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Risk of Cervical Cancer Associated with *Chlamydia trachomatis* Antibodies by Histology, HPV Type, and HPV Cofactors

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Abstract

Human papillomavirus (HPV) is the central etiologic factor for cervical cancer, and prior studies suggested *C. trachomatis* may act as an HPV cofactor. We examined the *C. trachomatis*—cervical cancer association by serotype, histology, HPV type in the tumor, and other HPV cofactors. We conducted a population-based study in the Seattle-Puget Sound area of 302 women with invasive squamous cell carcinomas (SCC), 185 women with adenocarcinomas of the cervix (AC), and 318 HPV seropositive control women. The risk of SCC associated with antibodies to *C. trachomatis* was increased (OR 1.6, 95% CI 1.1–2.2) but not for AC (OR 1.0, 95% CI 0.6–1.5). This association was independent of HPV type in the SCC tumor tissue. There was an association between specific serotypes of *C. trachomatis* and SCC for 6 of the 10 serotypes: B (OR 3.6, 95% CI 1.5–8.4), D (OR 2.1, 95% CI 1.2–3.5), E (OR 2.4, 95% CI 1.4–3.9), G (OR 3.0, 95% CI 1.1–7.9), I (OR 4.2, 95% CI 1.5–11.7), and J (OR 2.3, 95% CI 1.0–5.1), but not for the 4 types (C, F, H, and K) that were present at very low prevalence in this population. There was an increased risk of SCC, but not AC, associated with antibodies to *C. trachomatis* that was not serotype specific.

Keywords

Chlamydia trachomatis; HPV; cervical cancer; histologic type; microimmunofluorescence

Certain oncogenic genital human papillomavirus types (HPV) that are sexually transmitted have been shown to be a necessary but not sufficient cause of cervical cancer.[1] These oncogenic HPVs most often result in transient infections of the cervix, and only a small proportion of exposed women eventually progress to cervical cancer.[2] Since few women

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exposed to sexually transmitted HPV get cervical cancer, we are interested in cofactors in addition to HPV that promote the development of cancer. One such possible cofactor is *Chlamydia trachomatis*, which is highly prevalent among sexually active young women and can infect the cervix for long periods of time. Risk factors for *C. trachomatis* infection are similar to those for genital HPV infections, and include a history of multiple sexual partners, a recent new sexual partner, early age at first intercourse, and sporadic use of condoms or other barrier methods of contraception.[3,4]

C. trachomatis often causes cervicitis, which is a chronic infection of the endocervical cells of the transformation zone. Such inflammation may predispose women to other STDs, including genital HPV infection, by damaging epithelial integrity.[5] Recent studies suggested that a history of *C. trachomatis* infection was associated with persistence of oncogenic HPV infections,[6,7] and other studies have shown that persistent HPV infections are necessary for progression to high-grade cervical intraepithelial neoplasia (CIN) and carcinoma.[2,8] Thus, chronic cervical inflammation by *C. trachomatis* could increase the risk of transformation of cervical cells that are persistently infected with oncogenic types of HPV.

C. trachomatis antibodies are not used for clinical assessment of infection because the baseline prevalence in sexually active populations is high even among *C. trachomatis* culture negative asymptomatic patients.[5] However, *C. trachomatis* antibodies have been useful in epidemiologic studies as a measure of prior exposure. *C. trachomatis* antibodies are measured either with an enzyme-linked immunosorbent assay (EIA) or by microimmunofluorescence (MIF) that is serotype specific. Previous studies have demonstrated an association between *C. trachomatis* antibodies and increased risk of CIN and cervical cancer.[4,9–11] In a nested case-control study, Anttila et al. found an increased risk of cervical cancer associated with *C. trachomatis* antibodies (adjusted OR 2.1, 95% CI 1.3–3.5), with the strongest risks associated with three out of ten serotypes tested by MIF.[9] These results extend an earlier study in the same cohort: Koskela et al. found that *C. trachomatis* serotypes grouped into 3 classes of serotypes were associated with an elevated risk of cervical cancer.[10] In a hospital-based case-control study conducted in the by the International Agency for research on cancer (IARC), there was a 2-fold increased risk of invasive cervical cancer associated with *C. trachomatis* among HPV DNA positive subjects (OR 1.8, 95% CI 1.3–2.5).[12] This latter study confirmed the overall association of *C. trachomatis* and cervical cancer while controlling for HPV DNA.

