



Colposcopic characteristics and Lugol's staining differentiate anal high-grade and low-grade squamous intraepithelial lesions during high resolution anoscopy

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ABSTRACT

Background: Anal squamous intraepithelial lesions (SIL) and cancers are increased in immunocompromised populations. Based upon anatomic and histologic similarities, the cervix is used as the model for anal screening. During cervical colposcopy, acetic acid (AA) and Lugol's staining (LS) result in characteristic changes that help distinguish low-grade (L)SIL from high-grade (H)SIL. Lesion characteristics were evaluated for their ability to distinguish anal (a)LSIL from anal (a)HSIL during high-resolution anoscopy after application of AA and LS.

Methods: AA-stained lesions were described using standard cervical colposcopic criteria. LS was then applied and lesions were characterized as Lugol's-negative (L-), Lugol's-partial (L+/-), or Lugol's positive (L+) and then biopsied. Biopsies were characterized as benign, squamous atypia, LSIL or HSIL.

Results: 835 anal lesions were analyzed. Sensitivity and positive predictive value (PPV) for aHSIL were highest for characteristics associated with cervical (c)HSIL. L- was independently associated with aHSIL (OR=4.7, 95% CI=3.4–6.7). In multiple logistic regression analysis, significant predictors of aHSIL were flat contour (OR=2.24, 95% CI=1.3–3.8), mosaic pattern (OR=2.0, 95% CI=1.4–2.9), vascular punctuation (OR=1.5, 95% CI=1.1–2.1) and L- (OR=2.3, 95% CI=1.5–3.4). L- staining improved the PPV of aHSIL almost twofold in lesions that otherwise had a colposcopic impression of LSIL.

Conclusions: Evaluating acetowhite lesions for contour, surface, vascularity, and LS may maximize the likelihood of identifying aHSIL.

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1. Introduction

Anal cancer is rare, but the incidence of anal human papillomavirus (HPV) infection, anal low-grade squamous intraepithelial lesions (LSIL), anal high-grade squamous intraepithelial lesions (HSIL), and anal cancer are elevated in immune-compromised populations. In addition, the incidence of anal cancer is increased among men who have sex with men (MSM) in general, and women with a history of gynecologic cancers [1–7]. Based on similarities in the anatomy of the cervix and anal canal, and in the pathophysiology of HPV-related disease, the cervix has been used as the model for screening and diagnosis of HPV-induced

abnormalities of the anal canal, including LSIL and HSIL. The same HPV types affect both cervix and anus [8]. Cervical HSIL and anal HSIL are considered to be the cancer precursor lesions in the cervix and anus, respectively [9,10]. Like the anus the cervix is composed of squamous epithelium adjacent to columnar epithelium in a transformation zone that undergoes metaplasia. During this dynamic process, metaplastic squamous cells are susceptible to transformation by oncogenic HPVs. The majority of cervical HSIL, anal HSIL and cancers originate in their respective transformation zones [11–13].

Colposcopic examination is the standard procedure to identify cervical lesions for biopsy, usually following detection of abnormal cells on cytology. The colposcope's magnification and fiber-optic lighting along with application of 3–5% acetic acid and/or Lugol's staining result in recognizable epithelial and vascular changes or characteristics associated with cervical SIL that would otherwise be invisible. The mechanism of action by which acetowhitening

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occurs is incompletely understood but may be caused by light scattering in dysplastic tissues, osmotic shifts, and/or changes in cytokeratin expression causing lesions to turn white when acetic acid applied and can thus be recognized as potentially abnormal and targeted for biopsy [14,15]. Like acetic acid, Lugol's staining is used in cervical colposcopy to help differentiate cHSIL from cLSIL and to determine the lesion margins for treatment. On the cervix, Lugol's stain is taken up by normal glycogenated squamous epithelium, inducing a dark mahogany stain. Conversely, abnormal cells of SIL, particularly those with high-grade morphology, lack glycogen and thus do not stain [12]

Lesion characteristics, acetowhitening and Lugol's stain patterns are used to guide the selection of cervical biopsy sites to maximize the likelihood of finding the most advanced disease, including cervical HSIL or cervical cancer. Histopathologic assessment of colposcopic-directed biopsies is considered to be the gold standard for the diagnosis of lesion severity and is used to guide treatment. Previous work has described the colposcopically-defined lesion characteristics that help distinguish cervical (c)LSIL from cervical (c)HSIL [12,13]. Although the sensitivity of cervical colposcopy to predict the grade of disease varies widely, these lesion characteristics create a colposcopic impression used to select lesions to biopsy.

