Evidence for Chlamydia trachomatis as a Human Papillomavirus Cofactor in the Etiology of Invasive Cervical Cancer in Brazil and the Philippines

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Chlamydia trachomatis infection was examined as a cause of invasive cervical cancer (ICC) among women with human papillomavirus (HPV) infection. In total, 499 women with incident ICC (ICC patients) and 539 control patients from São Paulo, Brazil, and Manila, the Philippines, were included. C. trachomatis antibodies were detected by microimmunofluorescence assay. Presence of HPV DNA in cervical specimens was determined by a polymerase chain reaction–based assay. C. trachomatis seropositivity was associated with sexual behavior but not with HPV infection. C. trachomatis increased the risk of squamous cervical cancer among HPV-positive women (odds ratio, 2.1; 95% confidence interval, 1.1–4.0). Results were similar in both countries. There was a suggestion of increasing squamous cancer risk with increasing C. trachomatis antibody titers. This large study examined C. trachomatis and cervical cancer, taking into account the central role of HPV infection. C. trachomatis infection was found to be a possible cofactor of HPV in the etiology of squamous cervical cancer, and its effect may be mediated by chronic inflammation.

Extensive data show that human papillomavirus (HPV) is the central cause of cervical cancer, and high-risk HPV types are associated with a greater disease risk [1]. Only a small proportion of women infected with HPV infection progress to invasive cervical cancer (ICC). Thus, the development of ICC depends on other cofactors acting in conjunction with HPV, such as other sexually transmitted infections (STIs), smoking, hormones, nutritional deficiencies, or host-genetic/immunologic responses.

Clinical observations in the 1970s indicated that genital infection with Chlamydia trachomatis was associated with cervical atypia [2] and cervical neoplasia [3]. Genital C. trachomatis infections may result in chronic cervicitis, pelvic inflammatory disease, and endometritis, whereas ocular C. trachomatis infections may cause trachoma and may result in blindness in some cases. Interest in genital C. trachomatis infection as a potential etiologic factor for ICC has been related to its asymptomatic nature, persistence if left untreated, and induction of metaplasia [4] and chronic inflammation [5].

Two epidemiologic studies examined C. trachomatis in the etiology of ICC and in the context of HPV by using sensitive polymerase chain reaction (PCR)–based assays for HPV DNA detection [6, 7]. In a study of cervical neoplasia [6], C. trachomatis seropositivity was a significant risk factor for carcinoma in situ in Spain and Colombia after adjusting for HPV DNA. However, when combined country analyses were restricted to HPV DNA–positive case and control patients, C. trachomatis antibodies were not significantly associated with either in situ carcinoma or ICC. Some misclassification of HPV DNA resulting from the use of a first-generation PCR–based assay could not be ruled out. Another study reported no association between C. trachomatis antibodies and cervical neoplasia after adjusting for HPV DNA among women in Honduras [7]. Results restricted to HPV DNA–positive participants were not presented, although an ELISA known to have C. trachomatis/C. pneumoniae cross-reactivity was used. To further examine the role of C. trachomatis infection as a cofactor of HPV in the etiology of ICC, we present results from 2 International Agency for Research on Cancer...
(IARC) ICC case-control studies conducted in São Paulo, Brazil, and Manila, the Philippines.

Methods

This analysis is based on 2 cervical cancer case-control studies of 499 women with incident ICC (ICC patients) and 539 control patients in São Paulo and Manila, selected as described elsewhere [8, 9]. Both studies were part of an IARC multicenter case-control study and used similar protocols and questionnaires for recruitment and data collection.

All women with ICC were newly diagnosed and had received no previous treatment, and their ICC was histologically confirmed. Hospital-based control patients were selected and were frequency age-matched to the case patients by quinquennium of age. Women were not eligible to participate as control patients if they had received any previous cervical cancer treatment or if they had diseases possibly sharing risk factors with cervical cancer (e.g., cardiovascular or cerebrovascular disease, chronic bronchitis, emphysema, or neoplasia of the breast, reproductive or respiratory organs, anus, oral cavity, esophagus, bladder, or liver).

