

Evolution of Untreated Anal High-Grade Squamous Intraepithelial Lesions in High-Risk Men: A Prospective 5-Year Clinical Follow-up in the ANRS-EP57-APACHES Study

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Background. Histologically confirmed anal high-grade squamous intraepithelial lesions (hHSILs) are targets for diagnosis and treatment in anal cancer screening programs. However, the long-term natural history of untreated hHSILs remains poorly understood.

Methods. In the APACHES study, 513 human immunodeficiency virus (HIV)-positive men who have sex with men and were aged ≥ 35 years underwent 3 annual visits for anal cytology, human papillomavirus (HPV) testing, and high-resolution anoscopy with biopsies to detect hHSILs. hHSILs were not treated but were monitored every 6 months for up to 5 years. Methylation markers, *ASCL1* and *ZNF582*, were assessed in biopsy specimens at hHSIL diagnosis. The hHSIL regression probability was estimated using Cox proportional hazards models, adjusted hazard ratios, and Kaplan-Meier survival curves.

Results. Of 127 untreated hHSILs, 21% regressed within 1 year, 41% within 2 years, and 75% within 5 years. None progressed to cancer during follow-up. Regression was significantly less likely for hHSILs with concurrent high-grade findings at anal cytology (adjusted hazard ratio, 0.41 [95% confidence interval, .19–.88]), 12-month persistent HPV-16 infection in anal swab samples (0.46 [.21–.97]), or positivity for both *ASCL1* and *ZNF582* (0.39 [.18–.82]). The 5-year regression probabilities ranged from approximately 30% for *ASCL1/ZNF582*-positive hHSILs with high-grade cytology, to approximately 90% for *ASCL1/ZNF582*-negative hHSILs without high-grade cytology. hHSIL regression was not influenced by age, smoking, or HIV-related markers.

Conclusions. Only one-quarter of untreated hHSILs persisted at 5 years, representing lesions with the highest anal cancer risk. The methylation markers *ASCL1/ZNF582* were the strongest predictors of persistent hHSIL and, combined with HPV testing and cytology, may inform prioritization for diagnosis and treatment in anal cancer screening programs.

Keywords. HIV; HPV; host methylation markers; anal cancer; screening.

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Anal cancer screening is increasingly recommended for high-risk groups [1, 2], most notably persons living with human immunodeficiency virus (HIV; PLHIV), among whom men who have sex with men (MSM) and are living with HIV (MSM-LHIV) have the highest anal cancer incidence rates, reaching >100 cases per 100 000 person-years [3]. Human papillomavirus (HPV) infection is considered the necessary cause of anal squamous cell carcinoma [4, 5], with HPV-16 responsible for approximately 70% of cases in PLHIV [6].

Following primary screening using HPV and/or anal cytology, the key element of anal cancer screening is diagnosis,

followed by treatment, of histologically confirmed anal high-grade squamous intraepithelial lesions (hHSILs) under the guidance of high-resolution anoscopy (HRA) [7]. This approach has been given huge impetus by the Anal Cancer HSIL Outcomes Research (ANCHOR) trial, which established that treatment of HRA-diagnosed hHSILs can prevent anal cancer [8].

However, little is known about the natural history of hHSILs, which can be detected in the majority of PLHIV [8] and so are clearly not all true precancers [9] (ie, lesions with potential to progress to invasive cancer, as opposed to those that regress or remain indolent). One previous study reported on the 3-year natural history of untreated composite anal high-grade squamous intraepithelial lesions (HSILs; diagnosed by means of cytology and/or histology) in MSM [10], but, to our knowledge, no study has ever reported on long-term regression of hHSILs at the lesion level.

Understanding how many, and which, hHSILs will regress, versus those that will persist and progress to anal cancer, is key to improving the efficiency of anal cancer screening programs, which are in their public health infancy. This is because HRA is costly, difficult to perform, and burdensome for patients, requires medical expertise that is sometimes lacking, and therefore should be reserved only for those at the highest cancer risk [11–13]. Methylation markers, *ASCL1* and *ZNF582*, have been shown to be particularly promising in identifying the subset of hHSILs that are true anal cancer precursors [14–17].

The ANRS-EP57 APACHES study was designed to describe and evaluate the determinants and long-term regression/persistence of hHSILs over 5 years in MSM-LHIV. Long-term hHSIL persistence can be considered as a surrogate for anal cancer risk and is therefore a useful benchmark to identify markers for improving the efficiency of anal cancer screening programs, by (1) avoiding unnecessary overtreatment of hHSILs and (2) increasing screening capacity by reserving limited HRA only for those at highest anal cancer risk.

