

# An Association of Cervical Inflammation with High-Grade Cervical Neoplasia in Women Infected with Oncogenic Human Papillomavirus (HPV)<sup>1</sup>

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## Abstract

Previous reports of genital conditions, such as nonspecific genital infection/sore or vaginal discharge associated with cervical cancer (L. A. Brinton *et al.*, *J. Natl. Cancer Inst. (Bethesda)*, 79: 23–30, 1987; C. J. Jones *et al.*, *Cancer Res.*, 50: 3657–3662, 1990), suggest a possible link between either genital tract inflammation or changes in bacteria flora consistent with bacterial vaginosis (BV) and cervical cancer. To test whether changes in vaginal bacterial flora or the degree of cervical inflammation are associated with women having a human papillomavirus (HPV) infection or with women infected with oncogenic HPV having high-grade cervical lesions (high-grade squamous intraepithelial lesions or cancer), we conducted a case-control study of women <50 years old enrolled in the Costa Rican natural history study of HPV and cervical neoplasia. To test whether BV and inflammation were associated with HPV DNA positivity, Analysis 1 was restricted to women with no or mild (low-grade or equivocal) cytological abnormalities, and the degree of inflammation and Nugent score (a measure of BV) were compared between women infected ( $n = 220$ ) and not infected ( $n = 130$ ) with HPV. To test whether BV and inflammation were associated with high-grade lesions,

Analysis 2 was restricted to women infected with oncogenic HPV, and the degree of inflammation and Nugent score were compared between women with ( $n = 95$ ) and without ( $n = 158$ ) high-grade cervical lesions. In Analysis 1, BV and cervical inflammation were not associated with HPV infection. In Analysis 2, BV was not associated with high-grade lesions. However, we found a marginally significant positive trend of increasing cervical inflammation associated with high-grade lesions in oncogenic HPV-infected women, ( $P_{\text{trend}} = 0.05$ ). Overt cervicitis was associated with a 1.9-fold increase in risk of high-grade lesions (95% confidence interval, 0.90–4.1). The results of this study suggest that cervical inflammation may be associated with high-grade lesions and may be a cofactor for high-grade cervical lesions in women infected with oncogenic HPV.

## Introduction

Laboratory and epidemiological investigations have strongly implicated HPV<sup>4</sup> as the central cause of cervical cancer. Indeed, recent evidence suggests that HPV DNA can be detected in most (1), if not virtually all (2), cervical cancers. However, few HPV infections progress to cervical cancer; the vast majority cause no or only mild cytological abnormalities that may go undetected and subsequently regress to normalcy.

Although the causal role of HPV in cervical cancer has been established, the factors that determine whether an HPV infection will resolve to normalcy or progress to high-grade lesions (HSILs or cervical cancer) is incompletely understood. To address this question, we have recently analyzed data from the enrollment phase of a large, population-based cohort in Guanacaste, Costa Rica (3), in a search for non-HPV factors associated with HSILs and cancer. In HPV-infected women, smoking, parity, and oral contraceptive use in women with <3 pregnancies were associated with high-grade lesions (4).

STIs other than HPV have been proposed as etiological cofactors for cervical cancer, but no single agent has been shown to be consistently important (5, 6). In the absence of a specific pathogen, BV and cervical inflammation are intriguing as possible cofactors for high-grade lesions in HPV-infected women. Epidemiological studies of cervical cancer have focused on behavioral variables such as “female hygiene” (7) and douching (7–9) that might be related to genital infections. Earlier reports of nonspecific genital infection/sore associated with cervical cancer (10, 11) suggested a link between genital tract inflammation and cervical cancer, although neither study

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<sup>4</sup> The abbreviations used are: HPV, human papillomavirus; BV, bacterial vaginosis; HSIL, high-grade squamous intraepithelial lesion; STI, sexually transmitted infection; OR, odds ratio; CI, confidence interval; IL, interleukin.

