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ORIGINAL ARTICLE

Evaluating the performance of anal cytology and high-risk HPV genotyping for detecting anal HSIL in a clinic-based sample of people living with and without HIV in Puerto Rico

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Abstract

Background: Given the disproportionately elevated anal cancer risk in high-risk populations, it is important to assess the performance of commonly used anal cancer screening tools to improve the effectiveness of detection and treatment methods. This study evaluates 1) the concordance between anal cytology and histology results and 2) the performance of cytology and high-risk human papillomavirus (HR-HPV) genotyping as screening tools for detecting histologically confirmed anal high-grade squamous intraepithelial lesions (HSIL).

Methods: Data from the Anal Neoplasia Clinic in Puerto Rico (2014–2021; $n = 466$) was used. The clinical performance of anal cytology and HR-HPV genotyping to detect HSIL was compared to the gold standard: high-resolution anoscopy-guided biopsy. Sensitivity, specificity, positive predictive value, negative predictive value, and κ coefficients were calculated.

Results: A total of 66.95% of the patients were men, 74.0% were people living with HIV, 76.2% had anal HR-HPV infection, and 40.34% had histologically confirmed anal HSIL. The weighted κ statistic between the tests (cytology and histology) was 0.25 ($p < .001$). The sensitivity and specificity of cytology alone to detect anal HSIL were 84.3% (95% confidence interval [CI], 78.3%–89.1%) and 36.0% (95% CI, 30.3%–42.0%), respectively. Anal HR-HPV genotyping had higher sensitivity (92.2%; 95% CI, 87.4%–95.6%) and similar specificity (34.8%; 95% CI, 29.2%–40.7%) compared to cytology. The two tests combined (positive results following cytology or HR-HPV test) improved sensitivity to detect anal HSIL (97.9%; 95% CI, 94.8%–99.4%), but specificity was compromised (19.2%; 95% CI, 14.7%–24.4%).

Conclusion: Although HR-HPV genotyping improved the detection of anal HSIL, HR-HPV testing had lower specificity than anal cytology alone.

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KEYWORDS
anal cancer screening, anal cytology, high-grade squamous intraepithelial lesions, HR-HPV genotyping, HR-HPV infection, HR-HPV test, Puerto Rico

INTRODUCTION

Anal cancer incidence in the United States has risen steadily over the past decade, averaging a 2.2% annual increase.[1-3] Mortality trends are rising as well, at a rate of 3.9% per year.[3] In 2020, 30,000 cases of squamous cell carcinoma of the anus (SCCA), the most common type of anal cancer, were diagnosed worldwide (nearly 20,000 in women and 10,000 in men).[4] Notably, although 20% of these new SCCA cases around the world were diagnosed in men living with HIV, 25% of the new SCCA cases in Puerto Rico were diagnosed in men living with HIV, showing a greater contribution of HIV to the SCCA burden for men in Puerto Rico.[4] A study in Puerto Rico found that from 2000 to 2016 the incidence of SCCA among people living with HIV (PLWH) was 27.7 per 100,000 person-years in comparison with the general population, which was 1.1 per 100,000 person-years. Men who have sex with men (MSM) displayed the highest incidence rate at 60.5 per 100,000 person-years.[2]

The increasing incidence rates of anal cancer raise the importance of developing effective screening and treatment strategies to prevent anal cancer and its precancerous lesions (i.e., anal high-grade squamous intraepithelial lesions [HSIL]), particularly among PLWH. Anal cancer shares many similarities with cervical cancer like their clinical manifestations, causative agents, and histology.[5] These similarities have served as a template to develop analogous screening and treatment methods for the early detection and prevention of anal cancer.[5,6] Yet, further optimization is needed to strengthen the knowledge and evidence on the effectiveness and cost-effectiveness of the screening methods for anal cancer.[6]

Anal cytology is currently the most used detection method for anal squamous intraepithelial lesions (SIL) and anal cancer.[7] However, similar to cervical cytology, anal cytology has low sensitivity, which may increase the need for a repeat evaluation.[8-14] Thus, there is a growing interest in studying the potential role of high-risk human papillomavirus (HR-HPV) genotyping (in particular, as a cotesting method or a triage test) as a screening test for SIL. High-resolution anoscopy (HRA), the gold standard for detecting SIL, is recommended in clinical settings following any abnormal cytology results or positive HR-HPV biomarkers, to determine whether anal SIL is present and how severe it is.[15] No standardized guidelines currently exist for anal cancer screening. However, experts in the field have developed their own approaches based on clinical experience, published data, and available resources in their communities.[10] Evidence shows that anal cytology alone is not the most effective screening method for HSIL, and that it should be used with other complementary techniques such as oncogenic biomarker detection (HR-HPV genotyping) or HRA (with/without biopsy).[9,16]

In the present study, we aim to: 1) describe the prevalence of anal HR-HPV infection and anal HSIL; 2) determine the accuracy, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of anal cytology and the HR-HPV test as screening tools for detecting anal HSIL; and 3) use kappa (κ) coefficients to evaluate the concordance between anal cytology and HRA-guided histology results in men and women from a specialized clinic in Puerto Rico.