Prior studies have validated the use of anal cytology for screening of HPV-associated disease of the anus [16–18]. Examination of the anus with a colposcope, first described by O'Connor in 1977 [19] is now called high resolution anoscopy (HRA). Gradually, HRA has become essential in diagnosis and management of HPV-associated anal disease [20,21]. Previous work suggested that the use of acetic acid and the associated lesion changes characteristic of cervical HSIL have a sensitivity of 49–61% to detect anal HSIL [22,23]. However, 9–13% of lesions with colposcopic features consistent with anal LSIL also contained HSIL on biopsy [22,24]. In the present study, we sought to describe the lesion characteristics that help to distinguish anal LSIL from anal HSIL and to determine if the application of Lugol's solution in addition to acetic acid improved the sensitivity and positive predictive value (PPV) for anal HSIL compared with acetic acid alone.

2. Methods

We analyzed lesions from 399 HIV-infected and 172 HIV-uninfected men who have sex with men (MSM) who were enrolled in a natural history study of anal SIL between 1998 and 2000, and who received biopsies during routine bi-annual HRA examinations. Men were recruited via community outreach efforts. Characteristics of this cohort were described previously [17,25]. Excluded from this analysis were 137 HIV-infected and 81 HIV-

uninfected men who were biopsied but had a history of allergy to iodine and did not receive Lugol's staining, or patients whose biopsies were scant and could not be interpreted. Patients who had no anal lesions identified were not included. Perianal lesions were excluded because Lugol's staining is not useful on keratinized epithelium. The Committee on Human Research at the University of California San Francisco approved this study protocol.

2.1. Procedures

Prior to HRA, the anus was lubricated using 2% lidocaine gel mixed with KY-jelly while a digital anorectal exam was performed. A disposable anoscope was then inserted and a Q-tip wrapped in gauze and soaked in 3% acetic acid was placed in the anus. The anoscope was removed and the gauze was left in place for at least one minute to allow the acetic acid to saturate the mucosa of the entire anal canal. The gauze was then removed and the anoscope reinserted. HRA was performed with a Zeiss colposcope and additional acetic acid was liberally applied using small cotton-tipped swabs to identify colposcopic lesions. Each lesion was evaluated and coded for the presence or absence of specific lesion characteristics using colposcopic criteria commonly used in gynecology and previously validated for anal lesions [22]. Acetowhite epithelium (AWE) was defined as an area in the anus demarcated by the application of acetic acid. Each AWE lesion was described based on specific characteristics in the following four categories: contour, surface patterns, vascular patterns and margins. See Table 1.

Following acetic acid evaluation, full-strength Lugol's solution (1 part iodine, 2 parts potassium iodide, and 300 parts water) was applied to each lesion using a Q-tip. Staining patterns were categorized as Lugol's-negative (L–) if no staining occurred, Lugol's-partial (L+/-) if staining was variegated in color, or Lugol's-positive (L+) if staining was uniformly dark mahogany (Fig. 1). Lugol's stain was also applied to the entire transformation zone of participants who had prior abnormal cytology or history of aSIL, but no AWE was seen with application of acetic acid during HRA. The anus was evaluated for the presence of lesions that may only have been appreciated by Lugol's staining and areas of L– staining were biopsied. These areas of L– staining without AWE were categorized as 'non-AWE, L– lesions'.

In addition, a subset of AWE lesions with different Lugol's staining patterns within a single AWE lesion was evaluated from a convenience series of consecutive biopsies from 25 patients. Distinctly different areas were biopsied (e.g. one from the L– area and one from the L+ area) to determine if L– staining helped define the margins of HSIL to guide treatment. All examinations and biopsies were done by one of

Table 1
Description of lesion characteristics.

Category	Characteristic	Description
Contour	Flat	No elevation or minimal thickening, may be irregular or uneven
	Raised	Exophytic, verrucous, thickened, often in association with papillary changes
Surface patterns	Smooth	Even, without texture
	Granular	Irregular, coarse, or gritty
	Papillae	Thin, finger-like projections, often with warty looped vessels
Vascularity	Micropapillae	Slightly raised projections, similar to papillae but flattened in comparison, small capillary vessels may be present
	Punctation	End-on view of dilated capillary vessels creating dotted pattern, which may be fine or coarse
	Mosaic pattern	Tile-like pattern of connected vessels, even or uneven, fine or coarse and thickened
Margins	Warty vessels	Looped capillary vessels often within papillae or Verrucous lesions
	Distinct	Well-demarcated borders, sharply defined, may have internal margins
Lugol's stain	Regular	Symmetrical, straight or smooth outline
	Indistinct	Feathery, borders lack clarity
	Negative	No iodine uptake, yellow
	Partial	Variable iodine uptake, speckled appearance of yellow and brown
	Complete	Mahogany brown coloring, uniform uptake

two clinicians with 7–10 years experience in HRA at the time (JMB and NJ). All biopsies were sent for a histologic diagnosis. A single pathologist provided the histopathology diagnoses, blinded to the lesion descriptions, clinical impression and patient history. If multiple levels of SIL were found, the lesions were assigned the diagnosis based on the highest grade of disease.

