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Authors

Chiao, Elizabeth Y
Lensing, Shelly Y
Wiley, Dorothy J
et al.

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Screening strategies for the detection of anal high-grade squamous intraepithelial lesions in women living with HIV

Elizabeth Y. Chiao[a], Shelly Y. Lensing[b], Dorothy J. Wiley[c], Ashish A. Deshmukh[d], Jeannette Lee[b], Teresa M. Darragh[e], Mark H. Einstein[f], Naomi Jay[g], John Michael Berry-Lawhorn[g,h], Joel M. Palefsky[g,i], Timothy Wilkin[j], Luis F. Barroso[k], Ross D. Cranston[l], Rebecca Levine[m], Humberto M. Guiot[n,o], Audrey L. French[p], Deborah Citron[q], Masoumeh Katayoon Rezaei[r], Stephen E. Goldstone[s] and Elizabeth A. Stier[t]

Objective: HIV-infected women (WLHIV) have more than 10-fold higher risk for squamous cell cancer of the anus. Experts suggest cytology-based strategies developed for cervical cancer screening may prevent anal cancer by detecting anal cytologic or histological high-grade squamous intraepithelial lesion (hHSIL) for treatment. Currently, there is no consensus on anal-hHSIL screening strategies for WLHIV.

Design: Between 2014 and 2016, 276 WLHIV were recruited at 12 US AIDS Malignancy Consortium clinical trials sites to evaluate hHSIL prevalence and (test) screening strategies.

Methods: Participants completed detailed questionnaire, underwent anal assessments including high-risk human papillomavirus (hrHPV) testing using hrHPV-Hybrid Capture 2 (HC2) and hrHPV-APTIMA, anal cytology, and concurrent high-resolution anoscopy. Screening test characteristics for predicting hHSIL validated by central review of histologic diagnosis were estimated sensitivity, specificity, positive predictive value, and false-omission rate. Paired analyses compared sensitivity and specificity for hrHPV single tests to anal cytology alone.

Results: 83% (229/276) of enrolled WLHIV had complete anal assessment data and were included in this analysis. Mean age was 50, 62% black and 60 (26%) had hHSIL.

[a]Department of Epidemiology and General Oncology, University of Texas- MD Anderson Cancer Center, Houston, Texas, [b]Department of Biostatistics, University of Arkansas for Medical Sciences, Little Rock, Arkansas, [c]School of Nursing, University of California, Los Angeles, Los Angeles, California, [d]Department of Management Policy and Community Health, School of Public Health, The University of Texas Health Science Center at Houston, Houston, Texas, [e]Department of Pathology, UCSF Mt. Zion Medical Center, San Francisco, California, [f]Department of OB/GYN & Women's Health, Rutgers-NJMS, Newark, New Jersey, [g]Department of Medicine, Anal Neoplasia Clinic, Research, and Education Center, [h]Division of Hematology Oncology, [i]Department of Medicine, University of California, San Francisco, San Francisco, California, [j]Clinical Trials Unit, Department of Medicine, Cornell University, New York, New York, [k]Department of Internal Medicine (Infectious Diseases), Wake Forest University Health Sciences, Winston-Salem, North Carolina, USA, [l]University of Vic, Hospital Germans Trias i Pujol, Barcelona, Spain, [m]Department of Surgery, Montefiore, Bronx, New York, [n]Department of Medicine, [o]Department of Microbiology & Medical Zoology, University of Puerto Rico School of Medicine, San Juan, Puerto Rico, [p]Division of Infectious Diseases, CORE Center/Stroger Hospital of Cook County, Chicago, Illinois, [q]Department of Pathology, Baylor College of Medicine, Houston, Texas, [r]Department of Pathology, George Washington University, Washington, District of Columbia, [s]Department of Surgery, Icahn School of Medicine at Mount Sinai, New York, New York, and [t]Department of Obstetrics and Gynecology, Boston University School of Medicine, Boston, Massachusetts, USA.

Correspondence to Elizabeth Y. Chiao, MD, MPH, Department of Epidemiology and General Oncology, University of Texas MD Anderson Cancer Center, 1155 Pressler St., Unit 1340, Houston, Texas, USA.

Tel +1 713 792 1860; e-mail: eychiao@mdanderson.org
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Anal cytology (>atypical squamous cells of undetermined significance), hrHPV-HC2, and hrHPV-APTIMA sensitivity estimates were similarly high (83, 77, and 75%, respectively, P values > 0.2). Specificity was higher for both hrHPV-APTIMA and hrHPV-HC2 compared with anal cytology (67 vs. 50%, $P < 0.001$) and (61 vs. 50%, $P = 0.020$), respectively.

Conclusion: Anal hrHPV testing demonstrated similar sensitivity for anal cytology (>atypical squamous cells of undetermined significance) to predict anal hHSIL. Among tests with similar sensitivity, the specificity was significantly higher for hrHPV-APTIMA and hrHPV-HC2. Thus, anal hrHPV testing may be an important alternative strategy to anal cytology for anal hHSIL screening among WLHIV.

