Spontaneous Resolution of Asymptomatic *Chlamydia trachomatis* in Pregnancy

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OBJECTIVE: We sought to estimate the rate of spontaneous resolution of asymptomatic *Chlamydia trachomatis* in pregnancy and to evaluate factors associated with its resolution.

METHODS: A cohort of women enrolled in a large multicenter randomized bacterial vaginosis antibiotic trial (metronidazole versus placebo) that, when randomly allocated, had asymptomatic *C trachomatis* diagnosed by urine ligase chain reaction (from frozen archival specimens) between $16^{0/7}$ and $23^{6/7}$ weeks were included. The urine ligase chain reaction is a highly accurate predictor of genital tract chlamydial infection. A follow-up ligase chain reaction was performed between $24^{0/7}$ and $29^{6/7}$ weeks.

RESULTS: A total of 1,953 women were enrolled in the original antibiotic trial; 1,547 (79%) had ligase chain reaction performed both at randomization and follow-up. Women receiving antibiotics effective against *Chlamydia* between randomization and follow-up or having symptomatic *Chlamydia* infection were excluded (26 women). Of the 140 women (9%) who were diagnosed as positive via the initial ligase chain reaction assay, 61 (44%) had spontaneous resolution of *Chlamydia* by the follow-up ligase chain reaction assay. Factors associated with spontaneous resolution included older age ($P = .02$), more than 5 weeks from randomization to follow-up ($P = .02$), and a greater number of lifetime sexual partners ($P = .02$). Using a logistic regression model, maternal age and a greater-than-5-week follow-up interval remained significant; for every 5-year increase in maternal age, the odds of a positive result on the ligase chain reaction test at follow-up decreased by 40% (odds ratio 0.6; 95% confidence interval 0.4–0.9). Race, substance abuse, parity, and treatment with metronidazole were not associated with spontaneous resolution. Gram stain score and vaginal pH at randomization and follow-up also were not associated.

CONCLUSION: The prevalence of asymptomatic *C trachomatis* in pregnancy was 9%; infection resolved spontaneously in almost half of these women. The association of older age and increasing time interval to spontaneous resolution of *Chlamydia* is consistent with a host immune-response mechanism. (Obstet Gynecol 2005;105:557–62. © 2005 by The American College of Obstetricians and Gynecologists.)

LEVEL OF EVIDENCE: III

Chlamydia trachomatis is the most common bacterial sexually transmitted disease in the United States. In 2002, 834,555 new *Chlamydia* infections were reported to the Centers for Disease Control and Prevention (CDC). In women, the rate is now 455 cases per 100,000 population.[1] The prevalence of *Chlamydia* varies among the populations studied, ranging from 2% to 39%. Recently, surveillance of select prenatal clinics in the United States reported an overall prevalence of 7.4% (range, 1.5–14.4%).[1,2] *Chlamydia* rates are highest among young, sexually active women. Unfortunately, as many as 60–70% of women with *Chlamydia* are asymptomatic[3] and, if untreated, *Chlamydia* will persist for long periods of time. Like symptomatic *Chlamydia*, asymptomatic disease is associated with pelvic inflammatory disease, ectopic pregnancy, and infertility.[4–6] The CDC estimates that 3.5 million cases of asymptomatic *Chlamydia* occur every year, highlighting the public health impact of this disease.[7] Fortunately, many of the sequelae can be pre-

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vented or attenuated with treatment, which has prompted recommendations from the CDC, the U.S. Preventive Service Task Force, and the American College of Obstetricians and Gynecologists to annually screen all sexually active women up to the age of 25 and older women with risk factors.[1,2,8] Screening also is recommended at the first prenatal visit and again in the third trimester for pregnant women with risk factors for chlamydial infection. However, the high prevalence of *Chlamydia* in many populations makes treatment and follow-up a daunting task.

Several small studies have shown that up to 50% of persons spontaneously resolve asymptomatic *Chlamydia trachomatis* infection.[3,9] These studies were performed using culture techniques. Because cell culture techniques are only 65–85% sensitive, questions arise as to whether the observed clearance was a spontaneous cure or failure of the cell culture. One study using DNA amplification techniques confirmed that the clearance of asymptomatic *Chlamydia* does occur (28%) in both men and nonpregnant women. Resolution was associated with increasing age and time from diagnosis to follow-up studies, implicating a host–immune response to chlamydial infection.[10] This angle has not yet been evaluated in pregnant women with asymptomatic chlamydial infection–spontaneous resolution rates may be lower based on the potential for an altered host–immune response in pregnancy. The objectives of this analysis were to estimate the rate of spontaneous resolution (clearance) of asymptomatic *C trachomatis* in pregnancy and to evaluate the clinical factors associated with the spontaneous resolution.

