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Comparison of Nylon-Flocked Swab and Dacron Swab Cytology for Anal HSIL Detection in Transgender Women and Gay, Bisexual, and Other Men Who Have Sex With Men

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BACKGROUND: An anal histological high-grade squamous intraepithelial lesion (hHSIL) is an anal cancer precursor. Experts recommend Dacron swab anal cytology as a primary screen for anal hHSILs, especially among human immunodeficiency virus–infected and –uninfected men who have sex with men (MSM). Studies have shown that Dacron cytology inaccurately predicts anal hHSILs and results in unnecessary diagnostic procedures. Nylon-flocked (NF) swabs have been shown to trap pathogens and cells well. Thus, this study compared test characteristics of anal cytology using NF and Dacron swab collection protocols to predict anal hHSILs. **METHODS:** A single-visit, randomized clinical trial compared NF and Dacron swab anal cytology specimens to predict high-resolution anoscopy and biopsy–diagnosed anal hHSILs. Data for 326 gay men, bisexual men, other MSM, and male-to-female transgender women contributed descriptive and tabular statistics with which unadjusted and fully adjusted logistic regression models were constructed. The models estimated the odds of hHSILs, test accuracy (area under the curve [AUC]) and sensitivity, and specificity as well as the positive and negative predictive values of abnormal NF and Dacron cytology for predicting hHSILs. **RESULTS:** In the fully adjusted model, the sensitivities for NF and Dacron cytology were nearly equal (48% vs 47%), but the specificity was higher with NF cytology (76% vs 69%). Comparisons of the areas under receiver operating characteristic curves showed that NF cytology alone predicted hHSILs better than the covariate model (AUC, 0.69 vs 0.63; $P = .02$), but NF and Dacron cytology comparisons showed no statistically significant differences (AUC, 0.69 vs 0.67; $P = .3$). **CONCLUSIONS:** NF cytology and Dacron cytology provide modest sensitivity, but NF cytology has higher specificity and accuracy, and this is important for lowering the costs of population-based screening. ***Cancer Cytopathol* 2019;127:247-257**. © *2019 American Cancer Society.*

KEY WORDS: anal cancer screening; anal cytology; anal high-grade dysplasia; anal high-grade squamous intraepithelial lesion (HSIL) screening; cancer screening.

INTRODUCTION

Invasive anal cancer (IAC) disproportionately affects human immunodeficiency virus (HIV)–infected gay men, bisexual men, and other men who have sex with men (MSM), for whom current rates are higher than general male population estimates (130 vs 1.5 cases per 100,000 person-years), and some experts suggest that

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rates are higher in HIV-uninfected MSM.[1-5] Rates now exceed invasive cervical cancer rates when cervical cytology was introduced in the 1950s (approximately 50 cases per 100,000 person-years).[1,2,6]

Twelve human papillomavirus (HPV) genotypes are necessary but alone insufficient causes of human cancers.[7] Group 1/high-risk HPV carcinogens cause invasive cervical cancers, IACs, and other anogenital and aerodigestive cancers.[7,8] Risk factors for IACs and anal histological high-grade squamous intraepithelial lesions (hHSILs), which are precancers, include smoking, early-life or exclusive male-male sexual partnerships, receptive anal intercourse (RAI), anal high-risk HPV infections (especially HPV-16/18 among both men and women), and abnormal cervical cytology in women.[9-13] HIV infections and other immunosuppressive conditions such as organ transplants and a lifetime history of syphilis, chlamydia, gonorrhea, and anal warts are positively associated with IACs and anal hHSILs.[9-11]

HPV infections and disease are well described.[14,15] HPV infections may be asymptomatic or show cytological and histological low-grade squamous intraepithelial lesions and hHSILs. hHSILs may regress or remain stable, and few progress to cancer.[11-13] Meta-analyses suggest that the IAC incidence sharply rose after the introduction of combined antiretroviral therapy for HIV. For example, pre– and post–combined antiretroviral therapy era data show a 3.6-fold increase in the IAC incidence that is attributed to longer survival with HIV (21.8/100,000 vs 77.8/100,000 person-years).[16] The rate of progression from first clinical hHSIL detection to cancer may be 0.6% annually.[17]

Efficacious screening to identify anal hHSILs is consistent with other US secondary cancer prevention strategies. Currently, anal cytology is solely recommended by experts for anal cancer or hHSIL screening in high-risk populations.[18] Few head-to-head comparisons of anal cytology collection strategies have been published, especially with anal biopsy performed universally. Currently, experts recommend Dacron swab cytology specimen collection: data show that cytology samples marginally predict anal hHSILs.[18] To estimate the prevalence of abnormal cytology and to estimate the sensitivity, specificity, positive predictive value, and negative predictive value (SSPN) of 2 anal cytology collection protocols, we enrolled subjects into a single-visit randomized controlled trial.