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Keywords: anal cytology, anal dysplasia, high-risk human papillomavirus test, operating characteristics, screening, women living with HIV

Introduction

Persons living with HIV (PLWH) are at elevated risk of developing squamous cell carcinoma of the anus (SCCA) compared with the general population [1]. Compared with the general population, men who have sex with men (MSM) living with HIV have the highest SCCA risk (nearly 30–40-fold) followed by women living with HIV (WLHIV) (nearly 10-fold higher risk) [1,2]. There are differences but also pathophysiological similarities between cervical and anal cancer, including an etiologic association with persistent human papillomavirus (HPV) infection that if persist can progress to precancerous lesions, known as histologic high-grade squamous intraepithelial lesions (hHSIL) [3]. Because of high rates of SCCA among PLWH, several professional societies recommend algorithms similar to cervical cancer screening where anal cytology testing is followed by diagnostic high-resolution anoscopy (HRA) for anal hHSIL detection and subsequent treatment to prevent progression to SCCA [4].

The majority of prior studies have focused on determining the operating characteristics of anal cytology and high-risk HPV (hrHPV) testing in population-based studies of HIV-positive MSM [5]. While the use of anal cytology has been widely accepted as the optimal screening modality for HIV-positive MSM for anal precancers, the lack of studies measuring the performance characteristics for anal cancer screening among US WLHIV (i.e., cytology and/or HPV testing) hinders the ability to determine cost-effective screening strategies for these women [6]. Over the past 2 decades, hrHPV testing has been incorporated into cervical cancer prevention strategies in conjunction with cervical cytology to improve screening accuracy for the detection of cervical hHSIL [7]. However, to the best of our knowledge, no prior work has evaluated clinical performance of anal hrHPV testing in lieu of, or in addition to anal cytology for anal cancer screening for anal hHSIL in WLHIV.

An ongoing large randomized trial 'ANal Cancer/HSIL Outcomes Research' trial will address an important question regarding the efficacy of anal hHSIL treatment for SCCA prevention for PLWH [8]. However, understanding clinical performance of screening strategies to detect anal hHSIL among PLWH is crucial to develop efficient screening algorithms that will maximize cancer prevention and decrease unnecessary procedures. The AIDS Malignancy Consortium (AMC) study, 'AMC084: Screening HIV-positive women for anal cancer precursors (AMC084)', is a multicenter US-based national trial [9] that was designed to determine the performance characteristics of anal cytology and hrHPV testing in WLHIV. As such, unlike prior studies, all enrolled women underwent screening evaluations including anal cytology, anal HPV tests and concurrent HRA and biopsy (in contrast to prior studies where biopsies were only conducted in women with abnormal cytology), thus minimizing the potential for false negatives. In this article, we describe the performance characteristics of anal cytology and anal HPV tests alone and in combination compared with HRA-guided biopsies at the baseline visit for the cohort of women enrolled in the national study (AMC084).

Methods

Study design

AMC084 is a longitudinal, multisite national study of WLHIV to determine prevalence and incidence of anal HPV and hHSIL detected over a 2-year follow-up period. WLHIV were recruited at 12 US sites between 2014 and 2016. The clinicians responsible for performing HRA at each site were certified using a standardized approach focused on quality assessments developed and implemented by the AMC HPV Working Group [9]. The study protocol was approved by the US National Cancer

Institute, Cancer Therapy Evaluation Program and by institutional review boards for each participating institution. Potential participants were screened using a standardized questionnaire and medical records review. At baseline, all women were evaluated using a standard protocol. Women without hHSIL were then followed-up semiannually for 2 years. Follow-up was and discontinued if incident anal hHSIL, the primary endpoint, was diagnosed.

Sample and subjects

Eligible women were 18 years or older, living with HIV, had no history of anal hHSIL by cytology or histology, and had laboratory test results within the past 120 days showing absolute neutrophil and platelet count of more than 750 cells/μl and at least 75 000 cells/μl, respectively. Women with a history of pelvic radiation, anal or perianal cancer or treatment for anal or perianal condyloma or low-grade SIL (LSIL) within 4 months of study entry were ineligible.

Of 276 participants enrolled, 256 eligible participants had specimens available for central pathology review and comprised the baseline cohort. The cohort for evaluating test strategies consisted of the 229 study participants who had complete data on all tests: anal cytology and anal HPV results from both Hybrid Capture 2 (HC2; Qiagen, Inc, Valencia, California, USA) and HPV-Aptima Test (Hologic Corp, San Diego, California, USA). Participant characteristics were summarized for the complete baseline cohort and compared with the test strategy cohort and were not significantly different (data not shown).

Procedures

Clinical charts were reviewed and study participants queried for HIV information (date of diagnosis, current and nadir $CD4^+$ T-cell counts, current HIV viral load, and antiretroviral therapy history) and HPV-related information (past history of HPV-related anogenital diseases, including warts, abnormal cervical cytology, and colposcopy results). A baseline questionnaire was administered to the study participants by research staff to determine smoking status and recent sexual history. HIV viral load and $CD4^+$ cell count data were collected within 120 days of study enrollment, and if not available from clinical chart review were subsequently collected. All participants underwent a targeted physical exam, including exams of the vulva, vagina/cervix, anus, and perianus for signs of HPV-related lesions.

Anal specimen collection

Two anal specimens were collected for cytology and HPV testing. The swab for cytology was immersed in the liquid-based cytology (LBC) media required for local processing at each local institution (PreservCyt or SurePath). Because of concerns of the order of swab collection affecting the quality of the swab specimen on the analyses, anal swab specimens were collected from each study participant in the order as randomly assigned at the time of enrollment.

