Published in final edited form as:

Obstet Gynecol. 2014 November ; 124(5): 954–960. doi:10.1097/AOG.0000000000000429.

Biological and Behavioral Risks for Incident *Chlamydia trachomatis* Infection in a Prospective Cohort

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Abstract

Objective—To identify biological and behavioral risks for incident *Chlamydia trachomatis* among a prospective cohort of young women followed frequently.

Methods—Our cohort of 629 women from two outpatient sites was seen every 4 months (October 2000 through April 2012) for behavioral interviews and infection testing. *C trachomatis* was tested annually, and anytime patients reported symptoms or possible exposure using commercial nucleic acid amplification tests. Analyses excluded baseline prevalent *C trachomatis* infections. Risk factors for incident *C trachomatis* were assessed using Cox proportional hazards models. Significant risks ($p < 0.10$) from bivariate models were entered in a multivariate model, adjusted for four covariates chosen *a priori* (age, race or ethnicity, condom use, study site). Backwards step-wise elimination produced a final parsimonious model retaining significant variables ($p < 0.05$) and the four adjustment variables.

Results—The 629 women attended 9,594 total visits. Median follow-up time was 6.9 years (interquartile range 3.2–9.8), during which 97 (15%) women had incident *C trachomatis*. In the final multivariate model, incident *C trachomatis* was independently associated with HPV at the preceding visit ($p < 0.01$), smoking ($p = 0.02$), and weekly use of substances besides alcohol and marijuana ($p < 0.01$) since prior visit. Among 207 women with available colpophotographs (1,742 visits), cervical ectopy was not a significant risk factor (p range = 0.16–0.39 for ectopy as continuous and ordinal variables).

Conclusion—Novel risks for *C trachomatis* include preceding HPV, smoking, and substance use, which may reflect both biological and behavioral mechanisms of risk, such as immune modulation, higher-risk sexual networks, or both. Improved understanding of the biological bases for *C trachomatis* risk would inform our strategies for *C trachomatis* control.

Introduction

Chlamydia trachomatis is the most commonly reported bacterial sexually transmitted infection (STI), and the highest age-specific rates are found in young women 15–24 years

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Financial Disclosure: The authors did not report any potential conflicts of interest.

Presented at the annual scientific meeting of the Pediatric Academic Societies on May 4, 2013 (Washington, DC).

old.(1) The serious consequences of untreated infections include pelvic inflammatory disease (PID), tubal factor infertility, ectopic pregnancy, and chronic pelvic pain. Approximately 70-80% of infected women are asymptomatic, making targeted screening ineffective; hence the Centers for Disease Control and Prevention (CDC) advises at least annual screening of all sexuallyactive women 25 years and younger.(2) But despite expansion of *C trachomatis* screening, infection rates have not decreased and national strategies for control efforts are at a crossroads.(3) The natural history of infection is not well understood, and identification of women at greater risk for acquisition remains challenging. Older studies have identified risks including younger age, race or ethnicity, recent new sexual partners, but individual studies typically examine a limited number of risks, making it difficult to assess their independent influences. Biological vulnerability is another important issue. Since *C trachomatis* preferentially infects the columnar epithelium of the cervix, and younger women have greater areas of cervical ectopy (columnar epithelium visible on the ectocervix),(4) higher *C trachomatis* rates in younger women are commonly attributed to the assumed biological vulnerability of the ectopy. Unfortunately, rigorous evidence for this link is lacking, and this research is complicated by the frequent risky sexual behavior also found during adolescence and young adulthood. Our objective was to identify the biological and behavioral risk factors for incident *C trachomatis* infection among a prospective cohort of sexually active young women assessed frequently.