MATERIALS AND METHODS

Subject and Setting

Between 2013 and 2016, 347 MSM and 3 male-to-female transgender women provided written, informed consent to participate in a single-visit randomized controlled trial of 2 cytology collection protocols (Dacron and nylon-flocked [NF] swabs) and 4 HPV assays for predicting hHSILs determined by high-resolution anoscopy (HRA)–guided biopsy: the Improving Screening Tools for Anal Cancer [ISTA] study. ISTA compared the test characteristics of 2 anal cytology collection protocols with 2 high-threshold assays that measure group 1/high-risk HPVs in residual cytology specimens (https://clinicaltrials.gov/ct2/show/NCT02816879). In total, 325 MSM and 1 transwoman (n = 326) had complete cytology and biopsy data for cytology/histology. ISTA was approved by the University of California Los Angeles medical institutional review board (13-000997).

The study sample included community and Multicenter AIDS Cohort Study (MACS) participants. Previously, 67% of the MACS participants (1541 of 2311) had undergone anal (Dacron) cytology and HPV testing 1 or more times as part of a MACS substudy (2010-2015) described elsewhere.[19,20] Community recruitment used fliers placed at community clinics, social service organizations, drug treatment centers, and housing projects in Los Angeles and Riverside Counties.

Study Design

A block-randomization study design sorted the order of specimen collection into groups of 4. Examination and self-reported data and test specimens were gathered at a single visit.

Study Procedures

Cytology collection

Four anal swab specimens were collected from each subject. Randomization determined the swab order and then the preservative within each swab type (Dacron and NF swabs): PreservCyt (Hologic, Inc, Marlborough, Massachusetts) and SurePath (Becton, Dickinson, and Company, Franklin Lakes, New Jersey) for Dacron swabs and SurePath and an RNA preservative for NF swabs. The swab type tested the hypothesis that large–surface area (2120 mm^2) NF swab specimens would better

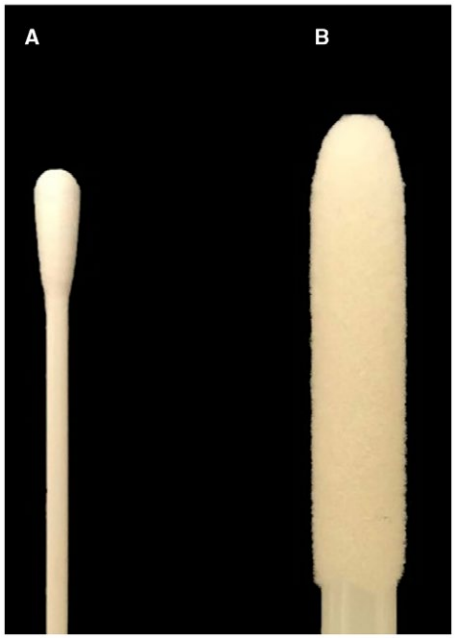

Figure 1. Comparison of Dacron and nylon-flocked swabs for cytology collection protocols with surface areas of approximately 130 and 2120 mm^2, respectively.

predict hHSILs than Dacron swab specimens (129 mm^2; Fig. 1). Differences between PreservCyt and SurePath for Dacron swab cytology specimens were evaluated. RNA-preserved NF swab specimens were reserved for future research.

Two cytology collection protocols were evaluated. For the first Dacron cytology collection, a lightly moistened swab was blindly inserted through the anal verge approximately 5 cm, approximated to the anal wall, rotated circularly over 30 seconds, withdrawn, and deposited into PreservCyt. To pass an NF swab, a disposable anoscope (CooperSurgical, Inc, Trumbull, Connecticut) was lightly lubricated across the leading edge (with a water-soluble lubricant) and inserted into the canal. After the verge was opened, the obturator was removed, and a dry rayon swab (Scopette, Jr; Owens & Minor, Mechanicsville, Virginia) removed excess lubricant. Once introduced, the NF swab (Copan Italia, Brescia, Italy) was approximated to the squamomucosal junction, twirled clockwise and counterclockwise to collect cells and fluids, withdrawn, and deposited into SurePath. Once placed, all subsequently collected swabs were collected through the anoscope and deposited into SurePath to prevent possible lubricant contamination. Swabs were agitated by hand and mechanically vortexed in a preservative solution before being removed. Specimen containers were sealed, conveyed to 1 Clinical Laboratory Improvement Amendments–certified laboratory, and evaluated (Tricore Reference Laboratories, Albuquerque, New Mexico).

Dacron swabs collected in PreservCyt and SurePath were routinely tested for cytology initially (n = 80), and Dacron and SurePath were additionally evaluated randomly thereafter. Herein, Dacron specimens paired with SurePath or PreservCyt (n = 145) were used to evaluate the effect of preservatives on cytology.

Cytology findings were classified as follows: negative for intraepithelial lesions (NIL); atypical squamous cells of undetermined significance (ASCUS) or atypical squamous cells, cannot rule out high-grade squamous intraepithelial lesion (ASC-H); low-grade squamous intraepithelial lesion; or high-grade squamous intraepithelial lesion (HSIL). Anal cytology showing ≥ASCUS is regarded as abnormal in clinical practice.[21] Dacron cytology/PreservCyt specimens showing fewer than 1 to 2 nucleated squames per high-powered field and NF cytology/SurePath samples showing fewer than 3 to 6 nucleated squames per high-powered field were evaluated as unsatisfactory for evaluation (unsatisfactory cytology).[22] No subject showed ASC-H or HSIL cytology with <hHSIL on biopsy. Thus, no composite diagnosis for HSILs was used for these analyses. We hypothesized that unsatisfactory cytology was costly, and we thus compared unsatisfactory and NIL cytology in these analyses.

