

## Is *Chlamydia trachomatis* a cofactor for cervical cancer?

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**Aim:** To review the literature to determine if an infection with *Chlamydia trachomatis* (CT) acts as a confounding factor in the pathogenesis of invasive cervical cancer (ICC) in women.

**Methods:** Web-based Medline and the Australian Institute of Health and Welfare (AIHW) search for key terms: cervical cancer (including neoplasia, malignancy and carcinoma), chlamydia, human papillomavirus (HPV) and immunology. The search was restricted to English language publications on ICC (both squamous and adenocarcinoma) and cervical intraepithelial neoplasia (CIN) between 1990-2010. **Results:** HPV is essential but not sufficient to cause ICC. Past and current infection with CT is associated with squamous cell carcinoma of the cervix of HPV-positive women. CT infection induces both protective and pathologic immune responses in the host that depend on the balance between Type-1 helper cells versus Type-2 helper cell-mediated immunity. CT most likely behaves as a cervical cancer cofactor by 1) invading the host immune system and 2) enhancing chronic inflammation. These factors increase the susceptibility of a subsequent HPV infection and build HPV persistence in the host. **Conclusion:** Prophylaxis against CT is significant in reducing the incidence of ICC in HPV-positive women. GPs should be raising awareness of the association between CT and ICC in their patients.



### Evidence for the role of HPV in the aetiology and pathogenesis of cervical cancer

HPV is a species-specific, non-enveloped, double stranded DNA virus that infects squamous epithelia and consists of the major protein L1 and the minor capsid protein L2. More than 130 HPV types have been classified based on their genotype and HPV 16 (50-70% of cases) and HPV 18 (7-20% cases) are the most important players in the aetiology of cervical cancer. [6,12] Genital HPV transmission is usually spread via skin-to-skin contact during sexual intercourse but does not require vaginal or anal penetration, which implies that condoms only offer some protection against CIN and ICC. [6] The risk factors for contracting HPV infection are early age at first sexual activity, multiple sexual partners, early age at first delivery, increased number of pregnancies, smoking, immunosuppression (for example, human immunodeficiency virus or medication), and long-term oral contraceptive use. Social customs in endemic regions such as child marriages, polygamy and high parity use may also increase the likelihood of contracting HPV. [13] More than 80% of HPV infections are cleared by the host's cellular immune response, which starts about three months from the inoculation of virus. HPV can be latent for 2-12 months post-infection. [14]

### Molecular Pathogenesis

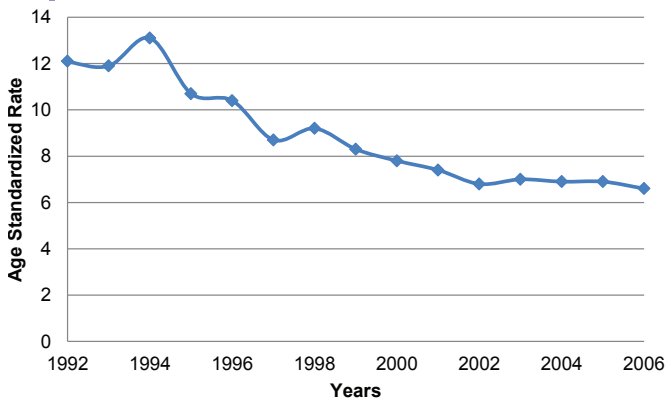
HPV particles enter basal keratinocytes of mucosal epithelium via binding of virions to the basal membrane of disrupted epithelium. This is mediated via heparan surface proteoglycans (HSPGs) found in the extracellular matrix and cell surface of most cells. The virus is then internalised to establish an infection mainly via a clathrin-dependent endocytic mechanism. However, some HPV types may use alternative uptake pathways to enter cells, such as a caveolae-dependent route or the involvement of tetraspanin-enriched domains as a platform for viral uptake. [15] The virus replicates in nondividing cells that lack the necessary cellular DNA polymerases and replication factors. Therefore, HPV encodes proteins that reactivate cellular DNA synthesis in noncycling cells, inhibit apoptosis, and delay the differentiation of the infected keratinocyte, to allow viral DNA replication. [6] The integration of viral genome in the host DNA causes deregulation of E6 and E7 oncogenes of high-risk HPV (HPV 16 and 18) but not of low risk HPV (HPV 6 and 11). This results in the expression of E6 and E7 oncogenes throughout the epithelium resulting in aneuploidy and karyotypic chromosomal abnormalities that accompany keratinocyte immortalisation. [5]

