Research Article

Cancer Epidemiology, Biomarkers & Prevention

A Composite Cytology–Histology Endpoint Allows a More Accurate Estimate of Anal High-Grade Squamous Intraepithelial Lesion Prevalence

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Abstract

Background: There is debate about the accuracy of anal cytology and high-resolution anoscopy (HRA), in the diagnosis of anal human papillomavirus (HPV)-related squamous intraepithelial lesions (SIL). Few studies have performed both simultaneously in a large sample of high-risk individuals.

Methods: At baseline in a community-based cohort of HIV-infected and uninfected homosexual men ages ≥35 years in Sydney, Australia, all men underwent anal swabbing for cytology and HPV genotyping, and HRA-guided biopsy. We evaluated the separate and combined diagnostic accuracy of cytology and histology, based on a comparison with the prevalence of HPV16 and other high-risk (HR) HPV. We examined trends in HPV prevalence across cytology–histology combinations.

Results: Anal swab, HRA, and HPV genotyping results were available for 605 of 617 participants. The prevalence of cytologically predicted high-grade SIL (HSIL, 17.9%) was lower than histologically diagnosed HSIL (31.7%, $P < 0.001$). The prevalence of composite-HSIL (detected by either method) was 37.7%. HPV16 prevalence was similar in men with HSIL by cytology (59.3%), HSIL by histology (51.0%), and composite-HSIL (50.0%). HPV16 prevalence was 31.1% in men with composite-atypical squamous cells suggestive of HSIL, to 18.5% in men with composite-low-grade SIL, to 12.1% in men with composite-negative results ($P_{trend} < 0.001$).

Conclusions: Significantly more HSIL was detected when a composite cytology–histology endpoint was used. Increasing grade of composite endpoint was associated with increasing HPV16 prevalence.

Impact: These data suggest that a composite cytology–histology endpoint reflects meaningful disease categories and is likely to be an important biomarker in anal cancer prevention. *Cancer Epidemiol Biomarkers Prev; 25(7); 1134–43. ©2016 AACR.*

Introduction

Anal cancer incidence has increased substantially in recent decades, and is highest among HIV-infected men who have sex with men (MSM), other people with HIV and other causes of immunosuppression, and women with previous human papillomavirus (HPV)-related anogenital disease (1–3). As with cervical cancer, anal cancer is largely caused by high-risk (HR) types of HPV (HR-HPV), particularly HPV16, and it is preceded by anal high-grade squamous intraepithelial lesions (HSIL; refs. 4, 5).

There is no clear agreement about the efficacy of screening and treatment of precursor lesions to prevent anal cancer (6). For cervical cancer, locations with population-based screening programs have seen a dramatic decline in cervical cancer incidence and mortality (7). Screening programs include cervical cytology [Papanicolaou (Pap) test], referral of women with abnormalities for colposcopy, and treatment of histologically proven cervical HSIL. Some researchers have advocated that a similar cytology-based screening and treatment program should be adopted for anal HSIL (8). However, the test performance of anal cytology continues to be debated (9). In particular, to achieve similar sensitivity to cervical screening, a lower abnormality threshold for referral for the diagnostic test [high-resolution anoscopy (HRA)-guided biopsy] is recommended by some (5). Partly because of the lower threshold for referral, anal cytology is generally less specific than cervical screening (5, 9). In addition, the diagnostic test, HRA-guided biopsy, is acknowledged as

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technically more difficult than cervical colposcopy and is therefore more likely to miss HSIL lesions, particularly if the anoscopist is relatively inexperienced with the procedure (10). Given this, some have suggested that the results of both the anal cytology and HRA may be necessary to obtain an accurate measure of true anal HSIL burden (11, 12).

To compare the separate and combined diagnostic accuracy of anal cytology and histology on HRA-guided biopsy, population-based studies that perform both tests on the same individuals at the same visit are required. However, only a handful of such studies has been reported (6, 13–15), perhaps because of reluctance to perform a relatively invasive procedure such as HRA in the entire at-risk population. We examined baseline data from a predominantly community-based cohort of homosexual men, in which both tests were performed on all participants at the same clinical visit. We evaluated categories of anal squamous intraepithelial lesion (ASIL) severity by examining the prevalence of the causative organism HPV16 and other HR-HPV in separate and combined categories of cytology and histology results.

Materials and Methods

Study population

The Study of Prevention of ANal Cancer (SPANC) is an ongoing prospective study of HIV-positive and HIV-negative homosexual men ages 35 years and older, based in Sydney, Australia. Participants were recruited mainly from community-based settings. About 35% of HIV-positive and 5% of HIV-negative men were recruited through medical clinics. The study was approved by the St Vincent's Hospital Ethics Committee (Sydney, Australia) and all participants gave written informed consent. The study protocol and the study's main objectives have been described in detail previously (16).

HPV detection, anal cytology, and HRA

The clinical and laboratory procedures performed in this study have been described in detail previously (16). In brief, a saline-moistened Dacron swab was inserted 3 to 5 cm into the anal canal without direct visualization, and then gradually withdrawn while applying firm circumferential pressure to the wall of the anal canal for approximately one minute. The swab was then agitated in a vial containing PreservCyt (Hologic Corporation). Before cytologic processing, an aliquot of the medium was transferred to a separate tube for HPV testing. The remaining sample was used for preparation of a slide for cytologic analysis. If the anal swab was deemed unsatisfactory for cytologic evaluation, a repeat anal collection was performed no less than 2 weeks from the first. This minimum time requirement was to allow sufficient time for the anal mucosal cells to regenerate after the initial swab. A "satisfactory" slide was defined as having at least 2,000 nucleated squamous cells.