In Brazil, 199 ICC patients (96.6% of eligible subjects) were recruited from 5 public hospitals and 2 cancer hospitals in São Paulo between June 1990 and June 1991. As hospital-based controls, 225 women (94.5% of those eligible) were interviewed and were selected from the 5 public hospitals. In total, 150 women with ICC (75.4% of participants; 137 with squamous and 13 with adenocarcinoma/adenosquamous cancer) and 173 control patients (76.9%) with chlamydia serology and HPV DNA results were included in this analysis. The main diagnostic categories of Brazilian control patients were diseases of the circulatory system (21.8%), infectious and parasitic diseases (12.9%), diseases of the digestive tract (12.3%), neoplasms (9.5%), and diseases of the nervous system (8.9%).

In the Philippines, 387 ICC patients (100% of those eligible) and 387 control patients (98.7% of those eligible) were identified among patients at the Philippine General Hospital in Manila between April 1991 and April 1993. A total of 349 women with ICC (90.2% of participants; 318 with squamous and 31 with adenocarcinoma/adenosquamous cancer) and 366 (94.6%) control patients with chlamydia serology and HPV DNA results were included in this analysis. The main diagnostic categories of Brazilian control patients were diseases of the circulatory system (21.8%), infectious and parasitic diseases (12.9%), diseases of the digestive tract (12.3%), neoplasms (9.5%), and diseases of the nervous system (8.9%).

Screening for HPV DNA was done by a PCR assay based on GP5+/6+ primer sequences at subpicogram levels. G5+/6+/C without additives. Histologic slides for cancer diagnosis were reviewed by an expert pathologist. The stage of disease was coded according to International Federation of Gynecology and Obstetrics standards. Cervical exfoliated cells were collected, by sampling the ectocervix with 2 wooden spatulas and the endocervix with 2 cytobrushes, and were placed in tubes with PBS, centrifuged, and stored at −70°C until shipment to a central laboratory for HPV DNA testing.

Laboratory procedures. Serum IgG antibody responses to C. trachomatis were determined by a microimmunofluorescence (MIF) assay, which, at present, is considered to be the most accurate serologic method [10]. The antigen panel consists of purified elementary bodies of C. trachomatis (serovar A and 3 pooled serovar groups of BDE, CJHI, and FGK) and C. pneumoniae [11]. C. pneumoniae was included in the antigen panel to monitor cross-reactive antibody responses and to test for specificity of the C. trachomatis findings. Clinically, C. trachomatis serovars D–K are primarily associated with genital chlamydial infections, whereas serovars A–C are associated with both hyperendemic trachoma and genital infection. Serologic testing was done without knowledge of case-control status.

All serum samples were screened for C. trachomatis at 1:8 dilution and were titrated to end point. With the exceptions mentioned below, an IgG titer ≥1:8 against any of the C. trachomatis serovar groups was considered to be evidence of past infection with C. trachomatis. An IgG titer ≥1:16 against C. pneumoniae was considered to be evidence of past C. pneumoniae infection. Serum samples that had identical titers for all C. trachomatis and C. pneumoniae species were also tested with C. psittaci (avian strain 6BC) to determine the presence of broad Chlamydia species cross-reactivity. Serum samples with identical titers for all chlamydial species (C. trachomatis, C. pneumoniae, and C. psittaci) were considered to be cross-reactive and were excluded from analyses: 7 Brazilian women (5 ICC patients and 2 control patients) and 4 Philippine women (2 ICC patients and 2 control patients) in this category were excluded. Serum samples from 9 ICC patients and 9 control patients who were seropositive for serovar A and negative for all other C. trachomatis serovars were considered to have evidence of past C. trachomatis ocular trachoma infections and were excluded from analyses.

A blinded reproducibility study was conducted by retesting a random sample of 10% of the serum specimens (n = 105) twice by MIF. C. trachomatis serovars A, BDE, CJHI, and FGK and C. pneumoniae had the following percentages of agreement: 83.9%, 87.5%, 84.8%, 83.9%, and 87.5%, respectively. The agreement for C. trachomatis positivity for the 2 repeat tests was 0.75 overall: 0.65 for case patients and 0.80 for control patients. The agreement for C. pneumoniae positivity was 0.6 for the 2 repeat tests overall: 0.56 for case and 0.65 for control patients.