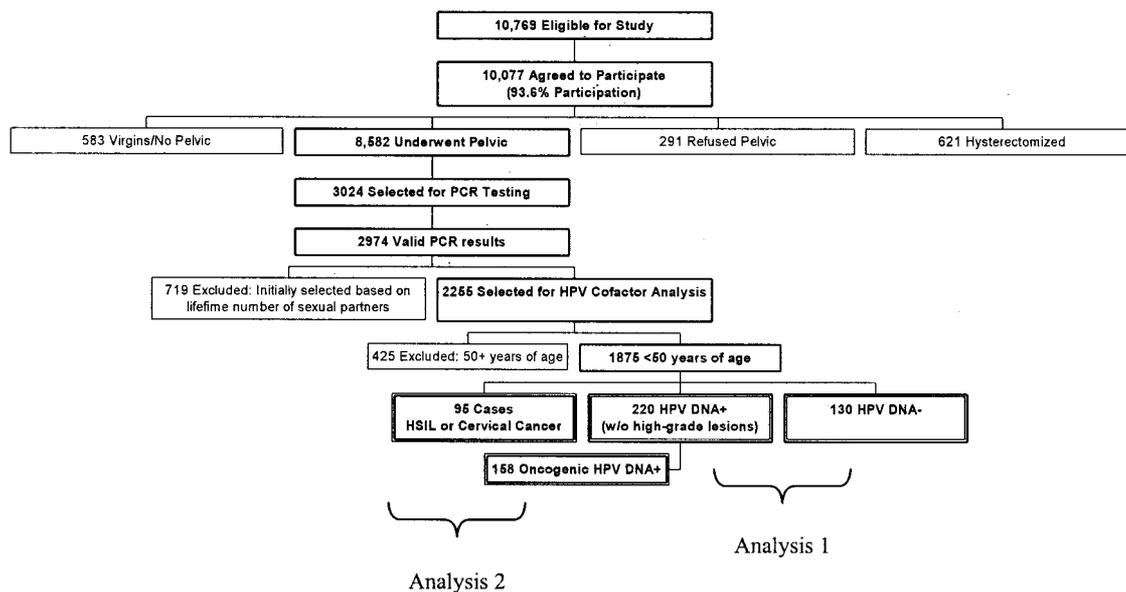


Fig. 1. Summary of study subject selection.

controlled for HPV. An association of self-reported abnormal vaginal discharge with cervical intraepithelial neoplasia (CIN) grade 1 in HPV-infected women (12) further suggests a link between genital tract anomalies and cervical cancer. A recent study with small numbers of women found elevated levels of inflammatory cytokines IL-6 and IL-8 in cervicovaginal lavages associated with cervical cancer, and elevated levels of IL-6 associated with cervical intraepithelial neoplasia (13). That inflammation has also been implicated as a risk factor for many cancers including squamous cell carcinomas (14), the type of carcinoma most commonly induced by HPV, supports the plausibility of cervical inflammation as a risk factor for cervical cancer in HPV-infected women.

In this study, BV and cervical inflammation were evaluated in enrollment Pap smears both for their association with HPV infection (Analysis 1) and with high-grade lesions in oncogenic HPV-infected women (Analysis 2).

The main clinical manifestation of BV is malodorous, thin homogenous, white, uniformly adherent vaginal discharge (15). For the purposes of this study, BV was defined as a change in vaginal microflora from predominately *Lactobacilli* to predominately anaerobic bacteria not normally present in high numbers in the lower genital tract. Cervical inflammation was defined as the increase in the average number of neutrophils in a microscope field.

## Materials and Methods

**Study Population.** A National Cancer Institute (NCI)-sponsored, NCI- and local IRB-approved population-based cohort of women in Guanacaste, Costa Rica was established in 1993–1994 for natural history studies of HPV and cervical cancer (Fig. 1; Refs. 3, 16). Briefly, 10,738 consenting women of the 11,742 women identified in a door-to-door survey residing in randomly chosen censal segments in Guanacaste were enrolled; 10,049 women (94%) participated in the enrollment interview. A final cohort of 8582 women was established after supplementing the number of cancer cases in the random sample by including all living women of Guanacaste diagnosed with cer-

vical cancer ( $n = 28$ ) and excluding women from this study based on previously published criteria (3, 16).

**Data and Specimen Collection.** A risk factor questionnaire that collected information on sociodemographic characteristics, sexual and reproductive practices, and cigarette smoking was administered to all of the subjects. During the pelvic exam for each participant, exfoliated cervical cells for Pap smear and ThinPrep cytology (Cytoc Corp., Boxborough, MA) were collected using a Cervex brush (Unimar, Wilton, CT). Additional ectocervical and endocervical cells were collected using a Dacron swab and stored in Specimen Transport medium (Digene, Silver Spring, MD) for HPV testing. Cervigrams were taken that were later developed at National Testing Laboratories (Fenton, MO) and interpreted by expert colposcopists.

