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CHLAMYDIA TRACHOMATIS AND INVASIVE CERVICAL CANCER: A POOLED ANALYSIS OF THE IARC MULTICENTRIC CASE-CONTROL STUDY

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To determine whether *Chlamydia trachomatis* infection is consistently associated with an increased risk of invasive cervical carcinoma (ICC) after accounting for the strong effect of human papillomavirus (HPV) infection, a case-control study of 1,238 cases of ICC and 1,100 control women from 7 countries was carried out (hospital-based studies in Thailand, the Philippines, Morocco, Peru, Brazil and population-based studies in Colombia and Spain, all coordinated by the International Agency for Research on Cancer, Lyon, France). *C. trachomatis* serum antibody detection was made by means of a microfluorescence assay. Among HPV DNA-positive cases and controls, the risk of squamous cell ICC was elevated in *C. trachomatis* seropositive women (OR = 1.8; 95% CI = 1.2–2.7) after adjustment for age, center, oral contraceptive use, history of Pap smears, number of full-term pregnancies and herpes simplex virus 2 seropositivity. The effect of *C. trachomatis* seropositivity on squamous cell ICC risk increased with increasing *C. trachomatis* antibody titers and was higher in women under 55 years of age. *C. trachomatis* antibodies were not associated with adeno- or adenosquamous cell carcinoma (OR = 1.0; 95% CI = 0.53–1.9) in HPV DNA-positive women. An association of *C. trachomatis* with squamous cell ICC was found among all cases and control women with or without adjustment for HPV.
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Key words: Chlamydia trachomatis*; cervical cancer; human papillomavirus*

Human papillomavirus (HPV) is the main, and likely necessary, cause of invasive cervical carcinoma (ICC).[1,2] Recent studies of the etiology of ICC aim to identify factors that may influence susceptibility to or progression of HPV infection to cervical neoplasia or ICC. Identification of cofactors acting in conjunction with HPV, such as exogenous hormones,[3] multiparity,[4] smoking and other sexually transmitted infections (STIs),[5] is important because these factors may be amenable to prevention.

Among STIs other than HPV, *Chlamydia trachomatis* may be an important HPV cofactor for cervical carcinogenesis. *C. trachomatis* are obligate intracellular bacteria that infect genital and ocular tissue. Genital *C. trachomatis* infections may induce chronic inflammation, epithelial tissue damage and pelvic inflammatory disease in some cases[6] and have been clinically associated with cytologic cervical atypia and the induction of cervical metaplasia,[7] which in turn may increase a woman's risk of cervical neoplasia.[8]

Positive associations between *C. trachomatis* microimmunofluorescence (MIF) seropositivity and ICC have been found in a case-control study from England (OR = 2.2)[9] and a pooled analysis[10] of cohort studies from Finland, Norway and Sweden (OR = 2.5)[11–13] that adjusted for HPV virus-like particle seropositivity. However, residual confounding due to HPV cannot be ruled out in these studies because the serologic methods used to measure HPV infection were of limited sensitivity and narrow spectrum of HPV types.[14] A Swedish cohort study indicated that *C. trachomatis* DNA was highly predictive of ICC development (OR = 17.1),[15] but the yield of HPV and chlamydial DNA was low from archived Pap smear slides.

We have previously reported findings on seropositivity for *C. trachomatis* antibodies and ICC from 2 studies in Brazil and the Philippines and have shown an OR of 2.1 (95% CI = 1.1–4.0).[5] To confirm and better quantify the association between prior *C. trachomatis* infection and ICC in different geographical settings, we expanded our pooled analyses and included 5 additional case-control studies of ICC coordinated by the International Agency for Research on Cancer (IARC).

MATERIAL AND METHODS

Contributing studies and data collection

The 7 countries in this pooled analysis include high-ICC-incidence populations in Morocco,[16] Brazil,[5,17] Peru[18] and Colom-

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bia;[19] intermediate-ICC-incidence populations in Thailand[20] and the Philippines[5,21]; and a low-ICC-incidence population in Spain.[19] Each study used a similar protocol and questionnaire for recruitment and data collection.

Methods and findings from each study have been described previously.[16–21] In summary, eligible cases were patients with incident, histologically confirmed, squamous cell, and adeno- or adenosquamous cell ICC. Cases had had no previous treatment for cervical carcinoma. Skilled pathologists reviewed the histologic slides of carcinoma cases and confirmed diagnosis. Clinical cancer stage was defined according to the International Federation of Gynecology and Obstetrics classification system.

Controls were population-based in Spain and Colombia[19] and hospital-based in the other countries and were frequency-matched to cases by quinquennium of age. For the hospital-based studies, women with conditions related to risk factors for ICC (*e.g.*, reproductive tract neoplasias or tobacco-related diseases) were not eligible to participate.

Personal interviews were conducted by trained interviewers using a standardized questionnaire including information on sociodemographic factors, smoking, sexual and reproductive history, history of Pap smears and selected STIs. Participants were asked to provide 10 ml of blood for the detection of *C. trachomatis* and herpes simplex virus type 2 (HSV-2) antibodies. Blood samples were processed by centrifugation at the site of collection. The separated serum was placed into vials, frozen at −20°C and shipped to IARC for storage. All protocols were cleared by the IARC and local ethical research committees in accordance with the Helsinki Declaration of 1983.