The HRA was performed immediately after the anal swab in a manner similar to established techniques of applying acetic acid (3% initially; ref. 16), but changed in January 2015 to 5% in response to evolving opinion that the higher concentration may allow better visualization of squamous intraepithelial lesion (SIL; ref. 10) and visualization using a colposcope on high magnification as published (10, 16). Following insertion of a plastic anoscope and application of 5% acetic acid, the anal canal was visualized under high-resolution magnification with further application of acetic acid, followed by Lugol iodine, to identify any abnormalities. Any abnormalities that were visually suggestive of ASIL were biopsied for histologic assessment. Men who had no visual abnormalities did not undergo biopsy.

The samples were transferred to a pathology laboratory (Douglass Hanly Moir Pathology, Sydney, Australia) for processing and assessment. Reporting of cytology and biopsy results were performed according to The Bethesda System TBS (17) and in accordance with criteria, terminology, and recommendations of the Lower Anogenital Squamous Terminology (LAST) Project (18), respectively, as described previously (14). In particular, when a diagnosis of HSIL-AIN2 was proposed, immunostaining for p16 INK4A (p16) was performed and only those with strong uniform staining of the basal layer were considered positive and thus given the diagnosis of HSIL-AIN2. If the result was negative, the lesion was downgraded to low-grade squamous intraepithelial lesions (LSIL) or negative for SIL, depending on other criteria. If multiple biopsies were taken, the result with the highest grade of disease was used for analysis.

HPV PCR amplification and reverse line blot detection were performed on the anal PreservCyt specimens using the Roche LINEAR ARRAY (Roche Molecular Systems) to detect 37 individual HPV types with modifications as described previously (16, 19). As an in-house modification, samples that were negative for HPV and internal control were retested with half the volume of eluted DNA to reduce the inhibition due to high bacterial DNA content.

Statistical analysis

We examined the prevalence of individual cytology and histology SIL categories, and the prevalence of HPV16 and other HR-HPV types (not including HPV16) within these categories. Within each histologic category, we calculated HPV16 and other HR-HPV prevalence in strata of cytologic result. In addition, within each cytologic category, we calculated HPV16 and other HR-HPV prevalence in strata of histologic result. We then constructed composite cytology–histology endpoints based on increasing prevalence of HPV16.

The exact binomial method was used to calculate 95% confidence intervals (CI) for prevalence values. We used χ^2 test for trend to analyze the association between prevalence of HPV16 and other HR-HPV types (not including HPV16) across cytology and histology disease categories, in addition to individual cytology and histology categories. Data analyses were performed using STATA version 12 (Stata Corporation).

Results

Among the 617 participants by the close of recruitment in August 2015, 397 (64.3%) were HIV negative and 220 (35.7%) were HIV positive. The median age at enrolment was 49 years (interquartile range, IQR: 43–56).

Of the 617 anal cytology results, 29 (4.7%) men had repeatedly unsatisfactory anal cytology, 241 (39.0%) had no ASIL, 103 (16.7%) had atypical squamous cells of undetermined significance (ASC-US), 47 (7.6%) LSIL, 88 (14.3%) ASC-H (atypical-squamous cells cannot exclude high-grade squamous intraepithelial lesion), and 109 (17.7%) HSIL (Table 1). HRA was performed on 616 men, and one man was unable to tolerate the procedure. Among the 616 HRA results, 117 (19.0%) men had a visually normal HRA and did not have any biopsies taken, 148 (24.0%) had biopsies showing no evidence of ASIL, 155 (25.2%) had LSIL,

Table 1. Cross-tabulation of cytologic and histologic anal SIL results from baseline visits in 617 men enrolled in the Study of the Prevention of Anal Cancer

	Histology							
Cytology	**No biopsy**	**Negative**	**LSIL**	**HSIL-AIN2**	**HSIL-AIN3**	**Missing**	**Total**	**Prevalence (95% CI)**
Unsatisfactory	2	13	9	0	5	0	29	4.7% (3.3–6.7)
Negative	72	96	40	14	18	1	241	39.0% (35.3–43.0)
ASCUS	17	17	40	9	20	0	103	16.7% (14.0–19.9)
LSIL	3	3	26	6	9	0	47	7.6% (5.8–10.0)
ASC-H	16	9	21	9	33	0	88	14.3% (11.7–17.3)
HSIL-AIN2	4	1	5	3	8	0	21	3.4% (2.2–5.2)
HSIL-AIN3	3	9	14	8	54	0	88	14.3 (11.7–17.3)
Total	117	148	155	49	147	1	617	
Prevalence (95% CI)	19.0% (16.1–22.3)	24.0% (20.8–27.6)	25.2% (21.9–28.8)	8.0% (6.1–10.4)	23.9 (20.7–27.4)			

Abbreviation: AIN, anal intraepithelial neoplasia.

and 196 (31.8%) had HSIL (Table 1). One man who had a superficially invasive anal cancer arising from an HSIL lesion was classified as HSIL in these analyses.

For the current analysis, data were included on 605 of the 617 (98.4%) participants who had each of anal swab, HRA, and HPV genotyping results available. Of the 12 excluded, 11 were participants with samples that were inadequate for HPV testing, in addition to the one man who was unable to tolerate HRA.