METHODS

Study Participants

The ANRS-EP57-APACHES study recruited MSM-LHIV aged ≥ 35 years between December 2014 and June 2016 in 6 French teaching hospitals (in Paris, Lyon, Rennes, Dijon, Marseille, and Montpellier). Participants underwent 3 annual visits (at 0, 12, and 24 months) with digital anal-rectal examination, anal cytology, HPV testing, and HRA with lesion-directed biopsies, as described elsewhere [11, 18–20]. Exclusion criteria were anal cancer history and hHSIL treated in the preceding year. MSM-LHIV with cytologically confirmed HSILs (cHSILs) or hHSILs (anal intraepithelial neoplasia grade 2 [AIN2] or grade 3 [AIN3]) detected by means of HRA-directed biopsies during

1 of the 3 annual visits were not treated (per protocol) but were monitored every 6 months with anal cytology and HRA, for up to a maximum of 72 months (median, 36 months [interquartile range, 19–56 months]), until June 2021.

Data and Sample Collection

A questionnaire was completed, including history of tobacco use, sexual habits, and previous proctological examination or treatment. HIV-related data were retrieved from medical records. At visits 0, 12, and 24 months, 2 anal cytological samples were collected using a swab (Rovers Anex brush) rinsed in PreservCyt medium (Hologic): 1 for cytology and 1 for HPV testing. At all other 6-monthly follow-up visits, a swab sample was taken for anal cytology only. Cytological samples were read by local cytopathologists and classified using revised Bethesda 2001 classification and LAST criteria, and cytological samples that did not meet the cellularity criteria were classified as unsatisfactory. Histology slides underwent blinded panel consensus review to establish the final hHSIL diagnosis.

HPV DNA and Methylation Marker Testing

Anal swab samples were tested for HPV DNA using the Cobas 4800 system (Roche Molecular Systems), providing genotyping for HPV-16, HPV-18, and other high-risk HPV types aggregated (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). hHSILs were tested for methylation markers *ASCL1* and *ZNF582* from formalin-fixed paraffin-embedded (FFPE) blocks of biopsy specimens obtained when the hHSIL was first diagnosed, in the Department of Pathology at the Amsterdam University Medical Center, according to a well-validated procedure [16]. In brief, whole tissue sections of FFPE tissue blocks containing hHSILs were sectioned according to the sandwich method, in which the first and last sections were stained for histological confirmation, and in-between sections were collected for DNA isolation using the QIAamp DNA FFPE tissue kit (Qiagen). For methylation analyses, isolated DNA was bisulfite converted using the EZ DNA Methylation Kit (Zymo Research), and bisulfite-converted sample DNA was analyzed for *ASCL1* and *ZNF582* methylation using a multiplex quantitative methylation-specific polymerase chain reaction assay, which also targeted the reference gene, β -actin (ACTB) [16]. Cycle threshold (Ct) values were measured at fixed thresholds for fluorescence. A Ct value of <32 for ACTB indicated sufficient DNA and DNA quality and adequate bisulfite conversion. $\Delta\Delta$ Ct ratios were computed using the comparative Ct method by comparing the target Ct values with the Ct values of ACTB and the internal quality control calibrator ($2 - \Delta\Delta$ Ct $\times 100$). Samples with a valid ACTB Ct (Ct <32) and no signal for *ZNF582* or *ASCL1* were considered negative for methylation. The DNA isolated from hHSIL biopsy specimens were also tested for HPV DNA using the QIAscreen test (Qiagen), providing genotyping for HPV-16, HPV-18, and other high-risk HPV

types aggregated (types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 67, and/or 68).

hHSIL Regression/Persistence End Point

The main outcome was regression of the hHSIL (which mirrors hHSIL persistence), monitored at the individual lesion level. At each 6-monthly follow-up HRA, the HRA practitioner reidentified, as far as possible, previously diagnosed lesions using images and cartography recorded at previous visits. hHSILs were defined as regressed (at the level of the lesion, not the patient) if either (1) the biopsy-proven histological grade of the same lesion was less than hHSIL or (2) there was visual disappearance of the lesion (after application of acetic acid and Lugol's iodine). Lesion years of follow-up in analyses were calculated from date of hHSIL diagnosis to the date of hHSIL regression and/or right censoring (see below).