Chlamydia trachomatis *antibody detection*

C. trachomatis IgG antibodies were determined by an MIF assay,[22] which is considered to be the reference standard for chlamydial serology. MIF assays were conducted without knowledge of case or control status. The antigen panel consisted of purified elementary bodies of *C. trachomatis* (serovar A and 3 pooled serovar groups of BDE, CJHI and FGK) and *C. pneumoniae*, which was included to monitor cross-reactive genus-specific antibody responses against all chlamydial species. Sera were screened for *C. trachomatis* at 1:8 dilution and titered to endpoint (1:8, 1:32, 1:128, ≥ 1:128). An IgG titer of ≥ 1:8 against any *C. trachomatis* serovar group was considered evidence of past *C. trachomatis* infection. An IgG titer of ≥ 1:16 against *C. pneumoniae* was considered evidence for past *C. pneumoniae* respiratory infections. Sera with identical titers for all *C. trachomatis* and *C. pneumoniae* species were also tested against *C. psittaci* (avian-strain 6BC) to determine the presence of genus-specific antibody responses. Sera from 6 cases and 11 controls with identical titers for *C. trachomatis*, *C. pneumoniae* and *C. psittaci* and from 20 case and 23 control participants that were seropositive for serovar A and negative for all other *C. trachomatis* serovars were considered noninformative as to *C. trachomatis* serologic status and not included in analyses. Their exclusion, however, did not significantly affect study results. This *Chlamydia* antibody detection strategy was identical to that previously reported in Brazil and the Philippines study,[5] and these results are thus included in this article. Conversely, sera from Spain and Colombia that had already been evaluated using a simplified MIF assay based on the L2 serovar[23] were retested using the MIF assay described above in order to improve the specificity of previous findings.

A blinded reproducibility study was conducted by retesting a random sample of 222 specimens (9.5%) twice. Percent agreement for *C. trachomatis* serovars A, BDE, CJHI and FGK and *C. pneumoniae* were 85.3%, 86.2%, 86.7%, 84.9% and 89.3%, respectively.

HPV DNA detection

Detailed descriptions of sample procedures and PCR-based assays used are given in the individual study publications.[16–21] Briefly, cervical exfoliated cells were collected from subjects by sampling the ectocervix and endocervix. Cervical cells were eluted and pelleted in phosphate-buffered saline and kept frozen at −70°C until shipment. Cervical biopsy specimens from cases were obtained and kept frozen at −70°C. HPV DNA testing was performed without knowledge of case or control status in a central laboratory. DNA quality was evaluated using β-globin primers. MY09/MY11 primers[19] were initially used for Spain and Colombia and GP5/6/TS primers for Brazil,[17] while the remaining studies employed $GP5^{+}/6^{+}$ primers. Specimens classified as unknown HPV types with the MY09/MY11 or GP5/6/TS primers were retested with $GP5^{+}/6^{+}$ primers. PCR products were assessed for HPV positivity by low-stringency Southern blot hybridization. HPV-positive samples were hybridized with oligonucleotide probes for HPV types 6, 11, 16, 18, 26, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 57, 58, 59, 61, 66, 68, 70, 72, 73, IS39, MM4, MM7, CP6108 and CP8304.[20,21] E7 primers for the 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) were used to reamplify case specimens positive for β-globin and classified as unknown HPV type or HPV-negative, as well as a subsample of control specimens that were β-globin-positive and HPV-negative.[18]

HSV-2 IgG antibody detection

Western blot (WB), the reference gold standard,[24] was used to detect HSV-2 antibodies in sera from Thailand, Morocco, Peru, Colombia and Spain. Sera from Brazil and the Philippines were screened for HSV-2 IgG antibodies with the Gull HSV-2 ELISA, and all sera with positive, equivocal or borderline negative results were retested with the WB to obtain type-specific results.[25]

Statistical analysis

Only cases and controls with available and valid *C. trachomatis* serology and HPV DNA laboratory results were included in the present analysis. The number of cases and controls thus slightly differ from those in the previous publications.[16–21]

Unconditional logistic regression models were fitted to individual data.[26] For squamous cell ICC, separate analyses for each study center were performed, then data from all centers were pooled together adjusted for center. Only pooled results adjusted for center are presented for adeno- or adenosquamous cell ICC due to the limited number of cases. Summary ORs and 95% CIs were computed from the above models, including terms for age, study center, history of Pap smears, oral contraceptive (OC) use, number of full-term pregnancies and HSV-2 seropositivity, plus number of lifetime sexual partners and age at first sexual intercourse when indicated. Women who reported hormonal contraceptive use were considered as oral contraceptive users because the use of injectable contraceptives was rare in study areas.[3]

Tests for trend were based on the likelihood ratio test between the models with and without a linear term for the variable of interest. To test for heterogeneity among the study centers, we compared the difference between the log likelihood of the model that estimated a common OR and the model that estimated an OR for each center to the chi-square distribution with degrees of freedom equal to the number of centers minus one.

Graphs are presented, displaying for each center a black square, whose center corresponds to the estimated OR and whose size is inversely proportional to the variance of the logarithm of the OR. The corresponding 95% CIs are drawn as a line. Diamonds plot the summary ORs for all squamous cell ICC or for all adeno- or adenosquamous cell ICC data together. The centers of the diamonds represent the OR and the extremes show the 95% CIs.