In this study we extended the evaluation of *C. trachomatis* to a population-based study in Seattle. Although all cases of cervical cancer contain HPV DNA in their tumor, but less than 50% of the cases are HPV serum antibody positive, we believe that a compromised ability to recognize HPV and form circulating antibodies may be important to the ability of HPV to evade immune surveillance, allowing genetic changes to accumulate. However, among controls, HPV seropositivity may act as a surrogate measure for prior exposure to HPV. Since HPV has been demonstrated to be the central etiologic agent of cervical cancer, we matched on HPV by using all cases and only HPV16 or HPV18 seropositive controls in this study.

Particular emphasis was placed on evaluating the association between specific serotypes of *C. trachomatis* and cervical cancer and how this relationship is potentially modified by histologic type of the tumor, HPV type in the tumor, and exposure to other recognized cofactors with HPV in cervical cancer (i.e, cigarette smoking, oral contraceptive use, and parity). The current study was able to include a much larger number of adenocarcinomas then other studies that have been conducted to date.

METHODS

Subject Eligibility, Identification, and Recruitment

The subjects in the present study were from a recent case-control study of cervical cancer conducted in Seattle, WA. Cases available for this analysis had to have a blood sample available and HPV DNA typing done: thus, there were 487 cases with blood available (out of 763 enrolled cases). Controls for this sudy were 318 women who were HPV16 or HPV18 antibody positive and had blood available (out of enrolled 1,264 controls). The response proportions for the original population-based study were 65.6% of eligible invasive cervical cancer cases, 74.9% of eligible in situ adenocarcinoma cases, and 62.6% of random-digit telephone dialed controls. More detailed study methods are given in prior reports,[13–15] and the basic study design is outlined here.

This population-based study had no measure of HPV DNA in the cervix at the time of the reference date for control subjects. As a surrogate of HPV exposure at a relevant time point to HPV-related cancer, we included only controls found to be positive for HPV16 or HPV18 L1 antibodies in the present study. In a subanalysis, we restricted the case group to also include only those cases that were HPV antibody positive (there were 140/302 SCC cases (46.4%) and 102/185 AC cases (55.1%) that were HPV16 or HPV18 antibody positive and available for this subanalysis). We found that the prevalence of *C. trachomatis* antibodies was quite similar in the case groups that were and were not HPV seropositive (i.e., 38.9% and 37.9% for SCC and 26.5% and 29.4% for AC, respectively).

Case subjects were women diagnosed between 1986 and 1998 with either incident invasive squamous cell cervical cancer (SCC; n=302) or incident in situ and invasive adenocarcinoma (AC; n=185) for whom HPV DNA typing and HPV serology had been completed and serum for Chlamydia testing was available. The International Classification of Disease for Oncology codes for histologic diagnoses was used, and all cases were coded as 801–807 for SCC or 814–838 for AC.[16] The histologic type of the tumor was determined by the community provider and verified by our pathology laboratory. All cases resided in the greater Seattle metropolitan area at the time of diagnosis and were ascertained by a population-based cancer registry (the Cancer Surveillance System, which is part of the Surveillance, Epidemiology and End Results Program of the National Cancer Institute, US). All of the control subjects included in this study (n=318) tested positive for antibodies to HPV16 or HPV18 by a virus-like particle assay[14] and had blood available for testing. Controls were recruited from the Seattle metropolitan area by the Waksberg-Mitofsky method for random-digit dialing and were frequency matched to case age in 5-year intervals.[17]

We compared key variables of the case subjects from the current study with those in the parent study stratified by histologic type. We found that the age distribution, number of partners, smoking history, and oral contraceptive use variables were all similar for SCC between the parent study and the current study. The AC cases were also similar except that AC cases in the present study were slightly younger than those in the parent study. Importantly, the controls in this study were all the HPV seropositive controls in the parent study; therefore, the controls were somewhat younger, had more sexual partners, and were more likely to use oral contraceptives then the controls who were HPV seronegative in the parent study.

Data Collection

In-person interviews were administered to all subjects, and blood was drawn at the time of interview. Blood samples were collected from 86.5% of cases and 88.3% of controls in the original study, and tissue blocks were available from 80.0% of interviewed cases. The Institutional Review Board of the Fred Hutchinson Cancer Research Center approved all research protocols.

