Evaluating the Association between Smoking Behaviors and Anal or Vulvar High-Grade Squamous Intraepithelial Lesion (HSIL) Recurrence

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Abstract

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Introduction: HPV-induced high-grade squamous intraepithelial lesions (HSILs) of the anus and vulva have high rates of recurrence after treatment. While many studies have demonstrated the positive association between smoking and primary lesion development, research on its association with anal and vulvar HSIL recurrence is limited.

Methods: We used data from a randomized controlled trial assessing the impact of HPV vaccination on the risk of anal and vulvar HSIL recurrence to design a longitudinal study with smoking as the primary exposure. To assess HSIL recurrence, participants were established at baseline screening as HSIL-negative and biopsies during the study were used to diagnose new HSIL. Participants were categorized as never, former, and current smokers at enrollment, and unadjusted and adjusted Cox-Proportional Hazard models were constructed to compare risk of HSIL recurrence by smoking status. Additional smoking variables were investigated to evaluate the risk of HSIL recurrence among regular smokers.

Results: During the study, there were 26 recurrences of anal HSIL and 11 recurrences of vulvar HSIL among the 188 enrolled participants. Current smokers accounted for 19.7% of the

participants, 18.1% former smokers, and 61.7% never smokers. Compared to never smokers, current smokers had 60% increased risk of HSIL recurrence (aHR: 1.60, 95% CI: 1.02, 2.52), and the risk estimate was higher in analyses limited to anal HSIL participants (aHR: 2.73, 95% CI: 1.37, 5.43). Former smokers did not have increased risk of overall HSIL recurrence, compared to never smokers (aHR: 0.91, 95% CI: 0.58, 1.41). Smoking risks were further elevated among participants who reported higher numbers of cigarettes smoked per day and longer duration of lifetime smoking. Among ever smokers, smoking more than 20 cigarettes per day increased risk of overall HSIL recurrence, compared to smoking less than 10 cigarettes per day (aHR: 1.37, 95% CI: 0.51, 3.71), but this increase was not statistically significant. Comparing participants who smoked between 3-14 years, those who smoked between 15-24 and 25-34 years did not have increased risk of HSIL recurrence, but those who smoked between 35-45 years had a marginally elevated risk of overall HSIL recurrence (aHR: 2.32, 95% CI: 1.00, 5.36). Among anal HSIL participants, compared to smoking 3-14 years, smoking for more than 14 years significantly increased risk of HSIL recurrence (15-24 years aHR: 3.48, 95% CI: 0.95, 12.67; 25-34 years aHR: 4.79, 95% CI: 1.13, 20.34; 35-45 years aHR: 8.00, 95% CI: 1.61, 39.77). Increased total years of smoking was found to be associated with increased risk of anal HSIL recurrence ($p = 0.05$) but not vulvar HSIL recurrence ($p = 0.38$).

Conclusions: Current smoking and total smoking years elevate the risk of anal and vulvar HSIL recurrence to varying degrees. Smoking is a modifiable risk and thus cessation should be highly encouraged, especially at primary lesion diagnosis.

Introduction

Human papillomavirus (HPV) is the highest incident sexually transmitted infection in the United States, with estimates that over 85% of US adults will acquire HPV by the age of 45[1]. Persistent HPV infection can become high-grade squamous intraepithelial lesions (HSIL) in a variety of tissues. Common sites of HSIL include the cervix, the oropharynx, and the lower genital tract, consisting of the vagina, vulva, and penis, and the anus[2]. Undetected or incompletely treated HSIL can progress to cancer, but studies have demonstrated the impact of therapeutic procedures in preventing this progression[3]. In contrast to cervical HSIL, which is routinely screened for by Pap or HPV tests, anal (AIN 2/3) and vulvar (VIN 2/3) HSILs are detected opportunistically. Data from the Surveillance, Epidemiology, and End Results (SEER) Program indicate modest (0.4%) to high (7.1%) increases in vulvar and anal HSIL annual incidence between 2000-2015[4]. In the United States in 2019, the incidence of anal cancer was 2.0 per 100,000 persons and the incidence of vulvar cancer was 2.3 per 100,000 women[4]. Between 1996-2012, the incidence of anal cancer in men who have sex with men (MSM) with HIV was 89.0 per 100,000 men[5].

The recurrence rates for anal and vulvar HSIL are high, ranging from 23-79% and highest among persons with HIV (PLWH) [6–20]. Prior studies of anal and vulvar HSIL recurrence overtime are summarized in Table 1. Studies of anal HSIL recurrence commonly followed at-risk populations such as PLWH and MSM for up to 3 years and found high (23-79%) recurrence rates (Table 1). On the other hand, studies of vulvar HSIL recurrence followed populations between 3 and 5 years and had 19-48% recurrence (Table 1). In these studies, recurrence is defined as the detection of a new HSIL of the same anatomical site (anal or vulvar) with histologic confirmation[6]. Recurrence places a substantial burden on patients to finance and undergo repeated surgery or surgeries, risking both functional impairments and compromised tissue appearance[6,7,19]. Recurrence also increases the chances of transformation into cancer[7].

Understanding risk factors for HSIL recurrence is crucial to early detection and management to prevent progression to malignancy.

In addition to the central role of HPV in HSIL development, there has been extensive research conducted on secondary risk factors, with an emphasis on smoking and immunosuppressive conditions[12,21–23]. It is hypothesized that tobacco smoke has a negative impact on the immune system's ability to clear an infection. Prior studies have demonstrated an association between tobacco smoke and a decrease in natural-killer cell activity, inflammation, and HPV antibody production[24–26]. Smoking may also play a role in increasing the vulnerability of cells to HPV infection and persistence[27,28]. Furthermore, *in vitro* studies have demonstrated the ability of byproducts of cigarette smoke to transform cells immortalized by HPV 16, a high-risk HPV genotype, activating their cancerous potential[29]. Epidemiologic studies have shown that current smokers are at an increased risk of developing anal and vulvar HSIL, and this risk is further augmented in the form of a dose-response association with number of cigarettes smoked per day[21,30,31].

The data on HSIL recurrence are limited, but potential factors correlated with risk of recurrence include HIV infection, smoking status, treatment modality of primary lesion (comparing surgical excision, ablation, and topical therapies), primary lesion size, and HPV antibody levels. PLWH are at a higher risk of developing anal HSIL. Smokers with HIV with a history of anal HSIL had a 70% increased risk of HSIL recurrence compared to PLWH who have never smoked (HR: 1.7, 95% CI: 1.1-2.4)[13]. Likewise, among women with vulvar HSIL, the odds of HSIL recurrence between current smokers and non-smokers was 1.61 (95% CI: 1.02, 2.55)[16].

Measures of smoking habits are often collected via self-reported surveys either administered by clinicians or the participant themselves. Researchers have questioned the accuracy of self-reporting compared to measuring cotinine levels in blood or saliva samples. On one hand, many studies have validated self-reporting, citing high sensitivity and specificity when compared to cotinine detection[32]. Additionally, high levels of second-hand smoke can produce

measurable levels of cotinine in saliva samples up to 5 days from exposure[33]. However, some reports have demonstrated possible mismeasurement from self-reporting, namely underestimating smoking prevalence[34,35]. To assess the accuracy of clinician reports versus self-reports, Convill et al. demonstrated that, among current smokers, clinician-reported smoking status was highly discordant with self-reported smoking status[36]. This trend was not as significant among former or never smokers[36]. These studies indicate both advantages of self-reported smoking habits, as a widely accepted approach with reduced second-hand smoke interference, and potential drawbacks.

In this study, we aimed to contribute to the data on the impacts of smoking and risk of anal and vulvar HSIL recurrence with respect to age, HIV status, time since primary lesion diagnosis, and type of medical insurance. If we confirm that smoking plays a role in HSIL recurrence, as it does in primary lesion development, further health messaging needs to be conveyed to persons with a history of anal and vulvar HSIL. In particular, it will be important to highlight that smoking is a modifiable risk factor for recurrence.

Methods

Study Design

We conducted a longitudinal study evaluating the association between smoking status at study enrollment and risk of anal or vulvar HSIL recurrence. The data came from a randomized controlled trial on the impact of HPV vaccination on anal and vulvar HSIL recurrence, entitled the “HPV Vaccine to Interrupt Progression of Vulvar and Anal Neoplasia” or the VIVA study[6].

Study Setting

The study took place at two locations, the University of Washington Virology Research Clinic in Seattle, WA, and the Gynecologic Oncology clinic associated with the University of Alabama at Birmingham in Birmingham, AL. Participants were seen in the clinic for initial

appointments of screening and vaccination with vaccine (Gardasil, 9-valent HPV vaccine) or placebo, and then attended screening follow-up visits over 36 months. Other visits were survey-based and were conducted remotely.