All cervical abnormalities identified by visual inspection, cytology, or cervicography were referred to colposcopy. Visible lesions identified by colposcopy were biopsied. A final diagnosis was assigned to each woman based on a review of cytology, cervigram, and histology: 168 women had high-grade lesions (40 with invasive cancer), 189 had low-grade lesions, 661 had equivocal lesions, and 7564 were judged normal (4).

**HPV DNA Testing.** On enrollment, cervical cell samples from each participant were assayed for HPV DNA using the Hybrid Capture Tube test (Digene Corp., Silver Spring, MD; Ref. 17). A subset of 3024 (of 8582) women were reassayed for HPV DNA using a more sensitive PCR-based L1 consensus primer HPV test (18, 19) because they had either an abnormal screening test ( $n = 1702$ ), a positive Hybrid Capture Tube test ( $n = 303$ ), were a random sample of the cohort ( $n = 295$ ), or were selected on a basis of their sexual behavior (4, 16). These women were selected for enrollment PCR testing because they were at the highest potential of developing cervical neoplasia during the follow-up phase of the study (16). Valid PCR results were obtained from 2974 samples; for this study, we used PCR results from 2255 women, excluding those women who were selected for PCR testing based exclusively on their sexual behavior to avoid biasing our analysis for sexually related

factors, such as sexually transmitted diseases, that might cause inflammation.

Proteinase K-digested exfoliated cervical cell specimens were tested for HPV by PCR using MY09/MY11 L1 consensus primers with primers for  $\beta$ -globin as the internal PCR control (16). Amplified DNA was separated by electrophoresis, transferred to a nylon membrane, and HPV DNA was detected by hybridization using radiolabeled generic probes for HPV. Fifty samples were invalid and excluded from analysis because of the absence of hybridization signal for both the generic HPV probe and the  $\beta$ -globin probe.

Specimens that were HPV positive by the generic probe were tested for specific HPV types by hybridization. Specific oligonucleotides were used to probe for 44 HPV types. Women were classified as infected with high-risk (oncogenic) types if the PCR test was positive for any of the most common 13 cancer-associated HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, or 68; Ref. 16). Women who were infected by other HPV types or were positive by the generic probe but negative for all specific probes were considered infected by low-risk (nononcogenic) types.

**Selection of Cases and Controls.** At and near the onset of menopause, reproductive tracts of women begin to atrophy, and levels of glycogen in the vaginal epithelium decrease significantly (20). In the absence of glycogen as a carbon source, the cervicovaginal environment is no longer able to support the growth of acidifying *Lactobacilli* associated with normal cervicovaginal ecology. In Guanacaste, there was a significant increase in the percentage of women who had elevated pH among older women, especially those women over 50 (21), which suggested decreased numbers of *Lactobacilli*. A feature of BV is decreased numbers of *Lactobacilli* in vaginal microflora (22). Thus, for this prevalent case-control study, we excluded women age 50 years and older from BV testing to minimize misclassification of BV status.

To evaluate associations of BV and cervical inflammation with HPV infection and with high-grade lesions, three study groups of women under the age of 50 years were selected from the group of 2300 tested for HPV by PCR (Fig. 1; Refs. 4, 16). One group consisted of a random sample of HPV DNA-negative women ( $n = 130$ ; 10% of HPV DNA-negative women) with low-grade lesions ( $n = 14$ ), or equivocal or no pathology ( $n = 116$ ). Those HPV DNA negative women who had low-grade lesions may have been misclassified by their cytology or HPV status. However, the results of analyses including these women in the control group were virtually the same as those that excluded them and, thus, they were included. A second group consisted of a random sample of HPV DNA-positive women with low-grade lesions, or equivocal or no pathology ( $n = 220$  with 158 infected with an oncogenic HPV type; 22% of HPV DNA-positive women and 21% of oncogenic HPV DNA-positive women). A final group of women consisted of all available cases of high-grade lesions with a Pap slide and an HPV PCR result ( $n = 95$ ; 65% of high-grade disease), 9 of whom were diagnosed with cervical cancer.

