

ORIGINAL RESEARCH

Spontaneous clearance of *Chlamydia trachomatis* accounting for bacterial viability in vaginally or rectally infected women (FemCure)

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ABSTRACT

Objectives Spontaneous clearance of *Chlamydia trachomatis* (CT) infections can occur between diagnosis and treatment. We followed CT patients to assess clearance using a conventional definition (no total CT-DNA, assessed by routine quantitative PCR methods) and a definition accounting for viability, assessed by viability PCR testing.

Methods Three outpatient STI clinics included CT-diagnosed women (The Netherlands, 2016–2017, FemCure study); participants had vaginal CT (vCT) and rectal CT (rCT) (group A: n=155), vCT and were rectally untested (group B: n=351), single vCT (group C: n=25) or single rCT (group D: n=29). Follow-up (median interval 9 days) vaginal and rectal samples underwent quantitative PCR testing (detecting total CT-DNA). When PCR positive, samples underwent V-PCR testing to detect 'viable CT' (CT-DNA from intact CT organisms; V-PCR positive). 'Clearance' was the proportion PCR-negative patients and 'clearance of viable CT' was the proportion of patients testing PCR negative or PCR positive but V-PCR negative. We used multivariable logistic regression analyses to assess diagnosis group (A–D), age, days since initial CT test (diagnosis) and study site (STI clinic) in relation to clearance and clearance of viable CT.

Results Clearance and clearance of viable CT at both anatomic sites were for (A) 0.6% and 3.9%; (B) 5.4% and 9.4%; (C) 32.0% and 52.0% and (D) 27.6% and 41.4%, respectively. In multivariate analyses, women with single infections (groups C and D) had higher likelihood of clearance than women concurrently infected with vCT and rCT ($p < 0.001$).

Of rectally untested women (group B), 76.9% had total CT-DNA and 46.7% had viable CT (V-PCR positive) at the rectal site.

Conclusions Of untreated female vCT patients who had CT also at the rectal site, or who were rectally untested, only a small proportion cleared CT (in fact many had viable CT) at their follow-up visit (median 9 days). Among single site infected women clearance was much higher.

Trial registration number NCT02694497.

INTRODUCTION

Chlamydia trachomatis (CT) infection is the most commonly reported treatable bacterial STI in high-income countries, with over 100 million people affected each year.^{1,2} CT disproportionately affects women in terms of its occurrence and burden of

sequelae, including pelvic inflammatory disease, ectopic pregnancy, tubal infertility and chronic abdominal pain.^{3–5} However, spontaneous clearance of urogenital CT has been reported,⁶ between 19%–54% within 1 year^{7,8} and 6%–44% between diagnosis and treatment,^{9–13} using quantitative PCR tests (or nucleic acid amplification tests (NAATs)). Studies on rectal clearance are scarce, reporting 18.2% in men and women (2/11; median interval 11 days)¹⁴ and 4.0% (1/25) in men and 18.4% (7/38) in women (median interval 8 days).¹³ Research demonstrated lower CT bacterial load as the only determinant for clearance.¹³

The highly sensitive assays that are widely used for diagnosing CT detect both CT-DNA from intact CT organisms (indicative of viable CT) and CT-DNA from non-viable CT (eg, nucleic acid remnants). As current tests are unable to distinguish viable from non-viable organisms, a positive result may reflect non-viable CT. Hence, we may propose another definition of clearance to the detection of viable CT only (ie, to consider clearance having occurred when there is no more viable CT and ignore remnant bacterial DNA). Recently, we validated a PCR method for assessment of CT viability (V-PCR) in clinical swab samples.^{15,16} This method combines the high sensitivity and specificity of PCR with the ability to exclude detection of nucleic acid remnants from non-viable CT. It does so by incorporating a sample pretreatment step with a membrane impermeable DNA intercalating dye prior to molecular analysis blocking amplification of remnant CT-DNA from non-viable bacteria. This allows the V-PCR analyses to detect CT-DNA originating from intact (ie, viable) bacteria. Assessment of CT viability as well as evaluating both the vaginal and rectal site is vital to fill the current knowledge gaps regarding spontaneous CT clearance in women. Here, we assessed clearance of total CT-DNA and of viable CT in women diagnosed with rectal CT (rCT) or vaginal CT (vCT) (FemCure)¹⁷ when they returned to the clinic for treatment.

METHODS

Regular care STI clinic setting

The study population consisted of participants of the FemCure study,¹⁷ recruited at the STI clinics of the Public Health Services South Limburg, Rotterdam-Rijnmond and Amsterdam. The STI

clinics apply the same procedures, using different NAATs with similar high sensitivity¹⁸ (Limburg: Roche Cobas 4800, CT/NG PCR assay (Roche Diagnostics, Basel, Switzerland); Amsterdam: Aptima combo 2 CT/NG TMA assay (Hologic Inc, San Diego, USA); and Rotterdam-Rijnmond: ProbeTec CT/NG SDA assay (Becton Dickinson, Franklin Lakes, USA)). All women were tested for vaginal CT. Women were also tested rectally if they reported anal sex or anal symptoms.¹⁹ At follow-up, women with a positive rectal result were treated with a 7-day course of oral doxycycline 100 mg twice daily.¹⁹ Women who were vaginally positive and either not rectally tested or rectally negative received a 1 g single oral dose of azithromycin.

Study population

Women who were diagnosed with a vaginal or a rectal CT infection (April 2016–September 2017) were included when they returned to the clinic for treatment (here called: follow-up visit). Inclusion criteria were age 18 years and older; negative for HIV, syphilis and *Neisseria gonorrhoeae*; no antibiotic use in the past month; and not being pregnant.¹⁷

Participants were categorised into diagnosis groups based on their initial STI clinic diagnosis: (A) vCT and rCT positive, (B) vCT positive and rCT untested, (C) vCT positive and rCT negative and (D) vCT negative and rCT positive.