High-resolution anoscopy and biopsy

Following anal cytology/HPV specimen collection, a digital anorectal exam was performed followed by HRA of the anal canal and perianus with at least two directed or random biopsies as previously described [9].

Laboratory testing

Cytology specimen processing

Local pathology departments processed the cervical and anal cytology specimens and evaluated the cytology using the Bethesda Classification System [10]. Cytology samples did not undergo Central Pathology Review as all of these sites had prior quality assurance for anal cytology from other anal cancer prevention trials.

Anal human papillomavirus analyses

Anal HPV-HC2 analysis was performed at the manufacturers' laboratories (Qiagen Corporation, Gaithersburg, Maryland, USA) as was HPV-Aptima (Hologic Inc., Marlborough, Massachusetts, USA) and results were reported to the investigators. HPV-HC2 is a signal amplification assay that detects at least 1 pg of HPV-DNA for a pool of 13 different hrHPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). HPV-Aptima assay is a nucleic acid amplification test that detects the HPV E6/E7 mRNA for a pool of 14 high-risk types of HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). HPV detection specimens underwent further analysis with the HPV-Aptima 16 18/45 Genotype Assay to identify specimens with HPV genotypes 16 and 18/45 (designated 16/18/45+).

Cytology specimen processing

Local pathology departments processed the cervical and anal cytology specimens and evaluated the cytology using the Bethesda Classification System [10]. Specimens were evaluated as negative for intraepithelial lesions or malignancy (NILM), atypical squamous cells (ASC) of undetermined significance (ASC-US), ASC, cannot rule out high-grade squamous intraepithelial lesion (ASC-H), and low-grade and high-grade squamous intraepithelial lesion (LSIL and HSIL, respectively). Cytology samples did not undergo Central Pathology Review as all of these sites had prior quality assurance for anal cytology from other anal cancer prevention trials.

Anal histology

The study outcome of interest was anal hHSIL (vs. less than hHSIL) as determined by the central pathology consensus review; all biopsy histopathology slides were reviewed by at least two independent pathologists. If there was disagreement between the two pathologists, a third pathologist review served as a tiebreaker. Biopsy specimens were evaluated using terminology and classifications

(including recommendations for p16 staining) from the Lower Anogenital Squamous Terminology Project [3]. Anal histology was reviewed by local and central pathology as previously described [9].

Statistical analysis

Log-linked binomial regression models were used to estimate risk ratios for anal hHSIL detection. Models were adjusted for race, current $CD4^+$ cell count and history of anal sex as these variables were found to be significantly associated with hHSIL diagnosis in our previous study [9]. The Kappa statistic was computed to measure the agreement between tests. Proportions with exact binomial confidence intervals (CIs) were computed for screening test characteristics, including sensitivity, specificity, positive predictive value (PPV), 1-negative predictive value (1-NPV) (also known as the False omission rate) for predicting hHSIL as determined by central pathology review as described above. McNemar's Chi-square test for paired categorical data was used to compare sensitivities and specificities for test strategies to cytology alone. The associations between swab collection order and HPV test results were evaluated using chi-square tests. *P* values less than 0.05 were deemed statistically significant. Analyses were conducted in SAS/STAT software, version 12.1 of the SAS System (SAS Institute, Cary, North Carolina, USA).

Results

The derivation of the 229 women included in the test strategy cohort from the full baseline cohort (256 women) is shown in Fig. 1. Enrollment occurred from February 2014 to June 2016. Table 1 summarizes the characteristics of 229 women included in the test strategy cohort. The median age of WLHIV in the test strategy cohort was 50 years [interquartile range (IQR): 44–55]. Women were predominantly non-Hispanic African Americans (62%) and current or former smokers (67%). Fifty-five percentage reported at least one lifetime male anal sex partner, and nearly half (48%) reported a prior sexual assault. Most were on cART (96%) with relatively high $CD4^+$ T-lymphocyte counts [median = 667 (IQR: 455–878 cells/μl)] and 86% had suppressed HIV viral load. Nearly 29% had abnormal cervical/vaginal cytology, with 27% testing positive for cervical/vaginal HPV-DNA.

There were a total of 60 prevalent hHSIL (26%) detected (Table 1). The prevalence of abnormal anal cytology