Statistical Analyses

hHSIL regression probability over time was estimated overall or stratified by significant determinants, using Kaplan-Meier survival curves truncated at 5 years after hHSIL diagnosis. Determinants of hHSIL regression were expressed as hazard ratios (HRs) and corresponding 95% confidence intervals (CIs), assessed using Cox proportional hazards models, at an individual lesion level, with hierarchical clustering of hHSIL lesions from the same visits and participants. HPV-16 persistence was assessed between the visit at which hHSIL was diagnosed and the visit 12 months later (and so was available only for those hHSILs detected at visits at 0, 6 or 12 months). The incidence of anal cancer was calculated at a person-year level.

RESULTS

Among 513 MSM-LHIV recruited in the APACHES study, 91 individuals were diagnosed with ≥ 1 hHSIL during the 2 years of follow-up and were eligible for per-protocol monitoring every 6 months. Their median age was 50 years (interquartile range, 46–56 years) and $>99\%$ were on combination antiretroviral therapy.

A total of 175 individual hHSILs were diagnosed during follow-up in the 91 MSM-LHIV with hHSILs. After exclusion of 29 hHSILs without any follow-up after hHSIL diagnosis and 19 hHSILs without a valid methylation result (16 with missing FFPE block and 3 with an insufficient methylation test result from the FFPE block), a total of 127 untreated hHSILs from 61 MSM-LHIV (contributing a total of 243 lesion-years) were included in the analytical dataset (Figure 1). Of the 127 lesions, 66 were observed to regress (33 by biopsy-proven histological grade less than hHSIL and 33 by disappearance on HRA), and others were right censored if the participant left the study early ($n = 10$), in the rare event that the hHSIL was treated outside of protocol ($n = 5$), or at the last visit before the study end in June 2021 ($n = 41$), whichever was earliest.

Of the 61 MSM-LHIV included, 26 had a single hHSIL, 14 had 2, 11 had 3, and 10 had ≥ 4 individual hHSILs diagnosed during their follow-up. hHSILs were newly diagnosed at month 0 ($n = 37$), during annual visits for all MSM-LHIV (28 at month 12 and 20 at month 24), or at 6-monthly follow-up visits in MSM-LHIV with a previous cHSIL or hHSIL (7 at 6 months, 9 at 18 months, 7 at 30 months, 6 at 36 months, 1 at 42 months, and 11 at ≥ 48 months). No anal cancers were diagnosed during the 300 person-years of follow-up in the 61 MSM-LHIV (nor in any

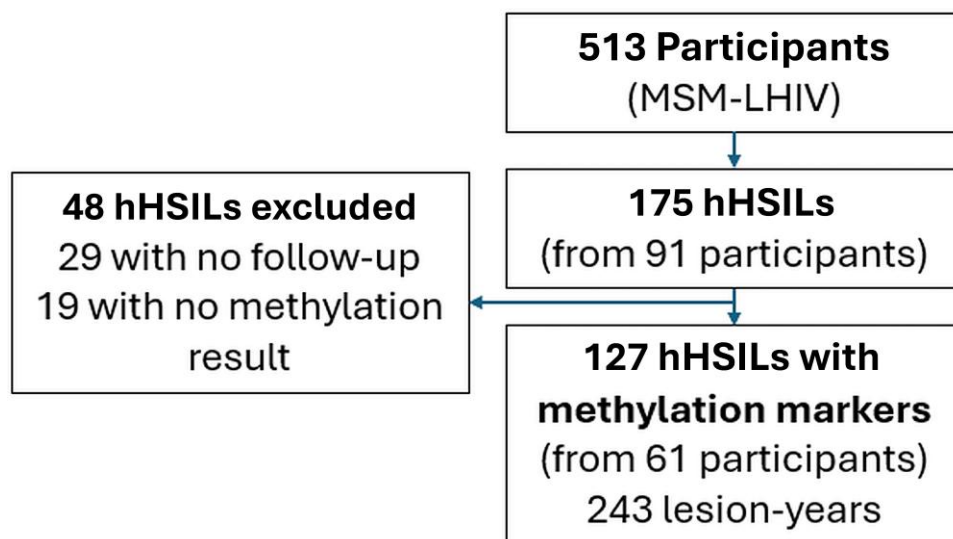


Figure 1. Flow chart of study population. Abbreviations: hHSILs, histologically confirmed anal high-grade squamous intraepithelial lesions; MSM-LHIV, men who have sex with men and are living with human immunodeficiency virus.

of the original 513 MSM-LHIV in the ANRS-EP57-APACHES study, irrespective of hHSIL).

