Smoking Is a Risk Factor for Cervical Intraepithelial Neoplasia Grade 3 among Oncogenic Human Papillomavirus DNA–Positive Women with Equivocal or Mildly Abnormal Cytology

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Abstract

Background: **Smoking is a potential risk factor for cervical cancer and its immediate precursor, cervical intraepithelial neoplasia grade 3 (CIN3), but few studies have adequately taken into account the possible confounding effect of oncogenic human papillomavirus (HPV) infection.**
Methods: **Women (n = 5,060) with minimally abnormal Papanicolaou smears were enrolled in the ASCUS and LSIL Triage Study, a clinical trial to evaluate management strategies, and were seen every 6 months for the 2-year duration of the study. Cervical specimens were tested for HPV DNA using both Hybrid Capture 2 and PGMY09/11 L1 consensus primer PCR with reverse line blot hybridization for genotyping. Multivariate logistics regression models were used to assess associations [odds ratio (OR) with 95% confidence intervals (95% CI)] between smoking behaviors and rigorously reviewed cases of cervical intraepithelial neoplasia grade 3 or cancer (≥CIN3) identified throughout the study (n = 506) in women with oncogenic HPV (n = 3,133).**
Results: **Current smoking was only weakly associated with increased HPV infection. Among infected women, current smokers (OR, 1.7; 95% CI, 1.4-2.1) and past smokers (OR, 1.7; 95% CI, 1.2-2.4) were more likely to be diagnosed with ≥CIN3 than nonsmokers. Greater smoking intensity (P_{Trend} < 0.0005) and duration (P_{Trend} < 0.0005) increased the strength of the association, with smoking ≥2 packs/d (OR, 3.3; 95% CI, 1.5-7.5) and smoking for ≥11 years (OR, 2.1; 95% CI, 1.5-2.9) most strongly associated with ≥CIN3 as compared to nonsmokers. The effects of intensity and duration seemed additive.**
Conclusions: **Women with oncogenic HPV and minimally abnormal Papanicolaou smears who smoke were up to three times more likely to be diagnosed with ≥CIN3 than nonsmokers. Smoking cessation trials targeting this population might be warranted.** (Cancer Epidemiol Biomarkers Prev 2005;14(5):1165–70)

Introduction

It is now understood that cervical infections by ~15 human papillomavirus (HPV) types are the necessary but not the sufficient cause of cervical cancer worldwide (1). HPV infection is an extremely common sexually transmitted infection (2) that occurs in most young sexually active women, only a small percentage of these infection go on to develop cervical cancer or its immediate precursor, cervical intraepithelial neoplasia grade 3 (CIN3; ref. 3). Multiple epidemiologic studies have identified secondary risk factors (HPV cofactors) that are associated with the development of CIN3 or cancer among cancer-associated (oncogenic) HPV infected women, including long-duration oral contraceptive use (4, 5), multiparity (5, 6), smoking (5, 7-9), host immune function (10), and possibly non-HPV sexually transmitted infections (11, 12). Smoking is of particular interest as a HPV cofactor because of the following reasons: (a) the consistency and strength of the association of smoking with ≥CIN3, (b) the biological plausibility including the observation of nicotine derived carcinogens in cervical mucus after smoking, and (c) the potential to modify smoking behaviors.

To examine the association of smoking in the development of CIN3 in young women, we undertook an analysis of oncogenic HPV DNA positive women with minimally abnormal Papanicolaou (Pap) tests recruited into the atypical

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squamous cells of unknown significance (ASCUS) low-grade squamous intraepithelial lesion (LSIL) Triage Study (ALTS; refs. 13-17), a 2-year randomized prospective trial to evaluate clinical management strategies. ALTS included thorough disease and HPV assessment based on intensive follow-up of patients, rigorous pathology review, and dual HPV DNA testing.