Laboratory Tests

The laboratories conducting the serologic assays were blinded to all characteristics of the study subjects. Testing for HPV DNA in tumor tissue of cases and HPV antibodies in serum from cases and controls were performed at the Fred Hutchinson Cancer Research Center in Seattle. Chlamydia serology was performed at the National Public Health Institute in Oulu, Finland.

HPV Antibodies—Sera were tested for HPV16 and HPV18 capsid proteins by using capture enzyme-linked immunosorbent assays, as previously described.[14] Capsids were produced using HPV16 L1 and HPV18 L1 recombinant vaccinia viruses and purified on cesium chloride gradients. Dr. Neil Christensen (University of Pennsylvania, Hershey Medical Center, Hershey, PA) kindly provided capture antibodies. Human sera were tested in triplicate with and without capsids. For each serum a value was calculated as follows: natural log of the average of the three wells containing antigen minus the natural log of the average of three wells with no antigen. The cutoff was determined using ROC analyses as previously described.[14]

Chlamydia Antibodies—Sera were screened for the presence of *C. trachomatis* by EIA.[9] Serum samples found to be positive by EIA were further analyzed by MIF for antibodies against the following serotypes: B, D, E, F, G, and J (American Type Culture Collection, Rockville, MD); and C, H, I, and K (Washington Research Foundation, Seattle, WA) that represent 10 of the 18 known *C. trachomatis* serotypes. MIF serology distinguishes serotypes by identifying type-specific epitopes residing on the major outer membrane protein, which are exposed on the surfaces of *C. trachomatis* elementary bodies. Elementary body antigens were prepared from *C. trachomatis* serotypes B, D, E, F, G, and J (grown in McCoy cells), and from serotypes C, H, I, and K (grown in HeLa-229 cells), and purified using conventional techniques.[18] A second MIF test was used to detect antibody to a non-sexually transmitted control antigen, *C. pneumoniae*. Titers of 16 or greater were considered

positive for *C. trachomatis* and titers of 32 or greater were considered positive for *C. pneumoniae*. Replicate blinded aliquots for 45 subjects were dispersed at random among the samples being tested for chlamydial antibodies. The paired repeats were 98.3% concordant between replicate samples for *C. trachomatis* EIA and 100% correlated for *C. pneumoniae* MIF. Among the 45 replicate samples, 12 samples were positive for one or more *C. trachomatis* MIF seroyptes: 7 were concordant for all serotypes, and 5 had one serotype that was discordant. Subjects who tested EIA screening assay positive and MIF negative (n=121) (i.e., those with serologic evidence of *C. trachomatis* infection that were not positive for any of the 10 serotypes assessed) are excluded from Table 4.

HPV DNA Typing—Polymerase chain reaction (PCR) methods were used to amplify HPV DNA extracted from paraffin-embedded tumor tissue so that each tumor could be classified by HPV type. To determine the adequacy of each specimen's DNA for typing, we used PCR to amplify 536-bp and 268-bp fragments of the β-globin gene. For all 487 case subjects, PCR was performed using primers specific for the E6 open reading frames of HPV16 and HPV18. The identity of the PCR products was confirmed by Southern hybridization. For 255 cases recruited in the earlier years of our study, consensus primers were also derived from the L1 open reading frame using MY09/MY11 primers.[19] For more recently assayed tumor specimens (232 cases), we used restriction fragment analysis to assign HPV types.[20] When the L1 products could not be assigned a type on the basis of restriction patterns, we assigned types by automated sequencing of the L1 consensus products. Among the 487 tumor samples typed, 297 were HPV16 positive, 110 were HPV18 positive, 41 were positive for HPV16 and HPV18, and 39 were positive for other types (1 HPV6/11, 8 HPV6, 9 HPV31, 11 HPV33, 3 HPV45, 1 HPV53, 1 HPV66, 5 type unknown).

Data Analysis

The relative risk of cervical cancer was estimated using the odds ratio approximation by exponentiation of coefficients obtained from multiple logistic regression models. Subjects with missing values for any variables in a model were excluded from that model. The following potential confounders were not included in final models because their inclusion did not substantially affect the odds ratios of interest: reference year, income (>$30,000, $15–30,000, <$15,000), alcohol use (never, former, current), years of education (13+, <13), marital status (married, not married), body mass index (<25, 25–29, 30+), and race (white, non-white). Two confounders were controlled in most analyses: age at reference date (continuous, linear) and lifetime number of sex partners (1, 2–4, 5+).To determine if the combined associations of *C. trachomatis* and another of the key cofactors for cervical cancer (i.e., parity, smoking, or oral contraceptive use) was greater than predicted from the individual risk factors, we calculated the relative excess risk for interaction (RERI) measure and confidence intervals under an additive relative risk model.[21,22] Polytomous regression was used to assess the difference between histologic types and *C. trachomatis* by examining the likelihood ratio test statistic.