One experienced examiner performed HRA for all subjects after the cytology specimen collection. A 5% acetic acid–soaked, gauze-padded swab, passed through an anoscope and subsequently withdrawn, allowed acetowhitening of anal epithelium. The anoscope was reintroduced with a 4% lidocaine cream/water-soluble lubricant. The anal canal was examined systematically under a bright light and magnification. Up to 0.5 mL of a 2% lidocaine/epinephrine (1:100,000) solution per quadrant was distributed evenly across the field for hemorrhoids obstructing the examination. Endoscopic or Tischler biopsy forceps were used to collect specimens in 10% neutral buffered formalin. Monsel's solution was applied to achieve hemostasis, as indicated.

For both cytology and histology, board-certified cytopathologists and histopathologists, blinded to clinical examination data, used standardized procedures. Cytology was classified with the Bethesda classification

system.[12,23,24] Histology specimens were classified according to international recommendations[25,26] and harmonized with expert recommendations:[12] anal intraepithelial neoplasia grades 2, 2/3, and 3 were classified as an hHSIL, and anal intraepithelial neoplasia grade 1 was classified as a histological low-grade squamous intraepithelial lesion.[12] When pathologists or providers were uncertain, block-positive $p16^{INK4a}$ immunostaining classified a specimen as an hHSIL.[12] Subjects showing 1 or more anal intraepithelial neoplasia grade 2, 2/3, or 3 lesions (hHSILs) were compared with those whose most severe finding was <hHSIL.[12,26]

Other covariates of interest

Other covariates of interest included the following: age (continuous), race (white vs nonwhite), HIV infection characteristics (HIV-uninfected, HIV-infected with <500 cells/mm^3, or HIV-infected with ≥500 cells/mm^3), smoking (former, current, or never smoker), number of male RAI partnerships reported for the 2 years before the examination (0, 1, 2-10, or >10), and swab collection order (first vs not).

Statistical analyses

Descriptive analyses, using Pearson chi-square, Kruskal-Wallis, and Student *t* test statistics, evaluated individual associations between covariates and hHSILs in the data. A stratified analysis using logistic regression contrasted SSPNs for first and subsequently ordered NF and Dacron cytology collection protocols; in addition, SSPN estimates, calculated with logistic regression, were summarized and adjusted for randomization order alone. To assess the odds of hHSILs, we also explored the effects of unsatisfactory cytology (vs NIL cytology) for both swab protocols. Final logistic regression models were adjusted for the effects of swab randomization alone.

Fully adjusted logistic regression models were used to estimate the odds of predicting hHSILs with cytology showing ≥ASCUS findings (vs NIL findings) from 2 swab collection protocols, with adjustments made for effects of other covariates of interest (other covariates). The final fully adjusted model evaluated ≥ASCUS versus NIL for predicting hHSILs, with adjustments made for the effects of unsatisfactory cytology (vs NIL) and other covariates. Odds ratios (ORs) with 95% confidence intervals (CIs) and areas under receiver operating characteristic curves were estimated from the data. Fully adjusted areas under the curve (AUCs) were used to assess each swab protocol's performance independently of the decision threshold to correctly classify those with and without hHSILs, with adjustments made for the effects of other covariates. The *U* statistic evaluated differences between models. Lastly, to estimate the effects of the preservative and slide preparation method on the ability of Dacron and NF swab cytology to accurately predict hHSIL and <hHSIL findings, AUCs from fully adjusted logistic regression models for 145 Dacron swab specimens collected in each preservative were compared.

RESULTS

Descriptive Analyses

The mean age of the participants was 55 years (standard deviation, 11.5 years), and most were white MSM (72%) and former (56%) or current tobacco smokers (18%). Nearly 40% of the subjects were HIV-uninfected, and among HIV-infected participants (60%), one-third showed fewer than 500 CD4+ T-lymphocytes/mm^3 (CD4+ count). More than half reported no RAI partners over the 2 years before the examination. HIV-infected subjects were nearly twice as likely as the uninfected to report minority race ($P = .04$) and current smoking ($P = .009$; Table 1).