2.2. Statistical analysis

Because of the potential bias that might occur with repeated sampling, only the first study visit in which a lesion was biopsied was included in this analysis. Multiple biopsies from the same patient were included if they occurred at the same visit and represented different lesions.

Data were analyzed using SPSS version 15.0 (SPSS, Chicago Ill.). For analysis, characteristics were dichotomized into categories hypothesized to be associated with either HSIL or < HSIL (i.e.,

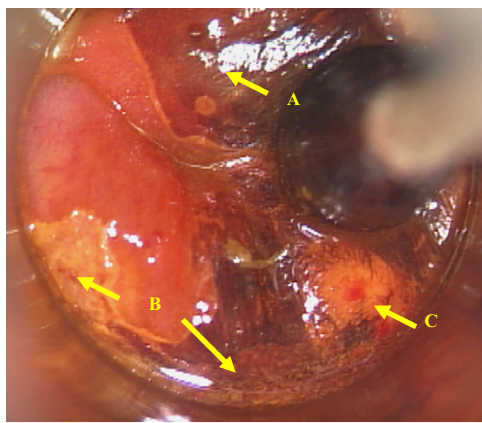


Fig. 1. Lugol's staining patterns in anal mucosa. Arrow A indicates positive uptake of Lugol's with mahogany brown uniform staining. Arrows B indicate partial or variable Lugol's uptake with areas of dark staining, or light brown uptake. Arrow C indicates a focal area of Lugol's negative staining

LSIL, squamous atypia or benign). Lugol's staining was dichotomized as L– versus L+/- or L+. Margins were dichotomized as distinct versus indistinct, or 'not scored' since raised lesions were not given a margin score. Lesions that presented with both characteristics of a dichotomized variable (for example flat and raised) were assigned to the category more commonly associated with LSIL. The dichotomized distribution of histology (HSIL versus < HSIL) and lesion characteristics were tested for significance using the Chi-square statistic. Sensitivity, specificity, positive predictive value (PPV), and negative predictive values (NPV) were calculated for each characteristic with HSIL as the dependent variable. To determine whether the addition of Lugol's staining had improved the performance of each characteristic to predict HSIL, a variable consisting of the 'characteristic plus L– staining' was derived in which the positive result (e.g. HSIL) had both the characteristic and L– staining. Contingency tables were calculated so that true positives were considered to be those characteristics with L– staining and HSIL while false positives were < HSIL. False negatives consisted of the remaining HSIL that did not have the characteristic or were not L–, and true negatives had neither the characteristic nor L– staining.

An analysis of the most common patterns of characteristics was done by calculating the frequency of patterns that presented with combinations of characteristics. All patterns that included at least 10 lesions were included in this analysis. The PPV was estimated as the proportion of the pattern that had a histologic outcome of HSIL.

Odds ratios (OR) were calculated for each of the characteristics individually using logistic regression. Multivariable logistic regression was used to model the cluster of characteristics associated with HSIL when characteristics had p values of < .05 in the univariate analyses.

3. Results

A total of 835 lesions representing biopsies taken from 399 HIV-infected and 172 HIV-uninfected men were included in these analyses. No differences were found in any demographic

Table 2
Colposcopic characteristics of anal lesions relative to histologic diagnosis.

Characteristic	Category	Anal HSIL N (%)	Anal LSIL N (%)	Atypia N (%)	Normal N (%)	Total N
Acetowhite		389 (47)	349 (42)	43 (5)	54 (6)	835
Contour	Flat	327 (60)	143 (26)	35 (6)	45 (8)	550
	Raised ^a	62 (22)	206 (72)	8 (3)	9 (3)	285
Surface	Smooth	374 (48)	322 (41)	42 (5)	49 (6)	787
	Not smooth	15 (31)	27 (56)	1 (2)	5 (10)	48
	Granular	27 (38)	38 (53)	1 (1)	6 (8)	72
	Not granular	362 (47)	311 (41)	42 (6)	48 (6)	763
	Papillae	23 (14)	141 (83)	4 (2)	1 (1)	169
	No papillae	366 (55)	208(31)	39(6)	54(8)	666
	Micropapillae	40 (35)	64 (56)	4 (4)	6 (5)	114
Vascular patterns	No micropapillae	349 (48)	285 (40)	39 (5)	48 (6)	721
	Punctuation	236 (60)	89 (23)	25 (6)	41 (11)	391
	No punctuation	153 (34)	260 (59)	18 (4)	13 (3)	444
	Mosaic pattern	176 (67)	50 (19)	18 (7)	17 (7)	261
	No mosaic pattern	213 (37)	299 (52)	25 (4)	37 (6)	574
	Warty vessels	61 (23)	189 (71)	8 (3)	7 (3)	265
	No warty vessels	328 (58)	160 (28)	35 (6)	47 (8)	570
Margins	Distinct	21 (44)	12 (25)	9 (19)	6 (12)	48
	Regular	96 (75)	47 (37)	3 (2)	6 (5)	152
	Indistinct	232 (58)	105 (26)	28 (7)	35 (9)	400
	Not scored ^b	40 (17)	185 (79)	3 (1)	7 (3)	235
Lugol's staining	Negative	335 (57)	175 (30)	40 (7)	38 (6)	588
	Partial	51 (24)	145 (68)	2 (1)	14 (7)	212
	Positive	3 (9)	29 (83)	1 (3)	2 (6)	35
	Total	389	349	43	54	835