Follow-up visit: CT-DNA and viable CT testing

Participants self-collected samples (swabs) first at the rectal site and then at the vaginal site just prior to antibiotic treatment. These samples were analysed for the current report.

For each anatomic site, a first sample was collected for viability testing, and a second sample was collected for CT-DNA testing. The sample for viability testing was placed in 4 mL sucrose phosphate (2SP) transport buffer and immediately stored at -80°C to maintain CT viability until laboratory analysis. The sample for CT-DNA testing was placed in COBAS buffer and used to test for total CT-DNA with PCR (Roche Cobas 4800), according to manufacturers' guidelines. Of the women who tested positive in PCR (ie, when total CT-DNA was detected), samples were subsequently tested for viability with V-PCR. A positive V-PCR test indicates presence of CT-DNA from intact (viable) CT organisms; a negative V-PCR test indicates that CT-DNA from intact (viable) CT organisms was not detected (thus indicates presence of remnant CT-DNA (non-viable CT) only).^{15 17}

Definitions of clearance

Clearance was defined as a sample testing negative by PCR (no total CT-DNA).

Clearance of viable CT was defined as a sample testing either negative by PCR or testing positive by PCR but negative by V-PCR (non-viable CT only).

It should be noted that 'clearance' infers a transition from a positive status (a diagnosis at first visit) to a negative status (at follow-up, just prior to treatment). We acknowledge that the CT positive status was not confirmed in all women as in group B, rCT was untested. Also viable CT status was unknown at diagnosis as viability testing was not possible on the type of swabs collected in routine care. 'Clearance' is used throughout for ease of reading and clarified to address the above limitation when needed.

Statistical analyses

First, we present clearance proportions at the level of anatomical site (infection level). Second, we present proportions at

patient level and separately for the diagnosis groups A–D, as these groups internationally reflect common STI clinic testing practices. We used univariable and multivariable logistic regression to evaluate the independent association between clearance outcomes and diagnosis group (A–D), age, time since initial STI clinic test (categories based on tertiles), study site (STI clinic) and self-reported history of CT, considering an overall p value of $p < 0.05$ as statistically significant (and between 0.05 and 0.07 as borderline significant). We also presented univariable analyses for education, background and number of sex partners in the last 3 months.¹⁷ We used IBM SPSS Statistics V.24 for analyses.

RESULTS

Study population

In total, 560 women participated in the FemCure study. Based on the diagnostic (first) clinic visit, 531 women had vCT (ie, groups A, B and C), and 184 women had rCT (ie, groups A and D). Group A had 155 women with vCT and concurrent rCT, group B had 351 women with vCT and who were rCT untested, group C had 25 women with single vCT (rCT negative) and group D had 29 women with single rCT (vCT negative) (online supplementary table 1). Median age was 22 years, that is, for groups A: 23 years (IQR 21–25), group B: 22 years (IQR 20–24), group C: 24 years (IQR 21–25) and group D: 25 years (IQR 22–28).

Women returned for follow-up after a median of 9 days, that is, for group A: 10 days (IQR 8–13), group B: 8 days (IQR 7–11), group C: 10 days (IQR 8–14) and group D: 10 days (IQR 8–13).

Clearance of vCT in 531 vCT diagnosed women (infection level)

Clearance was 6.0% (95% CI 4.2 to 8.4) and clearance of viable CT was 11.7% (95% CI 9.1 to 14.7). In multivariable logistic regression, clearance was higher in vCT single infected women (group C), compared with women with concurrent rCT (group A), and in participants at the Rotterdam-Rijnmond clinic, compared with the Limburg clinic. Clearance of viable CT was higher in single vCT infected women (group C), in women aged 21–23 or 24 years and older, compared with women 18–20 years and (borderline significantly higher) in women with a history of CT (table 1).

Clearance of rCT in 184 rCT diagnosed women (infection level)

Clearance was 15.8% (95% CI 10.8 to 21.8) and clearance of viable CT was 35.9% (95% CI 28.9 to 43.3). Clearance was borderline significantly higher in women with single rCT (group D) compared with women with concurrent vCT (group A) and in women with a history of CT (table 2).

Clearance at both anatomic sites in 560 vCT or rCT diagnosed women (patient level)

Clearance was 6.4% (95%CI:4.5 to 8.8) and clearance of viable CT was 11.4% (95%CI:8.9 to 14.4). In multivariable logistic regression, proportions of both outcomes were higher in rectally untested patients (group B), and substantially higher in vCT or rCT single infected women (groups C, D). Clearance was also higher in participants at the Rotterdam-Rijnmond clinic, compared with the Limburg clinic. Clearance of viable CT was higher in groups B,C, and D (compared with group A) and in women aged 21–23 years or 24 years and older (compared with 18–20 years) (table 3).