Materials and Methods

The participants were selected from an ongoing cohort study of the natural history of human papillomavirus (HPV) described elsewhere.(5) Briefly, during 2000-2006, women were recruited primarily from a family planning clinic and a college health center and were also allowed to refer their friends to enroll. Inclusion criteria for the original cohort were: 13-21 years old, newly sexually active (maximum of 5 years), nonpregnant and without history of immunosuppression, cervical neoplasia, or cervical procedures. HPV status was unknown at time of recruitment and not considered. Baseline visits were followed by 4-month interval visits. During follow-up, exclusion criteria were: development of cervical intraepithelial neoplasia (CIN)-2 or -3. This study includes the 629 women who attended at least 1 follow-up visit. Each woman gave written informed consent, and the Committee on Human Subject Research, University of California, San Francisco and San Francisco State University approved the study.

At all baseline and 4-month interval visits, we conducted an interview to assess medical history and behaviors; colpophotography to document the cervical epithelium; vaginal wet prep microscopy to detect *Trichomonas vaginalis*, yeast, and bacterial vaginosis (using Amsel's clinical criteria); and cervical lavages (10cc of sterile normal saline washed over the cervix, recollected and repeated 3 times) to detect HPV DNA(37 types) using Roche Reverse Line Blot assay.(6) Cervical swabs for *C trachomatis* and *Neisseria gonorrhoeae* were tested at annual visits at least, and also at any other visit when patients reported possible STI exposure or genital symptoms. *C trachomatis* was tested promptly upon swab collection, using commercial nucleic-acid amplification tests. LCx (Abbott Laboratories) was used in 2000-03; ProbeTec (Becton Dickinson) in 2003-10; and APTIMA Combo 2 (GenProbe) in 2011-12.

Cervical ectopy was measured quantitatively by computerized planimetry as previously described.(7, 8) Briefly, we (L.Y.H., A.B.M.) viewed the digitized colpophotographs (1024 × 1536 pixels) using Adobe® Photoshop CS2® (San Jose, CA) while blinded to all other patient data. Areas of ectopy were manually outlined, represented by pixel counts, and expressed as a percentage of the total cervical face. Due to our limited resources, ectopy measures were performed only for visits in 2000-2007, and primarily for the family planning site where the majority of *C trachomatis* infections occurred.

The main dependent variable was incident *C trachomatis*, defined as a *C trachomatis* positive visit during follow-up that was preceded by a *C trachomatis* negative visit. Upon an incident event, the woman's subsequent visits were right-censored. Incidence rate was defined as the number of incident infections divided by the at-risk time (person-years).

Cox proportional hazards models examined several independent variables suspected as risks for incident *C trachomatis*. Prevalent infections at baseline were excluded from analysis, but the subsequent *C trachomatis*- negative visit and follow-ups thereafter were eligible for analysis. Independent variables measured at baseline were race/ethnicity and age at first sex. All other independent variables were time-varying as follows. Dichotomous variables (yes/no) defined as current values (measured at the same visit as the incident *C trachomatis* test) were: condom use since prior visit, new partners since prior visit, partners since prior visit who have other partners, current smoking, substance use at least weekly since prior visit (assessed as 3 separate variables for alcohol use, marijuana use, and all other drugs), current or past pregnancy. Continuous variables defined as current values were: age (years), use of estrogen/progestin contraception (cumulative lifetime years) and use of progestin-only contraception (cumulative lifetime years). Dichotomous variables (positive/negative) defined as preceding values (measured at the nearest visit prior to the incident *C trachomatis* test) were: HPV and bacterial vaginosis, to examine these as preceding risks rather than as co-infections that commonly occur for all STIs. *N. gonorrhoeae* and *T. vaginalis* infections were too rare for analyses. Cervical ectopy (percentage of total cervical face) was defined as a preceding value. We avoided measuring ectopy as a current value since inflammation could interfere with our impression of ectopy. Ectopy was examined as a continuous variable, ordinal variable (ectopy <10% or ≥10%), and another ordinal variable (ectopy <10%, 10-39%, or ≥40%). We used <10% to represent lack of ectopy (rather than 0%) because of cervical eversion that is inherent to the speculum opening in a clinical exam.