**Evaluation of Bacterial Morphotypes.** Each Pap slide was assigned a score of 0 to 10 using a standardized scoring system (Nugent score) based on the relative proportion of bacterial morphotypes, which has been previously shown to be a reliable test for BV (23). Briefly, each bacterial morphotype, large gram-positive rods (*Lactobacillus*), small gram-negative or variable rods (*Gardnerella vaginalis* or *Bacteroides*), or curved gram-negative or variable rods (*Mobiluncus*), were quantified by averaging the number of organisms counted in 10 noncon-

secutive microscopy fields at  $\times 1000$ . A composite score was assigned based on the number of each bacterial morphotype, with a score of 0 corresponding to vaginal flora dominated by *Lactobacillus* and a score of 10 corresponding to vaginal flora dominated by *Gardnerella*, *Bacteroides*, or *Mobiluncus*. Those slides without any bacterial morphotypes were assigned a missing score (52 HPV DNA negatives and 105 HPV DNA positives in Analysis 1; 38 cases and 73 controls in Analysis 2). A score of 0–3 was judged as normal (*Lactobacillus*-dominated) flora, a score of 4–6 was judged as intermediate or reduced *Lactobacilli*, and a score of 7–10 was judged as BV-dominated.

**Evaluation of Cervical Inflammation.** Cervical inflammation was assessed by counting the number of neutrophils observed in microscope fields on Pap slides from each study subject. Enrollment Pap-stained smears were evaluated at  $\times 400$  initially to identify cervical mucus. Smears having no identifiable cervical mucus were considered invalid for evaluation ( $n = 1$ , a control in Analysis 2) because of uncertainties related to sampling adequacy. Valid slides were observed at  $\times 1000$  to identify neutrophils, identifiable by their multilobed nuclei. The numbers of neutrophils were counted in five nonadjacent fields not contaminated by squamous epithelial cells, and were averaged and scored as no inflammation (0–5 neutrophils/field), intermediate inflammation (6–30 neutrophils/field), and cervicitis ( $>30$  neutrophils/field). Also, no- and intermediate-inflammation levels were combined to create a binary variable (positive/negative) for cervicitis.

Importantly, to minimize bias, readings of Pap slides for cytopathology were done independently (M. E. S., M. L. H.) of the blinded assessments for Nugent score/neutrophils by collaborators (L. K. R., S. L. H.) who are not trained to evaluate cytopathology of cervical cells.

**Statistical Methods.** As an estimate of relative risk, ORs and corresponding 95% CIs were calculated using unconditional logistic regression analysis. To test for statistically significant dose-response relationships, Nugent score and inflammation levels were treated as continuous variables and tested as to whether the resulting  $\beta$  coefficient was non-zero.

First, we used standard contingency table methods and logistic regression to explore associations of sociodemographic, reproductive, and smoking variables with Nugent score and cervical inflammation levels.

Two main analyses were then performed using unconditional logistic regression (Fig. 1): estimating the association of BV and inflammation with HPV infection (Analysis 1) and estimating the association of BV and inflammation with high-grade lesions in oncogenic HPV-infected women (Analysis 2). Analysis 1 was restricted to women with low-grade lesions, or equivocal or no pathology. Women who were HPV DNA positive ( $n = 220$ ) were compared with women who were HPV DNA negative ( $n = 130$ ). The adjusted model in Analysis 1 included the age at first intercourse ( $<16$ , 16–19, 20+ years old) and the number of sexual partners (0–1, 2–3, 4+ partners), which are potential confounding variables for the risk of infection. Analysis 2 was restricted to women positive for oncogenic HPV to remove potential residual confounding and to evaluate only those women who were truly at risk for cervical cancer by virtue of having been infected with oncogenic HPV. Women who had a high-grade lesion ( $n = 95$ ) were compared with women who had less severe pathology ( $n = 158$ ). Nine cases of high-grade lesions that were HPV DNA negative were considered false negatives and treated as positives in our analysis (2). The adjusted model in Analysis 2 included age ( $<25$ , 25–30, 30–44, 45–49 years old), number of pregnancies (0–1, 2–3,