Prevalence of HR-HPV in cytologically and histologically defined ASIL

For cytologically defined endpoints, HPV16 prevalence progressively increased with increasing cytologic grade from 13.1%, 25.7%, 31.1%, 41.4%, 42.9%, and 63.2% ($P_{trend} < 0.001$) in men with negative, ASC-US, LSIL, ASC-H, HSIL-AIN2, and HSIL-AIN3 cytology, respectively. The prevalence of other HR-HPV types (not including HPV16) did not change significantly with increasing cytologic grade ($P_{trend} = 0.278$) as above (Fig. 1; Table 2). The prevalence of HPV16 and other HR-HPV types (not including HPV16) was 25.9% and 33.3%, respectively, in men with repeatedly unsatisfactory smears.

For histologically defined endpoints, the prevalence of HPV16 was 17.4% in men with normal appearance (no biopsy taken) and 17.6% in men with no ASIL on biopsy. This increased to 22.7%, 24.5%, and 60.1% ($P_{trend} < 0.001$) in men with LSIL, HSIL-AIN2, and HSIL-AIN3 histology, respectively. The prevalence of other HR-HPV types was increased in men with HSIL-AIN2 ($P = 0.002$), but overall the prevalence did not change across increasing histologic grade ($P_{trend} = 0.568$) as above (Fig. 1; Table 2).

To assess the potential value of combining cytology and histology results to improve the accuracy of HSIL detection, the prevalence of HPV16 and other HR-HPV types was analyzed in men with histologic disease endpoints. HPV prevalence was then analyzed within each histologic group, stratified by the results of their corresponding cytology diagnosis, as follows (Fig. 2 and Table 2).

Among 263 men with no histologic ASIL, the prevalence of HPV16 and other HR-HPV types was 13.3% and 33.3% in men with repeat unsatisfactory smears. Prevalence of HPV 16 increased

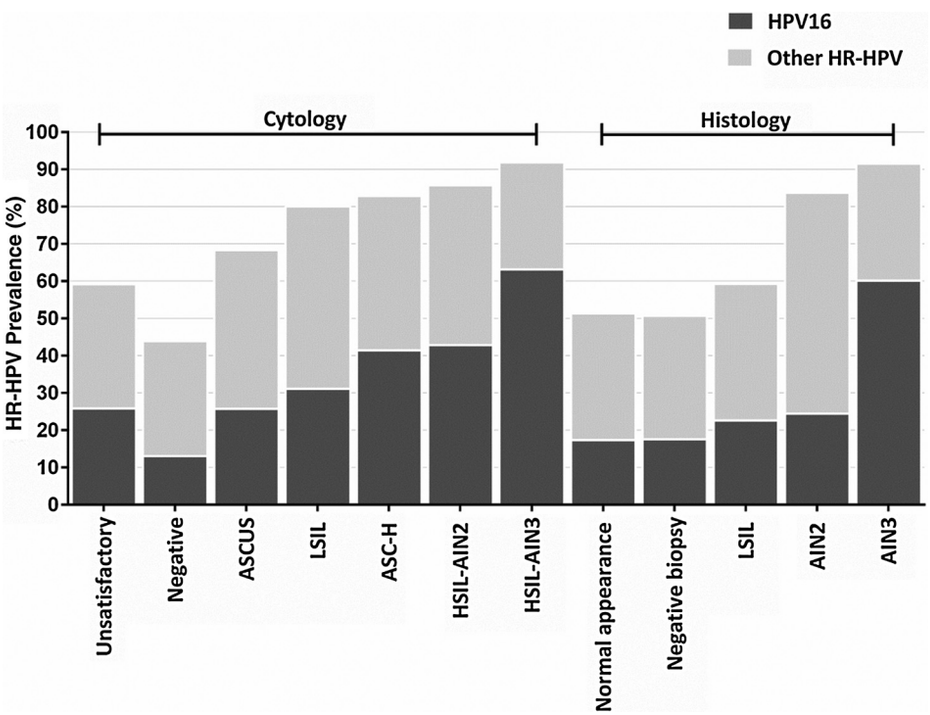

Figure 1.
Prevalence of HR-HPV DNA in ASIL diagnosed using anal Pap (Cytology) and HRA-guided biopsy of any visible abnormality (Histology) in 605 men with complete baseline results on cytology, histology, and HPV in the Study of the Prevention of Anal Cancer. Data for the prevalence of HPV16 (dark gray) and other HR-HPV types not including HPV16 (light gray) are presented for each disease category. AIN, anal intraepithelial neoplasia.

Table 2. Prevalence of HPV16, other HR-HPV types not including HPV16 (HR other), and LR-HPV only (LR-only) in all combinations of results obtained by cytology and histology in 605 men with complete baseline results on cytology, histology, and HPV in the Study of the Prevention of Anal Cancer