Unadjusted Cox proportional hazards models for incident CT each contained 1 independent variable. The adjusted models each contained 1 independent variable adjusted for race/ethnicity, age, and condom use since last visit, based on prior evidence of these 3 factors are associated with risk;(1, 9-13) and adjusted for study site since our 2 sites are geographically distinct and more infections occurred at the family planning clinic. Independent variables that were significant (p<0.10) in the unadjusted models were entered into a single multivariate model. We performed backwards-stepwise elimination to reach a final parsimonious model that retained all significant variables (p<0.05) and the 4 adjustment variables chosen *a priori* (race or ethnicity, age, condom use since last visit, study site).Analyses of ectopy included only the subset of visits with available colpophotographs. To compare ectopy <10% of the cervix versus ≥10%, we had 81% power to detect a

significant hazard ratio of 1.9 at $\alpha=0.05$ given our sample size and follow-up time. To compare ectopy <10%, 10-39%, 40%, we had 48% power given the same parameters.

Results

The final dataset included 629 women, 9,594 visits (during October 2000 through April 2012), and total at-risk person-time of 4,047 woman-years. Each woman attended a median of 17 visits (interquartile range (IQR) 8-25) and median follow-up time of 6.9 years (IQR 3.2-9.8) with 14 (2%) women lost to follow-up. At baseline, mean age was 18.6 years (SD, 2.2), and 23 (4%) women had prevalent *C trachomatis* infection. In follow-up, 97 (15%) women had incident *C trachomatis*, and the *C trachomatis* incidence rate was 2.4 per 100 woman-years (95% CI, 1.9-2.9). Table 1 shows the baseline characteristics of the women. Women were evenly divided between the 2 study sites, racially and ethnically diverse, and sexually experienced. At baseline, combined hormonal contraception (36%) was more commonly used than progestin-only contraception (4%). HPV infection was common (25%), BV was less common (10%) and *N. gonorrhoeae* and *T. vaginalis* were very rare (<1%). Ectopy measuring 10-39% of the total cervix was seen in 62 (30%) women, and ectopy 40% of the cervix was seen in 35 (17%). Figure 1 shows an example of a colpophotograph.

Table 2 shows risk factors for incident *C trachomatis*. In the unadjusted models, increased risk was associated with study site ($p<0.01$), African-American or Latina race or ethnicity (both $p<0.01$ compared to Caucasian), younger age ($p<0.01$), and condom use ($p=0.02$), which were the four variables we had chosen *a priori* for use in the adjusted models. The unadjusted models also showed increased risk to be associated with younger age at first sex, having new partners since prior visit, having partners who have other partners, smoking, weekly use of substances (besides alcohol and marijuana) since prior visit, history of pregnancy, and having HPV at prior visit ($p<0.01$ for all variables). In the adjusted models, all of these risks remained significant except for pregnancy. In the single multivariate model reached by backwards step-wise elimination (table 3), 3 factors remained independently associated with increased risk for incident *C trachomatis*: smoking ($p=0.02$), weekly use of substances (besides alcohol and marijuana) ($p<0.01$), and having HPV at prior visit ($p<0.01$). Significant interaction between 2 of our adjustment variables was seen ($p<0.01$). Specifically, when women had new partners, condom use was associated with less risk ($p=0.12$). When women reported no new partners, condom use was associated with more risk ($p<0.01$). Table 4 shows subset analyses of cervical ectopy for the 207 women (1,742 visits) who had available colpophotographs; their CT incidence rate was 3.6 per 100 woman-years (95% CI, 2.7-4.6). Ectopy was not a significant risk factor for incident CT, whether measured as continuous or ordinal variables (p range=0.16-0.39).

Discussion

Many findings from our prospective study are consistent with prior work, which is an important strength. The CDC has long cited recent new partners as a key *C trachomatis* risk that should prompt more frequent screening, (2) and is supported by more recent prospective studies. (9, 10) Our finding of risk from partners who have other partners is consistent with

cross-sectional studies of partners' concurrency being linked to more STIs,(14, 15) and with the concept of sexual networks, in which a woman's risk is influenced by her relationship position and STI rates in the greater network.(16) Similarly, frequent substance use as a risk may reflect the woman's involvement in a higher-risk sexual network or riskier partner and condom practices.(17) Another strength of our study is the consistency between findings from the adjusted models and the final multivariate model (Tables 2, 3).