MATERIALS AND METHODS

Data was obtained from medical records at the Anal Neoplasia Clinic (ANC) of the University of Puerto Rico Comprehensive Cancer Center in San Juan. The ANC is a specialized referral-based clinic that serves patients across the island and provides diagnostic, screening, and treatment services for anal lesions and anal cancer. Patients come to the ANC for various reasons: 1) they had an abnormal anal pap test at their primary clinic, 2) they have anal cancer symptoms (e.g., bleeding, a mass, itchiness, or hemorrhoids), 3) they are at higher risk for anal cancer (e.g. immunocompromised individuals or women with a history of lower genital dysplasia [cervical, vaginal, or vulvar]), or 4) they are concerned about their anal health (PLWH, immunocompromised individuals, and/or people with a history of HPV-related cancers). A detailed description of the ANC has been published in previous works by our team.[11,17] Of the six physicians who have provided clinical services to patients in the ANC, all have received training through the International Anal Neoplasia Society (IANS) to perform anal cytology and HRA, and four have been certified by the AIDS Malignancy Consortium (UM1-CA121947).

Secondary data analysis was performed on the cross-sectional data collected from patient medical records of baseline visits that took place at the ANC between October 2014 (when the clinic opened) and September 2021. A total of 974 men and women received services at the clinic during this period. To be included in this analysis, a patient record was required to contain complete information on the patient's HIV status, HR-HPV genotyping, anal cytology, *p16* immunostaining test (if applicable), and an HRA-guided biopsy (Figure 1). The final study sample was 466 patients, which comprised 48.0% of the ANC's patients. Data were entered and stored using the online storage platform REDCap.[18]

Anal cytology and HR-HPV genotyping

To perform anal cytology and HR-HPV genotyping during the baseline visit, a sample is obtained before the HRA is executed. Detection of anal cytological abnormalities and oncogenic HR-HPV genotypes is performed using a single collection of anal cells. Trained physicians obtain anal samples by blindly inserting a Dacron swab into the anal canal approximately 2 or 3 inches and rotating the swab 360° while applying lateral pressure to trace the entire surface of the anal wall.

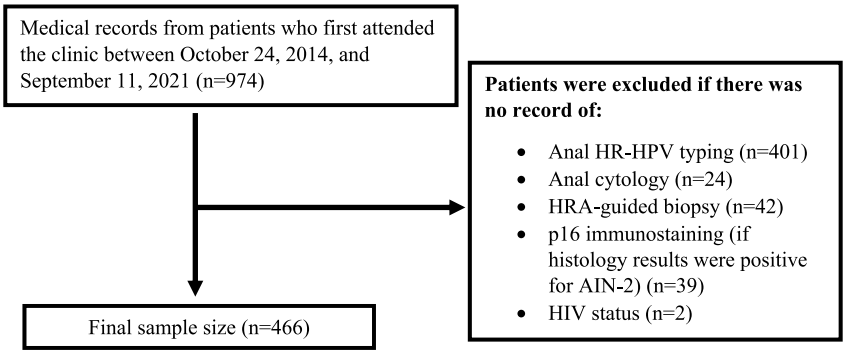


FIGURE 1 Eligibility criteria. HR-HPV indicates high-risk human papillomavirus; HRA, high-resolution anoscopy; AIN-2, anal intraepithelial neoplasia.

The swab is then removed and placed into a vial containing a solution for a few seconds.[17,19] Samples are then sent to the laboratory to be analyzed by trained pathologists. For standardized classification purposes, anal cytology samples are reported using the Bethesda System, which includes five categories: 1) negative for anal lesions; 2) atypical squamous cells of undetermined significance (ASCUS); 3) low-grade squamous intraepithelial lesions (LSIL); 4) atypical squamous cells, cannot exclude HSIL (ASC-H); and 5) HSIL.[19] Conversely, oncogenic biomarkers were detected using the Cobas 4800 test, which looks specifically for HR-HPV infections by amplifying target DNA through a polymerase chain reaction and nucleic acid hybridization. This test provides individual results for *HPV-16* and *HPV-18*, along with simultaneous, pooled results for other HR-HPV genotypes (including types *31*, *33*, *35*, *39*, *45*, *51*, *52*, *56*, *58*, *59*, *66*, and *68*).[20] Each sample was tested for these HR-HPV genotypes, and documentation was reported for those patients who tested positive.[17]