Screening for HPV DNA was done by a PCR assay based on GP5+/6+/TS-PCR and G5+/6+/6’ primers, as described elsewhere [8, 9]. In brief, amplification of a fragment of the β-globin gene served as an internal control for efficiency of each specimen for amplification. Specimens were labeled “HPV X” for HPV-positive specimens when a specific HPV type could not be determined. GP5+/6+/TS-PCR primers detect sequenced HPV types 6, 11, 16, 18, 31, and 33, and unsequenced HPV types at subpicogram levels. G5+/6+/6’ primers detect over 30 different HPV types including types 6, 11, 16, 18, 26, 31, 33–35, 39, 40, 42–45, 51, 52, 54, 56–59, 61, 66, 68, 70, 72, 73,
Results

In total, we studied 455 ICC patients with squamous ICC, 44 with adenocarcinoma/adenosquamous ICC, and 539 age-matched control patients from Brazil and the Philippines (table 1). *C. trachomatis* seropositivity was significantly higher among all squamous ICC patients (47.7%) than among control patients (22.1%; *P* < .0001) but was not significantly higher among adenocarcinoma/adenosquamous ICC patients (29.6%) than among control patients (22.1%; *P* = .3). The difference in seroprevalence between squamous ICC patients and control patients increased with increasing *C. trachomatis* antibody titer. Among control patients, *C. trachomatis* seropositivity was similar in Brazil (20.2%) and the Philippines (23.0%). *C. pneumoniae* seropositivity did not significantly differ between ICC patients and control patients but was lower among control participants in Brazil (68.2%) than in the Philippines (82.8%; *P* < .001). HPV DNA positivity was significantly higher among all ICC patients (93.8%) than among control patients (11.3%; *P* < .001).

The prevalence of *C. trachomatis* antibodies did not differ significantly by clinical cancer stage in either Brazil or the Philippines (data not shown) or for ICC patients from the 2 countries combined (combined countries): stage I, 40.2% (*n* = 87); stage II, 47.8% (*n* = 180); stage III, 47.5% (*n* = 200); and stage IV, 50.0% (*n* = 4; *P* trend = .3).

Table 2 shows that *C. trachomatis* seropositivity was significantly associated with sexual behavioral factors among control patients in the age-adjusted analyses. In the multivariate model,

### Table 1. Seroprevalence of *Chlamydia* antibodies and presence of human papillomavirus (HPV) DNA among invasive cervical cancer patients and control patients, by country and overall.

| Variable                  | Brazil                        | The Philippines     | Overall                        |
|---------------------------|-------------------------------|---------------------|-------------------------------|
|                           | SQ patients                   | AD patients        | All control patients          | SQ patients                   | AD patients        | All control patients |
| No. of women with results | 137                           | 13                 | 173                           | 318                          | 31                 | 366                  |
| Mean age, years           | 52.9                          | 47.8               | 52.5                          | 47.2                         | 48.5               | 46.5                 |
| *C. trachomatis* seropositive Titer | 38.0 | 30.8 | 20.2 | 51.9 | 29.0 | 23.0 | 45.5 | 29.6 | 22.1 |
| 8                        | 16.1                          | 15.4               | 13.3                          | 11.0                         | 9.7                | 9.0                  | 12.5 | 11.4 | 10.4 |
| 32                       | 12.4                          | 15.4               | 4.1                           | 25.8                         | 9.7                | 10.9                 | 21.8 | 11.4 | 8.7  |
| 128                      | 9.5                           | 0.0                | 2.9                           | 15.1                         | 9.7                | 3.0                  | 13.4 | 6.8  | 3.0  |
| *C. trachomatis* serovarA | 34.3                          | 30.8               | 17.9                          | 46.9                         | 25.8               | 20.2                 | 43.1 | 27.3 | 19.5 |
| BED                      | 36.5                          | 30.8               | 20.2                          | 51.3                         | 29.0               | 22.7                 | 46.8 | 30.0 | 21.9 |
| CHIJ                     | 30.7                          | 30.8               | 14.5                          | 46.2                         | 19.4               | 19.4                 | 41.5 | 22.7 | 17.8 |
| FGK                      | 26.3                          | 23.1               | 13.9                          | 44.7                         | 22.6               | 17.8                 | 39.1 | 22.7 | 16.5 |
| *C. pneumoniae* seropositive HPV DNA | 74.5 | 76.9 | 68.2 | 76.7 | 90.3 | 82.8 | 76.0 | 86.4 | 78.1 |
| Positive                 | 92.7                          | 76.9               | 16.2                          | 95.3                         | 90.3               | 9.0                  | 94.5 | 86.4 | 11.3 |
| High riskb               | 91.3                          | 100.0              | 60.7                          | 93.4                         | 92.9               | 63.6                 | 92.8 | 94.7 | 62.3 |