Table 1 Sociodemographics of women analyzed in Analysis 1 and Analysis 2

	Analysis 1			Analysis 2 <sup>a</sup>			Analysis 2 <sup>a,c</sup>		
	HPV <sup>-</sup>	HPV <sup>+</sup>	<i>P</i>	Controls	Cases <sup>b</sup>	<i>P</i>	Controls	Cases <sup>c</sup>	<i>P</i>
	<i>n</i> = 130	<i>n</i> = 220		<i>n</i> = 158	<i>n</i> = 95		<i>n</i> = 85	<i>n</i> = 57	
Age at enrollment (yr)	33.6	30.0	<0.01	29.4	32.2	<0.01	29.0	31.5	<0.01
Education (yr)	8.5	8.8	0.69	8.8	7.7	0.06	8.9	7.7	0.48
Average no. of pregnancies	3.9	3.2	0.03	3.1	3.6	0.07	3.1	3.8	0.18
Age at first intercourse (yr)	18.2	18.1	0.78	17.9	17.4	0.27	17.8	17.6	0.22
Average no. of sex partners, lifetime	3.3	3.3	0.99	3.2	4.5	0.18	2.1	4.1	0.19
% oral contraceptive use	95.4	88.6	0.22	87.8	91.2	0.41	84.5	87.7	0.59
% with yeast infection, ever	36.9	40.0	0.57	39.2	37.9	0.83	40.0	38.6	0.87
% ever smoked	10.0	6.8	0.67	5.7	18.9	<0.01	5.9	24.6	<0.01

<sup>a</sup> Restricted to women infected with oncogenic HPV types (see "Materials and Methods").

<sup>b</sup> Includes nine cases who were HPV DNA-negative.

<sup>c</sup> Excluding those subjects without a BV assessment.

4–5, 6+ pregnancies), and number of daily cigarettes (0, 1–5, 6+ cigarettes). Age is a standard confounding covariate for cancer. Number of pregnancies and number of cigarettes were found to be cofactors for the risk of high-grade lesions, both in the larger population-based study (4) and in this subgroup of women, and, thus, were included in models to eliminate any potential confounding. Oral contraceptive use was not associated with case-control status in this subset of women and did not alter the estimates of association and, therefore, was not included in any of the models.

The crude OR and adjusted OR for either HPV infection or high-grade lesions were nearly identical, regardless of how inflammation was categorized. For simplicity, only the values from the crude models will be discussed in the results unless otherwise noted. However, all of the models are presented in Tables 2–5.

In Analysis 2, controls with prevalent oncogenic HPV infection were chosen and may have biased our risk estimates unpredictably by overrepresentation in our control group of women with either persistent viral infection or recently acquired infections. Therefore, we conducted a parallel analysis that compared cases to all of the cohort non-case members in our population-based study, statistically adjusting for HPV. Results of this analysis did not differ from our main analysis and, therefore, are not presented.

## Results

### Correlates of Nugent Score and Cervical Inflammation.

Increasing vaginal pH (range, 4.0–5.5), measured in increments of 0.5 pH units as described previously (21), was strongly associated with an increase in Nugent score (decrease in *Lactobacilli*). Each 0.5-unit incremental increase in pH was associated with a 12-fold increased risk of reduced *Lactobacilli* (Nugent score, 4–6; OR, 12.2; 95% CI, 3.0–50.4) and a 29-fold increased risk of BV (Nugent score, 7–10; OR, 29.2; 95% CI, 9.6–88.4). No other covariates (*e.g.*, sociodemographic, reproductive, or smoking) were significantly associated with Nugent scores.

Woman who ever had a tubal ligation were less likely to have mild inflammation (OR, 0.31; 95% CI, 0.16–0.59) and cervicitis (OR, 0.22; 95% CI, 0.11–0.43). Woman who ever had a cesarean section were also less likely to have mild inflammation (OR, 0.31, 95% CI, 0.16–0.59) and cervicitis (OR, 0.20; 95% CI, 0.10–0.39). Current users of oral contra-

Table 2 ORs and corresponding 95% CIs for HPV infection associated with Nugent score in women with low-grade lesions, or equivocal or no pathology (Analysis 1)

Nugent score	<i>N</i>	Crude		Adjusted <sup>a</sup>	
		OR	95% CI	OR	95% CI
0–3 (normal)	60/37	1		1	
4–6 (reduced <i>Lactobacilli</i> )	14/9	0.96	0.38–2.4	1.0	0.40–2.7
7–10 (BV)	41/32	0.79	0.43–1.5	0.74	0.39–1.4
		<i>P</i> <sub>Trend</sub> = 0.46		<i>P</i> <sub>Trend</sub> = 0.36	
Unknown status <sup>b</sup>	105/52	1.3	0.73–2.1	1.3	0.74–2.2

<sup>a</sup> Adjusted for age at first intercourse and number of sexual partners.

