Test of Cure for Genital *Chlamydia trachomatis* Infection in Women

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Convenient, reliable tests of cure for genital chlamydial infections have not been evaluated. Cervical appearance, endocervical Gram stain, enzyme immunoassay, and culture for Chlamydia trachomatis *were evaluated during a pretreatment visit and at two subsequent randomized test-of-cure visits for 64 nongravid women with endocervical* C trachomatis *of 3544 patients screened. There were no useful correlations between* C trachomatis *resolution and cervical appearance. Endocervical Gram stain was determined to be unreliable for test-of-cure use. Both* C trachomatis *culture and enzyme-linked immunosorbent assay (ELISA) were shown to be effective for test-of-cure evaluation. The ELISA test became reliably negative 10 days after initiation of treatment and 1 to 5 days after the clearance of viable organisms detected by culture (P = .03). Convenience and cost considerations favor antigen detection methods. This study suggests that antigen detection methods can be used for situations in which test of cure is indicated, such as therapy noncompliance, circumstances supporting reinfection, pregnancy, complicated infections, requests for psychological reassurance, and evidence of persistent cervicitis.* **J Fam Pract 1990; 31:36-41.**

Genital *Chlamydia trachomatis* infection in women has emerged as a problem of major public health importance. Although the true incidence of genital *C trachomatis* is unknown, it is now thought to be the nation's most prevalent sexually transmitted disease.[1] Genital chlamydial infections have been estimated to cost the United States $1.4 billion annually.[2] The important clinical consequences familiar to family physicians include salpingitis, chronic pelvic pain, endometritis, ectopic pregnancy, and infertility. Unrecognized and inadequately treated *C trachomatis* during pregnancy may lead to infant conjunctivitis and pneumonia.

One problem with *C trachomatis* infections is that they are frequently subtle and patients are often asymptomatic. Yet *C trachomatis* infection may be suspected if several clinical factors are present.[3] Mucopurulent cervicitis classically consists of cervical ectropion and friability, and an endocervical mucopurulent discharge. These findings are caused by *C trachomatis* in 30% to 50% of cases.[4,5] These clinical conditions, however, are neither diagnostic of nor specific for *C trachomatis* infection.

A variety of laboratory tests have also been used. A Gram stain examination of an endocervical swab sample has been used to predict mucopurulent cervicitis and *C trachomatis* but is thought not to be diagnostic.[4] Serologic identification of *C trachomatis* confirms previous infection but has not been useful as a diagnostic tool for acute infection. Papanicolaou smears that demonstrate transformed lymphocytes and an increased number of polymorphonuclear cells and histiocytes are suggestive of *C trachomatis* infection[6] but are also not diagnostic. Vaginal wet smears and swab tests also fail to provide conclusive evidence for chlamydial infections.[7]

A culture is the most accurate means of diagnosing *C trachomatis* infection. Among the disadvantages of its use are high expense, lack of availability and timely results, concerns about viability during transport, and the need for a skilled microbiology laboratory.

Enzyme-linked immunosorbent assay (ELISA) detects antigen by two antibody reactions that enable a spectro-

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photometric reading. This technique offers the advantages of a short multitest processing time and usefulness for high-volume clinics. ELISA has a reported 85% to 89% sensitivity and 95% to 97% specificity.[8] The disadvantages of ELISA are the inability to assess whether an adequate sample has been obtained and the low predictive value of a positive test result in populations in which the prevalence of the disease is low.

Tests of cure for *C trachomatis*, although controversial, have been advocated for several reasons.[4,9] A significant posttreatment prevalence of infection exists, reported from 4.5% to 9.7%.[10–12] The organism also inflicts significant morbidity (infertility) and is occasionally a cause for mortality (ectopic pregnancy).

The Centers for Disease Control do not currently recommend universal test of cure for chlamydial infections[13]; however, test of cure for *C trachomatis* infection should be strongly considered in some situations. Patients with known or suspected noncompliance with therapy should receive test-of-cure evaluation.[14] There is a high risk of reinfection by contact with an untreated sexual partner. Noncompliance with instructions for sexual abstinence during treatment would indicate the performance of a test of cure. Resistance to tetracycline or doxycycline is not known to be a problem at present,[13] but resistance development in the future would be a reason to perform test of cure, as in the case of *Neisseria gonorrhoeae* posttreatment cultures. Pregnancy may also justify test of cure.[15]

Patients who exhibit evidence of continued posttreatment cervicitis probably require test of cure. Hobson et al[11] determined that posttreatment *C trachomatis* infections were not clinically apparent, and concluded that laboratory follow-up was essential for evaluation. Test of cure after treatment of patients with pelvic inflammatory disease should be considered even though cervical samples may not reflect endometrial or fallopian tube infection.[16,17]

Patients may request test of cure, particularly if the infection was diagnosed in the asymptomatic stage (eg, "If I never knew I had it, how will I know if it is gone?"). Similarly, clinicians cannot be confident of infection resolution just because clinical evidence for infection is absent.