NOTE. AD, adenocarcinoma/adenosquamous carcinoma; SQ, squamous carcinoma.

a Serovar positivity not mutually exclusive.
b Percentage of high-risk HPV types among HPV-positive participants.
Table 2. Prevalence of *Chlamydia trachomatis* and *C. pneumoniae* seropositivity by selected risk factors and associated odds ratios (ORs) among control women from Brazil and the Philippines.

| Risk factor                                      | No. of women | C. trachomatis seropositive | ORa (95% CI) | ORb (95% CI) | No. of women | C. pneumoniae seropositive | ORa (95% CI) | ORb (95% CI) |
|------------------------------------------------|--------------|-----------------------------|--------------|--------------|--------------|-----------------------------|--------------|--------------|
| Age, years                                      |              |                             |              |              |              |                             |              |              |
| <40                                             | 123          | 30.1                        | 1            | 1            | 123          | 71.5                        | 1            | 1            |
| 40–49                                           | 155          | 19.4                        | 0.6 (0.3–1.0) | 1            | 0.4 (0.2–0.8) | 0.4 (0.2–0.8)              | 155          | 80.0         |
| 50–59                                           | 158          | 18.4                        | 0.5 (0.3–0.9) | 0.5 (0.3–0.9)| 158          | 83.5                        | 2.3 (1.3–4.1) | 2.2 (1.2–4.2)|
| >60                                             | 103          | 22.3                        | 0.7 (0.4–1.3) | 0.7 (0.3–1.4) | 103          | 74.8                        | 1.6 (0.9–3.0) | 1.5 (0.8–3.0)|
| P trend = .2 P trend = .3                       |              |                             |              |              |              |                             |              |              |
| Country                                         |              |                             |              |              |              |                             |              |              |
| Brazil                                          | 173          | 20.2                        | 1            | 1            | 173          | 68.2                        | 1            | 1            |
| The Philippines                                 | 366          | 23.0                        | 1.1 (0.7–1.8) | 2.2 (1.2–3.9) | 366          | 82.8                        | 2.4 (1.6–3.7) | 2.2 (1.4–3.6)|
| Age at first sexual intercourse, years          |              |                             |              |              |              |                             |              |              |
| ≥21                                             | 286          | 18.2                        | 1            | 1            | 286          | 78.0                        | 1            | 1            |
| 17–20                                           | 190          | 24.2                        | 1.4 (0.9–2.2) | 1.1 (0.7–1.8) | 190          | 78.4                        | 1.1 (0.7–1.8) | 1.2 (0.7–2.0)|
| <17                                             | 63           | 33.3                        | 2.5 (1.3–4.6) | 1.7 (0.8–3.5) | 63           | 77.8                        | 1.2 (0.6–2.4) | 1.4 (0.7–3.1)|
| P trend = .02 P trend = .4                      |              |                             |              |              |              |                             |              |              |
| Total lifetime sex partners                      |              |                             |              |              |              |                             |              |              |
| 1                                               | 447          | 19.7                        | 1            | 1            | 447          | 79.9                        | 1            | 1            |
| >2                                              | 92           | 33.7                        | 2.4 (1.4–4.1) | 1.6 (0.9–2.9) | 92           | 69.6                        | 0.7 (0.4–1.3) | 0.7 (0.4–1.2)|
| Woman reported husband had other sex partners    |              |                             |              |              |              |                             |              |              |
| Never                                           | 356          | 14.6                        | 1            | 1            | 356          | 77.3                        | 1            | 1            |
| Ever/uncertain                                  | 183          | 36.6                        | 3.6 (2.3–5.5) | 3.2 (2.1–5.1) | 183          | 79.8                        | 1.3 (0.8–2.0) | 1.4 (0.9–2.3)|
| Parity                                          |              |                             |              |              |              |                             |              |              |
| 0–1                                             | 62           | 21.0                        | 1            | 1            | 62           | 77.4                        | 1            | 1            |
| 2–4                                             | 249          | 20.9                        | 1.1 (0.5–2.2) | 1.2 (0.6–2.5) | 249          | 76.3                        | 0.9 (0.4–1.7) | 0.8 (0.4–1.6)|
| ≥5                                              | 228          | 23.7                        | 1.6 (0.8–3.2) | 1.4 (0.6–3.0) | 228          | 80.3                        | 1.0 (0.5–2.0) | 0.9 (0.4–1.8)|
| P trend = .3 P trend = .4                       |              |                             |              |              |              |                             |              |              |
| Herpes simplex virus                            |              |                             |              |              |              |                             |              |              |
| Seronegative                                    | 433          | 18.7                        | 1            | 1            | 433          | 79.9                        | 1            | 1            |
| Seropositive                                    | 106          | 35.9                        | 3.3 (1.9–5.6) | 2.1 (1.1–3.8) | 106          | 70.8                        | 0.9 (0.5–1.4) | 0.8 (0.5–1.5)|