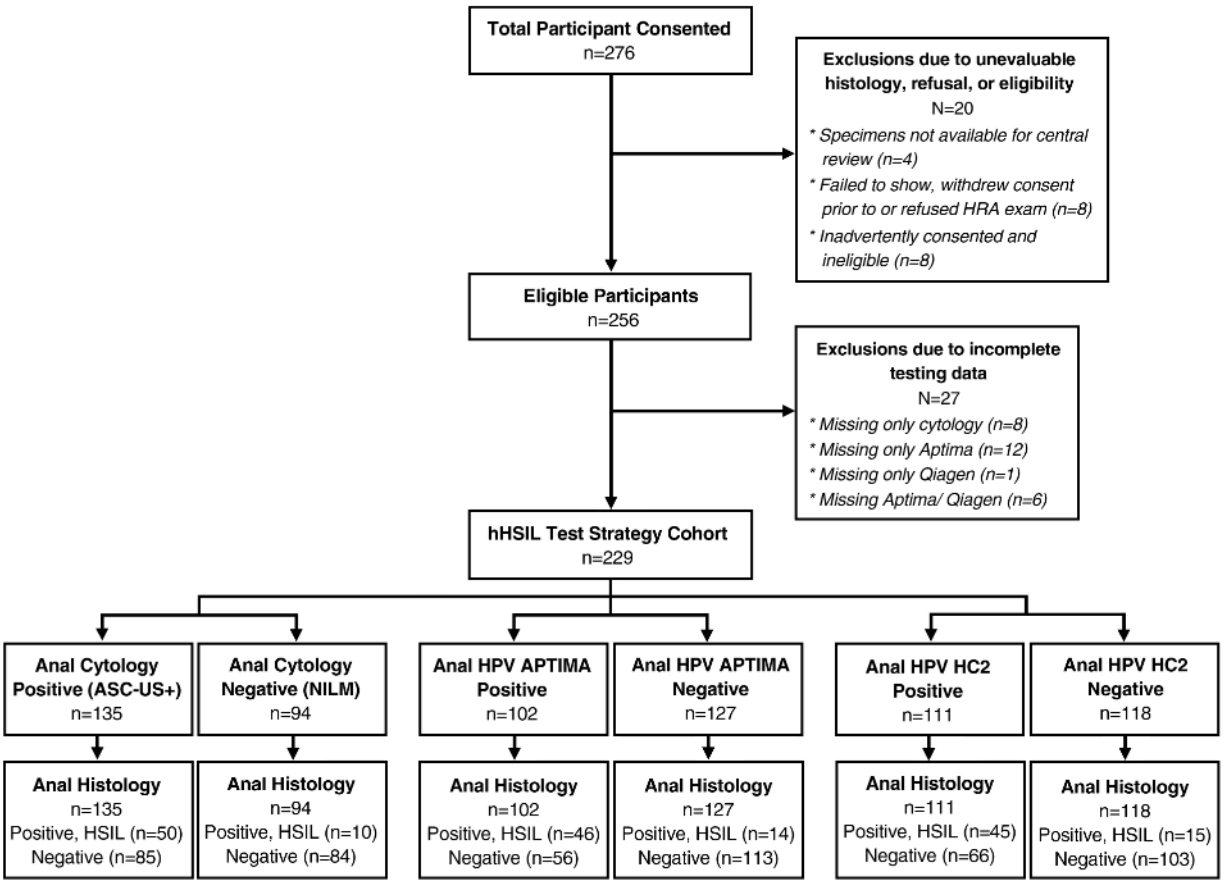

Fig. 1. Flow diagram for patient test strategy cohort.

Table 1. Participant characteristics[a].

Demographic characteristics	Test strategy cohort $N=229$[a] n (%)
Age, median (IQR)	50 (44–55)
Age	
<40 years	29 (13)
40–49 years	74 (32)
≥50 years	126 (55)
Race/Ethnicity	
NH Black	143 (62)
NH white or other	36 (16)
Hispanic	50 (22)
Smoking status	
Former/Current	149 (67)
Never	75 (33)
Education	
High school diploma or less	120 (54)
Some college or higher	101 (46)
Annual income	
<$20K	174 (82)
≥$20K	39 (18)
Marital status	
Married/Not married, living with someone	47 (21)
Divorced/Widowed/Single	179 (79)
HIV characteristics	
Current $CD4^+$ T-cell count, median (IQR)	667 (455–878)
Range	4–1961
Current $CD4^+$ T-cell count	
≤200 cells/μl	16 (7)
201–350 cells/μl	21 (9)
>350 cells/μl	192 (84)
Viral load	
Suppressed (≤200 copies/μl)	195 (86)
Unsuppressed (>200 copies/μl)	32 (14)
Nadir $CD4^+$ T-cell count	
≤200 cells/μl	110 (50)
>200 cells/μl	111 (50)
Current cART user	
Yes	216 (96)
No/Unsure	10 (4)
Reported clinical history	
Lifetime male anal sex partners	
0	99 (45)
1	65 (30)
≥2	55 (25)
History of anogenital warts	
Yes	60 (27)
No	161 (73)
History of abnormal cervical cytology	
Yes	123 (54)
No/Unsure/Declined	103 (46)
History of sexual assault	
Yes	106 (48)
No/Declined	115 (52)
Baseline cervical/Vaginal screening results	
Cervical/Vaginal cytology	
NILM[b]	158 (71)
ASC-US/LSIL	56 (25)
ASC-H/HSIL	10 (4)
Cervical/Vaginal HPV (HC2)	
HPV+	62 (27)
HPV−	166 (73)
Baseline anal screening results	
Anal cytology	
NILM	94 (41)
ASC-US/LSIL	114 (50)
ASC-H/HSIL	21 (9)
Anal HPV APTIMA	
HPV+	102 (45)
HPV−	127 (55)
Anal HPV HC2	
HPV+	111 (48)
HPV−	118 (52)
Baseline anal histology outcome	
Anal histology	
Benign	133 (58)
LSIL	36 (16)
hHSIL	60 (26)

ASC-US, atypical squamous cells of undetermined significance; cART, combined antiretroviral therapy; HC2, Hybrid Capture 2; HPV, human papillomavirus; IQR, interquartile range; LSIL, low-grade squamous intraepithelial lesion; NH, non-Hispanic; NILM, negative for intraepithelial lesions or malignancy.