^a Includes 48 lesions that were both flat and raised.
^b Raised lesions were not scored for margins.

characteristics between the men enrolled in the cohort study who did and did not undergo biopsy or whose biopsy results were excluded. A larger percentage of HIV-infected men (98%) was biopsied than HIV-uninfected men (92%)

3.1. Anal lesion characteristics

The distribution of lesion characteristics and Lugol's staining patterns in relationship to histology are listed in Table 2. The percentages of lesions diagnosed as HSIL (47%) and LSIL (42%) were similar. The low number of lesions diagnosed as squamous atypia (5%) and normal (6%) was congruent with the overall study goal to biopsy areas with an abnormal colposcopic appearance to determine the incidence of aSIL. Significant differences between lesions diagnosed with HSIL and < HSIL were found for all lesion characteristics ($p < .05$) except margins and surface granularity. HSIL was associated with the following characteristics: flat contour, smooth surface, punctation or mosaic pattern, and L– staining. In contrast, characteristics associated with LSIL were raised contour, granular, papillae or micropapillae surfaces, warty vessels and L+/- or L+ staining.

All margin characteristics were found more frequently with HSIL but no single margin characteristic was significantly associated with a specific histologic diagnosis. Indistinct margins were found in 70% of all lesions. Thirty-one percent of lesions were not scored for margins because they had a raised contour, and these were mostly LSIL (Table 2).

For all histologic diagnoses, the majority of lesions were L–. However, among the L– lesions, 57% of the lesions were diagnosed as HSIL, compared with 30% diagnosed as LSIL. In contrast, among the L+/- lesions 24% were HSIL compared with 68% diagnosed LSIL. Among the L+ lesions, 8.6% were HSIL compared with 83% diagnosed with LSIL (Table 2).

3.2. Sensitivity, specificity and predictive value of lesion characteristics and Lugol's staining for anal HSIL and LSIL

Characteristics with the highest sensitivities for HSIL were flat, smooth, punctation, and L– staining (Table 3). While having the highest sensitivity the surface characteristic 'smooth' also had the lowest specificity and a higher NPV than PPV, indicating that it was not able to discriminate between HSIL and LSIL. Margins also were a poor predictor of histologic result. The sensitivity of L– staining for HSIL was 86% and the specificity was 43%. The characteristics with the highest sensitivity for LSIL were

Table 3
Sensitivity and specificity of lesion characteristics for anal HSIL and LSIL.

Characteristic	Sensitivity		Specificity	
	LSIL	HSIL	LSIL	HSIL
Contour				
Flat	45.8	84.1	9.9	50.0
Raised	59.0	15.9	83.7	50.0
Surface				
Smooth	92.2	96.1	4.3	7.4
Granular	10.9	6.9	93.0	89.9
Papillae	40.4	5.9	94.2	67.3
Micropapillae	18.3	10.3	89.7	83.4
Vessels				
Punctation	24.1	60.7	36.8	65.2
Mosaic pattern	14.3	45.2	56.6	80.9
Warty	52.7	15.7	83.3	54.3
Margins				
Distinct	13.5	5.4	78.4	93.9
Indistinct	30.1	59.6	39.3	62.3
Lugol's				
Negative	50.1	86.1	15.0	43.3
Pos./Partial	55.3	13.9	88.9	56.7

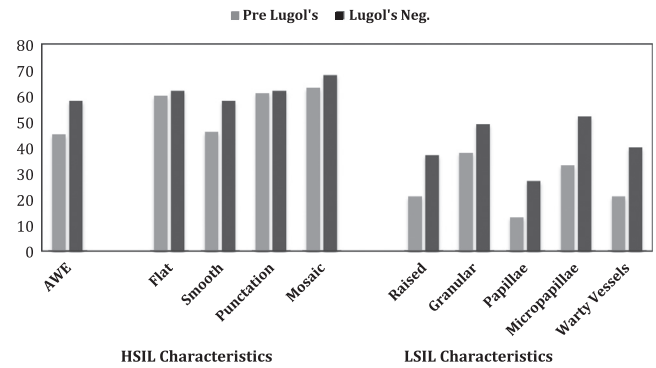


Fig. 2. The positive predictive value of lesion characteristics before and after Lugol's staining.