NOTE. CI, confidence interval.

a Adjusted by age and country of residence.

b Adjusted by age, country of residence, and all other factors in the table.
Table 3. Odds ratios (ORs) of human papillomavirus (HPV) DNA positivity among control women by *Chlamydia trachomatis* and *C. pneumoniae* seropositivity in Brazil and the Philippines combined.

| Parameter | HPV-positive/HPV-negative control women | OR (95% CI) |
|-----------|----------------------------------------|-------------|
| *C. trachomatis* IgG seronegative | 45/375 | 1 |
| *C. trachomatis* IgG seropositive | 16/103 | 1.4* (0.7–2.7) |
| Seropositive titer | | |
| 8 | 7/49 | 1.2* (0.5–2.9) |
| 32 | 8/39 | 1.6* (0.7–3.7) |
| 128 | 1/15 | P for trend = .2 |
| *C. pneumoniae* | | |
| Seronegative | 15/103 | 1b |
| Seropositive | 46/375 | 1.0b (0.5–2.0) |

NOTE. CI, confidence interval.

*C. pneumoniae* seropositivity was not associated with HPV DNA positivity among control patients.

To determine whether past *C. trachomatis* infection may increase the risk of being a carrier of HPV infection, we examined associations between *C. trachomatis* antibodies and HPV DNA among control patients (table 3). Results were similar in Brazil and the Philippines (data not shown). Neither *C. trachomatis* seropositivity (OR, 1.4; 95% CI, 0.7–2.7) nor high *C. trachomatis* antibody titers (OR, 1.6; 95% CI, 0.7–3.7) were significantly associated with HPV DNA detection. *C. pneumoniae* seropositivity was not associated with HPV DNA positivity among control patients.

In relation to cervical cancer, *C. trachomatis* seropositivity was significantly associated with squamous ICC after adjusting for age, HPV DNA positivity, HSV-2 seropositivity, the reported sexual behavior of a woman’s husband (for the combined countries, OR, 2.5; 95% CI, 1.5–4.1), and elevated (>32) *C. trachomatis* antibody titers (OR, 3.9; 95% CI, 2.1–7.3; data not shown).

Table 4. Odds ratios (ORs) of invasive squamous cervical cancer among human papillomavirus (HPV) DNA–positive study participants, by *Chlamydia trachomatis* and *C. pneumoniae* seropositivity.

| Parameter | HPV-positive patients/HPV-positive control women | OR (95% CI) |
|-----------|--------------------------------------------------|-------------|
| *C. trachomatis* IgG | | |
| Brazil Seronegative | 78/22 | 1* |
| Seropositive | 49/6 | 2.5* (0.9–6.9) |
| The Philippines Seronegative | 147/23 | 1* |
| Seropositive | 156/10 | 1.6* (0.7–3.7) |
| Both countries combinedb | | |
| Seronegative | 225/45 | 1* |
| Seropositive | 205/16 | 2.1* (1.1–4.0) |
| Seropositive titer | | |
| 8 | 56/7 | 1.4* (0.6–3.3) |
| 32 | 94/8 | 2.7* (1.2–5.9) |
| 128 | 55/1 | P for trend = .01 |
| *C. pneumoniae*, both countries combinedb | | |
| Seronegative | 102/15 | 1c |
| Seropositive | 328/46 | 1.2c (0.6–2.3) |

NOTE. CI, confidence interval.

*C. pneumoniae* infection was not associated with squamous or adenosquamous ICC in either Brazil or the Philippines (data not shown) or in the combined analysis of both countries. The associ-
ations between \textit{C. trachomatis} and squamous cervical cancer were similar for \textit{C. pneumoniae}—seropositive and —seronegative women (data not shown).

