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Time to clearance of *Chlamydia trachomatis* ribosomal RNA in women treated for chlamydial infection

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Abstract. ***Background*:** The dynamics of chlamydia clearance after treatment administration for chlamydial urogenital infection are unknown. We estimated the time to clearance of *Chlamydia trachomatis* (CT) ribosomal RNA (rRNA) after administration of azithromycin for cervical chlamydial infection using APTIMA Combo 2 (Gen-Probe, Inc., San Diego, CA, USA). ***Methods*:** A total of 115 women diagnosed with urogenital chlamydial infection, defined as a positive APTIMA urine or endocervical specimen, were enrolled in the present study. Vaginal swabs on the day of treatment (Day 0) and on Days 3, 7, 10 and 14 after treatment with 1 g of azithromycin were self-obtained by participants. Specimens were tested in a single laboratory. Our analysis was limited to women who were CT-confirmed by vaginal swab at baseline, who returned all follow-up swabs, and who reported sexual abstinence during the follow-up period ($n = 61$). ***Results*:** Among 61 participants, 48 (79%) had a negative APTIMA at Day 14. Subjects with a negative APTIMA at each time-point were as follows: 0/61 (0%) on Day 0, 7/61 (12%) on Day 3, 28/61 (46%) on Day 7, 40/61 (66%) on Day 10, and 48/61 (79%) on Day 14. Multiple linear regression analysis predicted time to clearance at 17 days (95% confidence interval, 16–18 days) after administration of azithromycin. Seventeen of the 94 participants (18.1%) who screened positive for chlamydia had a negative vaginal swab on Day 0, indicating possible spontaneous clearance of CT. ***Conclusions*:** After treatment, CT rRNA declined with time. As rRNA was still detectable in 21% of the women 14 days after treatment, APTIMA should not be used as a test-of-cure in the 14-day period following azithromycin administration.

Additional keywords: APTIMA, test-of-cure, transcription-mediated amplification.

Introduction

Genital infection due to *Chlamydia trachomatis* (CT) is the most common reportable infectious disease in the USA, with the number of reported cases in 2007 exceeding 1.1 million per year. In women, undiagnosed and/or untreated infection can lead to pelvic inflammatory disease, ectopic pregnancy, infertility or chronic pelvic pain. The Centers for Disease Control and Prevention (CDC) recommends that sexually active women under the age of 26 years and women 26 years or older with risk factors (e.g. new or multiple sex partners) receive annual CT screening.[1] The CDC advises that patients diagnosed with CT abstain from sexual intercourse for 7 days after treatment with single-dose azithromycin or until completion of the 7-day course of doxycycline, to ensure adequate time for the antibiotic to take effect in order to prevent further disease transmission. Repeat CT testing after treatment (i.e. test-of-cure) is not recommended except in cases of pregnancy, persistent symptoms, or if there is concern for repeat exposure or medical non-adherence. Notably, the CDC recommendation for a 7-day period of abstinence following administration of azithromycin is based on expert opinion and clinical trial data, which informed the recommended 7-day course for doxycycline, as the duration of the persistence of viable organisms after azithromycin treatment is unknown.

True duration of infectivity after treatment for chlamydial infection is not clearly defined. Test-of-cure analyses have been difficult, as the gold standard test, chlamydia culture, has low sensitivity. Polymerase chain reaction (PCR) has been found to remain positive for a longer duration than the presumed period of active infection, and is therefore not recommended for use as a test-of-cure during the first 3 weeks following treatment.[2–5] In this regard, it is not possible to draw conclusions about infectivity in the setting of a positive PCR test following treatment, as the positive test result may simply represent the presence of non-viable and therefore non-infectious organisms.

Second generation nucleic acid amplification tests (NAATs) such as the APTIMA system, which detects ribosomal RNA

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(rRNA), have shown promise as potential tests-of-cure in women with chlamydial infection.[2] APTIMA has an improved sensitivity for CT detection compared with first generation NAATs, since at least 2000 copies of rRNA exist per bacterium in contrast to the approximate 10 copies of DNA per cell and lower false-negative rates due to specimen inhibition.[6] The APTIMA Combo 2 (Gen-Probe, Inc., San Diego, CA, USA) is Food and Drug Administration (FDA)-cleared for use on urine, endocervical, and vaginal swab specimens. The APTIMA system has not yet been evaluated as a test-of-cure for cervical chlamydial infection, and the excellent characteristics of this test lend to a potential role in this context.

