Original article

Recurrence of urogenital *Chlamydia trachomatis* infection evaluated by mailed samples obtained at home: 24 weeks' prospective follow up study

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Objectives: To evaluate the rate of recurrence of genital *Chlamydia trachomatis* infection after antibiotic therapy in a population of patients drawn from general practice, and to evaluate whether retesting after antibiotic therapy was advisable and, if so, whether it could be based on a strategy involving samples obtained at home and mailed to the laboratory for analysis.
Methods: Prospective follow up study of 42 patients with genital *C trachomatis* infection drawn from general practice. Patients at or above the age of 18, with a positive urogenital swab sample obtained by a general practitioner were invited to participate. Follow up testing was based on LCR testing (LCx, Abbott diagnostics) of first void urinary and vaginal flush samples taken by the patients at home and mailed to the laboratory at weeks 2, 4, 8, 12, and 24 after antibiotic therapy.
Results: Cumulated incidence of recurrent infection was calculated to 29% (95% CI: 12%–46%) during the 24 weeks of follow up. Previous or present sexually transmitted diseases other than *C trachomatis* were significantly associated with recurrence (OR 6.1, p=0.03). 89% of patients tested negative at week 2, and all patients tested negative at some point during the first 4–8 weeks. 84% of the test kits mailed to the patients were returned to the laboratory for analysis.
Conclusions: Recurrence of *C trachomatis* after antibiotic treatment is a substantial problem. Retesting should be carried out, but not sooner than 12–24 weeks after treatment. Requiring patients to take tests at home appears to be a promising method for retesting.
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Keywords: recurrent infection; ligase chain reaction; *Chlamydia trachomatis*

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Introduction

Chlamydia trachomatis is the most common sexually acquired bacterial disease among adolescents.[1] The possible consequences are pelvic inflammatory disease, which may lead to chronic abdominal pain, ectopic pregnancy, or female infertility.[1]

The need for test of cure after antibiotic therapy is debatable. Previous studies[2–4] have found rates of recurrence of 32%, 16.8%, and 38.4%, respectively. However, these studies were carried out on high risk patients or other selected groups of patients, and involve only a limited number of tests during the follow up period. Furthermore, existing follow up studies are based on samples taken in doctors' offices. It has been demonstrated that the use of a system involving patients taking samples at home and then mailing them to a diagnostic laboratory for analysis[5] will result in more individuals being tested and more infections being detected compared with the conventional system of analysing swab samples obtained at doctors' offices.[6 7] Test of cure based on samples taken at home and then mailed to a diagnostic laboratory for analysis may thus be a reliable and convenient strategy.

The aim of this study was to evaluate the rate of recurrence of genital *C trachomatis* infection in a population of patients drawn from general practice, and, subsequently, to evaluate whether follow up testing was advisable and, if so, whether it could be based on a strategy involving patients taking samples at home. The phrase "recurrent infection" is defined as any infection after antibiotic therapy regardless of aetiology—that is, relapse of persisting infection, reinfection, etc.

Materials and methods

The study took place in the county of Ringkjøbing, Denmark, at the department of clinical microbiology at Herning County Hospital, which carries out approximately 10 000 *C trachomatis* tests per year. Approximately 5% of these were positive in 1997.

STUDY POPULATION AND RECRUITMENT

In the period 30 July to 16 December 1997, the department of clinical microbiology identified positive endocervical or urethral swab samples from 141 patients from general practice at or above 18 years, who had not been treated with antibiotics during the previous 4 weeks. Of these 141 patients, the general practitioners obtained informed consent to take part in the study from 12 males (mean 22.3 years) and 30 females (mean 22.3 years).

MICROBIOLOGICAL TESTING

The initial swab samples taken during the recruiting phase were tested by an enzyme immunoassay (EIA) (Syva, California) with confirmation by ligase chain reaction (LCR) (LCx, Abbott Diagnostics, Chicago) in a similar manner, to that described previously.[8] The

samples collected at home and mailed to the laboratory during the follow up phase were processed and analysed by LCx, as described previously.[5]

DIAGNOSIS AT BASELINE AND DURING FOLLOW UP

After written consent was obtained and immediately before treatment was given, females were requested to mail a vaginal flush sample and a first void urine sample taken at home, as described previously.[5] Males were requested to mail a first void urine sample. The general practitioner gave the test kits to the patients. The test results recorded at this stage were designated "baseline results."

During follow up, the sampling kits—consisting of a vaginal pipette for taking a vaginal flush sample for females, and a tube for taking a first void urine sample for both sexes—were mailed directly to the patients' addresses at 2, 4, 8, 12, and 24 weeks after antibiotic treatment. The samples were taken by the patients at home and mailed by ordinary mail to the department of clinical microbiology in prepaid and preaddressed envelopes. All patients with positive test results at 12 or 24 weeks were prescribed a new course of antibiotic treatment by their general practitioners.

A total of 210 test kits (150 kits for female patients and 60 for male patients) were sent by the laboratory, of which 176 (84%) (87% for female patients and 75% for male patients) were returned to the laboratory for testing. Twenty six of the 42 patients completed all tests.