On the basis of data from 44 ICC patients, \textit{C. trachomatis} seropositivity was not associated with adenocarcinoma/adenosquamous ICC risk in Brazil or the Philippines (data not shown) or in the combined country analysis (OR, 0.8; 95% CI, 0.3–2.2) among HPV DNA–positive participants after controlling for age, HSV-2 seropositivity, and a woman’s reported history of her husband’s sexual behavior.

Analyses were conducted for each \textit{C. trachomatis} serovar group (A, BED, CHJ, and FGK). The association between \textit{C. trachomatis} and squamous or adenocarcinoma/adenosquamous ICC was similar in all serovar groups (data not shown).

**Discussion**

We believe that our study is the largest of \textit{C. trachomatis}, HPV, and ICC to date that takes into account the strong effect of HPV and type-specific \textit{C. trachomatis} serology results. These data indicate a moderate, but significant, association between \textit{C. trachomatis} infection and ICC in the presence of HPV DNA. \textit{C. trachomatis} seropositivity was consistently associated with an increased risk (~2-fold) of squamous ICC among HPV DNA–positive participants, after adjusting for confounding factors. A pattern of increasing squamous ICC risk with increasing \textit{C. trachomatis} titers was observed, which suggests a dose-response effect. \textit{C. trachomatis} was not clearly associated with adenocarcinoma/adenosquamous carcinoma, although this observation was based on a small number of cases.

The positive associations of squamous ICC and \textit{C. trachomatis} infection in this study (OR, 2.1) are consistent with results from case-control studies of ICC that have used less sensitive methods than PCR for HPV detection, namely, Southern blotting (OR, 4.8) [15] and HPV serology (OR, 1.7–2.2) [16, 17]. Nested case-control studies have primarily found significant positive associations between \textit{C. trachomatis} serum antibodies and ICC (OR, 1.5–2.5) [18–20], although one smaller study found a nonsignificant 3-fold increase in ICC risk (OR, 3.0; 95% CI, 0.7–13.4) [21]. However, residual confounding due to HPV in these studies cannot be excluded, because less sensitive HPV serologic assays were used to assess HPV status [22].

Due to the notable association between HPV and ICC, a prerequisite to evaluating an HPV cofactor is to accurately assess HPV infection. The PCR-based assay used is one of the most accurate methods to assess cervical HPV infection [23]. Given the notably high prevalence of HPV DNA in cervical cancer worldwide (~99%) [12], we concentrated on analyses restricted to HPV DNA–positive women to reduce the likelihood of residual HPV confounding, so as to assess \textit{C. trachomatis} as an HPV cofactor. However, an association between \textit{C. trachomatis} antibodies and squamous ICC also emerged among HPV DNA–negative case and control patients.

Our data did not show a significant association between \textit{C. trachomatis} seropositivity and HPV DNA positivity, which may be due to the different nature of these 2 markers. Whereas HPV DNA indicates both current or persistent HPV infection, \textit{C. trachomatis} seropositivity represents a more cumulative measure of exposure to \textit{C. trachomatis} infection.

The use of species-specific MIF serologic testing allows the ascertainment of past \textit{C. trachomatis} infection while differentiating \textit{C. pneumoniae} antibodies from \textit{C. trachomatis} serovar groupings [11]. Unlike \textit{C. pneumoniae}, \textit{C. trachomatis} antibodies were significantly associated with sexual behavior and with squamous ICC. Results from our blinded reproducibility test showed a high agreement between seropositivity to the different \textit{C. trachomatis} serovars (>83%) and “substantial” $\kappa$ agreement for \textit{C. trachomatis} positivity [24] for both case and control participants. \textit{C. trachomatis} IgG antibodies may persist for years among women with acute infections [25], and antibody persistence and elevated titers are considered to be related to longer, more severe, and recurrent chlamydial infections [26]. Reliable data on the natural history and long-term persistence of \textit{C. trachomatis} antibodies are, however, lacking, although the disappearance of IgG \textit{C. trachomatis} serum antibodies is considered to be rare in women [10, 27].

Among the potential limitations of our study is the use of hospital-based control patients. This could have led to biased results if \textit{C. trachomatis} seroprevalence among the control patients was not representative of the population source for the ICC patients. Control participants in these studies, however, had a wide range of diagnostic categories and were recruited in tertiary public hospitals with wide reference populations. \textit{C. trachomatis} seropositivity did not differ significantly by any major diagnostic category among control patients. Although the case-control design did not elucidate the temporal association between \textit{C. trachomatis} seropositivity and ICC, all ICC patients in our study were newly diagnosed and had not received previous cervical cancer treatment and the frequency of \textit{C. trachomatis} seropositivity did not vary by the clinical stage of ICC, thus reducing the likelihood that our findings may be attributed to the development of \textit{C. trachomatis} antibodies following the onset of invasive disease.