In the present study, we used APTIMA Combo 2 vaginal swabs to test for CT at four time-points during the 14-day period after women received treatment for chlamydial infection, to determine optimal timing for using the APTIMA system as a test-of-cure for chlamydia urogenital infection.

Methods

Between January 2007 and November 2008, women who had a CT-positive urine or endocervical screening test (APTIMA Combo 2 (Gen-Probe, Inc., San Diego, CA, USA)) were recruited from one of four outpatient adolescent clinic sites in San Mateo County, California. Not all women with CT-positive tests were symptomatic. The women presented to those clinics for various health reasons, including pregnancy testing, birth control, sexually transmissible infection (STI) screening and annual Papanicolaou smears. Pregnant women, women under the age of 12 years, women who did not agree to abstain from sexual intercourse for 14 days after treatment, women who were unable to attend a final appointment following specimen collection, and women who were given empiric antimicrobial treatment at the time of initial screening were excluded from participating in the study.

At the treatment clinic visit (Day 0), written consent was obtained and an interviewer-administered questionnaire was performed in order to obtain demographic data, history of prior STI, number of sex partners and patterns of condom use. A single interviewer administered the questionnaires to all of the participants at the initial study visit. Single-dose azithromycin (1 g) was administered under direct observation during the baseline visit. Expedited treatment of partners with single-dose azithromycin was offered for up to three sex partners. Subjects were counselled to abstain from sexual intercourse for 14 days after administration of azithromycin. Condoms were available in clinic exam rooms for participants to take, but were not specifically provided to participants to use during the recommended 14-day abstinence period after azithromycin administration.

All of the participants were given both verbal and written instruction on self-administration of the APTIMA vaginal swabs. During the baseline study visit (Day 0), each individual performed the initial vaginal swab. Participants were then given four additional vaginal swabs and asked to self-administer the swabs on Days 3, 7, 10 and 14. A 14-day follow-up period was predetermined *a priori* because this was the maximum number of days felt appropriate to ask women to abstain from sexual intercourse, 1 week beyond the abstinence duration recommendation of the CDC. To facilitate accurate specimen collection, swabs were labelled with the appropriate collection day (3, 7, 10, 14) and the corresponding date and the day of the week. As a reminder, the interviewer telephoned each participant on the scheduled days of specimen collection. The specimens were placed in the APTIMA media and were stored at room temperature at the individual's home.

After the 14-day follow-up period, each subject returned to the clinic with the Day 3, 7, 10, and 14 swabs for a follow-up clinic appointment. The swabs were collected at that time, and participants were given a second interviewer-administered questionnaire to inquire about genital symptoms, adherence with performing the swabs on the appropriate dates, partner treatment, and sexual activity during the study duration; if they reported sexual activity during the 14-day period, they were also asked about condom use and if their partners were known to have an STI. The same interviewer who conducted the initial questionnaire also administered the questionnaire at the follow-up clinic appointment. For compensation, participants were given a $20 store credit at the initial visit and a $30 store credit at the final study visit. It was emphasised to the individuals before administration of the second questionnaire that they would receive the final payment regardless of whether they carried out the swabs correctly or if they abstained from intercourse during the 14-day study period.

The APTIMA Combo 2 vaginal swab specimens were processed according to the manufacturer's instructions by the San Mateo County Department of Public Health Laboratory. In order to minimise the risk of sample contamination, each step of specimen processing (i.e. target capture, transcription-mediated amplification and the dual kinetic assay protocol) was performed in a separate designated area of the laboratory.

Only women who had a positive confirmatory CT test at baseline (Day 0), who self-administered all five vaginal swabs and who reported abstinence for the 14-day study period were included in the analysis. Women were excluded if their baseline APTIMA swab was negative, if they reported that they did not abstain from sexual contact during the follow-up period, or if a swab result was missing. Demographic and sexual behaviour characteristics – median age, ethnicity, history of STI, number of sex partners, and frequency of condom use before enrolment in the study – were compared between the analysis and excluded groups to determine possible distribution differences. For analysis of categorical variables, we compared proportions using χ^2 and, when appropriate, Fisher's exact tests. For continuous variables, we compared medians using the Wilcoxon rank sum test.