Of 127 untreated hHSILs, an estimated 21% regressed by 1 year, 41% by 2 years and 75% by 5 years (Figure 2). Regression did not vary significantly by age, smoking, sexual behavior, or HIV-related markers (Table 1). Confirmed AIN3 lesions were significantly less likely than AIN2 lesions to regress (crude HR, 0.48 [95% CI, .26–.88]), but this difference lost statistical significance in the adjusted model (adjusted HR [aHR], 0.51 [.22–1.13]). The largest hHSILs tended to regress less, but this effect did not reach statistical significance (aHR, .46 [95% CI, .15–1.40]). hHSILs with concurrent cHSILs from anal swab samples were significantly less likely to regress in both crude (HR, 0.59 [.35–.99]) and adjusted (aHR, 0.41 [.19–.88]) models (Table 1). No significant differences were observed between HPV status at the time of hHSIL diagnosis and hHSIL regression, neither from anal swab samples, nor from biopsy specimens. However, regression was significantly less likely in the presence of persistent HPV-16 between the first and subsequent visits at 12 months, in both the crude (HR, 0.46 [95% CI, .23–.93]) and the adjusted (aHR, 0.46 [.21–.98]) models (Table 1).

Methylation markers in biopsy specimens were the strongest predictors of hHSIL regression/persistence, with adjusted HRs of 0.36 (95% CI, .17–.74) for *ASCL1*-positive hHSILs, 0.38 (.23–.64) for *ZNF582*-positive hHSILs, and 0.39 (.18–.82) for hHSILs positive for both *ASCL1* and *ZNF582* combined.

The distribution of *ASCL1/ZNF582* according to other significant predictors is shown in Supplementary Table 1 (eg, 24 of 39 hHSILs with persistent HPV-16 were positive for both *ASCL1* and *ZNF582*, of which only 8 [20.5%] regressed).

The probability of hHSIL regression over time, up to 5 years, according to independently significant determinants (methylation markers and anal cytology) is displayed alone and in combination in Figure 3A–3C. The highest regression probability was observed for hHSILs both without *ASCL1/ZNF582* methylation and without HSILs at anal cytology (approximately 90% regression at 5 years), and the lowest regression probability was observed for hHSILs positive for both *ASCL1/ZNF582* methylation and HSILs at anal cytology (approximately 30% regression at 5 years) or for HSILs both positive for *ASCL1/ZNF582* and with persistent HPV16 infection (approximately 30% regression at 5 years). Persistent HPV-16 status, although a statistically significant determinant of hHSIL regression, was available only for a subset of hHSILs, and strata were too few to be meaningfully described using Kaplan-Meier curves.

DISCUSSION

This first description of the long-term evolution of untreated anal hHSIL showed that 75% of hHSILs will regress by 5 years. The strongest predictors of hHSIL regression were absence of the methylation markers *ASCL1* and *ZNF582*, which predicted persistent hHSIL better than anal cytology alone.