raised, smooth, and warty vessels. The sensitivity of L+/- staining for LSIL was 50% and the specificity of L+/- staining for LSIL was 95%. The PPV for HSIL are summarized in Fig. 2. The characteristics with the highest PPV for HSIL were flat, punctation, mosaic pattern and L– staining. The characteristics with the highest PPV for LSIL were raised papillae, wart vessels and L+/- (data not shown). The NPV of L– staining for HSIL was 78% and the NPV of L+/- for LSIL was 70%. When Lugol's staining was negative, the PPV for HSIL increased for all characteristics. Characteristics with the highest PPV for HSIL when also L– stained were AWE, flat, smooth, punctation, and mosaic pattern (Fig. 2). However the characteristics with the largest increases in PPV when also L– stained were those typically associated with LSIL (e.g. raised, papillae and warty vessels). The NPV decreased for those characteristics typically associated with HSIL while it increased for those typically associated with LSIL when L– staining was present. The characteristics with the highest PPV for LSIL were raised, papillae, warty vessels and L+/- staining. The specificity and NPV of L+/- and L+ for LSIL were 86% and 70% respectively.

3.3. Logistic regression analyses

The results of the univariate logistic regression analyses indicating the relationships between lesion characteristics and HSIL are shown in Table 4. Flat contour, punctation, mosaic pattern, smooth surface, and L– were all significantly associated with HSIL ($p < .0001$). Warty vessels, papillae and micropapillae were associated with reduced risk of HSIL ($p < .05$). A smooth surface was associated with HSIL but was not statistically significant, and both granular surface and margins were protective of HSIL but were not statistically significant. A multiple logistic regression analysis that included all variables is shown in Table 5a. The characteristics that remained significant were flat, punctation, mosaic pattern, distinct margin, and L–. A final multiple logistic regression analysis with variables that remained significant is shown in Table 5b and it is considered the parsimonious model of lesion characteristics associated with HSIL.

3.4. Combinations of lesion characteristics

Most anal lesions present in a combination of characteristics. Patterns were determined using a matrix analysis of all possible combinations of characteristics. Of the 835 lesions, 720 presented in 10 patterns with a minimum of 10 lesions per pattern (Table 6). The highest PPVs (67–68%) for HSIL were in patterns that included a flat contour, mosaic pattern, and smooth surface with or without vascular punctation (Fig. 3a and b). The PPV for HSIL for all lesion patterns increased when L–. But lesions with characteristics typically associated with LSIL such as raised contour, smooth

Table 4
Univariate logistic regression analysis of anal lesion characteristic's ability to predict anal HSIL.

Characteristic	Odds ratio	95% Confidence limit		p Value
		Low	High	
Contour				
Flat	5.27	3.80	7.32	< .0001
Vessels				
Mosaic pattern	3.51	2.58	4.78	< .0001
Punctation	2.90	2.19	3.84	< .0001
Warty vessels	.22	.16	.31	< .0001
Surface				
Smooth	1.99	1.07	3.73	.0310
Granular	.67	.40	1.09	.11
Papillae	.13	.08	.21	< .0001
Micropapillae	.58	.39	.87	.0086
Margins				
Distinct	.89	.49	1.59	.68
Lugol's negative	4.73	3.36	6.67	< .0001

Table 5a
Multiple logistic regression analysis of anal lesion characteristics ability to predict HSIL.

Characteristic	Odds ratio	95% Confidence limit		p Value
		Low	High	
Contour				
Flat	2.24	1.32	3.79	.003
Vessels				
Mosaic pattern	2.03	1.43	2.88	< .0001
Punctation	1.53	1.07	2.06	.019
Warty vessels	1.07	.56	2.06	.83
Surface				
Smooth	1.77	.59	5.31	.30
Granular	1.91	.74	4.88	.18
Papillae	.55	.25	1.21	.14
Micropapillae	.94	.51	1.74	.84
Margins				
Distinct	.43	.22	.82	.010
Lugol's negative	2.27	1.52	3.40	< .0001

Table 5b
Final logistic regression model for anal characteristics predictive of HSIL.

Characteristic	Odds ratio	95% Confidence limit		p Value
		Low	High	
Flat	2.75	1.89	3.99	< .0001
Mosaic pattern	2.01	1.44	2.81	< .0001
Punctation	1.47	1.06	2.03	.0200
Lugol's Negative	2.32	1.57	3.42	< .0001

surface, warty vessels, and papillae or micropapillae had two-to-threefold increases in the PPV for HSIL when L– was present. Only 14% of these lesions were HSIL while 29% of those with L– were HSIL.