A test of cure by *C trachomatis* culture may be slightly more reliable than ELISA, especially in a low-prevalence population and if rapid results are not required. Tests of cure obtained primarily for legal cases and documentation would be best collected by culture method.

Clinical research on *C trachomatis* to date has focused on improving the cost-effectiveness of nonculture screening and diagnostic techniques. There has been little research regarding the clinical approaches to the posttreatment patient, however. In particular, pretreatment clinical screening methods and diagnostic tests have not been adequately evaluated for use as tests of cure.

As documented by McCoy cell culture testing, chlamydial infection usually resolves after compliant therapy. The use of antigen detection for test of cure of genital *C trachomatis* is problematic, as antigen tests detect nonviable organisms. In the posttreatment patient, therefore, positive antigen detection does not necessarily imply cervical infection. To use antigen detection properly for test of cure and to avoid false-positive results, the duration of *C trachomatis* antigen at the cervix following treatment must be determined. This critical interval has been unknown. Clinical and laboratory methods of posttreatment test of cure for women with genital *C trachomatis* infection were evaluated.

METHODS

Patient Population

During routine annual pelvic examinations, 373 college-aged women patients of the East Carolina University Student Health Service tested positive for *C trachomatis* by ELISA and were asked to participate in this study. Of these, 75 agreed and were enrolled in the study. Inclusion criteria were that the women could not be pregnant, that they have cervical *C trachomatis* infection, and that they be willing to participate in the 6-week follow-up.

Clinical Evaluation

During each visit one of four clinicians obtained a short standardized history and performed a pelvic examination. At the first visit the patient completed a brief questionnaire regarding medical history and menstrual cycle. Three endocervical swabs were obtained during each visit in a random order, one each for Gram stain, enzyme immunoassay, and McCoy cell culture. Cervical characteristics were noted. If a significant vaginal discharge was present, a specimen for saline and potassium hydroxide microscopic examination was obtained. All patients were previously screened by culture for *Neisseria gonorrhoeae* and found to have negative results.

Each subject was asked to complete therapy with 500 mg of tetracycline four times a day for 7 days, and to refrain from sexual intercourse and douching for the duration of the study. The patient also received a *C trachomatis* contact treatment card to give to her sexual partner(s).

Study Design

Each patient was seen three times, first for an initial pretreatment visit and twice for randomized follow-up visits. Uncertainty as to when *C trachomatis* antigen is cleared from the endocervix provided the rationale for a two-phase investigation. In phase 1, the initial 13 subjects received the first test-of-cure visit by random assignment 1 to 3 weeks from the initiation of therapy. The second test-of-cure visit was 3 to 6 weeks after therapy initiation to accommodate a full menstrual cycle. Phase 1 demonstrated that all subjects tested for *C trachomatis* antigen after 10 days remained negative. Therefore, phase 2 included the remaining 62 subjects and narrowed the investigation to days 3 through 10 after treatment began. The first test-of-cure visit was randomized to days 3 through 6, and the second test-of-cure visit randomized to days 7 through 10 after the initiation of treatment.

Laboratory Methods

Gram-stain analysis of endocervical specimens was performed by standard methods. Results were recorded as greater or fewer than 10 polymorphonuclear leukocytes per high-power microscopic field.

After collection from the patient, the culture transport media were refrigerated at 4°C and shipped by same-day courier in a cold pack to the Virology Branch of the North Carolina State Public Health Laboratory. Standard isolation procedures were used.[18] The specimens were inoculated onto McCoy cell monolayers that had been grown on round coverslips, centrifuged to assist phagocytosis, treated with cycloheximide media, and incubated at 37°C for 48 to 72 hours. Specimens then were stained for confirmation with fluorescein-labeled monoclonal antibodies. A positive diagnosis was made by observing microscopic fluorescent green inclusion bodies.