High-resolution anoscopy

An HRA procedure was performed during the baseline visit after the anal swab was taken. A disposable, clear, plastic Sani-Scope anoscope (CooperSurgical Inc, Trumbull, Connecticut)[17] is inserted in the anus; then the obturator is removed from the anoscope. A swab wrapped in gauze soaked in 3% acetic acid is introduced into the anoscope, then the anoscope is removed, and the gauze is left inside the anus. After 2 min, the gauze is removed from the anus, and the anoscope is reintroduced so that the anal transformation zone can be visualized using a Zeiss colposcope (Carl Zeiss Meditec AG, Jena).[17] Swabs with acetic acid are reapplied as needed to assess the epithelium. Staining with Lugol's iodine can help further characterize and identify any lesions. Areas with suspected anal SIL are biopsied and sent to the laboratory.[21] With the help of *p16* immunostaining in cases with anal intraepithelial neoplasia 2 (AIN-2), anal histology results are categorized using the Lower Anogenital Squamous Terminology (LAST) guidelines: 1) no SIL; 2) LSIL (defined as condyloma, LSIL, and *p16* negative AIN-2); and 3) HSIL (including *p16* positive AIN-2).[22] The most severe histopathology result from each patient is used for this classification.

Statistical analysis

Patients' sociodemographic information (age, sex assigned at birth, marital status), behavioral factors (lifetime tobacco use, sexual risk group, lifetime number of sexual partners, history of anal sex), and clinical data (HIV status, years living with HIV, use of antiretroviral therapy) were extracted from the clinic's medical records. Frequency distributions and measures of central tendency were used to describe the study population.

Chi-square test statistics (χ^2) were used to assess differences in study variables by HIV status. The weighted κ coefficient was used to assess the agreement between cytological and histological samples. For this particular analysis, anal cytology results were categorized as follows: no SIL (negative), LSIL (ASCUS/LSIL), or HSIL (ASC-H/HSIL).[13,14] Similarly, histology results were categorized as No SIL, LSIL, or HSIL+. The agreement was classified using the Landis and Koch guide as follows: poor ($0 \leq \kappa$), slight ($0 < \kappa \leq 0.2$), fair ($0.2 < \kappa \leq 0.4$), moderate ($0.4 < \kappa \leq 0.6$), substantial ($0.6 < \kappa \leq 0.8$), or almost perfect ($0.8 < \kappa \leq 1.0$).[23]

Validity measures such as sensitivity, specificity, and positive and negative predictive values were calculated using 95% confidence intervals (CIs) to compare the performance of anal cytology and HR-HPV genotyping against HRA-guided biopsy. For this analysis, histology results were dichotomized as no HSIL (No SIL/LSIL) or HSIL (HSIL/cancer); whereas anal cytology was categorized using different cutoff points: 1) ASCUS+: cytology results of ASCUS or worse; 2) LSIL+: cytology results of LSIL or worse; and 3) ASC-H/HSIL: cytology results of ASC-H or HSIL.[24] Analyses were done using Stata for Windows, Version 17 (Stata Corporation, College Station, Texas).

This study was approved by the institutional review board of the University of Puerto Rico, Medical Science Campus.

RESULTS

The mean age of patients was 43 years old (SD, 13.26) and the majority were PLWH (74.03%), single (54.04%), assigned male sex at birth (66.95%), and MSM (61.47%). Among the demographic and clinical

TABLE 1 Socio-demographic, behavioral, and clinical characteristics of a clinic-based sample of Hispanics by HIV status (n = 466).

	HIV (−) (n = 121)		HIV (+) (n = 345)		Overall (n = 466)		
Characteristic	**No.**	**(%)**	**No.**	**(%)**	**No.**	**(%)**	***p***
Age (years)							
Mean ± SD	44 ± 14.75		43 ± 12.70		43 ± 13.26		
Median (25th and 75th percentiles)	43 (31–55)		44 (31–54)	44 (31–54)	44 (31–54)		
<30	23	19.01	63	18.26	86	18.45	0.953
31–39	29	23.97	79	22.90	108	23.18	
40–49	23	19.01	74	21.45	97	20.82	
≥50	46	38.02	129	37.39	175	37.55	
Sex at birth							
Female	48	39.67	106	30.72	154	33.05	0.072
Male	73	60.33	239	69.28	312	66.95	
Marital status[a]							
Single	59	50.86	182	55.15	241	54.04	0.425
Partner	57	49.14	148	44.85	205	45.96	
Lifetime tobacco use[b]							
No	63	53.85	132	40.12	195	43.72	<0.05
Yes	54	46.15	197	59.88	251	56.28	
Lifetime number of sexual partners[c]							
≤10	66	58.41	157	49.53	223	51.86	0.105
>10	47	41.59	160	50.47	207	48.14	
History of anal sex							
No	39	32.23	74	21.45	113	24.25	**<0.05**
Yes	82	67.77	271	78.55	353	75.75	
Sexual risk group (lifetime)[d]							
MSM	65	55.08	211	63.75	276	61.47	
MSW	6	5.08	16	4.83	22	4.90	0.234
Women	47	39.83	104	31.42	151	33.63	
Years living with HIV (n = 345)[e]	–	–			–	–	
Mean ± SD			11 ± 9.74				
<5	–	–	120	35.61	–	–	
5-9	–	–	60	17.80	–	–	–
10-14	–	–	40	11.87	–	–	
≥15	–	–	117	34.72	–	–	
HAART therapy (n = 345)[f]							
No	–	–	3	0.93	–	–	–
Yes	–	–	321	99.07	–	–	