We calculated CT clearance as the percentage of women with a negative APTIMA Combo 2 among all women in the analysis group at the four time-points following administration of azithromycin (Days 3, 7, 10, 14). Predicted time-to-clearance (in days) of all participants was estimated using simple linear regression. Multiple linear regression was used to measure associations between time-to-clearance and demographic and sexual behaviours determined at baseline. Covariates in the linear regression model included age, Latina ethnicity, past history of a STI, reporting 'always' using condoms with sex partners during the year before the survey, reported vaginal

discharge at baseline and whether the respondent reported multiple male sex partners (≥2) during the year before the survey. SAS 9.1 software (Cary, NC, USA) was used to perform the data analysis. The study protocol was approved by the Institutional Review Board at both Stanford University School of Medicine and Mills-Peninsula Health Services/San Mateo Medical Center.

Results

Of the 115 women with a positive CT urine or endocervical APTIMA test, 96 (83.5%) returned for their follow-up appointment. Of the 96 participants, 77 (80.2%) had a positive confirmatory APTIMA vaginal swab at baseline. Of the remaining 19 women, 17 had a negative baseline (Day 0) swab result, suggesting possible spontaneous clearance of the organism, and the baseline CT results for two were not performed. These 19 women were excluded from the analysis. Four additional women were excluded because they did not return vaginal swabs for all of the study time-points (Days 3, 7, 10, 14). Another 12 women were excluded because they reported sexual activity during the 14-day study period. A total of 61 (53%) women were included in the analysis. Among respondents where partner treatment history was known, 81% (75/93) reported that at least one sex partner received patient delivered partner therapy. In the analysis group, 77% (47/61) reported delivering therapy to at least one sexual partner.

Table 1 compares characteristics between the 61 women included in the analysis and the 54 excluded women. No statistical differences in ethnicity, age, or sexual histories were observed between the two groups. Table 1 also shows the demographic characteristics of the 17 women with putative spontaneous clearance; that is, women who were initially CT-positive but were found to have a negative vaginal swab on their baseline (Day 0) visit. For women with spontaneous CT resolution, the median number of days between the initial screen and the enrolment APTIMA vaginal swab was 5 days (range 1–55 days) compared with 8 days (range 1–61 days) for the analysis group ($P = 0.03$). Sixteen of these women had negative swab results for all days in the study (Days 0, 3, 7, 10, 14) and one participant had negative swab results on Days 0, 3, 7, and 10, but had a positive result on Day 14. No significant differences in patient characteristics were observed between the baseline negative swab group and the analysis group. Of note, women with possible spontaneous clearance of CT had a lower percentage (but not significantly different) of reported past STI than our analysis group ($P = 0.21$), and a marginally significantly lower percentage of reported past CT infection than the analysis group ($P = 0.08$).

At Day 14, 48 of the 61 participants (79%) had a negative APTIMA vaginal swab. The number of individuals with a negative APTIMA at each of the five time-points was as follows: 0 of 61 (0%) on Day 0, 7 of 61 (12%) on Day 3, 28 of 61 (46%) on Day 7, 40 of 61 (66%) on Day 10, and 48 of 61 (79%) on Day 14 (see Fig. 1). During the course of the 14-day follow-up period, a total of 11 (18.0%) women were found to revert to a positive CT result following a negative result. Specifically, five women had a positive swab on Day 10 that followed a negative swab on Day 7, two women had a positive swab on Day 14 that followed a negative swab on Day 10, and

Table 1. Comparison of analysis group ($n = 61$) and women who were excluded due to a negative baseline (Day 0) APTIMA, baseline test not collected, lack of submission of all follow-up vaginal swabs, or due to sexual activity during the follow-up period ($n = 54$)

The analysis group is also compared with a subset of women who were screened *Chlamydia trachomatis* (CT) positive but were APTIMA negative at baseline (day 0) ($n = 17$). STI, sexually transmissible infection