	Histology				
	Negative/normal	**LSIL**	**HSIL-AIN2**	**HSIL-AIN3**	**Total**
Cytology	***n* (%)**	***n* (%)**	***n* (%)**	***n* (%)**	***N* (%)**
Unsatisfactory					
n	15	8	0	4	27
Negative	4 (26.7)	1 (12.5)	0 (0.0)	0 (0.0)	5 (18.5)
HPV16	2 (13.3)	2 (25.0)	0 (0.0)	3 (75.0)	7 (25.9)
HR other	5 (33.3)	3 (37.5)	0 (0.0)	1 (25.0)	9 (33.3)
LR only	4 (26.7)	2 (25.0)	0 (0.0)	0 (0.0)	6 (22.2)
Total HPV	11 (73.3)	7 (87.5)	0 (0.0)	4 (100.0)	22 (81.5)
Negative					
n	166	40	14	17	237
Negative	51 (30.7)	6 (15.0)	1 (7.1)	0 (0.0)	58 (24.5)
HPV16	20 (12.0)	3 (7.5)	1 (7.1)	7 (41.2)	31 (13.1)
HR other	45 (27.1)	12 (30.0)	9 (64.3)	7 (41.2)	73 (30.8)
LR only	50 (30.1)	19 (47.5)	3 (21.4)	3 (17.6)	75 (31.6)
Total HPV	115 (69.3)	34 (85.0)	13 (92.9)	17 (100.0)	179 (75.5)
ASCUS					
n	34	39	9	19	101
Negative	5 (14.7)	4 (10.3)	0 (0.0)	1 (5.3)	10 (9.9)
HPV16	7 (20.6)	9 (23.1)	1 (11.1)	9 (47.4)	26 (25.7)
HR other	16 (47.1)	13 (33.3)	7 (77.8)	7 (36.8)	43 (42.6)
LR only	6 (17.6)	13 (33.3)	1 (11.1)	2 (10.5)	22 (21.8)
Total HPV	29 (85.3)	35 (89.7)	9 (100.0)	18 (94.7)	91 (90.1)
LSIL					
n	6	24	6	9	45
Negative	0 (0.0)	1 (4.2)	0 (0.0)	0 (0.0)	1 (2.2)
HPV16	0 (0.0)	7 (29.2)	2 (33.3)	5 (55.6)	14 (31.1)
HR other	4 (66.7)	11 (45.8)	3 (50.0)	4 (44.4)	22 (48.9)
LR only	2 (33.3)	5 (20.8)	1 (16.7)	0 (0.0)	8 (17.8)
Total HPV	6 (100.0)	23 (95.8)	6 (100.0)	9 (100.0)	44 (97.8)
ASC-H					
n	25	20	9	33	87
Negative	1 (4.0)	1 (5.0)	0 (0.0)	0 (0.0)	2 (2.3)
HPV16	9 (36.0)	5 (25.0)	3 (33.3)	19 (57.6)	36 (41.4)
HR other	10 (40.0)	9 (45.0)	6 (66.7)	11 (33.3)	36 (41.4)
LR only	5 (20.0)	5 (25.0)	0 (0.0)	3 (9.1)	13 (14.9)
Total HPV	24 (96.0)	19 (95.0)	9 (100.0)	33 (100.0)	85 (97.7)
HSIL-AIN2					
n	5	5	3	8	21
Negative	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	1 (4.8)
HPV16	2 (40.0)	1 (20.0)	1 (33.3)	5 (62.5)	9 (42.9)
HR other	3 (60.0)	2 (40.0)	1 (33.3)	3 (37.5)	9 (42.9)
LR only	0 (0.0)	2 (40.0)	0 (0.0)	0 (0.0)	2 (9.5)
Total HPV	5 (100.0)	5 (100.0)	2 (66.7)	8 (100.0)	20 (95.2)
HSIL-AIN3					
n	12	14	8	53	87
Negative	0 (0.0)	1 (7.1)	0 (0.0)	2 (3.8)	3 (3.4)
HPV16	6 (50.0)	7 (50.0)	4 (50.0)	38 (71.7)	55 (63.2)
HR other	5 (41.7)	5 (35.7)	3 (37.5)	12 (22.6)	25 (28.7)
LR only	1 (8.3)	1 (7.1)	1 (12.5)	1 (1.9)	4 (4.6)
Total HPV	12 (100.0)	13 (92.9)	8 (100.0)	51 (96.2)	84 (96.6)
Total					
n	263	150	49	143	605
Negative	61 (23.2)	14 (9.3)	2 (4.1)	3 (2.1)	80 (13.2)
HPV16	46 (17.5)	34 (22.7)	12 (24.5)	86 (60.1)	178 (29.4)
HR other	88 (33.5)	55 (36.7)	29 (59.2)	45 (31.5)	217 (35.9)
LR only	68 (25.9)	47 (31.3)	6 (12.2)	9 (6.3)	130 (21.5)
Total HPV	202 (76.8)	136 (90.7)	47 (95.9)	140 (97.9)	525 (86.8)

from 12.0% in men with negative cytology to 47.1% in men with HSIL cytology ($P_{trend} < 0.001$). The prevalence of other HR-HPV also increased significantly with increasing grade of cytology (27.1%, 47.1%, 66.7%, 40.0%, and 47.1% in men with negative, ASC-US, LSIL, ASC-H, and HSIL cytology, respectively, $P_{trend} = 0.004$). Among 150 men with LSIL histology, the prevalence of HPV16 and other HR-HPV types was 25.0% and 37.5% in men with repeat unsatisfactory smears. Prevalence of HPV16 increased from 7.5% in men with negative cytology to 42.1% in men with HSIL cytology ($P_{trend} = 0.003$). The prevalence of other HR-HPV types did not change significantly with increasing grade of cytology ($P_{trend} = 0.245$). Finally, among 192 men with HSIL