RESULTS

A total of 1,139 squamous cell ICC, 99 adeno- or adenosquamous cell ICC cases and 1,100 controls were included (Table I). Most squamous cell ICC (94.6%) and adeno- or adenosquamous cell ICC cases (90.9%) were HPV DNA-positive, in contrast to

TABLE I – DISTRIBUTION OF CASES OF SQUAMOUS CELL AND ADENO- OR ADENOSQUAMOUS INVASIVE CARCINOMA OF THE CERVIX AND CORRESPONDING CONTROLS ACCORDING TO SELECTED FACTORS

Study center	Number			HPV DNA, %			Median age[1]			*Chlamydia trachomatis* seropositive, %			*Chlamydia pneumoniae* seropositive, %		
	Squamous cell carcinoma	Adeno- or adenosquamous carcinoma	Controls	Squamous cell carcinoma	Adeno- or adenosquamous carcinoma	Controls	Squamous cell carcinoma	Adeno- or adenosquamous carcinoma	Controls	Squamous cell carcinoma	Adeno- or adenosquamous carcinoma	Controls	Squamous cell carcinoma	Adeno- or adenosquamous carcinoma	Controls
Thailand	201	20	76	97.0	85.0	15.8	51	44	44	59.7	60.0	31.6	73.6	70.0	67.1
The Philippines	318	31	368	96.2	90.3	9.2	48	48	47	51.9	29.0	22.8	76.7	90.3	82.6
Morocco	149	9	161	98.0	100.0	21.7	50	55	41	65.1	33.3	42.9	63.8	77.8	54.0
Peru	169	25	169	95.3	92.0	17.2	47	49	49	55.0	44.0	40.8	64.5	52.0	61.0
Brazil	155	14	180	96.8	92.9	16.7	51	44	48	37.4	28.6	19.4	74.8	78.6	68.9
Colombia	64		64	82.8		25.0	46		48	71.9		50.0	40.6		25.0
Spain	83		82	80.7		9.8	55		56	32.5		31.7	56.6		50.0
All centers	1,139	99	1,100	94.6	90.9	14.9	49	48	47	53.2	39.4	30.8	68.9	73.7	66.0

[1]Including HPV-positive women only.

14.9% of controls. Overall, *C. trachomatis* antibodies were found more frequently in squamous cell ICC cases (53.2%) or adeno- or adenosquamous cell ICC cases (39.4%) than in control participants (30.8%). In contrast, *C. pneumoniae* seropositivity was similar in the 3 groups. *C. trachomatis* seropositivity varied significantly by country among control participants, ranging from 19.4% in Brazil to 50% in Colombia.

In control participants, *C. trachomatis* seropositivity was the highest in women under 35 years of age (Table II) and was significantly associated with indicators of sexual behavior. Independent risk factors were having no education, being single, separated, divorced or widowed, having a first sexual intercourse before 17 years of age, having 2 or more lifetime sexual partners and HSV-2 seropositivity. In contrast, *C. pneumoniae* seropositivity was not significantly associated with the indicators of sexual behavior available (data not shown), but was modestly associated with *C. trachomatis* seropositivity (OR = 1.4; 95% CI = 1.0–1.9; data not shown).

Figure 1 shows the ORs for ICC in HPV DNA-positive cases and controls according to *C. trachomatis* seropositivity by center and overall. The pooled OR was 1.80 (95% CI = 1.22–2.66) for

TABLE II – ODDS RATIOS OF *CHLAMYDIA TRACHOMATIS* SEROPOSITIVITY AND CORRESPONDING 95% CONFIDENCE INTERVALS AMONG CONTROL PARTICIPANTS BY SELECTED RISK FACTORS

	Number	*C. trachomatis* (%)	OR[1] (95% CI)
Age (years)			
< 35	162	35.2	1[2]
35–44	315	33.3	0.77 (0.49–1.21)
45–54	311	26.1	0.55 (0.33–0.91)
55–64	221	29.0	0.72 (0.42–1.25)
⩾ 65	91	35.2	0.70 (0.34–1.41)
Education			
Secondary or more	452	26.1	1[2]
Primary	455	29.7	1.01 (0.70–1.46)
None	191	45.0	1.67 (1.01–2.74)
Marital status			
Married	751	27.0	1[2]
Cohabiting	103	43.7	1.19 (0.70–2.02)
Separated, divorced, or widowed	246	37.0	1.43 (1.00–2.06)
Smoking			
Never	933	30.4	1[2]
Ever	159	32.1	1.07 (0.69–1.65)
Number of full-term pregnancies			
0	70	18.6	1[2]
1–2	259	33.6	1.75 (0.86–3.56)
3–4	326	27.6	1.38 (0.67–2.83)
⩾ 5	444	33.6	1.77 (0.85–3.69)
Chi-square trend (*p*-value)			0.80 (0.37)
Age at first intercourse (years)			
⩾ 21	498	23.5	1[2]
17–20	381	31.0	1.06 (0.75–1.52)
< 17	213	48.4	1.58 (1.01–2.48)
Chi-square trend (*p*-value)			3.49 (0.07)
Total lifetime number of sexual partners			
⩽ 1	838	25.3	1[2]
2	161	47.2	1.90 (1.25–2.89)
⩾ 3	101	50.0	2.25 (1.31–3.86)
Chi-square trend (*p*-value)			11.91 (<0.001)
Oral contraceptive use			
Never	753	29.1	1[2]
< 5 years	218	31.2	1.14 (0.78–1.66)
⩾ 5 years	128	40.6	1.25 (0.79–1.97)
Chi-square trend (*p*-value)			1.07 (0.30)
Herpes simplex virus 2			
Seronegative	806	25.1	1[2]
Seropositive	278	47.8	2.21 (1.58–3.11)
HPV DNA			
Negative	936	29.8	1[2]
Positive	164	36.6	1.28 (0.86–1.89)