MATERIALS AND METHODS

This study is a secondary analysis of data obtained during a randomized, double-masked multicenter clinical trial conducted by The National Institute of Child Health and Human Development Maternal–Fetal Medicine Units Network. In the randomized trial, 21,965 pregnant women were screened for bacterial vaginosis between 8 weeks, 0 days and 22 weeks, 6 days of gestation. Screening, eligibility, and exclusion criteria for the cohort have been described previously.[11] Briefly, women were ineligible for screening if they reported vaginal discharge with itching, burning, or odor; an allergy to metronidazole; ethanol abuse; antibiotic therapy within the previous 14 days; plans to deliver at another institution; cervical cerclage; preterm labor before screening; fetal death or life-threatening anomaly; multifetal gestation; or medical illness requiring long-term or intermittent drug therapy. Women who were found to have asymptomatic bacterial vaginosis were randomly assigned between 16 weeks, 0 days and 23 weeks, 6 days of gestation to 1 of 2 treatment groups: 2 g of metronidazole or a lactose placebo. The dose was repeated at 48 hours and then again at a follow-up visit between 24 weeks, 0 days and 29 weeks, 6 days of gestation. The primary aim of the trial was to estimate whether the treatment of asymptomatic bacterial vaginosis decreased the risk of preterm delivery. The results of this trial previously have been reported.[11]

During the study period, voided urine samples were collected before treatment at the randomization and at the follow-up visits. The urine specimens were frozen at −70°C and archived at 1 institution (University of Alabama at Birmingham). Analysis was performed subsequent to completion of the trial. A ligase chain reaction for genitourinary *C trachomatis* infection was performed in this central location. The ligase chain reaction is specific for *C trachomatis* plasmid DNA. Ligase chain reaction has been shown to be a highly effective, sensitive, and specific assay for genitourinary *C trachomatis*.[12–15] Specifically, ligase chain reaction of urine in pregnant women has been reported to be 84% sensitive and 99.5% specific,[12] which is a marked improvement over standard culture techniques. The results of the ligase chain reaction assays were not available to clinicians managing the women, and no one standardized *Chlamydia* screening program was mandated at the participating centers.

Women who did not have a randomization and a follow-up urine ligase chain reaction were excluded from this analysis, as were women with symptomatic *C trachomatis* genital infections. Symptoms included vaginal discharge and pruritus. Antibiotic use for any indication was recorded in the database. Women receiving antibiotics between randomization and follow-up reported to be effective for *Chlamydia*, specifically amoxicillin, penicillin derivatives, clindamycin, azithromycin, chloramphenicol, any fluoroquinolone, erythromycin, doxycycline, and rifampin, also were excluded. The study was approved by the institutional review boards of the participating centers. Informed consent was obtained from all women before randomization.

The data were analyzed using the Fisher exact, χ^2, or Mantel–Haenszel tests where appropriate. Logistic regression also was performed, and odds ratios (ORs) with 95% confidence intervals (CIs) were determined. Nominal P values were reported and adjustments were not made for multiple comparisons. A 2-sided $P < .05$ was considered significant. All analyses were performed using SAS 8 (SAS Institute, Cary, NC).

RESULTS

A total of 1,953 women were randomly assigned into the bacterial vaginosis trial published previously (Fig. 1).[11] Of these, 406 (21%) women did not have a randomiza-

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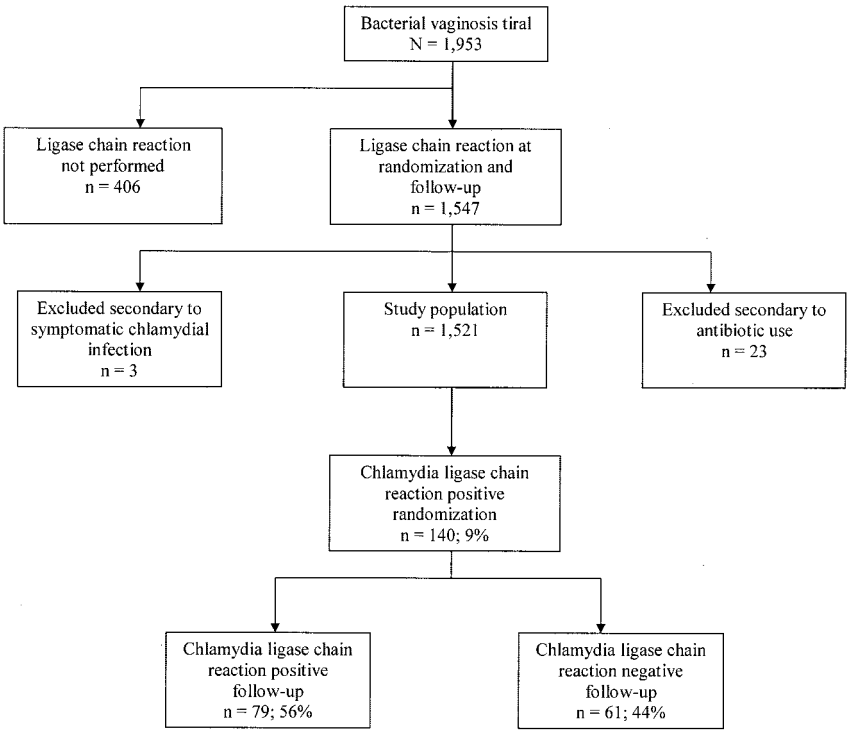

