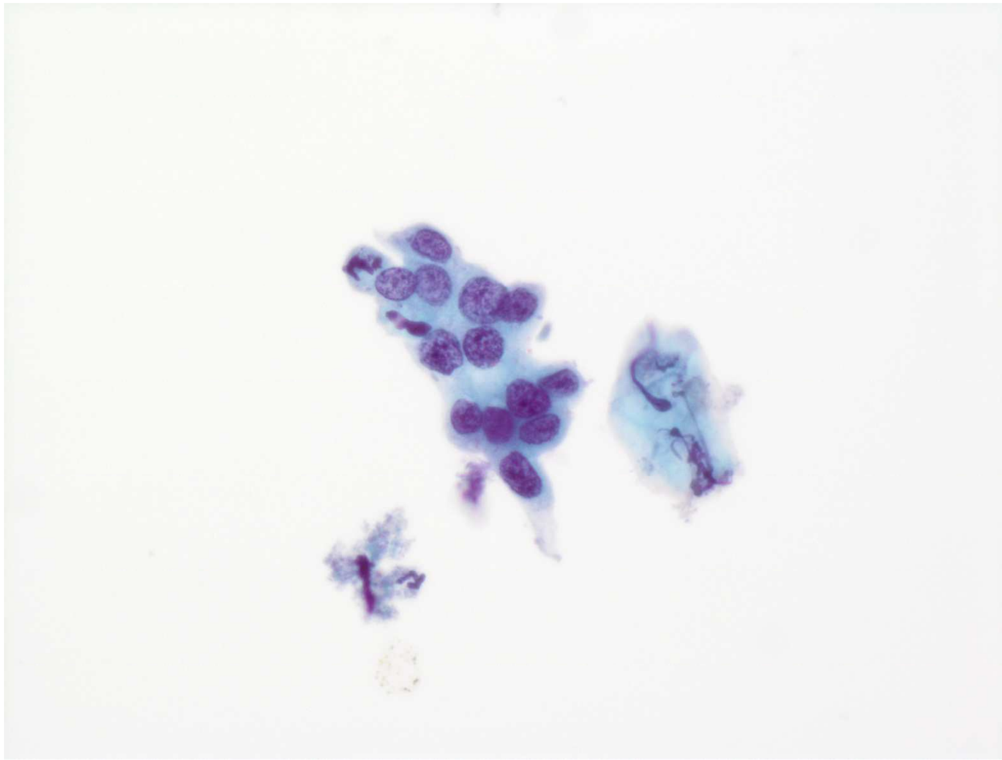

Figure 1: High-grade squamous intraepithelial lesion. Crowded atypical cells with hyperchromatic nuclei, nuclear membrane irregularities and high nuclear:cytoplasmic ratio.

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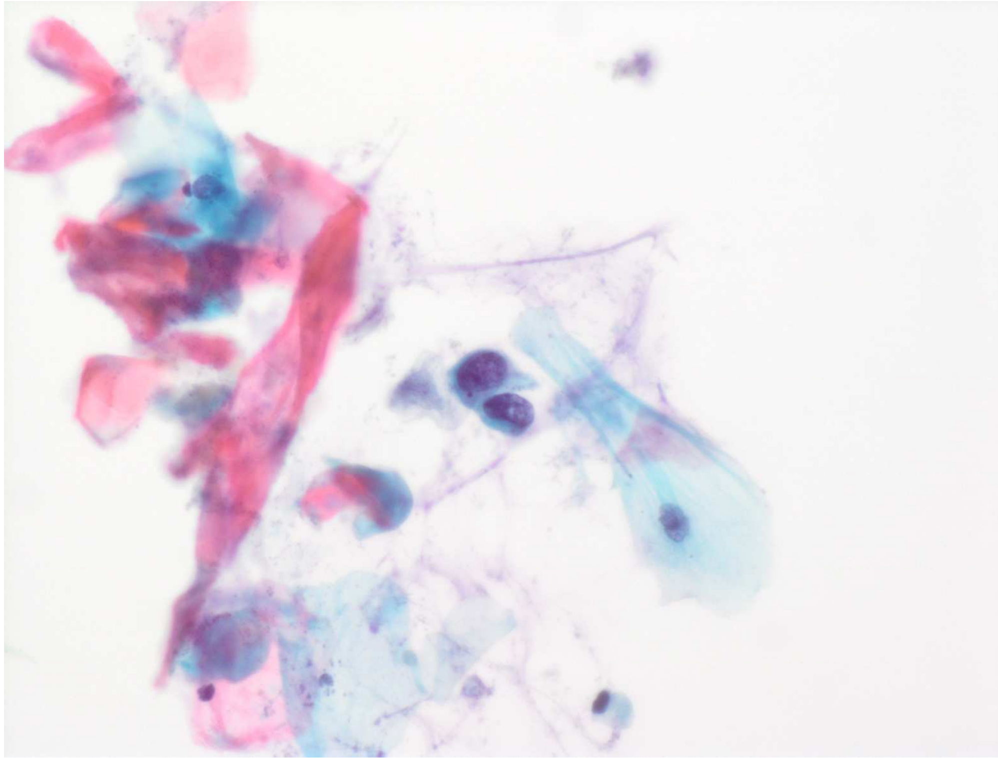

Figure 2: Atypical squamous cells, can’t exclude HSIL. Sparsely cellular preparation with two concerning but somewhat degenerate cells.