CLINICAL DATA

The patients and the general practitioner filled out a baseline questionnaire together as part of the consultation at which partner tracing and treatment were discussed. Patients received a new questionnaire together with the home sampling kit supplied at week 24. Table 1 lists the questions asked.

DEFINITIONS

Females were considered "test positive" if the urine sample, the vaginal flush sample or both samples tested positive. Males were considered test positive if the urine sample tested positive. Recurrence of genital *C trachomatis* infection was defined as a positive test following one or more negative tests.

STATISTICS

Data were analysed in a life table, taking into account censored cases, to estimate the cumulated incidence in the study population. A confidence interval of approximately 95% was calculated using the Greenwood formula. Patients were considered at risk of acquiring a recurrent infection after one negative test. Test results were included in the calculation until recurrent positive infection or "test not performed." From the time when one test was not performed, the patient in question was considered a censored case for the rest of the study period.

The data from the questionnaires were analysed using a Mantel–Haenszel analysis, stratified by time. In the statistical analysis, follow up time was calculated as the period from week 0 to the week including one of the following events: recurrent infection as defined above, end of study at week 24, or test not performed.

ETHICS

The study was approved by the local ethics committee of the county of Ringkjøbing. Informed consent was obtained from all patients and the study met the requirements of Helsinki Declaration II.[9]

Results

Test results for the entire study population are shown in table 2.

Six patients (three males and three females) had negative test results at baseline—that is, the samples taken by the subjects at home before the start of antibiotic therapy. In addition, one female patient had a negative urine sample but a positive vaginal flush sample at baseline, and the reverse was the case for one other female patient (data not shown).

Of 37 patients available for assessment at week 2, four (three females, one male), (11%), still tested positive. The sample from one of these was still positive at week 4 but negative when tested 8 weeks after antibiotic therapy.

Table 1 Patient data and data from questionnaires

	Patients with recurrent infection	*Patients with no recurrent infection*	*OR/p value*
Mean age (years)	22.3	22.3	0.67*/0.61
Sex, number of females	7/8 (88%)	23/34 (68%)	2.3/0.41
Questionnaire, week 0:			
(1) Symptomatic infection?	5/8	17/34	1.5/0.63
(2) Antibiotic treatment with azithromycin?	7/8	28/34	0.38/0.38
(3) Previous infection with *C trachomatis*?	3/8	12/34	1.2/0.84
(4) Other sexually acquired disease, previous or present?	3/8	3/34	6.1/0.03
(5) Regular intimate relationship?	5/8	24/34	0.52/0.37
Questionnaire, week 24:			
(1) Use of condom?	1/8	5/25	0.65/0.68
(2) Use of condom 1 week after treatment?	3/7	15/22	0.71/0.68
(3) Regular intimate relationship?	7/8	19/25	2.0/0.51
(4) Partner tracing performed?	6/7	22/23	0.39/0.37
(5) Number of partners during the past 24 weeks?	2.00	1.64	1.8†/0.44
(6) New regular intimate relationship during follow up?	3/7	2/12	3.2/0.15

The data were analysed using a Mantel–Haenszel analysis stratified by time. Not all patients completed the questionnaire at week 24

*Divided into two groups: first group younger than 23 years of age, second group 23 years or older.

†Divided into two groups: one group with two or more partners, and the other with fewer than two partners.

Table 2 Complete presentation of patient data

No of patients	*Baseline*	*Week 2*	*Week 4*	*Week 8*	*Week 12**	*Week 24*
Females						
12	+	−	−	−	−	−
3	+	−	−	−	−	+
2	+	−	−	ND	ND	ND
2	+	−	+	+	+	−
1	+	−	−	−	ND	−
1	+	+	+	−	ND	ND
1	+	+	ND	ND	ND	ND
1	+	ND	−	−	−	ND
1	+	−	+	−	+	−
1	−	−	−	−	−	+
1	+	−	−	−	−	ND
1	−	−	ND	−	−	−
1	+	−	ND	−	−	−
1	+	+	−	−	−	−
1	−	ND	−	−	−	−
Males						
4	+	−	−	−	−	−
1	−	−	−	−	+	−
1	+	−	−	ND	ND	−
1	−	−	−	−	−	−
1	+	−	−	−	−	ND
1	−	ND	ND	ND	ND	ND
1	+	+	ND	−	−	−
1	+	ND	−	−	−	−
1	+	ND	ND	ND	ND	ND

ND = not done.
*New antibiotic treatment given if the patient tested positive at week 12.
Samples collected by the patients themselves before antibiotic therapy are designated "Baseline." For the women, a positive test result (marked +) means a positive urine sample, a positive vaginal flush sample, or both. For men, it means a positive urine sample.

Recurring infection was identified in three patients at week 4, in none at week 8, in one at week 12, and in four at week 24.

Recurrent infection was thus observed in eight of the 42 patients during the 24 weeks of follow up, thereby resulting in a cumulated incidence of 29% (95% CI: 12%–46%).