### Introduction

The most recent epidemiological publication on the worldwide burden of cervical cancer has reported that cervical cancer (0.53 million cases) was the third most common female cancer reported in 2008 after breast (1.38 million cases) and colorectal cancer (0.57 million cases). [1] Cervical cancer is the leading source of cancer-related death among women in Africa, Central America, South-Central Asia and Melanesia, indicating that it remains a major public health problem in spite of effective screening methods and vaccine availability. [1]

The age-standardised incidence of cervical cancer in Australian women (20-69 years) has decreased by approximately 50% from 1991 (the year the National Cervical Screening Program was introduced) to 2006 (Figure 1). [2,3] Despite this drop, the Australian Institute of Health and Welfare estimated an increase in cervical cancer incidence and mortality for 2010 by 1.5% and 9.6 % respectively. [3]

Human papillomavirus (HPV) is required but not sufficient to cause invasive cervical cancer (ICC). [4-6] Not all women with a HPV infection progress to develop ICC. This implies the existence of cofactors in the pathogenesis of ICC such as smoking, sexually transmitted infections, age at first intercourse and number of lifetime sexual partners. [7] *Chlamydia trachomatis* (CT) is the most common bacterial sexually transmitted infection (STI) and it has been associated with the development of ICC in many case-controlled and population based studies. [8-11] However, a clear cause-and effect relationship has not been elucidated between CT infection, HPV persistence and progression to ICC as an end stage. This article aims to review the literature for evidence that CT acts as a cofactor in the development of ICC and HPV establishment. The understanding of CT as a risk factor for ICC is crucial as it is amenable to prevention.



**Figure 1.** Incidence of cervical cancer in Australia from 1991-2006 (Adapted from AIHW, 2010). [2]

*Natural History of HPV infection and cervical cancer*

Low risk HPV infections are usually cleared by cellular immunity coupled with seroconversion and antibodies against major coat protein L1. [5,6,12] Infection with high-risk HPV is highly associated with the development of squamous cell and adenocarcinoma of the cervix, which is confounded by other factors such as smoking and STIs. [4,9,10] The progression of cervical cancer in response to HPV is schematically illustrated in Figure 2.

**Chlamydia trachomatis and the immune response**

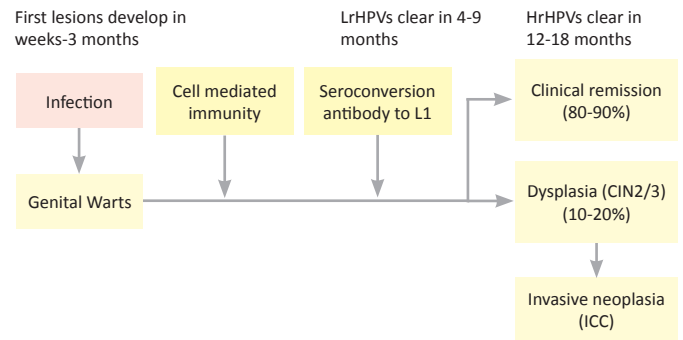
CT is a true obligate intracellular pathogen and is the most common bacterial cause of STIs. It is associated with sexual risk-taking behaviour and leads to asymptomatic and therefore undiagnosed genital infections due to the slow growth cycle of CT. [16] A CT infection is targeted by innate immune cells, T cells and B cells. Protective immune responses control the infection whereas pathological responses lead to chronic inflammation that causes tissue damage. [17]