RESULTS

The prevalence of *C. trachomatis* antibodies by key descriptive characteristics of the study participants are provided in Table 1. The antibodies to *C. trachomatis* declined with age for controls and also for both case groups, though this pattern was not as strong among the controls. As expected for a sexually transmitted disease, the prevalence of antibodies increased with number of sexual partners for cases and controls. The proportion positive for the non-sexually transmitted control antigen, *C. pneumonia*, did not increase with number of partners (data not shown).

The prevalence of *C. trachomatis* antibodies as measured by EIA was 38.4% in women with SCC, 28.1% in women with AC, and 26.1% in control women (Table 2). There was a significantly increased risk of SCC associated with *C. trachomatis* (OR 1.6, 95% CI 1.1–2.2). This elevated risk was not present for AC (OR 1.0, 95% CI 0.6–1.5). The difference between the risk estimates by histologic types was significant in a polytomous model (p=.01).

Samples positive by EIA were further tested by MIF, and the risk of SCC was 2-fold for those positive for one of the 10 serotypes compared to those negative by EIA (OR 2.3, 95% CI 1.5–3.7). There was an increased risk of SCC associated with high titers of *C. trachomatis* (OR 3.1, 95% CI 1.6–6.0) that was less pronounced and not significant for AC (OR 1.6, 95% CI 0.8–3.5).

There were significant associations with SCC for 6 of the 10 serotypes investigated (Table 3): specifically, serotypes B (OR 3.6, 95% CI 1.5–8.4), D (OR 2.1, 95% CI 1.2–3.5), E (OR 2.4, 95% CI 1.4–3.9), G (OR 3.0, 95% CI 1.1–7.9), I (OR 4.2, 95% CI 1.5–11.7), and J (OR 2.3, 95% CI 1.0–5.1). None of the individual *C. trachomatis* serotypes was associated with AC (data not shown). The most commonly occurring serotypes among all subject groups were types D and E. Together, types D and E accounted for 11.9% (32/318) of control, 23.9% (61/302) of SCC, and 13.8% (22/185) of AC positives. Approximately 77% of subjects positive for serotypes D or E were positive for both D and E.

Other risk factors for cervical cancer include high parity, current smoking, and long-term OC use. We found no clearly significant joint effects as assessed by the relative excess risk of interaction (data not shown). However, there were some interesting features of the increased risk of SCC associated with parity and current smoking. For example, the elevated risk of SCC associated with parity was significantly increased only for women positive for *C. trachomatis*. This is in contrast to the risk associated with current smoking, which was significantly elevated for women with and without *C. trachomatis* antibodies. Since there was no significant joint effect, we examined the risk of SCC and AC in fully adjusted models that simultaneously fit the *C. trachomatis*, parity, smoking, and oral contraceptive use data (and were adjusted for age and number of sexual partners). When this model was fit for SCC the risk associated with *C. trachomatis* remained elevated 2-fold (OR 2.2, 95% CI 1.4–3.5) and there was no increased risk associated with AC (OR 1.1, 95% CI 0.6–2.1).

In Table 4 the HPV type in the tumor tissue was used to subset the cases into tumors that contained HPV16 (but not HPV18) and those that contained HPV18 (but not HPV16). There

were consistently elevated risks for SCC containing HPV16 (OR 2.3, 95% CI 1.4–3.7) and HPV18 (OR 2.8, 95% CI 1.1–7.2) that were not seen for AC (HPV16: OR 0.9; HPV18: OR 1.2, respectively).

DISCUSSION

Oncogenic mucosotropic types of HPV are the central etiologic agents of both squamous cell cancer and adenocarcinoma of the cervix, but there are key differences in their cofactors with HPV. Such differences between histologic types are important an important indicator that uncontrolled confounding of HPV does not account for the *C. trachomatis* –cervical cancer relationship. Since both adenocarcinoma and squamous cell carcinoma are HPV-related, it seems likely that HPV confounding would act in the same direction for both histologic types. We have shown in this study that there is an increased risk of SCC, but not AC, of the cervix associated with *C. trachomatis* antibodies.