Study Subjects

Participants for this study were identified through the local SEER database or through physician referral. Eligibility criteria included men or women aged 27-69 at enrollment with a history of anal or vulvar HSIL diagnosed on or after 1/1/2014. If a participant had a history of HSIL at both sites, they were categorized based on the lesion with the most recent treatment. Additional criteria included at least 2 months since last therapy for HSIL and no clinical evidence of HSIL during the screening evaluation. Sexually active women of child-bearing potential agreed to use an effective contraceptive for the first 7 months of the study, and PLWH must have taken anti-retroviral therapy for at least 6 months prior to study enrollment. Participants were excluded if they were currently pregnant, were receiving chemotherapy, had a prior history of invasive anogenital or oropharyngeal cancer, had an unstable medical condition, or had previously received HPV vaccination. The study enrolled 170 participants from the Seattle site and 18 participants from the Birmingham site, for a total of 188 participants.

Data Collection

Participants had an initial screening visit, an enrollment visit and first vaccine at month 0, and then subsequent visits at months 2, 6, 7, 12, 18, 24, 36, and 42. Baseline data on smoking habits were collected at enrollment, as well as through self-administered computer-based questionnaires at months 6, 18, and 36, and telephone-based questionnaires at months 12, 24, and 42. Data were entered in a REDCap database for analysis.

Screening

The primary outcome in this study was anal and vulvar HSIL recurrence. Anoscopies and vulvoscopies were performed by expert clinical investigators on this study at baseline, 18, and 36 months. If the study clinician suspected a lesion, a biopsy was performed to confirm HSIL recurrence. Slides from biopsies taken at a study visit or between visits by a community provider were retrieved and reviewed by the study pathologist.

Exposures

The primary exposure of interest for this study was smoking status. At baseline, participants were asked to identify how long it had been since they smoked a cigarette. 'Regular' smoking is defined as smoking at least 100 cigarettes in a lifetime as well as smoking at least 1 cigarette a day for a period of time[37]. Participants were categorized as 'never' smokers if they had never smoked regularly, never smoked more than 100 cigarettes (5 packs) in their lifetime or had not smoked regularly for more than 16 years. Participants were categorized as 'former' smokers if they smoked more than 100 cigarettes in their lifetime, had a history of regular smoking, and smoked more than 1 year and less than 16 years prior to study enrollment. The remaining participants were categorized as 'current' smokers. Current smokers smoked more than 100 cigarettes in their lifetime, had a history of regular smoking, and noted smoking within the current year from study enrollment. Participants who noted a history of regular smoking but were unsure when they last smoked were categorized as 'former' smokers. Likewise, participants who noted uncertainty in ever smoking more than 100 cigarettes were categorized as 'former' smokers. While some participant smoking status did vary over the course of the 3-year study, smoking habits from the time of primary lesion development and prior to study enrollment are the most likely to have an association with the risk of HSIL recurrence. As such, we used the baseline assessment for the main analysis.

We assessed additional smoking variables such as the number of cigarettes smoked per day and total years of smoking to compare different categories of “ever” smokers. These participants include current and former smokers, as well as those who discontinued smoking more than 16 years ago (who were categorized as never smokers for main analyses). For the number of cigarettes, a categorical variable was created based on the sample range with 3 levels: 0-10 cigarettes (up to half a pack), 11-20 cigarettes (half to a full pack), and 21-30 cigarettes. For the total years, a categorical variable was created based on the sample range with 4 levels: 3-14 years, 15-24 years, 25-34 years, and 35-45 years.

Data Analysis

Demographic and clinical characteristics were calculated as counts and percentages for categorical variables, mean and standard deviation (SD) for normally distributed continuous variables, and median and interquartile range (IQR) for skewed distribution continuous variables. Cox-Proportional Hazard models were fit to assess the impact of smoking status at enrollment on risk of HSIL recurrence. Time-to-event data were modified to account for censoring due to loss to follow-up and event occurrence. Analyses were further stratified based on qualifying HSIL site to evaluate whether the association between smoking status and risk of HSIL recurrence differed by anatomical site.

Prior to performing adjusted analyses, smoking exposure variables and potential confounding variables were added individually to Cox-Proportional Hazard models to assess their roles in risk of HSIL recurrence. Potential confounding variables were determined *a priori* and were accounted for in all adjusted Hazard Ratio (aHR) calculations, including age at enrollment (continuous), number of months between primary HSIL diagnosis and study enrollment (continuous), medical insurance type (binary), HIV status (binary), and HSIL anatomical site (binary). These variables will hereby be referred to as ‘potential confounders’. One participant who noted having no medical insurance was included in the group defined by

public insurance. Additional smoking exposure variables were added to models comparing ever smokers, including total years of smoking (categorical) and number of cigarettes smoked per day (categorical). Kaplan-Meier survival curves were plotted to visualize HSIL recurrence or "failure" overtime. Trend tests were used to compare models with and without the additional smoking variables. All analyses were conducted in R (version 4.2.2) and evaluated at a two-sided significance level of 0.05.

Results

Study Population

Study participants were 46.3% men, with a median age of 55 years old (IQR: 48, 62 years), and 78.7% were white. Most participants had greater than 12 years of schooling, private medical insurance, reported average health, and were monogamous in the past year (Table 2). Of the 188 participants in the study, 104 (55%) had a history of anal HSIL and 84 (45%) had a history of vulvar HSIL. Most persons with anal HSIL were men (83.7%) and among the men, 84.4% had HIV. In contrast, a majority of women with a history of vulvar HSIL did not have HIV infection (97.6%). Anal HSIL participants entered the study after a longer period of time since primary lesion diagnosis (median 26.8 months) compared to vulvar HSIL participants (median 15.3 months). The study population was followed for a median of 23.9 months (IQR: 10.8, 40.0 months).

Participants reported their smoking status at enrollment as 19.7% current smokers, 18.1% former smokers, and 61.7% never smokers. Compared to former and never smokers, current smokers included more women (62.2%), a higher number of median years of smoking (26.0 years), and a larger proportion with new sexual partners in the past year (40.5%) (Table 2). Interestingly, vulvar HSIL participants reported a markedly higher median number of cigarettes smoked per day (17.5 cigarettes) compared to anal HSIL participants (10.0 cigarettes) (Table 2). Current and former smokers had a lower median number of months

between primary lesion diagnosis and study enrollment (18.2; 18.4 months) compared to never smokers (23.2 months). Former and never smokers reported higher income compared to current smokers. Other demographic and clinical characteristics, such as age at enrollment, distribution of HSIL anatomical site, and age first smoked were similar between groups.

Univariate associations with risk of HSIL recurrence

There were 37 instances of HSIL recurrence in the study, 26 among persons with anal HSIL (25.0%) and 11 among women with vulvar HSIL (13.1%). In assessing unadjusted models for HSIL recurrence, the only variable that seems to marginally increase overall risk of HSIL recurrence is time between primary lesion diagnosis and study enrollment (HR: 1.02, 95% CI: 1.01, 1.03) (Table 3). HIV status and sex were collinear with one another and because vulvar HSIL recurrence cannot be adjusted for sex, HIV status was chosen to be added into adjusted models. PLWH had a non-significant 14% increased risk of overall HSIL recurrence compared to persons without HIV (HR: 1.14, 95% CI: 0.81, 1.60). This increased risk appears to be elevated among vulvar HSIL participants (HR: 5.48, 95% CI: 1.28, 23.43). However only two women with HIV with a history of vulvar HSIL participated in the study, and both had a vulvar HSIL recurrence. Lastly, compared to participants with private insurance, those with public medical insurance had a non-significant 24% elevated risk of overall HSIL recurrence (HR: 1.24, 95% CI: 0.88, 1.75).

Risk of HSIL recurrence by smoking status

Compared to never smokers, current smokers had a non-significant 40% increased risk of HSIL recurrence (HR: 1.40, 95% CI: 0.93, 2.13) and former smokers had no increased risk of HSIL recurrence (HR: 0.91, 95% CI: 0.60, 1.38, Figure 1a). Compared to never smokers and adjusting for potential confounders, current smokers had a 60% increased risk of HSIL

recurrence (aHR: 1.60, 95% CI: 1.02, 2.52) and former smokers had no increased risk of HSIL recurrence (aHR: 0.91, 95% CI: 0.58, 1.41) (Table 4).

In an analysis restricted to participants with anal HSIL, current smokers had an increased risk of HSIL recurrence (HR: 2.10, 95% CI: 1.11, 3.99) that was not seen among former smokers who had no increased risk of anal HSIL recurrence (HR: 0.69, 95% CI: 0.38, 1.28, Figure 1b). Adjusting for potential confounders, current smokers had a strongly elevated risk of HSIL recurrence (aHR: 2.73, 95% CI: 1.37, 5.43), whereas former smokers had no increased risk of anal HSIL recurrence (aHR: 0.72, 95% CI: 0.38, 1.37) (Table 4).