<sup>b</sup> Estimates for slides for which Nugent score could not be assessed when added to the model as a separate category of exposure.

ceptives were at a nonsignificant increased risk (OR, 1.7; 95% CI, 0.81–3.6) of mild inflammation and at a significant increase risk (OR, 2.9; 95% CI, 1.4–5.9) of cervicitis.

**Analysis 1: Associations with HPV Infection.** Among women without high-grade lesions, HPV DNA-negative women and HPV DNA-positive women were similar in their baseline characteristics except for age at enrollment and average number of pregnancies (Table 1). HPV DNA-negative women were older than HPV DNA-positive women were, 33.6 to 30.0 years. They also averaged more pregnancies, 3.9 *versus* 3.2, primarily as the result of the age differences. However, these covariates were not associated with inflammation and, thus, were not considered further in Analysis 1.

Relative changes in vaginal bacterial morphotypes (normal, reduced *Lactobacilli*, BV, or unknown status) were not associated with HPV infection (*P*<sub>Trend</sub> = 0.46; Table 2). Increasing severity of cervical inflammation (none, mild inflammation, cervicitis) was nonsignificantly, negatively associated with HPV infection (*P*<sub>Trend</sub> = 0.20; Table 3). In stratified analyses, no effect of oral contraceptive use, barrier methods, and number of recent partners was observed on these relationships.

**Analysis 2: Association with HSIL/Cervical Cancer in Oncogenic HPV-infected Women.** Women with high-grade lesions were older yet less educated, had more pregnancies, and were more likely to smoke than women without high-grade lesions (Table 1). Number of pregnancies and number of cigarettes were included in logistic models because previous anal-

**Table 3** ORs and corresponding 95% CIs for HPV infection associated with cervical inflammation in women with low-grade lesions, or equivocal or no pathology (Analysis 1)

Neutrophils/field	N	Crude		Adjusted <sup>a</sup>		N <sup>b</sup>	Crude <sup>b</sup>		Adjusted <sup>a,b</sup>	
		HPV <sup>+</sup> /HPV <sup>-</sup>	OR	95% CI	OR		95% CI	HPV <sup>+</sup> /HPV <sup>-</sup>	OR	95% CI
0–5 (normal)	43/21	1		1		18/7	1		1	
6–30 (mild inflammation)	92/50	0.90	0.48–1.7	0.93	0.49–1.7	47/34	0.54	0.20–1.5	0.49	0.18–1.3
30+ (cervicitis)	84/59	0.70	0.37–1.3	0.70	0.37–1.3	50/37	0.53	0.20–1.4	0.53	0.20–1.4
		<i>P</i> <sub>Trend</sub> = 0.20		<i>P</i> <sub>Trend</sub> = 0.20			<i>P</i> <sub>Trend</sub> = 0.30		<i>P</i> <sub>Trend</sub> = 0.37	
<30 (referent)	135/71	1		1		65/41	1		1	
30+ (cervicitis)	84/59	0.75	0.48–1.2	0.74	0.47–1.2	50/37	0.85	0.48–1.5	0.92	0.50–1.7

<sup>a</sup> Adjusted for age at first intercourse and number of sexual partners.

<sup>b</sup> Excluding those subjects without a BV assessment.

**Table 4** ORs and corresponding 95% CIs for high-grade lesions (cases) associated with Nugent score in oncogenic HPV-infected women (Analysis 2)

Nugent score	N	Crude		Adjusted <sup>a</sup>	
		Cases/Controls	OR	95% CI	OR
0–3 (normal)	33/45	1		1	
4–6 (reduced <i>lactobacilli</i> )	4/11	0.50	0.15–1.7	0.44	0.11–1.7
7–10 (BV)	20/29	0.94	0.46–1.9	0.84	0.37–1.6
		<i>P</i> <sub>Trend</sub> = 0.80		<i>P</i> <sub>Trend</sub> = 0.60	
Unknown status <sup>b</sup>	38/73	0.71	0.42–1.2	0.69	0.40–1.2

<sup>a</sup> Adjusted for age, number of pregnancies, and number of cigarettes.

<sup>b</sup> Estimates for slides for which Nugent score could not be assessed when added to the model as a separate category of exposure.

ysis of our Costa Rican cohort of women suggested that these are risk factors for high-grade lesions (4); age was also included in models.