Materials and Methods

Study Design and Population. ALTS was a randomized trial conducted by the National Cancer Institute (NIH, Rockville, MD) comparing three triage strategies for women with ASCUS or LSIL; details of the design, methods, and primary results of ALTS have been published elsewhere (13-17). Briefly, women with ASCUS or LSIL cytology were recruited to participate in the study at four clinical centers: University of Alabama at Birmingham (Birmingham, AL), Magee-Womens Hospital of the University of Pittsburgh Medical Center Health System (Pittsburgh, PA), the University of Oklahoma (Oklahoma City, OK), and the University of Washington (Seattle, WA). The National Cancer Institute and local institutional review boards approved the study. A total of 5,060 women who were eligible and provided informed consent were enrolled in the study from November 1996 to December 1998: 3,488 women with ASCUS cytology (mean age = 28.8 years, median age = 26 years, age range = 18-81 years) and 1,572 with LSIL cytology (mean age = 24.8 years, median age = 23 years, age range = 18-68 years). Routine follow-up visits were scheduled every 6 months for the 2-year duration of the study. Women exiting the study underwent a colposcopic evaluation; >80% of women underwent an exiting exam and a colposcopic evaluation. Routine follow-up and exit visits concluded in January 2001.

At enrollment, women in each arm received the same enrollment pelvic examination with collection of two cervical specimens, the first in PreservCyt for ThinPrep cytology (Cytyc Corp., Boxborough, MA) and the second in specimen transport medium (Digene Corp., Gaithersburg, MD). Each ALTS participant was interviewed at enrollment and follow-up to collect information on demographic, lifestyle, and medical history. We refer readers to other references for details on randomization, examination procedures, patient management, and laboratory and pathology methods (13, 14, 17).

HPV DNA Testing. Hybrid Capture 2 (Digene) using the probe set B (henceforth, called HC2) is a DNA test for 13 oncogenic HPV types. HC2 relies on the formation of target HPV DNA-RNA probe heteroduplexes during the hybridization step in specimens positive for one or more oncogenic HPV types (HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV68), and the chemiluminescence detection of these hybrids by using an alkaline phosphatase-conjugated monoclonal antibody specific to DNA-RNA complexes with dioxetane substrate in a 96-well ELISA format. After liquid-based, ThinPrep (Cytyc) cytology slides were prepared, 4-mL aliquots of the residual in the PreservCyt vials were used for HPV DNA testing by HC2. Signal strengths in relative light units were compared with 1 pg/mL HPV type 16 DNA positive controls (relative light units/PC). The Food and Drug Administration–approved 1.0 relative light units/PC (~1 pg/mL) was used as the threshold for a positive result (18). Of the 5,060 women enrolled into ALTS, we had valid HC2 results on 4,819 (95.2%).

We also used L1 consensus primer PGMY09/11 PCR amplification and reverse line blot hybridization for type-specific detection (19) on cervical specimens collected into specimen transport medium (Digene) from each patient. Specimens were thawed, and one aliquot (150 μL) was digested by adding 7.5 μL of digestion solution [20 mg/mL proteinase K, 10% laureth-12, 20 mmol/L Tris, and 1 mmol/L EDTA (pH 8.5)] and incubating at 60°C for 1 hour. DNA from a 150-μL aliquot of the digested material was precipitated by adding 1.0 mL of absolute ethanol containing 0.5 mol/L ammonium acetate, incubating the mixture overnight at −20°C, and centrifuging for 30 minutes at 13,000 × g. The supernatant was discarded immediately, and the crude DNA pellet was dried overnight at room temperature. The pellet was resuspended in 50 μL of 20 mmol/L Tris and 1 mmol/L EDTA (pH 8.5).

We amplified 5 μL of each sample by using the PGMY09/11 L1 consensus primer system and AmpliTaq gold polymerase (Perkin-Elmer, Wellesley, MA). Amplifications were done in a thermal cycler (model 9600; Perkin-Elmer) using the following algorithm: 9-minute AmpliTaq gold activation at 95°C followed by 40 cycles of 1-minute denaturation at 95°C, 1-minute annealing at 55°C, and 1-minute extension at 72°C, and a 5-minute final extension at 72°C.