Among women with a history of vulvar HSIL, compared to never smokers, current smokers had 27% increased, but not statistically significant, risk of vulvar HSIL recurrence (HR: 1.27, 95% CI: 0.72, 2.24) and former smokers had no increased risk of HSIL recurrence (HR: 1.07, 95% CI: 0.59, 1.96, Figure 1c). Adjusting for potential confounders, compared to never smokers, current smokers had a non-significant 23% increased risk of vulvar HSIL recurrence (aHR: 1.23, 95% CI: 0.64, 2.36) and former smokers had no increased risk of HSIL recurrence (aHR: 1.07, 95% CI: 0.57, 2.0) (Table 4).

Among PLWH, compared to never smokers and adjusting for potential confounders, current smokers had significantly elevated risk of overall HSIL recurrence (aHR: 2.76, 95% CI: 1.26, 3.02) and former smokers did not have increased risk of overall HSIL recurrence (aHR: 0.73, 95% CI: 0.35, 1.52). Among persons with a history of anal HSIL and with HIV, the risks of anal HSIL recurrence of current and former smokers, compared to never smokers, were similar to the risk estimates of overall HSIL recurrence.

Risk of HSIL recurrence by number of cigarettes per day

To assess variations in HSIL recurrence among ever smokers, the data were restricted to only include those with a history of regular smoking (84 participants). Compared to those who reported smoking up to 10 cigarettes per day, those who reported smoking between 11-20

cigarettes per day had no increased risk of HSIL recurrence (HR: 1.10, 95% CI: 0.66, 1.86), and those who reported smoking more than 20 cigarettes had a non-significant 59% increased risk of HSIL recurrence (HR: 1.59, 95% CI: 0.61, 4.12). Adjusting for potential confounders, compared to those who reported smoking up to 10 cigarettes per day, those who reported smoking between 11-20 cigarettes per day had no increased risk of HSIL recurrence (aHR: 1.00, 95% CI: 0.56, 1.78), and those who reported smoking more than 20 cigarettes had 37% increased risk of HSIL recurrence that was not statistically significant (aHR: 1.37, 95% CI: 0.51, 3.71). In Table 5, risk estimates for number of cigarettes per day were not significantly elevated and showed no significant dose-response (p = 0.83 for trend), overall or by anatomic site (Table 5, Figure 2).

Risk of HSIL recurrence by years smoked

Compared to those who reported 3-14 total years of smoking, those who reported 35-45 years had a non-significant 80% increased risk of HSIL recurrence (HR: 1.80, 95% CI: 0.87, 3.70). Adjusting for potential confounders, compared to those who reported 3-14 total years of smoking, those who reported 35-45 years had marginally increased risk of HSIL recurrence (aHR: 2.32, 95% CI: 1.00, 5.36) (Table 6).

Among anal HSIL participants, compared to those who reported 3-14 total years of smoking, those who reported 15-24 years had higher risk of HSIL recurrence that was not statistically significant (HR: 2.40, 95% CI: 0.86, 6.69), those who reported 25-34 years had significantly increased risk of HSIL recurrence (HR: 3.52, 95% CI: 1.07, 11.52), and those who reported 35-45 years had markedly elevated risk of HSIL recurrence (HR: 5.83, 95% CI: 1.56, 21.88). Adjusting for potential confounders, compared to those who reported 3-14 total years of smoking, those who reported 15-24 years had a non-significant elevated risk of HSIL recurrence (aHR: 3.48, 95% CI: 0.95, 12.67), those who reported 25-34 years had significantly elevated risk of HSIL recurrence (aHR: 4.79, 95% CI: 1.13, 20.34), and those who reported 35-45 years had

markedly increased risk of HSIL recurrence (aHR: 8.00, 95% CI: 1.61, 39.77). Although not all individual estimates were statistically significant, there was a strong indication of an underlying trend in increasing risk with increased amount of time smoked (p = 0.05) (Figure 3).

Among women with a history of vulvar HSIL, compared to those who reported 3-14 total years of smoking, those who reported 35-45 years of smoking had 97% elevated, but not statistically significant, risk of vulvar HSIL recurrence (aHR: 1.97, 95% CI: 0.61, 6.42). However, in contrast to anal HSIL recurrence, there was no indication of a trend with duration of smoking (p = 0.38) (Figure 3).

Discussion

We conducted a longitudinal study to assess the association of smoking status on anal and vulvar HSIL recurrence. Our data indicate that current smoking increases the risk of anal and vulvar HSIL recurrence. Former smoking habits had no influence on risk of anal and vulvar HSIL recurrence when compared to the risk of HSIL recurrence among persons who never smoked. In analyses restricted to anal HSIL participants, these trends are maintained, and current smokers have a significantly higher risk of anal HSIL recurrence. Supplementary analyses on anal HSIL participants with HIV reveal a pointedly increased risk of anal HSIL recurrence comparing current to never smokers. These results agree with Gaisa et al. who found a 70% increased risk in anal HSIL recurrence comparing current versus never smokers among PLWH[13]. These findings demonstrate the important role current tobacco smoking may play in anal HSIL recurrence, particularly among PLWH.

When looking solely at vulvar HSIL recurrence, current smoking was not associated with significantly increased risks of HSIL recurrence, compared to never smokers. These results are in contrast to what Fehr et al. found with increased risk of both vulvar HSIL recurrence and progression to invasive cancer associated with smoking. However, Fehr et al. categorized smokers based on whether vulvar HSIL patients currently smoked more than 10 cigarettes a

day[16]. This creates different classification groups of women with vulvar HSIL defined as smokers compared to our analyses, which defined smoking status based on participant smoking history. Fehr et al. also controlled for treatment type, which they found to be significantly associated with the risk of vulvar HSIL recurrence[16]. We, however, were not able to adjust for treatment modality as 95.7% of lesions in our study were excised compared to 68.9% in the Fehr study[16].

Within our dataset, smoking more than 20 cigarettes per day did increase the risk of HSIL recurrence, as well as in restricted populations of anal and vulvar HSIL participants, but these increases were not statistically significant. This suggests that the quantity of cigarettes smoked may not be associated with risk of anal and vulvar HSIL recurrence or, more likely, that our study lacks statistical power to detect this association. These results are similar to what Madeleine et al. found with increased, but not statistically significant risk of VIN3 recurrence with smoking after initial diagnosis (HR: 2.1, 95% CI: 0.8, 5.4)[7]. However, Madeleine et al. did not assess the quantity of cigarettes smoked, so it is possible that quantity of cigarettes associated with risk of HSIL recurrence needs to be studied further.

In reference to the total number of years of smoking, compared to 3-14 years of smoking, smoking 35-45 years strongly elevated the risk of overall HSIL recurrence. Restricted to anal HSIL recurrence, each increase in smoking years category, compared to 3-14 years of smoking, was associated with subsequent higher and significant increases in risk of anal HSIL recurrence. There was a significant trend of increased risk of anal HSIL recurrence associated with increased years of smoking. From this, we can conclude that total lifetime years of smoking is a significant factor in elevating risk of anal HSIL recurrence. Limited to vulvar HSIL recurrence, compared to 3-14 years of smoking, participants who noted 35-45 years of smoking had an increase in, but not statistically significant, risk of vulvar HSIL recurrence. The suggested but not statistically significant trend is likely due to lack of power to detect these associations with vulvar HSIL recurrence. Nevertheless, the increases in risk estimates of anal and vulvar

HSIL recurrence comparing participants with 35-45 years of smoking to 3-14 years of smoking demonstrate the impact that high numbers of smoking years can have on risk of recurrence. The strong trends in anal HSIL recurrence demonstrate lifetime years of smoking as important predictors of anal HSIL recurrence.

We also confirmed that increased time between primary lesion diagnosis and study enrollment slightly increased the risk of HSIL recurrence. Because study enrollment criteria specified the earliest date of primary lesion diagnosis, this is further evidence of a time window for HSIL recurrence. While we did not find that smoking risks associated with HSIL recurrence are elevated with increased time between primary lesion and study enrollment, this time period may represent a critical period for managing risk factors associated HSIL recurrence.

This study has several limitations. The small sample size restricted the number of smokers for our analysis as well as our ability to detect smaller, but potentially clinically significant risks. For instance, we found evidence of increased risk of vulvar HSIL recurrence associated with women with HIV, but low power led to imprecise risk estimates that need to be confirmed by additional studies. Further, the multiple strategies for collected data (clinician, self, and telephone interviews) and different questions posed in each instrument led to inconsistent data collection over time and only allowed the use of enrollment data for smoking habits. For this reason, there may be some inaccuracies and mismeasurements in the smoking status categorizations. As vulvar HSIL participants largely did not have HIV, we were not able to account for the increased risk of HSIL recurrence that HIV poses, potentially biasing or overestimating risk estimates. Lastly, because HPV typing is not routinely performed on lesions, we were not able to account for increased likelihood of recurrence with high-risk HPV types.