[a]The denominators do not sum to 229 for some variables due to missing responses; variables with the most missing data are annual income ($n=16$), lifetime male sex partners ($n=10$), and education ($n=8$).

[b]No intraepithelial lesion or malignancy.

(ASC-US+), and detection of anal HPV was high. The prevalence of ASC-US+ on anal cytology was 59%. ASC-H/HSIL was found in only 9% (21/229). Almost half tested positive by one or both anal hrHPV tests: 45% (102/229) were positive by HPV-Aptima, 48% (118/229) by HC2. HPV test and anal cytology results were unaffected by swab order (all $P>0.25$ for overall Wald test comparing three orderings, data not shown).

Tabular statistics and unadjusted risk ratios for anal hHSIL by anal cytology, anal HPV testing, cervical cytology, and cervical-HPV testing, adjusted for selected demographic and clinical variables are summarized in Table 2. Compared with women with NILM anal cytology, women with anal cytology of ASC-H or HSIL showed statistically significant increased risk of hHSIL as did those with anal ASC-US or LSIL but to a lesser extent [unadjusted risk ratio = 7.2 (95% CI: 3.8–13.5) and risk ratio = 2.8 (95% CI: 1.5–5.4), $P<0.05$, respectively]. Cervical cytologic ASC-H or HSIL was strongly associated with anal hHSIL compared with normal cervical cytology [risk ratio = 3.1 (1.9–5.1)], whereas cervical cytology of LSIL or ASC-US was not [risk ratio = 1.3 (95% CI: 0.8–2.2)]. Testing positive for HPV in the anus or cervix were all associated with anal hHSIL: anal HPV by HC2 (risk ratio = 3.6, 95% CI: 1.9–5.4), anal HPV by HPV-Aptima (risk ratio = 4.1, 95% CI: 2.4–7.0), or cervical HPV by HC2 (risk ratio = 1.7, 95% CI: 1.1–2.6). In the multivariable model, which adjusted for race, current $CD4^+$, and history of anal sex, abnormal anal cytology, detection of anal HPV, ASC-H/HSIL cervical cytology, and detection of cervical HPV by HC2 were still associated with anal hHSIL.

Test performance characteristics

Sensitivity, specificity, and predictive value of PPV and false omission rate (1-NPV) tests.

Table 2. Positivity for individual anal and cervical screening tests according to anal hHSIL histology in the test strategy cohort.

	Total	Anal hHSIL	Unadjusted RR	Adjusted RR[a]
Anal cytology	*N*	*n* (%)	(95% CI)	(95% CI)
NILM	94	10 (11)	1	1
ASC-US+	135	50 (37)	3.48 (1.86, 6.51)	3.35 (1.65, 6.82)
ASC-US/LSIL	114	34 (30)	2.80 (1.46, 5.37)	1.49 (1.06, 2.08)
ASC-H/HSIL	21	16 (76)	7.16 (3.80, 13.49)	2.33 (1.58, 3.43)
Anal HPV APTIMA				
Negative	127	14 (11)	1	1
Positive for any hrHPV	102	46 (45)	4.09 (2.39, 7.01)	2.02 (1.35, 3.02)
HPV+, 16/18/45−	55	17 (31)	2.80 (1.49, 5.28)	3.17 (1.63, 6.17)
16/18/45+	47	29 (62)	5.60 (3.25, 9.63)	5.75 (3.15, 10.50)
Anal HPV HC2				
Negative	118	15 (13)	1	1
Positive	111	45 (41)	3.55 (1.89, 5.38)	1.77 (1.23, 2.54)
Cervical cytology[b]				
NILM	158	36 (23)	1	1
ASC-US/LSIL	56	17 (30)	1.33 (0.82, 2.17)	1.29 (0.74, 2.23)
ASC-H/HSIL	10	7 (70)	3.07 (1.87, 5.05)	3.41 (2.04, 5.69)
Cervical HPV HC2[b]				
Negative	166	36 (22)	1	1
Positive	62	23 (37)	1.71 (1.11, 2.64)	1.84 (1.15, 2.95)
Total	229	60 (26)		

ASC-US, atypical squamous cells of undetermined significance; CI, confidence interval; HC2, Hybrid Capture 2; hrHPV, high-risk human papillomavirus; LSIL, low-grade squamous intraepithelial lesion; NILM, negative for intraepithelial lesions or malignancy; RR, risk ratio.
[a]Adjusting for race (non-Hispanic Black vs. all others), current $CD4^+$ ($\leq$200, 201–350, >350), and history of anal sex (0 vs. 1+ partners). 10 Women were missing data for at least one of these covariates so that the adjusted models included at most 219 women.
[b]Five women had missing data for cervical cytology and one for cervical HPV testing.