Reverse line blotting using HPV genotyping strips (Roche Molecular Systems, Alameda, CA) was used to detect 27 HPV genotypes [HPV6, HPV11, HPV16, HPV18, HPV26, HPV31, HPV33, HPV35, HPV39, HPV40, HPV42, HPV45, HPV51-59, HPV66, HPV68, HPV73 (PAP238A), HPV82 (W13B), HPV83 (PAP291), and HPV84 (PAP155)] and a β-globin internal control. For 2,857 women, we tested for 11 additional nononcogenic genotypes HPV61, HPV62, HPV64, HPV67, HPV69-72, HPV81, HPV82v (IS39), HN89 (CP6108). Of the 5,060 women enrolled into ALTS, we had valid PCR tests on 4,915 (97.1%).

HPV Classification. Using both HC2 and PCR data, we classified HPV DNA status as positive or negative for oncogenic types (20): oncogenic HPV positive if positive by HC2 or by PCR for HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, or HPV68; otherwise, negative for oncogenic HPV. Among the women negative for oncogenic HPV, we classified as nononcogenic HPV positive those who had a positive PCR result for any HPV type other than the 13 oncogenic types listed above. We conservatively reclassified women (n = 202) as having a nononcogenic HPV type if they were HC2 positive but PCR negative for oncogenic types and positive for either HPV6, HPV53, HPV66, HPV67, HPV70, and HPV81, recognizing that HC2 occasionally cross-reacts with these types especially in cervical specimens from women with cytologic abnormalities (21). Of the 5,060 women enrolled into ALTS, 5,052 (99.8%) women had at least one test result and 4,682 (92.5%) had both tests; women with only one HPV test result were classified accordingly using the results available.

Pathology. Clinical management was based on the clinical center pathologists' cytologic and histologic diagnoses. In addition, all referral smears, ThinPreps, and histology slides were sent to the Pathology QC group (QC pathology) based at the Johns Hopkins Hospital for review and secondary diagnoses.

Our outcome of interest was defined as ≥CIN3, including histologic CIN3 and the very few (n = 7) cases of cancer cumulatively detected either at enrollment or during the 2-year follow-up as diagnosed by the QC pathology review. That is, we treated all cases diagnosed during the duration of ALTS as prevalent. We used this rigorous definition of cases in recognition that CIN3 detected within 2 years of an HPV DNA positive test is more likely to be a missed prevalent case than a true incident case, given that a single colposcopic evaluation with biopsy and histologic evaluation is not perfectly sensitive for detection of CIN3 and cancer (15), and CIN3 rarely develops from an HPV infection within 2 years. In contrast,

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CIN2 is a poorly reproducible diagnosis (22) that may represent an admixture of CIN1 and CIN3. We therefore included CIN2 into the multivariate models (described below) as an intermediate outcome, excluded from the primary case definition (CIN3 including the few cancers) and from controls (women with oncogenic HPV and <CIN2), thereby creating a conceptual ''buffer zone'' between infection and CIN3. In this analysis restricted to women who were positive for oncogenic HPV at enrollment (n = 3,133), 506 of 542 (93.4%) ≥CIN3 and 361 of 397 (90.9%) CIN2 diagnosed in ALTS were included, demonstrating the extraordinarily strong relationship between oncogenic HPV detection and diagnoses of ≥CIN2 (i.e., 7.7% of ≥CIN2 detected over 2 years were HPV DNA negative at enrollment).