This study had numerous strengths. All participants who entered the study had a prior HSIL diagnosis and were determined to have no detectable lesion at enrollment. This enabled us to establish a temporal relationship between smoking status at enrollment and HSIL recurrence during the study. The long follow-up period (median of 23.9 months) increased the

chances of HSIL recurrence in the study period. The longitudinal study design allowed for the continuous collection of data associated with HSIL recurrence, creating a vast dataset. Since cancer registries document broad populations, recruitment of participants from a local cancer registry increases the sample representativeness and consequential generalizability of our results.

Our findings demonstrate the importance of smoking cessation upon primary HSIL diagnosis, particularly among anal HSIL patients, to decrease the risk of HSIL recurrence. They also show the dual roles that smoking can play in increasing the risk of primary HSIL development and HSIL recurrence. While HPV-related lesions and cancers are not thought of as being directly related to tobacco smoking, it is clear that there may be mechanistic changes contributing to increased cellular vulnerability, impaired immune response, and activation of HPV-mediated cell proliferation. Future *in-vivo* studies on these mechanisms should be prioritized, as well as public health messaging on smoking risks.

Further cohort studies on anal and vulvar HSIL recurrence with larger sample size should be undertaken to confirm and extend our findings. With the knowledge that risk of vulvar HSIL recurrence increases with lower levels of HPV antibodies, designing statistical analyses to assess whether serology and smoking interact with each other would be useful in identifying cofactors that may increase risk of anal and vulvar HSIL recurrence to an even greater extent[7].

Tables

Table 1: Previous studies of anal and vulvar HSIL recurrence

Author	Design	Primary Diagnosis	HIV Status	Follow-up Years	N	Recur, n	Recur, %
Anal HSIL							
Swedish[9]	Retrospective Cohort	AIN2/3	Negative (MSM)	2	114	35	30.7%
Goldstone[11]	Prospective Cohort	AIN2/3	Negative (MSM)	3	271		66.0%
			Positive (MSM)	3	456		77.0%
Stier[12]	Retrospective Cohort	AIN2/3	Positive (WLWH)	1	45		29%
				2	45		52%
				3	45	22	79%
Gaisa[13]	Retrospective Cohort	AIN	Positive	Median 12.2 mo	303	148 (local) 198 (overall)	45% 60%
Burgos[14]	Prospective Cohort	AIN2/3	Positive (MSM)	1 2	100 100		23.5% 53.5%
Kobayashi[15]	Retrospective Cohort	AIN	Positive	Median 35 mo	61	30	49%
Vulvar HSIL							
Fehr[16]	Retrospective Cohort	VIN		34 mo	411	123	29.9%
Jones[17]	Prospective Cohort	VIN2/3		42 mo	382	122	31.9%
Madeleine[7]	Retrospective Cohort	VIN3		3 5	65 65	17 21	26.2% 32.3%
Wallbillich[18]	Retrospective Cohort	VIN2/3			303	87	28.7%
Satmary[8]	Retrospective Cohort	VIN		Median 89 mo	650	171	26.3%
Herod[19]	Retrospective Cohort	VIN		4	103	55	48%
Ghelardi[20]	Prospective Cohort	VIN non-vaccinated VIN vaccinated			76 42	24 8	32% 19%
van Seters[10]	Meta-analysis	VIN3			480	138	28.8%

Table 2: Baseline characteristics and demographics of the study sample, overall, by anatomic site of qualifying HSIL, and smoking status at enrollment.

		Qualifying HSIL		Smoking Status (at enrollment)		
	Overall	**Anal**	**Vulvar**	**Never**	**Former**	**Current**
N	188	104	84	116	34	37
Sex (\%)						
Men	87 (46.3)	87 (83.7)	0 (0.0)	57 (49.1)	16 (47.1)	14 (37.8)
Women	101 (53.7)	17 (16.3)	84 (100.0)	59 (50.9)	18 (52.9)	23 (62.2)
Age at enrollment (median [IQR])	55.0 [48.0, 62.3]	55.0 [49.8, 61.3]	56.0 [46.8, 63.3]	57.0 [51.0, 63.3]	52.0 [44.3, 57.0]	53.0 [46.0, 56.0]
Age at qualifying lesion (median [IQR])	53.0 [46.8, 61.0]	53.0 [47.8, 60.0]	55.0 [45.5, 61.3]	56.0 [49.0, 61.3]	49.5 [43.5, 55.0]	52.0 [43.0, 55.0]
Categorical age at qualifying lesion (\%)						
27-36	16 (8.5)	8 (7.7)	8 (9.5)	5 (4.3)	6 (17.6)	5 (13.5)
37-48	40 (21.3)	20 (19.2)	20 (23.8)	23 (19.8)	9 (26.5)	8 (21.6)
49-59	76 (40.4)	49 (47.1)	27 (32.1)	46 (39.7)	13 (38.2)	17 (45.9)
60-69	56 (29.8)	27 (26.0)	29 (34.5)	42 (32.6)	6 (17.6)	7 (18.9)
Months between diagnosis and study enrollment (median [IQR])	20.9 [10.8, 39.9]	26.8 [14.2, 42.4]	15.3 [8.5, 30.6]	23.2 [12.5, 40.6]	18.4 [9.3, 38.4]	18.2 [8.7, 38.2]
Time since qualifying HSIL (\%)						
Greater than or equal to 12 months	145 (77.1)	83 (79.8)	62 (73.8)	91 (78.4)	28 (82.4)	25 (67.6)
Less than 12 months	43 (22.9)	21 (20.2)	22 (26.2)	25 (21.6)	6 (17.6)	12 (32.4)
Race (\%)[a]						
White	148 (78.7)	76 (73.1)	72 (85.7)	93 (80.2)	25 (73.5)	29 (78.4)
Other	33 (17.6)	25 (24.0)	8 (9.5)	19 (16.4)	8 (23.5)	6 (16.2)
Hispanic (\%)[b]						
Yes	8 (4.3)	5 (4.8)	3 (3.6)	5 (4.3)	3 (8.8)	0 (0.0)
No	176 (93.6)	96 (92.3)	80 (95.2)	110 (94.8)	30 (88.2)	35 (94.6)
Income status (\%)[c]						
Less than $20,000	32 (17.0)	23 (22.1)	9 (10.7)	12 (10.3)	7 (20.6)	9 (24.3)
$20,000 to 50,000	32 (17.0)	17 (16.3)	15 (17.9)	18 (15.5)	5 (14.7)	9 (24.3)
$51,000 to 100,000	63 (33.5)	28 (26.9)	35 (41.7)	46 (39.7)	8 (23.5)	13 (35.1)

Over $100,000	59 (31.4)	35 (33.7)	24 (28.6)	38 (32.8)	14 (41.2)	6 (16.2)
Educational attainment status (\%)[d]						
<=High School	26 (13.8)	13 (12.5)	13 (15.5)	12 (10.3)	4 (11.8)	10 (27.0)
Some College/Associates	77 (41.0)	39 (37.5)	38 (45.2)	44 (37.9)	13 (38.2)	20 (54.1)
Bachelors+	84 (44.7)	51 (49.0)	33 (39.3)	59 (50.9)	17 (50.0)	7 (18.9)
Medical insurance status (\%)[e]						
Private	118 (62.8)	63 (60.6)	55 (65.5)	75 (64.7)	22 (64.7)	21 (56.8)
Public	65 (34.6)	39 (37.5)	26 (31.0)	38 (32.8)	10 (29.4)	16 (43.2)
Baseline health (\%)[f]						
Excellent	31 (16.5)	22 (21.2)	9 (10.7)	26 (22.4)	3 (8.8)	2 (5.4)
Very Good	92 (48.9)	54 (51.9)	38 (45.2)	61 (52.6)	16 (47.1)	14 (37.8)
Good	53 (28.2)	23 (22.1)	30 (35.7)	21 (18.1)	14 (41.2)	18 (48.6)
Fair	10 (5.3)	4 (3.8)	6 (7.1)	7 (6.0)	1 (2.9)	2 (5.4)
Poor	1 (0.5)	1 (1.0)	0 (0.0)	1 (0.9)	0 (0.0)	0 (0.0)
HIV Status (\%)						
Positive	72 (38.3)	70 (67.3)	2 (2.4)	43 (37.1)	14 (41.2)	15 (40.5)
Negative	116 (61.7)	34 (32.7)	82 (97.6)	73 (62.9)	20 (58.8)	17 (59.5)
Transplant status (\%)						
Transplant	5 (2.7)	2 (1.9)	3 (3.6)	5 (4.3)	0 (0.0)	0 (0.0)
No Transplant	183 (97.3)	102 (98.1)	81 (96.4)	111 (95.7)	34 (100.0)	37 (100.0)
New sex partners in the past year (\%)						
0	136 (72.3)	63 (60.6)	73 (86.9)	86 (74.1)	27 (79.4)	22 (59.5)
1	12 (6.4)	5 (4.8)	7 (8.3)	5 (4.3)	2 (5.9)	5 (13.5)
2	11 (5.9)	8 (7.7)	3 (3.6)	6 (5.2)	2 (5.9)	3 (8.1)
3+	26 (13.8)	26 (25.0)	0 (0.0)	18 (15.5)	3 (8.8)	5 (13.5)
Unknown	3 (1.6)	2 (1.9)	1 (1.2)	1 (0.9)	0 (0.0)	2 (5.4)
Age first smoked (mean (SD))	15.7 (4.8)	15.8 (5.5)	15.4 (3.7)	16.2 (4.7)	15.4 (3.9)	15.3 (5.6)
Total years of smoking (median [IQR])	21.0 [13.0, 30.0]	21.0 [12.0, 25.0]	21.0 [13.5, 32.3]	16.5 [8.5, 23.8]	20.0 [11.0, 22.5]	26.0 [20.0, 40.0]
Number of cigarettes smoked per day (median [IQR])	10.0 [9.25, 20.0]	10.0 [8.0, 20.0]	17.5 [10.0, 20.0]	20.0 [10.0, 23.8]	15.0 [9.3, 20.0]	10.0 [8.0, 20.0]