Abbreviations: HAART: highly active antiretroviral therapy; MSM, men who has sex with men; MSW, men who has sex with women.

[a]Missing value: 20.

[b]Missing value: 20.

[c]Missing value: 36.

[d]Missing value: 17.

[e]Missing value: 8.

[f]Missing value: 21.

characteristics evaluated, differences by HIV status were only seen in history of anal sex (HIV−: 67.77% vs. HIV+: 78.55%, $p < .05$) and history of tobacco use (HIV−: 46.15% vs. HIV+: 59.88%, $p < .05$). No other statistically significant differences were seen by HIV status (Table 1).

The overall prevalence of anal HR-HPV infection was 76.18%. Prevalence of the specific HR-HPV genotypes *16* and *18* was 34.65% and 17.18%, respectively. The prevalence of anal lesions by cytology was 45.49% for ASCUS, 13.52% for LSIL, 10.52% for ASC-H, and 2.79% for HSIL. The prevalence of anal lesions confirmed by histology was 42.92% for LSIL, 40.34% for HSIL, and 0.64% for anal cancer. A higher prevalence of anal HR-HPV infection (HIV−: 63.64% vs. HIV+: 80.58%, $p < .001$) and of histologically confirmed HSIL (HIV−: 30.58% vs. HIV+: 43.77%, $p < .001$) was observed among PLWH (Table 2).

The weighted κ between the cytology and histology results showed a concordance of 0.25 (95% CI, 0.17–0.33) in all the study samples. Concordance did not vary when stratifying by HIV status (PLWH [κ = 0.27 (95% CI, 0.11–0.44)] vs. people without HIV [κ = 0.22 (95% CI, 0.13–0.32)]). Additionally, 77.4% of cases with cytological HSIL results were confirmed by histology, but results suggest that anal cytology is not a good predictor of HSIL, because approximately 23% of people with a normal anal cytology had a diagnosis of HSIL in their histology results (Table 3).

In terms of the clinical performance of the tests, anal cytology to detect HSIL showed a sensitivity of 84.4% (95% CI, 78.5%–89.2%) and a specificity of 36.0% (95% CI, 30.3%–42.0%) in this study. The HR-HPV test showed a sensitivity of 92.1% (95% CI, 87.4%–95.6%) and a specificity of 34.9% (95% CI, 29.3%–40.9%). When combining anal cytology and HR-HPV (where both screening tests are positive), the sensitivity decreased to 78.5% (95% CI, 72.0%–84.1%) when using the ASCUS+ and HR-HPV+ cutoff point, and the specificity increased to 51.6% (95% CI, 45.6%–57.7%). Meanwhile, when using cotesting to detect HSIL (where at least one screening test is positive), the sensitivity increased to 97.9% (95% CI, 94.7%–99.4%), but the specificity decreased to 19.3% (95% CI, 14.8%–24.4%) (Table 4). For PLWH, the combination of both tests being positive (anal cytology and HR-HPV) using the cutoff point of ASCUS+ and HR-HPV+ had a similar sensitivity (82.5%; 95% CI, 75.5%–88.1%) and a higher specificity (45.5%; 95% CI, 38.3%–52.9%) compared with cytology or HR-HPV genotyping individually (Table 4). For people without HIV, the results were similar to those seen in the overall sample (Table 4).

TABLE 2 Prevalence of anal HR-HPV infection and SIL in a clinic-based sample of Hispanics by HIV status ($n = 466$).