Analysis. Standard contingency table methods, with Pearson χ^2 tests or, when appropriate, the Mantel extension test for trend, were used to assess the following: (a) possible associations of categorical variables with being oncogenic HPV DNA positive at enrollment in controls [i.e., <CIN2; n = 3,710 women; n = 2,366 (55.1%) with oncogenic HPV] and (b) possible associations of categorical variables with having a CIN2 or ≥CIN3 diagnosis (versus <CIN2) among oncogenic HPV DNA positive women. Odds ratios (OR) and 95% confidence intervals (95% CI) adjusted for relevant variables (e.g., identified as part of preliminary data analysis) were determined with stepwise logistic regression for detection of oncogenic HPV DNA among controls with stepwise multinomial logistic regression modeling for ≥CIN3 and CIN2 compared with controls. Smoking behaviors at enrollment were classified as smoking status (never, former, and current), smoking intensity (never, former, current using <1 pack/d, current using 1 to <2 packs/d, and current using ≥2 packs/d), and smoking duration (never, former, current using <6 years, current using 6 to <10 years, and current using ≥10 years). Final models to examine the associations of these factors with being HPV DNA positive upon enrollment among women with <CIN2 adjusted for age (18-19, 20-24, 25-29, 30-34, and ≥35 years), recent and lifetime numbers of sexual partners (0 recent/0-2 lifetime, 0 recent/≥3 lifetime, 1 recent/0-2 lifetime, 1 recent/≥3 lifetime, ≥2 recent/0-2 lifetime, and ≥2 recent/≥3 lifetime), and study center. Other covariates did not appreciably alter the associations of smoking exposures and detection of HPV DNA. Final models to examine the associations of smoking behavior upon enrollment and ≥CIN3 and CIN2 among women who were oncogenic HPV-positive at enrollment included adjustment for education (less than a high school diploma, high school diploma and post high school education less than a college degree, and a college degree or more education) and whether a woman had an HPV16 infection at enrollment. Other variables such as age, number of sexual partners, and reported cofactors such as oral contraceptive use and parity were not included in the final models relating smoking to ≥CIN3 or CIN2 because they were uninformative. Dose-response relationships (P_{Trend}) were assessed in the models by treating ordinal variables as continuous (which assumes a linear trend). Finally, we examined the interaction of smoking intensity and duration by examining the effect of smoking duration in strata defined by smoking intensity in multivariate models. A likelihood ratio test was done to determine statistically whether the interaction was multiplicative.

Results

In the entire cohort of women with minimally abnormal Paps (n = 5,060), 53% were never smokers, 35% were current smokers, and 12% were former smokers at enrollment. By comparison, among those who were positive for oncogenic HPV DNA (n = 3,131), 52% were never smokers, 39% were current smokers, and 9% were former smokers at enrollment. By comparison, an estimated 22.6% of women in the general U.S. population are smokers, and 35.4% of women with 9 to 11 years of education and 33.3% of women living below the poverty level are smokers (National Heath Interview Survey, 1998, Centers for Disease Control and Prevention, http://www.cdc.gov/nchs/nhis.htm). Thus, the percentage of smokers in this population of women with minimally abnormal Pap smears was above the national average, and 55.3% of women with less than a high school education smoked. In multivariate models adjusting for age, center, and sexual behavior, greater smoking intensity and duration were marginally associated with being oncogenic HPV DNA positive upon enrollment among controls (women without CIN2, CIN3, or cancer; Table 1).

Although we treated all cases of ≥CIN3 diagnosed during enrollment, follow-up, and at exit as prevalent, and therefore smoking behaviors during the follow-up phase of the trial were not considered in these analyses, most women did not change their smoking habits during the study. Of those who self-reported being nonsmokers upon enrollment, 91.7% remained nonsmokers. Of those who self-reported being current smokers at enrollment, 74.4% continued to smoke, 14.4% quit and did not resume smoking, and 11.2% reported mixed patterns of stopping and starting smoking during the study.

Among women with an oncogenic HPV infection at enrollment, self-reported current (OR, 1.7; 95% CI, 1.4-2.1) and past smoking (OR, 1.7; 95% CI, 1.2-2.4) upon enrollment

Table 1. Association of smoking behavior with detection of oncogenic HPV DNA upon enrollment among control women (n = 1,344 HPV-negative women and n = 2,366 oncogenic HPV-positive women)