3.5. Lugol's staining in the absence of AWE

When there were no acetowhite lesions seen during HRA, Lugol's staining of the transformation zone was done when a patient was considered at high-risk for aSIL (e.g. HIV-infected MSM or previous treatment for HSIL), or had a history of aSIL on prior anal cytology. 92 biopsies of these non-acetowhite L– stained areas were obtained from 92 patients. While the majority of these biopsies were normal (40%) or atypical (20%), 19% were

HSIL and 21% were LSIL. These lesions were only visible with L– staining.

3.6. Lugol's staining to define the borders of a lesion

25 lesions were biopsied to determine whether Lugol's staining defined the borders for acetowhite lesions with indistinct margins. In these areas of L– staining, the L– lesions were biopsied as well as the adjacent L+/- or L+ stained areas. 76% of the L– areas were HSIL while 12% of the L+/- or L+ were HSIL. Of the 24% L– areas that were not HSIL, 4 were normal and only 2 were LSIL. None were adjacent to HSIL in the L+/- or L+ areas. Of the lesions that were L+/- and were HSIL, all 3 were adjacent to HSIL in the adjacent L– stained areas.

4. Discussion

This study demonstrates that the colposcopic criteria used to distinguish cLSIL from cervical HSIL may also be used to distinguish between analLSIL and HSIL. The anal transformation zone and AWE lesions were discernible after application of 3% acetic acid to the anus. The colposcopic characteristics commonly used to describe cervical lesions were visible in anal lesions and most were associated with the expected histopathologic grade. Lugol's staining distinguished HSIL from LSIL, and helped define lesion borders, similar to its utility in detection of cervical disease. Lugol's staining has also been found to be useful for detection of esophageal disease [26–28].

Colposcopic detection of anal lesions has been reported by several groups [16,19,22,29,30]. O'Connor first described the use of the colposcope for detection of anal disease and used acetic acid minimally but did not use Lugol's solution [19]. Others have used the colposcope with acetic acid [29] or modified colposcopic terminology [24,30]. In one study the colposcopic appearance of a lesion was not predictive of histology, but lesions were categorized only by contour [30]. Scholefield et al. [24] reported correlations between colposcopic features and histology in 213 women but used a histologic classification system different from current standards. Friedlander et al. [16] used HRA in 32 patients and reported an 81% correlation between HRA impression and histology but did not report the criteria used for colposcopic impression. A recent study adapted vulvar terminology and found similar PPV for combinations of these adapted lesion characteristics including L– staining in a series of 168 lesions [31]. In the largest study to date of 385 biopsied anal lesions from 152 men, the colposcopic appearance of anal lesions was shown to be similar to cervical lesions and correlated with expected grade of disease but did not include Lugol's staining [22]. The current study represents the largest series of patients and lesions described, biopsied, and correlated with histologic diagnoses using standard colposcopy techniques including acetic acid and Lugol's staining.

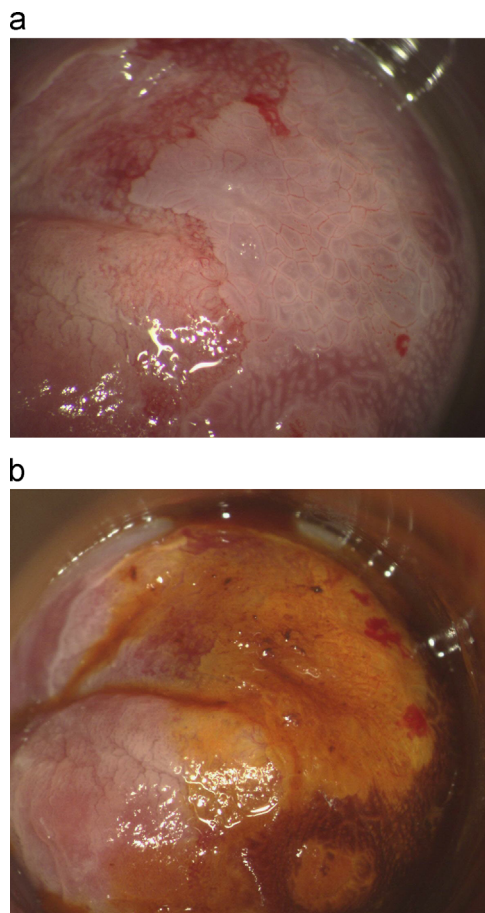
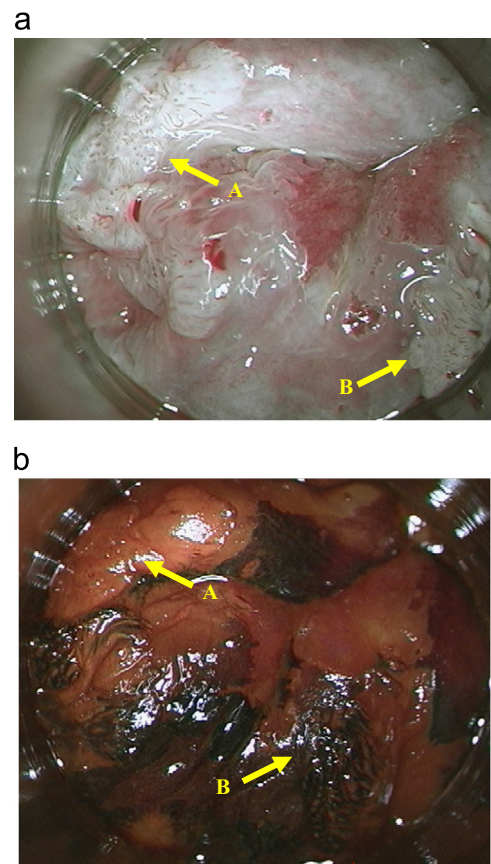
Characteristics in four of five categories (i.e., contour, surface, vascular patterns, and Lugol's staining) were found to broadly distinguish between LSIL and HSIL. Those associated with cHSIL: flat contour, smooth surface, punctation or mosaic vessels, and L– staining were all associated with HSIL both as individual characteristics and in combinations of characteristics. However, 'smooth' did not remain significant in the multivariable logistic regression. Characteristics associated with cLSIL: raised contour, granular, papillae and micropapillae surfaces, warty vessels, and LP or L+ staining were all associated with LSIL.

