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Human Papillomavirus Genotypes Predict Progression of Anal Low-Grade Squamous Intraepithelial Lesions

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Background. High-risk human papillomavirus (hrHPV)–induced anal low-grade squamous intraepithelial lesions (LSILs) have the potential to progress to high-grade squamous intraepithelial lesions (HSILs). We investigated whether anal hrHPV infections, particularly types 16 and 18, predict LSIL-to-HSIL progression.

Methods. One hundred forty-six human immunodeficiency virus (HIV)–infected and 22 HIV-uninfected patients with anal LSILs underwent cytology, HPV genotyping (16, 18, and pooled 12 hrHPV types), and high-resolution anoscopy-guided biopsy at baseline and surveillance. The associations between the rate of LSIL-to-HSIL progression and HPV types as well as longitudinal HPV-16/18 status were assessed by fitting separate Cox regression models.

Results. At baseline, 91% of patients harbored hrHPV: HPV-16/18 (44%) and non-16/18 (86%). Upon follow-up (median, 20 [range, 6–36] months), 41% developed HSIL (84% at the same anatomic location as the initial LSIL and 16% at a different location). Baseline HPV-16/18–positive patients had greater probability of progression than patients with non-16/18 types or negative (67%, 25%, and 7%, respectively; $P < .001$). Persistent HPV-16/18 conferred the highest probability of progression (70%), followed by intermittent HPV-16/18 positivity (52%). In unadjusted and adjusted analyses, baseline and persistent HPV-16/18 were significantly associated with LSIL-to-HSIL progression.

Conclusions. Anal LSIL patients who are positive for hrHPV, especially HPV-16/18, have an increased risk of developing HSIL. Type-specific HPV testing could serve as a risk stratification tool, providing prognostic information.

Keywords. human papillomavirus; low-grade squamous intraepithelial lesion; HIV; HPV genotyping.

Human papillomavirus (HPV) infection of the anal canal can cause low-grade squamous intraepithelial lesions (LSILs). Specific populations such as men who have sex with men (MSM), human immunodeficiency virus (HIV)–infected individuals, and women with cervical dysplasia are disproportionately affected [1–3]. Depending on host and viral factors, LSILs regress, persist, or progress to high-grade squamous intraepithelial lesions (HSILs), the precursors of anal squamous cell carcinoma [4, 5]. The LSIL-to-HSIL progression rate has been reported to be as high as 62% in HIV-infected men and 36% in HIV-uninfected men within 2 years [6]. Providing adequate surveillance for LSIL patients constitutes a critical component of anal cancer prevention. Given the growing demand for screening and limited healthcare resources, managing these patients might need to be prioritized based on individual risk of progression.

Anal high-risk HPV (hrHPV) infection is prevalent in >90% of MSM, a fact that largely offsets the utility of pooled HPV DNA tests in anal cancer screening [7, 8]. As an alternative, type-specific HPV genotyping may provide valuable prognostic information, especially considering the highly diverse biology and carcinogenicity of papillomaviruses [9]. HPV-16 and -18 are the most carcinogenic among the 40 sexually transmitted types; they more frequently result in persistent infection and lower clearance rates [10, 11]. The prevalence of HPV-16/18 increases with the severity of anal lesions (27% in LSIL, 69% in HSIL, and 72% in cancer), indicating a strong link between HPV-16/18 infection and disease progression [12].

There are limited data pertaining to the natural history of anal LSIL and the risk of LSIL-to-HSIL progression. Likewise, the role of HPV genotyping for anal HSIL detection has yet to be fully explored. Herein, we conducted a longitudinal study of patients with anal LSILs who underwent serial HPV genotyping and high-resolution anoscopy (HRA)–guided biopsy, aiming to explore the natural history of anal LSILs and to evaluate whether hrHPV infection, particularly types 16 and 18, is associated with an increased risk of disease progression.