Fig. 1. Original study population.

Sheffield. Spontaneous Chlamydia Clearance. Obstet Gynecol 2005.

tion and/or follow-up urine ligase chain reaction results for *C trachomatis*. Of the remaining 1,547 women, 23 (1.5%) were excluded secondary to antibiotic use effective against *C trachomatis*, and 3 were excluded secondary to symptomatic *Chlamydia* infection. The final population for this study included 1,521 pregnant women without symptoms of genitourinary *Chlamydia*. Approximately 9%, or 140 women, were positive for *C trachomatis* at randomization and thus were diagnosed with asymptomatic chlamydial infection. Sixty-one of the women who initially tested positive (44%) were negative for *C trachomatis* on the follow-up urine ligase chain reaction, indicating spontaneous clearance of their asymptomatic infection.

The *C trachomatis* ligase chain reaction positive rate at randomization was not different between the metronidazole and placebo groups (9.3% and 9.1%, respectively, $P = .08$). Also, no significant difference was found between the 2 groups with regard to *Chlamydia* resolution rates (41% and 46%, respectively, $P = .51$). Therefore, the data in the 2 treatment arms were combined for the remaining analyses.

Table 1 details the demographic data of the ligase chain reaction–positive women who spontaneously cleared *C trachomatis* compared with those with persistent infection. Although race, nulliparity, and insurance status did not differ between the 2 groups, age was a significant factor. Increasing age was associated with a greater likelihood of a negative follow-up *C trachomatis* ligase chain reaction result ($P = .01$ for age as a continuous variable).

Factors previously reported to be associated with spontaneous *Chlamydia* clearance in animals, men, and nonpregnant women are listed in Table 2. A greater number of lifetime sexual partners and a testing interval of more than 5 weeks from randomization to follow-up ligase chain reaction testing were associated with spontaneous clearance of *C trachomatis* in the univariant model ($P = .02$ and 0.02, respectively). No women in either

Table 1. Demographic Data of the Study Cohort

	Follow-up Ligase Chain Reaction (−) (N = 61)	Follow-up Ligase Chain Reaction (+) (N = 79)	*P*
Race			
Black	54 (89)	70 (89)	
Caucasian	3 (5)	6 (8)	.67
Hispanic	4 (7)	3 (4)	
Nulliparity	27 (44)	44 (56)	.18
Maternal age (y)			
< 20	22 (36)	42 (53)	
20–25	24 (39)	28 (35)	.01
≥ 26	15 (25)	9 (11)	
Government assistance	59 (97)	72 (91)	.30

Data are reported as n (%).

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Table 2. Risk Factors Associated With Spontaneous Resolution of *Chlamydia*

	Follow-up Ligase Chain Reaction (−) (N = 61)	Follow-up Ligase Chain Reaction (+) (N = 79)	P
Lifetime sexual partners			
1	3 (5)	9 (11)	
2–4	22 (36)	39 (49)	.02
≥ 5	36 (59)	31 (39)	
Alcohol use	4 (7)	4 (5)	.73
Tobacco use	7 (11)	6 (8)	.43
Randomization to follow-up interval (wk)			
Up to 5	16 (26)	36 (46)	.02
≥ 5	45 (74)	43 (54)	

Data reported as n (%).

group had a diagnosis of human immunodeficiency virus (HIV). Alcohol and tobacco use also did not differ between the groups.

The vaginal milieu, characterized by using Gram stain Nugent criteria scoring and vaginal pH at randomization and follow-up, had no effect on chlamydial clearance (Table 3). Bacterial vaginosis is defined as a Gram staining score of 7 or greater in conjunction with a vaginal pH of more than 4.5. These parameters were used to assess whether changes in the vaginal milieu, consistent with bacterial vaginosis, would affect *Chlamydia* clearance.