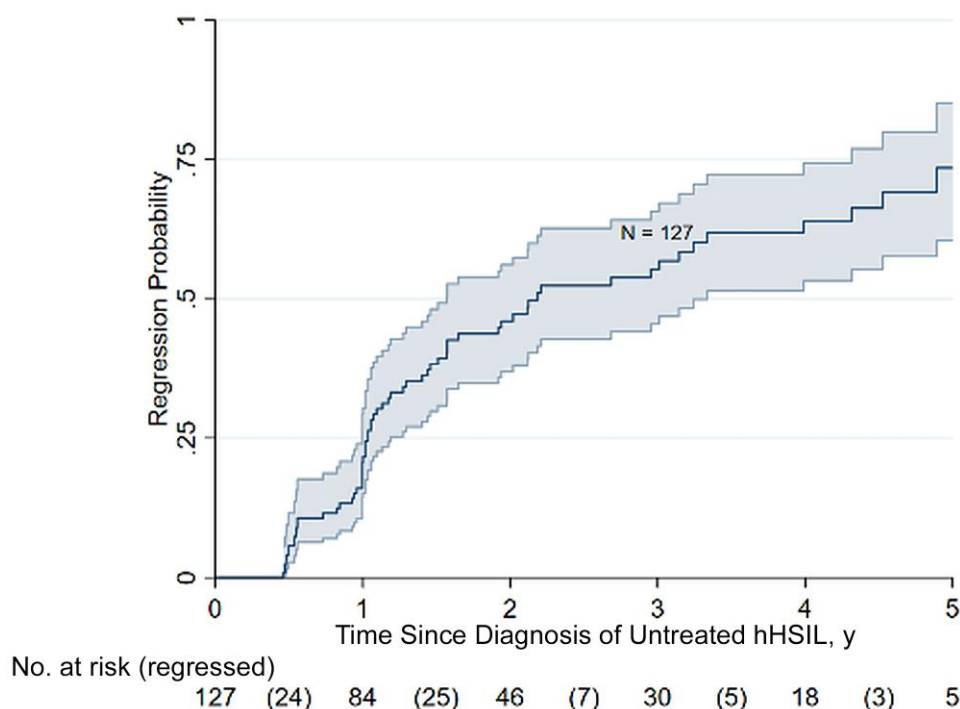


Figure 2. Regression probability in 127 untreated histologically confirmed anal high-grade squamous intraepithelial lesions (hHSILs) over time.

Table 1. Determinants of Regression Among 127 Untreated Histologically Confirmed Anal High-Grade Squamous Intraepithelial Lesions

Characteristic	Category	Total hHSILs, No.	hHSILs Regressed		aHR (95% CI) ^{a,b}
			No. (%)	Crude HR (95% CI) ^a	
Patient-level variables					
Age group	35–45 y	27	13 (48.1)	Reference	Reference
	45–55 y	58	27 (46.5)	0.84 (.34–2.05)	1.21 (.39–3.71)
	>55 y	42	26 (61.9)	0.99 (.39–2.54)	1.83 (.62–5.44)
Center	Paris	68	32 (47.1)	Reference	Reference
	Other	59	34 (57.6)	1.66 (.92–2.99)	1.75 (.79–3.85)
Smoking status	Never	33	19 (57.6)	Reference	Reference
	Ever	94	47 (50.0)	0.92 (.50–1.68)	0.93 (.38–2.26)
No. of sexual partners in last 12 mo	0	22	12 (54.5)	Reference	Reference
	1–4	53	26 (49.1)	1.07 (.42–2.69)	0.61 (.23–1.63)
	≥5	52	28 (53.8)	1.23 (.61–3.08)	0.54 (.20–1.45)
Years since HIV diagnosis	<15 y	49	23 (46.9)	Reference	Reference
	>15 y	75	40 (53.3)	1.04 (.53–2.01)	1.81 (.96–3.42)
Nadir CD4 cell count	<200/μL	39	8 (47.1)	Reference	Reference
	200–350/μL	30	46 (53.5)	1.07 (.51–2.25)	1.05 (.38–2.88)
	>350/μL	54	9 (45.0)	0.77 (.40–1.47)	1.11 (.45–2.67)
hHSIL-level variables					
Visit of hHSIL diagnosis	Prevalent (0 or 6 mo ^c)	44	31 (70.4)	Reference	Reference
	Incident (≥12 mo)	83	35 (42.2)	0.77 (.46–1.29)	0.86 (.40–1.84)
hHSIL size ^d	Small (1 clockface hours)	91	48 (52.7)	Reference	Reference
	Medium (2 clockface hours)	24	12 (50.0)	0.97 (.41–2.27)	1.25 (.54–2.86)
	Large (≥3 clockface hours)	12	6 (50.0)	0.56 (.29–1.06)	0.46 (.15–1.40)
hHSIL histology	AIN3 ^e	33	13 (39.4)	0.48 (.26–.88) ^f	0.51 (.22–1.13)
	AIN2 ^g	94	53 (56.4)	Reference	Reference
Anal cytology at hHSIL diagnosis	cHSIL	71	29 (40.9)	0.59 (.35–.99) ^f	0.41 (.19–.88) ^f
	ASCUS/cLSIL	44	29 (65.9)	Reference	Reference
	Normal	5	2 (40.0)	0.67 (.16–2.85)	1.61 (.55–4.71)
HPV, from anal swab sample at hHSIL diagnosis	Negative	2	1 (50.0)	1.19 (.27–5.21)	0.62 (.08–5.04) ^h
	Other high-risk only	36	23 (63.9)	Reference	Reference
	HPV-16	66	31 (47.0)	0.72 (.36–1.40)	0.72 (.41–1.29) ^h
HPV-16 persistence in anal swab sample (between hHSIL diagnosis and visit 12 mo later) ⁱ	Yes	39	16 (41.0)	0.46 (.23–.93) ^f	0.46 (.21–.98) ^f
	No	28	21 (75.0)	Reference	Reference
HPV, from hHSIL biopsy specimen	Negative	6	5 (83.3)	1.51 (.66–3.45)	1.65 (.61–4.44) ^h
	Other high-risk only	59	31 (52.5)	Reference	Reference
	HPV-16	55	26 (47.3)	0.94 (.53–1.66)	1.15 (.66–1.99) ^h
Methylation markers in hHSIL biopsy specimens					
ASCL1	Negative	48	31 (64.6)	Reference	Reference
	Positive	79	35 (44.3)	0.39 (.21–.71) ^f	0.36 (.17–.74) ^f
ZNF582	Negative	55	38 (69.1)	Reference	Reference
	Positive	72	28 (38.9)	0.40 (.23–.69) ^f	0.38 (.23–.64) ^f
Both ASCL1 and ZNF582	Negative	63	43 (68.2)	Reference	Reference
	Positive	64	23 (35.9)	0.34 (.20–.60) ^f	0.39 (.18–.82) ^f