[1]Adjusted for study center and all the variables in the table.– [2]Reference category.

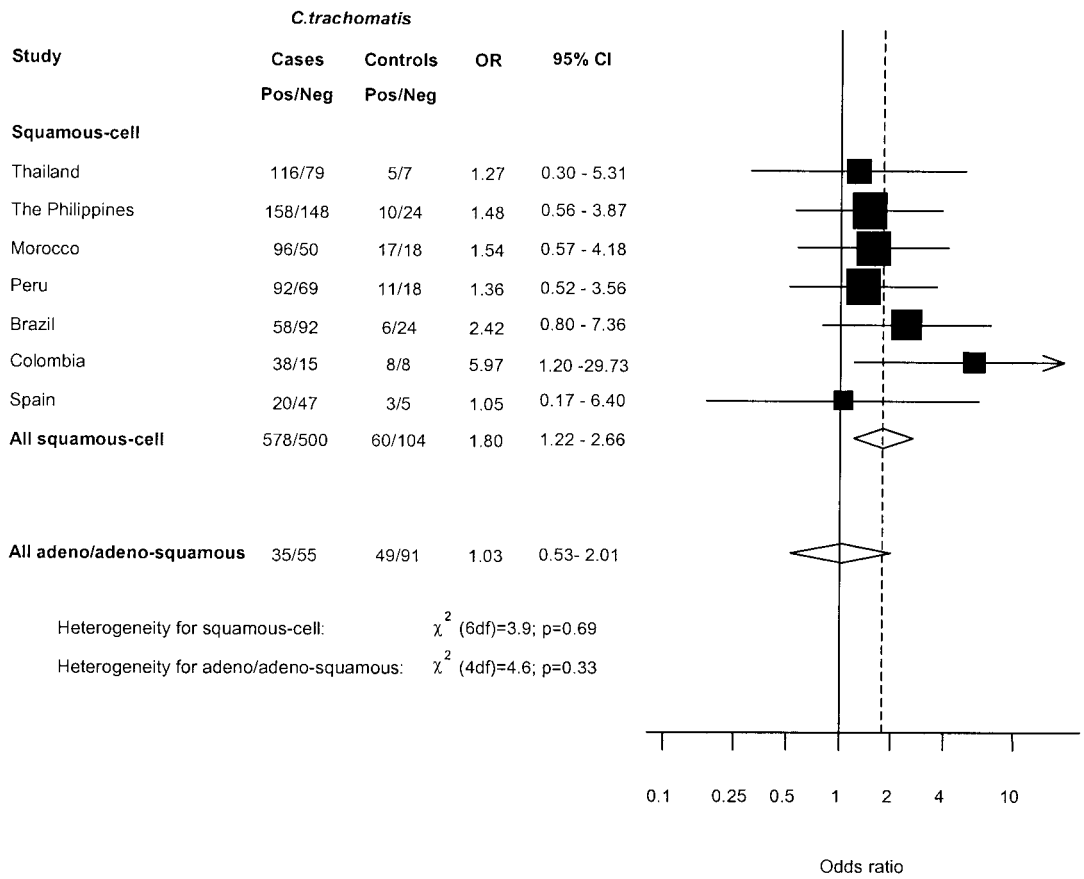

FIGURE 1 – Odds ratios of invasive cervical carcinoma and corresponding 95% confidence intervals among HPV-positive women according to *C. trachomatis* seropositivity. Odds ratios relative to *C. trachomatis* seronegative; adjusted for center, age, history of Pap smear, oral contraceptive use, number of full-term pregnancies and herpes simplex virus 2 seropositivity.

squamous cell ICC and 1.03 (95% CI = 0.53–2.01) for adeno- or adenosquamous cell ICC. Especially strong associations emerged in Brazil (OR = 2.42) and Colombia (OR = 5.97), whereas the OR was 1.05 in Spain. No significant heterogeneity between centers was present for either squamous cell or adeno- or adenosquamous cell ICC (Fig. 1). If the data from Colombia and Spain (*i.e.*, those centers where earlier, less sensitive tests for HPV detection were used) were eliminated, the association between *C. trachomatis* seropositivity and squamous cell ICC would persist (OR = 1.77; 95% CI = 1.16–2.70).