Smoking exposure	OR* (95% CI)
Never	1.0 (reference)
Former	**0.6 (0.5-0.8)**
Current	1.2 (1.0-1.4)
P_{trend}	0.2
Never	1.0 (reference)
Former	**0.6 (0.5-0.8)**
Current, <1 pack/d	1.1 (0.9-1.3)
Current, 1 to <2 packs/d	**1.4 (1.0-1.8)**
Current, ≥2 packs/d	1.6 (0.7-4.0)
P_{trend}	0.06
Never	1.0 (reference)
Former	**0.6 (0.5-0.8)**
Current, <6 y	1.0 (0.8-1.4)
Current, 6-10 y	1.1 (0.8-1.4)
Current, ≥11 y	**1.3 (1.0-1.7)**
P_{trend}	0.04
Never	1.0 (reference)
Former	**0.6 (0.5-0.8)**
Current, <1 pack/d	
Current, <6 y	0.9 (0.7-1.3)
Current, 6-10 y	1.2 (0.9-1.7)
Current, ≥11 y	1.1 (0.8-1.6)
Current, 1 to <2 packs/d	
Current <6 y	1.9 (0.9-3.9)
Current, 6-10 y	0.8 (0.5-1.4)
Current, ≥11 y	**1.5 (1.1-2.2)**
Current, ≥2 packs/d	
Current, <6 y	NA
Current, 6-10 y	1.3 (0.0-11.0)
Current, ≥11 y	1.7 (0.6-4.4)

NOTE: ORs with 95% CI from a multivariate logistic regression model comparing oncogenic HPV-positive versus HPV-negative women. Values in bold indicate ORs for which the lower or upper confidence bound does not include 1.00. Restricted to women with a diagnosis of <CIN2 during the 2-year study period.

Abbreviation: NA, not applicable.

*Adjusted for age, study center, and recent and lifetime numbers of sexual partners (0 and 0-2, 0 and ≥3, 1 and 0-2, 1 and ≥3, ≥2 and 0-2, ≥2 and ≥3).

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were equally associated with a diagnosis of ≥CIN3 at any time during the study (upon enrollment, during follow-up, or at the exit colposcopy) compared with women who never smoked. These estimates took into account HPV risk stratification (i.e., being DNA positive for HPV16 infection) and educational status (Table 2). Past smoking was only marginally associated with a CIN2 diagnosis (OR, 1.5; 95% CI, 1.0-2.1) and current smoking was not associated with a CIN2 diagnosis (OR, 1.2; 95% CI, 0.95-1.6).

Among women who self-reported to be current smokers at enrollment, increased smoking intensity and duration both elevated the risk of ≥CIN3. Smokers using <1pack/d (OR, 1.5; 95% CI, 1.2-2.0), 1 to <2 pack/d, (OR, 2.0; 95% CI, 1.4-2.7), and ≥2 packs/d (OR, 3.3; 95% CI, 1.5-7.5) were more likely to be diagnosed with ≥CIN3 than women who never smoked (P_{Trend} < 0.0005). Smokers who smoked for <6 years (OR, 1.3; 95% CI, 0.94-1.7), 6-10 years (OR, 2.0; 95% CI, 1.5-2.8), and ≥11 years (OR, 2.1; 95% CI, 1.5-2.9) were more likely to be diagnosed with ≥CIN3 than women who never smoked (P_{Trend} < 0.0005). Increasing smoking intensity and duration among current smokers were not associated significantly with having a CIN2 diagnosis.

Restricted to women who were HPV16 DNA positive upon enrollment, enrollment smoking status, smoking intensity, and smoking duration was also strongly associated with having ≥CIN3 (Table 3). These estimates did not differ significantly from those for all oncogenic HPV positive women. However, among HPV16 DNA–positive women, current smokers were more likely to have a CIN2 diagnosis (OR, 1.7; 95% CI, 1.1-2.8). However, finer distinctions of exposure (intensity and duration) did not appreciably alter the association of current smoking with CIN2 in HPV16 DNA–positive women.

Finally, we considered the interaction of enrollment smoking intensity and duration among current smokers (Fig. 1). Among women who smoked <1 pack/d, ORs of smoking for <6 years and ≥6 with ≥CIN3 were 1.2 (95% CI, 0.89-1.7) and 1.8 (95% CI, 1.4-2.5), respectively, compared to nonsmokers. Among women who smoked ≥1 packs/d, ORs of smoking for <6 years and ≥6 with ≥CIN3 were 1.5 (95% CI, 0.89-2.6) and 2.3 (95% CI, 1.6-3.2), respectively, compared to nonsmokers. The likelihood ratio test for a multiplicative interaction between intensity and duration was not significant (P = 0.8).