Abbreviations: aHR, adjusted hazard ratio; AIN2, anal intraepithelial neoplasia grade 2; AIN3, anal intraepithelial neoplasia grade 3; ASCUS, atypical squamous cells of undetermined significance; cHSIL, cytologically confirmed anal high-grade squamous intraepithelial lesion; CI, confidence interval; cLSIL, cytologically confirmed low-grade squamous intraepithelial lesion; hHSIL, histologically confirmed anal high-grade squamous intraepithelial lesion; HPV, human papillomavirus; HR, hazard ratio.

^aAdjusted for significant variables in the multivariate models—hHSIL histology, hHSIL cytology, HPV-16 persistence, and methylation markers (both *ASCL1* and *ZNF582*).

^bClustered by ID.

^chHSILs diagnosed at 6 months are considered prevalent as the 6-month visit often is provoked by HSIL cytology (but normal high-resolution anoscopy [HRA] findings) at month 0.

^dIn HRA examinations in France, lesions are localized using a clockface system. Accordingly, lesion size in our study was recorded as circumferential extent, defined by the number of clockface hours involved.

^eAIN3 was defined as AIN3 diagnosed by the APACHES consensus histological review committee and confirmed as AIN3 in a biopsy specimen tested for methylation markers/HPV.

^fSignificant at $P < .05$.

^gAIN2 was defined as AIN2 diagnosed by the APACHES consensus histological review committee or ≤AIN2 in the biopsy specimen tested for methylation markers/HPV.

^hNot adjusted for HPV-16 persistence, due to colinearity. Data were missing for nadir CD4 cell count for 4 hHSILs, for time since testing human immunodeficiency virus positive for 3, for anal cytology at hHSIL diagnosis for 7, for HPV from anal cytology at hHSIL diagnosis for 13, for HPV-16 persistence for 60, and for HPV at hHSIL biopsy for 7.

ⁱAvailable only for hHSILs diagnosed by month 12.