	HIV− ($n = 121$)		HIV+ ($n = 345$)		Overall ($n = 466$)		
Test	**No.**	**(%)**	**No.**	**(%)**	**No.**	**(%)**	***p***
HR-HPV							
Negative	44	36.36	67	19.42	111	23.82	<.0001
Positive	77	63.64	278	80.58	355	76.18	
HR-HPV 16 ($n = 355$)							
Negative	52	67.53	180	64.75	232	65.35	.650
Positive	25	32.47	98	35.25	123	34.65	
HR-HPV 18 ($n = 355$)							
Negative	65	84.42	229	82.37	294	82.82	.674
Positive	12	15.58	49	17.63	61	17.18	
Other HR-HPV[a] ($n = 355$)							
Negative	13	16.88	26	9.35	39	10.99	.061
Positive	64	83.12	252	90.65	316	89.01	
Anal cytology[b]							
Negative	47	38.84	82	23.77	129	27.68	.005
ASCUS	54	44.63	158	45.80	212	45.49	
LSIL	8	6.61	55	15.94	63	13.52	
ASC-H	9	7.44	40	11.59	49	10.52	
HSIL	3	2.48	10	2.90	13	2.79	
Anal histology							
No SIL	28	23.14	47	13.62	75	16.09	.016
LSIL	56	46.28	144	41.74	200	42.92	
HSIL	37	30.58	151	43.77	188	40.34	
Cancer	0	0.00	3	0.87	3	0.64	

Abbreviations: ASC-H, atypical squamous cells, cannot exclude HSIL; ASCUS, atypical squamous cells of undetermined significance; HR-HPV, high-risk human papillomavirus; HSIL, high-grade squamous intraepithelial lesions; LSIL, low-grade squamous intraepithelial lesions; SIL, squamous intraepithelial lesions.

[a]Other types of HR-HPV include *31*, *33*, *35*, *39*, *45*, *51*, *52*, *56*, *58*, *59*, *66*, and *68*.

[b]Classification using Bethesda System.

DISCUSSION

To our knowledge, few studies have assessed the performance of anal cytology in comparison with HRA-guided biopsy in high-risk Hispanic/Latino populations.[26] A high prevalence of HR-HPV infection (76.18%) was found in our clinic-based study population; this prevalence was significantly higher among PLWH (HIV+: 80.58% vs. HIV−: 63.64%, $p < .001$). Similar HR-HPV infection prevalence was seen in other studies with similar settings.[10,25]

Regarding the prevalence of anal lesions, HSIL prevalence as identified through anal cytology was 13.25% (HSIL/ASC-H) in comparison to a prevalence of 41.03% through anal histology. A significantly higher prevalence of anal HSIL identified through histology was seen in PLWH in comparison to people without HIV (HIV+: 47.77% vs. HIV−: 30.58%, $p < .05$). A fair concordance between anal cytology and histology was observed (κ = 0.25; 95% CI, 0.17–0.33), but 23% of people with a normal anal cytology had HSIL in the histology result. This finding is consistent with previous studies that have shown that anal cytology is not a good predictor of disease severity.[9-14]

A meta-analysis conducted in 2022 used information from 39 studies to assess the accuracy of anal cancer screening tests in populations at higher risk of anal cancer.[26] Most of the studies included were from the United Sates ($n = 17$) and Europe ($n = 11$)[26] and focused on PLWH ($n = 29$) and MSM living with HIV ($n = 18$). The

TABLE 3 Comparison of anal cytology with histopathology results in the detection of anal SIL by HIV status.

	Anal histology Overall (n = 466)				
Anal cytology	**No SIL, No. (%)**	**LSIL, No. (%)**	**HSIL, No. (%)**	**Total**	**Weighted κ coefficient (95% CI)**
No SIL	47 (36.43)	52 (40.31)	30 (23.26)	129	0.25 (0.17–0.33) $p < .001$
LSIL	27 (9.82)	135 (49.09)	113 (41.09)	277	
HSIL	1 (1.61)	13 (20.97)	48 (77.42)	62	
Total	75 (16.01)	200 (42.92)	191 (40.99)	466	
	HIV+ (n = 345)				
No SIL	27 (32.93)	36 (43.90)	19 (23.17)	82	0.27 (0.11–0.44) $p < .001$
LSIL	19 (8.92)	98 (46.01)	96 (45.07)	213	
HSIL	1 (2.00)	10 (20.00)	39 (78.00)	50	
Total	47 (13.62)	144 (41.74)	154 (44.64)	98	
	HIV− (n = 121)				
No SIL	20 (42.55)	16 (34.04)	11 (23.40)	47	0.22 (0.13–0.32) $p < .001$
LSIL	8 (12.90)	37 (59.68)	17 (27.42)	62	
HSIL	0 (0.0)	3 (25.00)	9 (75.00)	12	
Total	28 (23.14)	56 (46.28)	37 (30.58)	121	

Abbreviations: CI, confidence interval; HSIL, high-grade squamous intraepithelial lesions; LSIL, low-grade squamous intraepithelial lesions; SIL, squamous intraepithelial lesions.

TABLE 4 Performance of anal cytology individually and in combination with anal HR-HPV typing for the detection of histologically confirmed HSIL by HIV status.