Age, number of lifetime sexual partners, and the interval of time between each ligase chain reaction test all were associated with spontaneous clearance of *C trachomatis*. When all 3 variables were included in a logistic regression model, maternal age (OR = 0.6, 95% CI 0.4–0.9 per 5 years; $P = .02$) and follow-up interval of more than 5 weeks (OR = 0.4, 95% CI 0.2–0.9; $P = .03$) remained significant. For every 5-year increase in maternal age, the odds of ligase chain reaction positivity at follow-up decreased by 40%.

Table 3. Effect of Vaginal Milieu on *Chlamydia* Clearance

	Follow-up Ligase Chain Reaction (−) (N = 61)	Follow-up Ligase Chain Reaction (+) (N = 79)	P
Gram stain ≥ 7			
Randomization	53 (87)	69 (87)	.94
Follow-up	27 (46)	40 (51)	.57
pH ≥ 4.5			
Randomization	57 (93)	78 (99)	.17
Follow-up	39 (64)	57 (72)	.3

Data reported as n (%).

DISCUSSION

Spontaneous resolution of *C trachomatis* using a sensitive DNA assay occurred in 44% of pregnant women with asymptomatic *C trachomatis*. Maternal age was the strongest predictor of resolution–the older the woman, the more likely she was to have spontaneous resolution. Increasing time interval between testing and the greater number of lifetime sexual partners also were associated with resolution.

A number of previous studies have described the occurrence of spontaneous clearance of *C trachomatis* in both animals[16,17] and humans.[3,9,10] The limitations of these earlier studies was the sensitivity of the tissue culture-based testing (reported to be 65–85%).[13,18] The argument can be made that the chlamydial infection did not resolve but that the cell culture failed. The advent of DNA amplification technology (polymerase chain reaction and ligase chain reaction) has made available highly sensitive and specific assays for *C trachomatis*.[12,15] These tests using both cervical and urine specimens have been validated in pregnancy[12] and have been proven to be more cost-effective than tissue culture techniques.[19] To address the validity of the tissue culture-based studies, Parks et al[10] described a cohort of men and nonpregnant women with chlamydial infection using DNA amplification techniques. In this study, 28% of patients spontaneously resolved their infection, which is similar to the resolution rate described in our cohort of pregnant women with asymptomatic disease. These 2 studies confirm that spontaneous resolution of *Chlamydia* frequently does occur.

The activation of a host immune response is the most likely etiology for the spontaneous resolution of *Chlamydia*. Over the course of time, untreated animals have been found to resolve chlamydial infection.[16,17] In humans, increasing age and increasing time interval between testing have been associated with spontaneous resolution, indicating either prior chlamydial infection with the development of memory T cells or time to mount an immune response.[10] Pregnancy has long been assumed to be associated with suppression of humoral and cellular immunity to allow tolerance of the fetus. The possibility of this altered immune function in pregnancy could have decreased the clearance rate. However, we also found maternal age and time interval to be associated with resolution, as was a greater number of lifetime sexual partners (increasing the chance of prior chlamydial infection). These associations are all consistent with a host immune–response mechanism.

The role of *Chlamydia* infection in pregnancy complications, such as preterm delivery and preterm rupture of membranes, has been controversial. Reports have varied

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based on sample size, study population, and diagnostic techniques used. Spontaneous clearance of chlamydial infections may also explain some of the varied results and should be addressed in subsequent analyses.

This was a secondary analysis of a large randomized controlled trial and, as such, has limitations that must be addressed. The sensitivity and specificity of the ligase chain reaction are not 100% (as with any laboratory test), and therefore the possibility of false-positive and false-negative results remain. However, this DNA test is markedly better than previous culture techniques, and DNA amplification technology has become the "gold standard." A second limitation is that the power calculations were performed for the original study, not this secondary analysis. However, the numbers reported in this work are adequate to address the rate of spontaneous clearance of *Chlamydia* and to determine risk factors associated with this finding.

The public health impact of *Chlamydia* infection in the United States is without question. The rate of asymptomatic chlamydial infection in this cohort of pregnant women of 9% stresses the importance of screening all women during the prenatal period. Screening and subsequent treatment will not only decrease the risk of neonatal complications but also decrease the rate of long-term sequelae. Defining the spontaneous clearance rate (44%) and factors associated with resolution are important to our understanding of the pathophysiology of chlamydial infection. It also has major public health implications. Although 44% of infections resolved, most women with asymptomatic *Chlamydia* who were not treated had persistent infection. The importance of adequate treatment and follow-up remains a high priority for clinicians.

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APPENDIX

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