Characteristic	Analysis group ($n = 61$)		Excluded group ($n = 54$)		*P*-value	Screening CT+/APTIMA baseline vaginal swab – ($n = 17$)		*P*-value
	n/median	%	*n*/median	%		*n*/median	%	
Total	61	–	54	–	–	17	–	–
Ethnicity								
Latina	31	50.8	17	31.5	0.08	7	41.2	0.94
Asian/Pacific Islander	14	23.0	19	35.2		5	29.4	
African American	8	13.1	5	9.3		2	11.8	
White	5	8.2	4	7.4		2	11.8	
Mixed	3	5.0	9	17.7		1	5.7	
Median age (years)	18	–	18	–	0.99	17		0.55
Past STI history								
Any STI history	21	34.3	14	25.9	0.32	3	17.7	0.21
Chlamydia infection	20	32.8	12	22.6	0.23	2	11.8	0.08
Gonorrhoea	3	4.9	2	3.8	0.77	0	0.0	–
Sexually active 2-months before baseline visit	59	96.7	50	92.6	0.32	16	94.2	0.89
Median number of male sex partners during 2-months before baseline	1	–	1	–	0.32	2	–	0.54
Median number of male sex partners during 12-months before baseline	2	–	2	–	0.35	1	–	0.58
Condom use with sex partners before baseline								
Always/almost always	7	11.6	6	11.3	0.30	2	11.8	0.57
Sometimes	39	63.9	27	50.9		7	41.2	
Never	15	24.6	20	37.7		8	47.1	

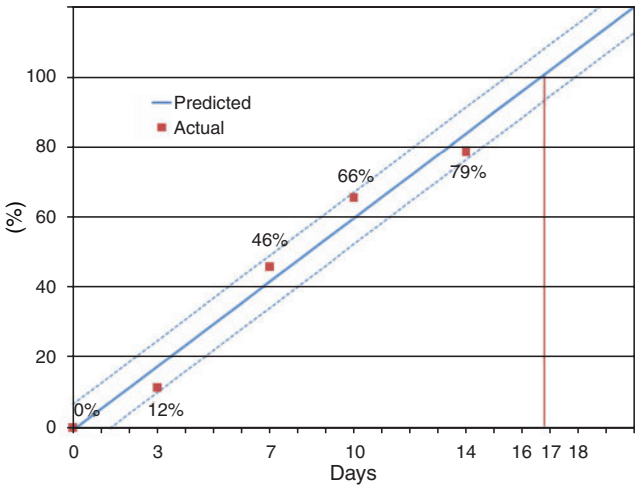

Fig. 1. Actual and predicted probabilities of *Chlamydia trachomatis* clearance.

four women had a positive swab result on Day 14 that followed a negative swab on Days 7 and 10. It is unclear in these women who reverted to CT positivity after a negative test, if they were re-infected or if vaginal swabs were detecting CT in semen from an infected partner (exposed but uninfected female partner).

The predicted time to 100% clearance of all subjects was estimated to be 17 days (95% confidence intervals (CI), 16, 18 days) (Fig. 1). Women who reported a previous CT infection were marginally significantly more likely to clear CT (100% clearance = 15 days, 95% CI, 13, 18 days) sooner than women without history of an STI ($P = 0.07$).

Women who reported any STI were significantly more likely to clear CT (100% clearance = 15 days, 95% CI, 13, 17 days) sooner than women without history of an STI ($P = 0.006$). Self-reported vaginal discharge at baseline was not significantly associated with clearance time. Time-to-clearance was estimated to be the same for women who reported vaginal discharge (18/61, 29%) compared with those who did not.

Discussion

In this prospective study of women treated for confirmed CT infection, although the number of women with detectable CT rRNA using APTIMA Combo 2 declined with time, we found that approximately one in five participants tested positive for CT rRNA 14 days after administration of azithromycin. This finding indicates that APTIMA Combo 2 should not be used as a test-of-cure for chlamydial infection in the 14-day period following administration of azithromycin. These results differ from those reported by Morré *et al.* whose findings on cervical smears (8% CT-positive at 7 days, 0% CT-positive at 14 days) and urine samples (6.7% CT-positive at 7 days, 0% CT-positive at 14 days) suggested that using nucleic acid amplification to detect CT rRNA could have value as a test-of-cure in women treated for chlamydial infection as early as 2 weeks after CT treatment.[2] Of note, however, it is possible that the earlier generation of the rRNA amplification technology used in this study influenced the time-to-clearance results.