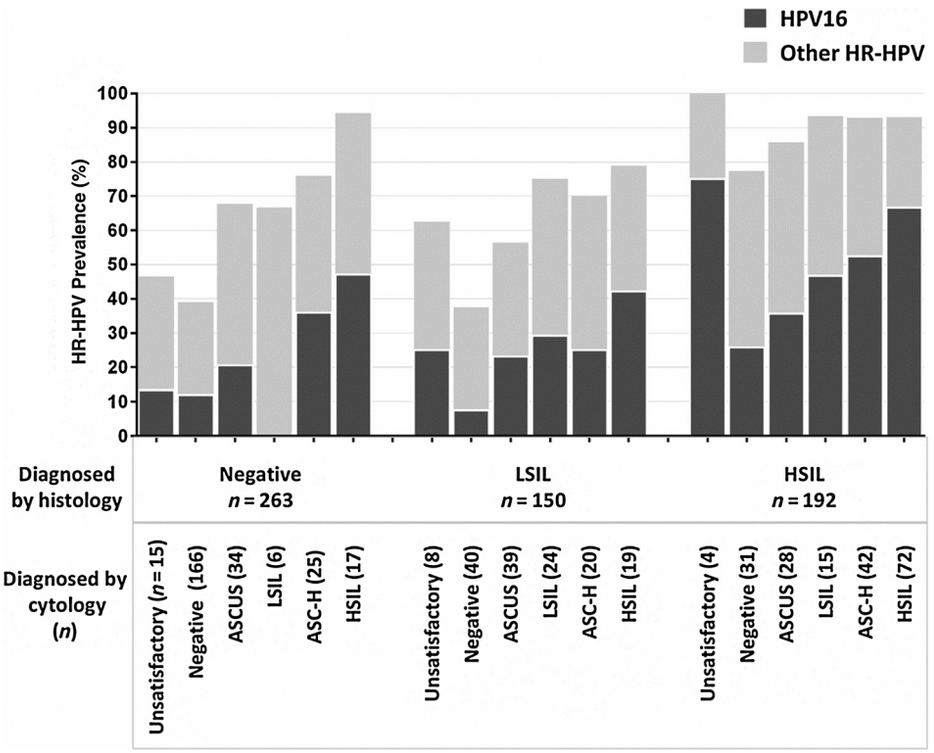

Figure 2.
HR-HPV DNA prevalence (HPV16 in dark gray and other HR-HPV types in light gray) in categories of histology, stratified by cytology results in 605 men with complete baseline results on cytology, histology, and HPV in the Study of the Prevention of Anal Cancer. AIN, anal intraepithelial neoplasia.

diagnosed histologically, the prevalence of HPV16 and other HR-HPV types was 75.0% and 25.0% in men with repeat unsatisfactory smears. Prevalence of HPV16 increased from 25.8% in men with negative cytology to 66.7% in men with HSIL cytology ($P_{trend} < 0.001$). The prevalence of other HR-HPV types decreased significantly with increasing grade of cytology (51.6%, 50.0%, 46.7%, 40.5%, and 26.4% as above, $P_{trend} = 0.004$, Fig. 2; Table 2).

Thus, within each histologic category, increasing grade of cytology was associated with an increasing prevalence of HPV16. Increasing grade of cytology was associated with increasing prevalence of other HR-HPV types only in men with no histologic ASIL. In men with histologic LSIL, the prevalence of other HR-HPV types remained stable with increasing grade of cytology, while for histologic HSIL, increasing grade of cytology was associated with a lower prevalence of other HR-HPV types.

Next, the prevalence of HPV16 and other HR-HPV types was examined in men within each cytologic result, stratified by their corresponding histology diagnosis, as follows (Fig. 3 and Table 2). Among 27 men with unsatisfactory cytology results, the prevalence of HPV16 increased significantly from 13.3% in men with negative histology to 75.0% in men with HSIL histology ($P_{trend} = 0.038$). The prevalence of other HR-HPV types did not increase significantly with increasing grade of histology ($P_{trend} = 0.908$). Among 237 men with a negative cytology result, prevalence of HPV16 did not increase significantly ($P_{trend} = 0.270$). The prevalence of other HR-HPV increased significantly with increasing grade of histology (27.1%, 30.0%, and 51.6% in men with negative, LSIL and HSIL histology, respectively, $P_{trend} = 0.028$). Among 101 men with ASC-US cytology, the prevalence of HPV16 increased from 20.6% in men with negative histology to 35.7% in men with HSIL histology, but the increase was not significant ($P_{trend} = 0.193$). The prevalence of other HR-HPV types did not change with increasing grade of histology ($P_{trend} = 0.918$). Among 45 men with LSIL cytology, the prevalence of HPV16 increased significantly from 0% in men with negative histology to 46.7% in men with HSIL histology ($P_{trend} = 0.045$). The prevalence of other HR-HPV types did not change with increasing grade of histology ($P_{trend} = 0.570$). Among 87 men with ASC-H cytology, the prevalence of HPV16 increase from 36.0% in men with negative and 25.0% in men with LSIL histology to 52.4% in men with HSIL histology, but the increase was not significant ($P_{trend} = 0.105$). The prevalence of other HR-HPV types did not change ($P_{trend} = 0.970$). Finally, among 108 men with HSIL cytology, the prevalence of HPV16 increased significantly from 47.1% in men with negative histology and 42.1% in men with LSIL to 66.7% in men with HSIL histology ($P_{trend} = 0.036$). The prevalence of other HR-HPV types decreased from 47.1% through to 26.4% ($P_{trend} = 0.089$, Fig. 3 and Table 2).

Thus, for each category of cytology, except HSIL, increasing grade of histologic result was associated with a higher HR-HPV prevalence (HPV16 and other HR-HPV types), but this difference did not always reach statistical significance. When the cytology result was HSIL, increasing grade of histology was associated with a significantly higher prevalence of HPV16 and a lower prevalence of other HR-HPV types.

Definition of composite endpoints based on positive cytology or histology results

Given the significant association between composite cytology-histology endpoints and HPV16, four composite endpoint outcomes were constructed, representing categories of increasingly close association with HPV16 (Table 3).