METHODS

Patient Selection and Demographics

The Institutional Review Board of the Icahn School of Medicine approved this study. Our HRA database was queried from January 2011 to January 2017 for patients with biopsy-proven

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anal LSILs. To meet inclusion criteria, patients were required to have anorectal cytology (ARC), HPV genotyping, and HRA-guided biopsy upon initial and surveillance visits. The interval between visits ranged from 6 to 36 months. Individuals with HSIL or atypical squamous cells, cannot exclude HSIL (ASC-H) on initial cytology were excluded. Patient demographic variables were extracted from medical records: age, gender, race/ethnicity, HIV status, $CD4^+$ T-cell count, HIV type 1 plasma RNA load, and smoking history.

Anorectal Cytology and HPV Genotyping

ARC and HPV genotyping results were limited to samples collected concurrently or within 3 months of HRA. ARC diagnoses were rendered by cytopathologists from the Mount Sinai Hospital using the 2001 Bethesda System criteria and categories: negative; atypical squamous cells of undetermined significance (ASC-US); LSIL; ASC-H; and HSIL [13]. Using remaining liquid cytology fluid, HPV genotyping was performed with the Roche Cobas HPV kit (Roche Diagnostics) following the manufacturer's instructions, capable of detecting HPV-16, HPV-18, and pooled results for 12 additional hrHPV types: 31/33/35/39/45/51/52/56/58/59/66/68.

High-Resolution Anoscopy and Biopsy

Following previously described techniques, author M. M. G. performed all HRA procedures and biopsies [14]. After treatment with 3% acetic acid and Lugol iodine, the perianal region, distal anal canal, and squamocolumnar junction were examined using a high-resolution colposcope at 15-fold magnification to look for abnormal vascular patterns and other signs of dysplasia or cancer, including ulceration, mass effect, and friability. Areas suspicious for dysplasia or cancer were biopsied. We divided the anal canal into octants and defined lesional locations as either anterior, right anterior, right lateral, right posterior, posterior, left posterior, left lateral, or left anterior. Random biopsies of benign-appearing tissue were not pursued for this study.

Histopathology Diagnosis

Author Y. L. diagnosed all biopsies based on hematoxylin-and-eosin slides using lower anogenital squamous terminology criteria [15]. The designation of normal squamous epithelium required the absence of any viral-induced cytological abnormalities (eg, nuclear enlargement, coarse chromatin, irregular nuclear membrane). When such abnormalities were present within the lower one-third of the epithelium, lesions were graded LSIL; when present in the middle or top third of the epithelium, lesions were graded HSIL. P16 immunohistochemistry was used on a subset of cases to confirm the diagnosis, whereby strong and diffuse positive staining supports the diagnosis of HSIL, while weak, patchy, or negative staining correlates with LSIL or benign mucosa [16].

Outcome Measurement

Based on surveillance HRA examination and biopsy results, outcomes were categorized as "progression" when 1 or more HSILs were detected, or "nonprogression" when biopsies revealed LSILs or normal mucosa. We designated HPV-16/18 status as "persistent infection" when HPV-16/18 was detected in both baseline and follow-up cytology samples, or as "intermittent infection" when HPV-16/18 was detected only once, either at baseline or follow-up.

Statistical Analysis

We first compared baseline characteristics for patients with LSIL-to-HSIL progression to those without progression using the *t* test for age and the χ^2 test for categorical variables. We then compared the probability of LSIL progression among baseline HPV categories (16/18; coinfection of 16/18 and other hrHPV; exclusively non-16/18 hrHPV; or no hrHPV) using the χ^2 test. To evaluate the association between longitudinal HPV-16/18 status (persistent infection, intermittent infection, and never infected with HPV-16/18) and disease progression, we then compared the incidence of HSIL among these groups, testing for significance with the χ^2 test. Last, we fit Cox proportional hazard regression models to determine the association of hrHPV groups, age (categorized into <40 years, 40–50 years, and >50 years to reflect differences in progression risk by age observed in prior studies [17]), gender, HIV, and smoking status on LSIL progression risk. Each risk factor was first included in an unadjusted model and then included in multivariable models; the first adjusted model included the presence of baseline HPV-16/18 types and non-16/18 types as primary predictors while the second model evaluated the association of longitudinal HPV-16/18 status adjusted for all covariates. Event time was calculated using the date of the index examination until LSIL progression or final follow-up examination; subjects were censored if progression had not occurred by the final follow-up. All analyses were performed in Stata version 13 software (StataCorp).