Table 1 Proportions and odds ratios of clearance of total CT-DNA and clearance of viable CT at the vaginal site in 531 women who were diagnosed with vCT (FemCure)

	Clearance of total CT-DNA			Clearance of viable CT		
	n/N (%)	OR (95% CI)	aOR (95% CI)	n/N (%)	OR (95% CI)	aOR (95% CI)
Total	32/531 (6.0)			62/531 (11.7)		
Diagnosis group						
(A) vCT and rCT	3/155 (1.9)	1***	1***	10/155 (6.5)	1***	1***
(B) vCT (rCT not tested)	21/351 (6.0)	2.22 (0.95 to 10.98)	3.35 (0.93 to 12.04)	39/351 (11.1)	1.81 (0.88 to 3.73)	2.14 (0.99 to 4.62)
(C) vCT (rCT negative)	8/25 (32.0)	23.84 (5.77 to 98.49)	21.81 (4.94 to 96.27)	13/25 (52.0)	15.71 (5.70 to 43.26)	15.63 (5.37 to 45.51)
Years of age						
18–20	7/159 (4.4)	1	1	9/159 (5.7)	1*	1*
21–23	10/197 (5.1)	1.16 (0.43 to 3.12)	1.20 (0.43 to 3.37)	28/197 (14.2)	2.76 (1.26 to 6.04)	2.79 (1.24 to 6.30)
≥24	15/175 (8.6)	2.04 (0.81 to 5.13)	1.78 (0.66 to 4.78)	25/175 (14.3)	2.78 (1.26 to 6.15)	2.47 (1.07 to 5.70)
Days since initial CT diagnosis						
1–7	11/200 (5.5)	1	1	22/200 (11.0)	1	1
8–11	12/194 (6.2)	1.13 (0.49 to 2.63)	1.22 (0.50 to 2.97)	21/194 (10.8)	0.98 (0.52 to 1.85)	0.97 (0.49 to 1.90)
≥12	9/137 (6.6)	1.21 (0.49 to 3.00)	1.42 (0.52 to 3.91)	19/137 (13.9)	1.30 (0.68 to 2.51)	1.31 (0.63 to 2.73)
Study site (STI clinic)						
Limburg	6/188 (3.2)	1*	1*	17/188 (9.0)	1	1
Rotterdam-Rijnmond	17/160 (10.6)	3.61 (1.39 to 9.38)	3.71 (1.33 to 10.33)	22/160 (13.8)	1.60 (0.82 to 3.14)	1.60 (0.77 to 3.22)
Amsterdam	9/183 (4.9)	1.57 (0.55 to 4.50)	1.78 (0.58 to 5.48)	23/183 (12.6)	1.45 (0.75 to 0.81)	1.49 (0.71 to 3.10)
Self-reported history of CT						
Yes	15/151 (9.9)	2.36 (1.44 to 4.85)	1.55 (0.70 to 3.42)	28/151 (18.5)	2.32 (1.34 to 4.00)	1.74 (0.96 to 3.15)
No†	17/380 (4.5)	1**	1	34/380 (8.9)	1**	1‡
Education						
Low	16/195 (8.2)	1	na	28/195 (14.4)	1‡	na
Middle†	8/197 (4.1)	0.42 (0.17 to 1.02)		20/197 (10.2)	0.57 (0.29 to 1.12)	
High	8/139 (5.8)	0.77 (0.32 to 1.85)		14/139 (10.1)	1.13 (0.58 to 2.22)	
Background						
Western	27/489 (5.5)	1	na	54/489 (11.0)	1	na
Non-Western	5/42 (11.9)	2.31 (0.84 to 6.36)		8/42 (19.0)	1.90 (0.83 to 4.31)	
No. of sex partners last 3 months						
0 or 1	15/186 (8.1)	1		21/186 (11.3)	1	na
2 or 3	13/257 (5.1)	0.61 (0.28 to 1.31)		29/257 (11.3)	1.00 (0.55 to 1.84)	
≥4	4/88 (4.5)	0.54 (0.18 to 1.69)		12/88 (13.6)	1.24 (0.58 to 2.65)	

Clearance of total CT-DNA: at follow-up, no total CT-DNA detected at the vaginal site; clearance of viable CT: at follow-up, no total CT-DNA or only non-viable CT at the vaginal site.

*Overall p value 0.01 to <0.05, **overall p value 0.001 to <0.01, ***overall p value <0.001.

†The few cases with unknown information are attributed to this category in analyses.

‡Overall p value 0.05 to <0.07.

This model includes diagnosis group, age, days since initial CT test (diagnosis), and study site (STI clinic); CI: Confidence interval; viability polymerase chain reaction (V-PCR) is used to test for viable CT; na: variable not entered into the multivariable model to calculate adjusted odds ratio.

aOR, OR in the multivariable analyses; CT, *Chlamydia trachomatis*; OR, odds ratio in univariable analyses; rCT, rectal *Chlamydia trachomatis*; vCT, vaginal *Chlamydia trachomatis*.

Distribution of test results at follow-up (patient level)

While some women cleared total CT-DNA (see above), most women tested PCR positive at follow-up, and many tested V-PCR positive (indicating viable infections) (table 4). The proportion of vCT diagnosed women testing V-PCR positive at both the vaginal and rectal site at follow-up was 62.6% in group A (concurrent rCT) and 46.7% in group B (rectally untested). Of single vCT diagnosed women (rCT negative, group C), 36.0% (9/25) women rectally had CT-DNA at follow-up (two had viable CT).

DISCUSSION

In untreated women with a vaginal or rectal CT diagnosis visiting STI clinics, we assessed the spontaneous clearance on their follow-up visit for treatment (after a median of 9 days), using a conventional definition (no total CT-DNA) and a definition accounting for viability (no CT-DNA or only remnant

CT-DNA (non-viable CT)). This is the first prospective study in a large group of women evaluating both anatomic sites within the same patient and including viability testing. We demonstrated low proportions of clearance in vCT patients who had a concurrent rCT diagnosis or who were rectally untested. This was in contrast to proven single site infected women of whom many showed clearance. Almost half of women who were rectally untested in regular STI clinic care showed viable CT at the rectal site at the follow-up visit.