Relative changes in vaginal bacterial morphotypes (normal, reduced *lactobacilli*, BV, or unknown status) were not associated with high-grade lesions in women infected with oncogenic HPV (Table 4). Increasing severity of cervical inflammation (normal, mild inflammation, cervicitis) was significantly, positively associated with high-grade lesions (*P*<sub>Trend</sub> = 0.05; Table 5), and cervicitis (<30 neutrophils/field) was non-significantly associated with high-grade lesions (OR, 1.9; 95% CI, 0.90–4.1) compared with women without any evidence of cervical inflammation (0–5 neutrophils/field). There was a statistically significant 70% increase of high-grade lesions associated with cervicitis (OR, 1.7; 95% CI, 1.0–2.8) treated as a binary variable.

We also investigated whether BV might be an effect modifier of the association of inflammation with high-grade lesions based on a previous report (24). We did not find that BV modified the association. However, when we stratified analyses by whether or not we could evaluate bacterial morphotypes, the relationship of inflammation and high-grade lesions in women infected with oncogenic HPV differed considerably between those who did not have an assessment (38 cases, 73 controls) versus those women who did (57 cases, 85 controls). In women without a Nugent score, there was no association between cervical inflammation and high-grade lesions. However, in those women with a Nugent score, increasing severity of cervical inflammation was positively associated with high-grade lesions (*P*<sub>Trend</sub> = 0.01 unadjusted; *P*<sub>Trend</sub> = 0.03 adjusted), and cervicitis (<30 neutrophils/field) was strongly associated with high-grade lesions, OR, 6.3; 95% CI, 1.3–30 (OR, 5.4; 95% CI, 0.9–29, adjusted) compared with women without any evidence

of cervical inflammation (0–5 neutrophils/field). There was a statistically significant 2-fold increase of high-grade lesions associated with cervicitis (OR, 2.2; 95% CI, 1.1–4.4 unadjusted; OR, 2.0; 95% CI, 0.94–4.4 adjusted) treated as binary variable.

## Discussion

Inflammation is a relatively nonspecific physiological response to tissue injury caused by exogenous factors such as microbial infections or chemical irritants (25). Hallmarks of the inflammatory response include migration of natural killer cells and phagocytes (e.g., neutrophils and macrophages) that release inflammatory mediators (e.g., IL-1 and IL-8). Inflammation, often in response to chronic infection, results in the production of nonspecific protective antimicrobial oxidants that can also cause oxidative damage to host DNA, leading to cancer (14). The association of inflammation with many cancers suggests that inflammation may be a universal risk factor for carcinogenesis.

HPV infection of the cervix is not believed to be inflammatory (14). Nevertheless, there is some epidemiological evidence, albeit weak, to suggest that inflammation might be linked to cervical cancer, perhaps as a HPV cofactor (7–12). Furthermore, there is an ecological association of greater levels of cervical inflammation in populations with higher incidence of cervical neoplasia. In Guanacaste, Costa Rica, where high rates of cervical cancer exist, unusually high levels of unexplained cervical inflammation have also been observed (Tables 3 and 5).

In the analysis for associations with high-grade lesions, we restricted our evaluation to women who were infected with oncogenic HPV types in an attempt to better define women who were at risk of disease, and we chose controls who were without high-grade lesions. Perhaps better choices for controls would be women who have been previously infected but unlike the cases, resolve their infection. However, detection of past infection and, therefore, identification of this subset of women has not been possible. In selecting oncogenic HPV-infected women as controls, we likely biased our control group toward women who have newly acquired HPV infections in which there has been insufficient time for progression to high-grade lesions, or women who have persistent HPV infections. Newly infected women are more likely to have undergone a change in sexual behavior that has led to infection, but it is unclear whether the inclusion of these women would attenuate or inflate our risk estimates. Persistent HPV infection is a prerequisite for progression to high-grade lesions, and the inclusion of women with persistent infection as controls may have attenuated our find-

Table 5 ORs and corresponding 95% CIs for high-grade lesions (cases) associated with cervical inflammation in oncogenic HPV-infected women (Analysis 2)