Data from the questionnaires are presented in table 1. Recurrent infection was more likely to occur in patients with a current or previous sexually acquired disease other than *C trachomatis*, 3/8 compared with 3/34 (OR 6.1, p=0.03) of the subjects previously or currently had such an infection. In all cases but one, the disease in question was condyloma acuminatum, the remaining case being herpes genitalis. There was no statistical difference between the two groups concerning any other clinical data from the two questionnaires—that is, number of patients with symptomatic infection, antibiotic treatment, previous infection with *C trachomatis*, regular intimate relationship, use of condom, partner tracing, and number of partners. Partner tracing was carried out in most cases in both groups. Six of seven patients with recurrent infection and 22 of 23 patients without recurrent infection confirmed that partner tracing had been performed. The remaining 12 patients did not answer this question. Partners were either treated blind—that is, they were given antibiotic treatment without previous testing, or tested for infection and treated if they tested positive.

Discussion

A large proportion of the patients in our study experienced a recurrent infection several weeks or months after antibiotic therapy and after having produced a negative test. Thus, the cumulated incidence of recurrent *C trachomatis* infection was 29% during 24 weeks of follow up.

These findings support those of previous studies. A retrospective study from a prenatal clinic found a recurrent infection rate of 32% during pregnancy and the puerperal period, as opposed to 5.7% in a control group of patients who tested negative at baseline.[2] Other investigators have found recurrence rates of 16.8% during the same pregnancy[3] and 38.4% primarily within 9 months of the initial infection.[4] A recent retrospective study of patients attending a sexually transmitted disease clinic concluded that prevention programmes should recommend repeated testing of all women with previous *C trachomatis* infection.[10]

Opinion is divided regarding at what time after treatment retesting should be performed. In this study, 89% of the patients tested negative for *C trachomatis* 2 weeks after treatment and all patients tested negative at some point during the first 4–8 weeks of follow up. Our data concerning short term follow up—that is, 2–4 weeks after treatment, are similar to the findings of two recent studies.[11 12] All recurrent infections in our study would have been detected by testing the patients at weeks 12 and 24 only. Since all patients with positive test results were given a new course of antibiotic treatment at week 12, we were unable to evaluate whether, without treatment, they would have continued to test positive at week 24. On the basis of these data we recommend that retesting should be carried out no sooner than 12 and/or 24 weeks after antibiotic therapy.

Eighty four per cent of the test kits sent to the patients were returned to the laboratory. The strategy for follow up—that is, involving either samples taken by a doctor or samples collected by the patients themselves and mailed to the laboratory, needs further evaluation. However, the high level of compliance in our study suggests that patients found the option of taking the samples themselves to be convenient. This may thus be a promising strategy for retesting.

A significant strength of our study is the study population itself, which consists of otherwise unselected patients from general practice. In Denmark, as in many other countries, nearly all *C trachomatis* infections are diagnosed and treated in general practice. Furthermore, this is a prospective study in which the patients are tested several times during 24 weeks of follow up.

A drawback to the study is the size of the study population—only 42 out of 141 patients initially identified as positive. This may be a consequence of the procedure required for inclusion in the study—that is, a visit to the doctor's office instead of a telephone call, or it may reflect unwillingness to participate in a study regarding sexually transmitted infections. Symptomatic infection may well have been a factor that motivated patients to participate in the study, as more than half of the patients who enrolled displayed symptoms. This is a higher number than expected and may constitute a bias, as most *C trachomatis* infections are asymptomatic.

Six of the 42 patients tested negative at baseline—that is, the samples taken by the

patients themselves before the initiation of antibiotic therapy. All patients had tested positive before entering the study—that is, they had tested positive to the tests taken by the doctor, since this was a primary inclusion criterion. The reason for this difference is not obvious, but if all six patients with negative baseline results were excluded, the cumulated incidence would still be high: 25% (95% CI: 7%–42%).

The cause of recurrence is unknown. Reinfection from the same partner(s), new infection acquired from new partner, or relapse after insufficient antibiotic treatment may all be explanations.

Taking into account the data from our questionnaires, a likely explanation of recurrence is higher risk behaviour. Although not significant, we found the recurrence group to contain a tendency towards patients with a higher number of partners, as well as a significantly higher number of patients with other sexually acquired diseases. Partner tracing is important in the prevention of reinfection and was carried out in most cases. Nevertheless, reinfection from the same partner(s) may have been an important source of reinfection. As regards relapse after antibiotic treatment, several studies[13 14] have shown that macrolides and tetracyclines are effective against *C trachomatis*, although the patients involved in these studies were considered cured if the organism could not be detected 4 weeks after therapy, and this follow up period may be too short. Further clarification of the question surrounding reinfection from the same partner, reinfection from a new partner, or treatment failure might be provided by genotyping *C trachomatis*.[15 16]

In conclusion, regardless of the mechanisms of recurrence, the incidence of recurrence is so high that retesting is advisable, but should be carried out no sooner than 12 and/or 24 weeks after antibiotic therapy. Home sampling is a promising strategy for retesting.

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