Our more intriguing findings include the increased risk associated with smoking and preceding HPV, and the varying effects of condom use depending on whether there were new partners. We hypothesize that smoking may have behavioral and biological mechanisms, as earlier initiation of smoking has been linked to riskier sexual behaviors and more STIs;(18) and smoking is correlated to depressed levels of cervical cytokines (IFN- γ , IL-10, IL-6, IL-1 β). (19) Of note, IL-6 and IFN-gamma are key cytokines in *C trachomatis* defense.(20) Similarly, we hypothesize that HPV preceding the incident *C trachomatis* may reflect the high risk of the woman's partnerships, or may act via suppressive effects on innate immunity. HPV16 has been linked to down-regulation of toll-like receptor(TLR)9 transcription *in vitro*, (21) and dampened mRNA expression of TLR2, TLR3, TLR7, TLR8, and TLR9 *in vivo*. (22) *In vitro* and animal studies have supported the roles of TLR2 and TLR9 in *C trachomatis* defense.(23, 24) Another intriguing finding regarding our adjustment variables was that condom use appeared protective in the setting of new partners, but was paradoxically risky when women reported no new partners in the visit interval. We postulate that in the setting of familiar partners, condom use may reflect the woman's perception of a riskier partner. Prior work has described strong associations between condom use and higher suspicion of the partner.(25) Another prospective study of incident *C trachomatis* among young women also reported higher *C trachomatis* rates among condom users, although non-significant.(11) If our findings regarding substance use, smoking, preceding HPV, and condoms are corroborated in future studies, this data could inform our clinical care. For example, patients reporting substances and smoking may prompt more careful STI evaluations. Counseling about HPV vaccines may extend to discussions of relationships between HPV and *C trachomatis*. For patients who use condoms inconsistently, clinicians may explore how the patient perceives their partners' level of risk and which scenarios prompt condom use versus non-use.

The lack of association between ectopy and *C trachomatis* appears contrary to popular assumptions. However, prior work has yielded mixed results likely related to the lack of precision in defining ectopy or cross-sectional designs making it difficult to distinguish ectopy from cervical inflammation.(26-30) Our study strengths were the colpophotography that enabled quantitative analyses, and the availability of the visit preceding the infection. Given the non-parametric distribution of ectopy among our women, we analyzed ectopy as both continuous and ordinal measures to capture the possibility of significance; yet we did not observe a significant association. An important limitation is our sample size and lack of power to detect a possibly small effect size. Alternatively, we speculate that biological vulnerabilities of younger women may be more strongly based on the lack of prior *C trachomatis* exposures and lack of immune memory. The topic of immunity was beyond the scope of our current study.

Limits of our study include the recruitment of younger women from a college clinic and family planning clinic, such that our findings may not be widely generalizable to a wider population, and the lack of biochemical markers or quantitative measures of smoking. Given the link found between smoking and incident *C trachomatis*, if the mechanism is biological, future studies should examine the possibility of a dose-response relationship or threshold for effect.

In summary, this prospective study confirmed risk factors for incident *C trachomatis* found in prior cross-sectional studies and highlighted novel risks including smoking, substance use, and preceding HPV, which may reflect both biological and behavioral mechanisms of risk. Interestingly, cervical ectopy was not a significant risk when measured quantitatively, suggesting that biological risks may lie in other mechanisms such as differences in immune defense rather than differences in cervical epithelium.

Acknowledgments

Supported by the National Institutes of Health (NIH): National Cancer Institute, #R37CA51323; National Institute of Allergy and Infectious Disease, #K23AI076670; National Center for Research Resources, UCSF-CTSI #UL1 RR024131.