	Histologically confirmed HSIL[a]			
Screening tests (cytology and HR-HPV typing)	**Sensitivity % (95% CI)**	**Specificity % (95% CI)**	**PPV % (95% CI)**	**NPV % (95% CI)**
Overall (n = 466)				
Single test				
ASCUS+	84.3% (78.3%–89.1%)	36.0% (30.3%–42.0%)	47.8% (42.3%–53.3%)	76.7% (68.5%–83.7%)
LSIL+	44.0% (36.8%–51.3%)	85.1% (80.3%–89.1%)	67.2% (58.2%–75.3%)	68.6% (63.4%–73.5%)
ASC-H/HSIL	25.1% (19.1%–31.9%)	94.9% (91.6%–97.2%)	77.4% (65.0%–87.1%)	64.6% (59.7%–69.3%)
HR-HPV+	92.1% (87.4%–95.5%)	34.9% (29.3%–40.9%)	49.6% (44.3%–54.9%)	86.5% (78.7%–92.2%)
HPV16+	41.5% (34.1%–49.1%)	72.1% (64.9%–78.5%)	59.3% (50.1%–68.1%)	55.6% (49.0%–62.1%)
HPV18+	18.8% (13.3%–25.3%)	84.4% (78.2%–89.3%)	54.1% (40.8%–66.9%)	51.4% (45.5%–57.2%)
Both screening tests positive				
ASCUS+ and HR-HPV+	78.5% (72.0%–84.1%)	51.6% (45.6%–57.7%)	53.0% (47.0%–58.9%)	77.6% (70.9%–83.4%)
LSIL+ and HR-HPV+	42.9% (35.8%–50.3%)	86.2% (81.5%–90.0%)	68.3% (59.2%–76.5%)	68.5% (63.3%–73.4%)
ASC-H/HSIL and HR-HPV+	24.1% (18.2%–30.8%)	95.3% (92.1%–97.5%)	78.0% (65.3%–87.7%)	64.4% (59.5%–69.0%)
Cotesting (at least one positive screening tests)				
ASCUS+ or HR-HPV+	97.9% (94.7%–99.4%)	19.3% (14.8%–24.4%)	45.7% (40.8%–50.7%)	93.0% (83.0%–98.1%)
LSIL+ or HR-HPV+	93.2% (88.6%–96.3%)	33.8% (28.2%–39.7%)	49.4% (44.2%–54.7%)	87.7% (79.9%–93.3%)
ASC-H/HSIL or HR-HPV+	93.2% (88.6%–96.3%)	34.5% (28.9%–40.5%)	49.7% (44.4%–55.0%)	88.0% (80.3%–93.4%)

TABLE 4 (Continued)