Neutrophils/field	N Cases/Controls	Crude		Adjusted <sup>a</sup>		N <sup>b</sup> Cases/Controls	Crude <sup>b</sup>		Adjusted <sup>a,b</sup>	
		OR	95% CI	OR	95% CI		OR	95% CI	OR	95% CI
0–5 (normal)	13/31	1		1		2/13	1		1	
6–30 (mild inflammation)	33/65	1.2	0.56–2.6	1.3	0.54–2.9	19/35	3.5	0.72–17	3.1	0.58–17
30+ (cervicitis)	49/61	1.9	0.90–4.1	2.0	0.86–4.7	36/37	6.3	1.3–30	5.4	1.0–29
		$P_{\text{Trend}} = 0.05$		$P_{\text{Trend}} = 0.07$			$P_{\text{Trend}} = 0.01$		$P_{\text{Trend}} = 0.03$	
<30 (referent)	46/96	1		1		21/48	1		1	
30+ (cervicitis)	49/61	1.7	1.0–2.8	1.7	1.0–3.0	36/37	2.2	1.1–4.4	2.0	0.94–4.4

<sup>a</sup> Adjusted for age, number of pregnancies, and number of cigarettes.

<sup>b</sup> Excluding those subjects without a BV assessment.

ings. This bias, however, cannot explain the positive association of inflammation with high-grade lesions. Furthermore, the association of inflammation with high-grade lesions was similar when all of the non-cases, regardless of HPV status, were included as controls, and adjusting for HPV was achieved through statistical means ( $P_{\text{trend}} = 0.05$ ; for cervicitis, OR, 1.9; 95% CI, 0.91–4.1). This suggests that the association of inflammation with high-grade lesions may be robust.

We found no association of BV with high-grade lesions in the women DNA positive for oncogenic HPV. These results could be attenuated if BV were associated with HPV persistence. Another possible explanation could be the gross misclassification of the Nugent score. However, we found that BV was strongly associated with elevated vaginal pH, consistent with clinical manifestations of BV (22), which suggested that misclassification of BV was not a significant problem in our study.

High-grade lesions were associated with increasing levels of cervical inflammation, the highest level of which was associated with a 2-fold increased risk. These data suggest that cervical inflammation may contribute to the progression of HPV infections to high-grade lesions. In combination with the high prevalence of cervical inflammation in Guanacaste, Costa Rica (Table 5), this association suggests that cervical inflammation may be an important contributor to the incidence of high-grade lesions in this locale. However, a prospective study is needed to ascertain whether inflammation is truly an HPV cofactor.

When we stratified our analysis of inflammation and high-grade lesions as to whether there was an adequate Pap smear for a Nugent score, we found no relationship in those in whom we were unable to assess a Nugent score, and we found a strengthened, albeit more statistically unstable, association in those that we were able to assess. Stratification on levels of Nugent score categories indicated no effect modification of the relationship of cervical inflammation and high-grade lesions (data not shown), which suggests that the modification of our risk estimates, if real, was limited to whether or not there was a Nugent score. This apparent effect modification could have arisen by chance. However, if for unknown reasons, this subset of women with Nugent scores have a more accurate assessment of inflammation, the relationship between inflammation and high-grade lesions may be more profound, and cervical inflammation may be relatively important to the progression of HPV infection to neoplasia.

There are a number of limitations of this study. First, the relatively small numbers of cases led to a limited statistical power, which was diminished further by missing assessments of bacterial morphotypes. Second, a change in the number of *Lactobacilli* in vaginal microflora may be an age-associated

decrease (21) rather than BV-related change; in some women, these changes in vaginal microflora, as indicated by changes in vaginal pH, occur before the age of 50. Third, the temporality of the inflammation with respect to HPV infection is uncertain. Inflammation associated with high-grade lesions may be a result, rather than a cause, of high-grade lesions. Also, the length of exposure to inflammation could not be assessed, but it seems possible that effect of chronic exposure may differ from that of acute exposure.

To address some of these concerns, future studies in Costa Rica will prospectively investigate incident cases of high-grade lesions using several indices of inflammation, including IL-1 and IL-8. We will also investigate whether other STIs via an inflammatory mechanism may be cofactors for cervical cancer. *Chlamydia trachomatis*, for example, is a well-known cause of cervicitis (15). In this study, no associations were observed with self-reported STIs but self-reporting of STI is typically unreliable. Future studies will rely on biological assays to better ascertain current infections and past exposures. STI remain a compelling explanation for inflammation-induced progression, given that STI might be transmitted concurrently with HPV and more often go untreated in developing countries than in developed countries.

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## An Association of Cervical Inflammation with High-Grade Cervical Neoplasia in Women Infected with Oncogenic Human Papillomavirus (HPV)

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