The clearance of CT-DNA at the vaginal site (6.0% in vCT diagnosed women; groups A, B and C) or at the rectal site (15.8% in rCT diagnosed women; groups B and D) is comparable with previous studies.^{6–14} Employing the definition of clearance of viable organism data increased proportions to 11.7% for the vaginal site and 39.5% for the rectal site. The higher clearance proportions observed at the rectal site (compared with the vaginal site) are notable. Possibly initial load at the rectal site is

Table 2 Proportions and odds ratios of clearance of total CT-DNA and clearance of viable CT at the rectal site in 184 women who were previously RCT diagnosed (FemCure)

	Clearance of total CT-DNA			Clearance of viable CT		
	n/N (%)	OR (95% CI)	aOR (95% CI)	n/N (%)	OR (95% CI)	aOR (95% CI)
Total	29/184 (15.8)			66/184 (35.9)		
Diagnosis group						
(A) vCT and rCT	21/155 (13.5)	1†	1†	54/155 (34.8)	1	1
(C) vCT (rCT negative)	8/29 (27.6)	2.43 (0.95 to 6.19)	2.74 (0.97 to 7.70)	12/29 (41.4)	1.32 (0.59 to 2.97)	1.34 (0.57 to 3.19)
Years of age						
18–20	6/41 (14.6)	1	1	12/41 (29.3)	1	1
21–23	10/63 (15.9)	1.10 (0.37 to 3.30)	1.14 (0.36 to 3.57)	25/63 (39.7)	1.59 (0.69 to 3.69)	1.67 (0.70 to 3.96)
≥24	13/80 (16.3)	1.13 (0.40 to 3.24)	0.96 (0.31 to 2.99)	29/80 (36.3)	1.37 (0.61 to 3.10)	1.31 (0.55 to 3.07)
Days since initial CT diagnosis						
1–7	7/43 (16.3)	1	1	16/43 (37.2)	1	1
8–11	13/72 (18.1)	1.13 (0.41 to 3.11)	0.89 (0.30 to 2.65)	29/72 (40.3)	1.14 (0.52 to 2.48)	0.91 (0.40 to 2.06)
≥12	9/69 (13.0)	0.77 (0.26 to 2.25)	0.58 (0.18 to 1.89)	21/69 (30.4)	0.74 (0.33 to 1.65)	0.57 (0.24 to 1.35)
Study site (STI clinic)						
Limburg	3/46 (6.5)	1	1	12/46 (26.1)	1	1
Rotterdam-Rijnmond	6/35 (17.1)	2.97 (0.69 to 18.82)	3.66 (0.79 to 16.99)	12/35 (34.3)	1.48 (0.57 to 3.86)	1.55 (0.57 to 4.17)
Amsterdam	20/103 (19.4)	3.45 (0.97 to 12.28)	4.43 (1.16 to 16.91)	42/103 (40.8)	1.95 (0.91 to 4.20)	2.25 (1.00 to 5.06)
Self-reported history of CT						
Yes	15/59 (25.4)	2.70 (1.21 to 6.06)	2.41 (1.04 to 5.60)	26/59 (44.1)	1.67 (0.89 to 3.17)	1.57 (0.81 to 3.04)
No‡	14/125 (11.2)	1*	1*	40/125 (32.0)	1	1
Education						
Low	8/57 (14.0)	1	na	20/57 (35.1)	1	na
Middle‡	13/62 (21.0)	1.63 (0.62 to 4.27)		25/62 (40.3)	1.25 (0.59 to 2.63)	
High	8/65 (12.3)	0.86 (0.30 to 2.46)		21/65 (32.3)	0.88 (0.42 to 1.87)	
Background						
Western	28/171 (16.4)	1	na	62/171 (36.3)	1	na
Non-Western	1/13 (7.7)	0.43 (0.05 to 3.41)		4/13 (30.8)	0.78 (0.23 to 2.64)	
No. of sex partners last 3 months						
0 or 1	10/57 (17.5)	1	na	15/57 (26.3)	1	na
2 or 3	15/88 (17.0)	0.97 (0.40 to 2.33)		38/88 (43.2)	2.13 (1.03 to 4.39)	
≥4	4/39 (10.3)	0.54 (0.16 to 1.86)		13/39 (33.3)	1.40 (0.58 to 3.41)	

Clearance of total CT-DNA: at follow-up, no total CT-DNA detected at the rectal site; clearance of viable CT: at follow-up, no total CT-DNA or only non-viable CT at the rectal site.

This model includes diagnosis group, age, days since initial CT test (diagnosis) and study site (STI clinic); viability polymerase chain reaction is used to test for viable CT; na (variable not entered into the multivariable model to calculate adjusted ORs).

*Overall p value 0.01 to <0.05, **overall p value 0.001 to <0.01, ***overall p value <0.001.

†Overall p value 0.05 to <0.07.