The present study was able to test a much larger group of cervical AC than previous studies[9,10,12] (n=185 in the present study compared to n=32 in the Nordic study and n=60 in the IARC study). Such differences in risk factors by histologic type of cervical cancer have previously been reported for smoking and obesity.[13,23–25] In this study we also found that histologic type, not HPV DNA type, is a more important parameter in the determining association between *C. trachomatis* and cervical cancer. Similarly, in two recent prospective studies of the association between *C. pneumoniae* antibodies and lung cancer, elevated risks were found for squamous cell cancers but not adenocarcinomas of the lung,[26,27], though another study did not find this difference by histologic type.[28] It may be that the underlying pathogenic mechanisms of Chlamydia in cancer are specific to squamous cell cancers.

The MIF test is considered to be the most specific of the *C. trachomatis* serologic tests, though new enzyme immunoassays and immunoblot assays have been developed that have a higher sensitivity when compared to culture positive samples.[29,30] Several aspects of the MIF assay can be construed as increasing the specificity of the association with *C. trachomatis*. For example, the risk estimates for SCC increased with increasing titers of *C. trachomatis*, perhaps indicating an increased risk of SCC with chronic *C. trachomatis* infection. In the serotype specific associations, we included cases that were MIF positive for multiple antibody types as a separate category to isolate the risk estimates associated with having only one serotype. This approach demonstrated elevated (though not necessarily significant) risk estimates for 6 of the 10 serotypes examined, serotypes B, E, J, G, I, and D. The large nested case-control study of Attila et al.[9] likewise reported increased risk estimates for serotypes G, I, and D, with marginal associations for serotypes B, E, and J. Also, neither study reported increased risks associated with serotypes C, F, or K. Thus, the present study and the Nordic studies found the same 6 serotypes to be associated with elevated risks of cervical cancer. Further, the ability of both studies to detect associations with the other serotypes (C, F, or K) was probably hampered by their low prevalence (<6%).

In this study, there were 121 EIA screen positives that did not have elevated MIF titers and for whom there was no excess risk of cervical cancer. These EIA+/MIF− results may represent untyped serotypes that have different association with or, alternatively, no

association with the risk of cervical cancer. The current study suggests that the extra expense of serotype testing is unlikely to aid in discerning the relationship between *C. trachomatis* and cervical cancer risk.

Accounting for HPV infection in a study of cervical cancer and *C. trachomatis* is important to avoid confounded results. Testing for HPV DNA in tumors is an effective method for measuring exposure among women with cervical cancer because expression of HPV oncogenes is necessary to maintain the malignant phenotype. The value of measuring HPV DNA in cervices of controls, however, is much less clear: most HPV infections are transient and natural history studies have shown that HPV DNA is detectable in cells from the cervix for less than a year in most infected women.[31] Therefore, the presence or absence of HPV DNA at a single time point in an otherwise healthy woman is a poor indicator of her lifetime exposure. Further, a report that determined the distribution of 37 genital HPV types in women in the general population found that fewer than 5% of normal women over 35 years of age had any detectable HPV DNA in the genital tract.[32] Although HPV serology is not a perfect measure of HPV exposure, it nonetheless captures some of the information regarding a woman's history of persistent HPV infection,[33] which is the type of exposure we would ideally like to measure.

HPV antibodies have been shown to correlate with the detection of HPV DNA in a type-specific fashion and to correlate with the lifetime number of sexual partners.[14,34] Among individuals who develop responses, antibodies persist for many years.[35–37] However, antibodies are imperfect indicators of lifetime HPV exposure because serum antibodies to HPV16 capsids are undetectable in 20% to 40% of women who test positive for HPV16 DNA in cells from the cervical mucosa.[33,34] When we restricted our analyses to HPV seropositive cases, the results were similar to those presented for all cases.