[a] Missing: Overall 7 (3.7), Anal 3 (2.9), Vulvar 4 (4.8), Current 2 (5.4), Former 1 (2.9), Never 4 (3.4), [b] Missing: Overall 4 (2.1), Anal 3 (2.9), Vulvar 1 (1.2), Current 2 (5.4), Former 1 (2.9), Never 1 (0.9), [c] Missing: Overall 2 (1.1), 1 (1.0), 1 (1.2), Never 2 (1.7), [d] Missing: Overall 1 (0.5), Anal 1 (1.2), Never 1 (0.9), [e] Missing: Overall 5 (2.7), Anal 2 (1.9), Vulvar 3 (3.6), Former 2 (5.9), Never 3 (2.6), [f]Missing: Overall 1 (0.5), Vulvar 1 (1.2), Current 1 (2.7)

Table 3: Univariate hazard ratios for potential variables impacting risk of HSIL recurrence, combined and stratified by qualifying HSIL site

Variable	Overall HSIL recurrence *HR (95% CI)*	Anal HSIL recurrence *HR (95% CI)*	Vulvar HSIL recurrence *HR (95% CI)*
Smoking status (at enrollment)			
Never	*Ref*	*Ref*	*Ref*
Former	0.91 (0.60, 1.38)	0.69 (0.38, 1.28)	1.07 (0.59, 1.96)
Current	1.40 (0.93, 2.13)	2.10 (1.11, 3.99)	1.27 (0.72, 2.24)
Months between primary lesion diagnosis and study enrollment	1.02 (1.01, 1.03)	1.02 (1.01, 1.03)	1.01 (1.00, 1.03)
Medical insurance			
Private	*Ref*	*Ref*	*Ref*
Public	1.24 (0.88, 1.75)	1.11 (0.70, 1.76)	1.40 (0.83, 2.35)
Age at study enrollment	1.00 (0.98, 1.02)	1.01 (0.98, 1.04)	0.99 (0.97, 1.02)
HIV Status			
Negative	*Ref*	*Ref*	*Ref*
Positive	1.14 (0.81, 1.60)	1.06 (0.66, 1.69)	5.48 (1.28, 23.43)
Sex			
Women	*Ref*	*Ref*	
Men	1.01 (0.79, 1.53)	1.06 (0.60, 1.88)	
HSIL site			
Anal	*Ref*		
Vulvar	0.91 (0.65, 1.26)		
Cigarettes per day			
≤10	*Ref*	*Ref*	*Ref*
11-20	1.10 (0.66, 1.86)	1.03 (0.40, 2.65)	1.09 (0.55, 2.17)
21+	1.59 (0.61, 4.12)	1.48 (0.49, 4.48)	3.24 (0.40, 26.18)
Years of smoking			
3-14	*Ref*	*Ref*	*Ref*
15-24	1.14 (0.61, 2.12)	2.40 (0.86, 6.69)	0.80 (0.32, 1.98)
25-34	2.04 (0.98, 4.25)	3.52 (1.07, 11.52)	1.40 (0.45, 4.34)
35-45	1.80 (0.87, 3.70)	5.83 (1.56, 21.88)	1.26 (0.48, 3.31)

HR, Crude/Unadjusted Hazard Ratio; CI, Confidence Interval; Ref, Reference category

Table 4: Hazard ratios for the association of smoking status and risk of HSIL recurrence, combined and stratified by qualifying HSIL site

Smoking Status (at enrollment)	Never	Former	Current
Overall HSIL Recurrence			
aHR (95% CI)[a]	*Ref*	0.91 (0.58, 1.41)	1.60 (1.02, 2.52)
Anal HSIL Recurrence			
aHR (95% CI)[b]	*Ref*	0.72 (0.38, 1.37)	2.73 (1.37, 5.43)
Vulvar HSIL Recurrence			
aHR (95% CI)[b]	*Ref*	1.07 (0.57, 2.0)	1.23 (0.64, 2.36)

[a] Adjusted for age, HIV status, time between primary lesion diagnosis and study enrollment, medical insurance, and HSIL anatomical site
[b] Adjusted for age, HIV status, time between primary lesion diagnosis and study enrollment, and medical insurance
aHR, Adjusted Hazard Ratio; CI, Confidence Interval; Ref, Reference category

Table 5: Hazard ratios for the association of cigarettes per day and risk of HSIL recurrence, combined and stratified by qualifying HSIL site among ever smokers

Cigarettes per day	≤10	11-20	21+	Trend test
Overall HSIL Recurrence				
aHR (95% CI)[a]	*Ref*	1.00 (0.56, 1.78)	1.37 (0.51, 3.71)	p = 0.83
Anal HSIL Recurrence				
aHR (95% CI)[b]	*Ref*	0.84 (0.28, 2.47)	1.25 (0.39, 3.98)	p = 0.84
Vulvar HSIL Recurrence				
aHR (95% CI)[b]	*Ref*	1.15 (0.53, 2.49)	1.92 (0.20, 18.31)	p = 0.85

[a] Adjusted for age, HIV status, time between primary lesion diagnosis and study enrollment, medical insurance, and HSIL anatomical site
[b] Adjusted for age, HIV status, time between primary lesion diagnosis and study enrollment, and medical insurance
aHR, Adjusted Hazard Ratio; CI, Confidence Interval; Ref, Reference category; p, p-value

Table 6: Hazard ratios for the association of total years of smoking and risk of HSIL recurrence, combined and stratified by qualifying HSIL site among ever smokers

Total years of smoking	3-14	15-24	25-34	35-45	Trend test
Overall HSIL Recurrence					
aHR (95% CI)[a]	*Ref*	1.19 (0.59, 2.43)	1.69 (0.68, 4.18)	2.32 (1.00, 5.36)	p = 0.21
Anal HSIL Recurrence					
aHR (95% CI)[b]	*Ref*	3.48 (0.95, 12.67)	4.79 (1.13, 20.34)	8.00 (1.61, 39.77)	p = 0.05
Vulvar HSIL Recurrence					
aHR (95% CI)[b]	*Ref*	0.81 (0.29, 2.29)	0.97 (0.23, 4.06)	1.97 (0.61, 6.42)	p = 0.38

[a] Adjusted for age, HIV status, time between primary lesion diagnosis and study enrollment, medical insurance, and HSIL anatomical site
[b] Adjusted for age, HIV status, time between primary lesion diagnosis and study enrollment, and medical insurance
aHR, Adjusted Hazard Ratio; CI, Confidence Interval; Ref, Reference category; p, p-value