If only women positive for 14 high-risk HPV types were included, the OR for *C. trachomatis* seropositivity would have been 1.89 (95% CI = 1.18–3.02) for squamous cell ICC (based on 990 cases and 104 control women) and 0.95 (95% CI = 0.44–2.07) for adeno- or adenosquamous cell ICC (based on 85 cases and 85 control women). Among 1,031 HPV-positive squamous cell ICC where the clinical stage of ICC was known, the effect of *C. trachomatis* seropositivity was similar for stage I or II cancers (OR = 1.58; 95% CI = 1.04–2.40) and stage III or IV cancers (OR = 1.85; 95% CI = 1.19–2.90; data not shown).

Table III shows that elevated *C. trachomatis* antibody titers were particularly associated with an increased risk of squamous cell ICC in HPV DNA-positive women (p for trend < 0.001), with the highest risk in women with antibody titers ≥ 128 (OR = 3.58). The effect of *C. trachomatis* was similar for the different *C. trachomatis* serovar groups (OR = 2.17 for A, 2.21 for BED, 1.65 for CJHI and 1.87 for FGK). The presence of 2 or more serovar groups was associated with a nonsignificantly elevated OR of 1.60 (95% CI = 0.84–3.03) compared to the presence of a single serovar group of BED, CJHI or FGK (data not shown). *C. pneumoniae* seropositivity was not associated with an increased risk of squamous cell ICC (OR = 1.03).

When the association between *C. trachomatis* infection and squamous cell ICC was analyzed by categories of age at diagnosis (Fig. 2), the effect of *C. trachomatis* appeared to be attenuated in older-age women, with no increased risk observed over 55 years of age (OR = 0.95).

Analyses were conducted to examine the association of *C. trachomatis* seropositivity and squamous cell ICC in strata of selected indicators of socioeconomic status (data not shown). The effect of *C. trachomatis* tended to be nonsignificantly lower ($p = 0.43$) in women with less education (no education: OR = 1.07, 95% CI = 0.47–2.39; primary education: OR = 1.74, 95% CI = 0.95–3.19; secondary or more education: OR = 2.55, 95% CI = 1.15–5.67). Results were consistent in women with rural (OR = 1.24; 95% CI = 0.54–2.84) and urban (OR = 1.84; 95% CI = 1.08–3.07) residence.

Table IV shows the combined effect of *C. trachomatis* and selected indicators of sexual behavior and oral contraceptive use on the risk of squamous cell ICC in HPV-positive women. The association between *C. trachomatis* infection and squamous cell ICC (Fig. 1) was slightly attenuated, but remained significant, after further adjustment for number of sexual partners and age at first intercourse (OR = 1.69; 95% CI = 1.16–2.51). After additional adjustment of *C. trachomatis* in the fully adjusted model, a woman's reported number of sexual partners was not significantly associated with squamous cell ICC risk (OR = 1.29; 95% CI = 0.83–2.0), whereas young age at first intercourse (OR = 2.44 for < 17 *vs.* ≥ 21 years) and ≥ 5 years oral contraceptive use (OR = 2.69) remained statistically significant risk factors.

TABLE III – ODDS RATIOS OF SQUAMOUS CELL INVASIVE CARCINOMA OF THE CERVIX AND CORRESPONDING 95% CONFIDENCE INTERVALS AMONG HPV-POSITIVE WOMEN ACCORDING TO *CHLAMYDIA TRACHOMATIS* AND *CHLAMYDIA PNEUMONIAE* SEROPOSITIVITY OVERALL AND IN STRATA BY AGE GROUP AT DIAGNOSIS

	HPV$^+$ cases (%)	HPV$^+$ controls (%)	OR[1] (95% CI)
Chlamydia trachomatis			
Titers			
Seronegative	500 (46.4)	104 (64.4)	1[2]
8	176 (16.3)	25 (15.2)	1.28 (0.76–2.14)
32	239 (22.2)	24 (14.6)	1.71 (1.01–2.87)
≥ 128	163 (15.1)	11 (6.7)	3.58 (1.73–7.39)
Chi-square trend (*p*-value)			13.59 (< 0.001)
Serovars			
A			
Seronegative	640 (59.4)	123 (75.0)	1[2]
Seropositive	438 (40.6)	41 (25.0)	2.17 (1.42–3.32)
BED			
Seronegative	525 (48.7)	114 (69.5)	1[2]
Seropositive	553 (51.3)	50 (30.5)	2.21 (1.49–3.29)
CJHI			
Seronegative	652 (60.5)	120 (73.2)	1[2]
Seropositive	426 (39.5)	44 (26.8)	1.65 (1.09–2.49)
FGK			
Seronegative	665 (61.7)	124 (75.6)	1[2]
Seropositive	413 (38.3)	40 (24.4)	1.87 (1.22–2.87)
Chlamydia pneumoniae			
Seronegative	322 (29.9)	61 (37.2)	1[2]
Seropositive	756 (70.1)	103 (62.8)	1.03 (0.70–1.51)

[1]OR adjusted for age, study center, history of Pap smear, oral contraceptive use, number of full-term pregnancies and herpes simplex virus 2 seropositivity.–[2]Reference category.