Dacron cytology showed a higher prevalence of ≥ASCUS than NF cytology (Table 1). Dacron cytology nearly equally showed NIL (37%), ≥ASCUS (34%), and unsatisfactory findings (29%). NF cytology showed NIL (62%) 3 times more often than ≥ASCUS (20%). Unsatisfactory specimens were more common with Dacron cytology (29%) than NF cytology (18%); also, the prevalence of unsatisfactory cytology was positively associated with the number of Dacron swabs collected ($r = 0.36$; $P < .0001$), but NF cytology was not ($P = .32$). Unsatisfactory NF cytology occurred approximately 10% more often among HIV-infected subjects than uninfected subjects ($P = .04$). The prevalence of unsatisfactory Dacron swab cytology specimens collected randomly was 11%, 15%, 35%, and 48% (from first to fourth). Nearly 14%, 20%, 15%, and 26% of the first to fourth collected NF swab cytology specimens were unsatisfactory. The odds of unsatisfactory specimens were statistically significantly greater for third and fourth collected Dacron cytology specimens alone

TABLE 1. Randomized Controlled Trial Comparing Two Cytology Collection Protocols for Predicting hHSILs for 326 Men Who Have Sex With Men and Transgender Women

	Total (n = 326)		HIV-Uninfected (n = 131)		HIV-Infected (n = 195)			≤hHSIL (n = 177)		hHSIL (n = 149)		
Variable	No.	%	No.	%	No.	%	*P*[a]	No.	%	No.	%	*P*[a]
Randomization							.73					.86
Copan	168	51.53	66	50.38	102	52.31		92	51.98	76	51.01	
Dacron	158	48.47	65	49.62	93	47.69		85	48.02	73	48.99	
Tobacco smoking							.01					.37
Never	84	25.77	31	23.66	53	27.18		49	27.68	35	23.49	
Former	182	55.83	85	64.89	97	49.74		100	56.5	82	55.03	
Current	60	18.40	15	11.45	45	23.08		28	15.82	32	21.48	
HIV infection characteristics												.27
HIV-uninfected	131	40.18	131	100	0	0		78	44.07	53	35.57	
HIV+, CD4 ≥ 500 cells/mm^3	131	40.18	0	0	131	67.18		68	38.42	63	42.28	
HIV+, CD4 < 500 cells/mm^3	64	19.63	0	0	64	32.82		31	17.51	33	22.15	
Race							.04					.83
Nonwhite (0)	90	27.61	28	21.37	62	31.79		48	27.12	42	28.19	
White (1)	236	72.39	103	78.63	133	68.21		129	72.88	107	71.81	
RAI partnerships 24 mo before HRA							.16					
0	186	57.06	85	64.89	101	51.79		147	66.1	69	46.31	
1	58	17.79	16	12.21	42	21.54		26	14.69	32	21.48	
2-10	60	18.40	22	16.79	38	19.49		26	14.69	34	22.82	
>10	11	3.37	4	3.05	7	3.59		3	1.69	8	5.37	
Missing	11	3.37	4	3.05	7	3.59		5	2.82	6	4.03	
Dacron swab cytology							.5					.0009
NIL	121	37.12	47	35.88	74	37.95		81	45.76	40	26.85	
≥ASCUS	112	34.36	42	32.06	70	35.9		48	27.12	64	42.95	
Unsatisfactory	93	28.53	42	32.06	51	26.15		48	27.12	45	30.2	
Nylon-flocked swab cytology							.39					<.0001
NIL	202	61.96	87	66.41	115	58.97		131	74.01	71	47.65	
≥ASCUS	66	20.25	24	18.32	42	21.54		18	10.17	48	32.21	
Unsatisfactory	58	17.79	20	15.27	38	19.49		28	15.82	30	20.13	
Variable	Mean	SD	Mean	SD	Mean	SD	*P*[b]	Mean	SD	Mean	SD	*P*[b]
Age, y	54.73	11.46	57.54	12.38	52.84	10.41	.0004	55.63	11.4	53.66	11.48	.12

Abbreviations: ASCUS, atypical squamous cells of undetermined significance; hHSIL, histological high-grade squamous intraepithelial lesion; HIV, human immunodeficiency virus; HRA, high-resolution anoscopy; NIL, negative for intraepithelial lesions; RAI, receptive anal intercourse; SD, standard deviation.
[a]Pearson chi-square test.
[b]Student *t* test.

(OR for third vs first, 4.4; 95% CI, 1.7-11.3; OR for fourth vs first, 7.5; 95% CI, 3.1-18.0).

All participants underwent 1 or more biopsies (mean, 3.1; standard deviation, 1.4; median, 3; range, 1-8), and 46% showed hHSILs. hHSIL-affected subjects reported more RAI partners over the 24 months before HRA ($P = .007$). An hHSIL was positively associated with ≥ASCUS and unsatisfactory cytology for NF and Dacron swab protocols ($P \leq .0009$; Table 1). Providers/pathologists infrequently requested p16 immunostaining: 62% (16 of 26) showed anal intraepithelial neoplasia grade 2, and 15% (4 of 26) and 23% (6 of 26) showed low-grade squamous intraepithelial lesion and NIL findings, respectively.