Discussion

We found enrollment smoking behaviors were strongly associated with a diagnosis of CIN3 or cancer in women with minimally abnormal Pap smears and oncogenic HPV upon enrollment who were participating in ALTS. Greater smoking intensity and duration increased the risk of ≥CIN3, with a possible additive effect of the two. Intense and long-duration smoking was related to being HPV DNA positive among controls; by restricting this analysis to oncogenic HPV DNA–positive women, we attempted to minimize confounding by HPV status. The strength of our analysis lay in the large numbers of outcomes, rigorous pathology review including a 2-year follow-up ascertainment to capture missed disease, and dual HPV testing to minimize misclassification. Limiting this analysis to just cases detected at enrollment did not significantly alter these observations (data not shown). These data are consistent a few other sizeable studies that have shown an association of smoking with CIN3 and cancer among women with oncogenic HPV DNA (7-9). We infer from our data that smoking in younger women (median age of 25 years, 25-75% interquartile range of 21-31 years, and 90% under the age of 40 years or younger) increases the risk of a precancerous cervical lesion. Although there were very few cancers diagnosed in this study, five of seven women diagnosed with cancer (age range of 23-41 years) were currently smoking at enrollment, suggesting that smoking was also associated with cancer in ALTS, perhaps by first increasing the likelihood of developing CIN3.

Interestingly, we did not find smoking associated with a CIN2 diagnosis among all oncogenic HPV positive women, except in women with HPV16 infections. It is not surprising that the correlation between smoking dose and duration was stronger for women with ≥CIN3 or worse compared with CIN2. Lesions diagnosed as CIN3 are likely to represent true cancer precursors whereas CIN2 lesions are a heterogeneous group of diagnoses, which, in addition to precancer, includes transient productive HPV infection, that looks unusually severe. Moreover, the interobserver variation for diagnosing CIN2 is significantly greater than for diagnosing CIN3 (22), further suggesting that CIN2 is subject to greater misclassification and is more heterogeneous in nature than CIN3. For these analyses, we adopted the a priori strategy of treating CIN2 as a separate outcome from ≥CIN3 rather

Table 2. Association of smoking habits with CIN2 and ≥CIN3 among oncogenic HPV DNA–positive women

		<CIN2	CIN2*		≥CIN3*	
	n (%)	*n* (%)	*n* (%)	OR† (95% CI)	*n* (%)	OR† (95% CI)
Never	1,623 (52)	1,255 (56)	173 (48)	1.0 (reference)	195 (39)	1.0 (reference)
Former	290 (9)	195 (9)	40 (11)	**1.5 (1.0 -2.1)**	55 (11)	**1.7 (1.2 -2.4)**
Current	1,212 (39)	809 (36)	147 (41)	1.2 (1.0 -1.6)	256 (51)	**1.7 (1.4 -2.1)**
P_{Trend}						**<0.0005**
Never	1,623 (52)	1,255 (56)	173 (48)	1.0 (reference)	195 (39)	1.0 (reference)
Former	290 (9)	195 (9)	40 (11)	**1.5 (1.0 -2.1)**	55 (11)	**1.7 (1.2 -2.4)**
Current, <1 pack/d	804 (26)	560 (25)	93 (26)	1.1 (0.9 -1.5)	151 (30)	**1.5 (1.2 -2.0)**
Current, 1 to <2 packs/d	376 (12)	231 (10)	52 (14)	**1.4 (1.0 -2.0)**	93 (18)	**2.0 (1.4 -2.7)**
Current, ≥2 packs/d	21 (1)	18 (1)	2 (1)	0.7 (0.2 -2.9)	12 (2)	**3.3 (1.5 -7.5)**
P_{Trend}						**<0.0005**
Never	1,623 (52)	1,255 (56)	173 (48)	1.0 (reference)	195 (39)	1.0 (reference)
Former	290 (9)	195 (9)	40 (11)	**1.5 (1.0 -2.1)**	55 (11)	**1.7 (1.2 -2.4)**
Current, <6 y	509 (16)	358 (16)	62 (17)	1.1 (0.8 -1.6)	89 (18)	1.3 (0.9 -1.7)
Current, 6-10 y	374 (12)	242 (11)	41 (11)	1.1 (0.8 -1.7)	91 (18)	**2.0 (1.5 -2.8)**
Current, ≥11 y	328 (10)	209 (9)	43 (12)	1.4 (1.0 -2.0)	76 (15)	**2.1 (1.5 -2.9)**
P_{Trend}						**0.0005**