RESULTS

Patient Characteristics and HPV Status at Baseline

A total of 168 patients met inclusion criteria. The median age was 42 (range, 21–73) years, 146 (87%) were HIV infected, and 34 (20%) were current smokers. One hundred fifty-four (92%) were male and all self-reported as MSM. At baseline, the median $CD4^+$ cell count was 582 (range, 30–1982) cells/mm^3; 115 (79%) HIV-infected patients had HIV RNA load <100 copies/mL. Forty-three percent of patients were white, 22% African American, 30% Hispanic, and 5% of other or unknown race or ethnicity. Baseline cytology results were negative for intraepithelial lesion (n = 25), ASC-US (n = 95), LSIL (n = 44), and unsatisfactory (n = 4). Upon initial HRA, an average of 3 biopsies (range, 1–8) were taken for each patient, revealing 1 or 2 LSILs (n = 131) or 3–5 LSILs (n = 37).

At baseline (Table 1), 15 patients (9%) were negative for hrHPV and 153 (91%) were positive. HPV-16 and -18 were detected in 74 (44%) patients in the following combinations: HPV-16 and/or -18 (n = 8); HPV-16 and/or -18 plus other hrHPV types (n = 66). Non-16/18 hrHPV types were detected in 145 (86%) patients (79 limited to non-16/18 types exclusively and 66 combined with HPV-16/18).

LSIL-to-HSIL Progression

Upon follow-up (median, 20 [range, 6–36] months), HRA-guided biopsy revealed HSIL in 69 (41%) patients constituting the progression group, LSIL in 86 (51%), and benign epithelium in 13 (8%). For statistical purposes, patients with LSIL or benign epithelium on follow-up were combined in the nonprogression group. The number of HSILs detected per patient was 1 (n = 42), 2 (n = 19), and 3–5 (n = 8). Of the 69 HSILs, 58 (84%) developed within the same octant as the index LSIL, whereas the remaining 11 HSILs (16%) developed in a different octant. None of the patients developed invasive carcinoma during the study period.

Age, race/ethnicity, and smoking status were similar between the progression and nonprogression groups (Table 2). MSM had a higher proportion of progression than female patients (43% vs 21%; P = .1), whereas HIV-infected patients had a greater proportion of progression than uninfected ones (45% vs 18%; P = .02). For HIV-infected patients, median $CD4^+$ cell count and HIV viral load distribution were similar between the 2 groups.

Correlation Between Baseline HPV Type and Incidence of Progression

As shown in Table 1, the probability of progression was similar among HPV-16/18–positive patients with or without coinfection by other hrHPV types (67% vs 50%). HPV-16/18–positive patients had a significantly greater probability of progressing than patients with non-16/18 types or negative hrHPV at baseline (67%, 25%, and 7%; P < .001).

Association Between HPV-16/18 Status Over Time and LSIL Progression

Among 74 patients with HPV-16/18 at baseline (Table 3), 54 (32%) patients remained infected upon follow-up (ie, persistent) and 20 (12%) changed from positive to negative. Additionally, 9 (5%) patients changed from negative to positive for HPV-16/18 at follow-up. Patients with persistent HPV-16/18 had the highest probability of LSIL-to-HSIL progression (70%). For patients with intermittent HPV-16/18 positivity, the proportion of progression was 52% overall, 50% (positive HPV-16/18 to negative), and 56% (negative HPV-16/18 to positive). For patients who tested negative for HPV-16/18 throughout the surveillance period, the probability of progression was markedly lower (19%). The difference in progression risk between longitudinal HPV-16/18 infection status groups was statistically significant (P < .001).