‡The few cases with unknown information are attributed to this category in analyses.

aOR, OR in the multivariable analyses; CI, Confidence Interval; CT, *Chlamydia trachomatis*; OR, OR in univariable analyses; rCT, rectal *Chlamydia trachomatis*; vCT, vaginal *Chlamydia trachomatis*.

lower, or mucosal lining cells are less susceptible to CT infection than vaginal mucosal cells.^{20 21}

At the patient level, clearance (at both anatomic sites) was low in vCT-diagnosed women who had concurrent rCT (clearance: 0.6%, clearance of viable CT: 3.9%). Clearance was also low in vCT-diagnosed women who were rCT untested (5.4%, 9.3%).²² Single vCT-infected (tested rCT negative) women, however, showed 32% clearance and 52% clearance of viable CT. It may be hypothesised that a diagnosed rCT is a marker for high viable CT loads at the vaginal site, when we assume that high vaginal CT loads are less likely to clear (as found previously¹³) and more likely to infect the rectal site (autoinoculation). Conversely, testing rCT negative would be a proxy for lower viable vaginal CT loads at diagnosis, with a higher likelihood of subsequent vaginal clearance. Unfortunately, we could not test this hypothesis as we did not have data on (viable) loads at diagnosis. Of note, two observations in our study (table 4) suggest autoinoculation from the vaginal to the rectal site: (1) viable CT at the rectal site was almost always observed in the copresence of viable CT at the vaginal site. (2) Of the 25 rCT negative and vCT positive patients (group C), 9 became rCT positive (6/9 reported no

recent anal sex), although this observation may also hint at the oral sex hypothesis.^{22 23}

Furthermore, age of 21–23 years or 24 years and older (compared with 18–20) and history of CT were independently associated with clearance of viable infections. These findings may reflect protective immunity that developed from repeated chlamydia exposures.¹¹ Finally, clearance proportions of vaginal total CT-DNA differed between study clinics, possibly explained by the different diagnostic NAAT tests used. Still, we consider this explanation unlikely as all used routine NAATs are highly sensitive and as STI clinics used the same NAAT for vaginal and rectal CT diagnostics (only differences in vaginal clearance were observed). Another unlikely explanation may be that clinics' served populations differed regarding their mean initial vaginal CT-DNA loads (as low load is predictive for clearance). We could not study this as information on initial CT load at time of diagnosis was unavailable.

The potential clinical implications of this study are that in the majority of female patients, visiting STI clinics, with vCT and a concurrent or untested rCT, the likelihood of clearance (median interval 9 days) is low. Thus, the risk of overtreatment

Table 3 Proportions and ORs of clearance of total CT-DNA and clearance of viable CT at the vaginal and the rectal site in 560 women who were previously diagnosed with vCT or rCT in FemCure

	Clearance of total CT-DNA			Clearance of viable CT		
	n/N (%)	OR (95% CI)	aOR (95% CI)	n/N (%)	OR 95% CI	aOR (95% CI)
	36/560 (6.4)			64/560 (11.4)		
Diagnosis group						
(A) vCT and rCT	1/155 (0.6)	1***	1***	6/155 (3.9)	1***	1***
(B) vCT (rCT not tested)	19/351 (5.4)	8.81 (1.17 to 66.43)	8.52 (1.10 to 65.96)	33/351 (9.4)	2.58 (1.06 to 6.28)	2.80 (1.11 to 7.10)
(C) vCT (rCT negative)	8/25 (32.0)	72.47 (8.54 to 614.95)	85.45 (9.49 to 769.34)	13/25 (52.0)	26.90 (8.67 to 83.46)	28.98 (8.83 to 95.12)
(D) rCT (vCT negative)	8/29 (27.6)	58.67 (6.98 to 492.82)	72.22 (8.18 to 637.54)	12/29 (41.4)	17.53 (5.83 to 52.71)	17.07 (5.49 to 53.10)
Years of age						
18–20	7/162 (4.3)	1#	1	7/162 (4.3)	1**	1*
21–23	10/204 (4.9)	1.14 (0.43 to 3.07)	1.11 (0.38 to 3.21)	26/204 (12.7)	3.23 (1.37 to 7.66)	3.36 (1.36 to 8.32)
≥24	19/194 (9.8)	2.40 (0.98 to 5.87)	1.58 (0.59 to 4.27)	31/194 (16.0)	4.21 (1.80 to 9.84)	3.20 (1.29 to 7.95)
Days since initial CT diagnosis						
1–7	12/207 (5.8)	1	1	23/207 (11.1)	1	1
8–11	16/205 (7.8)	1.38 (0.63 to 2.99)	1.58 (0.68 to 3.69)	24/205 (11.7)	1.06 (0.58 to 1.95)	1.02 (0.52 to 1.99)
≥12	8/148 (5.4)	0.93 (0.37 to 2.33)	0.93 (0.32 to 2.69)	17/148 (11.5)	1.04 (0.53 to 2.02)	0.92 (0.42 to 2.00)
Study site (STI clinic)						
Limburg	6/199 (3.0)	1**	1**	17/199 (8.5)	1	1
Rotterdam-Rijnmond	19/164 (11.6)	4.22 (1.64 to 10.82)	6.06 (2.13 to 17.25)	23/164 (14.0)	1.75 (0.90 to 3.39)	2.09 (0.99 to 4.40)
Amsterdam	11/197 (5.6)	1.90 (0.69 to 5.25)	2.43 (0.81 to 7.29)	24/197 (12.2)	1.49 (0.77 to 2.86)	1.63 (0.78 to 3.42)
Self-reported history of CT						
Yes	15/165 (9.1)	1.78 (0.89 to 3.55)	0.95 (0.44 to 2.09)	28/165 (17.0)	2.04 (1.20 to 3.47)	1.33 (0.73 to 2.42)
No†	21/395 (5.3)	1	1	36/395 (9.1)	1**	1
Education						
Low	16/203 (7.9)	1	na	29/203 (14.3)	1	na
Middle‡	11/208 (5.3)	0.65 (0.30 to 1.44)		18/208 (8.7)	0.57 (0.31 to 1.06)	
High	9/149 (6.0)	0.75 (0.32 to 1.75)		17/149 (11.4)	0.77 (0.41 to 1.47)	
Background						
Western	31/57 (6.0)	1	na	57/517 (11.0)	1	na
Non-Western	5/43 (11.6)	2.06 (0.76 to 5.61)		7/43 (16.3)	1.57 (0.67 to 3.69)	
No. of sex partners last 3 months						
0 or 1	15/195 (7.7)	1	na	21/195 (10.8)	1	na
2 or 3	17/269 (6.3)	0.81 (0.39 to 1.66)		31/269 (11.5)	1.08 (0.60 to 1.94)	
≥4	4/96 (4.2)	0.52 (0.17 to 1.62)		12/96 (12.5)	1.18 (0.56 to 2.52)	

Clearance of total CT-DNA: at follow no total CT-DNA detected at both anatomic sites; clearance of viable CT: at follow-up no total CT-DNA detected or only non-viable CT detected at both anatomic sites.