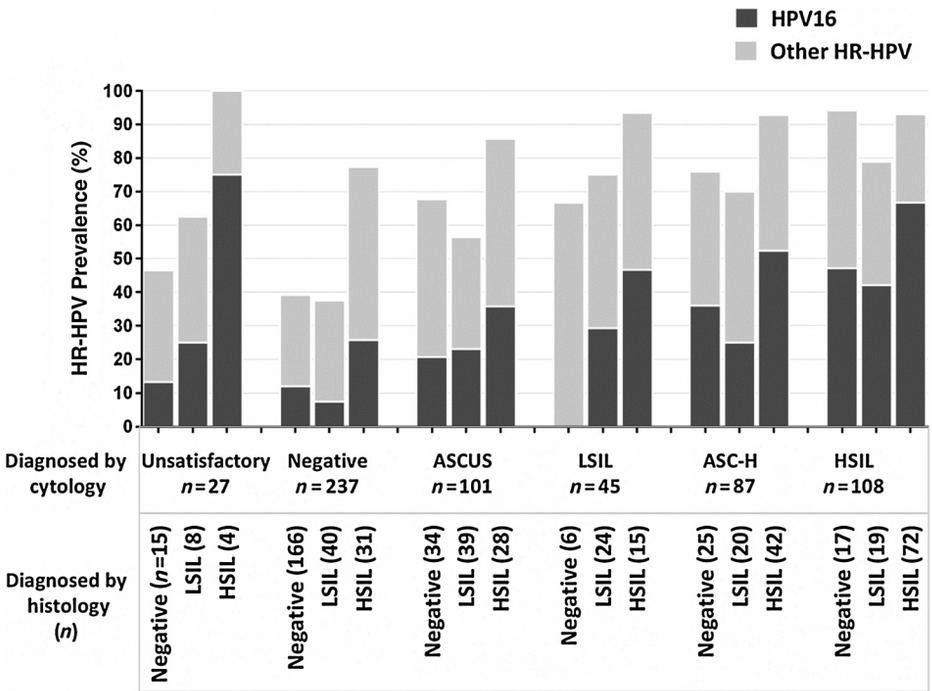

Figure 3.
HR-HPV DNA prevalence (HPV16 in dark gray and other HR-HPV types in light gray) in categories of cytology, stratified by histology results in 605 men with complete baseline results on cytology, histology, and HPV in the Study of the Prevention of Anal Cancer. AIN, anal intraepithelial neoplasia.

First, we defined a "composite-negative" category that included men who were negative for ASIL by both cytology and histology. Men with unsatisfactory cytology results who had normal histology were also included in this category, as the prevalence of HPV16 and other HR-HPV types was similar between the two groups. Almost one-third (29.9%; 95% CI, 26.3–33.7) of men in the SPANC cohort were in the composite-negative category. The prevalence of HPV16 and other HR-HPV types in men in this category was 12.1% and 27.6%, respectively. HPV16 prevalence in this composite category was lower than in either men with negative cytology or men with normal/negative histology endpoints considered separately. In addition, the prevalence of any HPV was lower in this group (Tables 2 and 3).

Second, we defined a "composite-LSIL" category that included men who had LSIL or ASC-US cytology and/or LSIL histology as their highest grade of ASIL. About one-quarter (25.0%, 95% CI, 21.6–28.6) of men were in this category. The prevalence of HPV16 and other HR-HPV types was 18.5% and 39.1%, respectively. Again, the prevalence of HPV16 in this composite endpoint was lower in men with the cytology and histology endpoints considered separately. In addition, a larger proportion of men with composite-LSIL had only LR-HPV DNA detected (31.1%), compared with 21.8% or 17.8% in men with ASC-US or LSIL cytology (Tables 2 and 3).

Third, we defined a "composite-HSIL" category that included men who had HSIL detected by either cytology and/or histology. Over one-third (37.7%, 95% CI, 33.8–41.7) of men were in this

Table 3. Prevalence of ASIL endpoints and the prevalence of HPV DNA within ASIL endpoints in the baseline visit in 605 men with complete baseline results on cytology, histology, and HPV in the Study of the Prevention of Anal Cancer

				Prevalence of HPV by ASIL endpoint		
		Number and proportion in each group		HPV16	All other HR-HPV types	LR-HPV only
Disease category	Definition	*N*	% (95% CI)	*n* (%)	*n* (%)	*n* (%)
Composite-negative	Visually normal HRA with no biopsy taken, or no evidence of ASIL on any biopsy, and normal or unsatisfactory cytology	181	29.9 (26.3–33.7)	22 (12.1)	50 (27.6)	54 (29.8)
Composite-LSIL	LSIL or ASCUS cytology and/or LSIL histology as the highest grade of ASIL	151	25.0 (21.6–28.6)	28 (18.5)	59 (39.1)	47 (31.1)
Composite-ASC-H	ASC-H cytology and no HSIL (≤LSIL) on histology	45	7.4 (5.5–9.8)	14 (31.1)	19 (42.2)	10 (22.2)
Composite-HSIL	HSIL detected by either cytology or histology	228	37.7 (33.8–41.7)	114 (50.0)	89 (39.0)	19 (8.3)
HSIL-AIN2	HSIL-AIN2 on cytology and/or AIN2 on histology without a diagnosis of AIN3 on either	51	8.4 (6.3–10.9)	11 (21.6)	31 (60.8)	7 (13.7)
HSIL-AIN3	HSIL-AIN3 on cytology or histology	177	29.3 (25.6–33.1)	103 (58.2)	58 (32.8)	12 (6.8)
HSIL cytology regardless of histology results		108	17.9 (14.9–21.1)	64 (59.3)	34 (31.5)	6 (5.6)
HSIL histology regardless of cytology results		192	31.7 (28.0–35.6)	98 (51.0)	74 (38.5)	15 (7.8)