Multivariable Analysis

In unadjusted analyses (Table 4, model 1), baseline HPV-16/18 was significantly associated with lesional progression (unadjusted hazard ratio [HR], 3.22 [95% confidence interval {CI}, 1.93–5.40]). There was no significant association between progression and other clinical variables, including non-16/18, age, male gender, HIV infection, and smoking status. In an adjusted model that included other potential confounders (HIV status, smoking status, and gender), baseline HPV-16/18 remained the only significant predictor of progression (adjusted HR, 3.25 [95% CI, 1.90–5.58]).

In unadjusted analyses of longitudinal HPV-16/18 status (Table 4, model 2), both persistent and intermittent infections were significantly associated with lesional progression (unadjusted HR, 5.57 and 2.14 compared to subjects without any hrHPV infection [95% CI, 3.09–10.04 and 1.05–4.37, respectively]). Persistent HPV-16/18 remained a significant predictor for progression in adjusted analyses (adjusted HR, 7.00 [95% CI, 3.69–13.27]).

DISCUSSION

In our study, a substantial number of patients (41%) with baseline anal LSIL developed HSIL during a median follow-up period of 20 months. In the majority (84%), HSIL developed at the initial LSIL location, consistent with the traditional theory that HPV-induced carcinogenesis is a stepwise progression

Table 1. Association Between Baseline High-Risk Human Papillomavirus Types and Progression to High-Grade Squamous Intraepithelial Lesions at Follow-up Visit

HPV Status at Baseline Visit			
Type 16/18	Other hrHPV[a]	No.	Progression to HSIL at Follow-up Visit, No. (%)
–	–	15	1 (7)
+	+	66	44 (67)[b]
+	–	8	4 (50)
–	+	79	20 (25)
Total		168	69 (41)

Abbreviations: HPV, human papillomavirus; hrHPV, high-risk human papillomavirus; HSIL, high-grade squamous intraepithelial lesion.

[a]Other hrHPV types: 31/33/35/39/45/51/52/56/58/59/66/68.

[b]HPV-16/18–positive patients had a significantly greater probability of progressing than patients with other types or negative hrHPV at baseline (67%, 25%, and 7%; P < .001).

Table 2. Patient Characteristics According to Low-Grade Squamous Intraepithelial Lesion Outcome (n = 168)

Characteristic	LSIL Outcome		*P* Value
	Progression to HSIL (n = 69)	LSIL or Benign Epithelium (n = 99)	
Age, y, mean (range)	44 (22–65)	44 (21–73)	.9
Gender			
Male	66 (43)	88 (57)	.1
Female	3 (21)	11 (79)	
Race/Ethnicity			
White	34 (49)	38 (38)	.3
African American	12 (17)	25 (25)	
Hispanic	21 (30)	29 (29)	
Other	2 (3)	7 (7)	
Smoking history			
Current	14 (20)	20 (20)	.5
Former	20 (29)	21 (21)	
Never	35 (51)	58 (59)	
HIV status			
Infected (n = 146)	65 (45)	81 (55)	.02
Uninfected (n = 22)	4 (18)	18 (82)	
$CD4^+$ T-cell count, cells/mm^3	(n=65)	(n=81)	
<1000	57 (88)	68 (84)	.5
≥1000	8 (12)	13 (16)	
HIV RNA load, copies/mL	(n=65)	(n=81)	
<100	48 (74)	67 (83)	.2
≥100	17 (26)	14 (17)	

Data are presented as number of cases (percentage) unless otherwise indicated.

Abbreviations: HIV, human immunodeficiency virus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.

from LSIL to HSIL. Meanwhile, a small subset of patients (6%) developed HSIL at a location different from the index LSIL, suggesting either subclinical LSIL that was not visualized at the index HRA, the possibility of rapid LSIL-to-HSIL evolution, or an alternative pathway in which HSIL arises de novo without a precursor LSIL stage. Such pathways have each been observed in HPV-related cervical carcinogenesis [18]. Further investigation of these carcinogenic pathways in the context of anal lesions will expand the current understanding of the natural history of anal HPV infection, likely guiding future anal cancer screening strategies.