NOTE: ORs with 95% CI from multinomial logistic regression models comparing women with a CIN2 or ≥CIN3 diagnosis to women with a <CIN2 diagnosis. Values in bold indicate ORs for which the lower or upper confidence bound does not include 1.00. Includes all women who were oncogenic HPV-positive upon enrollment (i.e. women with all diagnoses: <CIN2, CIN2, ≥CIN3) during the 2-year study period.

*Includes all cases diagnosed upon enrollment, during the 2-year follow-up, and at the exit colposcopy.

†Adjusted for HPV16 DNA positivity and education.

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Table 3. Association of smoking habits with CIN2 and ≥CIN3 among HPV16 DNA–positive women

		<CIN2	CIN2*		≥CIN3*	
	n (%)	*n* (%)	*n* (%)	OR† (95% CI)	*n* (%)	OR† (95% CI)
Never	365 (44)	221 (52)	42 (39)	1.0 (reference)	102 (35)	1.0 (reference)
Former	90 (11)	46 (11)	15 (14)	1.7 (0.9 −3.2)	29 (10)	1.4 (0.8 -2.3)
Current	373 (45)	161 (38)	52 (48)	**1.7 (1.1 −2.8)**	160 (55)	**2.0 (1.4 -2.8)**
P_{Trend}						**<0.0005**
Never	365 (44)	221 (52)	42	1.0 (reference)	102 (35)	1.0 (reference)
Former	90 (11)	46 (11)	15	1.7 (0.9 −3.2)	29 (10)	1.4 (0.8 -2.3)
Current, <1 pack/d	227 (27)	101 (24)	31	1.6 (1.0-2.8)	95 (33)	**1.9 (1.3 -2.8)**
Current, 1 to <2 packs/d	136 (16)	57 (13)	20	**1.9 (1.0 −3.5)**	59 (20)	**2.0 (1.3 -3.2)**
Current, ≥2 packs/d	10 (1)	3 (1)	1	2.0 (0.2 −20.0)	6 (2)	3.7 (0.9 -15.0)
P_{Trend}						**<0.0005**
Never	365 (44)	221 (52)	42 (39)	1.0 (reference)	102 (35)	1.0 (reference)
Former	90 (11)	46 (11)	15 (14)	1.7 (0.9 -3.2)	29 (10)	1.4 (0.8 -2.3)
Current, <6 y	164 (20)	78 (18)	26 (24)	1.8 (1.0 -3.1)	60 (21)	**1.5 (1.0 -2.3)**
Current, 6-10 y	117 (14)	47 (11)	15 (14)	1.8 (0.9 -3.5)	55 (19)	**2.4 (1.5 -3.8)**
Current, ≥11 y	92 (11)	36 (8)	11 (10)	1.6 (0.8 -3.5)	45 (15)	**2.5 (1.5 -4.1)**
P_{Trend}						**<0.0005**

NOTE: ORs with 95% CI from multinomial logistic regression models comparing women with a CIN2 or ≥CIN3 diagnosis to women with a <CIN2 diagnosis. Values in bold indicate ORs for which the lower or upper confidence bound does not include 1.00. Includes all women who were HPV16-positive upon enrollment (i.e., women with all diagnoses: <CIN2, CIN2, ≥CIN3) during the 2-year study period.
*Includes all cases diagnosed at enrollment, during the 2-year follow-up, and at the exit colposcopy.
†Adjusted for education.