This model includes diagnosis group, age, days since initial CT test (diagnosis), and study site (STI clinic); viability polymerase chain reaction is used to test for viable CT; na (variable not entered into the multivariable model to calculate adjusted OR).

*Overall p value 0.01 to <0.05, **overall p value 0.001 to <0.01, ***overall p value <0.001.

†Overall p value 0.05 to <0.07.

‡The few cases with unknown information are attributed to this category in analyses.

aOR, odds ratio in the multivariable analyses; CI, Confidence Interval; CT, *Chlamydia trachomatis*; OR, OR in univariable analyses; rCT, rectal *Chlamydia trachomatis*; vCT, vaginal *Chlamydia trachomatis*.

will be very low. When one is interested to restrict treatment to viable infections only, the lower viable CT detection in single site CT-infected women and in those aged >20 years is notable, but these predictors lack specificity to guide treatment policy. Perhaps the most important direct implication for clinical practice can be derived from our finding that in rectally untested women (group B), 87.5% (n=272/351) had CT-DNA and 48.4% (n=170/351) had viable rectal CT at follow-up (table 4). This is indicative of large numbers of missed (viable) rectal infections when women present for vaginal CT treatment. In clinical practice, most women remain rectally untested, missing two-thirds of rectal CT infections when diagnosing vCT.^{24 25} It may be a problem when these rectally untested (but rCT positive) women are treated with azithromycin for their vCT, as azithromycin is less effective than doxycycline for rCT.^{26 27} In some of the Dutch STI clinics, and in some international guidelines, universal doxycycline treatment for CT infections in women has

been adopted, potentially tackling the aforementioned issue. It should be noted that clinical implications of CT-DNA and viable CT at the rectal site (in terms of 'true infections', transmission-potential, morbidity) are yet unknown, and we also do not know whether the clinical impact of rectal infections would be affected by the underlying route of infection (eg, autoinoculation).

This study has several limitations. (1) We did not have information on viable CT at time of initial diagnosis. V-PCR analyses is not possible on routinely collected materials as it requires a specific transport buffer and immediate storage in -80°C, precluding the assessment of viability in current routine clinical care diagnosis. Therefore, as in all clearance studies, the starting point was CT diagnosis by regular care tests (assessing total CT-DNA). Thus, the observed proportions of non-viable CT may be an overestimation when interpreted as the transition from viable to non-viable. (2) As in all human clearance studies, the moment of infection is unknown. To aid interpretation,

Table 4 Distribution of CT-DNA and viable CT at the vaginal and the rectal site at the follow-up visit (just prior to treatment) a median of 9 days later, in 560 women who were previously CT diagnosed (FemCure)

	Group A	Group B	Group C	Group D
	vCT and rCT	vCT (rCT untested)	vCT (rCT negative)	rCT (vCT negative)
	n=155, n (%)	n=351, n (%)	n=25, n (%)	n=29, n (%)
CT-DNA (PCR positive) at follow-up				
No CT-DNA at vaginal and rectal site	1 (0.6)	19 (5.4)	8 (32.0)	8 (27.6)
CT-DNA detected at the vaginal site only	20 (12.9)	60 (17.1)	8 (32.0)	0 (0)
CT-DNA detected at the rectal site only	2 (1.3)	2 (0.6)*	0 (0)	21 (72.4)
CT-DNA detected at the vaginal and rectal site	132 (85.2)	270 (76.9)*	9 (36.0)†	0 (0)
Viable CT (PCR and V-PCR positive) at follow-up				
No CT-DNA or only non-viable CT at vaginal and rectal site	6 (3.9)	33 (9.4)	13 (52.0)	12 (41.4)
CT-DNA and viable CT at the vaginal site only	48 (31.0)	148 (42.2)	10 (40.0)	0 (0)
CT-DNA and viable CT at the rectal site only	4 (2.6)	6 (1.7)*	0 (0)	17 (58.6)
CT-DNA and viable CT at the vaginal and rectal site	97 (62.6)	164 (46.7)*	2 (8.0)†	0 (0)

CT-DNA is assessed with a quantitative PCR; viable CT is assessed with a viability PCR.

*The rectal site was initially not tested, thus could be positive or negative. The fact that at follow-up (just prior to treatment), some women rectally had CT-DNA or viable CT-DNA suggests that in those women the rectal site was initially positive (350/351 patients reported no anal sex in 2 weeks prior to treatment).