The risk of LSIL-to-HSIL progression conferred by HPV-16/18 was significantly greater compared to non-16/18 hrHPV types (65% vs 25%), especially in cases of persistent HPV-16/18, where the probability of HSIL development was very high (70%). Risk remained elevated even when positivity of HPV-16/18 fluctuated during surveillance. Our results indicate that HPV-16 and -18 are significant predictors of anal LSIL-to-HSIL progression, underscoring the potential clinical utility of type-specific HPV testing in the management of anal LSIL patients.

Our results are in line with a study by de Pokomandy et al, who reported that among HIV-infected MSM, the cumulative incidence of anal HSIL was 23.1% at 24 months and 36.6% at 36 months [17]. In their study, whether individually or in combination, HPV-16 and -18 were strongly associated with

Table 3. Association Between Human Papillomavirus Type 16/18 Status Over Time and Probability of Low-Grade Squamous Intraepithelial Lesion Progression

HPV-16/18 Positivity			N=168	
Status Over Time	Baseline	Follow-up	No. (%)	Progression to HSIL at Follow-up Visit, No. (%)
Persistent	+	+	54 (32)	38 (70)
Intermittent	+	−	20 (12)	10 (50)
Intermittent	−	+	9 (5)	5 (56)
Always negative	−	−	85 (51)	16 (19)

Abbreviations: HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion.

[a]The difference in probability of low-grade squamous intraepithelial lesion progression between longitudinal HPV-16/18 infection status groups was statistically significant ($P < .001$).

Table 4. Unadjusted and Adjusted Cox Proportional Hazard Regression Models Evaluating Predictors of Low-Grade Squamous Intraepithelial Lesion Outcomes

Model and Predictor	Unadjusted HR (95% CI)	*P* Value	Adjusted HR (95% CI)	*P* Value
Model 1: Baseline HPV				
Baseline HPV-16/18	3.22 (1.93–5.40)	<.001	3.25 (1.90–5.58)	<.001
Baseline non-16/18	2.05 (.82–5.09)	.12	1.72 (.67–4.41)	.26
Age, y				
<40	Reference		Reference	
40–50	0.52 (.28–1.0)	.05	0.83 (.42–1.63)	.58
>50	1.04 (.61–1.77)	.89	1.54 (.84–2.81)	.16
Male gender	2.24 (.70–7.12)	.17	2.23 (.67–7.43)	.19
HIV	1.82 (.66–5.01)	.25	1.44 (.51–4.10)	.50
Smoking history				
Never	Reference		Reference	
Former	1.70 (.98–2.96)	.06	1.44 (.79–2.60)	.23
Current	1.36 (.72–2.53)	.34	1.18 (.62–2.23)	.62
Model 2: Longitudinal HPV-16/18 status				
Longitudinal HPV-16/18				
Persistent	5.57 (3.09–10.04)	<.001	7.00 (3.69–13.27)	<.001
Intermittent	2.14 (1.05–4.37)	.04	2.02 (.97–4.19)	.06
Always negative	Reference		Reference	
Age, y				
<40	Reference		Reference	
40–50	0.52 (.28–.99)	.05	0.88 (.44–1.77)	.72
>50	1.04 (.61–1.77)	.89	1.64 (.88–3.04)	.12
Male gender	2.24 (.70–7.12)	.17	3.23 (.96–10.84)	.06
HIV	1.82 (.66–5.01)	.25	1.72 (.60–4.89)	.31
Smoking history				
Never	Reference		Reference	
Former	1.26 (.63–2.50)	.51	1.67 (.76–2.48)	.30
Current	0.74 (.39–1.38)	.34	0.82 (.42–1.61)	.57

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; HPV, human papillomavirus; HR, hazard ratio.

progression. Burgos et al reported similar trends as ours, albeit with relatively lower risks: 26% of their HPV-16/18–positive patients progressed within 2 years vs 12.8% of those with non-16/18 types [19]. While our cohort exclusively comprised biopsy-proven LSIL patients at baseline, theirs mainly comprised patients with normal cytology and HRA, thereby representing a lower-risk group. Differences in patient cohort notwithstanding, we reached similar conclusions in that the risk of progression for HIV-infected patients with anal HPV infection is primarily determined by the presence or absence of HPV-16/18.