ELISA was performed at the East Carolina University Student Health Service Laboratory according to manufacturers' specifications. Samples were read by spectrophotometer at 492 mm. If the absorbance reading exceeded the mean of three negative controls plus 0.10 unit, a positive result was recorded.

Statistical Analysis

The difference in time for cure (lag time) between the two detection methods (culture and ELISA) was recorded and tested for statistical significance by Gehan-Wilcoxon analysis.[19] Gehan-Wilcoxon analysis and the chi-square test were used to determine the effect on antigen clearance and cure intervals (as determined by cell culture) of the menstrual cycle, clinical factors, and menstrual sanitary products.

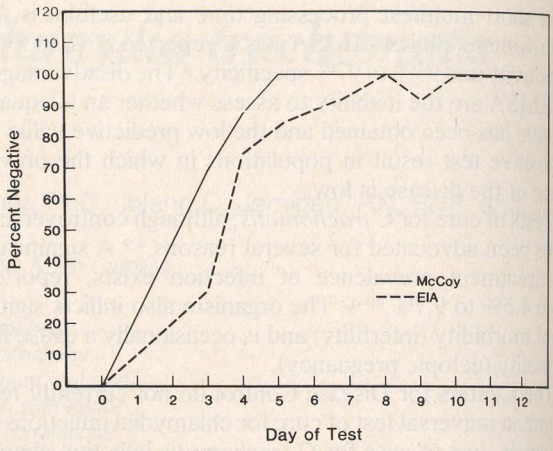

Figure 1. Plot of clearance of *C trachomatis* from the cervix as a function of the time after beginning antibiotic therapy. Continuous line denotes McCoy cell culture method of detection; interrupted line denotes enzyme-linked immunosorbent assay (ELISA) (N = 64).

RESULTS

A total of 3544 patients were screened during the study period from May 1987 to June 1988. The finding that 373 (10.5%) women were positive for *C trachomatis* by ELISA conforms to the prevalence rate for college health facilities and family planning clinics reported by other researchers.[3,20,21] Of the 75 (20%) positive patients who agreed to participate in the study, the rate of compliance for follow-up encounters was 98.6%.

Sixty-four (85%) of these subjects who were positive for *C trachomatis* by ELISA at the screening visit were positive by culture on the day of treatment. To resolve discrepant results, enzyme immunoassay specimen dilution buffer was examined for *C trachomatis* elementary bodies by direct immunofluorescent antibody technique. Elementary bodies were found in six specimen dilution buffer samples, which suggests a total of 70 true-positive subjects. The other five samples contained no elementary bodies. The study data, however, are based only upon those 64 patients with a positive culture and ELISA test result at initiation of therapy.

Resolution of endocervical chlamydial infection, as documented by McCoy cell culture and ELISA, is shown in Figure 1. Cell cultures were negative for viable organisms by day 5. All ELISA tests of cure were negative by day 10, demonstrating a lag time between culture cure and antigen clearance as measured by ELISA. In general, the chlamydial organism becomes nonviable 1 to 5 days be-

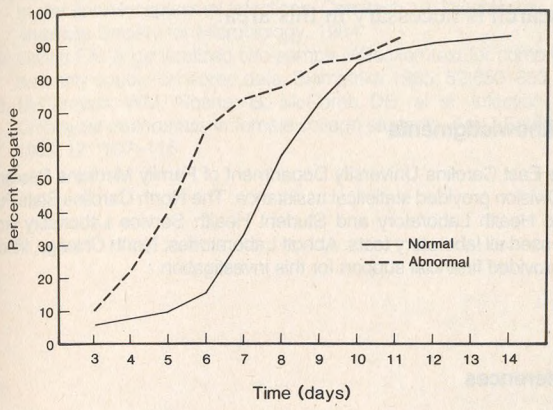

Figure 2. Plot of *C trachomatis* cervical infection cure for normal- and abnormal-appearing cervices. Continuous line denotes the normal-appearing cervices; interrupted line denotes the abnormal-appearing cervices.

fore the antigen clears. This delay is statistically significant by the Gehan-Wilcoxon test ($P = .03$).

Infection was shown to resolve faster for women whose cervices initially appeared abnormal (defined as demonstrating erythema, ectropion, or friability) than for women with normal-appearing cervices (Figure 2). This finding was statistically significant for both ELISA ($P = .0022$) and McCoy cell culture ($P = .0001$).