*Innate immunity*

The mucosal epithelium of the genital tract provides first line of host defence. If CT is successful in entering the mucosal epithelium, the innate immune system is activated through the recognition of pathogen-associated molecular patterns (PAMPs) such as the Toll-like receptors (TLRs). Although CT lipopolysaccharides can be recognised by TLR4, TLR2 is more crucial for signalling pro-inflammatory cytokine production. [18] This leads to the production of pro-inflammatory cytokines such as interleukin-1 (IL-1), IL-6, tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) and granulocyte-macrophage colony-stimulating factor (GM-CSF). [17] In addition, chemokines such as IL-8 can increase recruitment of innate-immunity cells such as macrophages, natural killer (NK) cells, dendritic cells (DCs) and neutrophils that in turn produce more pro-inflammatory cytokines to restrict CT growth. Infected epithelial cells release matrix metalloproteases (MMPs) that contribute to tissue proteolysis and remodelling. Neutrophils also release MMPs and elastases that contribute to tissue damage. NK cells produce interferon (IFN)- $\gamma$  that drives CD4 T cells toward the Th1-mediated immune response. The infected tissue is infiltrated by a mixture of CD4, CD8, B cells, and plasma cells (PCs). [17,19,20] DCs are essential for processing and presenting CT antigens to T cells and therefore linking innate and adaptive immunity.

*Adaptive Immunity*

Both CD4 and CD8 cells contribute to control of CT infection. In 2000, Morrison *et al.* showed that B cell-deficient mice, depleted of CD4 cells, are unable to clear CT infection. [21] However, another study in 2005 showed that passive transfer of chlamydia-specific monoclonal antibodies into B-cell deficient and CD4 depleted cells restored the ability of these mice to control a secondary CT infection. [22] This indicates a strong synergy between CD4 and B cells in the adaptive immune response to CT. B cells produce CT-specific antibodies to combat the pathogens. In contrast, CD8 cells produce IL-4, IL-5 and IL-13 that do not appear to protect against chlamydia infection and may



**Figure 2.** Steps and timeline in the development of ICC in response to HPV infection. HrHPVs: High risk HPV infection; LrHPVs: Low risk HPV infection; L1: major capsid protein.

even indirectly enhance chlamydia load by inhibiting the protective CD4 response. [23] A similar observation was made by Agrawal *et al.* who examined cervical lymphocyte cytokine responses of 255 CT antibody-positive women with or without fertility disorders (infertility and multiple spontaneous abortions) and of healthy control women negative for CT serum IgM or IgG. [20] The study revealed a significant increase in CD4 cells in the cervical mucosa of fertile women, compared with those with fertility disorders and with negative control women. There was a very small increase in CD8 cells in cervical mucosa of CT infected women in both groups. The results showed that cervical cells from the women with fertility disorders secreted higher levels of IL-1b, IL-6, IL-8, and IL-10 in response to CT; whereas, cervical cells from antibody-positive fertile women secreted significantly higher levels of IFN- $\gamma$  and IL-12. This suggests that a skewed immune response toward Th1 prevalence protects against chronic infection. [20]

The pathologic response to CT can result in inflammatory damage within the upper reproductive tract due to either failed or weak Th1 action resulting in chronic infection or an exaggerated Th1 response. Alternatively, chronic infection can occur if Th2 response dominates Th1 immune response and result in autoimmunity and direct cell damage which in turn will enhance tissue inflammation. Inflammation also increases the expression of human heat shock protein (HSP), which induce production of IL-10 via autoantibodies leading to CT associated pathology such as tubal blockage and ectopic pregnancies. [24]

**Evidence that Chlamydia trachomatis is a cofactor for cervical cancer**

Whilst it has been established that HPV is a necessary factor in the development of cervical cancer, it is still unclear why the majority of women infected with HPV do not progress to ICC stage. Several studies in the last decade have focused on the role of STIs in the pathogenesis of ICC and discovered that CT infection is consistently associated with squamous cell ICC.

In 2000, Koskela *et al.* performed a large-scale case-controlled study within a cohort of 530,000 Nordic women to evaluate the role of CT in the development of ICC. [10] One-hundred and eighty-two women with ICC (diagnosed during a mean follow-up of five years after serum sampling) were identified via linking data files of three Nordic serum banks and the cancer registries of Finland, Norway and Sweden. Microimmunofluorescence (MIF) was used to detect CT-specific IgGs and HPV16-, 18- and 33-specific IgG antibodies were determined by standard ELISAs. Serum antibodies to CT were associated with an increased risk for cervical squamous-cell carcinoma (HPV and smoking adjusted odds ratio (OR), 2.2; 95% confidence interval (CI), 1.3-3.5). The association remained also after adjustment for smoking both in HPV16-seronegative and seropositive cases (OR, 3.0; 95% CI, 1.8-5.1; OR, 2.3, 95% CI, 0.8-7.0 respectively). This study provided sero-epidemiologic evidence that CT could cause squamous cell ICC. However the authors were unable to explain the biological association between CT and squamous cell ICC.