The utility of HPV testing in anal cancer screening is still in question owing to the high prevalence of HPV in populations at risk [20]. As 91% of our patients were positive for hrHPV, we share the consensus that pooled hrHPV testing is of limited value in managing anal LSIL patients [21, 22]. Among all hrHPV types, HPV-16/18 constituted 44% of our cases, consistent with the prevalence reported by a meta-analysis of anal LSIL cases (55% for HIV-infected and 38% for HIV-uninfected men [23]). Importantly, 70% of patients who later progressed in our cohort were positive for HPV-16/18 at baseline. In other words, testing for HPV-16/18 identified two-thirds of patients who later progressed. As with co-testing in cervical cancer screening, anal swab samples can be used for cytology and HPV genotyping simultaneously, providing critical prognostic information that may guide subsequent surveillance [24].

All patients in our cohort underwent HPV genotyping at least twice, permitting us to further correlate progression risk with HPV-16/18 status over time. On consecutive anal cytology samples, type-specific HPV infection may be intermittent (ie, change from positive to negative or vice versa). Among our patients with HPV-16/18 at baseline, most persisted whereas a subset converted to negative upon follow-up. Though progression was most frequent for patients with persistent HPV-16/18 (70%), the probability for intermittently positive HPV-16/18 subjects was lower but still elevated (52%), suggesting that this is still a relatively high-risk group.

From a clinical perspective, we should be cautious in drawing the conclusion that a change from positive HPV-16/18 to negative indicates clearance of infection and therefore requires less surveillance. First, one must exclude false-negative results related to inadequate anal cytology samples or technical issues in DNA amplification [25, 26]. Second, studies on the HPV life

cycle suggest that intermittent positivity often corresponds with a state of viral latency or low viral load hovering close to the detection threshold, and thus may not indicate true clearance [27, 28]. When the host's immune system weakens, latent HPV can reactivate and initiate disease progression, a phenomenon that often occurs in cervical cancer cases among elderly or HIV-infected women [29, 30].

HPV latency and reactivation have not been explored in anal carcinogenesis. Immunocompromised HIV-infected MSM are likewise at increased risk for reactivation of latent HPV or, more commonly, for acquisition of new infection through repeat exposure [31]. Our findings suggest that the general understanding of what constitutes elevated risk of progression—namely, persistent HPV-16/18 positivity—should be expanded to include intermittent HPV-16/18 positivity, as this carries a comparable progression risk [32]. HIV-infected patients with single-point or short-term negative HPV-16/18 results might benefit from additional HPV genotyping and continuing surveillance. We believe that a large-scale study pertaining to the HPV life cycle in the anal microenvironment will grant a better understanding of this complex phenomenon and its clinical implications.

Our study benefited from a relatively large cohort of patients that included both HIV-infected and uninfected patients with longitudinal anal dysplasia surveillance and serial HPV testing. It was limited by its retrospective approach and by the clinical nature of data collection that generally is not as uniform and rigorous compared with prospective studies. Nonetheless, our study provides evidence that a significant portion of patients with LSIL progress to HSIL, especially when HPV-16/18 is present. Therefore, HPV genotyping may have an important application in anal cancer prevention, similar to its role in cervical cancer screening.

In summary, HPV genotypes are significant predictors of anal LSIL-to-HSIL progression, underscoring the clinical relevance of HPV genotyping in the management of anal LSIL patients. For those harboring HPV-16/18, whether persistent or intermittent, careful surveillance is particularly warranted. The incorporation of type-specific HPV testing into current anal cancer screening algorithms may enhance risk assessment and provide valuable prognostic information that could guide subsequent surveillance.

Notes

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