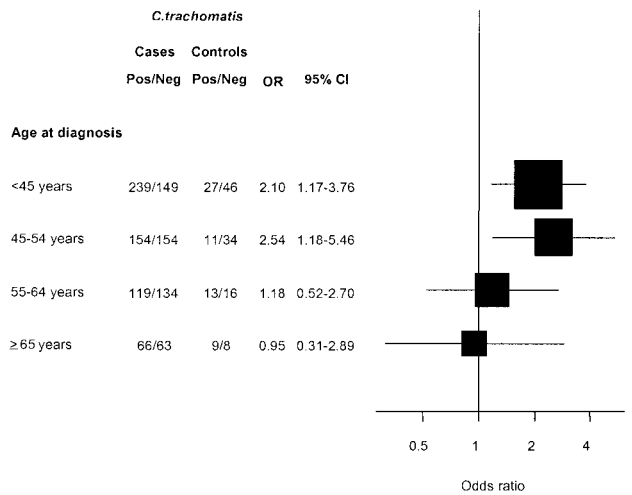

FIGURE 2 – Odds ratios of squamous cell invasive carcinoma of the cervix and corresponding 95% confidence intervals among HPV-positive women according to *C. trachomatis* seropositivity and age at diagnosis. Odds ratios relative to *C. trachomatis* seronegative; adjusted for center, age, history of Pap smear, oral contraceptive use, number of full-term pregnancies and herpes simplex virus 2 seropositivity.

The OR of *C. trachomatis* in all case and control women, after adjustment for the same factors as in Figure 1, was 2.19 (95% CI = 1.80–2.67) for squamous cell ICC and 1.35 (95% CI = 0.84–2.17) for adeno- and adenosquamous cell ICC (data not shown). After additional adjustment for HPV infection, the ORs slightly diminished: 1.83 (95% CI = 1.33–2.52) for squamous cell ICC and 1.14 (95% CI = 0.63–2.08) for adeno- or adenosquamous cell ICC, again with no significant heterogeneity between centers (Fig. 3). The risk of squamous cell ICC increased with higher antibody titers: OR = 1.19 for titers of 8, OR = 2.03 for titers of 32 and OR = 3.57 for titers of ≥ 128.

DISCUSSION

This study, based on data from 1,238 case and 1,100 control participants in 7 countries worldwide, shows that *C. trachomatis* serum antibodies were associated with a 1.8-fold increased risk of squamous cell ICC. An increased squamous cell ICC risk was consistently found in all countries considered, except for Spain, although *C. trachomatis* seropositivity in controls varied greatly by country. This risk was higher in women with elevated *C. trachomatis* antibody titers, and in women under 55 years of age. *C. trachomatis* and *C. pneumoniae* species-specific serum antibodies were differentiated using MIF assay, and an increased risk of squamous cell ICC was found in women with *C. trachomatis* but not with *C. pneumoniae* antibodies. Our study thus supports the possibility that *C. trachomatis* increases squamous cell ICC risk, after accounting for cervical HPV infection using highly sensitive PCR-based methods, and other risk factors for ICC. Our ability to restrict analyses to HPV DNA-positive case and control participants, albeit justified by the causal link between HPV infection and ICC, did not alter the relationship between *C. trachomatis* and squamous cell ICC risk. The effect of *C. trachomatis* was similar for HPV DNA-positive women and for all cases and controls after adjustment for HPV DNA status or HPV DNA type. After adjustment for major confounding factors, but not for HPV DNA status, the OR for squamous cell ICC was 2.2 (*i.e.*, slightly more elevated than after stratification or adjustment for HPV).

Our analysis of *C. trachomatis* in 99 adeno- or adenosquamous cell ICC cases is the largest to date, given the relative rarity of cervical adenocarcinoma. Our findings are consistent with another study of those from 32 adenocarcinoma cases[10] that found no effect due to *C. trachomatis*. Overall, these data support the notion that some HPV cofactors may differ according to the histologic type of ICC.

The reliability of our results is supported by the use of a multicenter study with a large sample size, a common protocol and standardized laboratory procedures for the ascertainment of HPV and *C. trachomatis* infections. To determine a woman's cumulative exposure rather than current *C. trachomatis* infection, we

TABLE IV – ODDS RATIOS OF SQUAMOUS CELL INVASIVE CARCINOMA OF THE CERVIX AND CORRESPONDING 95% CONFIDENCE INTERVALS AMONG HPV-POSITIVE WOMEN ACCORDING TO THE COMBINED EFFECT OF *CHLAMYDIA TRACHOMATIS* SEROPOSITIVITY AND SEXUAL BEHAVIOR

	C. trachomatis		OR[2] (95% CI)
	Seronegative cases/controls	Seropositive cases/controls	
Number of sexual partners			
1			
n	344/78	319/37	
OR[1] (95% CI)	1[3]	1.70 (1.05–2.76)	1[3]
≥ 2			
n	156/26	259/23	
OR[1] (95% CI)	1.30 (0.74–2.31)	2.16 (1.19–3.92)[4]	1.29 (0.83–2.00)
Age at first intercourse (years)			
≥ 21			
n	149/48	106/20	
OR[1] (95% CI)	1[3]	1.54 (0.80–2.97)	1[3]
17–20			
n	211/31	250/19	
OR[1] (95% CI)	2.15 (1.18–3.90)	3.64 (1.85–7.18)	2.22 (1.36–3.63)
< 17			
n	140/23	222/20	
OR[1] (95% CI)	2.25 (1.14–4.45)	4.18 (2.08–8.43)[5]	2.44 (1.41–4.23)
Oral contraceptive use			
Never			
n	347/69	387/39	
OR[1] (95% CI)	1[3]	1.70 (1.06–2.73)	1[3]
< 5 years			
n	81/28	92/15	
OR[1] (95% CI)	0.75 (0.41–1.36)	1.32 (0.65–2.67)	0.76 (0.47–1.23)
≥ 5 years			
n	71/6	95/6	
OR[1] (95% CI)	2.90 (1.14–7.40)	4.21 (1.64–10.85)[6]	2.69 (1.35–5.35)
OR (95% CI)	1[3]	1.69 (1.14–2.51)	