Figures

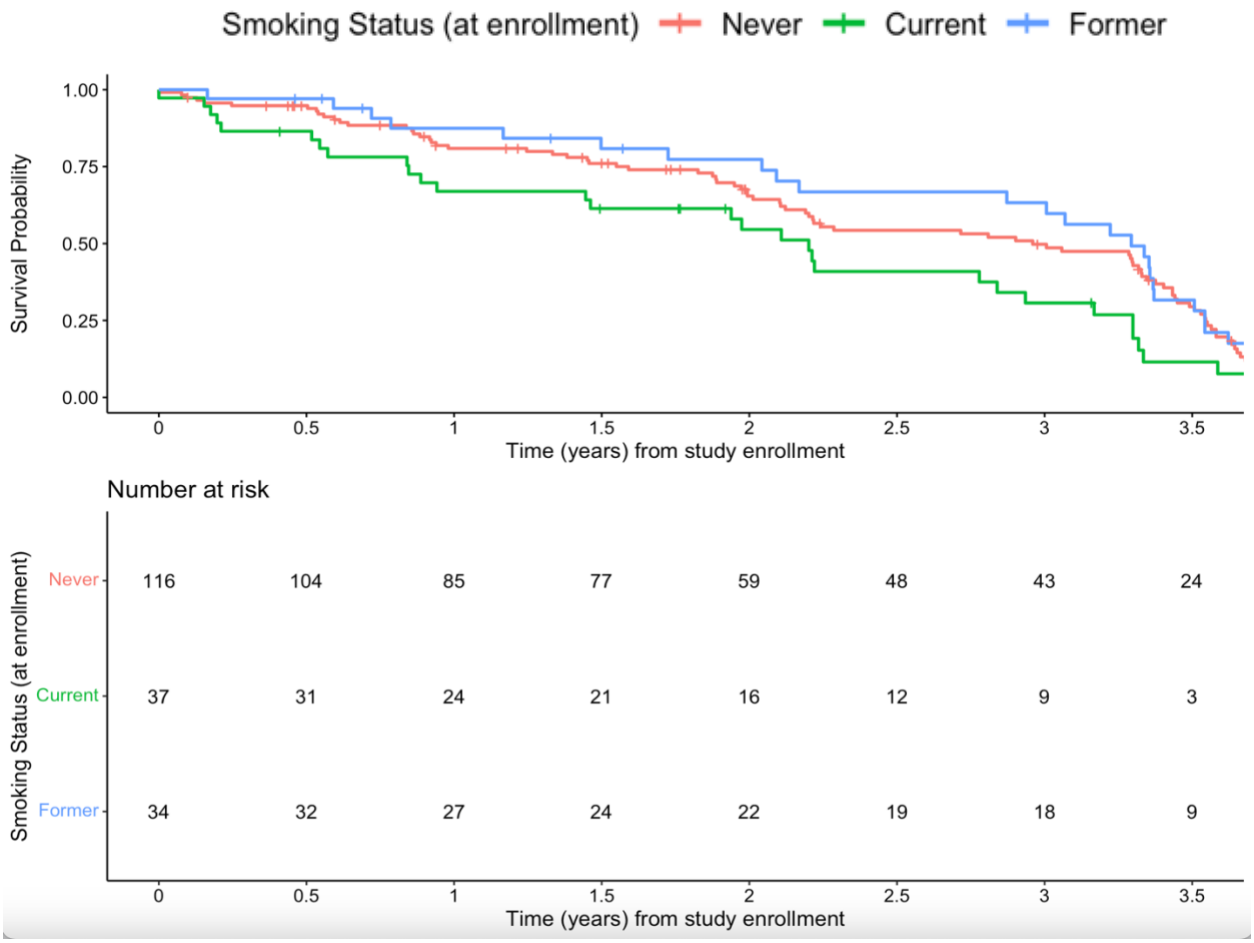

Figure 1a: Kaplan-Meier survival curve of overall HSIL recurrence, stratified by smoking status (Red: Never; Green: Current; Blue: Former)

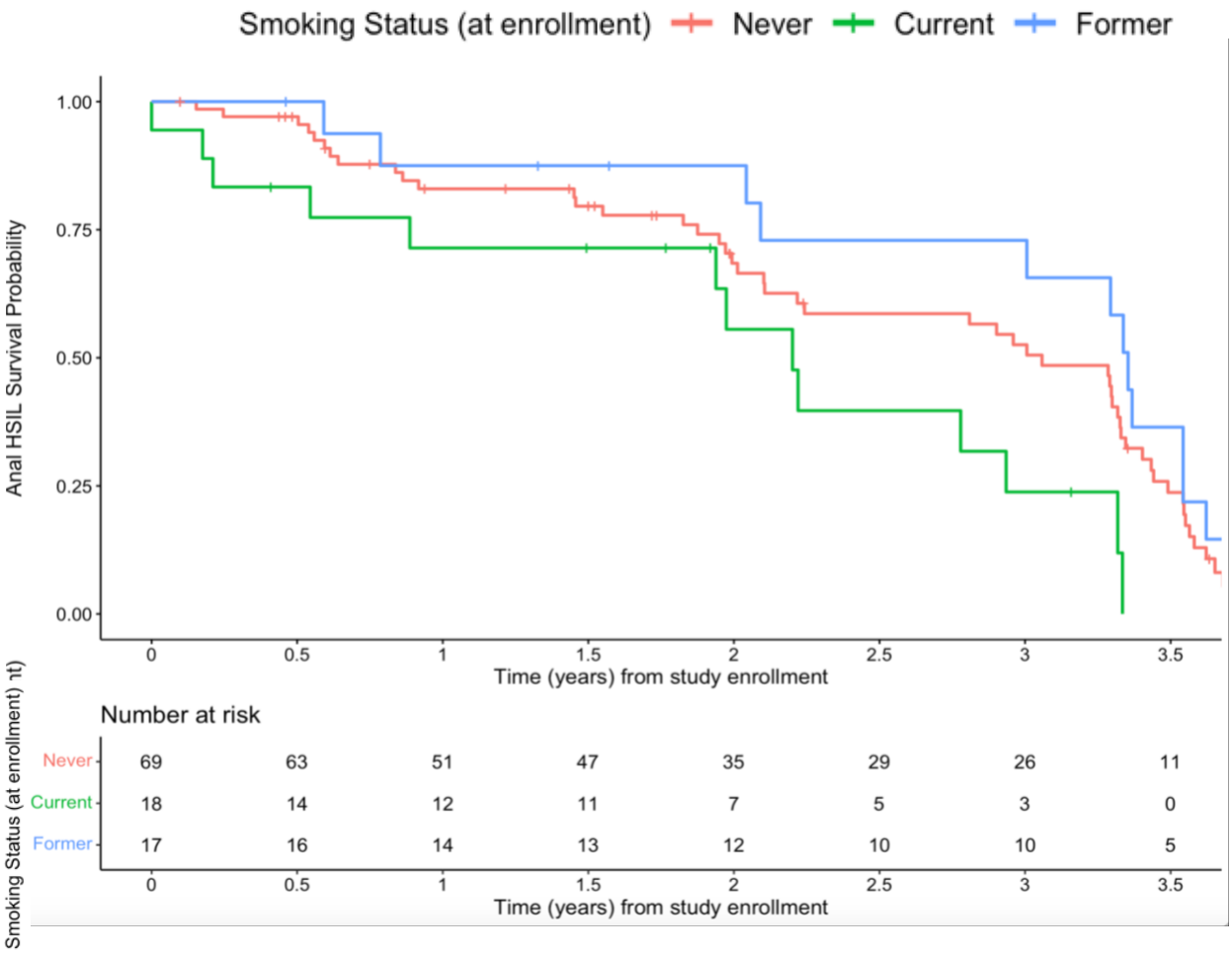

Figure 1b: Kaplan-Meier survival curve of anal HSIL recurrence, stratified by smoking status (Red: Never; Green: Current; Blue: Former)