Previously reported clinical predictive criteria for *C trachomatis*[3] (ie, mucopurulent discharge, friability, cervical appearance, and Gram stain) were evaluated to determine their usefulness as reliable test-of-cure indicators. Other factors, such as phase of menstrual cycle or use of menstrual sanitary products, which may correlate with the antigen clearance from the cervix, were also analyzed.

TABLE 2. PERCENTAGE OF *CHLAMYDIA TRACHOMATIS* CLINICAL INDICATORS OBSERVED DURING THERAPEUTIC MANAGEMENT OF CERVICAL INFECTION

Indicators	Pretreatment	During Treatment	After Treatment
Abnormal cervix (ectropy, edema, erythema, or friability)	43	35	19
Friability	12	4	3
Mucopus	9	11	3

Mucopurulent cervicitis, defined as greater than 10 polymorphonuclear leukocytes per high-power microscopic oil-immersion field by Gram stain, was shown not to influence antigen or viable organism clearance (Table 1). The presence or absence of cervical discharge was not a reliable indicator of the resolution of the infection. The form of contraception, phase of menstrual cycle, and the type of menstrual sanitary product were also found not to correlate with *C trachomatis* infection resolution as determined by ELISA or culture.

Changes in nonspecific clinical indicators were analyzed during the treatment course (Table 2). The data suggest decreases in rates of cervical abnormality, friability, and mucopus during and after therapy. The limited

TABLE 1. FACTORS THAT INFLUENCE OR REFLECT CHLAMYDIAL INFECTION RESOLUTION

Factors	Enzyme Immunoassay χ^2	Enzyme Immunoassay *P* Value*	Cell Culture χ^2	Cell Culture *P* Value*
Clinical				
Cervical appearance: normal vs abnormal	9.37	.0002	15.8	.0001
White cell count <10/hpf vs >10/hpf	.46	.49	.46	.66
Abnormal discharge: absent vs present	.42	.51	.43	.51
Contraception				
Oral contraceptive pill vs other methods	.58	.44	.002	.95
Barrier vs other methods	.78	.37	.008	.93
Menstrual cycle				
Phase: proliferative vs secretory (<14 days vs >14 days)	1.05	.30	.04	.84
Menstrual sanitary products				
Pad vs other methods	.06	.80	.07	.79
Tampon vs other methods	.60	.44	.04	.84

*Gehan-Wilcoxon method used.

rates of change and the small sample size, however, prevent conclusive results.

DISCUSSION

The intent of this investigation was to evaluate how clinical factors and laboratory techniques may be used for tests of cure of genital *C trachomatis* infection in women. The choice of methods depends on availability, personnel expertise, equipment, expense, processing time, prevalence, and purpose.[8]

The study described here was designed primarily to evaluate test-of-cure methods. Somewhat unexpected was the finding that cervices that initially appeared abnormal actually resolved infection more rapidly and cleared antigen more quickly than normal-appearing cervices. The presence of erythema, ectropion, and friability may be more indicative of inflammation than of severity of the infection. The inflammatory process implies the presence of appropriate immune cells,[6] which may provide the capacity for more rapid antigen clearance compared with the normal-appearing cervix without inflammation. Another plausible explanation for this finding is that the inflammatory process may improve antibiotic delivery because of an increase in capillary permeability and blood flow to the cervix. Moreover, the "persistent infection" associated with a normal-appearing cervix may actually reflect an upper genital tract infection, which may resolve more slowly. Previous investigators have documented the recovery of *C trachomatis* from the endometrium of patients with a negative cervical culture.[16,17] Cervical appearance following therapy remains an unreliable indicator of *C trachomatis* infection resolution as demonstrated by these investigation findings.

Saglio and Henley[22] questioned the use of ELISA for test-of-cure evaluations because these tests detect nonviable organisms. Soren and Willis[23] evaluated ELISA for test of cure in 29 patients and found a predictive value of 40%. That study, however, failed to account for the delay in antigen clearance shown in this study. Classic methods of calculating predictive value for ELISA are invalid because they do not account for delay in antigen clearance.

The investigation data establish that antigen detection by an ELISA test provides a reliable test of cure for genital *C trachomatis* infections in women when obtained 10 days following initiation of appropriate tetracycline therapy. The clinical relevance for rural family physicians is especially important and obvious. Implementation of selected tests of cure could contribute to decreasing the prevalence of this potentially devastating infection. This study design excluded pregnant patients and those with obvious pelvic inflammatory disease; therefore, further research is necessary in this area.

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