†The rectal site was initially tested CT-DNA negative. The fact that at follow-up (just prior to treatment), women rectally had CT-DNA or viable CT-DNA suggests that new infections may have occurred at the rectal site between diagnosis and treatment (6/9 patients reported no anal sex in the 2 weeks prior to treatment).

rCT, rectal *Chlamydia trachomatis*; vCT, vaginal *Chlamydia trachomatis*.

antibody detection could be useful to exclude individuals who were exposed to CT but who were not infected,²⁸ and CT load at diagnosis could help to predict spontaneous clearance,¹³ but samples to collect such data were unavailable. (3) The exact day of clearance is unknown, as we did not collect daily samples. (4) Clearance may differ by genotype,^{29–31} but genotyping was not performed on the diagnosis study samples. (5) Genotyping would also be valuable to exclude reinfections by other genotypes, as positive results at follow-up were classified as ‘not cleared’ or ‘viable’ in this study. (6) Possibly, some initial test-positive results might be false positive. Also, using a single sensitive PCR test to assess clearance could have resulted in detecting false positives or false negatives.³² ‘On off effects’, that is, a positive and negative test result in the same sample, might occur in samples with a low bacterial load around the detection limit. Related to this is the possibility of the initial test not being a ‘true infection’, but rather reflecting transient CT-DNA. This is also referred to as ‘passive infection’.^{33 34} We could not determine to what extent such a phenomenon was present in our STI clinics population and to what extent it may have affected our results. Furthermore, we could not estimate the duration of infection as,

at diagnosis, the date of last sex (ie, exposure) was unknown. (7) The women sampled first the swab for V-PCR testing, and second the swab for PCR testing. In theory, the second swab may have had a lower bacterial load affecting clearance rates. However, we feel that such risk is minimal as the PCR test for CT-DNA test is highly sensitive. (8) We cannot entirely rule out contamination of swabs during sampling, for example, contamination of a rectal swab with vaginal secretions, despite clear written and visual instructions. (9) In the FemCure study sample, women with high education, without a previous STI or non-Western migrant background were under-represented compared with CT infected STI clinic women.²⁷ However, these characteristics were not associated with clearance overall, and therefore, we think that these differences do not impact the internal validity of the study. (10) Our study population was confined by exclusion criteria, such as <18 years or STI coinfection, so inferences to excluded subsets are not possible. (11) Caution should be taken when making population inferences from the frequency distributions of FemCure’s baseline characteristics, as STI clinic populations may change over time due to policy changes. However, this may not be a substantial problem as we examined diagnosis groups, based on current testing practice. Lastly, STI clinic populations may not represent the general population.

To conclude, spontaneous clearance occurs at the vaginal and the rectal site and is more likely in women infected with CT at a single anatomic site. However, clearance is low in vCT diagnosed female patients who were concurrently diagnosed with rCT or were rCT untested. In fact, these patients commonly showed viable CT at the follow-up visit. These findings might have diagnostic and therapeutic implications for women tested for CT.

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Key messages

- ▶ In the understudied population of women (560 *Chlamydia trachomatis* (CT) diagnosed patients), we assessed spontaneous clearance (median follow-up of 9 days) of CT infections at the vaginal and the rectal site and including viability testing.
- ▶ Women infected with CT at a single anatomic site CT had the highest likelihood to clear CT-DNA and to clear viable CT.
- ▶ In vaginal CT diagnosed female patients, clearance is low when they also were concurrently diagnosed with rectal CT or when they were rectally untested; in fact these patients commonly showed viable CT at both anatomic sites at follow-up.
- ▶ These findings might have diagnostic and therapeutic implications for women tested for CT.