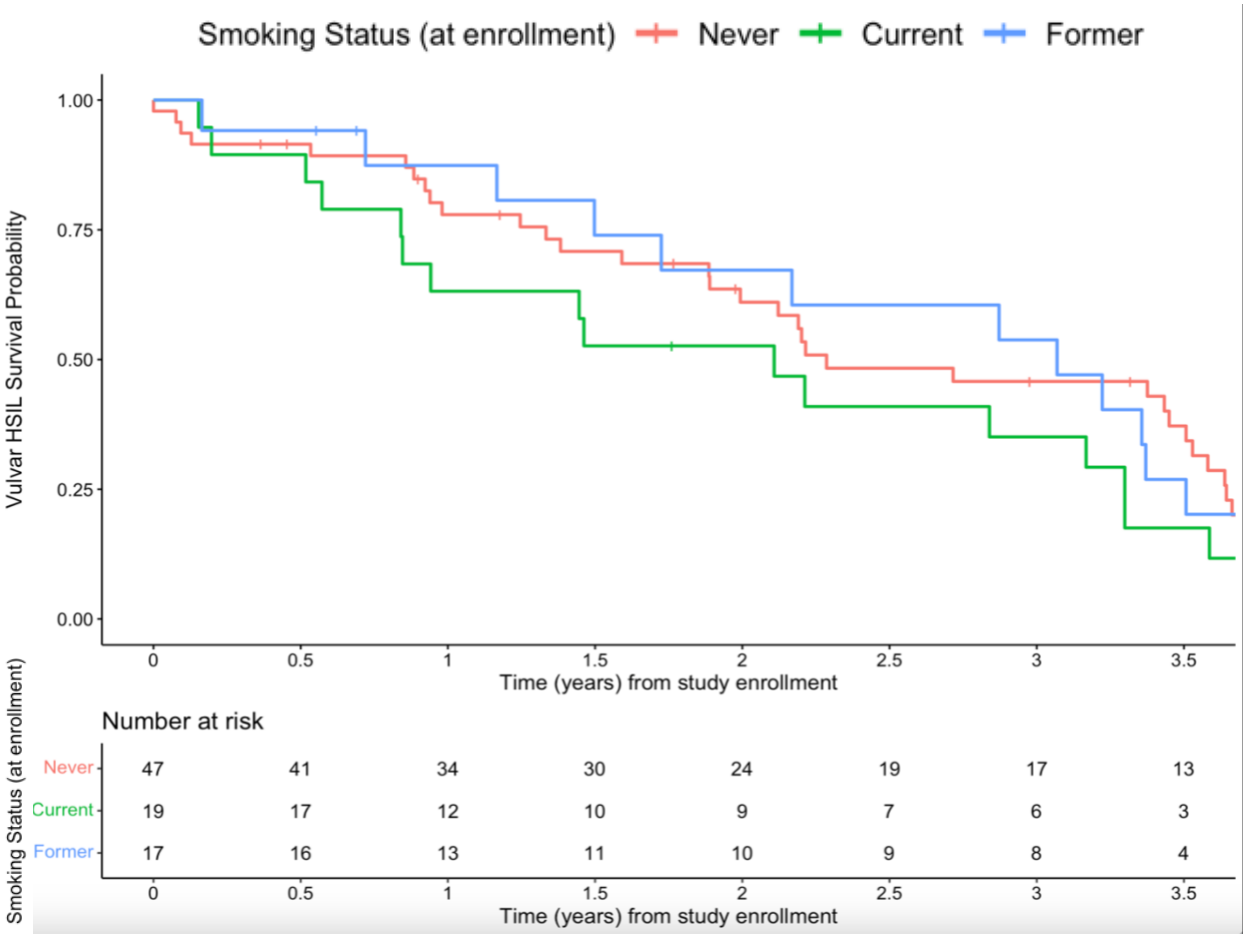

Figure 1c: Kaplan-Meier survival curve of vulvar HSIL recurrence, stratified by smoking status (Red: Never; Green: Current; Blue: Former)

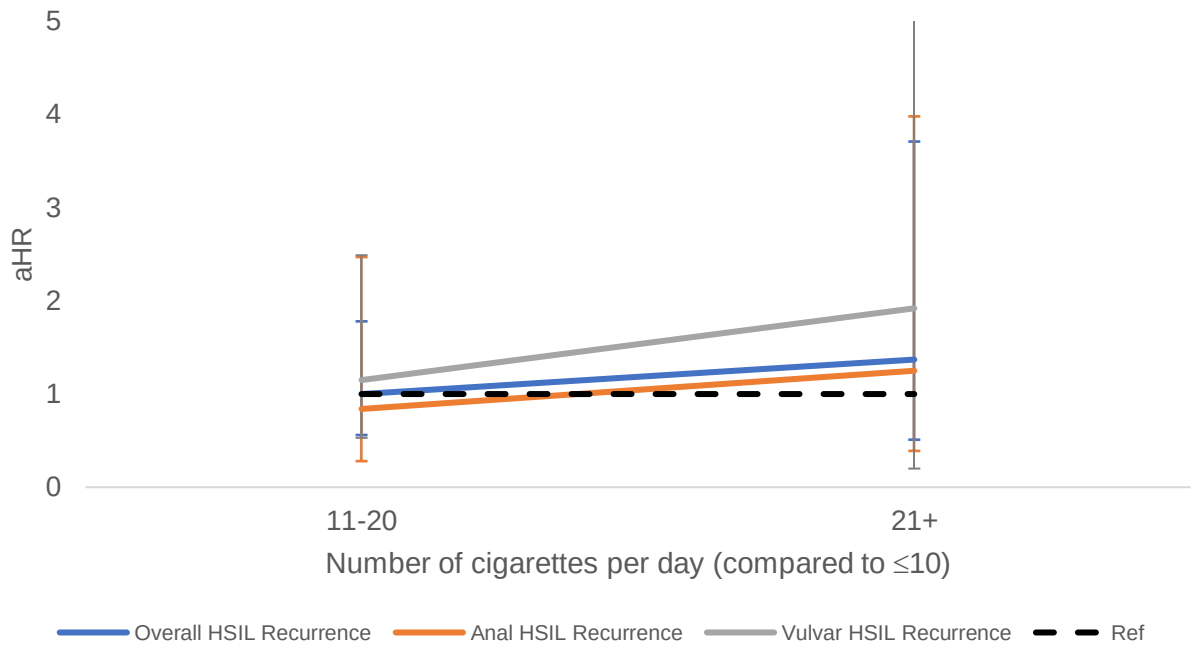

Figure 2: Trend in adjusted hazard ratios (aHR) associated with categories of number of cigarettes smoked, compared to smoking ≤ 10 cigarettes. Error bars represent 95% confidence intervals.
(Blue: Overall HSIL Recurrence; Orange: Anal HSIL Recurrence; Grey: Vulvar HSIL Recurrence; Dashed: Reference)

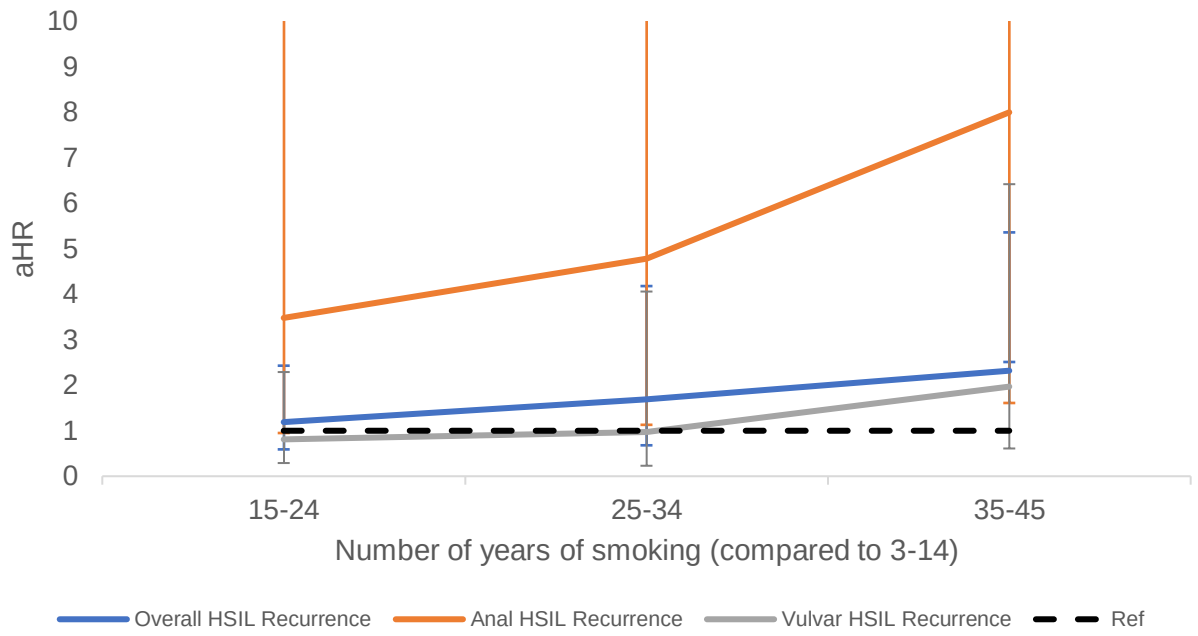

Figure 3: Trend in adjusted hazard ratios (aHR) associated with categories of years of smoking, compared to smoking between 3-14 years. Error bars represent 95% confidence intervals. (Blue: Overall HSIL Recurrence; Orange: Anal HSIL Recurrence; Grey: Vulvar HSIL Recurrence; Dashed: Reference)

References

1. Chesson HW, Dunne EF, Hariri S, Markowitz LE. The Estimated Lifetime Probability of Acquiring Human Papillomavirus in the United States. Sex Transm Dis. 2014 Nov;41(11):660–4.

2. Schiffman M, Doorbar J, Wentzensen N, de Sanjosé S, Fakhry C, Monk BJ, et al. Carcinogenic human papillomavirus infection. Nat Rev Dis Primers. 2016 Dec 1;2(1):1–20.

3. Palefsky JM, Lee JY, Jay N, Goldstone SE, Darragh TM, Dunlevy HA, et al. Treatment of Anal High-Grade Squamous Intraepithelial Lesions to Prevent Anal Cancer. New England Journal of Medicine. 2022 Jun 16;386(24):2273–82.

4. Surveillance, Epidemiology, and End Results Program [Internet]. SEER. [cited 2023 Mar 15]. Available from: https://seer.cancer.gov/index.html

5. Colón-López V, Shiels MS, Machin M, Ortiz AP, Strickler H, Castle PE, et al. Anal Cancer Risk Among People With HIV Infection in the United States. JCO. 2018 Jan;36(1):68–75.