[1]Adjusted for age, study center, history of Pap smear, oral contraceptive use, number of full-term pregnancies, herpes simplex virus 2 seropositivity, number of sexual partners and age at first intercourse.–[2]Adjusted as above and for *C. trachomatis* seropositivity.–[3]Reference category.–[4]Chi-square for interaction = 0.00 (1 df), $p = 0.95$.–[5]Chi-square for interaction = 0.16 (2 df), $p = 0.93$.–[6]Chi-square for interaction = 0.05 (2 df), $p = 0.70$.

measured *C. trachomatis* serum antibodies. The specificity of the observed MIF antibody responses was validated by the observation that *C. trachomatis*, unlike *C. pneumoniae*, antibody responses were correlated with indicators of sexual behavior.

The dose-response relationship between *C. trachomatis* MIF antibody titers and squamous cell ICC risk provides further support for an etiologic role for *C. trachomatis* in cervical carcinogenesis. Elevated *C. trachomatis* titers (≥ 128) are likely to be maintained by continued antigenic stimulation, which may be due to either repeated or chronic chlamydial infections.[27] Elevated *C. trachomatis* titers have also been associated with adverse reproductive sequelae such as tubal infertility,[28] pelvic inflammatory disease[29] and the presence of heat shock proteins (HSPs).[29,30] The latter finding is of particular interest given that HSPs appear to be involved in the immunopathogenesis of chlamydial infections.[29,31]

No specific *C. trachomatis* serovar group had a higher association with squamous cell ICC in our study. *C. trachomatis* serotypes fall into 2 major antigenic groups, the B complex (consisting of serovars of B, D and E) and the C complex (consisting of serovars of C, J, H and I), with serovars of F, G and K as bridging serovars between the 2 major antigenic complexes (*i.e.*, they share minor antigenic profiles with both major complexes in addition to unique antigenic characteristics). Serologic assays such as the MIF measure cumulative exposure of an individual to antigens of *C. trachomatis*. An individual who is infected with one serovar and then subsequently exposed to another serovar from a heterologous antigenic complex will exhibit broad antibody reactivity to multiple serovars.[32] A nested case-control study from Finland, Norway and Sweden[10] found that serovar G was most strongly associated with squamous cell ICC but the confidence intervals were broad, and it is unclear how an increased risk for squamous cell carcinoma may be attributable to a single serovar using serologic assays since complex antigenic relationships exist between serovars of *C. trachomatis*.[10,32]

The association between squamous cell ICC and *C. trachomatis* seropositivity appeared to be stronger in younger women (< 55 years of age), suggesting that cumulative exposure to chlamydial infection may be less important, or less-well measured by seropositivity after middle age. The prevalence of *C. trachomatis* IgG antibodies declined with age among squamous cell ICC cases but not among control women. Few reliable data are available on the natural history of *C. trachomatis* antibody profiles during persistent or acute infections. *C. trachomatis* antibodies have been shown to persist for years; however, a loss of *C. trachomatis* antibodies or a decrease in IgG antibody titers over time may occur,[29,33] particularly if there is no continued antigenic stimulation.[34] A case-control study showed a stronger association between *C. trachomatis* seropositivity and squamous cell ICC when the serum sampling was closer in time to ICC diagnosis.[13]

C. trachomatis infection may increase the risk of squamous cell ICC by increasing host susceptibility to HPV or enhancing the effects of HPV. Inflammation, resulting from a chronic *C. trachomatis* infection, may result in the production of reactive oxygen species that may cause DNA damage and increase the risk of HPV-associated carcinogenesis.[35,36] *In vitro* data suggest that *Chlamydia*-infected cells are also less likely to undergo the normal process of programmed cell death.[37] Other laboratory data indicate that *C. trachomatis* may disrupt the normal structure of cadherin-catenin junctions in cervical epithelial cells,[38] resulting in increased susceptibility to HPV and other infections. Alternatively, *C. trachomatis* seropositive women may be more likely to elicit a humoral-mediated (Th2) rather than cell-mediated (Th1) immune response to particular antigens.[30] In this case, women infected with