than grouping them together. Our results indirectly support the distinction of CIN2 from CIN3 and the classification as CIN3 as a cervical cancer precursor. Among HPV16 positive women, smoking was associated with a CIN2 diagnosis, perhaps suggesting that HPV16-positive CIN2 may be more likely to be precancer. However, it is noteworthy that there were similar associations with CIN2 for any category of smoking exposure. Thus, associations of smoking with CIN2 diagnoses among HPV16 positive may be the consequence of the selection bias for the study, recruiting women with evidence of equivocal or mildly abnormal cytology.

It seems that smoking affects the interaction between the virus and the host in some manner that increases the likelihood of premalignant change but the exact biological mechanism of this interaction is uncertain. Given the presence of smoke carcinogenic metabolites in cervical secretions, smoking could increase the risk of CIN3 either by increasing the chance of viral persistence via immune modulation (23, 24) or of genomic damage via genotoxins (5, 25). Increased risk may be the result of ''gene-environment'' interactions of genotoxic smoking metabolites

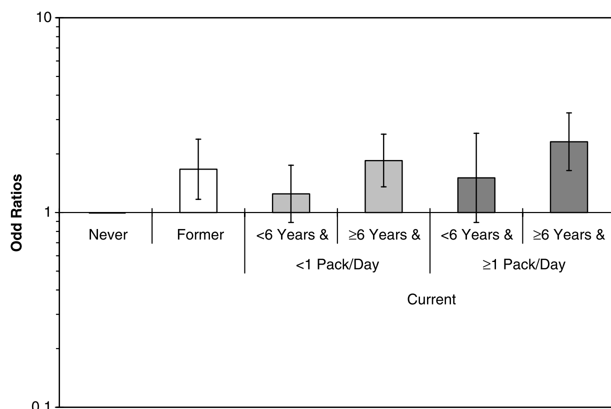

Figure 1. Results of multinomial logistic regression for the association of smoking duration (<6 and ≥6 years) and intensity (<1 and ≥1 packs/d) and ≥CIN3 among women positive for oncogenic HPV DNA, adjusted for HPV16 DNA positivity and education. *Bars,* 95% CIs.

and the inherited ability to detoxify them via metabolic pathways (26). Increased HPV prevalence among the most intense and longest duration current smokers is consistent with smoking-mediated immune modulation. Future analyses in ALTS will examine the relationship of smoking and viral persistence.

We note that women in ALTS who self-reported they were currently smoking at each visit were almost twice (20.8%) as likely to be lost to follow-up (i.e., did not have an exiting colposcopy) compared with those that never smoked (12.8%). Smokers might be more likely than nonsmokers to develop invasive cervical cancer from a precancerous lesion because of poorer participation in screening programs.

In ALTS, women with ASCUS cytology and a positive HC2 test (n.b., a group of women who will be readily identified as HPV DNA testing is integrated in to cervical cancer screening programs) or had LSIL who smoked were almost twice as likely to have ≥CIN3 (19.9%) than women who do not smoke (11.3%). Even successful treatment of CIN2 and CIN3 is not completely benign. These women are more likely to undergo ablative treatments (e.g., loop electrosurgical excision procedure), which have been associated with premature rupture of membranes and preterm delivery (27).

In summary, we have shown that smoking in women with oncogenic HPV and minimally abnormal Pap smears is associated with the development of CIN3, confirming in a high HPV prevalence population that smoking is an important secondary risk factor to oncogenic HPV infection. In addition to the other widely recognized negative health consequences of smoking (28), the clinical effect of smoking on the development of CIN3 and cervical cancer merits consideration. Whether closer surveillance of smokers in cervical screening program is warranted is unclear but perhaps it is warranted to counsel ASCUS/HC2 (oncogenic HPV) positive or LSIL women who have not developed treatable lesions to abstain from smoking and to encourage those who do smoke to participate in smoking cessation programs.

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