Unadjusted Analyses

Age, race, and tobacco use were associated with HIV infection. HIV-infected participants were younger than the HIV-uninfected (52.8 vs 57.54 years; $P = .002$), and whites were 1.7-fold less likely to be HIV-infected than minority participants (OR, 0.6; 95% CI, 0.3-1.0). HIV-infected participants were more likely to report current tobacco use (23% vs 11%) than comparators (Table 1).

hHSILs were not associated with age, race, smoking, or HIV infection characteristics (Table 1). Subjects reporting 1, 2 to 10, and more than 10 partners showed 2.6-, 3.1-, and 5.7-fold higher odds of hHSILs, respectively, than those reporting none ($P < .05$; Table 1). The odds of hHSILs were positively associated with both ≥ASCUS and unsatisfactory cytology (vs NIL) with either swab type. The odds of hHSILs for Dacron cytology showing ≥ASCUS were 2.7-fold greater than those for NIL findings (OR, 2.7; 95% CI, 1.6-4.6). NF cytology showing ≥ASCUS showed 4.9-fold higher odds than NIL for hHSILs (OR, 4.9; 95% CI, 2.6-9.1).

Unsatisfactory cytology findings (vs NIL) from either Dacron or NF cytology showed higher odds of hHSILs (ORs, 1.9 [95% CI, 1.1-3.3] and 2.0 [95% CI, 1.1-3.6], respectively; Table 2).

Comparisons for Abnormal and NIL Cytology

Unadjusted SSPN analyses

Unadjusted SSPN analyses showed that abnormal Dacron cytology was 1.5-fold more sensitive than NF cytology as primary screening for anal hHSILs: 62% versus 40% ($P = .02$; Table 2, Supporting Table 1A,B, and Fig. 2A,C). The specificity for abnormal Dacron cytology was lower than that for NF cytology: 63% versus 88% ($P < .0001$). The positive predictive value (PPV) for abnormal Dacron cytology was 1.3-fold lower than the PPV for NF cytology: 57% versus 73% ($P < .001$). Negative predictive value estimates for the 2 cytology collection protocols were similar.

Fully adjusted SSPN analyses

With adjustments made for the effects of age, race, HIV infection characteristics, swab randomization order, and the number of RAI sexual partners during the 2 years before the study visit, abnormal Dacron cytology was more sensitive for predicting hHSILs than NF cytology: 47% versus 42% ($P = .007$). The specificity was no greater (70% vs 81%; $P = .1$), although each showed specificity greater than 50% (P values <.0001). The PPV for Dacron cytology showing ≥ASCUS (56%) was 1.2-fold lower than the PPV for NF cytology (65%) for predicting hHSILs, and only NF cytology showed a PPV statistically significantly greater than 50% ($P = .005$).

The swab collection order affected the ability of abnormal Dacron cytology findings (vs NIL) alone to predict hHSILs. NF cytology showed similar sensitivity for first and second collected cytology specimens (40% vs 40%), but Dacron cytology sensitivity improved 1.2-fold when it was collected after an NF swab: 57% versus 68%. Nonetheless, the sensitivity for predicting hHSILs was greater than 50% for subsequently collected Dacron cytology swabs alone ($P = .01$). The specificity for NF cytology was high for both first specimens (83%) and subsequently collected specimens (93%), and both estimates were greater than 50% (H_0: specificity > 0.5; P values <.0001). The specificity of Dacron cytology for both first specimens (61%) and subsequently collected

TABLE 2. Logistic Regression Model Comparisons for 2 Cytology Collection Protocols Predicting hHSILs for 326 Men Who Have Sex With Men and Transgender Women

Test	Condition, OR (95% CI)			Test Characteristics		ROC Characteristics			
	≥ASCUS	Unsatisfactory	Randomization Order Alone	Sensitivity (%)	Specificity (%)	AUC (95% CI)	SE	*P*	*P*
Unadjusted model									
Dacron swab cytology	2.7 (1.6-4.6)	1.9 (1.1-3.3)	—	43.0	72.9	0.61 (0.55-0.67)	0.03	—	Reference
Nylon-flocked swab cytology	4.9 (2.7-9.1)	2.0 (1.1-3.6)	—	52.3	74.0	0.65 (0.59-0.70)	0.03	—	.4
Randomization order–adjusted model									
Randomization order (alone)						0.50 (0.45-0.56)	0.03	Reference	
Dacron swab cytology	2.7 (1.6-4.6)	2.0 (1.1-3.6)	0.9 (0.6-1.4)	43.0	67.2	0.62 (0.56-0.68)	0.03	.002	Reference
Nylon-flocked swab cytology	5.0 (2.7-9.2)	2.0 (1.1-3.6)	0.9 (0.6-1.4)	43.0	74.0	0.65 (0.59-0.71)	0.03	<.0001	.5
Fully adjusted model[a]									
Covariates (alone)						0.63 (0.56-0.69)	0.03	Reference	
Dacron swab cytology	2.6 (1.5-4.5)	2.1 (1.1-3.8)	—	47.0	68.9	0.67 (0.61-0.72)	0.03	.08	Reference
Nylon-flocked swab cytology	5.2 (2.8-10.0)	2.1 (1.1-4.0)	—	47.7	75.7	0.69 (0.64-0.75)	0.03	.02	.3

Abbreviations: ASCUS, atypical squamous cells of undetermined significance; AUC, area under the curve; CI, confidence interval; hHSIL, histological high-grade squamous intraepithelial lesion; OR, odds ratio; ROC, receiver operating curve; SE, standard error.