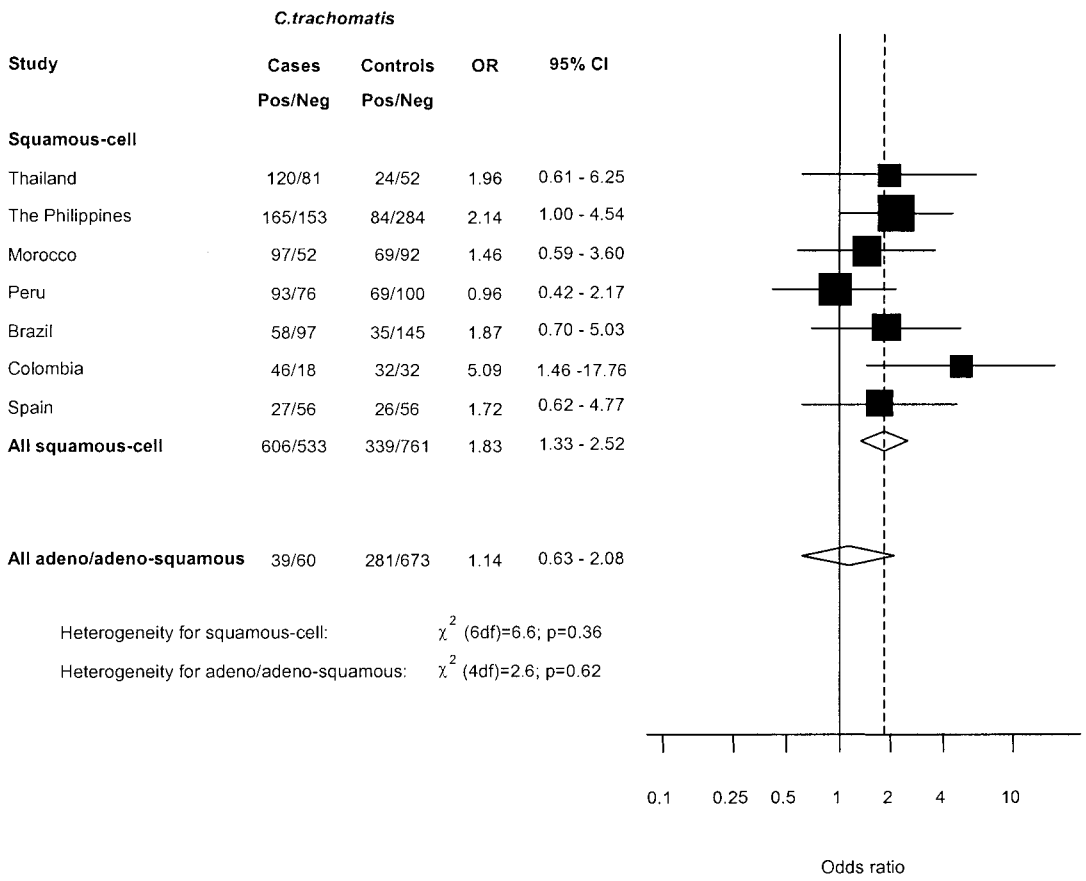

FIGURE 3 – Odds ratios of invasive cervical cancer and corresponding 95% confidence intervals among all cases and controls according to *C. trachomatis* seropositivity. Odds ratios relative to *C. trachomatis* seronegative; adjusted for center, age, history of Pap smear, oral contraceptive use, number of full-term pregnancies, herpes simplex virus 2 and HPV-DNA positivity.

C. trachomatis may have an impaired ability to clear an HPV infection or control HPV-induced cervical neoplasia. In a cohort study of Swedish women aged 32–38 years, self-reported history of *C. trachomatis* was associated with a 4-fold increased risk of HPV persistence after 12-month follow-up.[39]

We do not believe that our findings should be interpreted as *C. trachomatis* acting as a carcinogen of the cervix independently from HPV infection and note that no methods can distinguish transient from persistent HPV infection. The ascertainment of cervical HPV DNA is likely to have, for instance, a different meaning in case and control participants. HPV DNA in case women should indicate a persistent HPV infection, whereas some control women may be transiently infected or have been infected in the past and since cleared their infection. However, because the association between *C. trachomatis* and squamous cell ICC in HPV-positive women was not notably reduced after further adjustment or stratification for a woman's number of sexual partners, age at first intercourse and HSV-2 seropositivity, the effect of *C. trachomatis* is not likely to represent simply exposure to HPV or other STIs.

Another limitation of our study is that the MIF assay used does not differentiate a woman's exposure to genital or to ocular *C. trachomatis* infections. However, it is unlikely that our results are due to past exposure to ocular infections because endemic trachoma is rare or nonexistent in all study sites. Furthermore, the association between *C. trachomatis* and squamous cell ICC was consistent in strata of educational attainment and place of residence, although ocular infection should have been more frequent in low social classes and in rural areas. The use of hospital-based control subjects in 5 study sites may have also led to biased results if *C. trachomatis* seroprevalence in controls was not representative of the population source of the ICC patients. Hospital controls in our study, however, had a wide range of diagnoses and were ascertained in large public hospitals with reference populations similar to those where cases were identified.[16–18,20,21]

In conclusion, our results indicate that *C. trachomatis* infection may act in conjunction with HPV to increase the risk of squamous cell ICC, although this association is modest compared to the strong effect of HPV infection on ICC risk. Given that *C. trachomatis* is the most common bacterial STI worldwide, with over 12 million global cases estimated annually[40] and 700,000 cases reported in the United States,[41] additional data are needed to determine if screening and treatment of *C. trachomatis* infections may result in a lower incidence of low- and high-grade squamous intraepithelial lesions.

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