Our analysis indicated that predicted time to 100% clearance is ~17 days, with a shorter predicted time to clearance (15 days) in women with a past history of an STI. We speculate that women with a history of a prior STI had prior CT and had an immune response that could lead to faster clearance of the organism on re-exposure. However, women who may have spontaneously cleared CT did not have a higher frequency of reported STI than our analysis group; in fact, these women reported a lower prevalence of past CT infection.

In previous studies evaluating PCR as a test-of-cure for chlamydial infection, it appears that PCR may remain positive for up to 3 weeks following treatment.[2–4]

Workowski *et al.* found that PCR remained positive for a prolonged period compared with chlamydia culture; the authors presumed that PCR remained positive due to the persistent detection of DNA within non-infectious organisms.[7] It is highly likely that a persistently positive APTIMA test 14 days after treatment represents the detection of CT rRNA in non-viable organisms. In fact, because rRNA persists in the elementary bodies of CT, rRNA could potentially be detected in the absence of infectious organisms (J. Schachter, PhD, pers. comm., 2009). In this regard, it is not possible to infer infectivity based on a positive nucleic acid test result. Studies correlating positive APTIMA test results with chlamydia culture are needed to definitively answer the question regarding the correlation of positive nucleic acid amplification tests with the presence of infectious organisms. Unfortunately, chlamydia culture has notoriously poor sensitivity for the diagnosis of chlamydial infections, thereby rendering this type of study difficult to perform and interpret.

An unexpected finding was that 17 of the participants (18.1% of the subjects who returned for their follow-up appointment and for whom baseline vaginal swab results were available, $n = 94$) had an original positive screening urine or endocervical APTIMA for CT but were found to have a negative APTIMA vaginal swab before treatment, suggesting possible spontaneous clearance of CT before treatment. It is unclear if the individuals with five negative results did not perform the vaginal swabs correctly (thereby leading to false-negative results), if their original urine screening test was falsely positive (for example, due to CT measured in semen in women who were exposed but not infected), if they had isolated chlamydial urethritis, or if they truly spontaneously cleared their infection.

There are several limitations to our findings. Abstinence was assessed through self-report at the follow-up interview. Although a single interviewer conducted the questionnaires during both clinic visits for all of the participants and attempted to use identical tone and phrasing when asking the questions, there is potential for information and social desirability bias, particularly when asking about the sensitive issue of abstinence. Specifically, if an individual engaged in sexual activity during the study period but did not feel comfortable reporting it, an undetected potential for re-infection may have occurred in those subjects, which may have resulted in a positive test result not from the original chlamydial infection but from a repeat exposure. Similarly, data on prior STIs (including chlamydial infection) was obtained only through participant report, which may be less

reliable than chart review. Although the participants were given both verbal and written instruction regarding correct vaginal swab technique, false-negative results could have occurred in the setting of improperly obtained swabs. Since the swabs were self-collected without supervision, the specimens may not have been obtained on the exact schedule as specified in the study protocol, as was reported by seven (11.5%) of the 61 participants. Another methodological limitation is that we were only able to follow women for 14 days after treatment. Our follow-up period of 14 days was guided by the concern that many subjects would not abstain from sexual activity beyond 2 weeks following administration of azithromycin, given the study abstinence period is 1 week longer than is recommended by the CDC.[1] Since 21% of the women did not have a negative swab result at day 14, we could only estimate the time-to-clearance of CT infection in these women. Our estimated time-to-clearance is based on a fairly robust straight line from data on a large number of patients. It is unlikely this line would change slope dramatically. We did not collect data on oral contraceptive use and diagnostic evaluation for bacterial vaginosis was not done. Previous studies have found cervical ectopy mediated through oral contraceptive use and bacterial vaginosis may be risk factors for urogenital chlamydial infection.

In conclusion, we found that ~20% of women treated for chlamydial infection had a positive chlamydia APTIMA 14 days after treatment. Given these findings, APTIMA tests should not be used as a test-of-cure in the 14 days following administration of azithromycin. In our regression model, we estimated time to 100% clearance of CT rRNA was 17 days. We recommend when using highly sensitive assays for CT rRNA, at least 17 days should elapse before testing to evaluate response to treatment.

Conflicts of interest

None declared.

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