Figure 2 and Table 3 compare the operating characteristics of cytology to eight alternate screening strategies for a referral to HRA and anal hHSIL detection. The sensitivity for abnormal (ASC-US+) cytology was 83% and was not statistically significantly higher than sensitivity for detection of anal HPV-Aptima (HPV-Aptima+) and HPV-HC2+ (77 and 75%, respectively); 1-NPV for anal hHSIL were low for these tests and comparisons with cytology were all nonsignificant (all $P > 0.30$). While the specificity for ASC-US+ cytology was 50%, the specificity for HPV-Aptima+ was 67% ($P < 0.001$) and HPV-HC2+ was 61% ($P = 0.020$) demonstrating that the specificity of both hrHPV tests were significantly higher than cytology. The PPV for HPV-Aptima+ was significantly higher than PPV for ASC-US+ anal cytology (45 vs. 37%, $P = 0.011$), but PPV (41%) for HPV-HC2+ was not ($P = 0.27$). Anal cytology of ASC-H/HSIL showed the highest specificity (97%) but the lowest sensitivity (27%) compared with other screening tests (all $P < 0.001$). Aptima 16/18/45+ had the second highest specificity (89%, 95% CI 84%, 94%) but also the second lowest sensitivity (43%, 95% CI

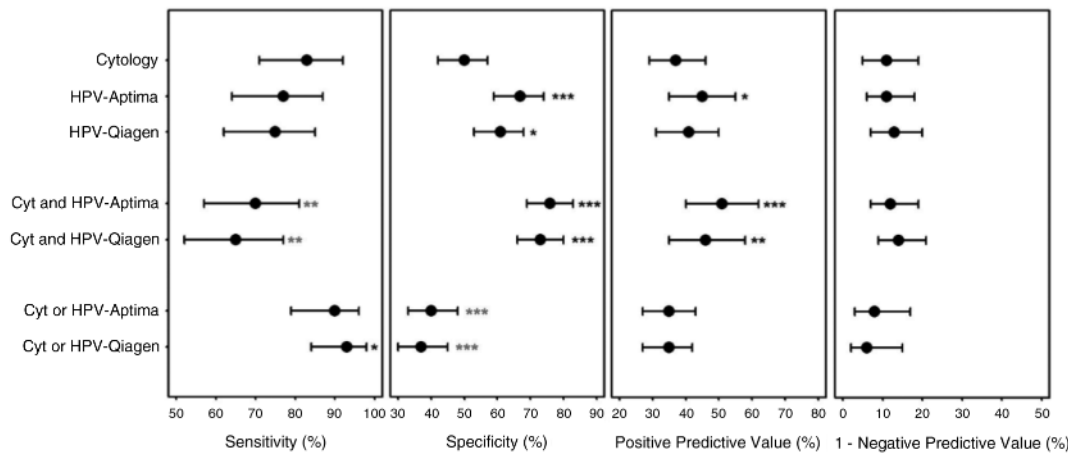

Fig. 2. Test characteristics for performance of cytology, human papillomavirus-Aptima, and human papillomavirus-Qiagen for predicting hHSIL in 229 women living with HIV. Paired comparisons with corresponding cytology test characteristic *P* value: *0.01–0.05, **0.001–<0.01, ***<0.001. Dot represent test characteristic estimate and error bars represent exact binomial confidence intervals.

Table 3. Operating test characteristics for anal cancer screening test strategies for predicting anal hHSIL in 29 women living with HIV.

Anal testing screening strategies	Positive test (HRA referral)	Number referred per hHSIL identified*	Youden's J[a]	SENS (%)	(95% CI)	SPEC (%)	(95% CI)	PPV (%)	(95% CI)	1-NPV[b] (%)	(95% CI)
Single test											
Cytology (ASC-US+)	135 (59%)	2.3	0.33	83	(71%, 92%)	50	(42%, 57%)	37	(29%, 46%)	11	(5%, 19%)
Cytology (LSIL+)	66 (29%)	1.1	0.40	58	(45%, 71%)	82	(75%, 87%)	53	(40%, 65%)	15	(10%, 22%)
Cytology (ASC-H/HSIL)	21 (9%)	0.3	0.24	27	(16%, 40%)	97	(93%, 99%)	76	(53%, 92%)	21	(16%, 27%)
APTIMA (positive any type)	102 (45%)	1.7	0.44	77	(64%, 87%)	67	(59%, 74%)	45	(35%, 55%)	11	(6%, 18%)
APTIMA 16/18/45+	47 (21%)	0.8	0.37	48	(35%, 62%)	89	(84%, 94%)	62	(46%, 75%)	17	(12%, 23%)
HC2	111 (48%)	1.8	0.36	75	(62%, 85%)	61	(53%, 68%)	41	(31%, 50%)	13	(7%, 20%)
Triage (both screening tests positive)											
ASC-US+ cytology and APTIMA	82 (36%)	1.4	0.46	70	(57%, 81%)	76	(69%, 83%)	51	(40%, 62%)	12	(7%, 19%)
ASC-US+ cytology and HC2	84 (37%)	1.4	0.38	65	(52%, 77%)	73	(66%, 80%)	46	(35%, 58%)	14	(9%, 21%)
(ASC-US and APTIMA+) OR LSIL+	100 (44%)	1.7	0.42	75	(62%, 85%)	67	(60%, 74%)	45	(35%, 55%)	12	(7%, 18%)
(ASC-US/LSIL and APTIMA+) OR ASC-H+	85 (37%)	1.4	0.45	70	(57%, 81%)	75	(67%, 81%)	49	(38%, 60%)	13	(8%, 19%)
ASC-US+ and APTIMA 16/18/45+	38 (17%)	0.7	0.36	43	(31%, 57%)	93	(88%, 96%)	68	(51%, 83%)	18	(13%, 24%)
APTIMA and HC2	91 (40%)	1.5	0.41	70	(57%, 81%)	71	(64%, 78%)	46	(36%, 57%)	13	(8%, 20%)
Cotesting (one or more positive screening tests)											
ASC-US+ cytology or APTIMA	155 (68%)	2.6	0.30	90	(79%, 96%)	40	(33%, 48%)	35	(27%, 43%)	8	(3%, 17%)
ASC-US+ cytology or HC2	162 (71%)	2.7	0.30	93	(84%, 98%)	37	(30%, 45%)	35	(27%, 42%)	6	(2%, 15%)
HC2 or APTIMA	122 (53%)	2.0	0.39	82	(70%, 90%)	57	(49%, 64%)	40	(31%, 49%)	10	(5%, 18%)