The authors thank Janet Jonte, B.S.N., Julie Jay, N.P., Evelyn N. Hanson, N.P., Cheryl Godwin de Medina, B.A., Susanna Miller Benningfield, B.A., and Robert Wilson for the compassionate patient care they provided at the clinical study sites and their careful work on study coordination, data collection, and data management.

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Figure 1.
Example of a colpophotograph evaluated by computerized planimetry. The areas of cervical ectopy are outlined manually by a solid black line.

Table 1
Baseline sociodemographics, clinical history, and behaviors for a prospective cohort of sexually active young women (N=629)

Characteristic	n (%)	Missing, n(%)
<i>Chlamydia trachomatis</i> infection (prevalent at baseline)	23 (4)	0 (0)
Study site		
College health center	305 (48)	0 (0)
Family planning clinic	324 (52)	
Race/Ethnicity		
Caucasian	185 (29)	0 (0)
African-American	74 (12)	
Latina	186 (30)	
Asian-American	137 (22)	
Other/Unknown	47 (7)	
Condom use ever in the past	585 (93)	12 (2)
Using combination estrogen/progestin contraceptives	229 (36)	7 (1)
Using progestin-only contraceptives	25 (4)	7 (1)
New partners in past 2 months	205 (33)	7 (1)
Most recent partner had other concurrent partners	98 (15.6)	7 (1)
Smoked in last 24 hours	159 (25)	7 (1)
Marijuana use at least weekly in past 2 months	138 (22)	7 (1)
Alcohol use at least weekly in past 2 months	149 (24)	7 (1)
Other substance use (besides marijuana and alcohol) at least weekly in past 2 months	10 (2)	7 (1)
HPV infection	160 (25)	0 (0)
<i>Neisseria gonorrhoeae</i> infection	2 (0.3)	0 (0)
<i>Trichomonas vaginalis</i> infection	4 (0.7)	24 (4)
Bacterial vaginosis	61 (10)	26 (4)
Pregnancy	18 (3)	7 (1)
Cervical ectopy, expressed as percentage of total cervix ^a		
Ectopy < 10% of cervix	110 (53)	422 (67)
Ectopy 10-39% of cervix	62 (30)	
Ectopy ≥ 40% of cervix	35 (17)	
	mean (SD)	

Characteristic	n (%)	Missing, n(%)
Age (years) at baseline	18.6 (2.2)	7 (1)
Age (years) at first sex	16.1 (1.9)	7 (1)
Lifetime number of partners	5 (6)	7 (1)

^a Analyses of cervical ectopy were restricted to the dataset of available colpophotographs.

Table 2
Risk factors for incident *Chlamydia trachomatis* infection in a prospective cohort of sexually active young women, unadjusted and adjusted models^a

	Unadjusted HR (95% CI)	Adjusted HR (95% CI)
Risk factors measured once at baseline		
Study site, family planning clinic	10 (4.6-19.6) **	n/a
Race/Ethnicity		n/a
Caucasian	1.0	
African-American	2.8 (1.4-5.4) **	
Latina	2.6 (1.5-4.5) **	
Asian-American	0.8 (0.3-1.7)	
Other/Unknown	1.0 (0.2-4.4)	
Age (years) at first sex	0.7 (0.6-0.8) **	0.9 (0.7-0.99) *
Time-varying risk factors measured at every 4-month interval visit		
Age (years)	0.8 (0.7-0.9) **	n/a
Condom use since prior visit	1.7 (1.1-2.7) *	n/a
New partners since prior visit	2.5 (1.7-3.7) **	2.5 (1.6-4.0) **
Partners since prior visit who have other partners	2.7 (1.7-4.3) **	1.9 (1.2-3.2) *
Current smoking	2.6 (1.8-4.0) **	1.8 (1.2-2.7) **
Alcohol use at least weekly since prior visit	0.8 (0.5-1.2)	n/a
Marijuana use at least weekly since prior visit	1.4 (0.9-2.2)	n/a
Other substance use (besides marijuana and alcohol) at least weekly since prior visit	6.1 (2.6-14.4) **	5.1 (2.2-12.0) **
Current or past pregnancy	1.9 (1.3-2.9) **	1.1 (0.7-1.8)
Combination estrogen/progestin use (cumulative lifetime years)	0.9 (0.8-1.1)	n/a
Progestin-only use (cumulative lifetime years)	1.1 (0.6-2.1)	n/a
HPV at nearest prior visit	1.8 (1.2-2.8) **	1.9 (1.2-2.9) **
Bacterial vaginosis at nearest prior visit	1.4 (0.8-2.6)	n/a