The PPV for HSIL of all characteristics increased with a L– stain pattern. Importantly, the largest increases were found in L– staining of lesions with otherwise typical LSIL appearances (see Fig. 4a and b). Prior studies have noted that approximately 10% of

Table 6

Combination of common lesion patterns: positive predictive value and logistic regression predictive probability for anal HSIL.

Pattern	Pattern Total N	# HSIL in pattern	PPV (%)	Lugol's-negative			Logistic regression of patterns when Lugol's-negative
				Pattern Total N	# HSIL in pattern	PPV (%)	
AWE ^a , flat, mosaic, smooth	88	60	68	75	53	71	.671
AWE, flat, mosaic, punctuation, smooth	144	97	67	137	92	67	.757
AWE, flat, punctuation, smooth	184	105	57	157	93	59	.606
AWE, flat, smooth	69	35	51	58	31	53	.502
AWE, flat, warty vessels, smooth	11	4	36	6	3	50	.519
AWE, flat, warty vessels, smooth, micropapillae	32	11	34	15	6	40	.503
AWE, raised, warty vessels, smooth, micropapillae	18	6	33	10	5	50	.311
AWE, raised, warty vessels, smooth, papillae	138	18	13	32	7	22	.209
AWE, raised, granular	20	2	10	10	1	10	.326
AWE, raised, warty vessels, smooth, papillae, micropapillae	16	0	0	1	0	0	.134
Total lesions with < 5 lesions per pattern	116	45	39	76	35	46	NA

^a AWE, acetowhite epithelium.**Fig. 3.** (A) and (B): an HSIL AWE lesion with coarse mosaic pattern and Lugol's negative staining.**Fig. 4.** (A) and (B): AWE lesions with raised warty pattern: In (a) lesions are seen prior to Lugol's staining. In (b) the same lesions are shown after Lugol's staining. Biopsied areas are indicated by arrows. Arrow A indicates a Lugol's negative stained area that was HSIL. Arrow B indicates area with Lugol's-positive/partial staining that was LSIL.

lesions with an LSIL appearance were HSIL [22,24]. In this series, 14% of lesions with LSIL characteristics were found to be HSIL on histology. However, this increased to 25% when L- compared with only 10% of the L+ or L +/- lesions. The PPV for HSIL was approximately two-fold higher when individual characteristics usually associated with LSIL were also L-. Findings from this study suggest that the addition of Lugol's staining to the HRA may help to differentiate HSIL lesions from LSIL with more precision than reliance upon the lesion's acetic acid appearance alone.

Specifically, lesions with a low-grade colposcopic impression should be considered for biopsy if that lesion is also L-.

Margin characteristics were not associated with the histologic grade of disease; this differs from cervical colposcopy. Cervical lesion margins are readily appreciated with the application of acetic acid, while anal lesion margins were difficult to evaluate and were frequently obscured or not fully seen. Since raised lesions, which were mostly LSIL, were not coded for margins in this study, this resulted in a falsely-inflated association between all

of the margin characteristics and HSIL. In some cases, the addition of LS allowed the clinician to see distinct margins when only indistinct margins were seen with acetic acid. In these situations Lugol's staining, may help define lesion margin for ablative treatment. Further research is needed to determine whether lesion margins in the anus can be better defined using Lugol's staining in addition to acetic acid.