Abbreviation: AIN, anal intraepithelial neoplasia.

category. The prevalence of HPV16 and other HR-HPV types was 50.0% and 39.0%, respectively. Within this category, we subcategorized HSIL as "composite-AIN2" (HSIL-AIN2 on cytology and/or AIN2 on histology without a diagnosis of AIN3 on either) and "composite-AIN3" (HSIL-AIN3 on either cytology or histology) with prevalence of 8.4% (95% CI, 6.3–10.9) and 29.3% (95%CI, 25.6–33.1), respectively. The prevalence of HPV16 in men with composite-AIN3 (58.2%) was more than twice that of men with composite-AIN2 (21.6%, $P = 0.002$), whereas the prevalence of other HR-HPV types was significantly lower in men with composite-AIN3 (32.8%) than in men with composite-AIN2 (60.8%, $P = 0.005$). The prevalence of HPV16 in this composite category was comparable with that of HSIL cytology and HSIL histology endpoints considered separately. Of note, the prevalence of LR-HPV types in the absence of detection of HR-HPV types decreased significantly with increasing grade of composite-endpoint ($P_{trend} < 0.001$; Tables 2 and 3).

Fourth, we defined a remaining category of men who had ASC-H reported on cytology and who had less than HSIL on histology. About one in 12 men (7.4%, 95% CI, 5.5–9.8) were in this "composite-ASC-H" category. The prevalence of HPV16 and other HR-HPV types in men in this category was 31.1% and 42.2%, respectively, and lay between those among men with composite-LSIL and men with composite-HSIL (Tables 2 and 3).

Last, we examined the association between HPV-16, other HR-HPV types, and LR-HPV only and increasing grade of composite endpoints by HIV status. Identical patterns to that seen in the overall study population were observed (Supplementary Table S1).

Discussion

In this study of predominantly community-recruited homosexual men undergoing anal cytology and HRA at the same clinical visit, the prevalence of cytologically predicted HSIL (18%) was substantially lower than that found on HRA-guided biopsy (32%). The use of a composite endpoint comprising either a cytologic and/or histologic diagnosis further increased HSIL prevalence to 38%. Composite-HSIL appeared to be a biologically meaningful category, with a very similarly high prevalence of HPV16 and other HR-HPV types (89% overall, with 50% HPV16, and 39% other HR-HPV) to that seen when histologic HSIL (90% overall, with 51% HPV16, and 39% HR-HPV) or cytologic HSIL (91% overall, with 59% HPV16, and 31% other HR-HPV) was seperately considered. Lower grades of composite endpoints were associated with lower rates of detection of HPV16 and other HR-HPV types. Composite-LSIL was associated with the highest rates of detection of LR-HPV in the absence of HR-HPV infection. Given the assocation of increasingly high prevalence of HPV16 across increasing grades of composite disease endpoint, these composite endpoints appear to represent biologically meaningful measures of significant HPV-related ASIL. The increased prevalence of HSIL found using the composite endpoint, combined with its strong relationship with HPV16, provides a rationale for using, where possible, composite cytology–histology endpoints in studies of the prevalence and risk factors for anal HSIL.

Our estimate of composite-HSIL prevalence was higher than the prevalence of biopsy-proven HSIL in the previous meta-analysis (6). This most likely reflects the improved sensitivity of a composite endpoint to detect HSIL compared with histology used alone. Composite cytology–histology endpoints have been applied in very few previous studies of ASIL. In three prospective studies, composite endpoints were reported in a subset of participants and visits (20–22). Only one study has previously reported composite cytology–histology endpoints on the entire population (13, 23–25). In that cross-sectional report of 363 HIV-positive MSM recruited from an anal cancer screening clinic in San Francisco, the prevalence of HSIL was 18% (95% CI, 14%–22%) using cytology alone, 22% (95% CI, 18%–27%) using histology alone, and 30% (95% CI, 26%–36%) using a composite cytology and/or histology endpoint (23). The prevalence of HPV16 of 55% (23) in participants with composite-HSIL was strikingly similar to our finding (50%). However, a higher prevalence of histologically proven HSIL (32%) was observed in our study, resulting in a higher composite endpoint prevalence. A possible reason for the higher HSIL prevalence is that in the San Francisco study, a maximum of two biopsies was performed (13), whereas in SPANC the number of biopsies was not limited, and in fact 28% of men had more than two biopsies taken. In addition, participants in SPANC reported higher levels of sexual risk behavior. In SPANC, nearly all men (98%) reported five or more lifetime male partners compared with 83% in the San Francisco study (13). This may have contributed to greater HR-HPV exposure and hence higher HSIL prevalence in our study.

Interestingly, within the composite-HSIL category, the prevalence of HPV16 in men with composite-AIN2 (22%) was less than half than that seen in men with composite-AIN3 (58%, $P = 0.002$). A similar, but less marked differential, was seen in the San Francisco study (47% vs. 61%; ref. 23). Considering a large majority of anal cancers are caused by HPV16 (26), this suggests that the composite-AIN3 category more accurately represents true "anal precancer" than the composite AIN2 category, as is the case in the cervix (27). In a recent Australian study of 112 anal cancer samples analyzed for the presence of HPV DNA, 108 (96%) were HPV DNA positive and, of those, 81 (75%) were positive for HPV16 (28).