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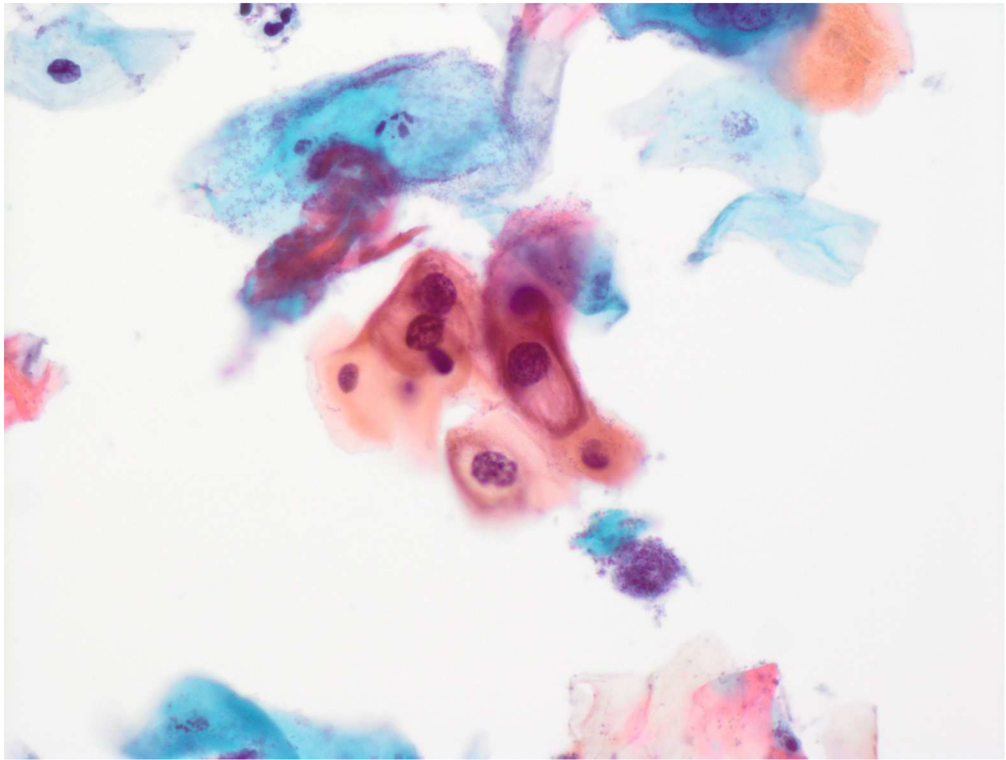

Figure 3: Low-grade squamous intraepithelial lesion. Typical koilocytes.

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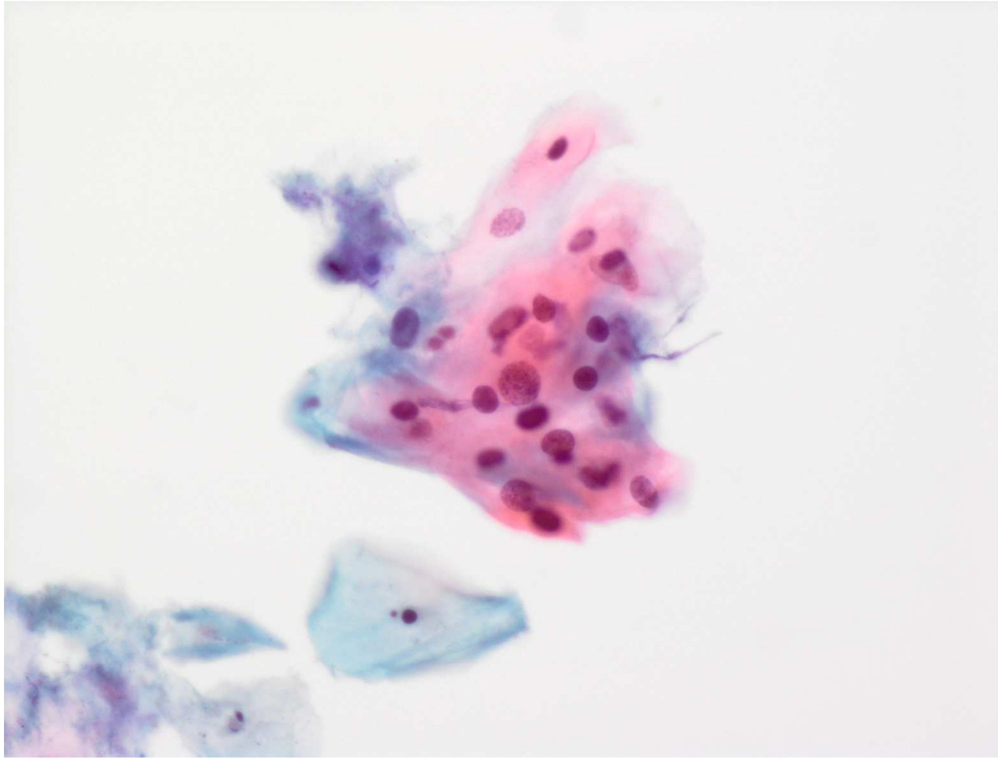

Figure 4: Atypical squamous cells of undetermined significance. Group of cells with nuclear enlargement and mild hyperchromasia but a low nuclear:cytoplasmic ratio.

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