The power of our analyses examining \textit{C. trachomatis} as an HPV cofactor in the etiology of cervical cancer is also limited because of the small number of HPV-positive control patients. Persistent HPV DNA infections have been associated with an increased risk of cervical neoplasia, particularly among women with high-risk HPV types [28]. In this study, a cross-sectional measurement of HPV DNA was taken among case and control patients. The extent to which this HPV DNA measurement among control patients >45 years old represents a persistent HPV infection merits further investigation. However, \textit{C. trachomatis} antibodies were associated with an increased squamous cancer risk when analyses were restricted to women who had not persisted, high-risk DNA types.

The increasing risk of squamous cervical cancer with increasing \textit{C. trachomatis} antibody titers gives further support to
the results found. High antibody titers may be a marker of persistent *C. trachomatis* infection, since women with long-term complications of chlamydial infection, such as pelvic inflammatory disease or tubal infertility, have significantly higher levels of MIF antibody than women with cervical chlamydial infection [29].

No specific *C. trachomatis* serogroup (A, BDE, CJHI, or FGK) was associated with a higher squamous cell cancer risk. The microbial risk factor associated with squamous cancer risk may not be serogroup specific. Alternatively, the statistical power to detect meaningful differences between the associations of different *C. trachomatis* serogroups and ICC by use of serologic testing may be limited because of common subspecies cross-reactivity and because women may have been infected with multiple serovars. The main shortcoming of this study is that, by use of BDE, CJHI, and FGK serogroup data in the MIF assay, it was not possible to distinguish clearly between exposure to *C. trachomatis* genital infections and hyperendemic ocular trachoma infections. Notwithstanding, it is difficult to believe that our results can be attributed to an effect of trachoma infection, because both studies were conducted in urban centers without a history of hyperendemic trachoma, and high *C. trachomatis* antibody titers were significantly associated with sexual behavior. Furthermore, when analyses were stratified by factors potentially associated with trachoma infection (i.e., place of residence or educational status), similar associations were found between *C. trachomatis* antibodies and ICC, which indicates that the associations found are not likely to be an effect of trachoma infection.

In this study of 44 patients with ICC, adenocarcinoma/adenosquamous carcinoma patients did not have a significantly higher prevalence of *C. trachomatis* antibodies than control patients. Koskela et al. [19] found similar results among 32 adenocarcinoma patients, which were contrary to expectations because of the tropism of *C. trachomatis* for endocervical cells. These results require confirmation with a larger sample size.

In terms of biologic plausibility, genital *C. trachomatis* infections are clinically characterized by cervical atypia and inflammation, as determined by cytologic, histopathologic, and colposcopic examinations [30, 31]. *C. trachomatis* infections may induce immature metaplasia [5], and both HPV and *C. trachomatis* may infect metaplastic tissue of the squamocolumnar junction, where cervical neoplasia arise [32]. The chronicity of *C. trachomatis* infection in conjunction with HPV may be a more pertinent factor mediating ICC risk.

Although a carcinogenic interaction between *C. trachomatis* and HPV has not been directly demonstrated, in vitro data show that *C. trachomatis* may inhibit cell apoptosis [33], a contributory element for carcinogenesis. Alternatively, inflammatory cytokine responses during a chlamydial infection may produce reactive oxygen species that might cause DNA damage or modification, providing a mechanistic link between chronic inflammation and malignant transformation [34]. Other bacterial or parasitic infections causing chronic inflammation have also been implicated in human cancer, such as *Helicobacter pylori* with stomach cancer and *Schistosoma haematobium* with bladder cancer.

Our results, based on a large number of newly diagnosed ICC patients, consistently indicate a potential etiologic role for *C. trachomatis* infection as an HPV cofactor in the development of squamous ICC. Further epidemiologic studies are needed to clarify the role of *C. trachomatis* in the etiology of cervical cancer. Additional prospective data are needed on the induction of infection by *C. trachomatis* and other STIs and on the effect of their relative timing, in conjunction with HPV infection, on the risk of cervical neoplasia, in addition to the effect of the treatment of *C. trachomatis* infection on the progression of cervical neoplasia.

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