Six hundred twenty nine women, 9,594 visits, and *C trachomatis* incidence rate of 2.4 per 100 woman-years (95% CI, 1.9-2.9).

^aCox proportional hazards models were constructed to examine each risk factor. Risk factors that were significant in unadjusted models were then adjusted for study site, race/ethnicity, age, and condom use since last visit. Adjusted models are not applicable for those variables already chosen *a priori* to serve as the adjustment variables.

HR, hazard ratio. CI, confidence interval.

*
 $p < 0.05$

**
 $p < 0.01$

Table 3
Risk factors for incident *Chlamydia trachomatis* infection in a prospective cohort of sexually active young women, single multivariate model^a

	HR (95%CI)
Study site, family planning clinic	8.8 (3.6-21.7) **
Race or Ethnicity	
Caucasian	1.0
African-American	1.9 (0.9-3.8)
Latina	1.6 (0.9-2.9)
Asian-American	0.7 (0.3-1.7)
Other/Unknown	0.7 (0.1-2.9)
Age (years)	1.1 (0.9-1.2)
Current smoking	1.7 (1.1-2.7) *
Other substance use (besides marijuana and alcohol) at least weekly since prior visit	3.7 (1.5-8.9) **
HPV at nearest prior visit	1.8 (1.2-2.8) **
Among visits at which women reported new partners ^b	
Condom use since prior visit	0.6 (0.3-1.2)
Among visits at which women reported no new partners ^b	
Condom use since prior visit	2.3 (1.2-4.4) **

Six hundred twenty nine women, 9,594 visits, and *C trachomatis* incidence rate of 2.4 per 100 woman-years (95% CI, 1.9-2.9).

^a A single Cox proportional hazards model was constructed. Backwards step-wise elimination produced a final model that also retained the variables for study site, race/ethnicity, age, condom use since prior visit.

^b The interaction term between new partners and condoms was significant at $p < 0.01$.

HR, hazard ratio. CI, confidence interval.

* $p < 0.05$

** $p < 0.01$

Table 4
Lack of associations between cervical ectopy^a and incident *Chlamydia trachomatis* infection among a prospective cohort of young women, unadjusted and adjusted models^b

	n (%)	Unadjusted HR (95%CI)	Adjusted HR HR (95%CI)
Ectopy, continuous variable (% of total cervix)	n/a	1.0 (0.99-1.03)	1.0 (0.99-1.03)
Ectopy < 10% of cervix	1277 (73)	1.0	1.0
Ectopy 10% of cervix	465 (27)	1.9 (0.7-4.8)	2.0 (0.7-5.3)
Ectopy < 10% of cervix	1277 (73)	1.0	1.0
Ectopy 10-39% of cervix	354 (20)	1.9 (0.7-5.2)	2.0 (0.7-5.9)
Ectopy 40% of cervix	111 (6)	1.9 (0.5-7.4)	1.8 (0.5-7.3)

Two hundred seven women, 1,742 visits, and *C trachomatis* incidence rate of 3.6 per 100 woman-years (95% CI, 2.7-4.6).

^a Cervical ectopy was defined as areas of columnar and early-mid squamous metaplasia, expressed as a percentage of total cervical face, measured at nearest visit prior to *C trachomatis* testing

^b Cox proportional hazards models examined ectopy in unadjusted models and models adjusted for study site, race/ethnicity, age, condom use since last visit.

HR, hazard ratio. CI, confidence interval.