Many more studies emerged in 2002 to investigate this association

between CT and ICC even further. Smith *et al.* performed a hospital case-controlled study of 499 ICC women from Brazil and 539 from Manila that revealed that CT seropositive women have a two-fold increase in squamous ICC (OR, 2.1; 95% CI, 1.1-4.0) but not adenocarcinoma or adenosquamous ICC (OR, 0.8; 95% CI, 0.3-2.2). [8] Similarly, Wallin *et al.* conducted a population based prospective study of 118 women who developed cancer after having a normal pap smear (average of 5.6 years later). [25] Women were followed up for 26 years. PCR analysis for CT and HPV DNA showed that the relative risk for ICC associated with past CT, adjusted for concomitant HPV DNA positivity, was 17.1. They also concluded that the presence of CT and of HPV was not interrelated.

In contrast, another study examining the association between CT and HPV in women with cervical intraepithelial neoplasia (CIN) found that there is an increase in CT rate in HPV-positive women (29/49) as compared to HPV-negative women (10/80), ( $p < 0.001$ ). [26] However, no correlation between HPV and CT co-infection was found and the authors suggested that the increased CT infectivity rate in HPV-positive women is presumably due to HPV-related factors, including modulation of the host's immunity. In 2004, a case-controlled study of 1,238 ICC women and 1100 control women in 7 countries coordinated by the International Agency for Research on Cancer (IARC), France also supported the findings of previous studies. [7]

Strikingly, a very recent study in 2010 confirmed that there was no association between CT infection, as assessed by DNA or IgG, and risk of cervical premalignancy, after controlling for carcinogenic HPV-positive status. [11] The authors have justified the difference in results from previous studies by criticising the retrospective nature of the IARC study, which meant that HPV and CT status at relevant times were not available. [7] However, other prospective studies have also identified the association between CT and ICC development. [9,25] Therefore, the results from this one study remain isolated from practically every other study that has found an association between CT and ICC in HPV infected women.

Consequently, it is evident that CT infection has a role in confounding squamous cell ICC in HPV infected women but it is not an independent

cause for ICC as previously suggested by Koskela *et al.* [10] Previous cause-and-effect association between CT and HPV are most likely from CT infection increasing the susceptibility to HPV. [9,11,27] The mechanisms by which CT can act as a confounder for ICC relate to CT induced inflammation (associated with metaplasia) and invasion of the host immune response, which increases susceptibility to HPV infection and enhances HPV persistence in the host. CT can directly degrade RFX-5 and USF-1 transcription factors that induce expression of MHC class I and MHC class II respectively. [17,28] This prevents recognition of both HPV and CT by CD4 and CD8 cells, thus preventing T-cell effector functions. CT can also suppress IFN-gamma-induced MHC class II expression by selective disruption of the IFN-gamma signalling pathways, hence evading host immunity. [28] Additionally, as discussed above, CT induces inflammation and metaplasia of infected cells, which predisposes them as target cells for HPV. CT infection may also increase access of HPV to the basal epithelium and increases HPV viral load. [16]

## Conclusion

There is sufficient evidence to suggest that CT infection can act as a cofactor in squamous cell ICC development due to consistent positive correlations between CT infection and ICC in HPV positive women. CT invades the host immune response due to chronic inflammation and it is presumed that it prevents the clearance of HPV from the body, thereby increasing the likelihood of developing ICC. More studies are needed to establish the clear biological pathway linking CT to ICC to support the positive correlation found in epidemiological studies. An understanding of the significant role played by CT as a cofactor in ICC development should be exercised to maximise efforts in CT prophylaxis, starting at the primary health care level. Novel public health strategies must be devised to reduce CT transmission and raise awareness among women.

## Conflicts of interest

None declared.

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