Screening tests (cytology and HR-HPV typing)	Histologically confirmed HSIL[a]			
	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
HIV+ (n = 345)				
Single test				
ASCUS+	87.7% (81.4%–92.4%)	33.0% (26.4%–40.1%)	51.3% (45.1%–57.5%)	76.8% (66.2%–85.4%)
LSIL+	45.5% (37.4%–53.7%)	81.7% (75.4% -86.9%)	66.7% (56.8%–75.6%)	65.0% (58.6%–71.0%)
ASC-H/HSIL	25.3% (18.7%–33.0%)	94.2% (89.6%–97.1%)	78.0% (64.0%–88.5%)	61.0% (55.2%–66.6%)
HR-HPV+	92.2% (87.6%–96.4%)	29.3% (23.0%–36.3%)	51.4% (45.4%–57.5%)	83.6% (72.5%–91.5%)
HPV16+	39.9% (31.8%–48.4%)	69.6% (61.1%–77.2%)	58.2% (47.8%–68.1%)	52.2% (44.7%–59.7%)
HPV18+	18.2% (12.2%–25.2%)	83.0% (75.5%–88.9%)	53.1% (38.3%–67.5%)	48.9% (42.3%–55.6%)
Both screening tests positive				
ASCUS+ and HR-HPV+	82.5% (75.5%–88.1%)	45.5% (38.3%–52.9%)	55.0% (48.3%–61.5%)	76.3% (67.4%–83.8%)
LSIL+ and HR-HPV+	44.2% (36.2%–52.4%)	82.7% (76.6%–87.8%)	67.3% (57.3%–76.3%)	64.8% (58.4%–70.7%)
ASC-H/HSIL and HR-HPV+	24.0% (17.5%–31.6%)	94.2% (89.9%–97.1%)	77.1% (62.7%–88.0%)	60.6% (54.8%–66.2%)
Cotesting (at least one positive screening tests)				
ASCUS+ or HR-HPV+	98.1% (94.4%–99.6%)	16.8% (11.8%–22.8%)	48.7% (43.0%–54.4%)	91.4% (76.9%–98.2%)
LSIL+ or HR-HPV+	94.2% (89.2%–97.3%)	28.3% (22.0%–35.2%)	51.4% (45.4%–57.4%)	85.7% (74.6%–93.3%)
ASC-H/HSIL or HR-HPV+	94.2% (89.2%–97.3%)	29.3% (23.0%–36.3%)	51.8% (45.8%–57.8%)	86.2% (75.3%–93.5%)
HIV− (n = 121)				
Single test				
ASCUS+	70.3% (53.0%–84.1%)	42.9% (32.1%–54.1%)	35.1% (24.4%–47.1%)	76.6% (62.0%–87.7%)
LSIL+	37.8% (22.5%–55.2%)	92.9% (85.1%–97.3%)	70.0% (45.7%–88.1%)	77.2% (67.8%–85.0%)
ASC-H/HSIL	24.3% (11.8%–41.2%)	96.4% (89.9%–99.3%)	75.0% (42.8%–94.5%)	74.3% (65.1%–82.2%)
HR-HPV+	89.2% (74.6%–97.0%)	47.6% (36.6%–58.8%)	42.9% (31.6%–54.6%)	90.9% (78.3%–97.5%)
HPV16+	48.5% (30.8%–66.5%)	79.5% (64.7%–90.2%)	64.0% (42.5%–82.0%)	67.3% (52.9%–79.7%)
HPV18+	21.2% (8.98%–38.9%)	88.6% (75.4%–96.2%)	58.3% (27.7%–84.8%)	60.0% (47.1%–72.0%)
Both screening tests positive				
ASCUS+ and HR-HPV+	62.2% (44.8%–77.5%)	65.5% (54.3%–75.5%)	44.2% (68.3%–88.4%)	79.7% (68.3%–88.4%)
LSIL+ and HR-HPV+	37.8% (22.5%–55.2%)	94.0% (86.7%–98.0%)	73.7% (48.8%–90.9%)	77.5% (68.1%–85.1%)
ASC-H/HSIL and HR-HPV+	24.3% (11.8%–41.2%)	97.6% (91.7%–99.7%)	81.8% (48.2%–97.7%)	74.5% (65.4%–82.4%)
Cotesting (at least one positive screening tests)				
ASCUS+ or HR-HPV+	97.3% (85.8%–99.9%)	25.0% (16.2%–35.6%)	36.4% (26.9%–46.6%)	95.5% (77.2%–99.9%)
LSIL+ or HR-HPV+	89.2% (74.6%–97.0%)	46.4% (35.5%–57.6%)	42.3% (31.2%–54.0%)	90.7% (77.9%–97.4%)
ASC-H/HSIL or HR-HPV+	89.2% (74.6%–97.0%)	46.4% (35.5%–57.6%)	42.3% (31.2%–54.0%)	90.7% (77.9%–97.4%)

Abbreviations: ASC-H, atypical squamous cells, cannot exclude HSIL; ASCUS, atypical squamous cells of undetermined significance; CI, confidence interval; HR-HPV, high-risk human papillomavirus; HSIL, high-grade squamous intraepithelial lesions; LSIL, low-grade squamous intraepithelial lesions; NPV, negative predictive value; PPV, positive predictive value; SIL, squamous intraepithelial lesions.

[a]HSIL (no: benign/LSIL; yes: HSIL); cutoff points: ASCUS+, cytology results of ASCUS or worse; LSIL+, cytology results of LSIL or worse; ASC-H/HSIL, cytology results of ASC-H or HSIL.

prevalence of high-grade anal intraepithelial lesions (grade +2) as determined through histology in this meta-analysis was 20.0% (95% CI, 17.0%–29.0%).[26]

In our study, anal cytology alone had a sensitivity of 84.4% (95% CI, 78.5%–89.2%) and a specificity of 36.0% (95% CI, 30.3%–42.0%) for detecting HSIL in the overall study population, whereas HR-HPV

genotyping had a higher sensitivity of 92.1% (95% CI, 87.4%–95.5%) and a specificity of 34.9% (95% CI, 29.2%–40.9%). The 2022 meta-analysis reported that anal cytology to detect anal HSIL had a pooled sensitivity and specificity of 71.0% (95% CI, 72.0%–87.0%) and 62.4% (95% CI, 54.0%–70.0%), respectively.[26] The same study found a pooled sensitivity and specificity for HR-HPV testing of 91.9% (95% CI, 87.0%–95.0%) and 41.8% (95% CI, 35.0%–49.0%), respectively.[26] Most of the studies used in this meta-analysis included a population of MSM living with HIV. For PLWH, it was observed that when both screening tests are positive using the cutoff point of ASCUS+ and HR-HPV+, the sensitivity did not vary significantly in comparison with the sensitivity of the cytology and HR-HPV tests individually but a higher specificity was seen.