[a]Adjusted for the effects of the following: age, race; human immunodeficiency virus infection, and, among the infected, CD4-T-lymphocyte count (≥500, <500 cells/mm^3); smoking, number of male receptive anal intercourse partnerships 2 years before the examination, and swab collection order.

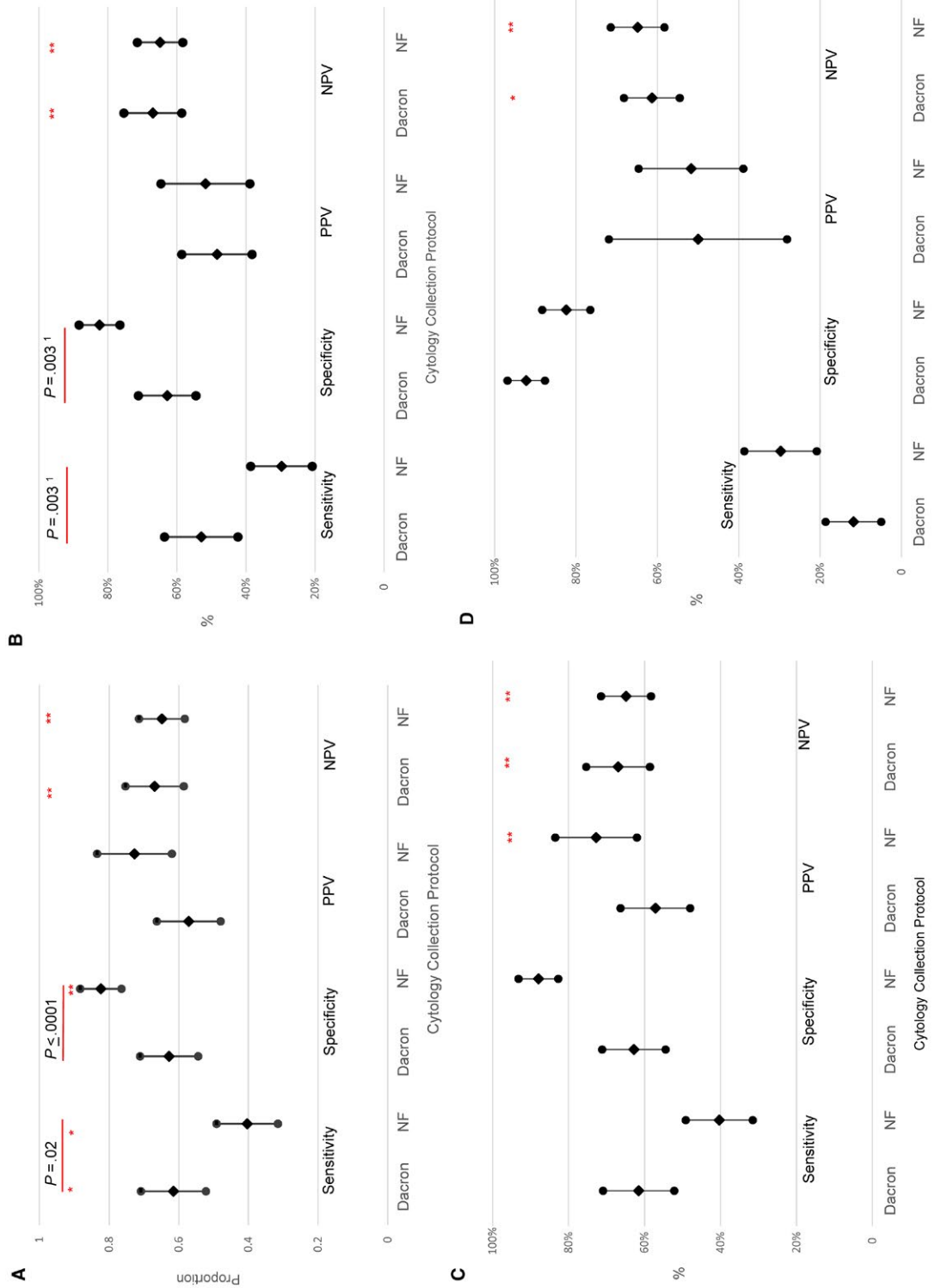

Figure 2. Comparison of the sensitivity, specificity, PPV, and NPV estimates for predicting anal hHSILs with NF and Dacron swab cytology collection protocols for 326 men who have sex with men and transgender women: (A) an unadjusted comparison of cytology showing ≥ASCUS versus NIL for predicting hHSILs, (B) an unadjusted comparison of cytology showing unsatisfactory findings versus NIL for predicting hHSILs, (C) a fully adjusted comparison of cytology showing ≥ASCUS versus NIL for predicting hHSILs, and (D) a fully adjusted comparison of cytology showing unsatisfactory findings versus NIL for predicting hHSILs. ASCUS indicates atypical squamous cells of undetermined significance; hHSIL, histological high-grade squamous intraepithelial lesion; NF, nylon-flocked; NIL, negative for intraepithelial lesions; NPV, negative predictive value; PPV, positive predictive value.

specimens (65%) was greater than 50% (P values <.05), and both were lower than NF cytology estimates. PPV estimates for first and successively collected NF cytology alone were greater than 50%: 66% and 82%, respectively (H_0: Pr > 50%; $P = .03$ and $P < .0001$).