Beyond potentially aiding the establishment or progression of HPV infections, we speculate that the inflammatory response and metaplasia triggered by *C. trachomatis* infection may encourage cell turnover and therefore the number of non-dividing differentiating cells that are needed for HPV replication and productive HPV infections. Further, persistent *C. trachomatis* infections may create an inflammatory environment conducive to HPV-induced carcinogenesis by increasing the chance of DNA replication errors that have been shown in vitro to lead to persistent disease and accumulation of genetically damaged cells.[38,39]

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Table 1

Key characteristics of the study population and prevalence of *Chlamydia trachomatis* EIA antibodies within descriptive categories for HPV seropositive controls, cases with invasive squamous cell cancer (SCC), and cases with in situ and invasive adenocarcinomas of the cervix (AC)

	Control (n=318)		SCC (n=302)		AC (n=185)	
	n (%) *C. trachomatis*,	%	n (%) *C. trachomatis* +		n (%) *C. trachomatis*,	%
Age						
18–33	80 (25.2)	27.5	80 (26.5)	51.2	63 (34.1)	31.7
34–40	84 (26.4)	29.8	63 (20.9)	41.3	52 (28.1)	28.8
41–50	82 (25.8)	29.3	77 (25.5)	37.7	48 (25.9)	27.1
51–74	72 (22.6)	16.7	82 (27.2)	24.4	22 (11.9)	18.2
Race						
White	292 (91.8)	25.0	278 (92.1)	38.8	171 (92.4)	26.9
Non-White	26 (8.2)	38.5	24 (7.9)	33.3	14 (7.6)	42.9
Education						
>H.S.	235 (73.9)	26.0	158 (52.3)	39.2	126 (68.1)	25.4
<=H.S.	83 (26.1)	26.5	144 (47.7)	37.5	59 (31.9)	33.9
No. of Sex Partners						
1	65 (20.7)	7.7	28 (9.3)	17.9	29 (15.8)	6.9
2–4	93 (29.6)	19.4	106 (35.1)	31.1	48 (26.1)	22.9
5–4	112 (35.7)	35.7	113 (37.4)	41.6	76 (41.3)	32.9
15+	44 (14.0)	45.5	55 (18.2)	56.4	31 (16.8)	45.2
Missing*	4		0		1	

*3 controls with 0 partners, 1 control who refused to answer, and one case who had 0 partners and a diagnosis of clear cell adenocarcinoma are excluded from subsequent analyses.

Table 2

Relative risk of squamous cell (SCC) and adenocarcinoma of the cervix (AC) associated with *C. trachomatis* by enzyme linked immunsorbent assay (EIA), *C. trachomatis* serotypes by micrommunoflourescence (MIF), and *C. pneumoniae* by MIF

	Controls		SCC Cases				AC Cases			
Antibody Test	**n**	**%**	**n**	**%**	**OR**	**(95% CI)**	**n**	**%**	**OR**	**(95% CI)**
C. Pneumoniae MIF										
Negative	239	75.2	238	78.8	1.0	Ref.	143	77.3	1.0	Ref.
Positive	79	24.8	64	21.2	0.8	(0.5–1.2)	42	22.7	0.9	(0.6–1.4)
C. trachomatis EIA										
Negative	235	73.9	186	61.6	1.0	Ref.	133	71.9	1.0	Ref.
Positive	83	26.1	116	38.4	1.6	(1.1–2.2)	52	28.1	1.0	(0.6–1.5)
C. trachomatis EIA and MIF										
EIA −	235	73.9	186	61.6	1.0	Ref.	133	71.9	1.0	Ref.
EIA+ MIF−	48	15.1	47	15.6	1.1	(0.7–1.8)	26	14.1	0.9	(0.5–1.5)
EIA+ MIF+	35	11.0	69	22.8	2.3	(1.5–3.7)	26	14.1	1.1	(0.6–2.0)
C. trachomatis number of serotypes detected by MIF*										
EIA −	235	87.0	186	72.9	1.0	Ref.	133	83.6	1.0	Ref.
1	8	3.0	17	6.7	2.6	(1.1–6.3)	3	1.9	0.5	(0.1–2.1)
2	11	4.1	17	6.7	1.8	(0.8–4.0)	10	6.3	1.4	(0.6–3.4)
3	9	3.3	14	5.5	1.9	(0.8–4.7)	7	4.4	1.1	(0.4–3.1)
4+	7	2.6	21	8.2	3.4	(1.4–8.4)	6	3.8	1.3	(0.4–4.1)
C. trachomatis MIF titers*										
EIA −	235	87.0	186	72.9	1.0	Ref.	133	83.6	1.0	Ref.
MIF 1:16	10	3.7	16	6.3	1.8	(0.8–4.0)	4	2.5	0.6	(0.2–2.1)
MIF 1:32	11	4.1	17	6.7	2.0	(0.9–4.4)	7	4.4	0.9	(0.3–2.4)
MIF 1:64	14	5.2	36	14.1	3.1	(1.6–6.0)	15	9.4	1.6	(0.8–3.5)

Odds ratios (OR) are adjusted for sex partners (1, 2–4,5+) and age at reference (continuous).