ASC-US, atypical squamous cells of undetermined significance; CI, confidence interval; HC2, Hybrid Capture 2; HRA, high-resolution anoscopy; LSIL, low-grade squamous intraepithelial lesion; NPV, negative predictive value; PPV, positive predictive value; SENS, sensitivity; SPEC, specificity.

[a]SENS + SPEC-1.

[b]1-NPV, or false omission rate.

*Based on an observed prevalence of 26% anal histological HSIL.

35%, 62%). Abnormal cytology (ASC-US+) compared with either HPV+ test had higher rates of referrals for HRA (59 vs. 45–48%) and higher number referred per hHSIL identified (2.3 vs. 1.7–1.8) (Table 3).

Figure 2 and Table 3 also show triage (or *in series*) and cotesting strategies (or *in-parallel* strategies) operating characteristics for referral for HRA for the detection of anal hHSIL. Triage screening strategies that combine ASC-US+ anal cytology with the detection of hrHPV demonstrated that the sensitivity of *cytology ASC-US+ as well as* HC2+ was 65% and that of cytology *ASC-US+ as well as* HPV-Aptima+ was 70%; both of these triage strategies were less sensitive than cytology *ASC-US+* alone ($P < 0.01$, for both). Accordingly, the specificities of both triage strategies were significantly higher than cytology alone; 73% for *ASC-US+* as well as HC2+ and 76% for *ASC-US+* as well as HPV-Aptima+ ($P < 0.001$ for both). Cotesting where positivity on either screening test (cytology *ASC-US+ or* detection of hrHPV) showed that having abnormal cytology *ASC-US+* or testing HPV-HC2+ was more sensitive (93%) than *ASC-US+* cytology alone ($P = 0.03$), whereas the sensitivity of abnormal cytology or HPV-Aptima+ (90%) was not (*P* value >0.1). The specificity of cotesting strategies significantly worse than cytology; 37% for both *ASC-US+* and HC2+ and 40% for both *ASC-US+* and HPV-Aptima+ ($P < 0.001$ for both). We also assessed if detection of hrHPV helped to triage minimally abnormal anal cytology [cytologic atypical squamous cells of undetermined significance (cASC-US), cytologic low-grade squamous intraepithelial lesion (cLSIL)] for HRA referral for the detection of anal hHSIL. Of women with cASC-US, 15/69 (22%) had underlying hHSIL, 34/69 (49%) of the ASC-US were HPV+, and 10/15 (67%) with hHSIL were both HPV+ with cASC-US. Of women with cLSIL, 19/45 (42%) had underlying hHSIL, 30/45 (67%) were HPV+, and 16/19 (84%) with hHSIL were both HPV+ and cLSIL (data not shown).

Screening test agreement

The test agreement of anal HPV detection with HPV-Aptima and HC2 was high [Kappa = 0.73 (0.64–0.82)]. However, the test agreement of cytology (ASC-US+) and HPV-Aptima [Kappa = 0.37 (0.26–0.49)] and HPV-HC2 [Kappa = 0.32 (0.20–0.44)] agreement was poor (data not shown).

Age-stratified analyses

In addition, age-stratified tabular analyses for these screening tests (individually and in combination) to predict hHSIL in WLHIV did not show differences between participants less than 45 years of age in comparison with those at least 45 but some comparisons were limited by small sample size, particularly for sensitivity (Supplementary Table 1, http://links.lww.com/QAD/B847).

Discussion

To our knowledge, this study is the first to compare the operating test characteristics for anal cytology and commercially available HPV assessments for the detection of anal hHSIL among WLHIV. In our cohort of 229 women, the majority (59%) had abnormal anal cytology (≥ASC-US) and fewer than 50% had anal HPV detected (HPV-Aptima 45% and HC2 48%). We found that abnormal anal cytology (ASC-US+) had similar sensitivity but lower specificity for the detection of anal hHSIL compared with either anal HPV test (HPV-Aptima or HC2) alone. We also evaluated combinations of the testing modalities, and of these testing modalities, and anal HPV testing (with either HPV-Aptima or HC2) alone demonstrated optimal screening characteristics. Our study provides evidence that the specificity of anal hrHPV testing is higher than anal cytology and, unlike screening for hHSIL in MSM living with HIV for whom anal hrHPV testing has poor test characteristics (low specificity, high false positive rate) due to the high prevalence of hrHPV infection in that population, anal hrHPV testing alone appears to be more appropriate for screening WLHIV for hHSIL.