Comparisons of Unsatisfactory and NIL Cytology

Unadjusted and fully adjusted SSPN estimates comparing unsatisfactory and NIL findings for Dacron and NF cytology are reported in tables and graphs (Table 2, Supporting Table 1A,B, and Fig. 2B,D). The prevalence of unsatisfactory findings was similar for Dacron and NF swab cytology: 15% and 21%, respectively ($P = .6$). Fully adjusted estimates showed that the sensitivity for unsatisfactory cytology to predict hHSILs was ≤50% ($P < .0001$). The specificity was lower for Dacron swabs than NF swabs: 79% and 85%, respectively (P values <.0001).

Effect of Preservatives on Dacron Swab Cytology

Paired, randomly ordered Dacron swab specimens that were intentionally separately collected in PreservCyt and SurePath suggested that the accuracies of cytology in predicting an hHSIL (vs <hHSIL) were similar. Accuracy estimates did not statistically significantly differ with AUC values of 0.698 and 0.691, respectively ($P = .83$).

Fully Adjusted Accuracy Analyses for Predicting hHSILs

Fully adjusted analyses incorporated all Dacron/PreservCyt and NF/SurePath cytology data. Analyses suggested that participants with ≥ASCUS on NF and Dacron cytology (abnormal) showed 5.2- and 2.6-fold higher odds of hHSILs (vs NIL), respectively (OR for NF cytology, 5.2; 95% CI, 2.8-10.0); OR for Dacron cytology, 2.6; 95% CI, 1.5-4.5). Overall, the models showed that the accuracies of abnormal Dacron and NF cytology for predicting hHSILs were closely approximated ($P > .3$) and differed modestly. The adjusted model that included abnormal NF cytology (vs NIL) more accurately predicted hHSILs than the covariates alone (age, race, HIV infection characteristics, swab randomization order, and number of RAI sexual partners during the 2 years before the study visit): the AUC for NF cytology was 0.69 ($P = .02$), whereas the AUC for the covariate was 0.63. However, abnormal Dacron cytology did not: the AUC for Dacron cytology was 0.67 ($P = .08$). No statistically significant differences were detected for Dacron or NF swabs showing ≥ASCUS (vs NIL) for predicting hHSILs in either HIV-infected or HIV-uninfected participants in comparison with a covariate model alone (Supporting Table 1A,B).

DISCUSSION

This head-to-head evaluation of 2 anal cytology collection protocols as a primary screen for anal hHSILs among HIV-infected and HIV-uninfected MSM and transgender women shows higher sensitivity but lower specificity and PPV values for Dacron cytology over NF cytology. The fully adjusted analyses suggest that only NF cytology improved overall accuracy for predicting hHSILs in comparison with covariates alone (swab randomization order and sociodemographic, sexual behavior, and HIV infection characteristics reported within 24 months of the examination). Higher specificity translates into fewer false-positive NF cytology tests, reduced diagnostic follow-up, and lowered costs of anal cytology screening for anal hHSILs.

Our adjusted sensitivity and specificity analyses for either swab fall within the range of published performances. Anal cytology instrumentation builds on more than 50 years of experience with cervical cytology screening strategies: cervical cytopicks; cotton, Dacron, rayon, and NF swabs; brooms; and cytobrushes with and without spatulas.[27-35] Cervical cytology shows wider variation in sensitivity (34%-96%; median, 64%) than specificity (92%-98%; median, 96%) for predicting hHSILs.[36] Anal cytology studies largely describe screening outcomes alone, and few report complete HRA/histology data. Among those that do, the sensitivity and specificity for Dacron (anal) cytology to predict hHSILs varied widely: 19%[37] to 89%[38] and 40%[38-40] to 88%,[37] respectively. Research reports, meta-analyses, and systematic reviews suggest that the sensitivity and specificity of anal cytology ≥ASCUS modestly predict hHSILs: 66% to 95% and 32% to 96%, respectively.[41-43] Comparatively, the sensitivity of cervical or anal cytology ≥ASCUS to predict cervical (91%) or anal hHSILs (90%) is high, but the specificity for cervical cytology (53%) is greater than that for abnormal anal cytology (33%).[43] Consequently, more false-positive tests may occur with abnormal anal cytology findings.[43]

Mechanistically, NF swabs trap more cells, microorganisms, and fluid during sampling. NF swab abrasiveness is demonstrated by the improved performance of Dacron cytology collected after NF cytology in this sample. One study of 23 NF cytology swabs showed a higher number of cells per slide in comparison with Dacron cytology ($P = .003$) but no greater DNA quantity or quality from NF swabs.[44] Others have reported 5- to 10-fold and 1.6- to 2.0-fold higher yields of cells and DNA, respectively, with NF swabs rather than cotton swabs for cervicovaginal sampling.[29]