*Subjects with screening assay positive and microimmunoflourescence assay negative results (EIA+ MIF−; n=121) were excluded from these analyses.

Table 3

Risk of Squamous Cell Cervical Cancer Associated with *C. trachomatis* Serotypes*

		Controls		SCC Cases			
		n	%	N	%	OR*	95% CI
Type B	EIA Negative	235	87.0	186	72.9	1.0	Ref.
	EIA+/Not Type B	27	10.0	47	18.4	2.0	(1.2–3.4)
	Type B	8	3.0	22	8.6	3.6	(1.5–8.4)
Type C	EIA+/Not Type C	32	11.9	67	26.3	2.5	(1.5–4.0)
	Type C	3	1.1	2	0.8	0.9	(0.1–5.2)
Type D	EIA+/Not Type D	7	2.6	19	7.5	3.3	(1.38.1)
	Type D	28	10.4	50	19.6	2.1	(1.2–3.5)
Type E	EIA+/Not Type E	6	2.2	11	4.3	2.2	(0.8–6.2)
	Type E	29	10.7	58	22.7	2.4	(1.4–3.9)
Type F	EIA+/Not Type F	27	10.0	55	21.6	2.5	(1.5–4.1)
	Type F	8	3.0	14	5.5	1.9	(0.8–4.8)
Type G	EIA+/Not Type G	29	10.7	54	21.2	2.2	(1.3–3.7)
	Type G	6	2.2	15	5.9	3.0	(1.1–7.9)
Type H	EIA+/Not Type H	33	12.2	64	25.1	2.3	(1.4–3.7)
	Type H	2	0.7	5	2.0	3.4	(0.6–18.8)
Type I	EIA+/Not Type I	30	11.1	51	20.0	2.0	(1.2–3.4)
	Type I	5	1.9	18	7.1	4.2	(1.5–11.7)
Type J	EIA+/Not Type J	25	9.3	49	19.2	2.3	(1.4–4.0)
	Type J	10	3.7	20	7.8	2.3	(1.0–5.1)
Type K	EIA+/Not Type K	33	12.2	66	25.9	2.4	(1.5–3.8)
	Type K	2	0.7	3	1.2	1.7	(0.3–10.1)
Grouped Serotypes**							
	Class B	32	11.9	63	24.7	2.3	(1.4–3.8)
	Intermediate	9	3.3	19	7.5	2.5	(1.1–5.7)
	Class C	11	4.1	28	11.0	3.0	(1.4–6.2)

* The reference group for each category in the table is composed of subjects negative by the EIA screening test (186 SCC cases and 235 controls). ORs are adjusted for sex partners (1, 2–4, 5+) and age at reference (continuous). Class B combines serotypes B, D, and E, the intermediate class combines serotypes F and G, and Class C combines serotypes C, H, I, J, and K. Subjects were positive

Table 4

Relative risk of squamous cell (SCC) and adenocarcinoma of the cervix (AC) associated with *C. trachomatis* antibodies, specific to HPV DNA type in the tumor and histology

	HPV16 Tumors			HPV18 Tumors		
	n (%+)	OR	95% CI	n (%+)	OR	95% CI
SCC	223 (27.3%)	2.3	(1.4–3.7)	27 (29.6%)	2.8	(1.1–7.2)
AC	64 (14.1%)	0.9	(0.4–2.1)	72 (16.7%)	1.2	(0.6–2.6)
Total	251 (23.9%)	1.9	(1.2–3.0)	99 (20.2%)	1.6	(0.8–2.9)

Odds ratios (OR) adjusted for sex partners (1, 2–4, 5+) and age at reference (continuous).

The subjects negative by the EIA *C. trachomatis* screening test (OD<1.4) are chlamydia negative and those positive by the EIA and MIF are chlamydia positive. HPV-specific tumor groups are mutually exclusive. Each case group is compared to 318 RDD controls (13% *C. trachomatis* positive).