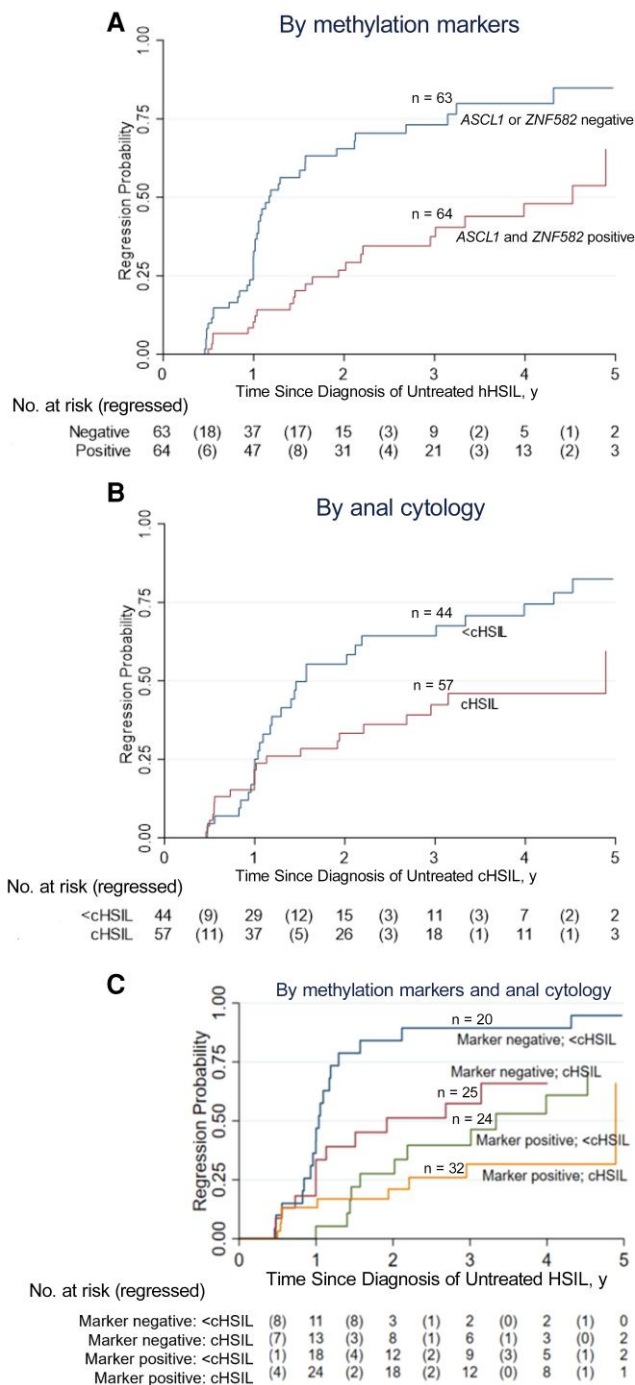


Figure 3. Regression probability in 127 untreated histologically confirmed anal high-grade squamous intraepithelial lesions (hHSILs) over time, stratified by methylation markers (A), anal cytology result (B), and their combination (C). Abbreviation: cHSIL, cytologically confirmed anal high-grade squamous intraepithelial lesion; <cHSIL, cytological grade less than cHSIL; HSIL, high-grade squamous intraepithelial lesion.

There are surprisingly few data on regression of untreated hHSILs with which to compare our results. The SPANC study reported high rates of untreated anal HSIL clearance over 3

years in 600 MSM, based on composite anal HSIL regression (diagnosed by means of histology and/or cytology) at a person, not lesion, level [10]. The ongoing MARINE study is monitoring 200 untreated hHSILs in MSM-LHIV for 2 years [21] and is more similar in design to APACHES, but its results are not yet available. The ANCHOR trial reported on anal cancer in the hHSIL monitoring arm (average follow-up, 27 months [8]) but has not reported on the probability, or determinants, of hHSIL regression. Our short-term estimate of 21% regression at 1 year, however, can be compared with the 28% reported in 60 PLHIV with hHSIL in the untreated control arm of a treatment trial [22].

hHSILs with concomitant cHSILs in anal swab samples were less likely to regress, corroborating a retrospective study showing that cHSILs are predictive for progression of hHSILs to anal cancer [14]. This suggests that hHSIL detected after a normal, or even an atypical squamous cells of undetermined significance/low-grade squamous intraepithelial lesion diagnosis on cytology, are less likely to represent true precancerous lesions and are lower priority for detection and treatment, corroborating its recommended use in triage for HRA [23]. Our data also suggested higher regression of AIN2 than of AIN3, consistent with previous data [10].

Larger hHSILs tended to be less likely to regress [10], consistent with the observation that larger untreated hHSILs have the greatest potential to progress to anal cancer [8], but numbers were too small to show a significant effect. Given that our rate of hHSIL detection in APACHES (approximately 22%) was only about half that in some other studies of MSM-LHIV (eg, >50% [8, 10]), we may have underdetected the smallest hHSILs (ie, those most likely to regress). It is also possible that biopsying the smallest lesions contributed to their regression.

ASCL1 and *ZNF582* were the strongest predictors of long-term hHSIL regression, stronger than anal cytology, establishing their potential to improve risk anal cancer risk stratification. These findings are consistent with other ad hoc evidence from anal cancer. In 10 anal cancer cases, hypermethylation of *ASCL1* and *ZNF582* was confirmed in all biopsy specimens collected before cancer diagnosis [16], and higher levels of *ASCL1* and *ZNF582* in anal swab samples in patients with histologically confirmed AIN3 ($n = 514$) were predictive for progression to anal cancer ($n = 22$) [14]. However, we observed regression in at least a third of hHSILs that were positive for both methylation markers, highlighting that there remains room for further risk stratification.

ASCL1 and *ZNF582* can predict the presence of hHSIL, when tested from biopsy specimens [15–17, 24] but more recently also from anal swab samples [25]. Tissue specificity means that methylation markers optimized for risk stratification of CIN3 and cervical cancer appear not to be discriminant for anal hHSILs [26].