Currently, several professional societies, as well as the New York State HIV guidelines recommend screening PLWH for anal cancer precursors using anal cytology. Of note, cervical cancer screening guidelines have incorporated hrHPV testing as an adjunct to cytology and in many countries hrHPV testing is now the primary screening test for cervical cancer [11]. Anal hrHPV detection has not been proposed as a viable screening option for MSM living with HIV because of high rates of prevalent hrHPV infection [12]. Recent meta-analyses of MSM living with HIV demonstrated that pooled sensitivity and specificity for anal cytology was 81–82% and 45–54.0%, respectively, and anal hrHPV testing had sensitivity and specificity of 91–95% and 24–27% [13,14], thus, making the specificity of hrHPV testing too poor to use in this population. Our findings demonstrating similar sensitivity but improved specificity for hrHPV compared with anal cytology for detection of anal hHSIL in WLHIV differs from these prior reports in MSM living with HIV and is likely due to the lower prevalence of hrHPV infection in WLHIV.

WLHIV have an increased incidence rate of anal cancer compared with women without HIV (standardized incidence ratio of 7.9–13.5) [1]. However, the risk is lower compared with MSM living with HIV (adjusted incidence rate ratio of 0.3). WLHIV appear have similar anal hHSIL prevalence compared with MSM living with HIV [27 (95% CI, 22–33%) [9] vs. 29% (95% CI 18–39%)] [12] but rates of anal hrHPV detection are substantially lower than those reported in MSM living with HIV [50 (95% CI, 16–85%) [15,16] vs. 73.5% (95% CI, 64–89%)] [12], respectively. Lesion size, infection

with multiple HPV types, and anatomic differences have been hypothesized to cause sensitivity and specificity differences. Thus, given the differences in the prevalence of anal hHSIL and anal hrHPV infection between WLHIV and MSM living with and without HIV, specific screening recommendations focused on hrHPV screening for WLHIV may be appropriate.

Significantly, unlike studies evaluating screening tests for cervical hHSIL, our study did not show improved sensitivity for anal hHSIL detection using hrHPV testing compared with cytology. One large meta-analysis conducted by Cochrane found that among 40 studies evaluating multiple methodologies for the detection of cervical hHSIL, the pooled sensitivity estimates for hrHPV HC2 (1 pg/ml threshold) vs. conventional cytology ASC-US+ or LBC (LBC−ASC-US+) were 89.9, 62.5, and 72.9%, respectively, and pooled specificity estimates were 89.9, 96.6, and 90.3%, respectively [17]. The differences in test performance between cervical and anal screening tests may be due to differences in the anatomic site, including differences in visual interpretation of cytologic findings based on expertise, and differences in test protocols and diagnostic thresholds between cervical and anal hrHPV specimens. Further research is necessary to determine if anal-specific protocols may improve the sensitivity for cytologic interpretations as well as hrHPV testing protocols.

In particular, hrHPV types 16 (and to a lesser extent 18) have been shown to correlate with progression to anal cancer [18]. Although hrHPV types have been detected in 100% of cervical cancers and 88% of anal cancers, HPV types 16 or 18 in particular are associated with 70% of cervical and 80% of anal cancers [19]. Furthermore, recent studies suggest that HPV 16 primarily drives the progression from anal HSIL to cancer (except in HIV-positive individuals where rates of other hrHPV types are commonly detected in invasive anal cancer specimens) [18]. Indeed, in our study, we found that Aptima 16, 18, or 45 had the second highest specificity (89%) and PPV (62%). Although Aptima 16/18/45 alone has high specificities and PPVs, the low sensitivity and higher false omission rates of the test make it a less optimal screening test for anal hHSIL.

We did not find any difference in test performance characteristics by age. This contrasts with Jin *et al.* [20] who found that the specificity of anal cytology improved with older age. They hypothesize that younger men may have more transient hrHPV and low grade lesions, thus decreasing the specificity for this population. In WLHIV, transient anal hrHPV infections may be less frequent. We have previously shown that the prevalence of anal hHSIL in our cohort did not change by age [9].

The strengths of this study include the racial and ethnic diversity of the participants and that all women enrolled in the study underwent HRA and biopsy (not only women with abnormal cytology). Furthermore, all the clinicians who performed HRAs underwent a rigorous certification process before study sites were activated. Our study is also different than other prior anal screening in that prior studies have either used referral populations (i.e., conducting HRA and biopsy only among women with ASC-US or greater anal cytology), or did not perform at least two anal biopsies from each patient, even if the HRA appeared negative. The limitations of the study include no central review of all the local cytology interpretations, and the specimens for cytology and hrHPV specimens were collected in separate specimen containers. However, the order of the specimen collection was randomly assigned to decreased any potential bias from fewer cells collected at the second specimen collection. Finally, the age range of the participants did not include many women under the age of 30, thus our power to detect the difference in screening test characteristics for that age group may be limited.

In conclusion, HC2 and HPV-Aptima anal hrHPV screening tests were found to have similar sensitivity and *improved* specificity compared with liquid-based anal cytology, for the detection of anal hHSIL in this multicenter cohort study of WLHIV. Because the risk of any anal hHSIL progression to invasive anal cancer is unknown, and not all anal hHSIL will progress to invasive cancer, there may not be any differential cancer risk for anal HSIL detected by the different screening strategies proposed. However, given the costs to patients and health care as well as the limited access to HRA, and the prolonged natural history of progression from anal hHSIL to invasive anal cancer, the value of noncytology-based tests, such as HPV biomarkers as the primary screening test could improve screening for anal HSIL in WLHIV and should be further investigated as part of anal screening programs for this population.

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Conflicts of interest

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