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REFERENCES

- Unemo M, Bradshaw CS, Hocking JS, *et al.* Sexually transmitted infections: challenges ahead. *Lancet Infect Dis* 2017;17:e235–79.
- Senior K. Chlamydia: a much underestimated STI. *Lancet Infect Dis* 2012;12:517–8.
- Haggerty CL, Gottlieb SL, Taylor BD, *et al.* Risk of Sequelae after *Chlamydia trachomatis* Genital Infection in Women. *J Infect Dis* 2010;201:134–55.
- Hoenderboom BM, van Benthem BHB, van Bergen JEAM, *et al.* Relation between *Chlamydia trachomatis* infection and pelvic inflammatory disease, ectopic pregnancy and tubal factor infertility in a Dutch cohort of women previously tested for chlamydia in a chlamydia screening trial. *Sex Transm Infect* 2019;95:300–6.
- den Heijer CDJ, Hoebe CJPA, Driessen JHM, *et al.* Chlamydia trachomatis and the risk of pelvic inflammatory disease, ectopic pregnancy, and female infertility: a retrospective cohort study among primary care patients. *Clin Infect Dis* 2019;69:1517–25.
- Geisler WM, untreated Dof. Duration of Untreated, Uncomplicated *Chlamydia trachomatis* Genital Infection and Factors Associated with Chlamydia Resolution: A Review of Human Studies. *J Infect Dis* 2010;201:104–13.
- Molano M, Meijer CJLM, Weiderpass E, *et al.* The Natural Course of *Chlamydia trachomatis* Infection in Asymptomatic Colombian Women: A 5-Year Follow-Up Study. *J Infect Dis* 2005;191:907–16.
- Morré SA, van den Brule AJC, Rozendaal L, *et al.* The natural course of asymptomatic *Chlamydia trachomatis* infections: 45% clearance and no development of clinical PID after one-year follow-up. *Int J STD AIDS* 2002;13 Suppl 2:12–18.
- Geisler WM, Wang C, Morrison SG, *et al.* The natural history of untreated *Chlamydia trachomatis* infection in the interval between screening and returning for treatment. *Sex Transm Dis* 2008;35:119–23.
- JOYNER JL, DOUGLAS JM, FOSTER M, *et al.* Persistence of *Chlamydia trachomatis* infection detected by polymerase chain reaction in untreated patients. *Sex Transm Dis* 2002;29:196–200.
- Sheffield JS, Andrews WW, Klebanoff MA, *et al.* Spontaneous resolution of asymptomatic *Chlamydia trachomatis* in pregnancy. *Obstetrics & Gynecology* 2005;105:557–62.
- Geisler WM, Lensing SY, Press CG, *et al.* Spontaneous resolution of genital *Chlamydia trachomatis* infection in women and protection from reinfection. *J Infect Dis* 2013;207:1850–6.
- van Liere G, Hoebe C, Dirks JA, *et al.* Spontaneous clearance of urogenital, anorectal and oropharyngeal *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in women, MSM and heterosexual men visiting the STI clinic: a prospective cohort study. *Sex Transm Infect* 2019.
- Apewokin SK, Geisler WM, Bachmann LH. Spontaneous resolution of extragenital chlamydial and gonococcal infections prior to therapy. *Sex Transm Dis* 2010;37:1–4.
- Janssen KJH, Hoebe CJPA, Dukers-Muijers NHTM, *et al.* Viability-PCR shows that NAAT detects a high proportion of DNA from non-viable *Chlamydia trachomatis*. *PLoS One* 2016;11:e0165920.
- Janssen KJH, Wolffs P, Lucchesi M, *et al.* Assessment of rectal *Chlamydia trachomatis* viable load in women by viability-PCR. *Sex Transm Infect* 2019;pii: sextans-2019-054002.
- Dukers-Muijers NHTM, Wolffs PFG, Eppings L, *et al.* Design of the FemCure study: prospective multicentre study on the transmission of genital and extra-genital *Chlamydia trachomatis* infections in women receiving routine care. *BMC Infect Dis* 2016;16:381.
- National Coordination for Infection Control (LCI). Procedure for the sexual health consultation. Available: <https://lci.rivm.nl/draaiboeken/consult-seksuele-gezondheid> [Accessed 4 Sep 2019].
- Lanjouw E, Ouburg S, de Vries HJ, *et al.* 2015 European guideline on the management of *Chlamydia trachomatis* infections. *Int J STD AIDS* 2016;27:333–48.
- Dukers-Muijers NHTM, Speksnijder AGCL, Morré SA, *et al.* Detection of anorectal and cervicovaginal *Chlamydia trachomatis* infections following azithromycin treatment: prospective cohort study with multiple time-sequential measures of rRNA, DNA, quantitative load and symptoms. *PLoS One* 2013;8:e81236.
- Dirks JAMC, van Liere GAFS, Hoebe CJPA, *et al.* Genital and anal *Chlamydia trachomatis* bacterial load in concurrently infected women: a cross-sectional study. *Sex Transm Infect* 2019;95:317–21.
- Yeruva L, Spencer N, Bowlin AK, *et al.* Chlamydial infection of the gastrointestinal tract: a reservoir for persistent infection. *Pathog Dis* 2013;68:88–95.
- Leenen J, van Liere GAFS, Hoebe CJPA, *et al.* A longitudinal study to investigate previous *Chlamydia trachomatis* infection as a risk factor for subsequent anorectal infection in men who have sex with men (MSM) and women visiting STI clinics in the Netherlands. *Epidemiol Infect* 2019;147:e214.
- Chandra NL, Broad C, Folkard K, *et al.* Detection of *Chlamydia trachomatis* in rectal specimens in women and its association with anal intercourse: a systematic review and meta-analysis. *Sex Transm Infect* 2018;94:320–6.
- Dukers-Muijers NHTM, Schachter J, van Liere GAFS, *et al.* What is needed to guide testing for anorectal and pharyngeal *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in women and men? evidence and opinion. *BMC Infect Dis* 2015;15:533.
- Geisler WM. Diagnosis and Management of Uncomplicated *Chlamydia trachomatis* Infections in Adolescents and Adults: Summary of Evidence Reviewed for the 2015 Centers for Disease Control and Prevention Sexually Transmitted Diseases Treatment Guidelines. *Clin Infect Dis*. 2015;61:S774–84.
- Dukers-Muijers N, Wolffs PFG, De Vries H, *et al.* Treatment effectiveness of azithromycin and doxycycline in uncomplicated rectal and vaginal *Chlamydia trachomatis* infections in women: a multicentre observational study (FemCure). *Clin Infect Dis* 2019.
- Bakshi RK, Gupta K, Jordan SJ, *et al.* Immunoglobulin-Based investigation of spontaneous resolution of *Chlamydia trachomatis* infection. *J Infect Dis* 2017;215:1653–6.
- Geisler WM, Black CM, Banda CI, *et al.* *Chlamydia trachomatis* OmpA genotyping as a tool for studying the natural history of genital chlamydial infection. *Sex Transm Infect* 2008;84:541–4. discussion 4–5.
- Götz HM, RJM B, Wolfers MEG, *et al.* Use of *Chlamydia trachomatis* high-resolution typing: an extended case study to distinguish recurrent or persistent infections from new infection. STI 2013.
- Versteeg B, Bruisten SM, Heijman T, *et al.* Monitoring therapy success of urogenital *Chlamydia trachomatis* infections in women: a prospective observational cohort study. *PLoS One* 2017;12:e0185295.

- 32 Dukers-Muijters NHTM, Morré SA, Speksnijder A, *et al.* Chlamydia trachomatis test-of-cure cannot be based on a single highly sensitive laboratory test taken at least 3 weeks after treatment. *PLoS One* 2012;7:e34108.
- 33 Price MJ, Ades AE, Soldan K, *et al.* The natural history of Chlamydia trachomatis infection in women: a multi-parameter evidence synthesis. *Health Technol Assess* 2016;20:1–250.
- 34 Lewis J, Price MJ, Horner PJ, *et al.* Genital Chlamydia trachomatis infections clear more slowly in men than women, but are less likely to become established. *J Infect Dis* 2017;216:237–44.