While it is relatively easy to define the different Lugol's staining patterns, Lugol's staining alone is not adequate for determining sites for biopsy. The low specificity (43%) and PPV (57%) for HSIL are not unique to anal lesions and have been seen with cervical and esophageal lesions [32–34]. It is therefore emphasized that a L– appearance cannot be the sole criterion for discriminating between potential LSIL and HSIL. In addition to the observation that LSIL commonly shows L– staining, several other situations can cause the anal mucosa to have a L– stain in the absence of HSIL. Glycogen is absent in scar tissue and in normal columnar epithelium and immature squamous metaplasia. Verrucous LSIL may appear to be L– if the stain is not applied carefully to the uneven surface and this may have contributed to a higher than expected rate of L– staining in the absence of HSIL. The distal canal and perianus will also appear L– as keratinized epithelium is not heavily glycogenated. Familiarization with these features will improve the PPV but even very skilled clinicians will obtain biopsies of L– lesions that are not HSIL.

In some cases Lugol's staining but not acetic acid revealed the presence of lesions. Biopsies of these L– stained areas yielded additional HSIL (19%) or LSIL (21%) in 92 lesions that may not have otherwise been biopsied. These findings suggest that L– staining may help identify HSIL when no acetowhite lesions are identified on HRA in high-risk situations such as after HSIL cytology.

Visual inspection of the cervix using Lugol's staining has been used in low resource settings in the absence of colposcopy. The sensitivity for CHSIL ranged from 60% to 87% while specificity ranged from 63% to 88% in these studies [35–37]. In this study, the sensitivity to detect HSIL was similar at 86% although specificity was lower (43%). However, the high NPV (78%) of L– for HSIL and the high PPV of L+/- or L+ for LSIL (75%) were notable. Only 14% of HSIL lesions were L+/- or L+, indicating that in the absence of other criteria, an L+/- or L+ lesion is unlikely to be HSIL.

There were several limitations in this study. Compared with prior published results [22] the higher prevalence of HSIL in this series may indicate an increased prevalence of HSIL in this cohort or that the clinicians became more skilled at distinguishing HSIL from LSIL. The high PPVs of lesion characteristics and combinations reported in this series may reflect the high proportion of the study population that was diagnosed with HSIL and the results may not be generalizable. However, the logistic regression analyses, a statistic less influenced by prevalence, indicated that the characteristics hypothesized to be associated with HSIL were significant indicators for HSIL and may be applicable even in a population with lower prevalence of HSIL. Our results may also not be generalizable to less experienced clinicians, as our own data indicated higher sensitivities and PPV in the lesions evaluated by more experienced clinicians (data not shown). The exclusion of scant biopsies or those in which Lugol's staining was not done may have introduced bias to the analysis. Finally, in this study 3% rather than 5% acetic acid was used and may have adversely affected lesion identification. The preponderance of L– lesions revealed a sampling bias. Systematic biopsies of L+ or LP+/- and L– areas could decrease sampling bias.

5. Conclusions

Colposcopic examination is considered standard practice to identify cervical SIL or cancer following an abnormal screening

cervical cytology. Application of acetic acid, evaluation of the lesion for different characteristics, and Lugol's staining are commonly used in gynecology to guide the clinician's choice for biopsy. Lugol's staining is routinely used to determine the margins for excision and treatment of HSIL in the cervix as well as the esophagus. This study represents the largest series of anal lesion descriptors with corresponding histology as well as the first systematic examination of Lugol's staining in anal lesions. The results indicate that colposcopic criteria developed for the cervix are useful to distinguish high-grade from low-grade anal lesions.

Patients with abnormal anal cytology may present with a large volume of disease and with varying types of lesions. It is not possible to biopsy every lesion. Clinicians must choose the lesions most likely to provide the highest grade of histopathology. A better understanding of the appearance and characteristics of these lesions will maximize the likelihood of obtaining the highest-grade lesions. Because clinicians typically assume that a lesion with warty appearance is LSIL, these are frequently not biopsied and may not be treated. These findings underscore the importance of biopsying lesions even when HSIL is not suspected. It has particular importance when cytology indicates the presence of HSIL but the lesions all have the appearance of LSIL. L– staining in these circumstances may provide the means to distinguish additional HSIL from LSIL when a lesion appears to be LSIL prior to application of Lugol's solution.

Evaluating lesions systematically for their contour, surface, vascular patterns and Lugol's staining can help guide the clinician in choosing lesions for biopsy to submit for histologic diagnosis. In particular, acetowhite lesions presenting with a pattern that includes punctation and mosaic vascular patterns and L– staining are likely to indicate the presence of HSIL. Standardization of HRA techniques including application of acetic acid and Lugol's staining may help maximize the likelihood of finding HSIL for diagnosis and treatment.

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