Some data suggest that cytology and HRA findings are modestly related. Four large studies performing both cytology and HRA with biopsies have reported that 12% to 25% of subjects with NIL cytology have hHSILs.[37,38,40,45] Three of the 4 studies have reported that a substantial fraction of HRA examiners using HRA miss hHSILs, with an average of 13% of subjects showing ASC-H or HSIL cytology with histological NIL findings or not undergoing biopsy during HRA.[37,38,40,45] Abnormal anal cytology is not associated with anal condyloma, a low-grade squamous intraepithelial lesion, in other reports.[38,46]

Our analyses may be limited. The prevalence of unsatisfactory cytology was higher than expected, and this was possibly related to the size of the NF swab. Also, collecting cytology through an anoscope for either swab may have impaired swab-to-epithelium contact. Our provider reported that subjects described NF swabbing as more abrasive (than Dacron). Our earlier published study[35] reported fewer unsatisfactory results with NF (7% [4 of 58]) and Dacron cytology (14% [8 of 58]) with the swabs employed herein. Nonetheless, published studies infrequently report or include unsatisfactory cytology in analyses. Several centers have reported that 6% to 7% of anal cytology specimens are unsatisfactory, and other studies with large samples have reported an unsatisfactory prevalence ranging from 10% to 17%.[47-50] The study protocol was developed and implemented as p16 immunostaining recommendations were published.[12] Testing herein was performed when the assay was requested to clarify diagnoses. Although our accuracy estimates have been adjusted for the effects of HIV infection, including CD4+ T-lymphocyte counts, the study was not adequately powered to detect differences in test characteristics or accuracy (AUC) estimates for HIV-infected and HIV-uninfected groups. Lastly, biases introduced by polychotomous, self-reported variables are difficult to predict.[51,52]

Our analyses suggest that NF cytology screening provides greater specificity than Dacron cytology. NF cytology showing ≥ASCUS showed 2-fold higher odds for hHSILs than (abnormal) Dacron cytology, and NF cytology alone demonstrated greater accuracy for predicting neoplasia than sociodemographic, sexual behavior, and HIV covariates alone. When screening-test findings are independent time over time, the probability of false-negatives (missing anal hHSILs) decreases steadily when screening is performed annually or semiannually in high-risk populations, such as HIV-infected MSM and transgender women.

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AUTHOR CONTRIBUTIONS

Dorothy J. Wiley: Research concept and design, acquisition and analysis of data, interpretation of data analyses, drafting of the manuscript, editorial support, revision of the manuscript, and approval of the manuscript for publication. **Hilary K. Hsu:** Research approach design, acquisition and analysis of data, editorial support, revision of the manuscript, and approval of the manuscript for publication. **Martha A. Ganser:** Detailed data analyses, iterative approaches to evaluating patterns, editorial support, revision of the manuscript, and approval of the manuscript for publication. **Jenny Brook:** Detailed data analyses, iterative approaches to evaluating patterns, editorial support, revision of the manuscript, and approval of the manuscript for publication. **David A. Elashoff:** Development of the analytic approach, iterative evaluation of data patterns, editorial support, revision of the manuscript, and approval of the manuscript for publication. **Matthew G. Moran:** Specimen collection approach concept and design, acquisition of specimens, analysis of data, interpretation of results, editorial support, revision of the manuscript, and approval of the manuscript for publication. **Stephen A. Young:** Development of the laboratory approach, implementation of quality assurance activities, interpretation of data analyses, editorial support, revision of the manuscript, and approval of the manuscript for publication. **Nancy E. Joste:** Development of histopathology and cytopathology quality assurance activities, interpretation of data analyses, editorial support, revision of the manuscript, and approval of the manuscript for publication. **Ronald Mitsuyasu:** Directed care project medical director, interpretation of data analyses, editorial support, revision of the manuscript, and approval of the manuscript for publication. **Teresa M. Darragh:** Implementation of histopathology and cytopathology quality assurance activities, interpretation of data analyses, editorial support, revision of the manuscript, and approval of the manuscript for publication. **David H. Morris:** Interpretation of data analyses, editorial support, revision of the manuscript, and approval of the manuscript for publication. **Otoniel M. Martínez-Maza:** Interpretation of data analyses, editorial support, revision of the manuscript, and approval of the manuscript for publication. **Roger Detels:** Interpretation of data analyses, editorial support, revision of the manuscript, and approval of the manuscript for publication. **Jian Yu Rao:** Implementation of histopathology and cytopathology quality assurance activities, interpretation of data analyses, editorial support, revision of the manuscript, and approval of the manuscript for publication. **Robert K. Bolan:** Interpretation of data analyses, editorial support, revision of the manuscript, and approval of the manuscript for publication. **Eric T. Shigeno:** Interpretation of data analyses, editorial support, revision of the manuscript, and approval of the manuscript for publication. **Ernesto Rodriguez:** Interpretation of data analyses, editorial support, revision of the manuscript, and approval of the manuscript for publication.

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