6. Stankiewicz Karita HC, Hauge K, Magaret A, Mao C, Schouten J, Grieco V, et al. Effect of Human Papillomavirus Vaccine to Interrupt Recurrence of Vulvar and Anal Neoplasia (VIVA): A Trial Protocol. JAMA Network Open. 2019 Apr 12;2(4):e190819.

7. Madeleine MM, Johnson LG, Doody DR, Tipton ER, Carter JJ, Galloway DA. Natural Antibodies to Human Papillomavirus 16 and Recurrence of Vulvar High-Grade Intraepithelial Neoplasia (VIN3). J Low Genit Tract Dis. 2016 Jul;20(3):257–60.

8. Satmary W, Holschneider CH, Brunette LL, Natarajan S. Vulvar intraepithelial neoplasia: Risk factors for recurrence. Gynecologic Oncology. 2018 Jan 1;148(1):126–31.

9. Swedish KA, Factor SH, Goldstone SE. Prevention of Recurrent High-Grade Anal Neoplasia With Quadrivalent Human Papillomavirus Vaccination of Men Who Have Sex With Men: A Nonconcurrent Cohort Study. Clinical Infectious Diseases. 2012 Apr 1;54(7):891–8.

10. van Seters M, van Beurden M, de Craen AJM. Is the assumed natural history of vulvar intraepithelial neoplasia III based on enough evidence? A systematic review of 3322 published patients. Gynecol Oncol. 2005 May;97(2):645–51.

11. Goldstone SE, Johnstone AA, Moshier EL. Long-term Outcome of Ablation of Anal High-grade Squamous Intraepithelial Lesions: Recurrence and Incidence of Cancer. Diseases of the Colon & Rectum. 2014 Mar;57(3):316.

12. Stier EA, Abbasi W, Agyemang AF, Valle Álvarez EA, Chiao EY, Deshmukh AA. Brief Report: Recurrence of Anal High-Grade Squamous Intraepithelial Lesions Among Women Living With HIV. J Acquir Immune Defic Syndr. 2020 May 1;84(1):66–9.

13. GAISA MM, LIU Y, DESHMUKH AA, Stone K, SIGEL K. Electrocautery Ablation of Anal High-grade Squamous Intraepithelial Lesions: Effectiveness and Key Factors Associated with Outcomes. Cancer. 2020 Apr 1;126(7):1470–9.

14. Burgos J, Curran A, Landolfi S, Guelar A, Miguel L, Dinares M, et al. Risk factors of high-grade anal intraepithelial neoplasia recurrence in HIV-infected MSM. AIDS. 2017 Jun 1;31(9):1245–52.

15. Kobayashi T, Sigel K, Kalir T, MacLeod IJ, Liu Y, Gaisa M. Anal Cancer Precursor Lesions in HIV-Infected Persons: Tissue Human Papillomavirus Type Distribution and Impact on Treatment Response. Dis Colon Rectum. 2019 May;62(5):579–85.

16. Fehr MK, Baumann M, Mueller M, Fink D, Heinzl S, Imesch P, et al. Disease progression and recurrence in women treated for vulvovaginal intraepithelial neoplasia. Journal of Gynecologic Oncology. 2013 Jul 1;24(3):236–41.

17. Jones RW, Rowan DM, Stewart AW. Vulvar intraepithelial neoplasia: aspects of the natural history and outcome in 405 women. Obstet Gynecol. 2005 Dec;106(6):1319–26.

18. Wallbillich JJ, Rhodes HE, Milbourne AM, Munsell MF, Frumovitz M, Brown J, et al. Vulvar intraepithelial neoplasia (VIN 2/3): Comparing clinical outcomes and evaluating risk factors for recurrence. Gynecologic Oncology. 2012 Nov 1;127(2):312–5.

19. Herod JJ, Shafi MI, Rollason TP, Jordan JA, Luesley DM. Vulvar intraepithelial neoplasia: long term follow up of treated and untreated women. Br J Obstet Gynaecol. 1996 May;103(5):446–52.

20. Ghelardi A, Marrai R, Bogani G, Sopracordevole F, Bay P, Tonetti A, et al. Surgical Treatment of Vulvar HSIL: Adjuvant HPV Vaccine Reduces Recurrent Disease. Vaccines (Basel). 2021 Jan 25;9(2):83.

21. Daling JR, Madeleine MM, Johnson LG, Schwartz SM, Shera KA, Wurscher MA, et al. Human papillomavirus, smoking, and sexual practices in the etiology of anal cancer. Cancer. 2004;101(2):270–80.

22. Benevolo M, Latini A, Rollo F, Giuliani M, Giglio A, Giuliani E, et al. Incidence of abnormal anal cytology in HIV-infected and HIV-uninfected men who have sex with men. Cancer Cytopathology [Internet]. [cited 2023 Jan 4];n/a(n/a). Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/cncy.22675

23. Chaturvedi AK, Madeleine MM, Biggar RJ, Engels EA. Risk of Human Papillomavirus–Associated Cancers Among Persons With AIDS. JNCI: Journal of the National Cancer Institute. 2009 Aug 19;101(16):1120–30.

24. Simen-Kapeu A, Kataja V, Yliskoski M, Syrjänen K, Dillner J, Koskela P, et al. Smoking impairs human papillomavirus (HPV) type 16 and 18 capsids antibody response following natural HPV infection. Scandinavian Journal of Infectious Diseases. 2008 Jan 1;40(9):745–51.

25. Shiels MS, Katki HA, Freedman ND, Purdue MP, Wentzensen N, Trabert B, et al. Cigarette Smoking and Variations in Systemic Immune and Inflammation Markers. J Natl Cancer Inst. 2014 Sep 30;106(11):dju294.

26. Jung YS, Park JH, Park DI, Sohn CI, Lee JM, Kim TI. Impact of Smoking on Human Natural Killer Cell Activity: A Large Cohort Study. J Cancer Prev. 2020 Mar 30;25(1):13–20.

27. Graham SV. The human papillomavirus replication cycle, and its links to cancer progression: a comprehensive review. Clinical Science. 2017 Aug 10;131(17):2201–21.

28. Aguayo F, Muñoz JP, Perez-Dominguez F, Carrillo-Beltrán D, Oliva C, Calaf GM, et al. High-Risk Human Papillomavirus and Tobacco Smoke Interactions in Epithelial Carcinogenesis. Cancers (Basel). 2020 Aug 6;12(8):2201.

29. Yang X, Jin G, Nakao Y, Rahimtula M, Pater MM, Pater A. Malignant transformation of HPV 16-immortalized human endocervical cells by cigarette smoke condensate and characterization of multistage carcinogenesis. Int J Cancer. 1996 Jan 26;65(3):338–44.

30. Malevolti MC, Lugo A, Scala M, Gallus S, Gorini G, Lachi A, et al. Dose-risk relationships between cigarette smoking and cervical cancer: a systematic review and meta-analysis. European Journal of Cancer Prevention. :10.1097/CEJ.0000000000000773.

31. Madeleine MM, Daling JR, Schwartz SM, Carter JJ, Wipf GC, Beckmann AM, et al. Cofactors With Human Papillomavirus in a Population-Based Study of Vulvar Cancer. JNCI: Journal of the National Cancer Institute. 1997 Oct 15;89(20):1516–23.

32. Patrick DL, Cheadle A, Thompson DC, Diehr P, Koepsell T, Kinne S. The validity of self-reported smoking: a review and meta-analysis. Am J Public Health. 1994 Jul;84(7):1086–93.

33. Cooke F, Bullen C, Whittaker R, McRobbie H, Chen MH, Walker N. Diagnostic accuracy of NicAlert cotinine test strips in saliva for verifying smoking status. Nicotine & tobacco research. 2008;10(4):607–12.

34. Scheuermann TS, Richter KP, Rigotti NA, Cummins SE, Harrington KF, Sherman SE, et al. Accuracy of Self-Reported Smoking Abstinence in Clinical Trials of Hospital-Initiated Smoking Interventions. Addiction. 2017 Dec;112(12):2227–36.

35. Connor Gorber S, Schofield-Hurwitz S, Hardt J, Levasseur G, Tremblay M. The accuracy of self-reported smoking: a systematic review of the relationship between self-reported and cotinine-assessed smoking status. Nicotine Tob Res. 2009 Jan;11(1):12–24.

36. Convill J, Blackhall F, Yorke J, Faivre-Finn C, Gomes F. The Role of Electronic Patient-Reported Outcome Measures in Assessing Smoking Status and Cessation for Patients with Lung Cancer. Oncol Ther. 2022 Dec 1;10(2):481–91.

37. NHIS - Adult Tobacco Use - Glossary [Internet]. 2019 [cited 2023 Jun 6]. Available from: https://www.cdc.gov/nchs/nhis/tobacco/tobacco_glossary.htm