Although anal cytology continues to be the primary screening tool for SIL, the results from our manuscript and other research studies have shown that anal cytology is not the best predictor of lesion grade when compared to histology results.[12,14] For this reason, we assessed the performance of the anal HR-HPV test as an additional screening tool for anal HSIL. The role of HR-HPV genotyping in anal cancer screening is still under investigation. However, studies have shown that using HR-HPV genotyping as the primary screening tool can increase sensitivity, but lower specificity.[26,27] Similarly, when both tests (anal cytology and HR-HPV genotyping) were combined in our study, the sensitivity increased (97.9%), but the specificity decreased (19.3%). Similar results were seen within each subset of people without HIV and PLWH. This finding is consistent with the meta-analysis recently performed by Clarke and colleagues,[26] which showed an increase in sensitivity (93.0%; 95% CI, 86.0%–97.0%) but a decrease in specificity (33.4%; 95% CI, 25.0%–43.0%) when anal cytology was combined with HR-HPV biomarkers.

Although HR-HPV genotyping had a high sensitivity, a low specificity was seen across all groups in our study. This low specificity could be explained by the high prevalence of anal HR-HPV in the study population. Given the high sensitivity of the HR-HPV test, another approach could be to use it as a triage method instead of as a primary screening test.[4,28,29] Triage with HR-HPV genotyping, targeted age, and recommendations based on risk instead of results has been used for cervical cancer screening, which could increase specificity without sacrificing sensitivity.[28,29] Further studies with additional biomarkers could help further optimize screening tests for anal HSIL in high-risk populations. This is currently being studied in the National Cancer Institute-funded ULACNet network.[30]

Recently, the randomized clinical trial known as the Anal Cancer/HSIL Outcomes Research (ANCHOR) demonstrated that treatment for anal HSIL can reduce the incidence of anal cancer among PLWH.[31] These findings highlights that it is important to continue studies on how to increase the efficacy of anal cancer screening tools for the detection of anal HSIL. ANCHOR and similar studies may promote the development of national guidelines for the prevention of anal cancer in higher-risk populations.

The major strengths of this study are that: 1) all participants were recruited from a clinic that specializes in the diagnosis and management of anal lesions; 2) it is standard that all patients in the clinic undergo an HRA during the first visit, independent of their cytology and HR-HPV test results; 3) all patient records in the analysis include HRA-guided histology results; 4) HRA-guided histology was used as the gold standard to evaluate anal cytology and HR-HPV genotyping as screening tools; 5) women and people without HIV were included in the study population (in addition to PLWH and MSM); and 6) clinical data was used to achieve the primary outcome. On the other hand, a main limitation is that the findings from this clinic-based sample might not be generalizable to the whole population of Puerto Rico and other Latino populations. Also, although all physicians completed the IANS HRA course before starting clinical procedures, some of these procedures were performed by physicians in their initial phases of clinical training, which could introduce some sampling error during the biopsy collection and affect lesion classification. In addition, it is important to mention that among the population included for this analysis, 51.9% were referred to the Anal Neoplasia Clinic because of an abnormal pap in their primary clinic, and 1.2% because of a positive HPV test result (data not shown). Although these tests were repeated on the baseline evaluation performed in the clinic, the proportion of referrals due to abnormal cytology was higher than for HPV testing.

In conclusion, the results from our study suggest that more studies are needed to understand the role of HR-HPV genotyping in anal cancer screening. Further investigation should be done in other high-risk populations to assess if the results are replicable and to assess the use of HR-HPV triage for detecting anal HSIL. Finally, educational efforts should continue among high-risk populations and their physicians to increased awareness about anal cancer prevention and screening.

AUTHOR CONTRIBUTIONS

Jeslie M. Ramos-Cartagena: Conceptualization, methodology, data curation, writing–original draft, formal analysis, and visualization. **Kandyce Keller:** Formal analysis, writing–original draft, and visualization. **Humberto M. Guiot:** Resources, conceptualization, investigation, and writing–review and editing. **Cristina Muñoz:** Resources, project administration, and investigation. **Vivian Colón-López:** Funding acquisition and writing–review and editing. **Ashish A. Deshmukh:** Writing–review and editing and funding acquisition. **Erick L. Suárez:** Methodology and writing–review and editing. **Maribel Tirado-Gómez:** Investigation, supervision, and funding acquisition. **Ana P. Ortiz:** Conceptualization, methodology, writing–review and editing, supervision, visualization, and funding acquisition.

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[Correction added on 15 July 2023, after first online publication: Acknowledgment section has been updated.]

CONFLICT OF INTEREST STATEMENT

Kandyce Keller reports grant funding from Universidad de Puerto Rico. The other authors declare no conflicts of interest.

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