Persistent HPV-16 infection in anal swab samples predicted hHSIL persistence, as previously suggested [10, 14]. However, no significant differences in regression probability were observed between HPV-16 and other high-risk HPV types when assessed cross-sectionally (neither in biopsy specimens nor in anal swab samples). While this has not been shown in any other study of hHSIL follow-up either, it might have been expected based on the evidence for an increase in attributable fraction of HPV-16 from hHSIL to anal cancer [6].

No anal cancers were observed during follow-up of untreated hHSILs in this study. Nevertheless, this zero incidence rate (95% CI, 0–2.5 cases) is compatible with the number of anal cancer cases that would be expected based on the anal cancer incidence among untreated hHSILs in PLHIV from the ANCHOR trial (1.3 case expected for 300 person-years) [8], from a population-based linkage study of AIN3 in Denmark (0.7 case expected) [27], and from the SPANC study of MSM, among whom HSILs were not treated (0.7 case expected) [10].

There are a number of limitations to be considered. First, we did not have individual HPV genotyping to assess the persistence of all individual high-risk HPV types, so we focused only on HPV-16. Furthermore, we have previously shown strong variations in HRA practices and HSIL diagnosis (by both cytology and histology) across centers and over time in APACHES [11], which mirrors large heterogeneity in hHSIL prevalence across the scientific literature on MSM living with HIV [28]. Thus, some incident hHSIL may have been missed by HRA at earlier visits (and detection helped by previous HSIL cytology), and this may be why we could not observe expected higher regression in incident than prevalent hHSILs, as reported elsewhere [10]. Moreover, if the HRA practitioner was unable to reidentify a stained lesion, regression was based on visual disappearance of the lesion without possibility of a histology reference.

However, once hHSIL was diagnosed in APACHES, HRA practitioners were competent and highly clinically motivated to carefully monitor these individual lesions over time. Indeed, when APACHES was initiated, leaving hHSILs untreated was considered highly controversial with respect to potential anal cancer risk. For these reasons, we did not analyze composite HSIL regression (ie, based on histology and/or cytology) at the level of individual MSM-LHIV [10], also because methylation markers, the most promising determinants of hHSIL regression were assessed from biopsy specimens and hence are lesion specific. While certain patient-level and visit-level variables were correlated among hHSIL from the same patient and/or visit, we accounted for this by hierarchical clustering in our statistical model. Finally, the lack of influence of HIV-related factors on hHSIL regression, despite their being established risk factors for anal cancer, while concurring with previous evidence [10], may be due to small sample size.

This new knowledge that the vast majority of hHSILs will regress highlights how maximizing cross-sectional sensitivity for

detection of hHSILs should not be the ultimate objective for evaluating markers to stratify anal cancer risk in screening programs. Rather, a good anal cancer risk stratifier should detect only a minority of hHSILs—namely, those that are more likely to persist. Thus, it is useful to see that methylation markers, in particular, may serve as an immediate cross-sectional surrogate for future hHSIL persistence, and so may be used to prioritize hHSIL treatment versus monitoring. Combining that with assessment of lesion size is likely to improve this stratification even further.

In order to avoid unnecessary HRA, treatments, and patient anxiety, it is preferable to avoid referral and diagnosis, of hHSILs that are destined to regress. Assessments in anal swab samples, namely, the presence of HSIL cytology, and HPV-16 persistence at 1 year, appear useful for discriminating long-term persisting hHSILs and so may be used to avoid HRA referral and treatment for patients with regressing hHSILs.

There is also data building to suggest that assessment of *ASCL1* and *ZNF582* methylation markers can be shifted from biopsy specimens to anal swab samples to predict hHSILs [25] and even to predict hHSILs progressing to anal cancer [14]. Thus, the use of methylation markers in primary screening with anal swab samples has huge potential to improve the efficiency of risk stratification in anal cancer screening programs. By avoiding HRA referral to detect and treat hHSILs that are destined to regress, precious HRA expertise can be focused on those at highest risk of anal cancer, with these gains in efficiency allowing more at-risk persons to be screened and more cancers prevented.

In conclusion, we show that hHSILs frequently regress under regular HRA surveillance, with most resolving within 5 years. Methylation markers, HPV-16 persistence, and HSIL cytology were strongly associated with the absence of hHSIL regression (ie, persistence) and may guide risk stratification and management protocols.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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