Although the grouping of cytology–histology endpoints in this study was guided by HPV16 prevalence, it must be acknowledged that there are many other potential groupings of composite endpoints. Our groupings are similar to those published by the San Francisco study (25), except in one regard: we created an additional category of men with ASC-H on cytology, but no HSIL on histology. We did this because this cytology–histology endpoint had a HPV16 prevalence (31%) that was clearly intermediate between that seen in composite-LSIL (19%) and composite-HSIL (50%). Limited data suggest that in women with cervical ASC-H who go on to have a normal or LSIL histology result at colposcopy, the risk of underlying HSIL may be substantial. In a prospective cohort study of 53 women with cervical ASC-H who underwent initial colposcopy without treatment, 24 (45%) were diagnosed with HSIL histology at follow-up (29). In the cervix, ASC-H is uncommon, accounting for approximately 0.2% to 0.5% of cytologic results (30, 31). In published studies, the prevalence of ASC-H in the anal canal is substantially higher (2%–11%; refs. 6, 23) than in the cervix and in SPANC was 14%. In this study, of all men with ASC-H cytology, 48% had HSIL histology (Table 2). The relatively high HPV16 prevalence in men with ASC-H in this cohort suggests that, as in the cervix, a high proportion of the remainder with composite ASC-H (7%) may

have undiagnosed HSIL, possibly reflecting small HSIL lesions not visualized on HRA.

This study has several limitations. First, the composite endpoints created in this study were based on cross-sectional data and not on prospective follow-up. As our follow-up of men with composite endpoints accrues, we will be able to make more definitive conclusions about the accuracy of the composite-HSIL diagnoses and their natural history. Second, the small sample size in some cytology–histology combinations limits the statistical power of the analysis. However, the statistically significant pattern of HPV16 detection supports the validity of the findings, and our report is almost double the size of the largest previous report of a combined cytology–histology endpoint. Third, although our anoscopists were well trained (16), most were relatively new to the field and it is possible that their performance in diagnosing HSIL at HRA will improve further with time. However, when we stratified baseline study visits by the degree of experience of the anoscopist, we found that the additional HSIL prevalence afforded by the composite endpoint was virtually identical between most- and lesser experienced anoscopists (data not presented). In a Dutch study, it has been reported that the diagnostic yield of HRA increases until an operator has performed approximately 200 HRAs (32). Fourth, it is important to note that HPV genotyping was performed on the ThinPrep aliquat collected before cytologic processing, and not on lesional tissue. Hence, in some instances, if the anal cytology missed an HSIL lesion during sampling, the associated (causative) HPV type may have also been missed. Given the high burden of HPV in this population, including the presence of multiple HPV types (33) and the concurrent presence of multiple lesions (of which the highest grade was taken as the endpoint), it cannot be inferred that the diagnosed lesions were definitely caused by the HPV types detected.

SPANC is one of only a small number of cohort studies globally and the largest published thus far, to perform anal cytology and HRA screening, as well as HPV genotyping at the same visit on all participants, with no limitation on the number of biopsies allowed. In addition, recruitment for the study was mostly from community-based settings with broad inclusion criteria, making the results more generalizable to a target screening population. Biopsy reporting was performed in accordance with the LAST Project recommendations (16, 18), limiting potential misclassification of histologic HSIL. There was a very high degree of inter-rater reliability and intra-rater repeatability in histologic diagnosis in the study (34).

This study demonstrates that epidemiologic studies of HSIL may underestimate the prevalence of HSIL if the results of anal cytology and HRA are not combined, and highlights the limitations of both techniques. Studies on the performance of anal cytology in detecting histologically proven HSIL in homosexual and bisexual men demonstrate that the procedure clearly underestimates the true prevalence of HSIL (35). HRA is also prone to considerable sampling and measurement error, and is much more technically demanding than cervical colposcopy (10). However, it has to be recognized that performing anal cytology and HRA on all participants, in epidemiologic studies and in clinical practice, will often not be practical, because HRA is an invasive and resource-intensive procedure. Research should focus on optimizing anal cytology sampling protocols to improve HSIL detection. Incorporating HPV and molecular biomarkers may prove useful in increasing the yield and diagnostic accuracy of anal cytology and targeting HRA to those most likely to have HSIL. It is also important to note, while composite endpoints may be useful in studies of HSIL prevalence to identify cases which are missed at HRA, they are not a substitute for HRA performed by well-trained individuals. Advanced training in HRA and attention to quality assurance programs, similar to those in cervical colposcopy (36) is likely to improve the diagnostic yield of HRA. When HRA is performed by the most experienced hands, composite endpoints may provide smaller additional benefit.

In summary, diagnosis of ASIL has been hindered by the limited sensitivity of both anal cytology sampling and HRA used alone. As a result, many published studies of cytology-only and histology-only ASIL endpoints likely underestimate the true prevalence of HSIL. This study has demonstrated that combining the results of anal cytology and HRA leads to the diagnosis of more biologically relevant disease. Until data on biomarkers that will improve diagnostic accuracy of cytology are available, epidemiologic studies of anal HSIL risk should, where possible, include data on composite cytology and histology endpoints in order to improve the sensitivity of ASIL detection.

Disclosure of Potential Conflicts of Interest

J.M Roberts and A. Farnsworth report receiving commercial research support from Hologic. S.M. Garland is the chief investigator in an HPV vaccine trial by GSK, investigator in a CSLBio Investigator-initiated grant, reports receiving commercial research grant from Merck Investigator initiated grant, has received speakers bureau honoraria from Merck, and is a consultant/advisory board member for Merck Global. C.K. Fairley has ownership interest (including patents) in shares in CSL Biotherapies. A.E. Grulich has received speakers' bureau honoraria from Merck. No potential conflicts of interest were disclosed by the other authors.

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